CHAPTER I

INTRODUCTION

The yields produced by crop plants can be substantially reduced by weeds, by organisms such as insect larvae or nematodes that feed on plants, and by disease organisms such as fungi, bacteria and viruses that attack the plant. Worldwide crop losses have been estimated to be 13.8 % from insects and other arthropods, 11.6 % from diseases and 9.5 % from weeds (Chrispeels and Sadava, 1994). There have been notable advancements in recent years towards the control of plant diseases. The economic losses due to plant diseases can be categorized into two groups. First, plant diseases are factors that reduce and localize an agricultural area or may be cultivated low price rotary crop. Second, plant diseases reduce quality and quantity of agricultural production. Pathogens causing plant diseases include bacteria, virus, viroid, mycoplasma, and especially fungus.

1.1 Plant diseases caused by fungi

Fungi are generally microscopic, lacking chlorophyll and conductive tissues. Most of the 100,000 fungus species known are strictly saprophytic, living on dead organic matter. More than 8,000 species of fungi cause diseases in plants (Agrios, 1997). All plants are attacked by some kinds of fungi. Each parasitic fungus can attack one or many kinds of plant. Almost all of plant pathogenic fungi spend a part of their lives on their host and the rest in soil. Some fungi spend all their lives on the host. Only spores may land on the soil where they remain dormant until they are again carried to a host, on which they grow and multiply. During their parasitic phase, fungi have influence to plant cells. The interaction between fungi and plant tissue deduces various types such as growing outside the host surface and sending feeding organs into the epidermal cells or growing between the cells in the intercellular spaces which may not send feeding organs into the cells. The survival of most plant pathogenic fungi depends on the prevailing conditions of temperature a moisture. The spores can resist broader range but require favorable conditions for their germination. The consequential majority of the plant pathogenic fungi spread from plant either to another plant or to different parts of the same plant. Fungi are disseminated primarily in the form of spores. After that spore germinates, develop hyphae and increases masses of mycelium. The fungi that cause diseases in plant are from a diverse group. Deuteromycetes or the imperfect fungi are a group of fungi that responsible for a major loss in food production. They cause diseases that may appear as leaf spot, blights, cankers, fruit pots, fruit rots, anthracnoses, stem rots, root rots, vascular wilt, and soft rots (Agrios, 1997).

The fungi can cause plant disease in field and produce latent infection that expresses disease symptom in postharvest and transportation process. The economic loss caused by the postharvest disease is more than 10 billion bath around the world. The critical cause of economic loss is resulting from fungi infection. The agricultural products damaged by postharvest diseases can be divided into two groups; low-moisture products such as crop grains and high-moisture products such as vegetables and fruits. *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp. damaged low-moisture products and produced toxin residue in product. The high-moisture products may be infected by *Alternaria* spp., *Helminthosporium* spp., *Botrytis* spp., *Phytophthora* spp. and *Rhizopus* spp. (Juangbhanich, 1982).

In this research, three common pathogenic fungi *Fusarium oxysporum, Alternaria* sp. and *Phytophthora* sp., causing disease in many Thai crops, were selected as model organisms.

Vascular wilts caused by *Fusarium oxysporum*

Vascular wilts caused by *Fusarium oxysporum* are widespread, very injurious and frightening plant disease appearing as more of less rapid wilting, browning and dying of leaves, shoots followed by final death of the whole plant. Wilts occur from the presence of the pathogen in xylem vascular tissues. Some vessels may be clogged with mycelium, spores or polysaccharides produced by the fungus. Clogging is further increased by accumulation of breakdown product of plant cells digested by fungus enzyme. The oxidation and translocation of breakdown products seem to also be responsible for the brown discoloration of affected vascular tissues (Agrios, 1997). The pathogen usually continues to spread internally through the xylem vessels until the entire plants are killed. *Fusarium oxysporum* causes vascular wilts in herbaceous perennial ornamentals, crops, annual vegetables, flowers and weeds.

The wilt fungus, *Fusarium oxysporum,* is soil inhabitants and infects plants *via* the roots or *via* the wounds. The mycelium is colorless but becomes cream-colored or pale yellow with age. At the optimum conditions it produced a pale pink or purplish colony. *Fusarium oxysporum* produces three kinds of asexual spores, microconidia, which are one or two, cells and usually produced under all conditions. Macroconidia are three to five cells and curved shapes. Chlamydospores are one or two cells, thick walled, round spores and produced terminally on the old mycelium. Special forms of *Fusarium* attack different host plants. Thus the fungus attacking tomato is designated as *F. oxysporum* f. sp. *lycopersici,* sweet potato *F. oxysporum* f. sp. *batatas,* banana *F. oxysporum* f. sp. *cubense,* onion *F. oxysporum* f. sp. *cepae,* carnation *F. oxysporum* f. sp. *dianthii,* chrysanthemum *F. oxysporum* f. sp. *chrysanthemi, etc. Fusarium* wilts are destructive in the warmer temperate regions and in the tropics and subtropics.

The development of vascular disease shows in Fig 1.1. The pathogen infests in soil are mycelium and all spores form but the most commonly are chlamydospores. They spread by water or contaminated farm equipment. When plants grow in contaminated soil, the germ tubes of spore or the mycelium sink in the root. The mycelium through the root cortex and enter the vessels. The ring of discolored vessels in secondary xylem will be present. After enlargement mycelium the cells were collapsed, vessels were distorted and plants will die.

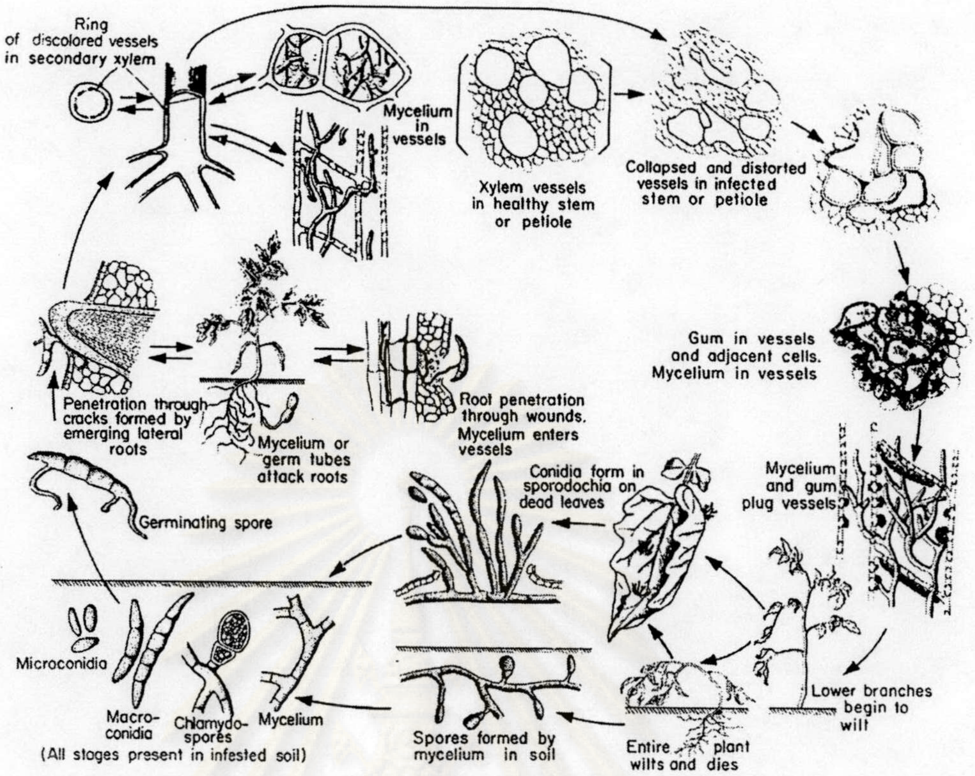


Fig. 1.1 Disease cycle of *Fusarium oxysporum* (Agrios, 1997)

Vascular wilts are difficult to control because the pathogen grows and spreads in the vessels. So the prevention of infection and posterior control with surface fungicides is practically impossible.

Alternaria diseases

Alternaria diseases usually emerge as leaf spots and blights, but they may also cause damping off of seeding, collar rots, tubers and fruit rots. *Alternaria* diseases occur in many plants such as potato, tomato, bean, tobacco, geranium, carrot, carnation, chrysanthemum, petunia, crucifers, squash, apple and many others.

The leaf spots are generally dark brown to black, often numerous and expansion. Lower, senescent leaves are usually attacked and the disease progresses upward and makes affected leaves go into a yellowish dry up and fall off. *Alternaria* attack in the fruit when they approach maturity. The spots are brown to black and may be small or they may enlarge to cover most of the fruit. An infected fruit is black, velvety surface layer of fungus growth and spores. In some fruits, such as tomato, a small lesion at the surface may indicate an extensive spread of the infection in the central core and segment of the fruit. If the fungus is carried with the seed, it may attack the seeding, usually after emergence, and cause damping off or stem lesion and collar rot. More frequently, spores are produced amply, especially during heavy dews and frequent rains. Spores are blown from mycelium and growing on plant debris or weeds. The germinating spores penetrate unprotected tissue through wounds and soon produce new conidia that are further spread.

Alternaria sp. has dark-colored mycelium and in older disease tissue it produced short, simple, vertical conidiophores that produce single or branched chains of conidia. The conidia are large, dark, long or pear shaped and multicellular with both horizontal and longitudinal cross walls. The conidia are detached easily and are carried by air currents. *Alternaria* spores are one of the most common fungal caused of allergies.

The development of disease (Fig 1.2) caused by *Alternaria* initiates from mycelium and spores infest in plant debris, on seeds and tubers. The mycelium germination is direct penetration or penetration through wound and leaf or stem are attacked and the symptom of disease will be present as collar rot damping off, stem rot, leaf rot and fruit rot.

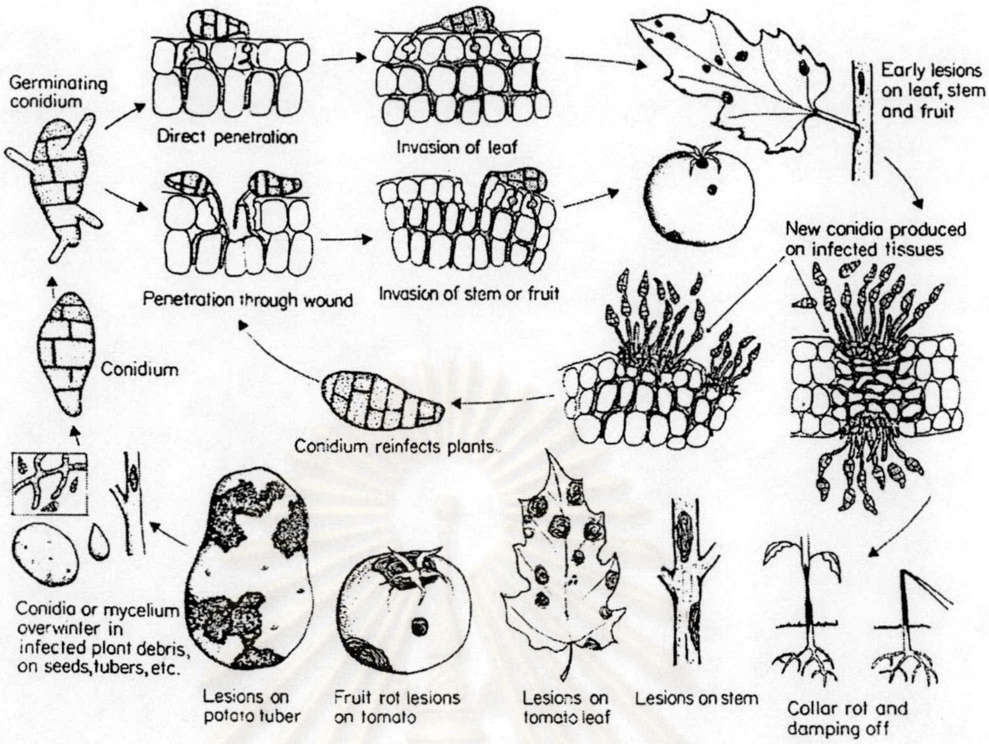


Fig. 1.2 Disease cycle of *Alternaria* sp. (Agrios, 1997)

Alternaria diseases are controlled primarily through the use of resistant varieties, of disease-free or treated seed and through chemical sprays with fungicides such as maneb, captafol, or maneb-zinc combination. The sprays should begin right after seedling emerge or transplanted and should be repeated within 1 to 2 weeks.

***Phytophthora* disease**

Phytophthora sp. cause diseases in many plants. Most species cause root rot, damping off of seedling, rot of lower stems, tubers, or may be caused rot of buds or fruits and foliar blights. The losses caused by *Phytophthora* are great, especially on trees and shrubs.

The plants suffering root rot showing symptom of drought starvation and become weakened and susceptible to attack by other pathogens. Known diseases caused by *Phytophthora* are briefly described below.

Phytophthora root rots suffusion where the soil becomes too wet, high soil moisture and atmosphere humidity, poorly drained area and temperature remain fairly low. Young seedling may be killed within a few days. While in older plant the killing of roots may be rapid or slow, depending upon the amount of fungus present in the soil and the prevalent environmental condition. Plants suffered by root rot, the small roots are dead, and necrotic brown lesions are present in the larger roots. The root system may decay and follow by rapid death of the plant.

Late blight of potatoes is one of diseases caused by this pathogen and may cause absolute destruction of all plants in a field within a week when optimum weather condition exists. The symptoms of disease become visible at first as circular water-soaked spots, usually at the edges of the lower leaves. The spot enlarges rapidly and forms blown blight areas. Under continually moistened condition, above ground parts of the plant blight and quickly rot. The rot continues to develop after the tubers are harvested and may be subsequently invaded by secondary fungi or bacteria.

The development of the disease is presented in Fig 1.3. The pathogen overwinters as oospore, chlamydospores, or mycelium in infest roots or in the soil. In the spring spore germinates by means of zoospores, while the mycelium grows further and produces zoosporangia which release zoospores. The zoospores swim around and infect root of susceptible hosts. Furthermore the mycelium spreads up the stem in the cortical region causing discoloration and collapse of the cells. When the mycelium reaches the aerial part of plants, it produces sporangiophores, which emerge through the stomata of the leave and extend into the air. The sporangia produced on the sporangiophores become dispersed by rain. When the sporangia land on the wet soil they incite to start next cycle disease.

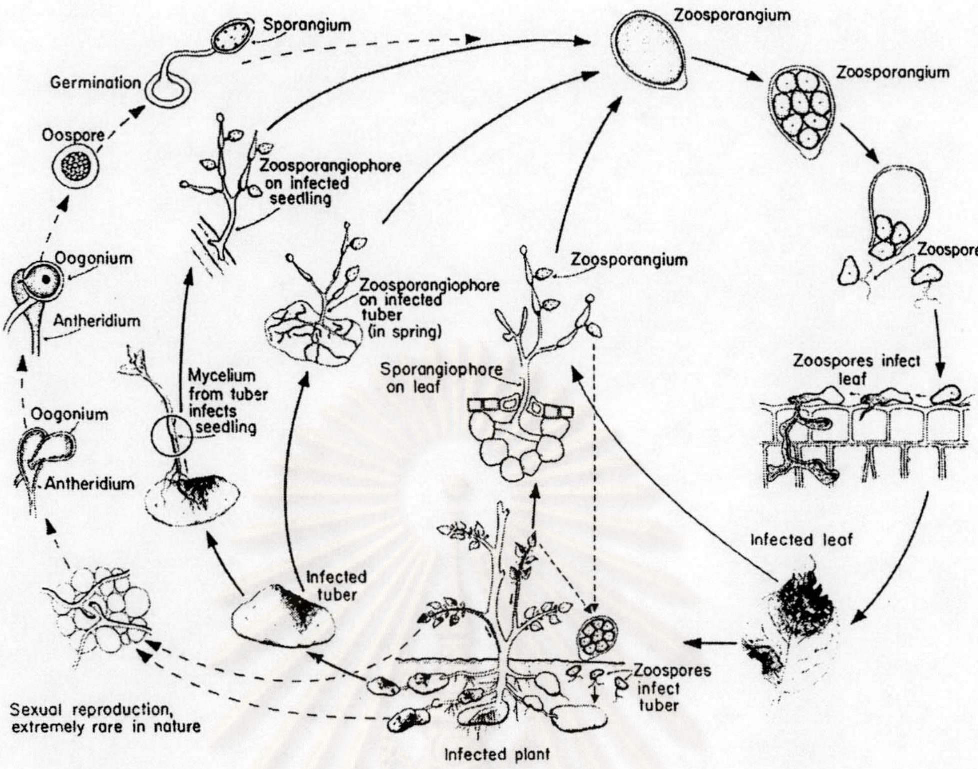


Fig. 1.3 Disease cycle of *Phytophthora* sp. (Agrios, 1997)

Control of *Phytophthora* disease can be applying ethazol to the soil, seeds and transplants. The application of a solution of copper oxychloride or Bordeaux mixture seems to greatly inhibit the growth of the fungus. Resistant varieties should be preferred, especially for poorly drained soils.

The fungicides employed for crop protection may be divided into two groups according to their mode of action. Protectant fungicides protect the plant against fungal infection on the surface and must be applied before the phenomena infections enter the host. Systemic fungicides act quite complicated, they translocate in plant, become generally distributed within it and render the tissue resistant to attack. For a fungicide to be useful, it must kill the pathogen or at least inhibit development, without causing substantial damage to the host. The fungicide must also be applied in such a way that it presents no hazard to those handing it, to consumers of the crop. In many countries there are now stringent regulations specifying the criteria which must be satisfied before a fungicide is used on any crop destined for human consumption.

Furthermore the persistence of fungicide is hazardous for environment ecosystems. Therefore, increased public concern about the level of fungicide residue prompts scientists to look for alternative approaches. The fungicide used in the future should fulfill the following requirement; high selectivity to target microorganism, low hazardous and complete degradability. Evaluation of antifungal activity from natural products could also possibly be used as antifungal agents to control plant disease.

1.2 Literature search on the antifungal constituents from higher plants

Fungal spores infecting on the leaf surface of plants have to oppose a complex series of defensive barriers induced in plants, before it germinates, grows into the plant tissue and causes damage. The strategy against the fungal includes physical barriers such as a thick cuticle and chemical ones such as the accumulation of antifungal metabolites. The antifungal metabolites can be divided into two types. These can be performed in the plant called 'constitutive antifungal substance' or they are induced after infection involving *de novo* enzyme synthesis, the 'induced antifungal constituents' or 'phytoalexin' (Renee and Jefferey, 1994).

Most of antifungal substances in plants are secondary metabolites. The classification of antifungal substances is based upon the chemical structure. They are terpenoid group (iridoids, sesquiterpenoids, saponins), nitrogen and/or sulphur-containing constituents (alkaloids, amines, amides), aliphatics especially long-chain and fatty acids, and aromatics (phenolics, flavonoids, stilbenes, bibenzyls, xanthones and benzoquinones). Examples of antifungal constituents in higher plants are collected as shown in Table 1.1 (Renee and Jefferey, 1994).

Table 1.1 Antifungal constituents in higher plants

Plant family	Species	Compounds	Chemical class	Microorganism studied
Anacardiaceae	*Mangifera indica*	5-(12-*cis*-heptadecenyl)-resorcinol	Alkylated-phenol	*Alternaria alternata*
		5-pentadecylresorcinol		
Burseraceae	*Commiphora rostrata*	2-decanone; 2-undecanone; 2-dodecanone	Alkanone	*Aspergillus* and *Penicillium* species
Cannabidaceae	*Humulus lupulus*	6-isopentenylnaringenin	Flavanone	*Trichophyton rubrum*
		xanthohumol	Chalcone	*T. mentagrophytes*
Combretaceae	*Combretum apiculatum*	4,7-dihydroxy 2,3,6-trimethoxyphenanthrene	Phenanthrene	*Penicillium expansum*
		2,7-dihydroxy-3,4,6-trimethoxydihydrophe-nanthrene		
		4,4′-dihydroxy-3,5-dimethoxydihydrostilbene	Dihydrostilbene	
Compositae	*Eupatorium riparium*	methylripariochromene	Chromene	*Clooectotrichum gloeosporioides*
	Helichrysum decumbens	phloroglucinol derivative	Prenylated phenol	*Cladosporium herbarum*
	Helichrysum nitens	chrysin dimethyl ether	Methoxylated-flavone	*C. cucumerinum*
		galangia trimethyl ether		
		baicalin trimethyl ether		
	Wedelia biflora	7,3′-di-*O*-methylquercetin	Flavonol	*Rhizoctonia solani*

Table 1.1 (continued)

Plant family	Species	Compounds	Chemical class	Microorganism studied
Cucurbitaceae	*Ecballium elaterium*	cucurbitacin I	Cucurbiacin	*Botrytis cinerea*
Cupressaceae	*Chamaecyparis pisifera*	pisiferic acid	Diterpene	*Pyricularia oryzae*
Dioscoreaceae	*Dioscorea batatas*	3-hydroxy-5-methoxybibenzyl 3,2′-dihydroxy-5-methoxybibenzyl	Oxygenated-bibenzyl	Examine against 24 fungi
		6-hydroxy-2,4,7-trimethoxyphenanthrene 6,7-dihydroxy-2,4-dimethoxyphenanthrene 2,7-dihydroxy-4,6-dimethoxyphenanthrene	Phenanthrene	
Dipterocarpa-Ceae	*Stemonoporus Canaliculatus*	canaliculatol	Stilbenetrimer	*Cladospporium cladosporioides*
Euphorbiaceae	*Croton lacciferus*	2,6-dimethoxybenzo-quinone	Benzoquinone	*C. Cladosporioides*
Gramineae	*Avena sativa*	avenacins	Triterpenoid-saponin	*Geumannomyces graminis* var. *tritici*
	Hordeum vulgare	gramine	Indole alkaloid	*Erysiphe graminis* f. sp. *hordei*
	Oryza officinalis	jasmonic acid	Modified fatty acid	*Pyricularia oryzae*

Table 1.1 (continued)

Plant family	Species	Compounds	Chemical class	Microorganism studied
Gramineae	*Oryza sativa*	epoxy-and hydroxylinoleic acid	Fatty acid	*Pyricularia oryzae*
	Sorghum cultivars	flavan-4-ols	Leucoanthocyanidin	*Fusarium moniliforme; Curvularia lunata*
	Triticum aestivum	α-triticene β-triticene	Alkadienal	*Cladosporium cucumerinum*
Grossulariaceae	*Ribes nigrum*	sakuranatin	Flavanone	*Botrytis cinerea*
Labiatae	*Rosmarinus officinalis*	2-(3,4-dihydroxyphenyl)ethenyl ester of caffeic acid	Enol ester of-hydroxy-cinnamic acid	*Cladospporium herbarum*
	Scutellaria violacea	clerodia; codrellin	Neo-clerodane diterpenoid	*Fusarium oxysporium* f. sp. *lycopersici*
Lauraceae	*Persea americana*	*cis-cis*-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene	Long-chain-alcohol	*Collectotrichum gloeospporioides*
Leguminosae	*Bauhinia manca*	isoliquiritigenin; isoquiritigenin-2′-methyl-ether; echinatin	Chalcone	*Botrytis cinerea; Saprolegnia asterophora*

Table 1.1 (continued)

Plant family	Species	Compounds	Chemical class	Microorganism studied
Leguminosae	*Lupinus albus*	iuteone; wighteone; iicoisoflavones A and B parvisoflavone	Isoflavone	*Cladosporium herbarum*
Molluginaceae	*Mollugo pentaphylla*	mollugenol A	Triterpenoid	*Cladosporium cucumerimum*
Myrsinaceae	*Rapanea melanophloeos*	sakurasosaponin	Triterpenoid-saponin	*C. cucumerimum*
Rosaceae	*Prunus yedoensis*	coumarin	Coumarin	*Cladosporium herbarum*
Rutaceae	*Glycosmis cyanocarpa*	sinharine; methylsinharine	Sulphur amide	*Cladosporium cladosporioides*
	Glycosmis mauritiana	ilukumbin; methylillkumbins A and B	Sulphur amide	*C. cladosporioides*
Solanaceae	*Lycopersicon esculentum*	tomatine	Steroidal alkaloid	*Fusarium solani*
	Nicotiana glutinosa	2-ketoepimanool	Diterpenoid	*Erysiphe cichoracerum*
	Nicotiana tabacum	α-and β-4,8,13-duvatriene-1,3-diols	Diterpenoid	*Peronospora tabacina*
Zingiberaceae	*Zingiber officinale*	gingerenones A, B and C; isogingerenone B	Diarylheptenone	*Pyricularia oryzae*

It could manifestly observe that antifungal constituents are all secondary metabolite, mainly being of terpenoids and phenolic compounds. These substances utilize as precursor for antifungal biosynthetic pathway. The aforementioned chemical structure aspects of antifungal compounds were quite promising to investigate for potent antifungal agents in terpenoid rich origins such as the volatile components in essential oils.

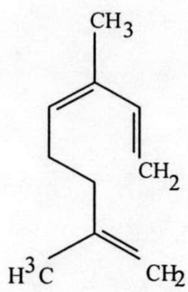
1.3 Biological characteristic, distribution and chemical constituents of essential oil

Essential or volatile oils are also secondary metabolite. Many essential oils have extremely useful properties and can be put to use in many aspects. For example, they can be utilized as antiseptic agent, perfume industry, food additives and aromatherapy. General methods for extraction of essential oils could be summerized as follows: expression, steam distillation, extraction with volatile solvents and resorption in purified fats. The separation of individual components is achieved by vacuum fractionation and by chromatographic methods. The major class of chemical substance present in essential oils is terpenes. The terpene essential oil can be divided into two subclasses, the mono- and sesquiterpenes (C_{10} and C_{15}), that differ in their boiling range (monoterpenes b.p. 140-180°C, sesquiterpenes b.p. > 200°C). In addition, phenolics and polyphenols are observed as one of antifungal compounds from plant. Phenolic compounds possessing a C_3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and cited as antifungal as well.

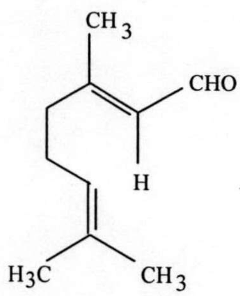
Monoterpenes

Monoterpenes are widespread and tend to occur as components in the majority of essential oils. Some compounds are regularly found in leaf oils, flowers and seed oils which tend to have special monoterpenes present. The monoterpenes can be subdivided into three groups: acyclic, monocyclic, and bicyclic. Some representative are shown in Fig 1.4 (Ikan, 1969).

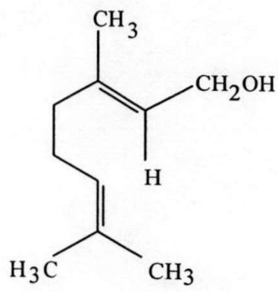
Acyclic monoterpenes



Ocimene

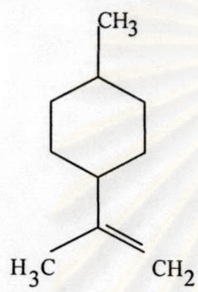


Geranial

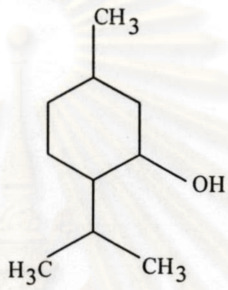


Geraniol

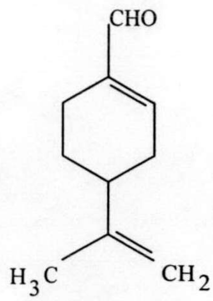
Monocyclic monoterpenes



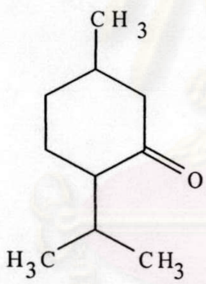
d-Limonenes



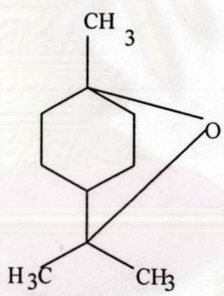
Menthol



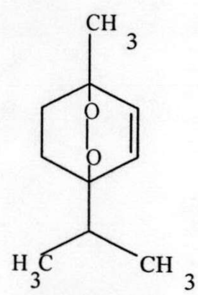
Perillaldehyde



Menthone

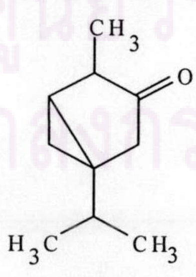


1,8-Cineole

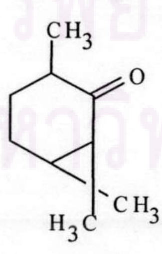


Ascaridole

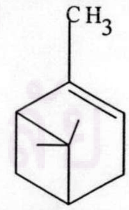
Bicyclic monoterpenes



Thujone



Carone



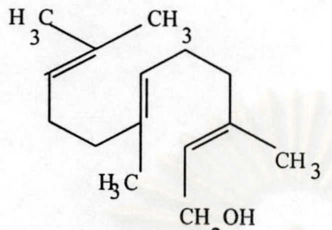
α-Pinene

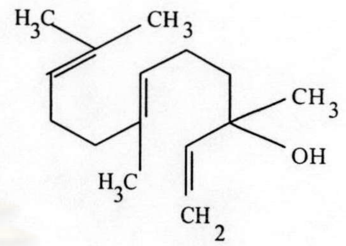
Fig. 1.4 Some common monoterpenes found in essential oils

Sesquiterpenes

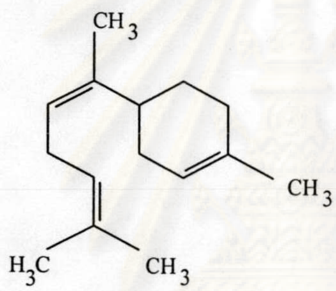
Sesquiterpenes are forming the higher-boiling fraction of the essential oils. Sesquiterpenes are unsaturated compounds and may be acyclic, monocyclic, bicyclic, and tricyclic. Some instances are presented as shown in Fig 1.5 (Ikan, 1969).

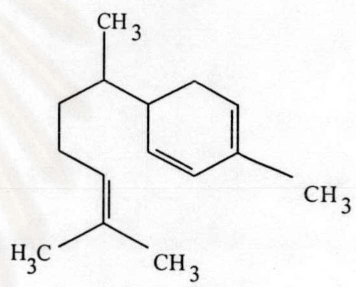
Acyclic sesquiterpenes


Farnesol

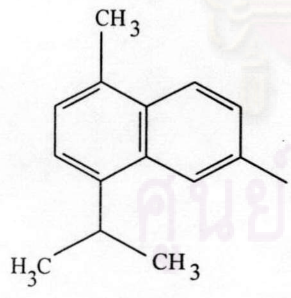

Nerolidol

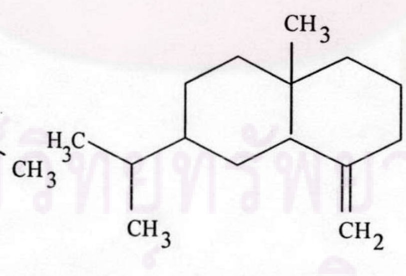
Monocyclic sesquiterpenes


α-Bisabolene


Zingiberene

Bicyclic sesquiterpenes


Cadalene


β-Selinene

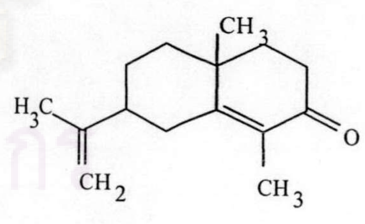

α-Cyperone

Fig. 1.5 Some common sesquiterpenes found in essential oils

Most essential oils consist of volatile steam-distillable fraction responsible for the characteristic fragrance found in many plants. Plant families, which are particularly rich in essential oils, include Compositae, Labiatae, Myrtaceae, Pinaceae, Rosaceae, Rutaceae and Umbelliferae, *etc.*

The most components in essential oil are one group of monoterpenes, which also exhibited strongly antimicrobial activity. In addition, volatile components have low molecular weight and their toxicity to mammals and environment is relatively low. Thereby, volatile components can positively be another alternative crop protecting agent as anti-phytopathogenic fungal in postharvest control.

From literature review, the antifungal activity of the essential oils is due to the action of their component. A study of the fungicidal activity of oil obtained from such genera as *Ocimum, Thymus, Origanum, Anethum, Eucalyptus, Foeniculum* and *Citrus* against several postharvest pathogens (Coccioni and Guizzardi, 1994). Certain volatile aromatic components produced by fruits during ripening also show antifungal activity (Marta and Guizzardi, 1998). Sydney Postharvest Laboratory studied the utilities of essential oil for control postharvest pathogen and found that the essential oil from tea tree oil, red thyme, clove oil and cinnamon leaf oil prevented the growth of *Botrytis cinerea.* Moreover, *Monarda citrodora* and *Melaleuca alternifolia* also exhibited antifungal activity against a wide range of common postharvest pathogen (Jenny, 2000). In addition, a number of volatiles that emanated from ripen peaches are fungicidal. It is possible to fumigate peaches with one of this volatile (benzaldehyde) and protect them against decay (Wilson *et al.* 1997). Most of the research involving this subject in the present time has been done by testing against the growth of fungi in the laboratory under ideal conditions. The methods for applying the oils are currently developed.

The precipitous withdrawal of methyl bromide as a fumigant is increases. It may be profitable to explore natural plant volatiles as alternatives antifungal treatment for fresh product. Especially, postharvest control causes by latent infection, which difficult to control because the pathogen residue localizes in an inactive state within host tissue. Perhaps volatile fungitoxic compounds from plant essential oils could be utilized to control postharvest disease of fruits and vegetables.

1.4 Objective of this research

The main objective of this study was to examine the antifungal activity of component in the crude essential oil from selected aromatic plants by bioautographic assay. Furthermore, the concentration effects of the crude essential oil extract on fungal growth were studied. The possibility of using the crude essential oil in postharvest disease control was also determined.