EFFECT OF INDOLE-3-ACETIC ACID (IAA) ON BIOMASS PRODUCTION OF OYSTER MUSHROOM (*Pleurotus sajor-caju*)

CHUAH PEI NING

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

CROP PRODUCTION PROGRAM
SCHOOL OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2011

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: EFFECT OF INDOLE-3-ACETIC ACID ON BIOMASS PRODUCTION OF OYSTER MUSHROOM (*Pleurotus sajor-caju*)

IJAZAH: DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

SAYA: CHUAH PEI NING (HURUF BESAR) SESI PENGAJIAN: 2010/2011

Mengaku membenarkan tesis * (LPSM/~~Sarjana/Doktor Falsafah~~) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

☐	SULIT	(Mengandungi maklumta yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana Penyelidikan dijalankan)
☑	TIDAK TERHAD	

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

Disahkan Oleh:

NURULAIN BINTI ISMAIL
LIBRARIAN
UNIVERSITI MALAYSIA SABAH

(TANDATANGAN PENULIS)

Alamat Tetap: BLOK 3H-1-1, TINGKAT PAYA TERUBONG 3, 11060 PULAU PINANG

Tarikh: 4/5/2011

(TANDATANGAN PENYELIA)

PROF. MADYA DR. MARKUS ATONG
PENASIHAT AKADEMIK
Sekolah Pertanian Lestari
Universiti Malaysia Sabah
(NAMA PENYELIA DAN COP)

Tarikh: 4/5/2011

Catatan: - * Potong yang tidak berkenaan.

** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak yang berkuasa/organisasi berkenaan dengan menyatakan sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT atau TERHAD.

Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara penyelidikan atau disertasi bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM)

DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree at this or any other university.

CHUAH PEI NING
BR07110070
20 April 2011

VERIFICATION

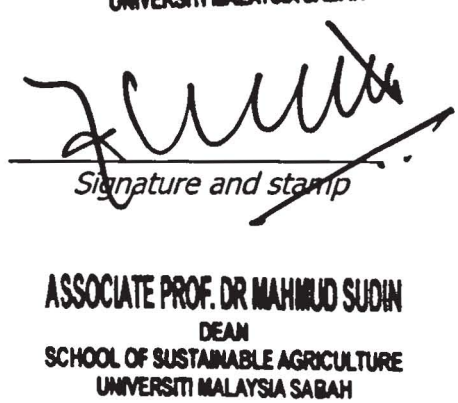
VERIFIED BY

1. Assoc. Prof. Dr. Markus Atong
   SUPERVISOR

2. Dr. Suzan Benedick
   EXAMINER 1

3. Assoc. Prof. Dr. Mahmud Sudin
   EXAMINER 2

4. Assoc. Prof. Dr. Mahmud Sudin
   DEAN
   SCHOOL OF SUSTAINABLE AGRICULTURE



ACKNOWLEDGEMENT

First of all, I would like to convey my deepest gratitude to my supervisor, Associate Prof. Dr. Markus Atong for guiding me throughout my project. Furthermore, it is my pleasure to acknowleged Assoc. Prof. Dr. Harpal Singh Saini, Ms. Chee Fong Tyng and Mr. Lum Mok Sam for their invaluable help, advice, idea, teachings and encouragement.

I also would like to express my sincere thanks to Mdm. Christina Kungin, Mdm. Ahjia bt Osman, Mr. Malcolm Boxey Jilimin, Mr. Zilpiree Jaabi and Mr. Ahmad bin Manik for their kind assistance and patience in helping me go through my project.

Besides that, I would like to dedicate my special thanks to all students and staff who involved direct and indirectly by giving me assistance, discussion and moral support along the progression of my final year project and dissertation wrtiting.

Last but not least, I would like to express my heartfelt thanks to my lovely and sweet family and friends for their endless supports, love and care. Their continuous support and encouragement have kept me going on throughout my hard time in the whole progress of this project. Without the help and support from all of you, I would not be able to finish this project. Once again, it was my pleasure and honor to have the support from all of you. Thank you very much!

ABSTRACT

Biomass parameters (mycelia diameter, weight and protein content) of oyster mushroom (*Pleurotus sajor-caju*) from Kota Kinabalu, Sabah were studied using Indole-3-Acetic Acid (IAA) treatments method. There were no significant differences ($P>0.05$) in terms of mycelia weight and protein content corresponding to different concentration of IAA treatments. Despite no significant difference, but treatment with 5 mg/L of IAA concentration give relatively, a maximum increase in mycelia diameter and protein content. The higher concentration of IAA treatment gives the most enhancements in increasing cell wall plasticity so as a better regulation of specific protein synthesis. These results warrant further study and analysis still need to be carried out in order to improve understanding on the effectiveness and economical concerns on the usage and application of IAA as a naturally occurring plant hormone in oyster mushroom production.

KESAN ASID INDOLE-3-ACETIC TERHADAP PENGELUARAN BIOJISIM CENDAWAN TIRAM (Pleurotus sajor-caju)

ABSTRAK

Parameter Biojisim (diameter, berat dan kandungan protein micelia) cendawan tiram (Pleurotus sajor-caju) dari Kota Kinabalu, Sabah dikaji dengan menggunakan kaedah rawatan Indole-3-Acetic Acid (IAA). Tidak ada perbezaan yang signifikan (P>0.05) dalam berat miselia dan kandungan protein sejajar dengan kepekatan rawatan IAA yang berbeza. Meskipun tidak ada perbezaan yang signifikan, namun secara relatifnya rawatan dengan 5 mg/L kepekatan IAA memberikan peningkatan yang paling tinggi dalam mencerminkan diameter miselia dan kadar protein. Kepekatan rawatan IAA yang tinggi akan memberikan peningkatan maksimum dalam meningkatkan keliatan (plasticity) dinding sel di mana ia menunjukkan pengawalan sintesis protein khusus yang lebih baik. Keputusan ini memberikan sokongan untuk kajian selanjutnya dan analisis masih perlu dilakukan untuk meningkatkan pemahaman tentang keberkesanan dan keprihatinan ekonomi pada penggunaan dan aplikasi IAA sebagai hormon tanaman semulajadi dalam produksi cendawan tiram.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, UNITS AND ABBREVIATION

µg	Microgram
µg/mL	Microgram per mililitre
μgcm^{-3}	Microgram per cubic centimetre
µL	Microlitre
ATP	Adenosine Triphosphate
ARFs	Auxin Response Factors
BSA	Bovine Serum Albumin
C/N	Carbon: Nitrogen ratio
EDTA	Ethylenediaminetetraacetic acid
FDACS	Florida Department of Agriculture and Consumer Services
g	Gram
IAA	Indole-3-Acetic Acid
KOH	Potassium Hydroxide
M	Mole
MEA	Malt-Extract Agar
mg	Milligram
mg/L	Milligram per litre
mL	Mililitre
mM	Milimole
mm	Millimetre
mM	Millimole
mm^2	Square millimetres
mRNA	Messenger Ribonucleic Acid
N	Normality
nm	nanometre
PDA	Potato Dextrose Agar
RM	Ringgit Malaysia
RPM	Revolutions per minute
SSA	School of Sustainable Agriculture
TDF	Total Dietary Fibre
tRNA	Transfer Ribonucleic Acid

CHAPTER 1

INTRODUCTION

1.1 Foreword

Mushrooms have been recorded as a source of vegetable and medicines for human beings throughout the world for thousands of year, involving a large number of edible species (Rehana *et al.*, 2007; Wong and Chye, 2009). In most countries, there is a well-established consumer acceptance for cultivated mushrooms, probably due to their unique flavour and texture. Nevertheless, an increase in the consumption of edible wild mushrooms has been observed, although they do not constitute a significant portion of the human diet (Valentao *et al.*, 2005). Edible wild mushrooms are traditionally used in many Asian countries as food and medicine (Manzi *et al.*, 1999; Sanmee *et al.*, 2003). Mushrooms are low in calories, lack cholesterol and virtually do not contain fat and sodium. Selnium and Niacin (essential minerals) that play important role in the immune system, the thyroid system and the male reproductive system and in cancer prevention are sufficiently contained in mushrooms (Ukoima *et al.*, 2009).

Being one of the most well known mushroom species, *Pleurotus sajor-caju* (oyster mushroom) is recognized as an excellent mushroom. The oyster mushroom is a popular edible mushroom which can be cooked or eaten raw. It is also an important ingredient of pizza and many other popular bakery dishes due to their nutty, subtle flavour that goes well in soups, stews, and sauces (Rehana *et al.*, 2007). For example oyster mushrooms taste best as chicken or seafood dish accompaniments or even as add-ons to soups or sauces. While cooking oyster mushrooms, it helps to stir fry with some unsalted butter and chopped onions or even herbs and spices in order to bring out the maximum amount of flavor (Spahr, 2009).

Oyster mushrooms are both delicious and nutritious. The sporophores of *P. sajor-caju* have 26.9% protein having high digestibility values, rich in vitamin C, B complex and all essential seventeen amino acids in good concentration. Vitamin B3 in oyster mushrooms is 5-10 times higher as compared to any other vegetable. Oyster mushrooms contain the majority of the mineral salts that are required by the human body. Calcium, phosphorus and iron content in oyster mushrooms is approximately double the amount available in beef, pork and chicken meat, yet oyster mushroom are fat and cholesterol free. Furthermore this mushroom also used for chronic catarrh diseases of the breast and hinges, lower the cholesterol level of blood, improves circulation, remedy for night sweating in tuberculosis, rheumatism, gout, jaundice, dropsy, intestinal worms and have anti-tumor, anti-viral and anti-cancer agents (Rehana *et al.*, 2007). The consumption of mushrooms would therefore need to be encouraged in order to enhance the intake of mineral nutrients by human beings (Ukoima *et al.*, 2009).

Mushroom demand of Malaysian in 2004 is 8,100 tonne/year however local production of mushroom is just up to 5,500 tonne/year. According to the statistics released by Department of Statistics Malaysia on the year of 2004, total imports of mushroom was up to 2,609 tonne which is equivalent to the value of RM 25.74 million (Department of Statistics Malaysia, 2004). This value reflects the production deficiency of mushroom in Malaysia. Research and developments are therefore required to improve the production and quality of mushroom in order to fulfil the huge local market demand for mushroom.

To date, the usage and applications of Indole-3-acetic acid (IAA) for the growth and quality enhancement have been studied in mushroom cultivation. IAA is the most abundant and important naturally occurring auxin. It is also known to be involved in many of the physiological and developmental processes in higher plants, including promoting growth in length of shoots and roots (Williams, 2010; Vessey, 2003; Qin *et al.*, 2005). Reported by Mukhopadhyay *et al.* (2004), IAA can enhances both the mycelia growth and protein content of edible oyster mushroom, *Pleurotus sajor-caju* grown in whey. The effects of phytohormones including indole-3-acetic acid (IAA) on mycelia growth and exopolysaccharide biosynthesis of medicinal mushroom *Pellinus linteus* also have been investigated (Guo *et al.*, 2009).

1.2 Justification

This study was carried out to improve the production and development of mushroom industry in Malaysia into a competitive and profit-generating industry through the enhancement in mushroom biomass and protein content. Enhancement of biomass production of *Pleurotus sajor-caju,* a well known mushroom species in local market will leads to higher mushroom production considerably without any prolong of production period in fulfilling local and international market demand. Besides that, yield improvement also can reduce the mushroom production deficiency in local market so as to reduce the import and reliability of mushroom from other countries.

Furthermore, the enhancement in protein content of *Pleurotus sajor-caju* will best suit customer demand nowadays since consumer have higher awareness and preference of food with rich nutrition and in this case the protein content has been improved. Therefore this study was carried out in order to examine the potential of implementing plant growth hormone of Indole-3-Acetic Acid (IAA) into the production of edible mushroom *Pleurotus sajor-caju* grown in sawdust as being practiced by most of the local mushroom's producers.

1.3 OBJECTIVE

To determine the effects of indole-3-acetic acid (IAA) in enhancing the mycelial growth and protein content of oyster mushroom, *Pleurotus sajor-caju*

1.4 Hyphothesis

H_0 : There is no significant effect of indole-3-acetic acid (IAA) on mycelial growth and protein content of *Pleurotus sajor-caju*

H_1 : There is significant effect of indole-3-acetic acid (IAA) on mycelial growth and protein content of *Pleurotus sajor-caju*

CHAPTER 2

LITERATURE REVIEW

2.1 Oyster Mushroom

The *Pleurotus* mushroom, which is more commonly known as "Oyster mushroom" worldwide has its origin from the Greek word "Pleuro" which means formed laterally or in a side way position, referring to the lateral position of the stipe (stem) in relation to its cap (Metzler and Metzler, 1992; Mukerji and Manoharachary, 2006). Another saying is that the naming of oyster mushroom (*Pleurotus spp.*) was due to the shape of the cap which looks much like oyster shells so as its flavor which tastes similar to oysters (Christensen, 1943; Metzler and Metzler, 1992; Mukerji and Manoharachary, 2006).

Today species of the genus *Pleurotus* are one of the most popular edible fungi in many countries in the subtropical and temperate zone (Chang and Quimio, 1982). The oyster mushroom is the second most important mushroom in production in the world, accounting for 25% of total world production of cultivated mushrooms. Oyster mushroom is grown worldwide, and it has been regarded as one of the most profitable cash crops in Korea, accounting for 65% of total domestic mushroom production (OECD, 2006). These groups of mushrooms have gained considerable importance recently, and are appreciated for their culinary properties and broad adaptability under varied agro climatic conditions. For example; when cultured artificially, they are able to grow well on most agricultural waste materials supplemented with additives (Chang and Quimio, 1982; Mukerji and Manoharachary, 2006).

The whole fruiting body of oyster mushrooms are soft and fleshy with three distinct parts- a fleshy shell or spatula shaped cap (pileus), a short lateral or central stalk called stipe or stem and long ridges and furrows underneath the pileus called gills or lamellae (Arora, 1986; Roy, 1998; Smith and Weber, 2001; OECD, 2006).

The color of the cap varies from white to gray, grayish-brown, tan, or dark brown (sometimes yellowish in old age) (Arora, 1986; Smith and Weber, 2001; Spahr, 2009). Its size may ranges from medium to large with up to 3-15 cm broad in diameter. Margin of the cap is inrolled when young, often wavy or lobes (Arora, 1986; Metzler and Metzler, 1992; Parjimo and Andoko, 2010).

Gills fairly close, broad, decurrent, white or tinged gray but often shows discolouring yellowish in old age and lose their firmness (Arora, 1986; Metzler and Metzler, 1992). The colour of the gills varies among species for example white, light grey, whitish ivory, tannish or brown. The gills stretch from the edge of the cap down to the stalk and bear the spores (Christensen, 1943; Smith and Weber, 2001; Spahr, 2009).

The stipe or stem is usually short (0-2 cm long), stout, and off-center or lateral of the cap, sometimes with light, fuzzy or downy hairs near the base. The stem is thick, solid, firm and dry with 0-5 mm in diameter. The stem attaches laterally to the wood, and clusters will often split open dead bark as the mushroom caps rise outward (Arora, 1986; Metzler and Metzler, 1992; Mcfarland and Mueller, 2009; Parjimo and Andoko, 2010).

The spores are smooth, oblong to elliptical in face view, hyaline in potassium hydroxide (KOH), inamyloid with white to pale lilac or lilac-gray spore print in air-dried deposit. The color of the sporophore is extremely variable character influenced by the temperature, light intensity and nature of substrate. It can germinate very easily on any kind of mycological media within 48-96 hours. The mycelium of *Pleurotus* is pure white in colour (Arora, 1986; Metzler and Metzler, 1992; Roy, 1998; Smith and Weber, 2001).

The oyster mushroom grows naturally on trees or dead woody branches of trees and hence is also known as "wood fungus" (Chang and Quimio, 1982). Oyster mushroom is a cellulose loving fungus and decaying wooden logs or sometimes on lying trunks of deciduous or conifers woods. It may also grow on decaying organic matter for example the decayed parts of living trees, especially willow and cotton wood (Roy, 1998; Metzler and Metzler, 1992). Oyster mushrooms are commonly found wild at anytime of the year in temperate forests and some species in tropical forests. Typically, they grow on dead logs, one relatively uncommon species attacks weak

living trees. They also can be found occasionally on the ground above buried roots or stumps. Nearly any deciduous tree species can be a host. Its preferred hosts include elm, cottonwood, alder, and sycamore. Oyster mushroom can be easily cultivated on wide variety of substrates, including compressed sawdust, and presumably coffee grounds (Christensen, 1943; Chang and Quimio, 1982; Arora, 1986; OECD, 2006; Rehana *et al.*, 2007; Mcfarland and Mueller, 2009)

In many countries, edible *Pleurotus* species are collected and cultivated as choice edibles and have been subjected of many taxonomic and genetic studies. *Pleurotus* has always been attractive to mushroom growers because of the ease with which most species can be cultivated. With over 25 recognized species and variety cultivated throughout the world, *Pleurotus* is also one of the diverse groups of cultivated edible fungi, which is being exploited commercially as a future mushroom especially in South East Asia region. Cultivated mushroom species of genus *Pleurotus* mainly include *P. ostreatus, P. sajor-caju, P. eryngii, P. cornucopiae, P. fossulatus, P. flabellatus, P. opuntae, P. citrinapileatus, P. membranaecus, P. platypus, P. eaus, P. sapidus, P. cystidious, P. columbines* and *P. pulmonarius*. Different species of *Pleurotus* are suited for growing within a temperature range of 15 to 30 °C. (Mukerji and Manoharachary, 2006).

2.1.1 *Pleurotus sajor-caju*

Pleurotus sajor caju are edible basidiomycete, which occur in both tropical and subtropical regions of the world. It was initially cultivated in India after the late of 1940's (OECD, 2006). In India, *Pleurotus sajor-caju* is one of the commonly growing species. It has been shown to exhibit hypotensive properties and reduce the rate of nephron deterioration, which may be useful for chronic renal failure patients (Sati, 2006). *Pleurotus sajor-caju* as one of the most successfully cultivated specialty mushrooms and is now considered to be a delicacy (Zhang *et al.*, 2002). It can be cultivated within a wide range of temperatures on different natural resources and agricultural wastes. *P. sajor-caju* can tolerate temperatures up to 28-30 °C, although it fruits faster and produces larger mushrooms at 25 °C during the cooler months of the year or in the highlands of the tropics. This species is among the most popularly cultivated mushroom in Malaysia (Chang and Quimio, 1982; Rehana *et al.*, 2007; Parjimo and Andoko, 2010).

The chemical composition of the mushroom and of the substrate used for growing shows that *Pleurotus sajor-caju* is effective in concentrating Nitrogen, Potassium, Phosphorus, Magnesium, Calcium, Sulphur, Sodium, Iron, Zinc, and Copper in their fruit bodies. This makes mushrooms of this genus to be good sources of minerals, for example calcium and phosphorus are essential for the formation of bones and teeth. Being low calorie contents, with small amount of lipids and high nutritious food, it is also recommended to counter obesity and especially against sugar troubles. For example the physic-chemical composition of *Pleurotus sajor-caju* consists of 88% moisture, 33.5% crude protein, 2.26% fat, 58.5% carbohydrate, and 8.25% ash. Furthermore, oyster mushroom also contains fairly high quantity of ascorbic acid (vitamin C) which helps in the treatment of scurvy (Chang and Miles, 1989; Moda *et al.*, 2005; Mukerji and Manoharachary, 2006; Rehana *et al.*, 2007; Ukoima *et al.*, 2009).

2.2 Mushroom Cultivation

Mushroom cultivation proceeds through three basic phases, regardless of the species of fungus: germination or isolation, expansion, and finally fruiting. The first stage involves isolating a mushroom culture from spores or from the tissue of a living mushroom. Spores germinated on nitrified agar in Petri dishes result (after mating) in a diversity of strains within the same culture, while tissue culture results in a genetically identical clone of the parent mushroom. The use of a semi-solid agar medium allows the cultivator to easily examine the culture for desired characteristics and to identify any contamination, if present. The mycelium can be propagated on agar more or less indefinitely and can be stored at this stage at cold temperatures for later retrieval (Royse, 2003; Nicholas and Ogame, 2006; Donsky, 2009).

Once a suitable pure clean culture has been isolated, the mycelium is then transferred to a secondary medium, usually sterilized whole grain in jars. The purpose of this stage is to expand the volume of mycelium (mycelia mass) to an amount that will support the desired amount of fruiting in the final phase. A small amount of mycelium on a wedge of agar is removed from a plate and inoculated the grain. When the grain is fully colonized, they are then used to inoculate larger containers of substrate. The material generated in this phase is generally known as spawn (Nicholas and Ogame, 2006).

Once a suitable amount of spawn has been generated, it is used to inoculate the final fruiting substrate. The exact ingredients of the fruiting substrate are depending on the mushroom species. Once a suitable medium has been prepared, it is mixed with spawn and left to colonize. After the fruiting substrate is colonized, fruiting is initiated. Eventually, if all goes accordingly to plan, mushroom form, first appearing as primordial, then enlarging to full size within a few days, at which point they begin to release their spores, and the cultivation cycle is complete (Beetz and Kustudia, 2004; Nicholas and Ogame, 2006).

Mushroom cultivation fits in very well with sustainable farming and has several advantages (Oei, 2005):

- It uses agricultural waste products
- A high production per surface area can be obtained
- After picking, the spent substrate is still a good soil conditioner

2.2.1 Tissue Culture

The first phase of mushroom cultivation is the isolation of a pure culture of mycelium which can be achieved by using the tissue culture technique. Tissue culture is recommended instead of the spores culture as the genetic characteristics of the parent mushroom can be preserved to the isolated mycelia. Moreover, spores are likely to yield a new strain and performance would be unpredictable. The parent mushroom or mother culture can be made from a fresh and healthy fruiting body (Royse, 2003; Oei, 2005; Donsky, 2009).

The two most commonly used nutrients agars for mushroom cultivation are Potato Dextrose Agar (PDA) and Malt-Extract Agar (MEA) which act as the medium for tissue culture of mushroom mycelium. PDA is most often used for tissue culture while Malt-Extract Agar is usually the medium of choice for spores. Most mushrooms prefer medium of neutral to slightly acid pH. That is a pH of 5.5 to 7.0. PDA medium just fit in within this pH range without further adjustment. Petri dishes, baby bottles and baby food jars all make good containers for agar (Oei, 2005; Donsky, 2009).

A clean environment is absolutely essential to the preparation of the tissue cultures. In particular, whenever the containers with sterilised medium need to be opened it must be done under aseptic conditions. The air carries numerous contaminants, which easily infect the sterilised medium. Young and vigorous mycelium

can be obtained from a young fruiting body using a scalpel, alcohol, sterilised agar slants, Petri dishes or bottles with agar, flame (non-smoking), and a clean table to work on, or preferably a laminar airflow cabinet or inoculation box. The inside of laminar airflow cabinet can be disinfected by cleaning with a 10% Clorox solution, or a 2% Formalin solution, or 70% ethyl alcohol (Oei, 2005; Parjimo and Andoko, 2010).

After inoculation, the Petri dishes with agar are incubated at 25 °C for about ten days. Within 3-4 days mycelium will cover the tissue and branch out on the agar. If no growth occurs on the agar, then possibly the parent mushroom was too old and therefore a fresher specimen should be try. Another possibility is that the scalpel did not cool down before taking the tissue sample thus over-heating the mycelium. The suitable mycelium should be pure white and grow out from the tissue. If yellow, blue, green or grey mycelia form on other places on the surface, then these are fungal contaminants while a creamy, shiny growth often indicates bacterial contamination. Once a pure culture of mycelium has been isolated, it is allowed to take over the agar dish. This can be done by inoculating two pure spore cultures into the same jar of grain (Oei, 2005; Parjimo and Andoko, 2010).

2.2.2 Mycelium

Mycelium (plural mycelia) is the vegetative part of a fungus, consisting of a fabric mass of interconnected, interwoven strands of cells. Fungal colonies composed of mycelia are found in soil and on or within many other substrates. A typical single spore germinates into a homokaryotic mycelium, which cannot reproduce sexually; when two compatible homokaryotic mycelia join and form a dikaryotic mycelium, that mycelium may form fruiting bodies such as mushrooms. A mycelium may be minute, forming a colony that is too small to see, or it may be extensive. This organism can be physically separated, and yet behave as one. The exquisite lattice-like structure of the mushroom mycelium often referred to as the mycelia network. Each colony extends long, complex chains of cells that fork repeatedly in matrix-like fashion, spreading to geographically defined borders (Elevitch, 2004; Miller *et al.*, 2010).

In order to feed, the mushroom mycelium absorbs nutrients from its surrounding environment. It does this in a two-stage process. First, the hyphae secrete extracellular enzymes onto or into the food source, which break down complex organic polymers into simpler compounds (generally various sorts of sugars). These newly

freed nutrients of monomers are then selectively absorbed through the hypal wall into the mycelia network by facilitated diffusion and active transport (Elevitch, 2004; Miller *et al.*, 2010).

Mycelium is vital in terrestrial and aquatic ecosystems for its role in the decomposition of plant material. It contributes to the organic fraction of soil, and its growth releases carbon dioxide back into the atmosphere. The mycelium of mycorrhizal fungi increases the efficiency of water and nutrient absorption of most plants and confers resistance to some plant pathogens. Mycelium is an important food source for many soil invertebrates (Miller *et al.*, 2010).

2.2.3 Mycelia Growth

As shown in figure 2.1, the leftmost shows a spore (the black dot) with the short germ tube growing out from it. The next figure shows the scene a little later, with several branching having occurred by now. The other two figures show later stages in the expansion of the mycelium. By repeated branching, the mycelium eventually assumes a circular form as shown in the rightmost of figure 2.1. That figure also shows that while the hyphae show a very marked outward growth, there are also cross-connections between the outward growing branches. The cross-connections between the radiating hyphae make it easy to move nutrients quickly around the growing mycelium, taking them to wherever they are most needed. The combination of radial growth, with branching hyphae growing out from behind the leading hyphae, means that a mycelium can explore and exploit a large area (Lepp, 2005).

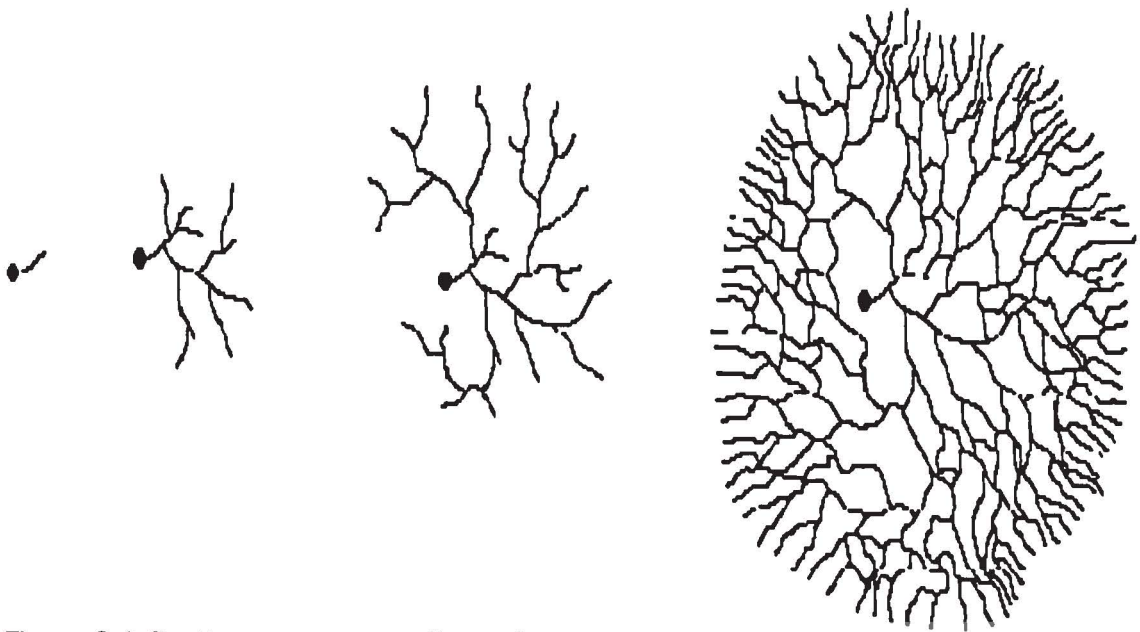


Figure 2.1 Continuous process of mycelia growth (Lepp, 2005).

2.2.4 Environment of Mushroom Cultivation

Environmental factors include temperature, relative humidity, light, carbon dioxide and acidity of substrate. As the growing room temperature is raised, relative humidity decreases. A higher temperature promotes fruitbody metabolism, which in turn, increases their respiration rate and results in high carbon dioxide production. Oyster mushrooms also need different environmental conditions at each growing stage. During incubation, appropriate relative humidity is 65-70% and water content of substrate is 65%. Optimal temperature for mycelial growth is 20-25 °C, but some thermophilic strains reach optimal growth at 25-35 °C. Mushroom mycelia are quite durable to high concentration of carbon dioxide during incubation (Chang and Quimio, 1982; Kang, 2004; Oei, 2005; Parjimo and Andoko, 2010).

Upon the completion of incubation, pinning induction follows. Pinning induction is made by worsening the environment in order that the mycelia cannot keep on with their vegetative growth and will therefore convert to a reproductive growth mode, which initiates fruitbody formation. Pinning induction includes cold shock, watering and lighting. Once the pins come out, growers stop pinning induction and maintain environmental conditions that are favourable to fruiting. Carbon dioxide concentration should be less than 800 ppm in its reproductive growth though the number differs according to strains. Fruitbody formation also requires high relative humidity up to 80-95% and lower temperature than optimal mycelial growth by 10 °C (Chang and Quimio, 1982; Kang, 2004; Oei, 2005; Parjimo and Andoko, 2010).

2.2.5 Mushroom Substrate

Mushrooms are not plants, and therefore require different conditions for optimal growth. Mushrooms derive all of their energy and growth materials from their growth medium, through biochemical decomposition processes (Chang and Miles, 1989). Substrate can be understood as the medium which provide the necessary growth materials for the growing of mushroom (Kang, 2004).

The main nutritional sources for oyster mushroom are cellulose, hemicellulose and lignin. C/N ratio is important factor for optimal substrate composition for oyster mushroom. Oyster mushroom requires much carbon and less nitrogen source than button mushroom (*Agaricus bisporus*) but most of main substrate materials such as cereal straw, cotton waste, sawdust need supplementation of nitrogen source such as

wheat and rice bran to reach optimal C/N ratio for oyster mushroom (Kang, 2004). The mycelial growth was best at a C/N ratio ranging from 18:1 to 36:1 with an optimumvalue at 24:1. On increasing the C/N ratio of the medium, both the amount of total dietary fibre (TDF) and chitin content in the mycelium decreased while glucan content increased. These results indicate that the proportions of the nitrogen and carbon source in a fermentation medium affect the biosynthesis of cell wall non-starch polysaccharide in mushroom mycelium. Inorganic materials are usually included in substrate materials and need not additional supplement. And amino nitrogen is used during spawn run, but it is not fit for fruiting, therefore, growers commonly do not need additional apply of amino nitrogen during mixing (Kang, 2004). Mushrooms grow well at relative humidity levels of around 95-100%, and substrate moisture levels of 50 to 75% (Chang and Miles, 1989).

2.2.5.1 Sawdust Substrate

Traditionally, local mushroom growers used tree stump as a substrate medium for growing mushroom however this practice is slowly impracticable due to the uncertain and expensive source of the tree stump. For the replacement, most of the local mushroom growers used sawdust which is cheaper and easily to get as the substrate medium for mushroom growing. Besides that, sawdust can provide faster growing rate and consequently shorter production and harvesting period (Parjimo and Andoko, 2010).

Before using the sawdust for mushroom growing, first sawdust needs to be sieve to get the uniform size and make free from impurities. This is because the sawdust source from wood industry usually posses different uniform level and sometimes mixed with wood chips, gravel or soil amendments. In order to supply all the necessary nutritional source for mushroom, sawdust need to mixed well with other supplements such as crushed rice, lime, gypsum, cotton, corn flour, potato flour and etc (Parjimo and Andoko, 2010). Among all these supplements, crushed rice, lime and gypsum are compulsory as they have the functions as below:

1. Crushed rice is rich with carbohydrate, carbon, nitrogen and vitamin B complex which are able to make faster for mycelium growth and encourage grow of mushroom's fruit body. Crushed rice of different rice varieties also can be used.

REFERENCES

Abel, S., Theologis, A. 1996. Early Genes and Auxin Action. *Plant Physiology* **111**:9–17

Acumedia Manufacturers, 2008

American Phytopathological Society, 1962. *Sourcebook of Laboratory Exercises in Plant Pathology.* San Francisco and London: W.H. Freeman and Company 369

American Public Health Association, 1960. *Standard Methods for the Examination of Dairy Products,* 13th Edition. New York: APHA

Arora, D. 1986. *Mushrooms Demystified: A Comprehensive Guide to the Fleshy Fungi.* California: Ten Speed Press.

Arteca, R.N. 1996. *Plant Growth Substances: Principles and Applications.* United States of America: Chapman & Hall

Basra, A. S. 2000. *Plant Growth Regulators in Agriculture and Horticulture: The Role and Commercial Uses.* New York: Food Products Press.

Beetz, A. and Kustudia, M. 2004. Mushroom Cultivation and Marketing: Horticulture Production Guide. *National Sustainable Agriculture Information Service.* 042905

Chandra, A. 1989. *Elsevier's Dictionary of Edible Mushrooms.* Elsevier Science

Chang, S.T. and Quimio, T.H. 1982. *Tropical Mushrooms: Biological Nature and Cultivation Methods.* Hong Kong: The Chinese University of Hong Kong.

Chang, S.T., and Miles, P.G. 1989. *Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact.* CRC Press.

Christensen, C.M. 1943. *Common Edible Mushroom.* Minneapolis: Lund Press

Davies, P.J. 2004. *Plant Hormones: Biosynthesis, Signal Transduction, Action! 3rd Edition.* New York: Kluwer Academic

Davies, P.J. 2010. *Plant hormones: Biosythesis, Signal Transduction, Action! Revised 3rd Edition.* London New York: Springer Dordrecht Heidelberg

Department of Statistics Malaysia, 2004

Donsky, M.A. 2009. *Cultivation Corner: Cultivation I.* Colorado: Colorado Mycological Society

Dundar, A., Acay, H., and Yildiz, A. 2008. Yield Performances and Nutritional Contents of Three Oyster Mushroom Species Cultivated on Wheat Stalk. *African Journal of Biotechnology* **7(19)**: 3497-3501

Eddleman, H. 1998. *Making Bacteria Media from Potato.* Indiana Biolab.

Elevitch, C.R. 2004. *Cultivating Connections with Trees.* Permanent Agriculture Resources.

Fishel, F. M. 2006. Plant Growth Regulators. *University of Florida.* PI-102

Goda, H., Sawa, S., Asami, T., Fujioka, S., Shimada, Y., Yoshida, S. 2004. Comprehensive Comparison of Auxin-regulated and Brassinosteroid-regulated Genes in *Arabidopsis. Plant Physiology* **134**:1555–73

Guilfoyle, T.J., Hagen, G. 2007. Auxin Response Factors. *Current Opinion in Plant Biology* **10**:453–60

Guo, X., Zou, X., and Sun, M. 2009. Effects of Phytohormones on Mycelia Growth and Exopolysaccharide Biosynthesis of Medicinal Mushroom *Pellinus linteus. Bioprocess and Biosystems Engineering* **32**:701-707

Gruen, H.E. 1959. Auxins and Fungi. *Annual Reviewed of Plant Physiology* **8**: 405-410

Kang, S. W. 2004. Part II. Oyster Mushroom by MushWorld. *Mushroom Growers' Handbook 1* **3**: 48-51

Kukavica, B., Mitrović, A., Mojović, M., and Jovanović, S.V. 2007. Effect of Indole-3-Acetic Acid on Pea Root Grpwth, Peroxidase Profiles and Hydroxyl Radical Formation. *Archives of Biological Science, Belgrade* **59(4)**: 319-326

Lepp, H. 2005. *The Mycelium.* Australian National Botanic Gardens.

Lorsch, J. 2002. *Protein Synthesis.* Macmillan Reference USA

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. *Journal Biological Chemistry* **193**: 265.

Manzi, P., Gambelli, L., Marconi, S., Vivanti, V., Pizzoferrato, L., 1999. Nutrients in Edible Mushrooms: An Interspecies Comparative Study. *Food Chemistry* **65(4)**: 477–482

McFarland, J. and Mueller, G.M. 2009. *Edible Wild Mushrooms of Illinois & Surrounding States: A Field-to-kitchen Guide.* Korea: University of Illinois Press

Metzler, S. and Metzler, V. 1992. *Texas Mushrooms: A Field Guide.* Texas: University of Texas Press

Miller, F.P., Vandome, A.F., McBrewster, J. 2010. *Mycelium.* Germany: VDM Publishing house Ltd.

Mockaitis, K. and Estelle, M. 2008. Auxin Receptors and Plant Development: A New Signaling Paradigm. *Annual Review of Cell and Development Biology* **24**: 55-80

Moda, E. M., Horii J., Spoto, M. H. F. 2005. Edible Mushroom *Pleurotus sajor-caju* Production on Washed and Supplemented Sugarcane Bagasse. *Scientia Agricola (Piracicaba, Brazil)* **62**: 127-132

Mukerji, K.G. and Manoharachary, C. 2006. *Current Concepts in Botany.* India: I.K. International Publishing House Pvt. Ltd.

Mukhopadhyay, R., Chatterjee, S., Chatterjee, B.P., Guha, A.K. 2004. Enhancement of Biomass Production of Edible Mushroom *Pleurotus sajor-caju* Grown in Whey by Plant Growth Hormones. *Process Biochemistry* **40**: 1241-1244

Naeem, M., Bhatti, I., Ahmad, R.H., and Ashraf, M.Y. 2004. Effect of Some Growth Hormones (GA_3, IAA and Kinetin) On the Morphology and Early or Delayed Initiation of Bud of Lentil (*Lens culinaris* medic). *Pakistan Journal of Botany* **36(4)**: 801-809

Nemhauser, J.L., Hong, F., Chory, J. 2006. Different Plant Hormones Regulate Similar Processes Through Largely Nonoverlapping Transcriptional Responses. *Journal of Cell Biology* **126**:467–75

Nicholas, L.G. and Ogame, K. 2006. *Psilocybin Mushroom Handbook: Easy Indoor and Outdoor Cultivation.* Canada: Quick Trading.

O'Connor, C. B. 1993. *Traditional Cheesemaking Manual.* ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia.

OECD, 2006. Oyster Mushroom. *Safety Assessment of Transgenic Organisms: OECD Consensus Documents* 1.

Oei, P. 2005. *Small-scale Mushroom Cultivation: Oyster, Shiitake and Wood Ear Mushrooms.* Wageningen: Agromisa Foundation and CTA.

Overvoorde, P.J., Okushima, Y., Alonso, J.M., Chan, A., Chang, C. *et al.* 2005. Functional Genomic Analysis of the AUXIN/INDOLE-3-ACETIC ACID gene family Members in *Arabidopsis thaliana. Plant and Cell Physiology* **17**:3282–300

Parjimo, H., and Andoko, A. 2010. *Perusahaan Tanaman Cendawan.* Malaysia: Synergy Media

Pushkar, K. 2008. Auxin: A Plant Growth Hormone. *Competition Science Vision.* November: 1207-1209

Qin, G., Gu, H., Zhao, Y., Ma, Z., Shi, G., Yang, Y., Pichersky, E., Chen, H., Liu, M., Chen. Z., and Qu, L.J. 2005. An Indole-3-Acetic Acid Carboxyl Mathyltransferase Regulates Arabidopsis Leaf Development. *The Plant Cell* 17: 2693–2704

Quimio, T.H. 2004. Part I Oyster Mushroom by MushWorld. *Mushroom Growers' Handbook 1* **1**: 4-12

Rehana, A., Tariq, M. and Rehman, T. 2007. Propagation of *Pleurotus sajor-caju* (Oyster Mushroom) through Tissue Culture. *Pakistan Journal of Bot*any **39(4)**: 1383-1386

Roe, S. 2001. *Protein Purification Techniques: A Practical Approach Second Edition.* England: Oxford University Press.

Roy, D. 1998. *Environment Management with Indian Experience.* New Delhi: S. B. Nangia A.P.H. Publishing Corporation.

Royse, D.J. 2003. *Cultivation of Oyster Mushroom.* Pennsylvania: The Pennsylvania State University.

Royse, D.J., Rhodes, T.W., Ohga, S., and Sanchez, J.E. 2004. Yield, Mushroom Size and Time to Production of *Pleurotus cornucopiae* (oyster mushroom) Grown on Switch Grass Substrate Spawned and Supplemented at Various Rates. *Bioresource Technology* **91(1)**: 85-91

Sanmee, R., Dell, B., Lumyong, P., Izumori, K., and Lumyong, S. 2003. Nutritive Value of Popular Wild Edible Mushrooms from Northern Thailand. *Food Chemistry* **82**: 527–532

Sati, S.C. 2006. *Recent Mycological Researches.* India: I.K. International Publishing House Pvt. Ltd.

Smith, A.H., and Weber, N.S. 2001. *The Mushroom Hunter's Field Guide.* Michigan, United States: The University of Michigan Press.

Spahr, D.L. 2009. *Edible and Medical Mushrooms of New England and Eastern Canada.* Berkeley, California: North Atlantic Books.

Taiz, L. and Zeiger, E. 2006. *Plant Physiology Fourth Edition.* Sunderland. United States: Sinauer Associates, Inc., Publishers

Tian Q., Uhlir N.J., Reed J.W. 2002. *Arabidopsis* SHY2/IAA3 Inhibits Auxin-regulated Gene Expression. *Plant Cell* **14**:301–19

Tomita, K., Murayama, T., and Nakamura, T., 1984. Effects of Auxin and Gibberellins on Elongation of Young Hyphae in Neurospora crassa. *Plant and Cell Physiology* **25**: 355–8

Tromas, A. and Perrot-Rechenmann, C. 2010. Recent Progress in Auxin Biology. *France: Comptes Rendus Biologies* **333**: 297–306

Ukoima, H.N., Ogbonnaya, L., Arikpo G.E. and Pepple, G.A. 2009. Nutritional, Organoleptic and Palatability Studies of Selected Edible Mushrooms in Nigeria. *World Applied Sciences Journal* **7(4)**: 479-484

Valentao, P., Lopes, G., Valente,M., Barbosa, P., Andrede, P.B., Silva, B.M., Baptista, P., and Seabra, R.M. 2005. Quantification of Nine Organic Acids in Wild Mushrooms. *Journal of Agricultural and Food Chemistry* **53 (9)**: 3626–3630

Vessey, J.K. 2003. Plant Growth Promoting Rhizabacteriia as Biofertilizers. Kluwer Academic Publishers. *Plant and Soil* **255**: 571–586

Walz, A., Park, S., Slovin, J.P., Ludwig-Müller, J., Momonoki, Y.S., and Cohen, J.D. 2002. A Gene Encoding A Protein Modified by the Phytohormone Indoleacetic Acid. USA: *Proceedings of the National Academy of Sciences* **99(3)**: 1718–1723

Williams, M.E. 2010. Introduction to Phytohormones. *The Plant Cell* **22**: 1-9.

Wilson, K. and Walker, J. 2010. *Principles and Techniques of Biochemistry and Molecular Biology, Seventh Edition*. Cambridge: Cambridge University Press

Wong, M.Y. and Chye, F.Y. 2009. Antioxidant Properties of Selected Tropical Wild Edible Mushrooms. *Journal of Food Composition and Analysis* **22**: 269–277

Yanagishima, N. 1963. Effect of Auxin and Antiauxin on Cell Elongation in Yeast. *Plant and Cell Physiology* **4**:257–64

Zhang, R., Li, X., and Fadel J.G. 2002. Oyster Mushroom Cultivation with Rice and Wheat Straw. *Bioresource Technology* **82**: 277-284