BIOLOGICAL CONTROL OF FRUIT FLIES (*Bactrocera correcta* Bezzi) BY PLANT EXTRACTS

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การควบคุมแมลงวันผลไม้ (*Bactrocera correcta* Bezzi) โดยชีววิธีด้วยสารสกัดจากพืช

นางสาวปาริชาต กรวยนอก

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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ปาริชาต กรวยนอก : การควบคุมแมลงวันผลไม้ (*Bactrocera correcta* Bezzi) โดยชีววิธีด้วยสารสกัดจากพืช (BIOLOGICAL CONTROL OF FRUIT FLIES (*Bactrocera correcta* Bezzi) BY PLANT EXTRACTS). อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.กรกช อินทราพิเชฐ, 147 หน้า.

งานวิจัยนี้ศึกษาการควบคุมแมลงวันผลไม้ (*Bactrocera correcta* Bezzi) โดยชีววิธีด้วยสารสกัดจากใบดาวเรือง สาบเสือ และผกากรอง และสารผสมของพืชเหล่านี้ พบว่าสารสกัดจากเอทานอลมีปริมาณสารประกอบฟีนอลิกทั้งหมดมากกว่าสารสกัดจากน้ำ ใบสาบเสือสกัดด้วยเอทานอลมีปริมาณสารประกอบฟีนอลิกทั้งหมดสูงที่สุด คือ 133.03 ± 3.48 มิลลิกรัม กาลิคแอซิด อิควิวาเลนท์/กรัม การตรวจสอบด้วยวิธีทินเลเยอร์โครมาโทกราฟี พบสารกลุ่มเทอร์พีน ซึ่งเป็นสารประกอบหลักในสารสกัด การทดสอบความเป็นพิษของสารสกัดในเบื้องต้นวิเคราะห์โดยวิธีบายชิมพ์ลีทัลลิตี ค่าความเข้มข้นของสารที่ทำให้สัตว์ทดลองตายไปครึ่งหนึ่ง (LC_{50}) สามารถบ่งบอกได้ว่าสารสกัดจากพืชอาจมีศักยภาพใช้สำหรับความคุมแมลง โดยเฉพาะอย่างยิ่งใบสาบเสือสกัดด้วยเอทานอลแสดงความเป็นพิษต่อบายชิมพ์ ซึ่งมีค่า LC_{50} 147.15 มคก./มล. ที่ 24 ชั่วโมง และมีศักยภาพสูงที่สุดในการควบคุมแมลงวันฝรั่งในทุกระยะของการเจริญเติบโต ใบสาบเสือสกัดด้วยเอทานอลมีผลสูงสุดต่อการฟักจากไข่ โดยยับยั้งร้อยละ 82.22 ± 6.19 ที่ 24 ชั่วโมง ด้วยค่าความเข้มข้นของสารที่ให้เกิดการตอบสนองร้อยละ 50 (EC_{50}) เท่ากับ 44.54 มก./มล. สำหรับประสิทธิภาพการควบคุมตัวอ่อน ใบสาบเสือสกัดด้วยเอทานอลมีการชักนำการตายของตัวอ่อนระยะที่สองสูงที่สุดร้อยละ 83.33 ± 1.92 โดยวิธีกิน ซึ่งคล้ายกันกับวิธีจุ่มที่ร้อยละ 87.78 ± 1.11 ค่า LC_{50} เท่ากับ 55.56 มก./มล. เมื่อทดสอบโดยวิธีกิน ในขณะที่วิธีจุ่มค่า LC_{50} เท่ากับ 52.99 มก./มล. ผลของสารสกัดจากพืชต่อการปรากฏของตัวเต็มวัยคล้ายกับผลของการฟักของไข่และการตายของตัวอ่อน การยับยั้งการปรากฏของตัวเต็มวัยโดยใบสาบเสือสกัดด้วยเอทานอลแสดงประสิทธิภาพสูงที่สุดร้อยละ 67.78 ± 2.22 ประสิทธิภาพการขับไล่ของสารสกัดจากพืชต่อแมลงวันตัวเต็มวัยทดสอบโดยโอลแฟคโตมิเตอร์ พบว่าใบสาบเสือสกัดด้วยเอทานอลแสดงการขับไล่สูงที่สุดร้อยละ 85.43 ± 3.90 ที่ 15 นาทีของการทดสอบ การขับไล่ของสารสกัดขึ้นอยู่กับความเข้มข้นและสวนทางกับเวลา ที่ 15 นาที ทุกการทดสอบสารสกัดมีความสามารถสูงในการขับไล่แมลงวัน หลังจาก 30 นาที ของการทดสอบ ประสิทธิภาพการขับไล่ลดลง พบว่าใบสาบเสือสกัดด้วยเอทานอลส่งผลให้เกิดการตายสูงที่สุดต่อแมลงวันตัวเต็มวัยด้วยเช่นกันที่ร้อยละ 80.00 ± 1.92 ค่า LC_{50} เท่ากับ 67.32 มก./มล. สำหรับการทดสอบด้วยสารสกัดผสมใบสาบเสือสกัดด้วยเอทานอลและใบดาวเรืองสกัดด้วยเอทานอลที่อัตราส่วน 3 ต่อ 1 มีประสิทธิภาพต่อการตายสูงที่สุดร้อยละ 72.22 ± 1.11 ที่ 24 ชั่วโมง สารสกัดผสมแสดงร้อยละอัตราการตายของ

แมลงวันตัวเต็มวัยน้อยกว่าสารสกัดเดี่ยว ความสัมพันธ์ระหว่างปัจจัยการทดลองในการควบคุมแมลงวันตัวเต็มวัยนั้นสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติ

กิจกรรมของไซโทโครม ซี ออกซิเดส ซึ่งเกี่ยวข้องกับการหายใจระดับเซลล์ทดสอบได้โดยใช้ของเหลวที่ได้จากการสกัดแมลงวันผลไม้ สารสกัดทุกชนิดรวมทั้งไซยาไนด์สามารถยับยั้งกิจกรรมของไซโทโครม ซี ออกซิเดสได้มากกว่าร้อยละ 50 และแสดงรูปแบบของการยับยั้งที่คล้ายคลึงกัน ดังนั้น สามารถสรุปได้ว่า สารสกัดจากใบดาวเรือง สาบเสือ และผกากรอง คาดหวังได้ว่าเป็นตัวเลือกที่น่าสนใจในการควบคุมแมลงวันฝรั่ง *B. correcta*

สาขาวิชาชีววิทยา
ปีการศึกษา 2556

ลายมือชื่อนักศึกษา_____
ลายมือชื่ออาจารย์ที่ปรึกษา _____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

PARICHAT KRUAINOK : BIOLOGICAL CONTROL OF FRUIT FLIES (*Bactrocera correcta* Bezzi) BY PLANT EXTRACTS. THESIS ADVISOR : ASSOC. PROF. KORAKOD INDRAPICHATE, Ph.D. 147 PP.

FRUIT FLIES (*Bactrocera correcta* Bezzi)/BIOLOGICAL CONTROL/MARIGOLD/ SIAM WEED/HEDGE FLOWER

Biological control of fruit flies (*Bactrocera correcta* Bezzi) using leaf extract of marigold (*Tagetes erecta* L.), Siam weed (*Chromolaena odorata* (L.) King & Robinson) and hedge flower (*Lantana camara* L.) and their combinations was investigated in this study. The results showed that the total phenolic compounds (TPC) of ethanol extracts were slightly higher than water extracts. The Siam weed leaf ethanol extract (SLE/e) contained highest total phenolic compound of 133.03 ± 3.48 milligrams gallic acid equivalents per gram (mg GAE/g). Thin layer chromatography (TLC) revealed the presence of terpenes group which was the major compounds in the extracts. The cytotoxicity of the extracts was preliminarily evaluated by brine shrimp lethality assay. The lethality concentration of 50% (LC_{50}) value indicated that the plant extracts could be potentially used for insect pest control. Especially, the SLE/e showed the highest cytotoxicity on brine shrimps with LC_{50} value of 147.15 µg/ml at 24 hours, and also was the most potent in controlling all stages of guava fruit flies development. The SLE/e exhibited the highest effect on egg hatching of 82.22 ± 6.19% inhibition at 24 hours with the effective concentration of 50% (EC_{50}) value of 44.54 mg/ml. For larvicidal efficacy, SLE/e was highly induced the morality of second instar larvae at 83.33 ± 1.92% by feeding assay, which was similar to dipping assay at 87.78 ± 1.11%. The LC_{50} value was 55.56 mg/ml in feeding assay while dipping assay, the LC_{50} value

was 52.99 mg/ml. The effects of the plant extracts on the adult emergence were similar to the effects on egg hatching and larva mortality. The antibiosis on fruit fly adult emergence by SLE/e exhibited highest efficacy of 67.78 ± 2.22% inhibition. The repellent efficacy of plant extracts on adult fruit flies was conducted by olfactometer. The result indicated that the SLE/e showed the highest repellency of 85.43 ± 3.90% at 15 minutes of treatment. The repellency of the extract was concentration dependent and inversed to time treatments. At 15 minutes, all of treatment extracts had high ability to repel the fruit flies. After 30 minutes of treatment, the repellent efficacy was declined. It was also found that the SLE/e produced the highest mortality effect on adult fruit flies at 80.00 ± 1.92% with LC_{50} value of 67.32 mg/ml. For the treatment with combination extracts, Siam weed leaf ethanol extracts and marigold leaf ethanol extracts at the ratio of 3:1 was highly effective at 72.22 ± 1.11% mortality at 24 hours. The combination extracts showed lower percent mortality of adult fruit flies than the individual extracts. The correlations among treatment conditions in biological control of adult fruit flies are significantly correlated.

The cytochrome c oxidase (COX) activity, related on cellular respiration, was investigated on the fruit flies lysate. All extracts as well as cyanide could inhibit COX activity with more than 50% and expressed the similar pattern of COX inhibition. Therefore, it could be concluded that the leave extracts of marigold, Siam weed and hedge flower are promising candidates for utilization as guava fruit flies *B. correcta* control agents.

School of Biology
Academic Year 2013

Student's Signature _____
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Parichat Kruainok

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LIST OF ABBREVIATIONS

ANOVA	=	Analysis of variance
BSLA	=	Brine shimp lethality assay
COX	=	Cytochrome c oxidase
CYT	=	Cytochrome c
DMSO	=	Dimethyl sulfoxide
DPPH	=	1,1,3,3-tetramethoxypropane and 1,1- diphenyl –2 – picryl – hydrazyl
EC_{50}	=	Effective concentration producing 50% response
HLE/e	=	Hedge flower leaf ethanol extract
HLE/w	=	Hedge flower leaf water extract
LC_{50}	=	Lethal concentration in 50% of the animals
MLE/e	=	Marigold leaf ethanol extract
MLE/w	=	Marigold leaf water extract
PBS	=	Phosphate-buffered saline
Rf	=	Retention factor
S.E.	=	Standard error
SLE/e	=	Siam weed leaf ethanol extract
SLE/w	=	Siam weed leaf water extract
TLC	=	Thin layer chromatography
TPC	=	Total phenolic compounds

CHAPTER I

INTRODUCTION

1.1 Background

Varieties of fruits grown in Thailand, including guava, mango, litchi, longan, peach, rose-apple and sapodilla, have great economic value and are important exports. Fruits are vulnerable to pests and germs. Insects are the most damaging pests of fruit crops affecting the valuable export trade of agricultural products of Thailand. Fruit flies are considered as a major pest causing damage to a wide variety of fruits and vegetable crops throughout the tropics and subtropics of the world, including Thailand. With increasing emphasis on quality of fruit and vegetable produce and with the possibility of expansion of trade in horticultural commodities, the countries importing as well as exporting are giving attention to fruit fly management at preharvest and postharvest levels (Drew, 1992). The economics losses due to fruit fly damage as estimated using domestic price showed hundred millions dollar (Asian Institute of Technology, 2010). The guava fruit fly, *Bactrocera correcta* (Bezzi), is one of the examples of economically important pests of fresh fruits of Thailand.

Thailand's trading partners have classified fruit flies as a primary quarantine pest. Exports market of fresh fruits such as Japan and the United State of America surveillance for fruit flies requires that postharvest disinfestations treatment prior transport fruits to markets. The cost of disinfestations depends on treatment methods

and varieties of fruits but is seen as a significant cost (Asian Institute of Technology, 2010). However, the quarantine restrictions imposed by the presence of fruit flies hinder the development of export markets. Fruit flies are attracted to host plants while their fruits are developing. Fruit flies feed and breed in their host plants. Adult fruit flies mostly lay their eggs in fresh fruits. The eggs hatch into larvae (maggots) which feed on the fruit pulp, causing the fruit to become a soft and mushy mess (Dekker and Messing, 1999). The secondary infections by bacteria or fungi sometimes follow the egg-laying and lead to further bad marking on the surface of the fruit (Cantrell, Chadwick and Cahill, 2002). Infested fruits quickly become rotten and inedible; this can induce fruit drop prior to harvest but inside of the fruits there is also destruction (Guamán, 2009), thus causing considerable losses in production or if harvested, the result of damage makes the fruit unsaleable both to export market and domestic market (Collins, 1998; Orankanok, Chinvinijkul, Thanaphum, Sitilob, and Enkerlin, 2007). Other major losses result from quarantine restrictions are imposed by importing countries to prevent the entry or establishment of unwanted fruit fly species. Considerable financial burdens are imposed on governments, farmers and exporters, who have choices but to implement quarantine surveillance systems, quality assurance schemes and acceptable post-harvest quarantine treatment if they wish to export fruit fly host products (Allwood and Drew, 1997).

Fruit fly management involves application of chemically synthetic insecticides, especially to commercial fruits. Although they are effective, it is reported that their use for several decades had disrupted the biological control system of natural enemies and led to outbreaks of insect pests including widespread development of resistance, undesirable effects on non-target organisms, and environmental and human health

concerns (Kim, Roh, Kim, Lee and Ahn, 2003). It is difficult to design chemicals which act specifically towards a given group of target insects (Well, Mongin and Bertsch, 1993). Besides hazardous effects on natural enemies, the limited availability, dangers and cost associated with the use of synthetic insecticides, there are problems regarding the resistance of the pest insect against these products (Boeke et al., 2004). The accumulation of insecticides in plants and animals can lead to long term human health problems as well. In this regard, the intensive uses of synthetic insecticides increased global concern to develop alternative sources of insecticides to use in plant safe management against pests and decrease environmental and toxicological risks (Mello and Silva-Filho, 2002).

A simple and safe technology has been developed to protect various fruits from the flies avoiding economic damage so that harmful environmental side effects are minimized (Stewart and McClure, 2013). There is a growing interest in the use of botanical insecticides to reduce the use of chemically synthetic insecticides and also to avoid problems of insecticide resistance (Thomas and Callaghan, 1999). The plant products that are traditionally used and produced by farmers in developing countries appear to be quite safe and promising for consumer health (Rajapakse and Van Emden, 1997). Many plants may provide potential alternatives to currently used insect control agents because they constitute a rich source of bioactive chemicals (Kim, Roh, Kim, Lee and Ahn, 2003). The interaction between plants and insects is chemically mediated by secondary metabolites (Pascual-Villalobos and Robledo, 1999).

Secondary metabolites of plants are important source for biopesticides, the development of new pesticides. The important role of secondary metabolites compounds was appreciated increased, especially in protective plants from pests. The

roles of secondary metabolites in plants are defending the plants against insect acting as insect repellents, feeding inhibitors and/or toxin (Mello and Silva-Filho, 2002; Kennedy and Wightman, 2011). Attention has recently been focused on the plants products investigation for plants protection because they are low mammalian toxicity, non-phytotoxic and easily biodegradable (Isman, 2006). Products of plants secondary metabolites may be used as alternatives pesticides for several applications approach in integrated plant protection (Dubey, Kumar, Singh and Shukla, 2009). In order to enlargement human health concerns the development of integrated and sustainable strategies for plant protection, which are safe to consumers, producers and the environment, the use of biopesticide need to be promoted (Margarita Stoytcheva, 2011). Since these are active against a limited number of species including specific target insect, biodegradable to non-toxic products, and potentially suitable for uses in integrated pest management, thus, they could lead to the development of new classes of safer insect control agents (Kim, Roh, Kim, Lee and Ahn, 2003).

This research focused on some plant extracts for potentially useful products as guava fruit fly (*Bactrocera correcta* Bezzi) control agents.

1.2 Research objectives

The main objectives of this study are as followings:

1.2.1 To observe the cytotoxicity of marigold (*Tagetes erecta* L.), Siam weed (*Chromolaena odorata* L.) and hedge flower (*Lantana camara* L.).

1.2.2 To control guava fruit fly (*Batrocera correcta* Bezzi) by the extracts of marigold (*Tagetes erecta* L.), Siam weed (*Chromolaena odorata* L.) and hedge flower (*Lantana camara* L.) on egg, larval, pupal and adult stages.

1.2.3 To evaluate the correlations among treatment conditions in the guava fruit fly (*Batrocera correcta* Bezzi) control experiments.

1.2.4 To investigate the mortality effect of plant extracts on insect cytochrome c oxidase.

1.3 Research hypothesis

Marigold, Siam weed and hedge flower extracts could control guava fruit fly eggs, larvae, pupae and adults.

1.4 Scope and limitations of the study

The leaf ethanol and water extracts of marigold (*Tagetes erecta* L.), Siam weed (*Chromolaena odorata* (L.) King & Robinson) and hedge flower (*Lantana camara* L.) were investigated for biological control of guava fruit fly (*Bactrocera correcta* Bezzi).

1.5 Expected result

This study will develop the biological control of guava fruit flies by the leaves of marigold, Siam weed and hedge flower to which the value is added. The plant-based insecticides can be used to replace synthetic insecticides which is beneficial to human health and sustain the environment.

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CHAPTER II

LITERATURE REVIEWS

2.1 Biology of guava fruit fly, *Bactrocera correcta* (Bezzi)

Fruit fly is know as a pest causing damage to various fruits and vegetable crops. The major fruit fly pests in Thailand belong to the genus *Bactrocera* (มนตรี จิรสุรัตน์, 2544; Kapoor, 2005; Liu and Ye, 2009; Puanmanee, Wongpiyasatid, Sutantawong and Hormchan, 2010). *Bactrocera correcta* (Bezzi), is one of the main insect pests of fruits affecting the valuable fruit production and quality and also impact exportation of agricultural products of Thailand. Adult females of fruit flies often lay their eggs in fruits and vegetables. The fruits that are infested by fruit flies obviously appear black spots on the fruit surface. The eggs hatch into larvae that feed on the flesh of the fruits. Feeding damage is cause of preharvest fruit drop and reduce quality of fruit production. *B. correcta* has potential to infest fruit from the early fruiting stage till to the harvest stage. Therefore, the investigation of effects is difficult in the early infestation. When infestations are not controlled, the fruit production may be damaged up to 100% (Guamán, 2009). The common routine of controlling fruit flies is insecticide spray. However, there are several disadvantages of the insecticide spray. Most insecticides are not only toxic to fruit flies but can also be toxic to humans or other life and persistence in environment (Gallo, 2007). To improve quality of fruit products, consumers, producers health and reduce environmental impacts, the

alternative techniques for fruit fly control by plant products emphasize on in natural pesticides.

2.1.1 Fruit fly, *Bactrocera correcta* (Bezzi)

B. correcta, known as the guava fruit fly , is one of the most destructive pests in the genus *Bactrocera*. *B. correcta* taxonomic is classified as follows;

Kingdom Animalia

Phylum Arthropoda

Class Insecta

Order Diptera

Family Tephritidae

Genus *Bactrocera*

Species *Bactrocera correcta*

Source: http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=671680.

2.1.2 Life cycle of the fruit fly, *Bactrocera correcta* (Bezzi)

The fruit fly has complete metamorphosis life cycle which is composed of four stages. Fruit fly life cycle depends on temperature. Cool temperature slows the developmental cycle and warm temperatures speeds it up. The development from an egg to an adult is in summer which requires about 16 days. Eggs are barely visible, they are white and elongate. They are laid on a food source of fermenting fruit or other moist organic materials and hatch into larvae approximately 24 hours after being laid. Larvae are pale white, feed constantly, and reach full size in 5 up to 6 days

(Lind, 1999). They are very difficult to be seen until they feed for a while and get larger. While feeding, they tunnel throughout the fruit, destroy the pulp and allow an entry of secondary infestation of bacteria and fungi (Vossen, Varela and Devarenne, 2004). Larvae feed on fungi and yeast organisms and grow in their food sources. Their feeding efforts turn their food into a semi-liquid mess. The larval stages complete developing in three larval instars before pupation.

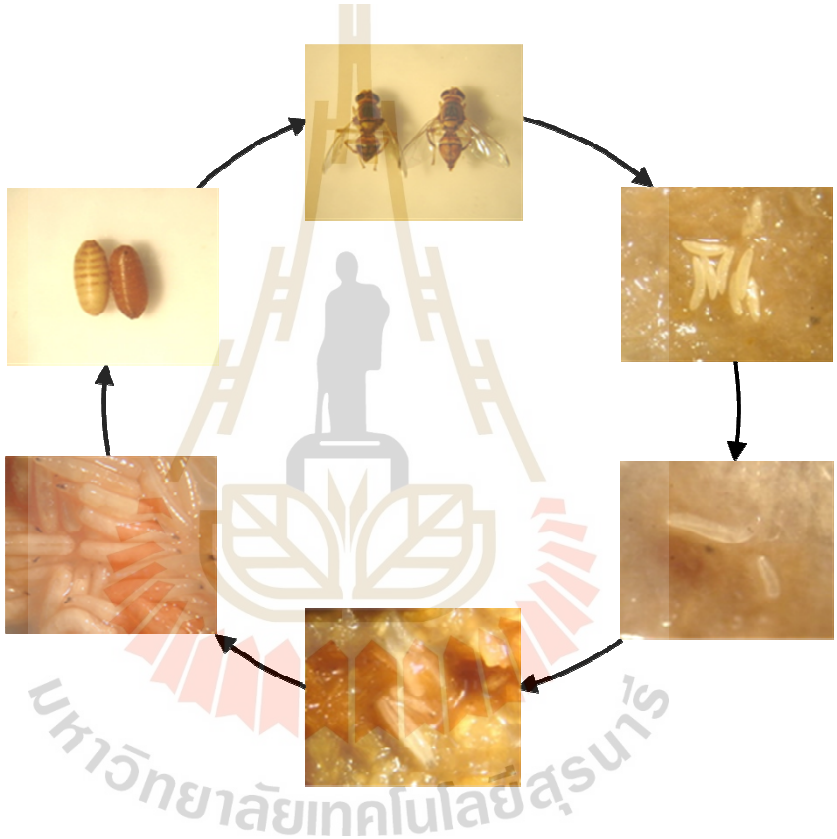

Figure 2.1 Life cycle of the fruit fly *Bactrocera correcta* (Bezzi).

When they fully grow, the larvae move to a drier area to pupate. Pupae are straw color and shape like small wheat grains. Emergence of the adults takes place after a few days when the adult fruit fly forces its way through the anterior end of the pupa. Shortly after emerging, the adult fly darkens in color, its abdomen expands, and it extends its wings. The adult is 1/8 – 1/4 inch long with clear wings, a dull brown body

(the female's abdomen is crossed by dark lines), and distinctive red compound eyes. Adult females can begin laying eggs within 48 hours after emerging from the pupae and begin mating within 12 hours. Adult fruit flies can live from a few days up to 30 days and the females lay approximately 500 eggs in their life span (Lind, 1999).

2.1.3 Distribution of fruit fly *Bactrocera correcta* (Bezzi)

B. correcta is widespread in South Asia and South East Asia (India, Sri Lanka, Burma, China, Taiwan, Laos, Vietnam, Cambodia) including Thailand (Kapoor, 2005; Liu and Ye, 2009; Asian Institute of Technology, On-line, 2010; Arakelian, 2011; Weems and Fasulo, 2012). *B. correcta* infestations in Thailand most occur in northern and central parts (มนตรี จิรสุรัตน์, 2544; Clarke et al., 2001).

2.1.4 Host plants

The fruit fly *B. correcta* is polyphagous with a wide host range, infesting the tropical and subtropical fruits of more than 62 hosts in 30 plant families (Allwood et al., 1999; Maynard, Hamilton and Grimshaw, 2004).

Recorded hosts plants such as *Citrus* spp., *Coffea canephora* Pierre ex Froehn. (as *Coffea robusta*), *Eugenia uniflora* L. (as *Eugenia mitchelli*), *Mangifera indica* L. (mango), *Prunus persica* L. (peach), *Psidium guajava* L. (guava), *Ricinus communis* L. (castor bean, castor-oil-plant, palma christi, wonder tree), *Santalum album* L. (sandalwood, white sandalwood), *Syzygium jambos* L. Alston (as *Eugenia jambos*) (roseapple), and *Ziziphus* spp., including *Ziziphus jujuba* Mill. (ber, jujube, Chinese date), *Annona squamosa* L. (Sugar apple) and *Manilkara achras* Fosberg

(Spodilla) are the most commonly attacked by *B. correcta* (Asian Institute of Technology, On-line, 2010; Weems and Fasulo, 2012).

2.1.5 Control of fruit flies

The fruit flies activity and population vary throughout the year and widespread in Thailand. Because of the potential losses from fruit fly infestations, control is typically carried out on routine basis, especially in commercial plantings. There are several application for fruit fly control. The most common methods for controlling fruit flies are cultural control, physical control, chemical control and biological control.

2.1.5.1 Cultural control

Cultural control is simple practice to reduce the fruit flies population, such as, protect fruit flies infestation by early harvesting. Crops are harvested at maturity stage when the crop products is not susceptible to fruit fly attack (Allwood, Leblanc, Vueti and Bull, 2001). The removal fallen fruit or over-ripe fruit that destruction of fruit flies infested are kept in sealed plastic bags then place under sun light for a period until larvae and other stages tends to die or bury to a depth of 1 meter and thick covering by soil could reduce the survival probability of larvae and other life stages of fruit flies reach to reduce fruit flies populations (Collins, 1998; Asian Institute of Technology, On-line, 2010).

2.1.5.2 Physical control

Physical control practices involve preventing female fruit flies laying their eggs on crop products (Asian Institute of Technology, On-line, 2010). The most common method is to bag or wrap fruit at early fruiting stage before crops

productions are susceptible to fruit fly attack. Practice of bagging can lessen damage from individual fruit (Collins, 1998). Bags are made from double layers of newspaper or brown paper. Bags may be carefully opened to check if the fruit inside is ripe when nearly harvest time. Plastic bags are not suit to be used, because the inside gets hot and moisture favors fungus growth (Allwood, Leblanc, Vueti and Bull, 2001).

2.1.5.3 Chemical control

Fruit fly control involves application of synthetic insecticides. The most common method for controlling fruit fly is a synthetic insecticide spray. Because this method has a potential to drop down fruit fly population in a minimum (Allwood and Drew, 1997). However, there are several disadvantages of the synthetic insecticide spray. The synthetic insecticide spray persist on the fruit surface and coverage thorough crop products (Allwood, Leblanc, Vueti and Bull, 2001). An indirect lost from the use of the synthetic insecticide spray is the impact on other insect species which are beneficial to production. These species include pollinators and natural parasites and predators of other fruit pests. The intensive use of synthetic insecticide spray can also elevate grower risk of exposure and the potential for long term health problems (Collins, 1998). In Thailand, pesticide residues have been found in soil, water and agricultural products (Thapinta and Hudak, 1998).

2.1.5.4 Biological control

The alternative methods for fruit fly control has been increasing for replace synthetic insecticide spray in recent years. The use of biological predators and parasitoids are commonly know as control agents. However, predators have little effect on fruit flies populations. The use of parasitoids to control fruit flies has a potential in reducing fruit fly populations (Allwood, Leblanc, Vueti and Bull, 2001).

The natural products have also been proved effective on fruit fly control. Some plants are especially rich in chemicals that can be extracted and used for fruit fly control. These plant products are known as botanical insecticides (Cranshaw, On-line, 2006). Botanical insecticides refer to plant secondary metabolites which include crude extracts and isolated or purified compounds from various plants species (Stoytcheva, 2011). The extracts of mintweed (*Hyptis suaveolens* L.), yam bean (*Pachyrhizus erosus* L.) and celery (*Apium graveolens* L.) has a potential to used as biological control agents on *Aedes aegypti* larvae and adults (Yongkhamcha and Indrapichate, 2012). The extracts of mintweed (*Hyptis suaveolens* L.), kitchen mint (*Mentha cordifolia* L.) and kaffir lime (*Citrus hystrix* L.) are highly toxic to adults and larvae of rice weevil *Sitophilus oryzae* infested in stored milled rice (Buatone and Indrapichate, 2011). Tanprasit (2005) reported that the efficiency of mintweed (*Hyptis suaveolens* L.) and hedge flower (*Lantana camara* L.) extracts as individual and combination extracts was able to control *Ae. aegypti*. Chokkhun (2011) demonstrated that the potential of kaffir lime (*Citrus hystrix* CD.) peel and papaya (*Carica papaya* L.) seeds extracts in biological control of *Ae. aegypti*. The extracts of mugwort (*Artemisia vulgaris*), mangosteen peel (*Garcinia mangostana*), croton (*Croton tiglium*), tobacco (*Nicotina tabacum*), Japanese poinsettia (*Pedilanthus tithymaloides*), pencil tree (*Euphorbia tirucalli*) and ginger (*Alpinia officinarum*) were reported to kill adult fruit flies (*Bactrocera dorsalis* H.) (รุจนี เล้ารัตนบูรพา, 2523). The efficacy to repellent adult fruit flies was found in the extracts from lipsticktree (*Bixa orellana* L.), neem (*Azadirachta indica* var. *siamensis* Veleton), Kaffir Lime (*Citrus hystrix*), melon (*Cucumis melo* L.), lemongrass (*Cymbopogon citraius* S.), heliotrope

(*Heliotropium indicum* R. Br.), shrubby basil (*Ocimum gratissimum* L.), orange gingerlily (*Hedychium occineum* var.), and castor-oil plant (*Ricinus communis* L.) (Areekul, Sinchaisri and Tigvatananon, 1978). Chuenwong (2006) showed that the potential of the extracts from neem (*Azadirachta indica* J.), sugar apple (*Anona squamosa* L.) and mintweed (*Hyptis suaveolens* L.) could control egg, larva, pupa and adult fruit flies (*Bactrocera dorsalis* H.).

2.2 Plants for *B. correcta* control

2.2.1 Marigold (*Tagetes erecta* L.)

Marigold (*Tagetes erecta* L.), a common garden plant grown throughout Thailand, is an herb of ancient medicinal repute (Cetkovic, Djilas, Canadanovic-Brunet and Thumbas, 2004). Marigold is classified as follows;

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Asterales

Family Asteraceae

Genus *Tagetes* L.

Species *Tagetes erecta* L.

Source: http://plants.usda.gov/java/profile?symbol=TAER.

Figure 2.2 Marigold (*Tagetes erecta* L.).

In traditional and homeopathic medicine it has been used for skin complaints, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularity, varicose veins, hemorrhoids, duodenal ulcers, etc. The yellow or golden-orange flowers of marigold are used as spice, tea and medicine. The pharmacological activity of marigold is related to the content of several classes of secondary metabolites such as essential oils, flavonoids, sterols, carotenoids, tannins, saponins, triterpene alcohols, polysaccharides and resin (Cetkovic, Djilas, Canadanovic-Brunet, and Thumbas, 2004). Marigold is typically planted as intercrops or in rotation with crops to control nematodes. Natarajan et al. (2006) found that population of tomato root knot nematode, *Meloidogyne incognita*, were reduced by cold aqueous extracts of marigold. Broussalis et al. (1999) demonstrated that the activity of marigold extracts by maceration with dichloromethane and methanol to against rice weevils (*Sitophilus*

oryzae). Sarin (2004) showed the potentiality of callus cultures of marigold to produce ascorbic acid as well as insecticidal pyrethrins. The pyrethrins extracted from the callus cultures can be safely used as an insecticide on flour beetles (*Tribolium* spp). In Thailand, marigold used locally as insecticides. The plants have a history of usage as folk remedies and are still used to kill or repel insect such as white flies, common cutworms and cabbage moths (ไพบูลย์ บุญชัย, On-line, 2546). Nikkon et al. (2009) Showed the insecticidal activity of crude extracts from the flower of marigold *Tagetes erecta* L. for against a stored product insect pest, *Tribolium castaneum* (Herbst). Islam and Talukder (2005) demonstrated that the potential of marigold (*T. erecta* L.) to against the red flour beetle (*Tribolium castaneum*) in stored products. Parugrug and Roxas (2008) showed that the efficacy to against maize weevil (*Sitophilus zeamais* M.) as repellency, adult mortality and antioviposition and growth inhibition.

2.2.2 Siam weed (*Chromolaena odorata* (L.) King & Robinson)

Siam weed (*Chromolaena odorata* (L.) King & Robinson), a perennial, is a diffuse, scrambling shrub that is mainly a weed of plantation crops and pastures in Asia and Western Africa (Thang, Patrick, Teik and Yung, 2001). Due to its fast growth rate, and prolific, wind-dispersed seed production, the plant can spread very easily (McFadyen and Skarratt, 1996). Siam weed, is classified as follows;

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Asterales

Family Asteraceae

Genus *Chromolaena* DC.

Species *Chromolaena odorata* (L.) King & H. Rob.

Source: http://plants.usda.gov/java/profile?symbol=chod.

Figure 2.3 Siam weed (*Chromolaena odorata* (L.) King & Robinson).

Traditionally, fresh leaves or a decoction of Siam weed leaves have been used throughout Vietnam for many years as well as in other tropical countries for the treatment of leech bite, soft tissue wound, burn wound, skin infection and dento-alveolitis (Thang, Patrick, Teik and Yung, 2001). Bouda, Tapondjou, Fontem and Gumedzoe (2001) reported that essential oil extracts from leaves of Siam weed has an insecticidal effect on the adult of maize grain weevils (*Sitophilus zeamais*). Leaf powder of Siam weed has been found to reduce infestation and damage caused by rice moth (*Corcyra cephalonica*). The powder showed a high efficacy against rice moth egg hatching into adult (Allotey and Azalekor, 2000). Siam weed extracts was used as domestic insecticide in Thailand. Leaves of Siam weed can used to control common cutworms, cabbage moths, aphids and beetles (อำนวย อิศรางกูร ณ อยุธยา, 2535).

2.2.3 Hedge flower (*Lantana camara* L.)

Hedge flower (*Lantana camara* L.) is commonly found in Thailand. It is also an important weed that infested farm and land around house. Sometime hedge flower found as an ornamental garden plant. Hedge flower, is classified as follows;

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Lamiales

Family Verbenaceae

Genus *Lantana* L.

Species *Lantana camara* L.

Source: http://plants.usda.gov/java/profile?symbol=laca2.

Figure 2.4 Hedge flower (*Lantana camara* L.).

In Africa, hedge flower play the role as medicinal plant. Leaves of hedge flower are used to treat skin itch, ulcers, hepatitis and rheumatism (Bouda, Tapondjou, Fontem and Gumedzoe, 2001). Moreover, essential oil extracts from leaves of hedge flower has an insecticidal effect on maize grain weevils (*Sitophilus zeamais*). Insecticidal activity of the essential oil can exploit for maize grain weevils (*S. zeamais*) control in stored products (Bouda, Tapondjou, Fontem and Gumedzoe, 2001). Hedge flower leaf extracts was found an effect against termites (*Microcerotermes beesoni*). The extracts exhibit excellent termites mortality (Verma, 2006). In Thailand, hedge flower has been used as folk insecticide. Leaves extracts of hedge flower has a strongly insecticidal activities against aphids and beetles (อำนวย อิศรางกูร ณ อยุธยา, 2535). Hedge flower leave extracts has potential to against *Ae.*

aegypti (Tanprasit, 2005). Kumar and Maneemegalai (2008) reported that the larvicidal effect of hedge flower leave extracts on larvae of mosquito species *Ae. aegypti* and *C. quinquefasciatus*. Adulticidal properties of hedge flower leaf extracts against adult mosquitoes *Aedes aegypti* L., *Culex quinquefasciatus* S., *Anopheles culicifacies* G., *An. fluviatilis* J. and *An. stephensi* Liston have been reported by Dua, Pandey and Dash (2010).

In this study, the leave extracts of marigold (*Tagetes erecta* L.), Siam weed (*Chromolaena odorata* (L.) King & Robinson) and hedge flower (*Lantana camara* L.) were investigated as the guava fruit fly (*Bactrocera correcta* Bezzi) control agents of the eggs, second instar larvae, pupae and the adults.

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CHAPTER III

PHYTOCHEMICAL PROPERTIES AND CYTOTOXICITY OF LEAF EXTRACTS OF MARIGOLD (*TAGETES ERECTA* L.), SIAM WEED (*CHROMOLAENA ODORATA* (L.) KING & ROBINSON) AND HEDGE FLOWER (*LANTANA CAMARA* L.)

3.1 Abstract

Marigold (*Tagetes erecta* L., MLE), Siam weed (*Chromolaena odorata* (L.) King & Robinson, SLE) and hedge flower (*Lantana camara* L., HLE) leaves were extracted in water and 70% ethanol. The total phenolic compounds (TPC) of the extracts were quantified by Folin-Ciocalteu method. The TPC contents of SLE/e, MLE/e and HLE/e were 133.03, 59.67 and 47.43 milligrams gallic acid equivalents per gram (mg GAE/ g), and in SLE/w, MLE/w and HLE/w were 84.87, 52.50 and 33.93 mg GAE/g, respectively. The major compound constituents of the extracts were analyzed by thin layer chromatography (TLC). Vanillin-sulphuric reagents detection indicated that the terpenes group was the major compounds in the extracts. The cytotoxicity of the extracts was investigated by brine shrimp lethality assay (BSLA). The cytotoxic efficacy at 24 hr of SLE/e, MLE/e and HLE/e were 147.15, 182.60 and

208.82 µg/ml, and SLE/w, MLE/w and HLE/w were 196.35, 256.32 and 273.29 µg/ml, respectively.

3.2 Introduction

Plant extracts have been used in controlling of insect pets. The insecticidal properties are the actions of to plant secondary metabolites. The active ingredients of plant secondary metabolites, such as minerals, vitamins, volatile oils, glycosides, alkaloids, flavonoids and other substances that are important for insecticidal properties (Obi, Nwanebu, Ndubuisi-Nnaji, Onuoha and Chiegboka, 2011; Kennedy and Wightman, 2011). The compounds of plant secondary metabolites have different various effects on insects (Boeke et al., 2004). Plant extracts contain several secondary compounds such as, phenolic and terpenes which have toxic activities against insects (Boudet, 2007).

Plants rich bioactive chemicals provide the potential to use as alternative insect control agents. Plant extracts from neem seed, *Parthenium hysterophorus* L. and eucalyptus leaves have been reportet the efficacy for controlling melon fruit fly, *Bactrocera cucurbitae* (Coquillett) in bitter gourd (*Momordica charantia* L.) (Ali et al., 2011). The effects of sweetflag (*Acorus calamus* L.), tumba (*Citrullus colocynthis* L.), turmeric (*Curcuma longa* L.), kuth (*Saussurea lappa* (Decaisne) C. B. Clarke), balchar (*Valeriana jatamansi* J.) and harmal (*Peganum harmala* L.) extracts in petroleum ether, acetone and ethanol were promising repellents against peach fruit fly *Bactrocera zonata* and suppressed the overall egg laying (Rehman, Jilani, Khan, Masih and Kanvil, 2009). The potential of leaf extracts of the hedgerow plant panax, *Polyscias guilfoylei* (B.), were reported that their attractiveness to control male and

female oriental fruit flies, *Bactrocera dorsalis* (Jang, Carvalho and Stark, 1997). The citronella grass methanolic extract has the potency to be used as a tool to protect mango from *Bactrocera carambolae* oviposition (Muryati, Trisyono, Witjaksono and Wahyono, 2012). The insecticidal activities of extracts from *Alpinia galanga* and *Cleome viscosaplant* using topical mist spray method showed the potential against *Bactrocera dorsalis* (Sukhirun, Bullangpoti and Pluempanupat, 2009). The plant extracts of harmal (*Peganum harmala* L.), rhizomes of kuth (*Saussurea lappa* C. B. Clarke) and balchar (*Valariana officianalis* L.) in petroleum ether, acetone and ethanol exhibited the repellent properties and growth inhibiting effect against *Bactocera zonata* Saunder (Khattak, Shahzad and Jilani, 2006).

The aim of this study was the control of guava fruit fly (*Bactrocera correcta*) by the extracts of marigold (*Tagetes erecta* L.), Siam weed (*Chromolaena odorata* L.) and hedge flower (*Lantana camara* L.) leaves. The information of this study is also expected for develop the biological control of guava fruit flies by the leaves of marigold, Siam weed and hedge flower to which the value is added. The plant-based insecticides can be used to replace synthetic insecticides which are beneficial to human health and sustainable the environment.

3.2.1 Plants for insect pest control

Marigold (*T. erecta* L.) is a common garden plant in Thailand. Marigolds have several compounds in their tissues which have biological activities against a range of organisms (Vasudevan, Kashyap and Sharma, 1997; Cetkovic, Djilas, Canadanovic-Brunet, and Thumbas, 2004). Marigolds have the potential to control plant pests including insects (Riga, 2009). The pharmacological activity of marigold is related to

the content of several classes of secondary metabolites such as essential oils, flavonoids, sterols, carotenoids, tannins, saponins, terpenoids, triterpene alcohols, alkaloids, polyacetylenes, fatty acids, polysaccharides, resin, alilanisol, anetol, limonene, methyl eugenol, and β-karyophyllene (Cetkovic, Djilas, Canadanovic-Brunet, and Thumbas, 2004; Salinas-Sánchez et al., 2012). The bioactivities of *T. erecta* extracts containing thienyls and terpenes have been studied extensively and known as nematocide, fungicide and insecticide (Vasudevan, Kashyap and Sharma, 1997). The major components of the essential oil of *T. erecta* were *cis*-ocimene (18.46%), (E)-oscimene (8.65%), l-limonene (11.16%), (E)-tagetone (10.56%), β-caryophyllene (6.9%) and dl-limonene (4.16%) (Tripathi, Bhatia, Walia and Kumar, 2012).

Siam weed (*C. odorata* L.) is widely spread weed in Thailand. *C. odorata* has been used as traditional medicine for the treatment of leech bite, soft tissue wound, burn wound, skin infection and dento-alveolitis (Thang, Patrick, Teik and Yung, 2001; Vaisakh and Pandey, 2012). Phytochemical screening of previous studies demonstrated compound of *C. odorata* extracts such as flavonoids, saponins, tannins steroids, triterpenes, alkaloids and essential oils. The oil from *C. odorata* also has been used as effective insecticides (Misra, 2009; Anyasor, Aina, Olushola and Aniyikaye, 2011; Alisi, Ojiako, Osuagwu and Onyeze, 2011; Félicien et al., 2012). The major chemical constituents in *C. odorata* ethanolic extract were protocatechuic, p-hydroxybenzoic, pcoumaric, ferulic and vanillic acids (Phan et al., 2001). The major components of essential oil from *C. odorata* analyzed by GC-MS were α-pinene (42.2%), β-pinene (10.6%), germacrene D (9.7%), β-copaen-4α-ol (9.4%), (E)-caryophyllene (5.4%), and geijerene/pregeijerene (7.5%) (Owolabi1a et al., 2010).

Hedge flower (*L. camara* L.) is commonly found as weed widely occur in Thailand and also found as an ornamental garden plant. Hedge flower has been commonly used as medicinal plant in Africa. Leaves of *L. camara* are used to treat skin itch, ulcers, hepatitis and rheumatism (Bouda, Tapondjou, Fontem and Gumedzoe, 2001). The major chemical constituents in *L. camara* leave were terpenoids, steroids, flavonoids, phenylethanoid glycosides, furanonaphthoquinones, iridoid glycosides and triterpenes (Sousa et al., 2012). In Thailand, *L. camara* has been commonly used as folk insecticide (อำนวย อิศรางกูร ณ อยุธยา, 2535). The major constituents of *L. camara* leaves extracted carried out by GC and GC-MS were Trans -β caryophyllene (17.65%), sabinene (9.11%), eucalyptol (7.53%), α-humulene (7.14%), bicyclogermacrene (5.77%), germacrene D (2.35%), β-elemene (2.24%), nerolidol (2.14%), davanone B (1.22%) β-caryophyllene (27.0%), α-humulene (11.8%), sabinene (9.7%), bicyclogermacrene (8.1%) and davanone (4.7%) (Singh and Tiwari, 2011; Saikia and Sahoo, 2011).

3.3 Materials and methods

3.3.1 Materials

Folin-Ciocalteus's reagent and sodium carbonate were purchased from Merck Chemical supplies (Darm-Stadt, Germany). Vanillin was purchased from BDH Chemicals Ltd. (Poole, England). Gallic acid was from Sigma (St. Louise, MO, U.S.A.). TLC plate of silica gel, 60 F-254 (thick 0.2 mm, 20 x 20 cm^2) was purchased from Merck Chemical supplies (Darm-Stadt, Germany). Dimethylsulfoxide (DMSO)

was purchased from Merck (Darm-Stadt, Germany). All chemicals were used analytical grade.

3.3.2 Collection for plants and extracts preparation

The fresh leaves of marigold, Siam weed and hedge flower were collected from the areas surrounding Suranaree University of Technology (SUT) campus and then dried by sun light for 2-3 days before extraction. Dried plant samples of 10 g were extracted in distilled water or 70% ethanol by Soxhlet extraction apparatus (Buchi model B811, Germany). The extracts were evaporated, dried by lyophilizer (Heto power dry LL3000, Wag Technology) and stored at -20°C until used. The dried extracts were dissolved in its original solvent during study.

3.3.3 Determination of total phenolic compounds availability

Phenolic compound was determined by the Folin-ciocalteau colorimetric method using gallic acid as a standard phenolic compound (Huang et al., 2004). A hundred micrograms of the extracts was dissolved in its original solvent. A hundred microliter of samples was mixed with 2 mL of 2% sodium carbonate and incubated for 2 min. Added Folin reagent (Folin:Methanol. 1:1 v/v) 100 µL and incubated for 30 minute. The absorbance was measured at 760 nm. The results were expressed as milligrams gallic acid equivalents (GAE) per gram extract. All samples were performed in triplicate.

3.3.4 Thin layer chromatography fingerprintings of plant extracts

TLC is a standard technique for separation of compound mixture. Its

sensitivity is high which allows separation of less than microgram amounts of material. Silica gel on a support material such as glass or aluminum is most widely employed (Harborne, 1998). TLC was used to obtain the fingerprinting of plant extracts in order to figure out the differences of their components. The extracts of 0.05 g were diluted in solvents, spotted on a TLC plate, silica gel 60 F_{254} (2 x 7.4 cm^2). The TLC system for marigold extraction used the mobile phase systems of ethyl acetate: methyl alcohol: carbon tetrachloride (3: 2: 5). The TLC system for Siam weed water extract used the mobile phase systems of ethyl acetate: carbon tetrachloride (3: 7). The TLC system for Siam weed ethanol extract used the mobile phase systems of toluene: chloroform: methyl alcohol (1: 7: 2). The TLC system for hedge flower water extract used the mobile phase systems of ethyl acetate: carbon tetrachloride (3: 7). The TLC system for hedge flower ethanol extract used the mobile phase systems of toluene: chloroform: methyl alcohol (1: 7: 2). The TLC plate was air dried and developed with different solvent. Bands developed were visualized under UV light at 366 nm (CAMAG UV cabinet). The relative migration of TLC bands was described by R_f value. The TLC plate was sprayed with Vanillin-sulphuric acid reagent, and then the TLC plate was heated at 100ºC until color appeared. The Vanillin-sulphuric acid reagent produced pink-red and blue spots (Cannell, 1998).

$$R_f = \frac{\text{Distance traveled by the center of substance spot from the origin (cm)}}{\text{Distance traveled by the solvent from the origin (cm)}}$$

where: R_f stands for ratio of front and its characteristics of any given compounds on each stationary phase using the appropriate mobile phase for the development of the plate.

3.3.5 Determination of cytotoxicity of plant extracts by brine shrimp lethality assay (BSLA)

The brine shrimp lethality assay was used for preliminary assessment of cytotoxicity (Solis, Wright, Anderson, Gupta and Phillipson, 1993). Brine shrimp (*Arthemia salina*) was cultured in artificial seawater (3.6 g of sea salt granules in 1 litter of distilled water). After egg hatching (24 hours), 10 first instars of brine shrimps were transferred to a 24-well plate. The various concentration of extract sample was selected based on the concentrations of preliminary tested. The plant extracts with various concentrations 10, 50, 100, 500, and 1000 µg/mL were added and 0.1% DMSO v/v was used as control. The number of mortality was observed and counted for 24 hr. The median lethal concentration (LC_{50}) was calculated by Probit analysis (Solis, Wright, Anderson, Gupta and Phillipson, 1993).

3.3.6 Data analysis

Data from all experiments were analyzed with a two-way analysis of variance (ANOVA) using program Statistical Package for the Social Sciences (SPSS) program for Windows v.17.0. All analyzes were at 95% confident level. The LC_{50} value was determined by Probit analysis (Finny, 1971).

3.4 Results

3.4.1 Total phenolic compounds

The total phenolic content of marigold, Siam weed and hedge flower leave extracts were determined by the Folin-ciocalteau colorimetric method and calculated as GAE. The results were shown in Table 3.1.

Table 3.1 Total phenolic compounds content of marigold, Siam weed and hedge flower leave extracts.

Plant	Extracts	Total Phenolic Compounds GAE(mg/g) ± S.E.
Tagetes erecta	MLE/w	52.50 ± 1.23 [b]
	MLE/e	59.67 ± 2.80 [c]
Chromolaena odorata	SLE/w	84.87 ± 2.05 [d]
	SLE/e	133.03 ± 3.48 [e]
Lantana camara	HLE/w	33.93 ± 0.77 [a]
	HLE/e	47.43 ± 0.40 [b]

The values are means of 6 observations, within a column followed by different letters are significantly different by Duncann's New Multiple Range Test ($P < 0.05$).

The total phenolic content of marigold, Siam weed and hedge flower ethanol extract (SLE/e, MLE/e and HLE/e) were 133.03 ± 3.48, 59.67 ± 2.80 and 47.43 ± 0.40 mg GAE/g, respectively. The total phenolic content of marigold, Siam weed and hedge flower water extract (SLE/w, MLE/w and HLE/w) were 84.87 ± 2.05, 52.50 ± 1.23 and 33.93 ± 0.77 mg GAE/g, respectively. The ethanol extracts of all plants contained the total phenolic compound was higher than that of the water extract.

3.4.2 Thin layer chromatography (TLC) analysis

TLC analysis was used to investigate some major components of plant extracts. The relative migration, Rf value and information of TLC bands were shown in table 3.2. The profile of marigold, Siam weed and hedge flower before and after

spraying with the vanillin reagent were shown in Figures 3.1-3.6, respectively. The different fluorescence bands were observed under UV light at 366 nm. The Vanillin-sulphuric acid reagent produced pink, red and blue spots, which indicated that the presence of terpenes.

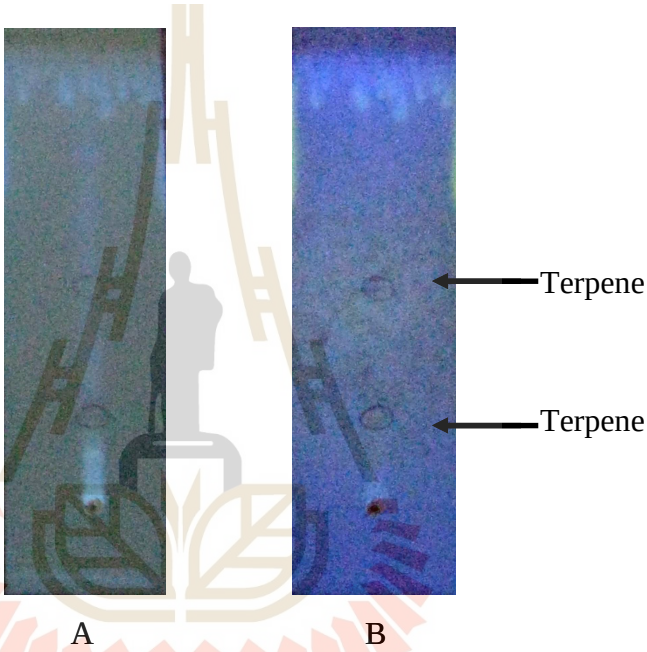


Figure 3.1 TLC chromatographs of marigold (*T. erecta*) water extract using a mobile phase of ethyl acetate: methyl alcohol: carbon tetrachloride at the ratio of 3: 2: 5.

A: before spraying with vanillin-sulphuric acid reagent

B: after spraying with vanillin-sulphuric acid reagent

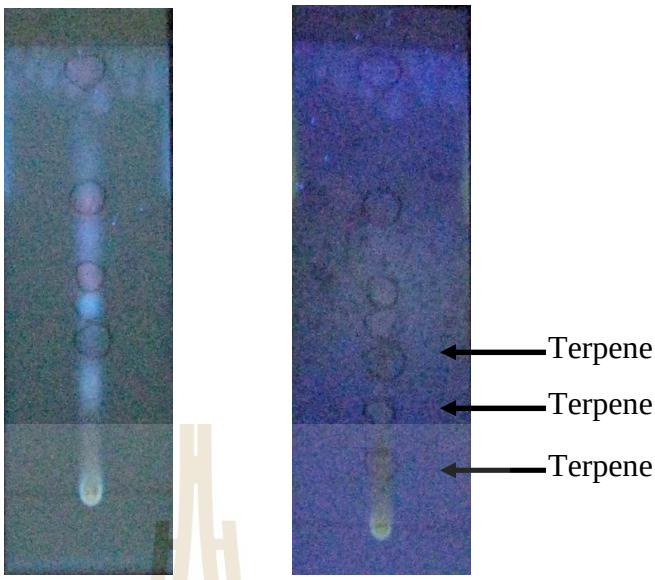


Figure 3.2 TLC chromatographs of marigold (*T. erecta*) ethanol extract using a mobile phase of ethyl acetate: methyl alcohol: carbon tetrachloride at the ratio of 3: 2: 5.

A: before spraying with vanillin-sulphuric acid reagent

B: after spraying with vanillin-sulphuric acid reagent

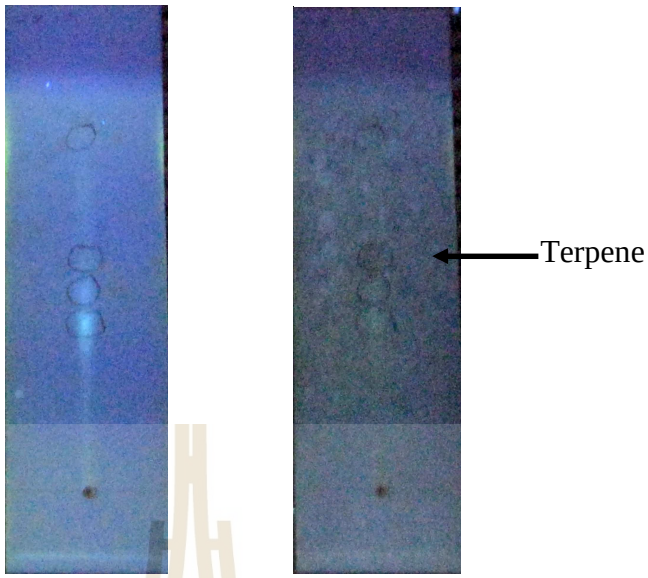


Figure 3.3 TLC chromatographs of Siam weed (*C. odorata*) water extract using a mobile phase of ethyl acetate: carbon tetrachloride at the ratio of 3: 7.

A: before spraying with vanillin-sulphuric acid reagent

B: after spraying with vanillin-sulphuric acid reagent

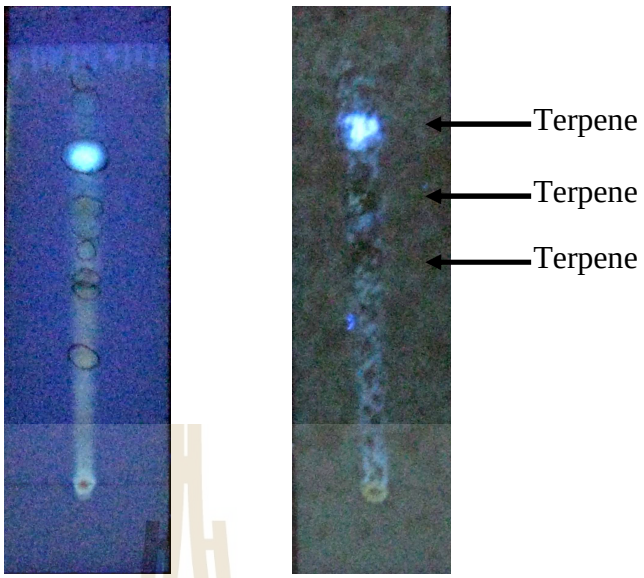
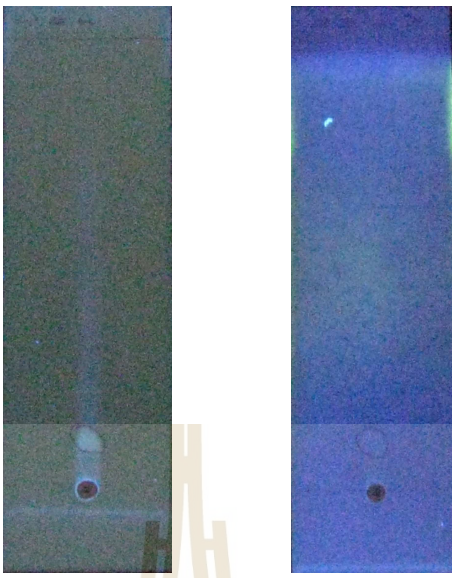


Figure 3.4 TLC chromatographs of Siam weed (*C. odorata*) ethanol extract using a mobile phase of toluene: chloroform: methyl alcohol at the ratio of 1: 7: 2.

A: before spraying with vanillin-sulphuric acid reagent

B: after spraying with vanillin-sulphuric acid reagent



A B

Figure 3.5 TLC chromatographs of hedge flower (*L. camara*) water extract using a mobile phase of ethyl acetate: carbon tetrachloride at the ratio of 3: 7.

A: before spraying with vanillin-sulphuric acid reagent

B: after spraying with vanillin-sulphuric acid reagent

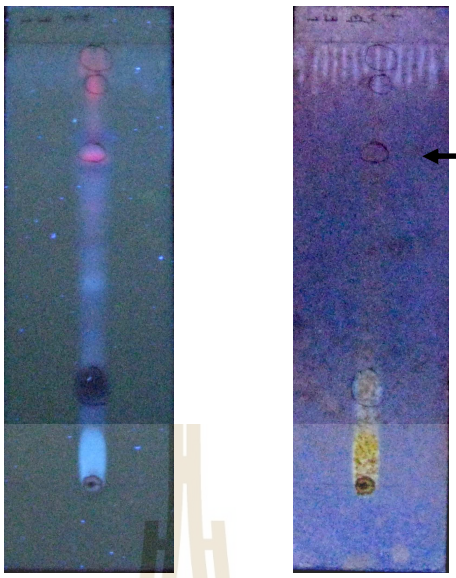


Figure 3.6 TLC chromatographs of hedge flower (*L. camara*) ethanol extract using a mobile phase of toluene: chloroform: methyl alcohol at the ratio of 1: 7: 2.

A: before spraying with vanillin-sulphuric acid reagent

B: after spraying with vanillin-sulphuric acid reagent

Table 3.2 The information detailed on TLC analyses of marigold, Siam weed and hedge flower leaf extracts.

Plant	Solvent	Mobile phase	Spraying reagent	No. of spots	R_f Values and color components
Tagetes erecta	H_2O	ethyl acetate: methyl alcohol: carbon tetrachloride (3: 2: 5)	Before	2	0.17 (deep red), 0.37 (light green)
			After	2	0.17 (deep red), 0.37 (pink)
	Alc	ethyl acetate: methyl alcohol: carbon tetrachloride (3: 2: 5)	Before	5	0.3 (green), 0.37 (blue), 0.43 (pink), 0.58 (pink), 0.87 (pink)
			After	3	0.1 (deep red), 0.2 (blue), 0.3 (deep red)
Chromolaena odorata	H_2O	ethyl acetate: carbon tetrachloride (3: 7)	Before	4	0.38 (light green), 0.5 (light blue), 0.57 (green), 0.8 (light green)
			After	1	0.57 (deep red)
	Alc	toluene: chloroform: methyl alcohol (1: 7: 2)	Before	9	0.27 (yellow), 0.38 (green), 0.4 (pink), 0.47 (light blue), 0.52 (light blue), 0.57 (pink), 0.68 (light blue), 0.8 (blue), 0.85 (pink)
			After	3	0.47 (deep blue), 0.57 (deep blue), 0.68 (light blue)

Table 3.2 The information detailed on TLC analyses of marigold, Siam weed and hedge flower leaf extracts (Continued).

Plant	Solvent	Mobile phase	Spraying reagent	No. of spots	R_f Values and color components
Lantana camara	H_2O	ethyl acetate: carbon tetrachloride (3: 7)	Before	1	0.1 (light green)
			After	0	-
	Alc	toluene: chloroform: methyl alcohol (1: 7: 2)	Before	4	0.2 (blue), 0.68 (red), 0.83 (pink), 0.88 (pink)
			After	1	0.68 (red)

3.4.3 Cytotoxicity

The cytotoxicity of the extracts was investigated by brine shrimp lethality assay (BSLA). The median lethal concentration (LC_{50}) of treatment was evaluated at 24 hours. The ethanol extracts of Siam weed (SLE/e) showed most prominent activity with LC_{50} of 147.15 µg/ml. The ethanol extracts of marigold and hedge flower exhibited potent lethality with LC_{50} of 182.60 and 208.82 µg/ml respectively. Whereas, the LC_{50} values at 24 hours of Siam weed, marigold and hedge flower water extracts (SLE/w, MLE/w and HLE/w) were 196.35, 256.32 and 273.29 µg/ml, respectively. The data represent the mean of LC_{50} values (Table 3.3).

Table 3.3 The cytotoxicity of the plant extracts performed by the brine shrimp lethality assay (BSLA).

Plant	**Extracts**	**LC_{50}(µg/ml)**
Tagetes erecta	MLE/w	256.32
	MLE/e	182.60
Chromolaena odorata	SLE/w	196.35
	SLE/e	147.15
Lantana camara	HLE/w	273.29
	HLE/e	208.82

Note: MLE/w, Marigold leaf water extract; MLE/e, Marigold leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract; HLE/w, Hedge flower leaf water extract; SLE/e, Siam weed leaf ethanol extract; SLE/w, Siam weed leaf water extract.

3.5 Discussion

Plant extracts contain several secondary compounds which have toxic activities to against insects (Boudet, 2007). The phenolics and their functional groups are considered as pest control (Makoi and Ndakidemi, 2007). The extracts of marigold (*T. erecta*), Siam weed (*C. odorata*) and hedge flower (*L. camara*) were investigated for phenolic compounds. The ethanolic extract of marigold, Siam weed and hedge flower showed higher phenolic content than water extract. The major components of plant extracts were analyzed by thin layer chromatography (TLC). It was mainly composed of terpenes detected by Vanillin-sulphuric reagent, similarly the previous literature that found terpenes in the extracts of marigold, Siam weed and hedge flower (Vasudevan, Kashyap and Sharma, 1997; Cetkovic, Djilas, Canadanovic-Brunet and Thumbas, 2004; Misra, 2009; Anyasor, Aina, Olushola and Aniyikaye, 2011; Alisi, Ojiako, Osuagwu and Onyeze, 2011; Félicien et al., 2012; Salinas-Sánchez et al., 2012; Sousa et al., 2012). The presence of terpenes in the extracts is believed to be insecticide (Vasudevan, Kashyap and Sharma, 1997).

3.6 Conclusion

In conclusion, this study indicates that the extracts of marigold, Siam weed and hedge flower possess insecticidal activities. The total phenolic compound contents of ethanolic extracts were higher than water extract in all plants. The presence of major components of the extracts was analyzed by TLC. In all extracts, except *L. camara* water extract, the Vanillin-sulphuric acid reagent produced pink, red and blue spots, which were likely to be terpenes. It is suggested that the extracts can be used as an insect control agents.

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CHAPTER IV

ANTIBIOSIS EFFECTS OF LEAF EXTRACTS OF MARIGOLD (*TAGETES ERECTA* L.), SIAM WEED (*CHROMOLAENA ODORATA* (L.) KING & ROBINSON) AND HEDGE FLOWER (*LANTANA CAMARA* L.) ON FRUIT FLIES (*BACTROCERA CORRECTA* BEZZI)

4.1 Abstract

The leaf ethanol and water extracts of marigold (*Tagetes erecta* L., MLE), Siam weed (*Chromolaena odorata* (L.) King & Robinson, SLE) and hedge flower (*Lantana camara* L., HLE) were investigated for antibiosis on eggs, larvae and pupae of guava fruit flies (*Bactrocera correcta*). The inhibition of egg hatching by SLE/e exhibited the highest effect of 82.22 ± 6.19% at 24 hours with EC_{50} value of 44.54 mg/ml. The inhibitory effects of all extracts on fruit flies egg hatching were ranged as SLE/e > HLE/e > MLE/e > SLE/w > HLE/w > MLE/w. The morality of second instar larvae were most induced by SLE/e at 83.33 ± 1.92% mortality with the LC50 value of 55.56 mg/ml by feeding assay, which was similar to dipping assay at 87.78 ± 1.11% with LC_{50} value of 52.99 mg/ml. The range of larvicidal efficacy of all extracts by feeding assay and dipping assay was equal. The efficacy was ranged as SLE/e > HLE/e > SLE/w > MLE/e > HLE/w > MLE/w. The effects of the three plant extracts on the adult emergence were similar to the effects of the egg hatching and the larva

mortality. The antibiosis on fruit fly adult emergence by SLE/e exhibited the highest efficacy with EC_{50} of 69.55 mg/ml, produced 67.78 ± 2.22% inhibition. However, the inhibition rates were ranged as SLE/e > MLE/e > SLE/w > HLE/e > MLE/w > HLE/w. It is concluded that the SLEs extracts were most potent in controlling eggs hatching, larvae and pupae mortalities of guava fruit flies *B. correcta*.

4.2 Introduction

Fruit flies are serious pests that cause economic losses of fruit and vegetable products in many countries. The major corps production export market of Thailand is fresh fruits. However, many crops especially citrus, carambola, guava, litchi, longan, peach, rose-apple, sapodilla and mango fruits are damaged by fruit fly attacks and losses may reach up to 100% (Dick and Drew, 1994). Adult fruit flies lay their eggs in the pericarp and larvae feed inside the fruits. The infestations not only cause severe damages by reducing both fruit production and quality, but also impacting on fresh fruit exportation due to the quarantine restrictions (Chen, Dong, Li and Liu, 2006). There are several strategies for controlling of fruit fly in commercial or domestic crops. The synthetic insecticides have been used globally to reduces economic losses and maintain agricultural products. The frequent use of those synthetic insecticides for fruit flies control has not resulted in sustainable management of the pest. Problems of chemical control are many residues of insecticides left in crops, health problems for farmers, contamination of water and soil, insecticide resistance development and decrease in natural enemy populations (Guamán, 2009). The plant products have been used as botanical insecticides are better ways to avoid problems of insecticide resistance. They are also biodegradable and harmless to the environment, pest-specific

and relatively harmless to non-target organisms including humans (Rehman, Jilani, Khan, Masih and Kanvil, 2009). One plant species may possess substances with a wide range of activities. Some plant products act as attractants while some plant products act as repellent, deterrent and anti-oviposition, like neem oil and cotton seed oil (Khattak, Shahzad and Jilani, 2006). There are several promising candidates of plant materials for future utilization as insects control agents. The marigold (*Tagetes erecta* L.), Siam weed (*Chromolaena odorata* (L.) King & Robinson) and hedge flower (*Lantana camara* L.) are plant from which several domestic insecticides have been considered worldwide.

Leaves and flowers methanol and ethanol extract of hedge flower *L. camara* have been found larvicidal effect on 3rd and 4th instar larvae of mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. The components of them were carried out by GC/MS analysis presence saponin, flavonoids, terpenoids and cardiac glycosides (Kumar and Maneemegalai, 2008). Essential oil from the leaves of *L. camara* showed adulticidal activity against important vectors of malaria, dengue, dengue hemorrhagic fever, yellow fever and chikungunya (Dua, Pandey and Dash, 2010). The flowers of *T. erecta* are very effective lavicide and could be useful against *Cx. quinquefasciatus* (Nikkon, Habib, Saud and Karim, 2011). The powder of Siam weed *C. odorata* showed a high efficacy against rice moth egg hatching into adult (Allotey and Azalekor, 2000). Therefore, this study aimed to find the use of plant extract for getting rid of fruit flies for biological safety to humans and the environment.

4.3 Materials and methods

4.3.1 Plants collection and preparation for extracts

Siam weed (*Chromolaena odorata* (L.) King & Robinson) and hedge flower (*Lantana camara* L.) leaves were collected on Suranaree University of Technology (SUT) campus. Marigold (*Tagetes erecta* L.) leaves were collected from a local farm surrounding Suranaree University of Technology (SUT) campus. All plant leaves were cleaned, sun dried and ground to powders. The dried plant powders of 10 g in a cellulose extraction thimble (Whatman International Ltd., Maidstone, England) were extracted in 150 ml of water or 70% ethanol in Soxhlet extraction apparatus (Buchi model B 811, Germany). The extracts were filtered, evaporated in rotary evaporator (Buchi instruments, Switzerland) and dried by lyophilizer (Freeze-zone 12 plus, Labconco Corporation, Missouri, USA). The extracts powders were stored at -20ºC until analysis. The dried extracts were dissolved in their original solvents for experiments.

Figure 4.1 The crude extracts of water and ethanol leaves of marigold, Siam weed and hedge flower.

4.3.2 Fruit fly rearing

Pupae of guava fruit fly (*Bactrocera correcta*) were obtained from the Thailand Institute of Nuclear Technology (Public Organization), Ministry of Science and Technology, Thailand. Adult flies emerge from the pupa cases in 7 days. Fruit flies were cultured in wire-net cages under laboratory conditions at 28 ± 2ºC, 65-70% relative humidity, and 12 hours light: 12 hours dark. Fruit fly fed with artificial food (Walker et al., 1997) and allowed to mate. The females were allowed to lay eggs in the egg dome (contain guava juice). After hatching, the larvae feed on artificial food and allow moving to pupa stage in wood chip trays.

4.3.3 Antibiosis to egg hatching

The antibiosis to egg hatching assay was modified from previous method (วรนาฎ คงตระกูล, 2544). Thirty eggs were gently moved and placed on Whatman filter paper discs #3 (42.5 mm in diameter), which were damped with 20, 40, 60, 80 and 100 mg/ml of the plant extracts. The papers were placed in the artificial food. Water and ethanol were used as controls. The eggs were allowed to hatch into larvae. The numbers of larvae were counted within 24 hours. The experiments were performed in triplicate. The median effective concentration, EC_{50}, was calculated by Probit analysis, used SPSS (Statistical Package for the Social Sciences) program for Windows v.17. The results were expressed as percentage of inhibition rate as

$$\% \text{ Inhibition} = \frac{\text{Number collected from control - Number collected from treated}}{\text{Number collected from the treated}} \times 100.$$

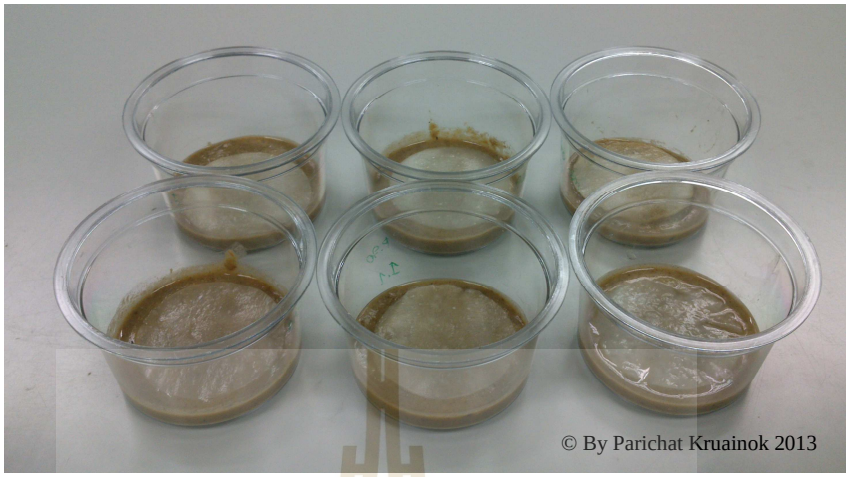

Figure 4.2 The preparation for testing eggs hatching.

4.3.4 Antibiosis to larval growth

4.3.4.1 Feeding assay

The antibiosis to larval growth by feeding assay was modified from previous methods (Chuenwong, 2006 and พรพิมล เตชะวัฒนเศรษฐ์, 2542). Thirty second-instar larvae of guava fruit fly were cultured in artificial food mixed with 20, 40, 60, 80 and 100 mg/ml of the plant extracts. Mortality of larvae were counted after 6, 12 and 24 hours. Water and ethanol were used as controls. The experiments were performed in triplicate. The mortality was corrected by Abbott's formula (Abbott, 1925). The mortality was calculated and analyzed for the LC_{50} by Probit analysis. The results were expressed as percentage of mortality as following formula;

$$\% \text{ Mortality } = \frac{\% \text{ Test mortality-\% Control mortality}}{100\text{-\% Control mortality}} \times 100 .$$

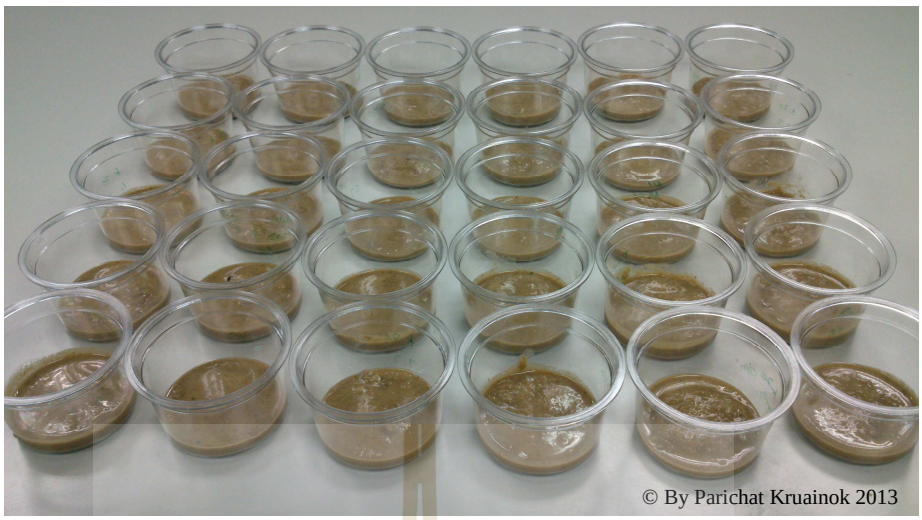

Figure 4.3 The preparation for larvicidial mortality tests by feeding assay.

Figure 4.4 Second instar larvae of fruit fly *B. correcta* fed on artificial food.

4.3.4.2 Dipping assay

The antibiosis to larval growth by dipping assay was modified from previous methods (Chuenwong, 2006, พรพิมล เตชะวัฒนเศรษฐ์, 2542 and สมบูรณ์ แสงมณีเดช และคณะ, 2548). Thirty second-instar larvae of guava fruit fly were dipped into

20, 40, 60, 80 and 100 mg/ml of the plant extracts for 3 seconds. Treated larvae were put in a 100-ml bottle, which the lid with a small hole was covered. The number of mortality larvae was counted after 6, 12 and 24 hours. Water and ethanol were used as controls. The experiments were performed in triplicate. The mortality was corrected by Abbott's formula (Abbott, 1925). The mortality was calculated and analyzed for the LC_{50} by Probit analysis. The results were expressed as percentage of mortality as previous formula.

4.3.5 Antibiosis to adult emergence

Thirty third-instar larvae of guava fruit fly were cultured in artificial food mixed with the three extracts at the concentrations of 20, 40, 60, 80 and 100 mg/ml. Water and ethanol were used as controls. The larvae were allowed to develop into pupae in the food. The numbers of molted adult flies were counted. The experiments were performed in triplicate. The median effective concentration, EC_{50}, was calculated by Probit analysis, used SPSS (Statistical Package for the Social Sciences) program for Windows v.17. The results were expressed as percentage of inhibition rate as previous formula.

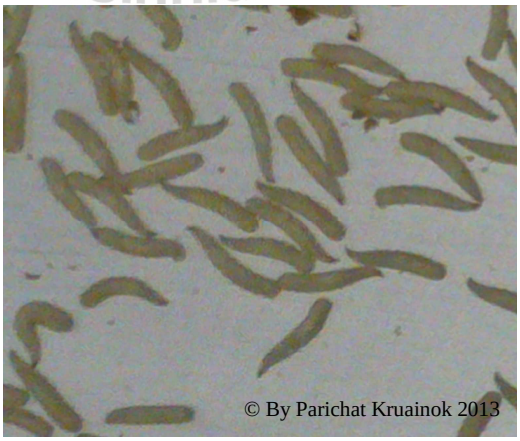

Figure 4.5 Third instar larvae of fruit fly *B. correcta* before cultured in artificial food.

A **B**

Figure 4.6 Pupae of guava fruit fly *B. correcta*.

A: Normal pupae

B: Pupae treated by plant extracts

4.4 Data analysis

All data were analyzed using analysis of variance (ANOVA) and in completely randomized design (CRD) using Statistical Package for the Social Sciences (SPSS) program for Windows v.17. The means were separated by the Duncan's Multiple Range Test (DMRT) when ANOVA was significant ($P<0.05$). Probit analysis (Finny, 1971) was used to estimate LC_{50} and EC_{50} value. The percentages of the mortality and inhibition rate were corrected by Abbott's formula (1925).

4.5 Results

4.5.1 Antibiosis to egg hatching

The eggs of fruit flies were treated with Siam weed (*C. odorata*, SLE) and hedge flower (*L. camara*, HLE) marigold (*T. erecta*, MLE) leaf extracts. The effects of the extracts were dose dependent. The SLE/e at the concentration of 100 mg/ml produced highest inhibitory effect of 82.22 ± 6.19% with EC_{50} value of 44.54 mg/ml (Table 4.1). The marigold leaf water extract, MLE/w was least effect on the egg hatching at 62.22 ± 1.11% inhibitory with EC_{50} value of 75.41 mg/ml. The ethanol extracts was much higher effect to egg hatching at the same concentration. The SLE/e produced 82.22 ± 6.19% inhibition, but the SLE/w produced only 76.67 ± 1.92% inhibition which was 1.08 fold lower. The HLE/e caused 80.00 ± 1.92% inhibition, while the HLE/w at the same concentration caused 66.67 ± 1.92% inhibition. The HLE/e was 1.2 fold more toxic than the HLE/w. Similarly, MLE/e produced 72.22 ± 1.11% inhibition and MLE/w showed 62.22 ± 1.11% inhibition which was 1.16 fold lower. Thus, the inhibitory effects of all extracts on fruit flies egg hatching were ranged as SLE/e > HLE/e > MLE/e > SLE/w > HLE/w > MLE/w (Figure 4.7).

Table 4.1 The egg hatching inhibition of guava fruit flies by leaf extracts of marigold, Siam weed and hedge flower.

Concentration mg/ml	% Inhibition (Mean ± SE)					
	Water extract			Ethanol extract		
	MLE/w	SLE/w	HLE/w	MLE/e	SLE/e	HLE/e
H_2O	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Ethanol	5.56 ± 1.11b	5.56 ± 1.11a	5.56 ± 1.11b	5.56 ± 1.11a	1.11 ± 1.11a	5.56 ± 1.11a
20	12.22 ± 1.11c	14.44 ± 2.22b	14.44 ± 2.94c	44.44 ± 2.94b	51.11 ± 5.56b	44.44 ± 2.94b
40	27.78 ± 1.11d	35.56 ± 4.84c	31.11 ± 1.11d	46.67 ± 0.00b	63.33 ± 3.33bc	46.67 ± 0.00b
60	44.44 ± 1.11e	48.89 ± 2.94d	45.56 ± 2.22e	57.78 ± 1.11c	70.00 ± 6.94bc	57.78 ± 1.11c
80	55.56 ± 1.11f	60.00 ± 1.92e	56.67 ± 0.00f	67.78 ± 4.84d	78.89 ± 6.76c	67.78 ± 4.84d
100	62.22 ± 1.11g	76.67 ± 1.92f	66.67 ± 1.92g	72.22 ± 1.11d	82.22 ± 6.19c	80.00 ± 1.92e
EC_{50} (mg/ml)	75.41	65.66	72.03	55.92	44.54	53.42

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

EC_{50}, median effective concentration

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract; MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

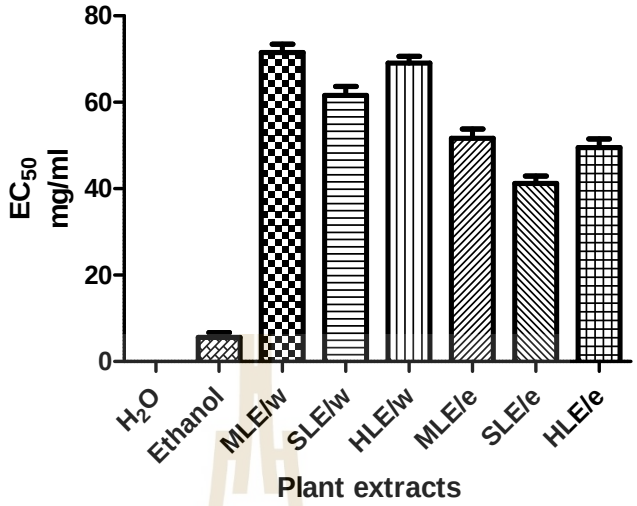

Figure 4.7 Egg hatching effects with EC_{50} values of the leaf water and ethanol extracts of marigold (*T. erecta*, MLE/w, MLE/e), the Siam weed (*C. odorata*, SLE/w, SLE/e) and hedge flower (*L. camara*, HLE/w, HLE/e) on *B. corrrecta*.

The relation among the conditions of treatment results shown in Table 4.2. It indicates that the fruit flies egg hatching inhibition with treatment of three plants (P) and two solvents (S), plant (P) and concentrations (C), solvents (S) and concentrations (C) is well correlate. However, the plants (P) and solvents (S) and concentration (C) are not significantly correlated. It can conclude that the inhibition of egg hatching of these 3 plant extracts are concentration dependent.

Table 4.2 Correlation among treatment conditions analyzed by analysis of variance for percent egg hatching inhibition of fruit flies with plant extracts, solvent and concentrations.

Variation	Degree of freedom	Mean square	F-value	Sig.
Plant (P)	2	2.523	71.234*	0.000
Solvent(S)	1	21.696	612.526*	0.000
P × S	2	0.255	7.210*	0.001
Concentration (C)	4	17.922	505.965*	0.000
P × C	2	0.255	7.210*	0.000
S × C	8	0.354	10.003*	0.000
P × S × C	8	0.051	1.432	0.188
Error	150	0.035		
Total	180			

* = Significant at $p \leq 0.05$
P = marigold, Siam weed and hedge flower S = water, ethanol
C = concentration of 20, 40, 60, 80, 100 mg/ml

4.5.2 Antibiosis to larval growth

4.5.2.1 Feeding assay

The second instar larvae of guava fruit fly *B. correcta* were cultured in artificial food mixed with the extracts. The effects of plant extracts on larval mortality were compared by the LC_{50} values at 24 h. The larvicidal efficacy of all extracts was ranged as SLE/e > HLE/e and SLE/w > MLE/e > HLE/w > MLE/w (Figure 4.8). It was evidently that the SLE/e expressed the highest effects on the second instar larvae at 83.33 ± 1.92% mortality with LC_{50} value 55.56 mg/ml (Table 4.3). The MLE/w efficacy was least with LC_{50} of 80.14 mg/ml causing 62.22 ± 2.22% mortality. The HLE/e and SLE/w toxicities were similar with LC_{50} values of 65.95 mg/ml. The mortality of fruit fly larvae was increased as the extract concentration increased and prolonged time (Tables 4.4 and 4.5). The ethanol extracts was much higher effect to larvae at the same concentration. The SLE/e produced 83.33 ± 1.92% inhibition, but the SLE/w produced only 74.44 ± 4.01% inhibition which was 1.1 fold lower. The HLE/e caused 74.44 ± 4.01% inhibition, while the HLE/w at the same concentration caused 65.56 ± 1.11% inhibition. The HLE/e was 1.1 fold more toxic than the HLE/w. Similarly, MLE/e produced 68.89 ± 1.11% inhibition and MLE/w showed 62.22 ± 2.22% inhibition which was 1.1 fold lower.

Table 4.3 The mortality effects of marigold, Siam weed and hedge flower on fruit fly larvae by feeding assay at 24 hours.

Concentration mg/ml	% Mortality (Mean ± SE)					
	Water extract			Ethanol extract		
	MLE/w	SLE/w	HLE/w	MLE/e	SLE/e	HLE/e
H_2O	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Ethanol	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
20	15.56 ± 1.11b	21.11 ± 1.11b	15.56 ± 1.11b	21.11 ± 1.11b	28.89 ± 1.11b	21.11 ± 1.11b
40	23.33 ± 1.92c	31.11 ± 1.11c	24.44 ± 2.22c	25.56 ± 1.11b	40.00 ± 1.92c	31.11 ± 1.11c
60	37.78 ± 2.94d	48.89 ± 1.11d	44.44 ± 1.11d	41.11 ± 2.94c	60.00 ± 1.92d	48.89 ± 1.11d
80	47.78 ± 1.11e	63.33 ± 1.92e	54.44 ± 2.94e	57.78 ± 2.22d	73.33 ± 1.92e	63.33 ± 1.92e
100	62.22 ± 2.22f	74.44 ± 4.01f	65.56 ± 1.11f	68.89 ± 1.11e	83.33 ± 1.92f	74.44 ± 4.01f
LC_{50} (mg/ml)	80.14	65.95	74.67	72.33	55.56	65.95

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

LC_{50}, median effective concentration.

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract; MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

Table 4.4 Mortality effects of marigold, Siam weed and hedge flower water leaf extracts on fruit fly larvae by feeding assay.

Extracts	Concentration mg/ml	% Mortality (Mean ± SE)		
		6 hours	12 hours	24 hours
MLE/w	20	7.78 ± 1.11b	11.11 ± 2.22b	15.56 ± 1.11b
	40	8.89 ± 1.11b	16.67 ± 1.92c	23.33 ± 1.92c
	60	17.78 ± 1.11c	31.11 ± 2.94d	37.78 ± 2.94d
	80	31.11 ± 2.94d	42.22 ± 1.11e	47.78 ± 1.11e
	100	43.33 ± 1.92e	55.56 ± 2.22f	62.22 ± 2.22f
SLE/w	20	13.33 ± 0.00b	13.33 ± 1.92b	21.11 ± 1.11b
	40	17.78 ± 1.11c	20.00 ± 1.92c	31.11 ± 1.11c
	60	32.22 ± 2.22d	40.00 ± 1.92d	48.89 ± 1.11d
	80	41.11 ± 1.11e	55.56 ± 2.94e	63.33 ± 1.92e
	100	56.67 ± 1.92f	66.67 ± 1.92f	74.44 ± 4.01f
HLE/w	20	10.00 ± 1.92b	12.22 ± 1.11b	15.56 ± 1.11b
	40	12.22 ± 1.11b	17.78 ± 1.11c	24.44 ± 2.22c
	60	18.89 ± 2.94c	37.78 ± 2.94d	44.44 ± 1.11d
	80	35.56 ± 2.94d	46.67 ± 1.92e	54.44 ± 2.94e
	100	47.78 ± 1.11e	60.00 ± 1.92f	65.56 ± 1.11f
H_2O		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract.

Table 4.5 Mortality effects of marigold, Siam weed and hedge flower ethanol leaf extracts on fruit fly larvae by feeding assay.

Extracts	Concentration mg/ml	% Mortality (Mean ± SE)		
		6 hours	12 hours	24 hours
MLE/e	20	11.11 ± 1.11b	15.56 ± 1.11b	21.11 ± 1.11b
	40	15.56 ± 2.22b	20.0 ± 01.92b	25.56 ± 1.11b
	60	24.44 ± 2.22c	34.44 ± 2.94c	41.11 ± 2.94c
	80	37.78 ± 2.94d	51.11 ± 2.22d	57.78 ± 2.22d
	100	52.22 ± 1.11e	61.11 ± 2.94e	68.89 ± 1.11e
SLE/e	20	14.44 ± 1.11b	21.11 ± 1.11b	28.89 ± 1.11b
	40	23.33 ± 1.92c	31.11 ± 1.11c	40.00 ± 1.92c
	60	38.89 ± 1.11d	48.89 ± 1.11d	60.00 ± 1.92d
	80	48.89 ± 1.11e	63.33 ± 1.92e	73.33 ± 1.92e
	100	61.11 ± 1.11f	74.44 ± 4.01f	83.33 ± 1.92f
HLE/e	20	14.44 ± 1.11b	11.11 ± 1.11b	21.11 ± 1.11b
	40	17.78 ± 1.11b	20.00 ± 1.92c	31.11 ± 1.11c
	60	27.78 ± 2.22c	40.00 ± 1.92d	48.89 ± 1.11d
	80	41.11 ± 2.94d	55.56 ± 2.94e	63.33 ± 1.92e
	100	54.44 ± 1.11e	64.44 ± 1.11f	74.44 ± 4.01f
Ethanol		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

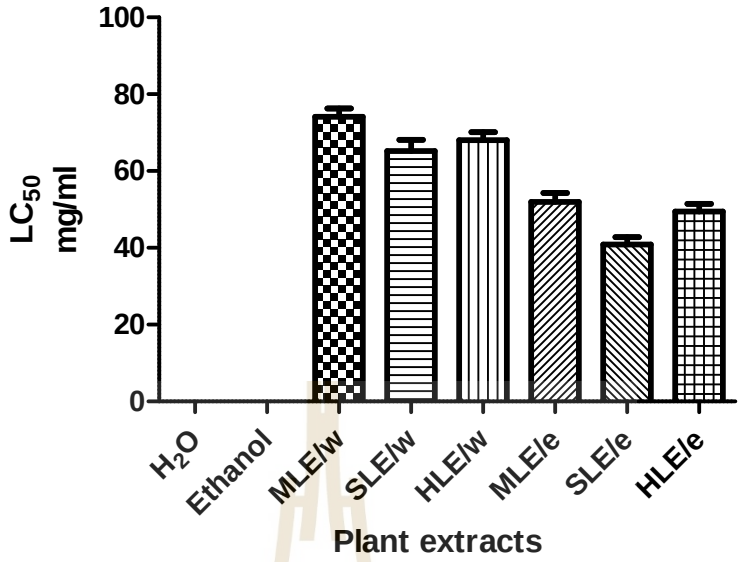

Figure 4.8 Mortality effects with LC_{50} values of the leaf water and ethanol extracts of marigold (*T. erecta*, MLE/w, MLE/e), the Siam weed (*C. odorata*, SLE/w, SLE/e) and hedge flower (*L. camara*, HLE/w, HLE/e) on second instar larvae *B. corrrecta* by feeding assay.

Table 4.6 demonstrated the correlations of the plant extracts and the experimental conditions. The plants (P), solvents (S), concentrations (C), and time periods (T) were not significantly correlated. The plants – times; and solvents - times are also not significantly correlated. However, the plants and solvents; plants and concentrations; solvents and concentrations; times and concentrations were significantly correlated. The plants and solvents and concentrations were well correlated. It can conclude that the mortality effect on fruit fly larvae of these 3 plant extracts are solvent and concentration dependent.

Table 4.6 Correlation among treatment conditions analyzed by analysis of variance for percent mortality of fruit fly larvae by feeding assay with plant extracts, solvent, concentrations and times.

Source of Variation	Degree of freedom	Mean square	F-value	Sig.
Plant (P)	2	1.645	3.778*	0.023
Solvent(S)	1	0.021	0.049	0.825
P × S	2	2.030	4.660*	0.010
Concentration (C)	4	8.116	18.636*	0.000
P × C	8	9.769	22.432*	0.000
S × C	4	2.262	5.194*	0.000
P × S × C	8	1.922	4.413*	0.000
Time(T)	4	22.156	50.875*	0.000
P × T	6	.826	1.896	0.079
S × T	4	.554	1.271	0.280
C × T	16	2.211	5.078*	0.000
P × S × C × T	34	0.088	0.203	1.000
Error	786	0.436		
Total	900			

* = Significant at $p \leq 0.05$
P = marigold, Siam weed and hedge flower S = water, ethanol
C = concentration of 20, 40, 60, 80, 100 mg/ml T = 6, 12, 24 hours

4.5.2.1 Dipping assay

The effects of plant extracts by dipping assay on mortality of fruit fly *B. correcta* larvae were observed. These toxic effects were similarly to the effects on the larval by feeding assay. The SLE/e was able to induce highest mortality over 87.78 ± 1.11% with LC_{50} value of 52.99 mg/ml, while the MLE/w was least induced 65.56 ± 1.11% mortality with LC_{50} value of 77.52 mg/ml (Table 4.7). The

effects of the extracts were dose and time dependent (Tables 4.8 and 4.9). The mortality effect of HLE/e was nearly equal to that the SLE/w which were 63.43 and 64.08 mg/ml and induced 67.78 ± 1.11% and 76.67 ± 1.92% death, respectively. The larvicidal efficacy of all extracts by dipping assay were ranged as SLE/e > HLE/e > SLE/w > MLE/e > HLE/w > MLE/w (Figure 4.9). The ethanol extracts more effective on fruit fly larvae than those of the water extracts. The HLE/e caused 67.78 ± 1.11% inhibition similarly the HLE/w with LC_{50} value of 63.43 and 71.77 mg/ml respectively. The SLE/e produced 87.78 ± 1.11% inhibition which was 1.1 fold greater than the SLE/w which produced only 76.67 ± 1.92% inhibition. Similarly, MLE/e produced 71.11 ± 1.11% inhibition and MLE/w showed 65.5665.56 ± 1.11% inhibition which was 1 fold lower.

Table 4.7 The mortality effects of marigold, Siam weed and hedge flower on fruit fly larvae by dipping assay at 24 hours.

Concentration mg/ml	% Mortality (Mean ± SE)					
	Water extract			Ethanol extract		
	MLE/w	SLE/w	HLE/w	MLE/e	SLE/e	HLE/e
H_2O	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Ethanol	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
20	15.56 ± 1.11b	22.22 ± 1.11b	16.67 ± 0.00b	22.22 ± 1.11b	30.00 ± 0.00b	16.67 ± 0.00b
40	24.44 ± 2.22c	32.22 ± 1.11c	26.67 ± 0.00c	26.67 ± 1.93c	42.22 ± 1.11c	26.67 ± 0.00c
60	40.00 ± 0.00d	51.11 ± 1.11d	46.67 ± 0.00d	43.33 ± 1.92d	61.11 ± 2.22d	46.67 ± 0.00d
80	48.89 ± 1.11e	64.44 ± 1.11e	57.78 ± 2.22e	58.89 ± 1.11e	75.56 ± 1.11e	57.78 ± 2.22e
100	65.56 ± 1.11f	76.67 ± 1.92f	67.78 ± 1.11f	71.11 ± 1.11f	87.78 ± 1.11f	67.78 ± 1.11f
LC_{50} (mg/ml)	77.52	64.08	71.77	70.29	52.99	63.43

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

LC_{50}, median effective concentration.

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract; MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

Table 4.8 Mortality effects of marigold, Siam weed and hedge flower water leaf extracts on fruit fly larvae by dipping assay.

Extracts	Concentration mg/ml	% Mortality (Mean ± SE)		
		6 hours	12 hours	24 hours
MLE/w	20	7.78 ± 1.11b	11.11 ± 1.11b	15.56 ± 1.11b
	40	10.00 ± 1.92b	17.78 ± 1.11c	24.44 ± 2.22c
	60	20.00 ± 0.00c	33.33 ± 1.93d	40.00 ± 0.00d
	80	31.11 ± 1.11d	44.44 ± 1.11e	48.89 ± 1.11e
	100	45.56 ± 2.22e	57.78 ± 2.22f	65.56 ± 1.11f
SLE/w	20	11.11 ± 1.11b	15.56 ± 1.11b	22.22 ± 1.11b
	40	17.78 ± 1.11c	22.22 ± 2.22c	32.22 ± 1.11c
	60	33.33 ± 1.93d	42.22 ± 1.11d	51.11 ± 1.11d
	80	44.44 ± 1.11e	57.78 ± 2.22e	64.44 ± 1.11e
	100	57.78 ± 2.22f	67.78 ± 1.11f	76.67 ± 1.92f
HLE/w	20	11.11 ± 1.11b	13.33 ± 0.00b	16.67 ± 0.00b
	40	13.33 ± 0.00b	18.89 ± 1.11c	26.67 ± 0.00c
	60	21.11 ± 2.22c	40.00 ± 1.92d	46.67 ± 0.00d
	80	38.89 ± 4.44d	46.67 ± 1.92e	57.78 ± 2.22e
	100	48.89 ± 1.11e	63.33 ± 1.92f	67.78 ± 1.11f
H_2O		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract.

Table 4.9 Mortality effects of marigold, Siam weed and hedge flower ethanol leaf extracts on fruit fly larvae by dipping assay.

Extracts	Concentration mg/ml	% Mortality (Mean ± SE)		
		6 hours	12 hours	24 hours
MLE/e	20	12.221.11b	15.561.11b	22.221.11b
	40	16.671.93c	21.111.11c	26.671.93c
	60	25.561.11d	35.562.22d	43.331.92d
	80	38.892.22e	52.221.11e	58.891.11e
	100	53.330.00f	62.222.22f	71.111.11f
SLE/e	20	16.670.00b	22.221.11b	30.000.00b
	40	24.452.22c	32.221.11c	42.221.11c
	60	38.891.11d	50.000.00d	61.112.22d
	80	51.111.11e	64.441.11e	75.561.11e
	100	62.221.11f	76.671.92f	87.781.11f
HLE/e	20	15.561.11b	12.221.11b	22.221.11b
	40	18.891.11b	21.112.22c	32.221.11c
	60	28.891.11c	42.221.11d	51.111.11d
	80	42.222.22d	57.781.11e	65.561.11e
	100	56.671.92e	65.561.11f	77.781.11f
Ethanol		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

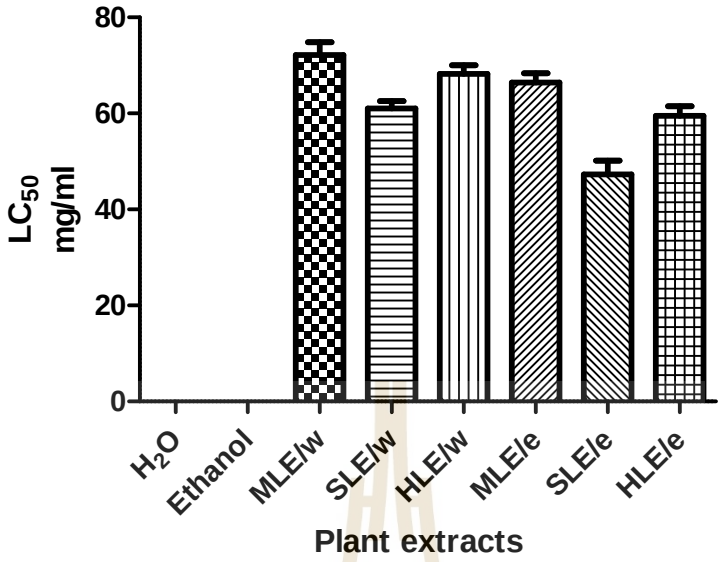

Figure 4.9 Mortality effects with LC_{50} values of the leaf water and ethanol extracts of marigold (*T. erecta*, MLE/w, MLE/e), the Siam weed (*C. odorata*, SLE/w, SLE/e) and hedge flower (*L. camara*, HLE/w, HLE/e) on second instar larvae *B. corrrecta* by dipping assay.

The correlations of the plant extracts and the experimental conditions were shown in table 4.10. The plants (P) are significantly correlated to the solvents (S) and the concentrations (C). However, the plants, the solvents, the concentrations, the time period (T) were not significantly correlated. It can conclude that the mortality effect on fruit fly larvae of these 3 plant extracts are solvent and concentration dependent.

Table 4.10 Correlation among treatment conditions analyzed by analysis of variance for percent mortality of fruit fly larvae by dipping assay with three plant extracts, at two solvent, with five concentrations and three times.

Source of Variation	Degree of freedom	Mean square	F-value	Sig.
Plant (P)	3	1.004	2.282	0.078
Solvent(S)	1	0.061	0.139	0.710
P × S	2	2.228	5.062*	0.007
Concentration (C)	4	8.173	18.569*	0.000
P × C	8	9.570	21.744*	0.000
S × C	4	2.349	5.337*	0.000
P × S × C	8	1.985	4.509*	0.000
Time(T)	4	21.623	49.130*	0.000
P × T	6	0.767	1.743	0.108
S × T	4	0.485	1.102	0.354
C × T	16	2.186	4.967*	0.000
P × S × C × T	34	0.073	0.166	1.000
Error	785	0.440		
Total	900			

* = Significant at $p \leq 0.05$

P = marigold, Siam weed and hedge flower S = water, ethanol

C = concentration of 20, 40, 60, 80, 100 mg/ml T = 6, 12, 24 hours

4.5.3 Antibiosis to adult emergence

The antibiosis on fruit fly adult emergence by the leave extracts of marigold, Siam weed and hedge flower were conducted. The inhibition of molted adult flies was observed. The effects of the extracts on the adult emergence were similarly to the effects of the egg hatching and the larvae. All ethanol extracts of plant leaves induced higher inhibition of molted adult flies than the water extracts. The inhibition rates of all extracts were ranged as SLE/e > MLE/e > SLE/w > HLE/e > MLE/w > HLE/w (Figure 4.10). The highest efficacy was SLE/e with EC_{50} of 69.55 mg/ml, produced 67.78 ± 2.22% inhibition which was similar to SLE/w (72.91 mg/ml, 66.67 ± 1.92% inhibition) (Table 4.11). Similarly, the MLE/e produced 67.78 ± 2.22% inhibition, but the MLE/w produced only 58.89 ± 1.11% inhibition which was 1.2 fold lower. The HLE/w produced lowest efficacy caused 55.56 ± 2.94% inhibition, while the HLE/e at the same dose caused 68.89 ± 2.94% inhibition. The HLE/e was 1.2 fold more toxic than the HLE/w.

Table 4.11 The inhibition of adult fruit flies emergence by leaf extracts of marigold, Siam weeds and hedge flower.

Concentration mg/ml	% Inhibition (Mean ± SE)					
	Water extract			Ethanol extract		
	MLE/w	SLE/w	HLE/w	MLE/e	SLE/e	HLE/e
H_2O	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Ethanol	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
20	12.22 ± 1.11b	18.89 ± 1.11b	13.33 ± 1.92b	15.56 ± 2.94b	15.56 ± 2.94b	23.33 ± 1.92b
40	27.78 ± 1.11c	34.45 ± 1.11c	25.56 ± 2.94c	30.00 ± 1.92c	30.00 ± 1.92c	37.78 ± 2.94c
60	41.11 ± 1.11d	45.56 ± 1.11d	36.67 ± 1.92d	44.44 ± 1.11d	44.44 ± 1.11d	46.67 ± 3.85d
80	53.33 ± 1.92e	51.11 ± 1.11e	47.78 ± 2.22e	55.56 ± 1.11e	55.56 ± 1.11e	54.44 ± 2.22e
100	58.89 ± 1.11f	66.67 ± 1.92f	55.56 ± 2.94f	67.78 ± 2.22f	67.78 ± 2.22f	68.89 ± 2.94f
EC_{50} (mg/ml)	78.38	72.91	83.44	72.49	69.55	77.23

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significantly difference, $P \leq 0.05$ Duncan multiple rang test (DMRT).

EC_{50}, median effective concentration.

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract; MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

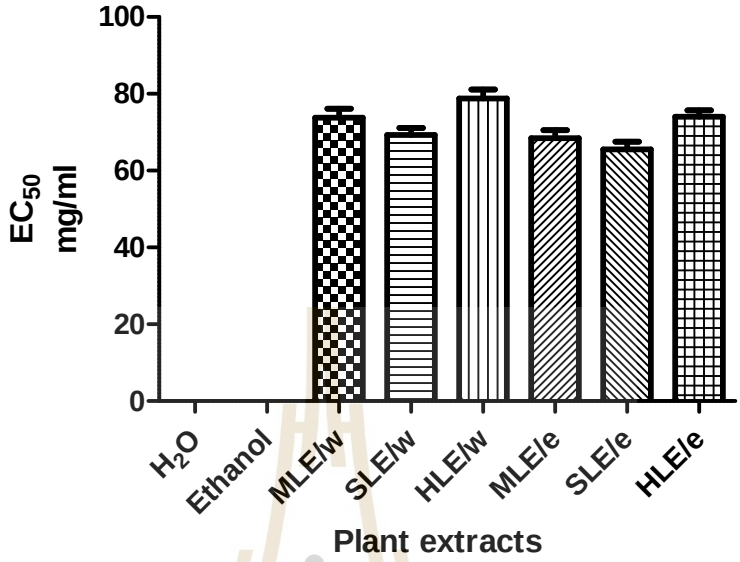

Figure 4.10 Adult fruit flies emergence effects with EC_{50} values of the leaf water and ethanol extracts of marigold (*T. erecta*, MLE/w, MLE/e), the Siam weed (*C. odorata*, SLE/w, SLE/e) and hedge flower (*L. camara*, HLE/w, HLE/e) on *B. corrrecta*.

The correlations of the plant extracts and the experimental conditions were shown in table 4.12. The plants (P) are significantly correlated to the concentrations (C). However, the plants (P), the solvents (S), the concentrations (C) are not significantly correlated. The solvents are also not significantly correlated to the plants and the concentrations. It can conclude that the inhibition of adult fruit flies emergence of these three plant extracts is concentration dependent.

Table 4.12 Correlation among treatment conditions analyzed by analysis of variance for percent inhibition of adult fruit flies emergence with three plant extracts, at two solvent, with five concentrations.

Source of Variation	Degree of freedom	Mean square	F-value	Sig.
Plant (P)	2	.869	61.252*	0.000
Solvent(S)	1	.956	67.378*	0.000
P × S	2	.018	1.278	0.282
Concentration (C)	4	14.544	1025.221*	0.000
P × C	8	.102	7.161*	0.000
S × C	4	.024	1.670	0.160
P × S × C	8	.023	1.650	0.115
Error	150	.014		
Total	180			

* = Significant at $p \leq 0.05$
P = marigold, Siam weed and hedge flower S = water, ethanol
C = concentration of 20, 40, 60, 80, 100 mg/ml

4.6 Discussion

The natural products have been increasingly used to replace synthetic insecticidal sprays as alternative methods for fruit fly control. Plant extracts have also been proved effective on fruit fly control. The present study demonstrated the effects of marigold, Siam weed and hedge flower extracts on eggs, larvae and pupae of guava fruit fly *B. correcta*. The leaf extracts of marigold (*T. erecta* L.), Siam weed (*C. odorata* (L.) King & Robinson) and hedge flower (*L. camara* L.) have a potential as the guava fruit fly control agents against eggs, second instar larvae and pupae. The marigold demonstrated that the activity against various insects. Sarin (2004) showed the potentiality of callus cultures of marigold to produce ascorbic acid as well as insecticidal pyrethrins for flour beetles (*Tribolium* spp) control. Natarajan et al. (2006) reported that population of tomato root knot nematode, *Meloidogyne incognita*, was reduced by cold aqueous extracts of marigold. Moreover, the marigold extracts by maceration with dichloromethane and methanol showed efficacy against rice weevils (*Sitophilus oryzae*) (Broussalis et al., 1999). Similarly, Parugrug and Roxas (2008) showed that the efficacy against maize weevil against maize weevil (*Sitophilus zeamais* Motsch) by repellence, adult mortality and antioviposition and growth inhibition. Nikkon et al. (2009) and Islam and Talukder (2005) also reported the insecticidal activity of crude extracts from the flowers of marigold against a stored product insect pest, *Tribolium castaneum* (Herbst). The powder of Siam weed showed a high efficacy against rice moth egg hatching into adults (Allotey and Azalekor, 2000). Leaves extracts of hedge flower have a strongly insecticidal activities against aphids, beetles and has potential against *Ae. Aegypti* (อำนวย อิศรางกูร ณ อยุธยา, 2535;

Tanprasit, 2005). Similarly, Kumar and Maneemegalai (2008) which found the larvicidal effects of hedge flower leaf extracts on larvae of mosquito species *Ae. aegypti* and *Culex quinquefasciatus*. Moreover, the essential oil extracts of hedge flower have insecticidal effect on maize grain weevils (*Sitophilus zeamais*) (Bouda, Tapondjou, Fontem and Gumedzoe, 2001).

These finding well supported the efficacy of leaf extracts of marigold, Siam weed and hedge flower on the guava fruit fly *B. correcta* control of the eggs, second instar larvae and pupae.

4.7 Conclusions

The leaf ethanol extracts of marigold, Siam weed and hedge flower were more potent for fruit fly *B. correcta* control than the water extracts at the same concentrations. The SLE water and ethanol extracts were extremely potent for controlling egg hatching, larval and pupal of guava fruit flies mortalities. The SLE/e produced highest inhibitory effect of 82.22 ± 6.19% with EC_{50} value of 44.54 mg/ml on egg hatching. The morality effects of SLE/e on second instar larvae were express at 83.33 ± 1.92% mortality with the LC50 value 55.56 mg/ml by feeding assay and express at 87.78 ± 1.11% with LC_{50} value of 52.99 mg/ml by dipping assay. The inhibitory effects of the extracts on the adult emergence were similarly to the effects of the egg hatching and the larval mortality. The SLE/e was high potent in controlling fruit fly adult emergence with EC_{50} of 69.55 mg/ml, produced 67.78 ± 2.22% inhibition. Therefore, the SLE extracts was best alternative for controlling egg hatching, larval and pupal development of guava fruit flies, *B. correcta*. However, the

correlations among treatment conditions in antibiosis effect on egg, larva and pupa of guava fruit flies are not significantly correlated.

4.8 References

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CHAPTER V

BIOLOGICAL CONTROL OF FRUIT FLIES (*BACTROCERA CORRECTA* BEZZI) BY LEAF EXTRACTS OF MARIGOLD (*TAGETES ERECTA* L.), SIAM WEED (*CHROMOLAENA ODORATA* (L.) KING & ROBINSON) AND HEDGE FLOWER (*LANTANA CAMARA* L.)

5.1 Abstract

The extracts of marigold (*Tagetes erecta* L., MLE), Siam weed (*Chromolaena odorata* (L.) King & Robinson, SLE) and hedge flower (*Lantana camara* L., HLE) were investigated for biological control of adult fruit flies (*Bactrocera correcta*). The ethanol and water extracts were applied to the adult fruit flies. The SLE/e was the most potent in repelling adults with EC_{50} 35.42 mg/ml and the MLE/w was the least with EC_{50} 43.23 mg/ml. The extracts of SLE/e at the concentration of 100 mg/ml produced the highest mortality effect at 80.00 ± 1.92% with LC_{50} of 67.324 mg/ml while, the MLE/w produced the least effects on fruit flies at 52.22 ± 1.11% with LC_{50} of 93.67 mg/ml. The combination extracts of SLE/e + MLE/e at the ratio of 3:1 was highly effective at 72.22 ± 1.11% mortality at 24 hours while the least effective pair was HLE/w and MLE/w at the ratio of 1:3 which was effective only 34.44 ± 4.01% mortality at 24 hours.

5.2 Introduction

The production of fruits and vegetables in Thailand generates important sources of income (Guamán, 2009). The insects are the most economically damaging pests of fruit and vegetable crops affecting the valuable export trade of agricultural products in Thailand. Fruit flies damage is caused of economics losses estimated using domestic price showed hundred millions dollar (Asian Institute of Technology, On-line, 2010). The guava fruit fly, *Bactrocera correcta* (Bezzi), is considered a very destructive insect that cause enormous economic losses of fruit products in Thailand and occurs throughout most countries in Southeast Asia, including Pakistan, India, Nepal, Burma, Sri Lanka, Vietnam China and Thailand (Drew and Raghu, 2002; Wang, Zhu, Zhou, Niu and Lei, 2006). The *B. correcta* is listed as a quarantine pest by most countries worldwide (Puanmanee, Wongpiyasatid, Sutantawong and Hormchan, 2010). The damages on crops consist on oviposition stings on the fruit surface causing fruit that drops early and be destructed inside of the fruits. This results in unmarketable crop (Guamán, 2009).

Insect pests controls are involve synthetic insecticides for their quick knock down effect (Khattak, Shahzad and Jilani, 2006). The frequent use of insecticides to fruit flies control for fruits and vegetable has not resulted in sustainable management of the pests (Guamán, 2009). Moreover, the use of insecticides as the only way to control pests in fruits and vegetables causes contamination of environmental and hygienic problems that represent a risk for people, animals, non-target insects and other organisms (Khattak, Shahzad and Jilani, 2006; Gallo, 2007). The alternative methods for controlling fruit flies has been developed to protect against various fruits

avoiding economic damage and protect the environment and as well as human health (Khattak, Shahzad and Jilani, 2006; Stewart and McClure, On-line, 2013).

The use of plants and plant-derived products are interested as botanical insecticides to reduce chemically synthetic insecticides and also to avoid problems of insecticide resistance (Thomas and Callaghan, 1999). Many plants may provide compounds that they use in preventing attack from insects pests and diseases (Kim, Roh, Kim, Lee and Ahn, 2003; Khattak, Shahzad and Jilani, 2006; Marta and Moore, 2011). Plant products have several uses to control insects due to plants derivatives are less toxic or non-toxic to mammals, vertebrates and invertebrates (Khattak, Shahzad and Jilani, 2006). The primary role of plant products is insecticide. Some plant products act as attractants while some plant products act as repellents (Khattak, Shahzad and Jilani, 2006).

Marigold (*Tagetes erecta* L.) Siam weed (*Chromolaena odorata* L. (King & Robinson) and hedge flower (*Lantana camara* L.) have been used as insecticides and are still used to kill or repel insects. Natarajan et al. (2006) reported that the inhibition of cold aqueous extracts from marigold on tomato root knot nematode, *Meloidogyne incognita*. Broussalis et al. (1999) showed that the activity of marigold extracts by maceration with dichloromethane and methanol to against rice weevils (*Sitophilus oryzae*). Sarin (2004) showed that the efficacy of marigold callus cultures produced ascorbic acid as well as insecticidal pyrethrins against flour beetles (*Tribolium* spp). Nikkon et al. (2009) and Islam and Talukder (2005) demonstrated the insecticidal activity of marigold *Tagetes erecta* L. against a stored product insect pest, *Tribolium castaneum* (H.). Parugrug and Roxas (2008) showed that the efficacy to against maize weevil (*Sitophilus zeamais* M.) was repellency, adult mortality and antioviposition

and growth inhibition. Bouda, Tapondjou, Fontem and Gumedzoe (2001) showed that the potential of essential oils from the leaves of *C. odorata and L. camara* for maize grain weevil, *Sitophilus zeamais* control in stored products. *Lantana camara* leaves chloroform extract were found termiticidal effects against adult termite *Microcerotermes beesoni* (Verma, 2006).

Therefore, the present study was conducted to investigate the effects of the water and ethanol leaf extracts of marigold (*Tagetes erecta* L., MLE/w, MLE/e), Siam weed (*Chromolaena odorata* L. (King & Robinson, SLE/w, SLE/e) and hedge flower (*Lantana camara* L. HLE/w, HLE/e) on adults of fruit flies (*Bactrocera correcta* Bezzi). Insecticidal, attractant and repellent activities were tested.

5.3 Materials and methods

5.3.1 Plant collection and preparation for extracts

The fresh leaves of Siam weed (*Chromolaena odorata* (L.) King & Robinson) and hedge flower (*Lantana camara* L.) were collected from surrounding of Suranaree University of Technology (SUT) campus. Marigold (*Tagetes erecta* L.) leaves were collected from a local farm near by Suranaree University of Technology (SUT) campus. All plant leaves were cleaned, dried by sunlight and ground to powder. Ten grams of dried plant powders in a cellulose extraction thimble (Whatman International Ltd., Maidstone, England) were extracted in 150 ml of water or 70% ethanol in Soxhlet extraction apparatus (Buchi model B 811, Germany). The extracts were filtered and evaporated in rotary evaporator (Buchi instruments, Switzerland) and dried by lyophilizer (Freeze-zone 12 plus, Labconco Corporation, Missouri,

USA). The extracts were stored at -20ºC until use. The dried extracts were dissolved in its original solvents for use in all experiments.

5.3.2 Fruit fly rearing

Pupae of guava fruit fly (*Bactrocera correcta*) were obtained from the Thailand Institute of Nuclear Technology (Public Organization), Ministry of Science and Technology, Thailand. Adult flies emerge from the pupa cases in 7 days. Fruit flies were cultured in wire-net cages (Figure 5.1) under laboratory conditions at 28 ± 2°C, 65-70% relative humidity, 12 hours light: 12 hours dark. Fruit fly fed with artificial food (Walker et al., 1997) and allowed to mate. The adult fruit flies aged 2 days after emerging were used for all experiments.

Figure 5.1 *Bactrocera correcta* rearing in laboratory.

5.3.3 Repellent tests

The repellent properties of the plant extracts were tested in an olfactometer (Figure 5.2), made of a-75 cm long plastic tube with 4 cm in diameter and a-29 mm diameter hole in the middle (Boeke et al., 2004).

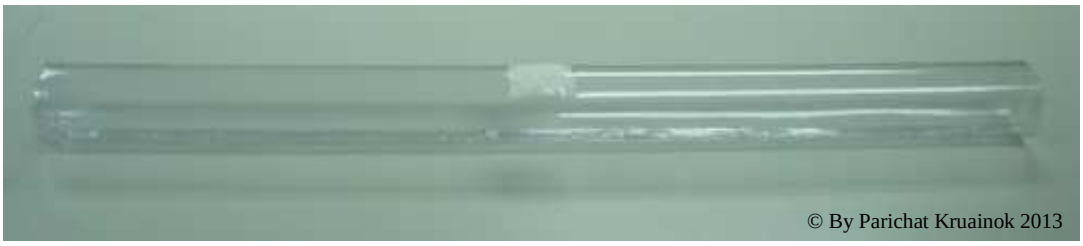

Figure 5.2 Olfactometer set up for repellent tests.

At one end of the tube, a 10-ml beaker containing 20, 40, 60, 80 and 100 mg/ml of the plant extracts. At another end of the tube, a 10-ml beaker containing 1 ml distilled water without plant extracts or ethanol were used as controls (Figure 5.3). The hole in the middle was covered with gauze, whereas the ends of the tube were covered. Individual female or male of fruit flies were introduced through the hole at the middle of the tube. The fly's behavior was observed for 15, 30 minutes and 1, 3 and 6 hours, the flies did not move immediately to either separate ends of tubes were counted. All repellent tests were repeated for 30 flies (15 females and 15 males). The median effective concentration, EC_{50}, was calculated by Probit analysis, used SPSS (Statistical Package for the Social Sciences) program for Windows v.17. The results were expressed as percentage of repellency as following;

$$\% \text{ Repellent} = \frac{\text{Number collected from control - Number collected from treated}}{\text{Number collected from the treated}} \times 100.$$

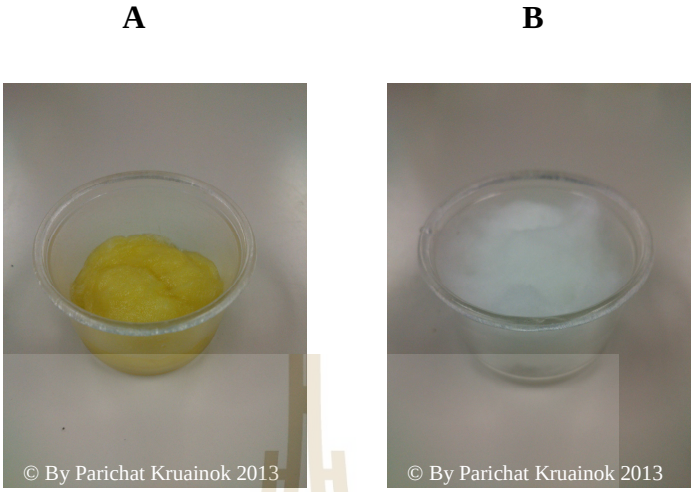

Figure 5.3 The extracts (A), distilled water (B) for repellent tests.

5.3.4 Insecticidal activity on adult guava fruit flies

Thirty adult guava fruit flies were placed into a plastic boxes (100×100×60 mm^3), which the lids are punched to make a hole and covered with gauze (Figure 5.4). The extracts of three plants at the concentrations of 20, 40, 60, 80 and 100 mg/ml as individuals or combinations (1:1, 3:1, 1:3) were spray directly on guava fruit fly (รุจนี เล้ารัตนบูรพา, 2523). Mortality of guava fruit flies was counted after 1, 6, 12 and 24 hours. Water and 70% ethanol were used as controls. The tests were performed in triplicate and repeated twice. The mortality was corrected by Abbott's formula (Abbott, 1925). The mortality was calculated and analyzed for LC_{50} by Probit analysis. The results were expressed as percentage of mortality as following;

$$\% \text{ Mortality} = \frac{\% \text{ Test mortality-\% Control mortality}}{100\text{-\% Control mortality}} \times 100.$$

Figure 5.4 The insecticidal activity test in a plastic box.

5.4 Data analysis

Data were analysis of variance (ANOVA) and in completely randomized design (CRD) using Statistical Package for the Social Sciences (SPSS) program for Windows v.17. The means were separated by the Duncan's Multiple Range Test (DMRT) when ANOVA was significant ($P < 0.05$). The LC_{50} and EC_{50} values were determined by Probit analysis (Finny, 1971). The percentage of repellence and the mortality were corrected by Abbott's formula (1925).

5.5 Results

5.5.1 Repellent tests

The repellent effects of the extracts of marigold (*Tagetes erecta*, MLE), Siam weed (*Chromolaena odorata*, SLE) and hedge flower (*Lantana camara*, HLE) on fruit flies were concentrations dependent (Table 5.1). The repellent activities of all extracts on the adult fruit flies could be compared by their EC_{50} values. The ethanol

extracts of all plant had higher potential than the water extracts. The SLE/e was the most effective in repelling the fruit flies as compared among the other which extracted in the same solvent, while the MLE/w was the least effective. The SLE/e exhibited EC_{50} of 35.42 mg/ml and the MLE/w exhibited 43.23 mg/ml. The repellent efficacy of the ethanol extracts were ranged as SLE/e > MLE/e > HLE/e and efficacy of the water extracts were ranged as SLE/w > HLE/w > MLE/w. At 15 minutes the EC_{50} values of the SLE/e, MLE/e and HLE/e were 35.42, 36.58 and 38.16 mg/ml respectively. The EC_{50} values of the water extracts of SLE/w, HLE/w and MLE/w were 37.66, 39.17, and 43.23 mg/ml respectively.

The repellent effects of the water extracts, SLE/w showed the highest repellency of 85.43 ± 3.90% at the concentration of 100 mg/ml at 15 minutes of treatment. The lowest repellency of MLE/w was 74.98 ± 4.18% (Table 5.2).

The repellent effects of the ethanol extracts, SLE/e showed the highest repellency of 88.46 ± 1.24% at the concentration of 100 mg/ml at 15 minutes of treatment. The lowest repellency of HLE/e was 75.03 ± 2.19% (Table 5.3).

The repellency of the extract was concentration dependent and inversed to time treatments. At 15 minutes, all of treatment extracts were able to repel the fruit flies highly. After 30 minutes of treatment, the repellent efficacy was declined. At the highest concentration of 100 mg/ml the repellent effects of MLE/w decreased from 74.98 ± 4.18% to 41.09 ± 8.20%; of SLE/w decreased from 85.43 ± 3.90% to 67.85 ± 5.15%; and of HLE/w decreased from 81.72 ± 2.97% to 39.90 ± 7.89% (Table 5.2). While the repellent effects of MLE/e decreased from 86.60 ± 2.54% to 42.21 ± 3.97%; of SLE/w decreased from 88.46 ± 1.24% to 67.13 ± 2.47%; and of HLE/e decreased from 75.03 ± 2.19% to 60.22 ± 5.17% (Table 5.3).

However, the repellent effects of all water extracts were slightly lower than those of the ethanol extracts of the same plants as well as of the same concentration. It is concluded that the SLE had the highest repellent activity on adult fruit flies as compared in Figure 5.4.

Table 5.1 The repellent effects of leaf extracts of marigold, Siam weed and hedge flower on guava fruit flies at 15-minutes treatment.

Concentration mg/ml	% Repellent (Mean ± SE)					
	Water extract			Ethanol extract		
	MLE/w	SLE/w	HLE/w	MLE/e	SLE/e	HLE/e
H_2O	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Ethanol	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
20	11.79 ± 9.06a	18.68 ± 5.91b	16.28 ± 7.53b	27.80 ± 9.14b	24.68 ± 5.87b	27.46 ± 6.64b
40	26.28 ± 4.59b	69.59 ± 2.25c	31.65 ± 3.95c	34.22 ± 1.70b	38.99 ± 3.45c	41.54 ± 3.42c
60	54.77 ± 1.44c	40.63 ± 1.73d	66.82 ± 3.12d	66.97 ± 2.65c	75.33 ± 2.94d	70.86 ± 2.12d
80	68.88 ± 1.88d	78.37 ± 4.87d	68.35 ± 3.95d	72.87 ± 7.19cd	81.20 ± 3.75de	75.03 ± 2.19d
100	74.98 ± 4.18d	85.43 ± 3.90e	81.72 ± 2.97e	86.60 ± 2.54d	88.46 ± 1.24e	87.43 ± 2.63e
EC50 (mg/ml)	43.23	37.66	39.17	36.58	35.42	38.16

Each value is the mean ± standard error, n = 6. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

EC_{50}, median effective concentration.

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract; MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

Table 5.2 Comparison of repellent activities of marigold, Siam weed and hedge flower leaf water extracts on adult guava fruit flies at designated times.

Extracts	Concentration mg/ml	% Repellent (Mean ± SE)				
		15 min	30 min	1 hours	3 hours	6 hours
MLE/w	20	11.79 ± 9.06a	29.22 ± 6.87b	9.13 ± 9.78a	0.80 ± 5.16a	1.04 ± 2.60a
	40	26.28 ± 4.59b	34.80 ± 8.39b	25.04 ± 7.72ab	13.27 ± 7.26a	9.74 ± 5.37ab
	60	54.77 ± 1.44c	63.18 ± 3.49c	49.71 ± 7.72c	35.02 ± 5.03b	21.45 ± 1.90bc
	80	68.88 ± 1.88d	75.60 ± 0.98cd	73.06 ± 2.55d	47.46 ± 5.59b	29.76 ± 6.77cd
	100	74.98 ± 4.18d	78.64 ± 1.33d	74.40 ± 1.25d	69.49 ± 4.46c	41.09 ± 8.20d
SLE/w	20	18.68 ± 5.91b	21.67 ± 3.80b	20.76 ± 1.62b	11.85 ± 4.05a	9.35 ± 2.69ab
	40	69.59 ± 2.25c	43.65 ± 5.20c	48.08 ± 8.88c	17.07 ± 6.35a	14.58 ± 2.33b
	60	40.63 ± 1.73d	62.09 ± 2.59d	63.76 ± 1.01d	37.30 ± 11.43b	42.71 ± 6.70c
	80	78.37 ± 4.87d	73.87 ± 3.60e	68.99 ± 3.56d	52.53 ± 7.54bc	48.10 ± 2.95c
	100	85.43 ± 3.90e	79.61 ± 1.34e	73.45 ± 1.74d	64.14 ± 2.53c	67.85 ± 5.15d
HLE/w	20	16.28 ± 7.53b	38.87 ± 5.63b	27.93 ± 7.00b	11.85 ± 4.05b	6.17 ± 6.17a
	40	31.65 ± 3.95c	45.84 ± 6.27b	29.15 ± 8.58b	16.43 ± 2.23b	18.72 ± 3.12ab
	60	66.82 ± 3.12d	64.58 ± 2.46c	48.23 ± 3.98c	32.24 ± 6.14c	26.70 ± 1.26bc
	80	68.35 ± 3.95d	75.97 ± 1.67d	64.13 ± 1.94d	53.52 ± 0.84d	33.88 ± 4.49cd
	100	81.72 ± 2.97e	83.08 ± 2.46d	71.73 ± 3.88d	63.06 ± 3.89d	39.90 ± 7.89d
H_2O		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Ethanol		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 6. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract.

Table 5.3 Comparison of repellent activities of marigold, Siam weed and hedge flower leaf ethanol extracts on adult guava fruit flies at designated times.

Extracts	Concentration mg/ml	% Repellent (Mean ± SE)				
		15 min	30 min	1 hours	3 hours	6 hours
MLE/e	20	27.80 ± 9.14b	29.46 ± 7.61b	20.55 ± 4.44b	3.61 ± 6.03a	10.46 ± 3.38b
	40	34.22 ± 1.70b	42.98 ± 2.86c	30.51 ± 7.48b	18.93 ± 7.40b	17.03 ± 3.24b
	60	66.97 ± 2.65c	68.19 ± 4.14d	61.80 ± 6.91c	38.72 ± 1.82c	28.92 ± 2.49c
	80	72.87 ± 7.19c,d	75.05 ± 3.78de	73.70 ± 2.77cd	52.90 ± 1.46d	32.92 ± 1.58c
	100	86.60 ± 2.54d	80.63 ± 0.32e	80.45 ± 3.86d	74.78 ± 3.05e	42.21 ± 3.97d
SLE/e	20	24.68 ± 5.87b	27.26 ± 6.46b	26.01 ± 3.17b	21.43 ± 6.27b	17.08 ± 2.55b
	40	38.99 ± 3.45c	48.03 ± 5.26c	53.44 ± 7.61c	36.19 ± 2.10bc	22.31 ± 3.26b
	60	75.33 ± 2.94d	73.20 ± 3.53d	62.93 ± 4.55c	42.60 ± 7.75c	48.77 ± 5.64c
	80	81.20 ± 3.75d,e	77.57 ± 3.63d	74.12 ± 1.61d	62.90 ± 7.78d	50.05 ± 3.02c
	100	88.46 ± 1.24e	85.29 ± 2.95d	79.35 ± 1.06d	73.15 ± 4.11d	67.13 ± 2.47d
HLE/e	20	0.00 ± 0.00a	48.31 ± 5.52b	33.44 ± 5.36b	17.05 ± 2.43b	10.46 ± 5.23a
	40	27.46 ± 6.64b	53.54 ± 6.27b	35.84 ± 7.89b	25.36 ± 0.36b	25.52 ± 4.14b
	60	41.54 ± 3.42c	73.54 ± 2.15c	55.85 ± 4.81c	37.86 ± 6.91c	26.80 ± 3.42b
	80	70.86 ± 2.12d	81.55 ± 1.40cd	70.27 ± 1.46d	59.91 ± 1.77d	39.58 ± 3.84c
	100	75.03 ± 2.19d	86.95 ± 2.38d	79.00 ± 1.46d	67.10 ± 3.82d	60.22 ± 5.17d
H_2O		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Ethanol		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 6. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT). MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract.

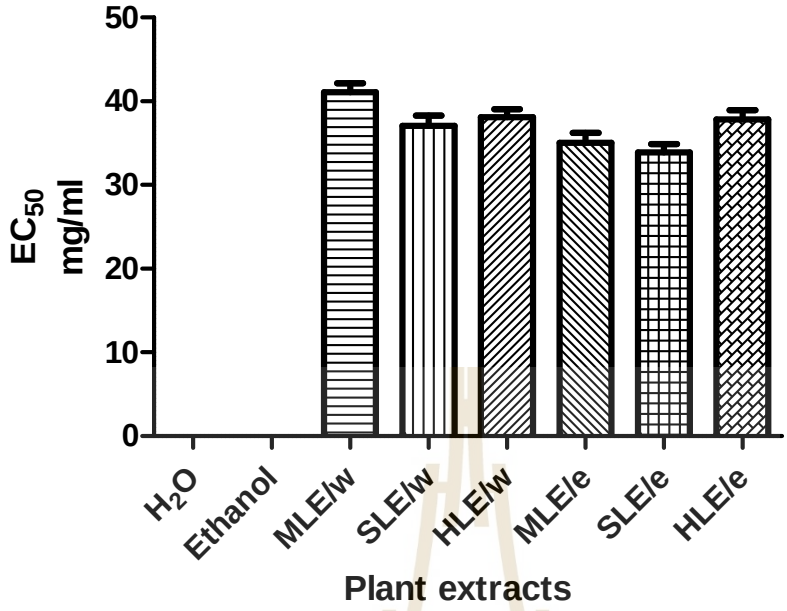

Figure 5.5 The repellent effects with EC_{50} values of the leaf extracts of the leaf water and ethanol extracts of marigold (*T. erecta*, MLE/w, MLE/e), the Siam weed (*C. odorata*, SLE/w, SLE/e) and hedge flower (*L. camara*, HLE/w, HLE/e) on adults of *B. corrrecta*.

The repellency of the extracts on the adult guava fruit flies by various conditions of treatments was analyzed. The analysis of variance shows the significances of effects for percent repellant of fruit flies by three plants (P), with two solvent extracts (S), with five concentrations (C) and five time periods (T) and interactions from plant extracts and concentrations, plant extracts and times, solvents and concentrations, concentrations and times, plants and solvents and concentrations, plants and solvents and concentrations and times. While, plant extracts and solvents, solvents and times show non significant effects. It can conclude that the repellent

effects of these three plant extracts is solvent, concentration and time dependent (Table 5.4).

Table 5.4 Correlation among treatment conditions analyzed by analysis of variance for percent repellant of adult fruit flies with three plant extracts, at two solvent, with five concentrations and five times.

Source of Variation	Degree of freedom	Mean square	F-value	Sig.
Plant (P)	2	4.586	123.307*	0.000
Solvent (S)	1	8.669	233.099*	0.000
P × S	2	0.006	0.165	0.848
Concentration (C)	4	2	4.586*	0.000
P × C	8	0.319	8.584*	0.000
S × C	4	0.162	4.360*	0.002
P × S × C	22	0.175	2.279*	0.001
Time (T)	4	51.935	1396.412*	0.000
P × T	8	0.708	19.033*	0.000
S × T	4	0.062	1.655	0.159
C × T	16	1.164	31.294*	0.000
P × S × C × T	80	0.175	4.711*	0.000
Error	750	0.037		
Total	900			

* = Significant at $p \leq 0.05$

P = marigold, Siam weed and hedge flower S = water, ethanol

C = concentration of 20, 40, 60, 80, 100 mg/ml T = 15, 30 minutes 1, 3, 6 hours

5.5.2 Insecticidal activity on adult guava fruit flies

The insecticidal effects the extracts of marigold (*Tagetes erecta*, MLE), Siam weed (*Chromolaena odorata*, SLE) and hedge flower (*Lantana camara*, HLE) by direct spraying was investigated (Table 5.5). The insecticidal effect on the adult fruit flies of all extracts could be compared by their LC_{50} values. The ethanol extracts of all plant extracts had higher efficacy in killing fruit flies than water extracts of the same plants as well as of the same concentration levels. The extracts of SLE/e produced the highest mortality effect, while the MLE/w produced the least effects on the fruit flies. The SLE/e exhibited LC_{50} of 67.32 mg/ml and the MLE/w exhibited 93.67 mg/ml. The mortality of fruit flies was increased as the extract concentration and time increased (Tables 5.6 and 5.7). The mortality effect of the ethanol extracts were ranged as SLE/e > MLE/e > HLE/e and efficacy of the water extracts were ranged as SLE/w > HLE/w > MLE/w. The LC_{50} values at 24 hours of the SLE/e, MLE/e and HLE/e were 67.32, 87.90 and 88.20 mg/ml, respectively. The LC_{50} values of the water extracts of SLE/w, HLE/w and MLE/w were 80.92, 89.88, and 93.67 mg/ml, respectively.

Table 5.6 and table 5.7 represented the dose dependent effects of water and ethanol extracts of marigold, Siam weed and hedge flower on the mortality percentage of adult guava fruit flies. At 24 hours, concentration of 100 mg/ml, the extracts showed maximum effect on the adults. The SLE/e exhibited highest mortality effect of 80.00 ± 1.92%. While, the MLE/w caused 52.22 ± 1.11% mortality of the flies. It was then concluded that the insecticidal activity of plant extracts was ranged as SLE/e > MLE/e > SLE/w > HLE/e > HLE/w > MLE/w (Figure 5.5).

Table 5.5 Mortality effects of leaf extracts of marigold, Siam weed and hedge flower on guava fruit flies at 24 hours.

Concentration mg/ml	% Mortality (Mean ± SE)					
	Water extract			Ethanol extract		
	MLE/w	SLE/w	HLE/w	MLE/e	SLE/e	HLE/e
H_2O	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Ethanol	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
20	16.67 ± 1.92b	10.00 ± 0.00a	5.56 ± 1.11b	11.11 ± 2.94b	5.56 ± 2.22a	5.56 ± 1.11b
40	20.00 ± 1.92b	23.33 ± 1.92b	11.11 ± 1.11c	20.00 ± 1.92c	16.67 ± 1.92b	11.11 ± 1.11c
60	28.89 ± 1.11c	41.11 ± 6.19c	31.11 ± 1.11d	27.78 ± 1.11d	53.33 ± 1.92c	31.11 ± 1.11d
80	37.78 ± 2.94d	50.00 ± 5.09cd	38.89 ± 2.22e	40.00 ± 5.09e	66.67 ± 3.33d	38.89 ± 2.22e
100	52.22 ± 1.11e	57.78 ± 4.01d	55.56 ± 2.94f	58.89 ± 2.22f	80.00 ± 1.92e	55.56 ± 2.94f
LC_{50} (mg/ml)	93.67	80.92	89.88	87.90	67.32	88.20

Each value is the mean ± standard error, n = 6. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

LC_{50}, median effective concentration.

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract; MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

Table 5.6 Mortality effects of marigold, Siam weed and hedge flower water leaf extracts on adult guava fruit flies at designated times.

Extracts	Concentration mg/ml	% Mortality (Mean ± SE)			
		1 hours	6 hours	12 hours	24 hours
MLE/w	20	1.11 ± 1.11a	11.11 ± 2.94b	13.33 ± 1.92b	16.67 ± 1.92b
	40	4.44 ± 1.11a	14.44 ± 2.22b	16.67 ± 1.92b	20.00 ± 1.92b
	60	15.56 ± 2.22b	21.11 ± 1.11c	23.33 ± 1.92c	28.89 ± 1.11c
	80	15.56 ± 2.22b	23.33 ± 1.92c	27.78 ± 1.11c	37.78 ± 2.94d
	100	33.33 ± 3.33c	42.22 ± 2.94d	47.78 ± 2.22d	52.22 ± 1.11e
SLE/w	20	2.22 ± 1.11ab	4.44 ± 1.11ab	7.78 ± 1.11a	10.00 ± 0.00a
	40	7.78 ± 1.11b	14.44 ± 1.11b	20.00 ± 1.92b	23.33 ± 1.92b
	60	16.67 ± 1.92c	27.78 ± 6.19c	34.44 ± 6.19c	41.11 ± 6.19c
	80	23.33 ± 1.92d	34.44 ± 6.19de	41.11 ± 4.84c	50.00 ± 5.09cd
	100	34.44 ± 4.01e	41.11 ± 2.94e	53.33 ± 1.92d	57.78 ± 4.01d
HLE/w	20	1.11 ± 1.11a	2.22 ± 1.11a	3.33 ± 0.00a	5.56 ± 1.11b
	40	4.44 ± 1.11a	8.89 ± 1.11b	10.00 ± 1.92b	11.11 ± 1.11c
	60	14.44 ± 2.22c	22.22 ± 2.22c	25.56 ± 2.94c	31.11 ± 1.11d
	80	20.00 ± 1.92c	31.11 ± 2.94d	32.22 ± 2.22d	38.89 ± 2.22e
	100	31.11 ± 4.84c	47.78 ± 2.22e	50.00 ± 0.00e	55.56 ± 2.94f
H_2O		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 6. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract.

Table 5.7 Mortality effects of marigold, Siam weed and hedge flower ethanol leaf extracts on adult guava fruit flies at designated times.

Extracts	Concentration mg/ml	% Mortality (Mean ± SE)			
		1 hours	6 hours	12 hours	24 hours
MLE/e	20	3.33 ± 0.00ab	4.44 ± 1.11a	5.56 ± 1.11b	11.11 ± 2.94b
	40	8.89 ± 1.11b	11.11 ± 2.94b	15.56 ± 1.11c	20.00 ± 1.92c
	60	20.00 ± 1.92c	22.22 ± 1.11c	24.44 ± 1.11d	27.78 ± 1.11d
	80	22.22 ± 2.94c	25.56 ± 1.11c	27.78 ± 1.11e	40.00 ± 5.09e
	100	37.78 ± 4.84d	43.33 ± 1.92d	48.89 ± 1.11f	58.89 ± 2.22f
SLE/e	20	3.33 ± 1.92ab	3.33 ± 1.92a	3.33 ± 1.92a	5.56 ± 2.22a
	40	10.00 ± 1.92b	12.22 ± 1.11b	12.22 ± 1.11b	16.67 ± 1.92b
	60	42.22 ± 2.94c	46.67 ± 1.92c	46.67 ± 1.92c	53.33 ± 1.92c
	80	51.11 ± 2.94d	55.56 ± 2.94d	55.56 ± 2.94d	66.67 ± 3.33d
	100	63.33 ± 3.33e	72.22 ± 1.11e	72.22 ± 1.11e	80.00 ± 1.92e
HLE/e	20	1.11 ± 1.11a	3.33 ± 1.92a	7.78 ± 1.11ab	8.89 ± 1.11b
	40	7.78 ± 1.11b	10.00 ± 0.00b	14.44 ± 1.11b	17.78 ± 2.94c
	60	16.67 ± 1.92c	18.89 ± 1.11c	22.22 ± 2.94c	26.67 ± 1.92d
	80	23.33 ± 1.92d	26.67 ± 1.92d	33.33 ± 5.09d	38.89 ± 1.11e
	100	32.22 ± 2.94e	34.44 ± 2.94e	55.56 ± 2.94e	60.00 ± 1.92f
Ethanol		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 6. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

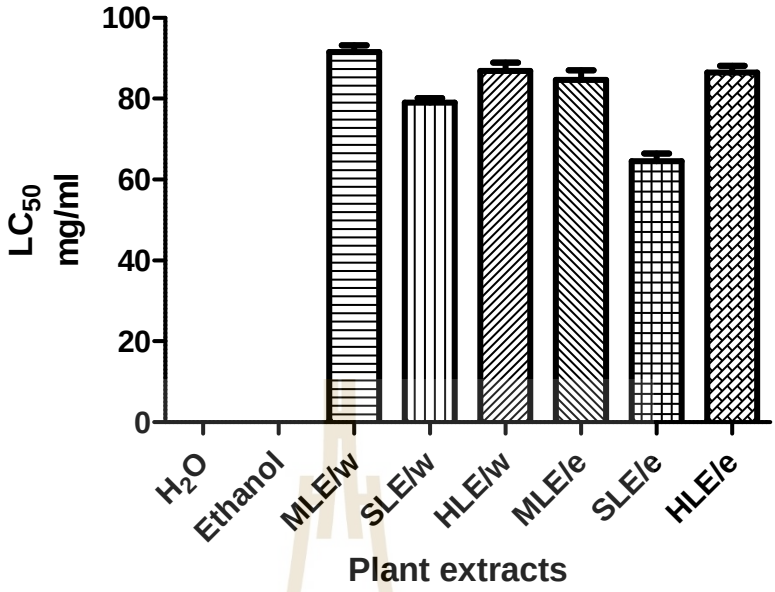

Figure 5.6 Mortality effects with LC_{50} values of the leaf water and ethanol extracts of marigold (*T. erecta*, MLE/w, MLE/e), the Siam weed (*C. odorata*, SLE/w, SLE/e) and hedge flower (*L. camara*, HLE/w, HLE/e) on adult *B. corrrecta*.

The correlations of the plant extracts and the experimental conditions were shown in table 5.8. It indicate that the insecticidal effect on fruit flies with treatment of three plants (P), two solvent extract (S), five concentration (C) and five time periods (T) are significantly correlated. It can conclude that the insecticidal effects of three plant extracts are solvent, concentration and time dependent.

Table 5.8 Correlation among treatment conditions analyzed by analysis of variance for percent mortality of fruit flies with three plant extracts, at two solvent, with five concentrations and four times.

Source of Variation	Degree of freedom	Mean square	F-value	Sig.
Plant (P)	2	51.175	289.008*	0.000
Solvent(S)	1	17.394	98.229*	0.000
P × S	2	8.773	49.543*	0.000
Concentration (C)	4	499.424	2820.445*	0.000
P × C	8	10.319	58.273*	0.000
S × C	4	4.509	25.464*	0.000
P × S × C	8	7.431	41.967*	0.000
Time(T)	3	79.874	451.081*	0.000
P × T	6	0.377	2.128*	0.049
S × T	3	5.030	28.404*	0.000
C × T	12	0.399	2.255*	0.009
P × S × C × T	66	0.561	3.166*	0.000
Error	600	0.177		
Total	720			

* = Significant at $p \leq 0.05$

P = marigold, Siam weed and hedge flower S = water, ethanol

C = concentration of 20, 40, 60, 80, 100 mg/ml T = 1, 6, 12, 24 hours

5.5.3 Effects of extract combinations on adult guava fruit flies

The effects of extract combinations at the ratio of 1:1, 3:1, 1:3 (v/v) were investigated for insecticidal activity against adult fruit flies *B. correcta*. The combinations made between the same extract solvent. The ethanol extracts of plant combinations exhibited higher insecticidal activity against fruit flies than those the water extracts. The effects of combination on the mortality of adult fruit flies by the three plant extracts were exhibited in Table 5.9 and table 5.10. The combinations between the water extracts, HLE/w + MLE/w at the ratio of 3:1 and SLE/w + HLE/w at the ratio of 1:3 were highly effective at 58.89 ± 2.94% and 58.89 ± 2.22% mortality at 24 hours respectively. The least effective pair was HLE/w and MLE/w at the ratio of 1:3 which was effective only 34.44 ± 4.01% mortality at 24 hours. While the combination between the ethanol extracts, SLE/e + MLE/e at the ratio of 3:1 was highly effective at 72.22 ± 1.11% mortality at 24 hours. The least effective pair was SLE/e + HLE/e at the ratio of 1:3 which was effective only 17.78 ± 2.94% mortality at 24 hours.

Table 5.9 Mortality effects of the combinations of marigold, Siam weed and hedge flower leaf water extracts on adult guava fruit flies.

Extract combination	Ratio mg/ml	% Mortality (Mean ± SE)			
		1 hours	6 hours	12 hours	24 hours
SLE/w + MLE/w	1 : 3	16.67 ± 1.92b	15.11 ± 3.62b	41.11 ± 2.94b	53.33 ± 1.92b
	1 : 1	23.33 ± 1.92b	27.78 ± 6.19b	34.44 ± 6.19b	50.00 ± 5.09bc
	3 : 1	34.44 ± 4.01c	34.44 ± 6.19b	41.11 ± 4.84c	57.78 ± 4.01c
SLE/w + HLE/w	1 : 3	4.44 ± 1.11a	8.89 ± 1.11a	10.00 ± 1.92a	58.89 ± 2.22d
	1 : 1	31.11 ± 4.84b	47.78 ± 2.22b	50.00 ± 4.01b	55.56 ± 2.94c
	3 : 1	20.00 ± 1.92c	31.11 ± 2.94c	32.22 ± 2.22c	38.89 ± 2.22d
HLE/w + MLE/w	1 : 3	3.33 ± 4.01a	4.44 ± 1.11b	5.56 ± 1.11b	34.44 ± 4.01c
	1 : 1	22.22 ± 2.94b	25.56 ± 1.11c	27.78 ± 1.11c	40.00 ± 5.09c
	3 : 1	37.78 ± 4.84c	43.33 ± 1.92d	48.89 ± 1.11d	58.89 ± 2.94d
H_2O		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 6. Numbers with different letters within the same column are significantly difference, $P \leq 0.05$ Duncan multiple rang test (DMRT).

EC_{50}, median effective concentration.

MLE/w, Marigold leaf water extract; SLE/w, Siam weed leaf water extract; HLE/w, Hedge flower leaf water extract.

Table 5.10 Mortality effects of the combinations of marigold, Siam weed and hedge flower leaf ethanol extracts on adult guava fruit flies.

Extract combination	Ratio mg/ml	% Mortality (Mean ± SE)			
		1 hours	6 hours	12 hours	24 hours
SLE/e + HLE/e	1 : 3	7.78 ± 1.11b	13.33 ± 1.92b	21.11 ± 1.11b	17.78 ± 2.94b
	1 : 1	16.67 ± 1.92c	18.89 ± 1.11c	22.22 ± 2.94b	27.78 ± 1.11c
	3 : 1	23.33 ± 1.92d	26.67 ± 1.92d	33.33 ± 5.09c	60.00 ± 1.92d
SLE/e + MLE/e	1 : 3	6.22 ± 1.18b	6.89 ± 0.11b	12.33 ± 1.45b	15.89 ± 0.48b
	1 : 1	12.22 ± 1.11c	12.22 ± 1.11c	16.67 ± 1.92b	55.56 ± 2.94c
	3 : 1	46.67 ± 1.92d	46.67 ± 1.92d	53.33 ± 1.92c	72.22 ± 1.11d
HLE/e + MLE/e	1 : 3	3.33 ± 0.21b	4.44 ± 1.11a	11.11 ± 2.94b	27.78 ± 1.18c
	1 : 1	8.89 ± 1.11c	11.11 ± 2.94b	15.56 ± 1.11c	45.56 ± 1.11c
	3 : 1	20.00 ± 1.92d	22.22 ± 1.11c	26.67 ± 0.51d	54.44 ± 2.22d
Ethanol		0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Each value is the mean ± standard error, n = 6. Numbers with different letters within the same column are significant difference, $P \leq 0.05$, analyzed by Duncan multiple rang test (DMRT).

MLE/e, Marigold leaf ethanol extract; SLE/e, Siam weed leaf ethanol extract; HLE/e, Hedge flower leaf ethanol extract.

5.6 Discussion

The present study on the leaf extracts of marigold, Siam weed and hedge flower expressed the repellent and insecticidal activity on adult guava fruit fly *B. correcta*. These results were supported by the efficacy of the extracts from Kaffir Lime (*Citrus hystrix*), melon (*Cucumis melo* L.), lipsticktree (*Bixa orellana* L.), neem (*Azadirachta indica* var. *siamensis* Veleton), lemongrass (*Cymbopogon citraius* Stapf.), heliotrope (*Heliotropium indicum* R. Br.), shrubby basil (*Ocimum gratissimum* Linn.), orange gingerlily (*Hedychium occineum* var.), and Castor-oil plant (*Ricinus communis* L.) that could be repellent adult fruit flies (Areekul, Sinchaisri and Tigvatananon, 1978). There is a variety of other aromatic plants were found their potential in mosquito repellence. (Kumar, Mishra, Malik and Satya, 2011; Tawatsin et al., 2001). Many plants have been repeat that their repellent properties was able to against insects such as clove, peppermint, citronella, turmeric, hairy basil, eucalyptus, lavender, peppermint, and catmint essential oils (Tawatsin et al., 2001). Parugrug and Roxas (2008) showed that the repellent efficacy marigold extracts against maize weevil (*Sitophilus zeamais* Motsch). These supports that the plant extracts could control insects by their repellent property.

According to Malik and Naqvi (1984), Adedire and Ajayi (1996), Olonisakin, Oladimeji and Lajide, (2006), some plants contained irritant and foul smelling chemicals to which strongly repelled insect pests. The repellent properties of kitchen mint and kaffir lime on insects supported that the strong choky odors exerted a toxic effect by disrupting normal respiratory activity of weevils resulting in asphyxiation and subsequent death. The constituents of plant extracts have been studied to possess their potentials as alternative chemicals for use as repellent and insecticide agents

(Shaaya, Kostjukovski, Eilberg and Sukprakarn, 1997). The biological control properties of plant extracts, including marigold, Siam weed and hedge flower, belong to monoterpenes which express toxic effects in a wide range on insects, such as ovicidal, larvicidal, repellent, deterrent, and antifeedant (Boeke et al., 2004; Coats, Karr and Drewes, 1991).

The leaf extracts of marigold, Siam weed and hedge flower in this study showed insecticidal activities against *B. correcta*. These results were supported by Parugrug and Roxas (2008) which showed that the efficacy of marigold to against maize weevil (*Sitophilus zeamais* Motsch) by repellenc, adult mortality and antioviposition and growth inhibition. Similary, Nikkon et al. (2009) and Islam and Talukder (2005) showed the insecticidal activity of crude extracts from the flower of marigold *Tagetes erecta* L. against a stored product insect pest, *Tribolium castaneum* (H.). Accordingly, marigold callus cultures produced ascorbic acid as well as insecticidal pyrethrins against flour beetles (*Tribolium* spp) (Sarin, 2004). Cold aqueous extracts of marigold could inhibited tomato root knot nematode, *Meloidogyne incognita* (Natarajan et al, 2006).

Moreover, marigold extracts by maceration with dichloromethane and methanol showed that the activity to against rice weevils (*Sitophilus oryzae*) (Broussalis et al., 1999). This finding was in agreement with the work of Bouda, Tapondjou, Fontem and Gumedzoe (2001) that reported the effect of essential oil extracts from leaves of Siam weed has an insecticidal activities on the adult of maize grain weevils (*Sitophilus zeamais*). Similary, adulticidal properties of hedge flower leaf extracts against adult mosquitoes *Aedes aegypti* L., *Culex quinquefasciatus* Say, *Anopheles*

culicifacies Giles, *An. fluviatilis* James and *An. stephensi* Liston that have been reported by Dua, Pandey and Dash (2010).

Additionally, Bouda, Tapondjou, Fontem and Gumedzoe (2001) showed that the potential of essential oils from the leaves of *L. camara* controlled maize grain weevil, *Sitophilus zeamais* in stored products. *L. camara* leaf chloroform extract was found containing termiticidal effects against adult termite *Microcerotermes beesoni* (Verma, 2006).

5.7 Conclusions

The extracts of marigold, Siam weed and hedge flower exhibited potent repellent and insecticidal activities against guava fruit fly *B. correcta*. The ethanol extracts of all plant had higher potential against fruit flies than water extracts of the same plants as well as of the same concentration levels. The SLE, water and ethanol extracts, were the most effective to control fruit flies by repellence and insecticide. They were strong repellent against the *B. correcta* within 15 minutes. The correlations among treatment conditions in biological control of adult fruit flies both of repellent and insecticidal activity are significantly correlated. Therefore, the present findings suggest that the leaf extracts of marigold, Siam weed and hedge flower could be promising candidates for biological control of fruit flies as repellent and adulticidal agents.

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CHAPTER VI

INHIBITION ACTIVITY OF CYTOCHROME C OXIDASE ON THE *BACTROCERA CORRECTA* BEZZI BY PLANT EXTRACTS

6.1 Abstract

The leaves extracts of Marigold (*Tagetes erecta* L., MLE), Siam weed (*Chromolaena odorata* (L.) King & Robinson, SLE) and hedge flower (*Lantana camara* L., HLE) were used for *Bactrocera correcta* Bezzi. control. The death of *B. correcta* was analyzed by the inhibition activity of extracts on cytochrome c oxidase (COX). The Cyanide, well known as COX inhibitor was used as control. The inhibition potential of the ethanol extracts on COX was slightly higher than the water extracts. The leave of hedge flower extracted with ethanol (HLE/e) was the most potent inhibition at 65.32 ± 0.78%. The inhibition of all extracts on COX were ranged as HLE/e (65.32 ± 0.78%) > SLE/e (58.26 ± 3.55%) > SLE/w (56.23 ± 5.80%) > MLE/e (55.35 ± 2.89%) > HLE/w (46.61 ± 4.82%) > MLE/w (41.73 ± 2.38%). It demonstrated that the marigold, Siam weed and hedge flower extracts were likely to be the biological insecticide for fruit flies control.

6.2 Introduction

Mitochondria are important organ which play roles in many physiological activities such as the metabolism of cabohydrates and lipids. The mitochondria structure was showed in Figure 6.1. In addition to playing roles in respiration and adenosine triphosphate (ATP) synthesis, mitochondria play important energy-dependent roles in the regulation of cellular function, including intermediate metabolism, ion regulation, ion transport, as well as in intracellular Ca_2^+ homeostasis and cell motility. The electron transport chain is central to the energy metabolism of the cell. The electrons from nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) are passed through the electron transport system, which are complex I, II, III and IV in the inner membrane of mitochondria. Finally, the end product of cellular respiration is the adenosine triphosphate (ATP) (Paula, Sucheta, Szundi and Einarsdóttir, 1999).

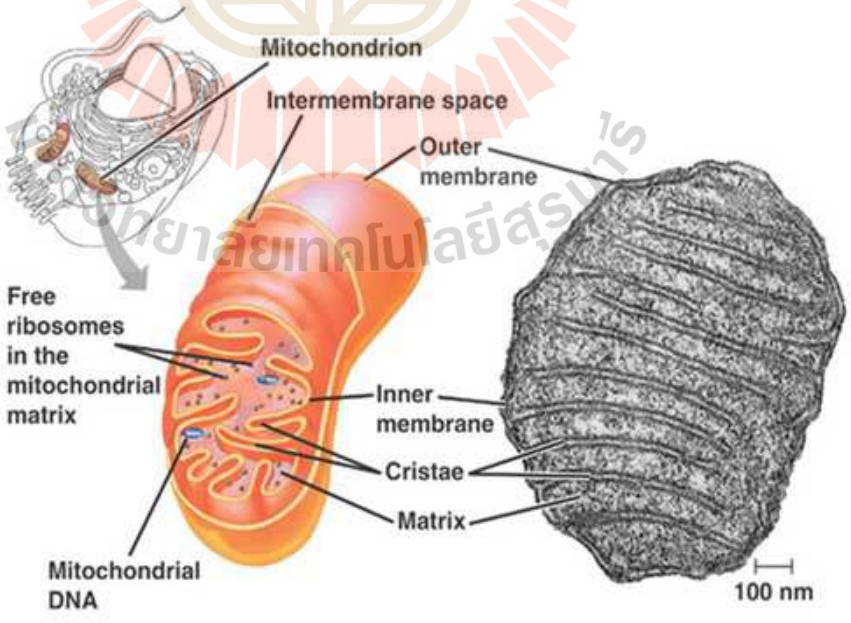

Figure 6.1 Structure of the mitochondria.

Source: http://quizlet.com/15522401/biology-chapter-6-flash-cards.html.

In higher organisms, the electron transport chain in the inner mitochondrial membrane is composed of four integral membrane enzyme complexes which are nicotinamide adenine dinucleotide (NADH): ubiquinone oxidoreductase (Complex I), succinate; ubiquinone oxidoreductase (Complex II), ubiquinol; cytochrome c oxidoreductase (Complex III); and cytochrome c oxidase (Complex IV). The energy released in this transport chain of electrons is used to generate a proton gradient across the inner mitochondrial membrane, which is consumed by ATP synthase (Complex V) for ATP production (oxidative phosphorylation) (Mather et al., 2007; Van Dooren, Stimmler and McFadden, 2006). The electron transport and oxidative phosphorylation was showed in Figure 6.2.

Cytochrome c oxidase (COX) is a large transmembrane protein embedded in mitochondrial inner membrane and is the terminal enzyme which functions as an electron carrier in the respiratory chain in reducing oxygen to water, producing ATP via oxidative phosphorylation and thus allowing energy and oxygen utilization by cells (Liénarda, Lassancea, Paulmierb, Picimbona and Löfstedta, 2006; Liu et al., 2007). It is a metalloprotein complex, catalyzes electrons transfer from reduces cytochrome c to a molecular oxygen, and preserves the free energy released in this exergonic reaction by maintaining the transmembrane proton gradient that is used to drive the synthesis of ATP or ion transport across the membrane. The activity of this enzyme is linked to the metabolic demand in the brain and reflects changes in neuronal activity; as a consequence, COX is used as an endogenous marker for neuronal activity (Wong-Riley, 1989). The enzyme is constituted by a variable number of subunits coded by the nuclear genome and three subunits coded by the mitochondrial genome that constitute the catalytic core (COXI, COXII and COXIII)

(Wikstrom and Casey, 1985; Capaldi, 1990). COXI contains the phosphorylation site, COXII interacts with cytochrome c in the electron transfer and COXIII is notably involved in the transmembrane proton pumping mechanism as well as protecting COX active sites (Namslauer and Brzezinski, 2004; Hosler, 2004).

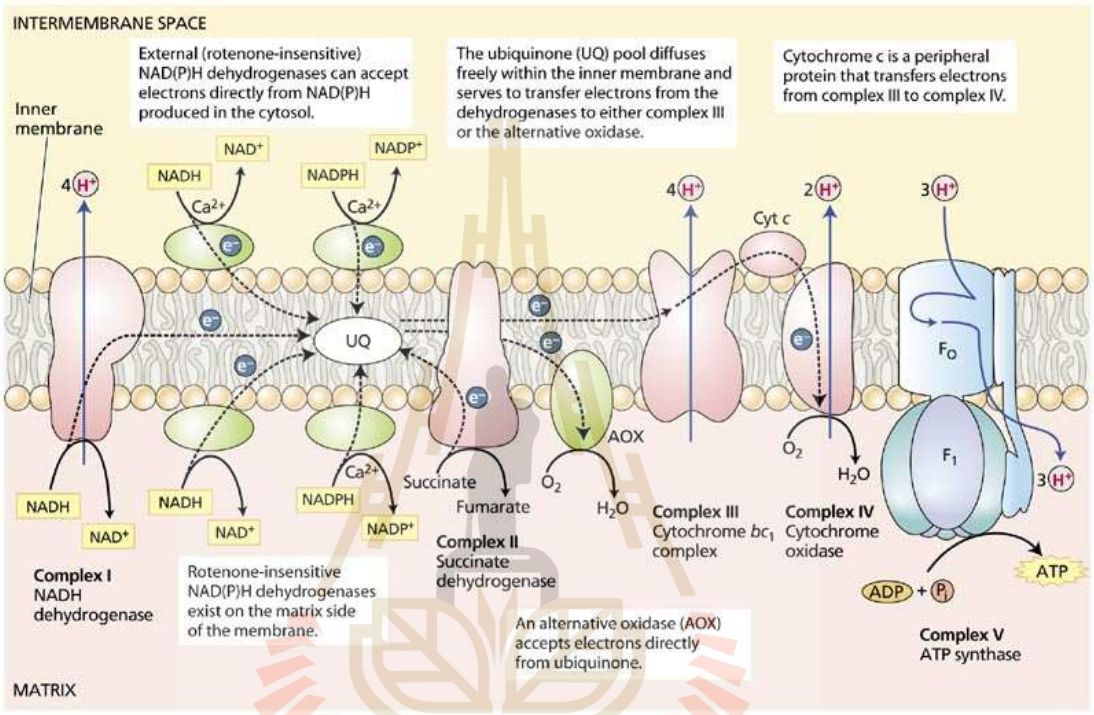

Figure 6.2 An overview of electron transport and oxidative phosphorylation. Source: http://ead.univ-angers.fr/~jaspard/Page2/COURS/Zsuite/1Respiration/Z999 suite/3ChaineRespiratoire/1ChainRespiratoire.htm.

The mitochondria disruption involved in the release of cytochrome c from mitochondria into the cytoplasm. Inhibition of cytochrome c oxidase prevents the utilization of molecular oxygen by cell leading to loss of cell function and then to cell death (Haritos and Dojchinov, 2003). The determination of cytochrome c oxidase is useful marker of mitochondria activities (Balaban, Mootha and Arai, 1996). Cytochrome c in a reduced form it is recognizable by its clear absorption spectrum

between 450 nm (minimum) and 590 nm (maximum). For oxidation of cytochrome c substance, it needs cytochrome oxidase. Inhibition of the oxidation of ferrocytochrome c was monitored as a function of cyanide concentration (Way, 1984). Cyanide binding to partially reduced forms produced by mixing cytochrome c oxidase with sodium dithionite was also examined.

$$4 \text{ ferrocytochrome c}^{(2+)} + 4H^{+} + O_2 \longrightarrow 4 \text{ ferriccytochrome c}^{(3+)} + 2H_2O$$

The inhibition of mitochondrial activity can be induced by pesticides which disrupt many sites of mitochondrial function and block the oxidative phosphorylation system. Plant products have naturally occurring toxic compound as mitochondrial respiration inhibitors. Rotenone and the piericidins have been known for a long time as insecticidal inhibitors of mitochondrial respiration and they have recently been increased of interest in this mode of action as a potential target for insecticides, acaricides, and fungicides. This has largely been due to the discovery of new compounds with good pesticidal activities in the inhibition of respiration (Jewess and Devonshire, 1998). As demonstrated recently by Haritos and Dojchinov (2003), the volatile formate esters caused inhibition of cytochrome c oxidase on the insect stored product pest *Sitophilus oryzae* (L.). Previous research on Brazilian flora that contained terpenoids, 1,8-cineole and limonene has documented insecticidal activities on insects *Sitophilus zeamais* (L.), *Sitophilus oryzae* (L.), *Rhyzopertha dommica* (F.) and *Tribolium castaneum* (H.) caused by their influence on the respiratory and digestive systems (Prates, Santos, Waquil, Fabris and Oliveira, 1998). Previously, Twenty naturally occurring monoterpenoids, high volatile compounds, were demonstrated as insecticidal fumigants to control rice weevil (*Sitophilus oryzae*), the

red flour beetle, (*Tribolium castaneum*), the sawtoothed grain beetle, (*Oryzaephilus surinamensis*), the house fly, (*Musca domestica*), and the German cockroach, (*Blattella germanica*) (Lee, Peterson and Coats, 2003). Recent examples, the identification of 20 volatiles from the steam distilled oil of the leaves from *Chamaecyparis obtusa* were successfully against adults of *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.) (Park, Lee, Choi, Park and Ahn, 2003).

In addition, the extracts of mintweed (*Hyptis suaveolens* L. Poit) kitchen mint (*Mentha cordifolia* Opiz) and kaffir lime (*Citrus hystrix* DC) leaves which contained aromatic, monoterpene and phenolic compound have been successfully used for the control of rice weevils (S. oryzae) by inhibiting the cytochrome c oxidase activity (Buatone, 2010). Moreover, the seed extracts of mintweed (*Hyptis suaveolens* (L.) Poit), yam bean (*Pachyrhizus erosus* L.) and celery (*Apium graveolens* L.) could control larvae and adults of *Aedes aegypti* L. the dengue hemorrhagic fever vector through the inhibition of cytochrome c oxidase activity (Yongkhamcha, 2010). These findings suggested that the mode of action of plant products involve in inhibition of mitochondrial cytochrome c oxidase of insects.

However, there are few data of the leaves extracts from marigold (MLE), Siam weed (SLE) and hedge flower (HLE) causes insect mortality at the cellular mechanism level. Interestingly, the compounds constituents of leaves extracts from MLE, SLE and HLE were group of terpenes (Vasudevan, Kashyap and Sharma, 1997; Cetkovic, Djilas, Canadanovic-Brunet and Thumbas, 2004; Misra, 2009; Anyasor, Aina, Olushola and Aniyikaye, 2011; Alisi, Ojiako, Osuagwu and Onyeze, 2011; Félicien et al., 2012; Salinas-Sánchez et al., 2012; Souza, Gomes, Vieites and Gomes, 2012) which had demonstrated to successfully control *B. correcta* (see Chapter IV

and V). The aim of this study was to investigate that the efficiency of leaves extracts from marigold (MLE), Siam weed (SLE) and hedge flower (HLE) mitochondria activity inhibit cytochrome c oxidase in mitochondria for supporting their mortality activities on *B. correcta* mortality.

6.3 Materials and methods

6.3.1 Materials

Cytochrome c from horse heart, *n*-dodecyl-β-D-maltoside, sodium dithionite, K_2HPO_4, KH_2PO_4, sucrose, disodium EDTA, magnesium sulfate, Tris buffer and bovine serum albumin (BSA) were purchased from Sigma Chemicals (St. Louis, MO, USA). Potassium hexacyanoferrate (III) was purchased from Fisher Scientific (Suwanee, Georgia, USA). Dimethylsulfoxide (DMSO) was purchased from Merck Chemical supplies (Darm-Stadt, Germany). All chemicals were analytical grade.

6.3.2 Plants collection and preparation for extracts

The fresh leaves of Siam weed (*Chromolaena odorata* (L.) King & Robinson, SLE) and hedge flower (*Lantana camara* L., HLE) were collected from surrounding of Suranaree University of Technology (SUT) campus. Marigold (*Tagetes erecta* L., MLE) leaves were collected from a local farm near by Suranaree University of Technology (SUT) campus. All plant leaves were cleaned, dried by sunlight and ground to powder. Ten grams of dried plant powders in a cellulose extraction thimble (Whatman International Ltd., Maidstone, England) were extracted in 150 ml of water or 70% ethanol in Soxhlet extraction apparatus (Buchi model B

811, Germany). The extracts were filtered and evaporated in rotary evaporator (Buchi instruments, Switzerland) and dried by lyophilizer (Freeze-zone 12 plus, Labconco Corporation, Missouri, USA). The extracts were stored at -20ºC until analysis. The dried extracts were dissolved in its original solvents for use in all experiments.

6.3.3 Fruit fly rearing

Pupae of guava fruit fly (*Bactrocera correcta*) were obtained from the Thailand Institute of Nuclear Technology (Public Organization), Ministry of Science and Technology, Thailand. Adult flies emerge from the pupa cases in 7 days. Fruit flies were cultured in wire-net cages, fed with artificial food (Walker et al., 1997), and allowed to mate. The females were allowed to lay eggs in the egg dome (contain guava juice). After hatching, the larvae feed on artificial food and allow moving to pupa stage in wood chip trays. The culture was continued for a week to allow the new adults emerged. The adult fruit flies aged 2 days after emerging were used for all experiments, were kept at -80^{o}C for making death to insects and preserving mitochondria until used.

6.3.4 Isolation for fruit flies mitochondria

The isolation for fruit flies mitochondria modified from the procedures described by Haritos and Dojchinov (2003) and Song and Scharf (2009). Five grams of whole *B. correcta* adults were manually homogenized in a tissue grinder on ice chilled with 10 ml isolation buffer containing 0.05 M Tris buffer (pH 7.4) 0.25 M sucrose, 0.001 M disodium Ethylenediaminetetraacetic acid (EDTA), 0.005 M magnesium sulfate, and 0.2% bovine serum albumin (BSA). The homogenate was

centrifuged at 500×*g* at 4ºC for 20 min to pellet the cellular debris. The supernatant was removed, transfered to a fresh tube and recentrifuged stepwise, at 3000×*g* and 10,000×*g* at 4^{o}C for 15 min. The supernatant was discarded. The mitochondrial pellets were resuspended in the 5 ml isolation buffer and centrifuged at 10,000×*g* at 4ºC for 15 min. The supernatant was discarded. The pellet was then resuspended in isolation buffer and kept at -80^{o}C until used.

6.3.5 Cytochrome c oxidase inhibition

Cytochrome c oxidase activities of fruit flies were measure using the spectrophotometric methods base on the method of Haritos and Dojchinov (2003) and Song and Scharf (2009). The cytochrome c from horse heart as a standard was dissolved in phosphate buffer and used for measurement of cytochrome c activity (Song and Scharf, 2009). Cytochrome c standard was reduced by adding a tiny crystal sodium dithionite immediately before use (Paula, Sucheta, Szundi and Einarsdóttir, 1999). One microgram per milliliter reduced cytochrome c (36 mM final concentration) was used as a substrate. The reaction buffer containing 40 mM phosphate buffer pH 6.2 containing 250 mM sucrose and 0.05% (w/v) lauryl maltoside. The concentration of extract sample was selected based on the concentrations of insecticidal activity tested. The extracts were obtained at 20, 40, 60, 80 and 100 mg/ml in a reaction well. The reduced cytochrome c was put into a 96-well plate, 20 µl/well, containing 20 µl reaction buffer. The extract sample (20 µl), and 0.1% DMSO (20 µl) were added. Lastly, the mitochondrial homogenate (20 µl) was added. The COX activity was immediately measured in spectrophotometer (ELISA microplate reader, Benchmark ^{plusTm}, Bio Rad) at 550 and 565 nm for 5

minutes at the most. Cyanide (0.003%) was used as control. The inhibition percentage was calculated as the following equation.

$$\% \text{ inhibition} = \left[\left(\frac{\text{Absorbance of the sample}}{\text{Absorbance of reduced cytochrome c}} \right) \times 100 \right]$$

6.3.6 Data analysis

The data were expressed as mean of three replicates and were calculated with one way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) statistical software. Values were statistically significant at $p < 0.05$.

6.4 Result

6.4.1 Inhibition of mitochondrial cytochrome c oxidase activity

The Inhibition of cytochrome c oxidase (COX) in *B. correcta* mitochondrial preparations by the ethanol and water leave extracts of marigold (*Tagetes erecta* L., MLE/w and MLE/e), Siam weed (*Chromolaena odorata* (L.) King & Robinson, SLE/w and SLE/e) and hedge flower (*Lantana camara* L., HLE/w and HLE/e) was investigated. The presence of the water leave extracts was obvious that the SLE/w expressed high potential of COX activity inhibition of *B. correcta* mitochondria. The SLE/w had higher activities than MLE/w and HLE/w which also inhibited COX. The COX inhibition induced by SLE/w, MLE/w, and HLE/w was 56.23, 46.61 and 41.73% respectively (Table 6.1). When compare to the control SLE/w 100 mg/ml exhibited a significant COX inhibition which was 1.52 fold lower.

Table 6.1 Inhibitory activity of cytochrome c oxidase (COX) on *B. correcta* adult by water extracts of *Tagetes erecta* (MLE/w), *Chromolaena odorata* (SLE/w) and *Lantana camara* (HLE/w).

Plant extracts	**Conc. (mg/ml)**	**% COX inhibition (Mean ± S.E.)**
MLE/w	20.00	26.93 ± 4.13[a]
	40.00	26.96 ± 6.08[a]
	60.00	27.16 ± 4.06[a]
	80.00	32.28 ± 2.73[ab]
	100.00	41.73 ± 2.38[b]
SLE/w	20.00	25.96 ± 4.18[a]
	40.00	27.22 ± 4.16[ab]
	60.00	30.32 ± 4.34[ab]
	80.00	41.75 ± 4.23[b]
	100.00	56.23 ± 5.80[c]
HLE/w	20.00	24.85 ± 3.44[a]
	40.00	28.92 ± 4.19[a]
	60.00	35.88 ± 7.79[ab]
	80.00	38.60 ± 1.09[ab]
	100.00	46.61 ± 4.82[b]
Cyanide	0.003 (mg/ml)	85.96 ± 0.97

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significantly difference ($P < 0.05$).

The ethanol leave extracts was demonstrated that their potency on cytochrome c oxidase inhibitory activity differ from the water leave extracts. HLE/e had slightly higher activities than SLE/e and MLE/e, the COX inhibition induced by HLE/e, SLE/e, and MLE/e was 65.32, 58.26 and 55.35% respectively (Table 6.2). When compare to the control MLE/e, SLE/e and HLE/e 100 mg/ml exhibited a significant COX inhibition which were 1.6, 1.5 and 1.3 fold lower respectively.

Table 6.2 Inhibitory activity of cytochrome c oxidase (COX) on *B. correcta* adult by water extracts of *Tagetes erecta* (MLE/e), *Chromolaena odorata* (SLE/e) and *Lantana camara* (HLE/e).

Plant extracts	Conc. (mg/ml)	% COX inhibition (Mean ± S.E.)
MLE/e	20.00	28.66 ± 1.65^{a}
	40.00	35.85 ± 4.90^{ab}
	60.00	49.18 ± 6.26^{bc}
	80.00	51.49 ± 4.59^{c}
	100.00	55.35 ± 2.89^{c}
SLE/e	20.00	24.21 ± 4.42^{a}
	40.00	27.46 ± 4.86^{a}
	60.00	31.29 ± 1.15^{ab}
	80.00	44.62 ± 8.40^{bc}
	100.00	58.26 ± 3.55^{c}
HLE/e	20.00	26.26 ± 1.83^{a}
	40.00	31.23 ± 4.53^{a}
	60.00	35.91 ± 2.08^{ab}
	80.00	44.04 ± 5.27^{b}
	100.00	65.32 ± 0.78^{c}
Cyanide	0.003 (mg/ml)	85.96 ± 0.97

Each value is the mean ± standard error, n = 3. Numbers with different letters within the same column are significantly difference ($P < 0.05$).

The profiles of COX inhibition by the water or the ethanol extracts are similar as in Figure 6.3. The potency of the extracts on COX inhibition was ranged as HLE/e > SLE/e > SLE/w > MLE/e > MLE/w > HLE/w. Therefore, it can conclude that the HLE/e is the highest inhibitor on cytochrome c oxidase in mitochondria in controlling *B. correcta*.

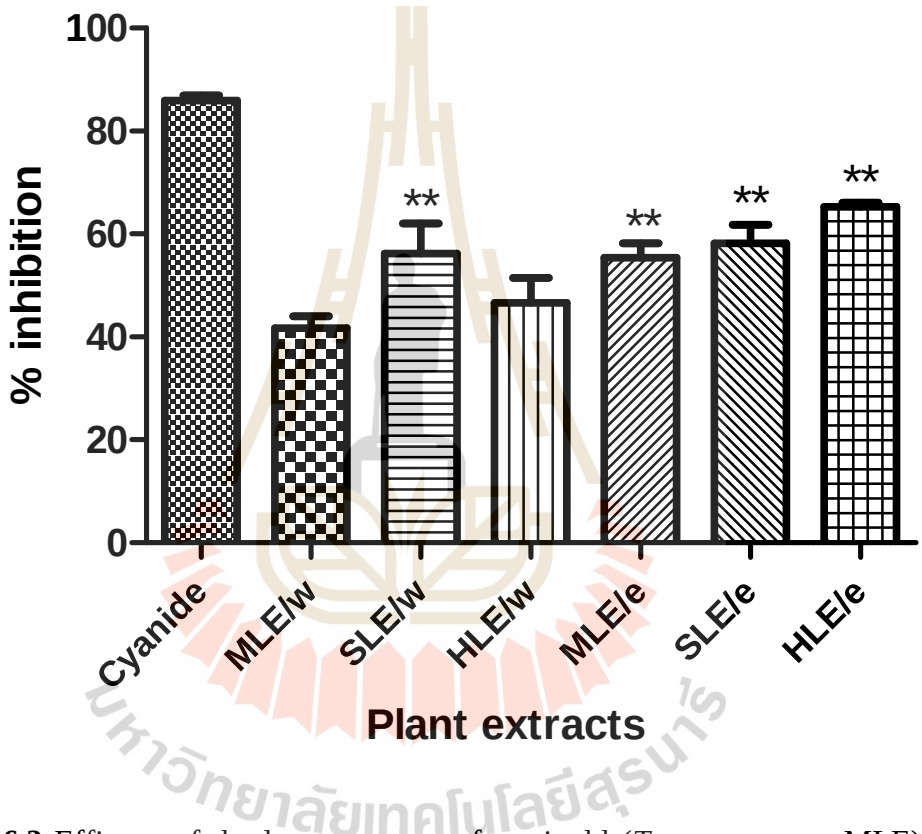

Figure 6.3 Efficacy of the leave extracts of marigold (*Tagetes erecta*, MLE), Siam weed (*Chromolaena odorata*, SLE) and hedge flower (*Lantana camara*, HLE) on the cytochrome c oxidase activity in mitochondria of fruit fly *B. correcta*, **P < 0.01.

The spectral profile of reduced–oxidized cytochrome c standard from horse heart was shown in Figure 6.4. It is typical to determine the extent of reduction of cytochrome c by measuring the difference in opical absorbance at 550 nm and 565 nm. The spectrum of reduced cytochrome c by the water leaf extracts was shown in Figure 6.5 and by the ethanol leaf extracts was shown in Figure 6.6. The difference in absorbance is denoted absorbance of COX, expressed as percent inhibition (% COX). This demonstrated that the leaf extracts inhibited COX activity in *B. correcta* mitochondria. The COX inhibition of the water extracts was ranged as SLE/w > MLE/w > HLE/w (Figure 6.5) and of the ethanol extracts was ranged as HLE/e > SLE/e > MLE/e (Figure 6.6).

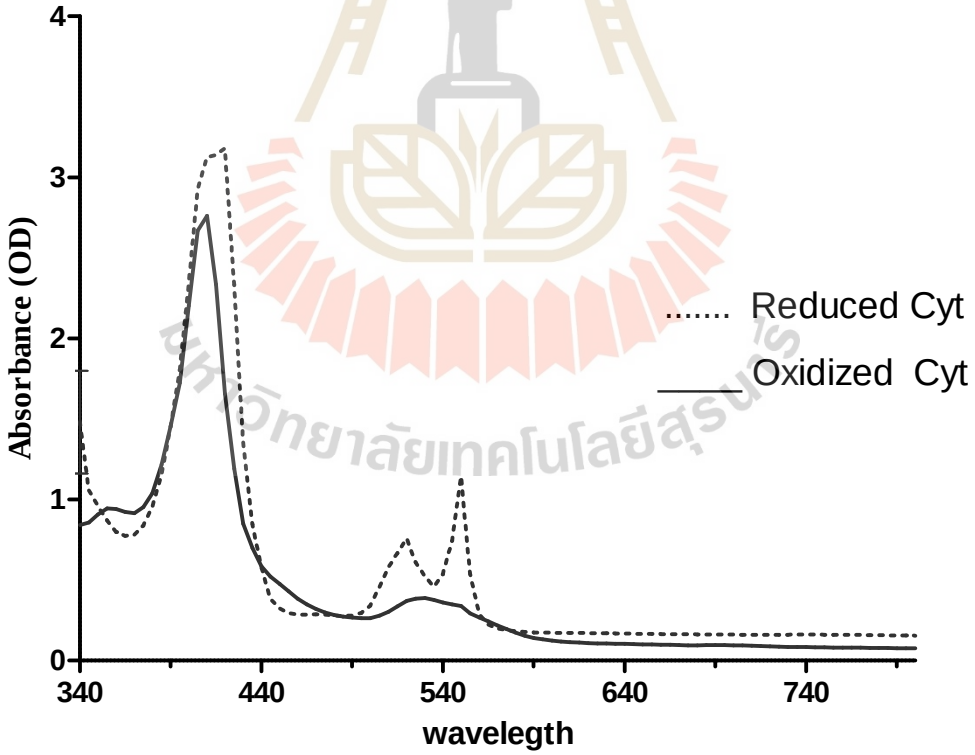

Figure 6.4 The spectral profile of reduced-oxidized cytochrome c from horse heart, the standard.

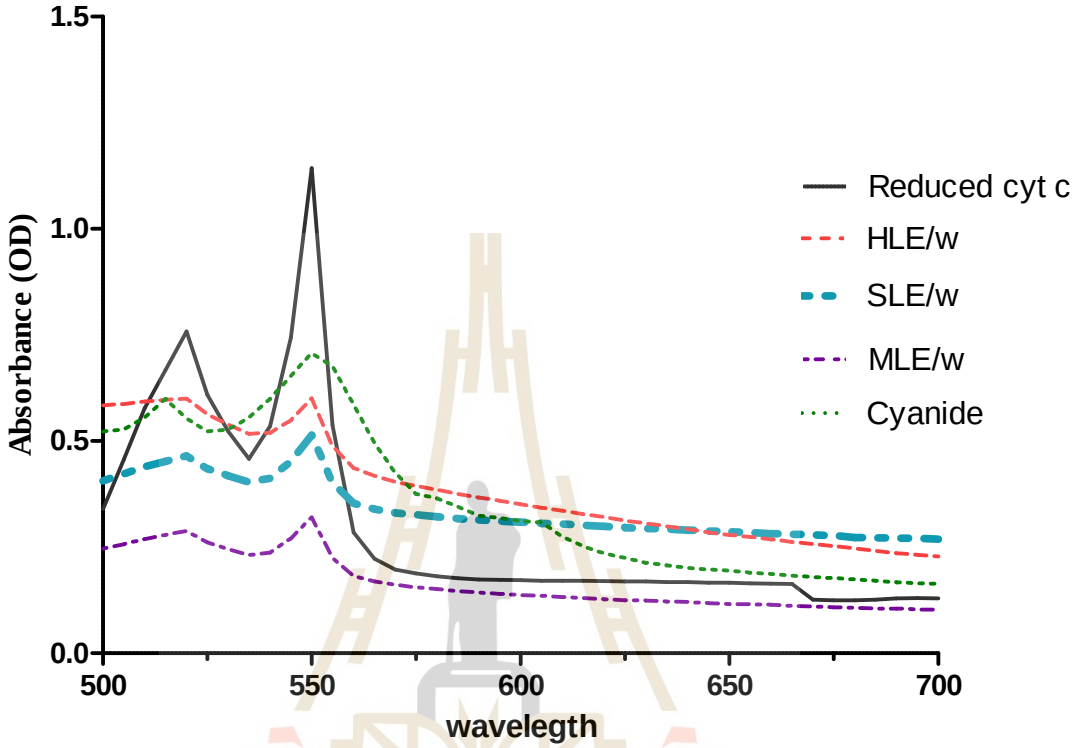

Figure 6.5 The action spectrum of reduced cytochrome c on fruit fly *B. correcta* mitochondria in the presence of the water extracts of marigold (*Tagetes erecta*, MLE), Siam weed (*Chromolaena odorata*, SLE) and hedge flower (*Lantana camara*, HLE).

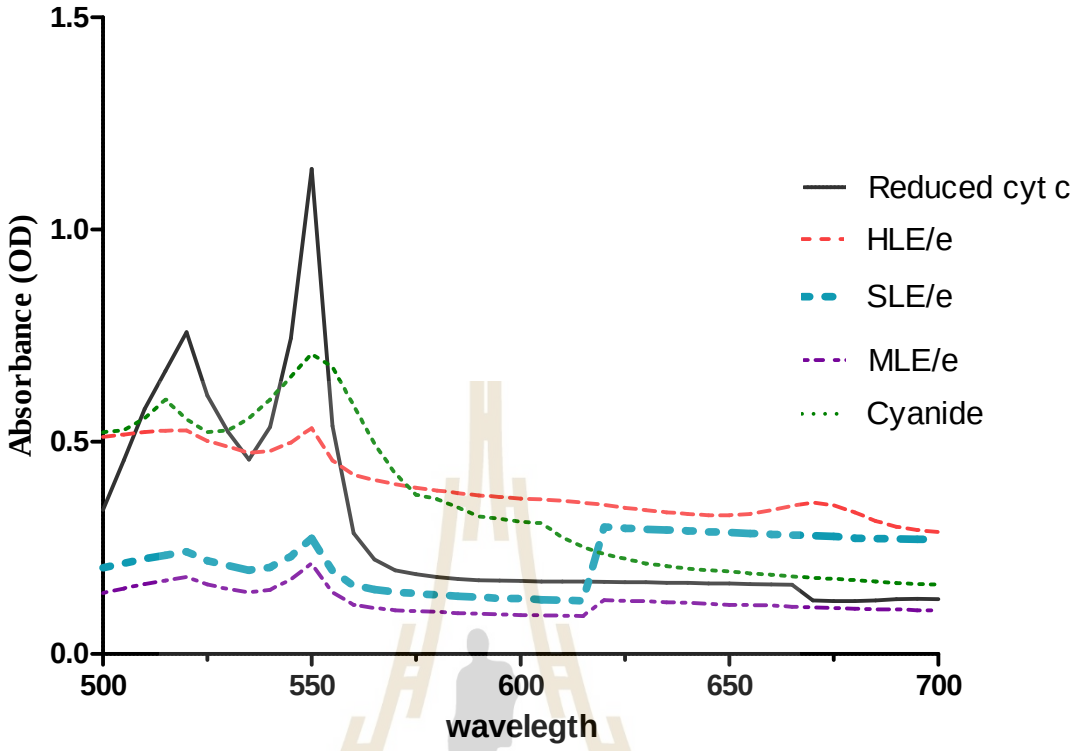

Figure 6.6 The action spectrum of reduced cytochrome c on fruit fly *B. correcta* mitochondria in the presence of the ethanol extracts of marigold (*Tagetes erecta*, MLE), Siam weed (*Chromolaena odorata*, SLE) and hedge flower (*Lantana camara*, HLE).

6.5 Discussion

Botanical pesticides tend to have broad-spectrum activity, are relatively specific in their mode of action and easy to process and use. The international literature on the biological properties of crude extracts and isolated secondary substances of plants against different insects and other organisms is abundant. Defense plant secondary metabolites belong to three categories: phenolics (such as tannins, flavones, flavonoids, glyceollin, and lignin), nitrogen compounds (such as sinalbin, sinigrin, dhurrin) and terpenoids (such as saponin, monooterpene). Overall categories of direct plant defenses against insect herbivores include limiting food supply, reducing nutrient value, reducing preference, disrupting physical structures, and inhibiting chemical pathways of the attacking insects (Kessler and Baldwin, 2002). Known major defense chemicals include plant secondary metabolites, protein inhibitors of insect digestive enzymes, proteases, lectins, amino acids and oxidases. Cytochrome c oxidase (COX) has been shown to be an interesting candidate for expression study in insects. Among those, differential expression of COX subunits has been found to be correlated with developmental modifications in insects. In *Apis mellifera*, distinct larval nourishment influences the developmental differentiation between queen and worker honeybees. Queen larvae have a higher respiration rate which is reflected by an over-expression of the COXI subunit (Corona, Estrada and Zurita, 1999).

The findings of this study provide information to confirm the impacts of plant extracts on mitochondrial. This study, the investigation which demonstrated that the extracts of marigold, Siam weed and hedge flower could be control agent of fruit flies which inhibit the cytochrome c oxidase activity. Similarly, Haritos and Dojchinov (2003) investigated COX inhibition of the volatile formate on the insect stored

product pest *Sitophilus oryzae* (L.) caused by inhibition of cytochrome c oxidase. Song and Scharf (2009) determinated *Drosophila melanogaster* COX inhibition in mitochondria by insecticidal materials, hydramethylnon and sodium cyanide caused of COX inhibitory activity. In addition, Buatone (2010) reported that the extracts of mintweed (*Hyptis suaveolens* L. Poit) kitchen mint (*Mentha cordifolia* Opiz) and kaffir lime (*Citrus hystrix* DC) leaves that contained aromatic, monoterpene and phenolic compound successfully used for the control of rice weevils (*S. oryzae*) by inhibiting the cytochrome c oxidase activity in the electron transport chain of cellular respiration in mitochondria. Moreover, Yongkhamcha (2010) demonstrated that the inhibition of cytochrome c oxidase activity of the electron transport chain in cellular respiration was induced by seed extracts of mintweed (*Hyptis suaveolens* (L.) Poit), yam bean (*Pachyrhizus erosus* L.) and celery (*Apium graveolens* L.) in controlling larvae and adults of *Aedes aegypti* L. the dengue hemorrhagic fever vector. This study is the first investigation of plant extracts in controlling fruit flies by interfering with the cellular respiration at the electron transport chain in mitochondria. This finding suggests that the extracts of marigold (*Tagetes erecta*, MLE), Siam weed (*Chromolaena odorata*, SLE) and hedge flower (*Lantana camara*, HLE) containing monoterpene and phenolic acid may be cytochrome c oxidase inhibitors against fruit flies. As the results showed that the plant extract potential to disrupted cytochrome c oxidase activities more than 50% inhibition.

6.6 Conclusion

The leaves ethanol extracts of *L. camara* (HLE/e) demonstrated the highest potency for cytochrome c oxidase inhibition in fruit fly mitochondria. The COX inhibition was 65.32 ± 0.78%. However, *C. odorata* (SLE) could also be candidate for cytochrome c oxidase inhibitor, since both of water and ethanol extracts had higher activities to inhibit COX equally. Their COX inhibitions were 58.26 ± 3.55% and 56.23 ± 5.80%. The extracts induced death by COX disruption and may be toxic on other systems. Therefore, this result may suggest that the extracts of *L. camara* and *C. odorata* extracted by water or ethanol can be the biological control agents for *B. correcta*.

6.7 References

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CHAPTER VII

CONCLUSION

The guava fruit fly, *Bactrocera correcta* (Bezzi), is one of the main economically important pests affecting the valuable fruit production and quality and also impacting the export trade of Thailand agricultural products. The effect of *B. correcta* is difficult to investigate in the early infestation, because it has potential to infest fruit from the early fruiting stage till to the harvest stage. Biological control is growing interest in the use as simple and safe technology to reduce the use of chemically synthetic insecticides and also to avoid several problems of insecticide. Therefore, the information for potentially useful products in the biological control of guava fruit flies is required. In the present study, the leave water and ethanol extracts of marigold (*Tagetes erecta* L.), Siam weed (*Chromolaena odorata* L.) and hedge flower (*Lantana camara* L.) were investigated for the efficiency in biological control of *B. correcta* in 4 stages: eggs, larvae, pupae and adults.

The total phenolic compounds (TPC) of ethanol extracts were slightly higher than water extract. The TPC contents of SLE/e, MLE/e and HLE/e were 133.03, 59.67 and 47.43 milligrams gallic acid equivalents per gram dry sample (mg GAE/ g), whereas SLE/w, MLE/w and HLE/w were 84.87, 52.50 and 33.93 mg GAE/g, respectively. The major constituents of the extracts were analyzed by thin layer chromatography (TLC). Vanillin-sulphuric reagents detection indicated that the terpenes group was the major compounds in the extracts. The cytotoxicity of the

extracts was investigated by brine shrimp lethality assay (BSLA). The lethality concentration of 50% (LC_{50}) at 24 h of SLE/e, MLE/e and HLE/e were 147.15, 182.60 and 208.82 µg/ml, and SLE/w, MLE/w and HLE/w were 196.35, 256.32 and 273.29 µg/ml, respectively. The LC_{50} which lower than 1,000 µg/ml indicated that the plant extracts could be potentially used for insect pest control.

The SLE/e exhibited the highest effect on egg hatching with 82.22 ± 6.19% inhibition at 24 hours. The inhibitory effect of all extracts on fruit flies egg hatching was ranged as SLE/e > HLE/e > MLE/e > SLE/w > HLE/w > MLE/w with EC_{50} value of 44.54, 53.42, 55.92, 65.66, 72.03 and 75.41 mg/ml, respectively. The SLE/e was the most efficient extract which could induce the morality of second instar larvae of 83.33 ± 1.92% by feeding assay and also on the dipping assay at 87.78 ± 1.11%. The range of larvicidal efficacy of all extracts by feeding assay and dipping assay showed a similar trend. The efficacy was ranged as SLE/e > HLE/e > SLE/w > MLE/e > HLE/w > MLE/w. By feeding assay, the LC_{50} values were 55.56, 65.95, 65.95, 72.33, 74.67 and 80.14 mg/ml while dipping assay, the LC_{50} values were 52.99, 63.43, 64.08, 70.29, 71.77 and 77.52 mg/ml, respectively. The effects of the three plant extracts on the adult emergence were similar to the effects of the egg hatching and the larva mortality. The antibiosis on fruit fly adult emergence by SLE/e exhibited highest efficacy of 67.78 ± 2.22% inhibition. Nevertheless, the inhibition rate was ranged as SLE/e > MLE/e > SLE/w > HLE/e > MLE/w > HLE/w with EC_{50} value of 69.55, 72.49, 72.91, 77.23, 78.38 and 83.44 mg/ml, respectively. The correlations among treatment conditions in antibiosis effect on egg, larva and pupa of guava fruit flies are not significantly correlated.

The repellent efficacy of plant extracts on adult fruit flies was conducted by olfactometer. The SLE/w showed the highest repellency of 85.43 ± 3.90% at 15 minutes of treatment. The repellent efficacy was ranged as SLE/e > MLE/e > SLE/w > HLE/e > HLE/w > MLE/w with EC_{50} value of 35.42, 36.58, 37.66, 38.16, 39.17 and 43.23 mg/ml, respectively. The SLE/e produced the highest mortality effect on adult fruit flies at 80.00 ± 1.92%. The mortality effect on the adult fruit flies was ranged as SLE/e > SLE/w> MLE/e > HLE/e > HLE/w > MLE/w with LC_{50} value of 67.32, 80.92, 87.90, 88.20, 89.88 and 93.67 mg/ml, respectively. The combination extracts of SLE/e + MLE/e at the ratio of 3:1 was highly effective with 72.22 ± 1.11% mortality at 24 hours while the least effective mixture was HLE/w + MLE/w at the ratio of 1:3 which was effective only 34.44 ± 4.01% mortality at 24 hours. Thus, it could be assumed that the combination extracts were antagonistic agent reducing the potential of SLEs in the biological control of adult guava fruit flies *B. correcta*. The correlations among treatment conditions in biological control of adult fruit flies both of repellent and insecticidal activity are significantly correlated. It is concluded that the SLE/e extracts were the most potent in controlling eggs hatching, larvae, pupae and adults mortalities of guava fruit flies *B. correcta*.

The death of *B. correcta* was investigated by the inhibitory activity of extracts on cytochrome c oxidase (COX). The leave ethanolic extract of hedge flower (HLE/e) was the most potent inhibition. The inhibition of all extracts on COX was ranged as HLE/e > SLE/e > SLE/w > MLE/e > HLE/w > MLE/w with % COX inhibition of 65.32 ± 0.78%, 58.26 ± 3.55%, 56.23 ± 5.80%, 55.35 ± 2.89%, 46.61 ± 4.82% and 41.73 ± 2.38%, respectively. It indicated that the inhibition of all extracts and cyanide on COX had the same pattern with more than 50% COX inhibition.

The results demonstrated that the marigold, Siam weed and hedge flower extracts were likely to be the biological insecticide for fruit flies control as well. The terpenes group, the major constituents of the extracts, could be a potent compound leading to the death of fruit flies. All plant extracts showed that the efficacy to against fruit flies as ovicides, antifeedants, larvicides, pupacide, repellents and adulticide, especially the Siam weed *C. odorata* leave extracts. Therefore, the applying of Siam weed *C. odorata* leave extracts as biological control agent for against guava fruit flies could be used to replace synthetic insecticides which is beneficial to human health and sustain the environment.

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