DOMESTICATION AND CULTIVATION OF WILD STRAINS OF *Pleurotus* AND *Lentinus* SPECIES

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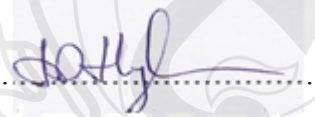

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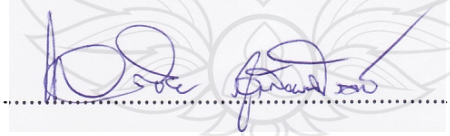

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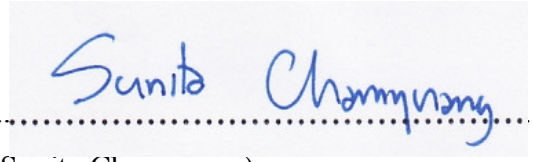

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Thesis Title	Domestication and Cultivation of Wild Strains of *Pleurotus* and *Lentinus* Species
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Degree	Master of Science (Biotechnology)
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ABSTRACT

In this study, three wild mushrooms, namely *Lentinus connatus*, *L. roseus*, and *Pleurotus giganteus*, were studied for possibility of domestication. Fruiting bodies of the three mushrooms were collected from forests in northern Thailand and morphologically characterized. In this study, attempts were made to investigate the optimal *in vitro* culture conditions of the mycelia of these mushrooms. Of seven media tested, black bean agar, red bean agar and soy bean agar were best for the mycelial growth of *L. connatus*, *L. roseus* and *P.giganteus*, respectively. The mushroom mycelia were able to grow at temperatures ranging from 20-30°C, with an optimal temperature of 30°C for *Lentinus* and 25°C for *Pleurotus*. These mushrooms grew fairly well in mild acidic, neutral and alkaline conditions (pH 5.0 – 8.0). The best mycelial growth and density of *P. giganteus* were observed in acidic media (pH 5.0 - 6.5) whereas those of *L. connatus* and *L. roseus* were obtained in slightly acidic to neutral pH (pH 5.0 – 7.0). For spawn production, three wild mushrooms could grow in all five substrates (red bean, black bean, mung bean, soy bean, and sorghum) to a varying extent. Of these, sorghum was selected for use in spawn preparation.

Recently a method for the cultivation of *Pleurotus giganteus* at the experimental level using saw dust as a substrate has been developed. The strain was isolated from a fresh fruiting body of *P. giganteus* (MFLU10 0154) using a piece of cap tissue and cultivated on potato dextrose agar. The cultivation method involves in two steps; i) inoculating on a saw dust substrate in polypropylene bags as the preliminary step, and ii) casing with soil, which is very important for fruiting. The developed method of growing *P. giganteus* is fully described in this Thesis.

The cultivation methods of *Lentinus connatus* involved on a saw dust substrate supplemented with poor and enriched substrates. Results showed that the enriched substrate took less time than poor substrates. Furthermore, yield of the mushroom production obtained from enriched substrate was 16.37 ± 1.71%, while the yield of poor substrate was 11.43±1.10%. However, both substrates could yield only one crop. Increased yields may be achieved by suitable substrate and conditions. Breeding methods may also result in high yields and this needs further study.

Lentinus roseus was also investigated on the fruiting bodies production in saw dust supplemented with poor and enrich substrates. Our results showed that, the mycelial growth occurred in both poor and enriched substrates but the enriched substrate took less time than poor substrates. In this study, however, *L. roseus* did not develop the fruiting bodies. A new strain might be chosen for the improvement on the cultivation methods.

Keywords: Casing/Cultivation/Enriched substrate/Mycelial growth/*Lentinus* species/*Pleurotus* species/Poor substrate

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CHAPTER 1

INTRODUCTION

Mushrooms are macrofungi with distinct fruiting bodies which can be either above or below ground and big enough to be seen with the naked eye. In terms of taxonomy, most mushrooms are classified as Basidiomycetes although some can be Ascomycetes. Generally, the mushrooms can be classified based on their distinct features, sexual spore, septate hyphae. For Basidiomycetes, clamp connection is present including the multicellular sporocarp called the basidioma or basidiocarps. Basidiospore is produced on a specialized cell called basidium.

1.1 Importance of Mushroom

Traditionally, mushrooms have been used as source of food, medicine, and often used in religious ceremonies (Chang & Miles, 2004). Edible mushrooms include wild and cultivated mushrooms. Some cultivated mushrooms are important in business as they can be produced in a large scale including *Agaricus bisporus*, *Lentinus edodes* and *Pleurotus* species. Wild mushrooms are also popular big their use is often limited due to low product and difficulty to cultivate.

1.1.1 As Foods

Generally, mushrooms are rich source of protein and contain all nine essential amino acids (Barros, Cruz, Baptista, Estevinho & Ferreira, 2008; Lee, Yun, Kim, Lim, Ham, Eum & Joo, 2011). Mushrooms contain higher protein amounts when compared with whole milk, skimmed milk, potatoes, broccoli, carrots, apple, banana and orange on a fresh weight basis (Çağlarırmak, 2007; Reis, Martins, Barros & Ferreira, 2012; Webb, 2012). They also contain low amount of fat and the content of unsaturated fatty acid which is more than saturated fatty acid (Barros, Baptista,

Correia, Casal, Oliveira & Ferreira, 2007(a); Barros et al., 2008; Sanmee, Dell, Lumyong, P., Izumori. & Lumyoung, S., 2003). Weigh content of saturated fatty acid presents in high level which may be harmful to human health (Chang & Miles, 2004). Several vitamins including ascorbic acid (vitamin C), folic acid (vitamin B9), Thiamin (B1), riboflavin (vitamin B2), niacin (vitamin B3), vitamin B12, vitamin D2, vitamin E are found in edible mushrooms (Çağlarırmak, 2007; Koyyalamudi, Jeong, Cho, & Pang, 2009; Mattila, Lampi, Ronkainen, Toivo & Piironen, 2002). Edible mushrooms are good source of minerals which contain both macronutrients and micronutrients (Agrahar-Murugkar & Subbulakshmi, 2005; Guo, Lin, J. Y. & Lin, J. F., 2007; Sanmee et al., 2003). In addition, their palatability includes of color, texture, flavor, and test are considered for consumption (Chang & Miles, 2004) in many type of meal (Table 1.1) (Kwon & Thatithatgoon, 2004; Reis et al., 2012).

1.1.2 As Medicine

Mushrooms and their extracts have been shown to provide health promoting benefits. Beta glucan or polysaccharide – protein complexes help to support immunomodulating and anti – tumor effects which appear to exert their anti – tumor effects by enhance immune activity via effects on the stimulating the macrophages, splenocytes, and thymocytes (Roupas, Keogh, Noakes, Margetts & Taylor, 2012). The consumption of β-glucan may help control plasma lipid concentrations and reduce the cardiovascular diseases by inhibiting the absorption of cholesterol and triglycerol (Guillamón, García-Lafuente , Lozano , D´Arrigo, Rostagno , Villares & Martínez, 2010). Atherosclerosis is a disease which is characterized by the accumulation of lipid and fibrous elements or connective tissue on the large arterial wall (Guillamón et al., 2010; Ley & Kim, 2008). These plaques are the critical factors to cause cardiovascular diseases by obstructing the blood flow, and the vessels impair the elastic and hydrodynamic stress (Guillamón et al., 2010; Ley & Kim, 2008). The extracts of various mushrooms have been shown to possess hypoglycemic activity and anti – hyperglycemic activity against diabetes – inducing compounds in obese and diabetic animal models (Roupas et al., 2012). Furthermore, mushrooms and their extracts have been reported to anti – microbial properties, anti – viral properties, support brain health (Roupas et al., 2012) (Table 1.2).

Table 1.1 Several types of edible mushrooms

Scientific name	Common name	Main uses
Pleurotus eryngii	King oyster mushroom/ Eryngii	- Served sautéed, grilled, braised, stewed, or boiled
Lentinula edodes	Shiitake	- Soups, sauces and salads.
Flammulina velutipes	Golden needle mushroom/ Enoki	- Soups and salads.
Agaricus bisporus	White mushroom/ Champignon	- Soups, gravies, stir-fries or in salads
Agaricus bisporus	Brown mushroom/ Portobello	- Soups, gravies, stir-fries or in salads
Pleurotus ostreatus	Oyster mushroom	- Soups, in stir-fry recipes with soy sauce or eaten stuffed
Volvariella volvacea	Straw mushroom	- Soups, stir-fries and boiled
Tremella fuciformis	Silver ear	- Soups, stir-fries, sweet dishes, or in salads
Pleurotus cystidiosus	Abalone mushroom	- Soups, in stir-fry recipes with soy sauce or eaten stuffed
Auricularia auricular	Wood ear	- Soups, stir-fries, sweet dishes, or in salads
Agrocybe cylindracea	Black poplar mushroom	- Soups, stir-fries, sweet dishes, or in salads

Table 1.2 Properties and mechanisms of bioactive compounds and mushrooms.

Biological activities	Mushrooms	Active compounds	References
Antimicrobial activities			
- *Escherichia coli*	*Pleurotus citrinopileatus*	Glucosylceramide	Meng et al. (2012)
- *Staphylococcus aureus*	*Pleurotus citrinopileatus*	Glucosylceramide	Meng et al. (2012)
- *Bacillus cereus*	*Lactarius piperatus*	Extracts	Barros et al. (2007 b)
- *Bacillus subtilis*	*Lactarius piperatus*	Extracts	Barros et al. (2007 b)
- *Pseudomonas aeruginosa*	*Lactarius piperatus* & *Lactarius deliciosus*	Extracts	Barros et al. (2007 b)
Antioxidant activities			
- Scavenging ability on DPPH radicals	*Inonotus obliquus*	Polyphenol	Lee et al.(2007)
- Scavenging ability on hydroxyl radical	*Boletus edulis*	Extracts	Vamanu & Nita (2013)
- Inhibition of lipid peroxidation	*Boletus edulis*	Extracts	Vamanu & Nita (2013)
- Chelating effect	*Boletus edulis*	Extracts	Vamanu & Nita (2013)
- Reducing power	*Boletus edulis*	Extracts	Vamanu & Nita (2013)

Table 1.2 (Continued)

Bioactive activities	Mushrooms	Active compounds	References
Anti – cancer activiries			
- Anti – cancer (breast)	*Ganerderma lucidum*	Polysaccharopeptides	Wan et al. (2008)
- Anti – cancer (gastric)	*Lentinus edodes*	Polysaccharide	Oba et al. (2009)
- Anti – cancer (skin)	*Lentinula edodes*	Extracts	Gu & Belury (2005)
- Anti – cancer (lung)	*Ganoderma lucidum*	Lucialdehydes A–C	Gao et al. (2002)
- Anti – cancer (bladder)	*Grifola frondosa*	Extracts	Louie et al. (2010)
Other therapeutic effect			
- Diabetes (type II)	*Agaricus blazei* Murill	Extracts	Hsu et al. (2007)
- Cardiovascular disease	*Pleurotus ostreatus*	Extracts	Khatun et al. (2007)
- Brain health and cognition	*Hericium erinaceus*	Extracts	Mori et al. (2009)
- Hepatitis B	*Ganoderma lucidum*	Ganopoly®	Zhou et al. (2005)
- Anti-viral (HIV)	*Ganoderma colossum*	Ganomycin B	el Dine et al. (2009)
- DNA damage	*Agaricus bisporus*	Heat-labile protein	Shi et al. (2002)
- Anti-arthritic	*Pleurotus ostreatus*	Beta-(1,3/1,6)-Dglucan	Bauerova et al. (2009)
- Wound healing	*Ganoderma lucidum*	Polysaccharide fractions	Gao et al. (2004)

1.2 Wild and Commercial Mushroom

Edible mushrooms can be defined in terms of a presence of palatability such as taste, aroma, color, and texture and an absence of toxic effects. Edible mushroom can be divided into two categories; those gathered in their wild habitat and in mushroom farm. Both mushrooms are consumed by human as nutritional food and their medicinal value. Cultivated and common wild mushrooms are often low cost in market. Furthermore, some mushrooms are more difficult to obtain shown higher price such as *Tricholoma magnivelare.*

1.2.1 Wild Mushroom

There are at least 2000 species belonging to 200 genera of macrofungi that show various degree of edibility. There are over 200 species have been collected from the wild and nearly 130 species of fungi can be cultivated whereas only 10 species grown commercially (Thawthong, Karunarathna, Thongklang, Chukeatirote, Kakumyan, Chamyuang, Rizal, Mortimer, Xu, Callac & Hyde, 2014; Mortimer, Karunarathna, Li, O., Gui, Yang, Yang, He, Ye, Guo, Li, H., Sysouphanthong, Zhou, Xu & Hyde, 2012; Sánchez, 2004; Sánchez, 2010; Vargas-Isla & Ishikawa, 2008). Several edible wild mushroom have important in many rural areas of some countries (Guillamón et al., 2010). Some species are very expensive because they are commonly found as symbiotically associated form those are known as a mycorrhiza. They are often available in market depending on the season (Guillamón et al., 2010). Furthermore, some edible wild mushrooms are not mycorrhiza such as *Pleurotus giganteus*. This mushroom used to be wild mushroom has long been a tradition in Malaysia, China, and Sri Lanka (Karunarathna, Yang, Raspe, Ko Ko, Vellinga, Zhao, Bahkali, Chukeatirote, Degreef, Callac & Hyde, 2012; Phan, Wong, David, Naidu & Sabaratnam, 2012). *Pleurotus giganteus* is now being cultivated as commercial mushroom in Malaysia (Phan et al., 2012). The approximate market information on popular wild mushrooms showed in Table 1.3 and Figure 1.1.

Table 1.3 Several types of edible wild mushrooms

Scientific names	Common name	Approximate wholesale price
Cantharellus cibarius	Chanterelle	22.50 US$/lb
Grifola frondosa	Maitake	13 US$/lb
Morchella esculenta	Dark Morel	46 US$/lb
Morchella esculenta	Blonde Morel	59.95 US$/lb
Laetiporus sulphureus	Chicken of the woods	13 US$/lb
Hypomyces lactifluorum	Lobster	11.95 US$/lb
Hydnum repandum	Hedgehog	27.50 US$/lb
Tricholoma magnivelare	Matsutake	25.50 US$/lb
Craterellus cornucopioides	Black Trumpet	28.50 US$/lb
Craterellus lutescens and *Craterellus tubaeformis*	Yellow foot	17.75 US$/lb

Note. The current exchange rate is as follow: 1 US$ = 32.57 Baht (Retrieved June 23, 2014 from http://www.bot.or.th/Thai/Pages/BOTDefault.aspx)

Source Bank of Thai (2014)

Source Earthy Delights (2014)

Figure 1.1 Representatives of known wild mushroom a. *Cantharellus cibarius* b. *Grifola frondosa* c. *Morchella esculenta* d. *Hypomyces lactifluorum* e. *Hydnum repandum* and f. *Tricholoma magnivelare*

1.2.2 Commercial Mushroom

Mushrooms have been used as food and medicinal properties for ancient time. *Auricularia auricular* was first cultivated on wood logs in China in 600 AD. Other wood logs cultivation such as *Flammulina velutipes* (800 AD) and *Lentinus edodes* (1000 AD) were grown in a similar method. The most advance in mushroom cultivation occur in France about 1600 when *Agaricus bisporus* was cultivated upon a composted substrate (Change & Miles, 2004). *Pleurotus ostreatus* was cultivated in the USA in 1900 (Sánchez, 2010). These mushrooms are the most cultivated mushroom worldwide. The cultivation of edible mushrooms continuously increases over the time due to their value as a nutritional and nutraceutical value (Change & Miles, 2004). The most commercially cultivated mushrooms worldwide and top ten country mushroom productions in the world are showed in Table 1.4 and Table 1.5. These mushrooms could be cultivated in a simple, cheap, shorter growth time, and also demand few environment controls (Reis et al., 2012).

Table 1.4 Commercially cultivated mushrooms (Sánchez, 2010)

Scientific names	Common name
Agaricus bisporus	Button mushroom, Champignon
Pleurotus species	Oyster mushroom
Lentinula edodes	Shiitake
Volvariella volvacea	Straw mushroom, paddy straw mushroom
Hericium species	Lion's head
Auricularia auricular	Jelly ear
Grifola frondosa	Maitake
Flammulina species	Golden mushroom
Pholiota nameko	Fat pholiota
Tremella species	White jelly

Table 1.5 World mushroom production (Modified from Northrop & Connor, 2009).

Country	Output in Tonnes (2008)	% of total World Output
China	1,608,219	45.9
United States	363,560	10.4
Netherlands	240,000	6.86
Poland	180,000	5.15
France	150,450	4.30
Spain	131,974	3.77
Italy	100,000	2.86
Canada	86,946	2.49
Ireland	75,000	2.14
Japan	67,000	1.92
Top 10 total (2008)	**3,003,149**	**100**

Source Medicinal Mushroom (2014)

Figure 1.2 Representative of commercial mushrooms a. *Agaricus bisporus* b. *Pleurotus ostreatus* c. *Lentinula edodes* and d. *Flammulina* species

1.3 Mushroom Cultivation

Mushroom cultivation methods varied considerably around the world. It depends on location of production. Some mushroom can be grown using indoor and outdoor method. Mushroom cultivation involved several different operation in a sequence that follows; selection of mushroom species, pure culture preparation, spawn preparation, the substrate preparation (compost and non-compost), spawn running, mushroom development, and postharvest management (Figure 1.3 and 1.4) (Chang & Miles, 2004; Pathak, Yadav & Gour, 2007).

Selection of mushroom species is an important factor to select the suitable species of mushroom to cultivate in climate condition in the area where the mushroom farm located and palatability who consume in the local area or more than commercial mushrooms (Chang & Miles, 2004). The first step is to obtain pure mycelium of the specific mushroom species. The mycelium can be obtained from a single or multi-spore culture or tissue cultures from its fruiting bodies (Chang & Miles, 2004; Pathak et al., 2007). To obtain inoculums (spawn), the pure mycelium is developed on a suitable substrate. The substrate material of spawn preparation is very important that they may be the same material used in the pure culture and they may be used as single or in combination. The substrates are generally used in the spawn preparation including various grains such as, rice straw, cotton waste, rice hull, cotton seed hull etc (Chang & Miles, 2004).

Edible mushrooms can be cultivated through a variety of methods depending on location of production. Many mushroom strain can be grown using ether indoor and outdoor or in compost and non – compost substrates. The good substrates should be balanced with carbon and nitrogen sources. The substrate should be complete sterilized or pasteurized to eliminate other organisms those contaminate and compete with mushroom mycelia (Chang & Miles, 2004). The substrate requires good aeration, suitable water content without becoming too wet, a proper pH and good drainage as the physical properties (Chang & Miles, 2004; Chang & Quimio, 1982; Pathak et al., 2007). The mushroom spawns are normally inoculated into the substrate or compost (Change & Miles, 2004). This step, the mushroom mycelium grows through the

substrate and produce extracellular enzyme that breakdown the macromolecules of the substrate into simple molecules and absorb into the mycelium for growth and development (Change & Miles, 2004). The mycelium running in this step is called the vegetative growth (Pathak et al., 2007). The suitable physical conditions during incubation must be maintained for mycelia growth. Different types of mushrooms require different conditions such as mushroom bed surfaces must not be allowed to dry out; they can be watered lightly with water sprinklers (Chang & Miles, 2004). Casing materials have essential functions in mycelium protection from the damaging effects of drying, to provide a suitable humid microclimate for the development of primordia, and act as a water reservoir for the maturing mushrooms or help for heavy flushes and help to support the growth of fruiting bodies and enhancing favorable microorganisms (Stamets & Chilton, 1983).

The substrate is colonized by mushroom mycelium under the suitable condition. The physical conditions like temperature, humidity and air ventilation need to be managed according to the different types of mushrooms. The first primodia develop into next stage until the fruiting bodies production. Fruiting bodies of mushrooms are harvested according to the species or the consumer preference and market value. It is not necessary to consider the postharvest technology if the mushroom farm is small but in large farms fresh fruiting bodies have to be packed, canned or dried to keep mushrooms in large quantities before sending to the market, as to avoid price increasing and to make them available all year (Chang & Miles, 2004; Pathak et al., 2007).

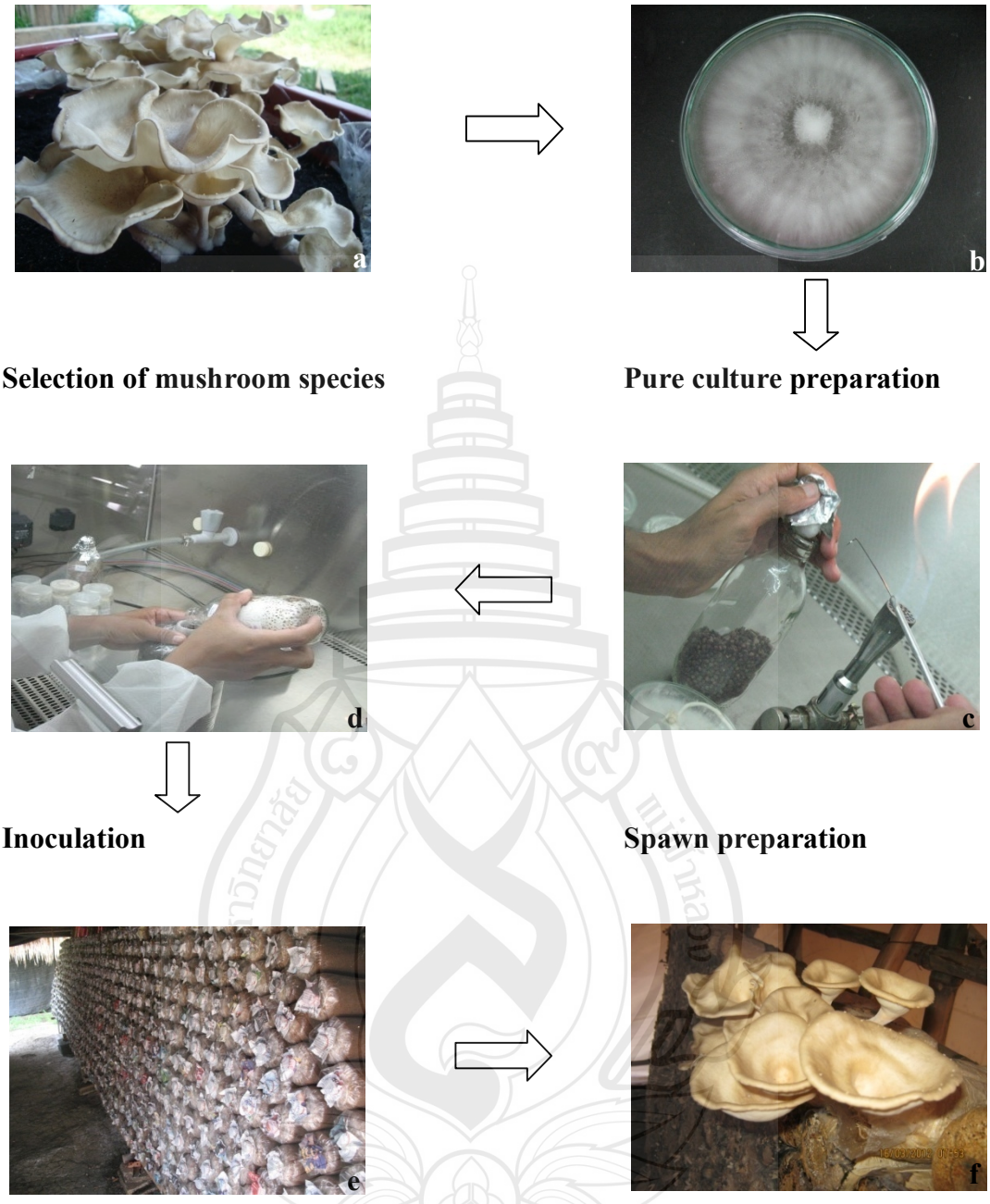


Figure 1.3 Overview procedure of mushroom cultivation

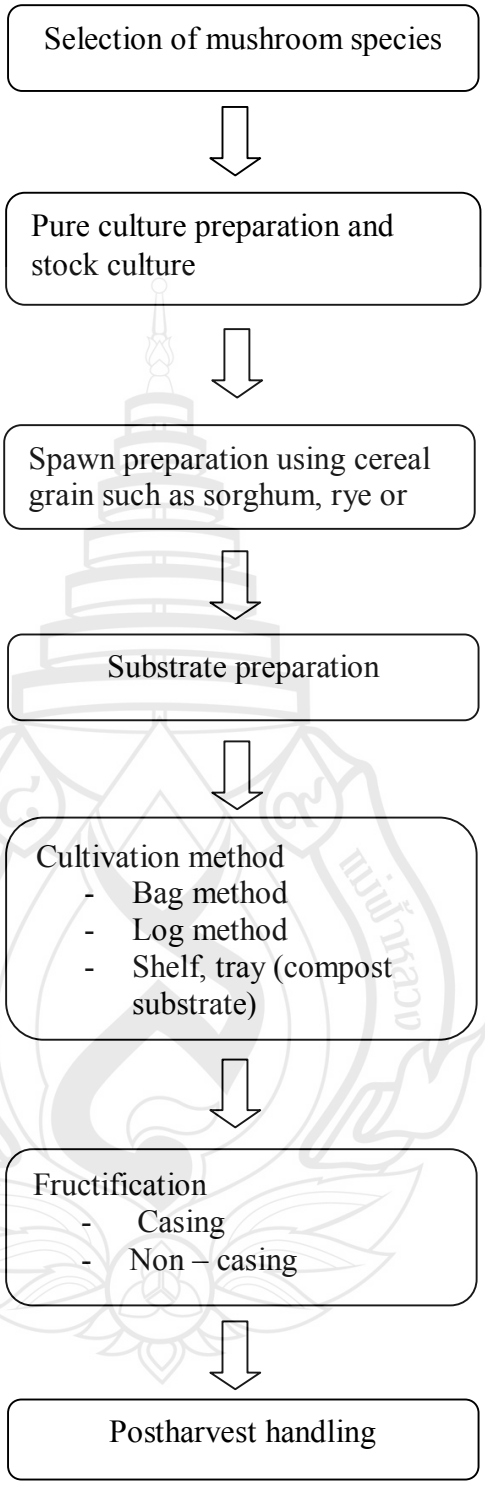


Figure 1.4 Overall procedure of mushroom cultivation

1.4 *Pleurotus* Species

Pleurotus belongs to the family of Pleurotaceae, the order of Agaricales in the Agaricomycetes class (Webster & Weber, 2007). A typical *Pleurotus* consists of a cap (pileus) that borne at the end of a stalk (stipe) (Stephenson, 2010). On the underside of cap, gills (lamellae) are located radiating from the stalk i.e. the basidia and basidiospores are produced in hymenium on the gills (Stephenson, 2010). The habitat of *Pleurotus* is typically on wood and several species are edible and commercially cultivated (Kirk, Cannon, Minter, & Stalpers, 2008; Largen, 1986).

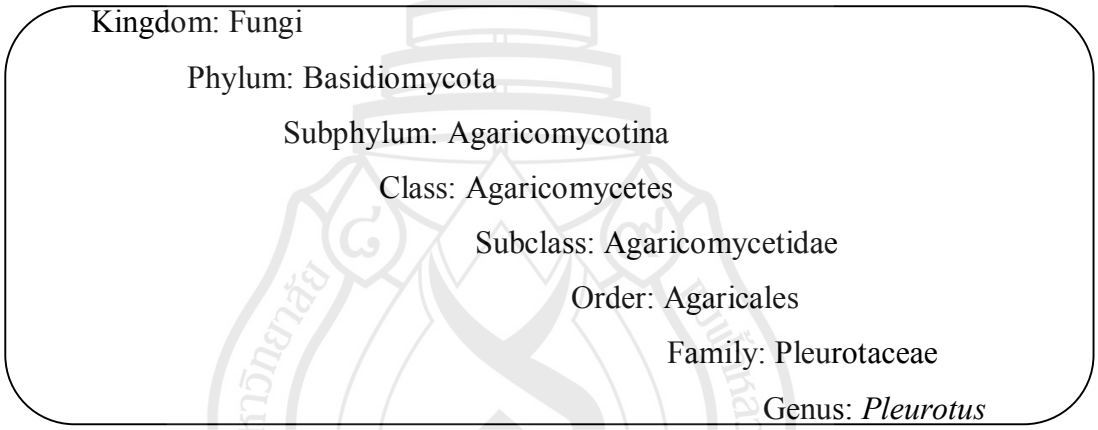


Figure 1.5 Taxonomy of *Pleurotus* mushroom

Pleurotus giganteus

Pleurotus giganteus was originally described from Sri Lanka in a typical of *Lentinus* (Berkeley, 1847). *Pleurotus giganteus* was revisited from *Panus*/*Lentinus giganteus* to *Pleurotus* based on molecular evidence (Karunarathna et al., 2012). This mushroom is edible and found in Sri Lanka, Malaysia, China, and Thailand. Apart from food benefit, there is a report described some useful medicinal properties of this mushroom. For example, phenolic compounds and triterpenoids that mimic nerve growth factor and are responsible for neurite stimulation (Phan et al., 2012).

In term of morphology, a typical of this mushroom is as follows (Figure 1.6): pileus 60–310 mm in diameter, strongly convex to applanate becoming slightly

depressed in the centre, dark brown (7F5), towards the margin light brown camel (6D4), grayish orange (5B4) at the marginal area, at centre fibrillose-scaly, surface initially uniformly dark, fuscous brown, fuliginous or black, then fading with age to pale ochraceous or yellowish brown (E8), with a darker centre although sometimes remaining dark, dry, distrupted into small, indefinite, radial, innate squamules, overlain by scanty, pale grey or blackish, verrucose-floccose, concentrically arrange remnants of the veil; margin strongly involute then straight, thin, slightly sulcate-striate. Lamellae moderately crowded with lamellulae of five lengths, decurrent, slightly interveined and anastomosing over the stipe apex, 2–3 mm broad, white to cream (3A2); edge entire, pale ochraceous or yellowish brown (E8) .Stipe up to 50–200 mm long, 7–10 mm broad at the apex, 10–15 mm at the base, fusiform, with radicating base, solid, with surface concolorous with the pileus, paler at the apex, finely tomentose with indefinite zones of paler velar remnants in the early stages; veil thin, floccose, pale to dark brown (6F6), soon reduced to floccose remnants but never forming an annulus on the stipe. Context 5–10 mm thick at the disk, submembranous at the pileal margin, white in pileus and stipe, fleshy-spongy, consisting of a dimitic hyphal system with skeletal hyphae .Generative hyphae 4–6 μm in diameter, inflating with a thick or slightly thickened wall, more or less radially parallel but frequently branching and with large clamp connections . Skeletal hyphae is 6–8 μm in diameter, hyaline of intercalary or terminal origin, becoming very thick-walled with a narrow lumen, tending to taper apically, occasionally with a limited lateral branch. Basidiospore 7–9 × 6–7 μm, broadly ellipsoid to ellipsoid, white in mass, smooth, with one large oil drop or multiguttulate, inamyloid, thin-walled). The large spores are not cylindrical but rather broadly ellipsoid, although the largest spores become oblong ellipsoid. Basidia are 25–40 × 8–10 μm, elongate, clavate, bearing 4 sterigmata .Lamella edge sterile with a broad layer of cheilocystidia 15–30 × 6–10 μm, more or less lecythiform with a ventricose base and a small capitellum (3–4 μm) subtended by a narrow neck, hyaline, thin-walled.

Note. a. Basidiocarps of *Pleurotus giganteus* from Sri Lanka, b–e: Basidiocarps of *P. giganteus* from northern Thailand. Scale bars: a = 20 cm; b, c = 10 cm

Source Karunarathna et al. (2012)

Figure 1.6 Basidiocarps of *Pleurotus giganteus*

1.5 *Lentinus* Species

Lentinus belongs to the family of Polyporaceae, the order of Polyporales in the Agaricomycetes class (Webster & Weber, 2007). *Lentinus* is a tropical species and rarely found in Polar Regions that can be extremely variable with basidiomes (Largent, 1986; Pegler, 1983). It can either slow growing or long – lived (Pegler, 1983). The fruiting body is centrally stipitate that the stipe may be shorter than the pileal diameter and may be exceptionally long and under the pileus compose of gills (Pegler, 1983). The habitat is typically on wood (Largent, 1986).

Kingdom: Fungi
Phylum: Basidiomycota
Subphylum: Agaricomycotina
Class: Agaricomycetes
Order: Polyporales
Family: Polyporaceae
Genus: *Lentinus*

Figure 1.7 Taxonomy of *Lentinus* mushroom

Lentinus connatus

Lentinus connatus was first reported by Berkeley (1842). This mushroom is edible and found in Thailand. Apart from food benefit, there is a report described some useful medicinal properties of this mushroom. For example, connatusins A showed significant cytotoxicity against human oral epidermoid carcinoma cells (KB), human breast cancer cells (BC), human lung cancer cells (NCI-H187), and Vero cells, as well as activity against the malarial parasite that (Rukachaisirikul, Tansakul, Saithong, Pakawachai, Isaka & Suvannakad, 2005).

In term of morphology, a typical of this mushroom is as follows (Figure 1.8):Karunarathna (data unpublished) has described *Lentinus connatus* Berk. which were collected in northern Thailand. Cap 2-15 (-20) cm in diameter, applanate

to deeply cyathiform, white to pale ochraceceous, white in context. Lamellae are deeply decurrent. Stipe is cylindrical, 2-15 cm x 3-15 mm, excentric or lateral, surface white soon becoming greyish brown or darker. Their smell looks like mushroomy and young fruiting bodies are edible. Cheilocystidia size is around 15-45 x 6-9 µm, clavate, narrowly clavate to cylindrical. Basidiospores are 6-8 x 3-4 µm, oblong to cylindrical, and brown in mass. Basidia are 15-45 x 6-9 µm, clavate, and have 4 spores.

Lentinus roseus

Lentinus roseus was also collected in northern Thailand as a new species. In term of morphology, a typical characteristic of this mushroom is as follow (Figure 1.8): basidiomes are relatively small. Pileus 5-5.5 cm in diameter, leathery, deeply cyathiform, when seen from above rounded flabelliform; margin eroded; surface pale yellow cream (4A3) at centre, darker towards margin and there reddish grey (11B2), not changing on bruising, dry, neither striate nor zonate. Lamellae deeply decurrent, with 5 tiers of lamellulae, 0.5 mm wide, reddish grey (11B2) close to pileus margin, pale yellow cream (4A3), towards stipe in old and young specimens, with entire edge .Stipe 1.5-2 × 0.5-0.7 cm, central, 1.5-2 × 5 mm at apex, expanding towards base 1.5-2 × 7 mm attached to a discoid base, solid, fusiform, yellowish white (4A2), floccose, leathery, solid, with white cottony context .Generative hyphae 4-5 µm diameter, inflated with a slightly thickened wall, more or less radially parallel but frequently branching and with large clamp connections .Skeletal hyphae 5-6 µm in diameter, hyaline, very thick walled with only a very narrow lumen, typically unbranched .Basidiospores 5-7 (-9) × 3-4 (-6) µm, ellipsoid to elongate, occasionally broadly ellipsoid or rarely subglobose, hyaline, thin walled, with few contents. Basidia are 18-24 × 5-7 µm, elongate clavate, bearing 4 sterigmata. Cheilocystidia 19-37 × 5.5-8 µm, clavate, hyaline, thin walled. Metuloids abundant to occasional on both sides and edges of the lamellae, 36-45 (-60) × 9-15 µm, mostly clavate with a broadly rounded apex, thick walled, hyaline. Clamp connections are prominent in generative hyphae

Note. a-b. Basidiocarps of *Lentinus connatus*. c–d. Basidiocarps of *Lentinus roseus*. Scale bars: a = 10 cm; c = 5 cm.

Source Karunarathna, Yang , Zhao, Vellinga , Bahkali, Chukeatirote & Hyde (2011)

Figure 1.8 Basidiocarps of *Lentinus connatus* and *Lentinus roseus*

1.6 Research Objectives

1.6.1 To determine an optimal condition for the mycelial growth of the three wild mushrooms; *Pleurotus giganteus*, *Lentinus connatus* and *Lentinus roseus*.

1.6.2 To evaluate a possibility in cultivating these three wild mushrooms using a sawdust substrate.

CHAPTER 2

OPTIMIZATION OF MYCELIUM OF THREE WILD MUSHROOMS

2.1 Introduction

Generally, mushrooms are regarded as 'functional food' due to their nutritive value and medicinal properties (Barros et al., 2008). Mushrooms are rich in protein and dietary fiber; and they also contain some vitamins and minerals such as vitamin B, vitamin D, potassium and magnesium (Chang & Miles, 2004; Sanmee et al., 2003). Several bioactive compounds are also found in mushrooms, for examples, eritadenine (known as hypocholesterolemic agent) is found in *Lentinus edodes* (Enman, Rova & Berglund, 2007) and bioactive compounds responsible for neurite stimulation can be found in *Pleurotus giganteus* (Phan et al., 2012). Glucosylceramide exhibiting antimicrobial activity is present in *Pleurotus citrinopileatus* (Meng, Ishikawa, Shimizu, Ohga & Kondo, 2012). At present, 650-700 mushroom species belong to 200 genera are known as edible but only about 130 mushroom species can be cultivated (Thawthong et al., 2014; Mortimer et al., 2012; Vargas-Isla & Ishikawa, 2008). Many edible mushrooms are wild collected and only available for limited period (i.e., in the raining season) (Sysouphanthong Thongkantha, Zhao, Soytong & Hyde, 2010), whereas some edible mushrooms are ectomycorrhizal and impossible to domesticate (Sanmee et al., 2003).It is therefore interesting if the new saprobic edible wild mushrooms could be cultivated for commercial purpose as then they are available for year around.

In 2011, three wild mushrooms namely *Pleurotus giganteus*, *Lentinus roseus*, and *Lentinus connatus*, were collected from Chiang Mai, Thailand (Karunarathna et al., 2011; Karunarathna et al., 2012). The cultivation of *Pleurotus giganteus* has recently been reported in China and Malaysia (Phan et al., 2012), whereas cultivation in northern Thailand has been partially successful (Klomklung, Karunarathna, Chukeatirote & Hyde, 2012). In contrast, there are no reports of the cultivation of the two *Lentinus* species (*Lentinus roseus* and *Lentinus connatus*). The discovery of *L. roseus* is quite recent and thus there is not much information except its morphological and phylogenetic data (Karunarathna et al., 2011). *Lentinus connatus* also occurs in the wild and only two reports describe its active compound (connatusin A) exhibiting antimalarial and cytotoxic activities (Rukachaisirikul et al., 2005). Due to limited data of growing *Pleurotus giganteus*, *Lentinus roseus*, and *Lentinus connatus* and potential use as food and medicine, our aim to further investigate optimal culture conditions for growing these three mushrooms. In this paper, an initial step was performed to determine favorable culture conditions for mycelial growth of *P. giganteus*, *L. connatus* and *L. roseus*.

2.2 Materials and Methods

2.2.1 Isolation of mushroom samples

Mushroom samples used in this study are shown in Table 2.1. For isolating the fungal mycelia, the sterile internal fungal tissues of their fruiting bodies were isolated and placed on potato dextrose agar (PDA). The mycelial culture was sub-cultured on PDA supplemented with 0.5% yeast extract and incubated at 30°C until the agar surface was fully covered with the fungal white mycelium.

Table 2.1 Mushroom samples

Mushrooms	Collection site
Pleurotus giganteus MFLU10 0154	Chiang Mai
Pleurotus ostreatus NK01	Phetchabun
Lentinus connatus MFLU08 1389	Chiang Mai
Lentinus roseus MFLU08 1376	Chiang Mai
Lentinus squarrosulus NK02	Phetchabun

2.2.2 Effect of culture media on mycelia growth

Several raw materials including *Phaseolus vulgaris* (red bean and black bean), *Phaseolus aureus* (mung bean), *Glycine max* L. (soybean), and *Sorghum biocolor* (L.) Moench (sorghum) were used in this study (Table 2.2 and Fig. 1). The modified agar media containing such raw materials were prepared i.e. initially; 50 g of each grain were separately soaked in 250 ml distilled water for 12 hours or overnight and boiled for 30 min. These grains were grounded using mortar and pestle, and then filtered through clean cheesecloth. Twenty grams of agar was added to each grain filtrate and the media volume was adjusted to 1 L by adding distilled water. The media were then autoclaved at 15 psi, 121°C for 15 min; 15 ml of each medium was poured into petri dishes. Malt extract agar (Difco) and PDA (Criterion) were also used for comparative study of the mycelial growth. Fungal mycelium discs (5 mm in diameter) were used as inoculums and transferred onto the center surface of each grain media. After 10 days of incubation at 30°C, the mycelial growth, density, and growth rate of the three mushrooms were measured.

Table 2.2 Raw materials

Raw materials	Sources
Red bean (*Phaseolus vulgaris*)	Tesco-Lotus, Chiang Rai
Black bean (*Phaseolus vulgaris*)	Tesco-Lotus, Chiang Rai
Mung bean (*Phaseolus aureus*)	Tesco-Lotus, Chiang Rai
Soy bean (*Glycine max* L.)	Tesco-Lotus, Chiang Rai
Sorghum (*Sorghum biocolor* (L.) Moench)	Chiang Rai local market

Note. a. Red bean b. Black bean c. Mung bean d. Soy bean e. Sorghum

Figure 2.1 Raw materials of culture media and spawn production

2.2.3 Effect of pH on mycelia growth

To screen an optimal pH for the mycelia growth, a mycelial agar disc (5 mm in diameter) was transferred to soy bean agar, red bean agar and black bean agar for *P. giganteus*, *L. roseus*, and *L. connatus*. The pH of the media was adjusted to a pH range of 5 – 8 with 1 M NaOH or HCl. The plates were incubated at 30°C for 12 days. The mycelial growth, density, and growth rate of the three mushrooms were measured.

2.2.4 Effect of temperature on mycelia growth

Four different temperatures 20, 25, 30, and 35°C were used to find the optimum temperature for mycelia growth of three wild mushrooms. Mycelia discs (5 mm in diameter) were taken from the petri dishes which were grown under suitable culture media and pH and then placed on the centre of a culture medium plate. Samples were incubated at four different temperatures for 10 days. The mycelial growth, density, and growth rate of the three mushrooms were measured.

2.2.5 Data collection and statistical analysis

A completely randomized design was used in this study. The data obtained for mycelial growth under different conditions were from five replicates. The mycelial growth of *Pleurotus ostreatus* and *Lentinus squarrosulus* were also determined for comparative purpose. The results were expressed as means and variance. Means were also compared using Duncan's multiple rang test by using SPSS-16 program.

2.3 Results and Discussion

2.3.1 Effect of culture media on mycelia growth

Seven different culture media were used to screen the optimal mycelial growth of three wild mushrooms (Table 2.3). After 10 days of incubation, *P. giganteus* was able to grow equally well on mung bean agar, black bean agar, red bean agar, sorghum agar, and soy bean agar. On these media, the mushrooms grew best on soy bean agar with the growth rate of 12.59 ± 0.34 mm/day (Table 2.3). In contrast, *P. giganteus* did not grow well when grow on PDA and MEA. This result, however,

was different from the result of Kumla, Suwannarach, Jaiyasen, Bussaban & Lumyong (2013), who reported that the best mycelial growth of *P. giganteus* was observed on PDA.

After one week of incubation, *Lentinus connatus* showed a very good equal growth on mung bean agar, black bean agar, red bean agar, sorghum agar, and soy bean agar (Table 2.3). The best mycelial growth and density of *L. connatus* was observed on black bean agar with the growth rate of 13.99 ± 0.33 mm/day (Table 2.3). On the other hand PDA and MEA showed the least mycelial growth rates. This result is related to Gbolagade, Fasidi, Ajayi & Sobowale (2006), who reported food materials such as yellow corn agar could support the mycelial growth of *Lentinus subnudus*.

After 8 days of incubation, *Lentinus roseus* grew equally well on mung bean agar, black bean agar, red bean agar, and soy bean agar. The best mycelial growth and density showed on red bean agar with the growth rate of 11.73± 0.25 mm/day (Table 2.3). The least mycelial growth rate and density were found on MEA. This result is related to Fasola, Gbolagade & Fasidi (2007), who reported food grains such as Ife brown beans, wheat, white corn and yellow corn agar could support mycelial growth of *Volvariella speciosa*.

Carbon source, nitrogen source, minerals (such as phosphorus, potassium and magnesium) and, vitamin (such as thiamin and biotin) are essential for mycelial growth of fungi (Chang & Miles, 2004). Five raw materials were also contained carbon, nitrogen, mineral and vitamin for mycelium growth with difference values (Berrios, Sqanson & Cheong, 1999; Habibullah, Abbas & Shah, 2007; Liu, Sun, Luo, Rao, Su, Xu & Yang 2012; Mubarak, 2005; Rani, Grewal & Khetarpaul, 2008; Sasanam, Paseephol & Moongngarm, 2011). Results showed that three wild mushrooms could be grown in all the media at 30 °C. Thus, the effect of culture media on mycelial growth varies according to the mushroom species.

Table 2.3 Effect of culture media on mycelial growth rate (mm/day) of three wild mushroom. Mycelial density was given in parentheses.

Mushrooms	Mycelial growth rate(mm/day) and density						
	Mung bean agar	**Black bean agar**	**Red bean agar**	**Sorghum agar**	**Soy bean agar**	**MEA**	**PDA**
Pleurotus giganteus	10.76± 0.25^{b}(+4)	11.96± 0.41^{c}(+3)	10.66±0.28^{b}(+2)	12.59± 0.38^{d}(+)	12.59± 0.34^{d}(+4)	4.69± 0.37^{a}(+4)	4.26±0.34^{a}(+)
Pleurotus ostreatus	14.56± 0.41^{cd}(+3)	16.43± 0.30^{f}(+4)	15.36± 0.32^{e}(+2)	14.26± 0.30^{c}(+)	14.79± 0.39^{d}(+3)	10.23±0.27^{a}(+2)	13.29± 0.27^{b}(+2)
Lentinus connatus	13.02± 0.27^{e}(+3)	13.99± 0.33^{f}(+4)	11.39± 0.54^{c}(+4)	12.49± 0.28^{d}(+)	10.89± 0.22^{b}(+2)	10.26± 0.36^{a}(+3)	11.43± 0.38^{c}(+4)
Lentinus roseus	10.43± 0.25^{d}(+4)	10.89± 0.38^{e}(+4)	11.73± 0.25^{f}(+5)	7.99± 0.16^{b}(+)	10.69± 0.39^{de}(+4)	5.93± 0.14^{a}(+3)	8.73± 0.27^{c}(+5)
Lentinus squarrosulus	19.33± 0.26^{f}(+4)	16.39± 0.25^{d}(+3)	16.53±0.35^{d}(+3)	15.69±0.21^{c}(+)	18.09±0.30^{e}(+2)	13.56±0.34^{a}(+3)	13.62± 0.39^{b}(+3)

Note. Mean followed by the same letters are not significantly different by Duncan's multiple range test ($P<0.05$). + very scanty, 2+ scanty, 3+ moderate, 4+ abundant, 5+ very abundant.

2.3.2 Effect of pH on mycelia growth

The effects of pH on mycelial growth of three wild mushrooms are shown in Table 2.4. Results showed that, there mushrooms grew fairly well in acidic, neutral and alkaline environments (pH 5.0 – 8.0). The best mycelial growth and density of *Pleurotus giganteus* were observed in acidic media of pH 5.0 - 6.5. This result is related to Kumla et al. (2013), who reported that the optimal pH for the mycelial growth of *P. giganteus* is at pH 7 but it normally grows well at pH range of 4-9.

The best mycelial growth and density of *Lentinus connatus* and *Lentinus roseus* were obtained in slightly acidic to neutral pH ranges from pH 5.0 – 7.0 (Table 2.4). These results are related to Gbolagade et al. (2006), who reported that the pH range from 4.0 – 8.0 could be supported the mycelial growth of *Lentinus subnudus* and the acidic medium (pH 5.0 – 5.5) is the best for mycelial growth.

The effect of pH is very important to choose the substrate for mushroom cultivation because the substrate could be a buffer to control the pH. Generally, calcium carbonate is used in mushroom cultivation to control pH of the medium (Change & Quimio, 1982).

Table 2.4 Effect of pH on mycelial growth rate (mm/day) of three wild mushroom. Mycelial density was given in parentheses.

Mushrooms	Mycelial growth rate (mm/day) and density						
	pH 5.0	**pH 5.5**	**pH 6.0**	**pH 6.5**	**pH 7.0**	**pH 7.5**	**pH 8.0**
Pleurotus giganteus	10.20± 0.41^{c}(+4)	9.36± 0.24^{b}(+4)	9.96± 0.24^{c}(+4)	9.63± 0.31^{b}(+4)	8.22± 0.36^{a}(+4)	8.29± 0.39^{a}(+4)	8.49± 0.33^{a}(+4)
Pleurotus ostreatus	15.92± 0.30^{c}(+4)	16.09± 0.14^{c}(+4)	16.09± 0.40^{c}(+4)	11.22± 0.93^{a}(+4)	11.89± 0.34^{b}(+4)	10.96± 0.36^{a}(+4)	11.89± 0.19^{b}(+4)
Lentinus connatus	10.89± 0.25^{c}(+4)	12.76±0.19^{d}(+4)	10.86± 0.24^{c}(+4)	9.76±0.34^{b}(+4)	9.76± 0.38^{b}(+4)	9.26±0.41^{a}(+4)	9.26± 0.43^{a}(+4)
Lentinus roseus	7.13± 0.31^{a}(+5)	8.43± 0.14^{c}(+5)	7.96± 0.24^{b}(+5)	7.93±0.36^{b}(+5)	8.36± 0.29^{c}(+5)	8.09± 0.32^{bc}(+5)	6.83± 0.23^{a}(+5)
Lentinus squarrosulus	21.23± 0.34^{d}(+2)	18.73± 0.19^{ab}(+2)	18.96± 0.29^{bc}(+2)	19.16± 0.26^{c}(+4)	18.76± 0.15^{ab}(+4)	19.33± 0.17^{c}(+4)	18.44± 0.39^{a}(+4)

Note. Mean followed by the same letters are not significantly different by Duncan's multiple range test ($P<0.05$). + very scanty, 2+ scanty, 3+ moderate, 4+ abundant, 5+ very abundant.

2.3.3 Effect of temperature on mycelia growth

Results were obtained when three wild mushrooms were under four different temperatures from 20 – 35°C (Table 2.5). The result showed that three mushrooms were able to grow at a temperature range of 20 – 30°C; however, three wild mushrooms did not grow at 35°C. The statistical analysis of *P. giganteus* showed that 25°C was the best temperature for mycelial growth with the growth rate of 9.66 ± 0.23 mm/day (Table 2.5). Our results agree with Kumla et al. (2013), who reported that the best temperature for mycelial growth of mushrooms is 25°C.

The best temperature for mycelial growth of *L. connatus* on black bean agar was observed at 30°C with the growth rate of 10.89 ± 0.19 mm/day (Table 2.5). *L. roseus* was able to grow well at a temperature range of 20 – 30°C. The best mycelial growth and density of *L. roseus* occurred at 30°C with the growth rate of 14.15± 0.28 mm/day (Table 2.5). This result is related to Gbolagade et al. (2006), who reported that the most suitable temperature for mycelial growth of *Lentinus subnudus* was 30°C.

Three wild mushrooms in this study were collected in the tropic region and the results showed that the temperature for mycelium growth of *P. giganteus* was 25°C and two *Lentinus* were 30°C. Temperature is one of the most important and critical physical factors on mycelium growth in mushroom cultivation. The optimum temperature is very important for growth, production of metabolic products and sporulation of mushrooms (Chang & Miles, 2004). Increasing temperature generally increase enzymatic activity but high temperatures inactive enzymes which affect on metabolism and growth of mushrooms (Chang & Miles, 2004). Several studies have shown *Pleurotus* and *Lentinus* species could be grown at 25°C or higher temperatures (45°C) (Chang & Quimio, 1982; Gbolagade et al., 2006; Vargas-Isla & Ishikawa, 2008).

Table 2.5 Effect of temperature on mycelial growth rate (mm/day) of three wild mushroom. Mycelial density was given in parentheses.

Mushrooms	**Mycelial growth rate (mm/day) and density**			
	20 °C	**25 °C**	**30 °C**	**35 °C**
Pleurotus giganteus	5.93± 0.25^{b}(+3)	9.66± 0.23^{d}(+4)	6.33± 0.31^{c}(+5)	0.00±0.00^{a}
Pleurotus ostreatus	10.36± 0.51^{b}(+2)	16.06± 0.32^{d}(+4)	15.33± 0.39^{c}(+3)	0.00± 0.00^{a}
Lentinus connatus	2.69± 0.21^{b}(+4)	8.39± 0.40^{c}(+4)	10.89± 0.19^{d}(+4)	0.00± 0.00^{a}
Lentinus roseus	13.25± 0.46^{b}(+5)	13.50± 0.25^{b}(+4)	14.15± 0.28^{c}(+4)	0.00± 0.00^{a}
Lentinus squarrosulus	8.63± 0.43^{b}(+2)	12.13± 0.46^{c}(+3)	17.59± 0.41^{d}(+4)	6.69± 0.36^{a}(+3)

Note. Mean followed by the same letters are not significantly different by Duncan's multiple range test ($P<0.05$). + very scanty, 2+ scanty, 3+ moderate, 4+ abundant, 5+ very abundant.

2.4 Conclusion

In this study, attempts were made to investigate the effect of raw materials (i.e., red bean, black bean, mung bean, soybean, and sorghum), pH, and temperature on the mycelium growth of *Pleurotus giganteus*, *Lentinus roseus* and *L. connatus* species. Our results showed that the best mycelia growths can be obtained on black bean agar and red bean agar for *L. conatus* and *L. roseus*, respectively, and both species grew well within a pH range of 5.0 – 8.0 (optimum 5.5 – 6.0) and their optimal temperature was 30°C. The optimal growth of *P. giganteus* was obtained on soybean agar within a pH range of 5.0 – 6.5 and the optimal temperature of 30°C. Since almost no researches have been done on growing *L. conatus* and *L. roseus*, these results will be a base for the future domestication research.

CHAPTER 3

DOMESTICATION OF WILD STRAIN OF *Pleurotus giganteus*

3.1 Introduction

Recently *Pleurotus giganteus* was transferred from *Lentinus* based on morphological and molecular evidences (Karunarathna et al., 2011). This species was previously named as *Lentinus giganteus* Berk. and was first described from Sri Lanka locally referred to as "Uru Paha" and classified in 'Decades of Fungi' (Berkeley, 1847). *Pleurotus giganteus* has been treated as a special food since ancient times as mentioned in Buddhist literature (Berkeley, 1847; Udugama & Wickramaratna, 1991). When fully grown, the basidioma is typically infundibuliform measuring up to 35 cm in diameter and 28 cm high (Berkeley, 1847; Udugama & Wickramaratna, 1991). The mushroom may be solitary but often forms in groups on the ground. *Pleurotus giganteus* has a thick, radicant stipe and subdistant broad lamellae which is typical of *P. giganteus* (Pegler, 1983; Karunarathna et al., 2011). It is a very popular mushroom because of its high protein content, excellent taste, bioactive components and the health-related functions (Udugama & Wickramaratna, 1991; Huang, 2005; Phan et al., 2012).

During studies of the genus *Lentinus* in northern Thailand we collected several species of *Lentinus* including three species new to science (Karunarathna et al., 2011).We also collected *Lentinus giganteus* and following molecular study found this taxon to be more closely related to *Pleurotus* (Karunarathna et al., 2012)

showing how molecular methods have revolutionized the study of taxonomy, systematics, phylogeny, biogeography, population and microevolutionary processes in basidiomycetes in the last two decades (Yang, 2011). Species of the genus *Pleurotus* are the best known of edible higher basidiomycetes as producers of the pharmacologic agent lovastatin (mevinolin) (Gunde-Cimerman, Friedrich, Cimerman, Benicˇki, 1993 (a); Gunde-Cimerman, Plemenitaš, Cimerman, 1993(b); Gunde-Cimerman & Cimerman, 1995). The presence of lovastatin was determined in four species: *P. ostreatus*, *P. cornucopiae*, *P. eryngii*, and *P. sapidus* (Wasser & Weis, 1999).

Pleurotus giganteus is one of the largest edible mushrooms in the world and can be grown on saw dust medium with supplements (Udugama & Wickramaratna, 1991). Saw dust from a mixture of wood species or Jak wood is preferred as the main substrate for *P. giganteus* growing (Udugama & Wickramaratna, 1991). *Pleurotus giganteus* is cultivated in China (Huang, 2005), Taiwan (Peng, 2006) and Malaysia (Phan et al., 2012), but even though it has a very good taste (Udugama & Wickramaratna, 1991), it is not yet cultivated in Thailand or Sri Lanka as a commercial mushroom (Karunarathna et al., 2012). The present experiment was undertaken to investigate the best conditions for domestication of wild *P. giganteus* using saw dust as a locally available substrate.

3.2 Materials and Methods

3.2.1 Spawning production

A pure culture of *Pleurotus giganteus* was maintained on soy bean agar as described in Section 2.2.1. Red bean, black bean, soy bean, mung bean and sorghum were used as substrates for spawn production. Materials were washed and soaked in distilled water for 12 hours, boiled for 5-10 minutes (almost cooked) and filtered through a clean white cloth. All materials were dried at room temperature. Forty grams of each substrate were placed in bottles and covered with lids and autoclaved. The mycelia from pure culture of *P. giganteus* were cut and inoculated into each grain material and incubated at the optimal temperature of 25ºC. The mycelial growth of each grain was recorded in order to choose the best raw material for spawn production.

3.2.2 Substrate composition, mushroom cultivation and fructification

Saw dust from rubber wood was used as the main substrate. For 1 kg of clean sawdust, 50 g of rice bran, 10 g of brewer's waste, 10 g of *Leucaena* leaf, 10 g of pumice sulfate, 10 g of calcium carbonate, and 10 g of flour were added to prepare the substrate. The components were mixed well and water gradually added until the moisture content was around 65–70%. Polypropylene bags (6.5 x 12.5 inch) were filled with 800 g prepared substrate and packed tightly. A hole (about 5 cm) was made with a PVC pipe at the centre for space to place the mycelial plugs. A plastic ring was used to make a "bottle neck" for easy handling. Plastic rings were used on the bags end, the bags end was pulled out through the ring, the pulled out part was folded down, tied with a rubber band and the hole plugged with cotton plug. The substrate bags were autoclaved at 15 psi for 15 min at 121°C or by using a steamer at 90–100°C for 3 h.

After sterilization the substrate bags were allowed to cool to room temperature, transferred with the spawn, normally using about 10 g or 0.5% to 5.0% of the weight of a substrate bag (Chang & Quimio, 1983), with *P. giganteus* under aseptic conditions. The bags were kept in a dark incubation house at room temperature and opened when the mycelia had completely colonized the substrate.

After the mycelia had completely grown in the substrate, the upper portions of the bags were opened. The opened surface of the substrate was scraped slightly with a sterile teaspoon to remove the thin whitish mycelia. The substrate bags were then placed on the shelf and covered with black cloth to give appropriate ventilation. To maintain 80–85% relative humidity in the culture house, water was sprinkled on the open end of the growing bags. Tap water spraying was carried out daily until pin heads and fruiting bodies developed.

When the fully covered with the polypropylene bags were completely removed and the contents transferred to the soil and buried or the top part of the growing bags was covered with soil and transferred to the growing house. Water spraying also was carried out daily until pin heads and fruiting bodies developed. The experiment was composed of 20 growing bags, ten bags were covered with soil and ten bags were kept without soil as controls.

3.2.3 Data collection

A completely randomized design (CRD) with ten replications was used in the experiment. Data were collected for time required for completion of mycelium running, duration from stimulation to harvest, number of mature fruiting bodies.

3.3 Results and Discussion

3.3.1 Grain spawn production

Mycelia of *Pleurotus giganteus* could grow in all five grain types to a varying extent. The best growth in terms of mycelial extension was obtained on soybean (12 days after inoculation) followed by black bean, red bean, mung bean and the poorest mycelia growth occurred in sorghum (Figure 3.1). In Thailand, mushroom growers normally use sorghum seeds as the spawn carrier because sorghum could be readily availability and low cost (Kwon & Thatithatgoon, 2004).

Note. a. soy bean b. black bean c. red bean d. mung bean e. sorghum

Figure 3.1 *Pleurotus giganteus* mycelial growth on different grain types for 12 days at 25 °C

3.3.2 Fructification

We observed that the mycelia of *Pleurotus giganteus* took 30-32 days to run from the top to the bottom of the substrate bags until pin heads developed. After 31 days of encasing, *P. giganteus* produced fruiting bodies in two bags and after 53 days in eight bags. In control bags, the fruiting was observed only in two bags after 33 days and until 53 days it was still only in two bags. Results of fruiting body development are shown in Table 3.1 and Figure 3.2. There was a significant difference of biological yield between the control group and the experimental group and biological yield of *P. giganteus* grown on control bags was poor (Table 3.1). According to Moorthy (1993), 25–28°C were found to be the optimum for *P. sajor-caju in vitro*. Cartwright & Findlay (1934) had observed that most of the fungi prefer a temperature range of 25–30°C for mushroom production. Total biological yield of the experimental group was 10 fruiting bodies/10 growing bags after 53 days (Table 3.1). The control group showed a very poor yield of only two fruiting bodies/10 bags even after 53 days (Table 3.1). Saw dust bags are mainly used for *Pleurotus* growing especially with *P. ostreatus*, whereas for *P. giganteus* growing it is essential to use soil casing in order to obtain a better yield. *Pleurotus giganteus* has been shown to have medicinal properties (Huang, 2005), and the protein content of *P. giganteus* in dry weight basis reported as 37.8%, which was the highest compared to most other cultivated popular mushrooms (Udugama & Wickramaratna, 1991). Our effort is to introduce *P. giganteus* to Thai markets as a new member, like Yanagi matsutake in Thailand (*Agrocybe cylindracea*) growing on saw dust substrate, which has a high demand and brings a handsome income to mushroom farmers (Kwon & Thatithatgoon, 2004). The lower yield and long time for *Pleurotus giganteus* to produce fruiting bodies would possibly make it an expensive mushroom. It is now important to develop better protocols for growing it at high yields which are produced quickly. It is also important to isolate other strains and find better strains for mushroom production. It may also be possible to breed hybrid strains using the methods described in Callac (1995). The study showed that it is possible to domesticate local strains of *P. giganteus* that can grow at a temperature consistent with Thailand farm productions.

In China it was successfully domesticated in the 1980's and strains are now extensively grown there (Chen & Hu, 2002; Wang-Qiu et al., 2006).

Table 3.1 Fruiting bodies production on saw dust media at 25°C

Mycelia running		**Fruiting bodies development**		
			Casing	**Non casing**
Days	**Bags**	**Days**	**Fruiting bodies No.**	**Fruiting bodies No.**
30	5	31	2	-
31	11	33	1	2
32	4	36	1	-
	20	42	2	-
		48	1	-
		53	3	-
			10	2

Note. a. *Pleurotus giganteus* in casing condition b. *Pleurotus giganteus* in non-casing condition

Figure 3.2 Fruiting bodies production of *Pleurotus giganteus*

3.4 Conclusion

The total biological yield of the experimental group was 10 fruiting bodies/10 growing bags after 53 days. Control group showed a very poor yield of only two fruiting bodies/10 bags even after 53 days. Saw dust bags are mainly used for *Pleurotus* growing especially with *P. ostreatus*, whereas for *P. giganteus* growing it is essential to use soil casing in order to obtain a better yield. *Pleurotus giganteus* has been shown to contains bioactive compounds that are responsible for neurite stimulation (Huang, 2005; Phan et al., 2012), and the protein content of *P. giganteus* in dry weight basis reported as 37.8%, which was the highest compared to most other cultivated popular mushrooms (Udugama & Wickramaratna, 1991). Our effort is to introduce *P. giganteus* to Thai markets as a new member, like other mushroom (*Agrocybe cylindracea*) in Thailand which has a high nutritional and medicinal value

(Kwon & Thatithatgoon, 2004). The low yield and long time for *Pleurotus giganteus* to produce fruiting bodies would possibly make it an expensive mushroom. It is now important to develop better protocols for growing it at high yields which are produced quickly. It is also important to isolate other strains and find better strains for mushroom production. It may also be possible to breed hybrid strains using the methods described in Callac (1995). The study showed that it is possible to domesticate local strains of *P. giganteus* that can grow at a temperature consistent with Thailand farm productions.

CHAPTER 4

DOMESTICATION OF WILD STRAIN OF *Lentinus connatus* AND *Lentinus roseus*

4.1 Introduction

The genus *Lentinus* is a genus of edible mushrooms that have a tough texture (Karunarathna et al., 2011). They are commonly consumed in the North-Eastern and Northern region of Thailand (Fangkrathok, Junlatat & Sripanidkulchai, 2013; Thetsrimuang, Khammuang, Chiablaem, Srisomsap & Sarnthima, 2011). Many previous studies were done on the properties of secondary metabolites from the crude extracts of *Lentinus connatus*. Results showed that extracts from *L. connatus* not only exhibited strong cytotoxicity activities against many human cancer cell lines such as oral epidermoid carcinoma cells (KB), human breast cancer cells (BC), human lung cancer cells (NCI-H187) but also exhibited activity against the malarial parasite (Bon, Banwell, Cade & Willis, 2011; Rukachaisirikul et al., 2005). The introduction of *L. connatus* cultivation might be a good choice of food and health. *Lentinus roseus* was found on rotten wood, in a forest with *Quercus*, *Castanopsis* and *Lithocarpus echinops* and in moist upper mixed deciduous forest. The color of mature fruiting bodies is rose (Karunarathna et al., 2011). This characteristic could be introduced to the commercial mushroom as a new choice for consumer.

In Thailand, *Lentinus* cultivation was less than *Pleurotus* species in number of species and quantity aspects (Karunarathna et al., 2011). Huge quantities of saw dust are freely available from the agro-forest and timber industries in Thailand that the grower prefers to use sawdust for mushroom cultivation (Fangkrathok et al., 2013; Kittitornkool, Bumroongsri & Kaewwongsri, 2013; Thetsrimuang et al., 2011).

The sawdust used in substrate does not have sufficient nitrogen and other components required for the fermentation process therefore, the compounding mixture is supplemented with nitrogen, carbohydrate, vitamin and mineral sources, such as rice bran, spent brewery grains, *Leucaena* leaf, to enhance this process (Gregori, Švagelj, Pahor, Berovič & Pohleven, 2008; Pathak et al., 2007). Spent brewery grains are by products with a low economic value of the brewing industry and also contained carbon and nitrogen source (Gregori et al., 2008). *Leucaena* leaf is the one substrate as a protein source which they were used on rumen diets for nitrogen source and were also used as nitrogen source in mushroom cultivation in Thailand (Kang, Wanapat, Pakdee, Pilajun & Cherdthong, 2012; Klomklung et al., 2012). This study was investigated the best conditions for domestication of wild *Lentinus* species using sawdust as a locally available substrate with spent brewer grain and *Leucaena* leaf.

4.2 Materials and Methods

4.2.1 Spawning production

A pure culture of *L. connatus* and *L. roseus* were maintained on black bean agar and red bean agar as described in Section 2.2.1. Red bean, black bean, soy bean, mung bean and sorghum were used as substrates for spawn production. Materials were washed and soaked in distilled water for 12 hours, boiled for 5-10 minutes (almost cooked) and filtered through a clean white cloth. All materials were dried at room temperature. Forty grams of each substrate were placed in bottles and covered with lids and autoclaved. The mycelia from pure culture of these mushrooms were cut and inoculated into each grain material and incubated at the optimal temperature (30ºC). The mycelial growth of each grain was recorded in order to choose the best raw material for spawn production.

4.2.2 Bag preparation, cultivation and fructification

Sawdust from rubber wood was used as the main substrate in two formulations with supplements (Table 4.1). The components were mixed well and water gradually added until the moisture content was around 65–70 %. Polypropylene bags (6.5 x 12.5 inch) were filled with 800 g prepared substrate and packed tightly.

A hole (about 5 cm) was made with a PVC pipe at the top of bags for space to place the mycelial plugs. A plastic ring was used to make a “bottle neck” for an easy handling. Plastic rings were used on the bags end, the bags end was pulled out through the ring, the pulled out part was folded down, tied with a rubber band and the hole plugged with cotton plug. The substrate bags were autoclaved at 15 psi for 15 min at 121°C or by using a steamer at 90–100°C for 3 h. After sterilization the substrate bags were allowed to cool to room temperature, inoculated with the spawn, normally using about 10 g or 0.5% to 5.0% of the weight of a substrate bag (Chang & Quimio, 1983), with *L. connatus* and *L. roseus* under aseptic conditions. The bags were kept in a dark incubation house at room temperature and opened when the mycelia had completely colonized the substrate.

After the mycelia had completely grown in the substrate, the upper portions of the bags were opened. The opened surface of the substrate was scraped slightly with a sterile teaspoon to remove the thin whitish mycelia. The substrate bags were then placed on the shelf and covered with black cloth to give appropriate ventilation. To maintain 80–85% relative humidity in the culture house, water was sprinkled on the open end of the growing bags. Tap water spraying was carried out daily until the fruiting bodies had fully developed. Fruiting bodies were cut when mature; wet weight was recorded for each formulation.

Table 4.1 Substrate formulation (Poor and Enriched substrates)

Poor substrate	Weight (g)	Enriched substrate	Weight (g)
Sawdust	1000	Sawdust	1000
Rice bran	30	Rice bran	50
Calcium carbonate	10	Spent brewery grain	10
		Leucaena leaf	10
		Flour	10
		Pumice sulfate	10
		Calcium carbonate	10

4.2.3 Data collection

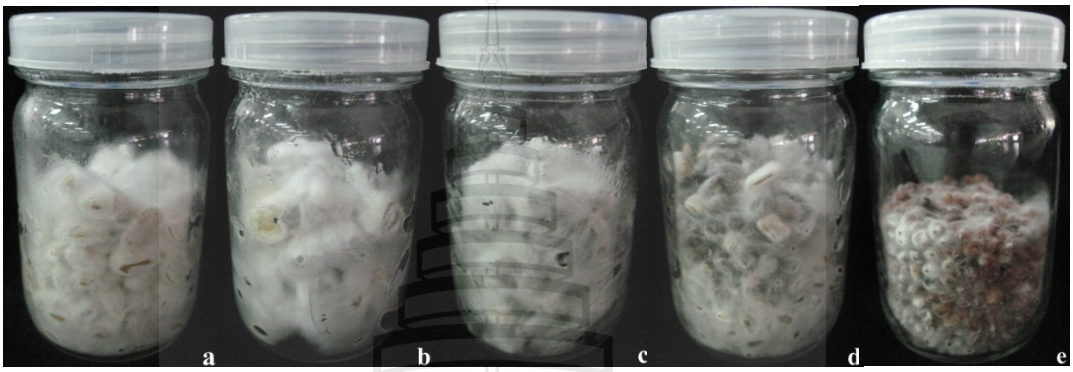
A completely randomized design (CRD) with ten replications was used in the experiment. Data were collected for time required for completion of mycelium running and fresh weight of mature fruiting bodies. The data were analyzed statistically mean by SPSS-16 program.

4.3 Results and Discussion

4.3.1 *Lentinus connatus* fructification

Spawning preparation: The mycelial growth of *L. connatus* in all five substrates were investigated and showed in Figure 4.1. After ten days of incubation, soy bean, red bean, black bean and mung bean had more thicker mycelial growth in comparison than sorghum by visually assess. The mycelial grew faster on soy bean, followed by red bean, black bean and mung bean. The best grain is a vehicle for evenly distributing the mycelium and also contains a nutritional supplement. Whole grain is used because each kernel becomes a mycelial capsule which smaller kernels of grain can be launched into bulk substrates (Stamets, 2000).

In Thailand, mushroom growers normally use sorghum seeds as the spawn carrier (Kwon & Thatithatgoon, 2004) but our results showed sorghum was the least suitable substrate. However, Thai farmer still used sorghum as substrate because of its readily availability, easily to find in the market and low cost.



Note. a. soy bean b. red bean c. black bean d. mung bean e. sorghum

Figure 4.1 *Lentinus connatus* mycelia growth on different grain type

Bag cultivation: The spawn production of *L. connatus* was inoculated into two types of substrates for a comparison on the fruiting bodies yield. In this study, *L. connatus* took 40 - 45 days (poor substrate) and 35 - 39 days (enriched substrate) for complete running in the substrate bags (Table 4.2 and Figure 4.2). The yield of *L. connatus* of poor substrate was 11.43 ± 1.10% and the enriched substrate was 16.37 ± 1.71%. The enriched substrate could be support the mushroom yield more than poor substrate because they contained high nitrogen, carbon, vitamin and mineral sources. The nitrogen sources are rice bran, spent brewer grain and *Leucaena* leaf and they also contain some vitamin and mineral (Anbarasu, Dutta, Sharma & Rawat, 2004; Gregori et al., 2008; Kang et al., 2012; Stamets, 2000).

Despite the sawdust, the carbon sources were came from spent brewer grain and flour (Gregori et al., 2008; Stamets, 2000). Mineral also contained from pumice sulfate and calcium carbonate. The supplements were used to enhance the best substrate, extra treatment is required to prevent a contamination and insure success (Stamets, 2000). Other problem of this study was both substrate formula could be produce only crop that the solution of this problem might be change the new stain, support by suitable substrate and condition or breeding methods (Stamets, 2000) that it need to future study.

Table 4.2 Mycelia running of *Lentinus connatus* on different substrate formulation

Substrate formulation I		**Substrate formulation II**	
Days	**Bags**	**Days**	**Bags**
40	1	35	2
41	1	36	2
42	-	37	3
43	3	38	1
44	3	39	2
45	2	40	-
46	-	41	-
	10		10

Note. a. Fruiting bodied in poor substrate b. Fruiting bodies of enrich substrate. Scale bars: A = 10 cm; B = 10 cm.

Figure 4.2 Fruiting bodies production of *Lentinus connatus*

4.3.2 *Lentinus roseus* fructification

Spawning preparation: Mycelia of *L. roseus* could grow in all five grain types to a varying extent. After ten days of incubation, red bean, mung bean, black bean and soy bean had more thicker mycelial growth in comparison than sorghum by visually assess.The best growth in terms of mycelia extension was obtained on red bean followed by sorghum, mung bean, black bean and the poorest mycelia growth occurred in soy bean (Figure 4.3). In this study, red bean and sorghum was support the spawn production but sorghum has lower price than red bean so it appropriate to use for spawn production than red bean.

Note. a. red bean b. sorghum c. mung bean d. black bean e. soy bean

Figure 4.3 *Lentinus roseus* mycelia growth on different grain type for 10 days at 30°C

Bag cultivation: The mycelia of *Lentinus roseus* took 32-35 days for formula I and 29-32 days for formula II to run from the top to the bottom of the substrate bags, no fruiting bodies were form after opened up (Table 4.3 and Figure 4.4). The components of enriched substrate are composed of high carbohydrate, nitrogen, vitamin and mineral source that support the mycelium growth (Change & Miles, 2004). The problem of pre-harvest period was mycelium grows but fails to produce fruiting bodies that might be cause by absence of clamp connection, bad strain, and mismatch of strain with substrate formula, was infected by other microorganism and nematode and inhibited by environmental toxins (Stamets, 2000). Preliminary test, *L. roseus* was cultivated on enrich substrate and the condition same as in this study that the fruiting bodies could be develop.

Other solution to improve the problem might be check for clamp connection because *L. roseus* is one species that contain large clamp connection (Karunarathna et al., 2011; Stamets, 2000).

Table 4.3 Mycelial running of *Lentinus roseus* on different substrate

Poor substrate		**Enriched substrate**	
Days	**Bags**	**Days**	**Bags**
29	-	29	2
30	-	30	6
31	-	31	1
32	1	32	1
33	3	33	-
34	4	34	-
35	2	35	-
	10		10

Note. a. Growing bag b. non-fruiting bodies c. on the top of growing bag

Figure 4.4 Fruiting bodies production of *Lentinus roseus*

4.4 Conclusion

Sorghum was the cheapest raw material for spawn production of *Lentinus connatus*. The mycelium growth occurs in poor and enrich substrate but in the enriched substrate took the less time than poor substrate. Further, the yields of *L. connatus* in enriched substrate were higher than poor substrate. *Lentinus connatus* could be introduced to new mushroom domestication in Thailand. The increased yield may be support by suitable substrate and condition and breeding methods are also support the high yield that it need to future study. Red bean was the best raw material for spawn production of *L. roseus* base on the mycelial growth. Sorghum grain was also a suitable substrate of spawn production of *L. roseus* based on the low cost. The mycelium growth occurred in both poor and enriched substrate. Furthermore, the mycelium took less time in enriched substrate than the poor substrate too. In this

study, *L. roseus* could not develop the fruiting bodies. The new strain might be choosing to improve the cultivation methods.

CHAPTER 5

Conclusion

Wild mushrooms have been consumed since ancient time. Some wild mushrooms like mycorrhizal mushroom (*Russula* sp. and *Cantharellus* sp.) are expensive because they can be collected only from the wild. Furthermore, another wild mushrooms that can be cultivated, but have not been developed for cultivation in mushroom house. *Pleurotus* and *Lentinus* species such as *Pleurotus ostreatus* and *Lentinus edodes* are also known as nutritional food. In this study, three wild mushrooms were studied to introduce then as new cultivated mushrooms.

5.1 Optimization of Mycelium of Three Wild Mushrooms

Three wild mushrooms namely; *Pleurotus giganteus*, *Lentinus connatus*, and *Lentinus roseus* were studied on cultivation on the seven different culture media (red bean agar, black bean agar, mung bean agar, soybean agar, sorghum agar, MEA and PDA) to obtain the best mycelial growth. *P. giganteus* was able to grow seven different culture media. On these media, the mushrooms grew best on soy bean agar. *Lentinus connatus* showed a very good equal growth on mung bean agar, black bean agar, red bean agar, sorghum agar, and soy bean agar. The best mycelial growth of *L. connatus* was observed on black bean agar. *L. roseus* also grew equally well on mung bean agar, black bean agar, red bean agar, and soy bean agar. The best mycelial growth showed on red bean agar.

The results showed that, three mushrooms grew fairly well in acidic, neutral and alkaline environments (pH 5.0 – 8.0). The best mycelial growth and density of *P. giganteus* were observed in acidic media of pH 5.0 - 6.5. The best mycelial growth and density of *L. connatus* and *L. roseus* were obtained in slightly acidic to neutral pH ranges from pH 5.0 – 7.0

Results showed that three mushrooms were able to grow at a temperature range of 20 – 30°C; however, three wild mushrooms did not grow at 35°C. Statistical analysis of *P. giganteus* showed that 25°C was the best temperature for mycelial growth. The best temperature for mycelial growth of *L. connatus* on black bean agar was observed at 30°C and the best mycelial growth and density of *L. roseus* also occurred at 30°C.

5.2 Domestication of Wild Strain of Three Wild Mushrooms

Three wild mushrooms were cultivated on the rubber saw dust substrate. The cultivation method of *P. giganteus* involves two steps, inoculating on saw dust substrate in polypropylene bags as the preliminary step and transferring to the soil as the second step, which is very important for fruiting. Total biological yield of the experimental group was 10 fruiting bodies/10 growing bags after 53 days. The control group showed a very poor yield of two fruiting bodies/10 bags even after 53 days.

Lentinus connatus and *L. roseus* were cultivated on nutrient poor and enriched substrates. *Lentinus connatus* took less time on enriched substrate than nutrient poor substrate. Further, the yields of *L. connatus* in enriched substrate were higher than nutrient poor substrate and the mycelium growth of *L. roseus* occurred in both nutrient poor and enriched substrates. Also, the mycelium took less time in enriched substrate than the nutrient poor substrate. In this study, *L. roseus* could not develop the fruiting bodies that might be due to the absence of clamp connection, bad strain, and mismatch of strain with substrate formula, was infected by other microorganism and nematode and inhibited by environmental toxins.

5.3 Further Work

In this study, *Pleurotus giganteus* and *Lentinus connatus* were able to produce the fruiting bodies. However, as compared with commercial mushrooms, both mushrooms gave lower yield. It is now important to develop better protocols for growing then for high yields which are able to fructify. It is also important to isolate other strains and find better strains for mushroom production. It may also be possible to breed hybrid strains using the methods. *Lentinus roseus* could not develop the fruiting bodies. The new strains might be choosen to improve the cultivation methods.

5.4 Publication from This Thesis

During this study, the experiment was related to optimization of wild mushrooms and cultivation of wild mushrooms. Publication obtained from my study is as follow:

Klomklung, N., Karunarathna, S. C., Chukeatirote, E. & Hyde, K. D. (2012).

Domestication of wild strain of *Pleurotus giganteus*. *Sydowia*, 64 (1), 39-54

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APPENDICS

APPENDIX A

EFFECT OF MEDIA ON MYCEIA GROWTH

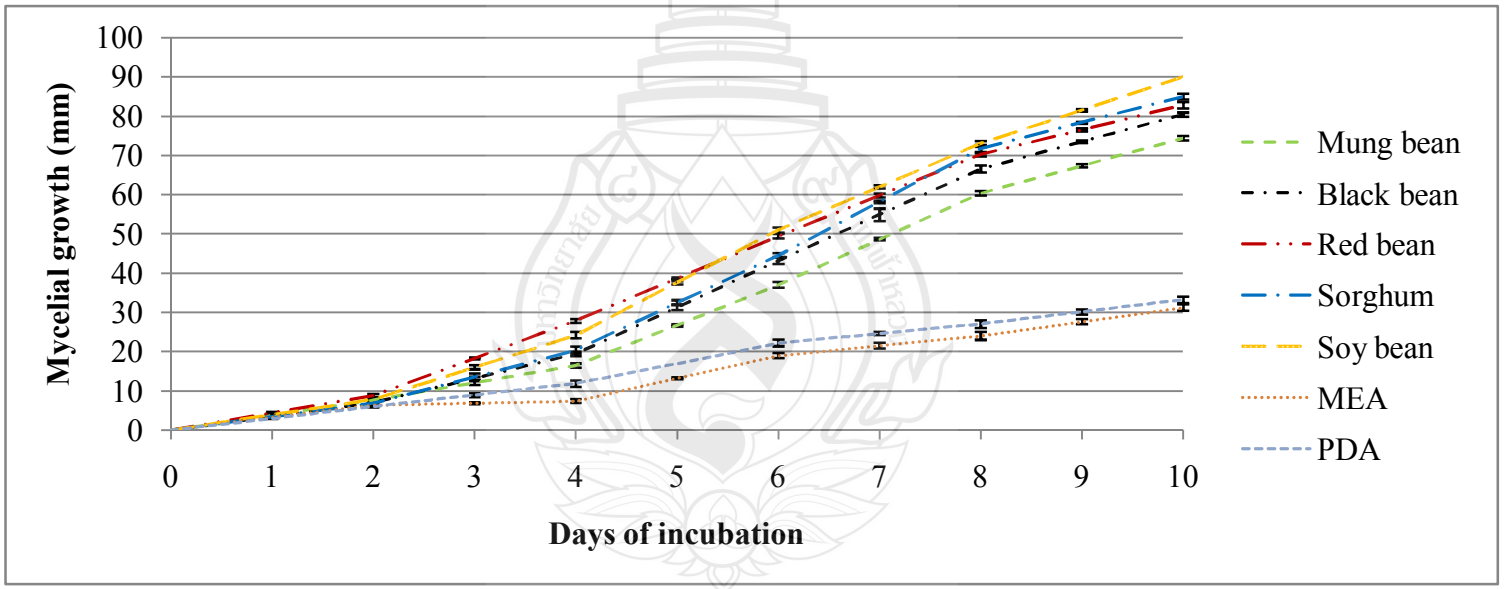

Figure A1 Effect of media on mycelia growth of *Pleurotus giganteus*

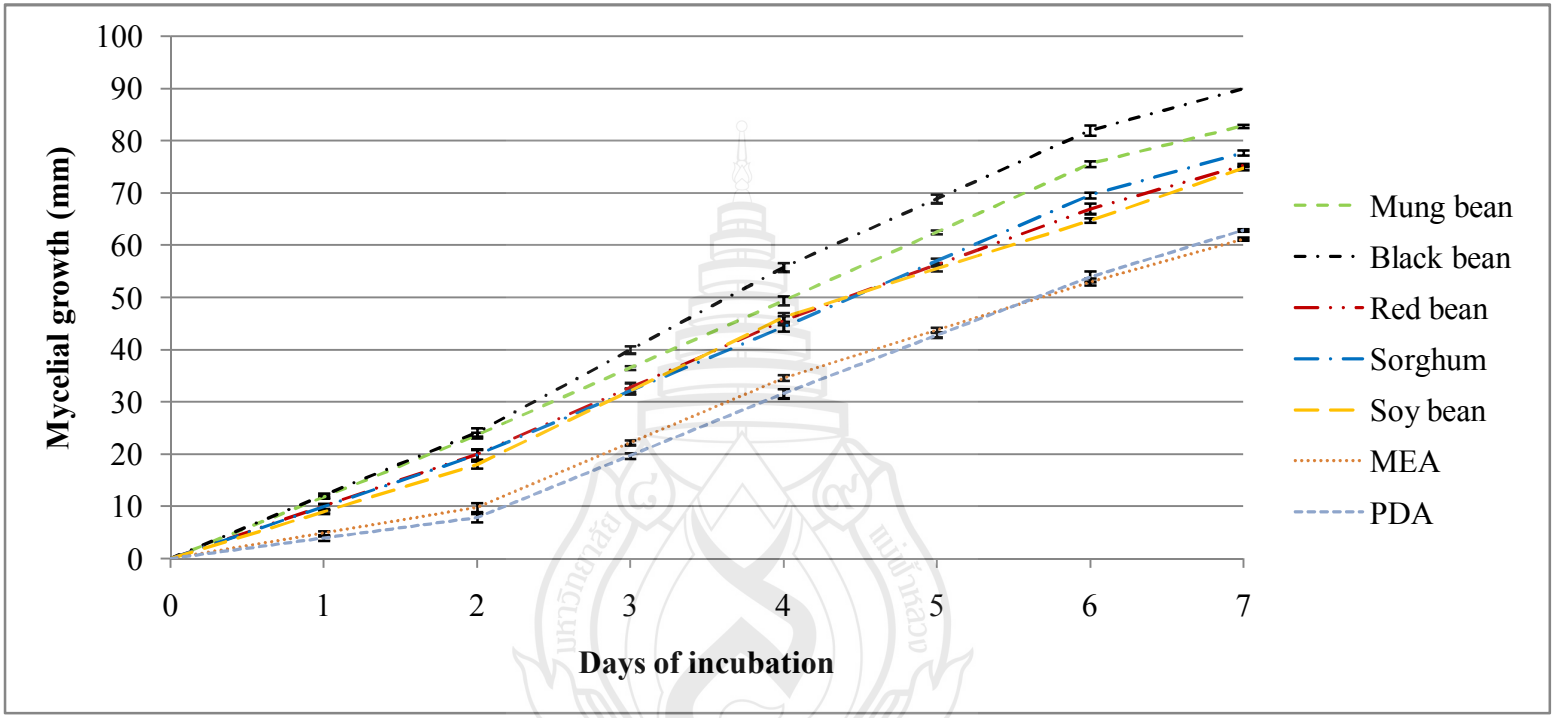

Figure A2 Effect of media on mycelia growth of *Lentinus connatus*

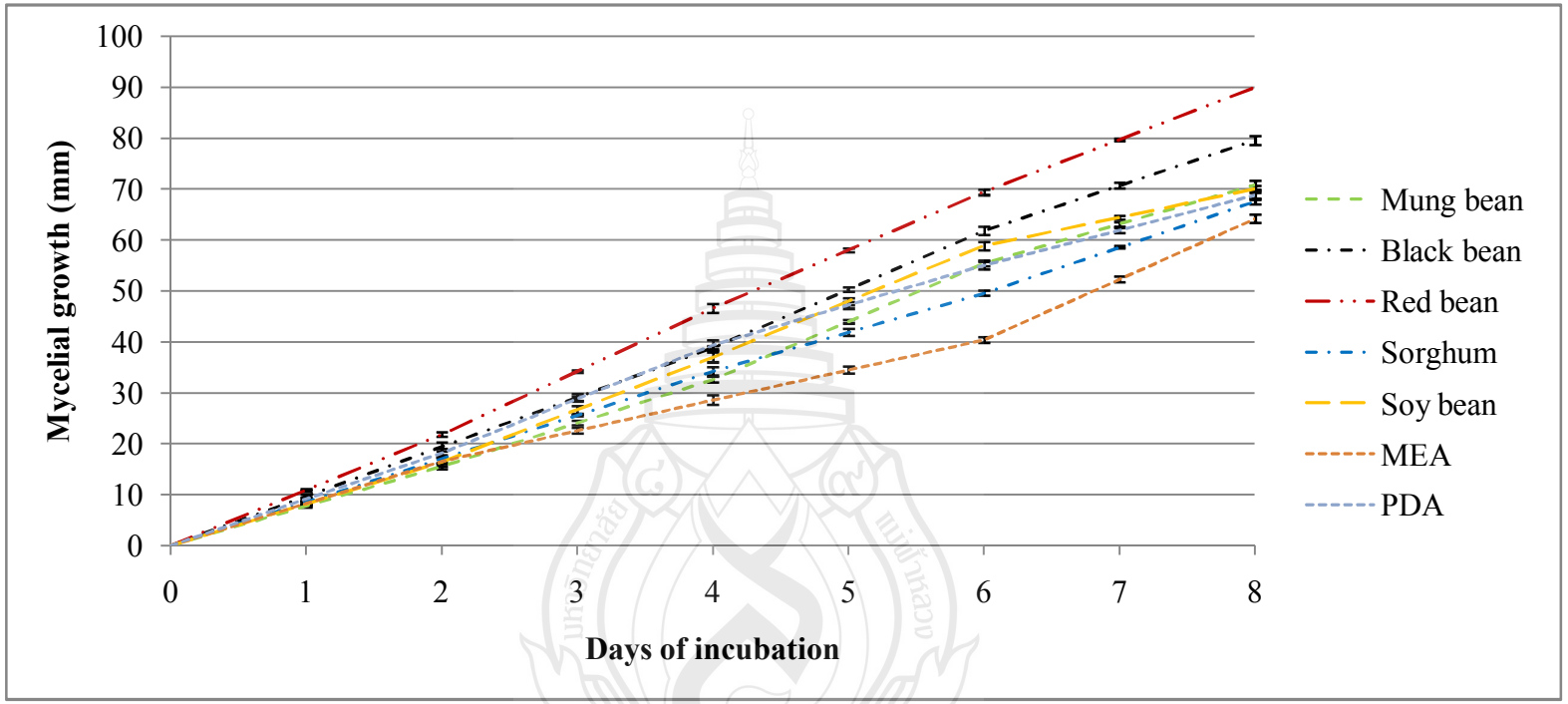

Figure A3 Effect of media on mycelia growth of *Lentinus roseus*

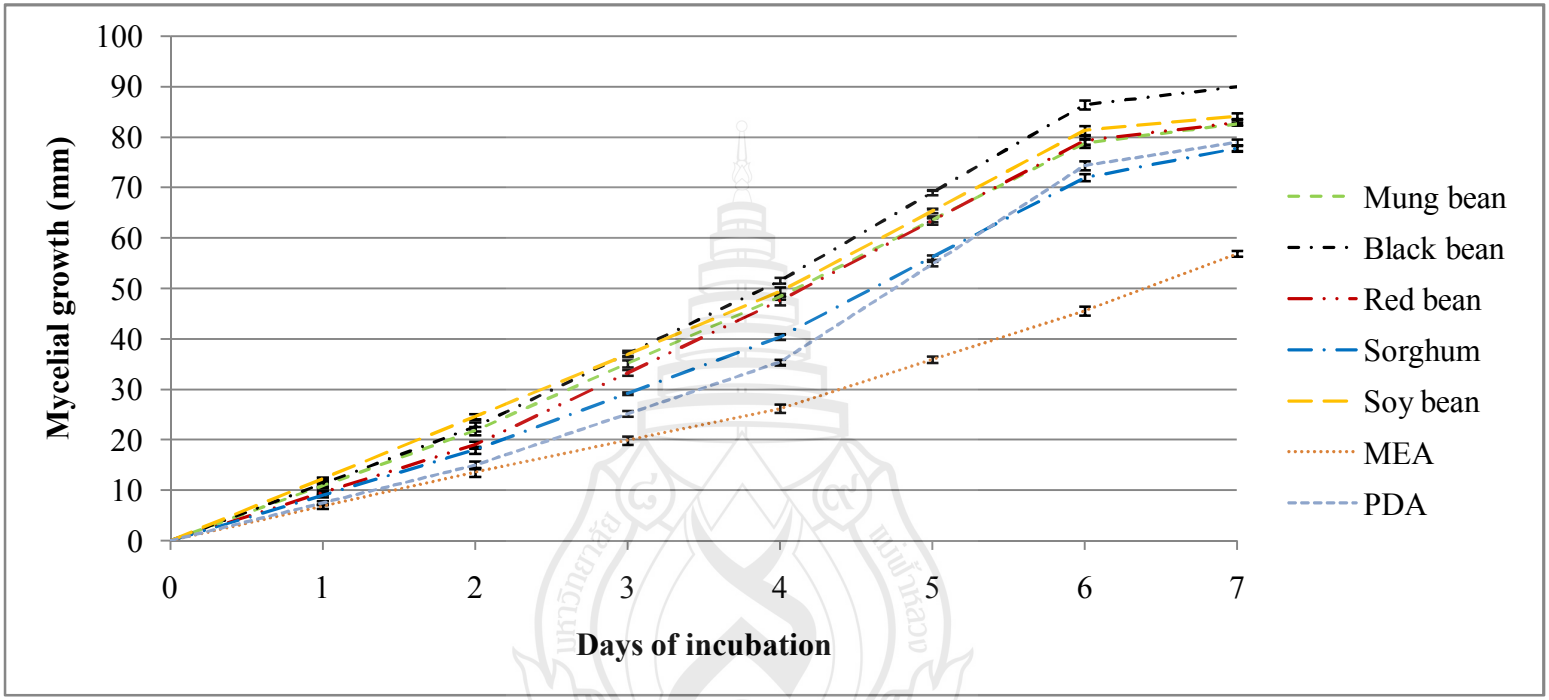

Figure A4 Effect of media on mycelia growth of *Pleurotus ostreatus*

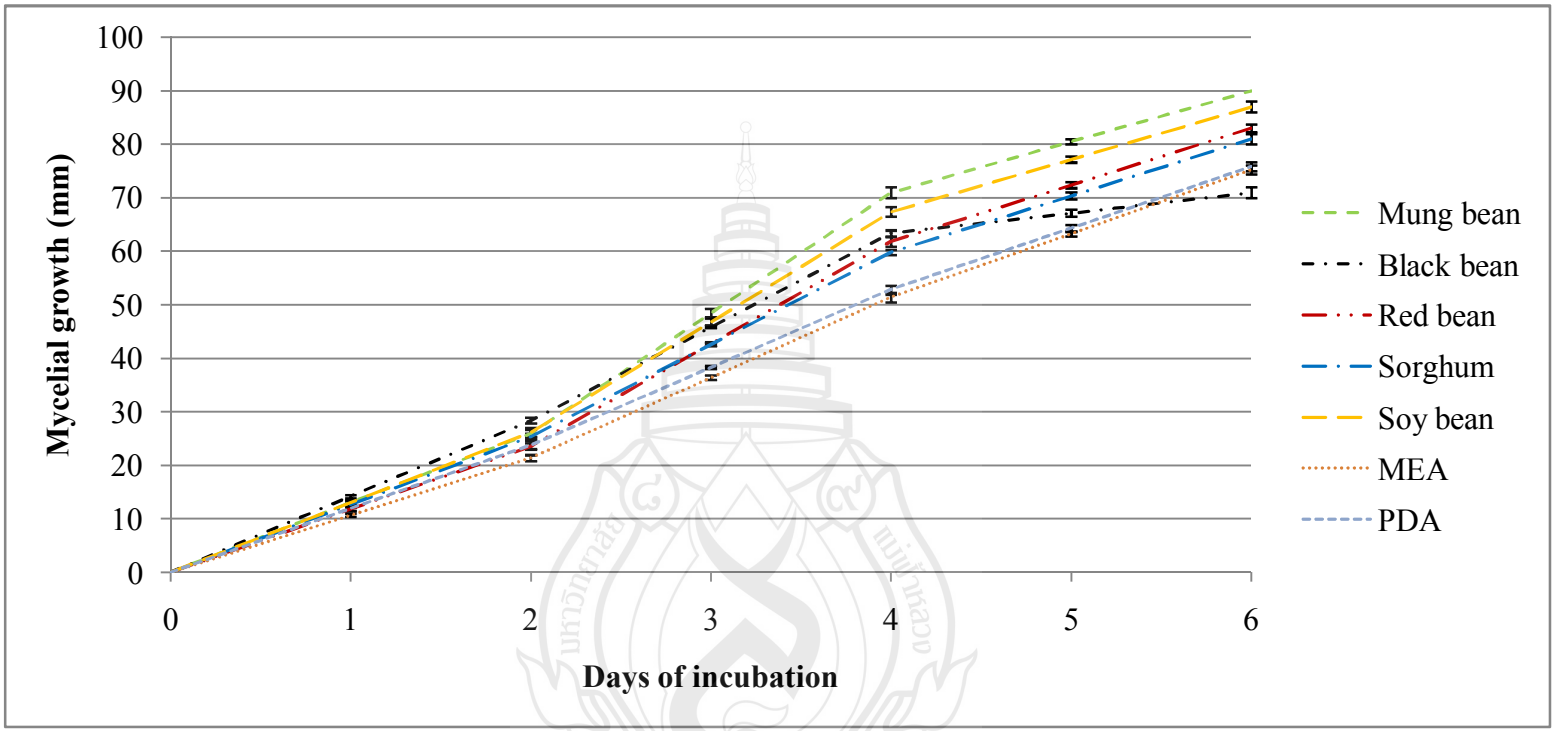

Figure A5 Effect of media on mycelia growth of *Lentinus squarrosulus*

APPENDIX B

EFFECT OF pH ON MYCELIA GROWTH

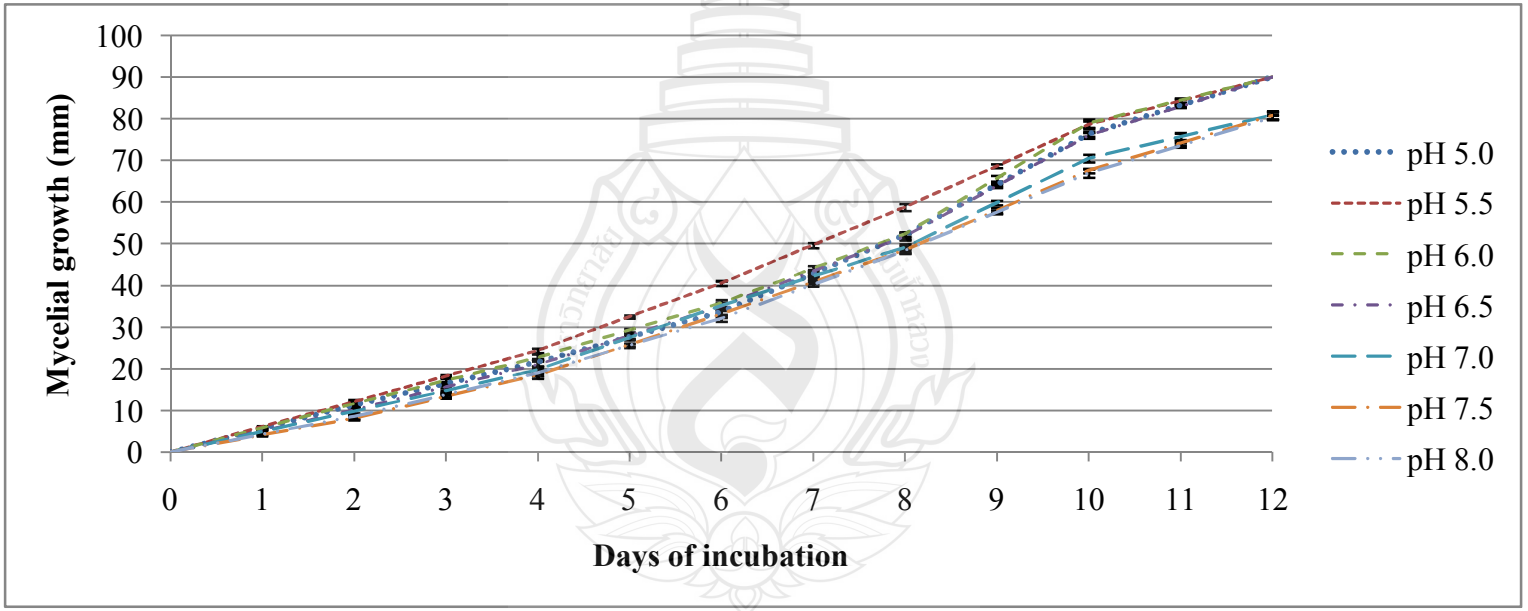

Figure B1 Effect of pH on mycelia growth of *Pleurotus giganteus*

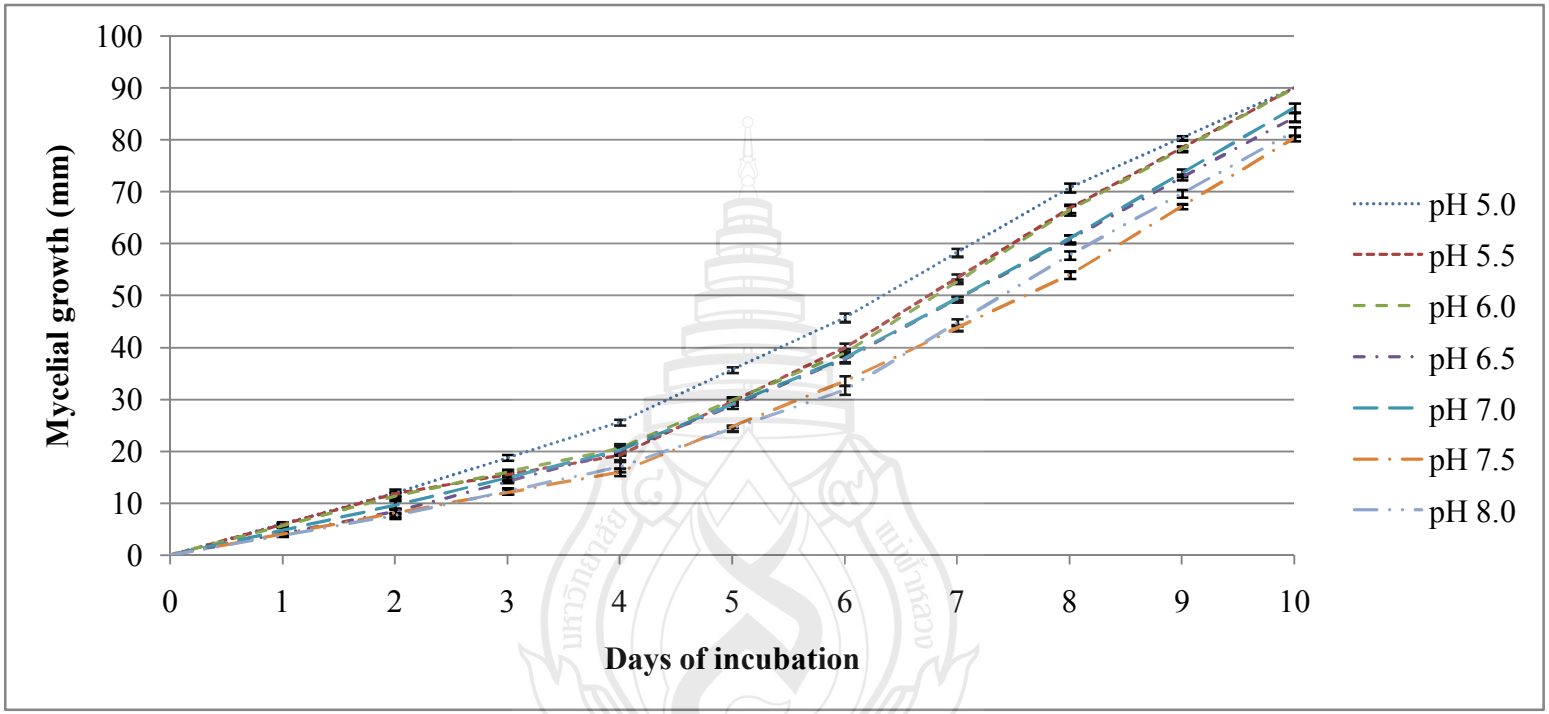

Figure B2 Effect of pH on mycelia growth of *Lentinus connatus*

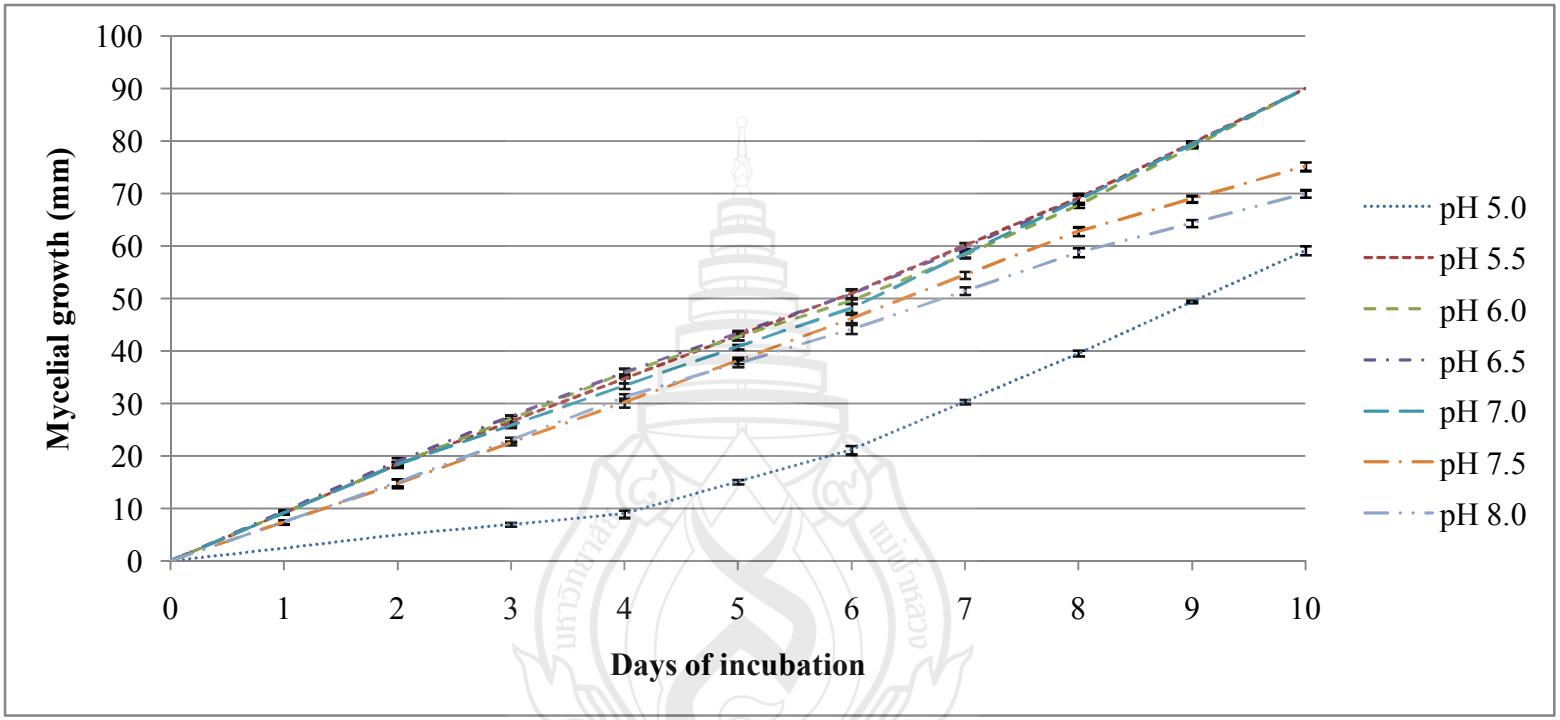

Figure B3 Effect of pH on mycelia growth of *Lentinus roseus*

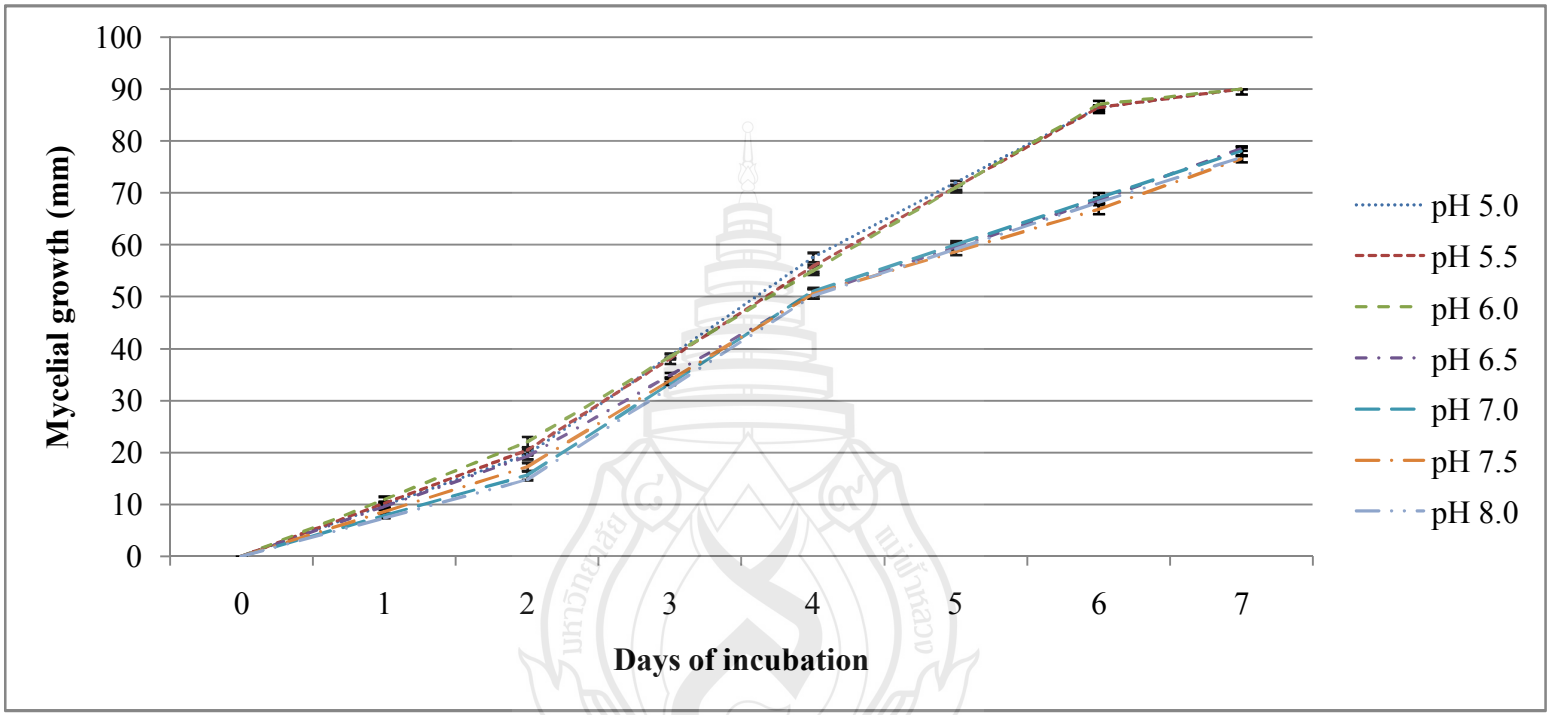

Figure B4 Effect of pH on mycelia growth of *Pleurotus ostreatus*

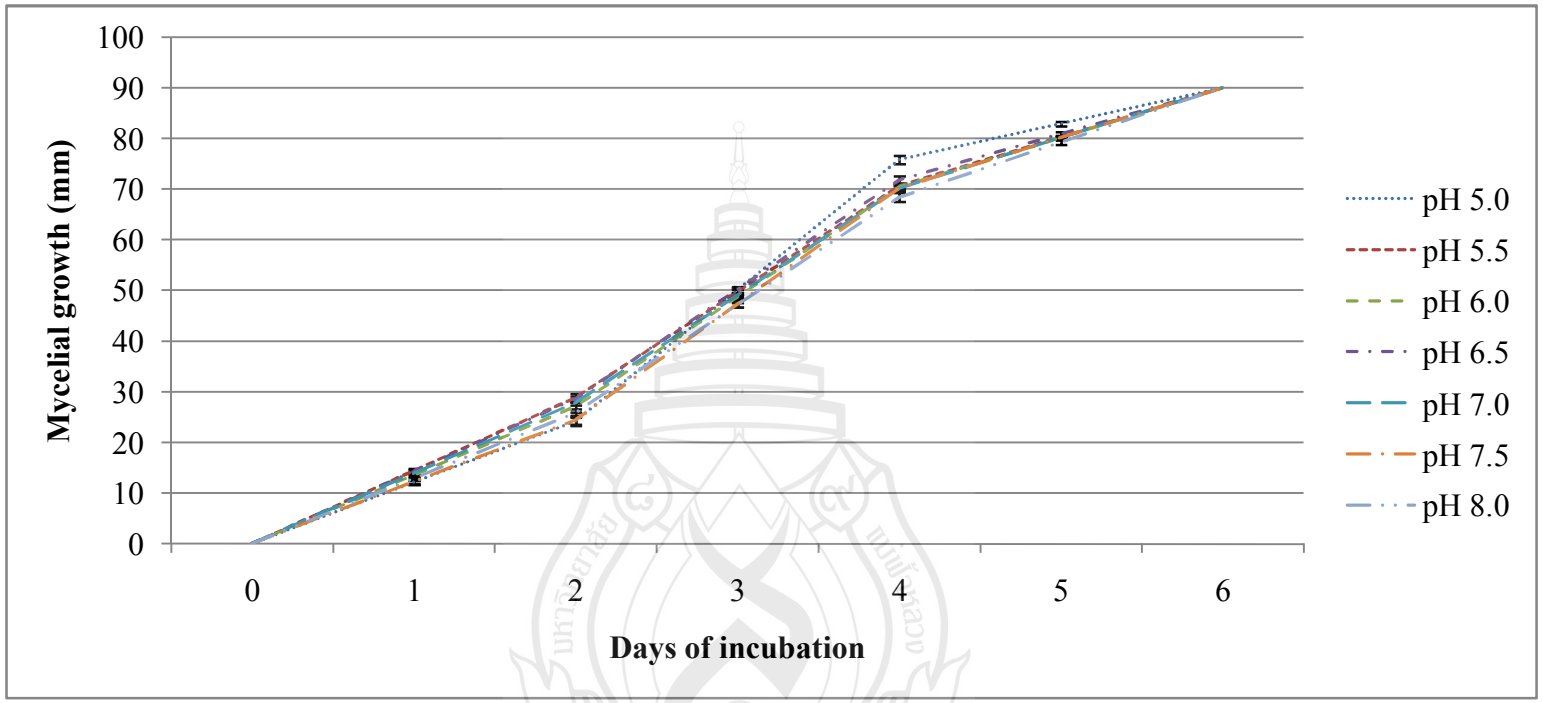

Figure B5 Effect of pH on mycelia growth of *Lentinus squarrosulus*

APPENDIX C

EFFECT OF TEMPERATURE ON MYCEIA GROWTH

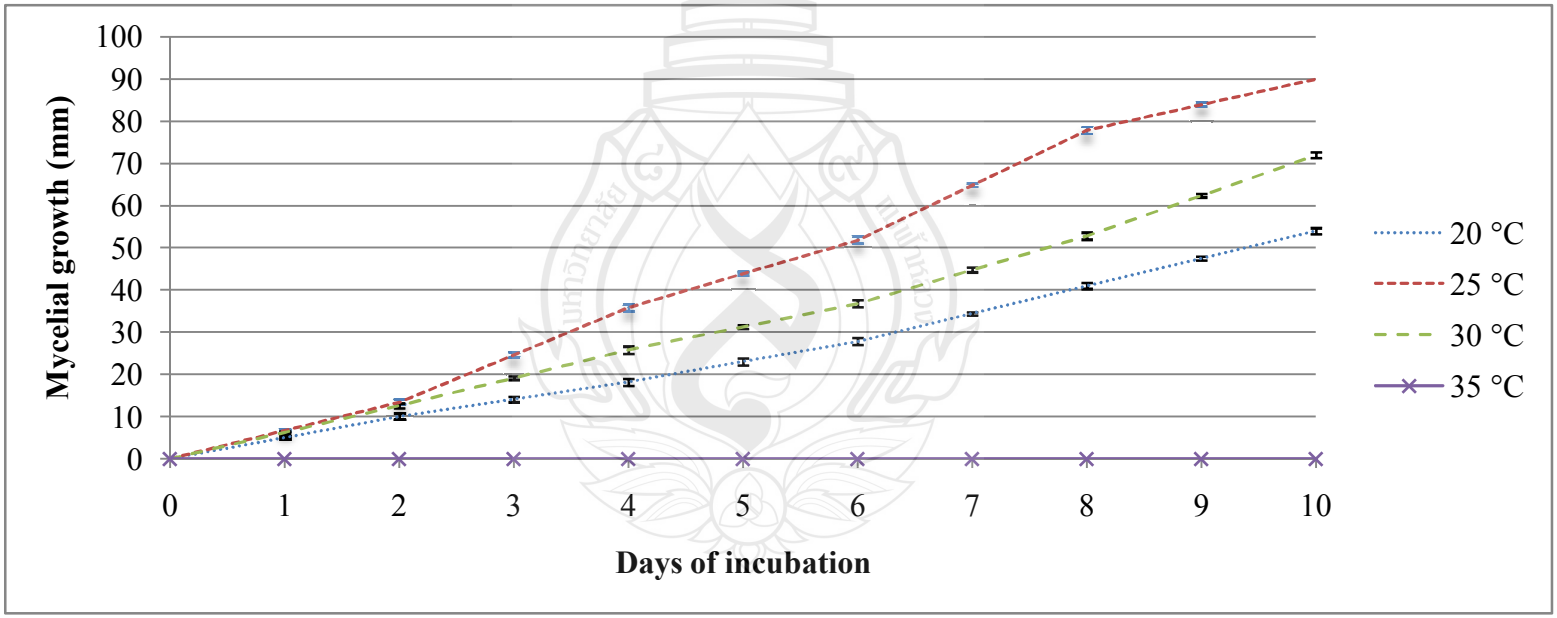

Figure C1 Effect of temperature on mycelia growth of *Pleurotus giganteus*

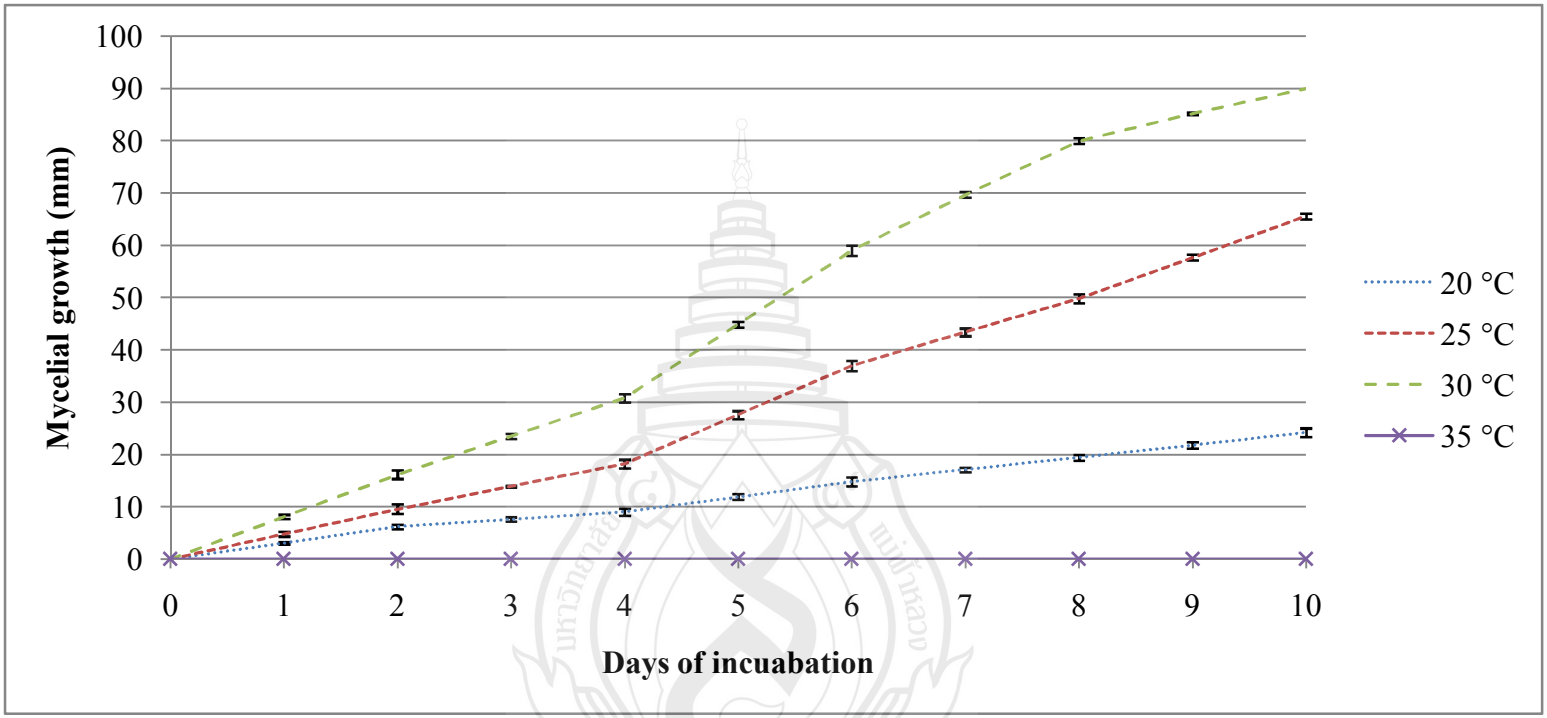

Figure C2 Effect of temperature on mycelia growth of *Lentinus connatus*

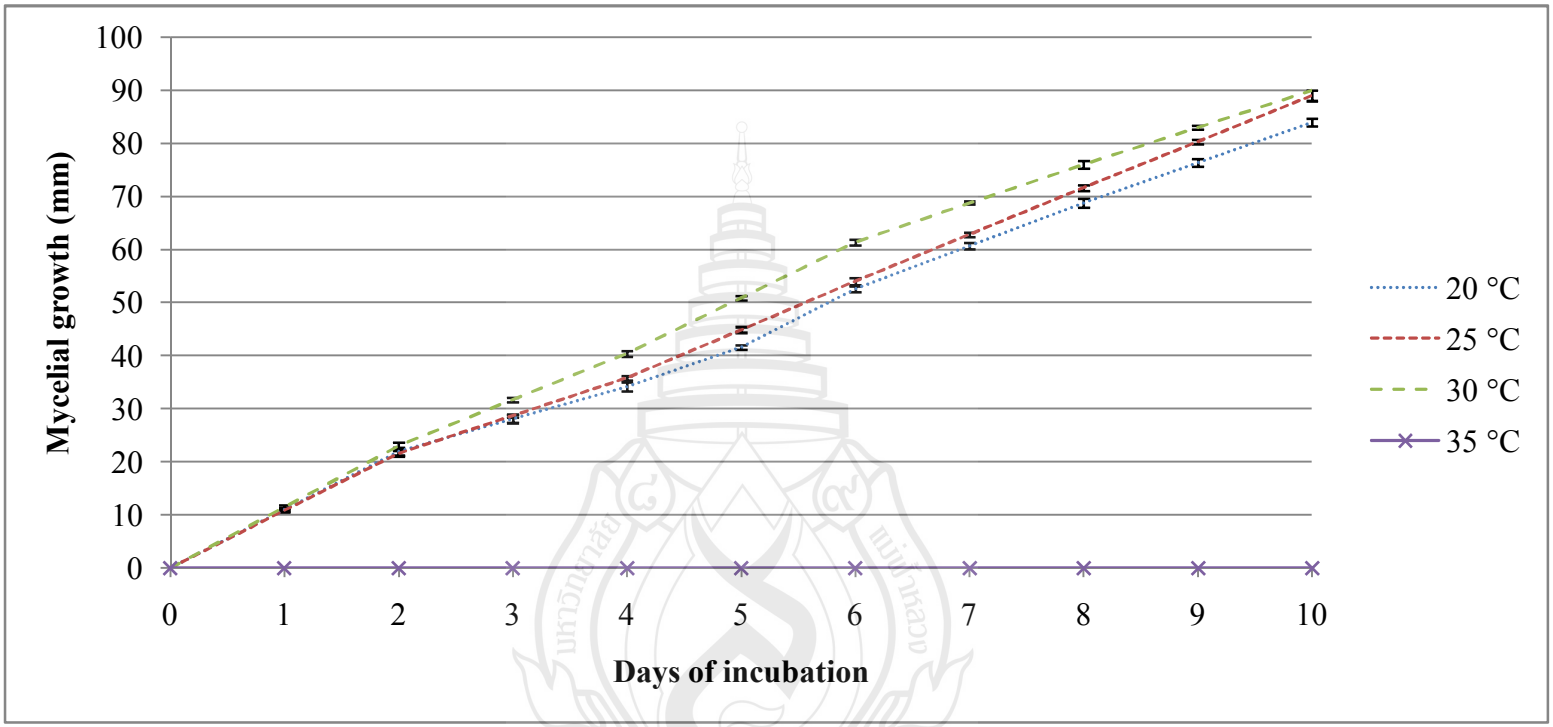

Figure C3 Effect of temperature on mycelia growth of *Lentinus roseus*

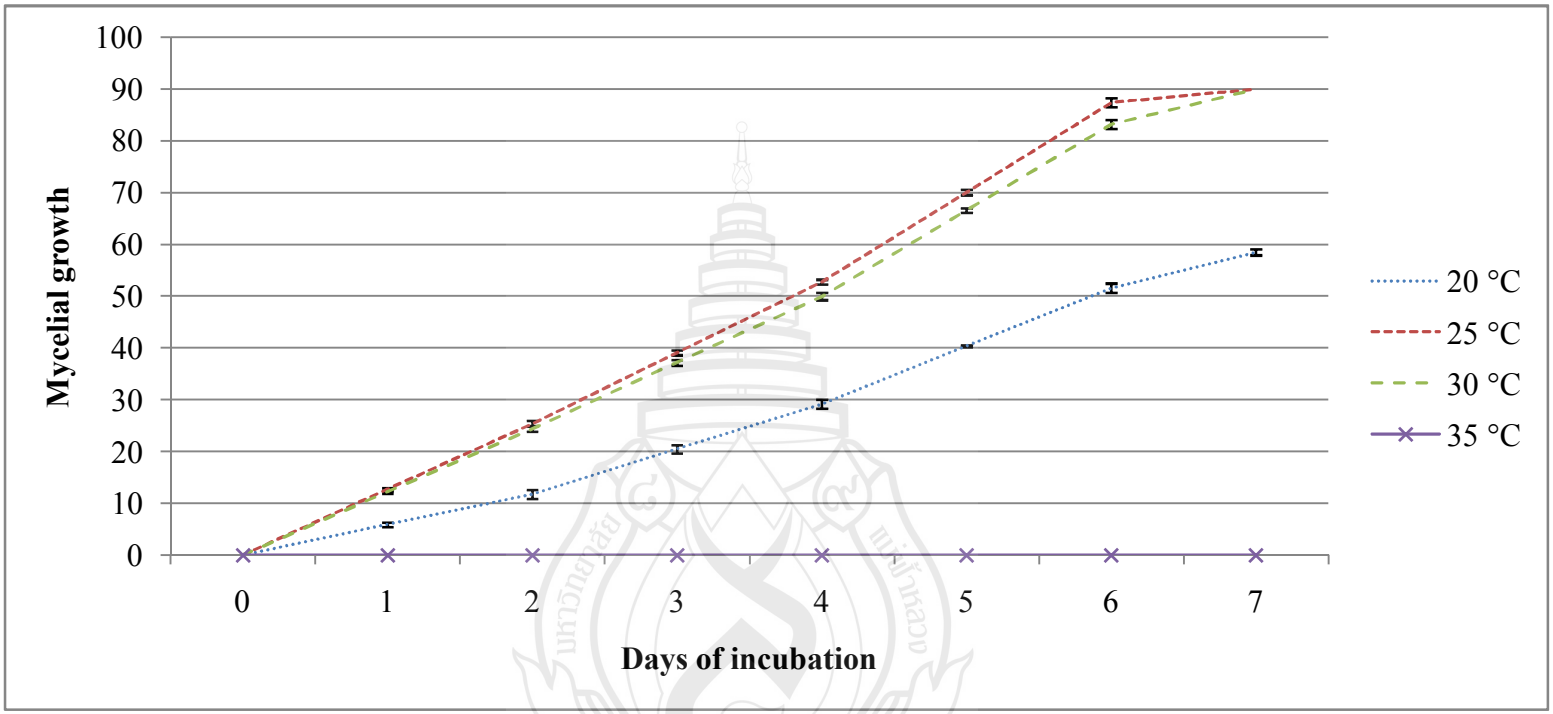

Figure C4 Effect of temperature on mycelia growth of *Pleurotus ostreatus*

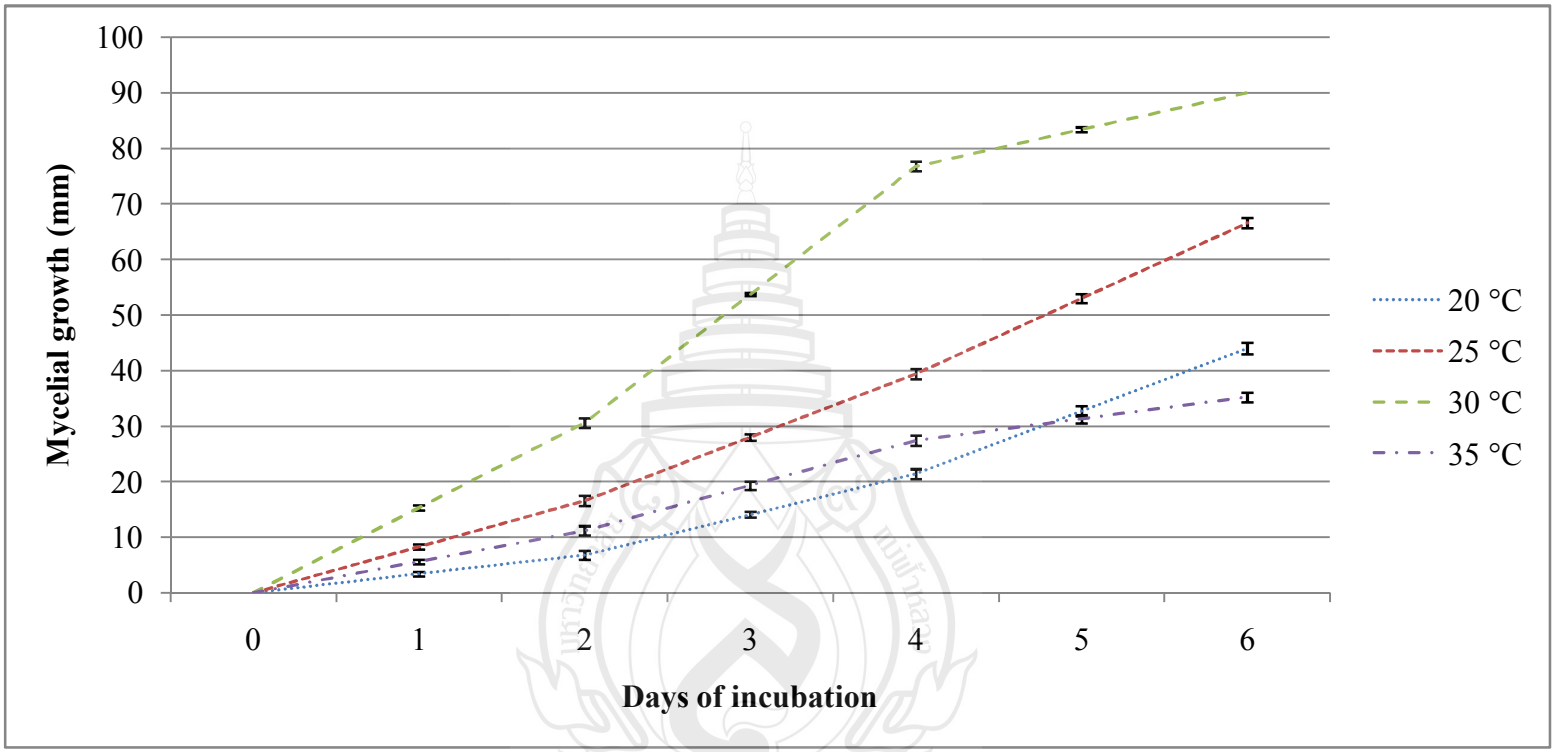

Figure C5 Effect of temperature on mycelia growth of *Lentinus squarrosulus*

APPENDIX D

FRUITING BODIES PRODUCTION OF *Lentinus connatus*

Poor substrate		Enriched substrate	
Weight	**Yield (%)**	**Weight**	**Yield (%)**
100	12.50	150	18.75
90	11.25	120	15.00
80	10.00	140	17.50
100	12.50	150	18.75
80	10.00	120	15.00
90	11.25	120	15.00
95	11.88	130	16.25
80	10.00	110	13.75
100	12.50	130	16.25
100	12.50	140	17.50
Mean	11.43±1.10	Mean	16.37±1.71

APPENDIX E

Publication

Domestication of wild strain of *Pleurotus giganteus*

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Pleurotus giganteus has relatively large fruiting bodies and is a saprobe in heavily rotting underground wood in forests; it is collected and widely consumed in many tropical countries including Thailand. Although *P. giganteus* is a popular edible mushroom, it is not cultivated in Thailand or Sri Lanka as a commercial mushroom. Recently a method for the cultivation of *P. giganteus* at the experimental level using saw dust as a substrate has been developed. The strain was isolated from a fresh fruiting body of *P. giganteus* (MFLU10 0154) using a piece of cap tissue and cultivated on Potato Dextrose Agar (PDA). Spawn was grown in sorghum (*Sorghum bicolor*) seeds. The cultivation method involves two steps, inoculating on a saw dust substrate in polypropylene bags as the preliminary step and transferring to the soil as a second step, which is very important for fruiting. The developed method of growing *P. giganteus* is fully described with all necessary steps.

Keywords: mushroom, polypropylene bags, saw dust substrate, tropical countries, underground wood.

Recently *Pleurotus giganteus* was transferred from *Lentinus* based on morphological and molecular evidences (Karunarathna *et al.* 2012). This species was previously named as *Lentinus giganteus* Berk. and was first described from Sri Lanka locally referred to as "Uru Paha" and classified in 'Decades of Fungi' (Berkeley 1847). *Pleurotus giganteus* has been treated as a special food since ancient times as mentioned in Buddhist literature (Berkeley 1847, Udugama & Wickramaratna 1991). When fully grown, the basidioma is typically infundibuliform measuring up to 35 cm in diameter and 28 cm high (Berkeley 1847, Udugama & Wickramaratna 1991). The mushroom may be solitary but often forms in groups on the ground. *Pleurotus giganteus* has a thick, radicant stipe and subdistant broad lamellae which is typical of *P. giganteus* (Pegler 1983, Karunarathna *et al.* 2012). It is a very popular mushroom because of its high protein content, excellent taste, bioactive components and the health-related functions (Udugama & Wickramaratna 1991, Huang 2005).

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CURRICULUM VITAE

CURRICULUM VITAE

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