Complete Report

Study on Agaricus in Eastern and Central Thailand and Bioactive Compound Test

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ABSTRACT

Sixty wild mushrooms were collected in five provinces of six points in Thailand. These were divided into 7 orders, 17 families. Three species of wild mushrooms were selected for screening antagonists.

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 µg/ml. Followed by crude hexane extract from *B. affinis* var. *maculosus* AJ2-3 and methanol crude extract from *Lactarius* spp. CH3-01 with percentage of inhibition of 55.95 and 76.13% which the effective dose (ED_{50}) at 75.19 and 98.66 µg/ml, respectively.

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 methanol crude extract from *Lactarius* spp. CH3-01 gave significantly highest inhibition for the spore production of *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 by 83.95% with the effective dose (ED_{50}) of 3.79 µg/ml and at the concentration of 1,000 µg/ml. Followed by ethyl acetate crude extract from *Clitocybe* spp AJ2-2 and ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 with percentage of inhibition of 83.90% and 79.71% 17.54 and 59.85 µg/ml, respectively. Therefore, methanol crude extract from *Lactarius* spp. CH3-01 was selected as a potent antagonist to control fusarium wilt of tomato.

Keyword: Mushroom; *Colletotrichum Coffeanum*; *Fusarium oxysporum* f. sp. *lycopersici*

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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. 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). 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* sp. species, respectively, as well as the French origin of *A. bisporus,* named champignon.

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).

1.1.2 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).

1.2 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. 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.

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). 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.

CHAPTER 2

OBJECTIVES

2.1 Research Background

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.

2.2 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 substance. 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 3

RESEARCH METHODOLOGY

3.1 Mushroom Collection and Identification

3.1.1 Survey Procedure

Collections were made in the forests 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.

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.

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

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.

3.2 Bioactive Compound Test 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.

3.2.3 Bioactive Compound tests

3.2.3.1. Bioactive Substance Extraction

The fungal metabolites from some species of Agaricales, as follows: 1. *Clitocybe* spp (AJ2-2); 2. *Boletus affinis* var. *maculosus* (AJ2-3); 3. , *Lactarius* spp. (CH3-01) 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).

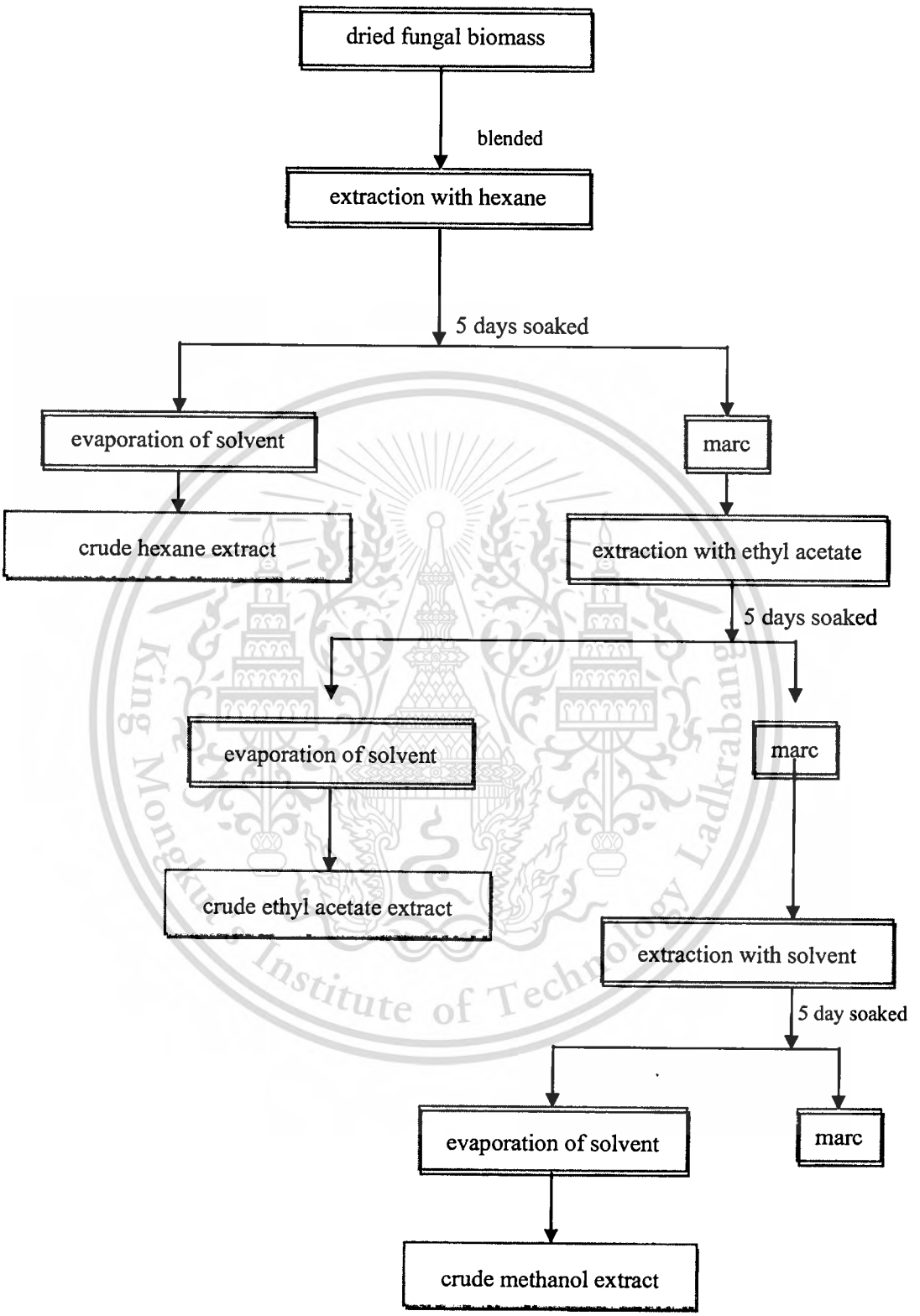

Fig. 3.1 Extraction method.

3.2.3.2 Bioactive Compound Tests against *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 race 2 Causing Tomato Wilt and *Colletotrichum coffeanum* Causing Coffee Anthracnose

The crude extracts of mushroom 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 $lbs/inch^2$ 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.

CHAPTER 4

RESULTS

4.1 Mushroom Collection and Indentification

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, Table 4.1.

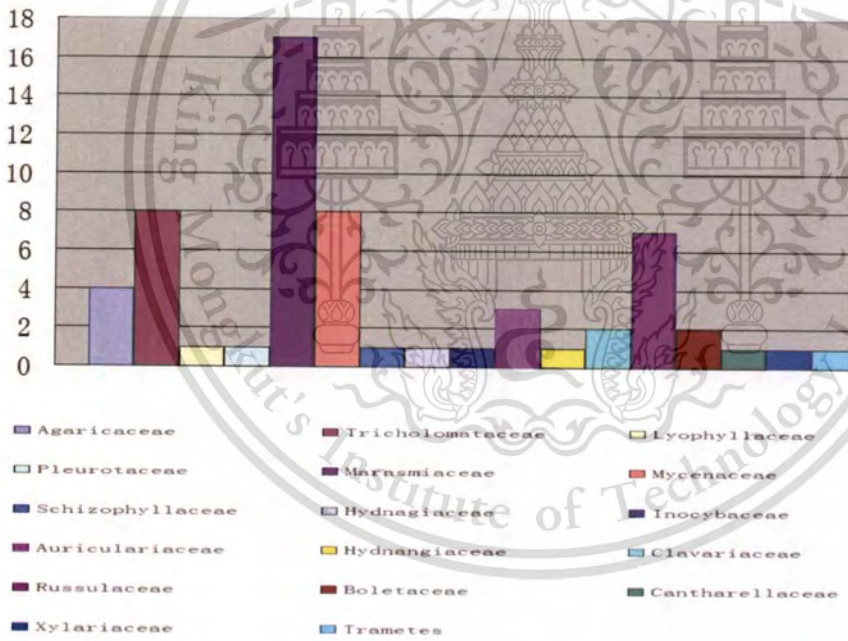

Fig.4.1 Collection of mushrooms

Table 4.1 Collection of mushrooms

No.	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)

No.	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)

No.	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)

No.	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, small 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.

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,* 0.2x1.2cm high, slender, same color with cap, smooth (Fig.4.3). Habit: scattered under leaves.

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.

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.

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.

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

A small flesh mushroom. *Cap,* 0.7 cm in diameter, bell-shaped to hemisphere, brown to buff, margin with clear streak, *Gill,* free, unequal, board, brown; *Stem,* 2.8 x 0.1 cm, upper white with half bottom brown, smooth (Fig.4.7). Habit: solitary on the fallen leaves.

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.

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 at first 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.

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

Cap, 3-3.2 cm in diameter, convex, densely covered dark small scales, dark center; *Gill,* free, white, unequal; *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 .

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.

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.

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: grow on humus.

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 .

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

Cap, flat, smooth, white; *Gills,* adnate, white; *Stem,* white, smooth, thin (Fig.4.15). Habit: scattered on fallen leaves .

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.

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 .

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.

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

Cap, 2.2 cm in diameter, pure white, flat ; *Gills,* free, unequal, white; *Stem,* 1.2 x 0.1 cm, white, smooth (Fig.4.19). Habit: solitary on the dead wood .

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 .

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 .

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.

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

Cap, 2-3.5 cm in diameter, white, flat or convex with slightly depress in the center, sticky, smooth ; *Gill,* white, unequal, free, close, dark when dry; *Stem,* 1.5-3.5 x 0.5-1 cm, white, cylindrical, smooth; *Spore print,* white (Fig.4.23). Habit: grow in clisters on rotting soil.

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

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

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.

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

Cap, 2.5 cm in diameter, yellowish brown, convex to flat ,smooth, wavy margin with clear lined ; *Gills,* free, unequal, buff; *Stem,* 1 cm, yellowish buff, cylindrical, smooth; *Spore print,* brown (Fig.4.26). Habit: solitary in soil.

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.

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

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

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.

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 0.7cm,dark brown, cylindrical, downy the part attach gills is red (Fig.4.30). Habit: solitary in soil.

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.

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, no attachments; *Gill,* free, pale-brown, *Stem,* 5.5 x1 cm, smooth, deep brown, black bottle, thick with a white membranous ring, easy to fall off; *Flesh,* white, thick (Fig.4.32). Habit: scattered in the wet solid.

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.

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.

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: grows in clusters in grassland.

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: grows in grassland or tea garden.

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.

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.

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

Cap, strong convex, pure white, smooth, wet, slightly line with margin; *Gill,* broad, 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.

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.

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.

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

A flesh , yellowish brown mushroom. *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.

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: grows in clusters on stems of the wood.

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.

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: grow in clusters on leaves.

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: grow in clusters on wood.

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: grow in clusters on stem of wood.

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: grow in clusters on wood.

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

Fruit body 2.5-9 cm a across, ear-shaped, out surface brown to dark, inner surface gray-brown to dark, minute hair, smooth sometimes with wrinkled (Fig.4.49). Habit: severally or numerously on rotting wood.

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: grow in clusters on dead branches.

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.

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.

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.

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.

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

Cap, 3-8 cm across, deeply convex then expanded with depressed disc; white to yellowish white or pale buff, 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.

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.

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.

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: grow in clusters.

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: grow in clusters.

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

Cap, 3-5 cm across, flat with the margin strongly inrolled, raising umbo in the crnter, 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: grow in clusters.

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: grow on rotting leaves.

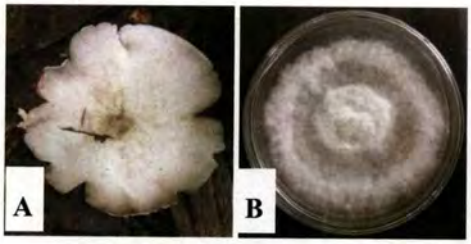

Fig. 4.2 *Agaricus macrosporus* (CH01)

A. fruiting body, B. culture on PDA

Fig.4.3 *Tricholoma* spp. (CH02)

A. fruiting body, B. culture on PDA

Fig.4.4 *Lactarius* spp. (CH3-01)

A. fruiting body, B. culture on PDA

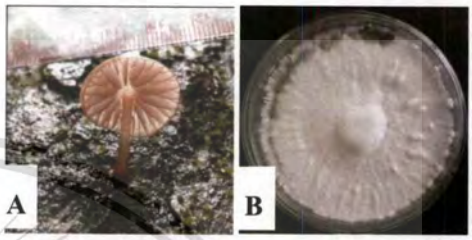

Fig.4.5 *Marasmius* spp. (CH3-02)

A. fruiting body, B. culture on PDA

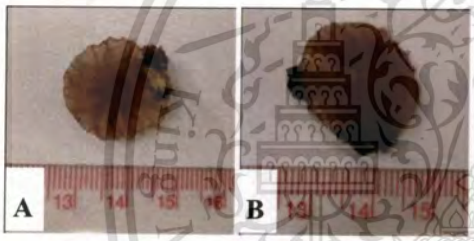

Fig.4.6 *Mycena rosella* (CH3-03)

A, B. fruiting body

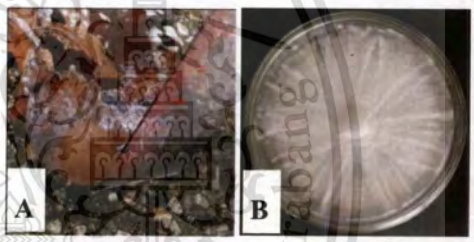

Fig.4.7 *Marasmius androsaceus* (CH3-04)

A, fruiting body, B. culture on PDA

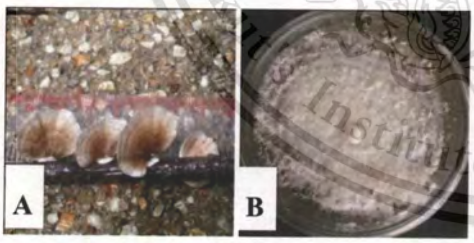

Fig.4.8 *Trametesversicolor* spp. (CH3-05)

A, fruiting body, B. culture on PDA

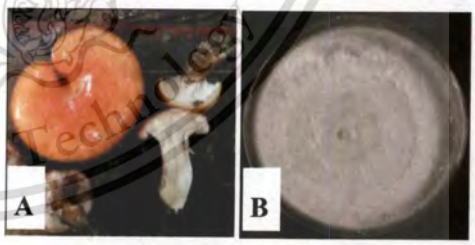

Fig.4.9 *Lactarius sanguifluus* (CH3-06)

A. fruiting body, B. culture on PDA

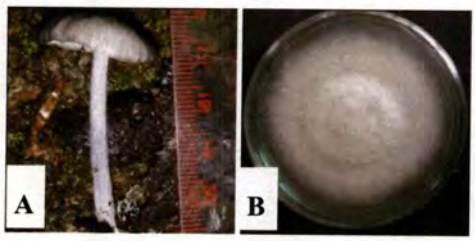

Fig.4.10 *Mycena subcaerulea* (CH3-07)

A. fruiting body,B. culture on PDA

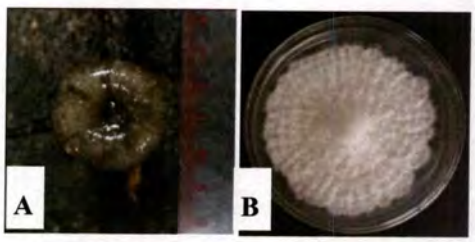

Fig.4.11 *Clitocybula atrialba* (CH3-08)

A. fruiting body, B.culture on PDA

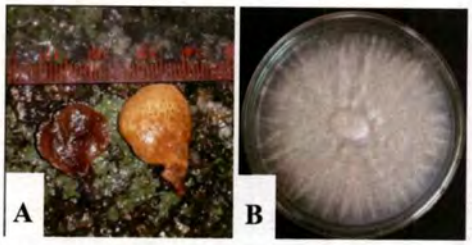

Fig.4.12 *Tremiscus* spp. (CH3-09)

A. fruiting body, B. culture on PDA

Fig.4.13 *Clavulinopsis helvola* (CH3-10)

A. fruiting body, B. culture on PDA

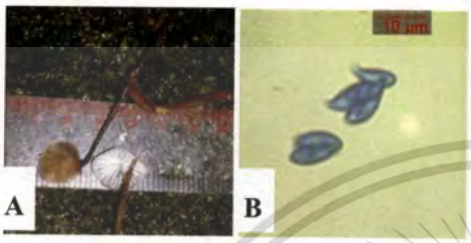

Fig.4.14 *Mycena inclinata* (CH3-11)

A. fruiting body, B. spore, Bar. B= 10 µm

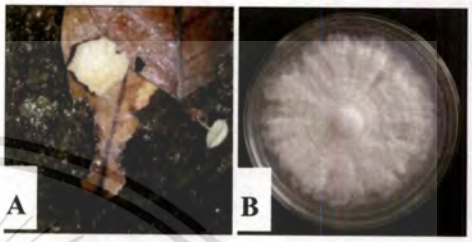

Fig.4.15 *Marasmiellus albuscorticis* (CH3-12)

A. fruiting body, C. culture on PDA

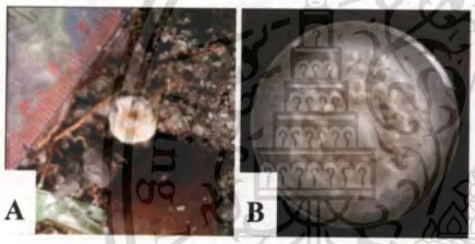

Fig.4.16 *Laccaria* spp. (CH3-13)

A. fruiting body, B. culture on PDA

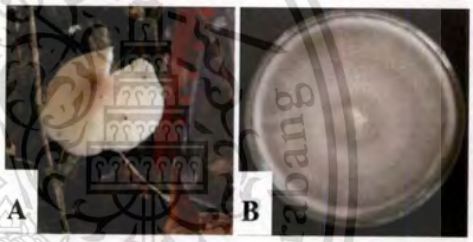

Fig.4.17 *Termitomyces microcarpus* (CH3-14)

A. fruiting body, B. culture on PDA

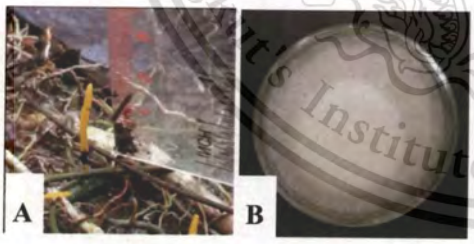

Fig.4.18 *Clavulinopsis fusiformis* (CH3-15)

A. fruiting body, B. culture on PDA

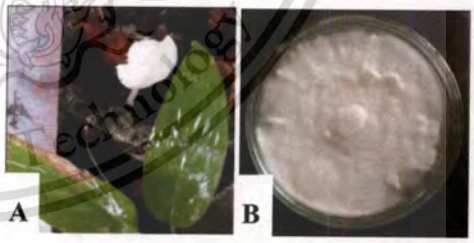

Fig.4.19 *Resinomycena rhododendri* (CH3-16)

A. fruiting body, B. culture on PDA

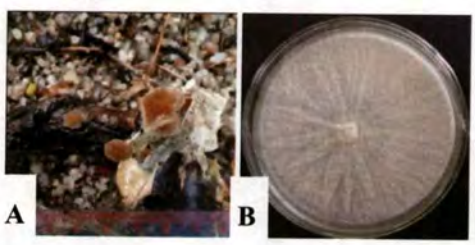

Fig.4.20 *Marasmius foetidus* (CH3-17)

A. fruiting body, **B.** culture on PDA

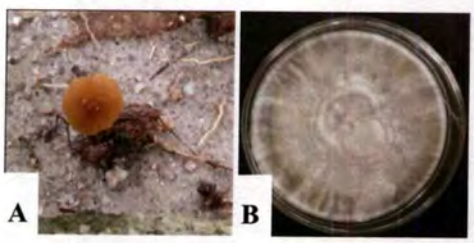

Fig.4.21 *Marasmius plicatulus* (CH3-18)

A. fruiting body, B. culture on PDA

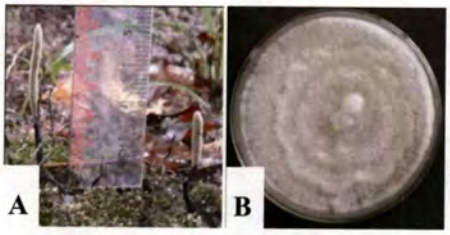

Fig.4.22 *Xylaria hypoxylon* (CH3-19)

A. fruiting body, B. culture on PDA

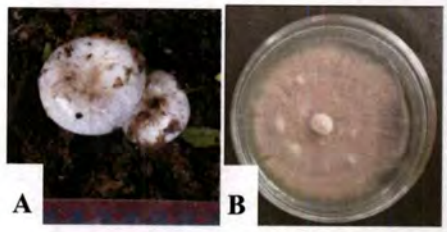

Fig.4.23 *Lactarius controversus* (CH3-20)

A. fruiting body, B. culture on PDA

Fig.4.24 *Marasmius scorodonius* (CH3-21)

A. fruiting body, B. culture on PDA

Fig.4.25 *Marasmius oreades* (CH3-22)

A. fruiting body, B. culture on PDA

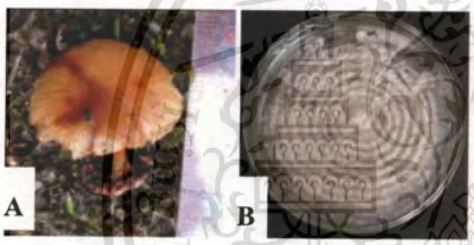

Fig.4.26 *Marasmius* spp. (CH3-23)

A. fruiting body, B. culture on PDA

Fig.4.27 *Lactarius* spp. (CH3-24)

A, B. fruiting body

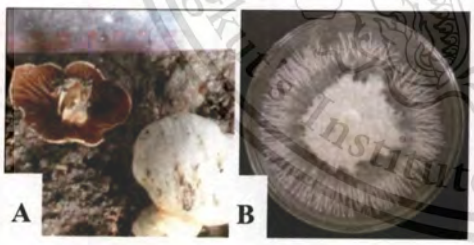

Fig.4.28 *Agaricus* spp. (CH3-25)

A. fruiting body, B. culture on PDA

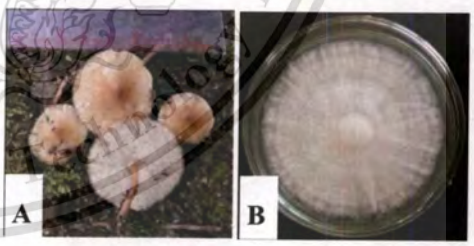

Fig.4.29 *Collybia dryophila* (CH3-26)

A. fruiting body, B. culture on PDA

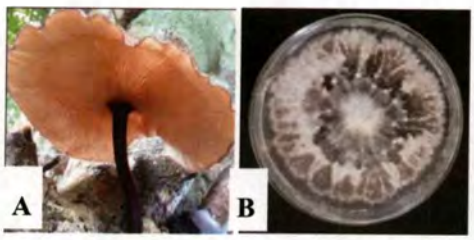

Fig.4.30 *Lactarius* spp. (CH3-27)

A, B. fruiting body

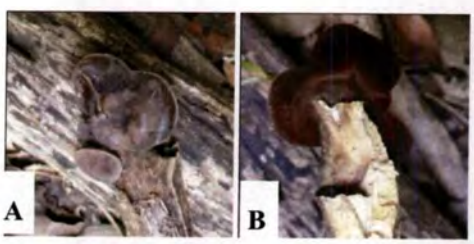

Fig.4.31 *Auricularia auricula* (PH01)

A, B. fruiting body

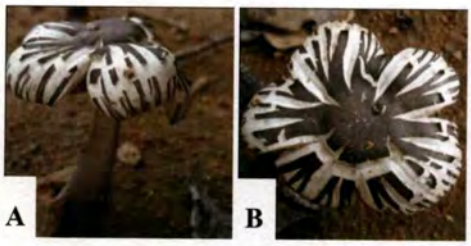

Fig.4.32 *Tricholoma* spp. (PH02)

A, B. fruiting body

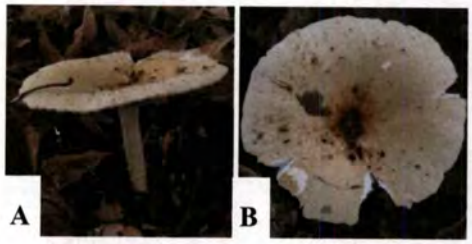

Fig.4.33 *Termitomyces* spp. (PH03)

A, B. fruiting body

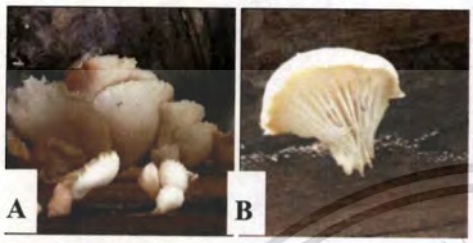

Fig.4.34 *Pleurocybella porrigens* (PH04)

A, B. fruiting body

Fig.4.35 *Pluerotus gigantenus* (PH05)

A, B. fruiting body

Fig.4.36 *Leucocoprinus fragilissimus* (PH06)

A. fruiting body, B. culture on PDA

Fig.4.37 *Collybia strictipes* (PH07)

A. fruiting body, B. culture on PDA

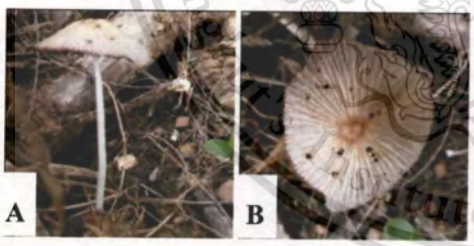

Fig.4.38 *Marasmius* spp. (PH08)

A, B. fruiting body

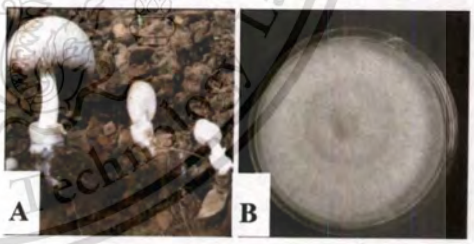

Fig.4.39 *Coprinus* spp. (PH09)

A. fruiting body, B. culture on PDA

Fig.4.40 *Marasmius* spp. (PH10)

A. fruiting body, B. culture on PDA

Fig.4.41 *Collybia iocephala* (PH11)

A. fruiting body, B. culture on PDA

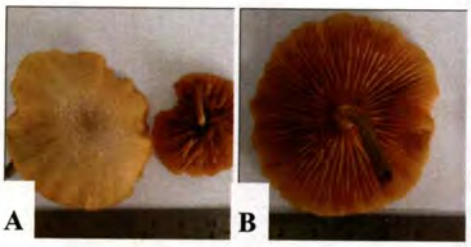

Fig.4.42 *Collybia* spp. (LB01)

A, B. fruiting body

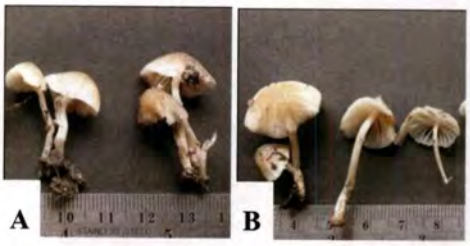

Fig.4.43 *Mycena* spp. (SY01)

A, B. fruiting body

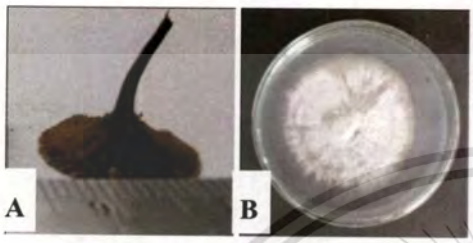

Fig.4.44 *Marasmius* spp. (SY02)

A. fruiting body, B. culture on PDA

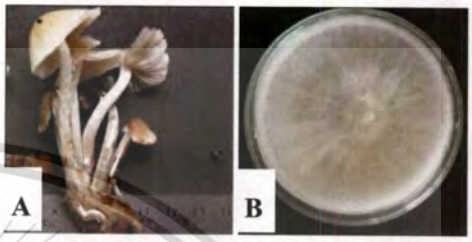

Fig.4.45 *Mycena* spp. (SY03)

A. fruiting body, B. culture on PDA

Fig.4.46 *Marasmius purpureostriatus* (SY04)

A. fruiting body, B. culture on PDA

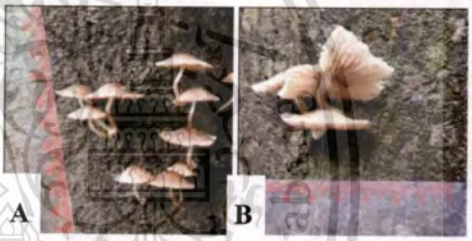

Fig.4.47 *Mycena* spp. (SY05)

A, B. fruiting body

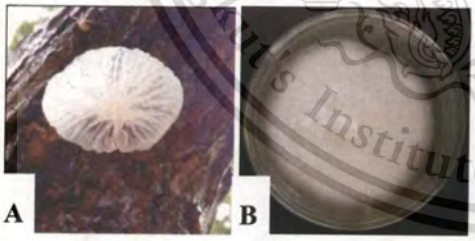

Fig.4.48 *Marasmiellus albuscorticis* (SY06)

A. fruiting body, B. culture on PDA

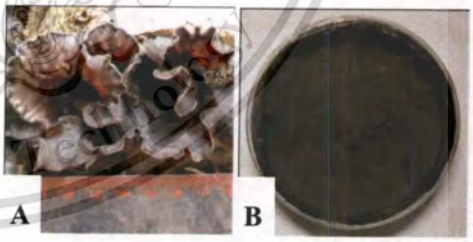

Fig.4.49 *Auricularia auricula* (SY07)

A. fruiting body, B. culture on PDA

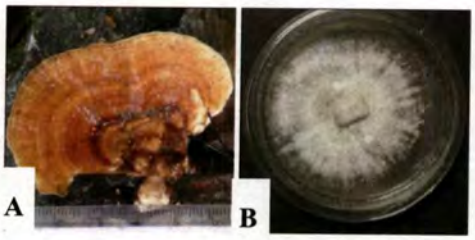

Fig.4.50 *Schizophyllum commune* (SY08)

A. fruiting body, B. culture on PDA

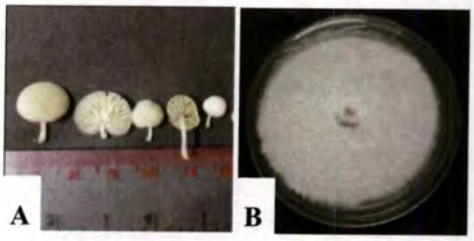

Fig.4.51 *Marasmillus ramealis* (SY09)

A. fruiting body, B. culture on PDA

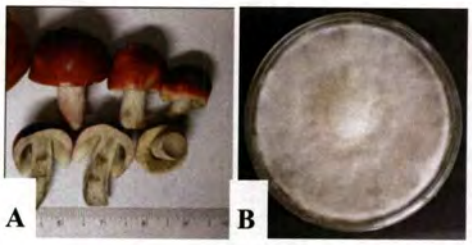

Fig.4.52 *Russula foetens* (AJ01)

A. fruiting body, B. culture on PDA

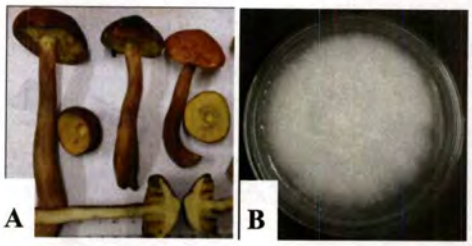

Fig.4.53 *Boletus retisporus* (AJ02)

A. fruiting body, B. culture on PDA

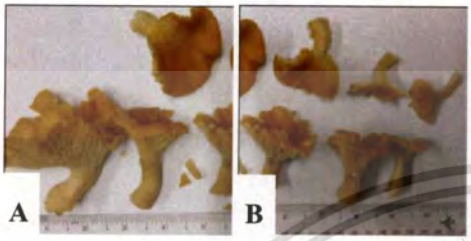

Fig.4.54 *Cantharellus cibarius* (AJ03)

A, B. fruiting body

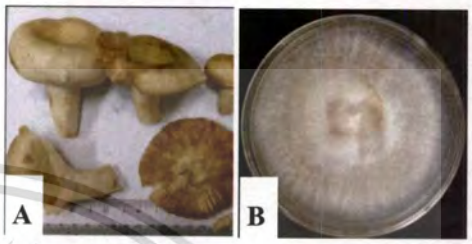

Fig.4.55 *Russula crassotunicata* (AJ04)

A. fruiting body, B. culture on PDA

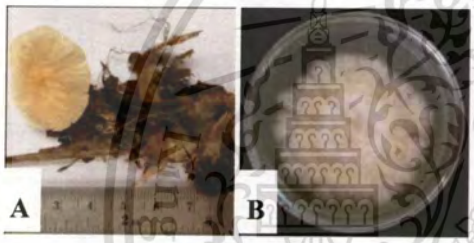

Fig.4.56 *Laccaria vinaceoavellanea* (AJ2-1)

A. fruiting body, B. culture on PDA

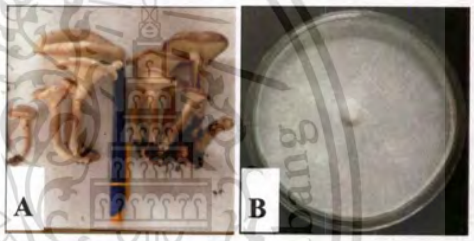

Fig.4.57 *Clitocybe* spp (AJ2-2)

A. fruiting body, B. culture on PDA

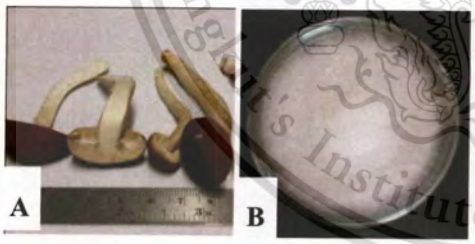

Fig.4.58 *Boletus affinis* var. *maculosus* (AJ2-3)

A. fruiting body, B. culture on PDA

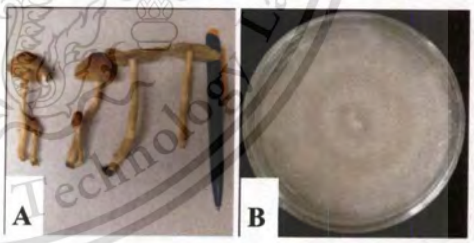

Fig.4.59 *Inocybe fastigiata* (AJ2-4)

A. fruiting body, B. culture on PDA

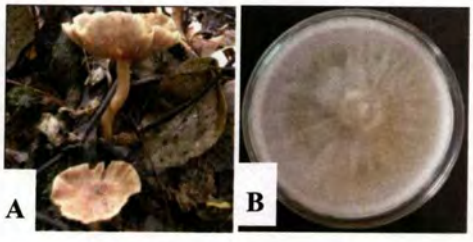

Fig.4.60 *Clitocybe* spp. (AJ2-5)

A. fruiting body, B. culture on PDA

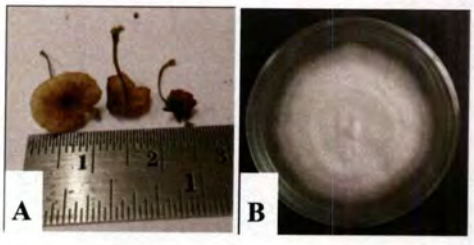

Fig.4.61 *Mycena vulgaris* (AJ2-6)

A. fruiting body, B. culture on PDA

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. Isolate was identified as *Colletotrichum.coffeanum* (Fig. 4.62).

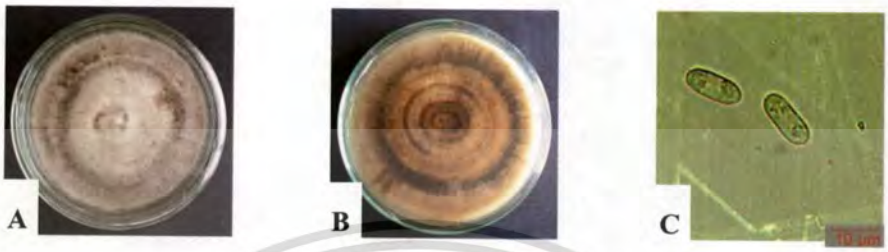

Fig.4.62 Three isolates of *Colletotrichum* spp. from leave anthtacnose of coffee Arabica A, B, C. *C.coffeanum*

4.2.2 Pathogenicity Test of *Colletotrichum coffeanum*

Pathogenicity test of *Colletotrichum coffeanum* wsa done in using plug inoculation method. The agar plug was inoculated over the wounded coffee leaves and incubated in moist chamber at room temperature (28-30°C) for 14 days. The result showed that the lesion size developed by *C. coffeanum* isolate was 27.25 mm which gave the highest virulent for disease incidence.

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.4.63) was obtained from Dr. Kasem Soytong, which confirmed by DNA sequencing.

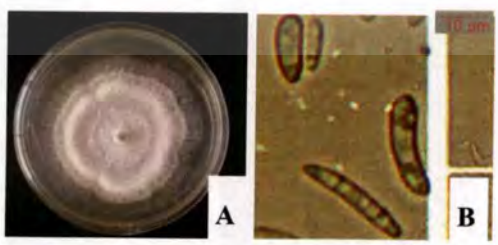

Fig 4.63 *F.oxysporum* f. sp. *lycopersici* race 2 A. 10-day-old culture on PDA, B. conidia

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* using a root dip inoculation method. The disease incidence was determined at 15 days. After test, the symptom of fusarium wilt disease showed the yellowing and wilting leaves . Wilted leaves usually dropped prematurely (Fig. 4.64). 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 4.64 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

Clitocybe spp (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01), were extracted their biological active substances as crude extracts and tested for their abilities to inhibit the growth *C. coffeanum* and *Fusarium 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 yields of crude extracts were recorded as shown in Table 4.2. With this, 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.

Table 4.2 Extraction of biological active substances

Taxon	Fresh weight(g)	Dry weight(g)	Organic solvents		
			Crude Hexane(g)	Crude EtOAc(g)	Crude MeOH(g)
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)	5230	91.56	0.39 (0.43%)	0.43 (0.47%)	4.87 (5.32%)
Lactarius spp (CH3-01)	1920	79.10	0.43 (0.54%)	1.68 (2.12%)	3.98 (5.03%)

4.4 Bioactive Compound 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 *Clitocybe* spp (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01) 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.

4.4.1 Biological Activity against Coffee Anthracnose Causing by *Colletotrichun. 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 4.3). Crude methanol 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 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 4.4).

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 μg/ml when compared to the control (Table 4.5). Crude ethyl acetate 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, followed by followed crude hexane inhibited 55.95% which the ED_{50} values was 75.19 μg/ml (Tables 4.6).

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 ppm when compared to the control (Table 4.7). Crude methanol 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, 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 4.8).

Table 4.3 Crude extracts of *Clitocybe* spp. AJ2-2 testing for growth inhibition of *Colletotrichum coffaenum* at 5 days

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 4.4 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 (ppm)	Number of spores [1] (10^6)	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 .

Table 4.5 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 4.6 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 (ppm)	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 .

Table 4.7 Crude extracts of *Lactarius* spp CH3-01 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[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 4.8 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 (ppm)	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. 4.65 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

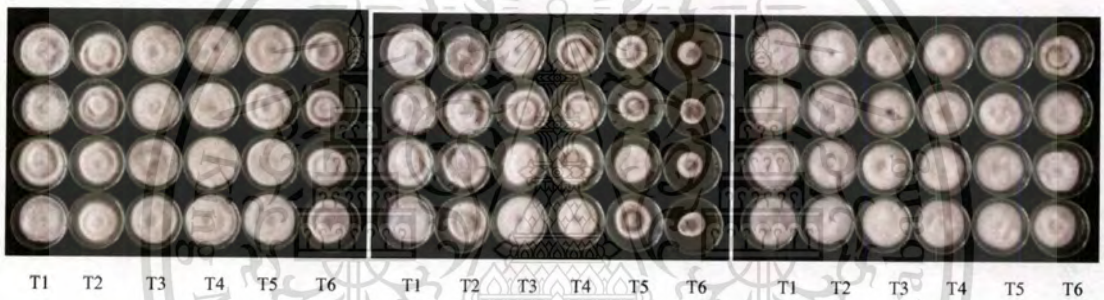

Fig.4.66 Five-day-old colony of *C. coffeanum* on PDA containing crude extracts from *B. affinis* var. *maculosus* AJ2-3 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH

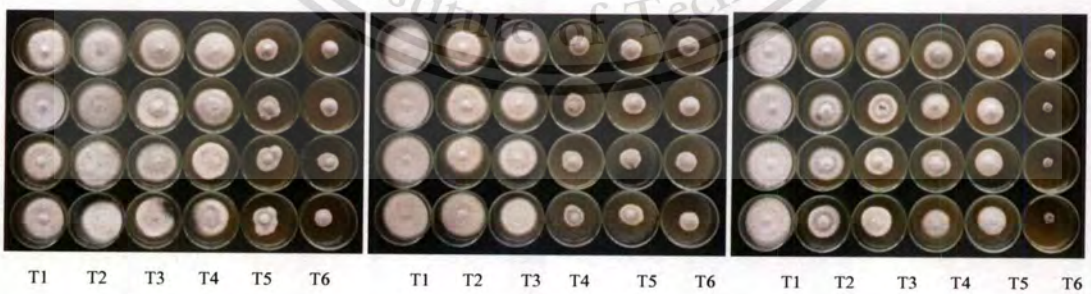

Fig.4.67 Five-day-old colony of *C. coffeanum* on PDA containing crude extracts from *Lactarius* spp CH3-01 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH

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

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

Results showed 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 1,000ppm when compared to the control (Table 4.9). 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, followed by crude methanol which inhibited 77.68% and crude hexane inhibited 68.95% (Tables 4.10).

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 4.11). 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, followed by crude hexane which inhibited 76.91% and crude methanol inhibited 64.36 % (Table 4.12).

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 4.13). 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, followed by crude hexane which inhibited 83.36% and crude methanol inhibited 76.31% (Tables 4.14).

Table 4.9 Crude extracts of *Clitocybe* spp. Aj2-2 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 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	4.27^{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 4.10 Spore production inhibition of crude extracts from *Clitocybe* sp AJ2-2 to *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 7days and effective dose (ED_{50}) values

Crude extracts	Concentration (ppm)	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 .

Table 4.11 Crude extracts of *Boletus affinis* var. *maculosus* AJ2-3 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 5days

Crude extracts	Concentration (ppm)	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 4.12 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 (ppm)	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 .

Table 4.13 Crude extracts of CH3-01 testing for growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 5days

Crude extracts	Concentration (ppm)	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 4.14 Spore production inhibition of crude extracts from *Lactarius* sp CH3-01 to *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 7 days and effective dose (ED_{50}) values

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

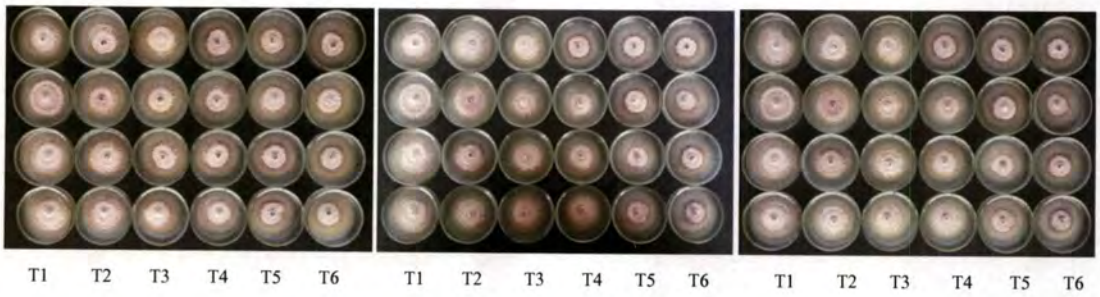

Fig. 4.68 Five-day-old colony of *F. oxysporum* on PDA containing crude extracts from *Clitocybe* sp AJ2-2 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH

Fig. 4.69 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

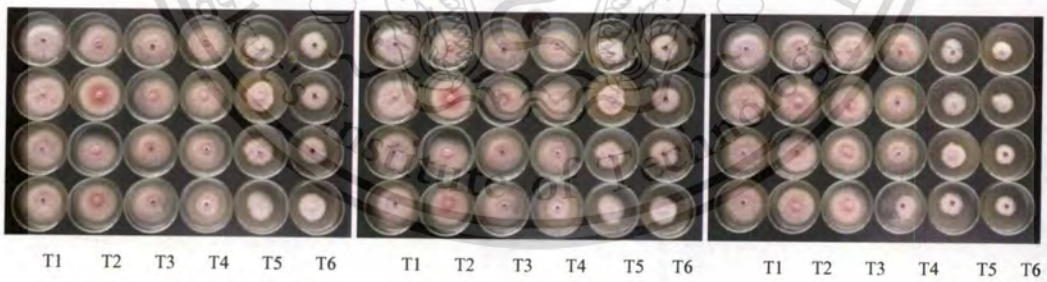

Fig. 4.70 Five-day-old colony of *F. oxysporum* on PDA containing crude extracts from *Lactarius* spp CH3-01 at T1=0, T2=10, T3=50, T4=100, T5=500 and T6=1,000 µg/ml concentrations. Left-Crude Hex; Middle-Crude EtOAc; Right- Crude MeOH

CHAPTER 5

DISCUSSION

All 60 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 (Srivastava, *et al.*, 2011). *Clitocybe* spp. AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3 and *Lactarius* spp. CH3-01 were described which these species reported to be found in Thailand (Konemann, 1998; Roger, 1991; States, 2004; Susan and Van, 2000).

In this study, *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 woundinoculated 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, *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 were reported for the first time that the metabolites from *Clitocybe* sp AJ2-2, *B. affinis* var. *maculosus* AJ2-3 and *Lactarius* spp. CH3-01could inhibit *C. coffaenum* causing coffee anthracnose. Meanwhile, the metabolites from *Clitocybe* spp. AJ2-2, *B. affinis* var. *maculosus* AJ2-3 and *Lactarius* spp CH3-01 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, 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. 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 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.

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.

C. coffeanum isolate gave the highest virulent for disease incidence awhich the lesion size developed by was 27.25 mm.

Three promising Agaricales *Clitocybe* spp. (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01 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 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. *Clitocybe* spp. (AJ2-2), *Boletus affinis* var. *maculosus* (AJ2-3), *Lactarius* spp. (CH3-01), gave the growth inhibition of *F. oxysporum* f. sp. *lycopersici* race 2 over 50%. Among them, crude extracts from *Lactarius* spp. CH3-01gave the best result to inhibit the growth of *F. oxysporum* f. sp. *lycopersici* race 2. The crude ethyl acetate from *Lactarius* spp. CH3-01gave 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.

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