น้ำมันหอมระเหยที่มีฤทธิ์เป็นสารรมต่อด้วงงวงข้าว *Sitophilus oryzae* (Linnaeus)

นางสาว วชิราภรณ์ ฟูนัน
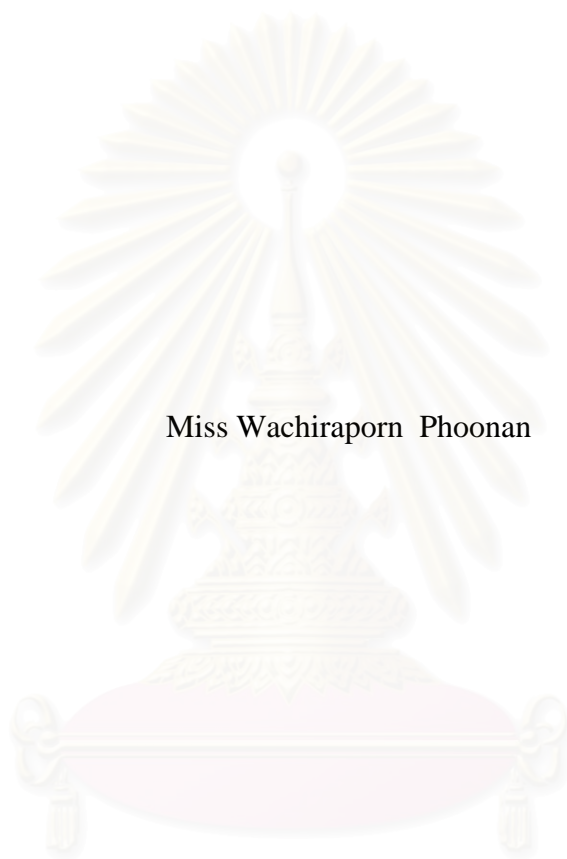
วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีชีวภาพ หลักสูตรเทคโนโลยีชีวภาพ

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2549

ESSENTIAL OILS AS FUMIGANT AGAINST RICE WEEVILS,

Sitophilus oryzae (Linnaeus)

Miss Wachiraporn Phoonan

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Biotechnology

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Thesis Title   ESSENTIAL OILS AS FUMIGANT AGAINST RICE WEEVILS, *Sitophilus oryzae* (Linnaeus)

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Field of Study   Biotechnology

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วชิราภรณ์ ฟูนัน : น้ำมันหอมระเหยที่มีฤทธิ์เป็นสารรมต่อด้วงงวงข้าว *Sitophilus oryzae* (Linnaeus) (ESSENTIAL OILS AS FUMIGANT AGAINST RICE WEEVILS, *Sitophilus oryzae* (Linnaeus)) อาจารย์ที่ปรึกษา : ผศ.ดร.วรินทร ชวศิริ, อาจารย์ที่ ปรึกษาร่วม : อ.ดร.ชัชวาล ใจซื่อกุล, 51 หน้า.

การประเมินฤทธิ์ในการเป็นสารรมของน้ำมันหอมระเหย 25 ชนิดต่อด้วงงวงข้าว *Sitophilus oryzae* Linn ซึ่งเป็นแมลงศัตรูในโรงเก็บที่สำคัญชนิดหนึ่ง พบว่าน้ำมันหอมระเหยจาก เมล็ดเทพธาโร (*Cinnamomum porrectum* Roxb Kosterm) แสดงฤทธิ์ในการเป็นสารรมต่อตัว เต็มวัยของด้วงงวงข้าวสูงที่สุดหลังการทดสอบเป็นเวลา 24 ชั่วโมง ตามด้วยน้ำมันหอมระเหยจาก สะระแหน่ญี่ปุ่น (*Mentha arvensis* Linn) และไพล (*Zingiber cassumunar* Roxb) ที่ค่า LC_{50} เท่ากับ 79, 138 และ 260 µL/L air ตามลำดับ ได้วิเคราะห์องค์ประกอบหลักในน้ำมันหอมระเหย แต่ละชนิดด้วยเทคนิคทางสเปกโทรสโคปิกและหลังจากนั้นทดสอบฤทธิ์ในการเป็นสารรม พบว่า safrole องค์ประกอบหลักในน้ำมันหอมระเหยจากเมล็ดเทพธาโรแสดงประสิทธิภาพในการเป็น สารรมต่อด้วงงวงข้าวมากที่สุด และมี LC_{50} = 57.9 µL/L air ที่ความเข้มข้น 100 µL/L air หลังการ ทดสอบเป็นเวลา 24 ชั่วโมง นอกจากนี้ ยังไม่พบสารตกค้างของ safrole บนข้าวที่ทำการรมแล้ว จากการศึกษาความสัมพันธ์ของประสิทธิภาพในการเป็นสารรมกับองค์ประกอบหลัก พบว่า menthol (LC_{50} = 95.4 µL/L air) เป็นสารออกฤทธิ์ในน้ำมันหอมระเหยจากสะระแหน่ญี่ปุ่น ในขณะที่ terpinen-4-ol (LC_{50} = 165.5 µL/L air) ซึ่งเป็นหนึ่งในองค์ประกอบหลักในน้ำมันหอม ระเหยจากไพลเป็นสารออกฤทธิ์ ดังนั้นน้ำมันหอมระเหยทั้งสามชนิดและองค์ประกอบหลัก เหล่านั้นอาจสามารถพัฒนาเป็นสารรมที่มีประสิทธิภาพสำหรับเมล็ดธัญพืชในโรงเก็บได้

หลักสูตร............เทคโนโลยีชีวภาพ.............ลายมือชื่อนิสิต........วชิราภรณ์ ฟูนัน.........

สาขาวิชา...........เทคโนโลยีชีวภาพ.............ลายมือชื่ออาจารย์ที่ปรึกษา..........................

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KEY WORD: FUMIGANT ACTIVITY / ESSENTIAL OIL / *Sitophilus oryzae* Linn
WACHIRAPORN PHOONAN : THESIS TITLE. ESSENTIAL OILS AS FUMIGANT AGAINST RICE WEEVILS, *Sitophilus oryzae* (Linnaeus) THESIS ADVISOR : ASSISTANT PROFESSOR WARINTHORN CHAVASIRI, Ph.D. THESIS CO-ADVISOR : CHATCHAWAN CHAISUEKUL, Ph.D., 51 pp.

The fumigant activity of twenty-five essential oils was evaluated on an important stored-product insect pest *Sitophilus oryzae* Linn. The essential oils from *Cinnamomum porrectum* (Roxb) Kosterm (Thep ta-ro) exhibited the highest fumigant toxicity against adults of *S. oryzae* after 24 h exposure, followed by those from *Mentha arvensis* Linn (Japanese mint) and *Zingiber cassumunar* Roxb (plai) with LC_{50} 79, 138 and 260 µL/L air, respectively. The main constituents of each essential oil were analyzed by spectroscopic techniques and then tested in fumigation bioassay. Safrole, the major component of the essential oil from *C. porrectum*, exhibited the most potent fumigant (LC_{50} = 57.9 µL/L air) at the concentration of 100 µL/L air after 24 h exposure. Additionally, the residue of safrole could not significantly be detected on the fumigated rice. The major component-fumigant activity relationship study revealed that menthol (LC_{50} = 95.4 µL/L air) was an active component of the essential oil from *M. arvensis*, while terpinen-4-ol (LC_{50} = 165.5 µL/L air), one of the major constituents of the essential oil from *Z. cassumunar*, was an active component. Thus, the essential oils from all three plant sources and their major constituents may be developed as a potent fumigant for storage grains.

Program of ......Biotechnology.........Student's signature...W. Phoonan...........

Field of study...... Biotechnology.......Advisor's signature........W. Chavasiri.......

Academic year.........2006..............Co-advisor's signature...Chatu Chisuhl.....

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CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

b.p.	boiling point
°C	degree Celsius
$CDCl_3$	deuterated chloroform
cm	centimeter
^{13}C-NMR	proton nuclear magnetic resonance
D	dry
F	fresh
g	gram (s)
GC	gas chromatography
GC-MS	gas chromatography – mass spectroscopy
h	hour (s)
^{1}H-NMR	carbon nuclear magnetic resonance
J	coupling constant
L	liter (s)
LC_{50}	the concentration which causes 50% mortality
L:D	light:dark
mg	milligram (s)
MHz	megahertz
min	minute (s)
mL	milliliter (s)
mm	millimeter (s)
n	number
ppm	part per million
R_f	retardation factor
R.H.	relative humidity
SE	standard error
w/w	weight by weight
μg/mL	microgram per milliliter
μL	microlitre (s)
δ	chemical shift

CHAPTER I

INTRODUCTION

Grain products form a large part of the diet of the world's population, 64% of all food sources in the world, which divide into 50% of cereals and 14% of pulses. Therefore, the grain culturing area constitutes or occupied 75% of total culturing area worldwide. The agricultural grain products are rice, maize, wheat, sorghum, mung bean, soybean and groundnut etc. (Sukprakarn *et al.*, 1996). In the Southeast Asia, post harvest losses of grains have been estimated at 10-30%, caused mainly by improper drying and pest infestation during crop storage and distribution, and under hot and humid conditions (Hayashi *et al.*, 2004).

Rice is the most importance crop and staple food in Thailand. The rice is cultivated in all part of Thailand at about 10 million hectares and the annual production is about 19.5 million tons. Nearly all of the grain and other agricultural products are kept in the mills, godowns or silos (Sukprakarn, 1989). Storage facility operators must manage and protect the stored grain from various damages. The damages of rice grains are resulted from two important factors. The first factor is the physical factor, such as temperature and humidity, and the second factor is biological factor, such as insects, mites, fungi, birds, and rodents. Among these biological factors, insects are the most important pest, which cause the most damage to rice grain products (Sukprakarn *et al.*, 1996).

Infestation of stored product insects results in a variety of damage and economic loss, including the physical loss of commodity by insect direct consumption, the spoilage and loss of commodity, the encouragement of mold growth, the contamination of commodities with insect bodies and waste products which may be toxic, repulsive or allergenic. Moreover, the infested commodities may be rejected by consumers (both human and animal), and the resultant social and legal costs, restriction of trade and damage to economies will follow (Rees, 2004).

These are very serious problems for all agricultural countries, including Thailand. Nevertheless, chemical control agents, known as pesticides, have been the

common practice of pest management and have contributed a major part in protecting crops from the damages inflicted by pests. In fact, most pesticides are obtained from synthetic agents. Most farmers usually employ synthetic agrochemicals since they are convenient, fast and easy to handle. However, synthetic agrochemicals have many drawbacks. The use of synthetic pesticides has caused some concerns regarding their adverse effects on the environment. These compounds are often not biodegradable and the residues are concentrated in food chains and accumulated in soil, water and plants, so they cause invariably environment pollution (Munakata, 1970). Furthermore, synthetic pesticides are generally toxic to human and non-target animals, and most of them are imported from foreign countries. According to the all of the previous reasons, novel alternatives from natural products seem to be promising to replace some synthetic chemicals. Naturally occurring compounds are considering friendly to environment because they are easy to decompose, low toxic to user, and inexpensive than imported pesticides (Lee *et al.*, 2001b).

1.1 General characteristics of *Sitophilus oryzae* Linnaeus

Many of the stored product insects are in the order Coleoptera at about 600 species (Sukprakarn *et al.*, 1996). The Curculionidae or true weevils is one of the largest families of beetles that can be found in a wide range of habitats, and many species are important pests of agriculture, horticulture and forestry. They can attack the stems, roots and seeds of plants, and some are wood borers. The head of adult weevil has a characteristic snout to bore into substrate. Members of the genus Sitophilus are among the most important pests of stored grain (Rees, 2004).

Sitophilus oryzae (Linnaeus) or rice weevil is a major important pest of paddy and polished rice in Thailand (Sukprakarn *et al.*, 1996) because this weevil is capable of successfully attacking and breeding in previously undamaged whole cereal and pulse grains. However, rice weevils are rarely successful on milled ground commodities. Rice weevils' infestation also allows the secondary pests to attack the damaged grains (Mutambuki, 2004). Therefore, the control of the weevil is necessary to manage the stored rice.

Morphological characteristics: Rice weevils vary in body color from reddish brown to nearly black and are usually marked on the back with four light- reddish or yellowish spots. The body length is 2.4-4.5 mm in adult. The size of adult varies

somewhat with the seed size from which it emerged (Hayashi *et al.*, 2004; Rees, 2004; Naunwat *et al.*, 2005).
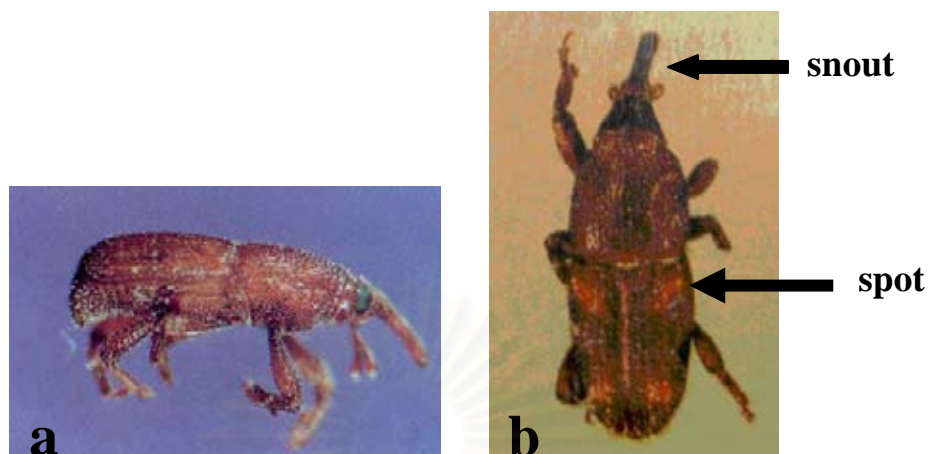
Figure 1.1 The adults of *Sitophilus oryzae* a) side view feature b) top view feature

Life cycle: Female rice weevil selects a spot on the grain surface then chews a small hole, into which she lays an egg. Eggs are laid singly into grains. The hole is then plugged with a waxy secretion. About 150-300 eggs are laid per female. The white legless larvae develop hiddenly within a cavity hollowed out within the grain. This stage takes approximately 25 days at 25 °C. Pupation takes place within the cavity made by the larva. Upon emergence from the pupa, the adult may spend several days within the cavity. Eventually, a newly molted adult will chew its way out, leaving a ragged hole. Adult weevils continue to feed on grain and may live about 4-12 months. The life cycle under favorable conditions can be completed within 30-35 days (Hayashi *et al.*, 2004; Rees, 2004; Naunwat *et al*., 2005).
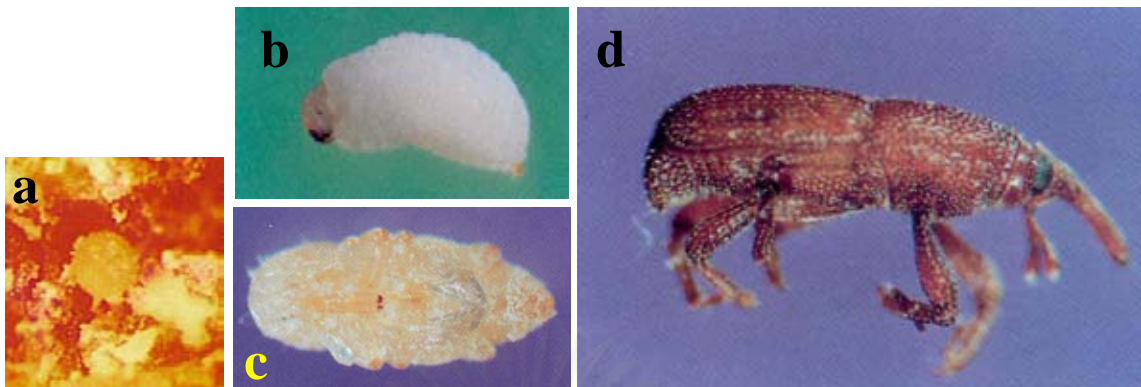
Figure 1.2 Different stages of *S. oryzae* a) egg b) larva c) pupa d) adult

(Ministry of Agriculture and Cooperatives, Rice Department, 2006)

Behavior: The adults can fly but not often. Their behavior is always hidden in the grain pile. They will move out from their habitat and climb to the top when they are disturbed (Naunwat *et al*., 2005). When they are threatened by enemies, they will feign death by enclosing their appendages with their body.

Infestation damage: Rice weevils are capable of infesting all cereal grains, but they prefer wheat, rice and other small grains. Feeding by larvae leaves large cavities inside grains and newly emerging adult leave behind large ragged emergence holes. Adults cause further damage by feeding, mainly by attacking previously damaged grain. Their infestations produce a lot of heat and moisture, and this encourages extensive quality loss, mold growth and growth of populations of other insect species (Rees, 2004).
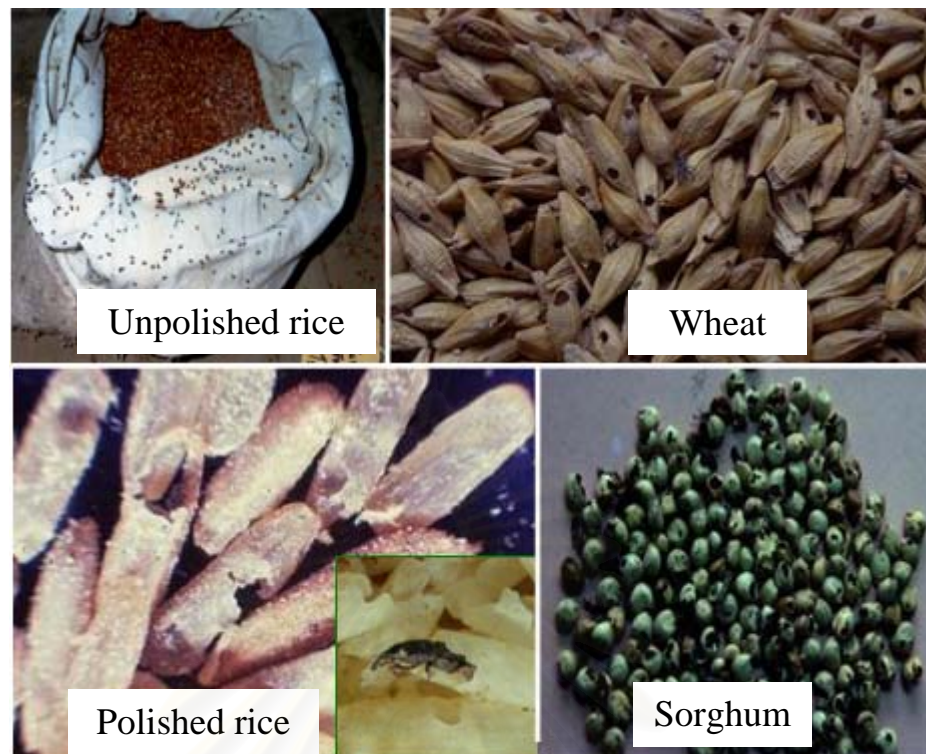
Figure 1.3 The infestations of *S. oryzae* (L.) in grains.

(Ministry of Agriculture and Cooperatives, Rice Department, 2006)

The insect control of storage pests: The insect control can be divided into two strategies.

1) Non-chemical control

- *Store hygiene*: The primary control tactic targets to minimizing insect pest infestation in stored grain through storage hygiene. Pit stores need to be thoroughly cleaned of insect pests prior to filling. Old grain should be checked, and if necessary, re-dried and cleaned to control existing infestations. Different products should be stacked separately. The storage structures should be closed off to prevent entry by pests, airtight silos with good thermal insulation offer the best protection (Mutambuki, 2004).

- *Repellent herbs*: Admixture the grain or seed with inert substances such as dust or plant parts could prevent the grains from insect damage for some period (Sukprakarn, 1989).

- *Temperature Control*: Since most stored product insects cannot tolerate extreme temperature, heating and cooling are logical approaches to insect control. To some extent, it has been a common practice to superheat some commodities for insect control. The temperatures of 55-60°C maintained for 10 to 12 hours are effective. Low temperature is probably the most important single factor in making long term storage possible and economical. The insects become inactive and eventually die at a temperature below 12°C (Sukprakarn, 1989).

- *Moisture Control:* Most of the stored grain insects are unable to survive and reproduce in grain whose moisture content is below 9%. Most favorable grain moistures for insect development ranges from 12 to 15%. Various means, such as drying, and refrigerator curing, can be employed to reduce and maintain the moisture below than favorable for reproduction and development to control the insects (Sukprakarn, 1989).

2) Chemical Control

    This strategy is commonly used in insect control. For the protection of stored produce against the insects, the following groups of pesticides are used:

    a) *Contact insecticides*: They can be either liquid or dust formulations. They can kill by penetrating the insect body being ingested or vapor inhalation. The lower toxicity insecticides in the group of organophosphate are fernitrothion (Sumithion®), chlorpyrifos methyl (Reldan®), methacrifos (Damfin®) and Dichlorvos. The commonly used synthetic pyrethroids are permithrin, cypermethrin (K-orthene®), deltamehrin (Ripcord®) and betacyfluthrin. These can be sprayed on coated or admixed with grain for long-term protection (Ministry of Agriculture and Cooperatives, Rice Department, 2006).

    b) *Fumigants*: These are chemicals which are toxic to insects in the vapour and smoke forms. They can be supplied in various formulations (solid, liquid or gas), when they are in contact with moist air, release highly toxic respiratory poison gases. In order for the fumigant to be effective, the commodity or space being treated must be properly sealed. The most common used fumigants in storage are 1) gasses fumigants and 2) liquid

fumigants. Gasses fumigants, such as methylbromide and phosphine are highly effective, but they have some constraints. Use of methylbromide will soon be restricted due to its potential ozone depleting properties (World Meteorological Organization [WMO], 1995). Moreover, it is highly toxic to warm-blooded animals including human (Dansi *et al.*, 1984) and reducing the rate of seed germination (Ministry of Agriculture and Cooperatives, Department of Agriculture, 2005). Phosphine fumigation, which is widely used, may become increasingly limited in use because the resistance of stored-grain insects to phosphine has now been discovered in more than 45 countries. In addition, phosphine has been argued to be genotoxic to occupationally exposed fumigators (Bell, 2000). Liquid fumigants, such as carbon tetrachloride or mixture of carbon disulphide, ethylene dibromide or ethylene dichloride, and carbon tetrachloride, are easier to handle as they are less toxic to man. The liquid is poured on the produce or left in trays to evaporate. Such a fumigation takes several days depending on the temperature and quantity of fumigant used (Sukprakarn, 1989).

In many storage systems, fumigants are the most economical and convenient tool for managing stored-grain insect pests not only because of their ability to kill a broad spectrum of pests but because of their easy penetration into the commodity while leaving minimal residues (Bell, 2000). Moreover, they have potential to kill all insects in a single application (Sukprakarn *et al.*, 1996). According to the constraints of the synthetic fumigants, we try to find the new safer fumigants from the natural products.

The synthetic standard compound we used in this study, Dichlorvos (dimethyl-2,2-dichlorovinyl phosphate, often referred to as DDVP), has high vapor pressure and high insecticidal activity in vapor phase comparing to other insecticides. It is widely used in household, food storage, for the treatment of companion animals and for the control stored-product pests. Dichlorvos has short residue stability, especially at higher temperatures and higher moisture levels. It is readily metabolized and rapidly excreted. Many researches usually use dichlorvos as the fumigant against the stored-insect pests (Lee *et al.*, 2003a; Rowlands, 1970).

1.2 Essential oils

Plant essential oils are secondary metabolites synthesized by plants. Plants containing essential oil belong to both Gymnospermae (Pines and Cycads) and Angiospermae (flowering plants). The essential oils are produced in glandular hair, schizogenous canals, ducts, cavities of heartwood, oil glands present in leaves, buds and in some cases, oleoresins. Essential oils occur commonly in the families Lamiaceae, Verbenaceae, Valerianaceae, Araliaceae, umbelliferae, Myrtaceae, Cistaceae, Violaceae, Thymelaceae, Anacardiaceae, Rutaceae, Burseraceae, Cneoraceae, Meliaceae, Magnoliaceae, Santalaceae, Betulaceae, Juglandaceae, Myricaceae, Salicaceae, Asteraceae, Ericaceae, Poaceae, Araceae, Pandanaceae, Cyperaceae, Zingiberaceae and Orchidaceae (Bhat, 2005). Many essential oils can be utilized as antiseptic agents, perfume industry, food additives and aromatherapy. Essential oils are abundant sources of terpenoids. They consist of a complex mixture of mono- and sesquiterpenes, alcohols, ketones, acids and esters. There are four general methods for the extraction of essential oils: expression, steam distillation, extraction with volatile solvents and resorption in purified fats (Ikan, 1969).

Classifications of essential oil

Monoterpenes: Monoterpenes are widespread and tend to occur as major components of essential oils (Ikan, 1969). They consist of two isoprene units. Their molecular formular is $C_{10}H_{16}$. The boiling point of monoterpenes is about 140-180°C (Ikan, 1969). The monoterpenes can be subdivided into three groups: acyclic, monocyclic and bicyclic (Ikan, 1969) as shown in Figure 1.4

Acyclic monoterpenes
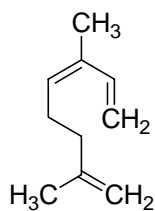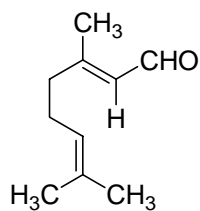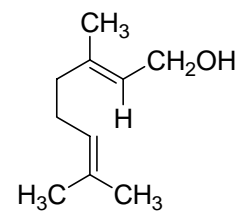
Ocimene   Geranial   Geraniol

Monocyclic monoterpenes
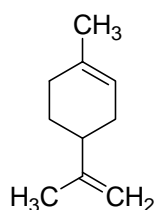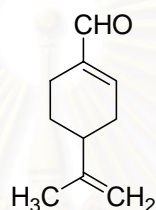
d-Limonene   Perillaldehyde   1,8-Cineole
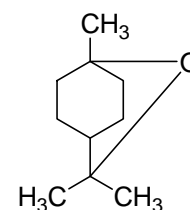
Bicyclic monoterpenes
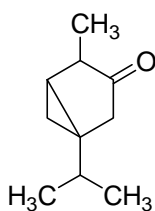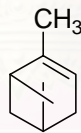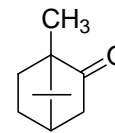
Thujone   α-Pinene   Camphor

Figure 1.4 Some common monoterpenes found in essential oils (Ikan, 1969)

Sesquiterpenes: Sesquiterpenes are forming the higher-boiling fraction of essential oils. They are formed by the union of three isoprene units. Sesquiterpenes are unsaturated compounds and may be acyclic, monocyclic, bicyclic and tricyclic (Ikan, 1969) (Figure 1.5).

Acyclic sesquiterpenes
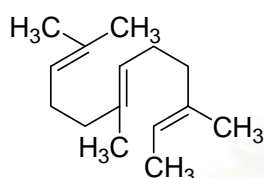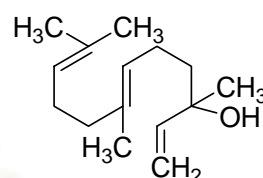
Farnesol   Nerolidol

Monocyclic sesquiterpenes
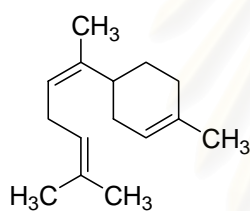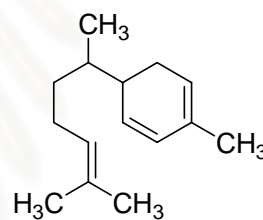
α-Bisabolene   Zingiberene

Bicyclic sesquiterpenes
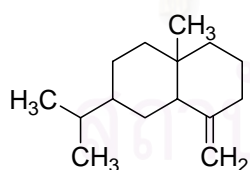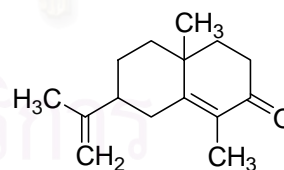
β-Selinene   α-Cyperone

Figure 1.5 Some common sesquiterpenes found in essential oils (Ikan, 1969).

1.3 Fumigants from plants against *S. oryzae* (L.)

Several essential oils have been previously reported to effectively kill adult rice weevils, such as *Labiatae* sp. oil ZP51 (Shaaya *et al.*, 1997), *Mentha arvensis* L. var *piperascens* (Lee *et al.*, 2001a), eucalyptus, rosemary (Lee *et al.*, 2001b), *Foeniculum vulgare* fruit (Kim and Ahn, 2001), cinnamon, horseradish, mustard (Kim *et al.*, 2003), *Acorus gramineus* rhizome (Park *et al.*, 2003b), and plants in family Myrtaceae; *Eucalyptus nicholii*, *E. conodocarpa*, *E. blakelyi*, *Callistemon sieberi*, *Melaleuca fulgens*, and *M. armillaris* (Lee *et al.*, 2004a; Lee *et al.*, 2004b).

Moreover, the fumigant activity of the essential oil constituents was also reported, such as menthone (Lee *et al.*, 2001a), 1,8-cineole, benzaldehyde (Lee *et al.*, 2001b), (*E*)-anethole, estragole and (+)-fenchone (Kim and Ahn, 2001), *l*-fenchone, limonene and pulegone (Lee *et al.*, 2003a), and (*Z*)-asarone (Park *et al.*, 2003b). This study investigated the fumigant toxicity of various essential oils and their constituents from Thai plants towards *S. oryzae*. From these reports, oxygenated monoterpenoids tend to exhibit higher activity than hydrocarbons (Papachristos *et al.*, 2004). Ketone compounds were found more toxic than other monoterpenoids (Lee *et al.*, 2003a).

1.4 Rationale

The use of synthetic fumigants for managing the stored-product insect pest at the present time normally causes harmful effects to the environment and other animals. Essential oils are considered to be the natural fumigant controlling stored-product insect pests because of their high volatility, toxicity to stored-grain insect pest, biodegradability and eco-friend. In this research, the fumigant activity of selected essential oils was examined against rice weevil, *Sitophilus oryzae* L and their major constituents were investigated comparing with the synthetic fumigant as standard.

1.5 The goals of this research

1. To study the fumigation activity of various essential oils against *S. oryzae*.

2. To study the fumigation activity of their major constituents against *S. oryzae*.

3. To analyze the residues of fumigants on the fumigated rice.

CHAPTER II

MATERIALS AND METHODS

2.1 Insects

Rice weevils were obtained from the colony maintained at the Department of Biology, Faculty of Science, Chulalongkorn University. Weevils were reared on the polished rice in the 8 ounce glass jar; 100 adult insects per 500 g of rice grain under conditions of 30 ± 2 °C, 70 ± 10 % relative humidity (R.H.) and a photoperiod of 12:12h (L:D). The rearing jars were sealed with the blotting paper to give air flow. Cultures were changed every two weeks and newly emerging adult rice weevils were transferred into a new jar containing fresh rice grain. The 7-14 days adults of emerging rice weevils were used as tested specimen.

2.2 Plant materials

Sources of plant samples and commercial-grade essential oils used in this study are presented in Table 2.1.

Table 2.1 Sources of essential oils

No	Family	Scientific name	Common name	Plant part
1	Annonaceae	*Cananga odorata* (Lamk.) Hook. P. et Th.	Ylang ylang	Flowers
2	Graminae	*Vertivevia zizanioides*	Vertiver	Fibrous root
3	Illiciaceae	*Illicium verum* Hook.f.	Star anise	Fruits
4	Lamiaceae	*Mentha arvensis* Linn.	Japanese mint	Flowering
5		*Rosemarinus officinalis* Linn.	Rosemary	Flowering tops
6	Lauraceae	*Cinnamomum cassia* Presl.	Cinnamon	Leaves
7		*Cinnamomum porrectum* (Roxb.) Kosterm	Thep Ta-ro	Seeds
8	Myrtaceae	*Eugenia caryophyllata* Thumb	Clove	Flowers
9				Leaves
10		*Melaleuca cajuputi* Powell	Cajeput	Leaves
11		*Eucalyptus globules* Labille	Eucalyptus	Leaves
12	Piperaceae	*Piper betle* Linn.	Betal vine	Leaves
13		*Piper nigrum* Linn.	Black pepper	Peppercorns
14	Rutaceae	*Citrus hystrix* DC.	Kaffir lime	Leaves
15				Fruits
16		*Citrus aurantifolia* Swing.	Lime	Fruits
17		*Zanthoxylum limonella* Alston	Ma khaen	Fruits
18		*Citrus reticulata* Blanco	Tangerine	Fruits
19	Scrophulariaceae	*Limnophila aromatica* Merr.	Phak khayaeng (Thai name)	Aerial part
20	Zingiberaceae	*Zingiber cassumunar* Roxb	Plai (Thai name)	Rhizomes
21		*Curcuma* spp.	Khamin dam (Thai name)	Rhizomes
22		*Zingiber* spp.	Plai khaew (Thai name)	Rhizomes

Table 2.1 (continued)

No	Family	Scientific name	Common name	Plant part
23	Zingiberaceae	*Alpinia nigra* (Gaertn.) B.L.Burtt	Ginger	Rhizomes
24		*Boesenbergia pandurata*	Lesser galangal	Rhizomes
25		*Curcuma domestica* Valeton.	Turmeric	Rhizomes

Note: The essential oils No 1-6, 8-9, 11-16, 18 and 24-25 were obtained from Thai-China Flavours and Fragrances industry Co., Ltd., Nontaburi and those of No 7 and 10 were supported by Royal Forest Department, Bangkok. The rest essential oils were gained by hydrodistillation.

2.3 Chemicals

Merck's TLC (aluminium sheet, silica gel 60 F254 pre-coated 20×20 cm, layer thickness 0.2 mm) was used for the compound separation. All solvents were purified by distillation, except diethyl ether and acetone which were analytical grade. Certain monoterpenes were purchased from Fluka Chemies A.G. (Switzerland).

2.4 General procedures for hydrodistillation

Some essential oils were obtained by hydrodistillation (Dean-stark distillation) (Vogel, 1980). Each sample was finely chopped and put into a 1000 mL round bottom flask. The deionized water was added into the flask to about 500 mL. The flask was connected to the Dean-stark apparatus for hydrodistillation. The hydrodistillation was carried out for approximately 4 h or until no oil come out with the distillate. After cooling, the distillate was extracted by diethyl ether twice. After extraction, the solvent phase was dried over anhydrous sodium sulfate and was concentrated by rotary evaporator. The obtained essential oil was then collected and stored in the dark at 4°C to avoid the oxidation until being tested for the fumigant activity.

2.5 Fumigation bioassay

The fumigation bioassay was adapted from the method used in Park *et al*. (2003a). Ten 7-14 days adults *S. oryzae* were put into the 2.5×2.5×2.0 cm plastic box together with 0.7 g of the polish rice. The mesh cloth was covered over the body part of the box by adhesive tape to prevent any direct contact of the weevils with the tested

compounds. Then, 50 μL of the essential oil or pure compound solution diluted by acetone for each concentration was applied into the 2.3×2.3 cm filter paper (Whatman No.1, Whatman International Ltd., Maidstone, England). The applied paper was left out in the fume hood for a minute to evaporate the solvent. After that, the paper was placed on the mesh cloth and then the lid was closed immediately. The parafilm was bound down the connection between the box body and the lid to prevent the leaking of fumigant and bound cover again with the adhesive tape. Then, tested boxes were turned over to allow the fumigant paper lay in the bottom side. The control groups were the boxes which did not have any treatment (control A) and the boxes which contained only solvent (control B). All treatments were replicated three times. Those tested boxes were observed at the same conditions with the insect rearing conditions. The mortality was collected after 24 h exposure.

$$\% \text{ mortality } = (O/T) \times 100$$

O : Observed number of the dead *S. oryzae*

T : Total number of *S. oryzae*

The dead adult *S. oryzae* was taken into the account by shaking the box or prodding with paintbrush to observe the moving of appendages of the insect. If its appendages did not move, that insect was considered to be dead. It revealed that the insect died (Kim and Ahn, 2001)

2.6 Screening of essential oils for fumigation bioassay

Twenty-five essential oils from Thai plants were tested as fumigants at the concentration of 1000 μL/L air as described above. The mortality percentage was observed after 24 h exposure.

The essential oils which caused 100% mortality were selected to test at lower concentrations (400 and 200 μL/L air). After that, the LC_{50} of the most potential essential oils were computed by Probit analysis (Finney, 1971).

2.7 Isolation of major constituents from the potent essential oils

A selected essential oil was fractionally distilled. Each fraction was collected upon boiling range and analyzed by gas chromatography (GC) comparing with the crude essential oil. The fractions containing major constituent were re-separated by column chromatography. The pure compounds were characterized by spectroscopic techniques including ^{1}H- and ^{13}C- NMR and reconfirmed the purity by GC.

2.8 Spectroscopic analysis

The GC analysis was performed on Varian, CP-3800 gas chromatograph. The essential oil mixture was subjected to the GC analysis using CP-sil 5 column. The temperature program was increased from 50 to 65°C with a rate of 1.5°C per min as step 1 and from 65 to 160°C with a rate of 15°C per min as step 2. The injection temperature and the detector temperature was 250°C. The components of essential oil were determined by comparing with external standards.

The ^{1}H and ^{13}C nuclear magnetic resonance including 2D-NMR experiments were carried out with a Jeol 400 MHz JNM-A500 FT-NMR spectrometer.

2.9   Fumigation test of pure compounds

The isolated constituents of the effective essential oils were tested for fumigation bioassay comparing with fumigant standard—dichlorvos at concentration of 100 μL/L air. The exposure time was 24 h. The LC_{50} of main components of each essential oil were determined, subsequently.

2.10 Residue determination

Residues in the polished rice were determined in menthol and safrole at 400 μL/L air. Three groups of rice were compared. The first group was the rice sample mixed with fumigant directly as positive control. The second group was negative control fumigated with only acetone and the last group was the fumigated rice sample. Each group was sampled twice at pre-aeration and 24 h aeration in fume hood. The rice samples (2.1 g) were extracted with 5 mL acetone for 1 h as adapted from Lee *et al*. (2003b). Analysis of fumigant residue was performed by GC under the same

program as previously described in 2.8. The residue quantity was determined from calibration curve of each compound.

2.11 Statistical analysis

The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Duncan's test at $P = 0.05$. The LC_{50} was calculated by Probit analysis (Finney, 1971). All computations were done using the SPSS program. Means ($\pm$ SE) of untransformed data are reported. Corrected mortality was calibrated by Abbott's formula as follows:

$$\text{Corrected \% mortality} = \frac{(T - C) \times 100}{100 - C}$$

T : % mortality of treatment group

C : % mortality of control group

CHAPTER III

RESULTS AND DISCUSSION

3.1 Hydrodistillation results

Some selected plants were obtained from hydrodistillation according to the procedure described in Chapter II. The results of hydrodistillation are presented in Table 3.1.

Table 3.1 The hydrodistillation of some selected plants

No	Scientific name	Plant part	plant weight[*] (g)	Oil weight (g), (% w/w)
1	*Alpinia nigra*	Rhizomes	2,000 F	1.64 (0.08%)
2	*Curcuma* spp.	Rhizomes	1,300 F	1.67 (0.13%)
3	*Limnophila aromatica*	Aerial part	2,400 F	1.92 (0.08%)
4	*Zanthoxylum limonella*	Fruits	700 D	3.49 (0.50%)
5	*Zingiber cassumunar*	Rhizomes	3,000 F	13.25 (0.44%)
6	*Zingiber* spp.	Rhizomes	1,100 F	0.85 (0.08%)

[*] F = fresh weight and D = dry weight.

The hydrodistillation of some selected Thai plants yielded variable amounts of essential oils ranging from 0.08 to 0.50% (w/w) of fresh (or dry) weight. *A. nigra*, *L. aromatica* and *Zingiber* spp. provided the lowest amount whereas the highest amount of essential oil was achieved from the fruits of *Z. limonella*.

3.2 Screening of essential oils for fumigation bioassay

Six essential oils extracted as aforementioned, together with nineteen commercial essential oils were preliminarily screened for fumigant activity against *Sitophilus oryzae* at 1000 µL/L air. The exposure time was at 24 h. The results are shown in Table 3.2.

Table 3.2 The fumigant activity of essential oils against *Sitophilus oryzae* adults at concentration of 1000 μL/L air after 24 h treatment.*

No	Scientific name	Mean ± SE**, *n* = 3	Toxicity Class***
1	*Cananga odorata*	0.0 ± 0.0 [a]	
2	*Vertivevia zizanioides*	0.0 ± 0.0 [a]	
3	*Piper betle*	0.0 ± 0.0 [a]	
4	*Zingiber* spp.	0.0 ± 0.0 [a]	Low
5	*Curcuma domestica*	0.0 ± 0.0 [a]	
6	*Curcuma* spp.	3.3 ± 3.3 [a]	
7	*Limnophila aromatica*	16.7 ± 12.0 [ab]	
8	*Citrus reticulata*	43.3 ± 21.9 [bc]	Medium
9	*Citrus hystrix* (fruits)	53.3 ± 17.6 [cd]	
10	*Cinnamomum cassia*	63.3 ± 6.7 [cd]	
11	*Piper nigrum*	63.3 ± 12.0 [cd]	
12	*Eugenia caryophellata* (flowers)	70.0 ± 10.0 [de]	Medium-high
13	*Zanthoxylum limonella*	70.0 ± 15.3 [de]	
14	*Citrus aurantifolia*	73.3 ± 8.8 [de]	
15	*Illicium verum*	90.0 ± 5.8 [ef]	
16	*Eugenia caryophellata* (leaves)	90.0 ± 5.8 [ef]	
17	*Melaleuca cajuputi*	93.3 ± 6.7 [f]	
18	*Boesenbergia pandurata*	93.3 ± 3.3 [f]	
19	*Mentha arvensis*	100.0 ± 0.0 [f]	
20	*Rosemarinus officinalis*	100.0 ± 0.0 [f]	High
21	*Cinnamomum porrectum*	100.0 ± 0.0 [f]	
22	*Eucalyptus globules*	100.0 ± 0.0 [f]	
23	*Citrus hystrix* (leaves)	100.0 ± 0.0 [f]	
24	*Zingiber cassumunar*	100.0 ± 0.0 [f]	
25	*Alpinia nigra*	100.0 ± 0.0 [f]	

* Each datum represents the means of three replicates, each set up with 10 adults.

** Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Duncan's test). Mortalities were transformed to arcsine square-root before ANOVA. Means (±SE) of untransformed data are reported.

*** The low toxicity class is < 25 % mortality; the medium toxicity class is 25-50 % mortality; the medium-high toxicity class is 51-74 % mortality and the high toxicity class is ≥ 75 % mortality.

From Table 3.2, twenty-five essential oils from Thai species were screened against *S. oryzae* by a fumigation method at 1000 µL/L air and %mortality was observed after 24 h treatment. Eleven essential oils: *I. verum*, *E. caryophellata* (leaves), *M. cajuputi*, *B. pandurata*, *M. arvensis*, *R. officinalis*, *C. porrectum*, *E. globules*, *C. hystrix*, *Z. cassumunar* and *A. nigra*, caused mortality in *S. oryzae* higher than 75%, ranging from 90-100%. This group exhibited high significant potent toward *S. oryzae*, thus was selected for further testing at the lower concentration of 400 µL/L air. The results are presented in Table 3.3.

Table 3.3 The fumigant activity of essential oils against *Sitophilus oryzae* adults at concentration of 400 µL/L air after 24 h treatment.*****

No	Scientific name	Mean $\pm$ SE**, $n = 3$	Toxicity Class***
1	*Melaleuca cajuputi*	17.8 $\pm$ 9.1 [ab]	Low
2	*Alpinia nigra*	30.0 $\pm$ 15.8 [ab]	
3	*Boesenbergia pandurata*	31.1 $\pm$ 23.3 [ab]	Medium
4	*Rosemarinus officinalis*	45.3 $\pm$ 23.2 [ab]	
5	*Citrus hystrix* (leaves)	56.9 $\pm$ 3.2 [ab]	
6	*Eugenia caryophellata* (leaves)	57.2 $\pm$ 3.9 [ab]	Medium-high
7	*Eucalyptus globules*	68.9 $\pm$ 19.4 [bc]	
8	*Illicium verum*	69.5 $\pm$ 6.3 [bc]	
9	*Mentha arvensis*	100.0 $\pm$ 0.0 [c]	
10	*Cinnamomum porrectum*	100.0 $\pm$ 0.0 [c]	High
11	*Zingiber cassumunar*	100.0 $\pm$ 0.0 [c]	

* Each datum represents the means of three replicates, each set up with 10 adults.
** Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Duncan's test). Mortalities were transformed to arcsine square-root before ANOVA. Means ($\pm$SE) of untransformed data are reported.
*** The low toxicity class is < 25 % mortality; the medium toxicity class is 25-50 % mortality; the medium-high toxicity class is 51-74 % mortality and the high toxicity class is $\geq$ 75 % mortality.

From Table 3.3, the essential oils from *M. arvensis, C. porrectum,* and *Z. cassumunar* resulted in 100% mortality after 24 h exposure. Five essential oils: *M. arvensis*, *C. porrectum, Z. cassumunar, I. verum* and *E. globules* were further selected for testing at lower concentration of 200 μL/L air. These were classified in the medium-high and the high class of toxicity. The results are presented in Table 3.4.

Table 3.4 The fumigant activity of essential oils against *Sitophilus oryzae* adults at concentration of 200 μL/L air after 24 h treatment.*****

No	Scientific name	Mean ± SE**, n = 3	Toxicity Class***
1	*Eucalyptus globules*	0.0 ± 0.0 [a]	Low
2	*Illicium verum*	20.0 ± 0.0 [a]	
3	*Zingiber cassumunar*	76.7 ± 23.3 [b]	High
4	*Cinnamomum porrectum*	83.3 ± 3.3 [b]	
5	*Mentha arvensis*	100.0 ± 0.0 [b]	

  * Each datum represents the means of three replicates, each set up with 10 adults.
  ** Means within a column followed by the same letter are not significantly different at
    $P = 0.05$ (Duncan's test). Mortalities were transformed to arcsine square-root before
    ANOVA. Means (±SE) of untransformed data are reported.
 *** The low toxicity class is < 25 % mortality; the medium toxicity class is 25-50 %
    mortality; the medium-high toxicity class is 51-74 % mortality and the high toxicity
    class is ≥ 75 % mortality.

From fumigation testing at concentration of 200 μL/L air, the results showed that three essential oils from *Z. cassumunar*, *C. porrectum* and *M. arvensis* exhibited the highest activity toward *S. oryzae* at exposure time 24 h. The essential oil from *M. arvensis* revealed the complete mortality while those of *Z. cassumunar* and *C. porrectum* showed 76.7 % and 83.3 % mortality, respectively. These three essential oils were categorized in the high toxicity class.

3.3 LC$_{50}$ determination

The concentration of effective essential oils which caused 50 % mortality of *S. oryzae* was investigated by fumigation bioassay. LC$_{50}$ was estimated by Probit analysis (Finney, 1971). Probit linear regression curves of three essential oils are shown in Figure 3.1.

M. arvensis

Y=8.89X-19.03

$R^2 = 0.845$
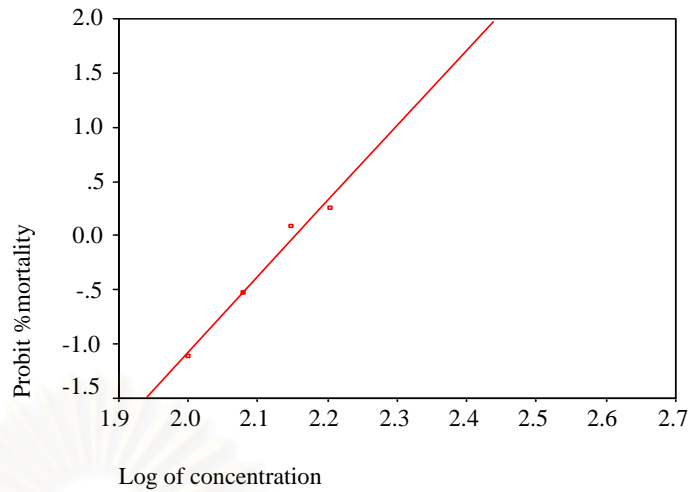
Z. cassumunar

Y=17.10X-41.30

$R^2 = 0.992$
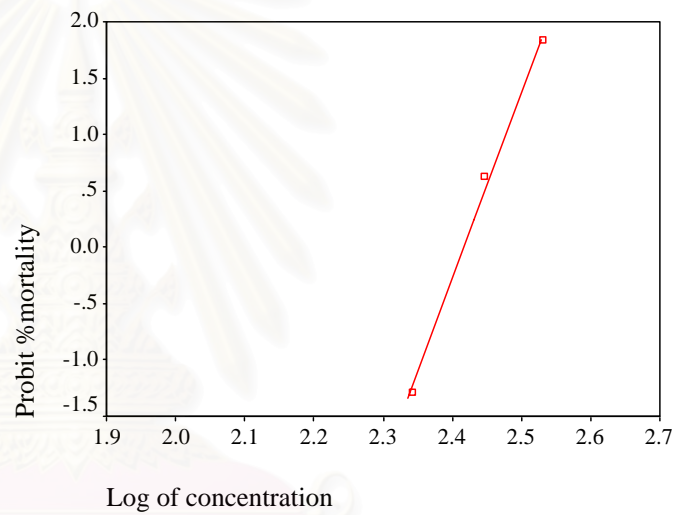
C. porrectum

Y = 2.65X-5.02

$R^2 = 0.893$
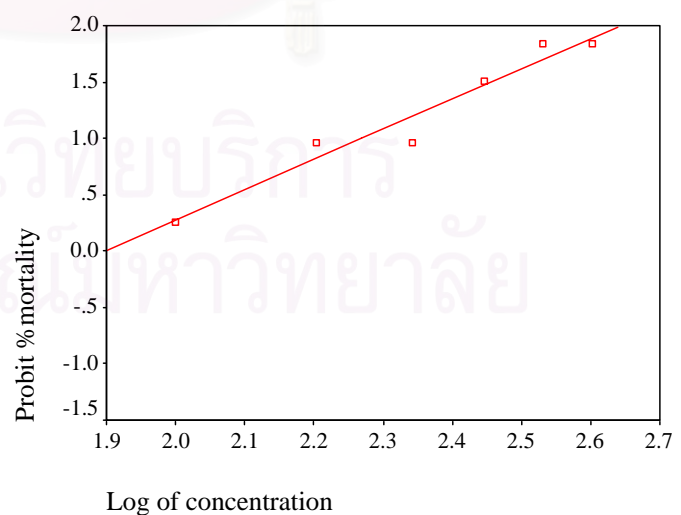
Figure 3.1 Linear regressions of concentrations of three highest potent essential oils

against *S. oryzae* at 24 h exposure.

According to the LC_{50} analysis curves (Figure 3.1), the concentration causing 50% mortality of *S. oryzae* after 24 h exposure and at 95% of confidential limit (P=0.05) of the essential oils from *M. arvensis*, *Z. cassumunar* and *C. porrectum* were 138, 260 and 79 μL/L air, respectively. Their regression equations of mortality were Y=8.89X-19.03, Y=17.10X-41.30 and Y=2.65X-5.02, respectively. The higher value of LC_{50} showed the lower efficiency of that compound. This result indicated that the essential oil from *C. porrectum* exhibited the highest activity against *S. oryzae* while that from *Z. cassumunar* displayed the lowest one.

The fumigant activity against *S. oryzae* of essential oil from *M. arvensis* has been reported by Lee *et al.* (2001a) and Singh *et al.* (1995), while essential oils from *C. porrectum* and *Z. cassumunar* had not been previously reported. Their studies reported that essential oil from *M. arvensis* gave high potent in fumigation against *S. oryzae* at 24 h exposure time. Its LC_{50} values were varied from 45.5 μL/L air (Lee *et al.*, 2001a) to 229.8 μL/L air (Singh *et al.*, 1995). The LC_{50} of essential oil from *M. arvensis* in our study were higher than that in the report of Lee *et al.* (2001a) but comparable to LC_{50} from the study of Singh *et al.* (1995) The reasons may be from different strain of *S. oryzae* or different in major constituents in *M. arvensis* essential oil. This discrepancy was comparable to that reported by Lee *et al.* (2000) that *Oryzaephilus surinamensis* L., a chlorpyrifos-methyl-resistant strain, was cross-resistant to essential oil from *Eucalyptus globules* Labill and its primary monoterpenes, 1,8-cineole. Compared to our study, the essential oils from *E. globules* and *Rosemary officinalis* which were rich in 1,8-cineole gave high value of LC_{50} (non effective). Another reason may derive from the difference in major constituents of essential oil. Thus, it is essential to examine fumigant activity of major constituents against *S. oryzae*.

3.4 Essential oil analysis

Essential oil was generally recognized to consist of a mixture of various compounds, particularly, terpenes (Ikan, 1969). Thus, it is important to analyze the active principle responsible for the interest biological activity. In this research, three effective essential oils are selected to examine for active ingredients effecting on *S. oryzae*. The GC and ^{1}H-NMR techniques were exploited.

3.4.1 The essential oil from *M. arvensis*

The gas chromatogram (Figure 3.2) of the essential oil from *M. arvensis* revealed the presence of two major components at retention times of 20.54 and 19.57 min, respectively. The comparison of the retention times of each component with those of standards revealed that these two major compounds were menthol and menthone, respectively. % Composition is displayed in Table 3.5.

Table 3.5 The compositions of essential oil from *M. arvensis*

Component	Retention time (min)	% composition
Menthol	20.54	76.64
Menthone	19.57	8.34
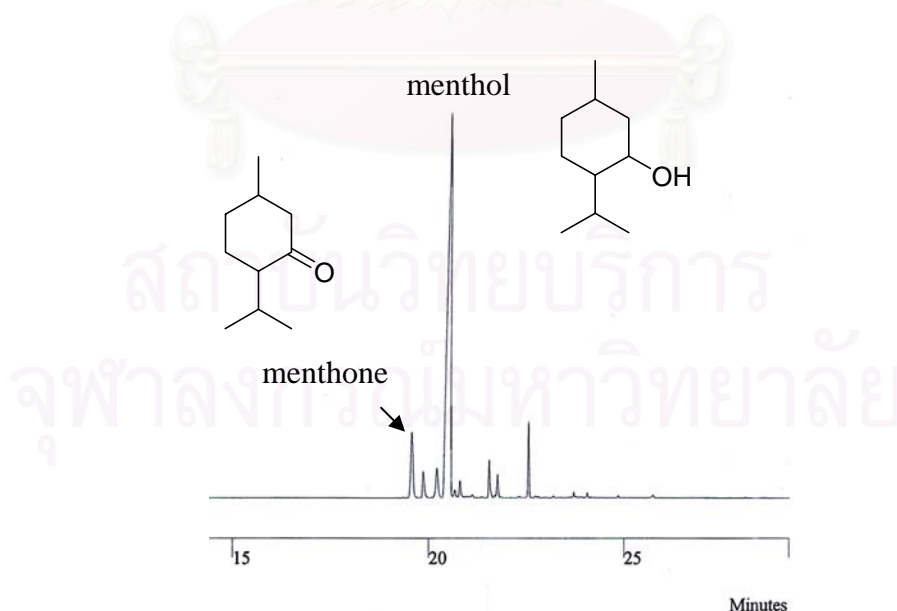
Figure 3.2 The gas chromatogram of the essential oil from *M. arvensis*

3.4.2 The essential oil from *Z. cassumunar*

The gas chromatogram (Figure 3.3) of the essential oil from *Z. cassumunar* revealed two major components at the retention times of 8.85 and 20.52 min. After comparing with authentic samples, the former was identified as sabinene, while the latter was terpinen-4-ol. The % composition is displayed in Table 3.6.
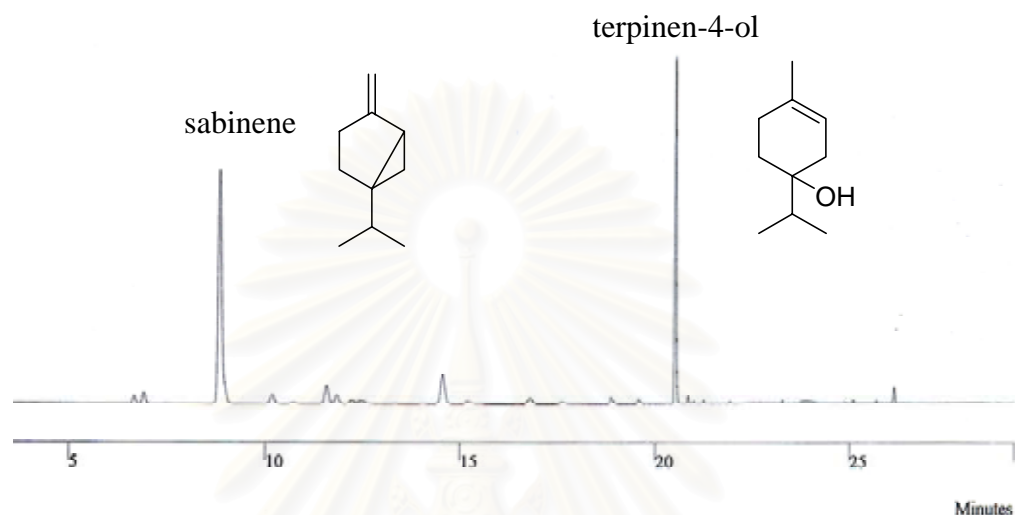
Figure 3.3 The gas chromatogram of the essential oil from *Z. cassumunar*

Table 3.6 The composition of the essential oil from *Z. cassumunar*

Component	Retention time (min)	% composition
Sabinene	8.85	47.36
Terpinen-4-ol	20.52	31.69

The essential oil from *Z. cassumunar* (450 g) was further fractionally distilled. Three fractions: 178 g, 86 g and 145 g with the boiling range of 160-170°, 170-200° and > 200°C were collected. The first fraction contained mainly sabinene according to the comparison with the standard compound. This fraction was carefully redistilled to collect the distillate with boiling range of 163-165°C. After analyzed by GC, more than 95% sabinene was obtained. The identity of this compound was confirmed by ^{1}H-NMR as shown in Figure 3.4 and Table 3.7.
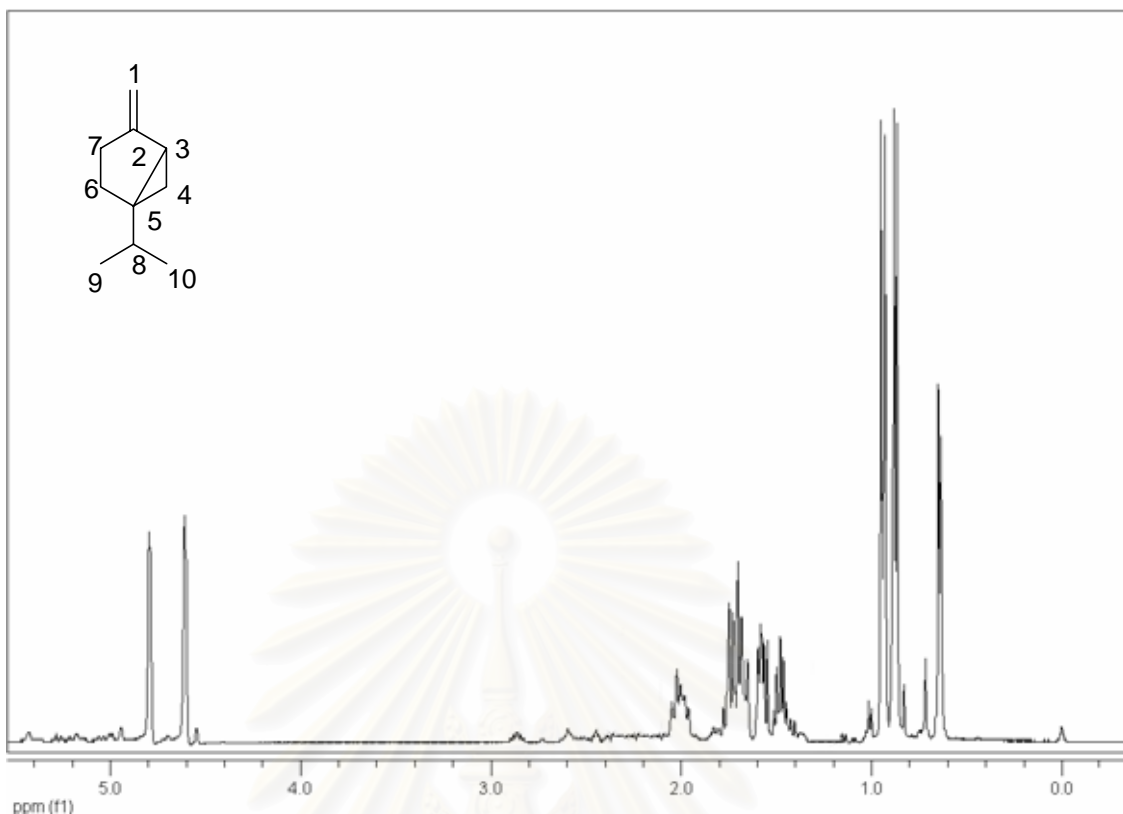
Figure 3.4 ^{1}H-NMR spectrum of compound **1** (sabinene)

Table 3.7 The ^{1}H-NMR chemical shift assignments of sabinene.

Position	Chemical shift (ppm)	
	Compound 1	Sabinene[a]
1	4.79 (1H, s)	4.73, 4.54 (2H, C=CH$_2$)
	4.61 (1H, s)	
3	1.59-1.54 (1H, m)	-
4	0.64 (2H, d, *J*=5.5 Hz)	0.66, 0.60 (2H, C-6 H's)
6	1.78-1.65 (2H, m)	-
7	2.05-1.96 (1H, m)	-
8	1.51-1.43 (1H, m)	-
9	0.94 (3H, d, *J*=6.9 Hz)	0.90 (3H, d, *J*=6.0 Hz, CH-CH$_3$)
10	0.87 (3H, d, *J*=6.9 Hz)	0.82 (3H, d, *J*=7.0 Hz, CH-CH$_3$)

[a] Reference from Fanta and Erman (1968).

The second fraction containing a mixture of compounds was analyzed by GC. This fraction was therefore not examined further for the constituents in details. The last fraction (50 g) was subjected to silica gel column chromatography using 1 : 1.5 of a mixture of CH_2Cl_2-hexane as an eluent to obtain the pure compound exhibiting on TLC at R_f 0.40 (50% hexane: CH_2Cl_2). The confirmation of this compound was performed by GC as terpinen-4-ol. The ^{1}H-NMR spectrum is displayed in Figure 3.5 and Table 3.8.
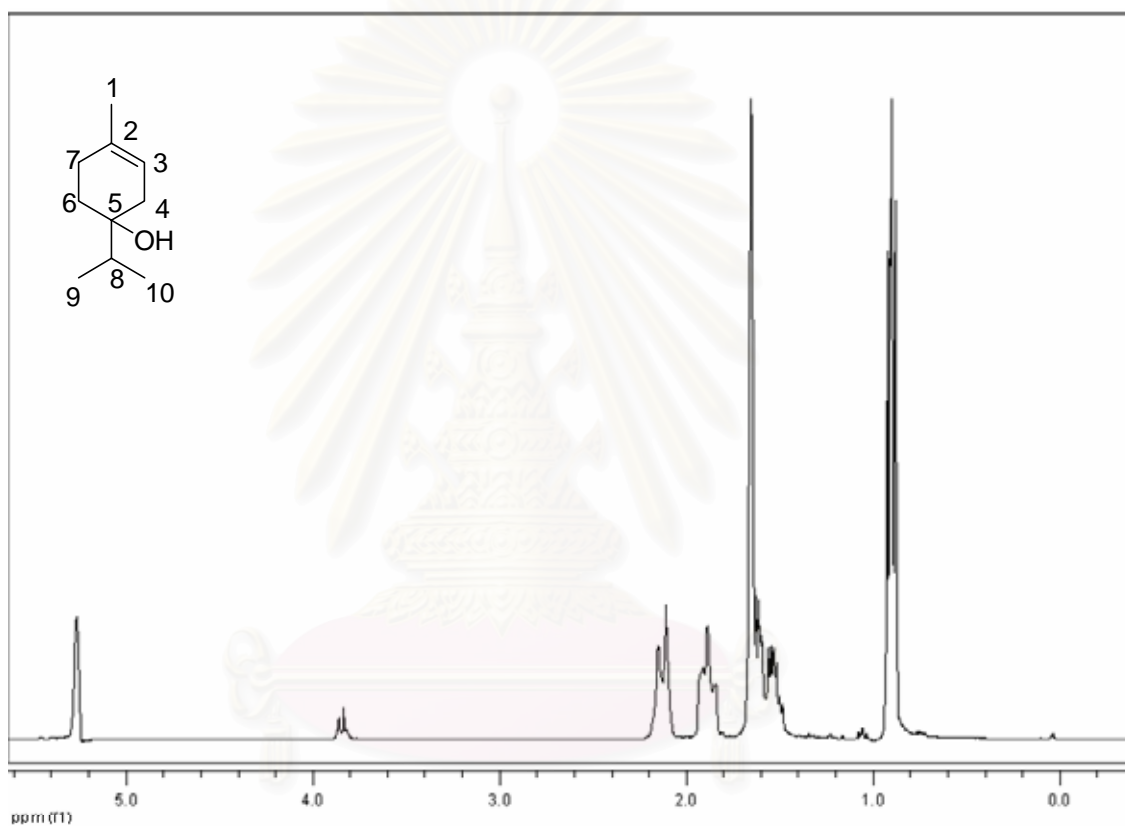
Figure 3.5 ^{1}H-NMR spectrum of compound **2** (terpinen-4-ol)

Table 3.8 The ^{1}H-NMR chemical shift assignments of terpinen-4-ol.

Position	Chemical shift (ppm)	
	Compound 2	**Terpinen-4-ol [a]**
1	1.65 (3H, s)	1.70 (3H, s)
3	5.27 (1H, s)	5.31 (1H, dd, J=2.3, 0.7 Hz,)
4	2.13 (2H, d, J=15.8 Hz)	-
6	1.56-1.49 (2H, m)	-
7	1.92-1.85 (2H, m)	-
8	1.63-1.60 (1H, m)	-
9, 10	0.90 (6H, dd, J=7.0, 10.0 Hz)	0.96 (3H, d, J=6.9 Hz)
		0.93 (3H, d, J=6.9 Hz)

[a] Reference from Ngo and Brown (1998)

3.4.3 The essential oil from *C. porrectum*

The gas chromatogram of the essential oil from *C. porrectum* revealed that a major component presented at the retention time of 22.40 min with 65.70% composition (Figure 3.6). The major peak was compared with authentic sample and found that it gave the same retention time as safrole. This essential oil was further purified by silica gel column chromatograph eluting with 1 : 1 of CH_2Cl_2 : hexane. The main compound, safrole was confirmed its identity by ^{1}H-NMR (Figure 3.7 and Table 3.9).
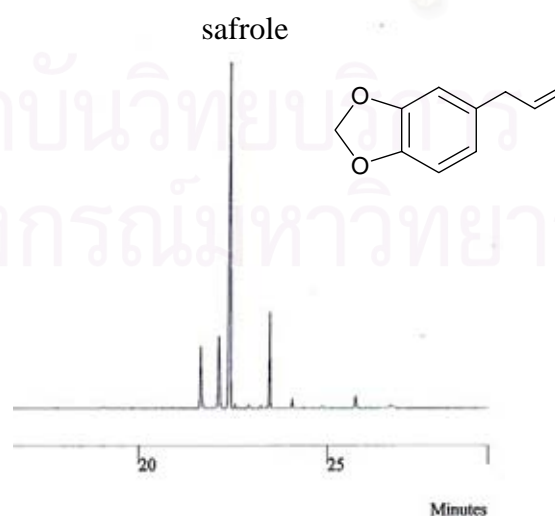
Figure 3.6 The gas chromatogram of the essential oil from *C. porrectum*
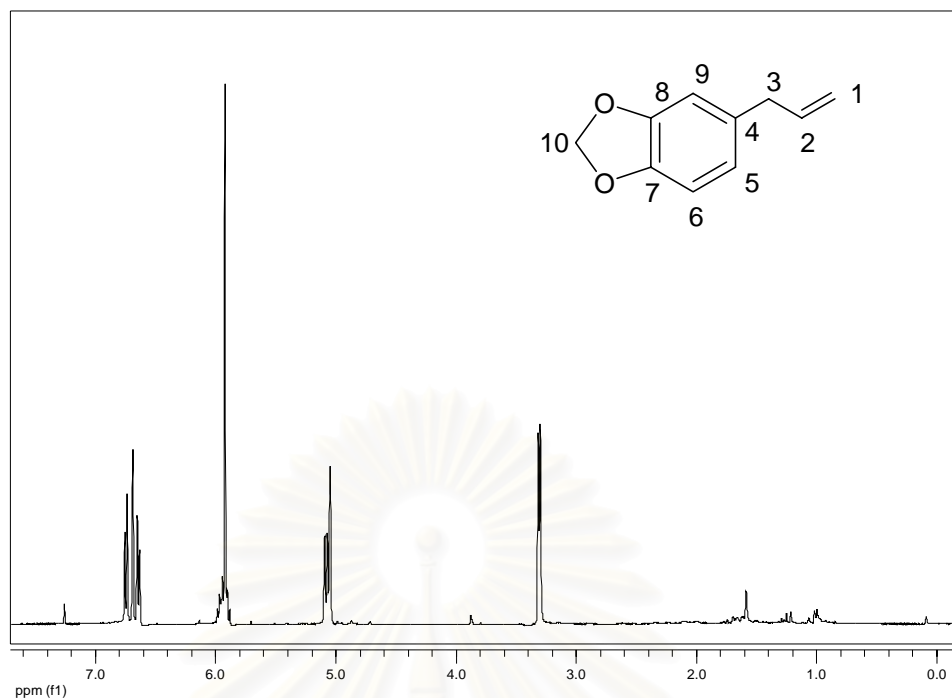
Figure 3.7 ^{1}H-NMR spectrum of safrole.

Table 3.9 The ^{1}H-NMR chemical shift assignments of safrole.

Position	Chemical shift (ppm)	
	safrole	safrole [a]
1	5.10-5.05 (2H, m)	5.10 (2H, m)
2	5.99-5.89 (1H, m)	5.96 (1H, m)
3	3.31 (2H, d, J=6.6 Hz)	3.34 (2H, d, J=6.7 Hz)
5	6.50 (1H, d, J=7.8 Hz)	6.67 (1H, dd, J=7.9, 1.7 Hz)
6	6.75 (1H, d, J=7.9 Hz)	6.78 (1H, d, J=7.9 Hz)
9	6.69 (1H, s)	6.72 (1H, d, J=1.4 Hz)
10	5.92 (2H, s)	5.95 (2H, s, O-CH$_2$-O)

[a] Reference from Mohottalage *et al.* (2007)

3.5 Fumigation test of constituents

Five main constituents found in the three effective essential oils, including ten commercial monoterpenoids consisted of each essential oil (Pappas, 2006; Bruneton, 1995), were tested against *S. oryzae* in fumigation bioassay described above in Chapter II. The concentration of 100 µL/L air was selected for this test. The synthetic

fumigant -- dichlorvos, was the standard chemical. The results are shown in Table 3.10 and Figure 3.8.

Table 3.10 The fumigant activity of essential oils against *Sitophilus oryzae* adults at the concentration of constituents at 100 μL/L air after 24 h treatment.*****

Compound	Mean ± SE**, *n* = 3	Toxicity Class***
1. menthol	60.0 ± 12.6 [c]	Medium-high
2. menthone	53.3 ± 11.7 [c]	
3. limonene	0.0 ± 0.0 [a]	Low
4. 1,8-cineole	0.0 ± 0.0 [a]	
5. carvone	88.3 ± 3.3 [d]	High
6. β-pinene	0.0 ± 0.0 [a]	Low
7. α-pinene	0.0 ± 0.0 [a]	
8. linalool	31.7 ± 7.3 [b]	Medium
9. sabinene	0.0 ± 0.0 [a]	Low
10. terpinen-4-ol	28.3 ± 4.4 [b]	Medium
11. α-terpinene	0.0 ± 0.0 [a]	Low
12. γ-terpinene	0.0 ± 0.0 [a]	
13. *p*-cymene	0.0 ± 0.0 [a]	
14. terpinolene	0.0 ± 0.0 [a]	
15. safrole	88.9 ± 1.1 [d]	High
16. dichlorvos	100.0 ± 0.0 [e]	

* Each datum represents the means of three replicates, each set up with 10 adults.

** Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Duncan's test). Mortalities were transformed to arcsine square-root before ANOVA. Means (±SE) of untransformed data are reported.

*** The low toxicity class is < 25 % mortality; the medium toxicity class is 25-50 % mortality; the medium-high toxicity class is 51-74 % mortality and the high toxicity class is ≥ 75 % mortality.
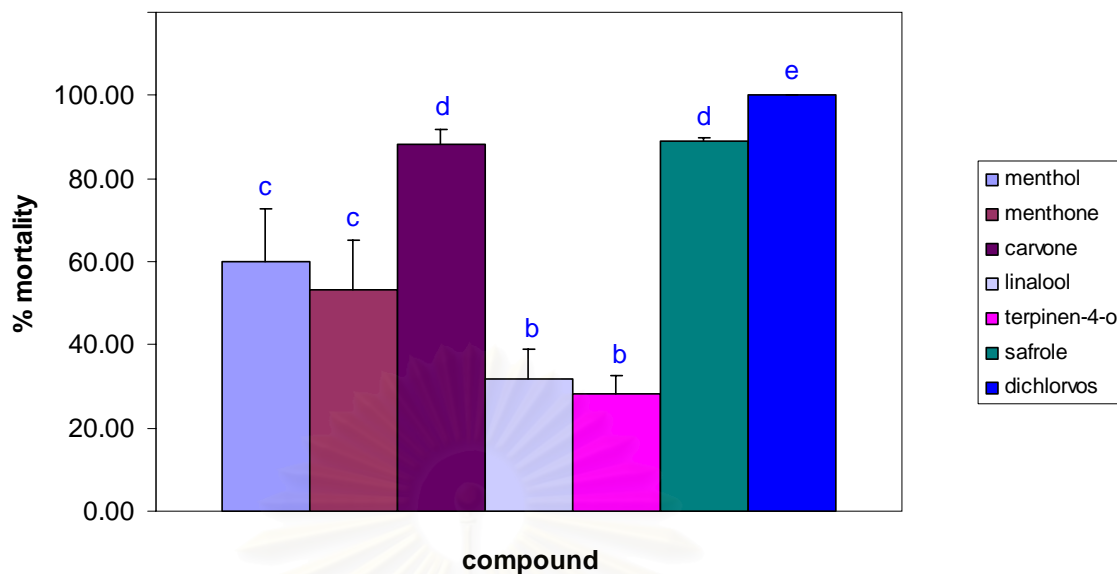
Figure 3.8 The mortality percentages of adult *S. oryzae* at the concentration of the
effective constituents at 100 µL/L air after 24 h treatment.

From Table 3.10, the compounds number 1-8 were found in essential oil from
M. arvensis (Bruneton, 1995) and compounds number 5, 7, 8 and 9-14 were found in
essential oil from *Z. cassumunar* (Pappas, 2006). The structures of these compounds
are shown in Figure 3.9.
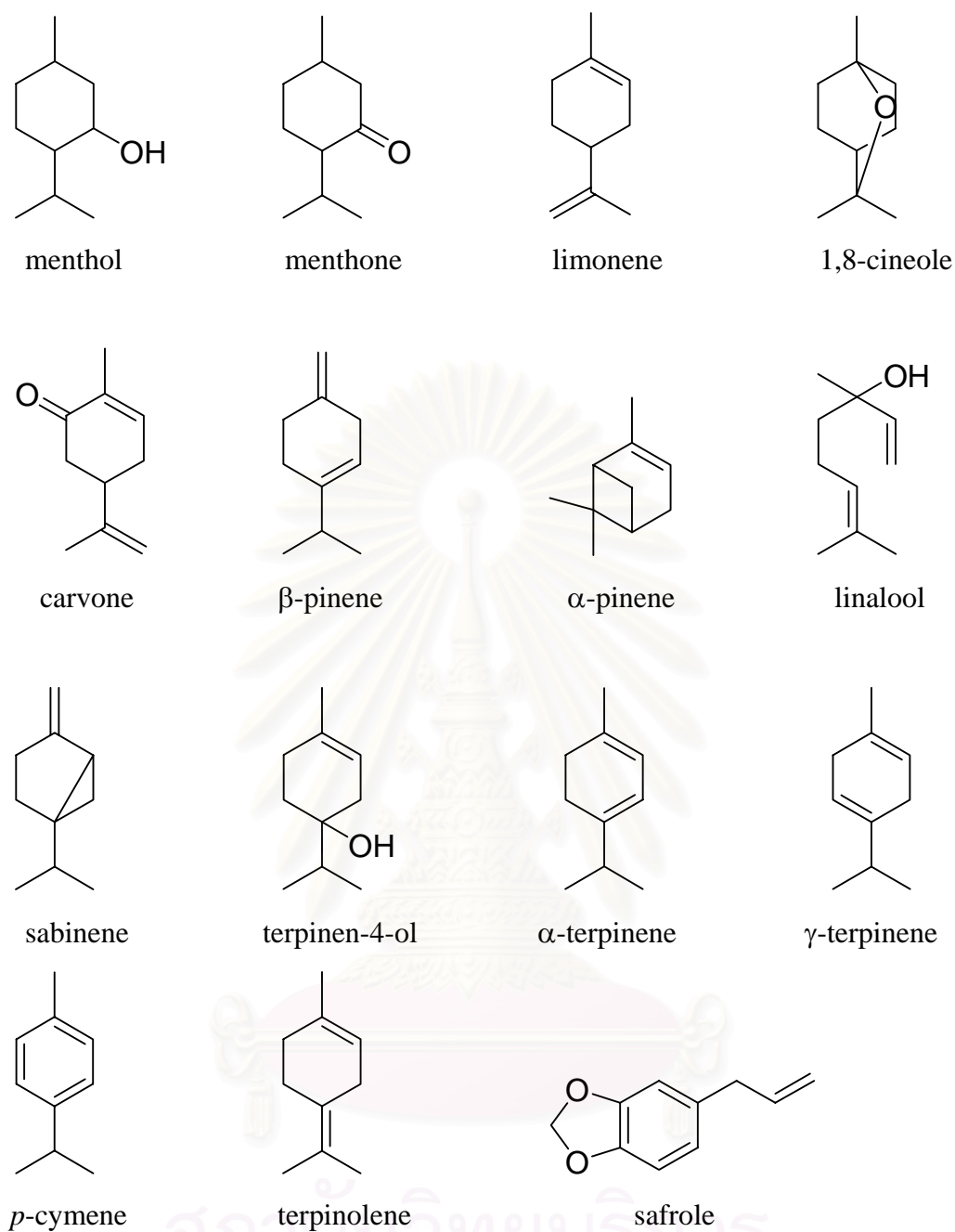
Figure 3.9 Structures of compounds used in this fumigation test

The results from Table 3.10 and Figure 3.8 showed the concentration of 100 μL/L air of safrole gave the highest mortality of *S. oryzae* among natural chemicals. In the other hand, safrole was not significantly different to carvone and showed activity against *S. oryzae* less than the synthetic fumigant, dichlorvos. Three compounds, safrole, carvone and dichlorvos, showed high toxicity, while limonene, 1,8-cineole, β-pinene, α-pinene, sabinene, α-terpinene, γ-terpinene, *p*-cymene and

terpinolene appeared non toxic to *S. oryzae* in fumigation. These results were comparable to the report of Papachristos *et al.* (2004) that oxygenated monoterpenoids exhibited higher activity than hydrocarbons. Six active compounds in this study (menthol, menthone, carvone, linalool, terpinen-4-ol and safrole) showed oxygen atom in their structures, especially safrole and dichlorvos which have two and four atom of oxygen, respectively and caused the higher mortality. It was possible that much more number of oxygen atoms in the compound structure might cause higher mortality of insect, so it was interested to further study about this point.

Moreover, there are four constituents (menthol, menthone, carvone and linalool) of essential oil from *M. arvensis* effecting toward *S. oryzae*, while only one constituent (terpinen-4-ol) of essential oil from *Z. cassumunar* effected to this insect. Thus, those resulted in the essential oil from *M. arvensis* had higher effective than *Z. cassumunar* oil. The reason might be from the synergistic phenomena of oxygenated monoterpenoids containing in the essential oil (Papachristos *et al.*, 2004)
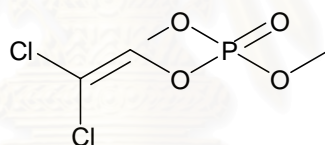
dichlorvos

Subsequently, the main constituents of each essential oil were evaluated LC_{50} against *S. oryzae* and compared the effect of first major component and second major component toward *S. oryzae*. The result is shown in Table 3.11.

Table 3.11 LC_{50} values of main components in three essential oils against *S. oryzae*.

compound	Concentration (μL/L air)						LC_{50}
	50	**100**	**160**	**220**	**280**	**700**	**(μL/L air)**
M. arvensis							
1. menthol	0.0 ± 0.0	60.0 ± 12.6	86.7 ± 6.7	93.3 ± 6.7	100.0 ± 0.0	100.0 ± 0.0	95.4
2. menthone	3.33 ± 3.3	53.3 ± 11.7	60.0 ± 5.8	93.3 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	104.8
Z. cassumunar							
1. sabinene	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	>700
2. terpinen-4-ol	0.0 ± 0.0	30.0 ± 11.5	53.3 ± 8.8	53.3 ± 6.7	83.3 ± 3.3	100.0 ± 0.0	165.5
C. porrectum							
safrole	46.7 ± 3.3	83.3 ± 3.3	86.7 ± 6.7	96.7 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	57.9

Safrole showed the highest effective against *S. oryzae* with LC_{50} of 57.9 μL/L air. Menthol, the first major component of *M. arvensis* essential oil, appeared non significant higher toxicity than menthone with LC_{50} of 95.4 and 104.8 μL/L air, respectively, while terpinen-4-ol, the second major constituent of *Z. cassumunar* oil caused significant higher mortality than sabinene (Duncan's test, $P = 0.05$). This result is in contrary with Lee *et al.* (2001a). Our result indicated that the major constituent was not necessarily to be the active ingredient of the essential oil.

3.6 Residue analysis

The levels of menthol and safrole residues in fumigated rice and control groups are shown in Figure 3.10. The residues of both fumigants were completely removed within 24 h aeration. In comparison with menthol, safrole left the lowest residue on the fumigated rice at pre-aeration. It was possible that safrole had lower absorption on surface rice. The results are consistent with those of wheat fumigated with 1,8-cineole (Lee *et al.*, 2001a). European Commission 2002 (2002) reported the acute oral LD_{50} of safrole for rats and mice to be 1950 and 2350 mg/kg of body weight, respectively. These indicated that safrole have low toxicity to mammals according to United States Environmental Protection Agency (2007). Additionally, maximum levels for safrole in foodstuffs and beverages can be added 1 mg/kg and the estimated average intake of safrole (for consumers) was assumed to be 1 mg/person/day (European Commission 2002, 2002). Thus, our study revealed that using safrole as fumigant for controlling *S. oryzae* should be safe to consumers because of no residues of safrole on rice after aeration for 24 h. In summary, safrole showed potential as the best alternative fumigant toward *S. oryzae* because it had more potential than other monoterpenes and in combination with low residue on the rice commodity.
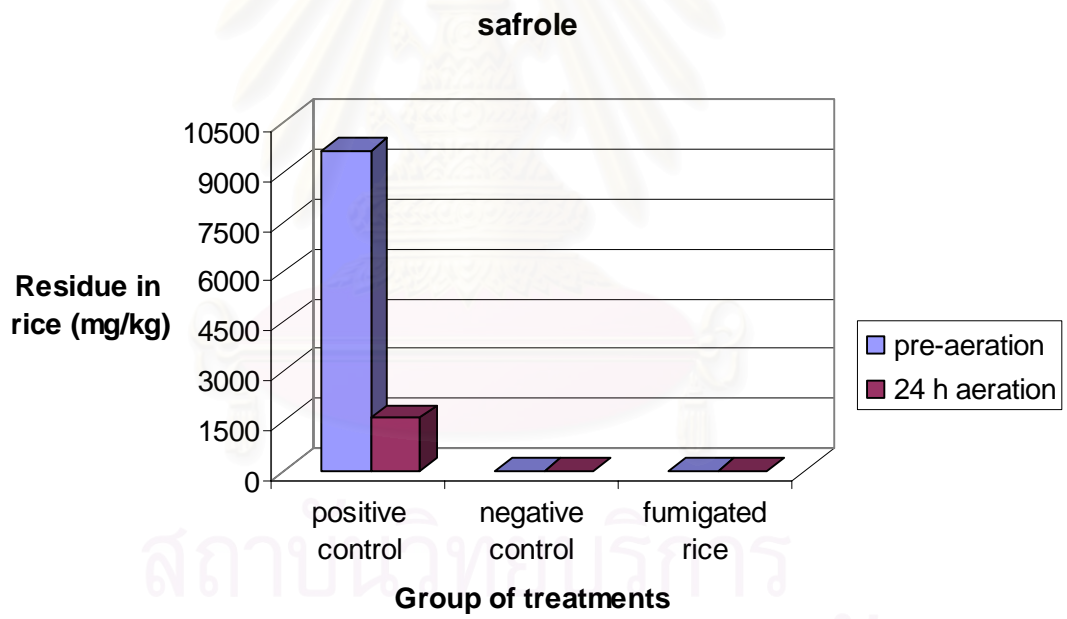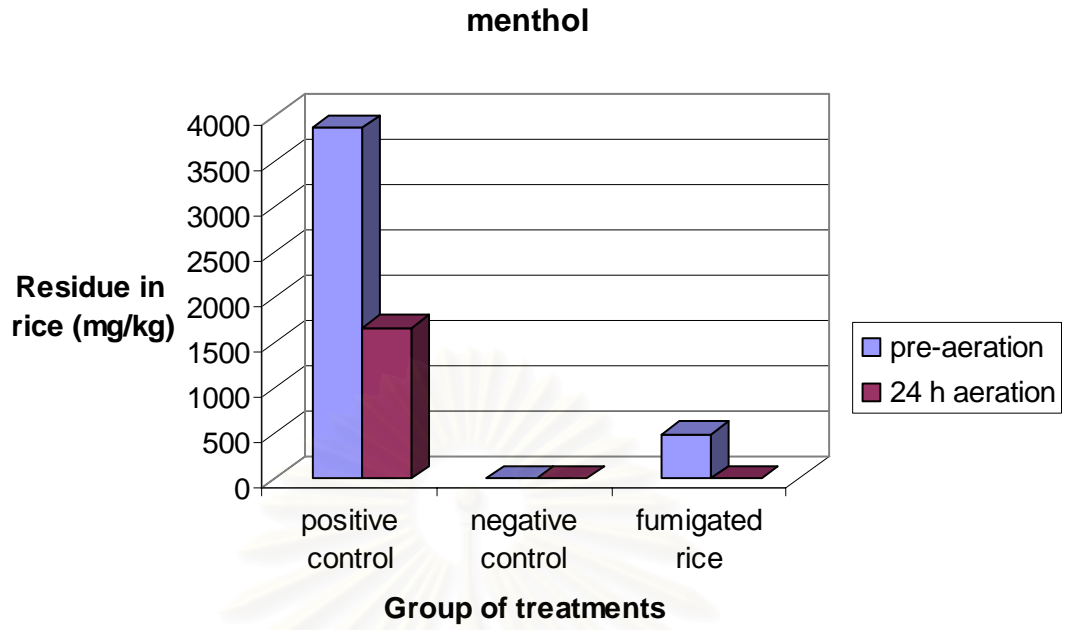
Figure 3.10 Residues of menthol and safrole (mg/kg) on fumigated rice comparison between groups of pre-aeration and 24 h aeration.

CHAPTER IV

CONCLUSION

During the course of this research, the fumigant activity of twenty-five essential oils from Thai plants was screened against a stored-product insect pest, *Sitophilus oryzae* Linn (Rice weevil). As the results, three essential oils from *Cinnamomum porrectum* (Roxb) Kosterm (Thep ta-ro) exhibited highest fumigant toxicity against adults of *S. oryzae* after 24 h exposure, followed by those from *Mentha arvensis* Linn (Japanese mint) and *Zingiber cassumunar* Roxb (plai), respectively. Their LC_{50} values ranged from 79 to 260 µL/L air.

Moreover, chemical constituents and fumigant activity of each active essential oil were examined, as well as, the active components in each active essential oil were identified, fractionalized and purified. The essential oil from *M. arvensis* was found to be rich in menthol and followed by menthone, while essential oil from *Z. cassumunar* was rich in sabinene and terpinen-4-ol, and safrole appeared to be the largest component of essential oil from *C. porrectum*. The results of fumigation bioassay of those components revealed that safrole exhibited the most potent fumigant against *S. oryzae* at concentration of 100 µL/L air after 24 h exposure.

The main component and its fumigant activity relationship were compared for mint and plai. The results revealed that menthol, the first major constituent, is active component of essential oil from *M. arvensis*, while terpinen-4-ol, the second major constituent of essential oil from *Z. cassumunar*, is its active component. Therefore, some major constituent was not necessarily to be the active ingredient.

In addition, the structures of compounds also affected to fumigant activity. Other essential oil's compound test indicated that the most of oxygenated monoterpenoids were more effective on *S. oryzae* than hydrocarbons. These effective oxygenated compounds were menthol, menthone, carvone, linalool, terpinen-4-ol and safrole. They tended to be much more effective depended upon the number of oxygen atom in their structures.

Residue analysis of two monoterpenes (menthol and safrole) revealed that the fumigated polished rice, which was not aerated, cannot be detected any residue of

safrole. However, both of them did not leave residues on the fumigated polished rice after 24 h aeration.

In summary, safrole and its essential oil from *C. porrectum* showed promise as the best alternative safety fumigants toward *S. oryzae* because it had more potential than other monoterpenes and together with lower residue on the rice commodity.

Proposal for future work

This research provided the screening of the fumigant activity of crude essential oils. Further studies should involve the elucidation of all active constituents from active crude essential oils, modify structure and thoroughly study on structure activity relationship. Moreover, mode of action of essential oil on *S. oryzae* has not been illuminated. In addition the essential oils are not as broad spectrum as synthetic fumigants, but their efficacy can be improved by using them in conjunction with carefully designed packaging and developing new application methodology such as control release or encapsulation or synthesizing their active components as safely commercial fumigants.

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APPENDIX
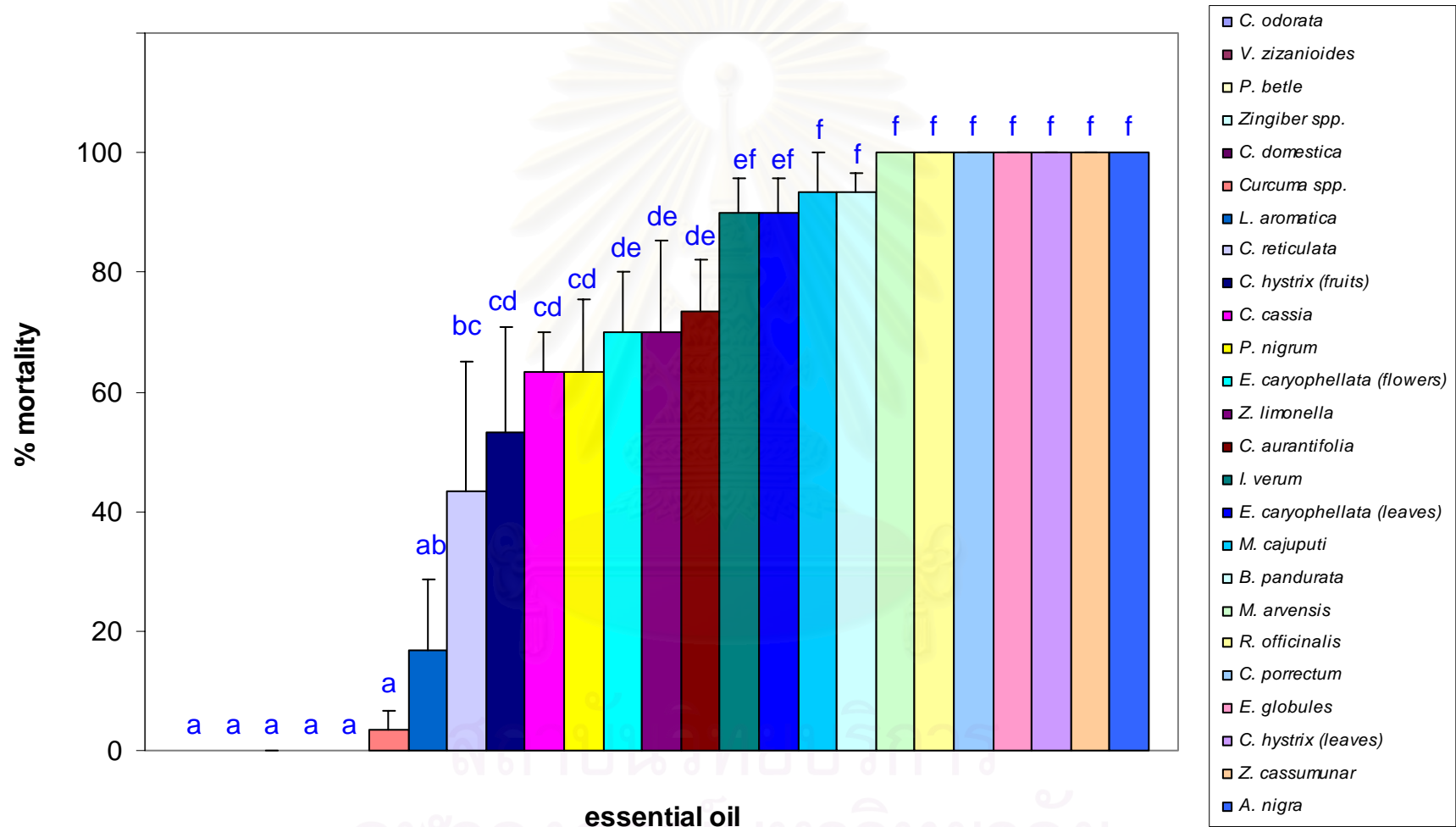
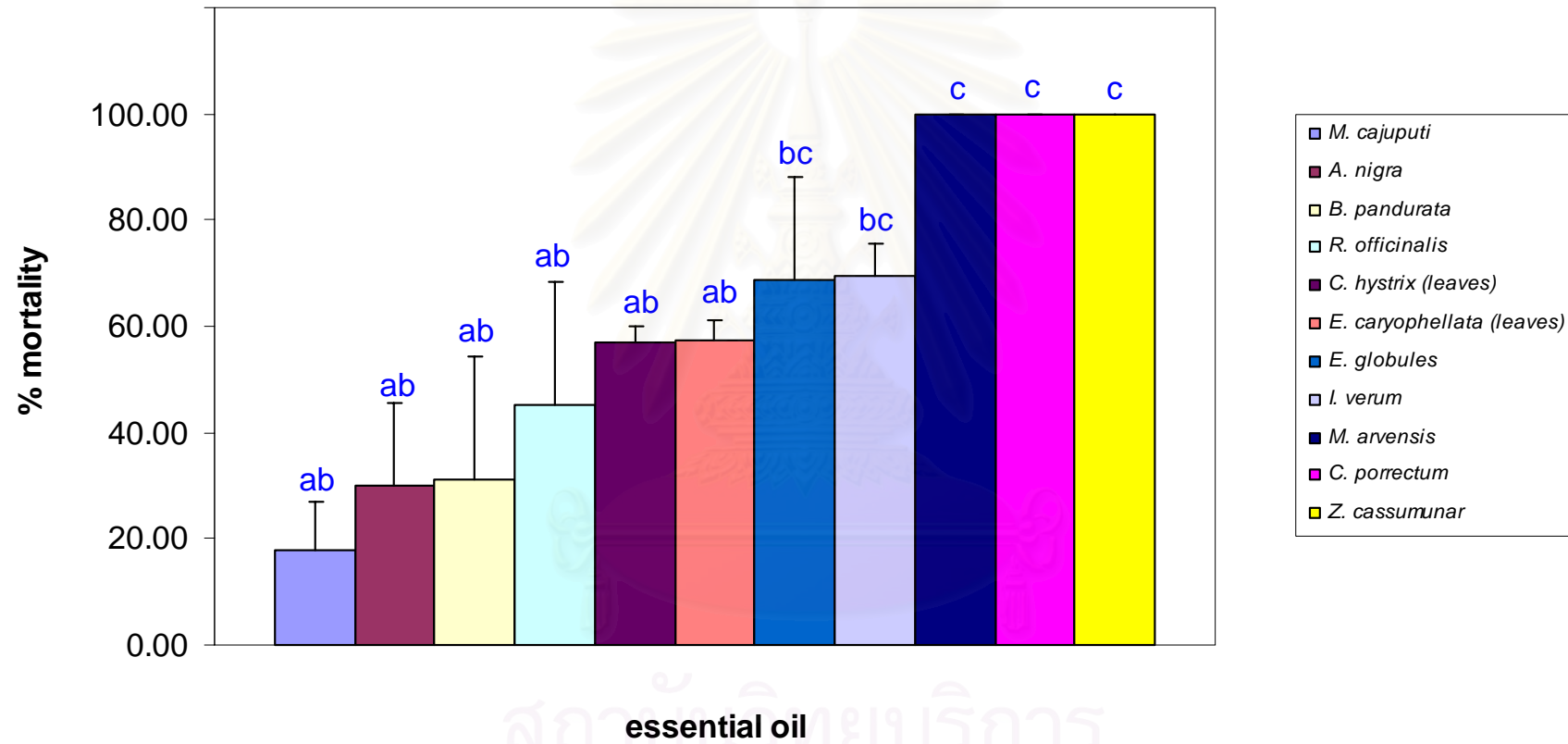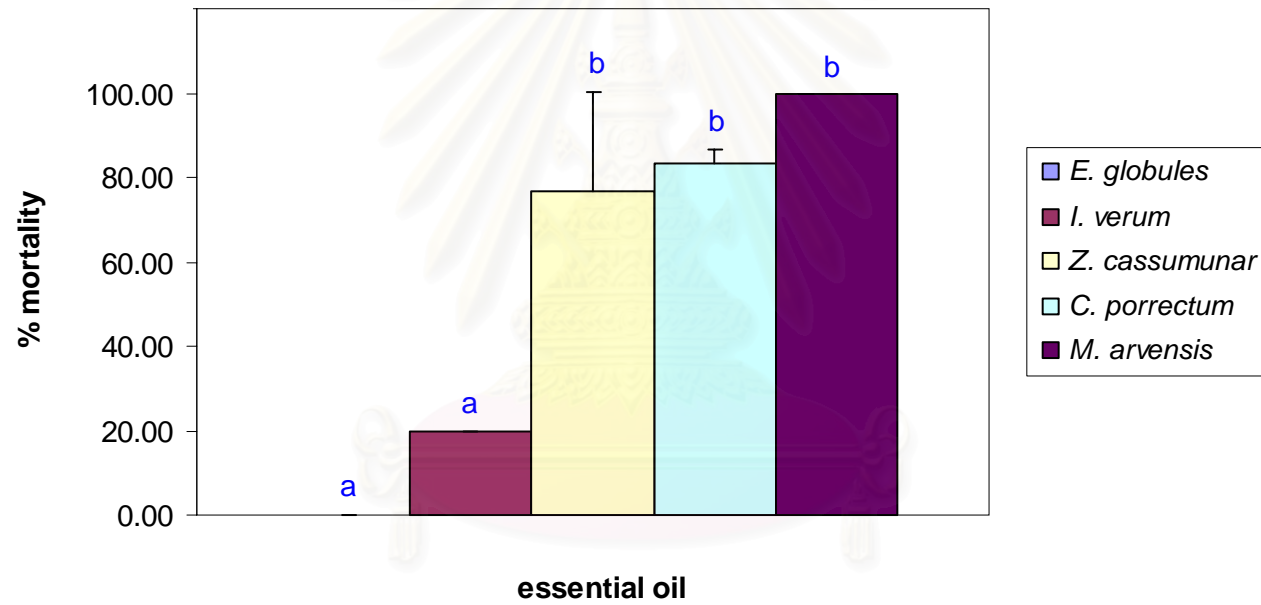**Figure A1** The fumigant activity of essential oils against *Sitophilus oryzae* adults at concentration of 1000 µL/L air after 24 h treatment.

Figure A2 The fumigant activity of essential oils against *Sitophilus oryzae* adults at concentration of 400 μL/L air after 24 h treatment.

Figure A3 The fumigant activity of essential oils against *Sitophilus oryzae* adults at concentration of 200 µL/L air after treatment for 24 h.

Table A1 The LC$_{50}$ of three effective essential oils against *S. oryzae*.

| plant | Mean of %Mortality ($\pm$SE), *n*=3 | | | | | | | | | LC$_{50}$[a] |
	50	100	120	140	160	220	280	340	400	(μL/L air)
M. arvensis	0.0 ± 0.0	13.3 ± 6.6	30 ± 5.8	53.33 ± 3.3	60.0 ± 20.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	138
Z. cassumunar	0.0 ± 0.0	0.0 ± 0.0	-	-	0.0 ± 0.0	10.0 ± 5.8	73.3 ± 12.0	96.7 ± 3.3	100.0 ± 0.0	260
C. porrectum	33.3 ± 8.8	60.0 ± 11.5	-	-	83.3 ± 3.3	83.3 ± 8.8	93.3 ± 6.6	96.7 ± 3.3	96.7 ± 3.3	79

[a] Mortality percentage of each concentration were calculated LC$_{50}$ using Probit analysis Program

Table A2 Residues of menthol and safrole (mg/kg) on fumigated rice comparison between groups of pre-aeration and 24 h aeration.

Groups	Residue in rice (mg/kg)			
	menthol		safrole	
	Pre-aeration	24 h aeration	Pre-aeration	24 h aeration
1. Positive control	3873.48	1660.28	9650.76	1592.83
2. Negative control	0.00	0.00	0.00	0.00
3. Fumigated rice	489.33	0.00	0.00	0.00

VITAE

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