STUDY ON AGARICALES IN RAIN FOREST AREAS AND THEIR INTERACTION AGAINST COFFEE ANTHRACNOSE AND FUSARIUM WILT OF TOMATO

YALING LUO

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Student	Miss. Yaling Luo
Student ID	56604018
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Thesis Advisor	Assist. Prof. Dr.Wattanachai Pongnak
Thesis Co-advisor	Assoc. Prof. Dr. Kasem Soytong
	Assist. Prof. Dr. Supattra Poeaim

ABSTRACT

Sixty wild mushrooms were collected in five provinces of six points in Thailand. These were divided into 7 orders (Agaricales, Auriculariales, Boletetaless, Cantharellales, Polyporaceae, Russulales, Xylariales), 17 families (Agaricaceae, Auriculariaceae, Boletaceae, Cantharellaceae, Clavariaceae, Exidiaceae, Hydnangiaceae, Inocybaceae, Lyophyllaceae, Marasmiaceae, Mycenaceae, Pleurotaceae, Polyporaceae, Russulaceae, Schizophyllaceae, Tricholomataceae, Xylariaceae). Six species of wild mushrooms were selected to extract biological activity substances.

All of the promising antagonists were tested for their abilities to control *Colletotrichum coffeanum* in bi-culture plate. Bi-culture antagonistic test for antagonism showed that methanol crude extract from *Clitocybe* spp. AJ2-2 gave significantly highest inhibition for the spore production of *C. coffaenum* by 89.08% with the effective dose (ED_{50}) of 9.65 µg/ml at the concentration of 1,000 ppm. Followed by crude hexane extract from *Boletus. affinis* var. *maculosus* AJ2-3 and crude methanol extract from *Lactarius* spp. CH3-01 with percentage of inhibition of 55.95 and 76.13% with the effective dose (ED_{50}) of 75.19 and 98.66 µg/ml, respectively. Moreover, the hyphae, oogonia and oospores of the pathogen formed abnormal protoplasm in cells and demonstrated uncommon shape implies antibiosis.

All of the promising antagonists were tested for their abilities to control *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2 in bi-culture plate. Bi-culture antagonistic test for antagonism showed that crude ethyl acetate extract from *Lactarius* spp. CH3-27 gave significantly highest inhibition for the spore production of *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 by 97.88 % with the effective dose (ED_{50}) of 1.81 µg/ml at the concentration of 1,000µg/ml. Followed by methanol crude extract from *Lactarius* spp. CH3-01 with percentage of inhibition of 83.95% and the effective dose (ED_{50}) of 3.79 µg/ml, respectively. Moreover, the hyphae, oogonia and oospores of the pathogen formed abnormal protoplasm in cells and demonstrated uncommon shape implies antibiosis. Therefore, crude ethyl acetate extract from *Lactarius* spp. CH3-27 and crude methanol extract from *Lactarius* spp. CH3-01 were selected as a potent antagonist to control fusarium wilt of tomato.

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CHAPTER 1

INTRODUCTION

1.1 General Introduction to Mushrooms

1.1.1 Modern Taxonomy of Agaricales

Agaricales comprises the so-called mushrooms and toadstools, and is the largest clade of mushroom-forming fungi. The number of mushrooms existing species in nature is estimated in around 10,000 from which approximately 10% are likely to be edible (Chye *et al.*, 2009). Worldwide, approximately twenty-five species are widely accepted as food, but only a few of them are commercially produced. It is reported that 2.4 million tons of mushrooms were produced in 2002, the major producers being China (708 billion tons), United States (390 thousand tons), The Netherlands and Japan, while main consumer countries where Germany, China, The Netherlands and Japan (FAO, 2009). Latin America has produced only 1.3% of the world total cultivated mushrooms that year, the largest producers being Mexico (58.6%), Chile (17.6%) and Brazil (10.6%) (Taveira *et al.*, 2009). Among the edible mushrooms produced worldwide, *Agaricus bisporus* is the most cultivated one (38%), followed by species of the genus *Pleurotus* (25%) and *Lentinula edodes* (10%) (Moda, 2008). There are clearly two mushrooms markets: one referring to the most commercialized species, champignon (*Agaricus bisporus*), and another that gathers exotic mushrooms, including species such as *L. edodes*, *Pleurotus* spp., *Auricularia* spp., *Flamulina velutipes*, *Grifola frondosa*, *Hypsizygus marmoreus*, *Pholiota nameko*, *Tremella fuciformis* and *Volvariella* spp. (Moda, 2008; Furlani, 2005). Recently, the species *Agaricus blazei*, known as "sun mushroom" has awakened the interest for consumption, as a food supplement, due to its potential medicinal properties (Furlani, 2005; Wang, 2010). Ingestion of mushrooms in some countries is contained by the high cost, little knowledge of their nutritional and medicinal properties and lack of expertise to distinguish beneficial from poisoning species. Mushrooms names are sometimes adopted in accordance to their provenience as for shiitake and hiratake, Japanese names adopted for *L. edodes* and *Pleurotus* spp. species, respectively, as well as the French origin of *A. bisporus,* named champignon.

The modern taxonomy of Agaricales began from Swedish botanist Elisa Magnus Fries (1794-1878). Fries system is a simple macro classification system. Systematics is one of the

major subdivisions of biology, and is as broad-based as genetics or molecular biology. In his classification 12 genera of gilled mushrooms (agarics) were recognized based on macroscopic features of basidiocarps and colour of spore prints (white, pink, brown, purple-brown and black). His system was widely used as it had the advantage that many genera could be identified on field characters. The system was relatively unchallenged until Fayod studied the anatomy and microscopic features of basidiocarps and consequently recognized 108 genera in 1889. The most influential systematic treatment of the Agaricales is the Agaricales in modern taxonomy by Singer (1986). Singer utilized Fayod's anatomic characters and Fries's macroscopic characters in reorganizing families and genera. The term "Agaricales" in his scheme refers to the order containing the type genus *Agaricus* and the type family Agaricaceae. In his system there were 3 majors groups in the order Agaricales: Agaricales, Boletales, and Russulales. Those 3 groups were accepted as the euagaric clades, bolete clade and russuloid clade based on molecular data (Hibbett and Thorn, 2001). Totally 18 families and 230 genera were distinguished in his system (Singer 1986).

Agaricales belongs to Eumycota, Basidiomycotina, Hymenomycetes (Alexopoulos and Mimx, 1979). Basidiomycota are characterized by a multi-layered cell walls, barrel-shaped structures or pulley wheel occlusions at the septa of hyphae (dolipore septa), an extended dikaryophase, clamp connections that often develop on septa, and the formation of meiosporangia (basidia) that produce meiospores (basidiospores) at the tips of sterigmata (Kendrick 2000). Almost 30,000 species had been described (Kirk *et al.*, 2001).

Historically Basidiomycota was treated as a subphylum Basidiomycotina and comprised 3 classes: Teliomycetes (rust and smut fungi), Hymenomycetes (mostly gilled mushrooms) and Gasteromycetes (puffballs, bird's nest fungi, earth stars, stinkhorns) in a 5 subphyla classification system (Ainsworth *et al.*, 1973). In the latest edition of the "Dictionary of Fungi", Basidiomycota comprise 3 classes: first, Basidiomycetes which including members of Hymenomycetes and Gasteromycetes, second, Urediniomycetes and Ustilaginomycetes, and third, "hymenomycetes" and "gasteromycetes" were taken as informal and not monophyletic categories (Kirk *et al.*, 2001).

With the exception of Urediniomycetes and Ustilaginomycetes that contain important plant pathogens, the Basidiomycetes mostly are saprobes and symbionts, and play ecologically important roles, such as oxygen, carbon and nitrogen cycling. Humans were first attracted to mushrooms since ancient times because of their edible or poisonous traits. Mushrooms are an

important group in the biosphere and their significance in diversity and conservation issues have been recognized extensively (Kaul 2001). Singer (1986) recognize the 17 families in the Agaricales, including Agaricaceae, Amanitaceae, Bolbitiaceae, Boletacea, Coprinaceae, Cortinariaceae, Crepidotaceae, Entolomataceae, Gomphidiaceae, Hygrophoraceae, Paxillaceae, Pleurotaceae, Pluteaceae, Russulaceae Schizophyllaceae, Strophariaceae, Tricholomataceae and others. More than 9000 species in more than 300 genera, and 26 families had been described. Mostly they are terrestrial, lignicolous and saprobic, and many are mycorrhizal (Kirk *et al.*, 2001). The Mycelium typically formed by the spores germinate primary hyphae, with primary anastomosis affinity forming secondary hyphae, no or a lock-like joint, with a septum and barrel bung hole cover. Some types of secondary mycelium may also be formed streptozotocin. Fruiting body fleshy, easily broken, rarely membranous or leathery. Typical fruiting bodies, including the cap, stipe, located below the cap gills or bacteria tube, located in the middle or upper part of stipe mushroom ring and base volva. Hymenium often in the initial stage of growth within the biofilm shedding covered completely exposed when ripe. No burden separated single spore spores, colorless or colored, its shape, size, color and ornamentation is an important basis for minutes (Arora, 1986).

1.1.2 Features of Agaricales

The main features of Boletaceae is the hymenium tube, but the classification is very complex. Its classification is based on the characteristics of a variety of macro, micro characteristics and certain chemical reactions. For example, the color of nature buns, arms control arrangement, the epidermis of caps, the color of the burden in different parts of the fruiting bodies. The main difference of Russulaceae with other Agaricales is that it has a typical medullary hyphae groups tied around the ball-shaped fruiting bodies of fungi cells of marrow. Spores white or yellow, ornamentation from starch reaction. Usually do not like joint locks, the fold and the stipe connected. The family includes only the genus *Russula* and *Lactarius.* The main characteristics of Hygrophoraceae is to produce a cylindrical burden, the burden to produce white, thin-walled spores. Tricholomataceae is a very large families, mainly produces white buns species composition. Better known genus includes *Termitomyces , Flammulina, Marasmius, Clitocybe, Tricholoma, Panellus, Laccaria, Omphalotus, Armillaria.* Amanitaceae, its main feature is a white spores, gill free with the ring and the drag. Singer (1986) divided Strophariaceae into 9 genus., the main feature is gills together with stipes, spores are purple. Corprinaceae produce dark spores, gills are easily liquefied to black, ink-like liquid.

1.1.3 A High Value Family- *Agaricus*

According Singer(1986), Agaicaceae is a large family, better known is *Agaricus*. The cup of *Agaricus* normally is white or grey, gill free with the ring and the volva; the stipe is easy to be separated from the cup. The majority of mushrooms are edible, medicinal or health care values. For example, *Agaricus bisporus* (Jelange) Imbach, occurs scaly mushrooms, *Agaricus crocopeplus* Berk, woodland mushrooms, *Agaricus silvaticus* Schaeff, large purple mushroom, *Agaricus augustus* Fr, white mushrooms, *Agricus bernardii* (Quél.) Sacc, big fat mushrooms, *Agaricus bitorquis* (Quél.) Sacc, and the four spore mushrooms as *Agaricus campestris* L. Which has been carried out in artificial cultivation in order for edible, *Agaricus subrufescens* Peck reported to do liquid fermentation and mycelia contains large amounts of polysaccharides and other biologically active substances (Genpei Yu and Jigui Bao, 2008), *Agaricus arvensis* Schaeff reported that involved in the human body's immune system regulating function has the good role in promoting, aroused people's great concern to the wild mushrooms. Brazil mushrooms *Agaricus blazei* Murr reported to be involved in lowering blood sugar, improved arteriosclerosis and cytotoxicity to some cancer cell lines (Xiaoping Luo and Junyan Wang, 2007). But toxic mushrooms of genus *Lepiota* often are mistaken for the edible mushrooms of genus *Macrolepiota* and thus are common cause of poisoning. Similarly *A. phalloides* can be misidentified as an edible species of genera *Amanita*, *Lepiota* or *Russula*, thus causing 8% of the total fungal poisonings in Italy (Assisi *et al.*, 2008).

1.1.4 General Introduction to Metabolites

A primary metabolite is a product essential to the survival of an organism. Primary metabolites are produced during growth; when the available nutrients are absorbed, growth ceases and the organism switches over to secondary metabolism. Mushrooms are the fruiting bodies of a vast mass of unseen myccorizhae that appear near the end of a growth cycle. When consumed, they usually contain both primary and secondary metabolites. All mushrooms produce certain metabolites in common. Fatty acids, proteins, sugars, carbohydrates and vitamins all aid in the growth of the organism. These metabolites make the mushroom a highly valued food. Fungi are such a good source of citric acid that they are used to produce it commercially. The complex fatty acids present in most mushrooms are being widely studied as dietary aids to enhance free radicals in the blood and as antioxidants. Wild mushrooms can be a gourmet's delight. While mushrooms are not a significant source of protein, they are much higher in protein and fatty acids than most vegetables. Wild mushrooms are sought after for their flavor and not for their health benefits,

although a study of the primary metabolites in such wild mushrooms as the Boletus species show that they are high in antioxidants. There are 10,000 species of mushroom worldwide and of those, only a few are edible, and only a handful of species are cultivated (Cedar Sanderson). Mushrooms are good sources of protein, carbohydrates and fibers and, conveniently, they have low lipid content, being considered healthy foods (Smiderle *et al.*, 2008). Kalač (2009) compiled nutrition information for fifteen species of edible mushrooms, including the centesimal composition, describing crude protein contents levels between 16.5-59.4% of dry matter (dm), carbohydrates between 30.6-75.0% dm, and lipids between 0.4-9.0% dm. Furlani (Furlani, 2004.) studied the centesimal composition of shiitake, champignon and oyster mushrooms, acquired in Brazil, that presented average protein levels ranging between 19.0-28.5%, carbohydrates between 54.1-69.6%, lipids between 4.3-5.4% and ash totals between 7.0-12.0%. Shibata and Demiate (Shibata and Demiate, 2003) reported that carbohydrates, proteins, lipids and ashes composition in the sun mushroom varied between 41.9-47.9%, 34.8-39.8%, 0.8-1.2% and 6.9-7.8%, respectively, according to the cultivation form. General profiles of edible mushrooms show presence of essential aminoacids such as lysine, valine and methionine (Len-Guzmn *et al.*, 1997; Mendez, 2005) essential fatty acids like linoleic (C18: 2n-6) and oleic (C18:1n-9) acids (Kavishree *et al.*, 2008) and carbohydrates like mannitol and trealoses (Manzi, Aguzzi, Pizzoferrato, 2001; Barros *et al.*, 2008). Most usual minerals found in mushrooms are calcium, magnesium, potassium, iron, zinc and copper (Genccelep *et al.*, 2009; Ouzouni *et al.*, 2009), but levels of selenium compounds in the edible species Albatrellus pes-caprae and Boletus edulis were reported by Slejkovec and collaborators (Šlejkovec *et al.*, 2000). Presence of vitamin C (ascorbic acid) and niacin was reported for *A. bisporus*, *L. edodes* and *P. ostreatus*. Vitamins B1 and B2 were reported for shiitake, champignon and oyster mushrooms by Furlani and Godoy (Furlani and Godoy, 2008) Vitamin D was reported in the fruit bodies of *Cantharellus cibarius* (chanterelle), an edible mushroom available in both pigmented and albino forms (Rangel-Castro, *et al.*, 2002) and in *A. bisporus* (Corey *et al.*, 2009). Levels of calcium, magnesium, zinc and iron were described for mushrooms from Finland, Malaysia and Turkey. The variation is due to several reasons like species, source (wild or cultivated), climatic variations, maturity level, conservation process, and substrate of cultivation. Level of calcium in *Pleurotus ostreatus* reached up to 126 mg% for a wild species collected in Erzurum province in Turkey. Magnesium levels varied from 130-300 mg while, for zinc, a small variation (8.3-14.9 mg%) was found among all analyzed samples from Finland and Turkey. Iron levels varied greatly, reaching 68.2 mg% for a *P. ostreatus* sample.

Malaysian mushrooms showed very low levels of zinc and iron, compared to the samples obtained from other countries. Differences found corroborate the influence of external parameters, including seasonal variations. In fact, Turkish *P. ostreatus* and *P. sajor-caju* as well as Finlandian samples were cultivated; Turkish *P. ostreatus* from Erzurum province was a wild sample, as well as the Malaysian *Pleurotus* specimen. Çağlarırmak evaluating the calcium, magnesium, zinc and iron contents of *L. edodes*, according to the growth period, reported a decrease on their levels with the time. The deadly *Amanita*. Even touching it can lead to toxic effects. Many wild mushrooms produce metabolites that are harmful or even deadly to the human body if ingested. Care must be taken when identifying and harvesting wild mushrooms. Eating the Amanita species can result in death, and if the patient survives, he suffers permanent damage to the liver and kidneys. False morels, so difficult to differentiate from the highly sought morel, can result in painful food poisoning symptoms. Mycotoxins may be a primary or secondary metabolite; the defining line is difficult to determine and one should never assume that a young mushroom is safer to eat than a fully developed one.

1.2 General Introduction to Bioactivity Compound

Bioactive compounds in plants are compounds produced by plants having pharmacological or toxicological effects in man and animals. Although nutrients elicit pharmacological or toxicological effects when ingested at high dosages (e.g. vitamins and minerals), nutrients in plants are generally not included in the 12 term bioactive plant compound. The typical bioactive compounds in plants are produced as secondary metabolites. Thus, a definition of bioactive compounds in plants is: secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals. (Bernhoft, 2010). Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs. Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So, their extraction, isolation, purification, characterization and synthesis of these bioactive ingredients from crude extracts by various

analytical methods become very important. Bioactive molecules are those chemical compounds which produced by living organism or synthesized in laboratory, that exert a biological effect on other organisms.

1.3 Coffee Diseases and Tomato Diseases

Coffee is belongs to Rubiaceae, a perennial evergreen shrub and a perennial horticultural crops. Leaves which are opposite elongated oval, glossy, at the end of a long branches, small branches, and flowers are white, open branches in the base of the petiole link. Once ripe, coffee "berries" are picked, processed, and dried to yield the seeds inside. The seeds are then roasted to varying degrees, depending on the desired flavor, before being ground and brewed to create coffee. The main active ingredient caffeine of coffee, have a strong central stimulant effect. People taking caffeine or caffeinated beverages often disappear drowsiness, fatigue mitigation, quick thinking. Dose increased, the central stimulant effects more obvious tensions, anxiety, restlessness, insomnia, tremor. Larger doses produce local or systemic spasm. There are many different diseases on coffee, for example, anthracnose: *Colletotrichum gloeosporioides* (teleomorph = *Glomerella cingulata* (Stonem.) Spauld and Shrenk.), *Colleotrichum kahawae;* armillaria root rot: *Armillaria mellea;* algal (red) leaf spot: *Cephaleuros virescens;* bacterial blight: *Pseudomonas syringae* pv garcae; bark disease: *Fusarium stilboides* (teleomorph = *Gibberella stilboides*); berry blotch: *Cercospora coffeicola*; black (Rosellinia) root rot: *Rosellinia* spp.; black (seedling) root rot: *Rhizoctonia solani*; blister spot: Virus (uncharacterised); brown blight: *Colleotrichum gloeosporioides* (teleomorph = *Glomerella cingulata*, *Colletotrichum kahawae;* brown eye spot: *Cercospora coffeicola;* brown leaf spot: *Phoma costarricensis;* canker: *Ceratocystis fimbriata*, *Phomopsis coffeae* Bondarzeva-Monteverde; collar rot: *Fusarium stilboides* (telemorph = *Gibberella stilboides*); coffee berry disease; *Colletotrichum kahawae*, *Ascochta tarda,* physiological effect of overbearing: often exacerbated by rust; dry root rot: *Fusarium solani;* hot and cold disease: physiologic effect of exposure to extremes of temperature – common at high altitudes; leaf blight: *Ascochyta tarda*; leaf spot: *Phyllosticta coffeicola*; pink disease: *Corticium salmonicola;* red blister disease (robusta coffee) *Cercospora coffeicola*; red root rot: *Ganoderma philippi*; root knot: *Meloidogyne* spp.; rust (orange or leaf rust): *Hemileia vastatrix*; rust: (powdery or grey rust) *Hemileia coffeicola*; south America leaf spot: *Mycena citricola*; thread blight: *Corticium koleroga;* tip blast: *Phoma costarricensis*; tracheomycosis (Wilt): *Gibberella xylarioides* (anamorph = *Fusarium*

eylarioides); wilt: *Ceratocystis fimbriata*, *Fusarium oxysporum* Schl. f. sp. coffea; warty berry: *Botrytis cinerea* (Waller, 1998). Especially coffee anthracnose mainly has three kinds pathogen : *Colletotrichum gloeosporioid* Penz, *C. coffeanum* Noack, and *C.kahawae*. Symptoms: when leaves victims, mostly in the incidence of leaf margin, the upper and lower leaf surfaces showing irregular light brown to dark brown spots. Lesion restricted by the veins, a diameter of about 3mm, later merged into a large number of lesions lesion , lesion central white , yellow edge , later gray, on which there are many small black dots (pathogen spores) are arranged in concentric wheels pattern. Branches after the victim was depressed lesion, followed by dead branches, grow small black dot on it. Ripe berries and green berries victims, initially presented nearly round berry surface water-soaked spots, followed by lesions become sunken, dark brown to gray-black big spots, grow pink sticky substance on it. Agricultural measures: 1 quarantine; 2 tending to strengthen the management. Chemical control: In the early stages, with 1% Bordeaux mixture or 0.4% copper oxide powder or 0.5% copper oxychloride, at the onset of the season once every 7 ~ 10 d spraying, even spray 2 or 3 times, the prevention branch dieback and leaves anthrax has a good effect. The tomato (*Lycopersicon esculentum*) is the edible, often red fruit/berry of the nightshade *Solanum lycopersicum*. Tomatoes have bleeding, blood pressure, diuretic, stomach and digestion, thirst, detoxification effect. Since the ratio of tomato vitamin A, vitamin C suitable, so eat can enhance the function of small blood vessels, prevent vascular aging. Tomato flavonoids, both reducing capillary permeability and prevent rupture of the role, as well as the prevention of hardening of the arteries of the special effects that can prevent cervical cancer, bladder cancer and pancreatic cancer and other diseases; Tomatoes help flattening wrinkles, make the skin smooth and delicate, inhibit bacteria. Eat tomatoes also less prone to dark circles, and not susceptible to sunburn. Tomato is an economically important vegetable crop, suffering from many fungal diseases (Ketelaar and Kumar, 2002). For bacterial diseases, including bacterial canker: *Clavibacter michiganensis* sub spp. *Michiganensis;* bacterial speck: *Pseudomonas syringae* pv. *tomato; b*acterial spot: *Xanthomonas campestris* pv. *vesicatoria; b*acterial stem rot and fruit rot: *Erwinia carotovora* subsp. *Carotovora; b*acterial wilt: *Ralstonia solanacearum*; pith necrosis: *Pseudomonas corrugata;* syringae leaf spot: *Pseudomonas syringae* pv. *syringae*. For fungal diseases, including alternaria stem canker: *Alternaria alternata* f. sp. *lycopersici; a*nthracnose: *Colletotrichum coccodes*, *Colletotrichum dematium*, *Colletotrichum gloeosporioides*, *Glomerella cingulata*; black mold rot: *Alternaria alternata*, *Stemphylium botryosum*, *Pleospora tarda*, *Stemphylium herbarum*, *Pleospora herbarum*, *Pleospora lycopersici*, *Ulocladium consortiale*,

Stemphylium consortiale; black root rot: *Thielaviopsis basicola*, *Chalara elegans*; black shoulder: *Alternaria alternata;* buckeye fruit and root rot: *Phytophthora capsici*, *Phytophthora drechsleri*, *Phytophthora nicotianae* var. *parasitica*, *Phytophthora parasitica*; cercospora leaf mold: *Pseudocercospora fuligena*, *Cercospora fuligena*; charcoal rot: *Macrophomina phaseolina;* corky root rot: *Pyrenochaeta lycopersici;* didymella stem rot: didymella lycopersici; early blight: *Alternaria solani;* fusarium crown and root rot: *Fusarium oxysporum* f. sp. *radicis-lycopersici;* fusarium wilt: *Fusarium oxysporum* f. sp. *lycopersici;* gray leaf spot: *Stemphylium botryosum* f. sp. *lycopersici*, *Stemphylium lycopersici*, *Stemphylium floridanum*, *Stemphylium solani;* gray mold: *Botrytis cinerea*, *Botryotinia fuckeliana*; late bright: *Phytophthora infestans*; leaf mold: *Fulvia fulva*, *Cladosporium fulvum*; phoma rot: *Phoma destructiva;* powdery mildew: *Oidiopsis sicula*, *Leveillula taurica*; pythium damping-off and fruit rot: *Pythium aphanidermatum*, *Pythium arrhenomanes*, *Pythium debaryanum*, *Pythium myriotylum*, *Pythium ultimum*; rhizoctonia damping-off and fruit rot: *Rhizoctonia solani*, *Thanatephorus cucumeris*, rhizopus rot: *Rhizopus stolonifer;* septoria leaf spot: *Septoria lycopersici*; sour rot: *Geotrichum candidum*, *Galactomyces geotrichum*, *Geotrichum klebahnii*; southern blight: *Sclerotium rolfsii*, *Athelia rolfsii*; target spot: *Corynespora cassiicola*; verticillium wilt: *Verticillium albo-atrum*, *Verticillium dahliae*; white mold: Sclerotinia sclerotiorum, Sclerotinia minor. (Vegetable MD Online, http:// vegetablemdonline. ppath.cornell. edu/factsheets/Tomato_List.htm). Especially *Fusarium oxysporum* f. sp. *lycopersici*, the fungus that causes Fusarium wilt, attacks only certain tomato cultivars. Plants infected by this soil-dwelling fungus show leaf yellowing and wilting that progress upward from the base of the stem. Initially, only one side of a leaf midrib, one branch, or one side of a plant will be affected. The symptoms soon spread to the remainder of the plant. Wilted leaves usually drop prematurely. Affected plants die early and produce few, if any, fruits. Splitting open an infected stem reveals brownish streaks extending up and down the stem. These discolored streaks are the water-conducting tissue, which becomes plugged during attack by the fungus, leading to wilting of the leaves. Plants are susceptible at all stages of development, but symptoms are most obvious at or soon after flowering. To minimize losses from fusarium wilt, it is advisable to plant resistant varieties, and many resistant varieties are available. The letter "F" following the variety name indicates resistance to one or more races of the Fusarium fungus. Resistant varieties may become infected, but disease will not be as severe as with susceptible varieties and a reasonable yield should still be obtained. In addition, plant disease-free seed or transplants in well drained, disease-free soil, rotate at least four years away from tomatoes to

reduce populations of the fungus in soil, and remove and destroy infected plant residue. In greenhouse or seedbeds, disinfest soil by treating with steam.

1.4 Research Background and Objectives

1.4.1 The Need for Mycological Knowledge from Tropical Areas

The magnitude of fungal diversity (including chromistan fungi, lichen-forming fungi, slime moulds and yeasts) has been estimated at 1.5-3 million species, and only 2.5-5% of that figure have been described (Hawksworth, 1991). Presently, 75-120,000 species are actually known to science (Kirk *et al.*, 2001). Although the figure of 1.5 million was generally accepted this figure has been questioned as being too high or too low (Hawksworth, 2001). This is because the estimation of global species numbers relied heavily on data from temperate UK and Europe, and much more basic data is needed from the tropics (Hyde, 2001). An increased inventory of tropical mycological taxa is a vital component of knowledge development (Hawksworth, 2001 and Subramanian, 1982).

Increase in knowledge of the geographic range of a fungal species might offer more phylogenetic information than before. Global geographic distributions of some fungal species defined by morphology have been reported. However, when these species are defined by phylogeny, they have been shown to comprise several to many endemic species (e.g. *Schizophyllum commune* in James *et al.*, 1999; James and Vilgalys, 2001; *Lentinula* in Hibbett 2001). Thus taxa reported from locations distant from their original distribution and those taxa reported as having a worldwide distribution based only on morphology must be viewed with caution (Taylor, 2006). Such discoveries also give mycologists a challenge to provide a more comprehensive recognition of morphospecies in tropical areas and give rise to the need for knowledge about their reproductive or genetic isolation.

1.4.2 Research Objectives

This research provides data on Agaricales in Thailand. As for some species of Agaricales are important cultivated edible species. Moreover, some of them are not only beautiful, bright colors, but also has ornamental value. Therefore, they have high economic value. In Thailand, the rich forest resources for the growth and development of mushrooms provide a good external conditions, and rainfall in the rainy season promote the growth of their saprophytic mushrooms. The seasonal climate of Thailand coupled with the complex topography has resulted in rich biodiversity, including of fungal diversity. Coffee anthracnose and fusarium wilt of tomato

are the common plant diseases. Chemical control is the traditional control method. Although chemical control methods is quick, but also has the effect of not lasting disadvantages. More importantly, it will cause some degree of environmental contamination. Thus, biological control methods came into being. But then there are any problems to be solved. In this test, the use of bioactive substances of mushrooms control the two diseases. As well known, bioactive substances of mushrooms is diverse, and the use of different solvents, different methods, different extraction conditions change each time of extraction in the extraction process, etc., may give different biological active substances. So choose a suitable extraction conditions require in-depth study of the problem lies. Moreover, in view of certain toxic mushrooms on the progress of work also brings some risks.

The objectives of this study were:

a. To study the distribution of Agaricales in Thailand;
b. To describe and identify Agaricales;
c. To extract biological active substances;
d. To test the efficacy of some biological active substances to inhibit plant pathogens –fusarium wilt and coffee anthracnose.

CHAPTER 2

LITERATURE REVIEW

2.1 Morphological Identification on Agaricales

Agaricales, the so called mushrooms. There is no golden rule when it comes to identifying unfamiliar mushrooms, edible or otherwise. Deadly poisonous toadstools can share the same characteristics as many edible fungi. Of course, many genera of mushrooms have familiar and reliable traits, but it is always best to know all specific features. Some species, such as the Giant Puffball, are extremely recognisable, but they are few and far between. The following information can help overcome basic identification obstacles and covers the common mushroom-shaped species – those with a typical cap and stem. Mainly on the morphological identification of specimens for morphological observation, recording, comparison, analysis and identification. Accumulation often requires expertise. However the common accepted instruction described by Largent (1986). The colour terms follow Kornberup and Wanscher (1978).

2.2 Biological Properties of Mushrooms

There is a growing interest in the use of medicinal plants to control plant diseases, only about 2,400 plant species among more than 250,000 higher plants have been screened for phytoactivity (Oluwalana and Adekunle, 1998; Oluwalana *et al.*, 1999; Khafagi and Dewedar, 2000). It has been known that macro fungi are used as a valuable food source and traditional medicines since Greek and Roman antiquity (Anke, 1989), Dioscorides, first century Greek physician, knew that Laricifomes (Fomitopsis) officinalis (Vill.) Kotl and Pouzar (Fomitopsidaceae) can be used for treatment of "consumption", a disease now known as tuberculosis (Stamets, 2002). It is believed that mushrooms need antibacterial compounds to survive in their natural environment. Antimicrobial compounds could be isolated from many mushroom species and some proved to be of benefit for humans (Lindequist, 2005). In early studies performed by Anchel, Hervey and Wilkins in 1941, diverse antibiotic activity was detected in basidiocarp or mycelia culture extracts of more than 2000 fungal species. In recent in vitro studies, screening for the antimicrobial activity of basidiomycete strains, some studies were done both of in basidiocarp and in submerged culture. Antimicrobial activities of basidiomycete

strains from (Rosa *et al.,* 2003; Wasser and Weis, 1999; Ezeronye, Daba, Okwujiako and Onumajuru, 2005). Similarly, Rosa *et al.* (2003) detected 14 mushroom isolates with significant activity against one or more of the target microorganisms (Rosa *et al.*, 2003). Zjawiony (2004) observed that 75% of polypore fungi that have been tested show strong antimicrobial activity (Zjawiony, 2004). Antibacterial activities of mushroom exopolysaccharides such as lentinan (from Lentinus edodes), schizophyllan (from Schizophyllum commune) and PSK ("Polysaccharide Kureha" from Trametes versicolor) have also been reported (Stamets, 2002; Chihara, 1992; Sakagami, Takeda 1993; Wasser and Weis, 1999; Ezeronye, Daba, Okwujiako, Onumajuru and 2005). For mushrooms, the common culture medias are potato dextrose broth (PDB) and yeast extract sucrose broth (YES). However, recent studies show that not every species is applicable to both media. Found in the study of Chomcheon p.et al., 1 mg of the crude extract from *P. citrinopileatus* culturing PDB demonstrated strong activities against *E. coli* ATCC 25922 (higher than activity of 10 g ampicillin) and *S. aureus* ATCC 25923 (less than activity of 10 g ampicillin).The crude extract from *T. crassum* culturing PDB showed weak and no activity against *S. aureus* ATCC 25923. YES broth is found to be inapplicable for promoting the production of antibacterial metabolite(s) by *Pleurotus citrinopileatus* and *Tricholoma crassum* Berk. (Chomcheon *et al.*, 2013), in contrast to previous reports of other fungi (Paterson, 1994). This finding suggests that (1) the effect of culture medium is highly unpredictable and variable, which is in agreement with previous report by Wiyakrutta and colleagues (Wiyakrutta *et al.,* 2004). (2) PDB used for submerged fermentation of *P. citrinopileatus* could induce the mushroom to secrete active metabolite(s) into the culture medium. Culture media have been known to affect the production of fungal secondary metabolites(Paterson., 1994, Wiyakrutt *et al.,* 2004). For example, the endophytic fungus mitosporic Dothideomycete spp. LRUB20 have been reported to produce different metabolites when cultivated in malt Czapek medium and Czapek yeast autolysate medium (Chomcheon *et al.*, 2006, Chomcheon *et al.*, 2009). Based on primary screening, the crude extract with best activity was sequentially assayed for the minimum inhibitory concentration (MIC) valves. The crude extract from *P. citrinopileatus* culturing PDB inhibited the growth of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 with MIC values of 5.0 mg/ml and 2.5 mg/ml. This implied that activities of the crude extract against Gram-negative bacteria and Gram-positive bacteria were comparable. In conclusion, submerged fermentation of *P. citrinopileatus* in PDB could be used to cultivate the mushroom for the production of antibacterial metabolites. This alternative way instead of direct extraction from mushroom

fruiting body might yield novel metabolites (Chomcheon *et al.*, 2013). However, Extraction parameters, including solvent type and concentration, extraction time and temperature are an important consideration when screening for phenolic profiles. Five types of solvents namely methanol, ethanol, acetone, water and hexane are usually used to determine the best extraction yield of phenolic profiles. Alcoholic solvents are commonly used for phenolic extraction, as they give the highest yield of total extract although they are not highly selective for phenols (Spigno, Tramell and Faveri, 2007). Higher yield of total phenols are obtained by using polar solvents, for instance, water, methanol and ethanol. Solvent concentration is one of the major investigated factors in the extraction of antioxidative compounds. A mixture of solvents, in particular aqueous alcohol, are preferred to using a mono-component solvent system, as they are more effective at extracting antioxidative compounds. The extraction time for phenolic compounds varies from a few minutes to 24 hours, e.g.one day is used to extract phenolic compounds from red currant marc (Lapornik, Prošek and Wondra, 2005), and not more than 3 hours are employed to extract the dried sage, *Salvia officinalis* (Durling, Catchpole, Grey, Webby, Mitchell, Foo and Perry, 2007). Spigno *et al.* (Spigno, Tramelli and Faveri, 2007) explained that in order to be cost effective in the manufacturing sector, one of the determinants in cost reduction is to optimize the extraction time. Extraction temperature is one of the major concerns as it would impact on the yield of extraction (Tabart, Keversm, Sipel, Pincemail, Defraigne and Dommes, 2007), and increased temperature promotes the solubility of solute as well as the diffusion coefficient (Pinelo, Rubilar, Jerez, Sineiro and Nunez, 2005; Spigno, Tramelli and Faveri, 2007). However, an increased temperature may denature certain bioactive compounds in particular phenolic compounds (Spigno, Tramelli and Faveri, 2007). As supported by Juntachote *et al.* (Juntachote, Berghofer, Bauer and Siebenhandl, 2006), extraction yield of lemon grass decreased with an increase in temperature. However, different samples may involve different processing steps that may affect the overall extraction yields according to Kim *et al.* (Kim, Jeong, Park, Nam, Ahn and Lee, 2006). The effect of temperature cannot be generalised as it strongly depends on the type of compounds with different characteristics (Spigno, Tramelli and Faveri, 2007). Similar trends have been reported by Herodez *et al.* (Herodez, Hadolin, Skerget and Knez , 2003) where the yield of extracted carnosic acid increases with a temperature increase from 0 to 20°C, but decreases thereafter. From the present study, the optimal temperature for all species is not more than 50°C, except for *H. conica* at 60°C.

2.3 Morphological Research in *Fusarium oxysporum*

Colonies fast-growing, reaching 4.5 -6.5 cm diam in four days at 25℃; aerial mycelium sparse to abundant and floccose, becoming felted, white or peach, but usually with a purple or violet tinge, more intense at the stomatic agar surface. Some isolates have a characteristic aromatic odour suggesting lilac, some produce sporodochia with an orange slime of marco-conidia. Micro-conidia generally abundant, mostly bornr on short (often reduced), simple, lateral phialides or form sparsely branched conidiphores, never forming chains, mostly 0-septate,ellipsoidal to cylindrical, straight or often curved, 5-12 x 2.3-3.5 um. Macro conidia fusiform, moderately curved , pointed at both ends, basal cells pedicellate, 3(-5)-septate, (20-)27-46(-60) x 3.0-4.5(-5.0) um. Chlamydospores terminal or intercalary in hryphae, often aoso in cinidia, hyaline, smooth-walled or roughened, 5-15 um diam. Sclerotial pustules present in some isolates, pale to green or deep violet (Domsch and Gams,1993).

However, in recently , Chopada *et al.*(2015) carried out a experiment to study the variability using 10 isolates of *Fusarium oxysporum* f. sp. *lycopersici* collected from different tomato growing areas of south Gujarat. Studies were made on cultural and morphological variation like mycelial colour, mycelial growth, dry mycelial weight, sporulation, conidial size and formation of chlamydospores. The isolates produced moderate, profuse fluffy, thin flat to slight fluffy and submerged growth with white, yellow, light pink, dark pink, orange and purple–orange pigmentation. Sporulation varied from 2.77×10^6 to 21.68×10^6 spores/ml. The maximum dry mycelial weight was observed in SGFOL-6 (193.33 mg), whereas the minimum dry mycelial weight was observed in isolate SGFOL-9 (120.67 mg). Dry mycelial weight varied from 55.33 to 88.33 mm. The size of macroconidia ranged from 15.46–21.8 × 4.91–5.45 to 21.42–44.28 × 7.35–9.14 µm with 1–6 septa in different isolates. The size of microconidia varied from 3.57–14.28 × 2.68–4.46 to 7.14–14.28 ×3.57–5.35 µm with 0–1 septa in different isolates.

2.4 Biological Control of Fuasarium Wilt

The wilt deseases caused by *Fusarium* spp. are difficult to control. Bord-spectrum biocides, such as methyl bromide have hazardous effects on the environment (Blacard, 1993). Similarly breeding cultivars that are resistant against soil-borne diseases is difficult due to multation in *Fusarium* spp.; therefore research has been focused on the biological control (Fravel *et al.*, 2003). A novel plant growth promoting fungus (EU0013) was recently isolated from eucalyptus roots (Teshima and Sakamoto, 2006). From morphological features of the

conidiophores and sequence data on the ITS region of rDNA, the EU0013 was identified as *Penicillium* spp. Howevèr, the report of Syed Sartaj AlamA *et al.* (2010) showed that efficacy of *Penicillium* spp. EU0013 on the growth of fusarium wilts pathogens.

In dual culture experiment, EU0013 formed an inhibition layer near CU1 *(Fusarium oxysporum* f. sp. *lycopersici*) and K124F (*Fusarium oxysporum*. f. sp. *conglutinans)* without any physical contacts. EU0013 resulted in 30.6% and 29.6% reduction radial of colony growth in CU1 and K124F, respectively, compared to the control. This inhibition zone formation indicates that EU0013 produces some antifungal compounds which inhibit the growth of these pathogens. The nature and composition of such compounds is not yet known applied the novel rooting-colonizing fugus, *Peniccilium* sp. EU30013 at inoculum concentration of 10^6 conidia /g to potting mix resulted in 78% reduction of the diseases in tomato. Prior application of EU0013 effectively reduced the development of fusarium wilt in tomato. (Syed Sartaj AlamA *et al.,* 2010). Dr. Sibounnavong (2008) made such a point of view d bioactive compound from *Emericella nidulans* strain EN against *F. oxysporum* f. sp. *lycopersici* gave a positive effect to inhibit the spore production of tested plant pathogen. The higher concentration gave better inhibition of spore production than the lower ones. It is indicated that the SKP02-bioactive compound from *Emericella nidulans* strain EN could inhibit the spore production of the pathogen. In 2010, it was reported (Sibounnavong *et al.*, 2010) that powder and oil formulations of *Emericella nidulans* gave highly significant at P = 0.01 to control fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* at 50 days after inoculated tomato seedlings with pathogen, the disease index were 1.75 and 2.00 where the wilt disease was decreased 63% and 57%, respectively. It was also noted that tomatoes treated with oil or powder forms of *Emericella nidulans* gave the highest root weight and followed by treated with filtrate of *Emericella nidulans*, procoraz and nontreated tomato with pathogen (12.50 g). It revealed that tomato treated with oil or powder forms of *Emericella nidulans* gave significantly highest total fruit weight per plant which were 582 and 533 g/plant and followed by tomatoes treated with filtrate of *Emericella nidulans*. And then, in 2012 Sibounnavong *et al.* (2012) found *F. oxysporum* f. sp. *lycopersici* VTS16 was isolated from infested tomato fields in Lao PDR and proved to be the most virulent isolate causing wilt of tomato var. Sida as confirmed by Sibounnavong *et al.*(2010). The isolate VTS16 was significantly highest disease index of tomato wilt caused by *F.oxysporum* f. sp. *lycopersici* var Sida which categorized as high virulent. It is showed that *Emericella nidulans* isolate L01 which cultured in PDB at pH 8 and mixed media of PDB and CWDB at pH6 produced more fungal

biomass which gave highly significant in fresh weight of fungal biomass than other treatments. *Emericella nidulans* isolate L01 is screened to be the most potential antagonistic fungus against *F. oxysporum* f. sp. *lycopersici* which inhibited spore production of 82.05%. *Emericella nidulans* isolate L01 developed as bio-agent formulation would be feasible to extend this biological fungicide to control tomato wilt in different tomato varieties where susceptible wilt incidence, especially in the field. Charoenporn *et al.* (2010) cited in the research that Dual culture test showed that *Chaetomium globosum* N0802, *Chaetomium lucknowense* CLT and *Trichoderma harzianum* PC01 could control fusarium wilt of tomato caused by *F. oxysporum* f. sp.*lycopersici*. In vivo testing, bio-agent formulations N0802 (*C. gl bosum*), CLT (*C. lucknowense*) and PC01 (*T. harz anum*) clearly demonstrated that these bio-agent formu lations gave a highly effective control of fusarium wilt of tomato caused by *F. oxysporum* f. sp *lycopersici*. The bio-agent formulations could reduce disease incidence of tomato wilt, leading to increase in yield. As a result of bio-agent formulations N0802 *(C. globosum)*, CLT (*C. lucknowense*) supported the previous work of Soytong *et al.* (2005) which showed that Chaetomium bio-products formulated from *C. globosum* and *C. cupreum* as powder formulation could control bud rot and basal stem rot of bottle palms caused by Thielaviopsis paradoxa in the field and reduce disease incidence by 75%. These new bio-agent formulations which act as concentration suspension in oil form were of different formulation but are effective in controlling fusarium wilt of tomato as seen in the report of Soytong *et al.* (2001) who showed that the biological products consist of *Chaeto mium* spp. (22 strains of *C. cupreum* and *C. globosum*) in biopellet and biopowder formulations which when applied to the soil could suppress the growth of *F. oxysporum* f. sp. *lycopersici* and reduce infection rate in tomato.

2.5 Morphological Research in *Colletotrichum* Species

Colletotrichum spp. are among the most important plant pathogens worldwide, causing the economically important disease anthracnose in a wide range of hosts, including cereals, legumes, vegetables and tree fruits (Bailey and Jeger, 1992). Usually producing intraepidermal acervuli (which in vivo resemble sporodochia) with a rather dense layer of subulate or cylindrical, hyaline phialidies supported by 1-3 short prismatic, hyalicne, or pigmented cells, sometimes interspersed , septate, moderately pointed aetae which may be long and arise from the basal stroma. Conidia cylindrical or falcate, 1-celled, hyaline,smooth-walled,aggregated in cream,orange, red, or brownish slimy masses. A characteristic feature of

genus are the brown, rounded, or lobed appressoria developed by germinating conidia; there are easily oberrved in water in hanging drop cultures.

Among these hosts, chilli (*Capsicum annuum*) considered the most important vegetable in Thailand (Poulos, 1992), is severely infected by anthracnose, with yield losses of up to 50% (Pakdeevaraporn *et al.*, 2005). Typical anthracnose symptoms on chilli fruits include sunken necrotic tissues, with concentric rings of acervuli that are often wet. Chilli pepper fruits with blemishes have reduced marketability (Manandhar *et al.*, 1995). Than *et al.* (2008) got 29 isolates of *Colletotrichum* spp. were obtained from infected chilli fruits, three from infected mango fruits and six from infected strawberry fruits, all showing symptoms of anthracnose. Distinct morphological types of cultures on PDA were observed in each morphological group after 7 days following subculturing. Isolates from group 1, mango *C. gloeosporioides*, produced colonies with little aerial mycelium in alternating concentric zones of light orange at the centre turning pale yellow towards the margin. Colonies produced by isolates from group 2, chilli *C. gloeosporioides*, varied from greyish-white to dark grey; some isolates (Ku1, Ku2, Ku3, Ku4, Ku5 and Ku8) showed diurnal zonation of pale grey to black aerial mycelium, whilst others (Ku6, Ku9 and Ku10) produced aerial mycelium in an even, felted mat. Isolates from group 3, strawberry *C. acutatum*, produced white to pale grey colonies showing diurnal zonation of dense and sparse development of aerial mycelia, sometimes with pinkish spore masses. Isolates from group 4, chilli *C. acutatum*, produced pale orange colonies with little aerial mycelium and a few orange conidial masses around the centre. Isolates from group 5, chilli *C. capsici*, produced colonies that were white to grey; most of the isolates showed the diurnal zonation of dense and sparse development of aerial mycelium, sometimes with beige-coloured spore masses. There was no significant difference in growth rate among isolates of the same species. However, a statistical difference was observed in the growth rate of the three different species. There were three types of conidia, viz. cylindrical, fusiform and falcate, observed in the three species of *Colletotrichum.* There were few differences in appressorial shape and size between groups. Most of the appressoria formed in slide cultures were irregularly shaped and only a few were ovoid. The identity of *Colletotrichum* species on *Coffea* (coffee) is not clearly understood. Prihastuti *et al.* (2009) mentioned in Differences in conidial morphology, colony characters and growth rates among the *Colletotrichum* isolates allowed them to be separated into three groups. Colonies produced by isolates from Group 1 varied from pale yellowish to pinkish with dense whitish-grey aerial mycelium and a few bright orange conidial masses near the inoculum point. Isolates from

Group 2 produced greyish green, not when first colour is qualified as grayish colonies in the centre, with sparse white aerial mycelium, green in reverse and orange conidial masses near the inoculum point. Colonies produced by isolates from Group 3 varied from grey to dark grey with dense pale grey aerial mycelium. Conidia produced by isolates of *Colletotrichum* Group 1 varied from fusiform with obtuse to slightly rounded ends to sometimes oblong. *Colletotrichum* Group 2 produced cylindrical conidia with obtuse ends (oblong) with narrowing at the centre. *Colletotrichum* Group 3 produced cylindrical conidia with obtuse to slightly rounded ends. There were statistically significant differences in length and width of conidia among the three groups. There was little distinction among the groups in size and shape of appressoria. Appressoria shape produced by slide cultures varied from ovoid, clavate or slightly irregular to irregular in shape .

2.6 Biological Control of Coffee Anthracnose

Anthracnose diseases, particularly those caused by *Colletotrichum* spp. are very common. Although severe everywhere, anthracnose diseases cause their most significant losses in the tropics and subtropics. In the report of Ajith (2010) showed all the selected *Trichoderma* sps has potential to inhibit the mycelial growth of *C.capsici.* The volatile compounds produced form all the selected *Trichoderma* species showed 30 to 67% inhibition of *C. capsici,* However non-volatile compounds or culture filtrate from *Trichoderma* viride at 3%-4% concentration shows complete mycelial inhibition of the test fungi. *Trichoderma harzianum, T. saturnisporum and T. reesei* also have the ability to control growth of *C. capsici* by 21 to 68% at a concentration of 50% culture filtrate. From the results it is clear that all the isolates taken were effective in controlling the pathogen in-vitro. Meanwhile, Vilavong Somlit and Kasem Soytong (2013) reported that *Ch. cupreum* can be antagonized *C. coffeanum* in dual culture plate after incubation for 30 days. The colony of *Ch. cupreum* grew over pathogen colony implies competition mechanism of control. This research finding proved that *C. coffeanum* causing anthracnose shown to be infected leaves and beans. *Ch. cupreum* proved to be effective antagonist against anthracnose pathogen. Further study needs to do biological activity of metabolites from *Ch. cupreum* for possible induction of plant immunity through phytoalexin in coffee and also application in the field experiments for organic coffee production in Lao PDR.

2.7 Crude Extracts from Mushrooms

The intensive and indiscriminate use of pesticides in agriculture has caused many

problems to the environment such as water, soil, animals and food contamination; poisoning of farmers; elimination of non-target organisms; and selection of phytopathogens, pest and weed insensitive to certain active ingredients (Stangarlin *et al.,* 1999). Aiming to minimize the negative effects of pesticides are been development the alternative control of plant disease, which includes the biological control, the induction of resistance and the use of natural products with induction of resistance and/or with direct antimicrobial activities.In recent years, more and more attention biological control, variable aspects now. Plants, fungi, or bacteria bioactive material is also widely used to control various diseases, pests. Plant extracts used to control of the phytopathogens have been obtained mainly from tree species (Tangarlin *et al.,* 1999). Aqueous extract (AE) of camphor controlled of anthracnose which caused by *Colletotrichum musae* in banana. The severity of anthracnose was reduced to 67% and 56% for camphor and fungicide treatments, respectively (Carré *et al.*, 2006). in vitro experiment, turmeric extract inhibited completely the cassava bacterial wilt in the concentration of 10% for the genotype from Mercedes, while for the Jaboticabal's turmeric there was a total control at 15% and for Mara Rosa at 20%. (Kuhn *et al.,* 2006). The effect of aqueous extract of ginger inhibited the antimicrobial activity of ginger with mycelial growth and sclerodia production (Rodrigues *et al.*, 2007). Aqueous extracts of the leaves of *Ocimum gratissimum* at 10, 25, 40 and 50% (w/v) concentrations induced the production of phytoalexins in soybean cotyledons and sorghum mesocotyls. The aqueous extracts also induced systemic resistance in cucumber against *Colletotrichum lagenarium*, reflected by reduction in disease incidence and an increase in chitinase production (Colpas *et al.,* 2009). Crude extract of wild basil at 5% was enough to provide inhibition of 100% on mycelial growth of *A. alternata* and *S. rolfsii* (Benini *et al,.* 2010). Crude extracts from *Chaetomium cupreum* CC, *C. globosum* CG, *Trichoderma harzianum* PC01, *T. hamatum* PC02, *Penicillium chrysogenum* KMITL44 and antibiotic substances Rotiorinol, Chaetoglobosin-C and Trichotoxin A50 inhibited the growth of *C. gloeosporioides* strain WMF01, with average ED_{50} values between 1 to 50 µg/ml. (Soytong, 2005). Chaetomium-mycofungicide inhibited pathogen growth by more than 53% over 10 days which caused by Phytophthora infestans (Soytong, 2005). *Chaetomium cupreum* RY202 was tested for ability to inhibit the growth of *Rigidoporus microporus*. The results showed that crude hexane extract from *Ch. cupreum* RY202 gave the highest inhibition of mycelial growth with inhibition of 82.0% and the effective dose (ED_{50}) of 170 µg/ml and crude ethyl acetate extract gave colony inhibition of 80.0 and 78.0% and the effective dose (ED_{50}) at 187 and 402 µg/ml, respectively. The bioactive

compound from *Ch. cupreum* named rotiorinol showed ability to inhibit the growth of *R. microporus* and inhibited the mycelial growth at 250 and 500 µg/l with effective dose (ED_{50}) 26 µg/ml. *Ch. cupreum* RY202 was formulated as powder and oil form. These formulations were applied to inhibit the growth of R. microporus. The results showed that *Ch. cupreum* RY202 could reduce the white root disease of rubber trees with disease reduction in the treatment of powder and oil from of 60 and 80%, respectively (Soytong, 2014).

Mushrooms are very appreciated as food in several countries and, besides their fine taste, they have great nutritional value as sources of essential fatty acids, minerals, proteins and biologically active metabolites. By the other hand, nonbasidiomycete filamentous fungi from several genera such as Rhizopus or Penicillium are commonly not associated with human diet. Nevertheless, these and other non-basidiomycete filamentous fungi have also been reported to produce several metabolites useful to be added to food for preservation, coloring, flavoring or even aiming at a biological effect such as antioxidants and essential fatty acids (Takahashi, 2010). Many mushrooms produce molecules that have biological properties like hipoglicemic and hipocolinesterolemic (Teichmann *et al.,* 2008, Diyabalanage *et al.,* 2008). Antitumor and antiregulatory polysaccharides, such as lentinan, extracted from *Lentinus edodes* species (Shiitake), which also have antifungal and antibacterial activities is also a good example (Wasser, *et al.,* 2002, Ngai *et al.*, 2003, Kupfahl *et al.*, 2006). Agrocibin isolated from the ethyl acetate extract of the species *Agrocybe perfecta*, presents imunossupressing, tripanocidal, cytotoxic and antifungal activities (Rosa *et al.,* 2006). Presence of nicotinic acid, and pyrazole-3(5)-carboxylic acid in *Volvariella volvacea* were related to the beneficial health effects of this edible specimen (Mallavadhani *et al.*, 2006). Mushrooms such as *A. bisporus, L. edodes, A. auricula* and many *Pleurotus* species have been shown to posses antagonistic effects against bacteria, fungi, viruses and cancer (Tochikura *et. al.*, 1988; Stamets, 1993; Jonathan and Fasidi, 2003). It was therefore the aim of this present study to screen *Auricularia polytricha, Corilopsis occidentalis*, *Daldinia concentrica*, *Daedalea elegans* and *Tricholoma lobayensis* for their antimicrobial activities. These will act as possible preventive agents against common bacteria and fungal infections of man. a member of the same genus *A. auricula* has been found to possess anibacterial property which could be used for the treatment of eye inflammatory disease (Well, 1994). In some medicinal mushrooms, often contain a lot of chemical anti-cancer COMPOUNDS. The use of medicinal mushroom extracts in the fight against cancer is well known and documented in China, Japan, Korea, Russia and now increasingly in the USA (Mizuno *et al.*, 1995). Many, if not all,

Basidiomycete mushrooms have been shown to contain biologically active antitumour and immunostimulative polysaccharides. In a recent review Reshetnikov *et al.* (2001) have listed 650 species and 7 intraspecific taxa from 182 genera of higher Hetero- and Homo-basidiomycetes that contain pharmacologicaly active polysaccharides that can be derived from fruit-bodies, culture mycelium and culture broths. IThe first definitive studies on these anticancer substances came in the late 1960s with the reports by Ikekawa *et al.* (1968, 1969) and Chihara *et al.* (1969,1970). They demonstrated that extracts of several different mushroom species exhibited remarkable host-mediating antitumour activities against xenographs, e.g.Sarcoma 180. Hot water extracts from *Grifola frondosa*, the Maitake mushroom, contain the D-Fraction which appears to be a highly active anticancer agent for both animals and humans (Jones, 1998; Maitake, 1999). According to reports, compounds 7, 10, and 12 of *Ganoderma* spp. KM01 exhibited antimalarial activity against Plasmodium falciparum in the range 6.0–10.0 μM (IC50) (Waranya Lakornwong *et al.*, 2013). Also, the indigenous *Ganoderma lucidum* mushrooms have a good potential for the production of useful bioactive metabolites and they may serve as a good source for antimicrobial drugs (Shikongo, 2013). The antagonistic activity of other 17 species of xylotrophic Basidiomycotina (*Coriolus versicolor, Flammulina velutipes, Ganoderma lucidum, Hypholoma fasciculare, H. sublateritium, Kühneromyces mutabilis, Lentinula edodes, Lentinus tigrinus, Pholiota alnicola, Ph. aurivella, Ph. destruens, Pleurotus ostreatus, P. cornucopiae, Polyporus squamosus, P. subarcularius, P. varius* and *Schizophyllum commune*) against 4 fungi (*Bipolaris sorokiniana, Fusarium culmorum, Gaeumannomyces graminis* var. *tritici and Rhizoctonia cerealis*), responsible for foot and root diseases of winter cereals, Almost all tested mushroom species markedly inhibited mycelial growth of the four phytopathogenic fungi, antagonistic activity of *P. ostreatus, H. fasciculare, G. lucidum, L. tigrinus* and *S. commune* being particularly strong (Susanna *et al.*, 2002). Some other edible mushrooms also have same function. *Pleurotus citrinopileatus* and *Tricholoma crassum* Berk, as two edible mushrroms,1 mg of the crude extractfrom *P. citrinopileatus* culturing PDB demonstrated strong activities against *Escherichia coli* ATCC 25922 (higher than activityof 10 g ampicillin) and *Staphylococcus. aureus* ATCC 25923 (less than activity of 10 g ampicillin) (Porntep Chomcheon *et al.*, 2013). In the report of evaluating of the phytochemical, antioxidant and antimicrobial in vitro assay of *Pleurotus pulmonarius*-LAU09 (JF736658), the antioxidant activity of extracts gave positive results with free radical scavenging activity found to be higher in all used in vitro methods. The result showed the potential of mushroom extract as a potent therapeutic agent and a food supplement. Also,

Gbolagade *et al.* (Fahey *et al.*, 2007) reported that *P. tuber-regium* extract had powerful medicinal importance by inhibiting the growth of *Bacillus cereus, E. coli, K. pneumoniae, S. aureus, Proteus vulgaris, and P. aeruginosa.* With the purpose of finding alternative ways to control *Bipolaris sorokiniana* and *Puccinia recondita* f. sp. *tritici*, the *in vitro* fungitoxic effect of *L. edodes* and *A. blazei* mushrooms was tested on these fungi. The aqueous extract of both mushrooms did not have significant effect on mycelial growth and spore germination of *B. sorokiniana*. On the other hand, the mushroom extracts inhibited the germination of *P. recondita* f. sp. *tritici* uredospores, particularly on the *L.edodes*, which exhibited the largest inhibition of spore germination (52%) (Fiori-Tutida *et al.*, 2007).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Mushroom Collection and Identification

3.1.1 Survey Procedure

Collections were made in the forest areas in five provinces, six sites of Thailand. Samples were collected during the raining season from July to October, 2013. The collected sites are as Table 3.1.

Table 3.1 Mushroom collection sites.

No.	Date	Location
1st	20th July 2013	Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″
2nd	13th August 2013	Chiangrai Province, Chiang Kong, N 20°15′36″, E 100°47′3″
3rd	17th August 2013	Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″
4th	3th September 2013	Kanchanaburi Province, Amphoe Mueang Kanchanaburi, N 14°0′12″, E 99°33′0″
5th	20th September 2013	Bangkok Province, Khet Lat Krabang(KMITL), N 13°43′24″, E 100°47′3″
6th	20th September 2013	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″,E 99°8′40 ″
7th	20th July 2013	Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″

3.1.2 Morphological Character Examination

Materials to be used for collection were as follows: rulers, knifes, recording papers, pH meter, Thermometer, plastic bags, spore print paper (black and white A4 paper with glue bonded together and cut into different sizes), rubber bands, camera.

Collection was divided into five steps as follows;- searching, processing, photography, recording and maintain specimens.

1) Search for fresh specimens: Mushroom specimens were collected and found to be grown in different conditions. For example, single, scattered, grows in group. The surrounding environment were observed to collect other specimens and young fruiting bodies would be appeared under the soil. Specimen must be carefully observed.

2) Processing fresh specimens were done spore print: The specimens were found in soil mud, leaves, small insects. For too moist specimens, towelled gently wipe excess water with tissue papers were done. According to the diameter specimens stipe, along the central bond of spore print, A4 sheet of paper was cut out just enough to stalk through the small hole. The stipe passed through the hole, fold or more parts of gills remained at the other side of the paper. If necessary, gently shaked cap, basidiospores would fall off the gills. The time of waiting fallen basidiospores varied due to specimens to get spore print.

3) Photography fresh specimens: Fresh specimens were photographed including the young and mature basidiocarps. The front, side, rear of specimens, the attachment of stem, spore print also would be photographed.

4) Recording features: Including two parts, the one was specimens characters and the other one was habitat. For the specimen characters, what should be noticed as shown in Table 3.2 and for habitat what should be noticed as shown in Table 3.3.

5) Maintain specimens: Each specimen was wrapped by foil or kept in plastic bag separately in order to avoid the mixture and crush and kept them in ice box to keep fresh. Young and mature basidiocarps were collected if them appearance; and the all part of basidiocarp were collected including the base of stipe and fell annulus.

The macrocharacters, chemical test and photograph of fresh sample were carried out as soon as possible after came back from the field trip which followed the instruction described by Largent (1986). If could not finish all samples in short time, the specimens were stored in the 4° C freezer waiting for examination. Then the specimens were divided into two groups. One put into the drier at least overnight to dehydration, sealed in the plastic bag, and kept in the herbarium. All of these specimens kept to work further for molecular phylogeny. The other group was maintained as spore print and made pure cultures for morphological identification.

Table 3.2 Specimens characters.

Structures	What should be noticed
Cap	Size; Shape; Color; Color change upon bruising; Attachment; Margin;
Flesh	Thickness; Color and color change upon bruising; Odor; Taste (not all);
Gills	Color; Attachment; Width; Size; Compactness; equeal or nor;
Ring	Existense; Color; Shape; Diameter; Location; Double or Single
Stipe	Shape; Color and Color change upon bruising; Solid or Hollow; Width; Height; Attachment;
Vlova	Existense; Siza; Shape; Color;
Rooting base	Existense;
Spore	Size; Shape;Color;
Spore print	Color;
Smell or Taste	Pleasant or Unpleasant;
The type of growing	Solitary; Scattered; Gregarious; Caespitose

Table 3.3 Habitat.

Items	**Description**
Location	province; elevation; coordinates
pH	Specific values
Symbiotic	Yes or No; Symbiotic plants
Growing land	Wood, Soil

3.1.3 Pure Culture Cultivation

Two methods were used to isolate into pure cultures by fresh tissue and single spore isolation.

1) Isolation into pure cultures by fresh tissue

Fresh specimens were cleaned with sterilize water, intercepted the stipe with a blade, quickly sterilized in 75% alcohol , the middle part of the stipe, which can be easier to obtain pure culture, then washed again in sterilize water, and slashed the surface portion of stipe with a knife which burnt alcohol lamp, removed the middle part, cut into small pieces, put them into water agar (WA), incubated for 2-5 days, and observed the growth of the mycelium, then transferred to

potato dextrose agar(PDA) until get pure culture.

2) Isolation into pure cultures by single spore

Two methods were used. For existing spore print, picked up a small amount of spores with a sterilized needle, directly transferred into the PDA. For the specimens which had not spores prints, kept the cap side up and gill folded side down, in the top of the prepared PDA medium shook specimens gently, also a small amount of basidiospores fell to the medium. Both methods were incubated at room temperature for a few days to obtain pure culture.

3.1.4 Morphological Identification

Specimens were identified roughly according to the color of spore print. The brief of some spore print colors were shown in Table 3.4. A small number of basidiospores were removed from spore print with needle, dipped in milk dripping with lactophenol or sterilize water slide and coverslip, then observed under 40X microscope spore shape (Globose, Ellipsoid, Oblong, Nodulose, Cylindric, Fusiform), size, color, surface ornamentation (Smooth, Warted, Spiny, Reticulate, Striate), recorded features, as morphological identification materials.

Pure cultures were made semi-permanent slides by small amount mycelia from the pure cultures with a needle and transferred to a pre-drops of lactophenol on glass slides, observed the mycelium structure under the 40 X compound microscope, as morphological identification materials.

Table 3.4 The brief of some spore print color.

The color of basidiospores in mass	Family
White	Hygrophoraceae
	Lepiotaceae
	Tricholomataceae
Pink	Pluteaceae
Brown	Agaricaceae
	Cortinariaceae
Black	Gomphidiaceae
	Coprinaceae
Purpuse	Strophariaceae

3.2 Fugal Metabolites from Some Mushrooms against *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2 Causing Tomato Wilt and *Colletotrichum coffeanum* Causing Coffee Anthracnose

3.2.1 Isolation of Pathogens

The disease samples were collected from coffee plantations of Arabica on leaves and brought to laboratory for isolation to be pure cultures. The symptom of coffee anthracnose which caused by *Colletotrichum* spp. was used to isolate the causal agent by using tissue transplanting technique. The advanced margin of lesion was surface disinfected with sodium hypocholite 10%, then cut with sterilized blade into small piece of 0.5 X 0.5 cm between advanced margin of healthly and infeted tussues on symptom of leaf, then soaked into 10% sodium hypocholite for a few minutes, and moved to sterilize distilled water, then placed in sterilized tissue paper to dry out, thereafter picked up with needle and placed onto water agar (WA), then incubated at room temperature approximately 27-30 ℃. The hyphal tip isolation was done by cutting with needle into small piece of hyphal tip and transferred onto potato dextrose agar (PDA), incubated at room temperature and observed growing colony until getting pure cultures. All isolates were morphologically identified into species by using binocular compound microscope.

Fusarium wilt pathogen was provided by Assoc. Prof. Dr. Kasem Soytong. Then transferred it into PDA and kept them for further work.

3.2.2 Pathogenicity Tests

Pathogenicity test for coffee anthracnose which caused by *Colletotrichum coffeanum* was conducted using detached leaf inoculated method. The experiment was done using Completely Randomized Design (CRD) with four replications. Treatments were inoculated into wounded leaves surface with an agar plug of pathogen and placed in moist chamber done in Petri dishes. Control treatment was done by transferring an agar plug of PDA alone onto wounded surface leaf. Data was collected as lesion size in mm and computed statistical analysis then compared treatment means using Duncan's Multiple Range Test (DMRT) at P = 0.05 and 0.01.

The pathogenicity test of fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2) that was tested in vivo to 15 day tomato seedlings. Tomato seeds were sown into coarse sand in plastic trays (10 × 15 × 5 cm) and were maintained for 2 weeks . Pathogenicity test was carried out using a root dip inoculation method. Tomato seedlings were uprooted gently and roots were washed with tap water to remove all sand (Bao *et al.*, 2002). The spore suspension for

inoculation was prepared by pouring 50 ml of sterile water into each of Petri dishes containing 10-day-old *Fusarium* isolate, stirring the mixture with a sterile glass stick, and pouring it into a glass. The concentration of conidia in the suspension was determined using Haemacytometer to adjust for spore number to $1x10^6$ conidia/ml. The 3-4 root tips were cut and soaked into spore suspension for 30 seconds. Control plants were sown in soil and treated with sterile distilled water. Incubation was performed at 22-25°C for 14 days. The disease severity was rated with follow the method of Sibounnavong (2012), as follows: 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting 81-100% or die. The most virulent isolate was selected for further experiment.

3.2.3 Bioassay Tests

3.2.3.1. Bioactive Substance Extraction

The fungal metabolites from some species of Agaricales, as follows: 1. *Leucocoprinus fragilissimus* (PH06) 2. *Collybidia strictipes* (PH07), 3. *Clitocybe* spp. (AJ2-2), 4. *Boletus affinis* var. *maculosus* (AJ2-3) 5. , *Lactarius* spp. (CH3-01), 6. *Lactarius* spp. (CH3-27) were cultured on PDB for 45 days. The extraction was performed using the method of Kanomedhakul *et al.* (2007) as seen in Fig. 3.1. Pure cultures were cultured to get fungal biomass. Each isolate was cut into 0.5cm x0.5cm pieces, picked up with the sterilized needle and transferred into 500 sterilized petri dishes. Each small piece of culture was cultured in 25-30 ml potato dextrose broth PDB at room temperature (28-30℃) for 45 days. Fungal biomass were removed from PDB, filtered through cheesecloth and air-dried. Fresh weight and dry weight of fungal biomass were weighted. Dried fungal biomass were ground with electrical blender, extracted with hexane (H) and shaken for 5 days at room temperature. The ground fungal biomass were separated by filtration through Whatman No.4 filter paper. The filtrates were evaporated in vacuo to yield crude extract. The marc would be further extracted with ethyl acetace (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. Each crude extract was weighted, then kept in refrigerator at 4℃ until use (Fig. 3.1).

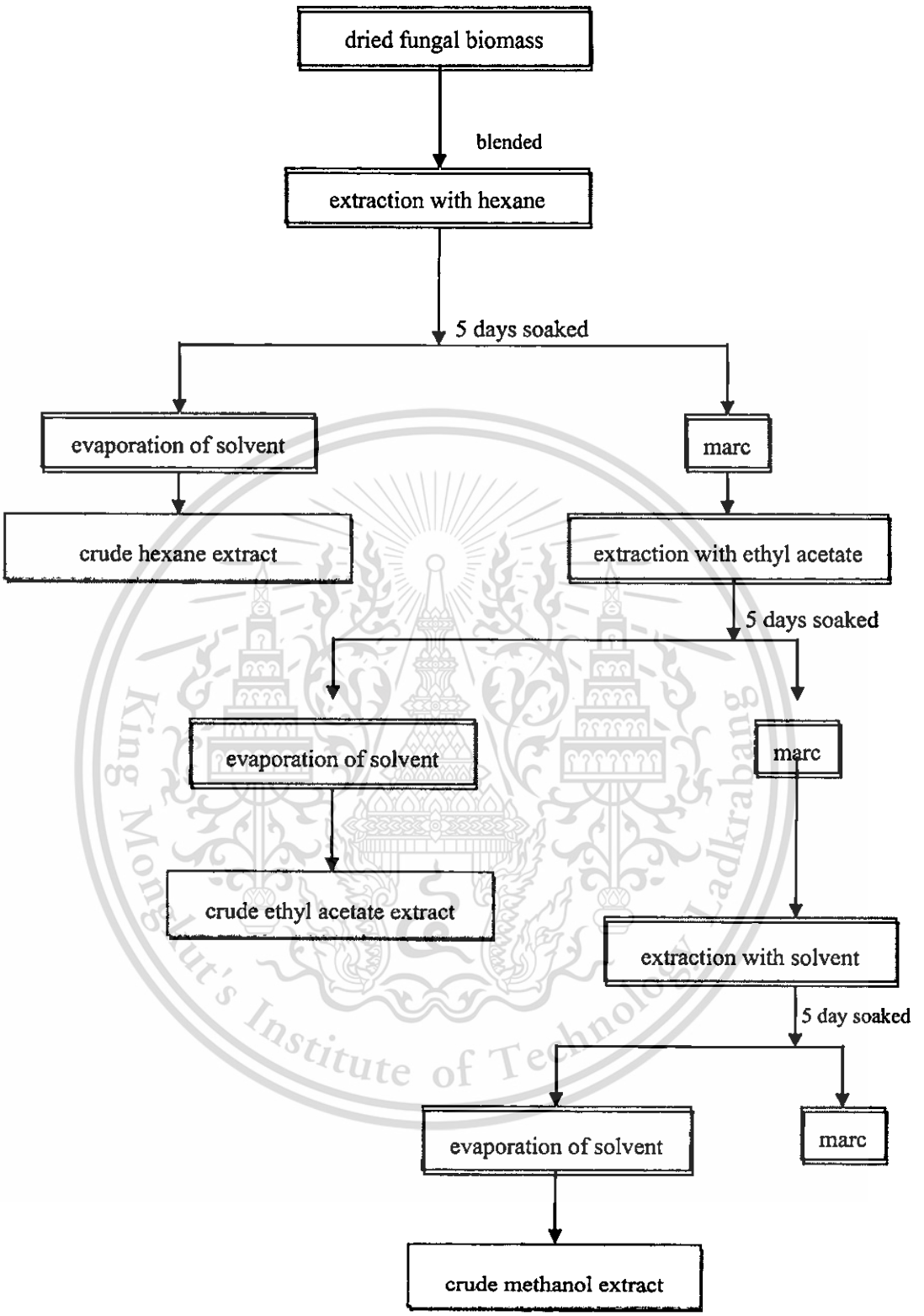

Fig. 3.1 Diagram of extraction method.

3.2.3.2 Bioassay Test against *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2 Causing Tomato Wilt and *C. coffeanum* Causing Coffee Anthracnose

The crude extracts of mushroom were tested for inhibition of the most aggressive isolate of *F. oxysporum* f. sp. *lycopersic and Colletotrichum coffeanum*. The experiment was conducted by using 3x6 factorial in Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts which was consisted of hexane crude, ethyl acetate crude and methanol crude and factor B represented the concentrations 0, 10, 50, 100, 500, and 1,000 µg/ml. Each crude extract dissolved in 2% dimethyl sulfoxiden (DMSO), then mixed into PDA before autoclaving at 121℃, 15 1bs/inch^2 for 30 minutes. The tested pathogen was cultured on PDA and incubated at room temperature for 5 days, then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen were transferred to the middle of PDA plate (5.0 cm diameter) in each concentration and were incubated at room temperature (28-30℃) for four days. Data were collected as colony diameter and number of conidia. Percentage of inhibition was computed. Data were statistically computed analysis of variance. Treatment means were computed with DMRT at P= 0.05 and P= 0.01. The effective does (ED_{50}) was computed by using probity analysis. The comparison between normal and abnormal propagates corneal dual-culture were observed under compound microscope.

3.2.3.3 Sporulation Conditions of *Colletotrichum coffeanum*

The experiment was conducted by using 3 factors factorial in Completely Randomized Design (CRD) with four replications. Factor A represented the different media which gave effect on spore production: A1= PDA media (potatoes, 200 g; dextrose, 15 g; agar, 20 g; H_2O, 1 L); A2= V8 juice media (V8 Juice, 200.0 ml; $CaCO_3$, 3.0 g; Agar, 15.0 g; Tap water to1.0 L; PH, 7.2). Factor B represented different light conditions: B1= constant illumination (continuous light; CL; 10000 lux, fluorescent lamp), B2= constant darkness (continuous darkness; CD), B3= 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp). Factor C represented the different volume of media: C1= 5ml media, C2=10ml media. 3 mm-diameter agar plug cut by sterilized cork borer from the pure cultures of *Colletotrichum coffeanum* placed in the center of 5 cm petri dishes which contained 5 ml of PDA media, 10 ml of PDA media, 5 ml of V8 media, 10 ml of V8 media, respectively.

Since 14th day, observed the sporulation every 7 days. The plate surface was washed 3 times with 10ml tap water to dislodge the fungal spores after different processing. Spore production was quantified using a hemacytometer. The data were calculated using EXCEL

software. In the study of individual factors, the data on sporulation were analyzed by Duncan's New Multiple Rrange Test (DMRT) and statistically computed analysis of variance. Treatment means were computed with DMRT at P= 0.05 and P= 0.01. The comparison between normal and abnormal propagates corneal dual-culture were observed under compound microscope.

CHAPTER 4

RESULTS

4.1 Mushroom Collection and Identification

4.1.1 Collection of Mushrooms

Sixty samples were collected in five provinces of six points in Thailand. These were divided into 7 orders (Agaricales, Auriculariales, Boletetaless, Cantharellales, Polyporaceae, Russulales, Xylariales), 17 families (Agaricaceae, Auriculariaceae, Boletaceae, Cantharellaceae, Clavariaceae, Exidiaceae, Hydnangiaceae, Inocybaceae, Lyophyllaceae, Marasmiaceae, Mycenaceae, Pleurotaceae, Polyporaceae, Russulaceae, Schizophyllaceae, Tricholomataceae, Xylariaceae) as seen in Fig. 4.1 and Table 4.1.

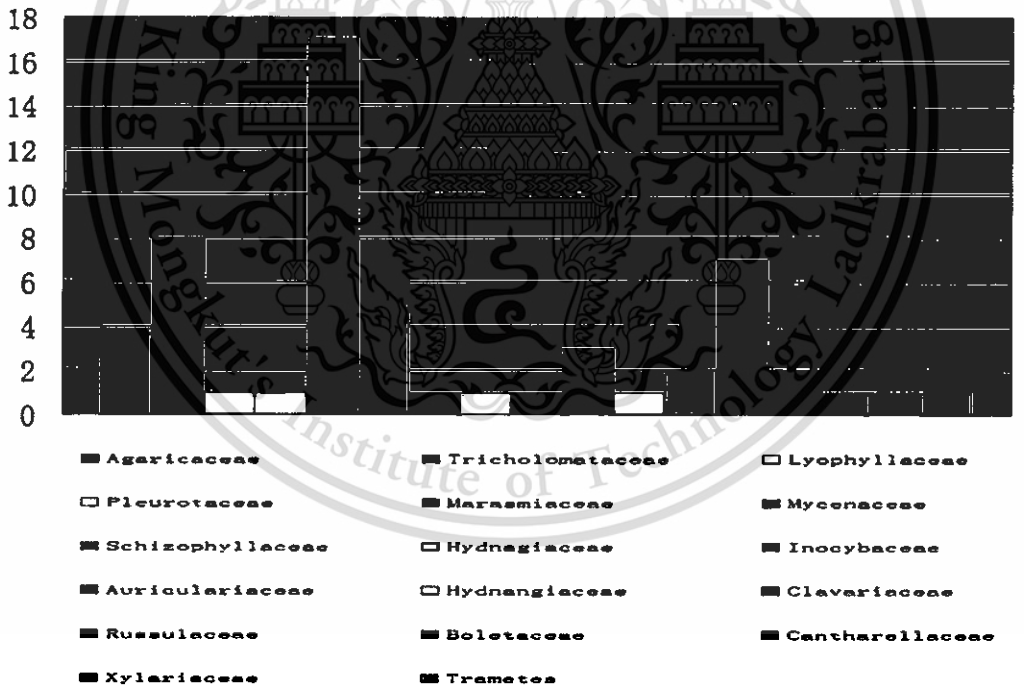

Fig.4.1 Collection of mushrooms.

Table 4.1 Collection of mushrooms.

Specimen number	Taxon	Family and Order	Location
CH01	*Agaricus macrosporus*	Agaricacea, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH02	*Tricholoma* spp.	Tricholomatacea, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-01	*Lactarius* spp.	Russulaceae, Russulale	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-02	*Marasmius* spp.	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-03	*Mycena rosella*	Mycenaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-04	*Marasmius androsaceus*	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-05	*Trametesversicolo* spp.	Polyporaceae , Trametes	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-06	*Lactarius sanguifluus*	Russulaceae, Russulale	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-07	*Mycena subcaerulea*	Mycenaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-08	*Clitocybula atrialba*	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-09	*Tremiscus* spp.	Exidiaceae, Auriculariales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-10	*Clavulinopsis helvola*	Clavariaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-11	*Mycena inclinata*	Mycenaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-12	*Marasmiellus albuscorticis*	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-13	*Laccaria* spp.	Hydnangiaceae Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-14	*Termitomyces microcarpus*	Tricholomataceae Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)

Table 4.1 (Continued)

Specimen number	Taxon	Family and Order	Location
CH3-15	*Clavulinopsis fusiformis*	Clavariaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-16	*Resinomycena rhododendri*	Mycenaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-17	*Marasmius foetidus*	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-18	*Marasmius plicatulus*	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-19	*Xylaria hypoxylon*	Xylariaceae, Xylariales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-20	*Lactarius controversus*	Russulaceae, Russulales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-21	*Marasmius scorodonius*	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-22	*Marasmius oreades*	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-23	*Marasmius* spp.	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-24	*Lactarius* spp.	Russulaceae, Russulales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-25	*Agaricus* spp.	Agaricaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-26	*Collybia dryopjila*	Marasmiaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
CH3-27	*Lactarius* spp.	Russulaceae, Russulales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
PH01	*Auricularia auricula*	Auriculariaceae, Auricuriales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH02	*Tricholoma* spp.	Tricholomataceae Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH03	*Termitomyces* spp.	Lyophyllaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)

Table 4.1 (Continued)

Specimen number	Taxon	Family and Order	Location
PH04	*Pleurocybella porrigens*	Marasmiaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH05	*Pluerotus giganteus*	Pleurotaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH06	*Leucocoprinus fragilissimus*	Agaricaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH07	*Collybia strictipes*	Tricholomataceae Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH08	*Marasmius* spp.	Marasmiaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH09	*Coprinus* spp.	Agaricaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH10	*Marasmius* spp.	Marasmiaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH11	*Collybia iocephala*	Tricholomataceae Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
LB01	*Collybia* spp.	Tricholomataceae Agaricales	Bangkok Province, Khet Lat Krabang(KMITL)
SY01	*Mycena* spp.	Mycenaceae Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY02	*Marasmius* spp.	Marasmiaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY03	Mycena spp.	Mycenaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY04	*Marasmius purpureostriatus*	Marasmiaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY05	*Mycena* spp.	Mycenaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY06	*Marasmiellus albuscorticis*	Marasmiaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY07	*Auricularia auricular*	Auriculariaceae, Auricuriales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)

Table 4.1 (Continued)

Specimen number	Taxon	Family and Order	Laction
SY08	*Schizophyllum commune*	Schizophyllaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY09	*Marasmillus ramealis*	Marasmiaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
AJ01	*Russula* spp.	Russulaceae, Russulales	Chiangrai Province, Chiang Kong
AJ02	*Boletus retisporus*	Boletaceae, Boletale	Chiangrai Province, Chiang Kong
AJ03	*Cantharellus cibarius*	Cantharellaceae, Cantharellales	Chiangrai Province, Chiang Kong
AJ04	*Russula crassotunicata*	Russulaceae, Russulales	Chiangrai Province, Chiang Kong
AJ2-1	*Laccaria vinaceoavellanea*	Hydnagiaceae, Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-2	*Clitocybe* spp.	Tricholomataceae Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-3	*Boletus affinis* var. *maculosus*	Boletaceae, Boletale	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-4	*Inocybe fastigiata*	Inocybaceae, , Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-5	*Clitocybe* spp.	Tricholomataceae Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-6	*Mycena vulgaris*	Mycenaceae, Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi

4.1.2 Isolation and Identification

For the specimen characters, the structures of the cap, flesh , gills ,tubes, ring, veil, stipe, volva, rooting base, spores, spore print, smell, taste, the type of growing were noticed.

Species description as follows:

1. ***Agaricus macrosporus*** (CH01)

A grey-white, flesh mushroom. *Cap,* 10 cm in diameter, convex , wavy margin, scales;

Gill, free, brown, wide, crowded; *Stem,* 4.5 x 1 cm, thick , acid, flesh, ring large, white, base bulb, smooth; *Flesh* , grass smell (Fig. 4.2).

Habit: gregarious in soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.6; Temperature 26℃; 20 Jul. 2013; collected by Yaling Luo.

2. ***Tricholoma* spp.** (CH02)

A flesh mushroom. *Cap,* 0.5-1.5 cm in diameter, convex with depressed centre, dark green; *Gill,* decurrent, whitish-dark green, distante; *Stem,* 0.2 x 1.2cm high, slender, same color with cap, smooth (Fig. 4.3).

Habit: scattered under leaves.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.6; Temperature 26℃; 26 Jul. 2013; collected by Yaling Luo.

3. ***Lactarius* spp.** (CH3-01)

A flesh mushroom, fruit body makes people think pf milk. *Cap*, 0.5-4 cm in diameter, convex , smooth, cream yellow with white, slight incurrent margin with not clearly lined, Color changes to buff when dry; *Gill*, free, close, cream yellow to pink; *Flesh*, white; *Stem,* 0.5-6 x 0.1-0.5 cm, white then becoming buff, smooth, having rooting base; S*pore print*, brown (Fig. 4.4).

Habit: scattered in sandy solid.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃;30 Oct. 2013; collected by Yaling Luo.

4. ***Marasmius* spp.** (CH3-02)

A small flesh mushroom, *Cap,* 2.2 cm in diameter, convex, smooth, , slight wavy margin with lined, *Gill,* free, fox, board, bronw; *Stem,* 1 x 0.25 cm, pale pinkish brown, smooth; S*pore print*, brown (Fig. 4.5).

Habit: solitary on the wood stem.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃;30 Oct. 2013; collected by Yaling Luo.

5. ***Mycena rosella*** (CH3-03)

A small flesh mushroom. *Cap*, 1.5-4 cm in diameter, convex , smooth; *Gill*, *adnate*, distant, brown, smooth, transparent; *Stem,* 0.7 x 0.15 cm, brown, smooth (Fig. 4.6).

Habit: solitary on the wood.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃;30 Oct. 2013; collected by Yaling Luo.

6. ***Marasmius androsaceus*** (CH3-04)

Cap, 0.7 cm, bell-shaped to hemisphere, brown to buff, margin with clear streak, *Gill*, free, unequal, brown; *Stem,* 2.8 x 0.1 cm, upper white with half bottom brown, smooth (Fig. 4.7).

Habit: solitary on the fallen leaves.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃;30 Oct. 2013; collected by Yaling Luo.

7. ***Trametesversicolor*** **spp.** (CH3-05)

Surface, 0.5-2.1 cm across, shell-shaped, brown to buff, faintly wrinkled, with well-defined brand of beige, grey; *Back surface,* with small white polies; *Stem,* short or none (Fig. 4.8).

Habit: solitary or gregatious in groups on the dead wood.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃;30 Oct. 2013; collected by Yaling Luo.

8. ***Lactarius sanguifluus*** (CH3-06)

Cap, 4.4-6.9 cm in diameter, flattened-convex with an irrolled margin, reddish to orange; *Gill,* decurrent, crowed, narrow, white, soon becoming purpose; *Stem* , 4.5-2.7x 1.1-1.7 cm, white then becoming blue when bruise, smooth; *Spore print,* yellowish brown (Fig.4.9).

Habit: scattered on humus.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃;30 Oct. 2013; collected by Yaling Luo.

9. ***Mycena subcaerulea*** (CH3-07)

Cap, 3-3.2 cm, convex, densely covered dark small scales; *Gill,* free, white; *Stem,* 5.5-6.0 x 0.3-0.5 cm, bluefish grey, smooth hollow with rooting base; *Spore print,* brown (Fig. 4.10).

Habit: scattered on humus.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

10. ***Clitocybula atrialba*** (CH3-08)

Cap, 1.3 cm in diameter, flat with depress in the center, smooth; *Gill,* decurrent, unequal, white, unequal; *Stem,* 2.0 x 0.1 cm, dirty white, smooth (Fig. 4.11).

Habit: scattered on humus.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

11. ***Tremiscus*** **spp.** (CH3-09)

Cap, 1.3-2.0 cm in diameter, fan-shaped, buff with stipes; *Tubes,* brown, small; ***Stem,*** extremely short (Fig. 4.12).

Habit: scattered on dead wood.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH5.8; Temperature 26℃; 20 Oct. 2013; collected by Yaling Luo.

12. ***Clavulinopsis helvola*** (CH3-10)

Fruitbody consists of slender clubs, 1-1.7cm height and 0.2-0.25 cm wide; cylindrical and unbranched, orange-yellow, in not being tufted (Fig. 4.13).

Habit: growed in cluster on humus.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

13. ***Mycena inclinata*** (CH3-11)

Cap, 0.5-1.0 cm in diameter, flatted to convex, brown, smooth; *Gill,* white, broad, unequal; *Stem*, extremely short or none; *Spore print,* white (Fig. 4.14).

Habit: scattered on dead wood .

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct.r 2013; collected by Yaling Luo.

14. ***Marasmiellus albuscorticis*** (CH3-12)

Cap, flat, smooth, white; *Gill,* adnate, white; *Stem,* white, smooth, thin (Fig. 4.15).

Habit: scattered on fallen leaves .

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

15. ***Laccaria* spp.** (CH3-13)

Cap, 1.5cm in diameter, flat with a raise center, smooth with a teeth-like margin ; *Gill,* free, unequal, brown; *Stem,* 1.1 x 0.3 cm, dirty white, smooth (Fig. 4.16).

Habit: scattered on humus.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

16. ***Termitomyces microcarpus*** (CH3-14)

Cap, 2.8 cm in diameter, white, convex with a buff raise center, smooth with a wavy margin ; *Gill,* free, unequal, white; *Stem,* 5.0 x 0.7 cm, yellowish white, smooth; *Spore print,* brown (Fig. 4.17).

Habit: solitary on humus .

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

17. ***Clavulinopsis fusiformis*** (CH3-15)

The fruitbody consists of tufts of bright yellow, slender clubs which are fused at their base; individual clubs are 0.1-0.5 cm wide, with a pointed tip (Fig. 4.18).

Habit: solitary or gregarious on humus.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

18. ***Resinomycena rhododendri*** (CH3-16)

Cap, 2.2 cm in diameter, pure white, flat ; *Gill,* free, unequal, white; *Stem,* 1.2 x 0.1 cm, white, smooth (Fig. 4.19).

Habit: solitary on the dead wood .

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

19. ***Marasmius foetidus*** (CH3-17)

Cap, 1-1.3 cm in diameter, brown, flat, sometimes incurrent, smooth ; *Gill*, brown; *Stem*, 1-1.5 x<0.1 cm, upper pale brown, half bottle dark brown, smooth (Fig. 4.20).

Habit: scattered on the dead wood .

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

20. ***Marasmius plicatulus*** (CH3-18)

Cap, 1-1.3 cm in diameter, buff, flat, smooth; *Gill*, white, unequal, free; *Stem*, 1.5 x<0.1 cm, reddish brown, thin, smooth (Fig. 4.21).

Habit: scattered on the dead wood .

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

21. ***Xylaria hypoxylon*** (CH3-19)

Fruitbody 4-5 cm high, cylindrical, upper fertile part 3-4cm high, 0.15-0.2 wide, dark grey ,cylindrical with a roughened surface which has dark spots; *Stem,* 1-2cm high, thin, dark (Fig. 4.22).

Habit: scattered in rotting soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

22. ***Lactarius controversus*** (CH3-20)

Cap, 2-3.5 cm, white, flat or convex with slightly depress in the center, sticky, smooth; *Gill,* white, unequal, free, close; *Stem,* 1.5-3.5 x 0.5-1 cm, cylindrical, smooth (Fig. 4.23).

Habit: growed in clusters on rotting soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

23. ***Marasmius scorodonius*** (CH3-21)

Cap, 1-1.5 cm in diameter, brown, convex with slightly lined, smooth; *Gill*, pinkish brown, unequal, free; *Stem*, 2-2.4 x 0. 1 cm, pale brown, smooth; *Spore print*, yellow (Fig. 4.24).

Habit: scattered in soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 October 2013; collected by Yaling Luo.

24. ***Marasmius oreades*** (CH3-22)

Cap, 2.5 cm in diameter, brownish red, convex smooth ; *Gill*, brown, unequal, adnate; *Stem*, 0.9 cm, yellowish white, cylindrical, smooth; *Spore print*, brown (Fig. 4.25).

Habitat: solitary in soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct.r 2013; collected by Yaling Luo.

25. ***Marasmius*** **spp.** (CH3-23)

Cap, 2.5 cm, yellowish brown, convex to flat, smooth, wavy margin with clear lined; *Gill*, free, unequal, buff; *Stem*, 1 cm, cylindrical, smooth; *Spore print*, brown (Fig. 4.26).

Habit: solitary in soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

26. ***Lactarius*** **spp.** (CH3-24)

Cap, 4.2 cm in diameter, cream white with pale yellowish, flat with incurrent wavy, very smooth; *Gill*, free, equal, pinfish white, close; *Stem*, 0.7 x 0.8 cm, white but becoming buff when dry, cylindrical, hollow, smooth; *Spore print*, brown (Fig. 4.27).

Habit: solitary in soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

27. ***Agaricus*** **spp.** (CH3-25)

Cap, 0.7-4.2 cm in diameter, cream white, convex to hemisphere , smooth; *Gill*, free, brown; *Stem* 3.5-1x 0.5-1.0 cm, cream white, smooth; *Spore print*, yellowish (Fig. 4.28).

Habit: scattered in soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 October 2013; collected by Yaling Luo.

28. ***Collybia dryopjila*** (CH3-26)

Cap, 1.5-3.0 cm in diameter, flat with a transparent wavy margin, buff with a brown raise in the center; *Gill,* adnate, white, close, unequal; *Stem,* 2-3 x 0.1-0.3 cm, buff, cylindrical, smooth; *Spore print,* pale yellow (Fig. 4.29).

Habit: scattered or in clusters in soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

29. ***Lactarius*** **spp.** (CH3-27)

Cap, 10 cm in diameter, flat with a white strongly depress in the center, reddish brown with lined, dark scales including the wavy margin; *Gill*, decurrent, pink, close, equal; *Stem*, 7 x 0.7 cm,dark brown, cylindrical, downy the part attach gills is red (Fig. 4.30).

Habit: solitary in soil.

Location: Thailand, Chanthaburi Province, Amphoe Khao Khichakut (Krating Waterfall), N 12°48′16″, E 102°6′53″; pH 5.8; Temperature 26℃; 30 Oct. 2013; collected by Yaling Luo.

30. ***Auricularia auricula*** (PH01)

A distinctive species recognized by the ear-shaped fruitbodies growing o n dead wood. *Fruitbody*, 1.9-2.6 cm across, irregularly ear-shaped, reddish black, gelatinous without a stalk; outer surface smooth with reddish black, inner surface reddish brown with short hair, velvet-like, *Flesh*, thin (Fig. 4.31).

Habit: on dead branches.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

31. ***Tricholoma*** **spp.** (PH02)

A white-black mushroom, recognized by the flowerlike shaped cap. *Cap*, 5.6 cm, white- black color, crack into flowerlike shaped, incurrent margin; *Gill*, free, pale-brown, *Stem*, 5.5 x1 cm, smooth, brown, thick with a white membranous ring; *Flesh*, white, thick (Fig. 4.32).

Habit: scattered in the wet solid.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

32. ***Termitomyces* spp.** (PH03)

A flesh mushroom. *Cap*, 13 cm, pale yellow-brown, flat, with a slightly depressed disc and minutely hairy toward the margin, Flesh white, *Gill*, adnexed, white, *Stem*, 14 x1.5 cm, oval-shaped basal bulb with whitish, smooth except small scales in the lower part, *Spore print*, buff (Fig. 4.33).

Habit: solitary in the fallen forestry.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

33. ***Pleurocybella porrigens*** (PH04)

It often forms large numbers of white clusters, with fan-shaped fruit bodies. *Cap*, 1.5-4.6 cm in diameter, fan-shaped, often erect, pure white, smooth, with an incurved margin; *Gill*, decurrently, unequal , creamy white, narrow, very crowed; *Stem*, none or very reduced; flesh thin, white, brittle (Fig. 4.34).

Habit: on rotting wood, prefers wet and colder regions.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

34. ***Pluerotus gigantenus*** (PH05)

A vey large stout, white mushroom. *Cap*, *11-25 cm* in diameter, convex soon becoming flatted with a inrolled margin, white, moist, smooth,, *Gill*, adnate, white with a slightly pink, *Flesh*, thick, white, close; *Stem*, 6-14x1.5x3.5 cm, white then becoming pale yellow (Fig. 4.35).

Habit: growed in clusters in grassland.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

35. ***Leucocoprinus fragilissimus*** (PH06)

A small, white or nearly transoarent,easy to crack mushroom. *Cap*, 2.4 cm in diameter, flat with a distinct yellow umbo, sometimes broadly bel-shaped, white, nearly transparent, margin clearly lined, thick, small yellow scales; *Gill*, free, white, unequal length; *Stem*, 3.5 x 0.1 cm, very slim, white, ring small, easily detachable in the lower part of the stem (Fig. 4.36).

Habit: growed in grassland or tea garden.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

36. ***Collybia strictipes*** (PH07)

A white, brittle mushroom. *Cap*, 4.5 cm in diameter, bell-shaped with margin remaining inrolled and clearly lined ,smooth; *Gill*, free, pink, broad, unequal length; Stem, 4.5 x 0.5 cm, white, flesh, smooth, peanut smell (Fig. 4.37).

Habit: scattered in grassland.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

37. ***Marasmius*** **spp.** (PH08)

A delicate ink-cap,frequently found lawns and recognized by the grooved cap and well-spaced gills. *Cap*, 2.5 cm in diameter, comvex soon becoming flattened, with a sunken brown centre, otherwise grey and radically pleated; *Gill*, free, narrow, black, well spaced; *Stem,* 9 x 0.5 cm, cylindrical, slender, fragile, hollow, white (Fig.4.38).

Habit: usually solitary on lawns and grass verges.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

38. ***Coprinus*** **spp.** (PH09)

Cap, strong convex, pure white, smooth, wet, slightly line with margin; *Gill*, broad, redish brown, well spaced; *Stem,* 5-1 x1-0.5 cm, cylindrical, pure white with a sac-like volva (Fig.4.39).

Habit: scattered in lawns.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.1; Temperature 28℃; 17 Aug. 2013; collected by Yaling Luo.

39. ***Marasmius*** **spp.** (PH10)

Cap, 4.4 cm in diameter, white to pale pink, nearly flat with a raised centre, smooth, slightly line with margin; *Gill*, free, *broad,* thick, pink, adnate , well spaced, unequal; *Stem*, 4 x1 cm, smooth, pure white with a small base bal (Fig. 4.40)

Habit: solitary in lawns.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.0; Temperature 28℃; 20 Aug. 2013; collected by Yaling Luo.

40. ***Collybia iocephala*** (PH11)

Cap, 5 cm in diameter, white to a slightly pink in margin, nearly flat with a raised centre, smooth, slightly line with margin; *Gill*, free, broad, thick, pink, adnate, well spaced, unequal; *Stem,* 4 x0.5 cm, smooth, pure white with a small base ball (Fig. 4.41).

Habit: solitary in lawns.

Location: Thailand, Phetchabuti Province, Amphoe Kaeng Krachan (Krating Waterfall) N 12°54′27″, E 99°38′53″; pH: 9.0; Temperature 28℃; 20 Aug. 2013; collected by Yaling Luo.

41. ***Collybia* spp.** (LB01)

Cap, 3-6 cm in diameter, convex with a wavy margin, covered with shaggy, erect scales in the centre *Gill*, free, brown, broad, decurrened; *Stem*, 1x0.5 cm, cylindrical, brown, silky membranous, hollow; *Flesh*, thick, soft and cream white (Fig. 4.42).

Habit: in clusters on fall woody.

Location: Thailand, Bangkok Province, Khet Lat Krabang(KMITL), N 13°43′24″, E 100°47′3″; pH 7.6; Temperature 28℃; 20 Sep. 2013; collected by Yaling Luo.

42. ***Mycena* spp.** (SY01)

Cap, 1.0-2.5 cm across, convex, slightly gray-brown or bright yellow ,becoming lighter toward the whitish margin, smooth; *Gill*, decurrent, almost distant, yellowish white; *Stem*, 1.5-3 x 0.1-0.2 cm, hollow, white (Fig. 4.43).

Habit: growed in clusters on stems of the wood.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall), N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 20 Sep. 2013; collected by Yaling Luo.

43. ***Marasmius* spp.** (SY02)

Cap, 0.2-0.35cm across, flat with depress center, remaining the incurved margin, dark brown, smooth, wet; *Gill*, decurrent, almost distant, buff, almost transparent; *Stem*, <0.5 cm, dark brown (Fig.4. 44).

Habit: scattered on dead wood.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 20 Sep. 2013; collected by Yaling Luo.

44. ***Mycena* spp.** (SY03)

Cap, 0.5-4.0 cm across, convex, bright yellow ,becoming lighter toward the whitish margin with minutely streak, smooth ; *Gill*, adnate, pure white, close; *Stem*, 0.5-6 x 0.3-0.1 cm, cylindrical, silky, white, cover some whitish down; *Flesh*, thin (Fig. 4.45).

Habit: growed in clusters on leaves.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 20 Sep. 2013; collected by Yaling Luo.

45. ***Marasmius purpureostriatus*** (SY04)

Cap, 2-5 cm in diameter, convex with a raise center, margin a little wavy, pinkish white, almost transparent, radially striated to ridges; *Gill*, decurrent to folded to stem, pinkish white, distant, broad; *Stem*, 1.5-4 x 0.2-0.6 cm, white, minutely (Fig. 4.46).

Habit: growed in clusters on wood.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 20 Sep. 2013; collected by Yaling Luo.

46. ***Mycena* spp.** (SY05)

A small mushroom with thin stem. *Cap*, 0.3-1.5 cm across, convex with an incurved margin and radically lined, grey with a dark grey and lined margin, ,becoming lighter toward the whitish margin; *Gill*, adnate, pinkish white, well-space; *Stem*, 2-3.5 cm height, cylindrical, white, smooth (Fig. 4.47).

Habit: growed in clusters on stem of wood.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 20 Sep. 2013; collected by Yaling Luo.

47. ***Marasmiellus albuscorticis*** (SY06)

Small, white cap with pure white gills, white stalk mushroom. *Cap*, 0.7-1.5 cm across, convex to flat, margin a little wavy, pure white, almost transparent; *Gill*, attached, distant, broad, white; *Stem*,1-1.5 cm in height, white, minutely (Fig. 4.48).

Habit: growed in clusters on wood.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 23 Sep. 2013; collected by Yaling Luo.

48. ***Auricularia auricula.*** (SY07)

Fruit body 2.5-9 cm in across, ear-shaped, out surface brown to dark, inner surface gray-brown to dark, minute hair, smooth sometimes with wrinkled; no stem or extremely short. (Fig. 4.49).

Habit: severally or numerously on rotting wood.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall), N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 23 Sep. 2013; collected by Yaling Luo.

49. ***Schizophyllum commune*** (SY08)

A common species, forming tiers of small, grey, hairy brackets on stumps. *Cap*, 0.2-1.5 cm in diameter, shell-shaped and laterally attached, pale grey or pure white in very dry conditions; *Gill,* radiating from a lateral attachment point, appearing to spit lengthwise along their edges and the side curling upwards, narrow, grey; *Stem*, none (Fig. 4.50).

Habit: growed in clusters on dead branches.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 23 Sept. 2013; collected by Yaling Luo.

50. ***Marasmillus ramealis*** (SY09)

Small tufts with slimy caps. *Cap*, 0.5-1.0 cm across, strongly convex, very slimy and nearly transparent; *Gill*, free, distant, broad, white; *Stem* 0.5-1x0.1 cm in height, hollow, white (Fig.4.51).

Habit: grow in clusters on stems of the wood.

Location: Thailand, Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″, E 99°8′40″; pH 8.6; Temperature 25℃; 23 Sep. 2013; collected by Yaling Luo.

51. ***Russula foetens*** (AJ01)

A bright red *Russula* .*Cap,* 3-7cm in diameter, bright red ,smooth, subglobose then convex to depress in the centre; *Gill,* free, cream white, close, equal, *Stem,* nearly cylindrical, white sometimes with a flush pink, gray to dark when crush, flesh, 2-6 x 0.5-2 .6cm; *Flesh*, white, thin (Fig. 4.52).

Habit: solitary or gregarious in sand soil, edible.

Location: Thailand, Chiangrai Province, Chiang Kong, N 20°15′36″, E 100°47′3″; pH 6.6; Temperature 28℃; 13 Aug. 2013; collected by Kesem Soytong.

52. ***Boletus retisporus*** (AJ02)

Fruit body, medium or large. *Cap,* with fine hair, purple, purplish red to red soil, the cap under the surface of the meat with red roses bacteria, wounded at. varying blue, flat hemispherical, later slightly flat, sticky when wet. *Flesh*, pale yellow. Tubes easily separated each other, childhood nozzle bright yellow to orange, the latter yellowish green polygon. *Stipe*, down gradually thick, internal solid (Fig. 4.53).

Habit: solitary or gregarious in sand soil.

Location: Thailand, Chiangrai Province, Chiang Kong, N 20°15′36″, E 100°47′3″; pH 6.6; Temperature 28℃; 13 Aug. 2013; collected by Kesem Soytong.

53. ***Cantharellus cibarius*** (AJ03)

Cap, egg yellow to orange, thick, fleshy, eventually becoming depressed. The margin inrolled, friable; *Gill*, fold-like, egg yellow color, which cover the hymenium are quite widely space, non-fluorescent, forked near the margin, and clearly decurrent the stem; uunequal length. *Stem*, nearly cylindrical, 2-3 x 0.9-2 cm, thick, not long, same color with cap and gills ; *Flesh*, white, thin (Fig. 4.54).

Habit: solitary or gregarious in sand soil, edible.

Location: Thailand, Chiangrai Province, Chiang Kong, N 20°15′36″, E 100°47′3″; pH 6.6; Temperature 28℃; 13 Aug. 2013; collected by Kesem Soytong.

54. ***Russula crassotunicata*** (AJ04)

Cap, 3-8 cm in across, deeply convex then expanded with depressed disc; white to yellowish white, staining yellowish brown; viscid when moist, usually dry and fealty; cuticle thick and rubbery, almost totally separable. *Gill*, adnate, quite distant, and narrow; pale yellow, staining yellow-brown when injured. Stem 35-50 x 9-20mm, equal, solid then spongy; white, staining yellow-brown; dry, dull, almost velvety. *Flesh*, firm; white, staining when cut (Fig. 4.55).

Habit: solitary or gregarious in sand soil, edible.

Location: Thailand, Chiangrai Province, Chiang Kong, N 20°15′36″, E 100°47′3″; pH 6.6; Temperature 28℃; 15 Aug. 2013; collected by Kesem Soytong.

55. ***Laccaria vinaceoavellanea*** (AJ2-1)

Cap, 2.5cm across, whitish yellow, flat with a depress in the center, grooves from centre towards margin ; *Gill*, adnate, white to yellow, well-space; *Stem*, 4.5cm, cylindrical, smooth, slender, reddishbrown when white on the upper (Fig. 4.56).

Habit: growssingly.

Location: Thailand, Kanchanaburi Province, Amphoe Mueang Kanchanaburi, N 14°0′12″, E 99°33′0″; pH 7.8; Temperature 26℃; 3 Sep. 2013; collected by Kesem Soytong.

56. ***Clitocybe*** **spp.** (AJ2-2)

Cap, 0.5-7 cm across, purplish to pink to pale brown, horn with strongly depress in the center and inrolled margin becoming wavy ; *Gill*, decurrent, white to olive-yellow; *Stem*, 3.5-9 cm, cylindrical, smooth, pink to dark brown (Fig. 4.57).

Habit: grows in clusters.

Location: Thailand, Kanchanaburi Province, Amphoe Mueang Kanchanaburi, N 14°0′12″, E 99°33′0″; pH 7.8; Temperature 26℃; 3 Sep. 2013; collected by Kesem Soytong.

57. ***Boletus affinis* var. *maculosus*** (AJ2-3)

Cap, 1-3.5 cm across, velvety redish-brown, dry shin, having a membraneous vein on the top part which promptly turns to tobacco color due to the falling spores.; *Gill*, adnate, white; *Stem*, 6-9 cm long, cylindrical, silky membranous, smooth (Fig. 4.58).

Habit: growed in clusters.

Location: Thailand, Kanchanaburi Province, Amphoe Mueang Kanchanaburi, N 14°0′12″, E 99°33′0″; pH 7.8; Temperature 26℃; 5 Sep. 2013; collected by Kesem Soytong.

58. ***Inocybe fastigiata*** (AJ2-4)

Cap, 3-6 cm across, conical at first, then the edges extend though a pointed umbo remains, the margin eventually cracks with ages, sometimes right to the umbo, the cuticle varies in color from pale yellow to ocher, the most striking feature of the cap is its striation with very noticeable fibrils; *Gill*, free, grayish-yellow with light green, brown when older; *Stem*, 6-9 cm long, cylindrical, silky membranous, smooth (Fig. 4.59).

Habit: growed in clusters.

Location: Thailand, Kanchanaburi Province, Amphoe Mueang Kanchanaburi, N 14°0′12″, E 99°33′0″; pH 7.8; Temperature 26℃; 5 Sep. 2013; collected by Kesem Soytong

59. ***Clitocybe* spp.** (AJ2-5)

Cap, 3-5 cm across, flat with the margin strongly inrolled, raising umbo in the cnter, pinkish brown when dry becoming yellowish gary, with clearly line towards the wavy margin; *Gill,* free, at first pink then buff, close; *Stem*, 7-8 cm, cylindrical, silky membranous, smooth, white then becoming yellowish white, sometimes dark at the bottom of stem (Fig. 4.60).

Habit: growed in clusters.

Location: Thailand, Kanchanaburi Province, Amphoe Mueang Kanchanaburi, N 14°0′12″, E 99°33′0″; pH 7.8; Temperature 26℃; 5 Sep. 2013; collected by Kesem Soytong.

60. ***Mycena vulgaris*** (AJ2-6)

A small, brittle mushroom. *Cap*, 0.5-1.0 cm across, convex to flat with slightly depress in the centre, yellowish brown slimy with a wavy margin; *Gill*, free, narrow, close, yellowish brown; *Stem*, 1-1.5 cm long, yellow to brown, smooth (Fig. 4.61).

Habit: growed on rotting leaves.

Location: Thailand, Kanchanaburi Province, Amphoe Mueang Kanchanaburi, N 14°0′12″, E 99°33′0″; pH 7.8; Temperature 26℃; 5 Sep. 2013; collected by Kesem Soytong.

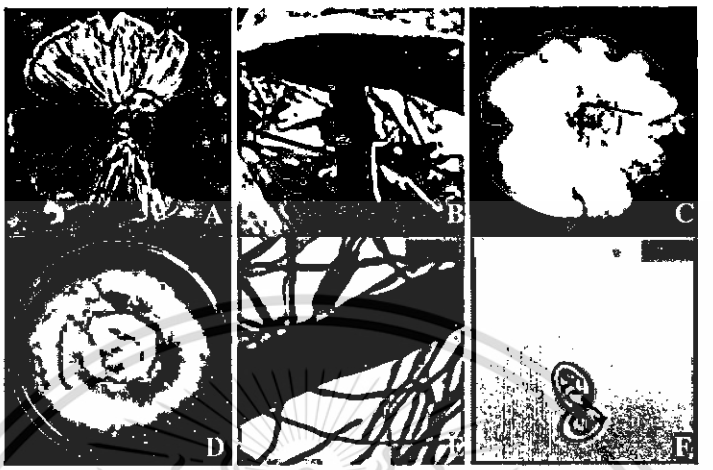

Fig. 4.2 *Agaricus macrosporus* (CH01) A , B, C. fruiting body, D. 15-day-old culture on PDA , E. mycelia, F. basidiospore, Bar. E, F= 10 μm.

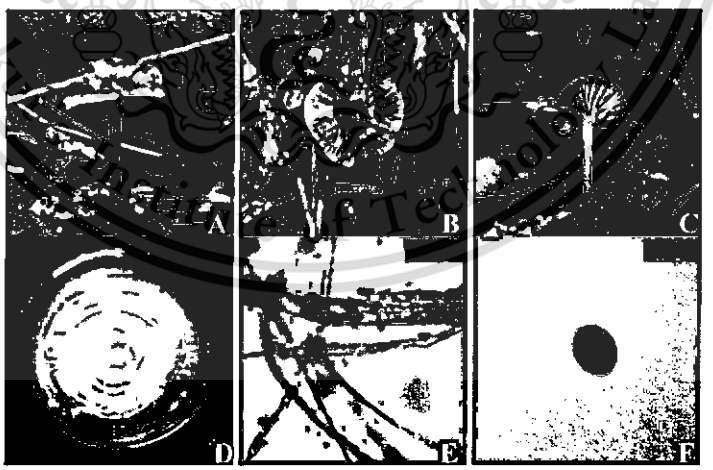

Fig.4.3 *Tricholoma* spp. (CH02) A , B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F= 10 μm.

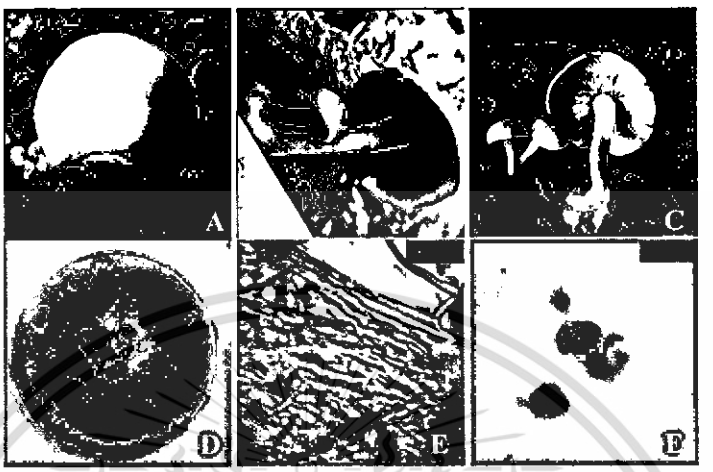

Fig.4.4 *Lactarius* spp. (CH3-01) A, B, C,D. fruiting body, E. 15-day-old culture on PDA, F. mycelia, G. basidiospore, Bar. F, G= 10 μm.

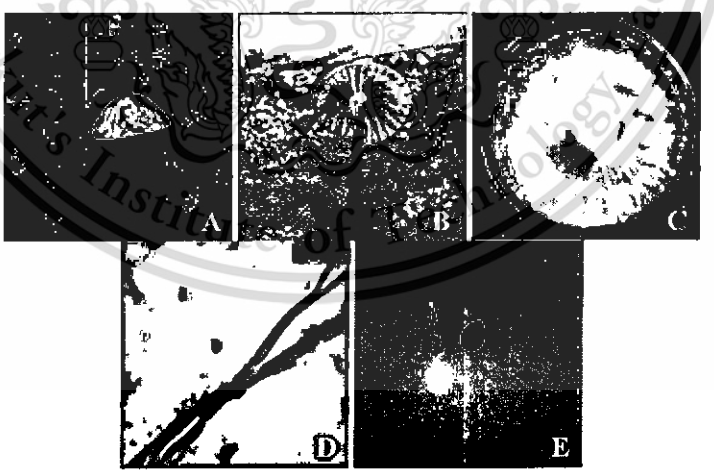

Fig.4.5 *Marasmius* spp. (CH3-02) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. basidiospore, Bar. D, E= 10μm.

Fig.4.6 *Mycena rosella* (CH3-03) A, B. fruiting body, C. mycelia, D. basidiospore, Bar. C, D= 10 μm.

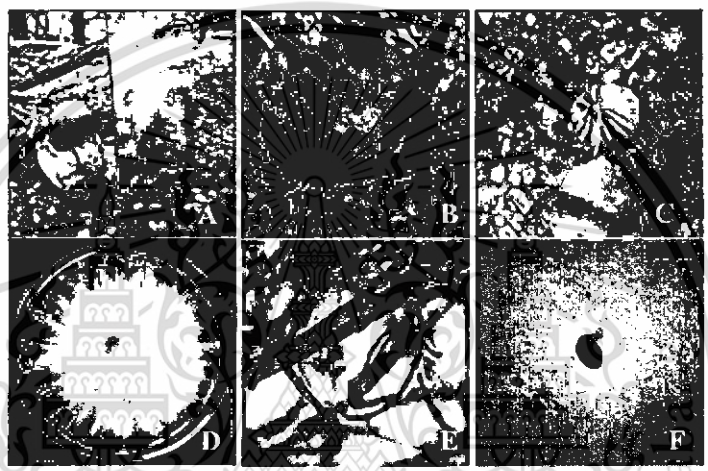

Fig.4.7 *Marasmius androsaceus* (CH3-04) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F= 10 μm.

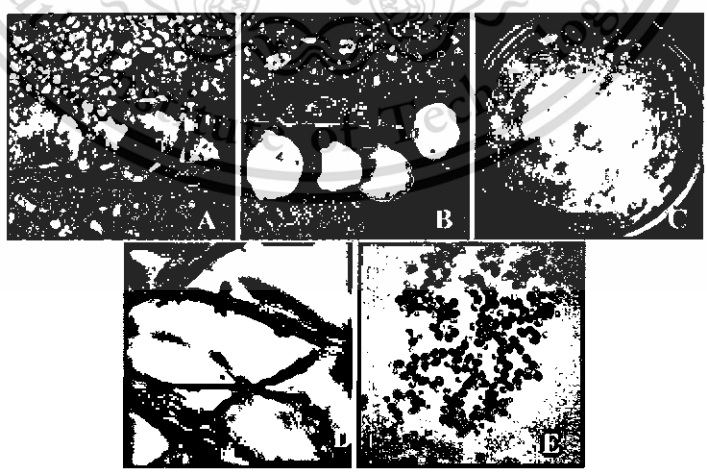

Fig.4.8 *Trametesversicolor* spp. (CH3-05) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E= 10 μm.

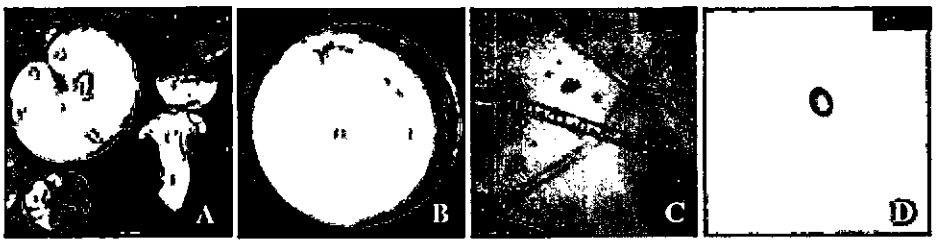

Fig.4.9 *Lactarius sanguifluus* (CH3-06) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, D. basidiospore, Bar. C, D= 10 μm.

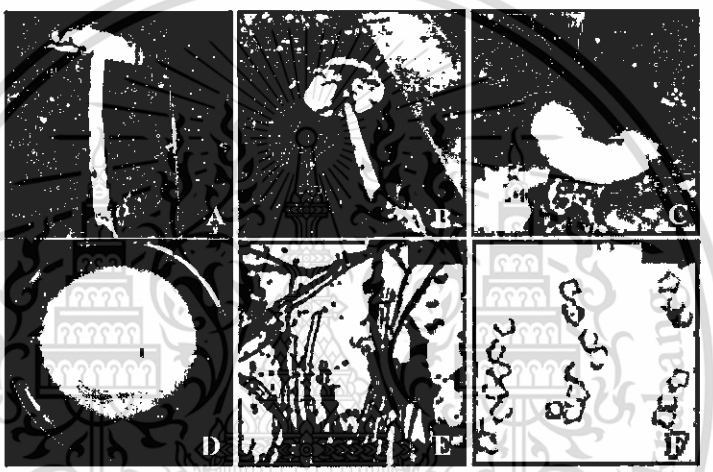

Fig.4.10 *Mycena subcaerulea* (CH3-07) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

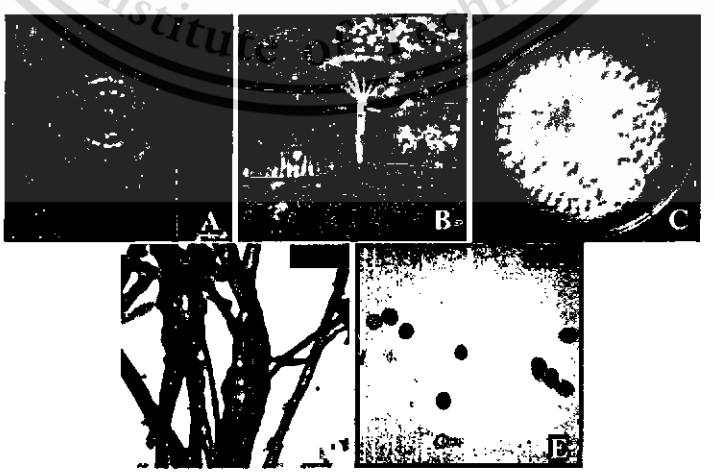

Fig.4.11 *Clitocybula atrialba* (CH3-08) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E = 10 μm.

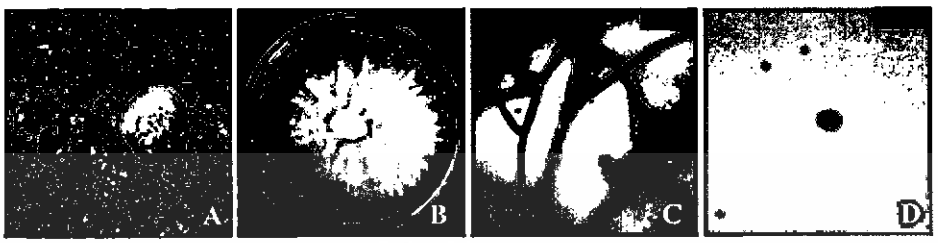

Fig.4.12 *Tremiscus* spp. (CH3-09) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, D. basidiospore, Bar. C, D = 10 μm.

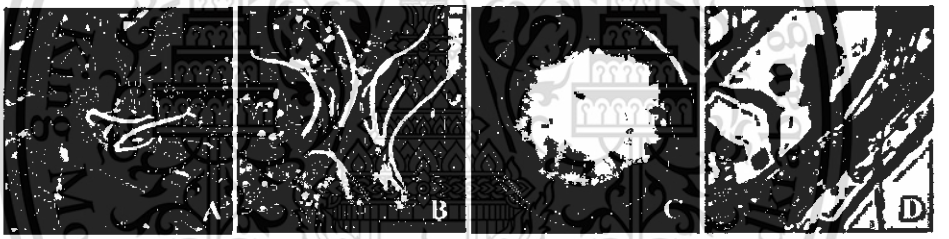

Fig.4.13 *Clavulinopsis helvola* (CH3-10) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, Bar. D =10μm.

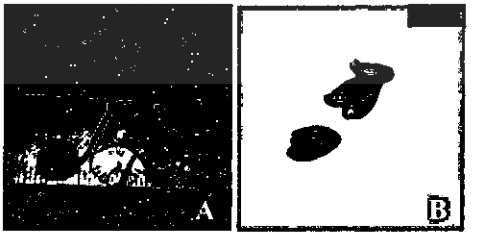

Fig.4.14 *Mycena inclinata* (CH3-11) A. fruiting body, B. basidiospore, Bar. B = 10 μm.

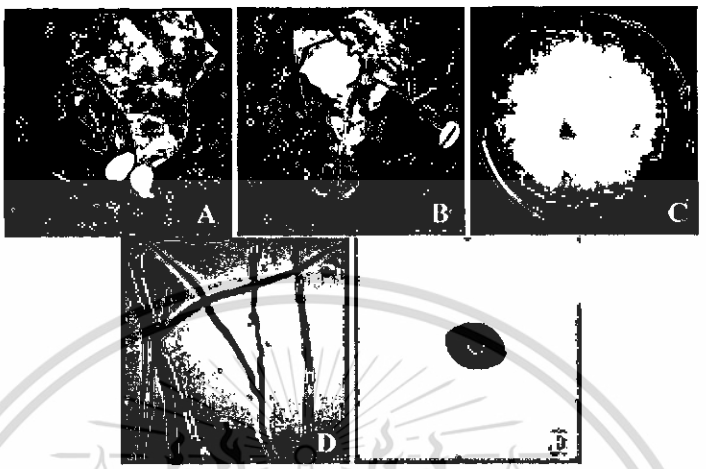

Fig.4.15 *Marasmiellus albuscorticis* (CH3-12) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E = 10 μm.

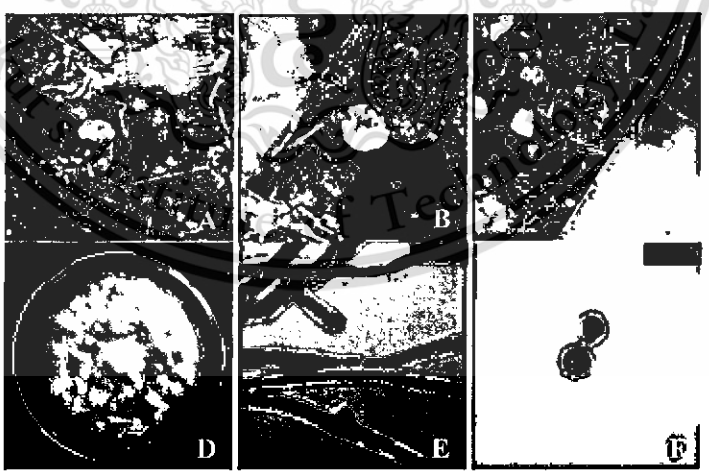

Fig.4.16 *Laccaria* spp. (CH3-13) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

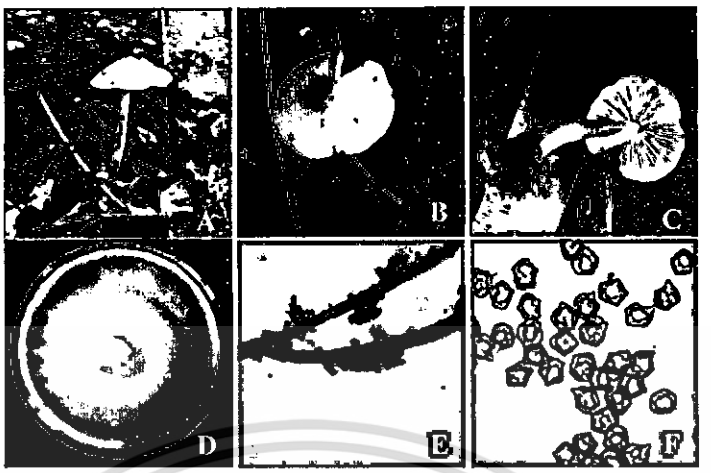

Fig.4.17 *Termitomyces microcarpus* (CH3-14) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

Fig.4.18 *Clavulinopsis fusiformis* (CH3-15) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, Bar. C =10μm.

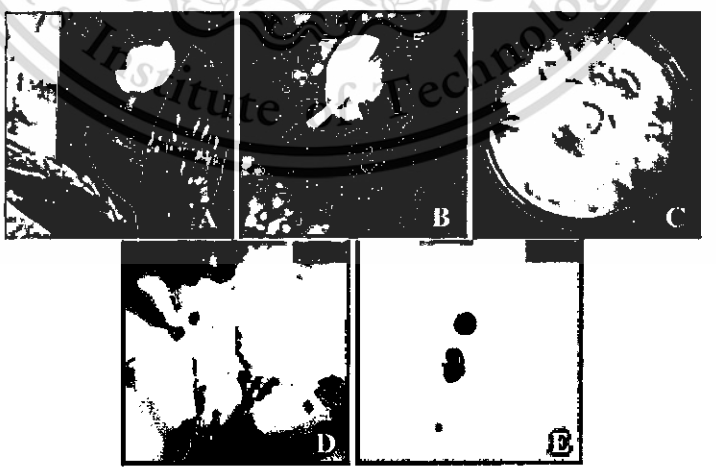

Fig.4.19 *Resinomycena rhododendri* (CH3-16) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E = 10 μm.

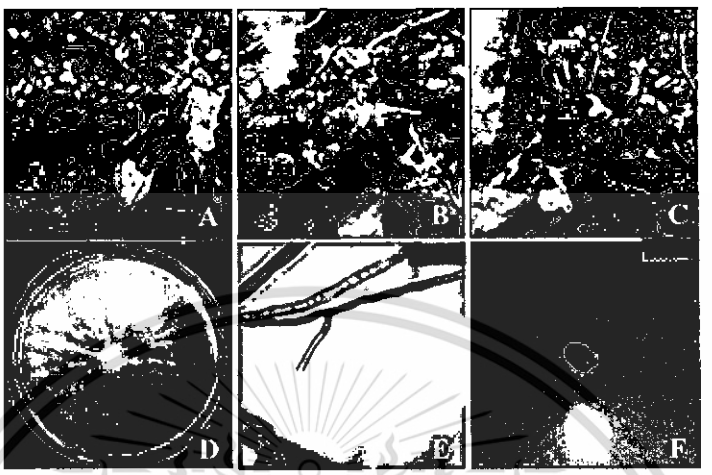

Fig.4.20 *Marasmius foetidus* (CH3-17) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

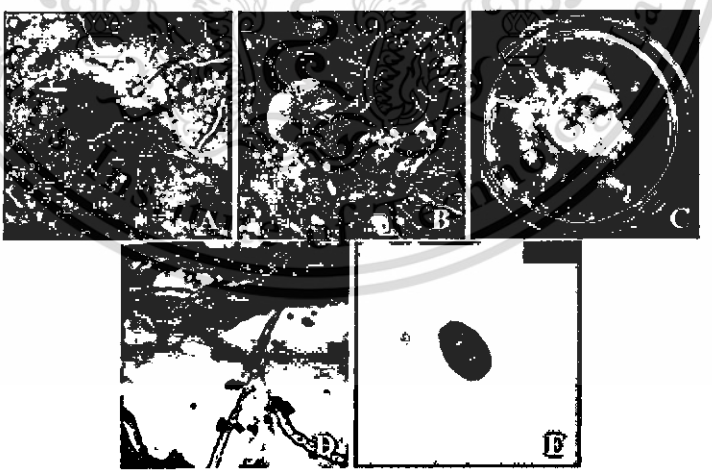

Fig.4.21 *Marasmius plicatulus* (CH3-18) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E = 10 μm.

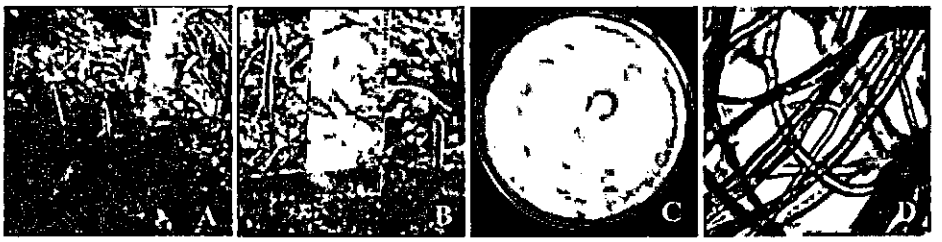

Fig.4.22 *Xylaria hypoxylon* (CH3-19) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia. Bar. D =10 μm.

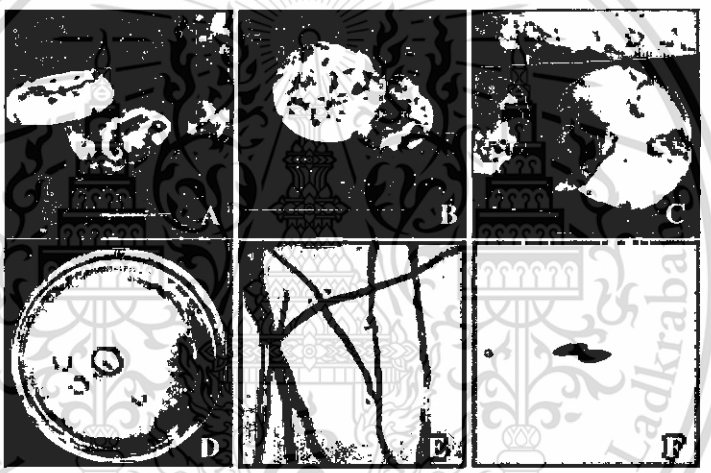

Fig.4.23 *Lactarius controversus* (CH3-20) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

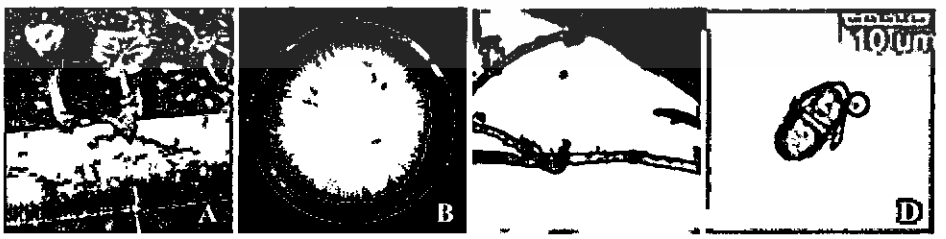

Fig.4.24 *Marasmius scorodonius* (CH3-21) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, D. basidiospore, Bar. C, D = 10 μm.

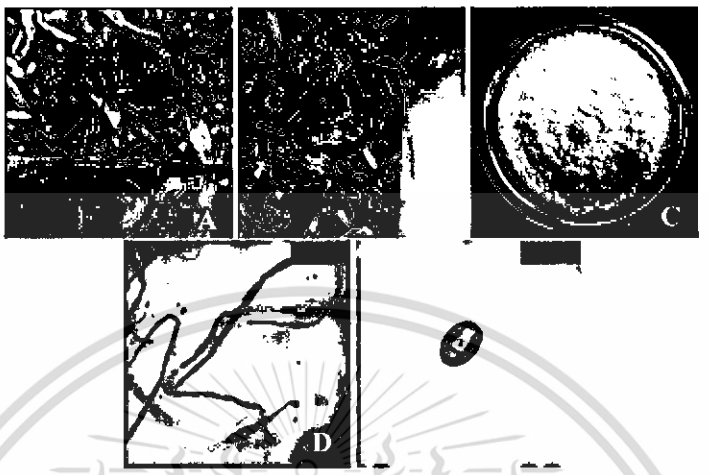

Fig.4.25 *Marasmius oreades* (CH3-22) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E = 10 μm.

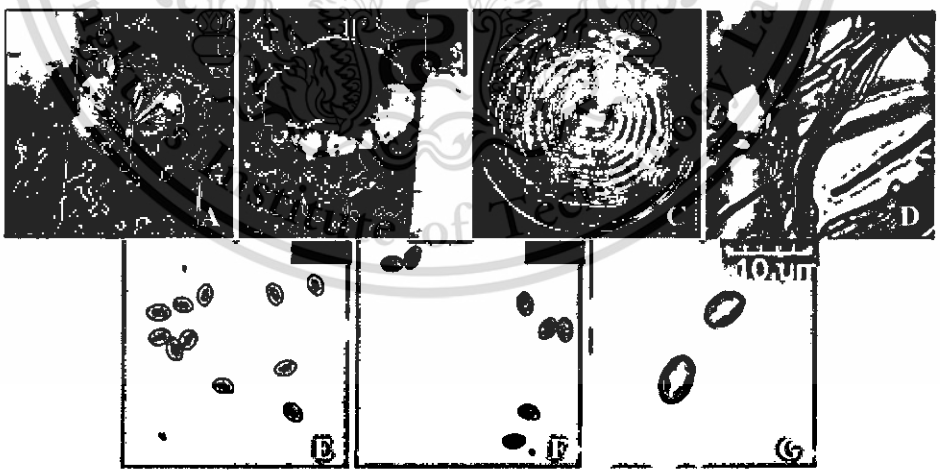

Fig.4.26 *Marasmius* spp. (CH3-23) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E, F,G. basidiospore, Bar. D = 100 μm, D, E, F, G = 10 μm.

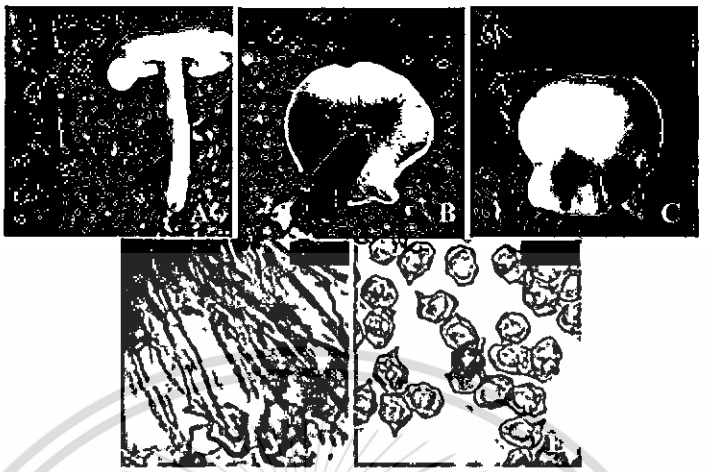

Fig.4.27 *Lactarius* spp. (CH3-24) A, B, C. fruiting body, D. mycelia, E. basidiospore, Bar. D, E = 10 μm.

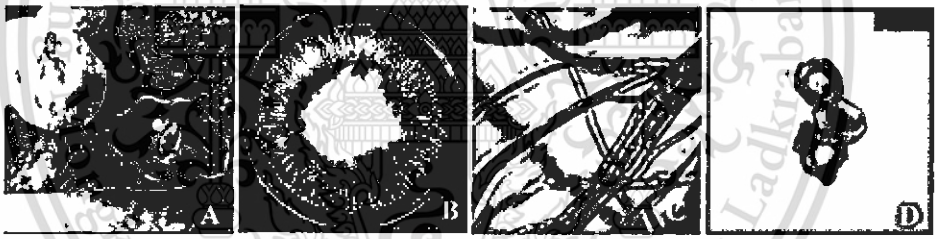

Fig.4.28 *Agaricus* spp. (CH3-25) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, D. basidiospore, Bar. C, D= 10 μm.

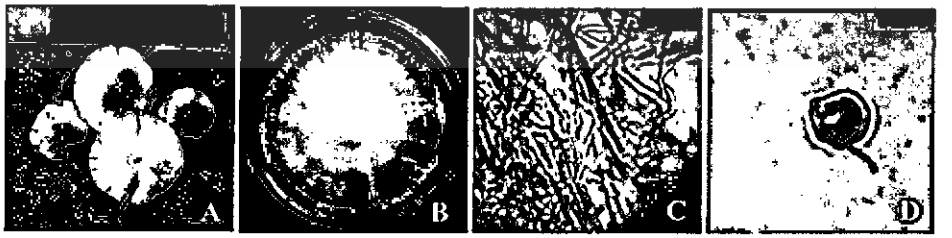

Fig.4.29 *Collybia dryophila* (CH3-26) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, D. basidiospore, Bar. C, D = 10 μm.

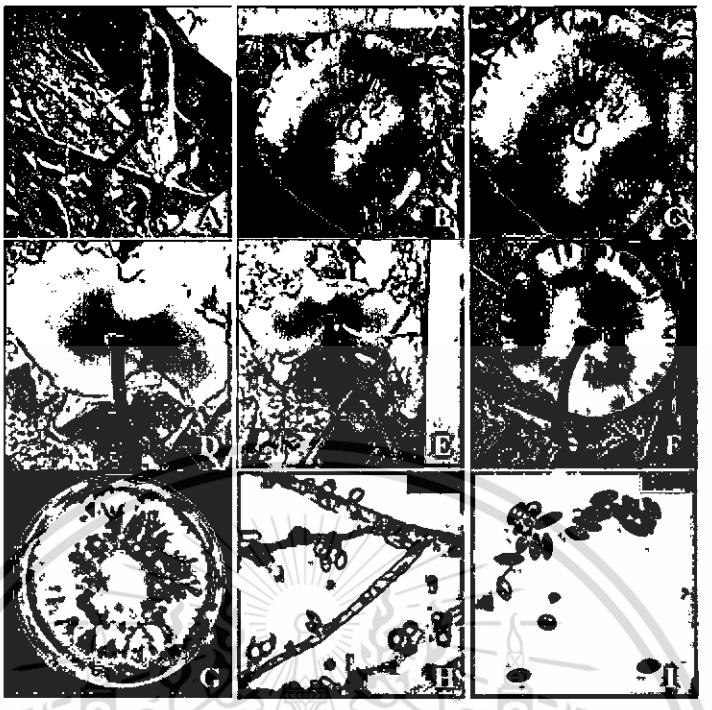

Fig.4.30 *Lactarius* spp. (CH3-27) A, B, C, D, E, F. fruiting body, G. 15-day-old culture on PDA, H. mycelia, I. basidiospore, Bar. H, I = 10 μm.

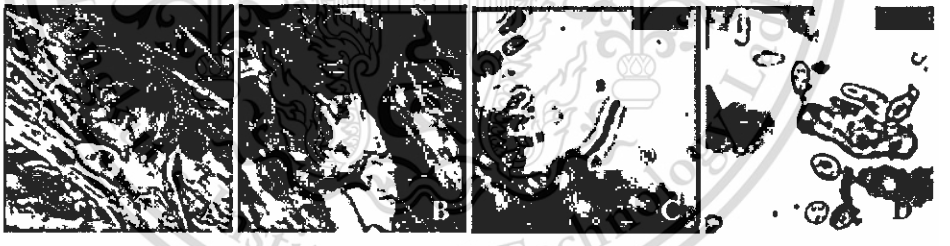

Fig.4.31 *Auricularia auricula* (PH01) A, B. fruiting body, C, D. basidiospore, Bar. C, D= 10 μm.

Fig.4.32 *Tricholoma* spp. (PH02) A, B, C, D, E. fruiting body.

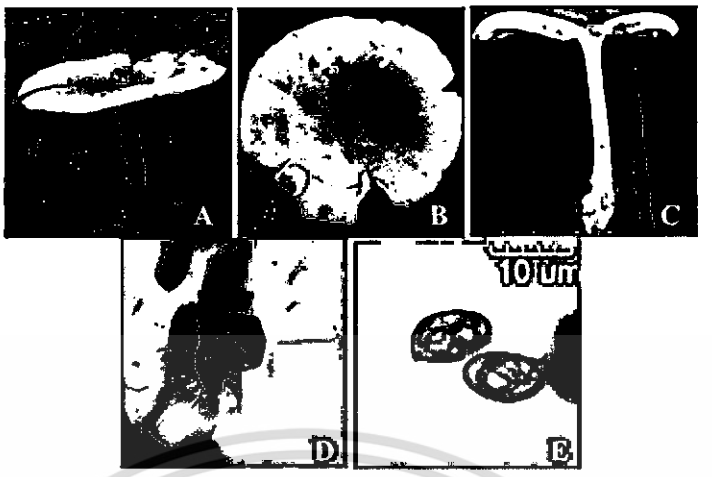

Fig.4.33 *Termitomyces* spp. (PH03) A, B, C. fruiting body, D. spore print, E. basidiospore, Bar. E = 10 μm.

Fig.4.34 *Pleurocybella porrigens* (PH04) A, B. fruiting body, C. basidiospore, Bar. C = 10 μm.

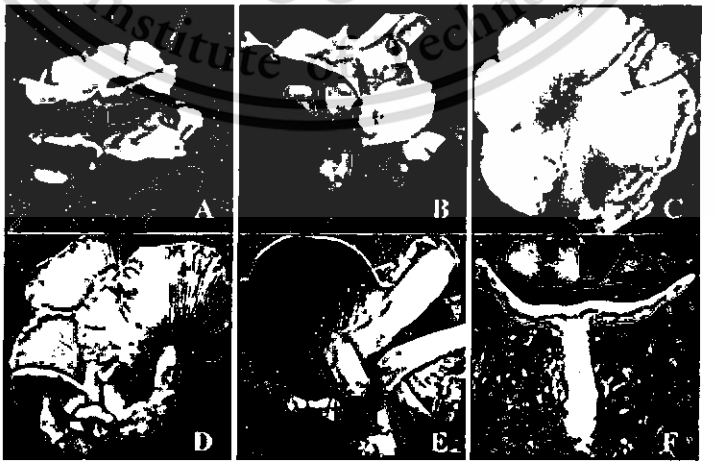

Fig.4.35 *Pluerotus gigantenus* (PH05) A, B, C, D, E, F. fruiting body.

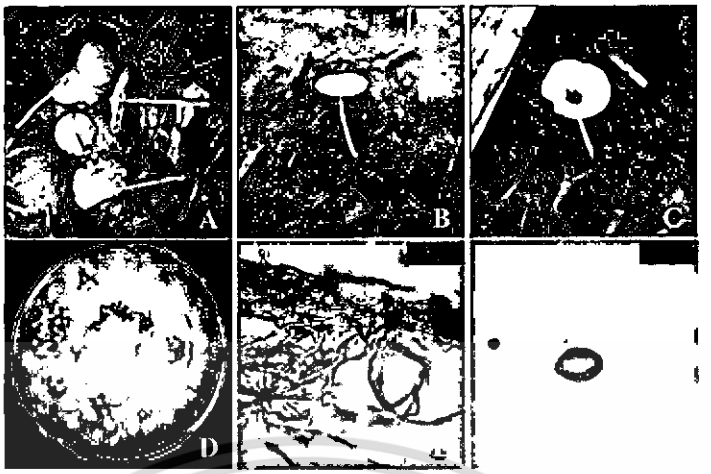

Fig.4.36 *Leucocoprinus fragilissimus* (PH06) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

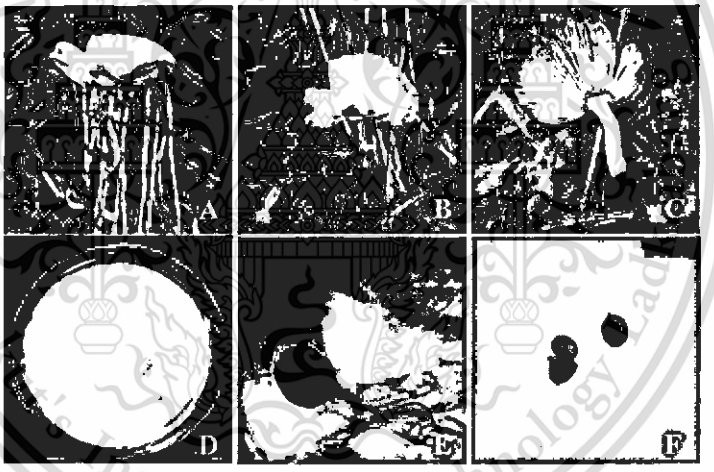

Fig.4.37 *Collybia strictipes* (PH07) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

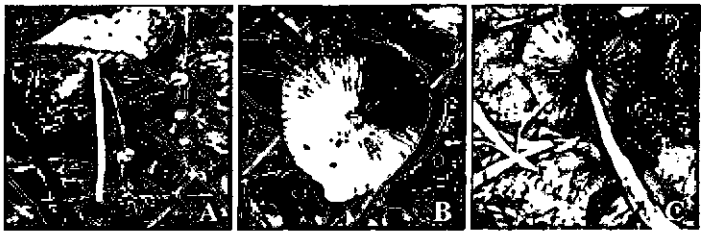

Fig.4.38 *Marasmius* spp. (PH08) A, B, C, D. fruiting body.

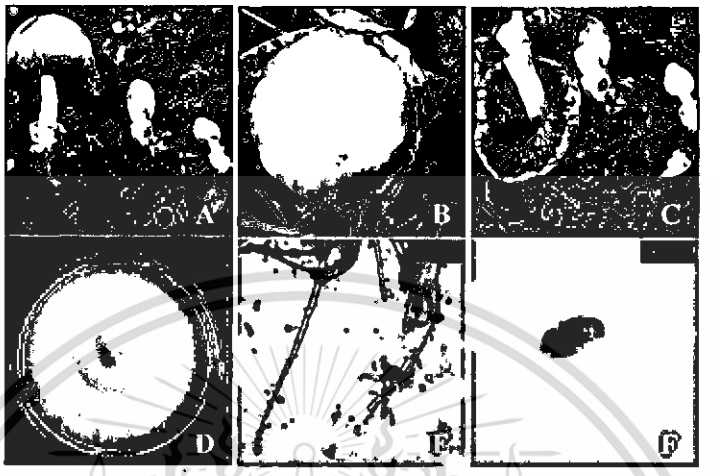

Fig.4.39 *Coprinus* spp. (PH09) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

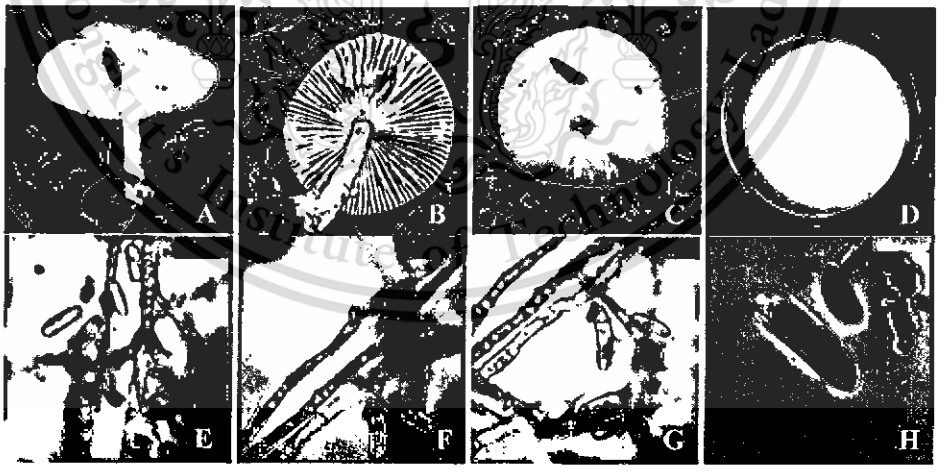

Fig.4.40 *Marasmius* spp. (PH10) A, B, C. fruiting body, D. 15-day-old culture on PDA, E, F, G. mycelia with spores, H. basidiospore, Bar. E, F, G =10 μm, H = 25 μm.

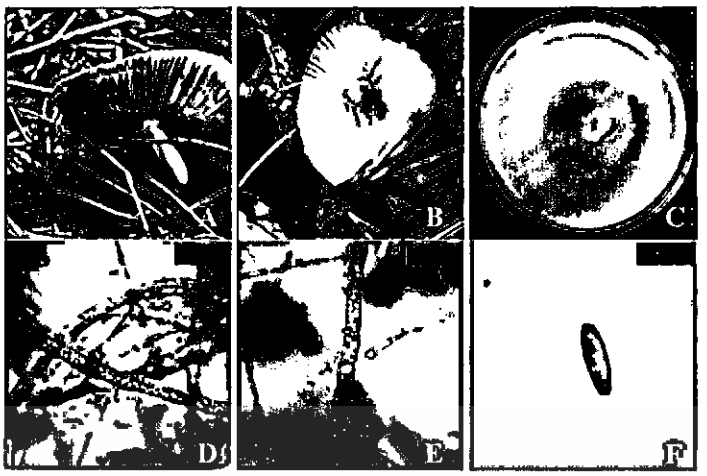

Fig.4.41 *Collybia iocephala* (PH11) A, B. fruiting body, C. 15-day-old culture on PDA, D, E. mycelia, F. basidiospore, Bar. D, E, F= 10 μm.

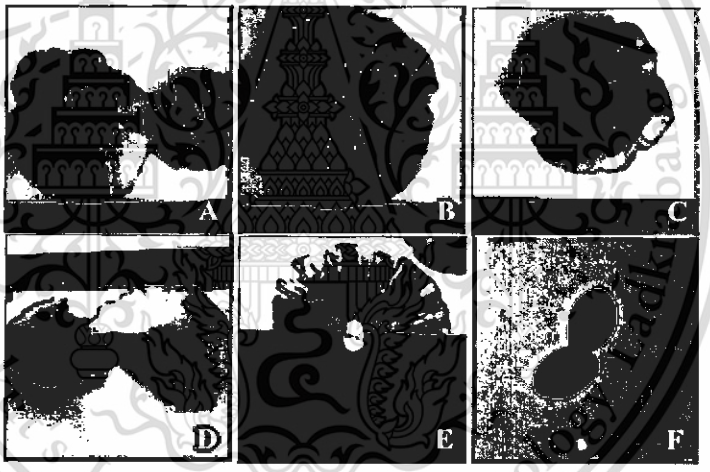

Fig.4.42 *Collybia* spp. (LB01) A, B, C, D. fruiting body, E. spore print, F. basidiospore, Bar. F= 10 μm.

Fig.4.43 *Mycena* spp. (SY01) A, B. fruiting body, C. basidiospore, Bar. C= 10 μm.

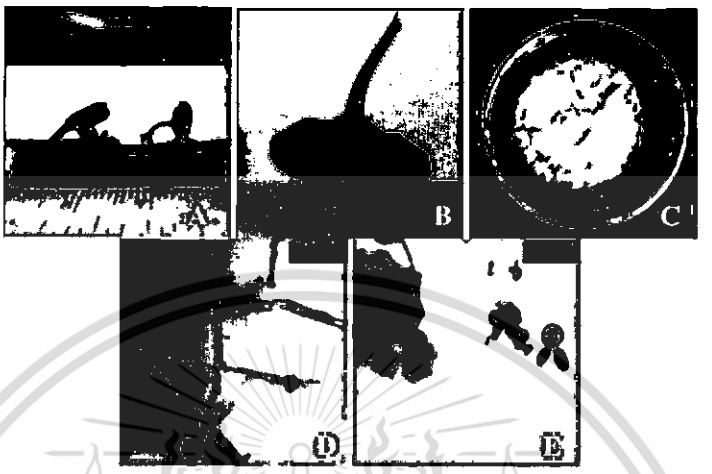

Fig.4.44 *Marasmius* spp. (SY02) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E= 10 μm.

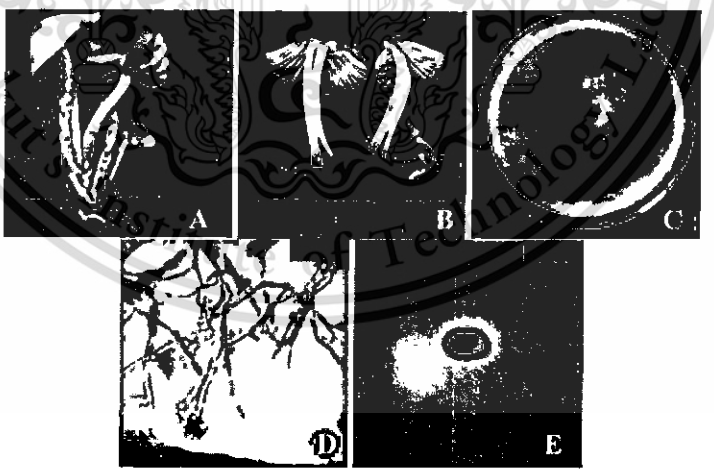

Fig.4.45 *Mycena* spp. (SY03) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D,E = 10 μm.

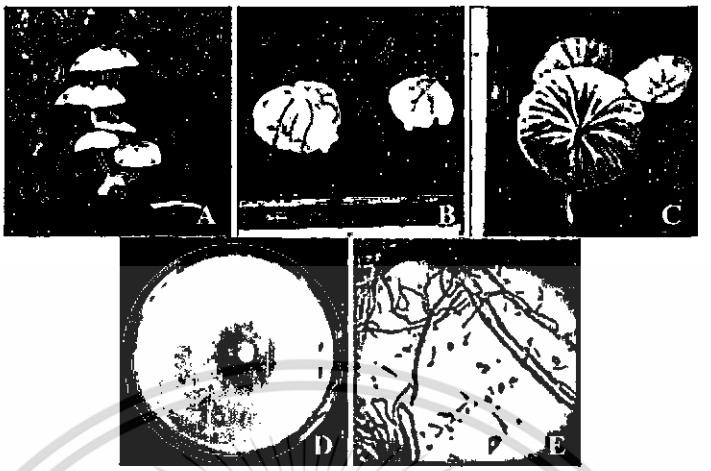

Fig.4.46 *Marasmius purpureostriatus* (SY04) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, Bar. E = 10 μm.

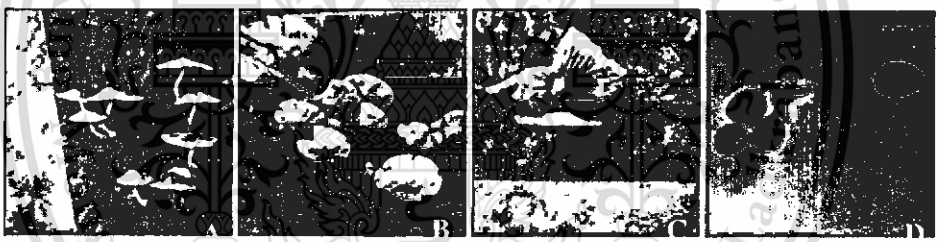

Fig.4.47 *Mycena* spp. (SY05) A, B, C. fruiting body, D. basidiospore, Bar. D = 10 μm.

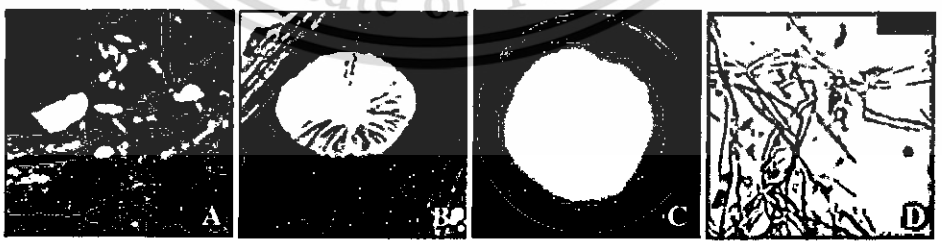

Fig.4.48 *Marasmiellus albuscorticis* (SY06) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, Bar. D = 10 μm.

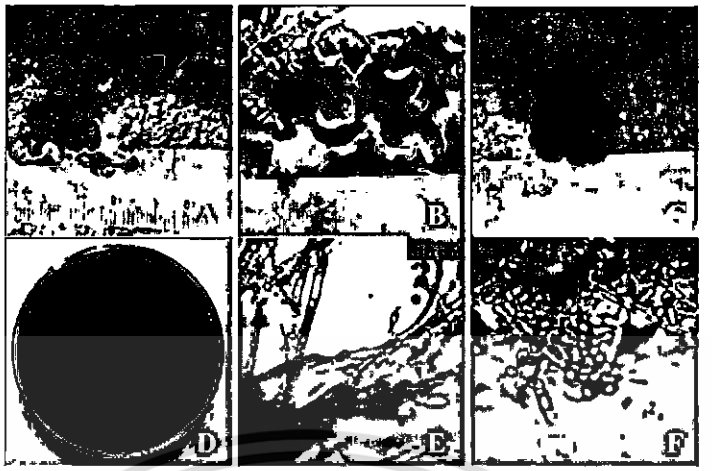

Fig.4.49 *Auricularia auricula* (SY07) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

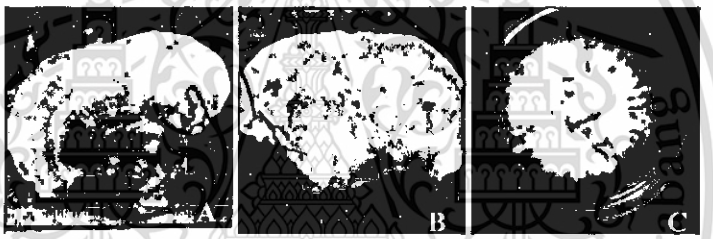

Fig.4.50 *Schizophyllum commune* (SY08) A, B. fruiting body, C. 15-day-old culture on PDA.

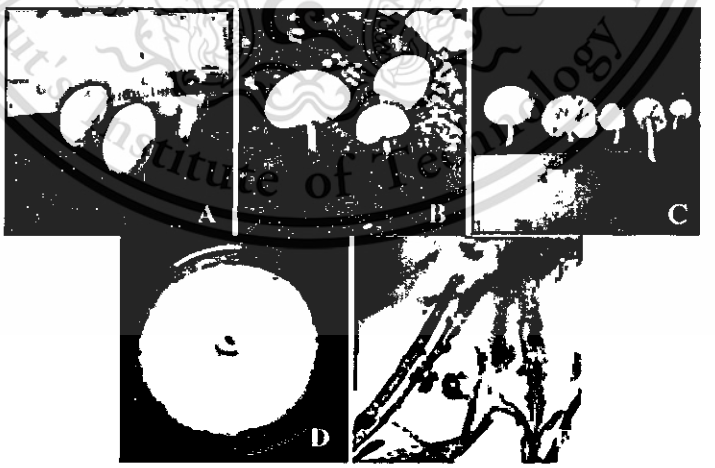

Fig.4.51 *Marasmillus ramealis* (SY09) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, Bar. E = 10 μm.

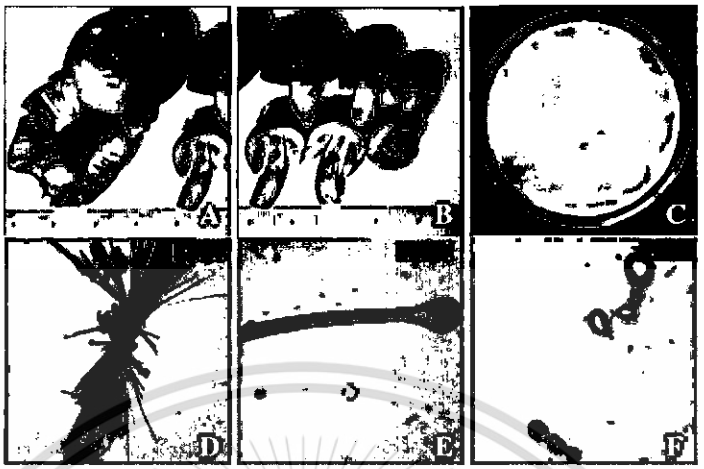

Fig.4.52 *Russula foetens* (AJ01) A, B. fruiting body, C. 15-day-old culture on PDA, D,E. mycelia, F. basidiospore, Bar. D, E, F = 10 μm.

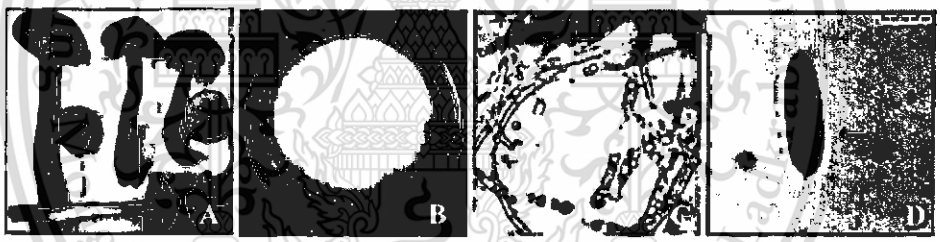

Fig.4.53 *Boletus retisporus* (AJ02) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, D. basidiospore, Bar. C = 10 μm, D = 25μm.

Fig.4.54 *Cantharellus cibarius* (AJ03) A, B. fruiting body, C. basidiospore, Bar. E, F = 10 μm.

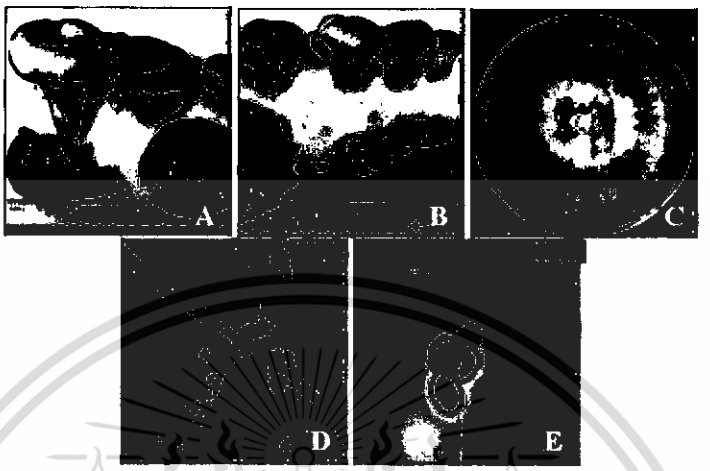

Fig.4.55 *Russula crassotunicata* (AJ04) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E = 10 μm.

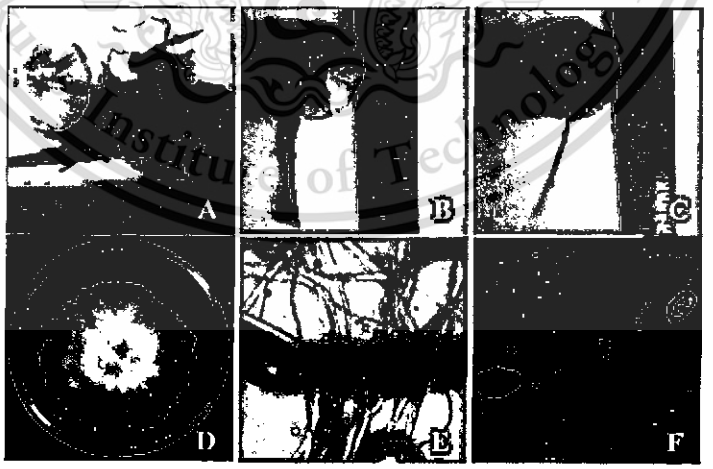

Fig.4.56 *Laccaria vinaceoavellanea* (AJ2-1) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, F. basidiospore, Bar. E, F = 10 μm.

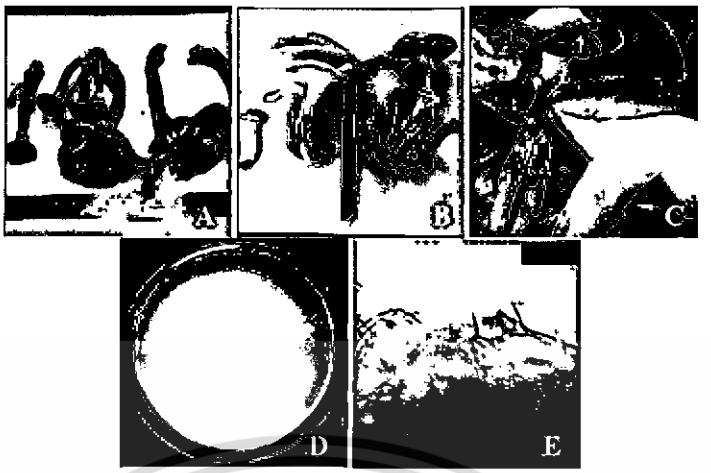

Fig.4.57 *Clitocybe* spp. (AJ2-2) A, B, C. fruiting body, D. 15-day-old culture on PDA, E. mycelia, Bar. E = 10 μm.

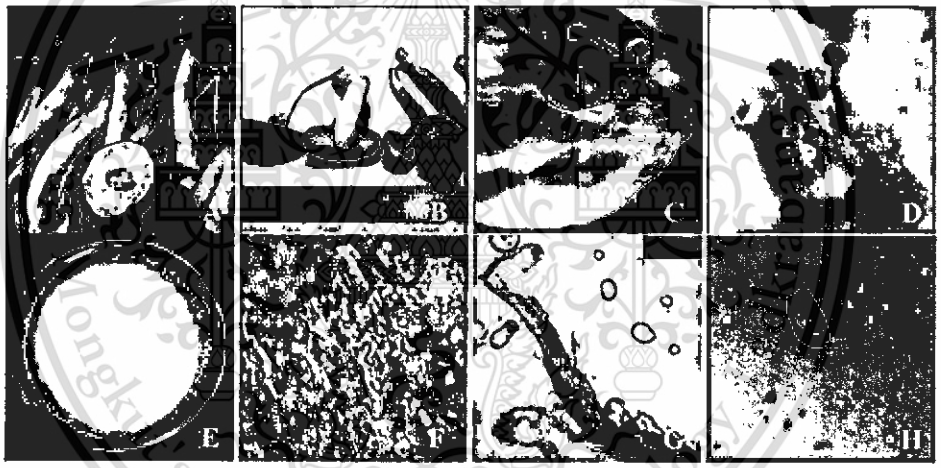

Fig.4.58 *Boletus affinis* var. *maculosus* (AJ2-3) A, B. fruiting body, C, D. fruiting body on PDB, E. 15-day-old culture on PDA, F,G. mycelia, H. basidiospore, Bar. F, G, H = 10 μm.

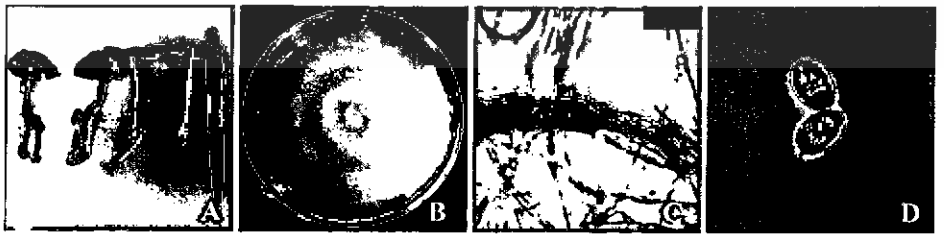

Fig.4.59 *Inocybe fastigiata* (AJ2-4) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, D. basidiospore, Bar. E, F = 10 μm.

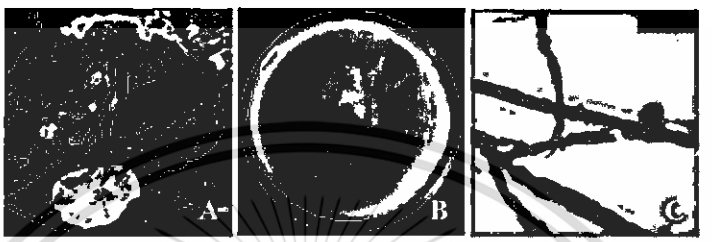

Fig.4.60 *Clitocybe* spp. (AJ2-5) A. fruiting body, B. 15-day-old culture on PDA, C. mycelia, Bar. C =10 μm.

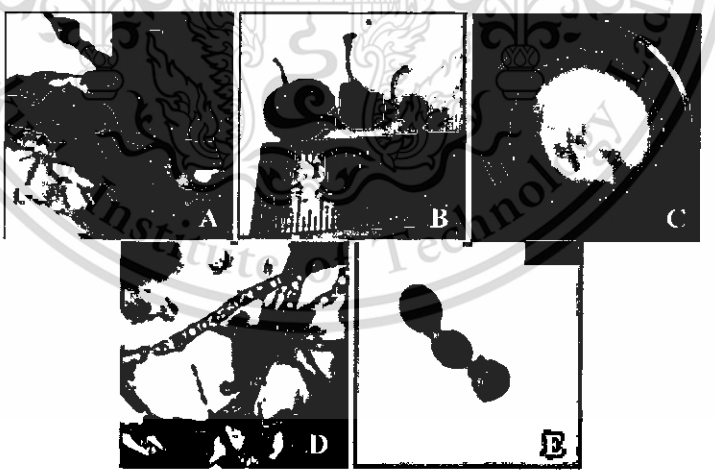

Fig.4.61 *Mycena vulgaris* (AJ2-6) A, B. fruiting body, C. 15-day-old culture on PDA, D. mycelia, E. basidiospore, Bar. D, E = 10 μm.

4.2 Pathogen Collection, Isolation and Pathogenicity Test

4.2.1 Isolation of Coffee Anthracnose Causing by *Colletotrichum* spp.

Three isolates of *Colletotrichum* were obtained from leave anthracnose of Arabica coffea. Of these, one isolate was identified as *Colletotrichum.coffeanum* (Fig. 4.62A, Fig. 4.62B, Fig. 4.62C), one isolate was identified as *Colletotrichum. gloeosporioides* (Fig. 4.62D, Fig. 4.62E, Fig. 4.62F) and one isolate was identified as *Colletotrichum.capsici* (Fig.4.62G, Fig. 4.62H, Fig. 4.62I).

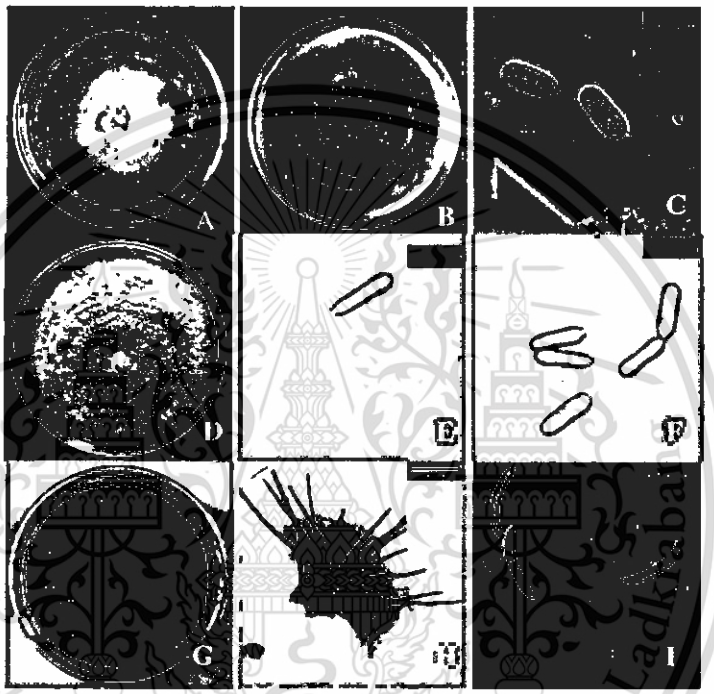

Fig.4.62 Three isolates of *Colletotrichum* spp. from leave anthtacnose of coffee Arabica A, B, C. *C. coffeanum*, D, E, F. *C .gloeosporioides*, G, H, I. *C. capsici* ; A, B, D, G. 15-day-old culture on PDA, C, E, F, I. conidia, H. ascomata, Bar. C, E, F, I= 10 μm, H= 100 μm.

4.2.2 Pathogenicity Test of *Colletotrichum* spp.

Pathogenicity tests of *Colletotrichum* spp. were separately done in each isolate using plug inoculation method. The agar plug of each isolate was inoculated over the wounded coffee leaves and incubated in moist chamber at room temperature (28-30°C) for 14 days. The results showed that the lesion size developed by *C. coffeanum* isolate was 27.25 mm, which gave the highest virulent for disease incidence and significantly differed from other isolates (P=0.05), and followed by *C. gloeosporioides* isolate which the lesion size were 4.50 mm. The *C. capsici* isolate did not develop any symptom when inoculated (Table 4.2; Fig. 4.63). The lesion size from

pathogenicity tests grouped into three categories as follows: high virulent group (*C. coffeanum*), low virulent group (*C. gloeosporioides*) and low virulent group (*C. capsici*). As a result, isolate *C. coffeanum* was selected for further study.

Table 4.2 Pathogenicity tests of *Colletotrichum* isolates using plug inoculation method.

Host	Isolate	Lesion diameter (mm)[2/]	Virulence[1/]
Arabica coffee	*C. coffeanum*	27.25[a]	H
Arabica coffee	*C. gloeosporioides*	4.50[b]	L
Arabica coffee	*C. capsici*	1.00[c]	L
Arabica coffee	Control	0.00[c]	N

[1/]Lesion size was assessed into five categories as follows: N, non-pathogenic = 0 mm; L, low virulent = 0.1-5.0 mm; M, moderately virulent = 5.1-10.0 mm; H, high virulent = >10.0 mm.

[2/]Average of four replications. Means followed by a common letter are not significantly different when compared to Duncan's Multiple Range Test (DMRT) at P = 0.05.

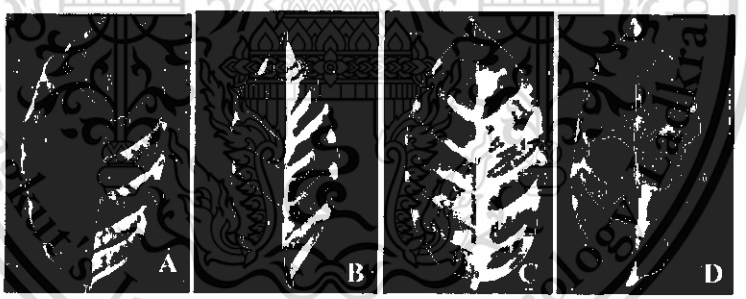

Fig.4.63 Pathogenicity tests of *Colletotrichum* isolates using plug inoculation method A, control; B, wounded leave with agar plug of *C. capsici*; C, wounded leave with agar plug of *C. gloeosporioides*; D, wounded leave with agar plug of *C. coffeanum*..

4.2.3 Isolation of Fusarium Wilt causing by *Fusarium oxysporum* f. sp. *lycopersici*

NKSC02 race 2

One isolate of *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2 (Fig.4.64) was obtained from Dr. Kasem Soytong, which confirmed by DNA sequencing.

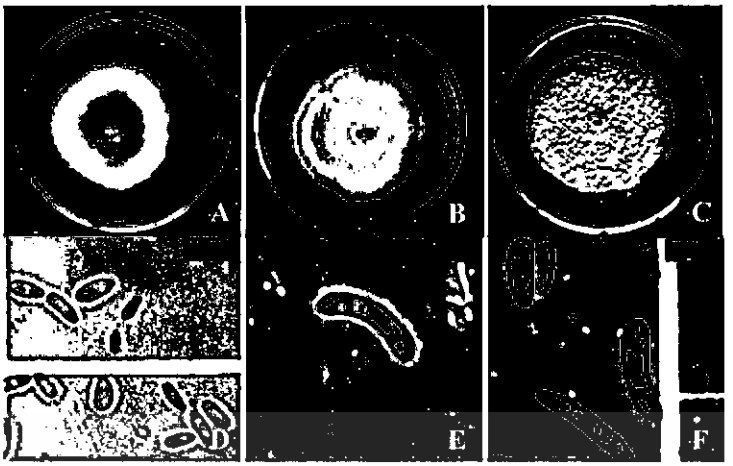

Fig 4.64 *F.oxysporum* f. sp. *lycopersici* race 2 A. 5-day-old culture on PDA, B. 10-day-old culture on PDA, C. 15-day-old culture on PDA, D, E, F. conidia, Bar. D, E, F= 10 μm.

4.2.4 Pathogenicity Test of *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2.

Pathogenicity test of *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2 was tested in vivo to 15 day tomato seedlings-*Lycopersicon esculentum* (Fig. 4.65) using a root dip inoculation method. The disease incidence was determined at 15 days. Disease index (DI) was recorded as follows: 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting 81-100% or die (Sibounnavong *et al.* 2010) .

After test, the symptom of fusarium wilt disease showed the yellowing and wilting leaves (level 6). Wilted leaves usually dropped prematurely (Fig. 4.66). Affected plants died early. Splitting opened an infected stem reveals brownish streaks extending up and down the stem. *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2 was confirmed as the virulent isolate.

Fig.4.65 15 day tomato (*Lycopersicon esculentum*) seedling.

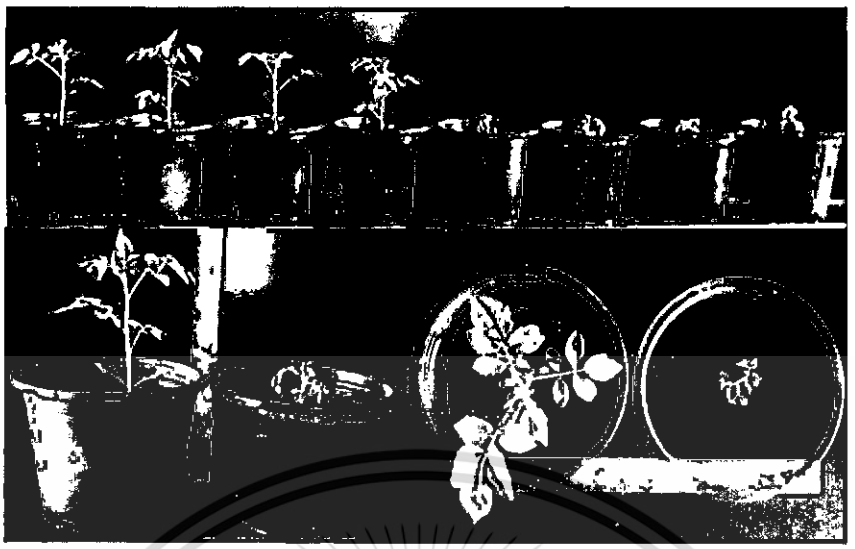

Fig 4.66 Pathologenicity test: upper right was four replications of the non-treated control and upper left was four replications of inoculated tomato seedlings ; lower right was side view of the non-treated control (left) and inoculated tomato seedling (right) and lower left was the non-treated control (left) and inoculated tomato seedling (right).

4.3 Biological Active Substances Extraction

Leucocoprinus fragilissimus (PH06), *Collybia strictipes* (PH07), *Clitocybe* spp. (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01), *Lactarius* spp. (CH3-27) were extracted their biological active substances as crude extracts and tested for their abilities to inhibit the growth *C. coffeanum* and *F. oxysporum* f. sp. *lycopersici* by plate assay. Dried fungal biomass were evaporately ground and extracted successively with solvents as follows: Hexane, Ethyl acetate (EtOAc) and Methanol (MeOH). The filtrates were evaporated to yield crude Hexane, EtOAc and MeOH extracts, respectively. The obtained crude extracts were presented in Fig. 4.67 and yields of crude extracts were recorded as shown in Table 4.3. The colors of crude Hex, crude EtOAc and crude MeoH were quite different, were pale yellow or red to dark brown depended on different species of Agaricales and extracting solvents (Fig. 4.67). Crude textures were oil, wax or solid (Fig. 4.67). The weight of crude Hexane, crude EtOAc and crude MeoH also were quite different. With this, the crude hexane, crude ethyly acetate and crude methanol from *L. fragilissimus* PH06 yielded 0.12, 1.12 and 4.06%, respectively. The crude hexane, crude ethyl acetate and crude methanol from *C. strictipes* PH07 yielded 0.36, 0.36 and 0.40%,

respectively. The crude hexane, crude ethyly acetate and crude methanol from *Clitocybe* spp. AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude ethyly acetate and crude methanol from *B. affinis* var. *maculosus* AJ2-3 yielded 0.43, 0.47 and 5.32%, respectively. The crude hexane, crude ethyl acetate and crude methanol from *Lactarius* spp. CH3-01 yielded 0.54, 2.12 and 5.03%, respectively. The crude hexane, crude ethyly acetate and crude methanol from *Lactarius* spp. CH3-27 yielded 3.88, 5.49 and 6.76%, respectively. The details were as follows (Table 4.3):

Table 4.3 Extraction of biological active substances.

Taxon	Fresh weight(g)	Dry weight(g)	Organic solvents		
			Crude Hexane(g)	Crude EtOAc(g)	Crude MeOH(g)
Leucocoprinus fragilissimus (PH06)	3,927	124.65	0.15 (0.12%)	1.39 (1.12%)	5.06 (4.06%)
Collybia strictipes (PH07)	2,010	55.00	0.2 (0.36%)	0.2 (0.36%)	0.22 (0.40%)
Clitocybe spp. (AJ2-2)	2500	72.10	4.27 (5.92%)	3.95 (5.48%)	4.32 (5.99%)
Boletus affinis var. maculosus (AJ2-3)	5,230	91.56	0.39 (0.43%)	0.43 (0.47%)	4.87 (5.32%)
Lactarius spp. (CH3-01)	1,920	79.10	0.43 (0.54%)	1.68 (2.12%)	3.98 (5.03%)
Lactarius spp.(CH3-27)	4,200	140.00	5.43 (3.88%)	7.69 (5.49%)	9.46 (6.76%)

The obtained rate of crude extracts was quite different. Crude MeOH extract from *Lactarius* spp. (CH3-27) gave the highest yield (9.46 g) followed by crude EtOAc extract from CH3-27 (*Lactarius* spp.), crude Hexane extract from *Lactarius* spp. (CH3-27), crude MeOH extract from *Leucocoprinus fragilissimus* (PH06). The rate of AJ 2-2 and CH3-27 are higher compared with PH07 which was lower obtained rate of crude extracts. Meanwhile, most of crude Hexane had the lowest obtained rate of crude extracts, as low as less than 0.12% compared with crude MeOH which had highest obtained rate, up to 6.76%. But the most stable obtained rate is AJ2-2 which crude Hexane, crude EtOAc, crude MeOH were similar rate.

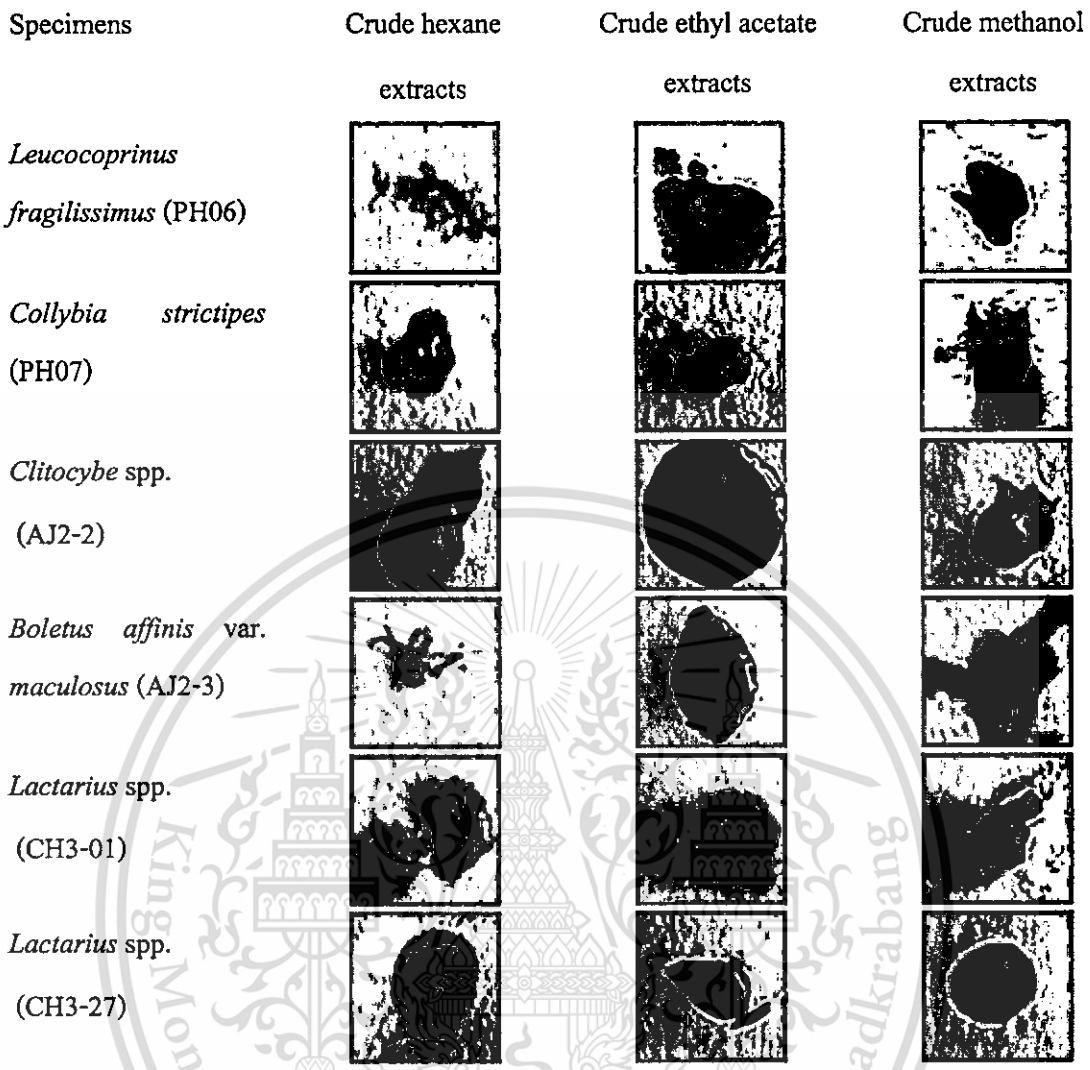

Fig 4.67 The characteristic of crude extracts.

4.4 Sporulation Conditions of *Colletotrichum coffeanum*

The isolate of *C. coffeanum* (Fig. 68) was obtained from leave athracnose of Arabica coffee. The results showed that the lesion size developed by *C. coffeanum* isolate was 27.25 mm, which gave the high virulent for disease incidence.

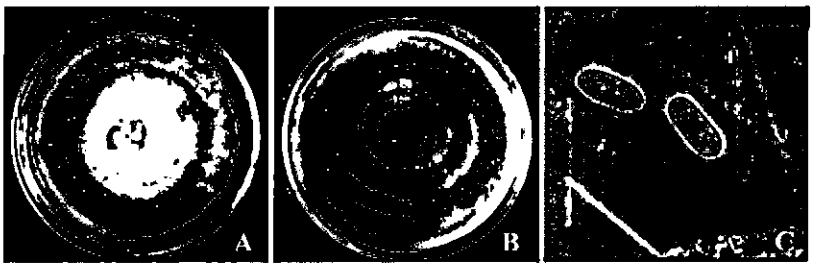

Fig. 68 *Colletotrichum coffeanum* A, B. *C. coffeanum*. C. spore. Bar. c= 10 μm.

Two kinds of media and two kinds of volume of media were tested to determine the effect of nutrients on sporulation. That was 5 ml of PDA media, 10 ml of PDA media, 5 ml of V8 media, 10 ml of V8 media, respectively. All of plates were incubated under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp), constant darkness (continuous darkness; CD) and a 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp), respectively. In these three different lighting conditions, continuous cultured 35 days. Spore production was quantified using a hemacytometer at each stage. Specific data are showed in Table 4.4.

Table 4.4 Effect of different factors on spore production of *Colletotrichum coffeanum*.

Media	Light conditions[1]	The volume of media	Spore production at different times (10^6)[2] 14 days	21 days	28 days	35 days
PDA	CL	5ml	3.75^{d}	11.00^{d}	34.00^{h}	90.50^{fg}
		10ml	5.75^{bc}	18.00^{bc}	55.25^{f}	111.50^{e}
	12:12 L:D	5ml	5.50^{bcd}	16.75^{bcd}	78.50^{cde}	175.25^{c}
		10ml	9.50^{a}	30.50^{a}	155.50^{b}	231.00^{a}
	CD	5ml	5.00^{bcd}	13.50^{cd}	41.25^{gh}	83.25^{g}
		10ml	6.50^{b}	22.75^{b}	75.75^{de}	152.25^{d}
V8	CL	5ml	4.25^{cd}	13.00^{cd}	44.00^{g}	100.00^{ef}
		10ml	5.75^{bc}	17.50^{bcd}	75.00^{e}	111.50^{e}
	12:12 L:D	5ml	5.75^{bc}	18.00^{bc}	86.25^{c}	192.50^{b}
		10ml	9.00^{a}	33.50^{a}	163.50^{a}	231.00^{a}
	CD	5ml	5.25^{bcd}	16.50^{bcd}	53.00^{f}	88.25^{fg}
		10ml	6.00^{bc}	23.25^{b}	83.25^{cd}	169.75^{c}

[1]/ CL=continuous light, 10000 lux, fluorescent lamp; CD=constant darkness, continuous darkness; 12:12 L:D= 12 h light/dark photoperiod , 10000 lux, fluorescent lamp.

[2]/Values are not signicantly different (P= 0.05) by Duncan's multiple range test.

The two kinds of culture medium, V8 juice agar was the best media for inducing sporulation (up to 2.31 $x10^8$) at 35th day, but it was not significantly different from PDA media. Fig. 4.69 showed effects of 5 ml PDA, 10 ml PDA, 5 ml V8, 10 ml V8, two different media in

different volume constant illumination (continuous light; CL; 10000 lux, fluorescent lamp), respectively. The best one was 10ml V8 juice agar under constant illumination. Under the same conditions as the volume, the spore production of V8 was higher than PDA. Fig. 4.70 showed effects of 5 ml PDA, 10 ml PDA, 5 ml V8, 10 ml V8, two different media in different volume under the same 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp), respectively. The best one was 10ml V8 juice agar under, too. Under the same conditions as the volume, the spore production of V8 was higher than PDA, but not significantly. Fig. 4.71 showed effects of 5 ml PDA, 10 ml PDA, 5 ml V8, 10 ml V8, two different media in different volume under constant darkness (continuous darkness; CD), respectively. The best one was 10ml V8 juice agar under, too. Under the same conditions as the volume, the spore production of V8 was higher than PDA, not significantly.

The results obtained from the three graphs also illustrate the above points. In short, PDA and V8 were able to induce the spore production of *Colletotrichum coffeanum*. But, V8 was better. However, this advantage was not obvious. Considering the cost and simplicity of the experimental, in future trials, preferred to choose PDA as medium for *Colletotrichum coffeanum.*

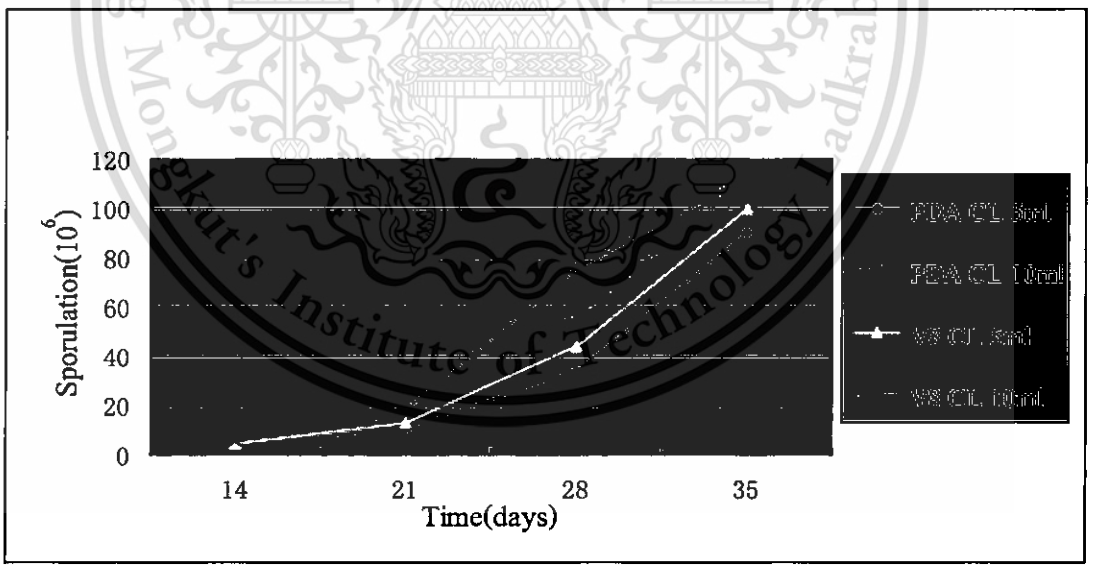

Fig.4.69 Effect of two kinds of media of different volume under constant illumination continuous light; CL; 10000 lux, fluorescent lamp) on spore production.

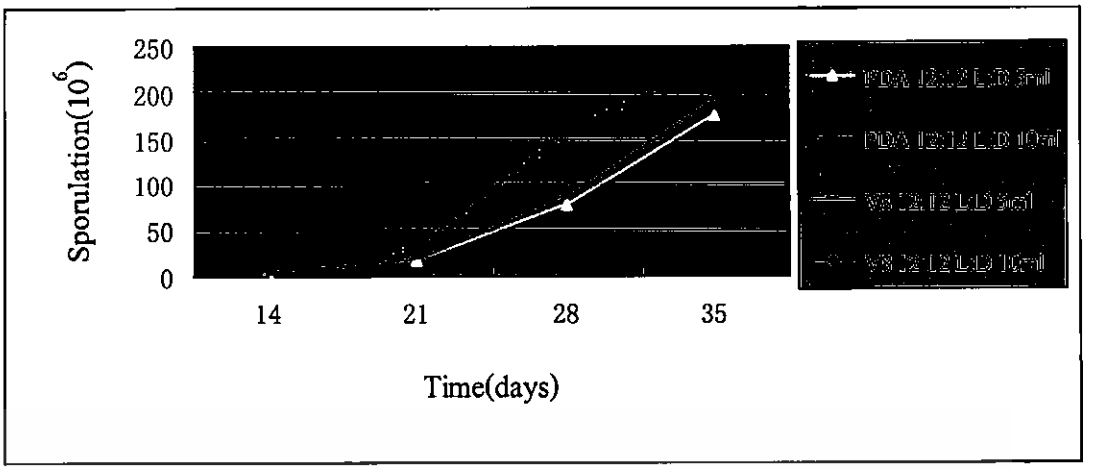

Fig.4.70 Effect of two kinds of media of different volume under 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) on spore production.

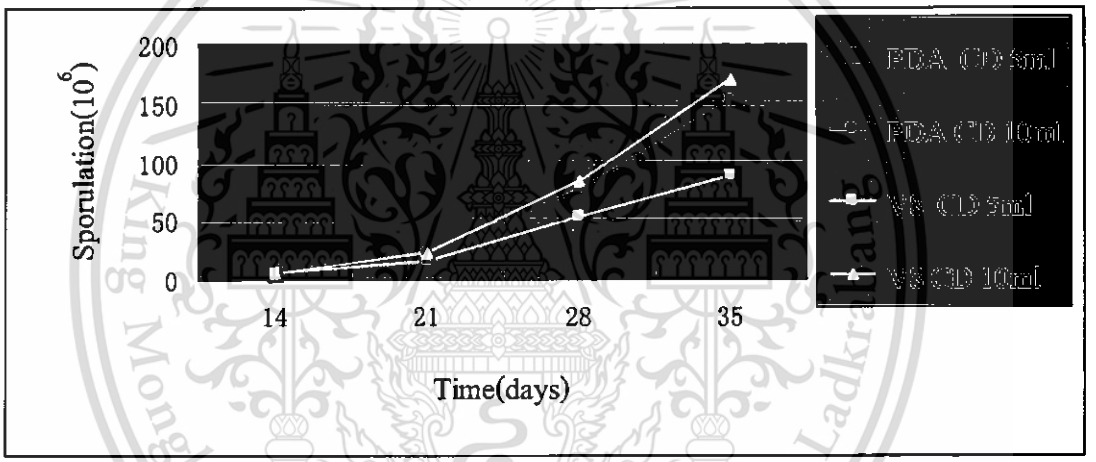

Fig.4.71 Effect of two kinds of media of different volume under constant darkness (continuous darkness; CD) on spore production.

In the life cycle of *Colletotrichum coffeanmum*, nutrition except for producing spores, there are also a part suitable for producing a wide variety of hyphae. V8 and PDA contain different nutrition, spore germination will have a different role. However, the same, even if the same medium, which contain the same nutrients, these nutrients can produce sufficient to induce spores and hyphae of an produce sufficient to induce, which is another important factor affecting spore production.

Fig. 4.72 showed the effect of 5 ml and 10 ml PDA under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp) on spore production, respectively. The best one was .10ml V8 juice agar under constant illumination. At the same time, 10 ml of the PDA

medium had a greater degree of inducing spores of *Colletotrichum coffeanmum*, compared with 5ml PDA medium. Fig. 4.73 and Fig.4.74 also showed the same conclusion, no matter under constant darkness (continuous darkness; CD) or a 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp). Moreover, the same conclusion in a case of V8 was verified (Fig. 4.75; Fig. 4.76; Fig. 4.77).

In conclusion, the capacity of medium for spore production of *Colletotrichum coffeanmum* played an important role. Under other conditions the same, increasing the content of the medium, the spores would follow a significant increase in yield.

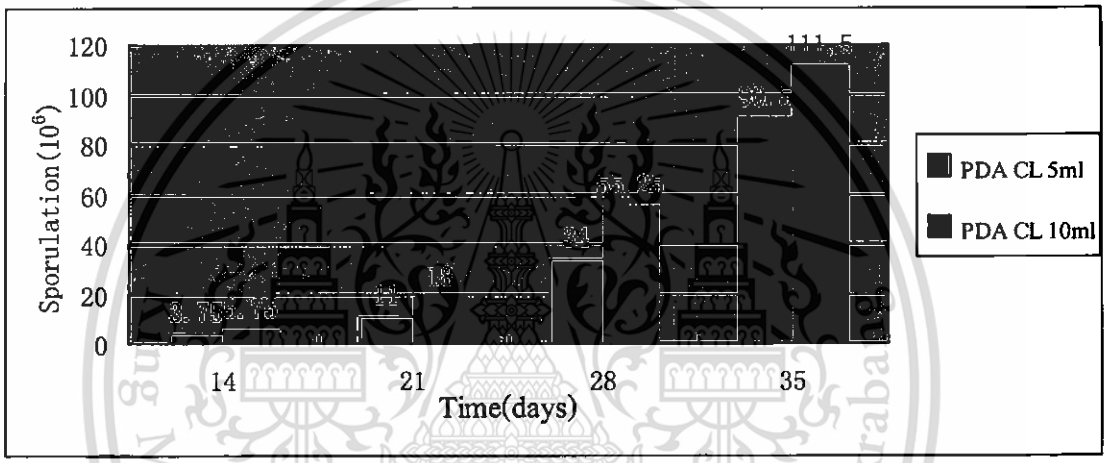

Fig. 4.72 Effect of different volume PDA under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp) on spore production.

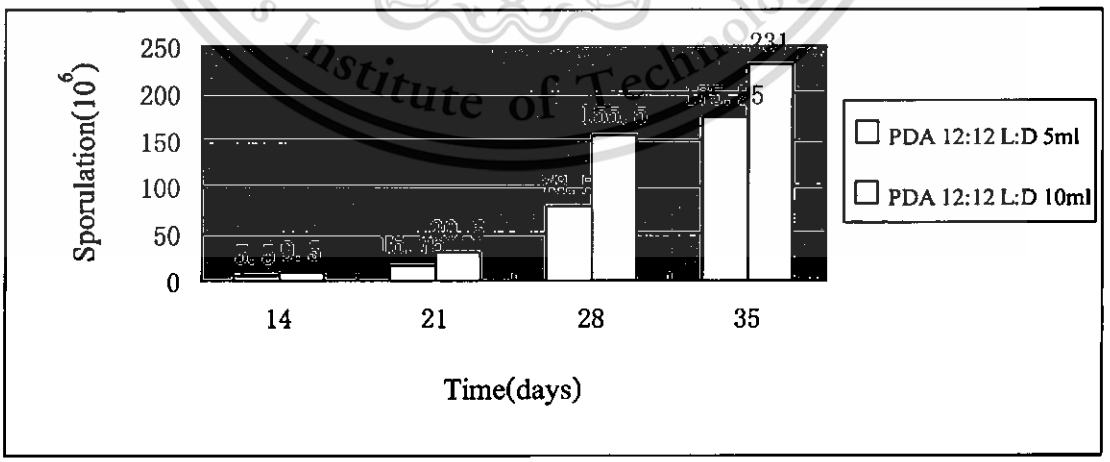

Fig. 4.73 Effect of different volume PDA under 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) on spore production.

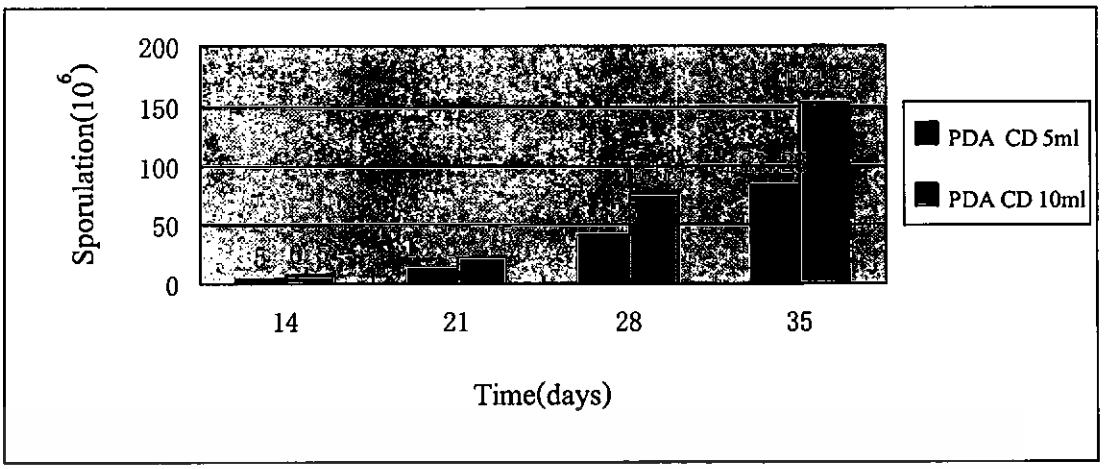

Fig. 4.74 Effect of different volume PDA under constant darkness (continuous darkness; CD) on spore production.

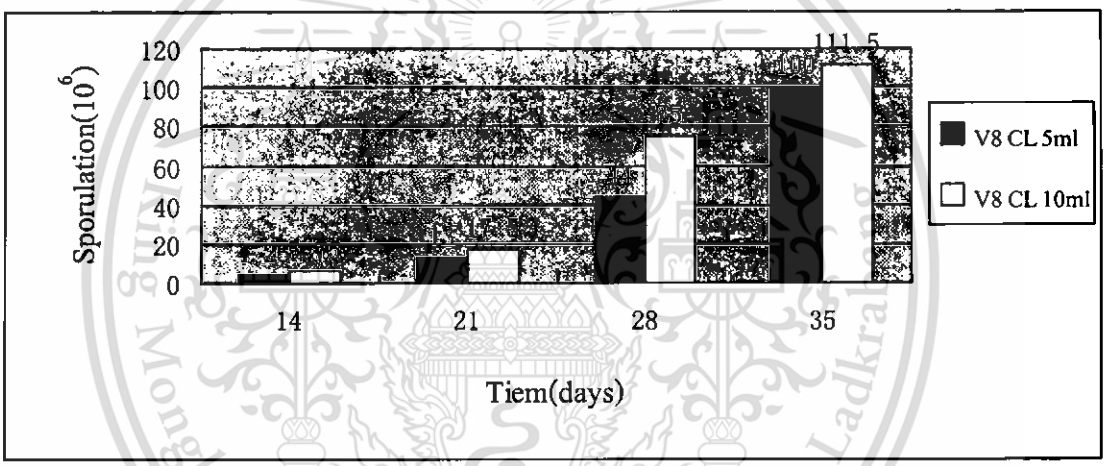

Fig. 4.75 Effect of different volume V8 under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp) on spore production.

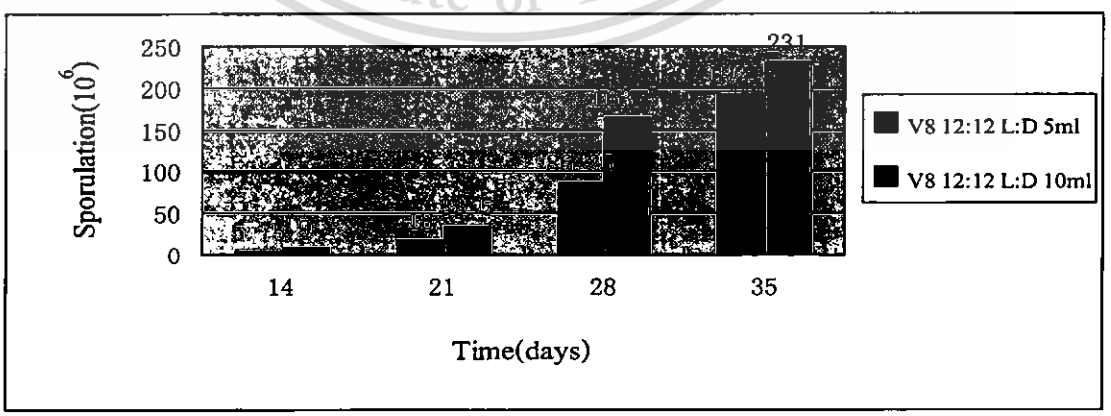

Fig. 4.76 Effect of different volume PDA under 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) on spore production.

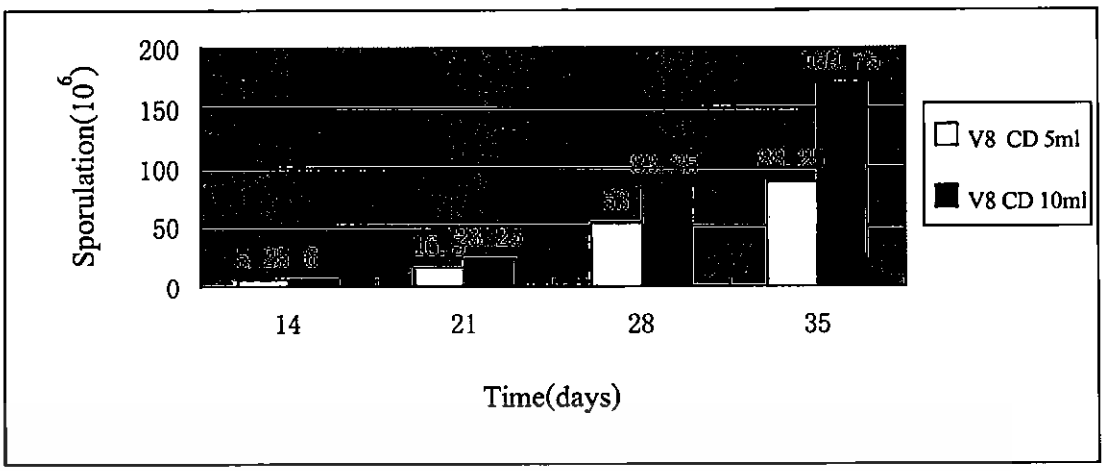

Fig. 4.77 Effect of different volume PDA under constant darkness (continuous darkness; CD) on spore production.

Fig. 4.78, Fig. 4.79, these two figures showed the effect of different light conditions in same volume PDA medium on spore production. In the early observation (before 21st day) found that there was no significant effect on spore production. However, at the 28th days of observation found that, only under 12 h light/dark photoperiod would significantly promote the induction of sporulation. Another two groups were still no significant difference. Same conclusion was confirmed by Fig. 4.80 and Fig. 4.81 which showed the effect of different conditions in same volume V8 medium on spore production. In conclusion, the length of illumination time, there was no significant effect on spore production, but in the late growth stage, light time would impact the spores yield in some extent. 12 h light/dark photoperiod gave the highest spores yield, compared with constant darkness and constant illumination. Especially, since 28th day, this difference was particularly prominent, the sporulation up to 155.5×10^6, until 35th day, the sporulation up to 231 $\times 10^6$.

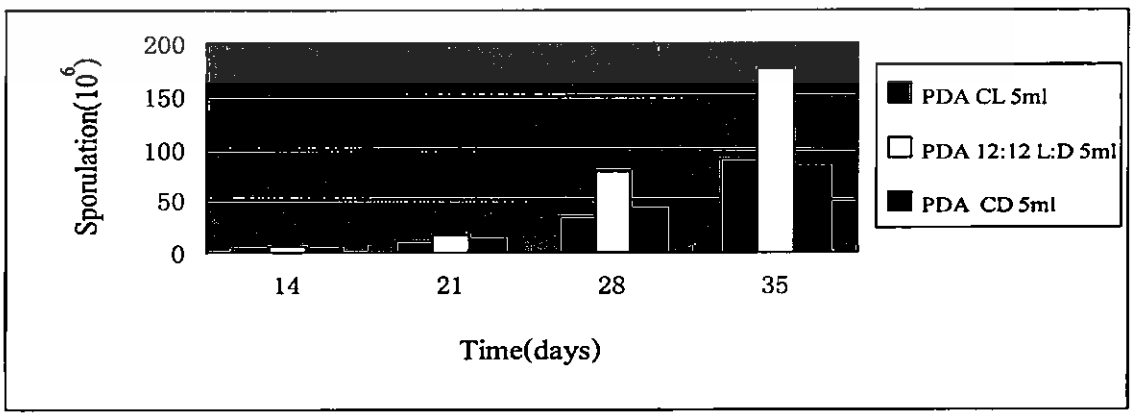

Fig. 4.78 Effect of different light conditions in 5ml PDA medium on spore production.

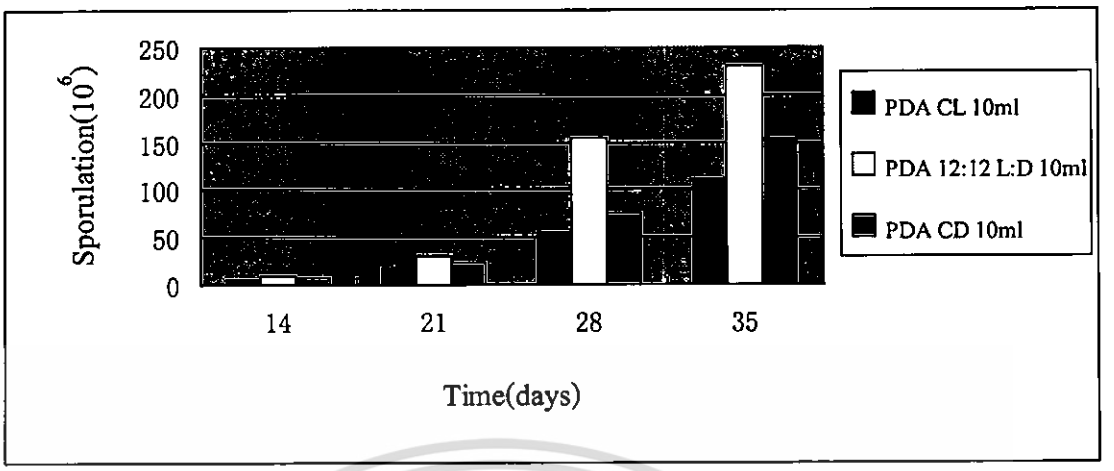

Fig. 4.79 Effect of different light conditions in 10ml PDA medium on spore production.

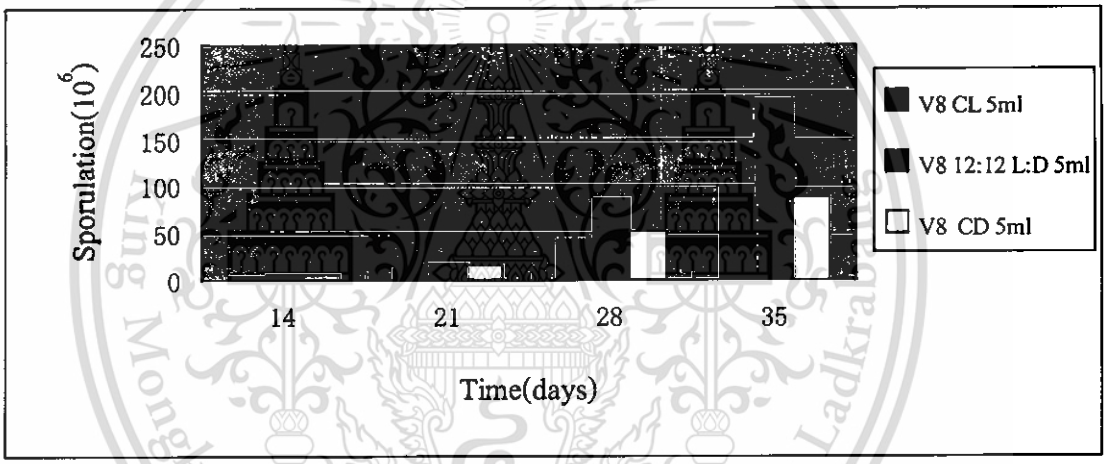

Fig. 4.80 Effect of different light conditions in 5ml V8 medium on spore production.

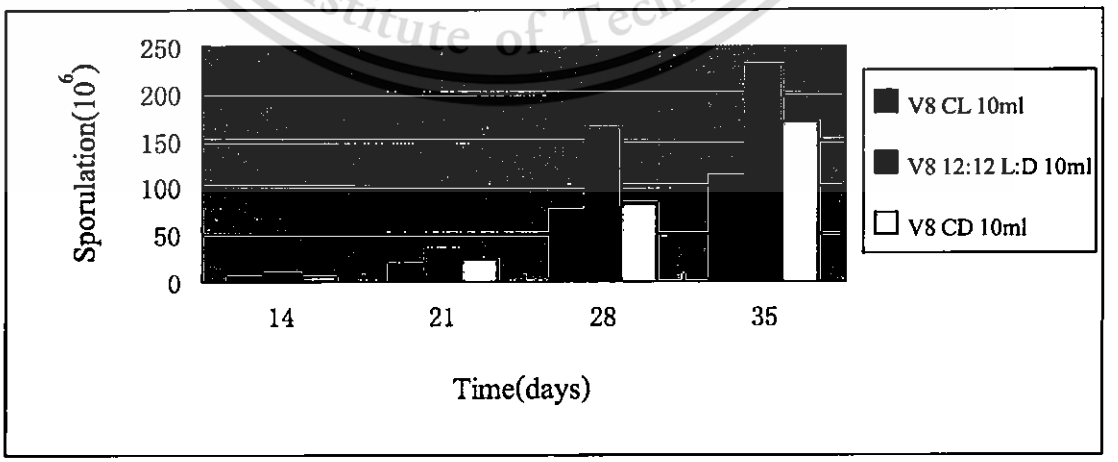

Fig. 4.81 Effect of different light conditions in 10ml V8 medium on spore production.

With the time passed by, the spores were increasing. From Fig. 4.82, Table 4.5, the highest growth rate was all occurred on the 28th day. Among them, 10ml PDA medium gave the highest growth rate, up to 4.10. Since the 28th day, the growth rate started to slow down.

Table 4.5 Growth rate of spore production of *Colletotrichum coffeanum.*

Media	**Light conditions**[1]	**The volume of media**	**Growth rate**		
			21st day[2]	**28th day**[3]	**35th day**[4]
PDA	CL	5ml	1.93	2.09	1.66
PDA	CL	10ml	2.13	2.07	1.02
PDA	12:12 L:D	5ml	2.05	3.69	1.23
PDA	12:12 L:D	10ml	2.21	4.10	0.49
PDA	CD	5ml	1.7	2.06	1.02
PDA	CD	10ml	2.5	2.33	1.01
V8	CL	5ml	2.06	2.38	1.27
V8	CL	10ml	2.04	3.29	0.49
V8	12:12 L:D	5ml	2.13	3.79	1.23
V8	12:12 L:D	10ml	2.72	3.88	0.41
V8	CD	5ml	2.14	2.21	0.66
V8	CD	10ml	2.88	2.58	1.03

[1]/ CL=continuous light, 10000 lux, fluorescent lamp; CD=constant darkness, continuous darkness; 12:12 L:D= 12 h light/dark photoperiod , 10000 lux, fluorescent lamp.

[2]/ Growth Rate=(Sporulation at 21st day - Sporulation at 14th day)/ Sporulation at 14th day.

[3]/ Growth Rate=(Sporulation at 28th day - Sporulation at 21st day)/ Sporulation at 21st day.

[4]/ Growth Rate=(Sporulation at 35th day - Sporulation at 28th day)/ Sporulation at 28th day.

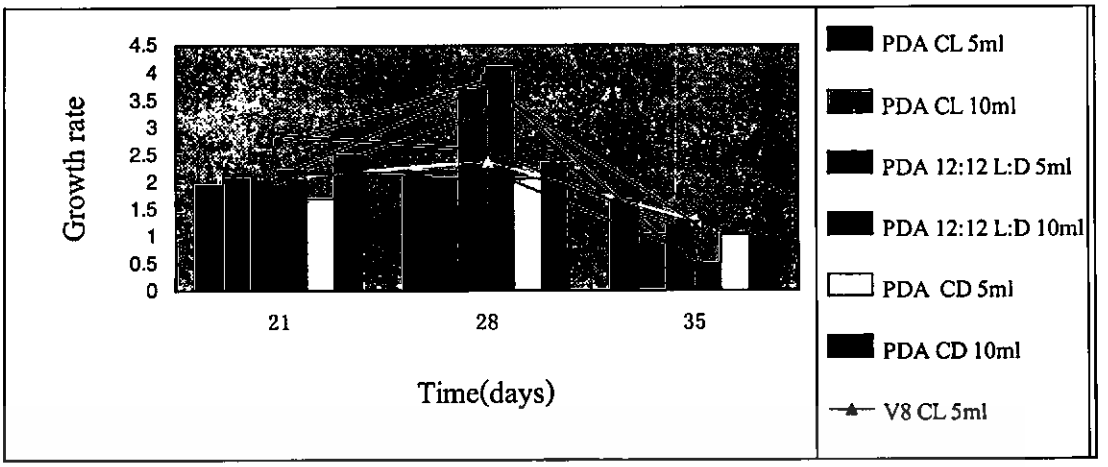

Fig. 4.82 Effect of incubation time on spore production.

Through the cross-analysis of a variety conditions which affected the *Colletotrichum coffeanmum* sporulation, the result showed that V8 and PDA were suitable for *Colletotrichum coffeanmum* as the medium. Although these two medium had different effects for sporulation, V8 better promoted sporulation. But this advantage was not very significant. On the contrary, the content of the medium gave a significant impact on spore yield. Increase media content, also would greatly improve the spore production. 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) was the most suitable light condition for *Colletotrichum coffeanmum* sporulation, compared with constant darkness (continuous darkness; CD) and constant illumination (continuous light; CL; 10000 lux, fluorescent lamp). Although with the growth of time, spore production was also growing. However, the growth rate in the 28th day to reach the fastest. Summing up the appeal, choose 10 ml PDA medium and incubated 28 days under natural light conditions, was the best time to observe spore production.

4.5 Bioassay Tests against Coffee Anthracnose Causing by *Colletotrichun coffeanum* and Fusarium Wilt Causing by *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2.

Crude extracts which extracted from *Leucocoprinus fragilissimus* (PH06), *Collybia strictipes* (PH07), *Clitocybe* spp. (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01), *Lactarius* spp. (CH3-27) were tested for their ability to control the growth of *C. coffeanum* and *Fusarium oxysporum* f. sp. *lycopersici*. Each crude extract was dissolved with 2% dimethylsulfoxide (DMSO), and then prepared in 6 concentrations (0, 10, 50, 100, 500 and 1,000

μg/ml) to test antifungal activities of each crude extract against mycelial growth and spore formation of *C. coffeanum* and *Fusarium oxysporum* f. sp. *lycopersici* on PDA at room temperature (Figs.4.83-4.100 and Tables 4.6-4.23).

4.5.1 Biological Activity against Coffee Anthracnose Causing by *C. coffeanum*

The crude extracts from *Clitocybe* spp. AJ2-2, *B. affinis* var. *maculosus* AJ2-3 and *Lactarius* spp. CH3-01 were selected for bioactivity test against coffee anthracnose caused by *C. coffaenum*.

Results showed that methanol crude extract from *Clitocybe* spp. AJ2-2 gave significantly highest inhibition of 30% for the colony growth of *C. coffaenum* at the concentration of 1,000 μg/ml when compared to the control (Table 4.6). Crude methanol extract from *Clitocybe* spp. AJ2-2 gave the significantly highest inhibition of the spore production of *C. coffaenum* as 89.08% at the concentration of 1,000 μg/ml which the ED_{50} values was 9.65 μg/ml (Table 4.7) and showed abnormal features of hyphae and spore (Fig. 4.84), and followed by crude ethyl acetate inhibited 86.48% and crude hexane 70.36% which the ED_{50} values was 11.10 μg/ml, 23.15 μg/ml, respectively (Table 4.7).

The ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 33.53% for the colony growth of *C. coffaenum* at the concentration of 1,000 μg/ml when compared to the control (Table 4.8). Crude ethyl acetate extract from *B. affinis* var. *maculosus* AJ2-3 gave inhibition of the spore production of *C. coffaenum* as 67.86% at the concentration of 1,000 μg/ml which the ED_{50} values was 7.66 μg/ml (Table 4.9) and showed abnormal features of hyphae and spore(Fig. 4.86), followed by followed crude hexane inhibited 55.95% which the ED_{50} values was 75.19 μg/ml (Tables 4.9).

The methanol crude extract from *Lactarius* spp. CH3-01 gave significantly highest inhibition of 76% for the colony growth of *C. coffaenum* at the concentration of 1,000 μg/ml when compared to the control (Table 4.10). Crude methanol extract from *Lactarius* spp. CH3-01 gave significantly highest inhibition of the spore production of *C. coffaenum* as 76.13% at the concentration of 1,000 μg/ml which the ED_{50} values was 98.66 μg/ml (Table 4.11) and showed abnormal features of hyphae and spore (Fig. 4.88), and followed by crude ethyl acetate inhibited 58.85% and crude hexane 41.15% which the ED_{50} values was 710.45 μg/ml,1621.32 μg/ml, respectively (Tables 4.11).

Table 4.6 Crude extracts of *Clitocybe* spp. AJ2-2 testing for growth inhibition of *Colletotrichum coffaenum* at 5 days.

Crude extracts	Concentration (µg/ml)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	4.97[a]	0.00[g]
	10	4.92[ab]	1.02[fg]
	50	4.90[ab]	1.53[fg]
	100	4.82[ab]	3.03[efg]
	500	4.70[bc]	5.54[ef]
	1000	4.57[cd]	8.04[de]
Crude EtOAc	0	4.98[a]	0.00[g]
	10	4.87[ab]	2.56[fg]
	50	4.72[bc]	4.27[efg]
	100	4.70[bc]	5.76[ef]
	500	4.42[d]	11.29[d]
	1000	4.17[e]	17.30[c]
Crude MeOH	0	5.00[a]	0.00[g]
	10	4.77[abc]	3.00[efg]
	50	4.85[ab]	4.75[efg]
	100	4.45[d]	12.50[d]
	500	3.85[f]	23.00[b]
	1000	3.50[g]	30.00[a]
C.V.(%)		3.05	27.68

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.7 Spore production inhibition of crude extracts from *Clitocybe* spp. AJ2-2 to *Colletotrichum coffaenum* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (µg/ml)	Number of spores [1](10⁶)	Inhibition(%)[2]	ED_{50}
Crude Hexane	0	7.38[a]	0.00[g]	23.15
	10	4.01[b]	45.62[f]	
	50	2.89[bc]	60.83[de]	
	100	2.19[bcd]	70.36[cd]	
Crude EtOAc	0	7.38[a]	0.00[g]	11.10
	10	3.69[b]	49.26[ef]	
	50	1.75[cd]	76.06[bc]	
	100	1.00[cd]	86.48[a]	
Crude MeOH	0	7.38[a]	0.00[g]	9.65
	10	3.69[b]	51.34[ef]	
	50	1.56[ef]	78.67[abc]	
	100	0.81[d]	89.08[a]	
C.V.(%)		3.05	31.43	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

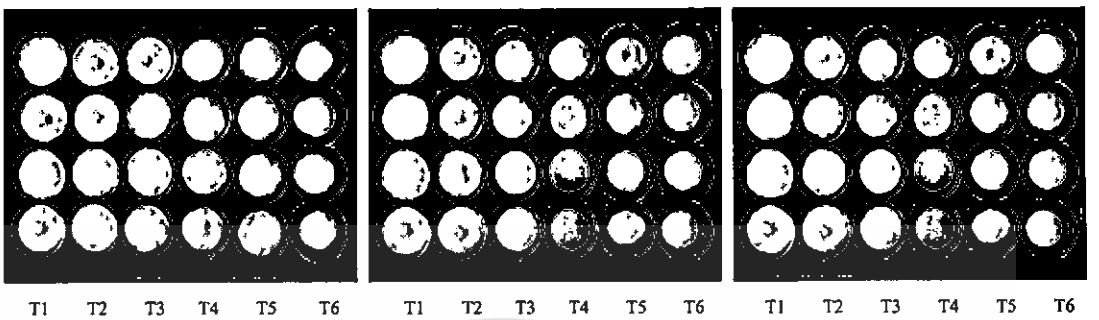

Fig. 4.83 Five-day-old colony of *C. coffeanum* on PDA containing crude extracts from *Clitocybe* spp. AJ2-2 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right-Crude MeOH.

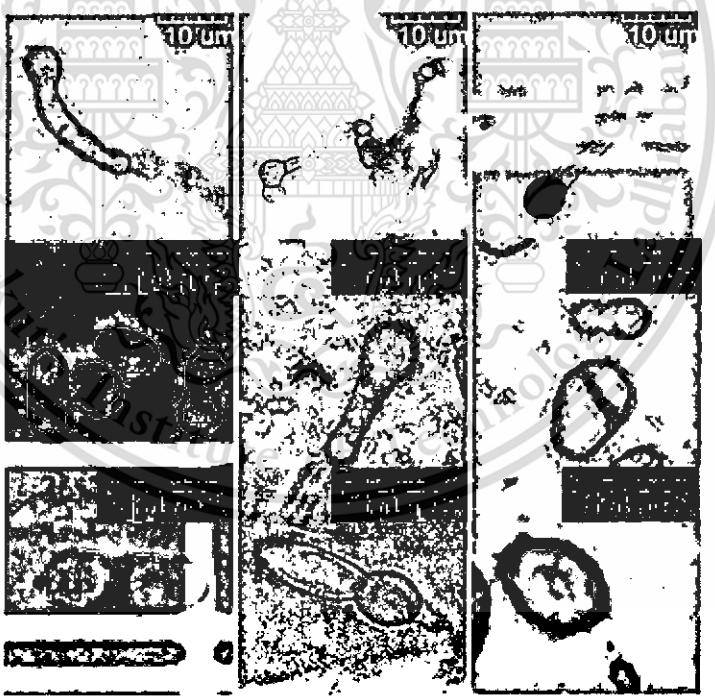

Fig. 4.84 Abnormal mycelia and spore of *Colletotrichum coffaenum* on PDA with crude MeOH from *Clitocybe* spp. AJ2-2 at the concentration of 1,000 µg/ml.

Table 4.8 Crude extracts of *Boletus affinis* var. *maculosus* AJ2-3 testing for growth inhibition of *Colletotrichum coffaenum* at 5 days.

Crude extracts	Concentration (µg/ml)	Colony diameter (cm)[1]	Growth inhibition(%)[2]
Crude Hex	0	5.00^{a}	0.00^{h}
	10	4.80^{bc}	4.00^{fg}
	50	4.72^{cd}	5.50^{fg}
	100	4.40^{e}	12.00^{e}
	500	4.15^{gh}	17.00^{cd}
	1000	3.82^{i}	23.50^{b}
Crude EtOAc	0	4.92^{ab}	0.00^{h}
	10	4.20^{fg}	14.72^{de}
	50	4.17^{fgh}	15.23^{cde}
	100	4.05^{h}	17.77^{cd}
	500	3.70^{i}	23.86^{b}
	1000	3.27^{j}	33.53^{a}
Crude MeOH	0	4.97^{a}	0.00^{h}
	10	4.80^{bc}	3.53^{g}
	50	4.62^{d}	7.03^{f}
	100	4.30^{ef}	12.06^{e}
	500	4.30^{ef}	12.06^{e}
	1000	4.07^{gh}	18.34^{c}
C.V.(%)		2.17	13.87

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.9 Spore production inhibition of crude extracts from *Boletus affinis* var. *maculosus* AJ2-3 to *Colletotrichum coffaenum* at 30days and effective dose (ED_{50}) values.

Crude extracts	Concentration (µg/ml)	Number of spores [1] (10^6)	Inhibition(%)[2]	ED_{50}
Crude Hexane	0	1.56[a]	0.00[b]	75.19
	10	1.13[cde]	27.98[ab]	
	50	0.75[de]	51.78[ab]	
	100	0.69[de]	55.95[ab]	
Crude EtOAc	0	1.56[a]	0.00[b]	7.66
	10	0.50[e]	67.86[a]	
	50	0.50[e]	67.86[a]	
	100	0.50[e]	67.86[a]	
Crude MeOH	0	1.56[a]	0.00[b]	267.66
	10	1.50[bc]	3.57[ab]	
	50	1.25[cd]	19.64[ab]	
	100	0.50[e]	67.86[a]	
C.V.(%)		19.67	12.63	

[1]/Average of four replications, Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control.

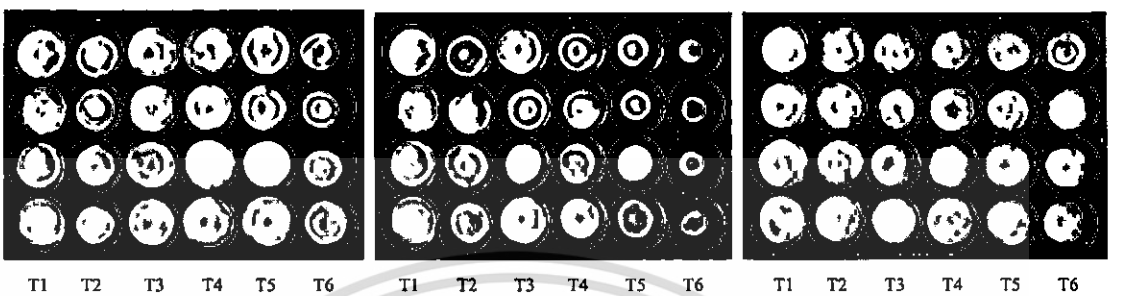

Fig.4.85 Five-day-old colony of *C. coffeanum* on PDA containing crude extracts from *B. affinis* var. *maculosus* AJ2-3 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH.

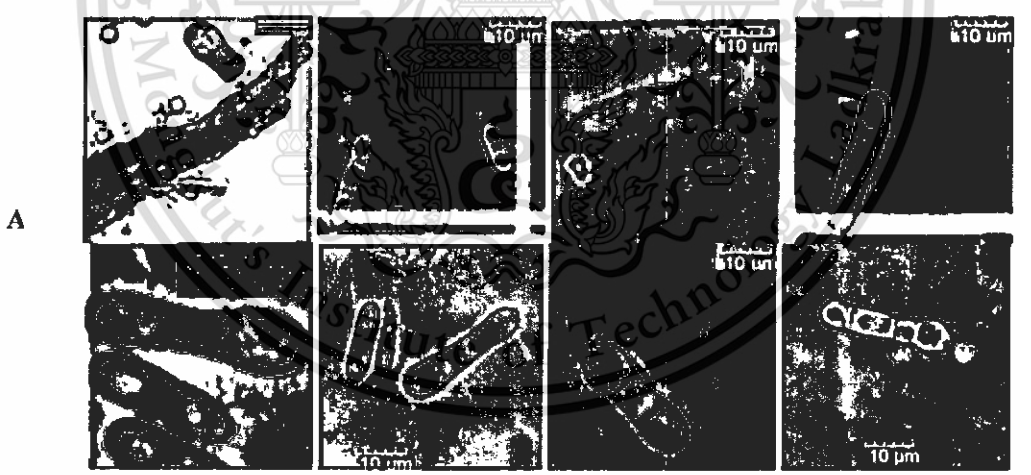

Fig.4.86 Abnormal mycelia and spore of *C. coffeanum* on PDA with crude ethyl acetate from *B. affinis* var. *maculosus* AJ2-3 at the concentration of 1,000 µg/ml. Bar.A=100 µm.

Table 4.10 Crude extracts of *Lactarius* spp. CH3-01 testing for growth inhibition of *Colletotrichum coffaenum* at 5 days.

Crude extracts	**Concentration (µg/ml)**	**Colonydiameter (cm)**[1]	**Growth inhibition(%)**[2]
Crude Hex	0	5.00[a]	0.00[m]
	10	4.93[b]	1.50[l]
	50	4.50[c]	10.00[k]
	100	4.30[e]	14.00[i]
	500	2.10[k]	58.00[c]
	1000	1.80[l]	64.00[b]
Crude EtOAc	0	5.00[a]	0.00[m]
	10	4.40[d]	12.00[j]
	50	4.30[e]	14.00[i]
	100	2.20[j]	56.00[d]
	500	2.10[k]	58.00[c]
	1000	1.80[l]	64.00[a]
Crude MeOH	0	5.00[a]	0.00[m]
	10	4.20[f]	16.00[h]
	50	4.10[g]	18.00[g]
	100	3.60[h]	28.00[f]
	500	3.40[i]	32.00[e]
	1000	1.20[m]	76.00[a]
C.V.(%)		0.64	1.56

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.11 Spore production inhibition of crude extracts from *Lactarius* spp. CH3-01 to *Colletotrichum coffaenum* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (µg/ml)	Number of spores [1] (10^6)	Inhibition(%)[2]	ED_{50}
Crude Hexane	0	2.43[a]	0.00[l]	1621.32
	10	2.35[b]	3.80[k]	
	50	2.12[c]	12.76[j]	
	100	2.01[d]	17.28[i]	
	500	1.59[f]	34.57[g]	
	1000	1.43[h]	41.15[e]	
Crude EtOAc	0	2.43[a]	0.00[l]	710.45
	10	2.00[d]	17.70[i]	
	50	2.00[d]	17.70[i]	
	100	1.44[h]	40.74[bc]	
	500	1.36[i]	44.03[d]	
	1000	1.00[k]	58.85[b]	
Crude MeOH	0	2.43[a]	0.00[l]	98.66
	10	1.75[e]	27.98[h]	
	50	1.53[g]	37.04[f]	
	100	1.21[j]	50.21[c]	
	500	1.19[j]	51.23[c]	
	1000	0.58[l]	76.13[a]	
C.V.(%)		0.56	36.33	

[1]/Average of four replications, Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

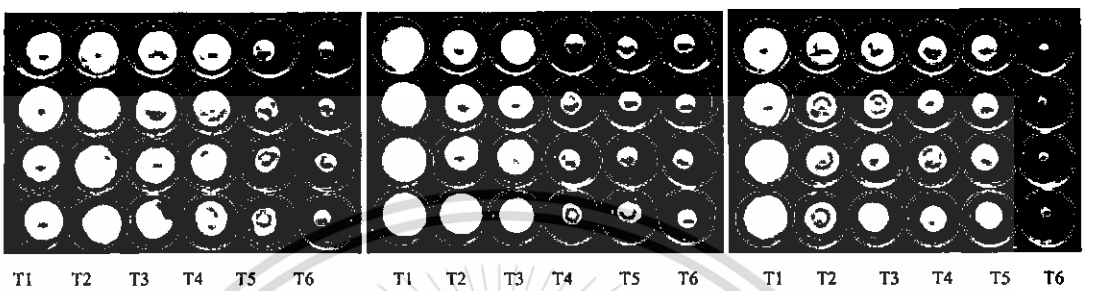

Fig.4.87 Five-day-old colony of *C. coffeanum* on PDA containing crude extracts from *Lactarius* spp. CH3-01 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH.

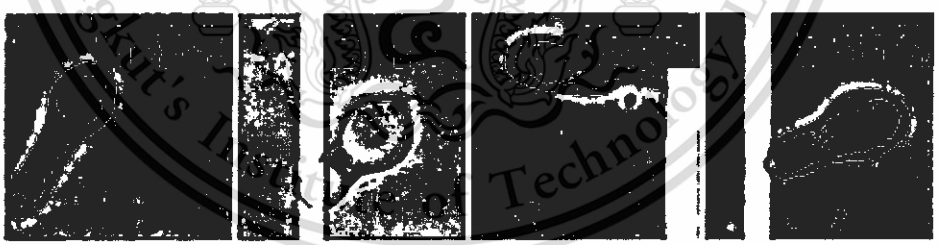

Fig.4.88 Abnormal mycelia and spore of *C. coffeanum* on PDA with crude MeOH from *Lactarius* spp. CH3-01 at the concentration of 1,000 µg/ml.

4.5.2 Bioactivity against Fusarium Wilt Causing by *F. oxysporum* f. sp. *lycopersici* NKSC02 race 2.

The crude extracts from *Leucocoprinus fragilissimus* (PH06), *Collybia strictipes* (PH07), *Clitocybe* spp. (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01), *Lactarius* spp. (CH3-27) were selected for bioactivity test against fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* race 2.

Results showed that Ethyl acetate crude extract from *Leucocoprinus fragilissimus* PH06 gave significantly highest inhibition of 75% for the colony growth of *F. oxysporum* f. sp. *lycopersici* race 2 at the concentration of 1,000 µg/ml when compared to the control (Table 4.12). Ethyl acetate crude extract from *Leucocoprinus fragilissimus* PH06 gave significantly highest inhibition of the spore production of *F. oxysporum* f. sp. *lycopersici* race 2 at 83.06% at the concentration of 1,000 µg/ml which the ED_{50} values was 52.78 µg/ml (Table 4.13) and showed abnormal features of hyphae and spore (Fig. 4.90), followed by crude methanol inhibited 75.21% and crude hexane which inhibited 67.97% (Tables 4.13).

Hexane crude extract from *Collybia strictipes* PH07 gave significantly highest inhibition of 52% for the colony growth of *F. oxysporum* f. sp. *lycopersici* race 2 at the concentration of 1,000 µg/ml when compared to the control (Table 4.14). Ethyl acetate crude extract from *Collybia strictipes* PH07 gave significantly highest inhibition of the spore production of *F. oxysporum* f. sp. *lycopersici* race 2 at 69.41% at the concentration of 1,000 µg/ml which the ED_{50} values was 79.42 µg/ml (Table 4.15) and showed abnormal features of hyphae and spore (Fig. 4.92), followed by crude hexane which inhibited 55.91% and crude methanol inhibited 54.80% (Tables 4.15).

Ethyl acetate crude extract from *Clitocybe* spp AJ2-2 gave significantly highest inhibition of 27% for the colony growth of *F. oxysporum* f. sp. *lycopersici* race 2 at the concentration of 1,000 µg/ml when compared to the control (Table 4.16). Ethyl acetate crude extract from *Clitocybe* spp. AJ2-2 gave significantly highest inhibition of the spore production of *F. oxysporum* f. sp. *lycopersici* race 2 at 83.90% at the concentration of 1,000 µg/ml which the ED_{50} values was 17.54 µg/ml (Table 4.17)and showed abnormal features of hyphae and spore (Fig.4.94), followed by crude methanol which inhibited 77.68% and crude hexane inhibited 68.95% (Tables 4.17).

The ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 35.50% for the colony growth of *F. oxysporum* f. sp. *lycopersici* race 2 at the

concentration of 1,000 µg/ml when compared to the control (Table 4.18). Ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of the spore production of *F. oxysporum* f. sp. *lycopersici* race 2 at 79.71% at the concentration of 1,000 µg/ml which the ED_{50} values was 59.85 µg/ml (Table 4.19) and showed abnormal features of hyphae and spore (Fig. 4.96), followed by crude hexane which inhibited 76.91% and crude methanol inhibited 64.36% (Table 4.19).

Crude methanol from *Lactarius* spp. CH3-01 gave significantly highest inhibition of 59.00% for the colony growth of *F. oxysporum* f. sp. *lycopersici* race 2 at the concentration of 1,000 µg/ml when compared to the control (Table 4.20). Ethyl acetate crude extract from *Lactarius* spp. CH3-01 gave significantly highest inhibition of the spore production of *F. oxysporum* f. sp. *lycopersici* race 2 at 83.95% at the concentration of 1,000 µg/ml which the ED_{50} values was 3.79 µg/ml (Table 4.21) and showed abnormal features of hyphae and spore (Fig. 4.98), followed by crude hexane which inhibited 83.36% and crude methanol inhibited 76.31% (Tables 4.21).

Ethyl acetate crude extract from *Lactarius* spp. CH3-27 gave significantly highest inhibition of 80% for the colony growth of *F. oxysporum* f. sp. *lycopersici* race 2 at the concentration of 1,000 µg/ml when compared to the control (Table 4.22). Ethyl acetate crude extract from *Lactarius* spp. CH3-27 gave significantly highest inhibition of the spore production of *F. oxysporum* f. sp. *lycopersici* race 2 at 97.88% at the concentration of 1,000 µg/ml which the ED_{50} values was 1.81µg/ml (Table 4.23)and showed abnormal features of hyphae and spore (Fig. 4.100), followed by crude hexane which inhibited 86.82% and crude methanol inhibited 73.55% (Tables 4.23).

Table 4.12 Crude extracts of *Leucocoprinus fragilissimus* PH06 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 5 days.

Crude extracts	Concentration (μg/ml)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	5.00^{a}	0.00^{j}
	10	4.35^{bc}	13.00^{hi}
	50	4.15^{d}	17.00^{g}
	100	4.10^{d}	18.00^{g}
	500	4.10^{d}	18.00^{g}
	1000	3.52^{e}	29.50^{g}
Crude EtOAc	0	5.00^{a}	0.00^{j}
	10	4.02^{d}	19.50^{g}
	50	3.38^{f}	32.50^{e}
	100	3.02^{g}	39.50^{d}
	500	2.32^{i}	53.50^{b}
	1000	1.22^{j}	75.00^{a}
Crude MeOH	0	5.00^{a}	0.00^{j}
	10	4.48^{b}	10.50^{i}
	50	4.30^{c}	14.00^{h}
	100	4.10^{d}	18.00^{g}
	500	3.07^{g}	38.5^{d}
	1000	2.55^{h}	49.00^{c}
C.V.(%)		1.80	5.43

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.13 Spore production inhibition of crude extracts from *Leucocoprinus fragilissimus* PH06 to *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 7days and effective dose (ED_{50}) values.

Crude extracts	Concentration (µg/ml)	Number of spores [1] (10^6)	Inhibition(%)[2]	ED_{50}
Crude Hexane	0	84.13[a]	0.00[g]	529.09
	10	78.56[a]	6.57[fg]	
	50	71.31[ab]	13.94[f]	
	100	50.00[cd]	39.54[d]	
	500	42.44[d]	48.89[cd]	
	1000	26.63[ef]	67.97[b]	
Crude EtOAc	0	84.13[a]	0.00[g]	52.78
	10	61.00[bc]	28.12[e]	
	50	45.88[d]	44.57[cd]	
	100	37.88[de]	54.28[c]	
	500	19.13[f]	77.13[ab]	
	1000	14.06[f]	83.06[a]	
Crude MeOH	0	84.13[a]	0.00[g]	99.94
	10	78.44[a]	6.01[fg]	
	50	70.31[ab]	15.16[f]	
	100	41.38[de]	50.09[cd]	
	500	38.38[de]	53.72[c]	
	1000	20.63[f]	75.21[ab]	
C.V.(%)		15.57	21.83	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

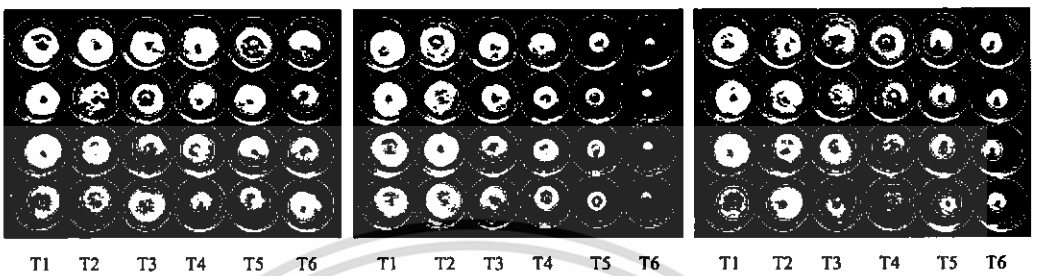

Fig.4.89 Five-day-old colony of *F. oxysporum* on PDA containing crude extracts from *Leucocoprinus fragilissimus* PH06 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH.

Fig.4.90 Abnormal spores of *F. oxysporum* on PDA with crude EtOAc from *Leucocoprinus fragilissimus* PH06 at the concentration of 1,000 µg/ml.

Table 4.14 Crude extracts of *Collybia strictipes* PH07 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 5 days.

Crude extracts	Concentration (µg/ml)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	5.00[a]	0.00[j]
	10	5.00[a]	0.00[j]
	50	5.00[a]	0.00[j]
	100	5.00[a]	0.00[j]
	500	3.75[e]	25.00[f]
	1000	2.40[j]	52.00[a]
Crude EtOAc	0	5.00[a]	0.00[j]
	10	4.15[c]	17.50[h]
	50	3.93[d]	21.50[g]
	100	3.02[f]	39.50[e]
	500	2.90[g]	42.00[d]
	1000	2.60[i]	48.00[b]
Crude MeOH	0	5.00[a]	0.00[j]
	10	5.00[a]	0.00[j]
	50	5.00[a]	0.00[j]
	100	4.30[b]	14.00[i]
	500	2.90[g]	42.00[d]
	1000	2.70[h]	46.00[c]
C.V.(%)		1.14	4.79

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.15 Spore production inhibition of crude extracts from *Collybia strictipes* PH07 to *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 7days and effective dose (ED_{50}) values.

Crude extracts	Concentration (µg/ml)	Number of spores [1](10^6)	Inhibition(%)[2]	ED_{50}
	0	190.18[a]	0.00[f]	
	10	157.31[b]	17.30[e]	
Crude Hexane	50	139.81[c]	26.47[de]	717.66
	100	125.69[cde]	33.89[cd]	
	500	116.25[e]	38.81[c]	
	1000	83.81[fg]	55.91[b]	
	0	190.18[a]	0.00[f]	
Crude EtOAc	10	128.00[cde]	32.60[cd]	
	50	120.13[de]	36.80[cd]	79.42
	100	76.94[fg]	59.54[b]	
	500	68.56[gh]	63.96[ab]	
	1000	58.19[fh]	69.41[a]	
Crude	0	190.18[a]	0.00[f]	
MeOH	10	134.81[cd]	28.19[cd]	
	50	133.13[cd]	28.96[cd]	732.21
	100	115.81[e]	28.96[cd]	
	500	84.75[f]	38.08[c]	
	1000	71.38[fgh]	54.80[b]	
C.V.(%)		6.44	14.72	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control.

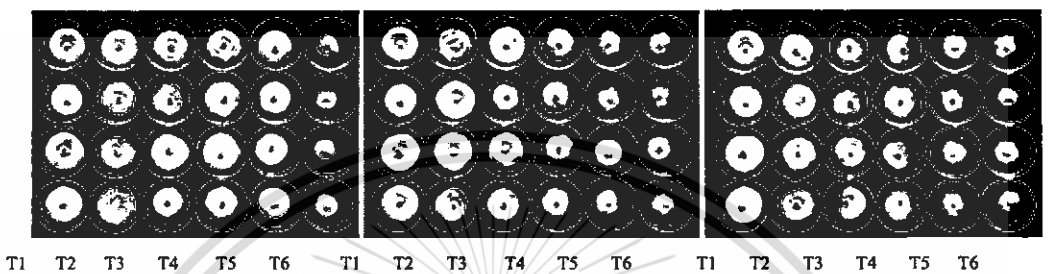

Fig. 4.91 Five-day-old colony of *F. oxysporum* on PDA containing crude extracts from *Collybia strictipes* PH07 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 μg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH.

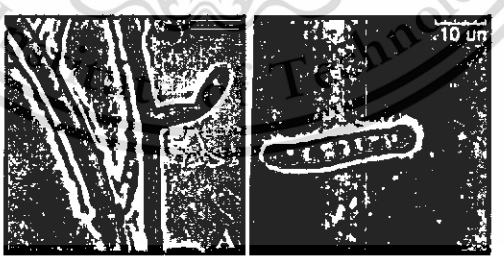

Fig.4.92 Abnormal mycelia and spore of *F. oxysporum* on PDA with crude EtOAc from *Collybia strictipes* PH07 at the concentration of 1,000 μg/ml. Bar. A =100 μm.

Table 4.16 Crude extracts of *Clitocybe* spp. Aj2-2 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 5 days.

Crude extracts	Concentration (µg/ml)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	5.00^{a}	0.00^{j}
	10	5.00^{a}	0.00^{j}
	50	5.00^{a}	0.00^{j}
	100	4.90^{a}	2.00^{i}
	500	4.80^{b}	4.00^{h}
	1000	4.62^{c}	7.50^{g}
Crude EtOAc	0	5.00^{a}	0.00^{j}
	10	4.35^{de}	13.00^{ef}
	50	4.27^{ef}	14.50^{de}
	100	4.20^{f}	16.00^{d}
	500	4.10^{g}	18.00^{c}
	1000	3.65^{i}	27.00^{a}
Crude MeOH	0	5.00^{a}	0.00^{j}
	10	4.40^{d}	12.00^{f}
	50	4.30^{ef}	14.00^{e}
	100	4.10^{g}	18.00^{c}
	500	4.00^{h}	20.00^{b}
	1000	4.00^{h}	20.00^{b}
C.V.(%)		1.12	9.68

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.17 Spore production inhibition of crude extracts from *Clitocybe* spp. AJ2-2 to *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 7days and effective dose (ED_{50}) values.

Crude extracts	Concentration (μg/ml)	Number of spores[1] (10^6)	Inhibition(%)[2]	ED_{50}
Crude Hexane	0	132.63[a]	0.00[i]	500.21
	10	114.75[b]	13.47[h]	
	50	107.81[b]	18.72[h]	
	100	88.88[c]	33.01[g]	
	500	72.19[de]	45.58[f]	
	1000	41.19[h]	68.95[c]	
Crude EtOAc	0	132.63[a]	0.00[i]	17.54
	10	67.25[de]	49.30[ef]	
	50	56.31[fg]	57.54[d]	
	100	55.50[fg]	58.15[d]	
	500	42.00[h]	68.36[c]	
	1000	21.38[ij]	83.90[a]	
Crude MeOH	0	132.63[a]	0.00[i]	26.43
	10	75.25[d]	43.32[f]	
	50	63.50[ef]	52.11[de]	
	100	48.3[gh]	52.11[de]	
	500	29.63[i]	63.59[c]	
	1000	16.88[j]	77.68[b]	
C.V.(%)		6.32	7.14	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control.

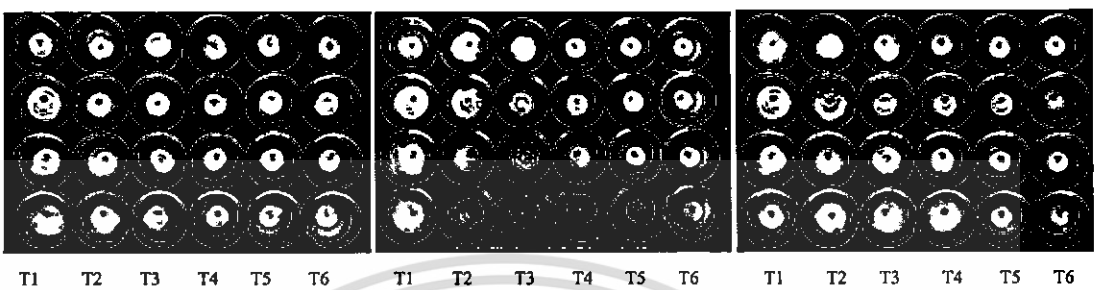

Fig. 4.93 Five-day-old colony of *F. oxysporum* on PDA containing crude extracts from *Clitocybe* spp. AJ2-2 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH.

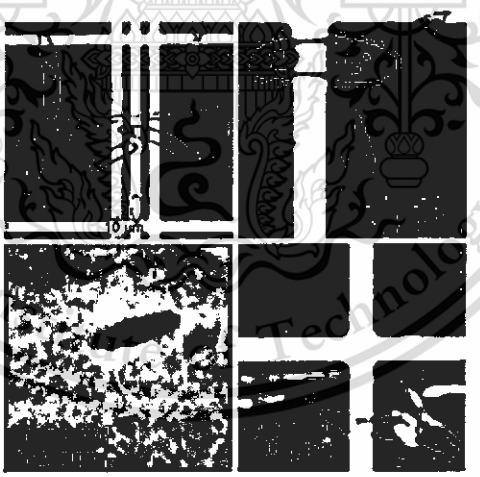

Fig. 4.94 Abnormal spores of *F. oxysporum* on PDA with crude EtOAc from *Clitocybe* spp. AJ2-2 at the concentration of 1,000 µg/ml.

Table 4.18 Crude extracts of *Boletus affinis* var. *maculosus* AJ2-3 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 5 days.

Crude extracts	Concentration (μg/ml)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	5.00[a]	0.00[d]
	10	5.00[a]	0.00[d]
	50	5.00[a]	0.00[d]
	100	5.00[a]	0.00[d]
	500	5.00[a]	0.00[d]
	1000	5.00[a]	0.00[d]
Crude EtOAc	0	5.00[a]	0.00[d]
	10	5.00[a]	0.00[d]
	50	5.00[a]	0.00[d]
	100	4.45[b]	11.00[c]
	500	4.25[c]	15.05[b]
	1000	3.22[d]	35.50[a]
Crude MeOH	0	5.00[a]	0.00[d]
	10	5.00[a]	0.00[d]
	50	5.00[a]	0.00[d]
	100	5.00[a]	0.00[d]
	500	5.00[a]	0.00[d]
	1000	5.00[a]	0.00[d]
C.V.(%)		1.12	0.83

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.19 Spore production inhibition of crude extracts from *Boletus affinis* var. *maculosus* AJ2-3 to *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 7 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (µg/ml)	Number of spores /1 (10^6)	Inhibition(%) /2	ED_{50}
Crude Hexane	0	83.00 a	0.00 i	151.44
	10	71.75 b	13.44 h	
	50	53.81 d	35.11 f	
	100	44.13 e	46.85 e	
	500	32.50 fg	60.89 bc	
	1000	19.25 h	76.91 a	
Crude EtOAc	0	83.00 a	0.00 i	59.85
	10	44.94 e	45.90 e	
	50	41.50 e	49.84 de	
	100	39.13 ef	52.86 de	
	500	28.63 g	65.50 b	
	1000	16.88 h	79.71 a	
Crude MeOH	0	83.00 a	0.00 i	131.90
	10	65.13 bc	21.45 g	
	50	59.06 cd	28.79 f	
	100	38.19 ef	28.79 f	
	500	29.63 g	54.29 cd	
	1000	19.50 h	64.36 b	
C.V.(%)		3.05	7.79	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control.

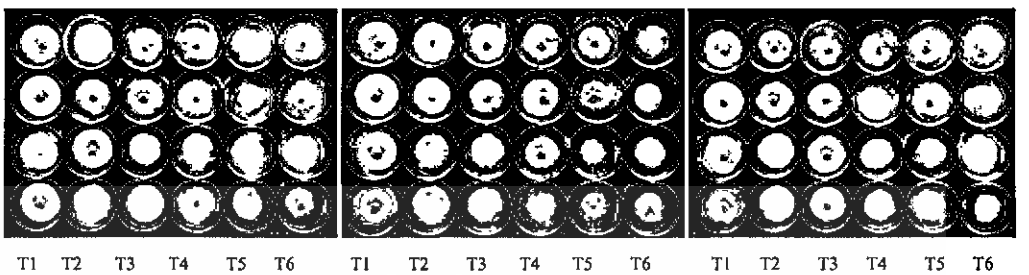

Fig. 4.95 Five-day-old colony of *F. oxysporum* on PDA containing crude extracts from *B. affinis* var. *maculosus* AJ2-3 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH.

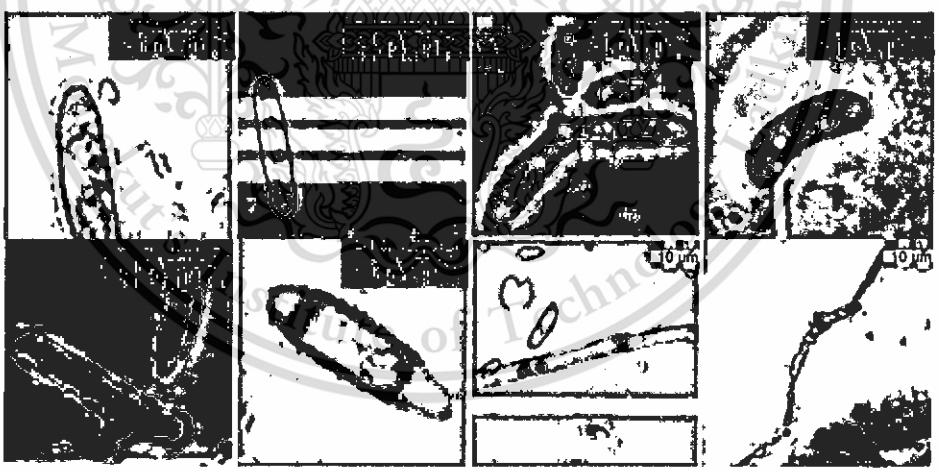

Fig.4.96 Abnormal spores of *F. oxysporum* on PDA with crude EtOAc from *B. affinis* var. *maculosus* AJ2-3 at the concentration of 1,000 µg/ml.

Table 4.20. Crude extracts of CH3-01 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 5 days.

Crude extracts	Concentration (µg/ml)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	5.00^{a}	0.00^{j}
	10	4.07^{c}	18.50^{h}
	50	4.02^{cd}	19.50^{gh}
	100	3.88^{d}	22.50^{g}
	500	2.80^{g}	44.00^{d}
	1000	2.65^{gh}	47.00^{cd}
Crude EtOAc	0	5.00^{a}	0.00^{j}
	10	4.02^{cd}	19.50^{gh}
	50	3.70^{e}	26.00^{f}
	100	3.02^{f}	39.50^{e}
	500	2.75^{g}	45.00^{d}
	1000	2.52^{hi}	49.50^{bc}
Crude MeOH	0	5.00^{a}	0.00^{j}
	10	4.65^{b}	7.00^{i}
	50	4.05^{c}	19.00^{h}
	100	3.95^{cd}	21.00^{gh}
	500	2.42^{i}	51.50^{b}
	1000	2.05^{j}	59.00^{a}
C.V.(%)		2.1539	5.78

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.21. Spore production inhibition of crude extracts from *Lactarius* spp. CH3-01 to *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 7 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (µg/ml)	Number of spores [1] (10^6)	Inhibition(%) [2]	ED_{50}
	0	57.13[a]	0.00[g]	
	10	28.86[bc]	49.04[e]	
Crude Hexane	50	23.56[cde]	58.30[d]	17.47
	100	25.73[bcd]	53.78[de]	
	500	12.88[f]	77.31[abc]	
	1000	9.63[f]	83.36[a]	
	0	57.13[a]	0.00[g]	
Crude EtOAc	10	26.18[bc]	54.57[de]	
	50	17.25[def]	69.76[c]	3.79
	100	12.88[f]	77.12[abc]	
	500	11.44[f]	79.64[ab]	
	1000	9.06[f]	83.95[a]	
Crude	0	57.13[a]	0.00[g]	
MeOH	10	34.06[b]	39.79[f]	
	50	22.31[cde]	60.31[d]	19.37
	100	16.38[ef]	60.31[d]	
	500	13.38[f]	70.93[bc]	
	1000	9.06[f]	76.31[abc]	
C.V.(%)		17.12	7.89	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control.

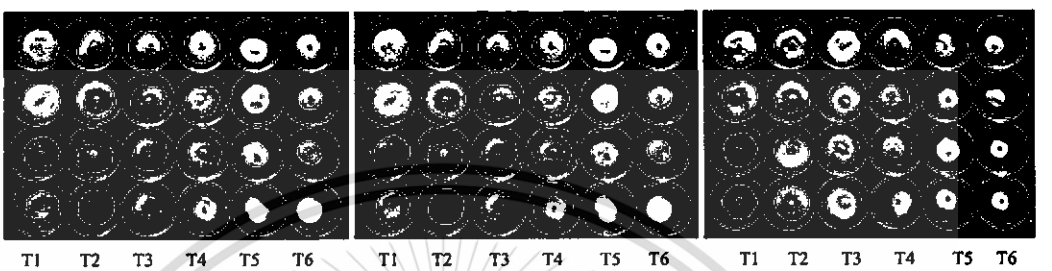

Fig. 4.97 Five-day-old colony of *F. oxysporum* on PDA containing crude extracts from *Lactarius* spp CH3-01 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH.

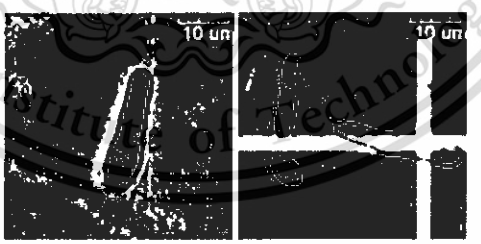

Fig. 4.98 Abnormal spore of *F. oxysporum* on PDA with crude EtOAc from *Lactarius* spp. CH3-01 at the concentration of 1,000 µg/ml.

Table 4.22. Crude extracts of *Lactarius* spp. CH3-27 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 5 days.

Crude extracts	**Concentration (µg/ml)**	**Colonydiameter (cm)**[1]	**Growth inhibition(%)**[2]
Crude Hexane	0	5.00[a]	0.00[k]
	10	5.00[a]	0.00[k]
	50	5.00[a]	0.00[k]
	100	5.00[a]	0.00[k]
	500	3.75[g]	25.00[e]
	1000	1.70[j]	66.00[c]
Crude EtOAc	0	5.00[a]	0.00[k]
	10	4.60[b]	8.00[j]
	50	4.10[e]	18.00[g]
	100	4.00[f]	20.00[f]
	500	2.95[h]	41.00[d]
	1000	1.00[k]	80.00[a]
Crude MeOH	0	5.00[a]	0.00[k]
	10	4.60[b]	8.00[j]
	50	4.307[c]	14.00[i]
	100	4.20[d]	16.00[h]
	500	2.93[b]	41.50[d]
	1000	1.50[j]	70.00[b]
C.V.(%)		0.77	2.62

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.23 Spore production inhibition of crude extracts from *Lactarius* spp. CH3-27 to *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 7days and effective dose (ED_{50}) values.

Crude extracts	Concentration (µg/ml)	Number of spores [1] (10^6)	Inhibition (%) [2]	ED_{50}
Crude Hexane	0	48.93[a]	0.00[l]	143.56
	10	42.31[b]	13.16[k]	
	50	31.25[d]	35.84[i]	
	100	27.16[d]	44.14[h]	
	500	20.31[e]	58.30[g]	
	1000	6.43g[h]	86.82[bc]	
Crude EtOAc	0	48.93[a]	0.00[l]	1.81
	10	14.31[f]	70.61[f]	
	50	10.56[fg]	78.50[de]	
	100	8.00[fgh]	83.58[cd]	
	500	4.31[hi]	91.25[b]	
	1000	1.06[i]	97.88[a]	
Crude MeOH	0	48.93[a]	0.00[l]	97.96
	10	38.43[bc]	21.10[j]	
	50	35.69[c]	26.75[j]	
	100	22.56[e]	26.75[j]	
	500	12.94[f]	53.70[g]	
	1000	4.69[hi]	73.55[cf]	
C.V.(%)		9.19	6.44	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control.

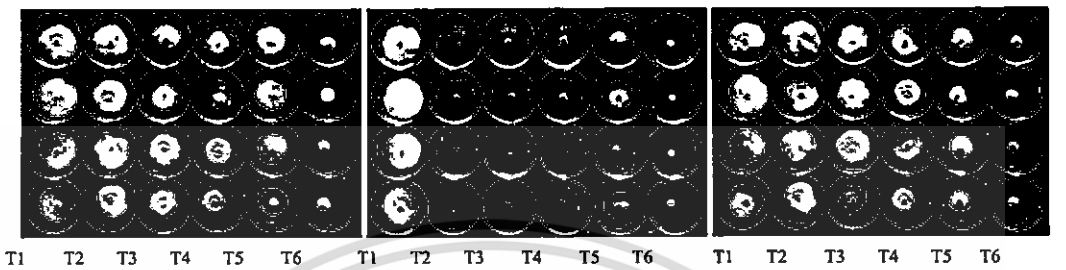

Fig. 4.99 Five-day-old colony of *F. oxysporum* on PDA containing crude extracts from *Lactarius* spp. CH3-27 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH.

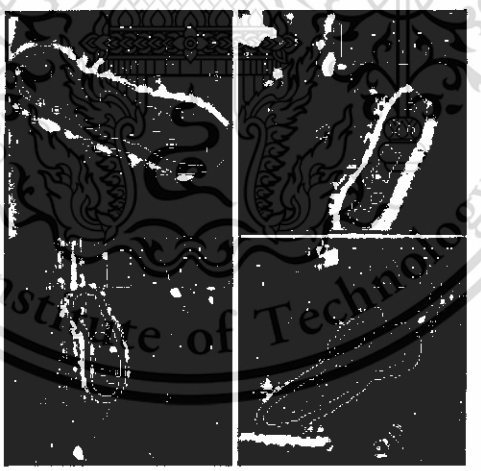

Fig. 4.100 Abnormal spores of *F. oxysporum* on PDA with crude EtOAc from *Lactarius* spp. CH3-27 at the concentration of 1,000 µg/ml.

CHAPTER 5

DISCUSSION

All sixty samples were collected in Thailand. With this, there were some literature reviews found those species in Thailand (Akom, 1996; David and Brian, 1992; Gary, 1981; Soytong, 1994; Konemann, 1998; Smith, 2001; Roger, 1991; States, 2004; Susan and Van, 2000). The surroundings mostly near natural water reserviour, rich forest vegetation. As previously mentioned, many Agaricales have edible and medical effects. In this collection of *Agaricus macrosporus* was reported as ediable (David Pegler and Brian Spooner, 1992), *Pluerotus giganteus* was reported as nutritional value and *Termitomyces* spp. was reported as edible mushrooms which are considered as healthy food because their mineral content is higher than that of meat or fish and most vegetables, apart from their nutritional value mushrooms have potential medicinal benefits (Srivastava *et al.*, 2011). *Leucocoprinus fragilissimus* PH06, *Collybia strictipes* PH07, *Clitocybe* spp. AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, *Lactarius* spp. CH3-01 and *Lactarius* spp. CH3-27 were described which these species reported to be found in Thailand (Konemann, 1998; Roger, 1991; States, 2004; Susan and Van, 2000).

For CH3-13 (Fig.4.16), Cap 1.5 cm in diameter, flat with a raise center, smooth with a teeth-like margin ; Gill free, unequal, brown; Stem 1.1 x 0.3 cm, dirty white, smooth; Habitat scattered on humun. And for CH3-27 (Fig. 4.30), Cap 10 cm in diameter, flat with a white strongly depress in the center, reddish brown with lined, dark scales including the wavy margin; Gills decurrent, pink, close, equel; Stem 7 x 0.7 cm, dark brown, cylindrical, downy the part attach gills is red; Habitat grows singly in soil. PH02 (Fig. 4.32), A white-black mushroom, recognized by the flowered shaped cap. Cap 5.6 cm,white- black color, crack into flowerlike shaped, incurrent margin, no attachments; Gill free, pale-brown, Stem 5.5 x1cm, smooth, deep brown, black bottle, thick with a white membranous ring, easy to fall off, Flesh white, thick; Habitat scattered in the wet soid.. All of them, no relative reports for them, they are presumed to new species.

In this study, three kind of *Colletotrichum* spp. were isolated from Coffee. They were *C. gloeosporioides C. coffeanum* and *C. capsici.* Among them, *C. coffeanum* gave the highest virulent. In the report of Than *et al.* (2008), Twenty-nine isolates of *Colletotrichum* spp. were obtained from infected chilli fruits, three from infected mango fruits and six from infected

strawberry fruits, in Thailand that showed typical anthracnose symptoms were identified as *C. acutatum*, *C. capsici* and *C. gloeosporioide.* Pathogenicity tests with the three *Colletotrichum* species isolated from infected chilli fruits showed that all the isolates were pathogenic on the susceptible Thai elite cultivar Bangchang. This result proved that these three species of *Colletotrichum* were casual agents of anthracnose infection on chilli. Non-infection of the resistant genotype *C. chinense* PBC 932 by *C. capsici* and *C. gloeosporioides* reconfirmed the importance of the resistance in this genotype to the interspecific breeding programme (Pakdeevaraporn *et al.*, 2005). The anthracnose symptoms produced by all three isolates of *C. acutatum* in wound inoculated fruits of PBC 932 indicated that *C. acutatum* was pathogenic and could overcome the resistance, but infection could not occur in PBC 932 without wounding, demonstrating the role of the cuticle in host resistance. Wounding was noticed to greatly enhance the ability of *Colletotrichum* to cause disease (Pring *et al.*, 1995). Oh *et al.* (1999) also showed the importance of cuticular wax layers of green and red pepper fruits to infection by *C. gloeosporioides*, where a negative correlation was found between cuticle thickness and disease incidence. Plant breeders need to be aware of the potential of *C. acutatum* to be a major pathogen when developing new chilli cultivars for resistance to anthracnose disease. The fact that *C. acutatum* from strawberry was a pathogen of chilli confirmed numerous reports about the cross-infection potential among different species of *Colletotrichum* on a multitude of hosts (Freeman *et al.*, 1998). In contrast to cross-inoculation studies by Sanders and Korsten (2003), who showed that isolates of *C. gloeosporioides* from mango could produce symptoms on other hosts such as guava, chilli pepper and papaya, isolates of *C. gloeosporioides* from mango did not show any symptoms on inoculated chilli fruits in the present study. Although mango isolates of *C. gloeosporioides* were highly pathogenic when re-inoculated onto mango fruits (data not shown), it is unclear why no symptoms were produced on chilli fruits by the mango isolates. Further microscopic work is needed to examine the host reaction to initial infection by these pathogens. Despite the high levels of infection potential on detached fruits, it is not known whether isolates could pose a threat in the field, since the inoculation studies were carried out under optimal conditions to induce infection by the pathogen (Sanders and Korsten, 2003). Further studies with different inoculation tests and different stages of ripeness are needed to confirm these results.

In this study showed that V8 juice agar was the more suitable medium for sporulation of *C. coffeanum,* compared with the PDA agar but not significant. The content of the medium gave a significant impact on spore yield. Increase media content, also would greatly improve the

spore production. 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) was the most suitable light condition for *Colletotrichum coffeanmum* sporulatio. Slade *et al.* (1987) reported that the effect of spore inoculum density, medium concentration, and temperature on slime-spot formation,spore yield, and mycelium production by *Colletotrichum gloeosporioides* on agar media were studied with a simple microplate assay. A steady-state spore yield (spore-carrying capacity) independent of inoculum density was reached only on media that supported good fungal growth and sporulation. The spore-carrying capacity was reached earlier, the denser the inoculum. On standard mycological media a high inoculum density (2.5 $x10^6$ spores per ml) resulted in a slimy mass of conidia forming a slime spot, a phenomenon associated with greatly reduced mycelium formation and indicative of microcycle conidiation. In contrast, for a similar inoculum density, enhanced mycelial growth preceded sporulation and overrode slime-spot formation on highly concentrated media; a very low medium concentration resulted in much less mycelium, but spore production was also decreased. Exposure to suboptimal growth temperatures of 36 to 48°C for up to 8 days did not induce microcycle conidiation from inocula that did not form a slime spot at 28°C. Microcycle conidiation, as indicated by rapid development of a slime-covered colony after fungal spore inoculation of solid media, occurred on diverse commonly used microbial media but only under conditions of a high inoculum density (25, ul, 2.5 x 106 spores per ml).Microcycle conidiation was most pronounced on media highly favorable for fungal growth, such as V8 and RV8 (both of which contain 20% V8 juice) and PDA. However, increasing the V8 concentration beyond the normal 20% level caused microcycle conidiation to be replaced by dense vegetative mycelial development. These data are consistent with microcycle induction being a function of (i) diffusionrestricted nutrient availability to the fungal colony (resulting from nutrient demand by a rapidly developing high inoculum colony outstripping nutrient resupply to the colony by diffusion from the surrounding agar), (ii) spore densitydependent accumulation of microcycle-inducing fungal metabolites, or both. High nutrient concentrations in the media (e.g., >20% V8) would attenuate diffusion-restricted nutrient availability and might also override metabolite induction of microcycle conidiation. The critical nutrient factor promoting mycelial growth rather than microcycle conidiation at high inoculum densities is not simply organic content, since for similar inocula slime spots are formed on RV8 (56 g of organic matter liter-') and on 20% V8 (8 gliter-1) but are substantially reduced on 40% V8 (16 g liter-1) and are not formed at all on 60% V8 (24 g liter-1) (Slade *et al.* 1987).

In this study, *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2 gave the most virulent for tomatoes. In the report of Juliano C. da Silva and Wagner Bettiol, race 2 of *Fusarium oxysporum* f. sp. *lycopersici* isolates C21A, TO11 and TO245 were found to be pathogenic to the cultivar Viradoro at all inoculum concentrations tested , causing a drastic reduction of plant height. The isolate TO245 was the most virulent, causing the maximum diseases severity in plants grown in substrate infested with 10^6 and 10^5 conidia ml^{-1} of substrate. These results agree with those of Andrade and Micherref (2000), who demonstrated that tomato plants of different cultivars, inoculated with 10^6 conidia ml-1 of isolates C-1, C-7, C-21A, and F-23 of *F. oxysporum* f. sp. *lycopersici* race 2, showed a 50% disease incidence. He *et al.* (2002) also showed that 10^6 CFU g-1 soil of *F. oxysporum* f. sp. asparagi caused the death of asparagus plants

The research findings are reported for the first time that the metabolites from *Clitocybe* spp. AJ2-2, *B. affinis* var. *maculosus* AJ2-3 and *Lactarius* spp. CH3-01could inhibit *C. coffaenum* causing coffee anthracnose. Meanwhile, the metabolites from *Leucocoprinus fragilissimus* PH06, *Collybidia strictipes* PH07, *Clitocybe* spp. AJ2-2, *B. affinis* var. *maculosus* AJ2-3 and *Lactarius* spp. CH3-01, *Lactarius* spp. CH3-27 could inhibit fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* race 2. Similar report from Badalyan *et al.* (2002) stated that the antagonistic activity of 17 species of Basidiomycotina (*Coriolus versicolor*, *Flammulina velutipes*, *Ganoderma lucidum*, *Hypholoma fasciculare*, *H. sublateritium*, *Kühneromyces mutabilis*, *Lentinula edodes*, *Lentinus tigrinus*, *Pholiota alnicola*, *Ph. aurivella*, *Ph. destruens*, *P. ostreatus*, *P. cornucopiae*, *Polyporus squamosus*, *P. subarcularius*, *P. varius* and *Schizophyllum commune*) could inhibit plant pathogens, *Bipolaris sorokiniana*, *Fusarium culmorum*, *Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia cerealis* that causing foot and root diseases of winter cereals. The potential of fungal metabolites from fungi have been usually reported to produce antibiotic substances against human and plant pathogens. Sibounnavong, P. (2012) reported that *Emericella nidulans* isolate L01 developed as bio-agent formulation would be feasible controlled this tomato wilt in different tomato varieties where wilt incidence in the field and reported that crude methanol of *E. nidulans* isolate L01 at 1000 µg/ml significantly inhibited *F. oxysporum* f. sp. *lycopersici* 84.40%, and followed by crude ethyl acetate and crude hexane which were 64.40 and 60.28%, respectively. Crude methanol of *E. nidulans* isolate L01 expressed antifungal activity against *F. oxysporum* f. sp. *lycopersici* at the ED_{50} of 112 µg/ml, and followed by crude ethyl acetate and crude hexane which were 379 and 915 µg/ml, respectively. Kanokmedhakul *et al.* (2003) reported that a macrofungi, *Scleroderma citrinum* produces a

bioactive triterpenoid and vulpinic acid derivatives that expressed against *Candida albicans*. Morober, Soytong *et al.* (2014) reported that the natural products were isolated from the fruiting bodies of *Scleroderma citrinum*. A new lanostane-type steroids were found namely 4,4'-Dimethoxymethyl vulpinate (DMV) and 4,4'-Dimethoxyvulpinic acid (DMVA). These compounds showed that 4,4'-Dimethoxyvulpinic acid inhibited *Colletotrichum gloeosporioides* than 4,4'-Dimethoxymethyl vulpinate at all tested concentrations. The effective dose (ED_{50}) of DMVA compound could inhibit the mycelium growth of *C. gloeosporioides* at the concentrations of 81 μg/ml, respectively. The ED_{50} of DMV compound for inhibition of mycelial growth was 2,114 and 5,231 μg/ml, respectively. The production of conidia of *C. gloeosporioides* was inhibited by both compounds which the ED_{50} of DMA and DMVA compounds were 45 and 68 μg/ml, respectively. Rieger *et al* (2010) reported that pure culture of Basidiomycete, *Carpia montagnei* produced caripyrin as a new pyridylooxirane that inhibited *Magnaporthe oryzae* causing rice blast pathogen. These investigations were found biological active substances from *Clitocybe* spp. AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 to inhibit coffee anthracnose caused by *C. coffaenum*. The control mechanism would be involved in bioactive compound producing from these mushroom which possible be elucidated in further search finding.

CHAPTER 6

CONCLUSION

Sixty samples were collected in five provinces of six points in Thailand during the raining season from July to October, 2013. The most belong to Marasmiaceae, up to 17%, followed by Tricholomataceae and Mycenaceae.

Three isolates of *Colletotrichum* spp. were obtained from infected coffee leaves (Arabica), which collected from Bangkok provinces. They were *C. gloeosporioides C. coffeanum C. capsici*. *C. coffeanum* isolate gave the highest virulent for disease incidence and significantly differed from other isolates (P= 0.05) which the lesion size developed by was 27.25 mm, and followed by *C. gloeosporioides* isolate which the lesion size were 4.50 mm. The *C. capsici* isolatedid not develop any symptom when inoculated. The tomato seeding showed the yellowing and wilting leaves, plants die early.

The color of crude extracts from *Leucocoprinus fragilissimus* (PH06), *Collybia strictipes* (PH07), *Clitocybe* spp. (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01), *Lactarius* spp. (CH3-27) were pale yellow or dark brown or dark. Crude textures were oil, wax or solid. Crude MeOH extract from *Lactarius* spp. CH3-27 gave the highest yield (9.46 g) followed by crude EtOAc extract from *Lactarius* spp. CH3-27, crude hexane *Lactarius* spp. CH3-27, Crude MeOH extract from *Leucocoprinus fragilissimus* PH06, Crude MeOH extract from *Boletus affinis* var. *maculosus* AJ2-3, *T.* that gave yield of crude extract 7.69, 5.43, 5.06 and 4.87 g, respectively. The yields of crude extracts varied according to fungal isolates, dry weight of mycelial mats and kind of solvents.

In a variety of conditions affecting sporulation, the media which contained 10ml V8 jiuce gave the highest of spore production at 35days (up to 192.5 $x10^6$/ml). Through the cross-analysis of a variety conditions which affected the *Colletotrichum coffeanmum* sporulation, the result showed that V8 and PDA were suitable for *Colletotrichum coffeanmum* as the medium. Although these two medium had different effects for sporulation, V8 better promoted sporulation. But this advantage was not very significant. On the contrary, the content of the medium gave a significant impact on spore yield. Increase media content, also would greatly improve the spore production. 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) was the most

suitable light condition for *C. coffeanmum* sporulation, compared with constant darkness (continuous darkness; CD) and constant illumination (continuous light; CL; 10000 lux, fluorescent lamp). Although with the growth of time, spore production was also growing. However, the growth rate was the 28th day to reach the fastest. Summing up the appeal, choose 10 ml PDA medium and incubated 28 days under natural light conditions, was the best time to observe spore production.

Six promising Agaricales *Leucocoprinus fragilissimus* (PH06), *Collybidia strictipes* (PH07), *Clitocybe* spp. (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01), *Lactarius* spp. (CH3-27) were tested for their ability to control the growth of *C.coffeanum* and *Fusarium oxysporum* f. sp. *lycopersici*. It was found that *Clitocybe* spp. AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, *Lactarius* spp. CH3-01 gave the growth inhibition of *C.coffeanum* over 50%. Among them, crude extracts from *Clitocybe* spp. AJ2-2 gave the best result to inhibit the growth of *C. coffeanum*. The crude methanol extract from *Clitocybe* spp. AJ2-2 gave the highest growth inhibition of *C. coffeanum* up to 89.08% and the effective dose (ED_{50}) at 9.65 µg/ml. Whereas, crude ethyl acetate and crude hexan extracts gave the inhibition at 86.48% and 70.45% and the effective dose (ED_{50}) at 9.97 and 14.09 µg/ml., respectively. Followed by crude methanol, crude ethyl acetate and crude hexan from *Lactarius* spp. CH3-01 gave inhibit the growth of *C. coffeanum* at 76.13%, 58.85%, 41.15% and the effective dose (ED_{50}) at 98.66, 710.45, 1621.32 µg/ml, respectively. *Leucocoprinus fragilissimus* (PH06), *Collybidia strictipes* (PH07), *Clitocybe* spp. (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01), *Lactarius* spp. (CH3-27) gave the growth inhibition of *F. oxysporum* f. sp. *lycopersici* race 2 over 50%. Among them, crude extracts from *Lactarius* spp. CH3-27 gave the best result to inhibit the growth of *F. oxysporum* f. sp. *lycopersici* race 2. The crude ethyl acetate extract from *Lactarius* spp. CH3-27 gave the highest growth inhibition of *F. oxysporum* f. sp. *lycopersici* race 2 up to 97.88 % and the effective dose (ED_{50}) at 1.81 µg/ml. Whereas, crude hexane and crude ethyl acetate extracts gave the inhibition at 86.82% and 73.55% and the effective dose (ED_{50}) at 97.96 and 143.56 µg/ml., respectively. Followed by The crude ethyl acetate from *Lactarius* spp. CH3-01 gave the highest growth inhibition of *F. oxysporum* f. sp. *lycopersici* race 2 up to 83.95% and the effective dose (ED_{50}) at 3.79 µg/ml. Whereas, crude hexane and crude ethyl acetate extracts gave the inhibition at 83.36% and 76.31% and the effective dose (ED_{50}) at 17.47 and 19.37 µg/ml., respectively.

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APPENDIX

PUBLICATIONS

LuoYaling, Pongnak Wattanachai and Soytong, Kasem. 2013. "Survey collection, isolation and morphological identification of Agaricales."56-85. In 2nd International Conference on Integration of Science and Technology for Sustainable Development. Bangkok : King Mongkut's Institute of Technology Ladkrabang.

Luo Yaling, Pongnak Wattanachai and Soytong, Kasem. 2014. "Mushroom and mocrofungi collection for screening bioactivity of some species to inhibit coffee antharcnose caesed by *Colletotrichum coffeanum*." Journal of Agricultural Technology 10(4) : 845-861.

LuoYaling, Pongnak Wattanachai and Soytong, Kasem. 2014. "Survey collection, isolation and morphological identification of mushrooms in Chanthaburi Province of Thailand" pp. 73. In Proceeding of the 3rd International Conference on Integration of Science & Technology for Sustainable Development. 27-28 November, Champasack Grand Hotel, Pakse, Champasack, Lao PDR.

LuoYaling, Pongnak Wattanachai and Soytong, Kasem. 2015. "Two edible mushrooms' interaction against fusarium wilt which caused by *F. oxysporum* f. sp. *Lycopersici*." Journal of Agricultural Technology 11(2) : 539-550.

LuoYaling, Pongnak Wattanachai and Soytong, Kasem. 2015. "Effect of different factors on sporulation of *Colletotrichum coffeanum*." Journal of Agricultural Technology 11(4) : 997-1012.

AUTHOR BIOGRAPHY

Name	Miss Yaling Luo
Date of birth	4 June 1990
Nationality	Chinese
Address	Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang , Bangkok 10520, Thailand.
Telephone	090-3080205
Education	
2009-2013	Bachelor of Science in Agriculture (Plant Conservation), Southwest Forestry University, Kunming Yunnan, PR China.
2009-2013	Bachelor of Law (Minor), Southwest Forestry University, Kunming Yunnan, PR China.
2013 up to now	Master of Science, Agriculture, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL) , Bangkok, Thailand.
Awards	2010, Awardes as the "Excellent League member"; " Roll student" in Southwest forestry university. 2012, Awarded the "Government scholarships", "Roll student " in Yunnan province. 2013, Awarded as the"Outstanding graduates" in Yunnan Province.
Publications	**International Conference**

(1) 2013, 28-29 November, attended 2nd **International Conference on Integration of Science and Technology for Sustainable Development as** an oral presentation entitled *Survey collection, isolation and morphological*

identification of Agaricales, held at KMITL, Bangkok, Thailand.

(2) 2014, 27-28 November, attended 3rd **International Conference on Integration of Science and Technology for Sustainable Development** as an oral presentation entitled *Two edible mushrooms' interaction against fusarium wilt which caused by F. oxysporum f. sp. Lycopersici.*

(3) 2014, 27-28 November, attended 3rd **International Conference on Integration of Science and Technology for Sustainable Development** as a poster presentation entitled *Survey collection, isolation and morphological identification of mushrooms in Chanthaburi Province of Thailand* held at Champasack Grand Hotel, Pakse, Champasack, Lao PDR.

International Publication:

(1) LuoYaling, Pongnak Wattanachai and Soytong, Kasem. 2013. "Survey collection, isolation and morphological identification of Agaricales."56-85. In 2nd **International Conference on Integration of Science and Technology for Sustainable Development.** Bangkok : King Mongkut's Institute of Technology Ladkrabang.

(2) Luo Yaling, Pongnak Wattanachai and Soytong, Kasem. 2014. "Mushroom and mocrofungi collection for screening bioactivity of some species to inhibit coffee antharcnose caesed by *Colletotrichum coffeanum*." Journal of Agricultural Technology 10(4) : 845-861.

(3) LuoYaling, Pongnak Wattanachai and Soytong, Kasem. 2014. **"Survey collection, isolation and morphological identification of mushrooms in Chanthaburi Province of**

Thailand" pp. 73. In Proceeding of the 3rd **International Conference on Integration of Science & Technology for Sustainable Development**. 27-28 November, Champasack Grand Hotel, Pakse, Champasack, Lao PDR.

(4) LuoYaling, Pongnak Wattanachai and Soytong, Kasem. 2015. "Two edible mushrooms' interaction against fusarium wilt which caused by *F. oxysporum* f. sp. *Lycopersici*." Journal of Agricultural Technology 11(2) : 539-550.

(5) LuoYaling, Pongnak Wattanachai and Soytong, Kasem. 2015. "Effect of different factors on sporulation of *Colletotrichum coffeanum*." Journal of Agricultural Technology 11(4) : 997-1012.

PROCEEDINGS

AATSEA

The 2nd International Conference on

Integration of Science and Technology for Sustainable Development (ICIST 2013)

And

Annual Meeting of AATSEA

"BIOLOGICAL DIVERSITY, FOOD AND AGRICULTURAL TECHNOLOGY"

Faculty of Agricultural Technology
King Mungkut's Institute of Technology Ladkrabang
Bangkok, Thailand
November 28-29, 2013

ICIST 2013, KMITL, Bangkok, Thailand
November 28-29, 2013

Survey collection, isolation and morphological identification of Agaricales

Luo, Yaling*; Pongnak, Wattanachai [1]and Soytong, Kasem[1]

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang , Bangkok 10520, Thailand

The research findings in this study, all specimens were collected from different locations in Thailand which morphological identified into 32 species. As follows: *Agaricus macrospores*, *Tricholoma* spp, *Termitomyces* spp, *Pluerotus gigantean*, *Leucocoprinus fragilissimus*, *Collybidia strictipes* , *Marasmius* spp, *Coprinus* spp, *Marasmius spp*, *Collybia iocephala*, *Collybia spp*, *Mycena spp*. *Marasmius* spp, *Mycena* spp (SY03), *Mycena* spp (SY05), *Marasmiellus ramealis*, *Marasmiellus albuscorticis*, *Marasmius purpureostriatus*, *Russula spp*, *Russula crassotunicata* ,*Cantharellus cibarius*, *Inocybe fastigiata*, *Clitocybe spp* (AJ2-05), *Mycena vulgaris*, *Pleurocybella porrigens*, *Laccaria vinaceoavellanea*, *Clitocybe* spp, *Auricularia auriculara*, *Auricularia auricula* (SY14), *Schizophyllum commune*, *Boletus retisporus* and *Boletus affinis var. maculosus*.

Key words: Agaricales , edible mushroom

Introduction

Agaricales comprises the so-called mushrooms and toadstools, and is the largest clade of mushroom-forming fungi. More than 9000 species in more than 300 genera, and 26 families had been described. Mostly they are terrestrial, lignicolous and saprobic, and many are mycorrhizal (Kirk *et al.* 2001).

Agaricales belongs to Eumycota、Basidiomycotina Hymenomycetes、Homobasidiomycitidae (Alexopoulos and Mimx, 1979). Mycelium typically formed by the spores germinate primary hyphae, with primary anastomosis affinity forming secondary hyphae, no or a lock-like joint, with a septum and barrel bung hole cover. Some types of secondary mycelium may also be formed streptozotocin. Fruiting body fleshy, easily broken, rarely membranous or leathery. Typical fruiting bodies, including the cap, stipe, located below the cap gills or bacteria tube, located in the middle or upper part of stipe mushroom

* Corresponding author: e-mail: *iamalinluo@163.com

ring and base volva. Hymenium often in the initial stage of growth within the biofilm shedding covered completely exposed when ripe. No burden separated single spore spores, colorless or colored, its shape, size, color and ornamentation is an important basis for minutes (Arora, 1986).

Agaricales including Pleurotaceae, Schizophyllaceae, Hygrophoraceae, Tricholomataceae, Amanitaceae, Pluteaceae, Agaricaceae, Coprinaceae, Bolbitiaceae, Strophariaceae, Cortinariaceae, Crepidotaceae, Entolomataceae, Paxillaceae, Gomphidiaceae, Boletacea, Russulaceae and others. *Agaricus* L. belonging to Agaricaceae. The majority of mushrooms are edible, medicinal or health care values. For example, *Agaricus bisporus* (Jelange) Imbach, occurs scaly mushrooms, *Agaricus crocopeplus* Berk, woodland mushrooms, *Agaricus silvaticus* Schaeff, large purple mushroom, *Agaricus augustus* Fr, white mushrooms, *Agricus bernardii* (Quél.) Sacc, big fat mushrooms, *Agaricus bitorquis* (Quél.) Sacc, and the four spore mushrooms as *Agaricus campestris* L. Which has been carried out in artificial cultivation in order for edible, *Agaricus subrufescens* Peck reported to do liquid fermentation and mycelia contains large amounts of polysaccharides and other biologically active substances (Genpei Yu and Jigui Bao, 2008), *Agaricus arvensis* Schaeff reported that involved in the human body's immune system regulating function has the good role in promoting, aroused people's great concern to the wild mushrooms. Brazil mushrooms *Agaricus blazei* Murr reported to be involved in lowering blood sugar, improved arteriosclerosis and cytotoxicity to some cancer cell lines (Xiaoping Luo & Junyan Wang 2007). The objective of this research project was preliminary conduced to survey, collect and identify Agaricales. Further research findings are to be biological activity tests againist some human and plant pathogens.

Methodology

Survey procedure

Collections were made in the forests and grass area in 5 provinces of Thailand. Samples were collected during the raining season. The collected sites and dates were as follows:-

1) 20th July 2013
   Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall), N 12°48′16″, E 102°6′53″.
2) 13th August 2013
   Chiangrai Province, Chiang Kong, N 20°15′36″, E 100°47′3″.
3) 17th August 2013,
   Phetchabuti Province,Amphoe Kaeng Krachan (Krating Waterfall)

N 12°54′27″, E 99°38′53″.

4) 3th September 2013

Kanchanaburi Province, Amphoe Mueang Kanchanaburi, N 14°0′12″, E 99°33′0″.

5)20th September 2013

Bangkok Province, Khet Lat Krabang(KMITL), N 13°43′24″, E 100°47′3″.

6) 20th September 2013

Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall) , N 14°6′56″,E 99°8′40″.

Collection of specimens

Materials to be used for collection as rulers, knifes, recording papers, pH meter, Thermometer, plastic bags, spore print paper (black and white A4 paper with glue bonded together and cut into different sizes), rubber bands, camera.

Collection was divided into five steps as follows;- searching, processing, photography, recording and maintain specimens.

1) Search for fresh speciments:- Different specimens found to be grown in different ways and conditions. For example, singly, scattered, grows in groups. Before the subsequent acquisition, the surrounding environment would be searched to see if there were other specimens exist; sometimes, young fruiting bodies wouldl be appeared under the soil, so in search of a specimen after the nearby soil under would be carefully observed.
2) Processing fresh specimens and did spore print:- get rid of the dirts on the specimens, such as soil mud, leaves, small insects. For too moist specimens, under the premise of without breaking each part for structure of specimens, towelled gently wipe excess water with tissue papers were done. In the case of over one specimen, taking one of them, with a knife from the cap center to the stalk base truncated to prepare for the subsequent observation. According to the diameter specimens stipe, along the central bond of spore print, A4 sheet of paper cut out just enough to stalk through the small hole. The stipe passed through the hole, the fold or more parts of gills remained at the other side of the paper. If necessary, gently shaked cap, basidiospores would fall off the gills. The time of waiting fallen basidiospores varied due to specimens, so foldded the part of paper which not beening covered by the cap and wrapped to protect basidiospores. Noted that the length of the spores to be slightly larger than

the printing paper three times the diameter of the cap, can the complete package, after falling basidiospores, then getting spore print. (Figure 1)

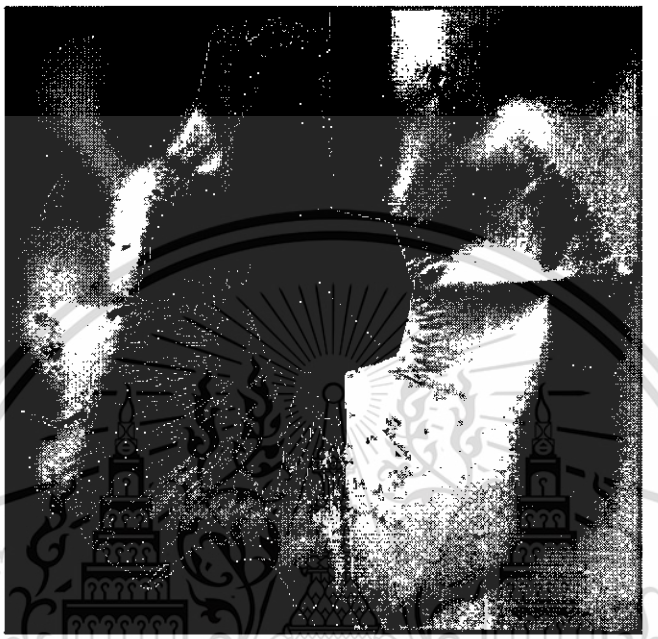


Figure. 1. Spore print

3) Photography fresh specimens:- Fresh specimens were photographed including the young and mature basidiocarps if them appeared. The front, side, rear of specimens, the attachment of stem, spore print also would be photographed. If some special features appeared such as the veil, appendages, volva and rooting base should be shot. Finally, each specimen in the shooting should place a ruler along to ensure the accuracy of the sample size.
4) Recording features-: Including two parts, the one was specimens characters and the other one was habitat. For the specimen characters, what should be noticed as shown in Table 1 and for habitat what should be noticed as shown in Table 2.

Table 1. Speciment characters

Structures	What should be noticed
Cap	Size; Shape; Color and color change upon bruising; Attachment; Margin;
Flesh	Thickness; Color and color change upon bruising; Odor; Taste(not all);
Gills or Tubes	Color; Attachment; Width; Size; Compactness; equeal or nor;
Ring or Veil	Existense; Color; Shape; Diameter; Location; Double or Single
Stipe	Shape; Color and color change upon bruising; Solid or Hollow; Width; Height; Attachment;
Vlova	Existense; Siza; Shape; Color;
Rooting base	Existense;
Spore	Siza; Shape;Color;
Spore print	Color;
Smell or Taste	Pleasant or Unpleasant;
The type of growing	Solitary; Scattered; Gregarious; Caespitose

Table 2. Habitat

Items	Description
Location	province; elevation; coordinates
pH	Specific values
Symbiotic	Yes or No; Symbiotic plants
Growing land	Wood, Soil

5) Maintain speciments: Each collection once collected was wrapped by foil or kept in plast bag separately in order to avoid the mixture and crush. As for the samples could not deal with as soon as possible, kept them with soil in ice box to keep fresh. In each collection the young and mature basidiocarps were collected if them appearance; and the all part of basidiocarp were collected including the base of stipe and fell annulus.

The macrocharacters, chemical test and photograph of fresh sample were carried out as soon as possible after came back from the field trip which followed the instruction described by Largent (1986). If could not finish all samples in short time, the specimens were stored in the 4° C freezer waiting for examination later. Then the specimens were divided into two groups. One put into the drier at least overnight to dehydration, sealed in the plastic bag, and kept in the herbarium. All of this specimens kept to work further for molecular phulogeny. The other group was maintained as spore print and pure cultures for morphological identify.

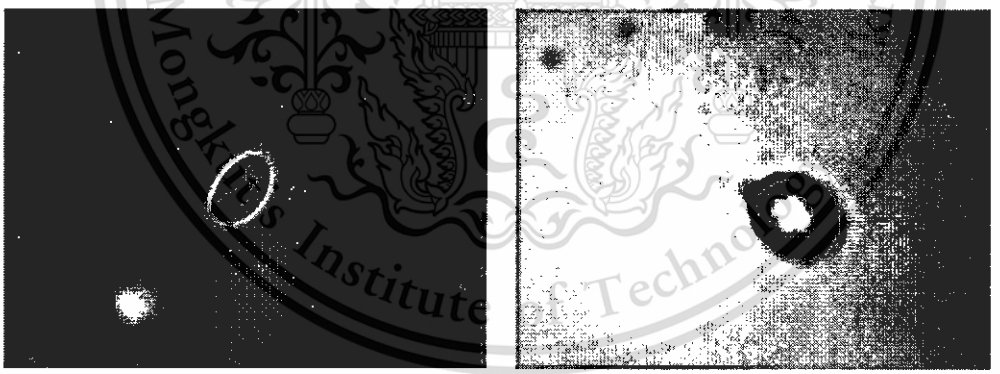
Morphological identification

1) Spores identify- Specimens were identified roughly according to the color of spore print. The brief of some sopre print colors was shown in Table 3.

A small number of basidiospores was removed from spore print with needle, dipped in milk dripping with lactophenol or sterilize water slide and coverslip, then observed under 40X microscope spore shape (Globose, Ellipsoid, Oblong, Nodulose, Cylindric, Fusiform), size, color, surface ornamentation (Smooth, Warted, Spiny, Reticulate, Striate), recorded features, as morphological identification materials which some basidiospores was shown in Figure 2.

Table 3. The brief of some spore print color

The color of basidiospores in mass	Family
White	Hygrophoraceae Lepiotaceae Tricholomataceae
Pink	Pluteaceae
Brown	Agaricaceae Cortinariaceae
Black	Gomphidiaceae Coprinaceae
Purpuse	Strophariaceae

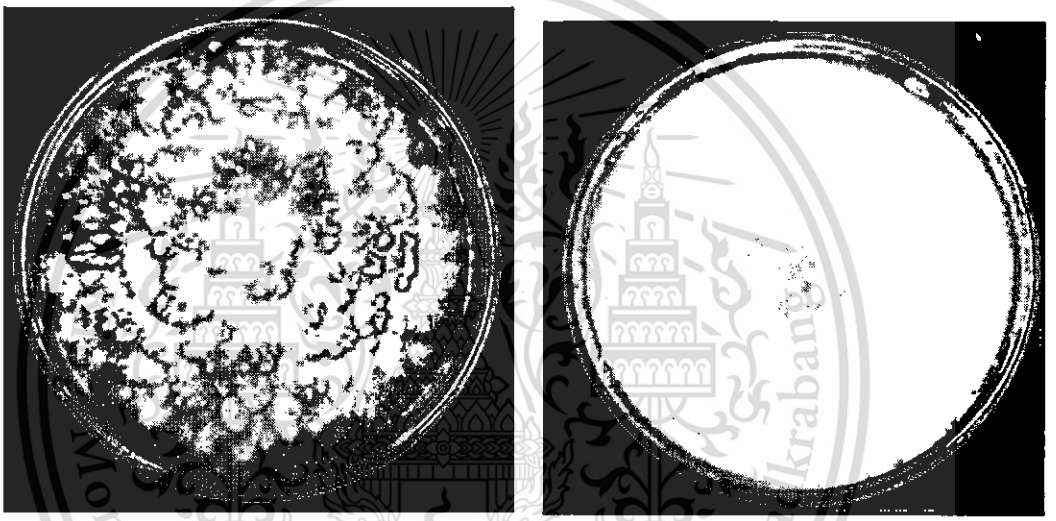


Mycena spp *Lepiota* spp

Figure. 2. showing basidiospores in some commobn species

2) Mycelium identify- As for this part, pure cultures were cultivated. Two methods were used to isolate into pure cultures by fresh tissue and by single spore isolation.
   - Isolate into pure cultures by fresh tissue

Fresh specimens were cleaned with sterilize water, intercepted the stipe with a blade, quickly sterilized in 75% alcohol , the middle part of the stipe cell activity, which can then be easier to obtain pure culture. Then washed again in sterilize water, and slashed the surface portion of stipe with a knife which burnt alcohol lamp, removed the middle part, cut into small pieces, put them into water agar (WA), incubated for 2-5 days, and observed the growth of the mycelium, then transferred to patoto dextrose agar(PDA) until get pure culture. Pure cultures of some common mushroom were shown in Figure 3.

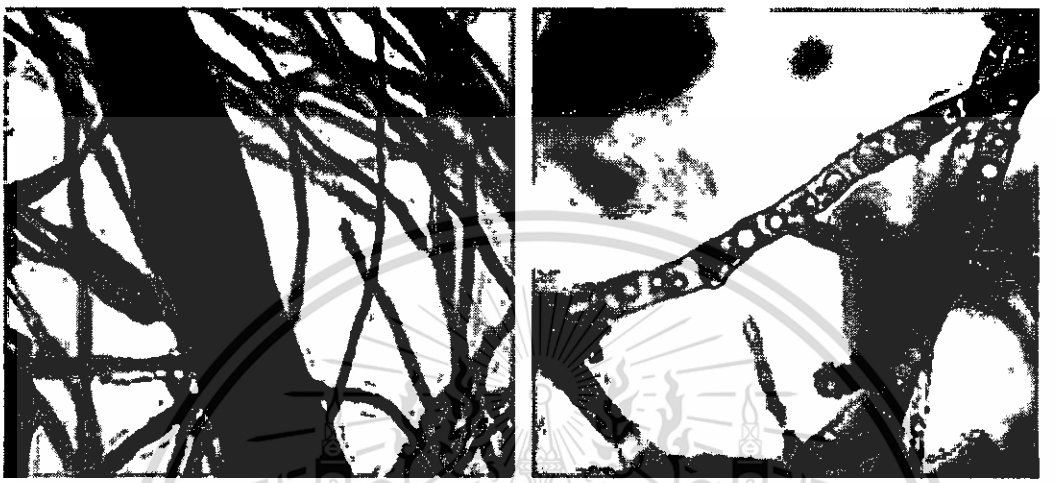


Figuer. 3. Pure cultures show

- Isolation into pure cultures by single spore isolation:- Two methods were used. For existing spore print, picked up a small amount of spores with a sterilized needle, directly transfrred into the PDA. For the specimens which had not spores prints, kept the cap side up and bacteria folded side down, in the top of the prepared PDA medium shaked specimens gently, also a small amount of basidiospores fell to the medium. Both methods were incubated at room temperature for a few days to obtain pure culture.

Pure culture in a colony was made semi-permanebt slide by small amount mycelia from the pure culture with a needle and transferred to a pre-drops of

lactophenol on glass slide, observed the mycelium structure under the 40 X compound microscope, as morphological identification materials (Figure 4).



Agaricus macrosporus *Marasmius* spp

Figuer 4. Showing mycelia in some common species

Results

From July 20th to September 20th, 32 specimens were collected in five provinces of six points in Thailand. These were divided into 3 orders, 13 families as seen in Table 4.

Table 4. Collection of specimens

Specimen number	Taxon	Family and Order	Location
CH02	*Agaricus macrosporus*	Agaricaceae, Agaricales	Chanthaburi Province, Amphoe Khao Khichakut(Krating Waterfall)
PH02	*Tricholoma* spp	Tricholomataceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)

(continued)

Table 4

Specimen number	Taxon	Family and Order	Location
PH03	*Termitomyces* spp	Lyophyllaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH05	*Pluerotus giganteus*	Pleurotaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH06	*Leucocoprinus fragilissimus*	Agaricaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH07	*Collybidia strictipes*	Tricholomataceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH08	*Marasmius* spp	Marasmiaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH09	*Coprinus* spp	Agaricaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH10	*Marasmius* spp	Marasmiaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH11	*Collybia iocephala*	Tricholomataceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH13	*Pleurocybella porrigens*	Marasmiaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
PH15	*Auricularia auricular*	Auriculariaceae, Auriculariales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
LB2	*Collybia* spp	Tricholomataceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
SY01	*Mycena* spp	Mycenaceae, Agaricales	Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)

			Waterfall)
			Phetchabuti Privince,Ampkoe Khao Khichakut(Krating Waterfall)
			Bangkok Province, Khet Lat Krabang(KMITL)
			Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY02	*Marasmius* spp	Marasmiaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
Sy03	Mycena spp	Mycenaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai
SY05	*Mycena spp*	Mycenaceae, Agaricales	Yok Waterfall)
SY09	*Marasmiellus ramealis*	Marasmiaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY13	*Schizophyllum commune*	Schizophyllaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
SY14	*Auricularia auricular*	Auriculariaceae, Auriculariales	Kanchanaburi Province, Amphoe Sai Yok (Sai
SY15	*Marasmiellus albuscorticis*	Marasmiaceae, Agaricales	Yok Waterfall)
SY16	*Marasmius purpureostriatus*	Marasmiaceae, Agaricales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
AJ01	*Russula spp*	Russulaceae, Russulales	Kanchanaburi Province, Amphoe Sai Yok (Sai Yok Waterfall)
AJ03	*Cantharellus cibarius*	Cantharellaceae, Cantharellales	Kanchanaburi Province, Amphoe Sai Yok (Sai
AJ06	*Russula crassotunicata*	Russulaceae, Russulales	Yok Waterfall)
AJ07	*Boletus retisporus*	Boletaceae, Boletales	Chiangrai Province, Chiang Kong

(continued)

Table 4

			Chiangrai Province, Chiang Kong
			Chiangrai Province, Chiang Kong
			Chiangrai Province, Chiang Kong
AJ2-1	*Laccaria vinaceoavellanea*	Hydnangiaceae, Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-2	*Clitocybe spp*	Tricholomataceae, Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-3	*Boletus affinis var. maculosus*	Boletaceae, Boletales	
AJ2-4	*Inocybe fastigiata*	Inocybaceae, Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-5	*Clitocybe* spp	Tricholomataceae, Agaricales	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
AJ2-6	*Mycena vulgaris*	Mycenaceae	Kanchanaburi Province, Amphoe Mueang Kanchanaburi
			Kanchanaburi Province, Amphoe Mueang Kanchanaburi

Discussion

All samples were collected in Thailand. The surroundings mostly near natural water reserviour, rich forest vegetation. As previously mentioned, many Agaricales have edible and medical effects. In this collection of *Agaricus*

macrosporus was reported as ediable (David Pegler and Brian Spooner , 1992), *Pluerotus giganteus* was reported as nutritional value and *Termitomyces* spp was reported as edible mushrooms which are considered as healthy food because their mineral content is higher than that of meat or fish and most vegetables, apart from their nutritional value mushrooms have potential medicinal benefits (Bobby, *et al*, 2011). The further research findling will be done on bioactive substances againist some human (*Escherichia coli* and *Candida albicans*) and plant pathogens (*Fusarium oxysporum* f sp *lycopersici*).

Acknowledgement

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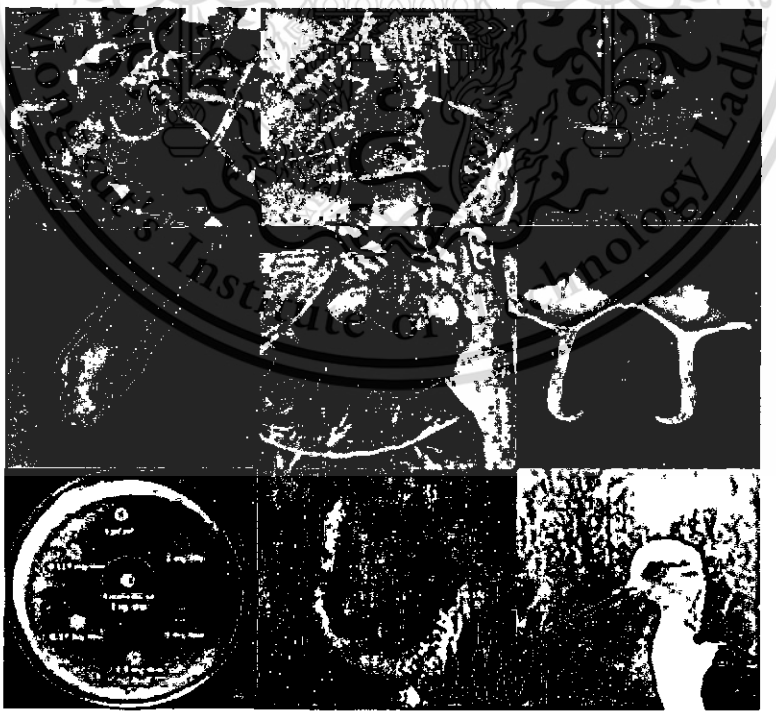

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Session 4: Biotechnology and Natural Products

Two edible mushrooms' interaction against fusarium wilt which caused by F. oxysporum f. sp. lycopersici

Yaling Luo, Wattanachai Pongnak and Kasem Soytong

Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
Corresponding author: iamalinluo@163.com

The majority of mushrooms are edible, medicinal or health care value, development value is high. The two collected specimens Clitocybe spp AJ2-2, Boletus affinis var. maculosus AJ2-3 were from the rain forest which located in Kanchanaburi Province, Amphoe Mueang Kanchanaburi ,Thailand (N 14°0'12", E 99°33'0"). Crude extracts were yielded from the two specimens. Results showed that the crude hexane, crude ethyly acetate and crude methanol from Clitocybe spp AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude ethyly acetate and crude methanol from B. affinis var. maculosus AJ2-3 yielded 0.43, 0.47 and 5.32 %, respectively. The crude extarcts from Clitocybe sp AJ2-2 and B. affinis var. maculosus AJ2-3 were selected for bioactivity test against fusarium wilt which caused by F. oxysporum f. sp. lycopersici. Result showed that crude ethyly acetate from Clitocybe sp AJ2-2 gave significantly highest inhibition of 83.90 % for spore production of F.oxysporum at concentration of 1000 ppm. Crude hexane from B. affinis var. maculosus AJ2-3 gave significantly highest inhibition of 76.91 % for the spore production ofF.oxysporum at the concentration of 1000 ppm. These investigations are also reported for the first that time that Clitocybe, B. affinis var. maculosus and have shown some antimicrobial substances against fusarium wilt which caused by F.oxysporum. Further investigation would be studies on chemical elucidation of these antagonistic substances.

Key words: mushroom, chemical constituents, bioassay

Antifungal substances from *Chaetomium cupreum* against *Pestalotia* spp. Causing gray blight disease of tea

Nguyen Huu Phong[1] , Pongnak Wattanachai[2] and Soytong Kasem[1]

[1] Department of Plant Protection Technology, [2] Department of Soil Science, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang , Bangkok 10520, Thailand
Corresponding author:

Tea is one of the most popular and lowest cost beverages in the world, next only to water. Tea trees are planted in 46 countries all over the world which range from Mediterranean-type climate to the hot humid tropics. Diseases on tea are very various infected. This research was focused on grey blight disease of tea caused by *Pestalotia* spp. Hexane, EtOAc and MeOH crude extract from *Chaetomium cupreum* were used to test antimicrobial substance against *Pestalotia* spp. The results showed that *Ch.cupreum* significantly inhibited *Pestalotia* spp. with the ED50 of 28.40 -154.12 µg/ml.

Key words: tea, antimicrobial substance test, *Pestalotia* spp., *Chaetomium cupreum*,

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Two edible mushrooms' interaction against fusarium wilt which caused by *F. oxysporum f. sp. lycopersici*

Luo, Yaling *, Pongnak, Wattanachai and Soytong, Kasem

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang , Bangkok 10520, Thailand

Luo, Yaling, Pongnak Wattanachai and Soytong Kasem (2015). Two edible mushrooms' interaction against *Fusarium* wilt which caused by *F. oxysporum* f.sp. lycopersici. Journal of Agricultural Technology Vol. 11(2): 539-550

The majority of mushrooms are edible, medicinal or health care value, development value is high. The two collected specimens *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3 were from the rain forest which located in Kanchanaburi Province, Amphoe Mueang Kanchanaburi, Thailand (N 14°0'12", E 99°33'0"). Crude extracts were yielded from the two specimens. Results showed that the crude hexane, crude ethyly acetate and crude methanol from *Clitocybe* spp AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude ethyly acetate and crude methanol from *B. affinis* var. *maculosus* AJ2-3 yielded 0.43, 0.47 and 5.32 %, respectively. The crude extarcts from *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were selected for bioactivity test against fusarium wilt which caused by *F. oxysporum f. sp. lycopersici.* Results showed that crude ethyly acetate from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 83.90 % for spore production of *F.oxysporum* at concentration of 1000 ppm. Crude hexane from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 76.91 % for the spore production of *F.oxysporum* at the concentration of 1000 ppm. These investigations are also reported for the first that time that *Clitocybe*, *B. affinis* var. *maculosus* and have shown some antimicrobial substances against fusarium wilt which canused by *F.oxysporum*. Further investigation would be studies on chemical elucidation of these antagonistic substances.

Key words: Agaricales, Edible mushroom, *Fusarium.oxysporum.*

Introduction

Agaricales comprises the so-called mushrooms and toadstools, and is the largest clade of mushroom-forming fungi. More than 9000 species in more than 300 genera, and 26 families had been described. Mostly they are terrestrial, lignicolous and saprobic, and many are mycorrhizal (Kirk *et al.* 2001).

* Corresponding author: e-mail: iamalinluo@163.com

Agaricales belongs to Eumycota、Basidiomycotina Hymenomycetes、Homobasidiomycitidae (Alexopoulos and Mimx, 1979). Mycelium typically formed by the spores germinate primary hyphae, with primary anastomosis affinity formin g secondary hyphae, no or a lock-like joint, with a septum and barrel bung hole cover. Some types of secondary mycelium may also be formed streptozotocin. Fruiting body is fleshy, easily broken, rarely membranous or leathery. Typical fruiting bodies, including the cap, stipe, located below the cap gills or bacteria tube, located in the middle or upper part of stipe mushroom ring and base volva. Hymenium often in the initial stage of growth within the biofilm shedding covered completely exposed when ripe. No burden separated single spore spores, colorless or colored, its shape, size, color and ornamentation is an important basis for minutes (Arora, 1986).

Agaricales including Pleurotaceae, Schizophyllaceae, Hygrophoraceae, Tricholomataceae, Amanitac eae, Pluteaceae, Agaricaceae, Coprinaceae, Bolbitiaceae, Strophariaceae, Cortinariaceae, Crepidotaceae, Ento lomataceae, Paxillaceae, Gomphidiaceae, Boletacea, Russulaceae and others. *Agaricus* L. belongs to Agaricaceae. The majority of mushrooms are edible, medicinal or health care values. For example, *Agaricus bisporus* (Jelange) Imbach, occurs scaly mushrooms, *Agaricus crocopeplus* Berk, woodland mushrooms, *Agaricus silvaticus* Schaeff, large purple mushroom, *Agaricus augustus* Fr, white mushrooms, *Agricus bernardii* (Quél.) Sacc, big fat mushrooms, *Agaricus bitorquis* (Quél.) Sacc, and the four spore mushrooms as *Agaricus campestris* L. Which has been carried out in artificial cultivation in order for edible, *Agaricus subrufescens* Peck reported to do liquid fermentation and mycelia contains large amounts of polysaccharides and other biologically active substances (Genpei Yu and Jigui Bao, 2008), *Agaricus arvensis* Schaeff reported that involved in the human body's immune system regulating function has the good role in promoting, aroused people's great concern to the wild mushrooms. Brazil mushrooms *Agaricus blazei* Murr reported to be involved in lowering blood sugar, improved arteriosclerosis and cytotoxicity to some cancer cell lines (Xiaoping Luo and Junyan Wang 2007). The seasonal climate of Thailand coupled with the complex topography has resulted in rich biodiversity, including of fungal diversity. The objective of this research project was preliminary conduced to test extracted biological activity substances that inhibit plant pathogens-*Fusarium.oxysporum.*

Tomatoes have bleeding, blood pressure, diuretic, stomach and digestion, thirst, detoxification effect. Since the ratio of tomato vitamin A, vitamin C suitable, so eat can enhance the function of small blood vessels, prevent vascular aging. Tomato flavonoids, both reducing capillary permeability and prevent rupture of the role, as well as the prevention of hardening of the arteries of the special effects that can prevent cervical cancer, bladder cancer and pancreatic cancer and other diseases; Tomatoes help flattening wrinkles, make the skin smooth and delicate, inhibit bacteria. Eat tomatoes also less prone to dark circles, and not susceptible to sunburn. There are also many diseases on tomatoes. Especially *Fusarium oxysporum* f. sp. *lycopersici*, the fungus that causes fusarium wilt, attacks only certain tomato cultivars. Plants infected by this soil-dwelling fungus show leaf yellowing and wilting that progress upward from the base of the stem. Initially, only one side of a leaf midrib, one branch, or one side of a plant will be affected. The symptoms soon spread to the remainder of the plant (Fig.1). Wilted leaves usually drop prematurely. Affected plants die early and produce few, if any, fruits. Splitting open an infected stem reveals brownish streaks extending up and down the stem (Fig. 2). These discolored streaks are the water-conducting tissue, which becomes plugged during attack by the fungus, leading to wilting of the leaves. Plants are susceptible at all stages of development, but symptoms are most obvious at or soon after flowering. To minimize losses from Fusarium wilt, it is advisable to plant resistant varieties, and many resistant varieties are available. The letter "F" following the variety name indicates resistance to one or more races of the Fusarium fungus. Resistant varieties may become infected, but disease will not be as severe as with susceptible varieties and a reasonable yield should still be obtained. In addition, plant disease-free seed or transplants in well drained, disease-free soil, rotate at least four years away from tomatoes to reduce populations of the fungus in soil, and remove and destroy infected plant residue. In greenhouse or seedbeds, disinfest soil by treating with steam.

Fig. 1. Fusarium wilt

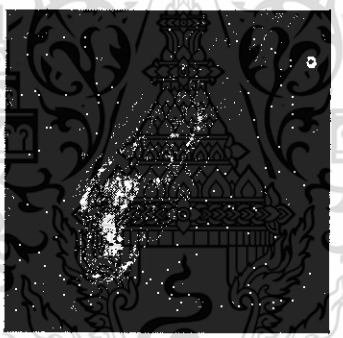


Fig. 2. Vascular browning caused by Fusarium wilt

Materials and methods

Collection and identification

Mushroom samples were collected during the raining season. Each collection site was recorded the macroclimates, chemical test and photograph of fresh specimens. Spore prinit was done as necessary in the collection sites. The specimens were brought to laboratory for further works, imorphologically identification and isolation to pure cultures.

Isolation of Pathogen and Pathogenicity Test

Fusarium oxysporum f. sp. *lycopersici* causing witl of tomato *Lycopersicon esculentum* was isolated from root symptom by tissue

transplanting techniques. The cultures were transferred into Potato Dextrose Agar (PDA) and incubated at room temperature. The morphological characteristic of *Fusarium* was studied under compound microscope. The pathogenicity test of fusarium wilt (*Fusarium oxysporum f. sp. lycopersici* NKSC02 race 2) was conducted in vivo to 15 day tomato seedlings. Tomato seeds were sown into coarse sand in plastic trays (10 × 15 × 5 cm) and maintained for 2 weeks. Pathogenicity test was carried out using a rootdip inoculation method. Tomato seedlings were uprooted gently and roots were washed with tap water to remove all sand (Bao J.R. *et al.*, 2001). The spore suspension for inoculation was prepared by pouring 50 ml of sterile water into each of Petri dishes containing 10-day-old Fusarium isolate, stirring the mixture with a sterile glass stick, and pouring it into a glass. The concentration of conidia in the suspension was determined using Haemacytometer to adjust the number of spores to $1x10^6$ conidia/ml. The 3-4 root tips will be cut and soaked into spore suspension for 30 seconds. Control plants were sown in soil and and be treated with sterile distilled water. Incubation was performed at 22-25°C for 14 days. The disease severity was rated with follow the method of Sibounnavong (2012), as follows: 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting 81-100% or die. The most virulent isolate was selected for further experiment.

Extraction of Biological Active Substances

The bioactive compounds were cultured in potato dextrose broth (PDB) at room temperature (28-30 C) for 45 days and extracted from the collected species of Agaricales as crude extracts. Fungal biomass were collected by moving from PDB, filtered through cheesecloth and air-dried overnight. Fresh and dried fungal biomass was recorded. Dried fungal biomass were ground with electrical blender, extracted with 200 ml hexane (H) and shaken for 5 days at room temperature. The filtrate from ground biomass was separated by filtration through Whatman No.4 filter paper. The filtrate was evaporated in *vacuum* to yield crude extract. The marc was further extracted with ethyl acetace (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. Each crude extract was weighted, and then kept in refrigerator at 4 C until use.

Bioactivity against tomato wilt which caused by Fusarium oxysporum f. sp. lycopersici

The crude extracts were tested for inhibition of the most aggressive isolat *C. coffeanum*. The experiment was conducted by using 3x6 factorials in Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100 and/or 500, and 1,000 µg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxiden (DMSO), mixed into potato dextrose agar (PDA) before autoclaving at 121C, 15 1bs/inch2 for 30 minutes. The tested pathogen were cultured on PDA and incubated at room temperature for 5 days, and then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA plate (5.0 cm diameter) in each concentration and incubated at room temperature (28-30C) for 5 days. Data were collected as colony diameter and computed the percentage of inhibition. Data were statistically computed analysis of variance. Treatment means were compared with DMRT at P=0.05 and P=0.01.

Results

Collection and Identification

Two specimens were collected from Thailand. They are *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, described as follows: ***Clitocybe* spp AJ2-2**: Cap 0.5-7 cm across, purperish to pink to pale brown, horn with strongly depress in the center and inrolled margin becoming wavy. Gills are decurrent, white to olive-yellow. Stem *3.5*-9 cm, cylindrical, smooth, and pink to dark brown. Habitat grows in clusters (Fig. 3). ***Boletus affinis* var. *maculosus* AJ2-3:** Cap 1-3.5 cm across, velvety redish-brown, dry shin, having a membraneous vein on the top part which promptly turns to tobacco color due to the falling spores. Gills are adnate, white. Stem is *6*-9 cm long, cylindrical, silky membranous, smooth. Habitat grows in clusters (Fig.4).

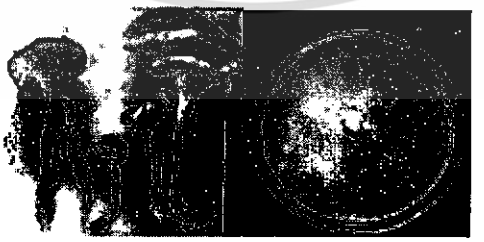


Fig. 3. *Clitocybe* spp

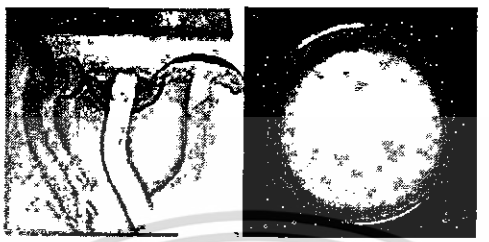
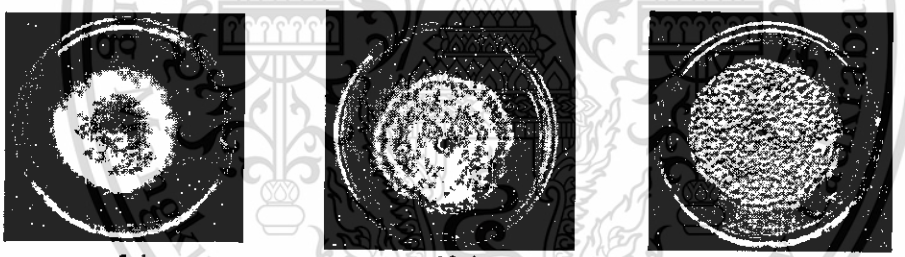


Fig. 4. *Lactarius sp*

Isolation of pathogen and pathogenicity test

With the tissue transplanting techniques, the pure cultures and spores of *F.oxysporum* were got, shown as fig. 5 and fig.6, respectively. After performing pathologenicity test followed Koch's Postulate 14 days, there were obvious sysmptoms on tomato. After careful observation, it was found that tomotoes which inoculated in spore suspension of F.oxysporum before planting wilted compared with non-treated group (Fig. 7).



5 days 10 days 15 days

Fig. 5. Pure cultures of *F.oxysporum*

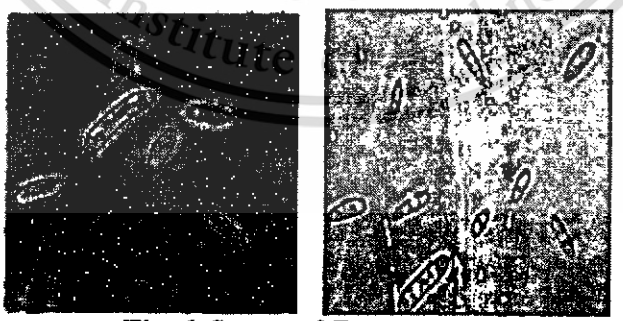


Fig. 6. Spores of *F.oxysporum*

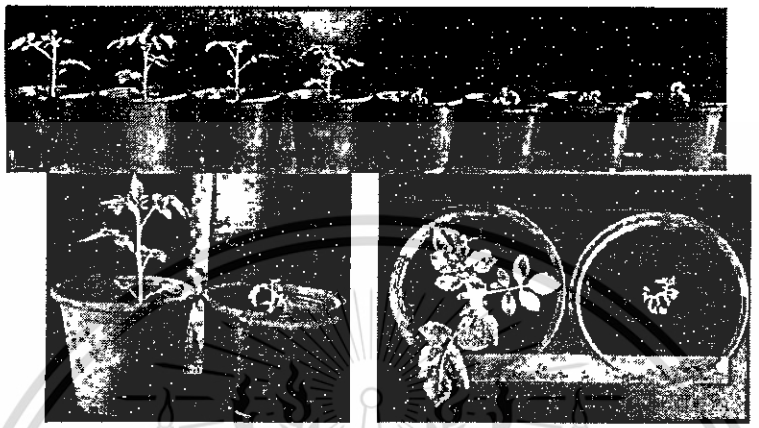


Fig. 7. Pathologenicity test
Where: C-Control (root soaked into sterilized water) T-Test (root soaked into spore suspection -10^6)

Extraction of Biological Active Substances

Each fungal biosmass was separately extracted to get crude hexane, crude ethyly acetate and crude methanol. The crude hexane, crude ethyly acetate and crude methanol from *Clitocybe* spp AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude ethyly acetate and crude methanol from *B. affinis* var. *maculosus* AJ2-3 yielded 0.43, 0.47 and 5.32 %, respectively.

Bioactivity against Fusarium oxysporum

The crude extarcts from *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were selected for bioactivity test against fusarium wilt caused by *F. oxysporum*. Results showed that methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 27 % for the colony growth of *F. oxysporum* at the concentration of 1,000 ppm when compared to the control (Table 1). Crude ethyl acetate from *Clitocybe* sp AJ2-2 gave significantly highest inhibited the spore production of *F. oxysporum* as 83.90 %, followed by crude methanol inhibited 77.68 % and crude hexane 68.95 % (Tables 2). The ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 35.50 % for the colony growth of *F. oxysporum* at the concentration of 1,000 ppm when compared to the control (Table 3). Crude ethyl acetate from B. affinis var. maculosus AJ2-3 gave significantly highest inhibited the spore production of *F. oxysporum* as 79.71 %

at the concentration of 1,000 ppm , followed by crude hexane inhibited 76.91% and crude methanol inhibited 64.36 % (Table 4).

Table 1. Crude extracts of *Clitocybe sp* Aj2-2 testing for growth inhibition of *Colletotrichum coffaenum* at 5days

Crude extracts	Concentration (ppm)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	5.00^{a}	0.00^{j}
	10	5.00^{a}	0.00^{j}
	50	5.00^{a}	0.00^{j}
	100	4.90^{a}	2.00^{i}
	500	4.80^{b}	4.00^{h}
	1000	4.62^{c}	7.50^{g}
Crude EtOAc	0	5.00^{a}	0.00^{j}
	10	4.35^{de}	13.00^{ef}
	50	4.27ef	14.50^{de}
	100	4.20^{f}	16.00^{d}
	500	4.10g	18.00^{c}
	1000	4.00h	20.00^{b}
Crude MeOH	0	5.00^{a}	0.00^{j}
	10	4.40^{d}	12.00^{f}
	50	4.27^{ef}	14.00^{e}
	100	4.10^{g}	18.00^{c}
	500	4.00^{h}	20.00^{b}
	1000	3.65^{i}	27.00^{a}

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 2. Spore production inhibition of crude extracts from *Clitocybe* sp AJ2-2 to *Fusarium oxysporum* f sp *lycopersici* at 7days

Crude extracts	Concentration (ppm)	Number of spores [1](10^6)	Inhibition (%)[2]	ED_{50}
Crude Hexane	0	132.63^{a}	0.00^{i}	*409.59*
	10	114.75^{b}	13.47^{h}	
	50	107.81^{b}	18.72^{h}	
	100	88.88^{c}	33.01^{g}	
	500	72.19^{de}	45.58^{f}	
	1000	0.25^{b}	68.95c	
Crude EtOAc	0	132.63^{a}	0.00^{i}	17.54
	10	67.25^{de}	49.3^{ef}	
	50	56.14^{fg}	57.68^{d}	
	100	56.31^{fg}	57.54^{d}	

	500	0.81[b]	68.36[c]	
	1000	21.38[ij]	83.90[a]	
Crude MeOH	0	132.63[a]	0.00[l]	26.43
	10	75.25[d]	43.32[f]	
	50	63.50[ef]	52.11[de]	
	100	48.3[gh]	52.11[de]	
	500	29.63[i]	63.59[c]	
	1000	16.88[j]	77.68[b]	
C.V.(%)		3.05	31.43	

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

Table 3. Crude extracts of *Boletus affinis* var. *maculosus* AJ2-3 testing for growth inhibition of *Fusarium oxysporum* f sp *lycopersici* at 5days

Crude extracts	Concentration (ppm)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	5.00a	0.00d
	10	5.00a	0.00d
	50	5.00a	0.00d
	100	5.00a	0.00d
	500	5.00a	0.00d
	1000	5.00a	0.00d
Crude EtOAc	0	5.00a	0.00d
	10	5.00a	0.00d
	50	5.00a	0.00d
	100	4.45b	11.00c
	500	4.25c	15.05b
	1000	3.22d	35.50a
Crude MeOH	0	5.00a	0.00d
	10	5.00a	0.00d
	50	5.00a	0.00d
	100	5.00a	0.00d
	500	5.00a	0.00d
	1000	5.00a	0.00d
CV(%)		1.12	0.83

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.
[2]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4. Spore production inhibition of crude extracts from *Boletus affinis* var. *maculosus* AJ2-3 to *Fusarium oxysporum* f sp *lycopersici* at 7 days

Crude extracts	Concentration (ppm)	Number of spores[1] (10^{x6})	Inhibition(%)[2]	ED_{50}
Crude Hexane	0	83.00[a]	0.00[i]	151.44
	10	71.75[b]	13.44[h]	
	50	50.69[ef]	35.11[f]	
	100	53.81[fde]	46.85[e]	
	500	32.50[hi]	60.89[bc]	
	1000	19.25[i]	76.91[a]	
Crude EtOAc	0	83.00[a]	0.00[i]	59.85
	10	44.94[fg]	45.90[e]	
	50	41.50[g]	49.84[de]	
	100	39.13[e]	52.86[de]	
	500	28.63[i]	65.50[b]	
	1000	16.88[j]	79.71[a]	
Crude MeOH	0	83.00[a]	0.00[i]	131.90
	10	65.13[bc]	21.45[g]	
	50	59.06[cd]	28.79[f]	
	100	38.19[gh]	28.79[f]	
	500	29.63[i]	54.29[cd]	
	1000	19.50[j]	64.36[b]	
C.V.(%)		3.05	7.79	9.12

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.
[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

Discussion

As results showed that methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 27 % for the colony growth of *F. oxysporum* at the concentration of 1,000 ppm, crude ethyl acetate gave significantly highest inhibited the spore production as 83.90 %, the effective dose (ED_{50}) could inhibit the spore growth of *F. oxysporum* at the concentrations of 17.54 ppm. The ethyl acetate crude extract from *B. affinis* var.

maculosus AJ2-3 gave significantly highest inhibition of 35.50 % for the colony growth of *F. oxysporum* at the concentration of 1,000 ppm, spore production as 79.71 % , and the effective dose (ED_{50}) could inhibit the spore growth of *F. oxysporum* at the concentrations of 59.85 ppm. In the literature reviews Phillips (1991) and States (2004) cited the two species *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were described which have been found in Thailand. The research findings are reported for the first time that the metabolites from Clitocybe sp AJ2-2 and B. affinis var. maculosus could inhibit *C. coffaenum* causing coffee anthracnose. Similar report from Badalyan *et al.* (2002) stated that the antagonistic activity of 17 species of Basidiomycotina (*Coriolus versicolor, Flammulina velutipes, Ganoderma lucidum, Hypholoma fasciculare, H. sublateritium, Kühneromyces mutabilis, Lentinula edodes, Lentinus tigrinus, Pholiota alnicola, Ph. aurivella, Ph. destruens, Pleurotus ostreatus, P. cornucopiae, Polyporus squamosus, P. subarcularius, P. varius* and *Schizophyllum commune*) could inhibit plant pathogens, *Bipolaris sorokiniana, Fusarium culmorum, Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia cerealis* that causing foot and root diseases of winter cereals. The potential of fungal metabolites from fungi have been usually reported to produce antibiotic substances against human and plant pathogens. Phoutthasone Sibounnavong (2012) reported that *E. nidulans* isolate L01 developed as bio-agent formulation would be feasible to extend this biological fungicide to control tomato wilt in different tomato varieties where susceptible wilt incidence, especially in the field. Crude methanol of *E. nidulans* isolate L01 at 1000 µg/ml significantly inhibited *F. oxysporum* f sp *lycopersici* 84.40 %, and followed by crude ethyl acetate and crude hexane which were 64.40 and 60.28%, respectively. Crude methanol of *E. nidulans* isolate L01 expressed antifungal activity against *F. oxysporum* f sp *lycopersici* at the ED50 of 112 µg/ml, and follwed by crude ethyl acetate and crude hexane which were 379 and 915 µg/ml, respectively.

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Mushroom and macrofungi collection for screening bioactivity of some species to inhibit coffee antharcnose caused by *Colletotrichum coffeanum*

Luo Yaling[1*], Wattanachai Pongnak[2] and Soytong Kasem[1]

[1]Department of Plant Production Technology, [2]Department of Soil Science, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang , Bangkok 10520, Thailand

Luo Yaling, Wattanachai Pongnak and Soytong Kasem (2014) Mushroom and macrofungi collection for screening bioactivity of some species to inhibit coffee antharcnose caused by *Colletotrichum coffeanum.* Journal of Agricultural Technology 10(4):845-861.

The 60 collected specimens from different locations in Thailand were morphological identified into 7 orders (Agaricales, Auriculariales, Boletetales, Cantharellales, Polyporales, Russulales, Xylariales), 17 Families (Agaricaceae, Auriculariaceae, Boletaceae, Cantharellaceae, Clavariaceae, Exidiaceae, Hydnangiaceae, Inocybaceae, Lyophyllaceae, Marasmiaceae, Mycenaceae, Pleurotaceae, Polyporaceae, Russulaceae, Schizophyllaceae, Tricholomataceae, Xylariaceae). Descriptions of *Leucocoprinus fragilissimus* PH06, *Collybia strictipes* PH07, *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01 and *Lactarius* sp CH3-27 were described. Crude extracts were yielded from *L. fragilissimus* PH06, *C. strictipes* PH07, *Clitocybe* spp AJ2-2, *B. affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01 and *Lactarius* sp CH3-27. Result showed that the highest obtained from crude MeOH of *Lactarius* sp CH3-27, up to 6.76 %. The crude extarcts from *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were selected for bioactivity test against coffee anthracnise caused by *Colletotrichum coffaenum.* Result showed that Methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 30 % for the colony growth of *C. coffaenum* at the concentration of 1,000 ppm, crude methanol from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 89.08 % for spore production of *C. coffaenum* at concentration of 100 ppm. Crude ethyl acetate from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 33.53 % for the colony growth of *C. coffaenum* at the concentration of 1,000 ppm. Crude methanol from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 76.69 % for the spore production of *C. coffaenum* at the concentration of 100 ppm. These investigations are also reported for the first that time that *L. fragilissimus*, *C. strictipes*, *Clitocybe*, *B. affinis* var. *maculosus* and *Lactarius* have shown some antimicrobial substances against coffee anthracnose canused by C. coffaenum. Further investigation would be studies on chemical elucidation of these antagonistic substances.

Keywords: Mushrooms, Agaricales, Crude extracts, Morphological identify

* Corresponding author: Luo Yaling; e-mail: iamalinluo@163.com

Introduction

Basidiomycota are macrofungi characterized by a multi-layered cell walls, barrel-shaped structures or pulley wheel occlusions at the septa of hyphae (dolipore septa), an extended dikaryophase, clamp connections that often develop on septa, and the formation of basidia that produce basidiospores at the tips of sterigmata (Kendrick, 2000). Almost 30,000 species have been found and described (Kirk *et al.*, 2001). Basidiomycetes are mostly being saprobes, symbionts and play ecologically important roles, such as oxygen, carbon and nitrogen cycling. Humans are first attracted to mushrooms since ancient times because of their edible or poisonous traits. Mushrooms are an important group in the biosphere and their significance in diversity and conservation issues have been recognized extensively (Kaul, 2001).

Agaricales comprises the so-called mushrooms and toadstools, and is the largest clade of mushroom-forming fungi. More than 9000 species in more than 300 genera, and 26 families had been described. Mostly they are terrestrial, lignicolous and saprobic, and many are mycorrhizal fungi (Kirk *et al.*, 2001). It is a class of widely distributed around the world, camp life and play an important economic saprophytic fungi. Their morphological characteristics were investigated through the canopy fleshy, smooth or scaly. Spores are oval or elliptical, smooth, dark brown or purple-brown (Hui and Changbiao, 2005). Field classification features are gill free, and easy separation with stipe, first as a white to pink, light brow, brown or dark brown when mature; a ring, single or double and spore print. They can grow in forests, grasslands, fields, farm, roadsides, gardens and other places (Hui, 2006, Rui-Lin *et al.*, 2008; 2012; 2013).

The majority of mushrooms are edible, medicinal or health care value, development value is high. For example, *Agaricus bisporus* (Jellange) Imbach, ocher scaly mushrooms *A. crocopeplus* Berk, woodland mushrooms *A. silvaticus* Schaeff, large purple mushroom *A. augustus* Fr, white mushrooms *A. bernardii* (Quél.) Sacc, big fat mushrooms *A. bitorquis* (Quél.) Sacc and the four spore mushrooms *A. campestris* L. They have long been carried out artificial cultivation in order to serve people to edible. *Agaricus subrufescens* Peck are reported to make a liquid fermentation and found the mycelia contains large amounts of polysaccharides and other biologically active substances for human body's immune system regulating function (Genpei and Jigui, 2008).

Moreover, the wild mushrooms *A. arvensis* Schaeff and Brazil mushrooms *A. blazei* Murr, etc. can affect in lowering blood sugar, improve arteriosclerosis and suppress cancer cell lines (Xiaoping and Junyan, 2007).

The objective was to collect and find out the metabolites from some mushrooms against coffee anthracnose caused by *Colletotrichum coffeanum*.

Materials and methods

Collection and identification

Mushroom samples were collected during the raining season from July, 2013 to October, 2013. Collection was made in the forests and grass areas in 5 provinces of Thailand, which are Chanthaburi, Chiangrai, Phetchabuti, Kanchanaburi and Bangkok Provinces. Each collection site was recorded the macroclimates, chemical test and photograph of fresh specimens. Spore prinit was done as necessary in the collection sites. The specimens were brought to laboratory for further works, imorphologically identification and isolation to pure cultures. The field trip was followed the instruction described by Largent (1986).

Isolation of pathogen and pathogenicity test

Colletotrichum coffeanum causing anthracnose of coffee var arabica was isolated from leaf symptom by tissue transplanting techniques and performed pathologenicity test followed Koch's Postulate.

Extraction of biological active substances

The bioactive compounds were extracted from some selected species of Agaricales as crude extracts. The extraction was performed using the method of Kanomedhakul *et al.* (2006). Some species of Agraricaeae were cultured in potato dextrose broth (PDB) at room temperature (28-30 C) for 45 days. Fungal biomass were collected by moving from PDB, filtered through cheesecloth and air-dried overnigrht. Fresh and dried fungal biomass was recorded. Dried fungal biomss were ground with electrical blender, extracted with 200 ml hexane (H) and shaken for 24 hour at room temperature. The filtrate from ground biomass was separated by filtration through Whatman No.4 filter paper. The filtrate was evaporated in *vacuo* to yield crude extract. The marc was further extracted with ethyl acetace (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. Each crude extract was weighted, and then kept in refrigerator at 4 C until use.

Biological activity against coffee anthracnose caused by C. coffeanum

The crude extracts were tested for inhibition of the most aggressive isolate of *C. coffeanum*. The experiment was conducted by using 3 x 6 factorial in Completely Randomized Design (CRD) with four replications. Factor A

represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100 and/or 500, and 1,000 µg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxiden (DMSO), then mixed into potato dextrose agar (PDA) before autoclaving at 121C, 15 1bs/inch^2 for 30 minutes. The tested pathogen were cultured on PDA and incubated at room temperature for 5 days, and then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA plate (5.0 cm diameter) in each concentration and incubated at room tmperature (28-30C) for 5 days. Data were collected as colony diameter and computed the percentage of inhibition. Data were statistically computed analysis of variance. Treatment means were compared with DMRT at P=0.05 and P=0.01.

Results

Collection and identification

57 specimens were collected from five provinces of six points in Thailand. These were morphologically identified into 7 orders (Agaricales, Auriculariales, Boletetales, Cantharellales, Polyporales, Russulales, Xylariales), 17 Families (Agaricaceae, Auriculariaceae, Boletaceae, Cantharellaceae, Clavariaceae, Exidiaceae, Hydnangiaceae, Inocybaceae, Lyophyllaceae, Marasmiaceae, Mycenaceae, Pleurotaceae, Polyporaceae, Russulaceae, Schizophyllaceae, Tricholomataceae, Xylariaceae). Thease were morphological identified into 57 species as follows:- *Agaricus macrosporus, Agaricus* spp., *Auricularia auricular, Boletus affinis var. maculosus, Boletus retisporus, Cantharellus cibarius, Clavulinopsis fusiformis, Clavulinopsis helvola, Clitocybula atrialba, Clitocybe* spp., *Collybia dryophila, Collybia iocephala, Collybia strictipes, Collybia* spp., *Coprinus* spp., *Inocybe fastigiata, Tricholoma* spp., *Lactarius controversus, Lactarius sanguifluus, Lactarius* spp., *Laccaria vinaceoavellanea, Laccaria* spp., *Leucocoprinus fragilissimus, Marasmiellus albuscorticis, Marasmiellus ramealis, Marasmius androsaceus, Marasmius foetidus, Marasmius purpureostriatus, Marasmius oreades, Marasmius plicatulus, Marasmius scorodonius, Marasmius* spp., *Mycena inclinata, Mycena rosella, Mycena subcaerulea, Mycena vulgaris, Mycena* spp., *Pleurocybella porrigens, Pluerotus giganteus, Resinomycena rhododendri, Russula crassotunicata, Russula* spp., *Schizophyllum commune, Termitomyces microcarpus, Trametesversicolor, Tremiscus* spp., *Termitomyces* spp., *Tricholoma* spp. and *Xylaria hypoxylon* as seen in Table 1.

Table 1. Collection of specimens

Species	Locations	Family/Order	Specimen No.
Agaricus macrosporus	Chanthaburi province, Amphoe Khao Khichakut	Agaricaceae, Agaricales	CH02
Agaricus spp	Chanthaburi province, Amphoe Khao Khichakut	Agaricaceae, Agaricales	CH3-25
Auricularia auricula	Phetchabuti Privince, Ampkoe Khao Khichakut	Auriculariaceae, Auricuriales	PH15
Boletus affinis var. *maculosus*	Kanchanaburi Province, AmphoeMueangKanchanaburi	Boletaceae, Boletale	AJ2-3
Boletus retisporus	Chiangrai Province, Chiang Kong	Boletaceae, Boletale	AJ07
Cantharellus cibarius	Chiangrai Province, Chiang Kong	Cantharellaceae, Cantharellales	AJ03
Clavulinopsis fusiformis	Chanthaburi province, Amphoe Khao Khichakut	Clavariaceae, Agaricales	CH3-15a
Clavulinopsis helvola	Chanthaburi province, Amphoe Khao Khichakut	Clavariaceae, Agaricales	CH3-15b
Clitocybula atrialba	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-08
Clitocybe spp	Kanchanaburi Province, AmphoeMueangKanchanaburi	Tricholomataceae Agaricale	AJ2-2
Clitocybe spp	Kanchanaburi Province, AmphoeMueangKanchanaburi	Tricholomataceae Agaricale	AJ2-5
Collybia dryopjila	Chanthaburi province, Amphoe Khao Khichakut	Tricholomataceae Agaricales	CH3-26
Collybia iocephala	Phetchabuti Privince, Ampkoe Khao Khichakut	Tricholomataceae Agaricales	PH11
Collybia strictipes	Phetchabuti Privince, Ampkoe Khao Khichakut	Tricholomataceae Agaricales	PH07
Collybia spp	Bangkok Province, Khet Lat Krabang(KMITL)	Tricholomataceae Agaricales	LB2
Coprinus spp	Phetchabuti Privince, Ampkoe Khao Khichakut	Agaricaceae, Agaricales	PH09
Inocybe fastigiata	Kanchanaburi Province, AmphoeMueangKanchanaburi	Inocybaceae, Agaricales	AJ2-4
Lactarius controversus	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-20
Lactarius sanguifluus	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-06
Lactarius spp.	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-01
Lactarius spp.	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-24
Lactarius spp.	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-27
Laccaria	Kanchanaburi Province,	Hydnangiaceae,	AJ2-1

vinaceoavellanea	AmphoeMueangKanchanaburi	Agaricales	
Laccaria spp	Chanthaburi province, Amphoe Khao Khichakut	Hydnangiaceae, Agaricales	CH3-13
Leucocoprinus fragilissimus	Phetchabuti Privince, Ampkoe Khao Khichakut	Agaricaceae, Agaricales	PH06
Marasmiellus albuscorticis	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-12
Marasmiellus ramealis	Kanchanaburi Province, Amphoe Sai Yok	Agaricaceae, Agaricales	SY09
Marasmius androsaceus	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-04
Marasmius foetidus	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-17
Marasmius purpureostriatus	Kanchanaburi Province, Amphoe Sai Yok	Marasmiaceae, Agaricales	SY16
Marasmius oreades	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-22
Marasmius plicatulus	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-18
Marasmius scorodonius	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-21
Marasmius spp.	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-02
Marasmius spp.	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-23
Marasmius spp.	Phetchabuti Privince, Ampkoe Khao Khichakut	Marasmiaceae, Agaricales	PH08
Marasmius spp.	Kanchanaburi Province, Amphoe Sai Yok	Marasmiaceae, Agaricales	SY02
Mycena inclinata	Chanthaburi province, Amphoe Khao Khichakut	Mycenaceae, Agaricales	CH3-11
Mycena rosella	Chanthaburi province, Amphoe Khao Khichakut	Mycenaceae, Agaricales	CH3-03
Mycena subcaerulea	Chanthaburi province, Amphoe Khao Khichakut	Mycenaceae, Agaricales	CH3-07
Mycena vulgaris	Kanchanaburi Province, AmphoeMueangKanchanaburi	Mycenaceae, Agaricales	AJ2-06
Mycena spp	Kanchanaburi Province, Amphoe Sai Yok	Mycenaceae, Agaricales	SY01
Mycena spp	Kanchanaburi Province, Amphoe Sai Yok	Mycenaceae, Agaricales	SY03
Mycena spp	Kanchanaburi Province, Amphoe Sai Yok	Mycenaceae, Agaricales	SY05
Pleurocybella porrigens	Phetchabuti Privince, Ampkoe Khao Khichakut	Marasmiaceae, Agaricales	PH13
Pluerotus giganteus	Phetchabuti Privince, Ampkoe Khao Khichakut	Pleurotaceae, Agaricales	PH05
Resinomycena	Chanthaburi province,	Mycenaceae,	CH3-16

rhododendri	Amphoe Khao Khichakut	Agaricales	
Russula crassotunicata	Chiangrai Province, Chiang Kong	Russulaceae, Russulales	AJ06
Russula spp	Chiangrai Province, Chiang Kong	Russulaceae, Russulales	AJ01
Schizophyllum commune	Kanchanaburi Province, Amphoe Sai Yok	Schizophyllaceae, Agaricales	SY13
Termitomyces microcarpus	Chanthaburi province, Amphoe Khao Khichakut	Tricholomataceae, Agaricales	CH3-14
Trametesversicolor spp	Chanthaburi province, Amphoe Khao Khichakut	Polyporaceae , Trametes	CH3-05
Tremiscus spp	Chanthaburi province, Amphoe Khao Khichakut	Exidiaceae, Auriculariales	CH3-09
Termitomyces spp	Phetchabuti Privince, Ampkoe Khao Khichakut	Lyophyllaceae, Agaricales	PH03
Tricholoma spp.	Chanthaburi province, Amphoe Khao Khichakut	Tricholomatacea Agaricales	CH2-09
Tricholoma spp	Phetchabuti Privince, Ampkoe Khao Khichakut	Tricholomataceae, Agaricales	PH02
Xylaria hypoxylon	Chanthaburi province, Amphoe Khao Khichakut	Xylariaceae, Xylariales	CH3-19

Descriptions of *Leucocoprinus fragilissimus* PH06, *Collybia strictipes* PH07, *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01and *Lactarius sp* CH3-27 are described as follows:-

***Leucocoprinus fragilissimus* PH06**

A small, white or nearly transoarent, easy to crack mushroom. Cap 2.4 cm in diameter, flat with a distinct yellow umbo, sometimes broadly bel-shaped, white, nearly transprrent, margin clearly lined, thick, small yellow scales. Gill free, white, unequal length. Stem 3.5 x 0.1 cm, very slim, white, ring small, easily detachable in the lower part of the stem; Habitat grows in grassland or tea garden (Fig.1).

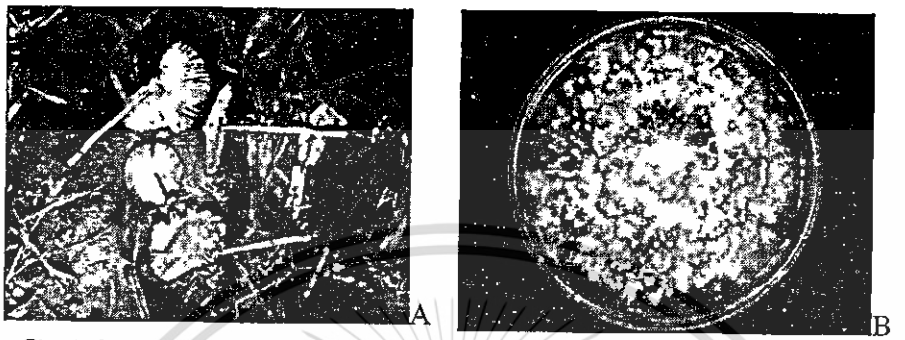

Fig .1. *Leucocoprinus fragilissimus;* A: Fruiting bodies in the field B: Pure culture

Collybia strictipes **PH07**

A white, brittle mushroom. Cap 4.5 cm in diameter, bell-shaped with margin remaining inrolled and clearly lined, smooth. Gill free, pink, broad, unequel lenghth. Stem 4.5 x0.5 cm, white, fresh, smooth, peanut smell. Habitat scattered in grassland (Fig.2).

Fig .2. *Collybia strictipes;* A: Fruiting body in the field B: Pure culture

Clitocybe **spp AJ2-2**

Cap 0.5-7 cm across, purperish to pink to pale brown, horn with strongly depress in the center and inrolled margin becoming wavy. Gills decurrent, white to olive-yellow. Stem *3.5*-9 cm, cylindrical, smooth, pink to dark brown. Habitat grows in clusters (Fig. 3).

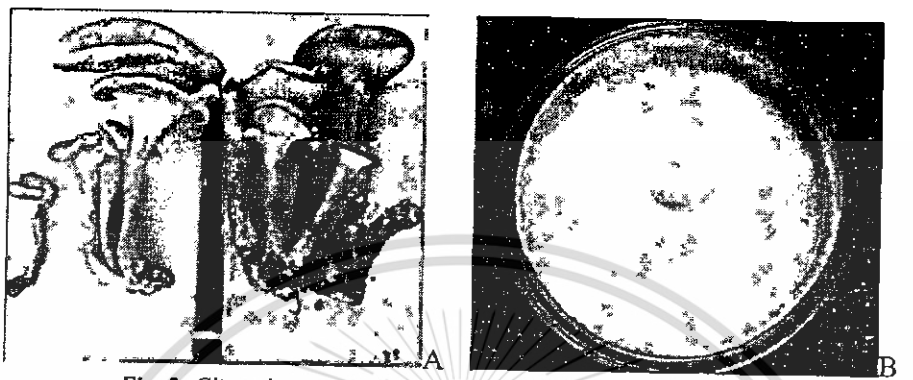

Fig. 3. *Clitocybe* spp; A: Fruiting bodies in the field; B:Pure culture

***Boletus affinis* var. *maculosus* AJ2-3**

Cap 1-3.5 cm across, velvety redish-brown, dry shin, having a membraneous vein on the top part which promptly turns to tobacco color due to the falling spores. Gills adnate, white. Stem 6-9 cm long, cylindrical, silky membranous, smooth. Habitat grows in clusters (Fig. 4).

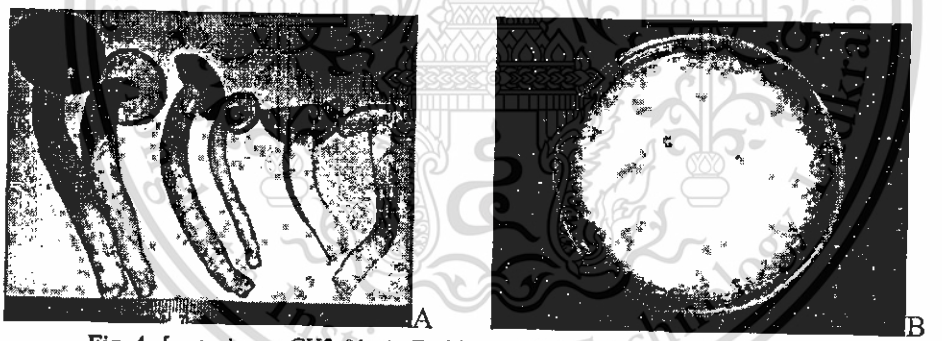

Fig. 4. *Lactarius* sp CH3-01; A: Fruiting bodies in the field B: Pure culture

***Lactarius* sp CH3-01**

A flesh mushroom, fruit body makes people think pf milk. *Cap* 0.5-4 cm in diameter, convex, smooth, cream yellow with white, slight incurrent margin with not clearly lined, Color changes to buff when dry; Gill, free, close, cream yellow to pink; Flesh white, Stem 0.5-6 X 0.1-0.5 cm, white then becoming buff, smooth, having rooting base, spore print brown. Habitat scatter in sandy soild (Fig. 5).

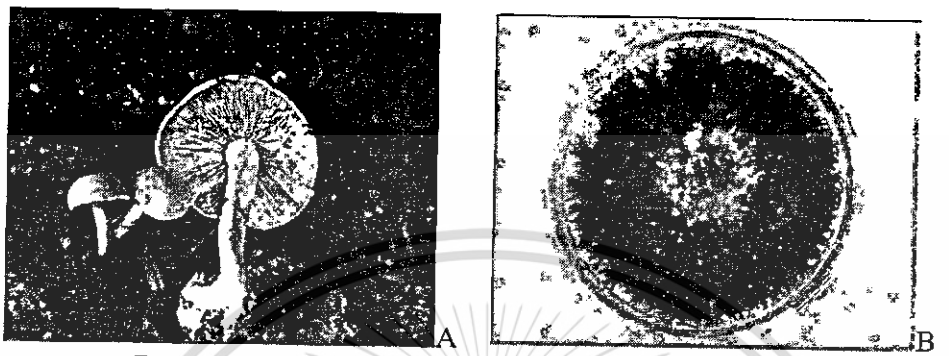

Fig. 5. *Lactarius* sp; A: Fruiting bodies in the field B: Pure culture

***Lactarius* sp CH3-27**

Cap 10 cm in diameter, flat with a white strongly depress in the center, reddish brown with lined, dark scales including the wavy margin. Gills decurrent, pink, close, equel. Stem 7x0.7cm, dark brown, cylindrical, downy the part attach gills are red. Habitat grows singly in soil (Fig. 6).

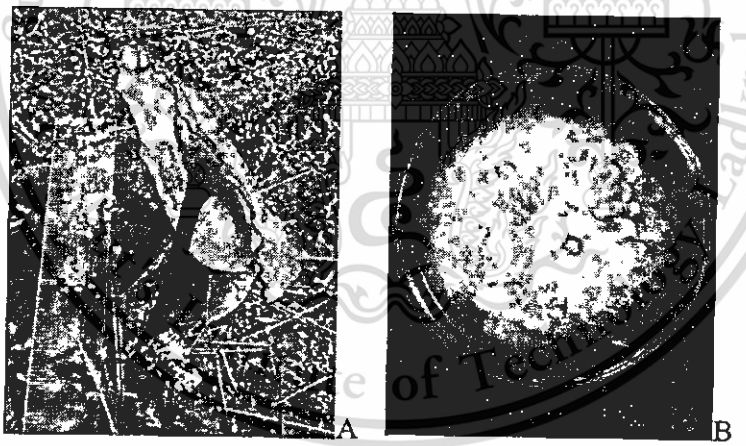

Fig.6. *Lactarius* sp. A: Fruiting body in the field B: Pure culture

Extraction of biological active substances

Pure cultures of *L. fragilissimus* PH06, *C. strictipes* PH07, *Clitocybe* spp AJ2-2, *B. affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01, *Lactarius* sp CH3-27 (Fig. 1-6) were isolated from fruting bodies and were separately cultured in PDB for 45 days. Each fungal biosmass was separately extracted to get crude hexane, crude ethyly acetate and crude methanol. With this, the crude

hexane, crude ethyly acetate and crude methanol from *L. fragilissimus* PH06 yielded 0.12, 1.12 and 4.06 %, respectively. The crude hexane, crude ethyl acetate and crude methanol from *C. strictipes* PH07 yielded 0.36, 0.36 and 0.40 %, respectively. The crude hexane, crude ethyly acetate and crude methanol from *Clitocybe* spp AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude ethyly acetate and crude methanol from *B. affinis* var. *maculosus* AJ2-3 yielded 0.43, 0.47 and 5.32 %, respectively. The crude hexane, crude ethyl acetate and crude methanol from *Lactarius* sp CH3-01 yielded 0.54, 2.12 and 5.03 %, respectively. The crude hexane, crude ethyly acetate and crude methanol from *Lactarius sp* CH3-27 yielded 3.88, 5.49 and 6.76 %, respectively (Table 2).

Table 2. Extraction of biological active substances from biomass culture for 45 days

Specimens	Fresh weight (g)	Fresh weight (g)	Yield[1], %	Crude Hexane(g)	Crude EtOAc(g)	Crude MeOH(g)
PH06 *L. fragilissimus*	3927	124.65	3.17	0.15 (0.12%)	1.39 (1.12%)	5.06 (4.06%)
PH07 *Collybia strictipes*	2010	55.00	2.73	0.2 (0.36%)	0.2 (0.36%)	0.22 (0.40%)
AJ2-2 *Clitocybe* spp	2500	72.10	2.88	4.27 (5.92%)	3.95 (5.48%)	4.32 (5.99%)
AJ2-3 *Boletus affinis var. maculosus*	5230	91.56	1.75	0.39 (0.43%)	0.43 (0.47%)	4.87 (5.32%)
CH3-01 *Lactarius* spp	1920	79.10	4.12	0.43 (0.54%)	1.68 (2.12%)	3.98 (5.03%)
CH3-27 *Lactarius* spp	4200	140.00	3.33	5.43 (3.88%)	7.69 (5.49%)	9.46 (6.76%)

[1](%)Yield = Weight after drying/ Weight before drying x 100%

Biological activity against coffee anthracnose caused by C. coffeanum

The crude extarcts from *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were selected for bioactivity test against coffee anthracnose caused by *C. coffaenum*. Result showed that methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 30 % for the colony growth of *C. coffaenum* at the concentration of 1,000 ppm when compared to the control (Table 3). Crude methanol from *Clitocybe* sp AJ2-2 gave significantly highest inhibited the spore production of *C. coffaenum* as 89.08 % and followed by crude ethyl acetate inhibited 86.48 % and crude hexane 70.45 % (Tables 4). The ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 33.53 % for the colony growth of *C.*

coffaenum at the concentration of 1,000 ppm when compared to the control (Table 5). Crude methanol and ethyl acetate from B. affinis var. maculosus AJ2-3 gave significantly highest inhibited the spore production of C. coffaenum as 67.86 % and followed by crude hexane inhibited 55.95 % (Tables 6).

Table 3. Crude extracts of *Clitocybe* sp AJ2-2 testing for growth inhibition of *Colletotrichum coffaenum* at 5days

Crude extracts	Concentration (ppm)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hexane	0	4.97^{a}	0.00^{g}
	10	4.92^{ab}	1.02^{fg}
	50	4.90^{ab}	1.53^{fg}
	100	4.82^{ab}	3.03^{efg}
	500	4.70^{bc}	5.54^{ef}
	1000	4.57^{cd}	8.04^{de}
Crude EtOAc	0	4.98^{a}	0.00^{g}
	10	4.87^{ab}	2.56^{fg}
	50	4.72^{bc}	4.27^{efg}
	100	4.70^{bc}	5.76^{ef}
	500	4.42^{d}	11.29^{d}
	1000	4.17^{e}	17.30^{c}
Crude MeOH	0	5.00^{a}	0.00^{g}
	10	4.77^{abc}	3.00^{efg}
	50	4.85^{ab}	4.75^{efg}
	100	4.45^{d}	12.50^{d}
	500	3.85^{f}	23.00^{b}
	1000	3.50^{g}	30.00^{a}
C.V.(%)		3.05	27.68

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4. Spore production inhibition of crude extracts from *Clitocybe* sp AJ2-2 to *Colletotrichum coffaenum* at 30days

Crude extracts	Concentration (ppm)	Number of spores [1](10^{5})	Inhibition(%)[2]
Crude Hexane	0	7.38^{def}	0.00^{bc}
	10	3.69^{cfg}	54.74^{ab}
	50	3.06^{fg}	63.81^{a}
	100	2.63^{fg}	70.45^{a}
Crude EtOAc	0	7.38^{def}	0.00^{bc}
	10	2.56^{fg}	65.55^{a}
	50	1.75^{g}	76.06^{a}
	100	1.00^{g}	86.48^{a}
Crude MeOH	0	7.38^{def}	0.00^{bc}
	10	3.69^{cfg}	51.34^{ab}
	50	1.56^{g}	78.67^{a}
	100	0.81^{g}	89.08^{a}
C.V.(%)		3.05	31.43

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.
[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

Table 5. Crude extracts of *Boletus affinis* var. *maculosus* AJ2-3 testing for growth inhibition of *Colletotrichum coffaenum* at 5days

Crude extracts	Concentration (ppm)	Colonydiameter (cm)[1]	Growth inhibition(%)[2]
Crude Hex	0	5.00^{a}	0.00^{h}
	10	4.80^{bc}	4.00^{fg}
	50	4.72^{cd}	5.50^{fg}
	100	4.40^{e}	12.00^{e}
	500	4.15^{gh}	17.00^{cd}
	1000	3.82^{i}	23.50^{b}
Crude EtOAc	0	4.92^{ab}	0.00^{h}
	10	4.20^{fg}	14.72^{de}
	50	4.17^{fgh}	15.23^{cde}
	100	4.05^{h}	17.77^{cd}
	500	3.70^{i}	23.86^{b}
	1000	3.27^{j}	33.53^{a}
Crude MeOH	0	4.97^{a}	0.00^{h}
	10	4.80^{bc}	3.53^{g}
	50	4.62^{d}	7.03^{f}
	100	4.30^{ef}	12.06^{e}
	500	4.30^{ef}	12.06^{e}
	1000	4.07^{gh}	18.34^{c}
C.V.(%)		2.17	13.87

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.
[2]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 6. Spore production inhibition of crude extracts from *Boletus affinis* var. *maculosus* AJ2-3 to *Colletotrichum coffaenum* at 30days

Crude extracts	Concentration (ppm)	Number of spores [1](10^x6)	Inhibition(%)[2]
Crude Hexane	0	1.56^{cde}	0.00^{b}
	10	1.13^{cde}	27.98^{ab}
	50	0.75^{de}	51.78^{ab}
	100	0.69^{de}	55.95^{ab}
Crude EtOAc	0	1.56^{cde}	0.00^{b}
	10	1.50^{cde}	3.57^{ab}
	50	1.25^{cde}	19.64^{ab}
	100	0.50^{e}	67.86^{a}
Crude MeOH	0	1.56^{cde}	0.00^{b}
	10	0.50^{e}	67.86^{a}
	50	0.50^{e}	67.86^{a}
	100	0.50^{e}	67.86^{a}
C.V.(%)		19.67	12.63

[1]Average of four replications, Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

Discussion

Thease were motphological identified into 49 species as follows:- *Agaricus macrosporus, Agaricus spp., Auricularia auricular, Boletus affinis var. maculosus, Boletus retisporus, Cantharellus cibarius, Clavulinopsis fusiformis, Clavulinopsis helvola, Clitocybula atrialba, Clitocybe spp., Collybia dryophila, Collybia iocephala, Collybia strictipes, Collybia spp., Coprinus spp., Inocybe fastigiata, Tricholoma spp., Lactarius controversus, Lactarius sanguifluus, Lactarius spp., Laccaria vinaceoavellanea, Laccaria spp., Leucocoprinus fragilissimus, Marasmiellus albuscorticis, Marasmiellus ramealis, Marasmius androsaceus, Marasmius foetidus, Marasmius purpureostriatus, Marasmius oreades, Marasmius plicatulus, Marasmius scorodonius, Marasmius spp., Mycena inclinata, Mycena rosella, Mycena subcaerulea, Mycena vulgaris, Mycena spp., Pleurocybella porrigens, Pluerotus giganteus, Resinomycena rhododendri, Russula crassotunicata, Russula spp., Schizophyllum commune, Termitomyces microcarpus, Trametesversicolor, Tremiscus spp., Termitomyces spp., Tricholoma spp. and Xylaria hypoxylon.* With this, there are some literature reviews found those species in Thailand (Akom, 1996; David and Brian, 1992; Gary, 1981; Soytong,

1994; Konemann, 1998; Smith, 2001; Roger, 1991; States, 2004; Susan and Van, 2000). *Leucocoprinus fragilissimus* PH06, *Collybia strictipes* PH07, *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01 and *Lactarius* sp CH3-27 were described which these species reported to be found in Thailand (Konemann, 1998; Roger, 1991; States, 2004; Susan and Van, 2000).

As result showed that methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 30 % for the colony growth of *C. coffaenum* at the concentration of 1,000 ppm. Crude methanol from *Clitocybe* sp AJ2-2 inhibited the spore production of *C. coffaenum* as 89.08 % and followed by crude ethyl acetate inhibited 86.48 % and crude hexane 70.45 %. It was also found that crude methanol and ethyl acetate of *B. affinis* var. *maculosus* AJ2-3 inhibited spore production of *C. coffaenum* 67.86 % and followed by crude hexane inhibited 55.95 %. The research findings are reported for the first time that the metabolites from Clitocybe sp AJ2-2 and B. affinis var. maculosus could inhibit *C. coffaenum* causing coffee anthracnose. Similar report from Badalyan *et al.* (2002) stated that the antagonistic activity of 17 species of Basidiomycotina (*Coriolus versicolor, Flammulina velutipes, Ganoderma lucidum, Hypholoma fasciculare, H. sublateritium, Kühneromyces mutabilis, Lentinula edodes, Lentinus tigrinus, Pholiota alnicola, Ph. aurivella, Ph. destruens, Pleurotus ostreatus, P. cornucopiae, Polyporus squamosus, P. subarcularius, P. varius* and *Schizophyllum commune*) could inhibit plant pathogens, *Bipolaris sorokiniana, Fusarium culmorum, Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia cerealis* that causing foot and root diseases of winter cereals.

The potential of fungal metabolites from fungi have been usually reported to produce antibiotic substances against human and plant pathogens. Kanokmedhakul *et al.* (2003) reported that a macrofungi, *Scleroderma citrinum* produces a bioactive triterpenoid and vulpinic acid derivatives that expressed against *Candida albicans*. Morober, Soytong *et al.* (2014) reported that the natural products were isolated from the fruiting bodies of *Scleroderma citrinum*. A new lanostane-type steroids were found namely 4,4'-Dimethoxymethyl vulpinate (DMV) and 4,4'-Dimethoxyvulpinic acid (DMVA). These compounds showed that 4,4'-Dimethoxyvulpinic acid inhibited *Colletotrichum gloeosporioides* than 4,4'-Dimethoxymethyl vulpinate at all tested concentrations. The effective dose (ED_{50}) of DMVA compound could inhibit the mycelium growth of *C. gloeosporioides* at the concentrations of 81 ppm, respectively. The ED_{50} of DMV compound for inhibition of mycelial growth was 2,114 and 5,231 ppm, respectively. The production of conidia of *C. gloeosporioides* was inhibited by both compounds which the ED_{50} of DMA and

DMVA compounds were 45 and 68 ppm, respectively. Rieger *et al* (2010) reported that pure culture of Basidiomycete, *Carpia montagnei* produced caripyrin as a new pyridylooxirane that inhibited *Magnaporthe oryzae* causing rice blast pathogen. These investigations were found biological active substances from *Clitocybe* spp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 to inhibit coffee anthracnose caused by *C. coffaenum.* The control mechanism would be involved in bioactive compound producing from these mushroom which possible be elucidated in further search finding.

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Effect of different factors on sporulation of *Colletotrichum coffeanum*

Luo Yaling[1*], Wattanachai Pongnak[2] and Soytong Kasem[1]

[1]Department of Plant Production Technology, [2]Department of Soil Science, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang , Bangkok 10520, Thailand

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The effect of different factors on sporulation of *Colletotrichum coffeanum* was investigated. Result showed that V8 and PDA media were suitable for the growth of *C. coffeanmum*. On the contrary, the content of the media gave a significant impact on sporulation. The media content increased then it would greatly increase the sporulation. 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) was the most suitable light condition for *C. coffeanmum* sporulation, compared with constant darkness (continuous darkness; CD) and constant illumination (continuous light; CL; 10000 lux, fluorescent lamp). Moreover, the growth rate in the 28th day showed the fastest growing culture. It is concluded 10 ml PDA medium and incubated 28 days under natural light conditions was the best time of sporulation.

Key words: Sporulation, *Colletotrichum coffeanum*

Introduction

Coffee belongs to Rubiaceae, is a perennial evergreen shrub and it is a perennial horticultural crop. Leaves which are opposite elongated oval, glossy, at the end of a long branches, small branches, and flowers are white, open branches in the base of the petiole link. Once ripe, coffee "berries" are picked, processed, and dried to yield the seeds inside. The seeds are then roasted to varying degrees, depending on the desired flavor, before being ground and brewed to create coffee. The main active ingredient caffeine of coffee, have a strong central stimulant effect. People taking caffeine or caffeinated beverages often disappears drowsiness, fatigue mitigation, quick thinking. Dose increased, the central stimulant effects more obvious tensions, anxiety, restlessness,

* Corresponding author: Luo Yaling; e-mail: iamalinluo@163.com

insomnia and tremor. Larger doses produce local or systemic spasm (Frey and Rebecca, 2003).

Coffee anthracnose which caused by *Colletotrichum coffeanum* is very common and one of the important disease of coffee trees. It causes economically important losses in coffee plantation. The symptom of coffee anthracnose disease is appeared irregular light brown to dark brown spots; lesion central white, yellow edge, later gray, many small black dots are arranged in concentric ring pattern in leaves Ripe berries and green berries show the symptom: water-soaked spots, lesions become sunken, dark brown to gray-black big spots (Brooklyn Botanic Garden, 2000). Therefore, the study of the use of bioactive substances to control coffee anthracnose has been interestingly increased. Ajith (2010) reported that all the selected *Trichoderma harzianum, Trichoderma saturnisporum* and *Trichoderma reesei* has potential to control growth of *Colletotrichum capsici* causing chilli anthracnose by 21 to 68% at a concentration of 50% culture filtrate. Meanwhile, Vilavong and Soytong (2013) reported that *Chaetomium cupreum* can be antagonized *C. coffeanum* in dual culture plate after incubation for 30 days. The colony of *Ch.cupreum* grew over pathogen colony implies competition mechanism of control. This research finding proved that *C. coffeanum* causing anthracnose shown to be infected leaves and beans. *Ch.cupreum* proved to be effective antagonist against anthracnose pathogen.

Under certain conditions some fungi undergo microcycle conidiation (Smith, 1981), whereby sporulation occurs directly after spore germination without, or with greatly reduced, mycelia growth. Microcycle conidiation of certain fungi may be induce by high-temperature stress (Anderson *et al.*, 1971; Anderson *et al.*, 1972; Sekiguchi *et al.*, 1975), nutrient depletion (Boosalis *et al.*, 1962; Mangenot *et al.*, 1976), or other factors inhibiting vegetative development (Park *et al.*, 1970; Rotem *et al.*, 1970). In the report of Slade (1987) mentioned that the effects of inoculum density, medium composition and concentration, and temperature on spore-carrying capacity (SCC) and microcycle conidiation by *Collectotrichum gloeosporioides* that was studied on solid media. For this fungus, spore production on solid media was similar to that in liquid media, so relationships found with the microplate method should provide useful information for spore production in analogous liquid systems.

The objective of this research finding was to study on the type of media, volume of media, light condition and incubation time for sporulation of *Colletotrichum coffeanum.*

Material and methods

Isolation of pathogen and pathogenecity test

Colletotrichum coffeanum was isolated by using tissue transplanting technique from coffee anthracnose on leaves var Arabica. The advanced margin of lesion was surface disinfected with sodium hypocholite 10 %, then cut with sterilized blade into small piece of 0.5 X 0.5 cm between advanced margin of healthly and infeted tussues on symptom of leaf, then soaked into 10 % sodium hypocholite for a few minutes, and moved to sterilize distilled water, then placed in sterilized tissue paper to dry out, thereafter picked up with needle and placed onto water agar (WA), then incubated at room temperature approximately 27-30 ℃. The hyphal tip isolation was done by cutting with needle into small piece of hyphal tip and transferred onto potato dextrose agar (PDA), incubated at room temperature and observed growing colony until getting pure cultures. All isolates were morphologically identified into species by using binocular compound microscope.

The experiment was conducted by using 3 factors factorial in Completely Randomized Design (CRD) with four replications. Factor A represented the different media whicht give effect on spore production: A1= PDA media (potatoes, 200 g; dextrose, 15 g; agar, 20 g; H_2O, 1 L); A2= V8 juice media (V8 Juice, 200.0 ml; CaCO3, 3.0 g; Agar, 15.0 g; Tap water to1.0 L; PH, 7.2). Factor B represented different light conditions: B1= constant illumination (continuous light; CL; 10000 lux, fluorescent lamp), B2= constant darkness (continuous darkness; CD), B3= 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp). Factor C represented the different volume of media: C1= 5ml media, C2=10ml media. 3 mm-diameter agar plug cut by sterilized cork borer from the pure cultures of *Colletotrichum coffeanum* placed d in the center of 5 cm petri dishes which contained 5 ml of PDA media, 10 ml of PDA media, 5 ml of V8 media, 10 ml of V8 media, respectively.

Data were collected and obsereved for sporulation every 7 days. The culture surface was washed 3 times with 10ml tap water to remove the fungal spores for counting. Spore production was quantified using a hemacytometer. Data was statistically computed analysis of variance. Treatment means were computed by Duncan Multiple's Range Test (DMRT) at P=0.05 and P=0.01. The comparison between normal and abnormal grew on corneal dual-culture were observed under compound microscope.

Pathogenicity test was conducted using detached leaf inoculated method. The experiment was done using Completely Randomized Design (CRD) with four replications. Treatments were inoculated into wounded leaves surface with an agar plug of pathogen and placed in moist chamber done in Petri dishes. Control treatment was done by transferring an agar plug of PDA alone onto wounded surface leaf.

Results

Colletotrichum coffeanum was isolated and identified causing anthracnose on coffee leaves var. Arabica (Fig.1).

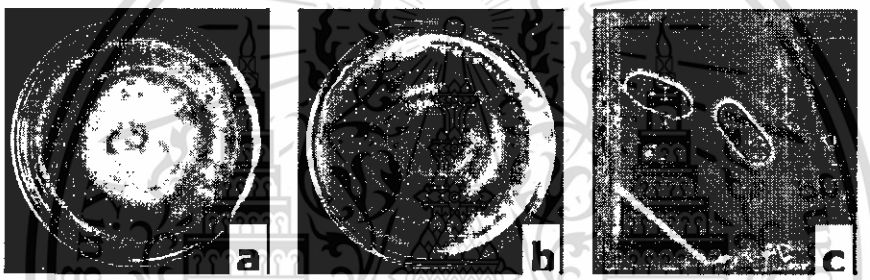

Fig. 1 *Colletotrichum coffeanum* isolated from coffee leaves anthracnose
a, b = Pure cultures of *C.coffeanum*. c = conidia. Bar. c= 10 μm

The results showed that the lesion size developed by *C. coffeanum* isolate was 27.25 mm, which gave the high virulent for disease incidence to prove pathogenicity test.

Two kinds of media and two kinds of volume of media were tested to determine the effect of nutrients on sporulation. That was 5 ml of PDA media, 10 ml of PDA media, 5 ml of V8 media, 10 ml of V8 media, respectively. All of plates were incubated under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp), constant darkness (continuous darkness; CD) and a 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp), respectively. In these three different lighting conditions, continuous cultured 35 days. Spore production was quantified using a hemacytometer at each stage. Specific data are shown in Table 1.

Table 1 Effect of different factors on sporulation of *Colletotrichum coffeanum*

Media	Light conditions[1]	volume of media	Spore production at different times (10^6)			
			14 days	21 days	28 days	35 days
PDA	CL	5ml	3.75^{dl}	11.00^{d}	34.00^{h}	90.50^{fg}
		10ml	5.75^{bc}	18.00^{bc}	55.25^{f}	111.50^{e}
	12:12 L:D	5ml	5.50^{bcd}	16.75^{bcd}	78.50^{cde}	175.25^{c}
		10ml	9.50^{a}	30.50^{a}	155.50^{b}	231.00^{a}
	CD	5ml	5.00^{bcd}	13.50^{cd}	41.25^{gh}	83.25^{g}
		10ml	6.50^{b}	22.75^{b}	75.75^{de}	152.25^{d}
V8	CL	5ml	4.25^{cd}	13.00^{cd}	44.00^{g}	100.00^{ef}
		10ml	5.75^{bc}	17.50^{bcd}	75.00^{e}	111.50^{e}
	12:12 L:D	5ml	5.75^{bc}	18.00^{bc}	86.25^{c}	192.50^{b}
		10ml	9.00^{a}	33.50^{a}	163.50^{a}	231.00^{a}
	CD	5ml	5.25^{bcd}	16.50^{bcd}	53.00^{f}	88.25^{fg}
		10ml	6.00^{bc}	23.25^{b}	83.25^{cd}	169.75^{c}

[1]/ CL=continuous light, 10000 lux, fluorescent lamp; CD=constant darkness, continuous darkness; 12:12 L:D= 12 h light/dark photoperiod , 10000 lux, fluorescent lamp

[2]/Average of four replications, Mean followed by the same letters are not signicantly different by Duncan's multiple range test at P= 0.05.

The type of Media

Result showed that V8 juice agar was the best media for inducing sporulation (up to 2.31 x10^8) at 35th day, but it was not significantly different from PDA media, and followed by media in different volume constant illumination (continuous light; CL; 10000 lux, fluorescent lamp), respectively (Fig.2). The best one was 10ml V8 juice agar under constant illumination. Under the same conditions as the volume, the spore production of V8 was higher than PDA. The effects of 5 ml PDA, 10 ml PDA, 5 ml V8, 10 ml V8, two different media in different volume under the same 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) were shown in Fig. 3. The best one was 10ml V8 juice agar. Under the same conditions as the volume, the spore production of V8 was higher than PDA, but not significantly. Thee effects of 5 ml PDA, 10 ml PDA, 5 ml V8, 10 ml V8, two different media in different volume under constant darkness (continuous darkness; CD) were shown in Fig. 4. The best one was also 10ml V8 juice agar. The sporulation of V8 was higher than PDA. In short, PDA and V8 were able to induce the spore production of *Colletotrichum coffeanum*. But, V8 was better than others.

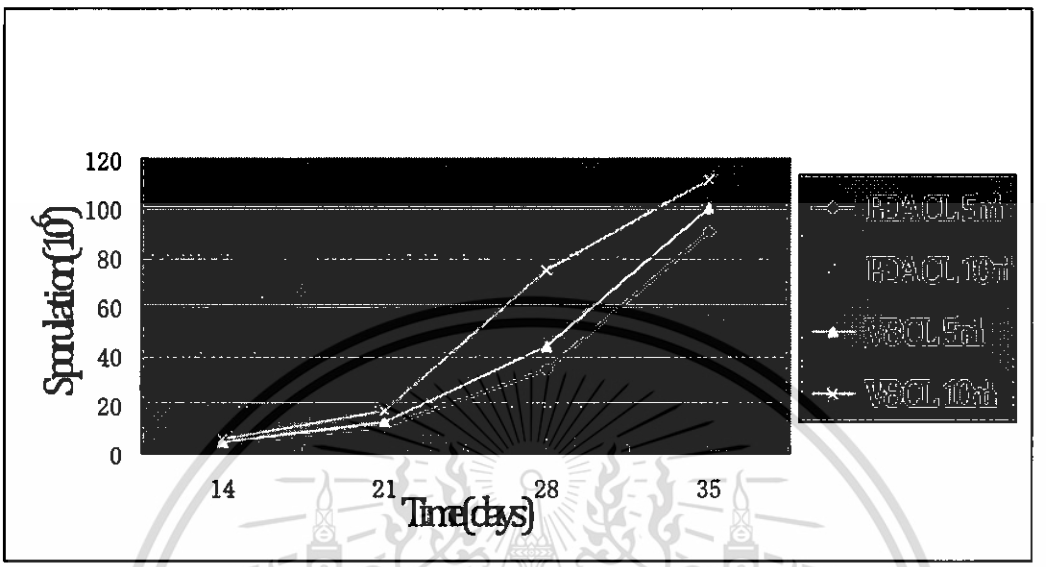

Fig.2 Effect of two kind of media of different volume under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp) on spore production

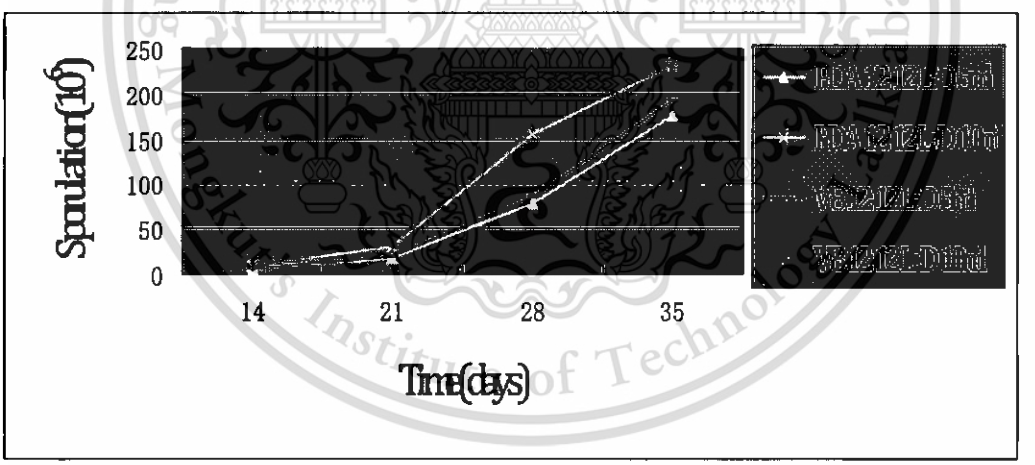

Fig.3 Effect of two kinds of media of different volume under 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) on spore production

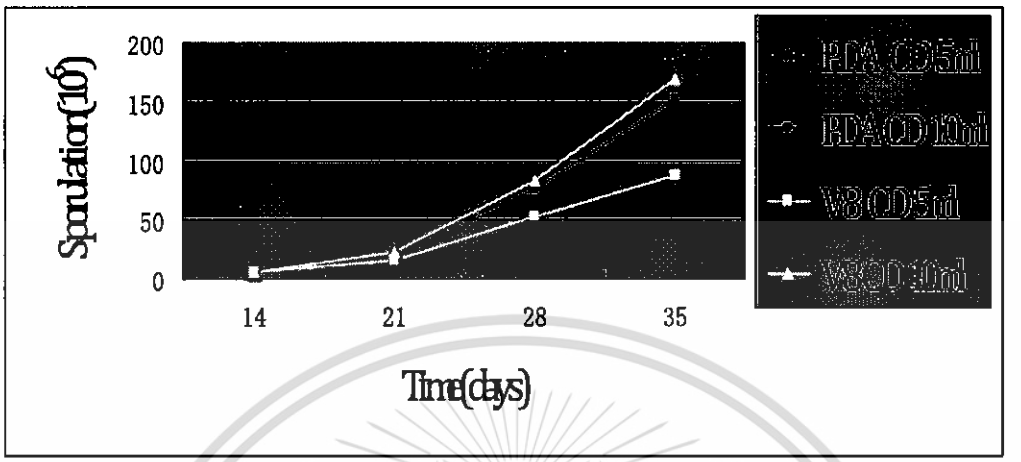

Fig.4 Effect of two kinds of media of different volume under constant darkness (continuous darkness; CD) on spore production

The volume of media

Result showed the effect of 5 ml and 10 ml PDA under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp) on sporulation were differed to each other (Fig.5). The best one was 10ml V8 juice agar under constant illumination. At the same time, 10 ml of the PDA medium gave a greater degree of sporulation of *Colletotrichum coffeanmum* than other treatmentswhen compared with 5ml PDA medium. The result of constant darkness (continuous darkness; CD) and a 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) were shown in Figs. 6 and 7.

The effect of different volume V8 under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp) on spore production, effect of different volume PDA under 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) on spore production and effect of different volume PDA under constant darkness (continuous darkness; CD) on spore production were shown in Figs. 8, 9 and 10.

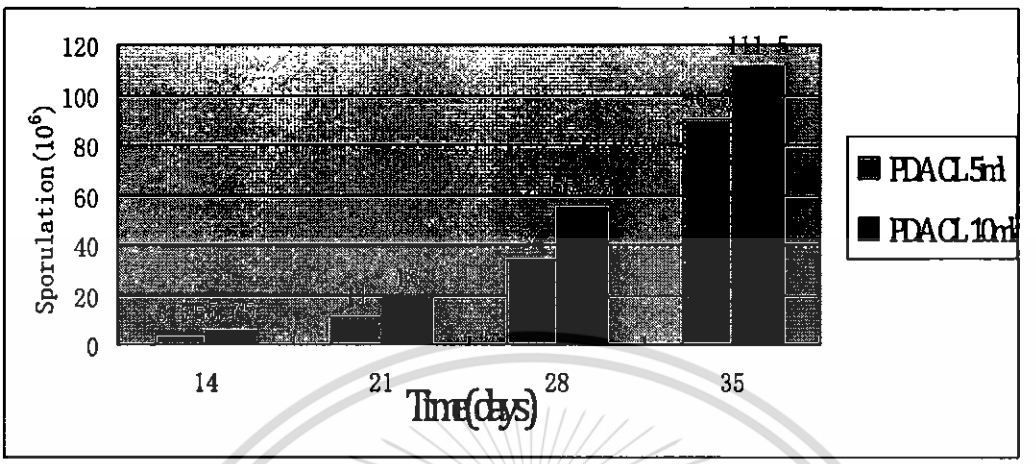

Fig. 5 Effect of different volume PDA under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp) on spore production

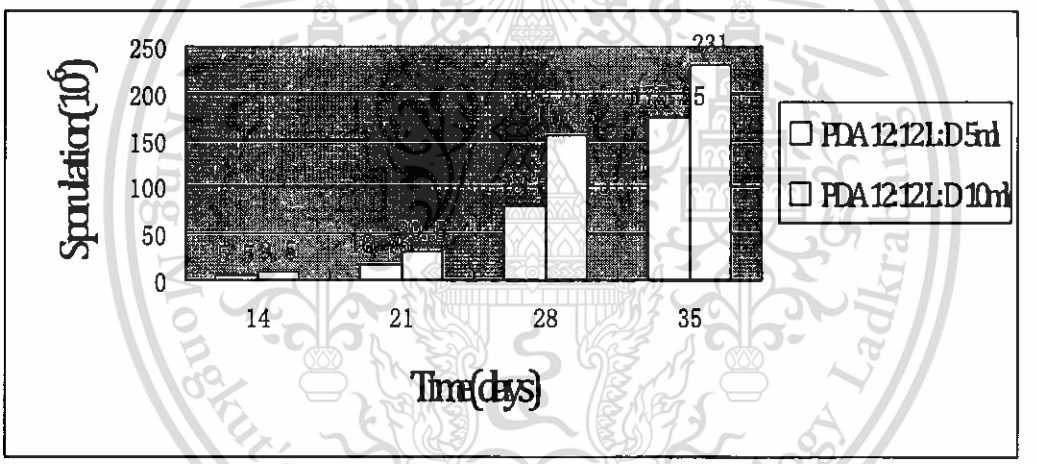

Fig. 6 Effect of different volume PDA under 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) on spore production

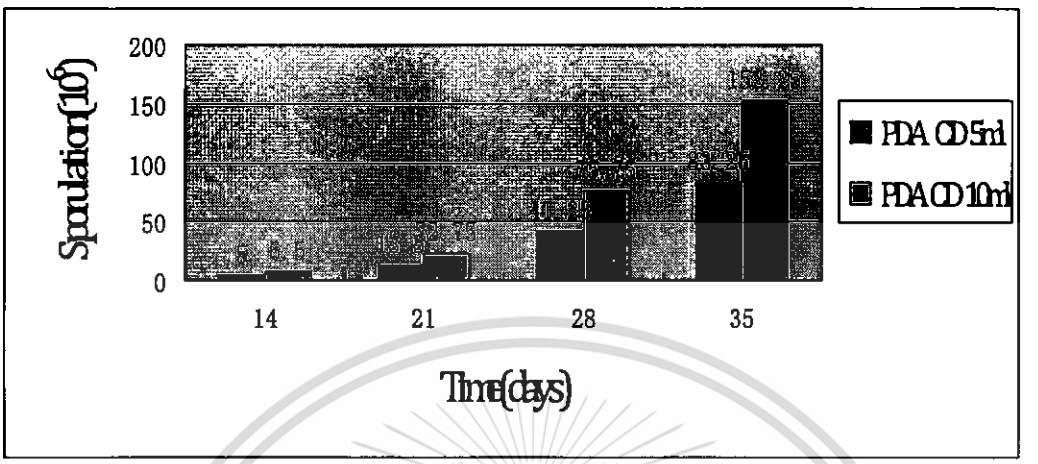

Fig. 7 Effect of different volume PDA under constant darkness (continuous darkness; CD) on spore production

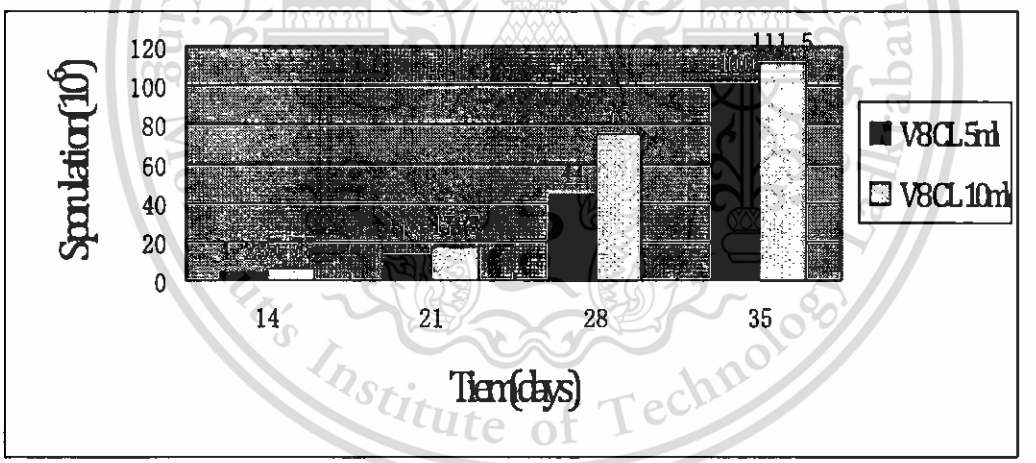

Fig. 8 Effect of different volume V8 under constant illumination (continuous light; CL; 10000 lux, fluorescent lamp) on spore production

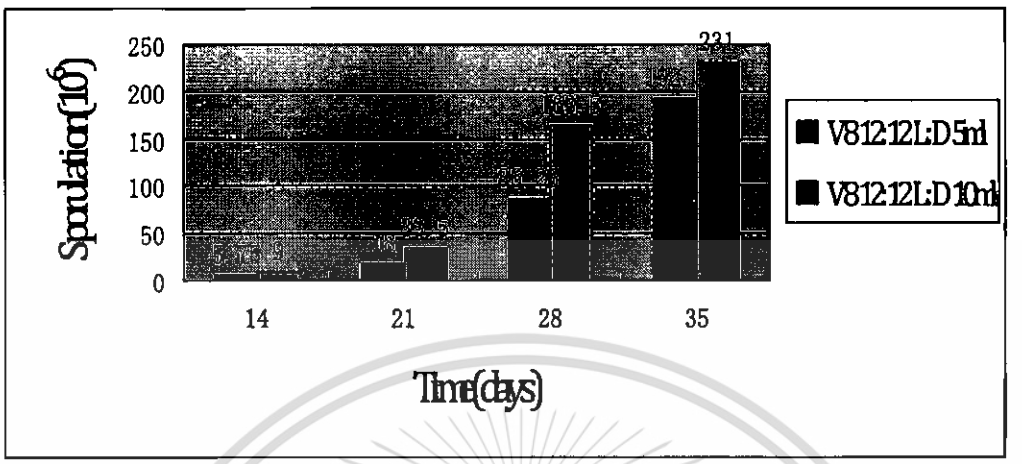

Fig. 9 Effect of different volume PDA under 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) on spore production

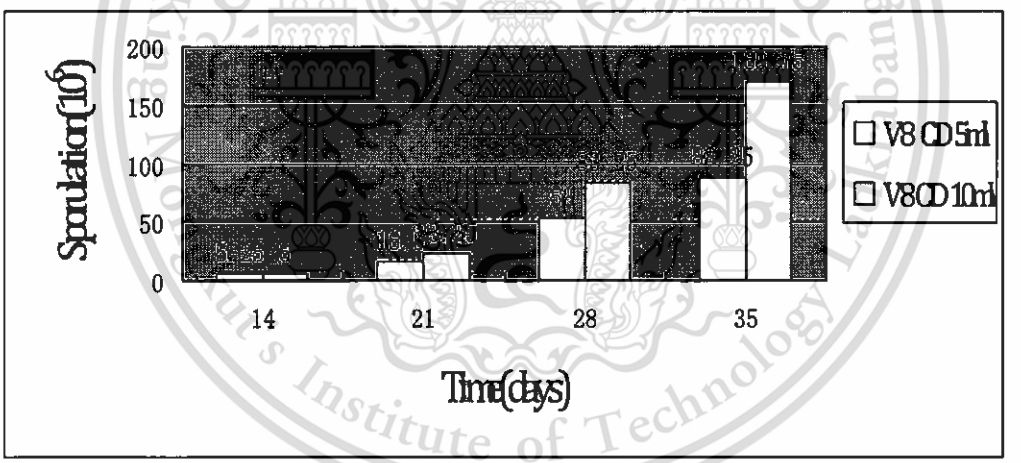

Fig. 10 Effect of different volume PDA under constant darkness (continuous darkness; CD) on spore production

Light conditions

Result showed the effect of different light conditions in same volume PDA medium on spore production were differed (Figs. 11 and 12). At early observation (before 21st day) found that there was no significantly afftected on sporulation. However, at the 28th days observation found that only under 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) would

significantly promote the induction of sporulation. Another two groups were still no significantly differed. The effect of different light conditions in 5ml and 10 ml V8 medium on sporulation was shown in Figs. 13 and 14.

In conclusion, the illumination time, there was no significantly afftected on sporulation, but in the late of growth stage, light time would impact the sporulation. 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) gave the highest sporulation when compared with constant darkness (continuous darkness; CD) and constant illumination (continuous light; CL; 10000 lux, fluorescent lamp). The 28th day incubation, the sporulation was 155.5x10^{6} and 35thday, the sporulation was 231 x10^{6}.

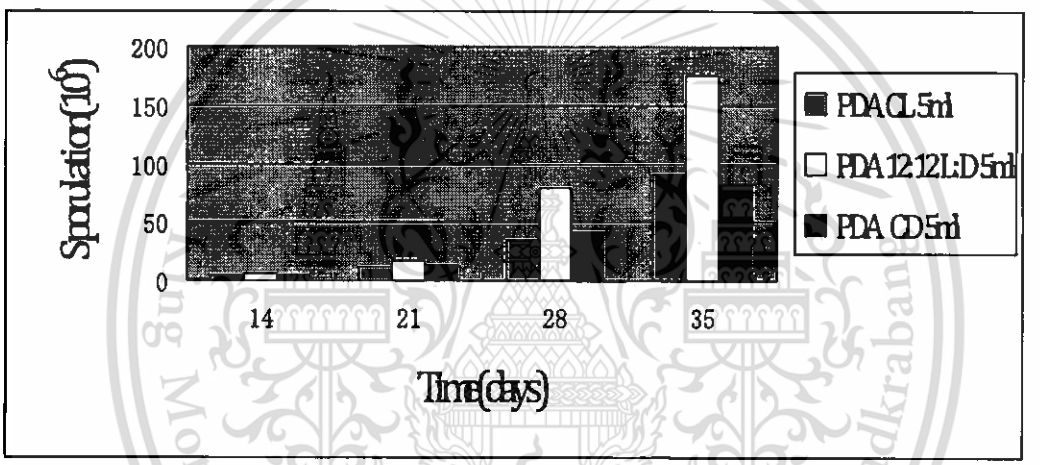

Fig. 11 Effect of different light conditions in 5ml PDA medium on spore production

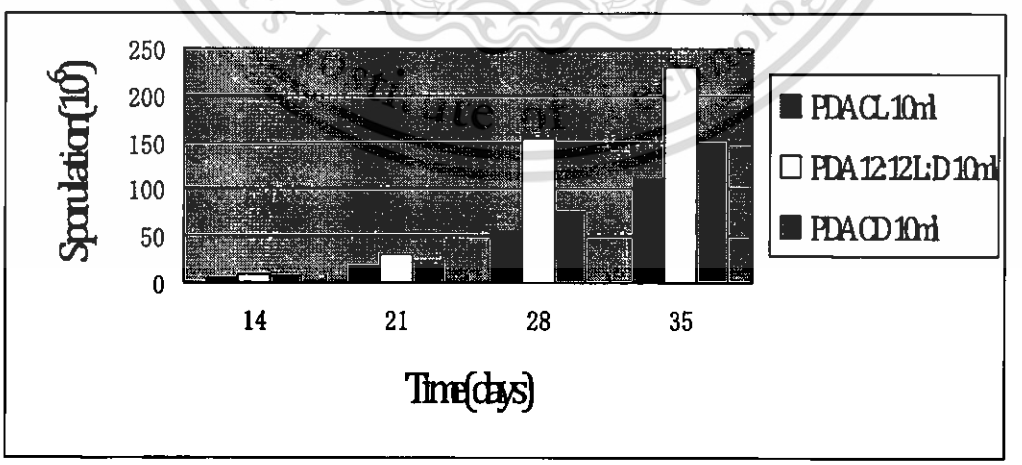

Fig. 12 Effect of different light conditions in 10ml PDA medium on spore production

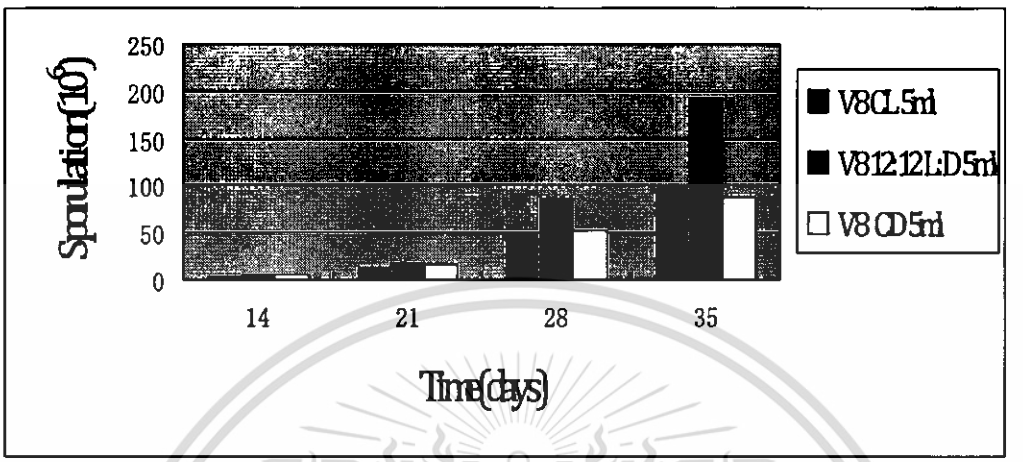

Fig. 13 Effect of different light conditions in 5ml V8 medium on spore production

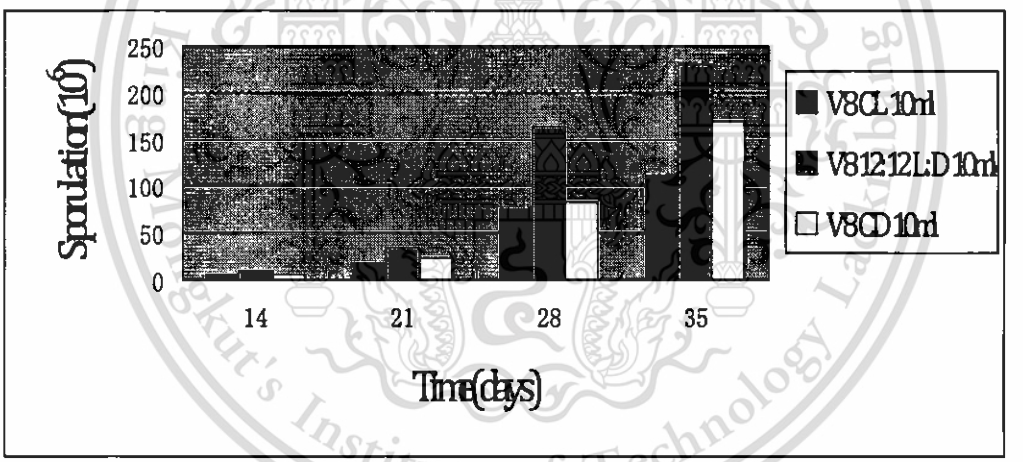

Fig. 14 Effect of different light conditions in 10ml V8 medium on spore production

Incubation time

The highest growth rate of *Colletotrichum coffeanmum* was shown on the 28th day incubation. Withthis, 10ml PDA medium gave the highest growth rate. At the 28th day, the growth rate started to decrease (Table 2, Fig.15).

Table 2 Growth rate of sporulation of *Colletotrichum coffeanum*

Media	Light conditions[1]	The volume of media	Growth rate		
			21st day [2]	28th day[3]	35th day[4]
PDA	CL	5ml	1.93	2.09	1.66
PDA	CL	10ml	2.13	2.07	1.02
PDA	12:12 L:D	5ml	2.05	3.69	1.23
PDA	12:12 L:D	10ml	2.21	4.10	0.49
PDA	CD	5ml	1.7	2.06	1.02
PDA	CD	10ml	2.5	2.33	1.01
V8	CL	5ml	2.06	2.38	1.27
V8	CL	10ml	2.04	3.29	0.49
V8	12:12 L:D	5ml	2.13	3.79	1.23
V8	12:12 L:D	10ml	2.72	3.88	0.41
V8	CD	5ml	2.14	2.21	0.66
V8	CD	10ml	2.88	2.58	1.03

[1]/ CL=continuous light, 10000 lux, fluorescent lamp; CD=constant darkness, continuous darkness; 12:12 L:D= 12 h light/dark photoperiod , 10000 lux, fluorescent lamp
[2]/ Growth Rate=(Sporulation at 21st day - Sporulation at 14th day)/ Sporulation at 14th day
[3]/ Growth Rate=(Sporulation at 28th day - Sporulation at 21st day)/ Sporulation at 21st day
[4]/ Growth Rate=(Sporulation at 35th day - Sporulation at 28th day)/ Sporulation at 28th day

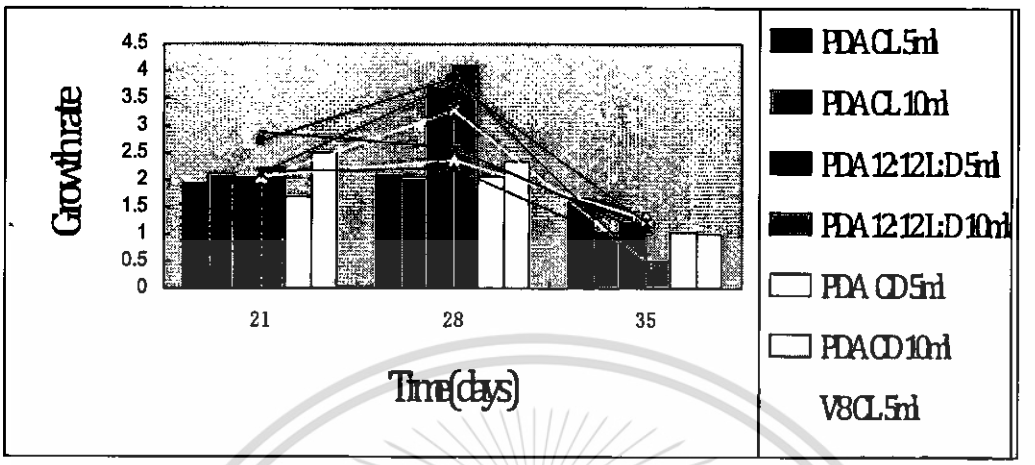

Fig. 15 Effect of incubation time on spore production

Result showed that V8 and PDA were suitable for the growth of *Colletotrichum coffeanmum* and not significantly differed when compare to the control. The media content gave significantly difference on sporulation. The increased media content, it also would increase sporulation in this research findings. 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) was the most suitable light condition for the growth of *Colletotrichum coffeanmum* sporulation, compared with constant darkness (continuous darkness,CD) and followed by the constant illumination (continuous light; CL; 10000 lux, fluorescent lamp). Although with the growth of time, spore production was also growing. However, the growth rate in the 28th day was the fastest sporulation with 10 ml PDA medium and incubated 28 days under natural light condition showed the best sporulation.

Discussion

In this study showed that V8 juice agar was the more suitable medium for sporulation of *C.coffeanum,* compared with the PDA agar but not significant. The content of the medium gave a significant impact on spore yield. Increase media content, also would greatly improve the spore production. 12 h light/dark photoperiod (12:12 L:D; 10000 lux, fluorescent lamp) was the most suitable light condition for *Colletotrichum coffeanmum* sporulatio. Slade *et al.* (1987) reported that the effect of spore inoculum density, medium concentration, and temperature on slime-spot formation,spore yield, and mycelium production by *Colletotrichum gloeosporioides* on agar media were studied with a simple microplate assay. A steady-state spore yield (spore-carrying capacity)

independent of inoculum density was reached only on media that supported good fungal growth and sporulation. The spore-carrying capacity was reached earlier, the denser the inoculum. On standard mycological media a high inoculum density (2.5 x10^6 spores per ml) resulted in a slimy mass of conidia forming a slime spot, a phenomenon associated with greatly reduced mycelium formation and indicative of microcycle conidiation. In contrast, for a similar inoculum density, enhanced mycelial growth preceded sporulation and overrode slime-spot formation on highly concentrated media; a very low medium concentration resulted in much less mycelium, but spore production was also decreased. Exposure to suboptimal growth temperatures of 36 to 48°C for up to 8 days did not induce microcycle conidiation from inocula that did not form a slime spot at 28°C. Microcycle conidiation, as indicated by rapid development of a slime-covered colony after fungal spore inoculation of solid media, occurred on diverse commonly used microbial media but only under conditions of a high inoculum density (25,ul, 2.5 x 106 spores per ml).Microcycle conidiation was most pronounced on media highly favorable for fungal growth, such as V8 and RV8 (both of which contain 20% V8 juice) and PDA. However, increasing the V8 concentration beyond the normal 20% level caused microcycle conidiation to be replaced by dense vegetative mycelial development. These data are consistent with microcycle induction being a function of (i) diffusionrestricted nutrient availability to the fungal colony (resulting from nutrient demand by a rapidly developing high inoculum colony outstripping nutrient resupply to the colony by diffusion from the surrounding agar), (ii) spore densitydependent accumulation of microcycle-inducing fungal metabolites, or both. High nutrient concentrations in the media (e.g., >20% V8) would attenuate diffusion-restricted nutrient availability and might also override metabolite induction of microcycle conidiation. The critical nutrient factor promoting mycelial growth rather than microcycle conidiation at high inoculum densities is not simply organic content, since for similar inocula slime spots are formed on RV8 (56 g of organic matter liter-') and on 20% V8 (8 gliter-1) but are substantially reduced on 40% V8 (16 g liter-1) and are not formed at all on 60% V8 (24 g liter-1) (Slade *et al.* 1987).

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