CONTROL OF INSECT PESTS OF VEGETABLES AND CUT ORCHID FLOWER BY FUMIGATION WITH PLANT ESSENTIAL OILS

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN AGRICULTURE
FACULTY OF AGRICULTURAL TECHNOLOGY
KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG
2016

KMITL-2016-AG-D-064-006

หัวข้อวิทยานิพนธ์	**การควบคุมแมลงศัตรูผักและกล้วยไม้ตัดดอกโดยการรมด้วยน้ำมันหอมระเหยจากพืช**
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บทคัดย่อ

ผักสดและกล้วยไม้ตัดดอกเป็นผลิตผลการเกษตรส่งออกที่สำคัญของประเทศไทย ซึ่งมักประสบปัญหาการปนเปื้อนแมลงศัตรูพืช อาทิเช่น เพลี้ยไฟ เพลี้ยแป้ง เพลี้ยอ่อน และแมลงหวี่ โดยปกติการกำจัดแมลงศัตรูพืชเหล่านี้มักใช้วิธีการรมหรือการจุ่มด้วยสารเคมีกำจัดศัตรูพืช ซึ่งแม้ว่าจะออกฤทธิ์รวดเร็วแต่ก็ ส่งผลกระทบต่อผู้บริโภคและสิ่งแวดล้อม ผลกระทบของสารเคมีต่อการเปลี่ยนแปลงทางสรีรวิทยาของพืช รวมทั้งปัญหาการต้านทานสารเคมีของแมลงศัตรูพืช ดังนั้นการศึกษาแนวทางป้องกันกำจัดแมลงในผักและกล้วยไม้ตัดดอกเพื่อการส่งออก โดยการใช้น้ำมันหอมระเหยจากพืช รวมทั้งการศึกษาผลต่อการเปลี่ยนแปลงทางสรีรวิทยาของพืชในการเก็บรักษาหลังจากการรมด้วยน้ำมันหอมระเหยจากพืช จึงเป็นทางออกที่จะช่วยแก้ปัญหาแมลงปนเปื้อนที่ปลอดภัย ไม่มีผลกระทบต่อคุณภาพของพืช ผู้บริโภค และ สภาพแวดล้อม

จากการทดสอบน้ำมันหอมระเหยจากพืชสมุนไพรเบื้องต้น 18 ชนิด ในการฆ่าตัวเต็มวัยเพลี้ยไฟ (*Frankliniella schultzei* (Trybom)) เพลี้ยอ่อน (*Aphis gossypii* Glover) และแมลงหวี่ขาว (*Bemisia tabaci* Gennadius) และตัวอ่อนของเพลี้ยแป้ง (*Pseudococcus jackbeardsleyi* Gimpel & Miller) โดยวิธีการรมในเครื่อง knockdown chamber พบว่าน้ำมันหอมระเหยจากอบเชย (*Cinnamomum bejolghota* (Buch.-Ham.) Sweet) กานพลู (*Syzygium aromaticum* (L.) Merr. & L.M. Perry) และตะไคร้บ้าน (*Cymbopogon citratus* (Dc.ex.Nees) Stapf) มีประสิทธิภาพในการฆ่าแมลงทั้ง 4 ชนิด มากที่สุด โดยมีค่า LC_{50} เท่ากับ 1.14-2.27 µl/L air ขณะที่น้ำมันหอมระเหยจากพืชทั้ง 3 ชนิด ที่ความเข้มข้น 3.0 µl/L air มีประสิทธิภาพในการฆ่าตัวเต็มวัยของเพลี้ยไฟ *Thrips palmi* Karny ได้ 100% ซึ่งสูงกว่าเพลี้ยไฟ *F. schultzei* (84.3-96.1%)

จากการศึกษาองค์ประกอบทางเคมีในน้ำมันหอมระเหยจากกานพลู อบเชย และตะไคร้บ้านด้วยเครื่อง GC-MS พบว่าน้ำมันหอมระเหยจากกานพลูและอบเชยมีสารประกอบ eugenol เป็นสารประกอบหลัก 97.10 และ 82.06% ตามลำดับ ส่วนน้ำมันหอมระเหยจากตะไคร้บ้าน มีสารประกอบ citral เป็นสารประกอบหลัก 69.73% (*trans*-citral และ *cis*-citral เท่ากับ 37.93

และ 31.80% ตามลำดับ) โดยสารมาตรฐาน eugenol และ citral มีประสิทธิภาพสูงในการฆ่าตัวเต็มวัยเพลี้ยไฟ (*F. schultzei*) โดยมีค่า LC_{50} เท่ากับ 1.31 และ 2.31 µl/L air ตามลำดับ และมีประสิทธิภาพสูงในการฆ่าตัวเต็มอ่อนของเพลี้ยแป้ง (*P. jackbeardsleyi*) โดยมีค่า LC_{50} เท่ากับ 1.20 และ 2.80 µl/L air ตามลำดับ

การศึกษาความเป็นพิษต่อกล้วยไม้ตัดดอก กระเพรา และมะเขือเปราะ จากการรมด้วยสูตรน้ำมันหอมระเหยจากพืช ในตู้แก้วขนาด 1 m^3 โดยการพิจารณาการค่าดัชนีการเปลี่ยนแปลงสี (ค่า L*, a* และ b*) ร้อยละของการสูญเสียน้ำ ปริมาณ phenolic กิจกรรมของเอ็นไซม์ polyphenoloxidase ปริมาณ anthocyanin (ในกล้วยไม้) ปริมาณความแน่นเนื้อ (ในมะเขือเปราะ) และปริมาณ chlorophyll (ในกระเพราและมะเขือเปราะ) พบว่าการรมด้วยสูตรน้ำมันหอมระเหยจากกานพลูและอบเชย อัตราส่วน 1:3 (Cl1Ci3) และสูตรน้ำมันหอมระเหยจากกานพลูและตะไคร้บ้าน อัตราส่วน 1:3 (Cl1Le3) ที่ความเข้มข้น 2.0 µl/L air เป็นเวลา 2-3 ชั่วโมง โดยมีการหมุนเวียนอากาศภายในตู้รม 15 นาที มีผลต่อการเปลี่ยนแปลงทางสรีรวิทยา ไม่แตกต่างกับกลุ่มควบคุม (ไม่ใช้สารเคมีสังเคราะห์)

การทดสอบประสิทธิภาพการรมด้วยสูตรน้ำมันหอมระเหยจากพืช สูตร Cl1Ci3 และ Cl1Le3 ต่อแมลงศัตรูพืช ที่ความเข้มข้น 2.0 และ 3.0 µl/L air รมเป็นเวลา 2 ชั่วโมง ในเครื่อง knockdown chamber พบว่าทุกสูตรและทุกความเข้มข้นสามารถฆ่าตัวอ่อนของเพลี้ยแป้งได้ 100% และสามารถฆ่าตัวเต็มวัยของเพลี้ยไฟ เพลี้ยอ่อน และแมลงหวี่ขาว เท่ากับ 84.5-97.6%, 82.2-95.7 และ 87.2-100.0% ตามลำดับ

จากการทดสอบภาคสนามถึงประสิทธิภาพการรมด้วยสูตรน้ำมันหอมระเหยจากพืช สูตร Cl1Ci3 และ Cl1Le3 ที่ความเข้มข้น 2.0 และ 3.0 µl/L air ต่อแมลงศัตรูพืช และความเป็นพิษต่อกล้วยไม้ตัดดอกและผัก (กระเพราและมะเขือเปราะ) เปรียบเทียบกับการรมกับ methyl bromide ที่ความเข้มข้น 20 และ 28 g/m^3 (อัตราคำแนะนำในการรมในผักและกล้วยไม้ตัดดอก ตามลำดับ) ใช้เวลาในการรม 2 ชั่วโมง ในตู้รม Department of Agriculture (DOA) chamber พบว่าการรมด้วยสาร methyl bromide ทุกความเข้มข้นมีประสิทธิภาพในการฆ่าแมลงได้ทุกชนิดทดสอบ 100% และสามารถฆ่าเพลี้ยไฟได้สูงกว่าสูตรน้ำมันหอมระเหยทุกสูตรได้อย่างมีนัยทางสถิติ ขณะที่สูตรน้ำมันหอมระเหยจากพืชทุกสูตรที่ความเข้มข้น 3.0 µl/L air สามารถฆ่าเพลี้ยแป้ง เพลี้ยอ่อน และแมลงหวี่ขาวได้ ไม่แตกต่างกับการใช้ methyl bromide โดยทุกการทดลองสามารถฆ่าตัวอ่อนของเพลี้ยแป้ง 100% อีกทั้งยังไม่พบการเปลี่ยนแปลงทางสรีรวิทยาของพืชอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับกลุ่มควบคุมที่ไม่ได้ใช้สารเคมีใดๆ อย่างไรก็ตามพบว่ากล้วยไม้ตัดดอกและมะเขือเปราะที่รมด้วยสาร methyl bromide ส่งผลให้เกิดลักษณะอาการพิษต่อพืช โดยมีอายุหลังการเก็บเกี่ยวเพียง 3 วันเท่านั้น ส่วนกระเพราแสดงอาการไหม้ที่เกิดจากการรมทันทีหลังการรม

คำสำคัญ: เพลี้ยไฟ เพลี้ยแป้ง เพลี้ยอ่อน แมลงหวี่ขาว กล้วยไม้ตัดดอก มะเขือเปราะ กระเพรา กานพลู อบเชย ตะไคร้บ้าน การรม

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Degree	Doctor of Philosophy
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Year	2016
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ABSTRACT

Fresh vegetables and cut orchid flower are important agricultural products from Thailand which are commonly found contaminating with insect pests such as thrips, mealybug, aphid, and whitefly. The insect contamination management is normally involved application of synthetic insecticidal products via fumigation and dipping methods. Although synthetic insecticides are considered highly efficient, the impacts on consumers and environment, changes in plant physiology, and problems of insect resistance are considered crucial. Therefore, the study of guidelines for the application of plant essential oils in insect management and the study of changes in physiology due to essential oil fumigations could be another potential solution which causes no harm on plants, consumers, and the environment.

Preliminary, insecticidal property of 18 medicinal plant essential oils (EOs) were examined against adults of thrips (*Frankliniella schultzei* (Trybom)), aphid (*Aphis gossypii* Glover) and whitefly (*Bemisia tabaci* Gennadius), and larvae of mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller) by fumigation in 25 L fumigation chamber. In general, EOs from cinnamon (*Cinnamomum bejolghota* (Buch.-Ham.) Sweet), clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry) and lemon grass (*Cymbopogon citratus* (Dc.ex.Nees) Stapf) showed the highest effective against the insects with LC_{50} at 1.14-2.27 µl/L air. All the EOs at 3.0 µl/L air commonly resulted in 100% mortality of *Thrips palmi* Karny and 84.3-96.1% of *F. schultzei*.

The examination of chemical components in cinnamon, clove and lemongrass EOs by GC-MS showed that cinnamon and clove EOs contained 97.10 and 82.06% of eugenol, respectively, while lemongrass EO contained 69.73% of citral (*trans*-citral and *cis*-citral at 37.93 and 31.80%, respectively). The results of standard chemicals fumigations showed eugenol and citral standards were highly toxic against adults of thrips (*F. schultzei*) with LC_{50} at 1.31 and 2.31 µl/L air, and larvae of mealybug (*P. jackbeardsleyi*) with LC_{50} at 1.20 and 2.80 µl/L air, respectively.

Physiological toxicity examination of EO formulas on cut orchid flower, holy basil and eggplant in 1 m^3 glass fumigation chamber were evaluated, by considering changes in color change parameters (L*, a* and b* values), percentage of weight loss, polyphenol oxidase (PPO) activity, phenolic content, texture condense (in eggplant), chlorophyll content (in holy basil and eggplant) and anthocyanin content (in orchid flower). The results showed that 2-3 h fumigations with 2.0 µl/L air EO formulas from clove and cinnamon at the ratio of 1:3 (Cl1Ci3) and clove and lemongrass at 1:3 (Cl1Le3) with 15-min air circulation resulted in no significantly different physiological changes on the cut orchid flower and vegetables (holy basil and eggplant) when compared to the control (no synthetic chemical treatment).

The fumigations of Cl1Ci3 and Cl1Le3 at 2.0 and 3.0 µl/L air for 2-3 hr against adults of thrips, aphid and whitefly, and larvae of mealybug in 25 L fumigation chamber were conducted. The results showed that the EO fumigation formulas killed 100% larvae of mealybug and 84.5-97.6%, 82.2-95.7% and 87.2-100.0% adults of thrips, aphid and whitefly, respectively.

In addition, on field fumigations with Cl1Ci3 and Cl1Le3 at 2.0 and 3.0 µl/L air were compared to methyl bromide fumigation at 20 and 28 g/m^3 (recommended concentrations for vegetable and flower fumigations, respectively) on the cut orchid flower and vegetables (holy basil and eggplant) in Department of Agriculture (DOA) fumigation chamber. Methyl bromide fumigations (at all concentrations) resulted in 100% mortalities of all insects. In particular, the mortalities of thrips fumigated by methyl bromide were significantly higher when compared to all EO fumigations. However, no significant differences in mortalities of mealybug, aphid and whitefly fumigated with all EO formulas at 3.0 µl/L were observed when compared to methyl bromide fumigation. All EO fumigations resulted in 100% mortalities of mealybug. Additionally, no significant physiological changes were observed on the flower and vegetables fumigated with EO, when compared to the control. On the other hand, the cut orchid flower and vegetables fumigated with methyl bromide showed destructive physiological changes on day 3 after fumigation. Particularly, burns were observed on holy basil immediately after the fumigations.

Keywords: thrips, mealybug, aphid, whitefly, cut orchid flower, eggplant, holy basil, clove, cinnamon, lemongrass, fumigation

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Asst.Prof.Dr.Ammorn Insung and co-advisor Asst.Prof.Dr.Lampan Khurnpoon for the continuous support of my Ph.D study and related research, for their patience, motivation, and knowledge. Their guidance really helped through my study. (I could not imagine having a better advisor and mentor.)

Besides, I would like to thank my thesis committee: Assoc.Prof.Dr.Warlardej Chantrasorn, Assoc.Prof.Dr.Tanimnun Jaenaksorn and Asst.Prof.Dr.Kanjana Saetiew, for not only their insightful comments and encouragement, but also for all comments and questions which inspired me to widen my research in various perspectives.

I thank my fellow lab-mates and colleagues for all discussions, for the sleepless nights we were working together, and for all the fun and happiness.

Last but not least, I would like to thank my family: my parents and my brother and sister for supporting me spiritually throughout my study.

This research was supported by the National Science and Technology Development Agency (NSTDA) grant P-12-00789.

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CHAPTER 1
INTRODUCTION

1.1 Statement and significance of the problems

Vegetables and flowers are important exported crops of Thailand with the total production of about 5.2 million tons each year. In 2014, the total export values of fresh vegetables from Thailand were 23,421 million baht, and 1,954 million baht for cut flowers (OAE, 2014a; 2014b). However, problems involving insecticide residues and insect contaminations have resulted in extensive losses and have continuously been threatening credibility of Thailand's exported products in many countries. In 2007, 149 notifications regarding insect pest contamination in fresh exported products from Thailand were reported, in which 75.2% involved contaminations in fresh vegetable and fruit, 16.8% in orchid flower, and 10.7% in other products. Moreover, 3,836 contaminations from 4,017 samples of the inbound contamination assessments were detected contaminating with insect pests, particularly thrips, mealybug, whitefly and aphid. Remarkably, the frequently contaminated products were holy basil, eggplant and cut orchid flower (OAR, 2010a, 2010b).

In 2009, Department of Agriculture, Ministry of Agriculture and Cooperation launched rigorous measures involving the management of export agricultural commodities, in response to the declaration of Agreement on Application of Sanitary and Phytosanitary Measures (SPS Agreement) proposed by World Trade Organization (WHO). The measures generally engaged pre-export inspection for insect contamination in fresh agricultural products. The contaminated products are required to accomplished appropriate insecticidal treatments prior to the shipment via different methods such as dipping, spraying and fumigation. Then, the Phytosanitary certification will only be issued for non-contaminated products (Bangkok Online, 2009).

In Thailand and other developing countries, insect pest management in fresh agricultural industry relies largely on different applications of synthetic insecticides. Particularly, methyl bromide and phosphine fumigations are among the most popular postharvest insect management methods (Misumi *et al.*, 2009). The chemical insecticide fumigation is widely accepted for its considerably high performance and time saving advantages. However, the applications of these chemical insecticides have recently been questioned for their impacts on the environment and plants. Methyl bromide has been reported as a cause of ozone-depleting in the atmosphere and

expected to be prohibited worldwide in 2015 (MBTOC, 2010), while phosphine has been frequently reported for insect resistance and damages on products (Daglish, 2004). Limam and Jemaa (2014) mentioned that continuing used of particular chemical pesticides usually caused resistance, and normally resulted in higher volume of use. Pesticide fumigations normally present extensive impacts on susceptible adult or mature insects. However, immature eggs or insects in pupal stages are protected by their feeding and breathing behaviors. Naturally, insect eggs and pupae have lower respiration when comparing to mature insects, so these immature insects are normally less susceptible to pesticide fumigation. Moreover, the sub-lethal dose of pesticide in the immature insect usually results in the development of resistance to the pesticide, even in higher concentration, when they become adults. These resistant adults will later survive insecticidal fumigation treatments, continue breeding, and pass the resistant genes on to other generations (Collins, 2009). In 1945, over 1,000 insect pest species were reported resisting to commercially available pesticides (Miller, 2004). Besides, overuse of pesticides has been considered a controversial issue among scientists and public. It has been estimated that over application of chemical pesticide causes almost $100 billion damage worldwide each year, and a total number of approximately 25 million tons of pesticides are used (Koul *et al.,* 2008). In 2015, over to 147,000 tons of pesticides were imported to Thailand with the total value was over 22,789 million baht (Thai-PAN, 2015). In general, environmental impacts of pesticides are mainly associated to their nonbiodegradable properties, which usually cause residues in soil, water, and crops. Basically, pesticidal activity, specificity, and degradability are considered 3 major indicators of pesticide's efficacy. However, pesticidal activity is usually considered the initial priority among other efficiency indicators. In fact, Pimentel (1995) reported that less than 0.1% of the applied pesticide reached the targeted pest, while 99.9% of pesticide normally residue in the environment. A threatening number of sickness caused by pesticide application were reported worldwide. In 2007-2012 around 17,340 people or 2.35 per 10,000 populations in Thailand were diagnosed as having pesticide related diseases (Siripanich, 2013), while rapid Alert System for Food (RASFF) of the European Union (EU) issued 55 notifications for pesticide contamination in Thailand's products in 2010-2011.

Thus, recent researches and studies have highlighted the development of biodegradable and environmental friendly pesticides to ensure public health security. Techniques that entail minimum pesticide application, while maintaining effective pest control and product quality have been required. Thus, nature oriented methods and

products are considered a prospectively efficient solution. The insect pest control technology today has continuously highlighted the development of "Green management" or more environmental friendly insecticidal, technology which specifically entails the property of natural degradability. Basically, green management involves natural control like applications of natural predators as well as plant derived insecticidal products such as plant extracts and essential oils (EOs). These products are generally high biodegradable and non-residual (Koul *et al.,* 2008). Many medicinal plant EOs have been extensively studied and used in insect pest control. There are many alternative insecticide products on market. However, application for industrial and export purposes is generally limited. Therefore, this study investigated of effective EOs against insect pests of commodities particularly, exported vegetables and cut orchid flower by fumigation method in order to solve the problems of insect contamination and secure environment as well as consumers' health.

1.2 Objectives of the study

1. To investigate effective essential oil fumigants against thrips, aphid, mealybug and whitefly.
2. To investigate the effective essential oil formulas and fumigation methods against the insect pests.
3. To examine the physiological changes in eggplant, holy basil and cut orchid flower due to essential oil fumigations.

1.3 Scope of the study

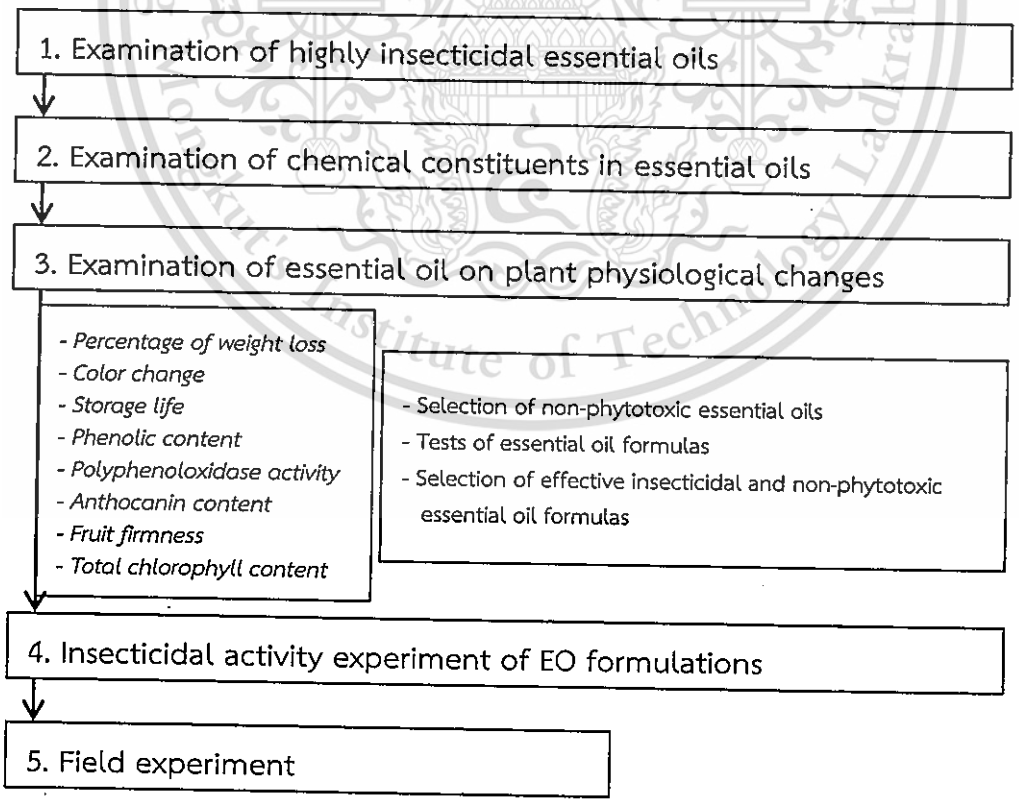
The fumigation assays in this study were divided into 5 main stages including 1) examination of highly insecticidal essential oils (EOs) 2) examination of EO's chemical constituents 3) examination of plant physiological changes due to EOs 4) insecticidal property experiment of EO formulations and 5) field experiment. In the first assay, fumigant toxicity of different EOs were examined, and the EOs that presented high insecticidal property against thrips, aphid, mealybug and whitefly were selected for further experiments. In the second assay, major chemicals in the selected EOs were examined. The obtained results were subsequently applied in the latter assays. In the third assay, different standards of the discovered chemicals were used in order to examine their physiological change or phytotoxic effects on the treated samples. Subsequently, EOs of which the major chemicals demonstrated

considerably low plant physiological changes were further selected for EO formulations. In this experiment, mixtures of different EOs were formulated and examined. In particular, the plant physiological changes examinations due to the mixtures were conducted according to different physiological change parameters in cut orchid flower and vegetables (eggplant and holy basil). Then, the EO formulas with lesss physiological changes in the treated samples were selected. In the fourth assay, fumigations of the selected less plant physiological changes EO formulas were conducted again on the earlier tested insects in order to examine their insecticidal activities. In this assay, EO formulas that presented high insecticidal activity with less physiological changes in the tested flower and vegetables were obtained. Finally, the most effective EO formulas were tested in field experiments.

The experiments in this study were conducted at Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, during the year 2011-2015 study period.

1.4 Process of the study

The experimental stages in this study were as follows;



CHAPTER 2
LITERATURE REVIEW

This chapter presents a review of related literature on essential oils (EOs) and insect pests normally invading orchid flower and vegetable. In addition, alternative methods in insect pest management will also be demonstrated. Essential oils have been studied in pest management for years. Thus, a review on related studies and effects of EOs on plants will also be presented, particularly in changes on characteristics on agricultural products and parameters indicating fresh product quality as stimulated by EO fumigation.

2.1 Insect pests

2.1.1 Thrips

Thrips are rasping-sucking insects with only one left mandible with a narrow stylet used in piercing the cell wall of plant tissues. The insects are relatively small insects with the approximate size between 0.5-5.0 mm long. They are sexual or asexual reproductrs, and females are more commonly found in nature (CUES, 2007a). Generally, thrips are polyphagous insects, which normally feed on a wide range of host plants (Paul, 2007). The insects normally hide in flowers, buds and leaf axils and usually go unnoticed until damages appear (Mahr *et al.*, 2001). In cut flower, thrips normally cause discoloration and deformities which directly affect on marketability of the products. In addition, thrips is a major transmitter of viral diseases such as tomato spotted wilt virus which is considered one of the most damaging viral diseases (CUES, 2007a).

2.1.2 Mealybugs

Mealybugs are small, soft-bodied, piercing-sucking insects. Adult females and nymphs are wingless and covered in white powdery or mealy wax secretion, particularly a series of white, lateral wax filaments on the margin of the body are most posteriorly prominent. The females normally feed on many parts of plants, while the adult males are short-lived, non-feeding and rarely collected. Mealybug invasion can cause considerable economic damages on agricultural and horticultural plants (Miller, *et al.*, 2002; 2005). Directs damages are usually a result of sap removal and toxins caused by the insect injection. Besides, indirect damages are associated

with sooty mold growth caused by honeydew stains on plants (Mibey, 1997). In addition, the effects of plant viruses transmitted by mealybugs are also reported. Feeding damage may cause leaf yellowing, defoliation, growth reduction, and death of plants. Besides, honeydew and sooty mold from the insect normally reduce marketability of plant products (Hoffmann, 2009).

2.1.3 Whiteflies

Whiteflies are small insects with the body length ranged approximately between 1.0-2.0 mm. There are more than 1,550 species (CUES, 2007b). Typically, whiteflies feed underside plant leaves by piercing and sucking into plant phloem. The saliva is toxic to plants, extensive infection can cause yellow, burned dry, or leaves fall off. Like mealybugs, whiteflies excrete honeydew which further causes black sooty mold and reduces marketability of the products. Moreover, whitefly is considered a major carrier of diseases which present serious impacts on global food production. This insect can rapidly gain resistance to chemical pesticides. Therefore, control and effective control measurement is considerably difficult and complex (Buranapanichpan, 2001; CUES, 2007a, 2007b).

2.1.4 Aphids

Aphids are important pest found breaking over economic crop fields in Thailand and many Asian countries. These insects are small. The body length is between 1.2-2.4 mm. Aphids are usually found piercing and sucking phloem on leaves, buds and flowers of plants, as they are both sexual and asexual reproductrs, aphids have high reproduction potential. Moreover, their short life cycles result in reproduction of many generations in a year. Infested plants are usually found having different levels of damage severity, such as stunted or decreased growth rate, molting, curling, yellowing, browning, wilting, low yields and death. In addition, aphid is also one of the most significant transmitters of many viruses which can cause extensive damages on commercial crops (Buranapanichpan, 2001).

2.2 Export agricultural products

2.2.1 Cut orchid flower

Cut orchid flower is one of the most important ornamental plants in the world market, and Thailand is among one of the biggest exporter country of this product which shares (OAE, 2014b), approximately 30% of the world total orchid

consumption (Lekawatana, 2010). The major importer countries of Thailand's cut orchid are Japan, Italy, USA, Taiwan and Germany (Saowalak, 2008). Recently, the Department of Agriculture and Cooperation has classified cut orchid flower as a product champion of the country. (Piluek and Wongpiyasatid, 2010). In 2014, the total export values of Thailand's cut flowers were 1,954 million baht, and *Denrdobium* flower is the most important orchid genera for export with the market value more than 90% of total orchid exports from Thailand (OAE, 2013b). *Dendrobium* flower is a popular red-purple hybrid with high growing rate, high productivity, and long vase life. However, insect contamination on this flower is frequently reported. Insect contamination normally results in product repudiations, and the entire products are rejected and burned, once contamination is detected (Suksomboon, 2004; Keinmeesuke *et al.*, 2008). Therefore, this problem requires immediate and effective management. In general, thrips is among the most frequently detected insects in export cut orchid flower (Piluek and Wongpiyasatid, 2010). Yano and Napompeth (1995) estimated that thrips probably occur at almost all orchid flower nurseries. The percentage of *Dendrobium* flowers attacked by thrips was 74% (84 from 113 flowers surveyed) in the nursery where no insecticide was applied (Yano and Napompeth, 1995), while the nurseries under heavy and regular application of insecticide also showed insects contamination.

2.2.2 Eggplant

Thai light round green is a variety of eggplant (*Solanum melongena* L.) in Solanaceae family commonly found in Southeast Asia. This variety is an open pollinated variety that typically grows, matures and completes the lifecycle over the course of one year. The bush is approximately 1.2 meters in high, and the fruit is usually oval or round in shape (2.5-5.0 cm in diameter and 20-40 g weight) (SAEDC, 2015). Eggplant is considered a major fresh export plant of Thailand to EU market. In 2007, more than 400 tons were export globally. However, more than 20 notifications of insect contamination were also reported and resulted in the product's categorization in a special contamination observation list (OCA, 2013).

2.2.3 Holy basil

Holy basil (*Ocimum sanctum* L.) is an annual crop in Labiatae family with the bush height about 1.2 meters. It has a strong anise-like, slightly musky and lemony taste with a camphoraceous aroma (Wangcharoen and Morasuk, 2007). This

herb also has been used by Asian people in traditional medicine. It is used for most stomach disorders, cramps, diarrhea, headaches, whooping cough and head colds (Uhl, 2000). It is a common commercial plant cultivated throughout the Southeast Asian tropic, particularly Thailand and Malaysia (Staples and Kristiansen, 1999). In addition to domestic consumption, this plant is also a major export flavoring plant in European markets (Pornsiriprathan, 2011). The Royal Project Foundation has been exporting holy basil, as well as other herbs and vegetables to Europe and other overseas markets (Bung-ila *et al.,* 2009).

2.3 Insect pests control

2.3.1 Conventional methods

In Thailand and other developing countries, insect pest management in fresh agricultural industry relies largely on different applications of synthesized insecticides. Particularly, methyl bromide and phosphine fumigations are among the most popular postharvest insect control management methods (Misumi *et al.,* 2009; Chu *et al.,* 2013). Chemical insecticide fumigation is widely accepted for its considerably high performances and time saving advantages. Insectcide fumigation has been playing a major role in insect pest elimination, owing to its suitability for large area application and management. However, resistances of insects against chemical pesticide and control failures in many field situations have been continually reported. Moreover, phytotoxicity or physiological changes in the products as caused by the chemical insecticides was also observed (Daglish, 2004; Athie and Mills, 2005; Pimentel *et al.*, 2008; Liu, 2011; Jamieson *et al.*, 2012). Furthermore, some other chemicals were found causing critical impacts on the environment. Particularly, the application of methyl bromide has become globally controversial for its influence on depletion of ozone layer and contamination of bromide residues (MBTOC, 2010). These problems have resulted in a complete ban of methyl bromide application in many countries since 2004. These limitations of chemical pesticides have, therefore, drawn interests in the application of other alternative methods.

2.3.2 Alternative methods

A current global interest has focused on the discovery of non-chemical strategies and environmental friendly pest control approaches (Isman, 2000; Zettler and Arthur, 2000). The approach include the application of biological methods such as predator or parasite strategies, physical method such as the use of CO_2 treatment

and thermol control, and bioinsecticide methods such as the application of plant extracts. Particularly, many plant extracted which are trationally used for medicinal purposes have been studied and developed for insect pest control purposes. Recently, there have been many reports on the effective use of plant extracts and EOs in insect pest management (Amiri *et al.*, 2013; Ateyyat *et al.*, 2009; Benelli *et al.*, 2012; Choi *et al.*, 2003; Gorski, 2004; Insung *et al.*, 2012; Ioannou *et al.*, 2012; Isik and Gorur, 2009; Jindapon *et al.*, 2010; Koul *et al.*, 2008; Muzemu *et. al.*, 2011; Pumnuan and Insung, 2012; Pumnuan *et al.*, 2012; Sittichok and Soonwera, 2012; Thanasirungkul *et al.*, 2012)

2.4 Plants with insecticidal properties of medicinal plant essential oils

Insect pest control technology today highlights the development of "green" methods, and plant EOs have been extensively studied and used in insect pest management for decades (Ayvaz *et al.*, 2008; Benelli *et al.*, 2012). Generally, plant EOs are recognized as an important natural source of monoterpenoids, which are well-known substances showing repellent, antifeedant and kill properties against insects (Ketoh *et al.*, 2005; Isman, 2006; Kumar *et al.*, 2011). Naturally, plants release a variety of volatiles including various alcohols, terpenes, and aromatic compounds in order to protect those selves from animals and invaders (Khater, 2012). These compounds are mainly active in the vapor phase via respiratory system (Tripathi *et al.*, 2009; Khater *et al.*, 2011), and may play as inhibition of acetylcholinesterase (AChE) (Seo *et al.*, 2014; Abou-Taleb, *et al.*, 2015; Yeom *et al.*, 2015) or adenosine triphosphatases (ATPases) (Abou-Taleb, *et al.*, 2015) as well as interfere with the GABA-gated chloride channels (Priestley *et al*, 2003) or neuromodulator octopamine of insects (Kostyukovsky *et al.*, 2002; Enan, 2005). These compounds are normally pest selective and harmless to non-target organisms (Isman, 2000) and mammals (Barnard and Xue, 2004). Therefore, plant EOs have been studied potential alternative insect control agents for their highly insect control properties, but low mammalian toxicity and persistence in the environment (Isman, 2000; Erler, 2005; Isikber *et al.*, 2006). Many reports presented toxicity of plant extracts or EOs from Apiaceae, Lauraceae, Cupressaceae, Lamiaceae, Rutaceae, Verbenaceae, Poaceae and Solanaceae plants against behaviors and mortality of thrips, mealybug, whitefly and aphid (Koschier and Sedy, 2001; Choi *et al.*, 2003; Gorski, 2004; Govindaiah *et al.*, 2006; Mbonu, 2006; Yi *et al.*, 2006; Ateyyat *et al.*, 2009; Cloyd *et al.*, 2009; Isik and Gorur, 2009; Muzemu *et. al.*, 2011; Fiaz *et al.*, 2012; Amiri *et al.*, 2013).

For example, Myrtaceae and Lauraceae plants were used in controlling stored product insects and stored product mites (Rozman *et al.*, 2007; Insung and Pumnuan, 2009; Pumnuan and Insung, 2012; Pumnuan *et al.*, 2012; Sittichok and Soonwera, 2012; Thanasirungkul *et al.*, 2012). There were also reports on the application of Piperaceae plant against bed bug (*Cimex hemipterus* L.) (Insung *et al.*, 2012), mushroom mite (*Luciaphorus perniciosus* Rack) (Pumnuan and Insung, 2012), common cutworm larvae (*Spodoptera litura* Fab.) and house fly larvae (*Musca domestica* L.) (Jindapon *et al.*, 2010). Zinggiberaceae plant is used in controlling mosquito (*Aedes aegypti* L.) (Kalaivani *et al.*, 2012), house dust mite (*Dermatophagoides pteronyssinus* (Trouessart)) (Insung and Pumnuan, 2009), and stored product mite (*Suidasia pontifica* Oudemans) (Pumnuan and Insung, 2012). Besides, there were reports on the use of Gramineae plant in controlling Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) (Benelli *et al.*, 2012), house fly (*Musca domestica* L.) (Sinthusiri and Soonwera, 2013) and fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) (Labinas and Crocomo, 2002). Rutaceae plant was also reported as having quality in controlling cockroach (*Periplaneta americana* L.) (Sittichok and Soonwera, 2012), bed bug (*C. hemipterus*) (Insung *et al.*, 2012), common cutworm (*Spodoptera littoralis* (Boisd.)) (Barakat, 2011), and Mediterranean fruit fly (*C. capitata*) (Ioannou *et al.*, 2012). Essential oils are applied in insect pest management by various methods such as direct spray, residue contact and fumigation (Choi *et al.*, 2003; Gorski, 2004; Koul *et al.*, 2008; Ateyyat *et al.*, 2009; Isik and Gorur, 2009; Muzemu *et. al.*, 2011; Amiri *et al.*, 2013).

Essential oils are by-products of plant metabolism in form of volatile oil at room temperature with distinctive odor. These oils can easily transform into gaseous state when slightly higher temperatures, even at an inconsiderable difference are obtained. In general, EOs can be found in every part of plant, depending on species. They are found in roots, ryzomes (ginger), wood (camphor), leaf (eucalyptus) and flowering parts (Labiatae family) (Pengelly, 1996). The EO content in plants is generally very low and rarely exceeds 1% (Bowles, 2003), but in some cases, for example in clove and nutmeg, the concentration reach more than 10% (Djilani and Dicho, 2012). Naturally, EO demonstrates various functions. There are cases that EOs help protect plants from extreme condition like heat or frost (Koul, *et al.*, 2008). In fruiting plants, EO plays a major role attracting insects for pollination. In addition, plants product EOs repel insects and invaders. Peppermint was found presenting repellant activity against ants, flies, lice, and moth, while lemon grass, blue gum,

rosemary, vetiver, clove and thyme are also dominant for their pest control properties (Kordali *et al.*, 2005). Moreover, EOs from cinnamon, lemon grass, lavender, tansy, sweet flag, clove, basil, wintergreen, cumin, black caraway, ajwain, sweet fennel, musk mallow, deodar cedar and pepper were also demonstrated various pest control properties (Koul *et al.*, 2008).

In fact, humans have been using EO for centuries as flavorings, perfumes, cosmetics and medicines. In addition, aromatic plants have been commonly used in stored product protection. In the world of advance technology, EOs have been discovered as presenting many pesticidal chemicals (Isman, 2000). Most EOs comprise of monoterpenes compounds. In particular, terpenes (such as myrecene, pinene, terpinene, limonene, *p*cymene, α- and β- phellandrene etc.) and terpenoids (such as acyclic monoterpene alcohols (geraniol, linalool), monocyclic alcohols (menthol, 4-carvomenthenol, terpineol, carveol, borneol,), aliphatic aldehydes (citral, citronellal, perillaldehyde), aromatic phenols (carvacrol, thymol, safrol, eugenol), bicyclic alcohol (verbenol), monocyclic ketones (menthone, pulegone, carvone), bicyclic monoterpenic ketones (thujone, verbenone, fenchone), acids (citronellic acid, cinnamic acid) and esters (linalyl acetate)) are normally found. Some EOs may also contain oxides (1,8- cineole), sulphur, methyl anthranilate and coumarins. However, higher terpenes can also be found as minor constituents. (Koul *et al.*, 2008).

Essential oils are complex mixture of natural organic compounds with monoterpenes as predominant constituents, and are more frequently used as insect fumigants. However, applications of other contact methods like direct spray, and dipping, topical and residue contacts are also employed. Previously, a number of studies have been conducted with a focus on the examination of insecticidal property of EOs and their potential constituents fumigation method, and many studies showed positive results. Bergamot mint (*Mentha citrata* Ehrhart) oil containing linalool and linalyl acetate has shown a significant fumigant effect to rice weevils (*Sitophilus oryzae* (L.)) (Singh *et al.*, 1989). *l*-carvone was reported presenting considerably more fumigant toxicity than contact toxicity against lesser grain borer (*Rhyzopertha domestica* (F.)) (Tripathi *et al.*, 2003). In addition, carvone and menthol was found being most effective fumigant against red flour beetle (*Tribolium castaneum* (Herbst)) and cowpea weevil (*Callosobruchus maculatus* (F.)). On the other hand, 1,8-cineole was found exhibiting, both contact and fumigant toxicity when tested against red flour beetle (*T. castaneum*) (Tripathi *et al.*, 2001). Besides, house fly (*M. domestica*) and red flour beetle (*T. castaneum*) have been determined

affected by carvacrol, carveol, geraniol, linalool, menthol, terpineol, thymol, verbenol, carvones, fenchone, menthone, pulegone, thujone, verbenone, cinnamaldehyde, citral, citronellal, and cinnamic acid (Rice and Coats, 1994). *Trans*-anethole, thymol, 1,8-cineole, carvacrol, terpineol, and linalool were evaluated as effective fumigants against red flour beetle (*T. castaneum*) (Koul *et al.,* 2008).

Combination of EOs and modification of fumigation conditions were also reported enhancing EO efficacy. Koul *et al.* (2008) reported that the combination of anethole and 1,8-cineole (1:1) demonstrated reduction in the population of red flour beetle (*T. castaneum*) by 100% at 50µl/L concentration, while a combination of four compounds reduced the progeny by 100%. Hummelbrunner and Isman (2001) studies synergism or additive effects of monoterpenoid binary mixtures against tobacco cutworm larvae (*S. litura*), and presented that thymol and *trans*-anethole synergized the effects of linalool (at 18 µg/larva dose, combined in 1:1 ratio). The application of binary mixer, adjustments of fumigation condition was also found resulting higher efficacy. For example, Koul *et al.* (2008) reported an improvement of mortality effect on red flour beetle (*T. castaneum*) treated by anethole when minimum heat treatment device was used.

2.4.1 Alternative management against thrips

Control of thrips population worldwide is largely dependent on repeated applications of conventional insecticides. However, the repetition has extensively disrupted natural biological control. Therefore, plant EOs and extracts are recently introduced for being potentially biodegrade and selective against pests (Isman, 2001). Many plants in different families such as Asteraceae, Apiaceae, Lauraceae, Lamiaceae, Myrtaceae, Meliaceae, Cucurbitaceae and Amaryllidaceae plants were found exhibiting toxicity against thrips. The extracts of bitter gourd (*Momordica charantia* L.), bakain (*Melia azedarach* L.) and neem (*Azadirachta indica* A. Juss.) were found effective in controlling the population of thrips (*Thrips tabaci* Lindeman) (Fiaz *et al.,* 2012). Mbonu (2006) used aqueous extract mixtures of gamhar (*Gmelina arborea* L.) and citron scented gum (*Eucalyptus citriodora* Denn) to control nean flower thrips (*Megalrothrips sjostedti* Trybom) by spray method under field condition and found no inferior results from synthetic insecticide. Yi *et al.* (2006) reported the fumigant toxicity of plants EOs from some plants against cowpea thrips (*Thrips palmi* Karny) and found that the armoise (*Artemesia vulgaris* L.), coriander (*Coriandrum sativum* L.), howood (*Cinnamomum camphora* Siebold), marjoram

(*Thymus mastichina* L.), niaouli (*Melaleuca viridiflora* Solander), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia sclarea* L.) EOs resulted in high mortality of the insect with LC_{50} at 17.29-40.46 mg/L air. In addition, Koschier and Sedy (2001) found that marjoram and rosemary EOs at 0.1–1.0% concentration showed satisfactory antifeedant effects against onion thrips (*T. tabaci*).

2.4.2 Alternative management against mealybugs

Aqueous leaf extracts of blue gum (*Eucalyptus globulus* Labill.) and betel vine (*Piper betle* L.) were examined against mortality of nymphs and adults of mealybug (*Maconellicoccus hirsutus* Green) under *in vitro* conditions by leaf dipping method. The findings showed that the extracts of *E. globulus* extract killed 100% of the nymphs and adults after 3 and 8 days after treatment (Govindaiah *et al.*, 2006). In addition, Cloyd *et al.* (2009) reported that EO products from Flower Pharm (cottonseed, cinnamon and rosemary oils) and Indoor Pharm (soybean, rosemary and lavender oils) provided more than 90% mortality of citrus mealybug under greenhouse experiments by direct spray.

2.4.3 Alternative management against whiteflies

Plant extracts have also been reported controlling whitefly infestation. There were many studies that exhibited toxicity of extracts or EOs from Brassicaceae, Asteraceae, Fabaceae, Myrtaceae, Umbelliferae, Rutaceae, Labiatae and Lauraceae plants against whitefly. For example, the aqueous extracts of garden cress (*Lepidiuim sativum* L.) killed 71% of the early stage nymphs of sweet potato whitefly (*Bemisia tabaci* Genn.) by contact method. Three plant extracts, garden cress (*L. sativum*), yarrow (*Achillea biebersteinii* Afan.) and white broom (*Retama raetam* (Forssk.)) were found preventing the development of pupae to adult development, and *R. raetam* extract killed adults, at non-significantly different levels when comparing to synthesized insecticide (Imidacloprid) by leaf dipping method (Ateyyat *et al.*, 2009). In addition, Choi *et al.* (2003) reported that bay (*Pimenta racemosa* (Mill.) J.W. Moore), caraway seed (*Carum carvi* L.), clove bud (*Eugenia caryophyllata* Thunb.), lemon eugalyptus (*E. citriodora*), bitter orange (*Citrus aurantifolia* Swing.), pennyroyal (*Mentha pulegium* L.), peppermint (*Mentha piperita* L.), rosewood (*Aniba rosaeodora* Ducke), spearmint (*Mentha spicata* L.), and thyme red (*Thymus vulgaris* L.) EOs were highly effective against adults, nymphs and eggs of greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) at 0.0023, 0.0093, and 0.0047 µl/ml air, respectively by

fumigation method. Similarly, Gorski (2004) revealed that EOs of basil, grapefruit, and sandalwood could be useful in the repelling greenhouse whitefly (*T. vaporariorum*).

2.4.4 Alternative management against aphids

Aphids are ather insect which shows high levels of pesticide resistance. In addition, owning to many recently reported drawbacks of conventional pesticides, many nature-based aphid management method have been studied. Isik and Gorur (2009) reported the use of EOs from Greek juniper (*Juniperus excelsa* M.-Bieb), prickly juniper (*Juniperus oxycedrus* L.), lorbeebaum (*Laurus nobilis* L.) and fennel (*Foeniculum vulgare* Miller) as an aphidicide in controlling cabbage aphid (*Brevicoryne brassicae* L.) population. Tomova *et al.* (2005) tested the biological activity of EO from Mexican marigold (*Tagetes minuta* L.) against aphid (*Acyrthosiphon pisum* (Harris), *Myzus persicae* (Sulzer) and *Aulacorthum solani* (Kaltenbach)), and demonstrated that *T. minuta* oil could significantly reduce the reproduction potential of the tested species. Jaastad (2007) showed that rapeseed oil significantly reduced damages by black cherry aphid (*Myzus cerasi* (F.)) on black cherry. Essential oils of cumin (*Cuminum cyminum* L.), anise (*Pimpinella anisum* L.), oregano (*Origanum syriacum* L. var. *bevanii*) and red gum (*Eucalyptus camaldulensis* Dehn.) were also reported as being effective fumigant in cotton aphid (*A. gossypii*) control (Tunc and Sahinkaya, 1998). Besides, EO of citronella grass (*Cymbopogon winterianus* Jowitt) at 1% (w/v) caused mortality in green peach aphid (*Myzus pesicae* (Sulzer)) at 96.9%, LC_{50} value was 0.36% by direct spray method (Pinheiro *et al.*, 2013).

2.5 Essential oils fumigation

Fumigation is considered as the most effective way in the management of pests in their most remote hiding places. The pest control method is highly suitable for the management in an enclosed area (Lorraine, 2014). Where fumigants reach target pests as gases. Fumigants are "wide-spectrum" pesticides, killing all species of arthropods. Volatile pesticides enter the insect's body through the body wall or breathing system.

The variety of EOs used in controlling insect pest, the EO application method also plays an important role affecting the effectiveness of insect pest management via fumigation method. Different types of insects normally have different living behaviors, therefore the selection of treatments importantly needs to consider the

particular morphology of the target insect pests, as well as the environment of the application areas. In closed system, EOs fumigation and contact methods showed high performance (Isman, 2000; Mahfuz and Khalequzzaman, 2007; Pumnuan *et al.*, 2012; Chu *et al,* 2013). Especially, Abramson *et al.* (2006) reported that in the closed system, the aphids were completely found dead when sprayed with EOs of citronella and alfazema but did not kill aphids when the arena was covered with a screen. The application of fumigants is limited to areas, spaces, items, or commodities that can be tightly enclosed. Fumigated structures must be sealed as air tight as possible and their occupants must left for seven days or longer. Some fumigants may damage items in the area being fumigated (Lorraine, 2014).

2.6 Changes in characteristics on agricultural products

Post-harvest treatments of agricultural products usually results in morphologically changes such as color, firmness, percentage of spoilage, weight loss, as well as shelf life. The application of postharvest insect pest management can have extensive side effects on agricultural products. In general, physiological changes on agricultural products can be observed in various damages such as degermination, defective seed growth and defective radical elongation (De Almeida *et al.*, 2010), for example, plant physiological changes of kenaf EO on lettuce seed (Kobaisy *et al.*, 2001) and hexanolic extract of flame lily (*Gloriosa superba* L.), cutch tree (*Acacia catechu* Willd) and jengkol (*Archidendron jiringa* Nielsen) on kale leaves (Pumnuan *et al.*, 2005). In cut flowers, problems involving physiological changes such as color and firmness, percentage of spoilage and weight loss after harvest have also been reported (Dahal, 2013). In addition, browning symptom caused by enzymatic or non-enzymatic reaction is also usually observed (Sapers, 1993). Almasi *et al.* (2012) mentioned that different species presented various degree of sensitivity against postharvest conditions.

2.6.1 Color changes

Colors change is an important indicator of plant longevity and usually influence purchasing decision. Normally, changing in color of fresh agricultural products is a result of changes in chlorophyll, carotenoid and anthocyanin.

Chlorophyll: Chlorophyll is green pigment considered vital for all living plants and animals, as it absorbs energy from sunlight which is photosynthesized into biochemical energy for living plants and consequently transferred to uphold the survival of plant and animal. In general, the highest absorbent of chlorophyll is at

430 nm (blue) and 660 nm (red) resulting in visible green light (Gross, 1987). Chlorophyll *a* is blue-green color, and chlorophyll *b*, with more polar, is yellow-green color (Siriphanich, 2007). Chlorophyll *a* and *b* are found at different ratios in plants resulting in various levels of green color in different parts. Normally, the ratio of chlorophyll *a* : *b* in parts is approximately at 3 : 1, and plants with more light exposure usually present higher amount of chlorophyll *a*. Chlorophyll molecules are non-stable and easily degraded by heat, oxygen and chemical. Chlorophyll degradation can occur during the senescence of fruit and leaf, or even during the growing stage. In addition, disease and insect infections can also result in plant stress and thereby chlorophyll degradation. Usually, chlorophyll degradation can be observed in the change of green into yellow or red color (Goodwin, 1988; Siriphanich, 2007). Moreover, Banhan *et al.* (2012) reported the observation of pale green leaves as an indicator of chlorophyll degradation in soybean.

Caroteniod: Carotenoids are red,-yellow pigments generally found in plants and photosynthestic organism (Bartley and Scolnik, 1995), especially in yellower-orange fruit and dark green vegetables (Bartley *et al.*, 1990). The pigments normally coordinate with chlorophyll in absorbing sun energy for photosynthesis. Moreover, carotenoids are an important provitamin a substrate.

Anthocyanin: Anthocyanins are pigments giving shade of redness and blueness in plants. These pigments are usually found changing throughout the growing process. The contents are generally unstable and easily deformed by many influencing factors such as pH, sunlight, temperature as well as combination of molecule in plant cells (Siriphanich, 2007). Threenet (1997) studied the development of anthocyanin content at different stages in Pompadouw orchid flowers, and reported the maximum increase of anthocyanin contents during the fruit week of budding period, and remained stable throughout blossom and early senescence. However, in cut flowers color degradation can normally be observed during postharvest storage, particularly intensive degradation in red-blue color flowers such as carnations and rosses in which red color turn darker into red-blue (bluing). This bluing symptom is also observed in white-colored flowers like jasmines. As a result of changes in anthocyanin content which are agueous solutions in the plant vacuole.

Color identification system: Color identification system is a standard academic method used in avoiding individually influenced identification of colors. In particular, CIE 1976 (L* a* b* color space) or CIE Lab is a widely acceptable standard which describes the coordinates of a specific color in a three dimensional space. The

values are demonstrated in three axies incliding L* for darkness (L*=0) to lightness (L*=100), b* for blueness (-b*) to yellowness (+b*), and a* for greenness (-a*) to redness (+a*). Any particular point in CIE Lab color space can specifically be described by using L*, a* and b* coordinates at that point. In addition, hue angle and chroma values can also be calculated. Hue angle and chroma are both related to human perception of colors. Hue angle is the angle observed when the line passing a particular point to zero origin. It is the attribute of color that is related to the perceived colors: red, yellow, green and blue or a combination of two of them. On the other hand, chroma is the length of the same line from the observed point to origin, defined as the chromatic intensity of a surface judged in comparison to a pure white. In the other words, it is colorfulness of a particular color. The colors below chroma present all the same lightness (L*) and the same hue angle. However, they present different chroma ranged from gray (chroma = 0) to brilliant red (chroma = 104) (X-Rite Incorporated, 2007).

2.6.2 Firmness changes and weight loss

The pattern of plant firmness is normally indicated by the patterns of cell wall, turgor and structure of the plant tissue. In addition, water is the major component dissolving. The solutes inside the rigid plant cell walls give supporting structure and firmness of fruits or vegetables (Schafer and Munson, 1990).

Therefore, water loss can induce decreases in quality of fresh fruit and vegetable products, which is usually observed in form of wilting, shriveling, flaccidness and soft. Furthermore, water loss can also accelerates senescence indicated by higher rates of membrane disintegration and leakage of cellular contents (Ben-Yehoshua and Rodov, 2003). In general, different products vary in potential for loss of water. Morphological differences such as cuticle thickness and composition are the important factors influencing rates of the loss. In addition, since transpiration is the major cause of loss in water compounds, damages or inexistence of stomata and lenticels, which are structures allowing gases and moisture transfer in plants are also the major water loss indicators. These morphological differences are different among varieties and even development stages in the same variety (Watkins *et al.*, 2012).

The senescence of vegetative tissue is exclusively a degradative process, where the compartmentalization failure of cells yields changes in membranes, cell walls, sub-cellular organelles, proteins, and cell death (Toivonen and Brummell, 2008) which consequently cause losses of plant firmness. In leafy vegetables, wilting and firmness loss can result in loss of visual appearance and

markatetability (Piagentini *et al.*, 2002) as the quality of freshness in vegetables is largely dependent on firmness, crispness and succulence (Wills *et al.*, 2007).

2.6.3 Phenolic contents and polyphenol oxidases

Enzymatic browning is a widespread color reaction which involves the interaction of oxygen, phenolic compounds and polyphenol oxidases (PPO) in fruits and vegetables. Fruits and vegetables, such as apple, pear, banana, peach, lettuce and potato, are especially susceptible to enzymatic browning. Particularly, treatments that changes in temperatures and levels of oxygen and carbon dioxide usually result in enzymatic browning which is a result of the contact between PPO and phenolic compounds. When cells are damaged, phenolic content in product will be regulated by PPO resulting the oxidation of phenolic compound into quinones and then polymerization to melanin (Siriphanich, 2007). Lee *et al.* (1990) reported the relationship between the occurrences of enzymatic browning and phenolic content in the products. Chuanjun and Ling (2006) reported positive correlation between PPO and phenol compound in early stage of *Phalaenopsis* explants browning. PPO is enzymatic browning catalyzes that changes monophenol into diphenol, and products quinones which are polymerized into browning pigments (Mayer and Harel, 1979; Carbonaro and Mattera, 2001). This activity can occur either with or without cuts on the skin (Jeong *et al.*, 2008). During processing and storage, browning not only has a negative effect on product appearance, but also impairs other sensory properties such as taste, odour and firmness, as well as nutritional value (Jiang, 2004; Komthong *et al.*, 2006).

2.7 Effects of essential oil fumigation on physiological changes of agricultural products

As customers are more concerned about health and environmental issues, EOs have recently been considered as an outstanding and more preferable alternative insect control agents than chemical insecticidal products. Many EOs were reported as having antimicrobial properties and have been used in food preservation and protection of postharvest symptoms in many agricultural products (Dorman and Deans, 2000). Several studies reported antioxidant activity of EOs in plants such as blueberries (Wang *et al.*, 2008) raspberries (Chanjirakul *et al.*, 2006) and leafy vegetables (Ponce *et al.*, 2004).

In general, there are many EO application method on postharvest agricultural products such as direct spray, residue contact and fumigation (Isman, 2000; Tripathi *et al.*, 2001; Choi *et al.*, 2003; Tripathi *et al.*, 2003;Abramson *et al.*, 2006; Mahfuz and Khalequzzaman, 2007; Koul *et al.*, 2008; Ateyyat *et al.*, 2009; Pumnuan *et al.*, 2012). In particular, fumigation is considered suitable for large scale application. However, EOs fumigation can cause changes in characteristics on plants, and the results are possibly preferable or unpreferable. Normally, EOs fumigation can result in changes of colors, PPO activity, and phenolic, anthocyanin and chlorophyll contents. In addition, Gao *et al.* (2014) reported the inhibition of senescence, decreased in browning index, increased in the accumulation of phenolic and the inhibition of PPO activity.

2.7.1 Effects of essential oils fumigation on color changes

Normally, color changes in agricultural products are extensively observed during the postharvest storage. The changes can be a result of senescence or other influencing factors, such as storage timing, plant species and temperatures. In addition, treatments with EOs can also contribute to risk of phytotoxicity (WSU, 2013). Changes in the external colors of plants can be indicated by parameters such as lightness, total color variation (ΔE) and browning index (BI) (Jiang, 2013). Gao *et al.* (2014) reported the changes in L value, ΔE and BI of button mushroom fumigated by clove, cinnamaldehyde and thyme EOs. The results of this study suggested that EOs fumigation presented significant impacts on L value, ΔE and BI. In addition, Valverde *et al.* (2005) reported significant influences of chemical compounds in plant EOs on color attributes of grape. However, there are no reported evidences on how chemical compounds in EOs work in changing color compounds in the products.

2.7.2 Effects of essential oils fumigation on firmness

Firmness is a critical factor influencing purchasing decision. Normally, changes in firmness are associated to changes in water content (Gao *et al.*, 2014). Softening in agricultural products is usually caused by degradation of cell wall or the leakage of intracellular matrix (Zivanovic *et al.*, 2000). There are many studies reporting the effect of EO fumigation on lower cell wall degradation. Gao *et al.* (2014) reported lower firmness diminution in button mushroom fumigated by EOs, and suggested that EOs could inhibit or reduce cell wall degradation caused by

microorganism activity. In addition, Kavoosi .(2014) reported lower firmness loss of grape fumigated by cumin and lemon grass EOs when compared to control.

2.7.3 Effects of essential oils fumigation on weight loss

Weight loss is one of the most important quality parameters of postharvest agricultural products. Weight loss in cold storage is generally the result of water loss. Normally, weight loss increases as the storage period progresses; nonetheless, there are cases that storage treatments influence different degrees in the loss of plant weights. Factors such as fungal contamination, respiration rate, lack of pre cooling, high temperatures and low relative humidity can also exacerbate the loss of water (Nelson, 2007). Gao *et al.* (2014) reported the weight loss reduction in button mushroom after fumigation with clove (2.13%), cinnamaldehyde (1.75%) and thyme (1.90%) when comparing to the control. However, in the study on effects of ammi plant EO fumigation on storage life of grapevine, Khezrzadeh *et al.* (2013) reported increase in the weight loss of the fumigation samples when comparing to the control.

2.7.4 Effects of essential oils fumigation on total phenolic contents

Changes in total phenolic contents in plants can be found either during the growing periods or after harvest conditions. In general, total phenolic contents increase when damages on plant cells occur (Siriphanich, 2007). Sharma and Tripathi (2006) hypothesized that the chemical compounds in EOs would trigger a signal that stimulates plant to product additional phenolic compound and subsequently increase antioxidant activity in respond to cell damages.

2.7.5 Effects of essential oils fumigation on polyphenol oxidase activity

Essential oils fumigation is also reported presenting some influences on PPO activity. Many studies reported changes in PPO activity during the storage period, either higher or lower, depending on the storage conditions and types or part of the products. Gao *et al.* (2014) reported lower PPO activity in button mushroom fumigated by clove, cinnamondehyde and thyme EOs. Sapers (1993) suggested that EOs fumigation can result in pH change and thereby inhibit PPO activity.

2.8 Insecticidal activity experiment of essential oil formulations

Combination of chemical compounds normally results in synergy or additive, while negative synergy or antagonism can also occur. Pesticide mixture can result in synergy and potentation of the compounds (Ware and Whitacre 2004; Warnock and Cloyd, 2005; Cloyd *et al.* 2007). Synergism involves the enhancement of a given pesticide by the addition of other pesticides or compounds (Ahmad, 2004). The process of synergy may also be related to potentation. Ahmad (2004) claimed that synergy is the process in which non or less toxic pesticides or compounds are mixed, while potentation involves the combination of similarly toxic pesticides. Synergy and potentiation of pesticides generally yield preferable pesticidal property and is normally used when higher insect pest control performance is required. However, combination of different compounds may also result in negative synergy or antagonism. Lindquist (2002) mentioned that antagonism reduced efficacy occurs as a result of combination of 2 or more pesticides, when compared to the application of individual pesticides. However, although there are benefits associated with pesticide mixtures, potential problems need to be considered when two or more pesticides are mixed together. These include phytotoxicity, pesticide incompatibility (Cloyd, 2001).

2.9 Field experiment

In the process of insecticide development and evaluation, on fields experiment is considered necessary as it reflects the conditions of real application. There are cases that high potential insecticides in laboratory experiments showed lower performances in field environment. Simply laboratory bioassays may not generally presume the actual impact of a pesticide on a field population of naturally infesting insects (Sarina *et al.*, 2007). However, appropriate dosage for field condition may be forecasted by using solid extrapolation of laboratory results. In addition, it is accepted that the minimum dosage that yields 90% mortality on field (MED 90) must be higher than laboratory estimated LD_{90}. Yaqoob and Arora (2005) applied the laboratory results in the field condition by multiplication with the supposed number that $MED90/LD_{90}$ was equal to one. In Haverty and Robertson (1982), the LD_{90} were multiplied by 3 to product a field dosage. However, there have not been any particular which feature relationships between laboratory, LD_{90} and field application dosages. Factors involving temperature, humidity and light are generally created or

controlled in the laboratory condition. However, these natural factors are almost impartible to be controlled on fields. The natural factors are almost impartible to be controlled on fields (Murage *et al.*, 2007). Therefore, field experiment is necessarily required, particularly when biodegradable natural derived insecticides are the subjects.

CHAPTER 3
METHODOLOGY

This study was aimed to investigate the effects of essential oil (EO) fumigations against insect pests of cut orchid flower and vegetables. The research methodology entailed the processes of insect culture and preparation, plant preparation, and fumigation assay. In the fumigation assay, different experiments were conducted in order to obtain EO fumigation formulas which presented significantly high mortality rates, while maintaining low phytotoxic effects on the treated plants. Finally, the formulas were applied in the insecticidal property experiment of EO formulation and on field fumigations in order to examine the quality of the EO fumigation formulas in the insect pest control and management.

3.1 Insect culture and preparation

The insect culture and fumigation assay in this study were conducted at Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. The insects tested in the study were common blossom thrips (*Frankliniella schultzei* (Trybom)), cotton thrips (*Thrips palmi* Karny), cotton aphid (*Aphis gossypii* Glover), cotton whitefly (*Bemisia tabaci* Gennadius) and Jack Beardsley mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller). Initially, the insects were collected from naturally infested sources which included thrips from lotus (*Nelumbo nucifera* Gaertn.), aphid from eggplant (*Solanum melongena* L.), whitefly from star gooseberry (*Phyllanthus acidus* (L.) Skeels) in Bangkok, Thailand, while mealybug was collected from naturally infested cassava leaves (*Manihot esculenta* Crantz) in Chachengsao province, Thailand.

The insects were cultured on the same type of the host plants as in the nature when cultured in insecticide free insectary (Figure 3.1 A-C), while mealybug was cultured on pumpkin fruits (*Cucurbita moschata* Decne) in laboratory, without insecticide application (Figure 3.1 D). In the insecticidal activity experiment of EO formulations and field experiments of cut orchid flowers, thrips (*T. palmi*) was the insect which naturally contaminated the collected flowers in Nakhon Pathom province, Thailand (Figure 3.1 E-F).

Prior to the fumigations, samples of 10-15 adults of thrips, aphid and whitefly were transferred onto petal of lotus, leaf of eggplant and leaf of star gooseberry, respectively, and placed in a plastic box (5x7x3 cm) with a thin net inserted in the cap (Figure 3.2 A-F). For mealybug, the samples of 10-15 nymph were transferred into an insect cage made of an acrylic sheet (3x5x0.5 cm) perforated into frustum of cone with the base (0.25 cm in diameter) covered with a filter paper (Whatman® No.1), and the top (0.5 cm in diameter) covered with a cover glass (2x2 cm) (Figure 3.2 G-H).

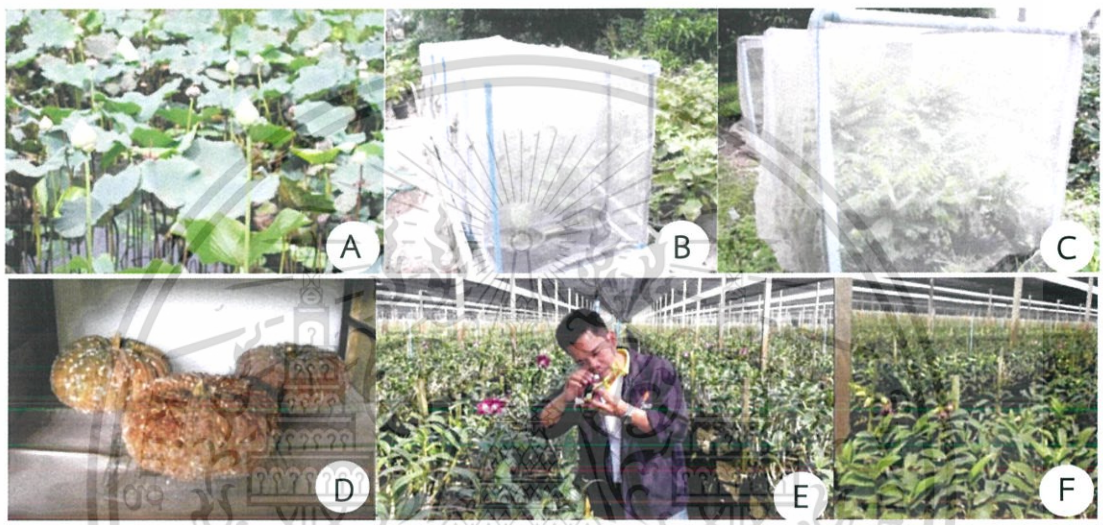


Figure 3.1 Insect culture conditions A: Thrips, B: Aphid, C: Whitefly, D: Mealybug, E-F: Orchid farm infested with thrips (*Thrips palmi* Karny).

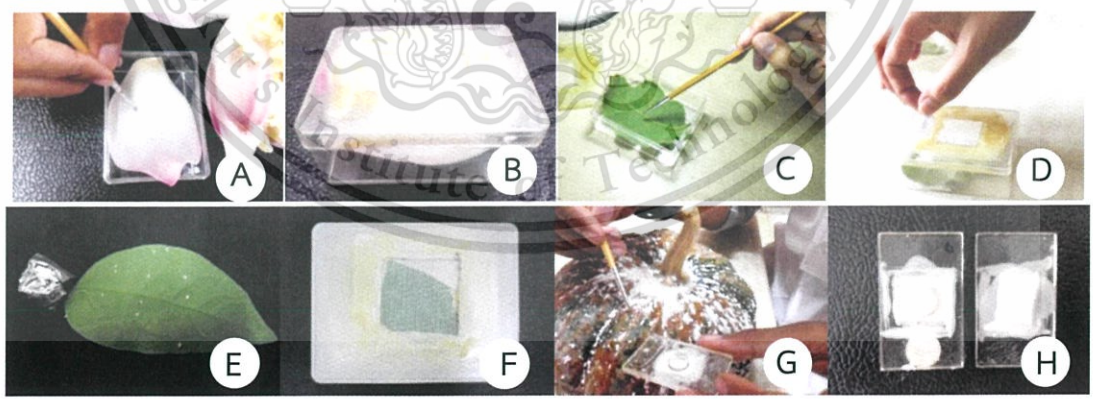


Figure 3.2 Insect preparation A-B: Thrips, C-D: Aphid, E-F: Whitefly, G-H: Mealybug.

3.2 Plant preparation

3.2.1 Cut orchid flower preparation

The orchid samples used in this study were *Dendrobium* 'Sonia' cut orchid flowers collected from an orchid farm in Nakorn Pathom province, Thailand.

The selected cut flower stalks were specifically 18 cm in length (from the cut to the first lower flower), with 4 blooming and 4 budding flowers. Initially, the flower stalks were weighed for pre-treatment weights. Subsequently, the cut ends of the stalks were covered with 5% glucose-soaked cotton put in plastic tubes filled with 8 ml of 5% glucose (Figure 3.3 A). Totally, 9 stalks of the cut orchid flower were prepared for each 3-replicated fumigation. Finally, the prepared flowers were stored at 25±1 °C until used.

3.2.2 Vegetable preparation

Holy basils (*Ocimum sanctum* L.) and eggplants (*Solanum melongena* L.) used in this study were collected from Taladthai Market, Pathum Thani province, Thailand. The selected stalks were approximately 25-30 cm in length and 5 g in weight, with observable fresh and high quality leaves (Figure 3.3 B). Totally, 15 stalks of the vegetable were prepared for each 3-replicated fumigation. The selected eggplants were of fresh and high quality with particularly 4 cm diameter (Figure 3.3 C). Totally, 15 eggplant fruits were prepared for each 3-replicated fumigation. Finally, the prepared vegetables were stored at 25±1 °C until used.

Figure 3.3 Sample plant preparation, A: cut orchid flower, B: eggplant, C: holy basil.

3.3 Essential oil extraction and preparation

In this study, EOs of eighteen medicinal plant species in eight different families (Table 3.1) were extracted by using water-distillation (Figure 3.4) method with a Clevenger-type apparatus, for the period of 6 h. The extracted oils were collected and dehydrated over anhydrous sodium sulphate and stored in amber-colored vials at 10-12 °C until the end of experiment. In addition, standard chemicals of major constituents in EOs were purchased from Sigma-Aldrich Co.LLC.

Table 3.1 Medicinal plants used for essential oil extraction.

Family / Scientific name	Common name	Plant part
MYRTACEAE		
1. *Syzygium aromaticum* (L.) Merr.&L.M. Perry	Clove	Dried flower bud
2. *Eucalyptus globulus* Labill.	Blue gum	Fresh leaf
LAURACEAE		
3. *Cinnamomum bejolghota* (Buch.-Ham.) Sweet	Cinnamon	Fresh leaf
PIPERACEAE		
4. *Piper nigrum* L.	Black pepper	Dried seed
5. *Piper betle* L.	Betel vine	Fresh leaf
ZINGIBERACEAE		
6. *Zingiber cassumunar* Roxb	Cassumunar ginger	Fresh rhizome
7. *Curcuma longa* L.	Turmeric	Fresh rhizome
8. *Alpinia nigra* (Gaertn.) Burtt	Galanga	Fresh rhizome
9. *Zingiber officinale* Roscoe	Ginger	Fresh rhizome
10. *Amomum krervanh* Pierre.	Cardamom	Dried seed
GRAMINEAE		
11. *Cymbopogon nardus* Rendle.	Citronella grass	Fresh leaf
12. *Cymbopogon citratus* (Dc.ex.Nees) Stapf	Lemon grass	Fresh leaf
RUTACEAE		
13. *Citrus aurantifolia* Swing.	Lemon	Fresh peel
14. *Citrus maxima* (Burm.) Merr.	Pummelo	Fresh peel
15. *Citrus reticulate* Blanco	Tangerine	Fresh peel
16. *Citrus hystrix* DC.	Kaffir lime	Fresh peel
LABIATE		
17. *Ocimum basilicum* L.	Sweet basil	Fresh leaf
COMPOSITAE		
18. *Eupatorium odoratum* L.	Bitter bush	Fresh leaf

3.4 Bioassay

The fumigation assays in this study were divided into 5 main stages including 1) examination of highly insecticidal EOs 2) examination of chemical constituents in EOs 3) examination of EO plant physiological changes 4) insecticidal activity experiment of EO formulations and 5) field experiment (Figure 3.5). In the first assay,

fumigation toxicities of different EOs were examined, and the EOs that presented high insecticidal activities against thrips, aphid, mealybug and whitefly were selected for further experiments. In the second assay, major chemicals in the selected EOs were examined. The obtained results were subsequently applied in the latter assay. In the third assay, different standards of the discovered chemicals were used in order to examine their original phytotoxic effects on the treated samples or caused physiological changes in tested plants. Subsequently, EOs whose major chemicals demonstrated considerably as low plant physiological changes were further selected for EO formulation. In this experiment, mixtures of different EOs were formulated and examined. The examinations of the mixtures on physiological changes were conducted on both cut orchid flower and vegetables. Subsequently, the EO formulas with less physiological changes in the treated samples were selected for next assay. In the fourth assay, fumigant toxicities of the selected EO formulas were examined again on the insects. In this assay, EO formulas that presented high insecticidal activity and showed less plant physiological changes in tested plants were obtained. Finally, the most effective EO formulas were tested in the field.

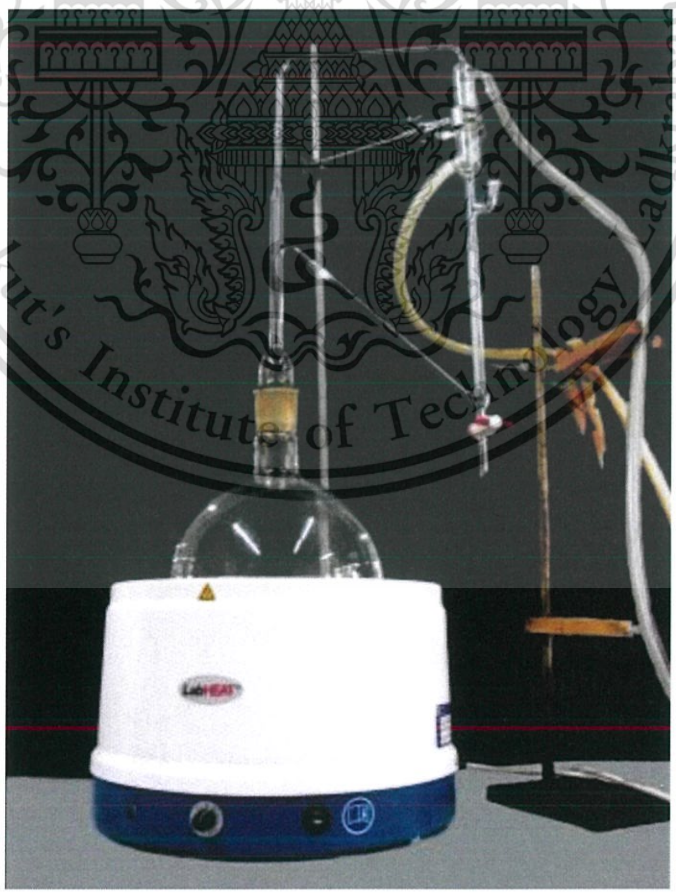


Figure 3.4 Water distillater.

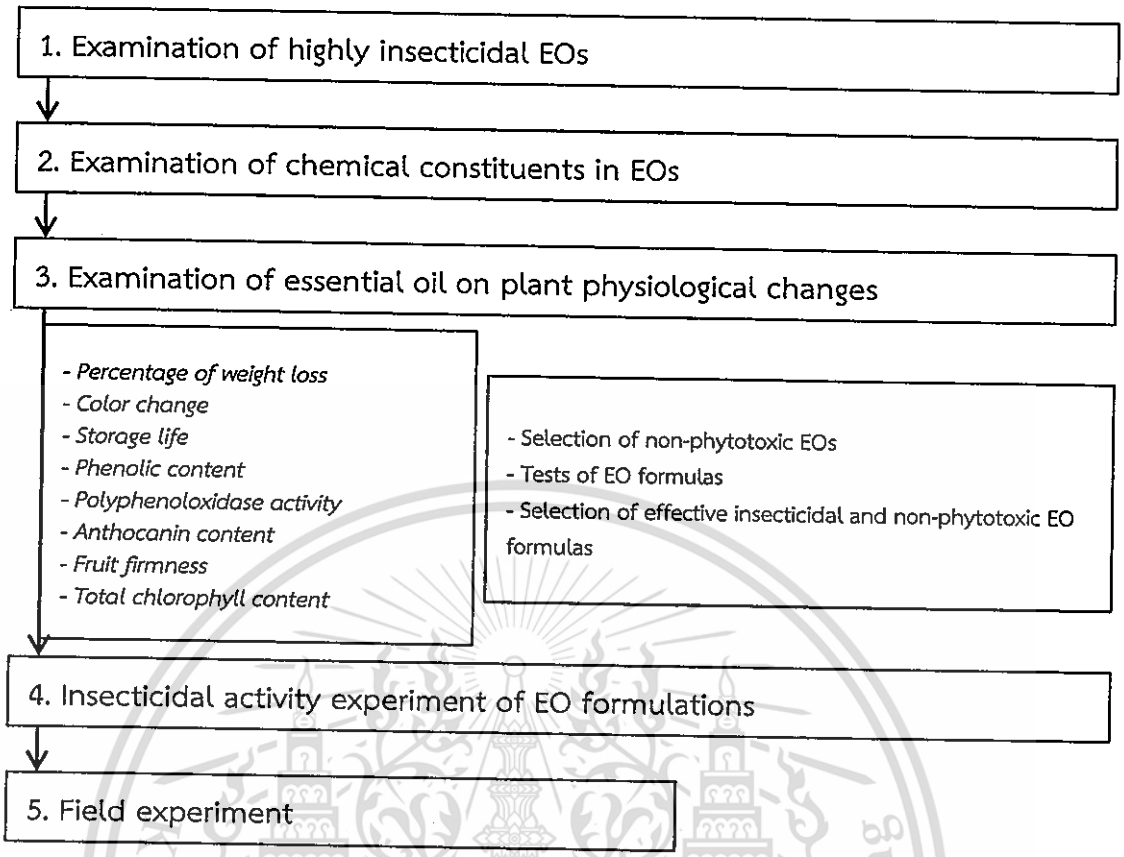


Figure 3.5 Experimental flow chart.

3.4.1 Examination of highly insecticidal essential oils

In this experiment, fumigant toxicities of different EOs were examined. Totally, the fumigations were conducted with EOs at 3 µl/L air, and 95% ethanol was used as the control. Initially, the prepared insect cages and plastic boxes (Figure 3.2) were simultaneously placed into a 25 L glass cylinder knockdown fumigation chamber (Burkard Co., England) (Figure 3.6). The samples were fumigated with different EOs (18 treatments) (Table 1) for 1 h, and then moved out and kept at 25±1°C. Mortalities of the insects were counted 24 h after treatment. Specifically, the insects were considered dead when no appendage motions were observed as probed with a small brush. The actual death rates were calculated via Abbot's formula (Abbott 1987). In general, the experiment was a completely 3-randomized replication design. Finally, the EOs with high insecticidal activity (demonstrating more than 80% mortality counts) were selected for the examination of effective concentrations.

Insecticidal activities of the selected EOs were examined again at a range of concentrations (0-5 µl/L air) in order to investigate the specific concentration that each EO demonstrated the highest insecticidal activity against the insects. Particularly,

the same procedures as in the earlier fumigations were applied. The data obtained were statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). In addition, lethal concentration of each EO needed in killing 50 and 90% of the insects (LC_{50} and LC_{90}, respectively) was calculated via probit analysis. Mortalities of the insects from each treatment were counted, and the EOs concentrations that presented considerably low LC_{50} and LC_{90} were considered having high insecticidal activity against the insects. Consequently, the EOs that commonly demonstrated high insecticidal activity against all insects were selected and examined for their major chemical constituents.

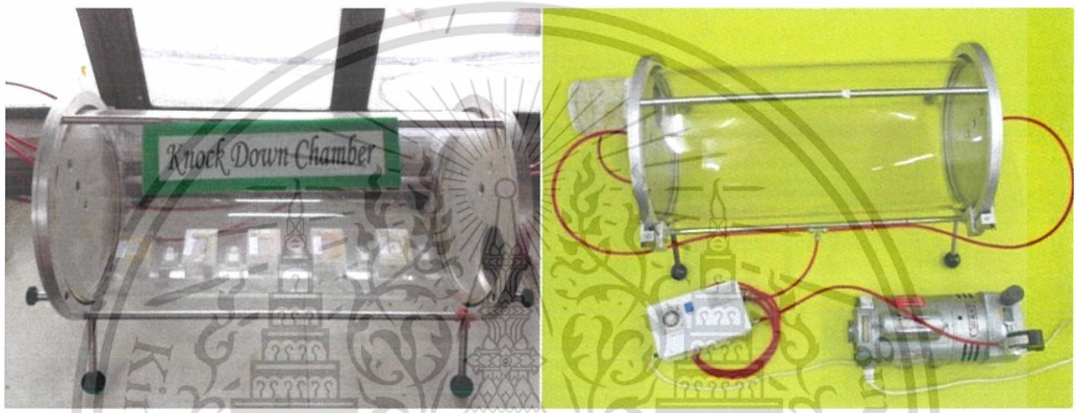


Figure 3.6 Glass cylinder knockdown fumigation chamber (25 L) (Burkard Co., England).

3.4.2 Examination of chemical constituent in essential oils

The EOs that were commonly toxic against all insect samples in 3.4.1 were selected and analyzed for their major components by using Gas Chromatography Mass Spectrometry (GC-MS) equipped with capillary column HP5MS (30 m x 0.25 mm i.d. and 0.25 µm film thickness). Direct injection with 0.4 µl as split mode (split ratio, 100:1) was made. Helium was used as carrier with ionizing voltage of 70 eV, mass ranged 50-500 m/z . Particularly, the injector and the detector were similarly maintained at 250°C. Then, standard chemicals of the major components found in the most effective EOs were examined for their plant physiological changes and toxicity against the insect pest in comparison to the EO formulas.

3.4.3 Examination of essential oil fumigation on plant physiological changes

This fumigation assay investigated phytotoxic effects of particularly high insecticidal activity EOs (from 3.4.1) and their standard chemicals (from 3.4.2) on cut orchid flower, eggplant, and holy basil. All fumigations in this assay were carried out

in 1 cubic meter glass chamber with 25 watt air circulator (7 inch-diameter) installed at the center. Initially, each EO at the concentrations of 0-5 µl/L air was injected using 10 pound per square inch (GAST® Model 1031-102A) atomizer air pump at 1 ml/7 sec. through the injection port (Figure 3.7). In particular, only one injection was applied for each concentration. The fumigation time was 1 h with all-time and 15-min air circulations. Subsequently, plant physiological change examinations on the treated samples were conducted on day 3 after fumigation. The plant physiological changes were considered based on color changing parameters (L*, a* and b* values) and percentage of weight loss. Then, the results from EO and chemical standard fumigations were compared in order to justify the active chemicals in the EOs. Subsequently, 2 particular EOs that presented the lowest physiological changes in the plants were selected for the examination EO formulas when the 2 EOs were mixed at different ratios (4:0, 3:1, 2:2, 1:3 and 0:4) at the concentration of the 0-5 µl/L air. The formula fumigations were conducted with all-time and 15-min air circulations. Plant physiological changes examinations of the formulas were conducted with a wider range of phytotoxicity. Particularly, in addition to color change parameters (L*, a* and b* values) and percentage of weight loss, polyphenol oxidase (PPO) activity and phenolic content, fruit firmness (in eggplant), chlorophyll content (in holy basil and eggplant) and anthocyanin content (in cut orchid flower) were also examined. The plant physiological change examinations were conducted immediately after the fumigations and every 3 days interval during the storage. The results were then compared to the controls (all-time and 15 min air-circulated fumigations with no EO injections).

Figure 3.7 Fumigation glass chamber (1 cubic meter).

3.4.3.1 Percentage of weight loss

The treated cut orchid flowers, eggplant, and holy basil were weighed immediately after fumigation and every 3-day interval, Then, percentages of

weight loss of the treated plants at each particular observation period were calculated as following;

Weight loss (%) = [(initial weight – observed weight) / initial weight] × 100.

3.4.3.2 Color changes

Color changes in the treated plants were measured using CIE L a b color space system by Color Flex spectrophotometer immediately after fumigation and every 3 day-interval after each treatment. In cut orchid flower, totally 9 petals form 3 opened flowers (3 petals form each flower) were sampled. In eggplant, a total of 9 spots on the treated fruits (3 spots from each fruit) were sampled. In holy basil, a total of 9 spots from 3 leaves were sampled. The color changes examinations were conducted with reference to the obtained L*, a* and b* values. Specifically, L* value demonstrated the degrees of lightness-darkness which were ranged from 0 for darkness to 100 for lightness. Then, a* value demonstrated shades of red-green color which were ranged from positive value (+a*) for the levels of redness to negative value (-a*) for the levels of greenness. Besides, b* value indicated shades of yellow-blue color, which were ranged from positive value (+b*) for the levels of yellowness to negative value (-b*) for the levels of blueness.

3.4.3.3 Storage life

In this study, storage life of the treated cut orchid flower, eggplant, and holy basil referred to the period when the plants remained unspoiled after treatment. In particular, the treated cut orchid flowers were considered spoilage when the petals of the first blooming droped. In addition, the treated holy basil were considered spoilage when more than 50% senescence on the leaves area. Besides, storage life of the treated eggplant was measured in reference to the firmness of the fruit (3.4.3.7). In particular, the treated eggplants were considered spoilage when more than 50% losses in fruit firmness were observed.

3.4.3.4 Phenolic content

Total phenolic content of the treated cut orchid flower, eggplant and holy basil in this study was examined using the methods described by Ketsa and Atantee (1998) and Singleton and Rossi (1965) with a slight modification. In this experiment, 1 g of small pieces from the treated flower petals was ground and

mixed with 10 ml 95% ethanol. The mixture was incubated at 80°C for 1 h. Subseqently, 0.8 ml extract was mixed with 2 ml of 10% sodium carbonate and 2-3 drops of 10% Folin-Ciocalteu reagent. The absorbance of the mixture was measured at 730 nm by spectrophotometer. Finally, the obtained value was compared to the standard curve of gallic acid at the concentration of 0-120 ppm.

3.4.3.5 Polyphenol oxidase (PPO) activity

PPO activity in the treated samples was examined using the methods modified from Benjamin and Montgomery (1973) and Coseteng and Lee (1987). In particular, 5 g of small pieces from the treated cut orchid flower petals, holy basil, and eggplant skin were mixed with 10 ml of 0.1 M phosphate buffer (pH 7.3). Each mixture was subsequently homogenized and centrifuged at 12,000 rpm at 4°C for 10 min. The supernatant (1.6 ml) was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.0) and 0.7 ml of 0.1 mM catechol. Subsequently, the mixture was measured for absorbance at 420 nm immediately after mixing (A_{420} initiation) and left for 3 min before the second measurement (A_{420}, 3 min). Finally, PPO activity was calculated in terms of unit per g protein as following;

$$\text{Unit} = (A_{420}\ \text{initiation} - A_{420}\ 3\ \text{min}) / (3 \times \text{Protein (mg)}).$$

This study applied the protein extraction and analysis methods proposed by Bradford (1976). In particular, 5 g of small pieces from the treated cut orchid flower petals, holy basil, and eggplant skin were mixed with 10 ml of 0.1 M phosphate buffer (pH 7.3). Then, each mixture was homogenized and centrifuged at 12,000 rpm at 4°C for 10 min. The supernatant (0.1 ml) was mixed with 2 ml of 0.01% Coomassie reagent. The mixture was measured for absorbance at 595 nm by spectrophotometer. Subsequently, the protein content was calculated as following;

$$\text{Protein (mg)} = (A_{595} \times \text{Supernatant volume (ml)} \times 10^{-3}) / \text{Slope of BSA}.$$

The standard curve prepared by reading the absorbance of BSA (bovine serum albumin) between 20-100 ppm in concentration.

3.4.3.6 Anthocyanin contents

In addition to other general plant physiological change tests, total anthocyanin contents were particularly examined in cut orchid flower. At each targeted period, the total contents of anthocyanin were measured with spectrophotometer, using the method proposed by Ragana (1997). In this test, 0.5 g

of small pieces from the treated orchid flower petals in each treatment were mixed with 10 ml of 85% ethanolic HCl (95% ethanol : 1.5 N HCl = 85:15). The mixtures were homogenized and kept at 4°C for 24 h. Then, the extractants were filtered through filter paper. Subsequently, 300 µl of each obtained extract was mixed with 3 ml of 85% ethanolic HCl and measured for the absorbance at wavelength 535 nm. Next, anthocyanin contents were calculated as following; Total anthocyanin = Total A / 98.2; Total A = (A_{535} * final volume (ml) * 100) / weight (g); A_{535} = absorbent at 535 nm]. The data was report in term of mg/100 g fresh weight.

3.4.3.7 Fruit firmness

Firmness of eggplant was measured with penetrometer. A plunger (1.11 cm in diameter) was pressed into the fruits, approximately 1 cm deep. The equipment was placed and pressed in 3 directions on each fruit and the results were reported in Newton (N).

3.4.3.8 Total chlorophyll content

In this study, total chlo rophyll contents in eggplant and holy basil were examined in order to indicate the levels of vegetable degradation. The chlorophyll contents including chlorophyll a, chlorophyll b and total chlorophyll were measured by spectrophotometer at wavelengths 666 and 653 nm using the methods proposed by Dere *et al.* (1998). The total amount of 0.5 g from each chopped vegetable was mixed with 10 ml of 95% methanol and kept at 6°C for 24 h. The vegetables were blended and sieved for the chlorophyll extracts. Then, the extracts were centrifuged at 12,000 rpm for 10 min for pure chlorophyll in methanol. The chlorophyll was measured by spectrophotometer at 666 and 653 nm, the chlorophyll a, b and total chlorophyll were calculated as following:

Chlorophyll a	$= 11.75 \times A_{666} - 2.350 \times A_{653}$
Chlorophyll b	$= 18.61 \times A_{666} - 3.960 \times A_{653}$
Total chlorophyll	= Chlorophyll a + Chlorophyll b;
A_{666}	= absorbent at 666 nm
A_{653}	= absorbent at 653 nm

3.4.4 Insecticidal activity experiment of essential oil formulation

Subsequent to the investigation of potent insecticidal EO formulas against thrips, aphid, mealybug and whitefly and the plant physiological change examinations, insecticidal activities of the EO formulas against the insects were

basically examined. In particular, similar insect samples and treatment method as in 3.4.1 were accomplished.

Additionally, the treatments were also tested on cotton thrips (*T. palmi*) which is also one of the most important insects of orchid. Consequently, the EO formulas that presented high mortality against the insects, while maintaining low physiological changes in the treated products in this examination were further tested for their insect pest control quality in field experiments.

3.4.5 Field experiment

Following the laboratory, effectiveness of the obtained EO formulas was consequently examined in the field application. In this assay, the fumigations were conducted in Department of Agriculture (DOA) fumigation chamber (Figure 3.8). The insect samples tested were mimic contaminating pest which were manually prepared on host plants, namely cut orchid flower, eggplant and holy basil leaf. In cut orchid flower, EO formula fumigations were also conducted on the naturally contaminated host plants. Then, the obtained insect mortalities were compared to the results from the conventional approach, methyl bromide fumigation.

3.4.6 Statistical analysis

In this study, Abbott's formula (Abbott, 1987) was applied for the insect actual death rate calculation. In general, the experiments were designed in three completely randomized replicates. The obtained data were statistically analysed by using analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). In addition, LC_{50} (median lethal concentration) was calculated by the probit method.

Figure 3.8 Field experiment in Department of Agriculture (DOA) fumigation chamber, A-B: sample preparations, C-E: methyl bromide fumigation, F-G: EO formula fumigations.

CHAPTER 4

RESULTS

4.1 Examination of essential oils with high insecticidal activity

4.1.1 Fumigant toxicity of essential oil against thrips

The preliminary results showed that 6 from 18 selected medicinal plant essential oils (EOs) including clove, cinnamon, cassumunar ginger, lemon grass, black pepper and cardamom oils presented distinctively high fumigant toxicity to adults of thrips (*F. schultzei*), with more than 80% mortalities. Particularly, clove and lemon grass EOs at the concentration of 3 µl/L air showed more than 95% mortality at 24 h after fumigation (Figure 4.1). In addition, the results showed that EO of clove presented the highest toxicity to adult of thrips with LC_{50} at 1.14 µl/L air, followed by EOs of lemon grass, cassumunar ginger, cinnamon, black pepper and cardamom with LC_{50} at 1.23, 1.46, 1.52, 1.55 and 1.84 µl/L air, respectively. Overall, clove, lemon grass and black pepper EOs at the concentration of 2.4 and 3.0 µl/L air were extremely toxic against thrips, with 78.4-90.2 and 90.2-98.0% mortality, respectively. No significant differences ($P<0.005$) among plant species were observed (Table 4.1, Figure 4.2).

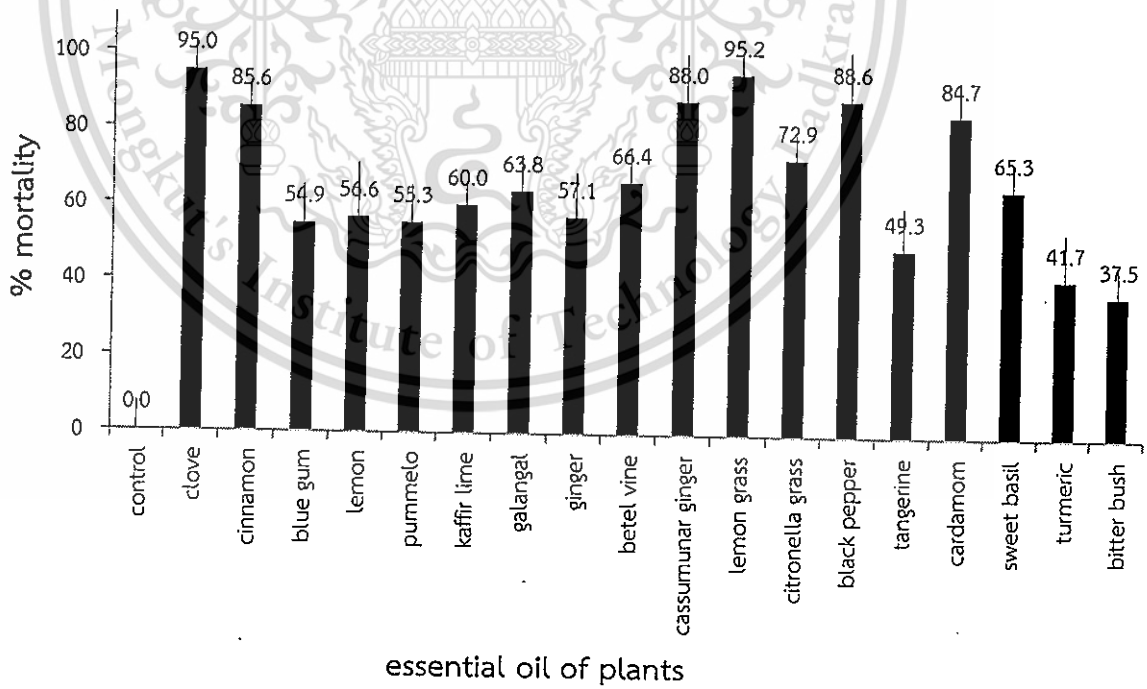


Figure 4.1 Mortality percentages of adult thrips (*Frankliniella schultzei* (Trybom)) at 24 h after fumigations with essential oil of medicinal plants at 3.0 µl/L air.

Table 4.1 Mortality percentages of adult thrips (*Frankliniella schultzei* (Trybom)) at 24 h after fumigations with essential oil of medicinal plants at various concentrations.

Plant essential oils	% Mortality [1/] ±SD						LC_{50} (µl/L air)	LC_{90} (µl/L air)	slope±SE
	Concentration (µl/L air)								
	0.0	0.6	1.2	1.8	2.4	3.0			
Black pepper	0.0±6.4	25.5±11.2^{a}	47.1±13.2^{b}	52.9±12.9^{c}	78.4±11.6^{ab}	90.2±13.8^{abc}	1.55	2.92	0.94±0.07
Cardamom	0.0±6.4	23.5±12.3^{a}	29.4±10.5^{c}	41.2±10.5^{d}	74.5±13.5^{b}	82.4±9.8^{c}	1.84	3.30	0.88±0.07
Cassumunar ginger	0.0±6.4	27.5±8.9^{a}	51.0±11.6^{b}	68.6±9.6^{ab}	76.5±10.5^{b}	86.3±4.8^{bc}	1.46	2.91	0.88±0.07
Cinnamon	0.0±6.4	35.3±6.4^{a}	47.1±12.3^{b}	60.8±6.1^{bc}	72.5±6.1^{b}	84.3±6.1^{c}	1.52	3.16	0.78±0.06
Clove	0.0±6.4	33.3±9.6^{a}	64.7±7.4^{a}	74.5±8.9^{a}	90.2±8.9^{a}	98.0±4.8^{a}	1.14	2.23	1.18±0.08
Lemon grass	0.0±6.4	33.3±6.1^{a}	52.9±10.5^{ab}	76.5±10.5^{a}	86.3±11.6^{ab}	96.1±6.1^{ab}	1.23	2.40	1.10±0.08

[1/] Means±SD in column followed by the same common letter were not significantly different (P<0.05) according to DMRT

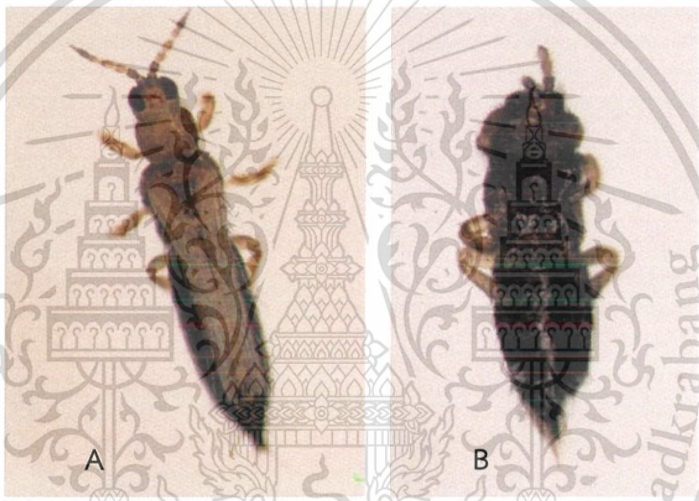


Figure 4.2 Thrips (*Frankliniella schultzei* (Trybom)), A: before essential oil fumigations, B: after essential oil fumigations.

4.1.2 Fumigant toxicity of essential oil against mealybugs

The preliminary results showed that 4 from the 18 selected medicinal plant EOs including clove, cinnamon, lemon grass and citronella grass oils presented high fumigant toxicity to the nymph of mealybug (*P. jackbeardsleyi*) with more than 80% mortality (Figure 4.3). Furthermore, the results showed that EO of clove presented the highest fumigant toxicity with LC_{50} at 1.23 µl/L air, followed by EOs of citronella grass, cinnamon and lemon grass with LC_{50} at 1.26, 1.32 and 1.75 µl/L air, respectively. No significant differences were observed from the EOs at the concentration of 1.8, 2.4 and 3.0 µl/L air (Table 4.2, Figure 4.4).

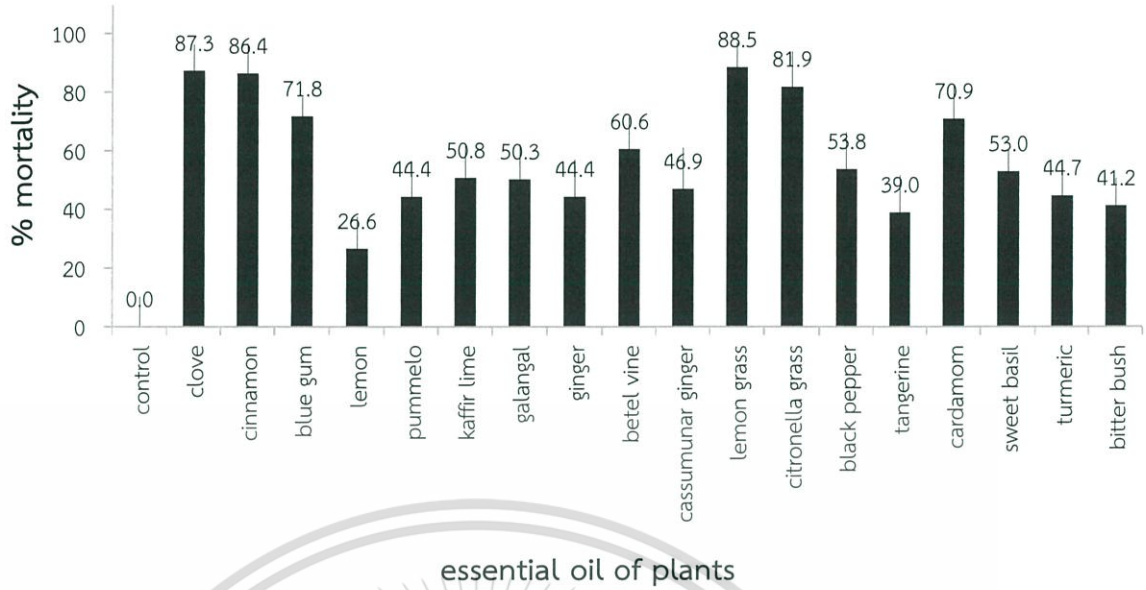


Figure 4.3 Mortality percentages of nymph of mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller) at 24 h after essential oil fumigations at the concentration of 3.0 µl/L air.

Table 4.2 Mortality percentages of nymph of mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller) at 24 h after essential oil fumigations at various concentrations.

Plant essential oils	% Mortality [1/] ±SD Concentration (µl/L air)						LC_{50} (µl/L air)	LC_{90} (µl/L air)	slope±SE
	0.0	0.6	1.2	1.8	2.4	3.0			
Cinnamon	0.0±9.2	40.8±9.2^{a}	53.1±9.2^{a}	69.4±10.2^{a}	77.6±9.2^{a}	87.8±10.9^{a}	1.32	2.86	0.84±0.06
Citronella grass	0.0±9.2	38.8±13.4^{a}	57.2±6.7^{a}	71.4±6.3^{a}	79.6±10.0^{a}	91.8±6.3^{a}	1.26	2.68	0.90±0.07
Clove	0.0±9.2	44.9±6.7^{a}	61.2±9.2^{a}	69.4±10.2^{a}	75.5±10.9^{a}	89.9±9.2^{a}	1.23	2.84	0.80±0.06
Lemon grass	0.0±9.2	18.4±6.3^{b}	36.8±9.2^{b}	55.1±10.0^{a}	71.2±6.2^{a}	83.7±6.3^{a}	1.75	3.16	0.90±0.07

[1/] Means±SD in column followed by the same common letter were not significantly different (P<0.05) according to DMRT

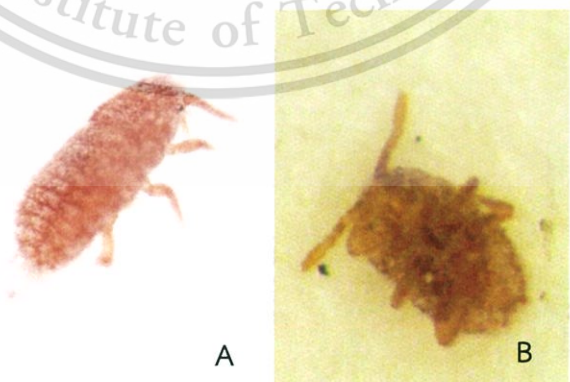


Figure 4.4 Mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller), A: before essential oil fumigations B: after essential oil fumigations.

4.1.3 Fumigant toxicity of essential oil against aphids

The preliminary results showed that 8 from the 18 selected medicinal plant EOs including galangal, cinnamon, blue gum, citronella grass, clove, cardamom, lemon grass and cassumunar ginger oils presented distinctively high fumigant toxicity to adults of aphid (*A. gossypii*) with more than 60% mortality (Figure 4.5). Furthermore, the results showed that, EO of lemon grass presented the highest toxicity to adults of aphids with LC_{50} at 1.70 µl/L air, followed by EOs of clove, cassumunar ginger, cinnamon, blue gum, cardamom, citronella grass and galangal with LC_{50} at 20.7-2.38 µl/L air. Approximately 85.0-96.7% mortalities were observed at the concentration of 3.6 µl/L air with no significant differences ($P<0.005$) when comparing among the 8 EOs (Table 4.3, Figure 4.6).

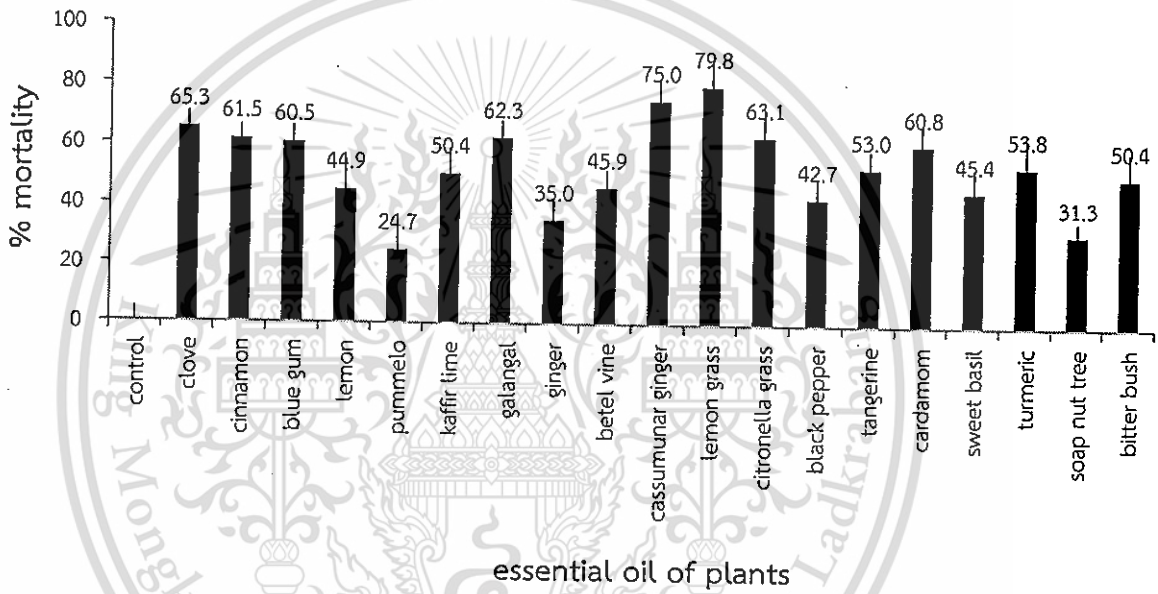


Figure 4.5 Mortality percentages of adult aphid (*Aphis gossypii* Glover) At 24 h after essential oil fumigations at 3.0 µl/L air.

Table 4.3 Mortality percentages of adult aphid (*Aphis gossypii* Glover) at 24 h after essential oil fumigations at various concentrations.

Plant essential oils	% Mortality [1/] ±SD							LC_{50} (µl/L air)	LC_{90} (µl/L air)	slope±SE
	Concentration (µl/L air)									
	0.0	0.6	1.2	1.8	2.4	3.0	3.6			
Blue gum	0.0±0.0	23.3±5.2^{abc}	30.0±6.3^{bc}	35.0±10.5^{b}	43.3±5.2^{cd}	63.3±5.2^{b}	86.7±8.2^{a}	2.30	4.27	0.65±0.05
Cardamom	0.0±0.0	11.7±7.5^{d}	26.7±5.2^{bc}	33.3±8.2^{b}	51.7±7.5^{bc}	61.7±4.1^{b}	85.0±5.5^{a}	2.35	4.08	0.74±0.05
Cassumunar ginger	0.0±0.0	20.0±8.9^{abcd}	30.0±8.9^{bc}	38.3±9.3^{b}	55.0±5.5^{b}	68.3±9.8^{b}	91.7±7.5^{a}	2.12	3.82	0.75±0.05
Cinnamon	0.0±0.0	18.3±7.5^{bcd}	28.3±7.5^{bc}	35.0±5.5^{b}	46.7±8.2^{bc}	65.0±5.5^{b}	88.3±7.5^{a}	2.27	4.07	0.71±0.05
Citronella grass	0.0±0.0	20.0±6.3^{abcd}	31.7±4.1^{bc}	40.0±6.3^{b}	36.7±8.2^{d}	61.7±7.5^{b}	85.0±10.5^{a}	2.35	4.38	0.63±0.05
Clove	0.0±0.0	26.7±5.2^{ab}	33.3±8.2^{c}	41.7±7.5^{b}	53.3±10.5^{bc}	66.7±5.2^{b}	90.0±8.9^{a}	2.07	3.98	0.67±0.05
Galangal	0.0±0.0	15.0±5.5^{cd}	23.3±5.2^{c}	36.7±8.2^{b}	43.3±8.2^{cd}	61.7±9.8^{b}	86.7±10.3^{a}	2.38	4.14	0.73±0.05
Lemon grass	0.0±0.0	28.3±7.5^{a}	43.3±5.2^{a}	53.3±5.2^{a}	66.7±8.2^{a}	78.3±11.7^{a}	96.7±5.2^{a}	1.70	3.33	0.79±0.05

[1/] Means±SD in column followed by the same common letter were not significantly different ($P<0.05$) according to DMRT

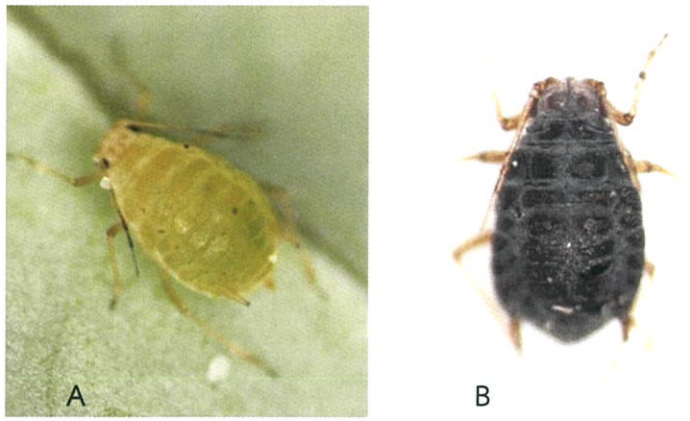


Figure 4.6 Aphids (*Aphis gossypii* Glover), A: before essential oil fumigations, B: after essential oil fumigations.

4.1.4 Fumigant toxicity of essential oil against whiteflies

The preliminary results showed that 5 from the 18 selected medicinal plant EOs including cassumunar ginger, cinnamon, citronella grass, lemon grass and clove oils presented distinctively high fumigant toxicity to adults of whitefly (*B. tabaci*) with more than 70% mortality (Figure 4.7). Furthermore, the results showed that, EO of clove presented the highest toxicity to adults of whitefly with LC_{50} at 1.36 µl/L air, followed by EOs of lemon grass, cinnamon, citronella grass and cassumunar ginger with LC_{50} at 1.47-1.64 µl/L air. Approximately 87.6-95.8% mortalities were observed at the concentration of 3.6 µl/L air with no significant differences ($P<0.005$) when comparing among the 5 EOs (Table 4.4, Figure 4.8).

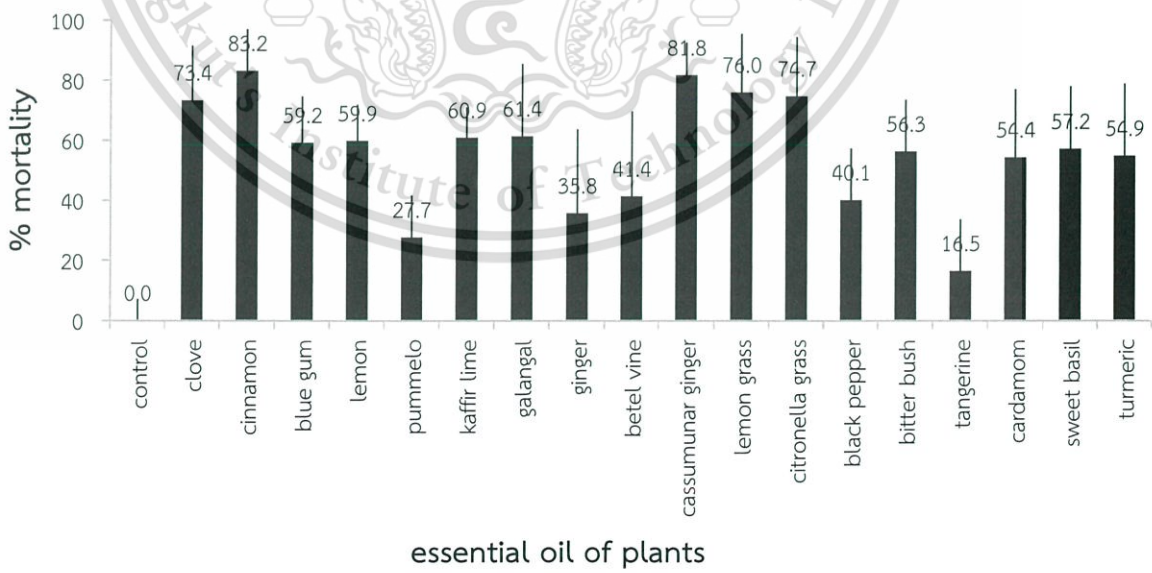


Figure 4.7 Mortality percentages of adult whitefly (*Bemisia tabaci* Genn.) at 24 h after essential oil fumigations at 3.0 µl/L air.

Table 4.4 Mortality percentages of adult whitefly (*Bemisia tabaci* Genn.) at 24 h after essential oil fumigations at various concentrations.

Plant essential oils	% Mortality [1/] ±SD							LC_{50} (µl/L air)	LC_{90} (µl/L air)	slope±SE
	Concentration (µl/L air)									
	0.0	0.6	1.2	1.8	2.4	3.0	3.6			
Cassumunar ginger	0.0±9.8	22.1±16.5^{a}	40.6±12.9^{c}	61.7±11.3^{a}	81.7±9.4^{a}	85.5±8.0^{a}	87.6±6.3^{a}	1.64	3.20	0.82±0.05
Cinnamon	0.0±9.8	22.1±20.8^{a}	52.7±9.2^{ab}	67.0±12.1^{a}	83.7±5.6^{a}	83.4±16.7^{a}	88.3±12.2^{a}	1.52	3.13	0.80±0.05
Citronella grass	0.0±9.8	24.9±17.4^{a}	46.9±14.5^{bc}	65.0±9.3^{a}	75.9±7.3^{a}	81.8±13.0^{a}	88.6±9.1^{a}	1.60	3.29	0.76±0.05
Clove	0.0±9.8	31.7±8.7^{a}	61.5±7.2^{a}	71.0±10.1^{a}	75.7±8.9^{a}	84.3±13.4^{a}	95.8±7.1^{a}	1.36	2.96	0.80±0.06
Lemon grass	0.0±9.8	35.3±20.4^{a}	54.8±12.0^{ab}	66.5±18.4^{a}	76.0±9.5^{a}	82.2±13.7^{a}	88.0±11.8^{a}	1.47	3.32	0.69±0.05

[1/] Means±SD in column followed by the same common letter were not significantly different (P<0.05) according to DMRT

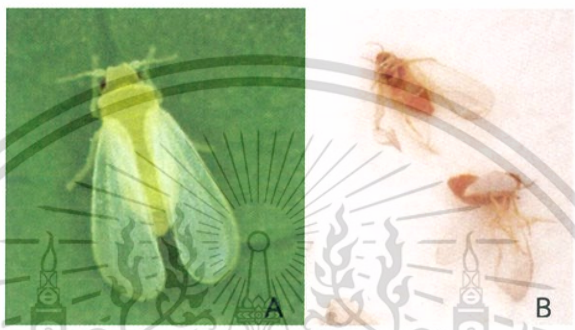


Figure 4.8 Whitefly (*Bemisia tabaci* Genn.), A: before essential oil fumigations, B: after essential oil fumigations.

In general examination of EOs fumigant toxicity against nymph of mealybug and adults of thrips, aphid and whitefly showed that EOs of clove, cinnamon and lemon grass were highly toxic against the insects. All EOs fumigations presented high fumigant toxicity to the nymph of aphid and thrips (data not shown). However, no EO fumigations were observed presenting toxicity against adults of mealybug and nymph of whitefly (data not shown) (Table 4.5).

Table 4.5 Fumigant toxicity of essential oils against insects.

Plant essential oils	Fumigant toxicity							
	Aphids		Thrips		Mealybugs		Whitefies	
	Nymph [1/]	Adult	Nymph [1/]	Adult	Nymph	Adult [1/]	Nymph [1/]	Adult
Cinnamon	√	√	√	√	√	X	X	√
Galangal	√	√	√	X	X	X	X	X
Blue gum	√	√	√	X	X	X	X	X
Citronella grass	√	√	√	X	√	X	X	√
Clove	√	√	√	√	√	X	X	√
Black pepper	√	X	√	√	X	X	X	X
Cardamom	√	√	√	√	X	X	X	X
Lemon grass	√	√	√	√	√	X	X	√
Cassumunar ginger	√	√	√	√	X	X	X	√

[1/] Data not shown, √ = highly toxic against the insect, X = lowly toxic against the insect

4.2 Examination of chemical constituents in essential oil and their fumigant toxicity against the insects

4.2.1 Major chemical compositions in insecticidal essential oils

The results showed that eugenol was the major component (97.10%) in clove EO, followed by *trans*-caryophyllene (1.69%). Likewise, eugenol was also the major compound in cinnamon EO (82.06%), followed by *trans*-caryophyllene (3.80%), 2-methoxy-4-propenylphenyl, acetate (3.53%) benzyl benzoate (3.52%) and cinnamaldehyde (1.52%). In lemon grass EO, the major compound was citral (69.73%; *trans*-citral (37.93%) and *cis*-citral (31.80%)), followed by lemonene (18.09%), 1,8-cineole (2.18%) and linalool (1.49%) (Table 4.6).

Table 4.6 Major chemical components in clove, cinnamon and lemon grass essential oils.

Plant essential oils	Chemical components	Concentration (%)
Clove	Eugenol	97.10
	trans-Caryophyllene	1.69
Cinnamon	Eugenol	82.06
	trans-Caryophyllene	3.80
	2-methoxy-4-propenylphenyl acetate	3.53
	Benzyl benzoate	3.52
	trans-Cinnamyl acetate	1.85
	Cinnamaldehyde	1.52
Lemon grass	*trans*-citral	37.93
	cis-citral	31.80
	Lemonene	18.09
	1, 8-cineole	2.18
	Linalool	1.49

4.2.2 Standard chemical and essential oil fumigant toxicities against the insects

The examination of standard chemicals and EOs fumigant toxicity against adults of thrips showed that the mortalities obtained from the fumigations with standard eugenol and clove EO at the concentrations of 2.4-3.0 µl/L air were significantly lower than those obtained from clove EO (90.2-98.0%). At the concentration of 3.6 µl/L air, mortality from standard eugenol fumigation was 93.40% with LC_{50} at 1.31 µl/L air, while clove and cinnamon EOs fumigations resulted in a complete mortality (100.0%) in adult thrips with LC_{50} at 1.14 and 1.48 µl/L air, respectively. The examination of citral fumigant toxicity against adults of thrips showed that the insect mortality from standard citral fumigation at 3.6 µl/L air (86.1% with LC_{50} at 2.31 µl/L air) was significantly lower than the value obtained from lemon grass EO fumigation (100% with LC_{50} at 1.23 µl/L air) (Table 4.7).

In the examination of standard chemicals and EOs fumigant toxicity against nymph of mealybug, the results showed that mortalities of clove, cinnamon and lemon grass EOs, and standard eugenol were significantly lower than standard citral fumigations. At the concentration of 3.6 µl/L air, mortality of clove, cinnamon and lemon grass EOs, and standard eugenol were 100.0% with LC_{50} at 1.12, 1.31, 1.71 and 1.20 µl/L air, respectively, while standard citral presented the lowest LC_{50} at 2.80 µl/L air (Table 4.8).

Table 4.7 Mortality percentages of adult thrips (*Frankliniella schultzei* (Trybom) at 24 h after fumigations with clove, cinnamon and lemon grass EOs, and standard chemicals at various concentrations.

Plant essential oils	% Mortality [1/] ±SD							LC_{50}	LC_{90}	
	Concentration (µl/L air)							(µl/L	(µl/L	slope±SE
	0.0	0.6	1.2	1.8	2.4	3.0	3.6	air)	air)	
Clove	0.0±6.4	33.3±9.6^{b}	64.7±7.4^{a}	74.5±8.9^{a}	90.2±8.9^{a}	98.0±4.8^{a}	100.0±0.0^{a}	1.14	2.22	1.19±0.08
Cinnamon	0.0±6.4	35.3±6.4^{ab}	47.1±12.3^{b}	60.8±6.1^{b}	72.5±6.1^{c}	84.3±6.1^{b}	100.0±0.0^{a}	1.48	3.00	0.85±0.06
Lemon grass	0.0±6.4	33.3±6.1^{b}	52.9±10.5^{ab}	76.5±10.5^{a}	86.3±11.6^{ab}	96.1±6.1^{a}	100.0±0.0^{a}	1.23	2.38	1.12±0.07
Eugenol	0.0±6.4	45.8±11.8^{a}	55.0±14.7^{ab}	68.5±11.6^{ab}	76.0±8.6^{bc}	84.6±10.2^{b}	93.4±5.4^{b}	1.31	3.10	0.72±0.05
Citral	0.0±6.4	13.6±3.9^{c}	29.4±8.1^{c}	39.7±7.1^{c}	50.4±7.3^{d}	58.9±5.0^{c}	86.1±6.5^{c}	2.31	4.13	0.70±0.05

[1/] Means±SD in column followed by the same common letter were not significantly different ($P<0.05$) according to DMRT

Table 4.8 Mortality percentages of nymph of mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller) at 24 h after fumigation with clove, cinnamon and lemon grass EOs, and standard chemicals at various concentrations.

Plant essential oils	% Mortality [1/] ±SD							LC_{50}	LC_{90}	
	Concentration (µl/L air)							(µl/L	(µl/L	slope±SE
	0.0	0.6	1.2	1.8	2.4	3.0	3.6	air)	air)	
Clove	0.0±9.2	44.9±6.7^{a}	61.2±9.2^{a}	69.4±10.2^{a}	75.5±10.9^{ab}	89.9±9.2^{a}	100.0±0.0^{a}	1.12	2.74	0.85±0.06
Cinnamon	0.0±9.2	40.8±9.2^{a}	53.1±9.2^{a}	69.4±10.2^{a}	77.6±9.2^{ab}	87.8±10.9^{a}	100.0±0.0^{a}	1.31	2.78	0.87±0.06
Lemon grass	0.0±9.2	18.4±6.3^{b}	36.8±9.2^{b}	55.1±10.0^{b}	71.2±6.2^{b}	83.7±6.3^{a}	100.0±0.0^{a}	1.71	3.03	0.97±0.06
Eugenol	0.0±9.2	49.9±5.4^{a}	57.2±8.9^{a}	70.1±6.1^{a}	87.5±3.6^{a}	93.5±7.1^{a}	100.0±0.0^{a}	1.20	2.47	0.72±0.05
Citral	0.0±9.2	8.1±5.7^{b}	19.5±8.2^{c}	23.1±8.9^{c}	29.0±8.2^{c}	50.7±2.7^{b}	79.5±5.1^{b}	2.80	4.60	0.72±0.06

[1/] Means±SD in column followed by the same common letter were not significantly different ($P<0.05$) according to DMRT

4.2.3 Examination of essential oils fumigant toxicity against thrips (*Frankliniella schultzei* (Trybom) and *Thrips palmi* Karny)

EOs of clove, cinnamon and lemon grass at 3.0 µl/L air were highly toxic against *F. schultzei* and *T. palmi*. All EOs resulted in a complete mortality in adults of *T. palmi* (100%), while 84.3-98.0% mortalities were obtained from adult of *F. schultzei* (Table 4.9).

Table 4.9 Mortality percentages of adult thrips (*Frankliniella schultzei* (Trybom) and *Thrips palmi* Karny) at 24 h after fumigations with clove, cinnamon and lemon grass EOs at 3.0 µl/L air.

Plant essential oils	% Mortality [1/]	
	F. schultzei	*T. palmi*
Clove	98.0±4.8[a]	100.0±0.0[a]
Cinnamon	84.3±6.[b]	100.0±0.0[a]
Lemon grass	96.1±6.1[a]	100.0±0.0[a]

[1/] Means±SD in column followed by the same common letter were not significantly different (P<0.05) according to DMRT

4.3 Examination of essential oils on plant physiological changes

4.3.1 Effect of essential oil on plant physiological changes in cut orchid flower

4.3.1.1 Primary test

Changes in color (L*, a* and b* values) of cut orchid flowers at 3 day after fumigation with clove, cinnamon, lemon grass and cassumunar ginger essential oils at 3.0 µl/L air for 1 h with all-time air circulation were examined. L* value of cut orchid flower fumigated by clove EO (24.59) was not significantly different from the control (21.81) and cinnamon EO (26.25), while significantly different results were observed in the flower fumigated with lemon grass and cassumunar ginger EOs (30.69 and 31.94, respectively). Besides, a* and b* values obtained from all EO fumigations were between 35.93 and 38.93, and -14.14 and -13.43, respectively, and the results were not significantly different from the control (38.98 and -13.37, respectively). All EO fumigations showed significant differences in percentages of weight loss (6.83-8.57%) when compared to the control (5.70%). (Table 4.10)

Table 4.10 The L*, a* and b* values of cut orchid flowers at 3 day after fumigation with clove, cinnamon, lemon grass and cassumunar ginger essential oils at 3.0 µl/L air.

Plant essential oils	Means[1/]±SD			
	L* value	a* value	b* value	%Weight Loss
Control (not treated)	21.81±1.39[c]	38.98±2.94[a]	-13.37±1.56[a]	5.70±0.40[c]
Clove	24.59±1.02[bc]	35.93±2.47[a]	-13.43±0.51[a]	6.83±0.54[b]
Cinnamon	26.25±0.81[b]	35.95±0.85[a]	-13.87±1.54[a]	7.80±0.45[ab]
Lemon grass	30.69±1.50[a]	38.62±2.32[a]	-14.14±0.81[a]	8.57±0.49[a]
Cassumunar ginger	31.94±2.66[a]	37.57±1.88[a]	-13.90±0.59[a]	8.40±0.29[a]

[1/] Means±SD in column followed by the same common letter were not significantly different (P<0.05) according to DMRT

In summary, the results of color change examination revealed that clove and cinnamon EOs presented non-significantly different physiological changes in the orchid when comparing to the control. Lower percentages of weight loss were observed in clove and cinnamon EO fumigations when compared to the other EOs. Consequently, phytotoxicities or physiological change effect of the 2 EOs at various concentrations were further examined in the secondary test.

4.3.1.2 Secondary test

The cut orchid flowers fumigated with clove and cinnamon EOs at concentrations of 3.0, 4.5 and 6.0 µl/L air were totally spoiled before day 6. Consequently, only the results from day 3 examinations were observed and presented (Table 4.11). In this test, the concentration that presented lowest physiological changes in the flowers would be selected for further experiments. Therefore, the observation focused only on the results that were not significantly different from the control.

Clove EO at the concentrations of 2.0 and 3.0 µl/L air (L^*= 23.10 and 23.31, respectively) and cinnamon EO at the concentration of 2.0 µl/L air (L^*=23.99) presented no significant differences ($P<0.05$) in L^* value when comparing to the control (L^*=23.50). In addition, a^* and b^* values from all treatments were generally ranged from 35.93 to 38.98 and -14.53 to -13.04, respectively, with no significant differences ($P<0.05$) when comparing to the control, nor among the different concentrations. Percentages of weight loss from clove EO at 2.0 and 3.0 µl/L air (6.74 and 6.44%, respectively) were not significantly different from the control (5.38%). On the other hand, the values obtained from cinnamon EO at all concentrations were significantly different from the control.

It could be summarized that the EO concentration of clove and cinnamon at 3 µl/L air was the minimum concentration that presented observable physiological changes in the cut orchid flowers in all parameters. Consequently, the EOs at 3 µl/L air were further applied in the tertiary test to investigate plant physiological changes of different EO formulas on the cut orchid flowers.

Table 4.11 The L*, a* and b* values and percentages of weight loss of cut orchid flowers on day 3 after fumigation with essential oils of clove and cinnamon at various concentrations.

Plant essential oils	Concentrations (µl/L air)	Means[1/] ±SD			
		L* value	a* value	b* value	%Weight Loss
Control	0	21.50±2.75^{d}	38.98±2.94^{a}	-13.28±1.28^{a}	5.38±0.31^{f}
Clove	2.0	23.10±0.88^{cd}	37.55±5.07^{a}	-13.82±2.47^{a}	6.74±0.55^{def}
	3.0	23.31±1.68^{bcd}	35.93±2.47^{a}	-13.10±1.68^{a}	6.44±1.20^{ef}
	4.5	24.87±1.24^{bc}	36.75±0.30^{a}	-13.04±1.12^{a}	10.61±0.54^{bc}
	6.0	32.25±1.54^{a}	37.25±1.54^{a}	-14.53±1.98^{a}	15.12±1.22^{a}
Cinnamon	2.0	23.99±1.35^{bcd}	36.05±1.91^{a}	-14.11±1.43^{a}	7.55±1.79^{de}
	3.0	25.10±1.45^{bc}	35.95±0.85^{a}	-14.20±0.27^{a}	8.67±0.43^{cd}
	4.5	26.31±0.59^{b}	38.62±2.32^{a}	-14.14±0.81^{a}	12.44±1.02^{b}
	6.0	32.57±1.88^{a}	37.57±1.88^{a}	-14.24±1.26^{a}	15.07±1.94^{a}

[1/]Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.

4.3.1.3 Tertiary test

In this experiment, effect of mixtures between clove and cinnamon EOs on plant physiological changes at different ratios (4:0, 3:1, 2:2, 1:3 and 0:4 represented by Cl4Ci0, Cl3Ci1, Cl2Ci2, Cl1Ci3 and Cl0Ci4, respectively) at 3.0 µl/L air and standard eugenol (Eu) were investigated. In general, L* and b* values from all formulas presented no significant differences (P<0.05) when comparing to the control. Non-significantly different a* values were obtained from formulas Cl4Ci0 (36.93) and Cl1Ci3 (37.95) when comparing to the control (38.98). Non-significant differences in percentages of weight loss was obtained from the formula Cl4Ci0 (6.44%) when comparing to the control (5.39%) (Table 4.12).

In summary, the results of all parameters from formula Cl4Ci0 were not significantly different from the control, whereas in formula Cl1Ci3, only L*, a* and b* values were not significantly different from the control. Although the percentage of weight loss obtained from formula Cl1Ci3 was significantly different from control, no significant differences were observed when comparing to formula Cl4Ci0. Consequently, these two formulas were selected for further experiment on examination of vase life and other plant physiological change parameters.

Table 4.12 The L*, a* and b* values and percentage of weight loss of cut orchid flowers on day 3 after fumigation with essential oil formulas and standard eugenol at 3.0 µl/L air.

Formulas[2/]	Means[1/] ±SD			
	L* value	a* value	b* value	%Weight Loss
Control	21.50±2.75^{a}	38.98±2.94^{a}	-13.28±1.28^{a}	5.39±0.31^{d}
Cl4Ci0	23.31±1.68^{a}	36.93±0.74^{abc}	-11.77±1.02^{a}	6.44±1.20^{cd}
Cl0Ci4	25.10±1.45^{a}	35.95±0.85^{bc}	-14.20±0.27^{a}	8.67±0.43^{b}
Cl1Ci3	22.58±1.09^{a}	37.95±1.01^{ab}	-12.35±1.70^{a}	6.75±0.11^{c}
Cl2Ci2	24.56±2.17^{a}	35.14±0.55^{c}	-12.11±0.94^{a}	10.37±0.37^{a}
Cl3Ci1	24.87±1.53^{a}	35.45±0.82^{c}	-12.66±1.43^{a}	9.26±1.02^{ab}
Eugenol	25.94±1.73^{a}	35.02±0.42^{c}	-13.09±1.73^{a}	10.22±0.44^{a}

[1/]Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.

[2/]Clove : cinnamon ratios at 4:0, 3:1, 2:2, 1:3 and 0:4 formulas represented by Cl4Ci0, Cl3Ci1, Cl2Ci2, Cl1Ci3 and Cl0Ci4, respectively.

4.3.1.4 Quaternary test

In this experiment, physiological changes in cut orchid flowers caused by the EO formulas Cl4Ci0 and Cl1Ci3 at 2.0 µl/L air was investigated. Remarkably, examinations of 3.0 µl/L air EOs were excluded since the primary test (4.3.1.1). Obtained result revealed that the flowers fumigated with EOs at this concentration were spoiled before day 6 observations. Figure 4.9 demonstrates plant physiological change due to formula Cl4Ci0 and Cl1Ci3 at 2.0 µl/L air from 1-4 h fumigations. Plant physiological changes was indicated by L*, a* and b* values and percentages of weight loss throughout the 12 days after fumigation (3 days interval observations). The results showed that the flowers fumigated with formula Cl4Ci0 for 1-4 h resulted in only 6 days vase life. The longest vase life was obtained from the flowers fumigated with of formula Cl1Ci3 for 1-3 h (12 days), while 4 h fumigation resulted in 9 days vase life. The L*, a* and b* values obtained from formula Cl1Ci3 on day 12 after treatment were ranged from 30.10 to 30.75, 25.36 to 32.47 and -15.13 to -9.11, respectively (Figure 4.9 A-C). The percentages of weight loss obtained from the formula on day 12 after the treatments (from 1, 2 and 3 h fumigations) were ranged from 25.26 to 32.76% (Figure 4.9 D). Consequently, 1-3 h fumigations of formula Cl1Ci3 were selected for the next experiment. The Figure 4.10 showed quality changes or symptoms of cut orchid flowers on day 0-12 after fumigations with EO formulas at 2.0 µl/L air.

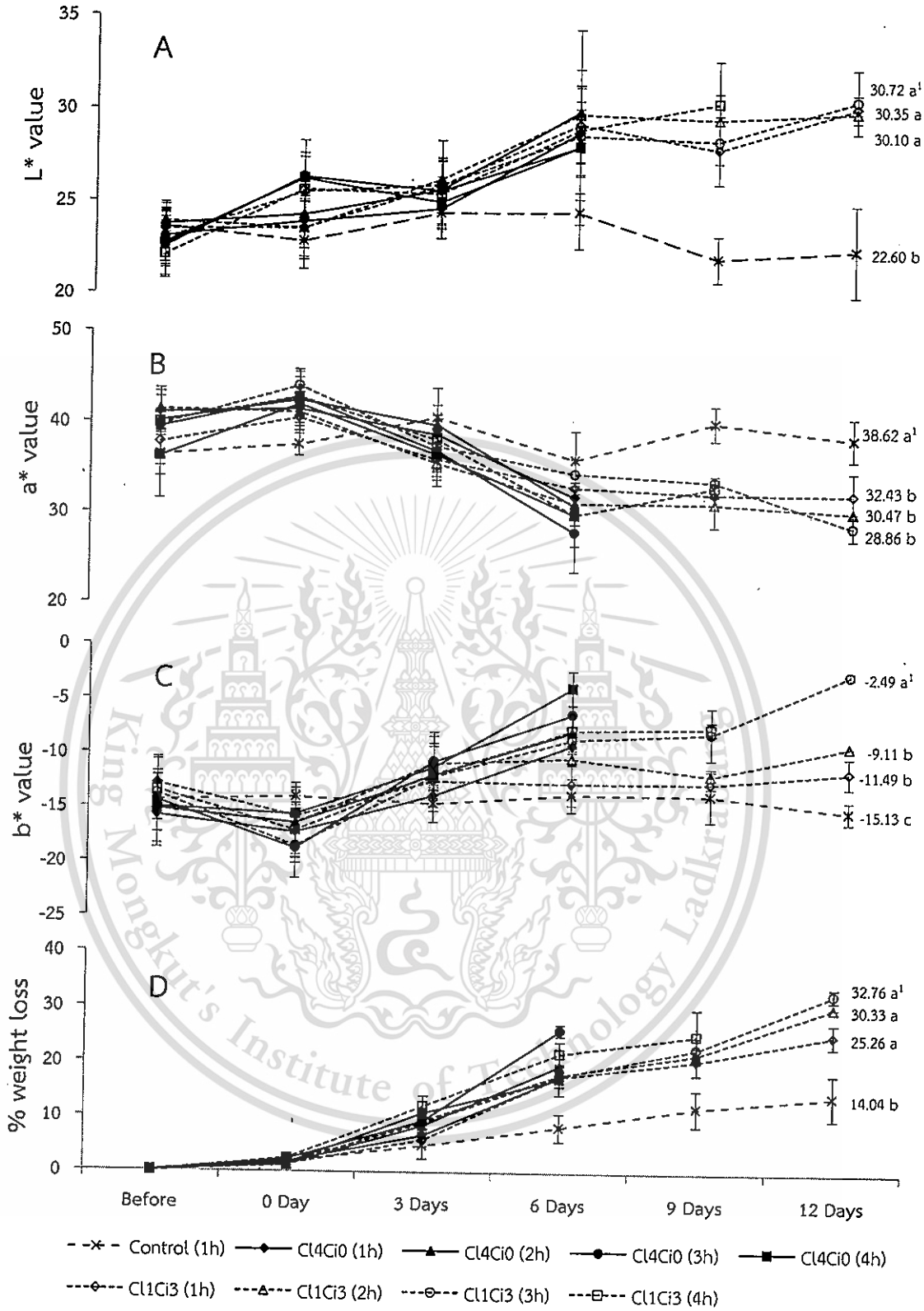


Figure 4.9 L*, a* and b* values (A-C) and percentage of weight loss (D) of cut orchid flowers on day 0-12 after fumigation with essential oil formulas at 2.0 µl/L air at various all-time air circulations. Clove : cinnamon ratios at 4:0 and 1:3 formulas represented by Cl4Ci0 and Cl1Ci3 respectively ([1]Means with the same common letter were not significantly different (P<0.05) according to DMRT).

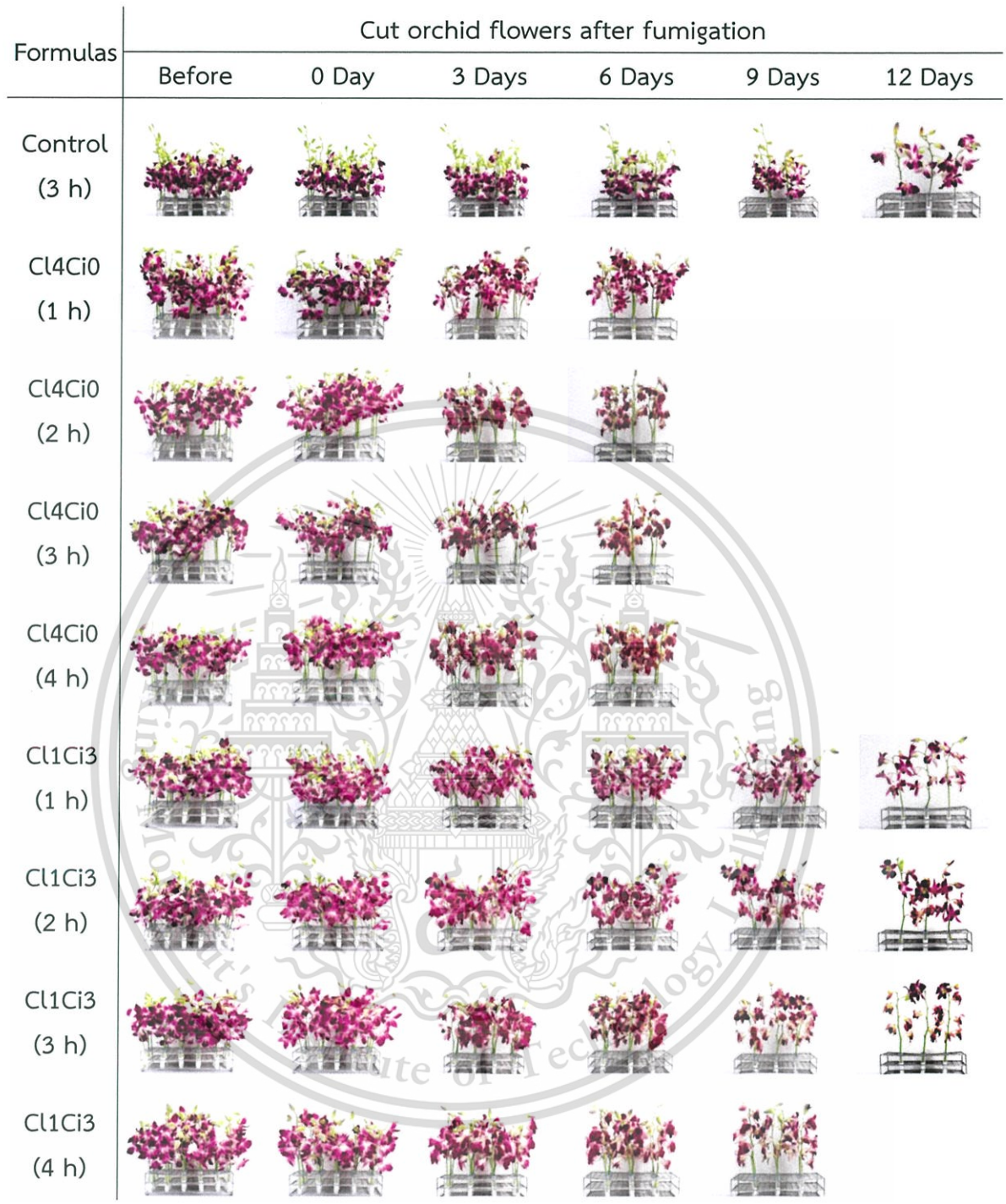


Figure 4.10 The cut orchid flower symptoms on day 0-12 after fumigations with essential oil formulas at 2.0 µl/L air at various all-time air circulations. Clove : cinnamon ratios at 4:0 and 1:3 formulas represented by Cl4Ci0 and Cl1Ci3 and Cl0Ci4, respectively.

4.3.1.4 Quinary test

It was hypothesized in the previous test that air circulation periods presented some effects of EO-fumigation on physiological changes in cut orchid flower. Therefore, the effects of different air circulation periods (15-min and all-time) were investigated. In particular, changes in L*, a* and b* values, percentages of weight loss, PPO activity, phenolic contents and anthocyanin contents of the flower fumigated with formula Cl1Ci3 at 2.0 µl/L air for 2 and 3 h were examined. The results showed that, L*, a* and b* values and percentage of weight loss obtained from 15-min air-circulated fumigation presented no significantly different physiological changes in the treated flower throughout the storage period (12 days) in vase solution when comparing to the control (Figure 4.11A-D). In contrast, all-time air-circulated fumigation presented higher plant physiological changes (as demonstrated earlier in the tertiary test).

Examinations of PPO activity, phenolic contents and anthocyanin contents of the cut orchid flower fumigated with formula Cl1Ci3 at 2.0 µl/L air with 15-min air-circulation for 2 and 3 h were conducted. The results showed that PPO activities and phenolic contents obtained on day 12 from all treatments were not significantly different when comparing to the control (Figure 4.12 A). Initially, the pre-treatment phenolic contents in the cut orchid flower were 5-10 mg/g fresh weight. On day 3 after fumigations, the phenolic content increased instantly to 15-20 mg/g fresh weight, and remained unchanged throughout the 12 days after fumigation (Figure 4.12 B). Similarly, total anthocyanin content obtained from all fumigations was constant at about 20-40 mg/100 g fresh weight throughout the 12 days in vase solution (Figure 4.12 C). Figure 4.13 shows quality changes of the cut orchid flower on 0-12 days after fumigation with EO formula Cl1Ci3 at 2.0 µl/L air.

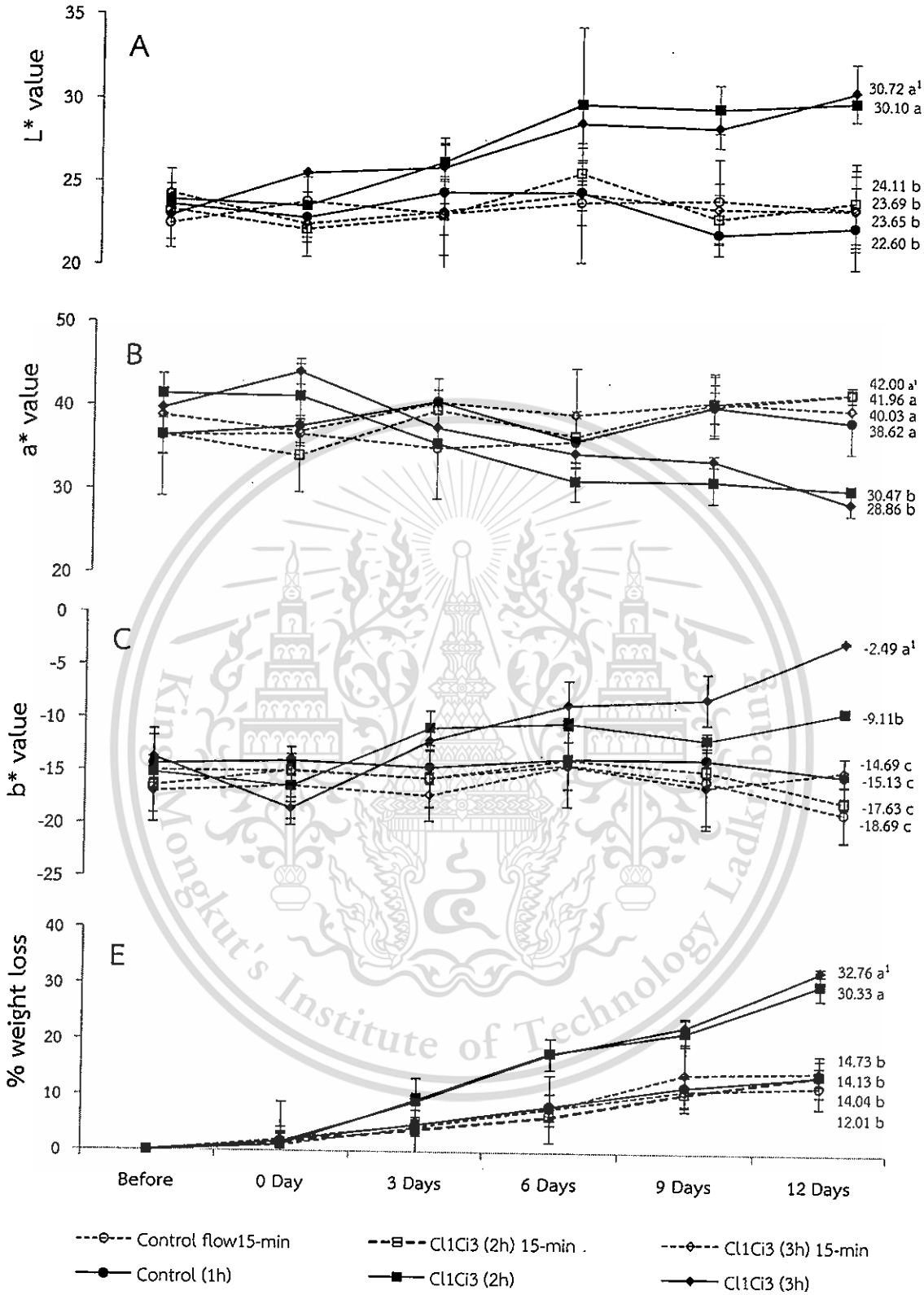


Figure 4.11 L*, a* and b* values (A-C) and percentage of weight loss (D) of cut orchid flowers at 0, 3, 6, 9 and 12 days after fumigations with essential oil formula Cl1Ci3 at 2.0 µl/L air (clove : cinnamon ratio 1:3), with all-time and 15-min air circulations ([1]Means with the same common letter were not significantly different ($P<0.05$) according to DMRT).

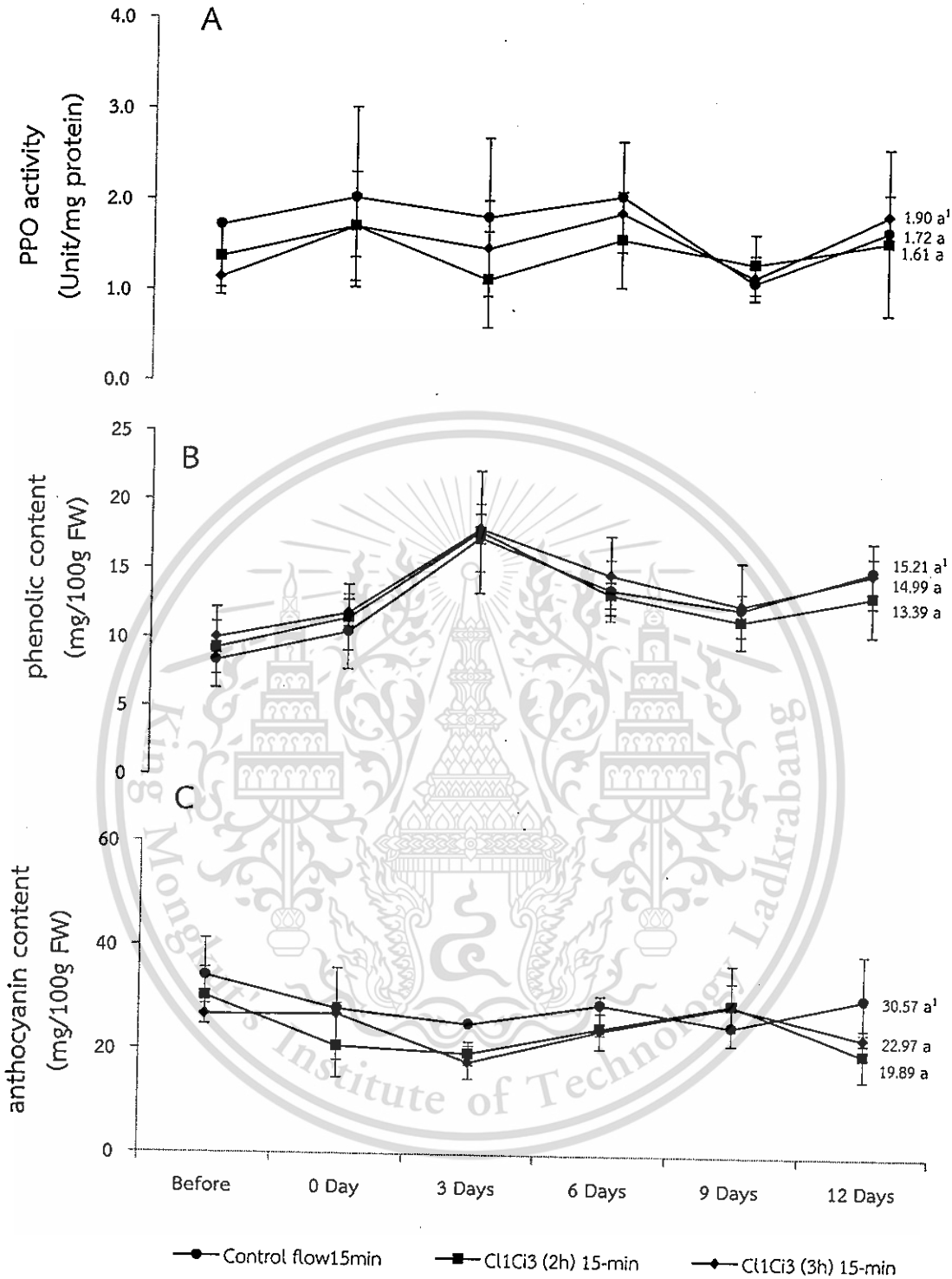


Figure 4.12 Polyphenol oxidase (PPO) activity (A), phenolic (B) and anthocyanin (C) contents of cut orchid flowers at 0, 3, 6, 9 and 12 days after fumigations with essential oil formula Cl1Ci3 at 2.0 µl/L air (clove : cinnamon ratio 1:3), with all-time and 15-min air circulations ([1]Means with the same common letter were not significantly different ($P<0.05$) according to DMRT).

Formulas	Cut orchid flowers after fumigation					
	Before	0 Day	3 Days	6 Days	9 Days	12 Days
Control (15min-3 h)						
Control (2 h)						
Cl1Ci3 (3 h)						
Cl1Ci3 (3 h)						
Cl1Ci3 (15min-2h)						
Cl1Ci3 (15min-3h)						

Figure 4.13 The cut orchid flower symptoms at 0, 3, 6, 9 and 12 days after fumigations with essential oil formula Cl1Ci3 (clove : cinnamon ratio 1:3) at 2.0 µl/L air by fumigation method, with the all-time and 15-min air circulations.

4.3.2 Effect of essential oil on plant physiological changes in holy basil and eggplant

4.3.2.1 Primary test

Changes in colors (L*, a* and b* values) of holy basil and eggplant were examined on day 3, after fumigations with EOs from clove, cinnamon, lemon grass and cassumunar ginger at the concentration of 3.0 µl/L air for 1 h with 15-min air circulation. It was found that b* values of holy basil fumigated with clove and lemon grass EOs were 24.67 and 24.33, respectively, and the results were not significantly different from the control (24.57). On the other hand, b* values obtained from holy basil fumigated with cinnamon and cassumunar ginger (22.32 and 23.40, respectively) were significantly different from the control. In addition, L* and a* values obtained from holy basil fumigated with all 4 EOs

(between 41.53 and 44.77, and -12.20 and -11.17, respectively) were not significantly different from the control (43.41 and -11.85, respectively) (Table 4.13)

Eggplants fumigated with EOs from clove, cinnamon and lemon grass presented a* values at -5.90, -5.70 and -5.97, respectively. These values were not significantly different from the control (-6.50). Similarly, the obtained b* values (20.37, 20.87 and 21.30, respectively) presented no significant differences when comparing to the control (20.87). However, a* and b* values (-5.20 and 19.53, respectively) obtained from the vegetables fumigated with cassumunar ginger EO were significantly different from the control. The L* values obtained from all EO-fumigations (between 73.10 and 73.67) were not significantly different from the control (73.87) (Table 4.13).

Table 4.13 The L*, a* and b* values of holy basil and eggplant on day 3 after fumigation with clove, cinnamon, lemon grass and cassumunar ginger essential oils at 3.0 µl/L air.

Plant essential oils	Means[1/]±SD					
	Holy basil			Eggplant		
	L* value	a* value	b* value	L* value	a* value	b* value
Control	43.41±1.05[a]	-11.85±0.79[a]	24.57±0.86[ab]	73.87±0.76[a]	-6.50±0.50[b]	20.87±0.71[a]
Clove	43.77±1.25[a]	-11.33±0.57[a]	24.67±0.61[a]	73.10±0.66[a]	-5.90±0.17[ab]	20.37±0.55[ab]
Cinnamon	44.77±1.00[a]	-11.17±0.76[a]	22.32±0.59[c]	73.17±0.57[a]	-5.70±0.66[ab]	20.87±0.12[a]
Lemon grass	42.53±0.50[a]	-11.20±0.20[a]	24.33±0.58[ab]	73.63±0.55[a]	-5.97±0.15[ab]	21.30±0.70[a]
Cassumunar ginger	41.53±1.75[a]	-12.20±0.85[a]	23.40±0.53[bc]	73.83±0.76[a]	-5.20±0.44[a]	19.53±0.61[b]

[1/]Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.

In summary, the results obtained from color change examinations in holy basil and eggplant fumigated with clove, cinnamon, lemon grass and cassumunar ginger at 3.0 µl/L air for 2 h with 15-min air circulation revealed that clove and lemon grass EOs presented non-significantly different physiological changes in the vegetables when comparing to the control. Thus these two EOs were selected for further experiments.

4.3.2.2 Secondary test

Color change parameters (L*, a* and b* values) of holy basil and eggplant fumigated with EOs formulas of clove and lemon grass (Cl4Le0, Cl3Le1, Cl2Le2, Cl1Le3 and Cl0Le4) with 15-min air circulation for 2 and 3 h were examined on day 3 after treatments. The results showed that L*, a* and b* values from all formulas were between 39.56 and 44.07, -10.87 and -9.63, and 23.51 and 36.13, respectively. The results were not significantly different when comparing to the control (38.85 and 40.69, -10.26 and -9.86,

respectively). However, when considering percentage of weight loss, it was found that EO formulas Cl4Le0, Cl2Le2 and Cl1Le3 presented 16.89-21.75% weight loss, which were not significantly different when comparing to the controls (16.41-17.79%) (Table 4.14).

In eggplant, the obtained L*, a* and b* values, and firmness were between 71.68 and 75.43, -7.56 and -5.97, 20.14 and 23.89, and 83.60 N and 94.50 N, respectively. The results were not significantly different when comparing to the controls (72.23 and 73.82, -6.04 and -5.75, 20.76 and 21.74, and 90.93 N and 98.11 N, respectively). However, when considering percentage of weight loss, it was found that only EO formulas Cl3Le1 and Cl1Le3 presented non-significantly different results (1.38-1.63%) when comparing to the controls (1.29-1.30%) (Table 4.15).

Table 4.14 The L*, a* and b* values and percentage of weight loss of holy basil on day 3 after fumigation with essential oil formulas at 3.0 µl/L air with 15-min air circulation for 2 and 3 h fumigation.

Formulas[2/], Fumigation time (h)	Means[1/]±SD			
	L* value	a* value	b* value	%Weight Loss
Control, 2 h	40.69±2.84^{a}	-10.26±0.64^{a}	23.69±1.98^{a}	16.41±4.29^{c}
Cl4Le0, 2h	44.07±1.19^{a}	-10.87±0.16^{a}	26.13±0.64^{a}	16.89±7.38^{c}
Cl3Le1, 2 h	42.37±1.12^{a}	-10.62±0.73^{a}	24.95±1.66^{a}	19.65±5.23^{bc}
Cl2Le2, 2 h	40.88±2.38^{a}	-9.92±1.11^{a}	23.90±2.81^{a}	20.35±5.65^{bc}
Cl1Le3, 2 h	41.94±2.46^{a}	-10.12±0.82^{a}	25.86±2.71^{a}	17.67±3.94^{c}
Cl0Le4, 2 h	41.50±3.19^{a}	-10.24±0.77^{a}	23.51±2.31^{a}	21.17±3.26^{bc}
Control, 3 h	38.85±1.64^{a}	-9.86±0.17^{a}	24.74±0.80^{a}	17.49±4.25^{c}
Cl4Le0, 3 h	42.41±1.76^{a}	-10.50±0.32^{a}	25.80±1.99^{a}	19.27±5.06^{bc}
Cl3Le1, 3 h	42.41±3.62^{a}	-10.02±0.59^{a}	26.04±2.85^{a}	28.56±4.39^{ab}
Cl2Le2, 3 h	42.34±2.15^{a}	-9.89±0.29^{a}	24.26±1.55^{a}	21.75±3.77^{bc}
Cl1Le3, 3 h	39.56±6.34^{a}	-10.12±0.97^{a}	25.83±3.25^{a}	18.05±2.63^{c}
Cl0Le4, 3 h	40.07±3.90^{a}	-9.63±0.63^{a}	25.32±1.12^{a}	31.38±9.51^{a}

[1/] Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.

[2/] Clove : Lemon grass ratio 4:0, 3:1, 2:2, 1:3 and 0:4 represented as formulas Cl4Le0, Cl3Le1, Cl2Le2, Cl1Le3 and Cl0Le4, respectively.

Table 4.15 The L*, a* and b* values, percentage of weight loss, and firmness of eggplant on day 3 after fumigation with essential oil formulas at 3.0 µl/L air.

Formulas[2/], Fumigation time (h)	Means[1/]±SD				
	L* value	a* value	b* value	%Weight Loss	Firmness (N)
Control, 2 h	73.82±3.19^{a}	-5.75±0.75^{a}	20.76±1.77^{a}	1.30±0.05^{c}	90.93±6.32^{a}
Cl4Le0, 2h	73.74±0.92^{a}	-7.35±0.37^{a}	22.53±1.27^{a}	1.59±0.23^{abc}	82.60±5.94^{a}
Cl3Le1, 2 h	71.68±1.11^{a}	-7.56±0.16^{a}	23.89±0.93^{a}	1.62±0.21^{abc}	90.57±4.65^{a}
Cl2Le2, 2 h	75.43±2.82^{a}	-5.97±0.53^{a}	20.14±1.56^{a}	1.62±0.20^{abc}	91.27±8.04^{a}
Cl1Le3, 2 h	72.89±2.76^{a}	-6.41±1.06^{a}	22.32±1.16^{a}	1.42±0.10^{bc}	87.06±8.06^{a}
Cl0Le4, 2 h	74.23±1.58^{a}	-6.50±0.52^{a}	21.79±0.22^{a}	1.75±0.16^{ab}	86.18±1.61^{a}
Control, 3 h	72.33±3.09^{a}	-6.04±1.05^{a}	21.74±1.93^{a}	1.29±0.04^{c}	98.11±9.67^{a}
Cl4Le0, 3 h	72.26±1.16^{a}	-6.47±0.64^{a}	21.94±1.35^{a}	1.71±0.26^{ab}	91.10±3.50^{a}
Cl3Le1, 3 h	71.68±1.88^{a}	-6.81±0.38^{a}	23.46±1.06^{a}	1.63±0.14^{abc}	94.50±10.65^{a}
Cl2Le2, 3 h	71.93±1.03^{a}	-6.29±0.50^{a}	21.87±0.77^{a}	1.78±0.28^{a}	89.30±2.95^{a}
Cl1Le3, 3 h	72.86±0.47^{a}	-6.43±0.14^{a}	22.25±0.48^{a}	1.38±0.09^{c}	91.87±6.05^{a}
Cl0Le4, 3 h	72.80±0.76^{a}	-6.48±0.59^{a}	22.57±1.20^{a}	1.78±0.14^{a}	85.54±6.50^{a}

[1/] Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.

[2/] Clove : Lemon grass ratio 4:0, 3:1, 2:2, 1:3 and 0:4 represented as formulas Cl4Le0, Cl3Le1, Cl2Le2, Cl1Le3 and Cl0Le4, respectively.

In summary, the results obtained from color change and firmness examinations of holy basil and eggplant fumigated with different EO formulas showed that Cl1Le3 presented the least physiological changes in the plants and the results were not significantly different when comparing to the controls. Consequently, this EO formula (Cl1Le3) was further tested for possible effects on color changes, %weight loss, firmness, total chlorophyll content, phenolic content and PPO activity of plants.

4.3.2.3 Tertiary test

Plant physiological changes examination of Cl1Le3 fumigation 2 and 3 h with 15-min air circulation on holy basil and eggplant presented that the observed shelf life of holy basil was not significantly different from the control (6 days). Similarly, L*, a* and b* values obtained from the treatments and the controls on day 6 (40.55 to 41.06, -9.20 to -8.45, and 24.45 to 25.94, respectively) were in the same ranges and not significantly different (Figure 4.14 A-C). In addition, it was found that percentage of weight loss became higher for longer shelf life. On day 6, the obtained percentages of weight loss were ranged between 18.48 and 21.57%, and these results were not significantly different when comparing to the control (Figure 4.14 D).

Considering plant physiological changes due to Cl1Le3 on eggplant, it was found that on day 9 after treatment the obtained L*, a* and b* values of the treatments and the control were between 73.30 and 74.71, -5.56 and -5.41, and 21.63 and 21.63, respectively, with no significant differences (Figure 4.15 A-C). In addition, the percentage of weight loss became higher for longer storage. On day 9, the obtained percentages of weight loss were ranged between 1.74 and 2.11% (Figure 4.15 D). Remarkably, the percentage of weight loss in eggplant was approximately 10 times lower than the loss in holy basil. Moreover, the results showed that fruit firmness of eggplant fumigated with Cl1Le3 became lower for longer storage. On day 9 after treatment the obtained firmness of the treatments and the controls were in non-significantly different range (74.06 and 83.30 N) (Figure 4.15 E).

Results of the examinations of total chlorophyll content, total phenolic content and PPO activity of holy basil fumigated with Cl1Le3 for 2 and 3 h with 15-min air circulation showed that all values were similar to the control. However, on day 6, chlorophyll content of holy basil fumigated for 2 h was significantly higher than the value obtained from the 3 h fumigation (196.8 and 182.0 µg/g FW, respectively). Moreover, the chlorophyll contents obtained from the treatments were also higher than from the controls (194.9 and 171.4 µg/g FW, respectively) (Figure 4.16 A). Similarly, total phenolic content obtained from 2 h fumigation was significantly higher than the content obtained from 3 h fumigation (1,367 and 1,211 mg/100g FW, respectively), and both values were higher than the controls (1,280 and 1,255 mg/100g FW, respectively) (Figure 4.16 B). Likewise, PPO activity of holy basil fumigated for 2 h was significantly higher than of the 3 h fumigation groups (1.00 and 3.75 Unit/mg protein, respectively), while the values obtained from the controls (2 and 3 h) were 1.00 and 1.13 Unit/mg protein, respectively (Figure 4.16 C).

In eggplant, total chlorophyll content, total phenolic content and PPO activity were in congruence with the control. On day 9 after treatment, the values were not significantly different when comparing to the control (3.40-4.28 µg/g FW, 17.32-209.4 mg/g FW and 1.42-1.82 Unit/mg protein, respectively) (Figure 4.17). The Figure 4.18 and 4.19 showed quality changes as plant symptoms of holy basil and eggplant.

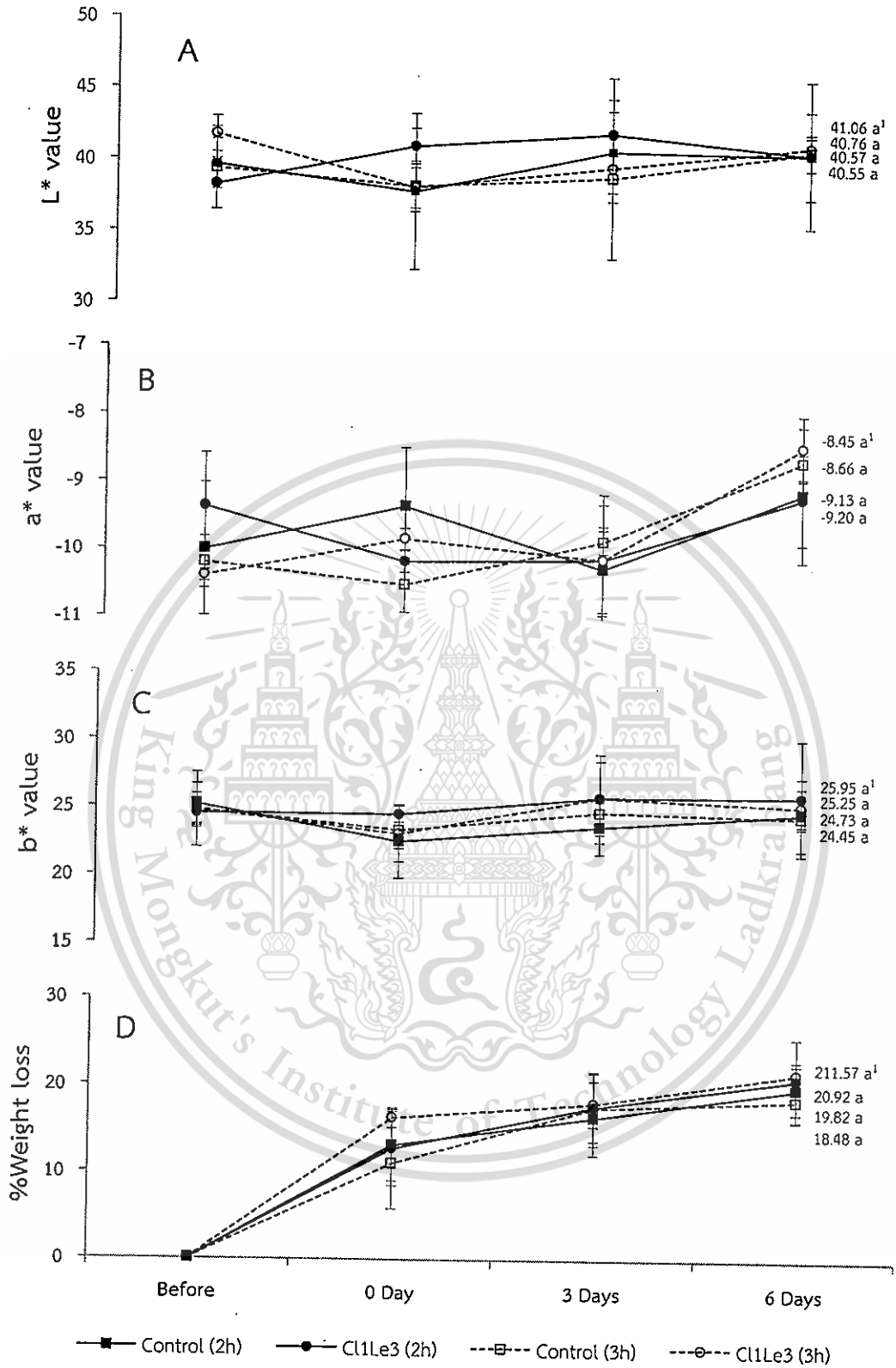


Figure 4.14 The L*, a* and b* values (A-C) and percentage of weight loss (D) of holy basil on day 0, 3 and 6 after fumigation with essential oil formula Cl1Le3 at 2.0 µl/L air (clove : lemon grass ratio 1:3), for 2 and 3 h with 15-min air circulation ([1]Means with the same common letter were not significantly different (P<0.05) according to DMRT).

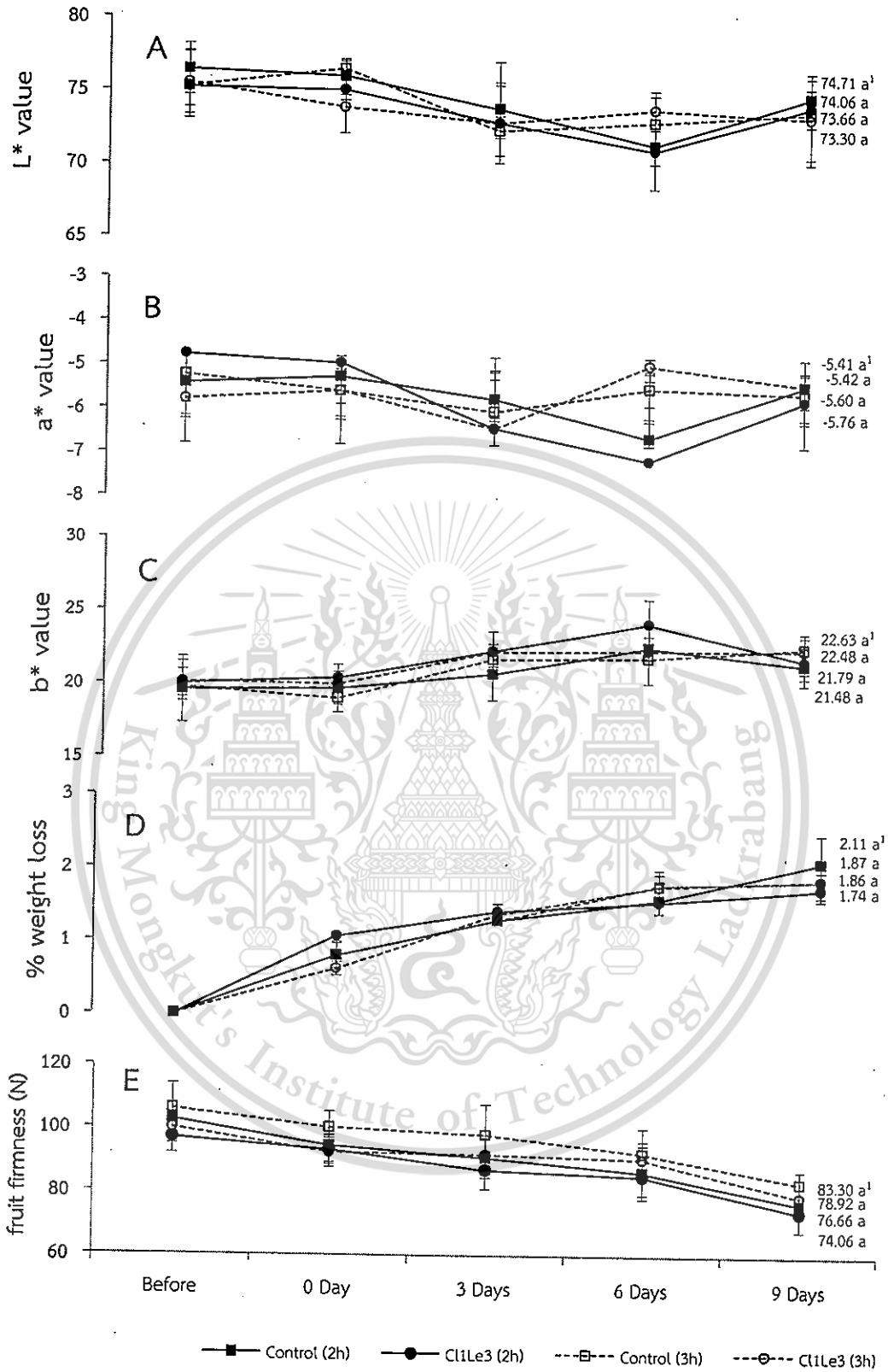


Figure 4.15 The L*, a* and b* values (A-C), percentage of weight loss (D), and firmness (E) of eggplant on day 0, 3, 6 and 9 after fumigation with essential oil formula Cl1Le3 at 2.0 µl/L air (clove : lemon grass ratio 1:3), for 2 and 3 h with 15-min air circulation ([1]Means with the same common letter were not significantly different ($P<0.05$) according to DMRT).

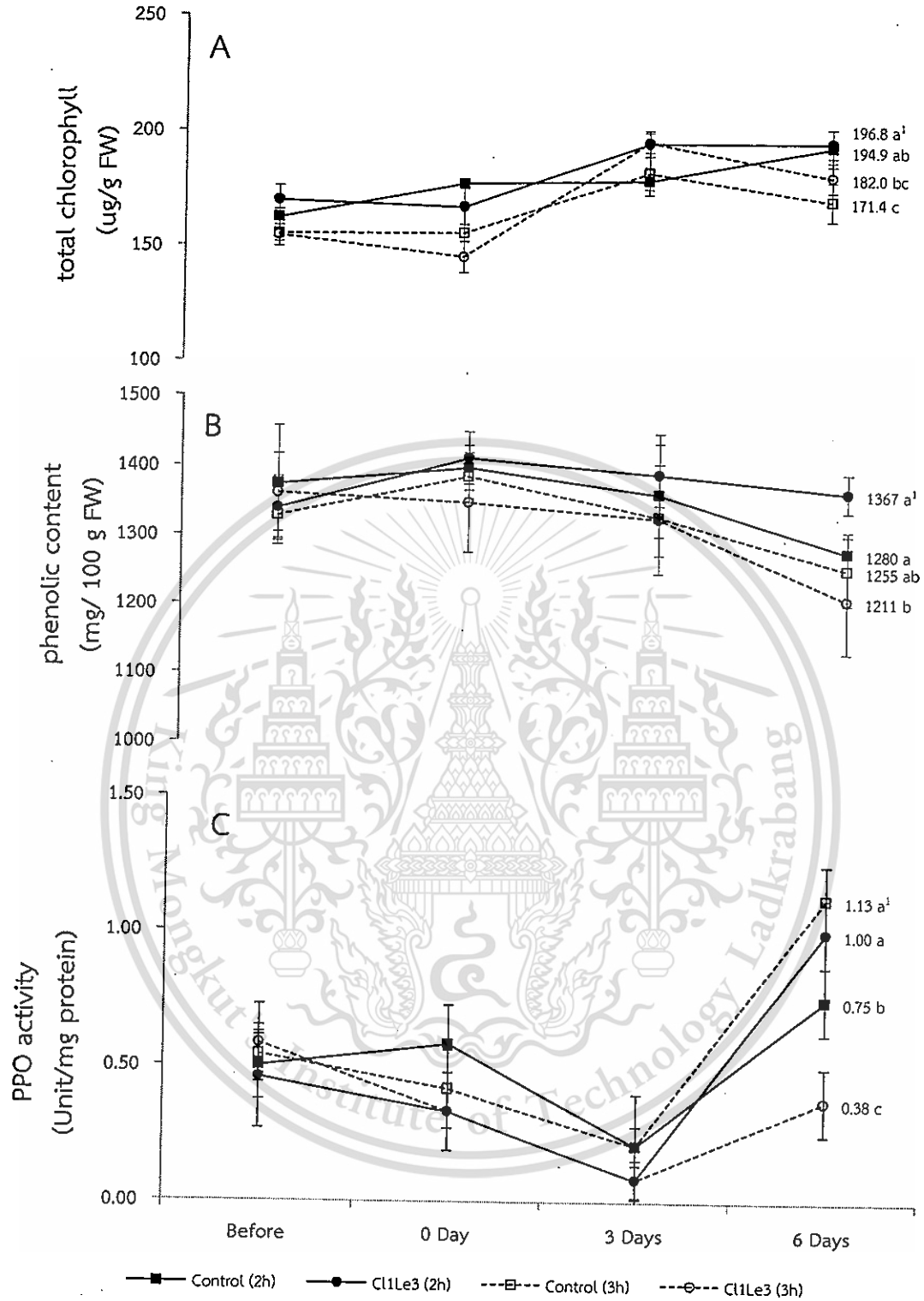


Figure 4.16 Total chlorophyll (A), phenolic (B) and polyphenol oxidase (PPO) activity (C) of holy basil treated with essential oil formula Cl1Le3 at 2.0 µl/L air (clove : lemon grass ratio 1:3) by fumigation method at 0, 3 and 6 days after treatment, for 2 and 3 h with 15-min air circulation ([1]Means with the same common letter were not significantly different ($P<0.05$) according to DMRT).

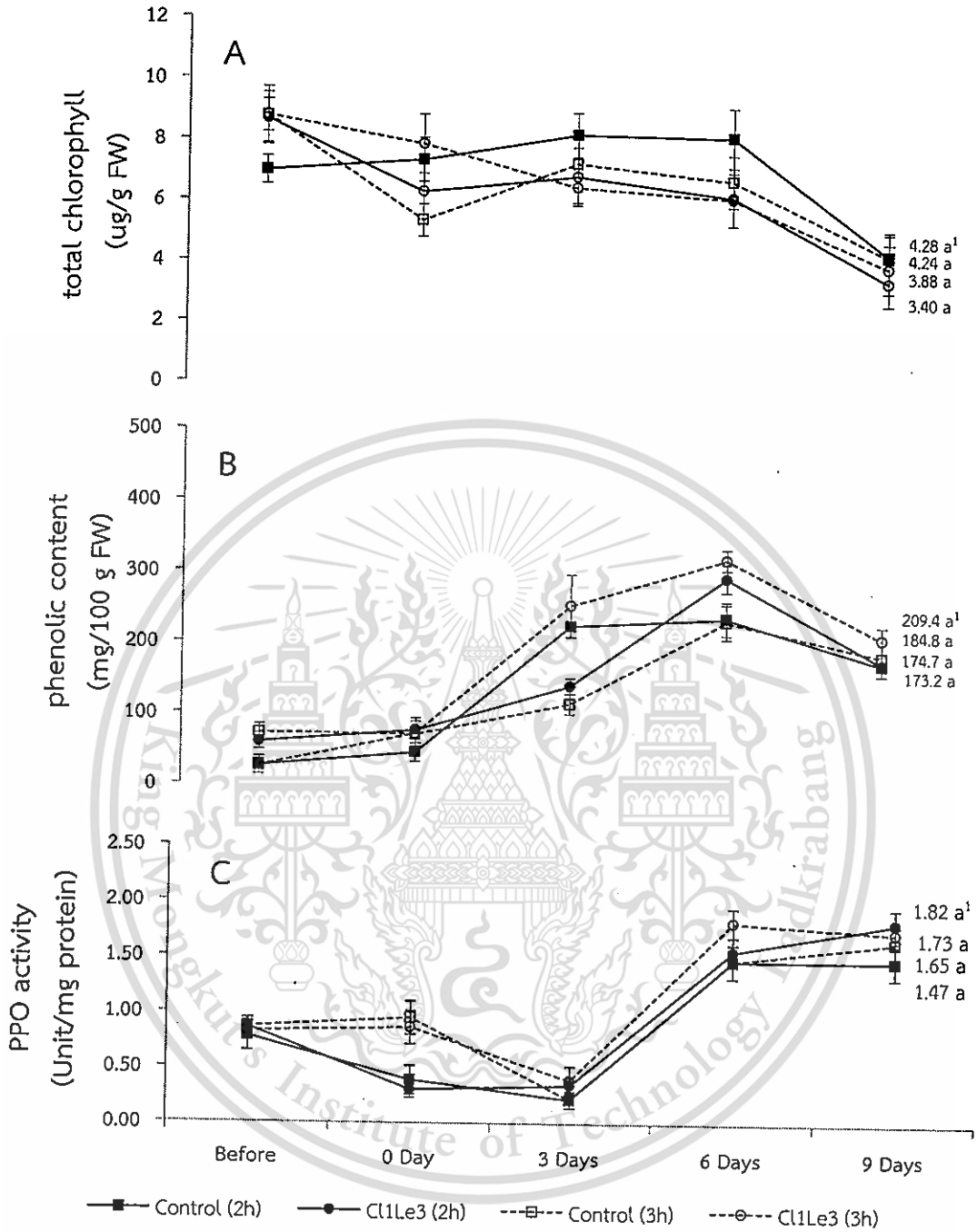


Figure 4.17 Total chlorophyll (A), phenolic (B) and polyphenol oxidase (PPO) activity (C) of eggplant on day 0, 3 and 6 after fumigation with essential oil formula Cl1Le3 at 2.0 µl/L air (clove : lemon grass ratio 1:3), for 2 and 3 h with 15-min air circulation ([1]Means with the same common letter were not significantly different ($P<0.05$) according to DMRT).

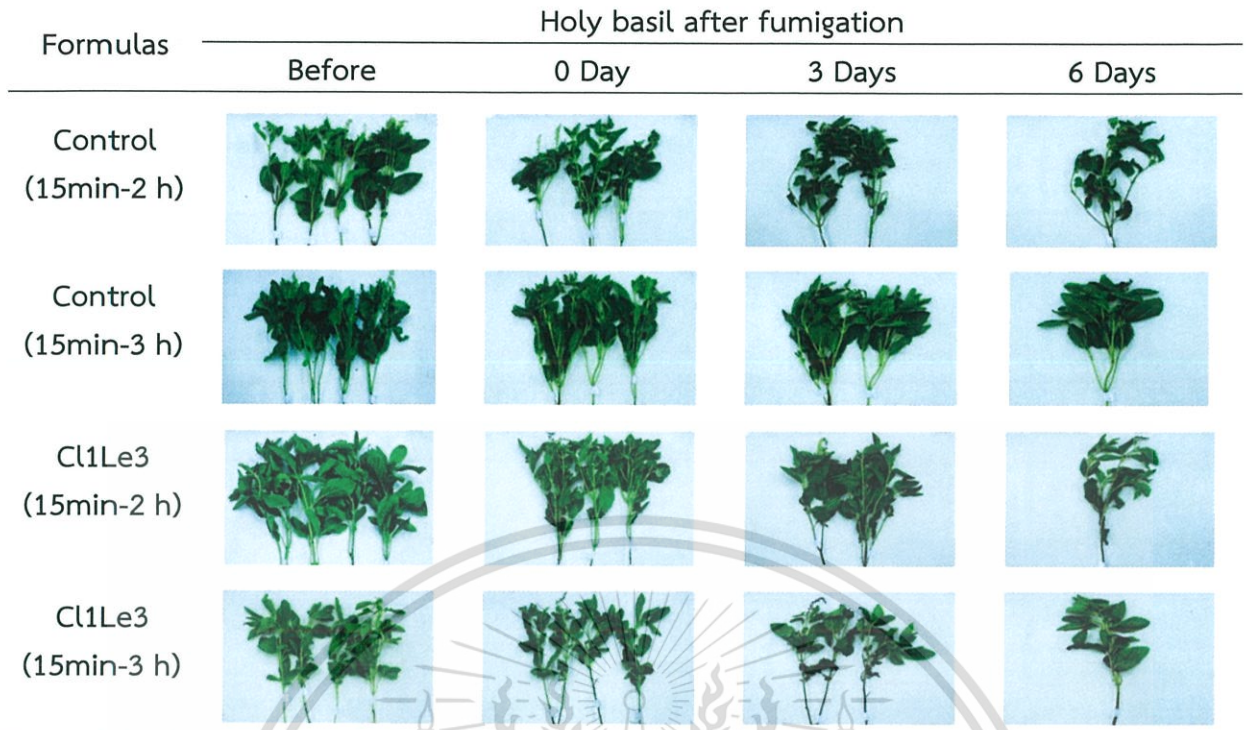


Figure 4.18 The holy basil symptoms on day 0, 3 and 6 after fumigation with essential oil formula Cl1Le3 at 2.0 µl/L air (clove : lemon grass ratio 1:3), for 2 and 3 h with 15-min air circulation by fumigation method.

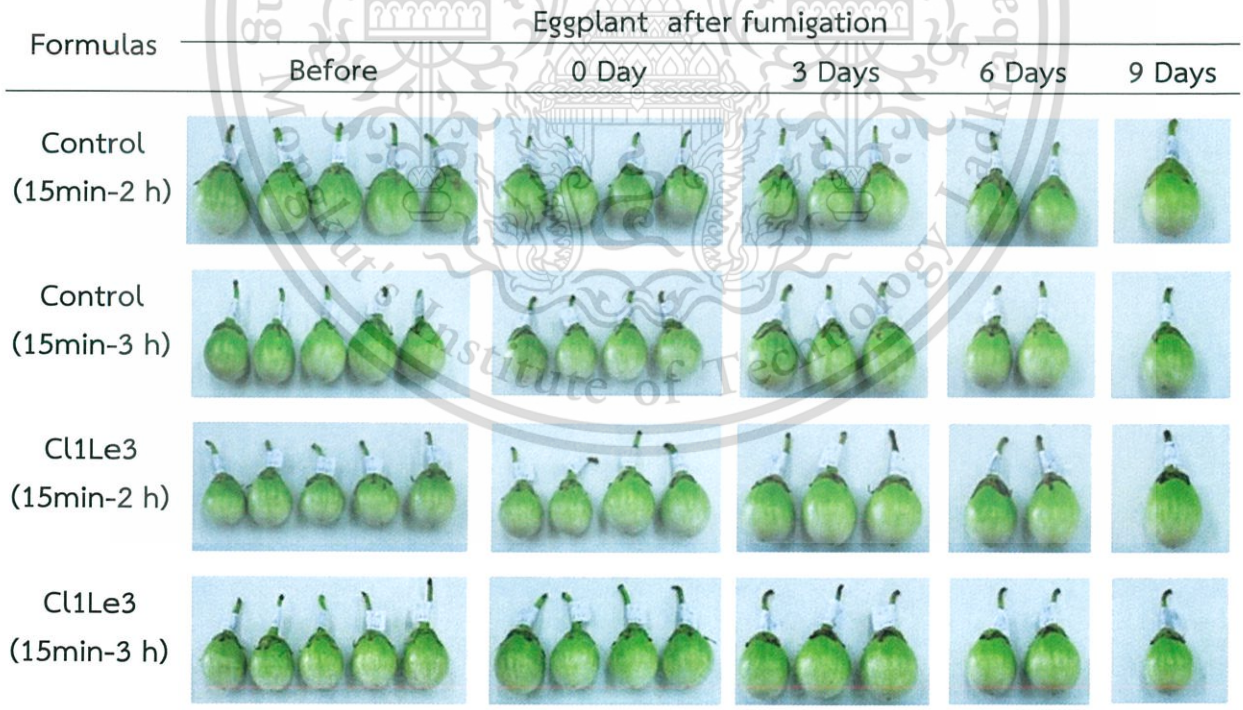


Figure 4.19 The eggplant symptoms on day 0, 3 and 6 after fumigation with essential oil formula Cl1Le3 at 2.0 µl/L air (clove : lemon grass ratio 1:3), for 2 and 3 h with 15-min air circulation by fumigation method.

4.4 Insecticidal activity experiment of essential oil formulation

Results of the fumigation bioassay conducted in fumigation chamber with the EO formulas Cl1Ci3 and Cl1Le3, which presented minimal physiological changes in orchid and vegetables in the previous experiment, at the concentration of 2.0 and 3.0 µl/L air for 2 h demonstrated a complete mortality in nymph of *P. jackbeardsleyi* fumigated with both EOs formulas at both concentrations. Likewise, no significant differences were obtained from each insect (*F. schultzei, A. gossypii*, and *B. tabaci*) at 84.5-97.6, 82.2-95.7 and 87.2-100.0%, respectively), regardless of formulas or concentrations. On the other hand, in adult thrips (*T. palmi*), it was found that all EO fumigations at the same concentrations presented non-significantly different results, regardless of the formulas. In particular, EO formulas Cl1Ci3 and Cl1Le3 at 2.0 µl/L air resulted in 93.3 and 86.7%, respectively, while both EO formulas at 3.0 µl/L air showed 100.0% mortality (Table 4.16).

Table 4.16 Mortality percentages of adult thrips (*Frankliniella schultzei* (Trybom) and *Thrips palmi* Karny), nymph of mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller), adult aphid (*Aphis gossypii* Glover), and adult whitefly (*Bemisia tabaci* Genn.) at 24 h after fumigations with essential oil formulas at 2.0 and 3.0 µl/L air in fumigation chamber (25 L) for 2 h.

Essential oil formulas[2/] (concentration)	% Mortality[1/]				
	F. schultzei (adult)	*T. palmi* (adult)	*P. jackbeardsleyi* (nymph)	*A. gossypii* (adult)	*B. tabaci* (adult)
Control	0.0±8.5^{b}	0.0±10.6^{c}	0.0±8.5^{b}	0.0±9.6^{b}	0.0±13.9^{b}
Cl1Cl3 (2 ul/L air)	89.9±2.8^{a}	93.3±5.8^{ab}	100.0±0.0^{a}	82.2±12.3^{a}	87.2±8.2^{a}
Cl1Cl3 (3 ul/L air)	97.6±4.2^{a}	100.0±0.0^{a}	100.0±0.0^{a}	93.1±6.0^{a}	96.7±5.8^{a}
Cl1Le3 (2 ul/L air)	84.5±12.5^{a}	86.7±5.8^{b}	100.0±0.0^{a}	90.0±3.1^{a}	88.5±6.1^{a}
Cl1Le3 (3 ul/L air)	94.8±6.3^{a}	100.0±0.0^{a}	100.0±0.0^{a}	95.7±5.9^{a}	100.0±0.0^{a}

[1/] Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.
[2/] Clove : Cinnamon and Clove : Lemon grass ratio 1:3 represented as formulas Cl1Ci3 and Cl1Le3, respectively.

4.5 Field experiment

Fumigant toxicity examination of Cl1Ci3 and Cl1Le3 at 2.0 and 3.0 µl/L air, and methyl bromide at 20 and 28 g/ m^3 (recommended dose in orchid and vegetable fumigations, respectively) demonstrated that methyl bromide fumigation at both concentrations resulted in 100.0% mortality in all insects. Besides, mortality percentages of *F. schultzei* and *T. palmi* obtained from methyl bromide fumigations were significantly higher than those from EO fumigations. However, all EO fumigations at 3.0 µl/L air of *P. jackbeardsleyi, A. gossypii* and *B. tabaci* presented no significantly different toxicity when

comparing to methyl bromide fumigation at both concentrations. Remarkably, a complete mortality in *P. jackbeardsleyi* was obtained from methyl bromide and EOs at all concentrations (Table 4.17).

Examination of plant physiological changes due to EO formulas and methyl bromide fumigation against *T. palmi* in both mimic and natural contaminated conditions in cut orchid flower were demonstrated. The fumigations of methyl bromide at both concentrations in mimic contaminated condition resulted in a complete mortality (100%), significantly higher than the results obtained from EO fumigations (73.9-83.3%) (P=0.05). Fumigation of methyl bromide and EO formulas in natural contaminated condition similarly resulted in a complete mortality (100%). However, it was remarked that the numbers of dead insects found in the bioassay were approximately 50% lower than the average pre-fumigation counts (10-15 insects / 10 flowers), and no living insects were observed (Table 4.18).

In field fumigation, effect of EO formulas (Cl1Ci3 and Cl1Le3), methyl bromide and control on plant physiological changes in cut orchid flower and vegetables were conducted in DOA fumigation chamber, demonstrated relatively similar symptoms as appeared some plant physiological changes after fumigations. However, cut orchid flower and eggplant fumigated with methyl bromide at recommend concentrations presented less than 3 days shelf lives and holy basil demonstrated immediate highly plant physiological changes as phytotoxic symptoms after the fumigations, while shelf lives the products fumigated with the EO formulas and the control were similar at 12 days (Figure 4.20-4.25).

Cut orchid flower fumigated with methyl bromide presented remarkably higher L* and b* values (lighter and yellowish in color) and a* value (increased greenness) when comparing to the control. On day 12, percentages of weight loss obtained from the flower fumigated with EOs were generally lower than 30%. In particular, color changes and percentages of weight loss in the flower fumigated with EOs at 2.0 µl/L air and the control were relatively similar, despite of having some minor differences (Figure 4.20 and 4.23).

In eggplant, the treated samples presented lower L* and b* values (increased darkness and blueness), while a* value was generally higher (increased redness) when comparing to the control. In addition, shelf life of all groups were over 9 days. The percentages of weight loss in the samples fumigated with EOs were lower than 5% on day 9, and lower densities were observed. Fumigation of the EO formulas at 3.0 µl/L air resulted in relatively similar changes in color, percentages of weight loss and densities, although minor differences were observed (Figure 4.21 and 4.24).

In holy basil, L* and b* values of the treatment group were lower (increased darkness and blueness), while a* value was higher (increased redness) when comparing to the control. In addition, shelf life of the EOs treatments and the control were over 6 days. On day 6, percentages of weight loss in the samples fumigated with EOs were lower than 25%, and lower densities were observed. In general, fumigation of the EO formulas at 3.0 µl/L air resulted in relatively similar changes in color and percentages of weight loss, although minor differences were observed (Figure 4.22 and 4.25).

Table 4.17 Mortality percentages of adult thrips (*Frankliniella schultzei* (Trybom) and *Thrips palmi* Karny), nymph of mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller), adult aphid (*Aphis gossypii* Glover) and adult whitefly (*Bemisia tabaci* Genn.) at 24 h after fumigations in field experiment with EOs formulas at 2.0 and 3.0 µl/L air and methyl bromide (MB) at 20 and 28 g/m^3 with 15-min air circulation in Department of Agriculture (DOA) fumigation chamber.

Essential oil formulas[2/] (concentration)	% Mortality[1/]				
	F. schultzei (adult)	*T. palmi* (adult)	*P. jackbeardsleyi* (nymph)	*A. gossypii* (adult)	*B. tabaci* (adult)
Control (not treated)	0.0±4.8^{c}	0.0±1.6^{d}	0.0±2.7^{b}	0.0±5.8^{c}	0.0±9.2^{c}
Methyl bromide (20g/m^3)	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}
Methyl bromide (28g/m^3)	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}
Cl1Cl3 (2 ul/L air)	66.1±13.2^{b}	73.9±6.7^{c}	100.0±0.0^{a}	82.2±7.1^{b}	91.7±6.7^{ab}
Cl1Cl3 (3 ul/L air)	74.6±6.7^{b}	83.3±2.9^{b}	100.0±0.0^{a}	96.0±6.9^{a}	96.7±5.8^{a}
Cl1Le3 (2 ul/L air)	64.6±4.9^{b}	72.2±9.6^{c}	100.0±0.0^{a}	82.4±6.6^{b}	88.5±6.1^{b}
Cl1Le3 (3 ul/L air)	65.3±13.3^{b}	75.0±5.0^{bc}	100.0±0.0^{a}	90.3±9.6^{ab}	100.0±0.0^{a}

[1/] Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.
[2/] Clove : Cinnamon and Clove : Lemon grass ratio 1:3 represented as formulas Cl1Cl3 and Cl1Le3, respectively.

Table 4.18 Mortality percentages of adult thrips (*Thrips palmi* Karny) at 24 h after fumigations in field experiment with EOs formulas at 2.0 and 3.0 µl/L air and methyl bromide at 20 and 28 g/m^3 with 15-min air circulation in Department of Agriculture (DOA) fumigation chamber.

Essential oil formulas[2/] (concentration)	% Mortality[1/]	
	T. palmi (adult)	
	mimic the contamination	contamination exists in nature
Control (not treated)	0.0±1.6^{d}	0.0±8.7^{b}
Methyl bromide (20g/m^3)	100.0±0.0^{a}	100.0±0.0^{a}
Methyl bromide (28g/m^3)	100.0±0.0^{a}	100.0±0.0^{a}
Cl1Cl3 (2 ul/L air)	73.9±6.7^{c}	100.0±0.0^{a}
Cl1Cl3 (3 ul/L air)	83.3±2.9^{b}	100.0±0.0^{a}
Cl1Le3 (2 ul/L air)	72.2±9.6^{c}	100.0±0.0^{a}
Cl1Le3 (3 ul/L air)	75.0±5.0^{bc}	100.0±0.0^{a}

[1/] Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.
[2/] Clove : Cinnamon and Clove : Lemon grass ratio 1:3 represented as formulas Cl1Cl3 and Cl1Le3, respectively. Numbers of dead thrips found in the bioassay were approximately 50% lower than the average pre-fumigation counts and no living insects were observed.

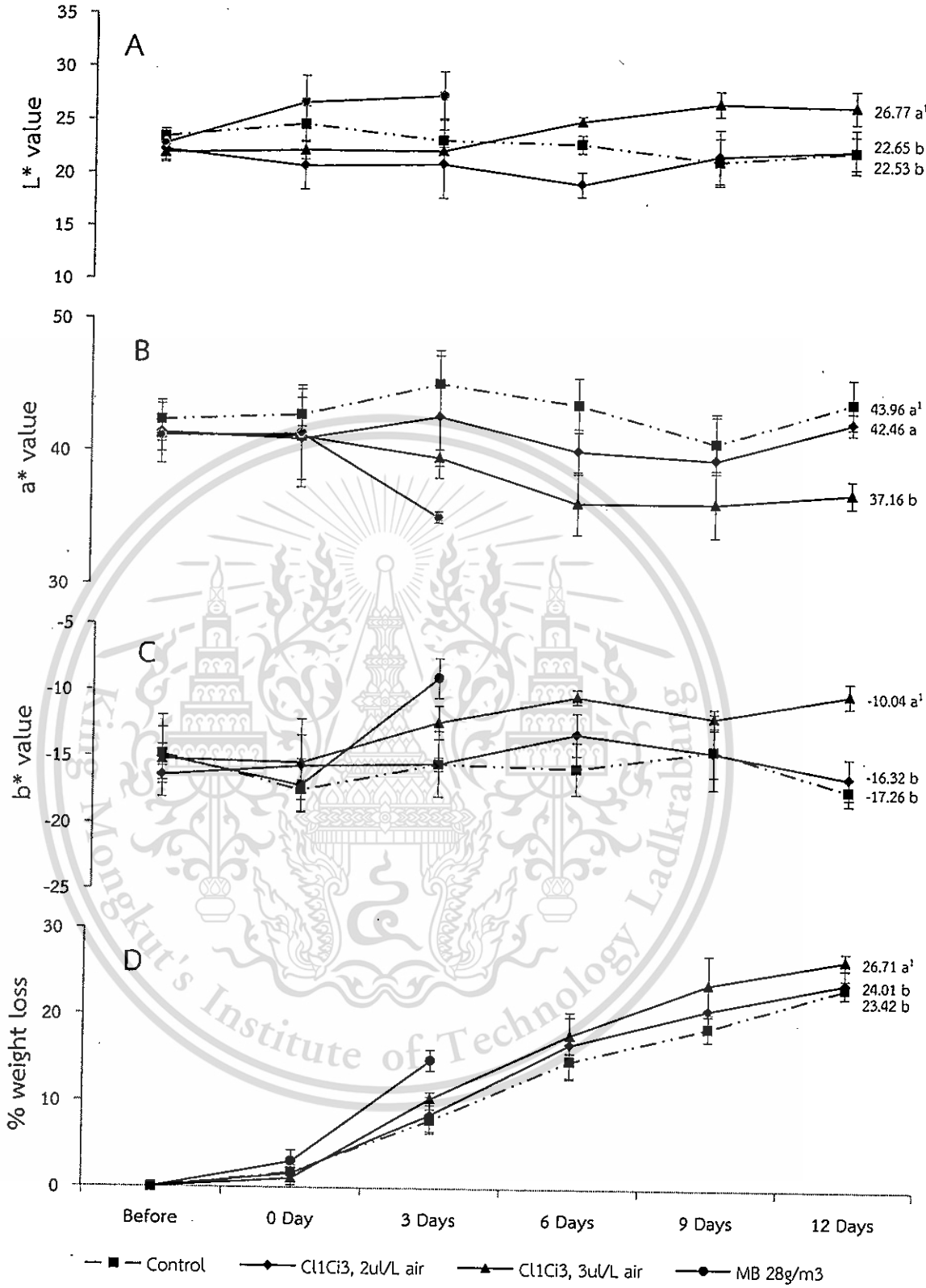


Figure 4.20 The L*, a* and b* values (A-C) and percentage of weight loss (D) of cut orchid flower on day 0, 3, 6, 9 and 12 after fumigation with essential oil formulas Cl1Ci3 (clove : cinnamon ratio 1:3) at 2.0 and 3.0 µl/L air, and methyl bromide (MB) at 28 g/m^3 for 2 h with 15-min air circulation. ([1]Means with the same common letter were not significantly different (P<0.05) according to DMRT).

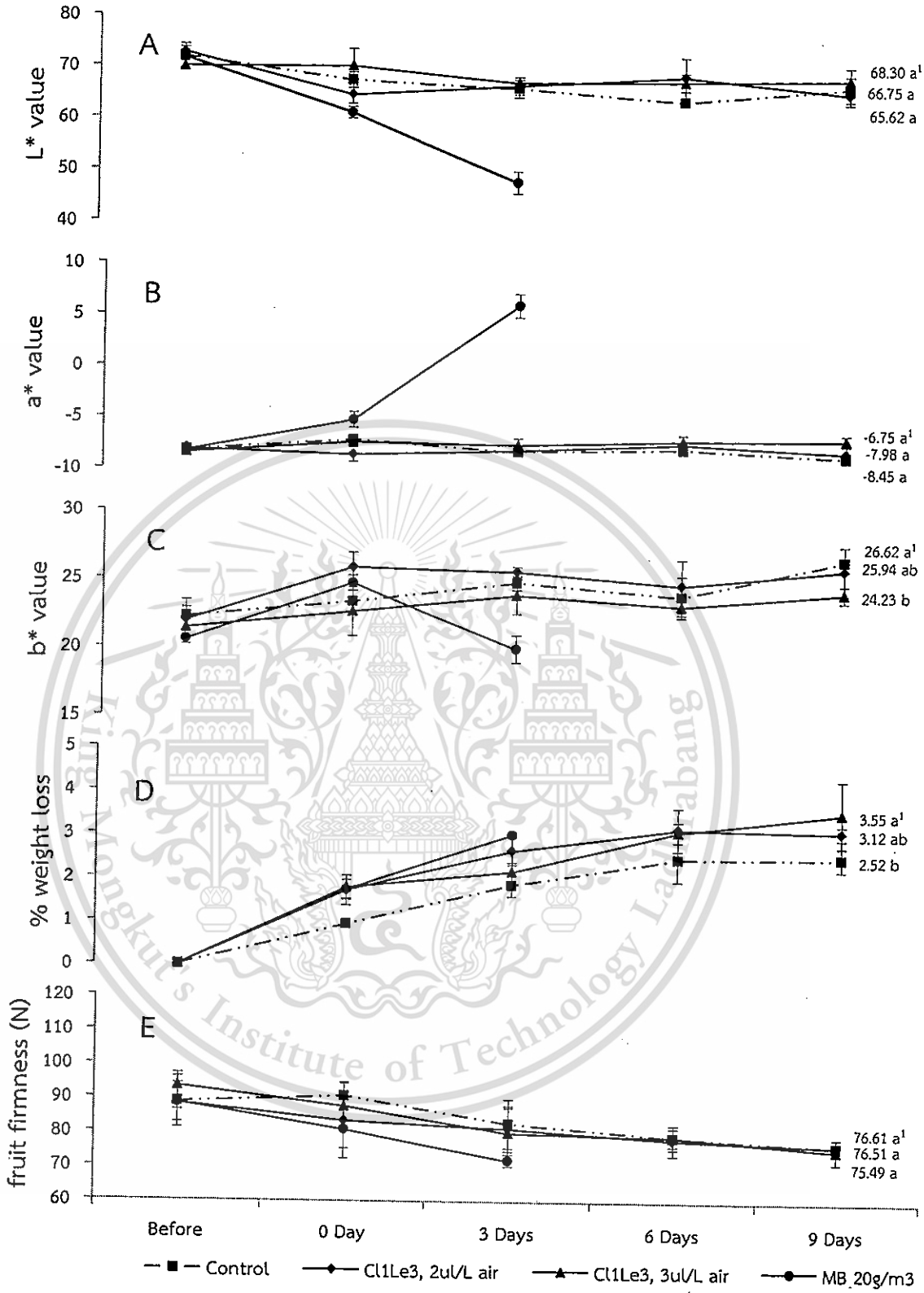


Figure 4.21 The L*, a* and b* values (A-C) and percentage of weight loss (D) of eggplant on day 0, 3, 6 and 9 after fumigation with essential oil formula Cl1Le3 (clove : lemon grass ratio 1:3) at 2.0 and 3.0 ml/m^3, and methyl bromide (MB) at 20 g/m^3 for 2 h with 15-min air circulation. ([1]Means with the same common letter were not significantly different ($P<0.05$) according to DMRT).

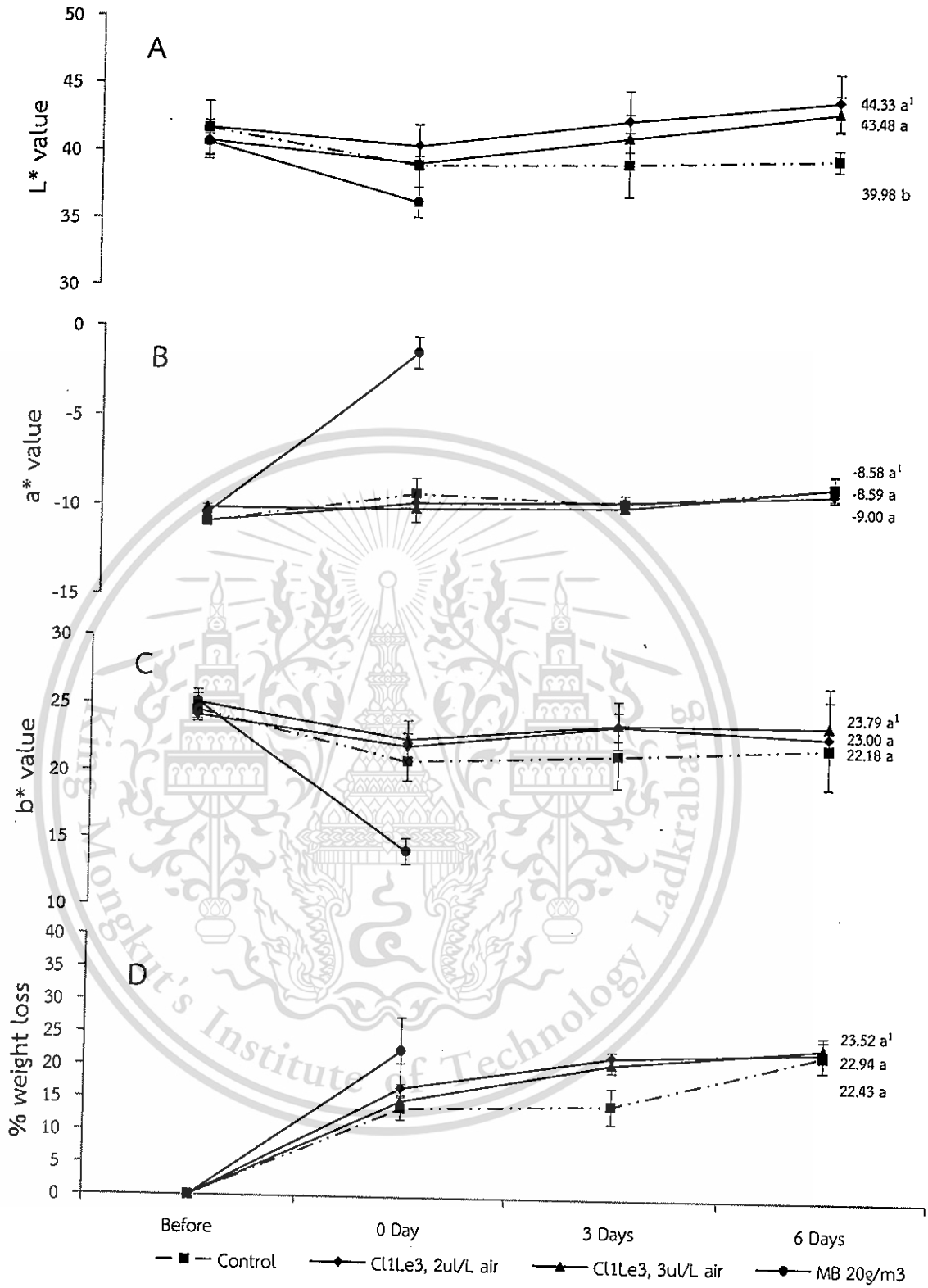


Figure 4.22 The L*, a* and b* values (A-C) and percentage of weight loss (D) of holy basil on day 0, 3 and 6 after fumigation with essential oil formula Cl1Le3 (clove : lemon grass ratio 1:3) at 2.0 and 3.0 µl/L air, and methyl bromide (MB) at 20 g/m^3 for 2 h with 15-min air circulation. ([1]Means with the same common letter were not significantly different ($P<0.05$) according to DMRT).

Figure 4.23 The cut orchid flower symptoms at 0, 3, 6, 9 and 12 days after fumigations with essential oil formula Cl1Ci3 (clove : cinnamon ratio 1:3) at 3.0 µl/L air, and methyl bromide at 28 g/m^3 for 2 h with 15-min air circulation.

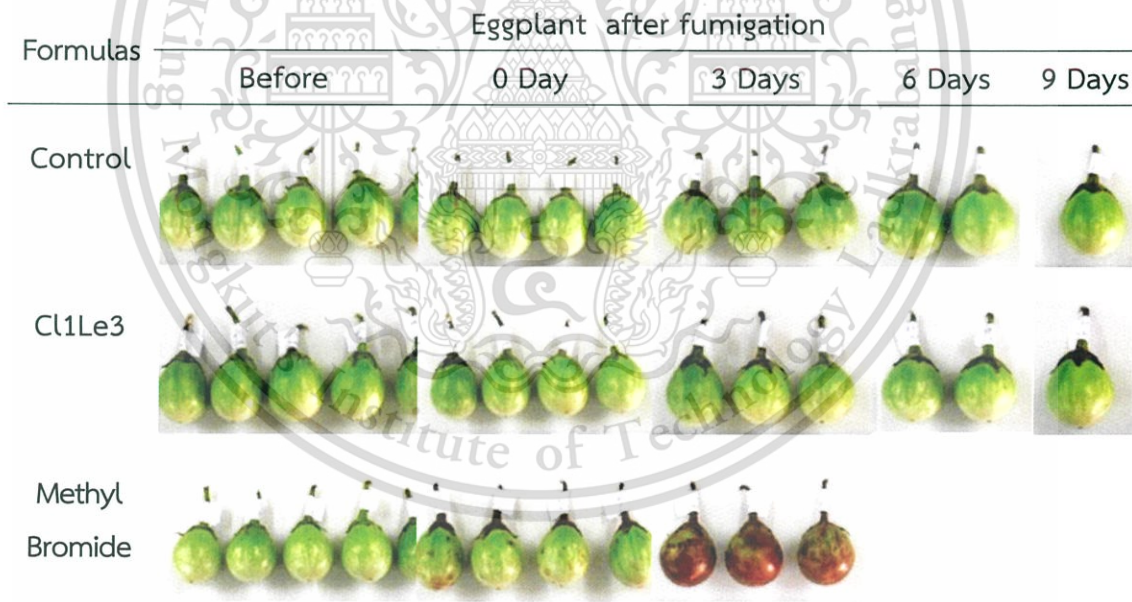


Figure 4.24 The eggplant symptoms at 0, 3, 6 and 9 days after fumigations with essential oil formula Cl1Le3 (clove : lemon grass ratio 1:3) at 3.0 µl/L air, and methyl bromide at 20 g/m^3 for 2 h with 15-min air circulation.

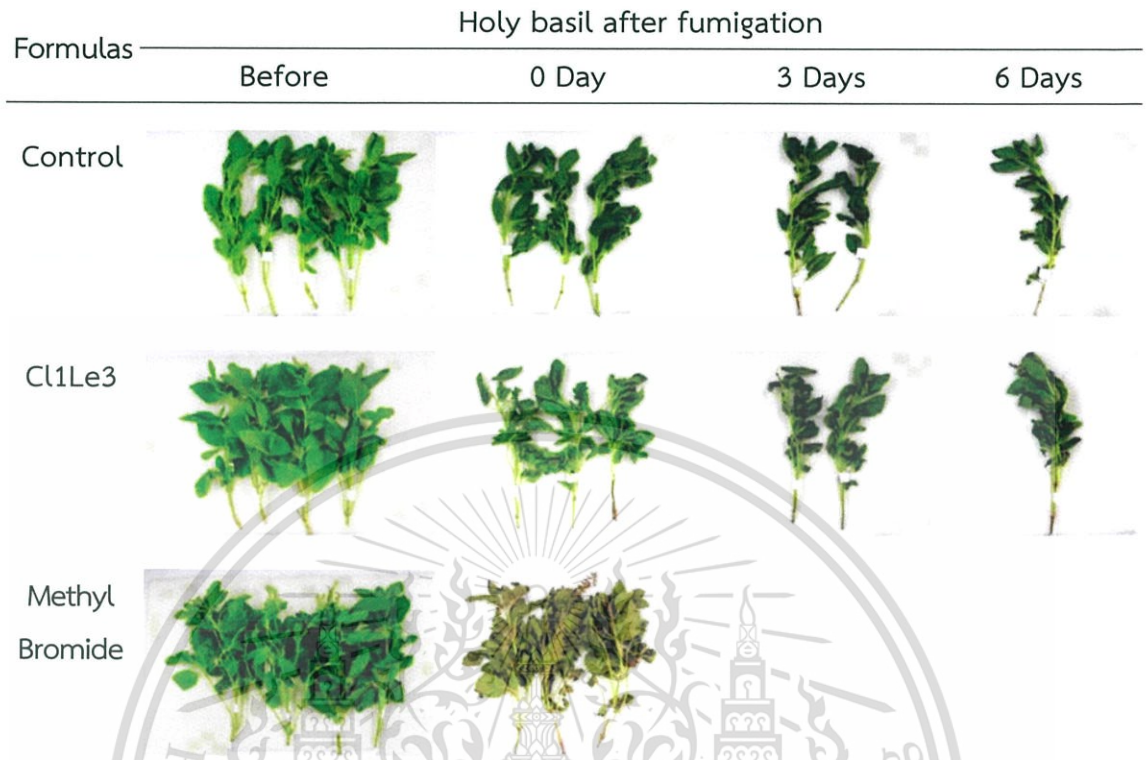


Figure 4.25 The holy basil symptoms at 0, 3 and 6 days after fumigations with essential oil formula Cl1Le3 (clove : lemon grass ratio 1:3) at 3.0 µl/L air, and methyl bromide at 20 g/m^3 for 2 h with 15-min air circulation.

CHAPTER 5
DISCUSSION

5.1 Examination of essential oil with high insecticidal activity

From the eighteen medicinal plants in this study, clove (*S. aromaticum*), cinnamon (*C. bejolghota*) and lemon grass (*C. citratus*) presented the highest mortalities against thrips (*F. schultzei*), mealybug (*P. jackbeardsleyi*), aphid (*A. gossypii*) and whitefly (*B. tabaci*). Recently, it has been reported that EOs present various insecticidal activities such as repellents, anti-feedants, growth regulation, anti-hatchability, and larval and adult mortality (Isman, 2000; Prakash and Rao, 1997; Papachristos and Stamopoulos, 2002). For example, the EO of clove showed insecticidal property against fruit fly (*Ceratitis capitata* (Wiedemann)) (Arancibia *et al.*, 2013), head louse (*Pediculus humanus capitis* (De Geer)) (Choi *et al.*, 2010), maize weevil (*Sitophilus zeamais* Motschulsky) (Pumnuan *et al.*, 2012), rice weevil (*S. oryzae*) (Ahmed and Salam, 2010) and pear psyllid (*Cacopsylla chinensis* (Yang & Li)) (Tian *et al.*, 2015). The EO of lemon grass showed insecticidal property against larger grain borer (*Prostephanus truncates* (Horn)) (Masamba *et al.*, 2003), rice weevil (*S. oryzae*) (Ahmed and Salam, 2010), cowpea bruchid (*C. maculatus*) (Paranagama *et al.*, 2003) and house fly (*M. domestica*) (Pinto *et al.*, 2015). The EO of cinnamon showed toxicity against rice weevil (*S. oryzae*) and cowpea weevil (*C. maculatus*) (Ahmed and Salam, 2010) and maize weevil (*S. zeamais*) (Pumnuan *et al.*, 2012). In addition, the EOs of cinnamon, clove and lemon grass were reported as having bioactivity against stored product mites (*Suidasia pontifica* Oudemans) (Pumnuan and Insung, 2011).

The results in this study indicated that a particular EO might show different levels of toxicities against different insect species. Lemon grass EO showed higher fumigant toxicity against thrips than mealybug, while cinnamon EO showed higher toxicity against mealybug than thrips. The EOs of clove and lemon grass showed higher toxicities against aphid and whitefly than the EOs of cinnamon. In particular, clove EO presented the highest toxicity against whitefly, while lemon grass EO showed the highest toxicity against aphid. Likewise, Ahmed and Salam (2010) reported that cinnamon EO was found showing higher fumigant toxicity against cowpea weevil (*C. maculatus*) and rice weevil (*S. oryzae*) than lemon grass, while the lemon grass EO showed high toxicity against brown planthopper (*Nilaparvata lugens*

(Stal)) (Chantawee *et al.*, 2012) and saw-toothed grain beetle (*Oryzaephilus surinamensis* (Linn.)) (Thanasirungkul *et al.*, 2012). The similar results were also found against maize weevil (*S. zeamais*) (Pumnuan *et al.*, 2012). These EOs were also highly toxic against many other insects and mites (Kim *et al.*, 2003; Masamba *et al.*, 2003; Kim *et al.*, 2006; Akhtar *et al.*, 2008; Hanifah *et al.*, 2011; Pinto *et al.*, 2015).

5.2 Examination of essential oils chemical constituents and their fumigant toxicity against the tested insects

In this study, general chemical compounds found in the plant EOs were monoterpenes. Monoterpenes have been well documented as highly active insecticidal fumigants for stored products insects for their high evaporating ability (Isman, 2006). These volatiles normally affect insect feeding behavior, growth and development, moulting, mating and oviposition behavior (Khater, 2012). Trans-cinnamaldehyde, cinnamyl acetate, benzaldehyde, eugenol, caryophyllene oxide, citral and T-cadinol which were the main compounds of EOs obtained from leaf of cinnamon also showed larvicidal activity against fourth-instar mosquito (*Aedes albopictus* (Skuse)) (Cheng *et al.*, 2009). Enan (2005) reported that cinnamic alcohol, eugenol, trans-antehole, and 2-phenethyl propionate showed high toxicity against fruit fly (*Drosophila melanogaster* Meigen). Insecticidal activity of citral (Jeon *et al.*, 2009), caryophyllene oxide and neral (Cheng *et al.*, 2009), limonene (Tripathi *et al.*, 2003) were also formerly reported. These monoterpinoid compounds interfered neurotoxic modes of action in insects (Koul *et al.*, 2008). In addition, Enan (2005) reported that carvacrol, coumarin, euvalyptol, eugenol and geraniol were found to interfere with octopamine receptor and acetylcholinesterase protein models of insects.

The examination of chemical components in the clove, cinnamon and lemon grass EOs revealed that the major component in clove and cinnamon EOs was eugenol. The percentage of eugenol in clove (97.100%) was found higher than in cinnamon (82.054%), and the EO of clove showed higher insecticidal property than the EO of cinnamon. The similar results were also reported by Pumnuan *et al.*(2008) and Ahmed and Salam (2010). It was highly possible that eugenol was the major chemical compound acting against the tested insects. However, the comparison of euginal based EOs and standard eugenol, the results showed that standard eugenol fumigations presented lower fumigant toxicity than the EOs with eugenol as the

major component (clove and cinnamon). Therefore, as mentioned by Hummelbrunner and Isman (2001), this study suggested that simply eugenol did not make the most effective insecticide, while a combination of different chemicals as in the EOs yielded considerably more acceptable results.

Moreover, lemon grass EO in this study showed high performance against the tested insects, while citronella grass which is another plant in the same genus, resulted in lower performance. This is perhaps related to the relatively low percentage of citral composition in citronella EO, when comparing to the significantly higher proportion in lemon grass EO. It was found in this study that citral was the major chemical found in lemon grass EO (69.730%). Other studies also showed that the citral composition in lemon grass EO was considerably high, in the range of 67-77% (Blanco *et al.*, 2009; Nonviho *et al.*, 2009; Sakirigui *et al.*, 2011; Tajidin *et al.*, 2012; Kpoviessi *et al.*, 2014). On the other hand, Oliveira *et al.* (2011) reported inexistence of citral in the EO extracted from citronella grass. Similarly, Kpoviessi *et al.* (2014) also reported no composition of citral in citronella grass EO extracted from Belgium species, while only 36.9% of citral was found in Southeast Asia citronella grass EO (Nakahara *et al*, 2003). In this study, the lemon grass EO showed higher toxicity against adults of thrips than the nymph of mealybug, and the similar results were obtained in the fumigation with standard citral. Therefore, it is highly possible that citral was the major chemical acting against thrips. However, when comparing the results of fumigant toxicity between lemon grass EO and standard citral against thrips, it was found that lemon grass EO showed higher fumigant toxicity than the standard citral. This finding also indicated that simply citral did not make the most effective insecticidal component, but rather a combination of different chemicals as in plant EOs.

Combinations of chemical compositions were reported to enhance EO efficacy. Chaubey (2008) reported that mixtures of different monoterpenes resulted in high synergistic property against many insect pests. The combination of anethole and 1,8-cineole (1:1) demonstrated reduction in the population of red flour beetle (*T. castaneum*) by 100% at the concentration 50µl/L (Koul *et al.*, 2008). In addition, Hummelbrunner and Isman (2001) examined synergism or additive effects of monoterpenoid binary mixtures against tobacco cutworm larvae (*S. litura*) and reported that thymol and *trans*-anethole synergized the effects of linalool (at 18 µg/larva dose, combined in 1:1 ratio). Lima *et al.* (2011) reported synergism of

different mixtures of carvacrol, 1,8-cineole and thymol against mealworm (*Tenebrio molitor* (L.)).

The comparison between the fumigant toxicity of standard eugenol and standard citral in this study suggested that standard eugenol showed higher fumigant toxicity against the adults of thrips and the nymph of mealybug than standard citral. Lee *et al.* (2001) reported that eugenol showed higher fumigant toxicity than citral against rice weevil (*S. oryzae*). Similarly, Cornelius *et al.* (1997) mentioned the higher fumigant toxicity of eugenol than citral against Formosan subterranean termite (*Coptotermes formosanus* Shiraki).

5.3 Examination of essential oil on plant physiological changes

5.3.1 Effect of essential oil on plant physiological changes in cut orchid flower

Cut orchid flower was more susceptible to particular EOs, while some other EOs showed significantly less plant physiological changes as different toxicity levels against cut orchid flower were observed. In general, orchid tissue is so delicate and easily damaged that inappropriate postharvest treatments usually result in the product quality problems involving color changes and loss of weight. In the primary test demonstrated that direct exposure EOs resulted in considerable deterioration. In addition, fumigations with plant EOs can increase loss of weight in plants. Batish *et al.* (2006) and Kohli *et al.* (1998) reported that fumigations with eucalyptus EO resulted in losses of weight and water content in weed plants, and increasing exposure period showed higher effects. Kordali *et al.* (2008) also reported a potent phytotoxic effect of *Origanum acutidens* (Hand.-Mazz) EO against *Amaranthus retroflexus* L., *Chenopodium album* L. and *Rumex crispus* L.

Nishida *et al.* (2005) mentioned that these damages can be an effect of monoterpenes which is a major component in botanic EOs. There have been no reports explaining the mode of phytotoxic activities of EOs on plants. However, in the study of bacteria and fungi, Solgi *et al.* (2009) and Tian *et al.* (2011) suggested that the mechanism of action involved membrane disruption as the low molecular weight and highly lipophilic terpine compounds could easily pass through cell membranes and induced biological responses of cells. In addition, Martinez-Romero *et al.* (2007) hypothesized that EOs might cause cell wall damage, morphological deformation and deterioration of the conidia and hyphae.

In this study, the cut orchid flowers fumigated with clove and cinnamon EOs at higher concentrations (4.5-6.0 µl/L air) were totally spoiled before day 6. The EOs of clove and cinnamon at the concentrations of 2.0 and 3.0 µl/L air presented less toxicity symptom than the higher concentrations, and no significant differences in color changes were observed when comparing to the control. The levels of EO concentrations were positively correlated to the degrees of changes in physiology of the treated orchid flowers. In addition, more color degradation was observed when higher concentrations of EOs was applied, particularly in L* values (lighter in color). This finding was in congruence with Gao *et al.* (2014) which reported an increase of L* value in button mushroom fumigated with clove, cinnamaldehyde and thyme EOs when higher concentrations were applied.

Combination of chemical compounds was also found to stimulate some property changes in the EO mixtures. The results of EO formula testing showed that different EO formulas at the same concentration demonstrated different levels of damages on the orchid flower, and different ratios of even the same EOs can result in different effects. However, formulas Cl4Ci0 and Cl1Ci3 showed no significant differences in L*, a* and b* values changes when comparing to the control. Miresmailli *et al.* (2006) mentioned that combinations of particular constituents could potentially result in changes in properties and effects of the EOs.

The findings revealed that air circulation period was another factor influencing physiological changes of the fumigated orchid flower. Particularly, changes in the percentages of weight loss are highly noticeable. A significant difference in percentages of weight loss between the fumigations with all-time and 15 min air circulation was obsererved, while the same air circulation periods showed no significant difference even when fumigation periods were varied. Van Meeteren and Van Gelder (1999) reported that loss of plant weight after harvest usually caused by the rate of transpiration and the in-vase rehydration capacity. However, 15 min air circulation in 2 and 3 h fumigation with Cl1Ci3 demonstrated no significant differences when compared to the control, while longer air circulation periods resulted in high deterioration. In general, the results indicated a positive relationship between fumigation periods and degrees of discoloration. The longer fumigation periods resulted in lighter color and higher water loss. Marshall *et al.* (2010) mentioned that flowers faded during senescence, and the degree of this physiological change was dependent upon factors such as temperature, light, nutrients, water availability and other surrounding conditions. In this study, the

changes in L*, a* and b* values indicated discoloration in the flower, particularly reduces in redness and blueness from the deep red-purple pre-fumigation color to paler and yellowish color.

In this study, PPO activity, phenolic and total anthocyanin contents detected in the treated cut orchid flowers were relatively constant throughout the 12 days in vase solution, when comparing to the pre-treatment value. Jeong *et al.* (2008) reported that a rapid increase in PPO activity, phenolic and total anthocyanin contents was generally observed as a result of increasing respiration rate stimulated by peeling and cutting of the plant tissue. However, as no cuttings or peelings were applied on the orchid flowers in this study, respiratory of the flower then probably remained stable and resulted in no observable changes in PPO activity. In addition the inhibition can also be a result of pH changes stimulated by the EOs (Sapers, 1993). Gao *et al.* (2014) reported the inhibition of browning index and PPO activity in button mushroom treated with EOs of clove, cinnamon and thyme. Lee *et al.* (1990) reported the positive correlation between enzymatic browning and phenolic content. Nevertheless in this study, an increase in phenolic content was observed despite the stable PPO activity. This increase was possibly a result of phenolic accumulation when stimulated by EOs fumigation (Gao *et al.*, 2014; Siriphanich, 2007). It was hypothesized in Sharma and Tripathi (2006) that damages on cells as caused by chemical compounds in EOs might trigger a signal that stimulated plant to product additional phenolic compound in antioxidant activity.

5.3.2 Effect of essential oil on plant physiological changes in holy basil and eggplant

In the primary test, the EOs of clove and lemon grass exhibited no significant physiological effects on holy basil and eggplant when compared to the control. Particularly, clove and lemon grass EO fumigations at 3.0 µl/L air showed no significant color changes on holy basil and eggplant. Normally, color changes in agricultural products can be extensively observed during the postharvest storage. The changes can be a result of senescence or other influencing factors, such as application timing, plant species, temperature, and EO type (WSU, 2013). In general, changes in the external colors were indicated by parameters such as lightness, total color variation and browning index (Jiang, 2013). Gao *et al.* (2014) reported the changes in color of button mushroom fumigated by clove, cinnamaldehyde and

thyme EOs. Valverde *et al.* (2005) reported different influences of plant EOs compounds on color attributes of grape.

In addition, there were reports on increases of weight loss (Kohli *et al.*, 1998; Batish *et al.*, 2006), and color changes in plants after fumigation with EOs (Castillo *et al.*, 2010). However, Plooya *et al.* (2009) argued that with the appropriate application, individual terpenoids which are the major compound in EOs can provide selective barriers as coatings against gas exchange, respiration and moisture loss in plants. Solgi and Ghorbanpour (2014) reported a potential coating property of gum Arabic which is a biopolymer obtained from stems and branches of Acacia tree.

Different ratios and concentrations of even the same EOs can result in different effects. The results in this study revealed that clove EO showed lower effect on plant physiological changes when compared to standard eugenol which is its major constituent. It is possible that mixtures of EOs might reduce concentration of phytotoxic constituents in the mixtures, while at the same time generates synergy effects against insects (Miresmailli *et al.*, 2006; Tripathi *et al.*, 2009; Choi *et al.*, 2010; Kim *et al.*, 2012). Maqbool *et al.* (2011) reported synergistic effects of the combination between gum Arabic and EOs of cinnamon and lemon grass against antracnose in bananas and papayas. Hummelbrunner and Isman (2001) studies synergism or additive effects of monoterpenoid binary mixtures against tobacco cutworm larvae and presented that thymol and *trans*-anethole synergized the effects of linalool (at 18 µg/larva dose, combined in 1:1 ratio). Lima *et al.* (2011) reported synergism of different mixtures of carvacrol, 1,8-cineole and thymol against mealworm.

Phytotoxic effects are normally dependent upon factors such as plant species, oil concentrations, volume of EO applied, and the size of plants (Bainard *et al.*, 2006; Boyd and Brennan, 2006). Jalili-Marandi *et al.* (2010) studied the effects of EOs from *Thymus kotschyanus* and *Carum copticum* for efficacy in the control of postharvest fungal decay in table grape and reported good inhibitory activity against fungal decay in oil-treated grapes, while showed negative impacts on the sensory quality of the grape. In contrast, Mohammadi and Aminifard (2012) researched the antifungal effects of the EOs against fungal pathogen of grey mould disease of peach and repored that anise, ammi, ziziphora and cinnamon EOs inhibited grey mould growth, while significantly decreased weight loss percentage. In addition, higher EO concentrations tend to exhibit the more physiological changes in plants (Meyer *et al.*, 2008).

5.4 Insecticidal activity experiment of EO formulation

Insecticidal activities of the EO formulas Cl1Ci3 and Cl1Le3 at 2.0 and 3.0 µl/L air for 2 h which presented no significantly different physiological changes in the flower and vegetables, respectively, were tested. In this study, the mixture of EOs from clove and cinnamon or clove and lemon grass have insecticidal property. The composite edible coating of clove EO combined with cinnamon and lemon grass showed the synergistic effects and the greatest potential to control insects. In addition the application of binary mixer, adjustments of fumigation condition were also found resulting higher efficacy. For example, Koul *et al.* (2008) reported an improvement of mortality effect on red flour beetle treated by anethole when minimum heat treatment device was used.

5.5 Field experiment

The results indicated that although methyl bromide fumigation demonstrated high insect control performance, the chemical fumigation caused serious physiological changes in the flower and vegetables. On the contrary, while yielding relatively similarly high insect control performance, the EO fumigation formulas showed no significant differences in physiological changes of the samples when comparing to the control. Applications of methyl bromide demonstrated on serious damages on plants physiology (Kostyukovsky and Shaaya, 2002). Normally, use of methyl bromide as a fumigant can reduce the quality of plants. Darker color was observed, particularly when higher methyl bromide concentrations were applied (Akagawa *et al.*,1997; Hansen *et al.*, 2000). Hansen *et al.* (2000) mentioned that methyl bromide fumigation generally resulted in a darker color for both fruit and stems of cherries. In contrary, many studies reported no impacts of EOs on cut flower (Kostyukovsky and Shaaya, 2002) and vegetables (Van Epenhuijsen *et al.*, 2008). In the end, use of mentioned plant essential oils as fumigant is a new alternative way to control the insect pest of vegetable and cut orchid flower, espicialy for those exported agricultural products.

CHAPTER 6
CONCLUSION AND SUGGESTION

6.1 Conclusion

The examination of 18 medicinal plant essential oils (EOs) against thrips, aphid, mealybug and whitefly showed that the EOs of clove, cinnamon, and lemon grass exhibited remarkably high insecticidal activity against the insects via fumigation method with LC_{50} at 1.14-2.27 µl/L air. In addition, the examination of the major chemical compounds in the three EOs with GC-MS revealed that eugenol was the major constituent in clove and cinnamon at 97.10 and 82.05%, respectively, while the major compound in lemon grass was citral at 69.73% (*trans*-citral and *cis*-citral at 37.93 and 31.80%, respectively). Moreover, fumigations with standard eugenol and citral also showed high insecticidal activities.

The formulation study revealed that 2-3 h fumigation with the mixture of clove and cinnamon at the ratio of 1:3 (Cl1Ci3) and the mixture of clove and lemon grass at the ratio of 1:3 (Cl1Le3) at the concentration 2.0 µl/L air, with 15-min air circulation presented no significant differences in physiological changes of cut orchid flower and vegetables (holy basil and eggplant), respectively, when comparing to the control. Cl1Ci3 and CliLe3 showed more than 65-100% insect mortalities on field application, while methyl bromide fumigation resulted in 100% mortalities. However, methyl bromide fumigation caused serious damages on the treated plants immediately after fumigation and remained marketable quality for only 3 days on shelf. On the other hand, no significant differences in physiological changes were observed in the plants fumigated with the EOs, when compared to the control.

6.2 Suggestion

The EO formulation guideline for using it to control contaminated insects of exported agricultural products by fumigation method, with no or less effect on physiological changes in the plants as follows;

1. It is recommended that the EOs of clove and cinnamon used in the EO formulas would contain more than 80% eugenol, and in lemon grass EO contains more than 60% citral.

2. It is recommended that the EOs should be sprayed thoroughly the fumigation chambers, and air pump is recommended. In addition, air-circulation in the chamber helps enhance efficacy of the fumigation. However, it is noted that only the initial 15-min air circulation is applied, since longer air circulation can result in severe damages on the treated plants.

3. The appropriate fumigation time for the EOs at the concentration of 2-3 µl/L air is 2-3 h as these durations resulted in no physiological changes in the plants. However, this formula may not yield satisfying results in all insect pests, and the increases in EO concentration can be taken into account, depending on physiology of the plants and targeted insects.

4. Essential oil fumigation requires no additional procedures. The similar processes as conventionally used in the fumigation of methyl bromide can be adopted. In addition, EO fumigation is less hazardous to user and environmental when compared to methyl bromide application.

5. The application of EO fumigation in postharvest products at the non-phytotoxic concentration might yield high but not 100% insect mortality when compared to the application of methyl bromide at the recommended rate. However, when considering the percentage of insect mortalities and plant damages, it can be concluded that EO fumigation is a potential alternative insect pest management method for export products which yields comparatively insect control property with less plant physiological changes when comparing to the conventional approach.

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2014	Application of Plant Essential Oils to Control Mushroom Mite, *Dolichocybe indica* Mahunka
2014	Application of Plant Essential Oils in Corporate with Petroleum Oil to Control Mealybug, *Pseudococcus jackbeardsleyi* Gimpel & Miller
2013-2014	Effect of fumigation of essential oil for controlling insect pest in export vegetables and flowers
2012	Application of black pepper, citronella grass and lemon grass essential oils to control mushroom mite, *Luciaphorus perniciosus* Rack.
2012	Control of Bed Bug, *Cimex hemipterus* by Plant Essential Oils
2012	Control of Corn weevil, *Sitophilus zeamais* by Essential Oil form Marigold
2010	Control of the stored product mite, *Suidasia pontifica* Oudemans by essential oils of medicinal plants
2009	Acaricidal activity of essential oils of medicinal plants against mushroom mites, *Luciaphorus perniciosus* Rack and *Formicomotes heteromorphus* Magowski

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