BIOLOGICAL CONTROL OF DENGUE FEVER MOSQUITOES (*AEDES AEGYPTI* LINN.) USING LEAF EXTRACTS OF CHAN (*HYPTIS SUAVEOLENS* (L.) POIT.) AND HEDGE FLOWER (*LANTANA CAMARA* LINN.)

Pisan Tanprasit

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การควบคุมยุงลาย (*AEDES AEGYPTI* LINN.) โดยชีววิธี ด้วยสารสกัดจากใบแมงลักคา (*HYPTIS SUAVEOLENS* (L.) POIT.) และใบผกากรอง (*LANTANA CAMARA* LINN.)

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ยุงเป็นที่รู้จักกันดีในนามของพาหะที่ทำให้เกิดโรคร้ายหลายโรค โดยเฉพาะยุงลาย *Aedes aegypti* Linn. เป็นที่รู้กันว่าเป็นพาหะนำโรคไข้เลือดออกและไข้เหลือง จึงได้เกิดความคิดที่จะกำจัดยุงลายพาหะ ซึ่งในการศึกษานี้ได้ทำการทดลองควบคุมยุงลายโดยชีววิธี ด้วยสารสกัดจากใบแมงลักคา สารสกัดจากใบผกากรอง และสกัดจากใบแมงลักคาผสมสารสกัดจากใบผกากรอง โดยทำการทดลองในระยะไข่ ลูกน้ำระยะที่สอง และ ระยะตัวเต็มวัย พบว่าสารสกัดผสมได้ให้ผลอย่างมีนัยสำคัญในการกำจัดยุงลายมากกว่าสารสกัดจากใบแมงลักคา และสารสกัดจากใบผกากรอง ซึ่งเมื่อเปรียบเทียบค่า LC_{50} ของสารสกัดเหล่านี้ พบว่าสารสกัดผสม (LC_{50} = 14.04%) มีผลในการกำจัดลูกน้ำยุงลายระยะที่สองมากกว่าสารสกัดจากใบแมงลักคา (LC_{50} = 20.24%) และสารสกัดจากใบผกากรอง (LC_{50} = 74.44%) เป็น 1.44 เท่า และ 5.30 เท่า ตามลำดับ ($P \leq 0.01$) นอกจากนั้นยังพบว่าสารสกัดจากใบแมงลักคาแสดงผลการเสริมฤทธิ์ต่อการตายของลูกน้ำยุงลายระยะที่สองเมื่ออยู่ในรูปของสารสกัดผสม ในยุงลายระยะตัวเต็มวัยก็เช่นกันสารสกัดผสมมีผลต่อเปอร์เซ็นต์การตายของยุงลายตัวเต็มวัยมากกว่าสารสกัดจากใบแมงลักคา และสารสกัดจากใบผกากรอง เมื่อเปรียบเทียบเปอร์เซ็นต์การตายของแต่ละสารสกัดที่ความเข้มข้น 50% พบว่าสารสกัดผสมมีผลต่อเปอร์เซ็นต์การตายของยุงลายตัวเต็มวัย (20.67%) มากกว่าสารสกัดจากใบแมงลักคา (10.67%) และสารสกัดจากใบผกากรอง (2.67%) เป็น 1.94 เท่า และ 7.74 เท่า ตามลำดับ ($P \leq 0.01$) แต่พบว่าสารสกัดทุกชนิดมีผลต่อการไม่ฟักออกของไข่ยุงลายน้อยมาก ทั้งนี้อาจเป็นเพราะว่าไข่ยุงลายที่ออกมาใหม่ๆ จะอ่อนนุ่มและมีสีขาวช่วงหนึ่งแต่ไม่นานไข่จะเริ่มมีเปลือกแข็งและมีสีดำหลังจากยุงวางไข่ เมื่อนำมาทดสอบกับสารสกัดทั้งสามชนิดทำให้สารสกัดแพร่ผ่านเข้าสู่เปลือกไข่ยุงได้ยาก ดังนั้นประสิทธิภาพของสารสกัดทั้งสามชนิดจึงมีผลน้อยมากในช่วงชีวิตของยุงช่วงนี้

สาขาวิชาชีววิทยา ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษา _____

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PISAN TANPRASIT : BIOLOGICAL CONTROL OF DENGUE FEVER MOSQUITOES (*AEDES AEGYPTI* LINN.) USING LEAF EXTRACTS OF CHAN (*HYPTIS SUAVEOLENS* (L.) POIT.) AND HEDGE FLOWER (*LANTANA CAMARA* LINN.) THESIS ADVISOR : ASSOC. PROF. KORAKOD INDRAPICHATE, Ph.D. 114 PP. ISBN 974-533-548-7

AEDES AEGYPTI/DENGUE FEVER MOSQUITO/BIOLOGICAL CONTROL/ HYPTIS SUAVEOLENS/CHAN/LANTANA CAMARA/HEDGE FLOWER/ SOXHLET EXTRACTION

Mosquitoes are well known as vectors of several diseases causing pathogens. *Aedes aegypti* is known to carry dengue and yellow fever. Renewed interest has been shown in the development of alternative strategies, including the use of suitable type of natural insecticides derived from traditional botanical pest control agents. Hence, biologically control of *Ae. aegypti* using leaf extract of chan, hedge flower and extract combination was investigated in this study. Studies were carried out to evaluate the effect of plant extracts on mosquito eggs, second instar larvae and adults. The results indicated that the mixed extracts possessed significant larvicidal activity against *Ae. aegypti* than those of *H. suaveolens* extract and *L. camara* extract. When compare LC_{50} of these among extracts, the mixed extract (LC_{50} 14.04%) had higher larvicidal activity than those of *H. suaveolens* extract (LC_{50} 20.24%) and *L. camara* extracts (LC_{50} 74.44%) about 1.44 and 5.30 times ($P \leq 0.01$), respectively. It was also found that *H. suaveolens* extract exhibited synergism effect on the mortality of *Ae. aegypti* larvae when presented in the combination extracts. Similarly, that the combination

extract also showed higher percent mortality of mosquito adults than those of *H. suaveolens* and *L. camara* extracts in all concentration levels. When compare percent mortality at 50% concentration among these three extracts, combination extract had higher mortality (20.67%) than those of *H. suaveolens* extract (10.67%), and *L. camara* extract (2.67%) about 1.94 and 7.74 times ($P \leq 0.01$), respectively. On the other hand, all extracts in this study i.e. *H. suaveolens* extract, *L. camara* extract and combination extracts had very low influenced on mosquito eggs. This could probably explain that mosquito eggs are soft and white at oviposition but undergo sclerotization during embryogenesis, becoming harder and darker. Hence, when the plant extracts exposed to the mosquito eggs, the extracts were difficult to diffuse to eggshell of mosquito. Therefore, the efficiency of plant extracts was very low in this stage of mosquito life cycle.

School of Biology

Academic year 2005

Student's Signature _____

Adviser's Signature _____

Co-adviser's Signature _____

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Pisan Tanprasit

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LIST OF ABBREVIATIONS

Abbreviation	
Ae. aegypti L.	*Aedes aegypti* Linneus
°C	degree Celsius
g	gram
h	hour
H. suaveolens L.	*Hyptis suaveolens* Linneus
in	inch
IU	International Unit
L. camara L.	*Lantana camara* Linneus
mg	milligram
mg/ml	milligram per milliliter
ml	milliliter
ppm	part per million
%	percent
SD	Standard Deviation
v/v	volume by volume
WHO	World Health Organization
w/w	weight by weight

CHAPTER I

INTRODUCTION

1.1 Background

Mosquitoes are the major vectors for many diseases such as dengue fever, yellow fever, malaria, filariavirus, Japanese encephalitis and other fevers (Service, 1983). There are many types of mosquitoes living in the tropical and sub-tropical regions of the world. They can be roughly divided into three groups, anopheles, aedes and culex (Donald, 2004) as shown in Figure 1. According to the World Health Organization (WHO), *Aedes aegypti* L. is one of the most important mosquitoes. It serves as the vector of dengue virus causing dengue fever and yellow fever in human. Yellow fever and dengue fever excruciating joint and muscle pain (it is dubbed "breakbone fever") that can, in some forms, progress to a deadly hemorrhagic syndrome.

Ae. aegypti L. (Figure 2) is a medium-sized blackish mosquito easily recognized by a silvery-white "lyre-shaped" pattern of scales on its scutum. Segments 1 to 4 of the hind tarsi possess broad basal white rings, segment 5 is white. The coloration of both male and female is similar. All mosquitoes have four stages of development. There are egg, larva, pupa, and adult. Larval and pupal stages develop in water as shown in Figure 3. The female mosquito will find specific substratum where she lays her eggs and searches diligently for suitable water for her offspring.

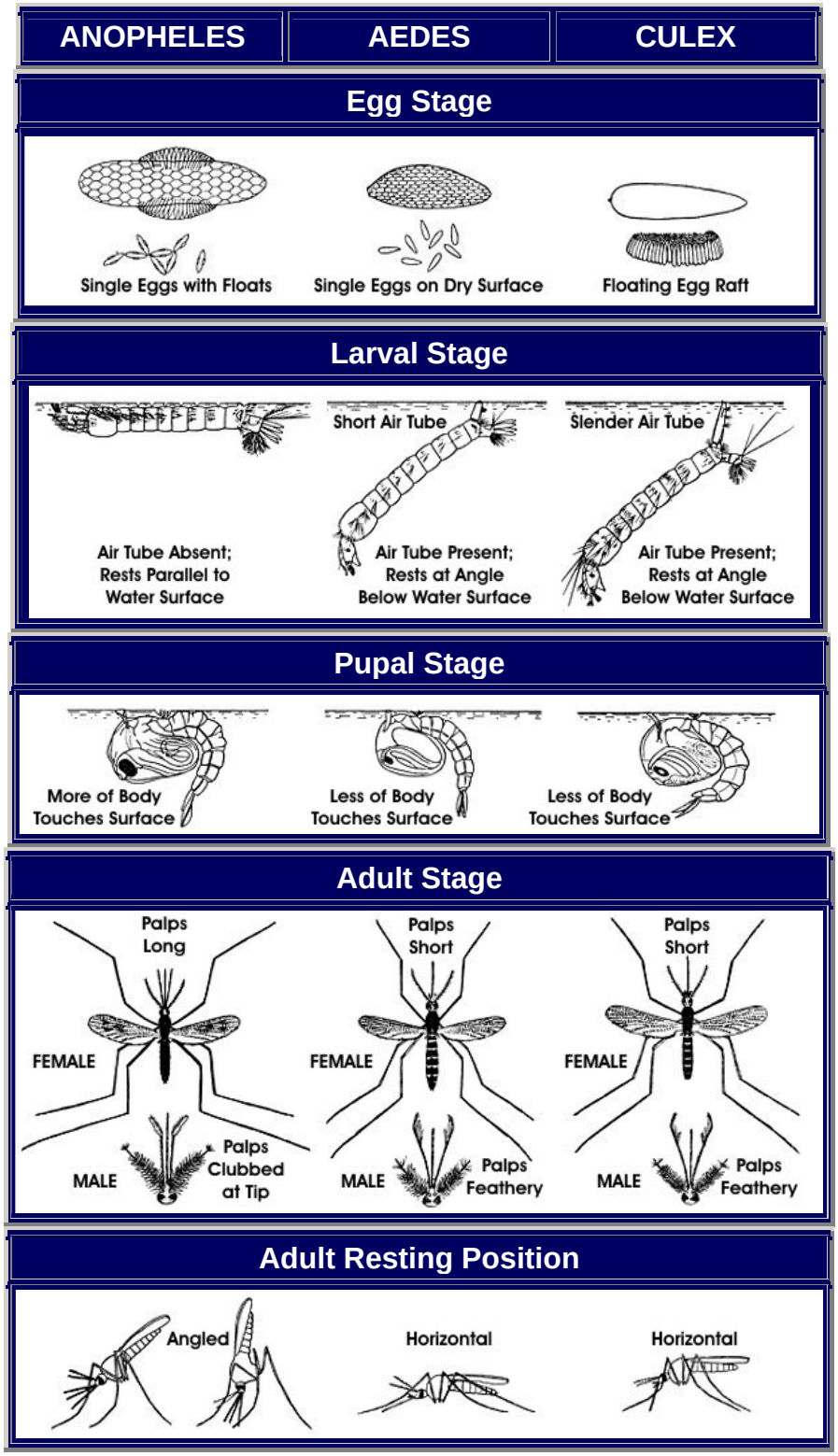

Figure 1 Principal characters for mosquito identification.

(http://entomology.unl.edu/urbanent/mosquito.htm)

Figure 2 Adult *Ae. aegypti* L.

(http://www.news.cornell.edu/stories/June05/denguefever.lm.html)

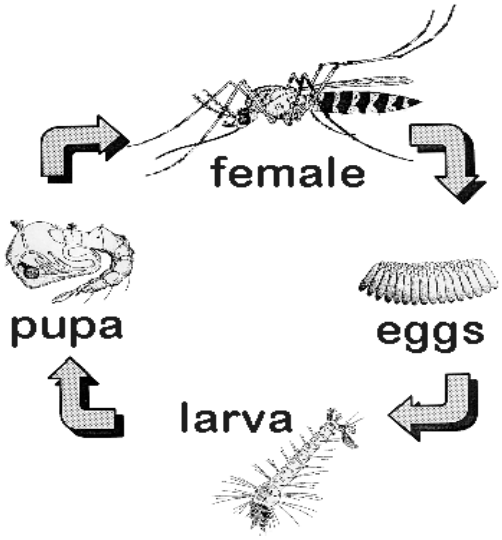

Figure 3 Life cycle of a mosquito.

(http://www.mosquitoes.org/LifeCycle.html)

Most of the mosquitoes that breed around the home require stagnant water rich decomposed organic material. This is not true for all mosquitoes that most pest species require clean, clear breeding habitats. Normally, female mosquitoes lay the 100-200 eggs to produce their next generations. Some species deposit their eggs directly on surface of water or in an area that will be flooded at a later date.

Immature stages of all types of mosquitoes are aquatic. They require water to complete their development. The eggs hatch in water and develop into larvae within 1-2 days. The larva is wingless, legless and wormlike in its appearance. Mosquito larvae are very active and move almost continuously as they shuttle to the surface to obtain oxygen and dive to the bottom to find food. Larval mosquitoes feed on organic matter in the water and grow rapidly. Within 7-8 days, they develop into pupa. The pupa has legs, wings and other characteristics as in the adult stage. When this stage is completed in one day, the fully formed adult emerges from the pupal case. The entire life cycle from egg to adult can be completed in less than 10 days when temperature is favorable.

1.2 Problem

Mosquito control is very necessary because mosquitoes are not only nuisance as biting insects, but are also involved periodically in transmitting disease to humans and animals. Dengue is the most important human viral disease transmitted by arthropod vectors. Annually, there are an estimated 50-100 million cases of dengue fever (DF), and 250,000 to 500,000 cases of dengue haemorrhagic fever (DHF) in the world (Rigau-Pérez et al., 1998). Over half of the world's population lives in areas at risk of infection, and these are popular tourist destinations too as shown in Figure 4. DF and DHF are caused by the four dengue viruses DEN 1, 2, 3 and 4, which are closely related antigenically. Infection with one serotype provides life-long immunity to that virus but not to the others. Dengue viruses are maintained in an urban transmission cycle in tropical and subtropical areas by the mosquito *Ae. aegypti*, a species closely associated with human habitation. In some regions other Aedes

species, such as *Ae. albopictus* and *Ae. polynesiensis* are also involved (Gubler, 1995 and 1997).

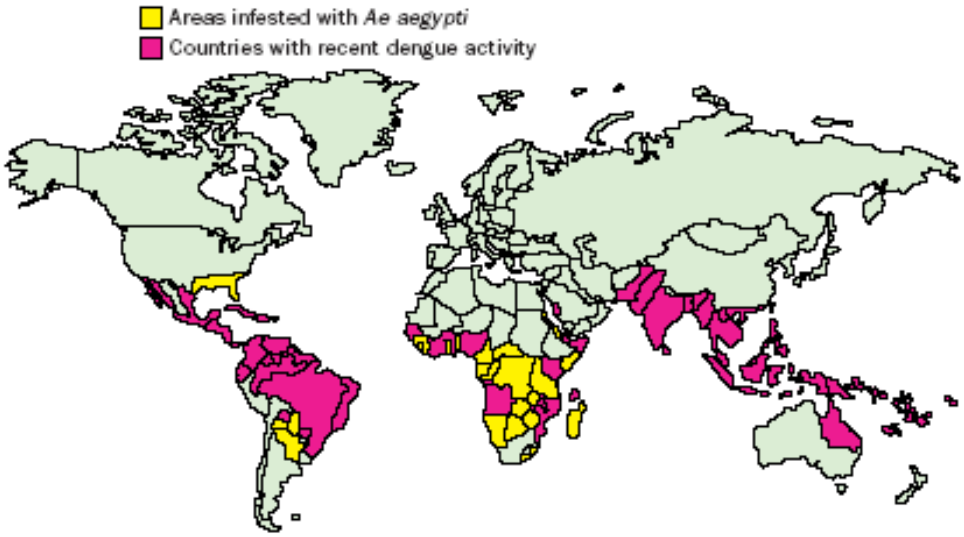

Figure 4 World distribution of dengue. (Rigau-Pérez et al., 1998)

Mosquito control which usually reduces mosquito populations can be achieved in various ways, including water management, biological control agents, and insecticides. The effectiveness of control is the control of larvae (larvicides) or mosquito adults (adulticides). However, mosquitoes are able to resist to chemically synthetic pesticides. There is growing concern about the potential health and environmental risks from these pesticides. Environmental protection agencies have banned or placed severe restrictions on the use of many pesticides which were formerly used in mosquito control programs (Rathburn, 1990).

Renewed interest has been shown in the development of alternative strategies, including the use of suitable type of natural insecticides derived from traditional botanical pest control agents. Hedge flower, *L. camara* L. is regarded both as a notorious weed and a popular ornamental garden plant. It has found various uses in folk medicine in many parts of the world. *L. camara* also produces a number of metabolites which some have been shown to posses useful biological activities (Ghisalberti, 2000). Mintweed or Chan, *H. suaveolens* has recently been shown to posses insecticidal properties (Singh and Upadhyay, 1993). Therefore, it is a challenging attempt to study these two plants for their phytotoxic acitivities as biological control agents.

In the present study, the biological control against *Ae. aegypti* by the two extracts from *H. suaveolens* and *L. camara* was investigated and evaluated for their potential of insecticide in the control of *Ae. aegypti*.

1.3 Research objectives

1) To study the effect of leave extracts of *H. suaveolens* and of *L. camara* on the hatching of eggs, survival of the second instars larva and adults of *Ae. aegypti* separately.

2) To study the effects of combination of *H. suaveolens* extract and *L. camara* on the hatching of eggs, survival of the second instars larva and adults of *Ae. aegypti*.

3) To investigate the optimal concentration of leaf-water extract of *H. suaveolens* extract and *L. camara* that can effectively criminate hatching of eggs, survival of the second instar larva and adults of *Ae. aegypti*.

1.4 Research hypothesis

The crude extracts of *H. suaveolens* and *L. camara* leaves in water, separately and combinationally, will be able to inhibit the hatching of eggs and to kill the second instar larva and the adults of *Ae. aegypti*.

1.5 Scope and limitations of the study

In this study, the observation will be limited to the followings:

1) The leaf of *H. suaveolens* and *L. camara* extracts.

2) The two plants will be collected on Suranaree University of Technology and its vicinity.

3) Plant extracts of *H. suaveolens* and *L. camara* leaves will be conducted in water as a solvent and in Soxhlet extraction apparatus.

4) The effects of the extracts on only 3 stages of mosquitoes, egg, the second instar larva and adult. Mosquito sample in the same stage has to be identical in size and age. In addition, it has to survive not more than 10% of mortal rate during treatment. All experiments will be carried on in the same place as well as the same environment.

5) *Ae. aegypti* is a kind gift from the Department of Control Disease by Insect 1, Saraburi, and Department of Disease Control**,** Ministry of Public Health, which is species of *Ae. aegypti* is identified.

6) All containers using in this experiment has to be the same size and shape.

1.6 Expected results

1) To obtain safely effective and potentially economic plant extracts; *H. suaveolens* and *L. camara*, in controlling of *Ae. aegypti* replacing the synthetic pesticides.

2) To develop and make value-added to weeds, pruning or trimming garden plants to farmers in application to use them as insecticidal agents.

3) To obtain useful data base of biological activities of plants extracts for further development for pest insect control.

4) To be plant-based pest control prototype system and be able to apply for further research for human health.

1.7 Definition of technical terms

1) Dead *Ae. aegypti* means un-hatching egg mosquito, abnormal swimming of larvae leading to lack of oxygen from water surface for breathing, non-movable larvae, or unable-to-fly adults.

2) Lethal concentration 50% end point (LC_{50}) refers to the concentration of plant extracts that can make the *Ae. aegypti* die 50 percent comparing to all samples within 24 or 48 hours.

3) Stop proceeding of experimentation is to stop the experiment when it was found that the mortal rate of larvae in container is less than 20%.

CHAPTER II

LITERATURE REVIEW

2.1 Biological control of *Aedes aegypti*

Plants may be an alternative source of mosquito repellent agents since they constitute a rich source of bioactive chemicals (Ahn, Chang and Kim, 2002). A number of research has been focused on plant extract or phytochemicals as potential sources of biological control agents and commercial mosquito repellent agents Venkatachalam and Jebanesen (2001) reported that the repellent activity of methanol extract of *Ferronia elephantum* leaves against *Ae. aegypti* was dose dependent.The repellent activity at 1.0 mg/cm^2 and 2.5 mg/cm^2 could protect up to 2 hours and could protect up to 4 hours, which was designated as 100% concentration of the extract. The total percentage protection of *F. elephantum* was 45.8% at 1.0 mg/ cm^2 and 59.0% at 2.5 mg/cm^2 for 10 houre. The larvicidal activity of commercial bark saponin extract (Sigma) from *Quillaja saponaria* was studied on 3rd-4th instar larvae of *Ae. aegypti* and *Culex pipiens* (Abramovich, Markus, Pelah and Wiesman, 2002). The larvae were exposed to a serial concentration (1000, 800, 500, 300, 100, 10, 1, 0.1 and 0.01 mg/l) of the extract for 1, 3, 5, 7 and 11 days. The results indicated that the commercial bark saponin is toxic, causing 100% larval mortality in *Ae. aegypti* and *C. pipiens* after 1 and 5 days at a dosage of 800 and 1000 mg/l, respectively. However, saponins had no effect on egg hatchability in either species. Ansari, Razda, Tandon and Vasudevan (2000a) investigated the larvicidal, growth inhibitor and repellent actions

of *Dalbergia sissoo* oil against *Anopheles stephensi, Ae. aegypti* and *Culex quinquefasciatus*. The oil was applied at 0.4-5 ml/m^2 on a water surface gave larval mortality to *Cx. quinquefasciatus* 100% *Ae. aegypti* 90% and *An. stephensi* 60% within 24 hours at 4 ml/m^2 the pupation of the species were totally inhibited. Adults that emerged from exposure to a sublethal dosage (2 ml/m^2) neither laid eggs (*Ae. aegypti*) nor hatch (*Cx. quinquefasciatus* and *An. stephensi*). The oil also showed strong repellent action 8-11 hours when 1 ml oil was applied on exposed parts of human volunteers. In addition, larvicidal activity of *Mentha piperita* L. oil (Peppermint oil) against these three species was studied . Ansari, Razda, Tandon and Vasudevan (2000b) *Cx. quinquefasciatus* was most susceptible, followed by *Ae. aegypti* and *An. stephensi*. Application of oil at 3 ml/m^2 on water surface area resulted in 100% mortality within 24 hours for *Cx. quinquefasciatus*, 90% for *Ae. aegypti* and 85% for *An. stephensi*. One hundred percent mortality of *Ae. aegypti* was achieved at 3 ml/m^2 in 48 hours or 4 ml/m^2 in 24 hours and of *An. stephensi* 100% mortality was observed at 4 ml/m^2 in 72 hours. The emergence of adults was also inhibited to a great extent when pupa were treated with 3 ml/m^2 of the oil. A few adults which emerged did not ovipost even after a blood meal. According to the study of Abdul, Arumugam, Geetha, Himalayan, and Saleem (2000), the acetone extract of *Feronia limonia* leaves and its chromatographic fractions were studied for larvicidal activity against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The active compound, identified as n-hexadecanoic acid, was effective with LC_{50} of 129.24, 79.58 and 57.23 ppm on *Cx. quinquefasciatus, An. stephensi,* and *Ae. aegypti* respectively.

The biological control of *Ae. aegypti* by *Argemone mexicana* L. seed was investigated (Sakthivadivel and Thilagavathy, 2003). The acetone fraction of the petroleum ether extract of *A. mexicana* seeds exhibited larvicidal and growth inhibiting activity against the 2nd instar larvae of *Ae. aegypti*. This activity occurred at higher concentrations (200, 100, 50 and 25 ppm). Chemosterilant activity, including reduction in blood meal utilization (27.70%), reduction in fecundity (19.00%), formation of larval-pupal intermediates, formation of pupal-adult intermediates, adult mortality and sterility of first generation eggs (100%), occurred at a low concentration (10 ppm) the *A. mexicana* seeds extract. The bioactivity of 14 essential oils from five plants: *Cinnamomum osmophleum*, *Taiwania cryptomerioides*, *Cunninghamia lanceolata*, *Cryptomeria japonica* and *Calocedrus formosana*, were studied using the brine shrimp lethality test and the *Ae. aegypti* larvicidal assay (Chang, Chang, Chen, Cheng and Tsai, 2003). All essential oils screened had LC_{50} values less than 200 µg/ml and showed significant lethality against brine shrimp. Nine essential oils showed toxicity against the 4th instar *Ae. aegypti* larvae in 24 hours with LC_{50} less than 100 µg/ml. The leaf and bark essential oil of *C. japonica* was most active against *Ae. aegypti* larvae with a LC_{50} of 48.1 µg/ml . Similarly, insecticidal activity of 11 extracts from nine South American medicinal plants was studied on *Ae. aegypti* larvae (Ciccia, Coussio and Mongelli, 2000). Eight extracts, which were *Aristolochia triangularis* Chem., *Baccharis coridifolia*, *Eupatorium hecatanthum*, *Pterocaulon polysachium*, *Pterocaulon purpurascens*, *Xanthium spinosum* L., *Jatropha isabelii*, *Minthostachys setosa* and *Abuta grandifolia* showed toxicity against the *Ae. aegypti* larvae (LC_{50} < 500 µg/ml). Dichloromethane extract from *A. grandifolia* and *M. setosa* demonstrated high larvicidal activity. The dichloromethane extract from *A.*

grandifolia, with was most active LC_{50} of 2.6 µg/ml (LC_{100} = 8.1 µg/ml). It was 2-fold higher than β-asarone, a natural botanical insecticide used as a positive control (LC_{100} = 16 µg/ml). That is the dichloromethane extract of *M. setosa* is potent against *Ae. aegypti* larvae with LC_{50} of 9.2 µg/ml (LC_{100} = 25.2 µg/ml). Moreover, 51 extracts from 29 plant species currently used in traditional medicine in Trinidad and the neighbouring Caribbean island was tested for antibacterial activity six bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* (Chariandy, Khambay, Phelps, Pollard and Seaforth, 1999). The extracts from eight the plants tested showed significant activity against one or more micro-organisms and the most susceptible bacterium was *S. aureus*. In the bioassays for toxicity towards the *Ae. aegypti* the most effective plant extracts were from *Justicia pectoralis*, *Manihot utilissima* and *Stachytarpheta jamaicensis*.

Goh et al. (2003) screened more than 60 Malaysian plants against two species of insects and found that *Melicope subunifoliolata* (Stapf) T.G. Hartley (Rutaceae) showed strong feeding deterrent activity against *Sitophilus zeamais* Motsch (Curculionidae) and strong larvicidal activity against *Ae. aegypti* One anti-insect compound, meliternatin (3,5-dimethoxy-3′,4′,6,7-bismethylendioxyflavone) and six other minor polyoxygenated flavones were isolated from *M. subunifoliolata*. The stem bark of *Microcos paniculata* contained a new alkaloid, *N*-methyl-6β-(deca-1′,3′,5′-trienyk)-3β-methoxy-2β-methylpiperidine, which showed good insecticidal activity against 2nd instar larvae of *Ae. aegypti* (Bandara, Jacobsson, Kumar and Molleyres, 2000). Afshan et al. (2000) isolated two new triterpenoids, 6α-*O*-acetyl-7-deacetylnimocinaol [24,25,25,27-tetra-norapotirucalla-(apoeupha)-6α-acetoxy-7α-

hydroxy-1,14,20,22-tetraen-21,23-epoxy-3-one] and meliacinol [24,25,26,27-tetranorapotirucalla-(apoeupha)-1α-trimethylacryloxy-21,23-6α,28-diepoxy-16-oxo-17-oxa-14,20,22-trien-3α, 7α-diol]were isolated from the methanolic extract of the fresh leaves of *Azadirachta indica* (neem). The first compound and nimocinal showed toxicity on 4th instar larvae of *Ae. aegypti* with LC_{50} of 21 and 83 ppm, respectively. The second compound had no effect even upto 100 ppm.

A granular formation, Vectobac-G®, of *Bacillius thuringiensis* var. *israelensis* (B.t.i.), and teflubenzuron (CME 134) were found highly effective in reducing *Ae. aegypti* larval populations at the mg/l and µg/l level under laboratory conditions (Chui, Tsoi and Wong, 1995). The LC_{95} (24 hours, 25 ± 2°C, L:D -14:10 hours) for Vectobac-G®, and of *Bacillius thuringiensis* and teflubenzuron against *Ae. aegypti* was 1.64 mg/l and 4.06 µg/l respectively. Bioassay showed that teflubenzuron has a higher degree of residual activity than Vectobac-G®. The efficacy of integrating LC_{95} concentrations of Vectobac-G® and teflubenzuron in different ratios against *Ae. aegypti* was studied as well. The Vectobac-G®:teflubenzuron combination ratios 1:9 and 9:1 have achieved effective mosquito control over 95%. Semi-field tests indicated that the 1:9 combinations of Vectobac-G® and teflubenzuron was 1.35 and 1.18 times more effective than the higher dosage LC_{95} concentrations of the two corresponding insecticides. Similary, Amalraj et al. (2000) studied the efficacy of aqueous suspension (AS) and granular (G) formulation of *B. thuringiensis* var. *israelensis* (Vectobac) against the immatures of mosquito vectors in the laboratory and under the field conditions. Laboratory tested showed that the aqueous suspension was relatively more effective against *Cx. quinquefasciatus* than *Ae. aegypti* and *An.*

stephensi, LC_{50} was 0.046, 0.060 and 0.190 mg/l. respectively. In stream pools, the application of Vectobac AS at 1.2 l/ha, more than 80% reduction in immature density of Anopheles larvae (*An. fluviatilis* and *An. culicifacies*) was observable during 2-8 days. The application at 2.4 l/ha was abservable during 3.5-9.0 days. At the dosage of 7.0 kg/ha of the granular formulation, a reduction in immature density by more than 80% was observed for 2-9 days. In polluted habitats such as cesspits, U-drains and cement tanks, the effectiveness of Vectobac AS lasted for 1-4 days when applied at 1.2 l/ha and 2.4 l/ha. The Vectobac G was effective for 1-3 days at application rates of 7.0 kg/ha and 14.0 kg/ha against *Cx. quinquefasciatus*. There was no significant difference in the effectivness between the two formulations and the two application rates.

Mosquito larvicidal activity of *Piper longum* fruit-derived material against fourth-instar larvae of *Ae. aegypti* was examined by Lee et al. (2002). A crude methanol extract of *P. longum* fruits was found to be active against the larvae, and the hexane fraction of the methanol extract showed a strong larvicidal activity of 100% mortality. The biologically active component of *P. longum* fruits was characterized as pipernonaline by spectroscopic analyses. The LC_{50} value of pipernonaline was 0.25 mg/l. The toxicity of pipernonaline is comparable to that of pirimiphos-methyl as a mosquito larvicide. In the tests with available components derived from *P. longum*, no activity was observed with piperettine, piperine, or piperlongumine. Ahn, Chang, Kim and Kim (2002) examined the repellent activity of materials derived from the methanol extract of fruits from *Foeniculum vulgare* against female *Ae. aegypti* using skin and patch tests and compared with that of the commercial *N*,*N*-diethyl-*m*-toluamide (deet) and (*Z*)-9-octadecenoic acid. The biologically active constituents of

the *Foeniculum vulgare* fruits were characterized as (+)-fenchone and (*E*)-9-octadecenoic acid by spectroscopic analyses. Responses varied according to compound, dose, and exposure time. In the skin test with female mosquitoes, at a dose of 0.4 mg/cm^2, (+)-fenchone and (*Z*)-9-octadecenoic acid exhibited moderate repellent activity at 30 min after treatment, whereas deet protect more than 1 hour at 0.2 mg/cm^2. (*Z*)-9-octadecenoic acid was more potent repellent agent than (*E*)-9-octadecenoic acid. (+)-Fenchone and (*E*)-9-octadecenoic acid merit further study as potential mosquito repellent agents or as active compounds.

The essential oils from leaves and inflorescences of *Hyptis martiusii* Benth were analyzed by GC-MS (Araújo et al., 2003). Twenty-six compounds representing 93.2% of the oil of leaves were characterized; Δ-3-carene (22.5%), 1,8-cineole (24.27%), β-caryophyllene (6.15%), and bicyclogermacrene (6.32%) were found as the major components. In the essential oil of inflorescences 27 compounds representing 87.7% of the oil were identified. The major components were Δ-3-carene (13.5%), α-pinene (5.78%), β-caryophyllene (6.59%), viridiflorene (8.25%), and germacrene B (5.21%). The essential oil of leaves and 1,8-cineole showed pronounced insecticidal effect against *Ae. aegypti* larvae and *Bemisia argentifolii*, the vectors of dengue fever and white fly fruit plague, respectively.

2.2 *Lantana camara* L.

L. camara L. (Verbenaceae) is a hairy shrub, native to tropical America. It is cultivated as an ornamental or hedge plant. Different parts of the plant are used in the folklore and traditional systems of medicine for treatment of various problems such as itches, cuts, ulcers, swellings, bilious fever, catarrch, eczema, tetanus, malaria, tumors, and rheumatism. (Sastri, 2000). Phytochemical studies undertaken by different groups of workers on different parts of the plant have resulted in the isolation of various terpenoids, steroids, and flavonoids (Begum, Qamar, Siddiqui and Wahab, 2000).

Figure 5 *Lantana camara* L. (Verbenaceae)

(http://www.floridata.com/ref/l/lant_c.cfm)

Begum, Firdous, Raza, Siddiqui and Siddiqui (1995) isolated seven pentacyclic triterpeonoids, camarinic acid, camaric acid, oleanolic acid, pomolic acid, lantanolic acid, lantanilic acid and lantic acid from the aerial parts of *L. camara*. The two new constituents, camarinic acid and camaric acid have been characterized as 22β-acetoxy-3,25-epoxy-3α-hydroxy-12-ursen-28-oic acid and 3,25-epoxy-3α-hydroxy-22β(2-methyl-2Z-butenoyloxy)-12-oleanen-28-oic acid, respectively. Also, a new urane was isolated from leaves of *L. camara* and is structure elucidated as 3,24-dioxo-urs-12-en-28-oic acid by means of spectral analysis (Tripathi and Yadav, 2003). The research work of Barre et al. (1997) discovered a novel trierpene 22β-acetoxylantic acid and the known triterpenes, lantic acid, 22β-dimethylacryloyloxy lantanolic acid, a mixture of 22β-dimethylacryloyloxy lantanolic acid and 22β-angeloyloxylantanolic acid and lantanolic acid. It was found that 22β-Acetoxylantic acid showed antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhi*. This compound and 22β-dimethylacryloyloxy lantanolic acid also showed antimutaganic activity. Moreover, two novel trierpenoids were isolated from the roots of *L. camara*: 3β,19α dihydroxy ursan-28-oic acid and 21,22β-epoxy-3β-hydroxy oleane-12-en-28-oic acid in its methyl ester form. Its leaves yielded an essential oil which is rich in sesquiterpenes. Oleanolic acid, which is thought to be a hepatoprotective compound, was isolated from *L. camara* roots and converted into its 28 → 13β lactone by a facile photo-oxidation reaction (Misra and Laatsch, 2000). In the study of Ngassoum et al. (1999), the essential oils of leaves and flowers of L. camara (Verbenaceae) from Cameroon and Madagascar were analysed by GC-FID and GC-MS. The oils are characterized by a high percentage of sesquiterpenes. The

major components in the oils from Cameroon are arcurcumene (25%), β-caryophyllene (13%) and caryophyllene epoxide (7%), while the main components of the oil from Mardagascar are davanone (15%) and β-caryophyllene (12%). The monoterpenes percentages are lower in the two essential oils and are represented by sabinene (1-9%), α-pinene (2-4%), 1,8-cineole (1-3%) and linalool (1-3%). Likewise, Khan, Naqvi, Singh, Srivastava and Syamasundar (2001) analyzed the chemical composition of leaves and flowers essential oils of *L. camara* from India by GC and GC-MS, which resulted in the identification of 71 and 64 costituents, representing 99.0% and 97.0% of the oils, respectively. The major constituents in the leaf oil were germacrene-D (20.5%), γ-elemene (10.3%), β-caryophyllene (9.4%), β-elemene (7.3%), α-copaene (5.0%) and α-cadinene (3.3%), while the major constituenets in the flower oil were β-elemene (14.5%), germacrene-D (10.6%), α-copaene (10.7%), α-cadinene (7.2%), β-caryophyllene (7.0%) and γ-elemene (6.8%). A comparison with the chemical composition of *L. camara* oils of different origins showed that their oils were significantly different from each other with respect to the major constituents. Also, Jose and Thoppil (2000, Ref 33) analyzed the essential oil of *L. camara* by gas liquid chromatography. It was found that the major components detected were β-caryophyllene, a sesquiterpene hydrocarbon, and geranyl acetate, an oxygenated monoterpene. Other components include terpinyl acetate, bornyl acetate, 1,8-cineole (all oxygenated monoterpenes) and D-limonen (monoterpene).

There was a report of Jose and Thoppil (2000) that the essential oil of *L. camara* remarklably inhibited the growth of most tested bacteria and fungi; *Pseudomonas aeruginosa*, *Aspergillus niger*, *Fusarium solani*, and *Candida albicans*

most sensitive. Ali-Emmanuel, Akakpo, Moudachirou and Quetin-Leclercq (2003) investigated the use of ointments made with ethanolic extracts of leaves of *Senna alata, L. camara* and *Mitracarpus scaber*, as topical treatments on chronic crusty or acute lesions of dermatophilosis, on bovine dermatophilosis. These ointments provoked the falling off of the crusts after 3-4 days of treatment. Hair grows on the treated areas, which heal without scarring, within 3-4 weeks after the end of treatment. The healed animals became free of dermatophiliosis without recurrence for more than 3 years and were in good health. Bouda, Fontem, Gumedzoe and Tapondjou (2001) investigated the efficacy of essential oil extracts from *Ageratum conyzoides, Chromolaena odorata* and *L. camara*, common weed species in Cameroon, on the mortality of the maize grain weevil, *Sitophilus zeamais* (Coleoptera, Curculionidae). They found that significant insect mortality was obtained with all the essential oil used. The mortality of *S. zeamais* increased with the concentration of the essential oils of the three plants and the duration of exposure of the weevils on the treated substrates. The essential oil extract of *A. conyzoides* was the most effective insecticide (LD_{50} = 0.09% in 24 hours), followed by *L. camara* oil (LD_{50} = 0.16%) and *C. odorata* oil (LD_{50} = 6.78%).

A partially purified fraction obtained from *L. camara* leaves containing seven chemicals Dawra, Makkar and Sharma (1987). Lantadene A and lantadene B were the major compounds and nontoxic to guinea pigs. However, petroleum ether and methanol extract of aerial of *L. camara* were toxic to *Callosobruchus chinensis* Dixit Harshan and Saxena (1992) the extacts showed 10-43% mortality at 5% concentrations, fecundity loss at higher doses. The antiovipositional values were 30 mg/100 g for petroleum ether extract and 40 mg/100 g of seed for methanol extract. A

hydroalcoholic extract from *L. camara* leaves Basto et al. (2003) on fertility did not interfere with overall weight or internal organ weights of male rats, but interfered with sperm count, daily sperm production and sperm morphology in a dose-dependent-manner. Two new constituents, lantanoside and lantanone, and the known compounds linaroside and camaric acid were isolated from the aerial parts of *L. camara* (Begum, Qamar, Siddiqui and Wahab, 2000). Lantanoside, linaroside and camaric acid were tested for nematicidal activity against root-knot nematode *Meloidogyne incognita* and showed 90, 85 and 100% mortality, respectively, at 1.0% concentration. The results were comparable to those obtained with the conventional nematicide furadan (100% mortality at 1.0% concentration).

2.3 *Hyptis suaveolens* L.

The genus *Hyptis* (Labiatae) is very large (400 species), and many of its reported species are known for their medicinal use as indigenous drugs (Almtorp, Hazell and Torssel, 1991; Kuhnt, Rimpler and Heinrich, 1994; Raja Rao and Rao, 1990). Such as antifungal, antibacterial, and anticonvulsant (Akah and Nwambi, 1993; Asekun, Ekundayo and Adevini, 1999) agents, gastrointestinal ailments and malaria (Pereda-Mianda and Gascon-Figueroa, 1988). In addition, many species of *Hyptis* are used against pest and other pest insects, stored product are commonly used against mosquitoes (Palsson and Jaeson, 1999). The leaves of those species are also largely used as potent insect repellents by native populations of many parts of the world (Aycard, Kini, Kam, Gaydou and Faure, 1993; Pereda-Miranda Garcia and Delgado, 1990; Pereda-Miranda and Delgado, 1990).

Figure 6 *Hyptis suaveolens* (L.) Poit

(http://members.iinet.net.au/~weeds/western_weeds/lamiaceae.htm)

Four species of Lamiaceae, namely, *Mentha cordifolia* Opiz ex Fresen, *Ocimum basilicum* L., *O. basilicum* L. forma *citratum* Back and *H. suaveolens* (L.) Poit were examined individually for the anitibacterial study and the synergistic effect against drugs-susceptible and drugs-resistant clinical isolates of bacteria Chitsomboon, Eumkeb, Nakkiew and Punopas (2003). All of them showed antibacterial activities against clinical isolated test bacteria. *H. suaveolens* individually displayed the best antibacterial activity against Methicilin-resistant *Staphylococcus aureus*. *H. suaveolens* in combination with *O. basillicum* showed

synergistic effect against Ciprofloxacin-resistant *Pseudomonas aeruginosa*. Dehydroabietinol isolated from *H. suaveolens* was found to inhibit growth of chloroquine-sensitive as well as chloroquine-resistant strains of *P. falciparum* cultivated in erythrocytes in vitro (IC_{50} 26-27 μm) (Christensen et al., 2002). However, erythrocytes exposed to dehydroabietinol were transformed in a dose-dependent manner towards spherostomatocytic forms with concomitant formation of endovesicles, as disclosed by transmission electron microscopy. Thus, dehydroabietinol incorporates into the erythrocyte membrane effecting the survival of *Plasmodium* parasites. The observed antiplasmodial effect of dehydroabietinol is presumably an indirect effect on the host cell. Because of these findings, microscopic investigations should be generally used to support claims of antimalarial effects of apolar natural products.

Azevedo et al. (2001) investigated the essential oils of *H. suaveolens* colleted from 11 localilites in the Brazilian Crerdo region by GC-MS. It was found that Sabinene, limonene, bicyclogermacrene, β-phellandrene and 1,8-cineole were the principal constituents. Three groups of essential oils to be distinguished with respect to the content of p-mentha-2,4(8)-diene, limonene/β-phellandrene/γ-terpinene and germacrene D/bicyclogermacrene. Geographically variation in essential oil composition indicated that the sesquiterpenes are mainly produced in the plant samples grown at lower latitudes. According to the study of Aguirre et al. (2004), a novel trypsin inhibitor purified from chan seeds was purified and characterized. Its apparent molecular mass was 8700 Da with an isoelectric point of 3.4. Its N-terminal sequence showed a high content of acidic amino acids (seven out of 18 residues). Its inhibitory activity was potent toward all typsin-like proteases extracted from the gut

of the insect *Prostehanus truncatus* (Coleoptera: Bostrichidae), a very important pest of maize. This acitivity was highly specific, because among proteases from seven different insects, only those from *P. truncatus* and *Manduca sexta* (Lepidotera: Sphingidae) were inhibited. Adeniyi, Asekun and Ekundayo (1999) found that the essential oil of *H. suaveolens* displayed significant inhibitory activity against two gram-positive and four gram-negative bacteria and was also very active against the fungus *Candida albicans*.

One interesting research work about *H. suaveolens* has been done by Peerzada (1997). It was reported that twenty three compounds were found and abundant enough to be identified by GC-MS as shown in Table 1 and Figure 5.

Table 1 The chemical composition of the essential oils from *H. suaveolens* Peerzada (1997).

Component	Percentage (%)
α-Thujene	0.3
α-Pinene	2.5
Camphene	0.02
Sabinene	3.9
β-Pinene	4.2
Myrcene	0.6
α-Phellandrene	2.0
1,8-Cineole	32
γ-Terpinene	0.7
α-Terpinolene	0.3
Linalool	0.06
Fenchol	0.3
4-Terpinenol	2.3
α-Terpineol	0.2
Eugenol	1.2
α-Copaene	1.8
β-Elemene	1.0
β-Caryophyllene	29
α-Bergamotene	1.6
α-Bergamotene	2.0
Aromadendrene	0.5
γ-cadinene	0.1
δ-cadinene	0.5

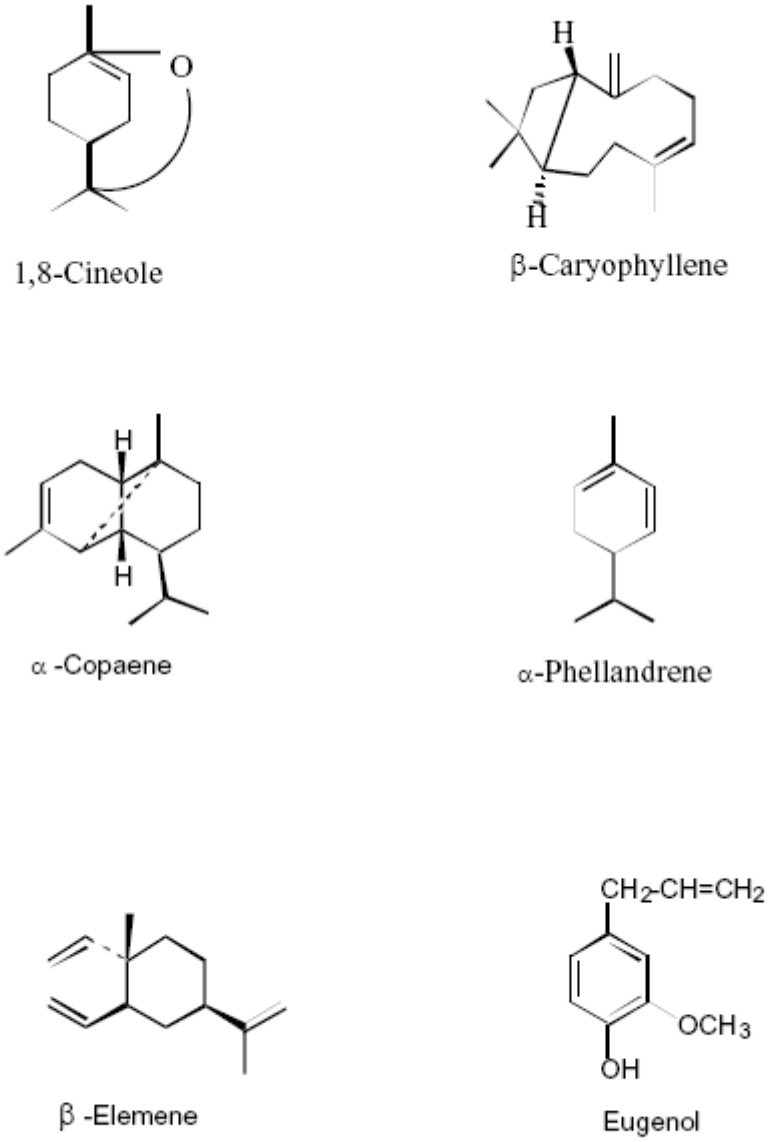

Figure 7 Major components, 1-8-cineole and β-caryophyllene and others only found in *H. suaveolens* Peerzada (1997).

.

CHAPTER III

MATERIALS AND METHODS

3.1 Plants

Leaves of *Hyptis suaveolens* and *Lantana camara* were collected on Suranaree University of Technology (SUT) campus and its vicinity. The leaves were cleaned, chopped, and dried in an oven at 45°C for 2 days. The dried samples were ground into fine powder. One hundred grams of sample were extracted in 500 ml distillation water as a solvent using Soxhelet extractor apparatus as shown in Figure 6. Extraction was carried out for 24 hours. The extract was filtered through Whatman filter paper No.1 and then was evaporated to the volume of 250 ml, approximately. This extract was defined as a 100% crude extract and kept at 4°C during study.

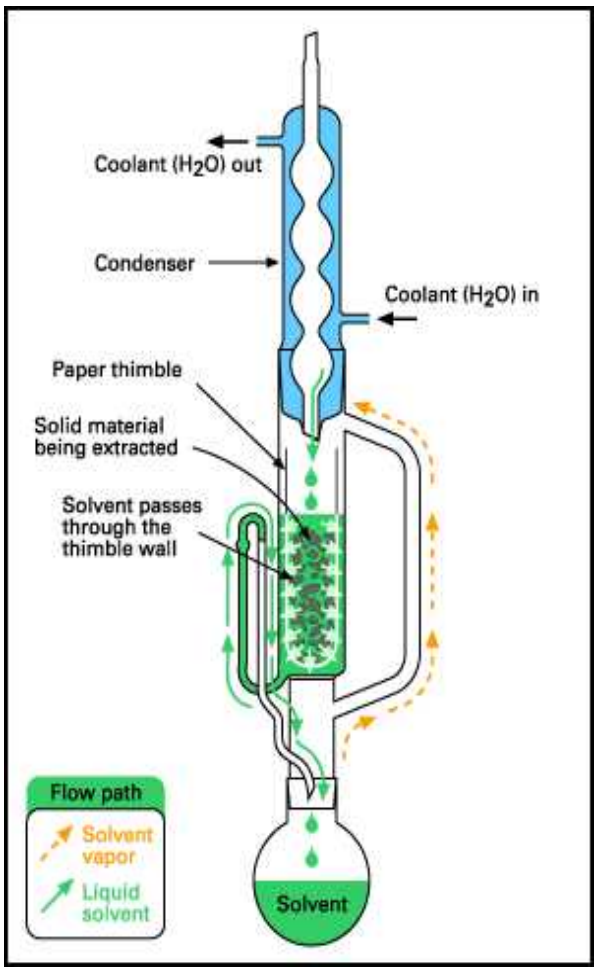

Figure 8 Set-up of Soxhlet extractor apparatus.

(http://www.anl.gov/OPA/logos16-2/extractor2.htm)

3.2 *Aedes aegypti* rearing

Adult of *Aedes aegtpti* were cultured in the steel-wire net-cages (Figure 9), dimension of 0.5 m × 0.5 m × 0.5 m. Male mosquitoes, *Ae. aegtpti*, were fed with vitamin syrup solution (APPENDIX A). Female mosquitoes were fed with guinea pig's blood, providing them protein for hatching by the guinea pig is back hairs were cut and shaved leaving bare skin and then put in the mosquito s 'cages.

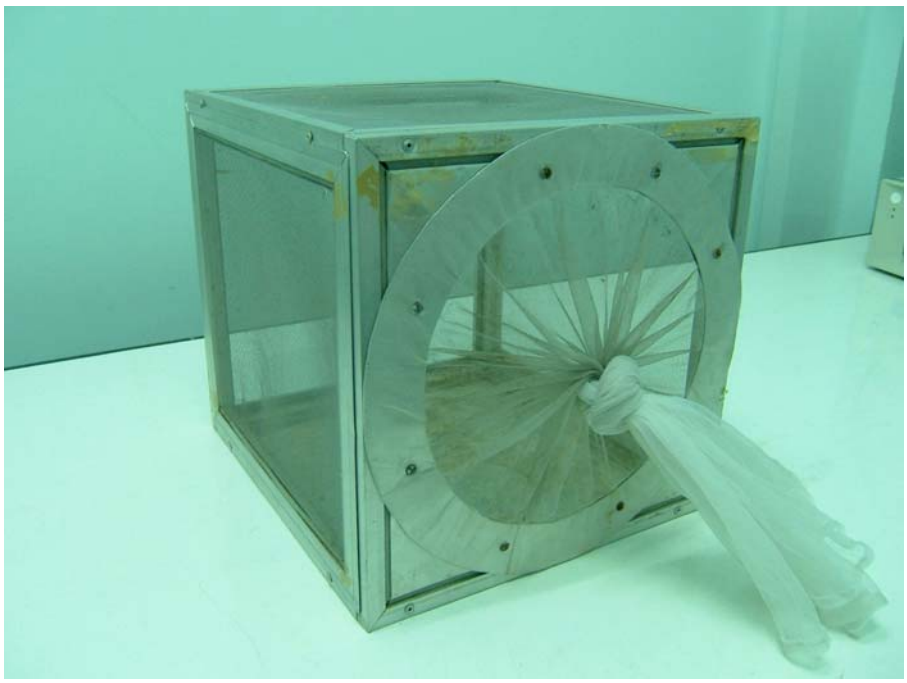

Figure 9 The steel-wire net-cages for *Aedes aegtpti* rearing.

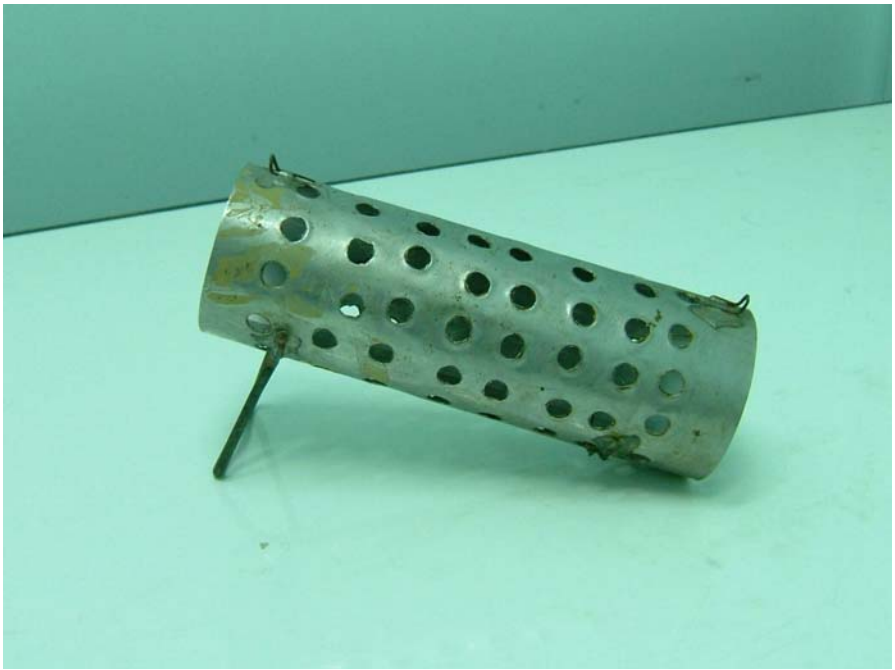

Figure 10 The cages of guinea pig.

3.3 *Aedes aegypti* culture

Male and female mosquitoes in the same cage, were allowed to mate. Female mosquitoes layed eggs on an earthenware bowl (Figure 11), 10 inches in diameter. On which a filter paper stripe was placed inside. The bowl was filled with water to nearly full. The filter paper will be served as a substrate for mosquito eggs. The filter paper stripe of eggs was brought to dry at room temperature and than kept at 20°C during study or stored in cool and dry humidity for long term up to one year.

Figure 11 The earthenware bowl for female mosquitoes layed eggs.

A number of eggs were collected and allowed to hatch at the same time in a filled water container in order to obtain larvae of the same age within 2-4 days. Typically, the yield of hatching was approximately 80%. There are four stages of larvae development. The larvae were fed with ground-pig food. Some of the second instar larvae were used to test with the extracts. The remaining larvae were become pupae within 10 days. The pupae were transferred to the net cage. They took 2-3 days to develop into adults. The adult mosquitoes were tested with the extracts. The mosquito culture was maintained by allowing males and females mated and produced eggs were kept at 4°C.

3.4 Experimental design

In the present study, the experiments were set up as completely randomized design (CRD). They consisted of three main parts and each part comprised of three experiments. Hence the total experiment units were nine as shown below and as in the scheme Figure 7(a-c): Each experiment was performed in triplicate of fifty samples.

Part I: Effects of extracts on mosquito eggs

Experiment 1: Effects of *H. suaveolens* extracts at different conditions on the hatching of mosquito eggs.

Experiment 2: Effects of *L. camara* extracts at different conditions on the hatching of mosquito eggs.

Experiment 3: Effects of extract combination (*H. suaveolens* extracts + *L. camara* extracts) at different conditions on the hatching of mosquito eggs.

Part II: Effects of extracts on mosquito larvae

Experiment 4: Effects of *H. suaveolens* extracts on mosquito second instar larvae.

Experiment 5: Effects of *L. camara* extracts on mosquito second instar larvae.

Experiment 6: Effects of extract combination on mosquito second instar larvae.

Part III: Effects of extracts on adult mosquito

Experiment 7: Effects of *H. suaveolens* extracts on adult mosquito.

Experiment 8: Effects of *L. camara* extracts on adult mosquito.

Experiment 9: Effects of extract combination on adult mosquito.

Part I: Effects of extracts on mosquito eggs

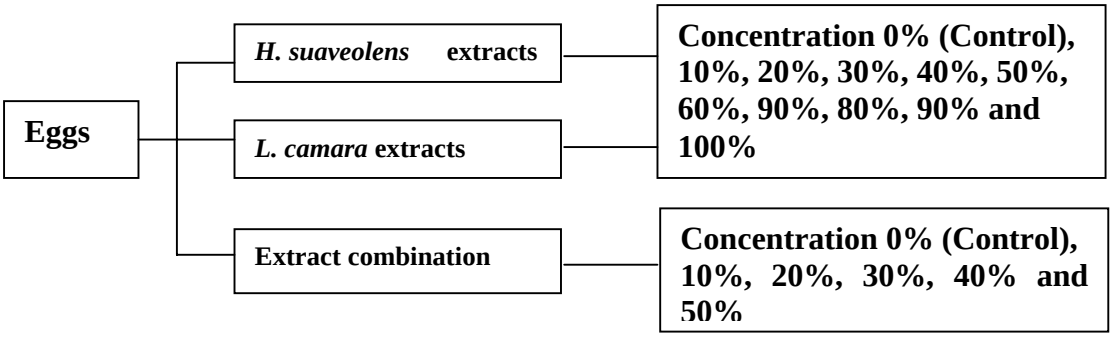

Figure 12(a)

Part II: Effects of extracts on mosquito larvae

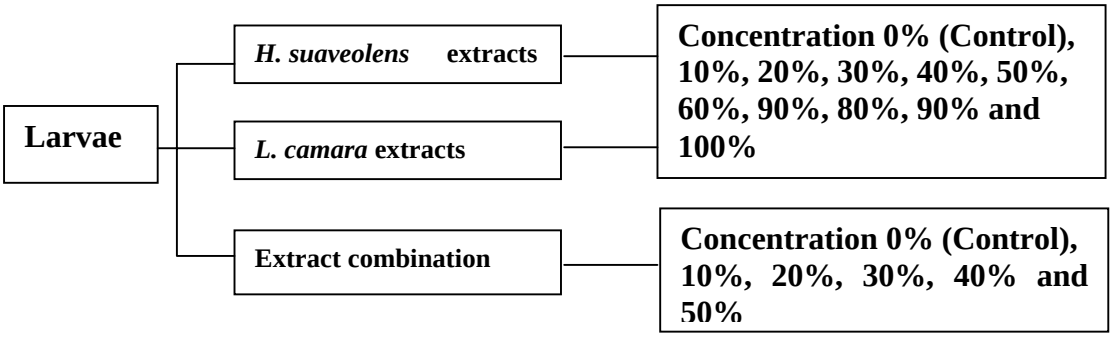

Figure 12(b)

Part III: Effects of extracts on adult mosquito

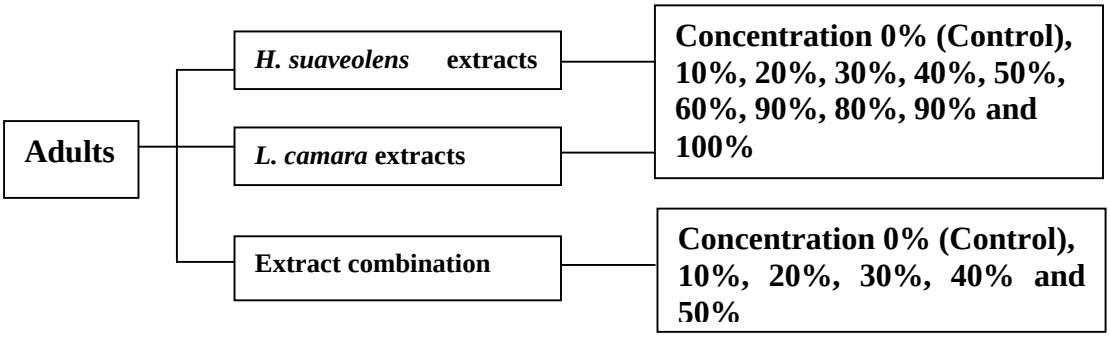

Figure 12(c)

Figure 12 Experimental design (a-c).

3.5 Materials

3.5.1 *Aedes aegypti* rearing

1. Guinea pigs
2. Earth-ware bowl (diameter 10 in)
3. Steel wire net cage (Mosquito cage) dimension of 0.5 m × 0.5 m × 0.5 m and 12 in × 12 in × 12 in
4. Guinea pig cage
5. Pig food
6. Vitamin syrup (composition in appendices)
7. Cotton cloth

3.5.2 Equipments

1. Soxhlet extractor apparatus	Buchi
2. Membrane filter, 47 mm. in Diameter	Whatman
3. Dry oven	
4. Blender	
5. Rotary evaporator	Buchi
6. Vacuum pump	Buchi
7. Beakers (50, 100, 250, 500 and 1,000 ml)	Pyrex
8. Conical flask (100, 250 and 500 ml)	Pyrex
9. Pipettes (1, 5 and 10 ml)	Brand
10. Measuring cylinders (10, 50, 100, 250 and 500 ml)	Brand
11. Petri dishes	Pyrex
12. Test tube (6 in × ¾ in)	Pyrex
13. Dropper	

14. Glass funnel Pyrex

15. Sprayer bottle

3.6 Methodology

3.6.1 Effects of extracts on the hatching of mosquito eggs

In this section, it was divided into three experiments according to *H. suaveolens* extracts, *L. camara* extracts and extract combination, as followings:

Experiment 1: The efficiency of *H. suaveolens* extracts on the inhibition of egg-hatching were investigated. In this experiment, it can be classified into eleven treatments according to the concentration of crude extracts, ranging from 0% (control sample using distilled water) to 100%. A strip of filter paper (5 cm × 5 cm) containg mosquito eggs was soaked in 10 ml of *H. suaveolens* extracts in a petri dish for 24 hours. The eggs strip was then transferred to fresh-clean water. Hatching ability of mosquito eggs was observed within 4 days. Non-hatching eggs were counted and calculated for LC_{50}.

Experiment 2: The efficiency of *L. camara* extracts on the inhibition of egg-hatching were investigated. In this experiment, it can be classified into eleven treatments according to the concentration of crude extracts, ranging from 0% (control sample using distilled water) to 100%. A strip of filter paper (5 cm × 5 cm) containg mosquito eggs was soaked in 10 ml of *L. camara* extracts in a petri dish for 24 hours. The eggs strip was then transferred to fresh-clean water. Hatching ability of mosquito eggs was observed within 4 days. Non-hatching eggs were counted and calculated for LC_{50}.

Experiment 3: The efficiency of extract combination on the inhibition of egg-hatching were investigated. In this experiment, it can be classified into eleven treatments according to the concentration of crude extracts, ranging from 0% (control sample using distilled water) to 50%. A strip of filter paper (5 cm × 5 cm) containg mosquito eggs was soaked in 10 ml of extract combination in a petri dish for 24 hours. The eggs strip was then transferred to fresh-clean water. Hatching ability of mosquito eggs was observed within 4 days. Non-hatching eggs were counted and calculated for LC_{50}.

3.6.2 Effects of extracts on mosquito second instar larvae

In this section, it was divided into three experiments according to *H. suaveolens* extracts, *L. camara* extracts and extract combination, as followings:

Experiment 4: The effect of *H. suaveolens* extracts on eliminating of second instar larvae were investigated. In this experiment, it can be classified into eleven treatments according to the concentration of crude extracts, ranging from 0% (control sample using distilled water) to 100%. Twenty ml of each concentration will be put into 50 ml beakers and then fifty second instar larvae will be dipped into these beakers. Observe the result second instar larvae at 48 hours by counting the number of dead second instar larvae. Calculate the percentage of mortality of second instar larvae from three replications of each treatment and determine lethal concentration (LC_{50}).

Experiment 5: The effect of *L. camara* extracts on eliminating of second instar larvae were investigated. In this experiment, it can be classified into eleven treatments according to the concentration of crude extracts, ranging from 0% (control

sample using distilled water) to 100%. Twenty ml of each concentration will be put into 50 ml beakers and then fifty second instar larvae will be dipped into these beakers. Observe the result second instar larvae at 48 hours by counting the number of dead second instar larvae. Calculate the percentage of mortality of second instar larvae from three replications of each treatment and determine lethal concentration (LC_{50}).

Experiment 6: The effect of extract combination on eliminating of second instar larvae were investigated. In this experiment, it can be classified into eleven treatments according to the concentration of crude extracts, ranging from 0% (control sample using distilled water) to 50%. Twenty ml of each concentration will be put into 50 ml beakers and then fifty second instar larvae will be dipped into these beakers. Observe the result second instar larvae at 48 hours by counting the number of dead second instar larvae. Calculate the percentage of mortality of second instar larvae from three replications of each treatment and determine lethal concentration (LC_{50}).

3.6.3 Effects of extracts on adult mosquitoes

In this section, it was divided into three experiments according to *H. suaveolens* extracts, *L. camara* extracts and extract combination, as followings:

Experiment 7: The effect of *H. suaveolens* extracts on eliminating of adult mosquitoes were investigated. In this experiment, it can be classified into eleven treatments according to the concentration of crude extracts, ranging from 0% (control sample using distilled water) to 100%. Five ml of each concentration will be put into sprayer bottles and then fifty adult mosquitoes, which were in a steel wire net cage

(dimension of 12 in × 12 in × 12 in), will be sprayed throughout the cage. Leave this cage for 12 hours and then the same dose of crude extracts will be repeatedly sprayed through the cage again. Observe the result adult mosquitoes at 48 hours by counting the number of dead mosquitoes. Calculate the percentage of mortality of adult mosquitoes from three replications of each treatment and determine lethal concentration (LC_{50}).

Experiment 8: The effect of *L. camara* extracts on eliminating of adult mosquitoes were investigated. In this experiment, it can be classified into eleven treatments according to the concentration of crude extracts, ranging from 0% (control sample using distilled water) to 100%. Five ml of each concentration will be put into sprayer bottles and then fifty adult mosquitoes, which were in a steel wire net cage (dimension of 12 in × 12 in × 12 in), will be sprayed throughout the cage. Leave this cage for 12 hours and then the same dose of crude extracts will be repeatedly sprayed through the cage again. Observe the result adult mosquitoes at 48 hours by counting the number of dead mosquitoes. Calculate the percentage of mortality of adult mosquitoes from three replications of each treatment and determine lethal concentration (LC_{50}).

Experiment 9: The effect of extract combination on eliminating of adult mosquitoes were investigated. In this experiment, it can be classified into six treatments according to the concentration of extract combination, ranging from 0% (control sample using distilled water) to 100%. Five ml of each concentration will be put into sprayer bottles and then fifty adult mosquitoes, which were in a steel wire net cage (dimension of 12 in × 12 in × 12 in), will be sprayed throughout the cage. Leave this cage for 12 hours and then the same dose of crude extracts will be repeatedly

sprayed through the cage again. Observe the result adult mosquitoes at 48 hours by counting the number of dead mosquitoes. Calculate the percentage of mortality of adult mosquitoes from three replications of each treatment and determine lethal concentration (LC_{50}).

3.7 Data analysis

Data from all experiments were analyzed by using Program SPSS for Window V.10. In the case of lethal concentration (LC_{50}), it was analyzed by using Probit analysis. In addition, the comparison of the relationships between-subjects effects the type of extracts and the concentration of extracts (Experiment 1-3, Experiment 4-6, and Experiment 7-9) were determined by using One-WAY-ANOVA of variance and 2-WAY-ANOVA of variance analysis.

3.8 Location of research

This study was conducted at the Center for Scientific and Technological Equipment, Building F2, Suranaree University of Technology, Nakhon Ratchasima.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effect of plant extracts on the hatching of *Aedes aegypti* eggs.

4.1.1 Effect of *Hyptis suaveolens* extracts on the hatching of *Aedes aegypti* eggs.

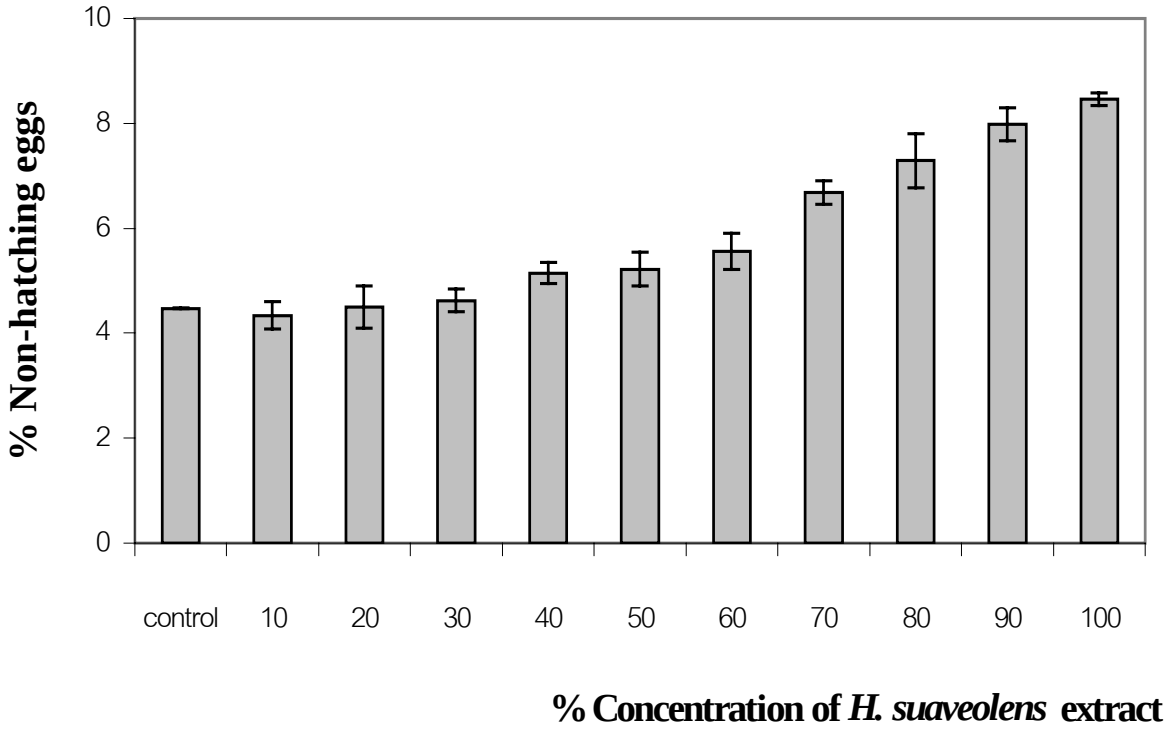

Figure 13 The effect of *H. suaveolens* extract at various concentrations on the hatching of *Ae. aegypti* eggs within 24 hours.

The effect of *H. suaveolens* extract on the hatching of mosquito eggs was conducted. The egg strips were soaked in the extract of various concentrations for 24 hours and then transferred onto clear water in petri-dishes. The non-hatched eggs were counted and expressed as to imply the inhibitory effect on hatching activity of the plant extracts. It was found that increasing concentration of the extract from 10-30% did not change the hatching of eggs significantly, that was 96% hatching or 4% non-hatching (Figure 13). While, the percentage of non-hatching of mosquito eggs slightly increased when the concentration of the extracts were increased from 40-100% ($P \leq 0.05$). LC_{50} of *H. suaveolens* on hatching of the eggs can not be depicted or may exceed 100% of the crude extract.

4.1.2 Effect of *Lantana camara* extracts on the hatching of *Aedes aegypti* eggs.

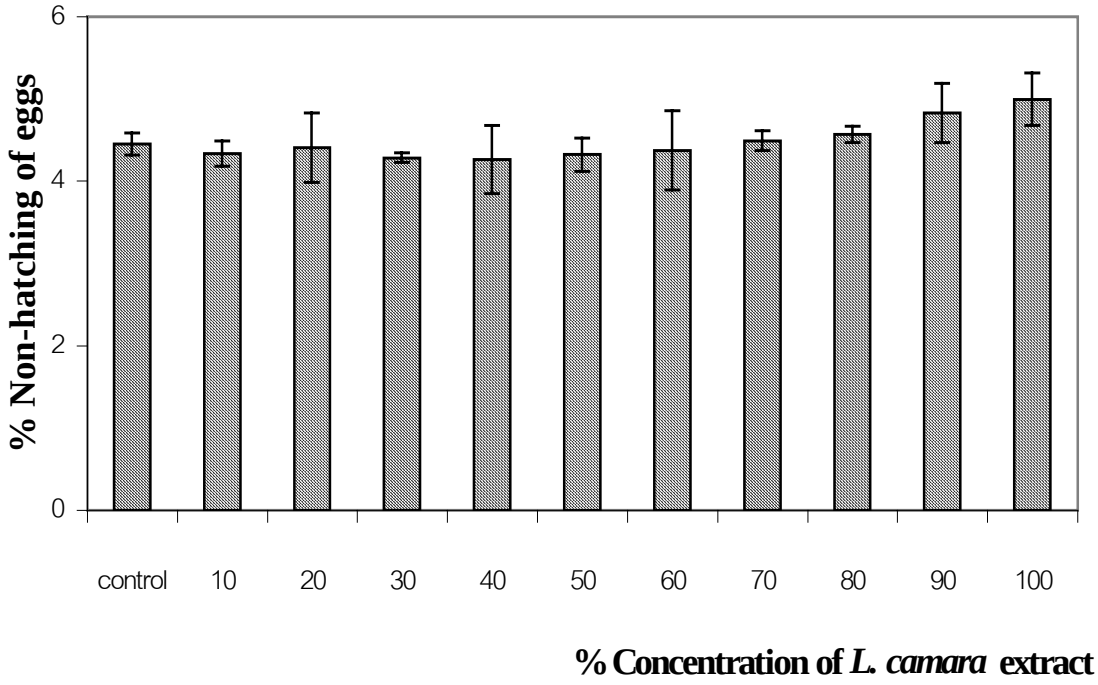

Figure 14 The effect of *L. camara* extract at various concentrations on hatching of *Ae. aegypti* eggs within 24 hours.

Figure 14 shows that the effect of *L. camara* at various concentrations on the hatching of mosquito eggs within 24 hours. *L. camara* of all concentrations, 0-100%, showed very slight inhibition on the egg hatching. Approximately, 5% eggs did not hatch, showed insignificantly different ($P > 0.05$) in all concentration ranges. In particular, although the 100% of *L. camara* extract was used, percent non-hatching egg was almost similar to the control.

4.1.3 Comparison between the effect of *Hyptis suaveolens* and *Lantana camara* extracts on the hatching of *Aedes aegypti* eggs.

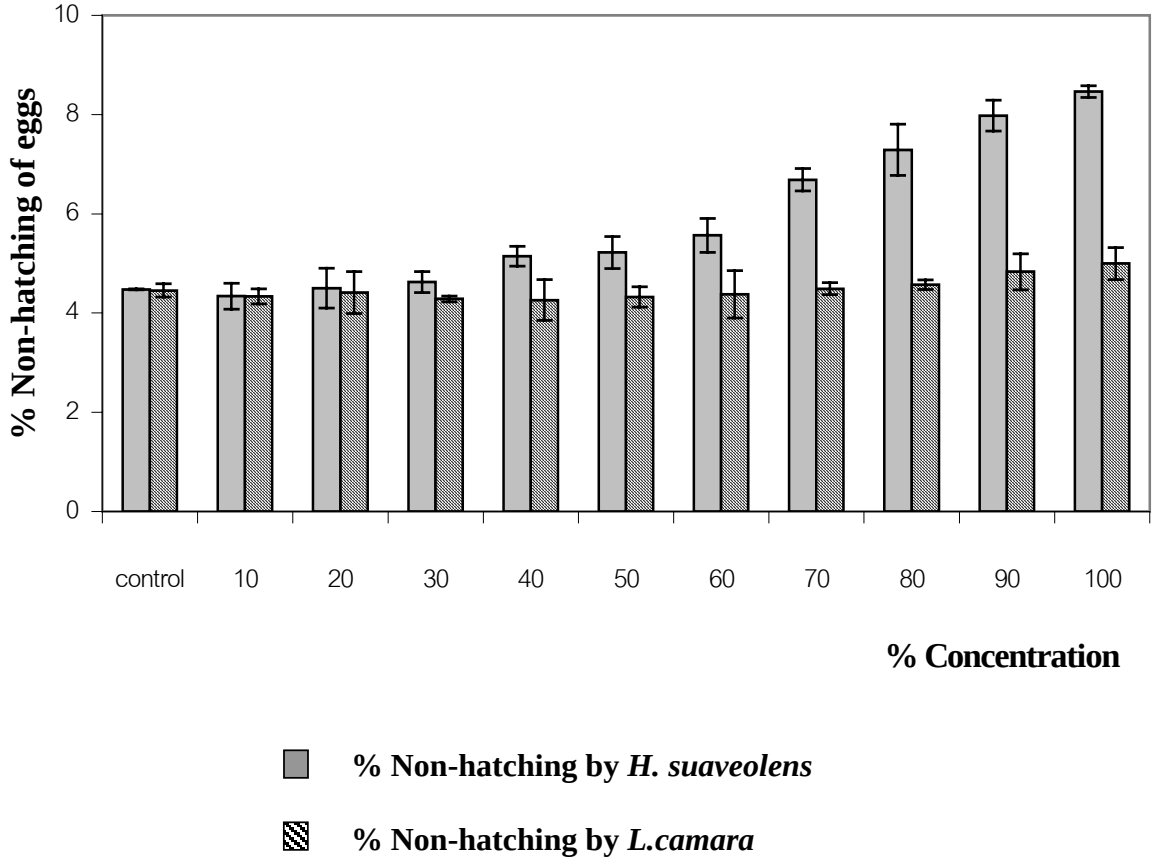

Figure 15 Comparison of the effect of *H. suaveolens* and *L. camara* extracts at various concentrations on hatching of *Ae. aegypti* within 24 hours. Shown as % non-hatching eggs.

The hatching effects between *H. suaveolens* and *L. camara* extracts on *Ae. aegypti* eggs were compared. At low levels of both extracts, 10-30%, there was no hatching inhibition of the mosquito eggs as compare to the control which was about 4-5% of non-hatched eggs obtained. The *L. camara* extract showed no hatching

inhibition at all concentrations of treatment. While 40-60% of *H. suaveolens* extracts showed slight inhibition, 5% of non-hatched eggs. The hatching inhibition was gradually increased when increasing the concentration levels of the *H. suaveolens* extract to 100%, non-hatched eggs was 8%, $P \leq 0.05$. As shown in Figure 15 the effect on hatching of eggs treated with 100% of the extracts, *H. suaveolens* extract was nearly two folds of eggs treated with *L. camara* extract. However, both plant extracts demonstrated a small inhibition effect on the hatching of mosquito eggs, that is no inhibition by *L. camara* and only about 2% inhibition by *H. suaveolens* as compared to control.

4.1.4 Effect of extract combination of *Hyptis suaveolens* : *Lantana Camara*, (1:1) on hatching of *Aedes aegypti* eggs.

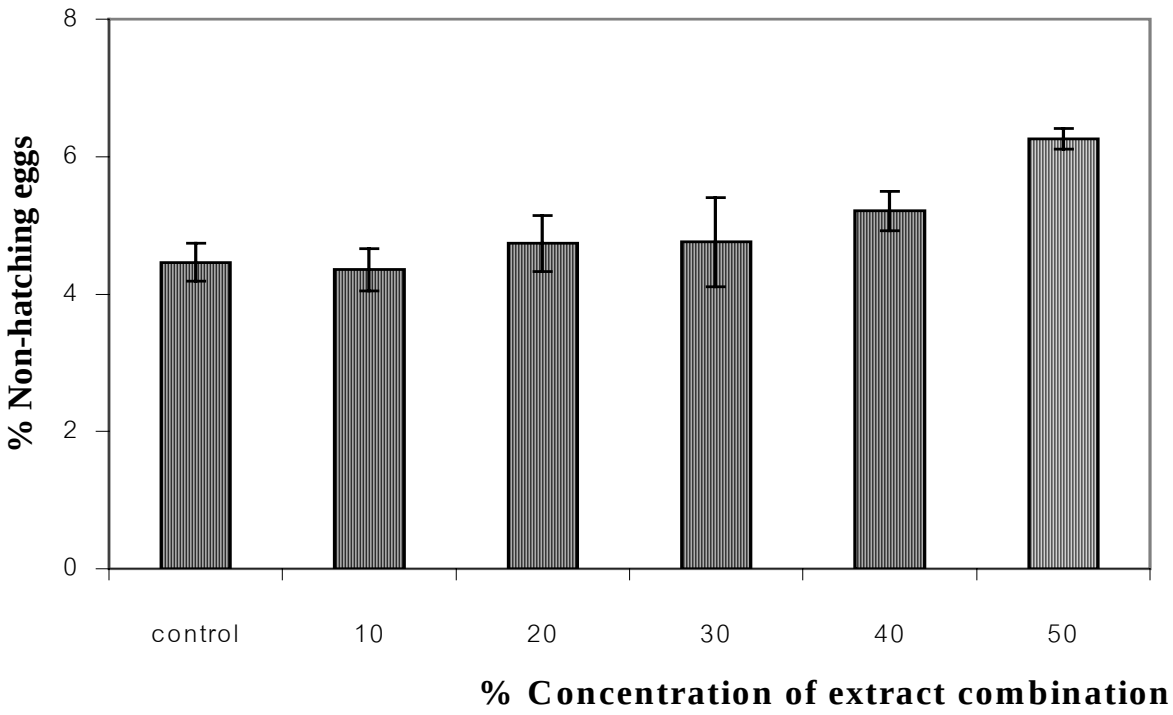

Figure 16 The effect of combination of *H. suaveolens* and *L. camara* extracts at 1:1 ratio on hatching of *Ae. aegypti* eggs within 24 hours. The combination was the mix between each individual extracts from concentration 10% to 50%.

The combination the two-plant extracts insignificantly inhibited the hatching of mosquito eggs as compared to the control ($P > 0.05$) and Figure 14. It is likely that the plant extracts do not affect the hatching activity of eggs, eventhough, the slight effect was observed, as shown in term of non-hatching of 6% (Figure 16), could lightly contribute from *H. suaveolens* extract in the combination. LC_{50} of *L. camara* extract on hatching inhibition of the eggs can not be obtained or exceed 100%.

4.1.5 Comparison among the effect of individual extracts of *Hyptis suaveolens* and *Lantana camara* and their combinations on *Aedes aegypti* eggs.

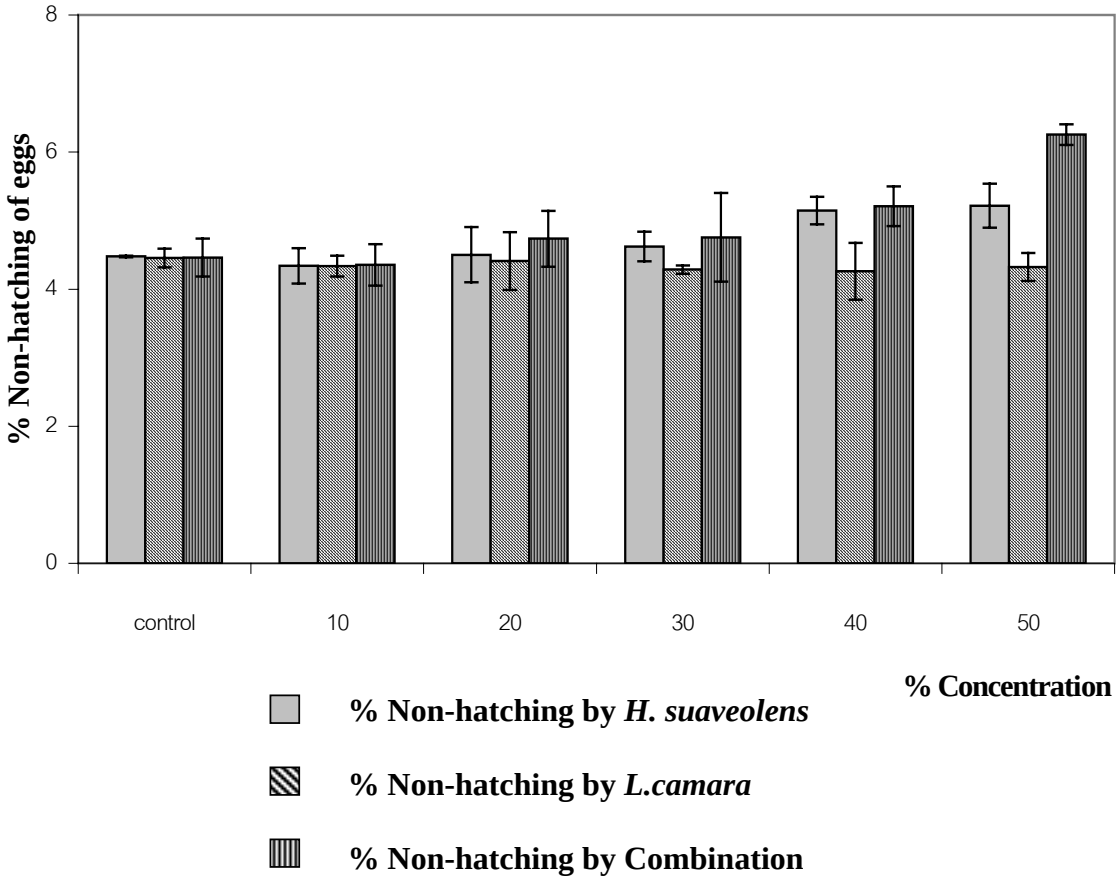

Figure 17 Hatching *Ae. aegypti* eggs, shown as % non-hatching, by *H. suaveolens*, *L. camara* and their combination (1:1) from 10-50%.

This comparison study revealed that all treatments of individual extracts and the combination of them, (10-50%), did not inhibit the hatching activity of *Ae. aegypti* eggs (Figure 17). Fifty percent extracts seemed to significantly inhibit the hatching of the mosquito eggs, as demonstrated in non-hatching of *H. suaveolens* = 6.25%, *L. camara* = 5.22%, and their combination = 4.32%, ($P \leq 0.05$).

4.1.6 Effect of the extract combination of *Hyptis suaveolens* and *Lantana camara* at various mixing ratios on *Aedes aegypti* eggs.

Table 2 The effect of extract combination of *H. suaveolens* and *L. camara* at various ratios on hatching of *Ae. aegypti* eggs within 12 hours.

Mixing Ratio of Extract Combination *H. : L.*	Non-hatching mosquito eggs									
	Rep 1			Rep 2			Rep 3			Average
	Total	Non Hatched	% Non Hatched	Total	Non Hatched	% Non Hatched	Total	Non Hatched	% Non Hatched	% ± SD
Control	145	6	4.138	167	8	4.790	159	7	4.403	4.444 ± 0.32 a
20% : 80%	150	7	4.67	164	8	4.878	174	8	4.598	4.714 ± 0.11 c
40% : 60%	165	9	5.455	157	9	5.732	156	9	5.769	5.652 ± 0.14 bcd
50% : 50%	148	9	6.081	173	11	6.358	185	13	7.027	6.489 ± 0.20 bcd
60% : 40%	157	10	6.369	187	14	7.487	142	11	7.746	7.201 ± 0.58 b
80% : 20%	192	15	7.813	165	13	7.879	183	13	7.104	7.598 ± 0.14 bd

Number with different letters (a,b) within the same column are significantly different ($P \leq 0.01$).

Number with different letters (c,d) within the same column are significantly different ($P \leq 0.05$).

The extract combination of *H. suaveolens* and *L. camara* in various and reverse ratios showed no inhibition of *Ae. aegypti* egg hatching, similar to direct combination (Table 2, Figure 16 and Figure 17). Low numbers of non-hatched eggs were observed in all combinations. At equal amount of the two extracts, non-hatched eggs were 6% as compared to 4% of control. As the amount of *H. saveolens* increased or the amount of *L. camara* decreased the non-hatched eggs was only 2-3% higher than the control (Table 2). The results indicated that all extracts in this study did not effect on the mosquito eggs.

This could probably explain that mosquito eggs which are soft and white at oviposition undergo sclerotization during embryogenesis. They become harder and darker. This property is associated with proteinaceous endochorin, and is derived from protein cross-linkage, a process dependent on the presence of N-acetyldopamine. This compound derived from tyrosine and one of the enzymes involved in its production (Boomsma et al., 1989). Moreover and Soumaré (2005) found that the mature eggs of *Ae. aegypti* are 500 mm in length and 150 mm in diameter. The ornamentations consisted of two or one cupola-shaped pillar fixed on a disk measuring 16 mm in diameter. The different disks were linked by a lacunar chorionic layer. Hence, when the mosquito eggs exposed to the plant extracts, the extracts were difficult to diffuse to eggshell of mosquito. Therefore, the plant extracts have very low effect on this stage of mosquito life cycle. Bradley and Colleagues (1996) found that egg clarion resisted to peroxidase treated for 48 hours. The mosquito egg clarion is consisted of double layer of exoclorion and endoclorion, which linked with lamellar layer (Adelaide et al., 1999; Soumare and Ndiaye, 2005). The egg clarion, therefore, protects the mosquito embryo from harmful chemicals.

4.2 Effect of plant extracts on mosquito second instar larvae of *Aedes aegypti.*

4.2.1 Effect of *Hyptis suaveolens* extracts on the second instar larvae of *Aedes aegypti.*

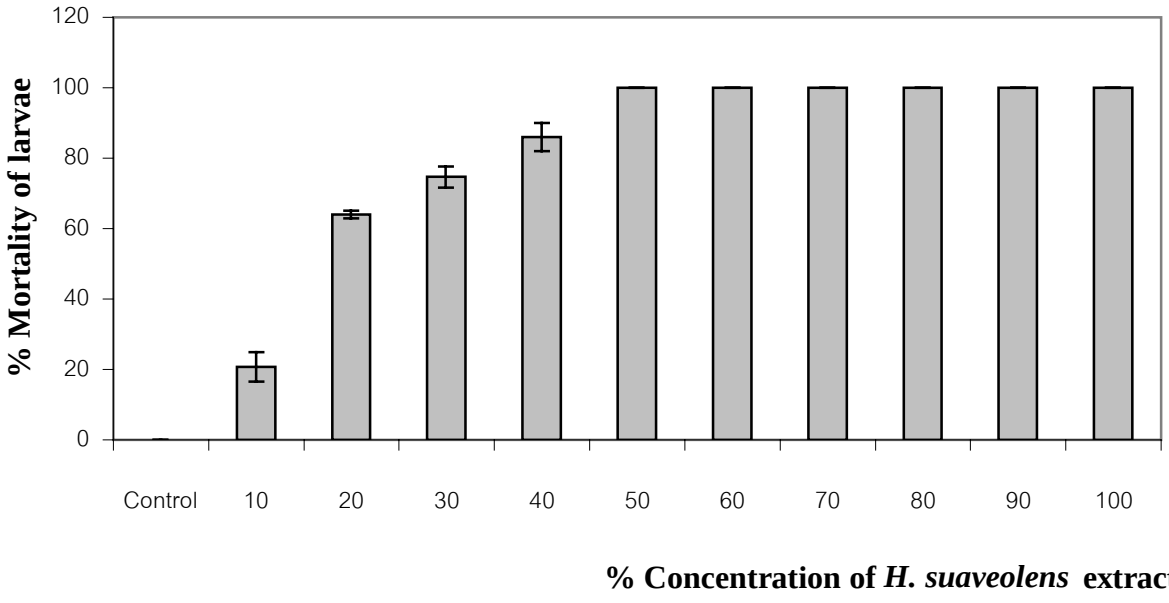

Figure 18 The effect of *H. suaveolens* concentrations on mortality of *Ae. aegypti* larvae within 48 hours.

Hyptis suaveolens extract showed insecticidal effect on the mosquito by killing the second instar larvae within 48 hours. It was found that 10% extract effectively killed 25% larvae. the mortality of the larvae was rapid increased with increasing the concentration of the extract. They were totally killed (100%) when treated with 50% extract, higher concentration and crude extract, $P \leq 0.01$, (Figure

18). The LC_{50} value of the *H. suaveolens* extract on the mosquito second instar larvae was 20.24%.

4.2.2 Effect of *Lantana camara* extracts on the second instar larvae of *Aedes aegypti.*

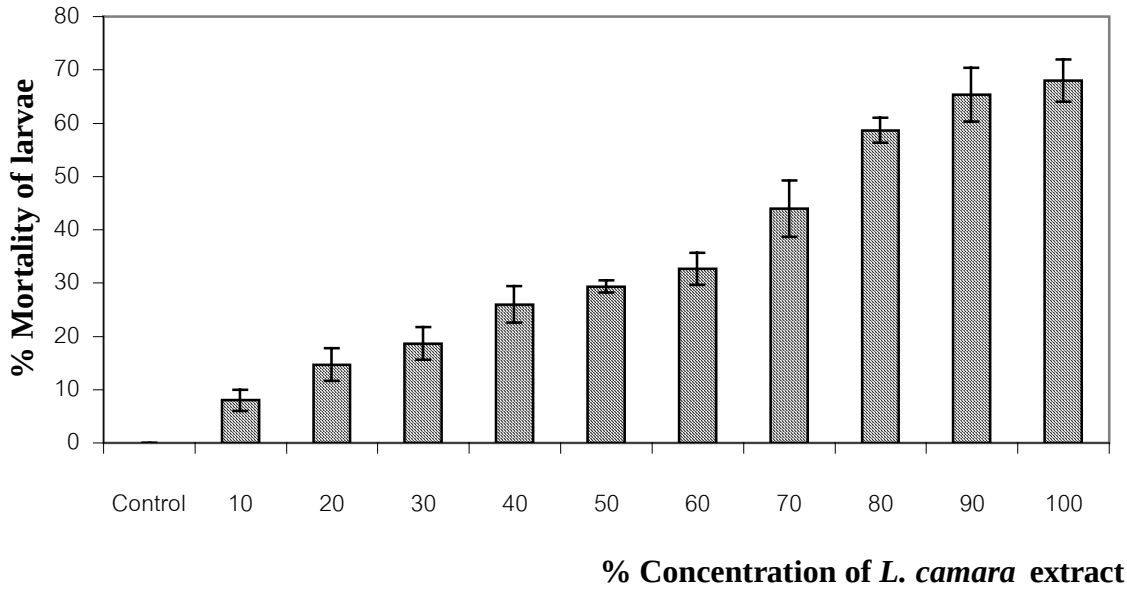

Figure 19 The effect of *L. camara* concentrations on mortality of *Ae. aegypti* larvae within 48 hours.

The effect of *Lantana camara* extracts on the mosquitoe second instar larvae gradually increased as a dose-dependent fashion. The mortality of the mosquito larvae at 48-hour treatment of the most diluted (10%) *L. camara* extract was only 10%, approximately. Fifty percent dilution of the extract killed 30% larvae and the crude extract killed the only 70% larvae, $P \leq 0.01$, (Figure 19). The LC_{50} value of *L. camara* extract on the mosquito second instar larvae was 74.44%.

4.2.3 The comparison between the effect of *Hyptis suaveolens* and *Lantana camara* extracts on the second instar larvae of *Aedes aegypti.*

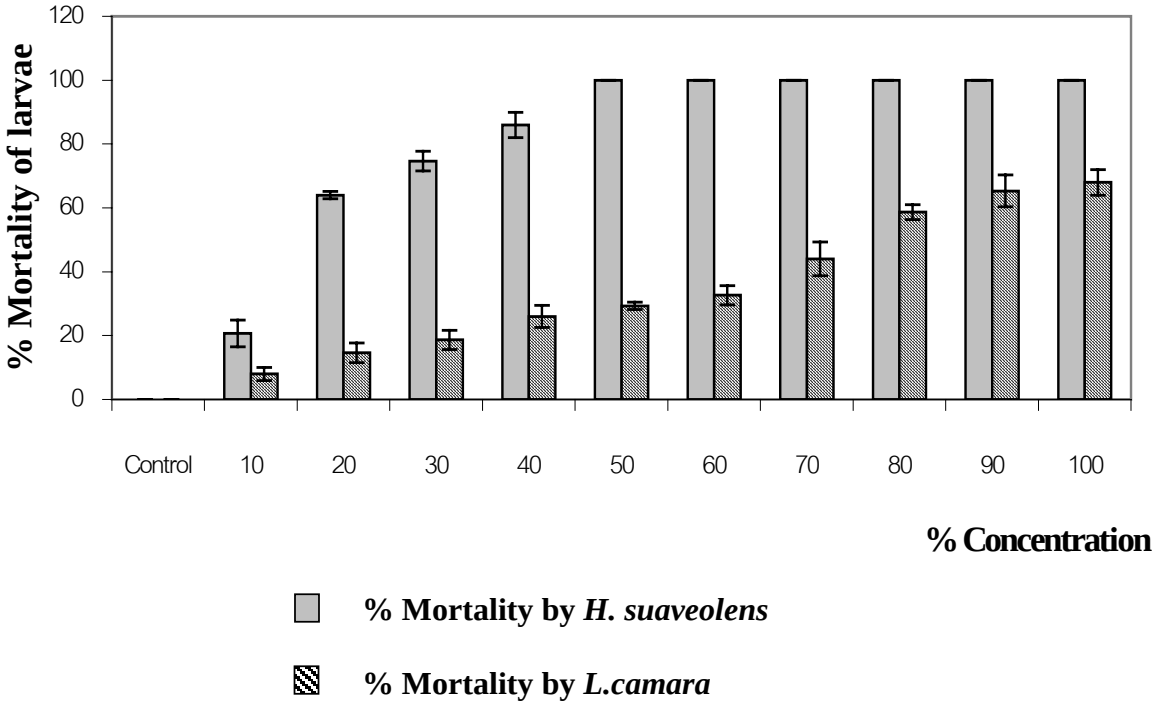

Figure 20 Comparison of larvicidal activity between *H. suaveolens* and *L. camara* extracts on *Ae. aegypti* second instar larvae at 48 hours.

The mortality of the mosquito second instar larvae was compared by drawing two pools of the data on the graph. It is obviously seen that at the same dilution of the extracts, the mortality of mosquito larvae caused by the *H. suaveolens* extract was higher than by the *L. camara* extract. At 10% dilution, the activity of the *H. suaveolens* extract was 2 fold of the *L. camara* extract. The larval mortality caused by *H. suaveolens* extract was increased 3-4 fold higher than by the *L. camara* extract among the dilutions of 20-70, $P \leq 0.01$. However, the crude extract of *H. suaveolens*

affected only 1.5 fold of the crude extract of *L. camara*. The mortality of the mosquitoes by the *L. camara* extracts never reached 100% (Figure 20). The larvicidal activity of *H. suaveolens* extract (LC_{50} = 20.24%) was about 3.68 fold stronger than that of the *L. camara* extracts (LC_{50} = 74.44%).

4.2.4 Effect of the extract combination of *Hyptis suaveolens* and *Lantana camara* extracts at a ratio 1:1 (v:v) on the second instar larvae of *Aedes aegypti.*

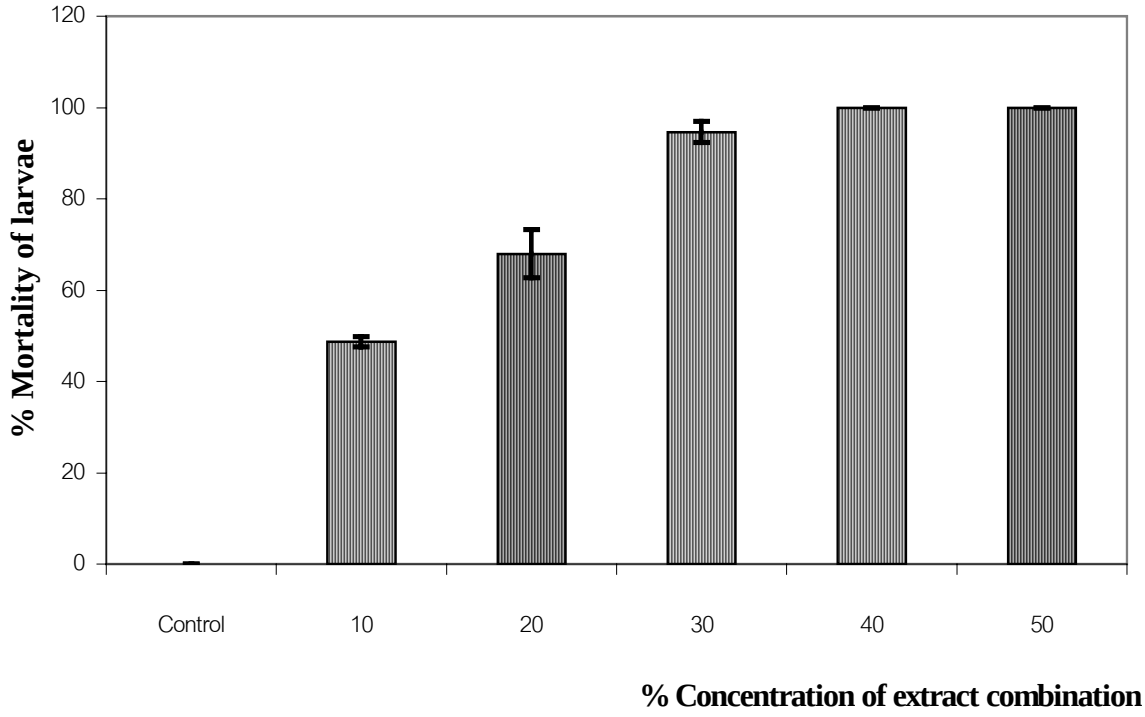

Figure 21 The effect of extract combinations on mortality of *Ae. aegypti* larvae within 48 hours.

The same volume of the same concentration of the two extracts was combined (1:1). Therefore, the concentration of the combination was able to conduct ranging

from 10% to 50%. The mortality of second instar larvae of *Ae. aegypti* increased as the higher concentration of the extracts were combined as shown in Figure 21. The 10% extract combination killed 50% larvae. The mortality of the larvae was nearly 100% at 30% extract combination, it reached 100% at 40% combination ($P \leq 0.01$) and then it leveled off. The LC_{50} value of the extract combination on the mosquito second instar larvae was 14.04%.

4.2.5 Comparison of the effects of *Hyptis suaveolens* and *Lantana camara* extracts and their combination on the second instar larvae of *Aedes aegypti.*

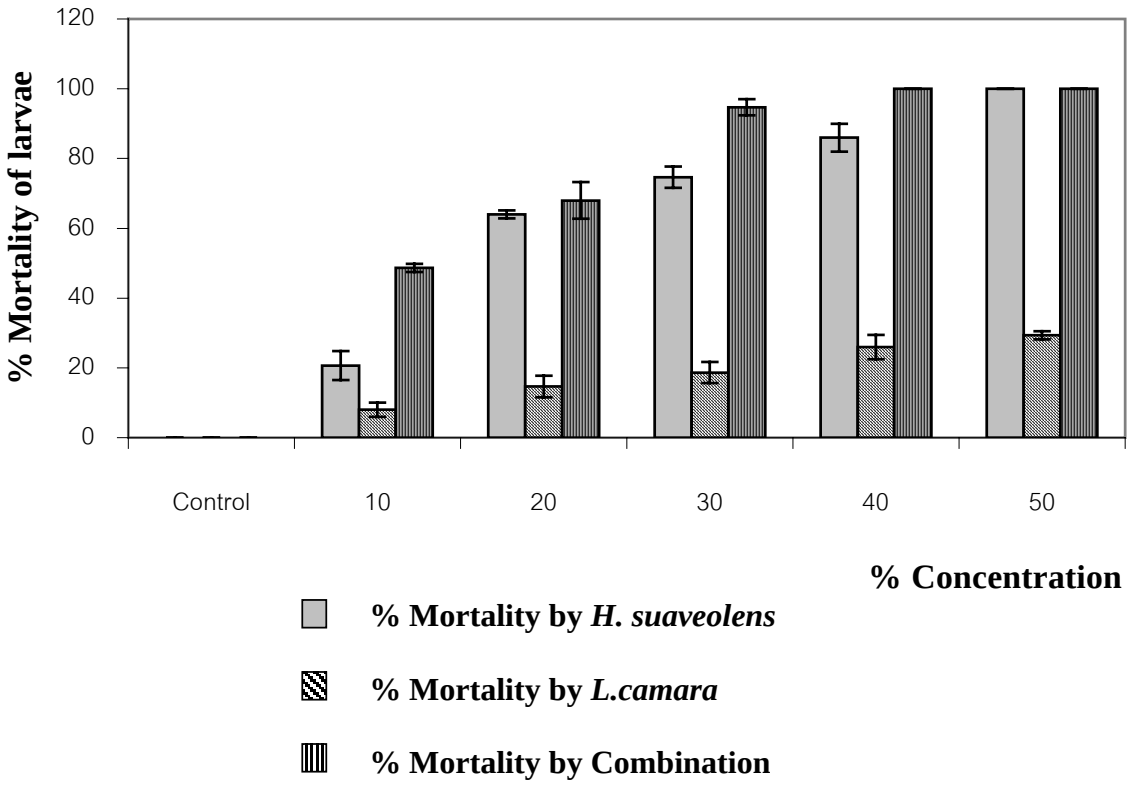

Figure 22 Comparison of larvicidal activity among *H. suaveolens* and *L. camara* extracts and their combinations on the second instar larvae of *Ae. aegypti*.

For clear comparison and explanation, three pools of data from 4.2.1, 4.2.2 and 4.2.4 were drawn in one graph, on purpose. According to the combination, the final concentration in the treatment was able to be done only up to 50% as demonstrated in Figure 20. It was obviously revealed that the combination of extracts possessed larvicidal activity against *Ae. aegypti*, significantly. Ten percent extract combination killed 50% larvae, while the individual extract of *H. saveolens* killed 20% larvae and of the *L. camara* killed only 10% larvae. The effect of extract combination was similar to that of the extract of *H. saveolens* from 20% dilution and higher up to 50%. The effect of *L. camara* extract was low and slightly increased from 10% up to 25% mortality as the highest, $P \leq 0.01$. The LC_{50} of these extracts and their combination was as followings.

The LC_{50} of *H. suaveolens* extract = 20.24%

The LC_{50} of *L. camara* extracts = 74.44%

The LC_{50} of the extract combination = 14.04%

Therefore, the larvicidal activities of the extract combination was about 1.44 and 5.30 fold higher than of the individual extracts, respectively.

4.2.6 Effect of the combination of *Hyptis suaveolens* and *Lantana camara* extracts at various mixing ratios on the second instar larvae of *Aedes aegypti.*

Table 3 The effect of the extract combination (*H. suaveolens* and *L. camara*) on mortality of *Ae. aegypti* larvae at various and reversely mixing ratios within 48 hours.

Combining Ratio of Extracts *H. : L.*	Percent Mortality of Second Instar Larvae of *Ae. aegypti*					
	Rep 1	Rep 2	Rep 3	Total	Average	%Mortality ± SD
Control	0	0	0	0	0	0 ± 0.00 a
20% : 80%	46	45	50	141	47	94 ± 2.64 b
40% : 60%	50	50	50	150	50	100 ± 0.00 b
50% : 50%	50	50	50	150	50	100 ± 0.00 b
60% : 40%	50	50	50	150	50	100 ± 0.00 b
80% : 20%	50	50	50	150	50	100 ± 0.00 b

Number with different letters (a,b) within the same column are significantly different ($P \leq 0.01$).

The combination extracts of *H. suaveolens* and *L. camara* were combined at reversely mixing ratios from 20% to 80% of each extract. All combinations showed very high larvicidal activity against *Ae. aegypti second* instar larvae as the mortality presented in Table 3. Most combinations completely killed the larvae, except the combination of 20% : 80% of *H. suaveolens* : *L. camara,* which killed 94% larvae. This indicated that the second instar larvae of *Ae. aegypti* were very susceptible to the combination extracts than to individual extracts of the two plants. In addition, it is

likely that the mortality of mosquito larvae was dependent on the *H. suaveolens* extracts. Or in another word, the *H. suaveolens* extracts contributed a stronger larvicidal effect to the combination than the *L. camara* extract.

4.2.7 Synergistic effect of *Hyptis suaveolens* extract to and *Lantana camara* extract on the second instar larvae of *Aedes aegypti.*

Table 4 The synergistic effect of *H. suaveolens* extracts in mixed extract on mortality of *Ae. aegypti* larvae within 48 hours.

Mixing Ratio of Extract Combination *H. : L.*	Mortality of mosquito larvae					
	Rep 1	Rep 2	Rep 3	Total	Average	%Mortality ± SD
Control	0	0	0	0	0	0.00 ± 0.00 a
25% : 75%	48	46	50	144	48	96.00 ± 2.00 ab
20% : 75%	40	40	38	118	39.33	78.67 ± 1.15 ab
15% : 75%	32	35	34	101	33.67	67.33 ± 1.52 abc
10% : 75%	28	35	32	95	31.67	63.33 ± 3.51 b
5% : 75%	30	32	29	91	30.33	60.67 ± 1.52 bd
0% : 75%	31	29	31	91	30.33	60.67 ± 1.15 bd

Number with different letters (a,b) within the same column are significantly different ($P \leq 0.01$).

Number with different letters (c,d) within the same column are significantly different ($P \leq 0.05$).

The synergistic effect of *H. suaveolens* and *L. camara* extracts on the mortality of *Ae. aegypti* larvae was tested according to the finding 80% of *L. camara* extract in the 20% : 80% (*H.* : *L.*) combination could not completely kill the larvae, but the reversed combination (*H.* : *L.* = 80% : 20%) could. The synergism effect test was conducted by fixing the concentration of *L. camara* extract at 75% which was at its LC_{50} value and then varying the concentration of *H. suaveolens* extract in the combination. Table 4 demonstrates that without *H. saveolens* extract or only *L. camara* extract, the mortality of the larvae was 60%. This was accounted as a base line. When increasing the concentration of *H. saveolens* extract, the mortality rate of the larvae was gradually increased from 10% : 75% (*H.* : *L.*). It reached the maximal mortality when 25% *H. saveolens* extract in the combination, that was *H.* : *L.* = 25% : 75%. Therefore, at the LC_{50} concentration of the *L.camara* extract, 75%, and in the present of the highest concentration of the *H. suaveolens* extract which was 25%, the *H. suaveolens* enhanced the mortality rate of the larvae about 1.6 fold as compare to without it.

This study obviously demonstrates that the leave-water extracts of *H. saveolens* and *L. camara* and the combination of them can kill the *Ae. aegypti* second instar larvae with their LC_{50} values of 20%, 75 % and 14%, respectively. The *H. saveolens* extract is about 3-4 fold more potent than the *L. camara* extract. The extract combination is about 5 fold more effective than the *L. camara* extract alone. Moreover, the *H. saveolens* extract can enhance the larvicidal activity up to 1.5 fold of the *L. camara* extract alone. Both plants can be used as biological agents to control the mosquito larvae which are widely distribute in water in the household and cause wide dispersal of hemorrhagic fever in communities of Thailand. The efficacy of *H.*

suaveolens extract could due to its terpinolene which is stronger than lantadene A and lantadene B in *L. camara* extract. According to the studies of Dharmagadda, Mittal, Naik and Vasudevan (2005), the mortality of mosquitoes was directly dependent on the concentration of *Tagetes patula* essential oil. The *T. patula* essential oil contained 11.2% terpinolene as found in the *H. suaveolens* extract. Our study agrees with the bioassays of Dharmagadda et al., which revealed that *T. patula* oil was most toxic against *Ae. aegypti* (LC_{50} = 13.57 ppm, LC_{90} = 37.91 ppm), followed by *Anopheles stephensi* (LC_{50} = 12.08 ppm, LC_{90} = 57.62 ppm) and *Culex quinquefasciatus* (LC_{50} = 22.33 ppm, LC_{90} = 71.89 ppm). In addition, the effect of *Dalbergia sissoo* oil against mosquitoes studied by Ansari, Razdan, Tandon and Vasudevan (2000) showed that larvicidal activity was directly proportional to dosages. One hundred percent mortality of *Cx. quinquefasciatus* larvae was observed within 24 hours at 5 ml/m^2, followed by *Ae. aegypti* (90%) and *An. stephensi* (60%) and pupation was totally inhibited. It could possibly be concluded that terpinolene in the *H. suaveolens* extract may be the chemical that enhanced the effect of the *L. camara* in their combination.

4.3 Effect of plant extracts on the adults of *Aedes aegypti*

The adult mosquitoes were reared in a 30 × 30 × 30 centimeter wire cage. The plant extract was sprayed at 5 ml/30 cm^3. Death mosquitoes were counted after 48 hours of treatment.

4.3.1 Effect of *Hyptis suaveolens* extract on the adults of *Aedes aegypti.*

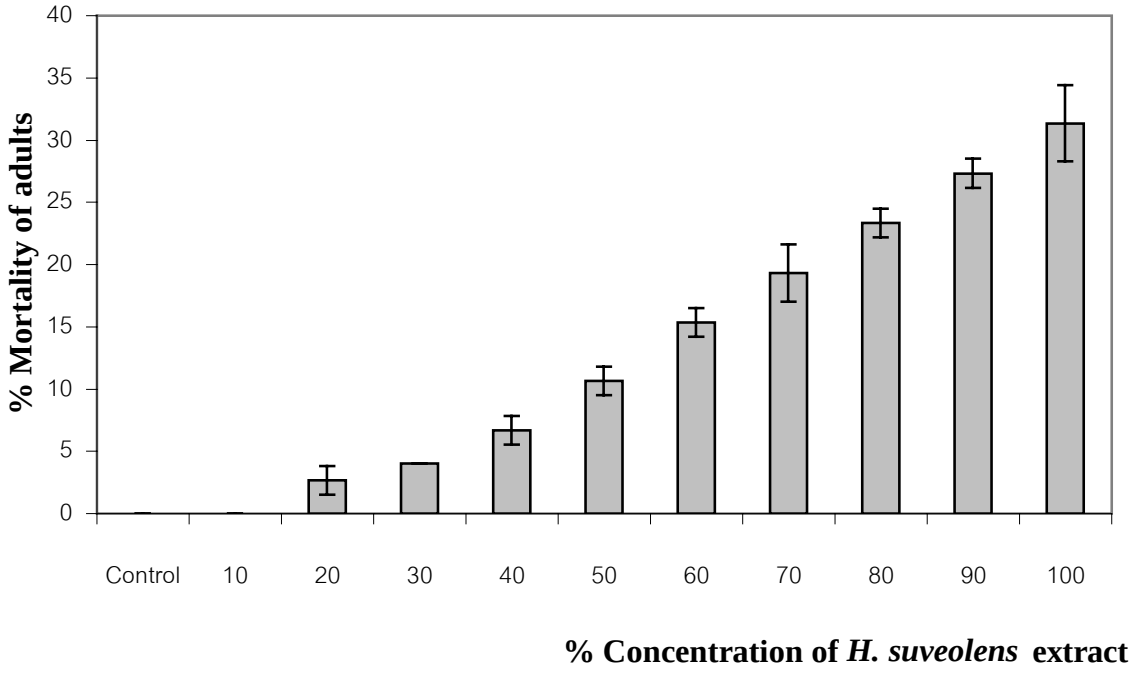

Figure 23 The effect of *H. suaveolens* at various concentrations on mortality of *Ae. aegypti* adults within 48 hours.

It was found that the effect of *H. Suaveolens* extract on the adult mosquitoes, *Ae. aegypti*, was dependent on the concentration of the extract. Ten percent of the extract did not kill the mosquitoes as compared to the control. The insecticidal effect of the *H. saveolens* extract could be observed from 20% with 3% mortality. Then, the

mortality was gradually increased to 32% by the crude extract, $P \leq 0.01$ (Figure 23). However, the mortality of mosquito adults never reached 50%, eventhough the highest concentration of the extract, which was crude, was used in the test.

4.3.2 Effect of *Lantana camara* extracts on the adults of *Aedes aegypti.*

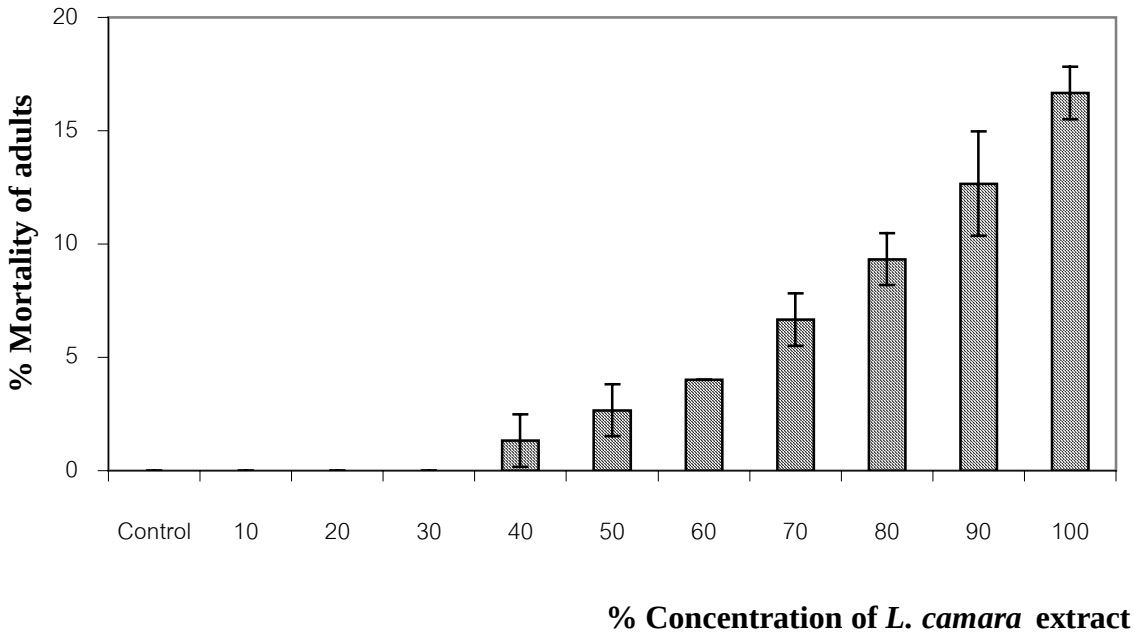

Figure 24 The effect of *L. camara* at various concentrations on mortality of *Ae. aegypti* adults within 48 hours.

Ten to thirty percent of *L. camara* extracts did not kill the adult mosquitoes. The killing effect of this extract showed from the concentrations of 40-100% with the mortality of 2-16%, $P \leq 0.01$, (Figure 24). Similarly, the mortality did not reach 50%

as found in the treatment of the *H. suaveolens* extract. It is obviously seen that the effect of *H. suaveolens* was stronger than that of *L. camara*.

4.3.3 Comparison the effect of *Hyptis suaveolens* and *Lantana camara* extracts on the adults of *Aedes aegypti.*

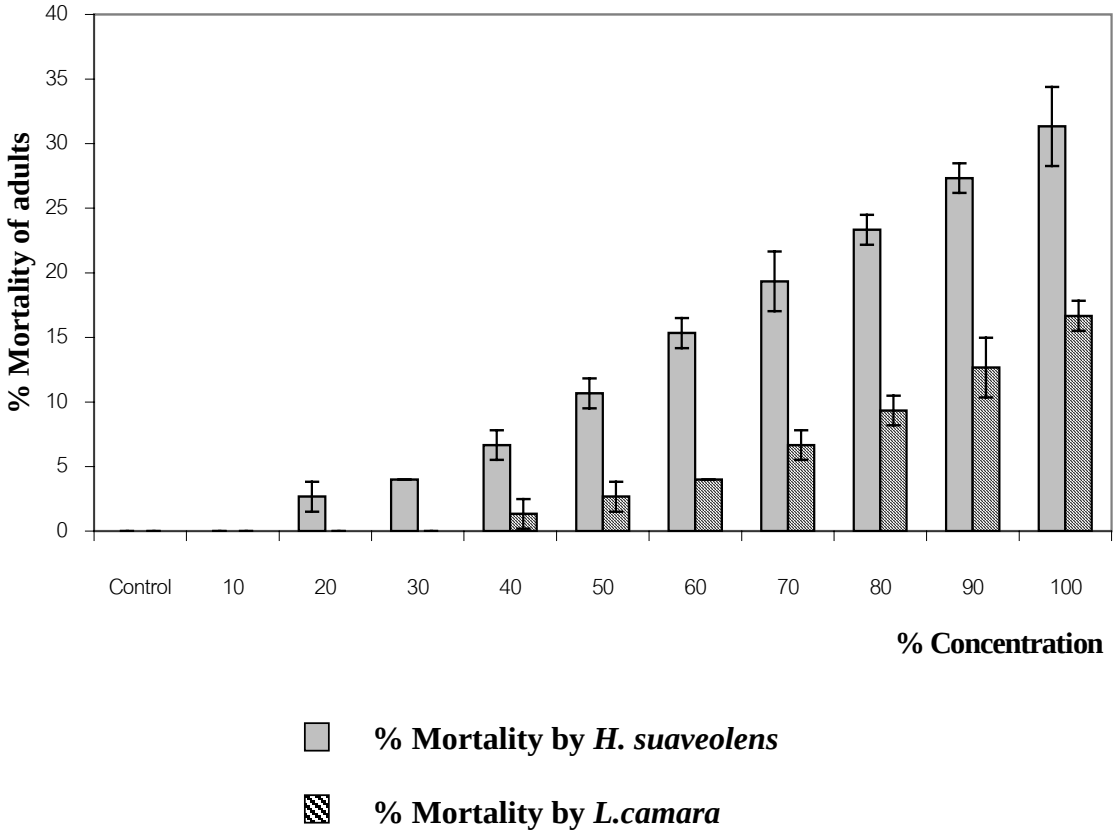

Figure 25 Comparison of the effects of *H. suaveolens* and *L. camara* extracts on the mortality of adult *Ae. aegypti*.

It was found that the extract of *H. suaveolens* was more effective on mortality of mosquito adults than that of *L. camara* in all concentrations of the treatments. The

extract of *L. camara* from 10-30% did not kill the adult mosquitoes. The comparable effect was obtained from 40% to 100% extracts of the two plants with the mortality ranging from 3-32% for the *H.saveolens* and 2-16% for *L. camara*, $P \leq 0.01$, (Figure 25). In this comparable range, the *H. saveolens* extract was approximately 2-4 fold more effective than the *L. camara* extract.

4.3.4 The Effect of the combination extracts of *Hyptis suaveolens* and *Lantana camara* (1:1) on the adults of *Aedes aegypti.*

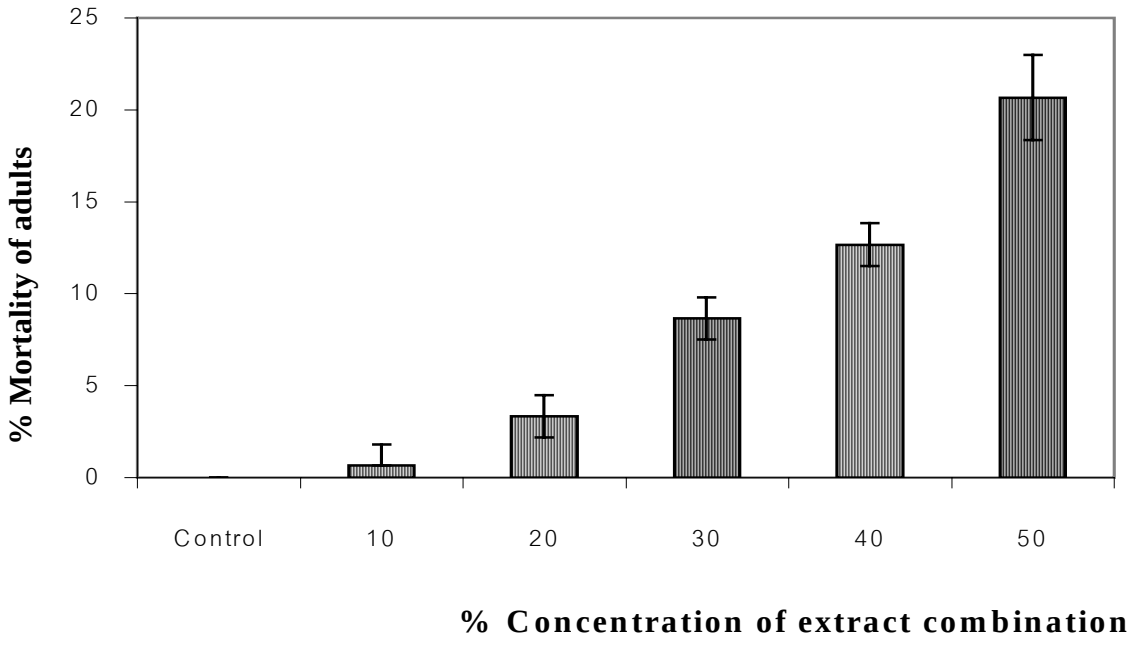

Figure 26 The effect of the combination extracts at various concentrations on mortality of *Ae. aegypti* adults within 48 hours.

The extracts of *H. suaveolens* and *L. camara* of the same concentration at the same proportion (1:1) were combined up to 50%. The combined solutions were tested

for the mortality of the adult mosquitoes. The mortality of *Ae. aegypti* adults increased with an increasing the concentration of the combination. The adult-mosquito mortality ranged from 1-22% of the combination of 10-50% concentrations, respectively, $P \leq 0.01$, (Figure 26). Therefore, the LC_{50} value cannot be depicted.

4.3.5 Comparison the effects of *Hyptis suaveolens* and *Lantana camara* extracts and their combination on the adults of *Aedes aegypti.*

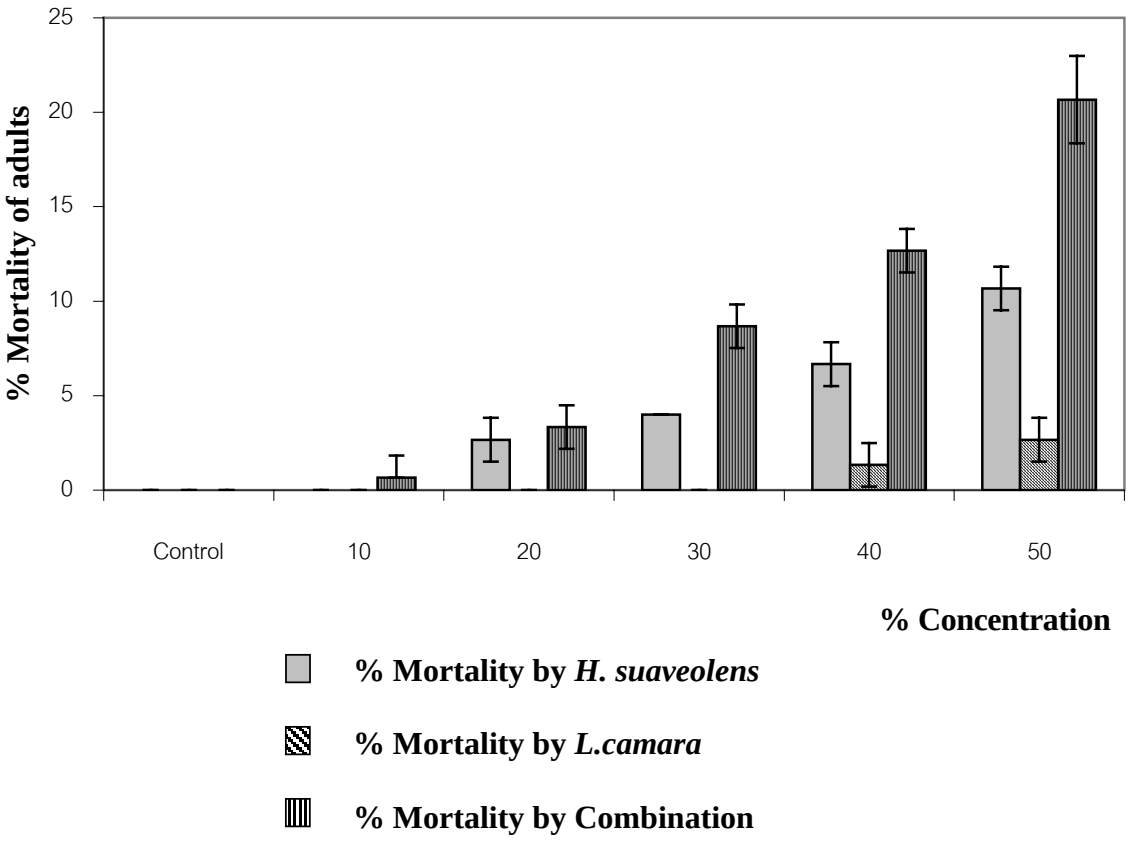

Figure 27 Comparison mortality of adult mosquitoes treated with of *H. suaveolens* and *L. camara* extracts individually and with the combination of the two extracts at 1:1 ratio.

It was found that the extract combination showed higher mortality of mosquito adults than those of *H. suaveolens* and *L. camara* extracts in all concentration levels. When compare the mortality at 50% concentration among three treatments, the extract combination showed highest mortality of 20.67%, while the individual treatment of *H. suaveolens* extract was 10.67%, and of *L. camara* extract was 2.67% , $P \leq 0.01$, (Figure 27). That is the effect of the combination extract is about 2 and 8 fold of *H. suaveolens* and *L. camara* extracts, respectively. However, when take consideration in Figure 25, the synergism effect of *H. sauveolens* extract in the combination also appeared for mortality of adult mosquitoes.

4.3.6 Effect of reversed combination of extracts of *Hyptis suaveolens* and *Lantana camara* at various on the adults of *Aedes aegypti.*

Table 5 The effect of mixed extract (*H. suaveolens* and *L. camara*) at various ratios on mortality of *Ae. aegypti* adults within 48 hours.

Mixing Ratio of Extract Combination *H. : L.*	Mortality of mosquito adults					
	Rep 1	Rep 2	Rep 3	Total	Average	%Mortality ± SD
Control	0	0	0	0	0	0.00 ± 0.00 a
20% : 80%	6	5	6	17	5.67	11.33 ± 0.57 b
40% : 60%	5	7	8	20	6.67	13.33 ± 1.52 bc
50% : 50%	9	11	10	30	10.00	20.00 ± 1.00 bd
60% : 40%	8	10	11	29	9.67	19.33 ± 1.52 bcd
80% : 20%	12	12	11	35	11.67	23.33 ± 0.57 bd

Number with different letters (a,b) within the same column are significantly different ($P \leq 0.01$).

Number with different letters (c,d) within the same column are significantly different ($P \leq 0.05$).

The extract combination of *H. suaveolens* and *L. camara* at various ratios and reverse concentrations of 20-80% showed mortality of adult mosquitoes, *Ae. aegypti* ranging of 11-23% as presented in Table 5. This could be suggested that the spraying method with a dosage of 5 ml/ m^2 did not produce ≥ 25% mortality of mosquito adults after 48 hours. The combination of *H. suaveolens* and *L. camara* extracts was more

effective than individuals. The mortality were 20.67%, 10.67% and 2.67% respectively.

However, Ansari, Razdan, Tandon and Vasudevan (2000) found that adult mosquito which emerged from exposure to a sublethal dosage (4 ml/m^2) of *Dalbergi sissoo* oil neither lay eggs (*Ae. aegypti*) nor hatch eggs (*Cx. quinquefasciatus* and *An. stephensi*). In their studies, they concluded that the larvicidal activity of *D. sissoo* oil was observed at higher dose, and the lower doses inhibited the reproductive potential of adults to great extent. Therefore, it can be claimed that the extract combination of *H. suaveolens* and *L. camara* could possibly affect the reproduction of *Ae. aegypti* as well.

In this experiment, LC_{50} of all extract treatments were not detected. The extract in the sprayed aerosol may not coat and penetrate the skin of the mosquito, which is covered by wax (Rather, 1990). Unlike dipping method that the extracts can coat the larval skin and possibly be eaten by the larvae (Ansari et al., 2000).

CHAPTER V

CONCLUSION

Previously, synthetic chemical spray, such as DDT, was most applied to kill or reduce the number of mosquitoes, which damaged the environment as well. Natural products from plants are explored to replace the chemically synthetic insecticides. *Hyptis suaveolens* and *Lantana camara*, therefore, are proposed for this purpose. This study aimed to use the extracts of Chan (*Hyptis suaveolens* (L.) Poit.) and Hedge Flower (*Lantana camara* Linn.) to biologically control dengue fever mosquito (*Aedes aegypti* Linn.) which is the vector of the yellow fever and the deadly hemorrhagic fever diseases. Hemorrhagic fever disease, caused by Dengue virus, which widely distributed throughout Thailand by this mosquitoes as the major means. Therefore, biologically control agents to get rid of this mosquito ought to seeking for. Leaves of the plants were extracted in water using Soxhlet extractor apparatus and evaporated to a certain volume, counted as a 100 percent crude extract. The extracts in liquid form were tested to observe three aspects in controlling the mosquitoes which are the larvicidal effect on larvae, the hatching activity of the eggs, and the mortality of the adult mosquitoes. The tests were conducted by using each individual extract, separately, and the combination of them in treatments on the mosquito subjects which were the second instar larvae, eggs and adult mosquitoes.

The larvicidal effect of the plant extracts were performed by dipping method. Larvae were dipped in the extracts at various concentrations for 48 hours. The dead

larvae were counted and the mortality was calculated and presented in percentage. LC_{50} was depicted by Probit analysis.

The *H. suaveolens* extract was found most effective to kill the second instar larvae. Its larvicidal activity was dose dependent fashion. The extract completely killed the second instar larvae at its concentration of 50% and its LC_{50} was 20.24%. While, the *L. camara* extract was less effective and its larvicidal activity was also dose dependent. The LC_{50} of it was 74.44%. Fifty percent extract killed 30% larvae and the 100% killed only 70% larvae, never reached 100 percent. The combination of both extracts possessed significant larvicidal activity against *Ae. aegypti* than each individual treatment. The LC_{50} of combined extracts of the same concentration was 14.04%. The combined extract had larvicidal activity about 1.4 and 5.3 fold ($P \leq 0.01$) higher than *H. suaveolens* extract and *L. camara* extract, respectively. It suggested that the *H. suaveolens* extract exhibited synergistic effect by enhancing the effect of *L. camara* in the extract combination. Therefore, *H. suaveolens* is more applicable to use as a biological agent to control mosquitoes at the larval stage than *L. camara*. The combination of them is even better in this control.

In case of mosquito eggs, *H. suaveolens* and *L. camara* extracts and their combination had no effect on mosquito eggs. Eventhough, *crude* extracts of *H. suaveolens* and *L. camara* showed slightly inhibition on egg hatching, 8% and 5% ($P \leq 0.05$) respectively. The egg is protected by a shell called the chorion. The chorion is constructed of three layers, making it strong and providing safety for the embryo inside the egg. Once the eggs are laid, the chorion becomes water resistant. This could probably explain that mosquito egg shell prevents any agents to penetrate inside and protect the embryo from danger. These extracts which were in water media could not

or slightly penetrate inside the egg shell to kill the mosquito embryo. Therefore, plant extract in water is not applicable in biological control of mosquitoes. Other kinds of media in extraction are suggested to further explored for the biological control of *Ae. aegypti* eggs.

Adult *Ae. aegypti* is an active flying insect and in the stage of disease transmission. This is another important stage needed to be got rid of. *H. suaveolens* and *L. camara* extracts and the combination of them were used to test for controlling the adult mosquitoes. Similar pattern of results to those of larvicidal activity was obtained. The *H. suaveolens* extract was more effective in killing the adult mosquitoes than the *L. camara* extect, significantly. The crude extract of *H. suaveolens* showed 2 fold mortality of the extract of *L. camara*, 32% as opposed to 16%. It was also found that the extract combination killed more adult mosquitoes. As compare at 50% dilution, the mortality was 2.6, 10.67 and 20.67% ($P \leq 0.01$) for the *H. suaveolens* and *L. camara* extracts and the combination, respectively. That is the effect of the combination was 2 and 8 fold of the *H. suaveolens* and *L. camara* extracts, respectively. However, the LC_{50} of all treatments was not able to depict. It is obviously that *H. suaveolens* and *L. camara* could be good as a biologically control agent for adult mosquitoes.

In summary, *Hyptis suaveolens* is effective to be a biological agent to control *Ae. aegypti* mosquitoes at larval and adult stages. It also can be used in combination with *L. camara* in order to enhance the morality effect of the later plant. However, the biological control of *Ae. aegypti* mosquitoes by natural plant products in this study has not yet thoroughly tested. The mosquito pupae are suggested to be studied, in additionally, since its rapid development to be adult mosquitoes.

REFERENCES

REFERENCES

Abdul, R.A., Arumugam, S., Geetha, G., Himalayan, B. and Saleem, G.B. (2000). Effect of *Feronia limonia* on mosquito larvae. **Fitoterapia.** 71: 553-555.

Abramovich, A., Markus, A., Pelah, D. and Wiesman, Z. (2002). The use of commercial saponin from *Ouillaja saponaria* bark as a natural larvicidal agent against *Aedes aegypti* and *Culex pipiens*. **Journal of Ethopharmacology.** 81: 407-409.

Adelaide, T.M., Denise, V., Jose, B.P., Maria, G.R. and Maurilio, J.S. (1999). Anopheles albitarsis eggs: ultrastructural analysis of chorion layers after permeabilization. **Journal of Insect Physiology**. 45: 915-922.

Adeniyi, B.A., Asekun, O.T. and Ekundayo, O. (1999). Antimicrobial activity of the essential oil of *seeds Hyptis suaveolens* leaves. **Fitoterapia.** 70: 440-442.

Afshan, F., et al. (2000). Two insecticidal tetranortriterpenoids *from Azadirachta indica*. **Phytochemistry.** 53: 371-376.

Aguirre, C., et al. (2004). A novel 8.7 kDa protease inhibitor from chan *seeds* (*Hyptis suaveolens* L.) inhibits proteases from the larger grain *borer Prostephanus truncatus* (Coleoptera: Bostrichidae). **Comparative Biochemistry and Physiology.** 138: 81-89.

Ahn, Y-J., Chang, K-S., Kim, D-H. and Kim, S-I. (2002). Repellent activity of constituents identified in *Foeniculum vulgare* fruit against *Aedes aegypti* (Diptera: Culicidae). **Journal of Agricultural and Food Chemistry.** 50: 6993-6996.

Akah, P.A. and Nwambi, A.I. (1993). Nigerian plants with anti-convulsant property. **Fitoterapia.** 62: 42-44.

Ali-Emmanuel, N., Akakpo, J.A., Moudachirou, M. and Quetin-Leclercq, J. (2003). Treatment of bovine dermatophilosis wish *Senna alata*, *Lantana camara* and *Mitracarpus scaber* leaf extracts. **Journal of Ethopharmacology.** 86: 167-171.

Almtorp, G.T., Hazell, A.C. and Torssel, K.B.G. (1991). A lignan and pyrone and other constituents from *Hyptis capitata*. **Phytochemistry.** 30: 2753-2756.

Amalraj, D.D., et al. (2000). Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis* (vectobac) against mosquito vectors. **Acta Tropica.** 75: 243-246.

Araújo, C.C., et al. (2003). Insecticidal activity and chemical composition of volatile oils from *Hyptis martiusii* Benth. **Journal of Agricultural and Food Chemistry.** 51: 3760-3762.

Ansari, M.A., Razda, R.K., Tandon, M. and Vasudevan, P. (2000a). Larvicidal and repellent actions of *Dalbergia sissoo* Roxb. (*F. Leguminosae*) oil against mosquitoes. **Bioresource Thechnology.** 73: 207-211.

Ansari, M.A., Razda, R.K., Tandon, M. and Vasudevan, P. (2000b). Larvicidal and mosquito repellent actions of peppermint (*Mentha piperita*) oil. **Bioresource Thechnology.** 71: 267-271.

Asekun, O.T., Ekundayo, O. and Adevini, B.A. (1999). Antimicrobial activity of the essential oil of *Hyptis suaveolens* leaves. **Fitoterapia.** 70: 440-442.

Aycard, J.P., Kini, F., Kam, B., Gaydou, E.M. and Faure, R. (1993). Isolation and identification of spicigera lactone: Complete H and C assignments using two-dimensional NMR experiments. **Journal of Natural Products.** 56: 1171-1173.

Azevedo, N.R., et al. (2001). Chemical variability in the essential oil of Hyptis suaveolens. **Phytochemistry.** 57: 733-736.

Bandara, K.A., Jacobsson, U., Kumar, V. and Molleyres, L-P. (2000). Insecticidal piperidine alkaloid from *Microcos paniculata* stem bark. **Phytochemistry.** 54: 29-32.

Barre, J.T., et al. (1997). A bioactive triterpene from *Lantana camara*. **Phytochemistry.** 45 (2): 321-324.

Bastos, F., et al. (2003). Effects of *Lantana camara* (Verbenaceae) on rat fertility. **Veterinary and Human Toxicology.** 45 (1): 20-23.

Begum, S., Firdous, S., Raza, S.M., Siddiqui, B.S. and Siddiqui, S. (1995). Pentacyclic triterpenoids from *Lantana camara*. **Phytochemistry.** 38 (3): 681-685.

Begum, S., Qamar, F., Siddiqui, B.S. and Wahab, A. (2000). Nematicidal constituents of the aerial parts of *Lantana camara*. **Journal of Natural Products.** 63: 765-767.

Bouda, H., Fontem, D.A., Gumedzoe, M.Y.D. and Tapondjou, L.A. (2001). Effect of essential oils from leaves of *Ageratum conyzoides*, *Lantana camara* and *Chromolaena odorata* on the mortality of *Sitophilus zeamais* (Coleptera, Curculionidae). **Journal of Stored Products Research.** 37: 103-109.

Bradley, A.H., Bruce, M.C. and Jianyoung, L. (1996). Involvement of peroxidase in chorion hardening in Ae*des aegypti* . **Insect Biochemistry and Molecular Biology**. 26 (3): 309-317.

Chang, H.T., Chang, S.T., Chen, W.J., Cheng, S.S. and Tsai, K.H. (2003). Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. **Bioresource Thechnology.** 89: 99-102.

Chariandy, C.M., Khambay, B.P.S., Phelps, R.H., Pollard, G.V. and Seaforth, C.E. (1999). Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. **Journal of Ethopharmacology.** 64: 265-270.

Chitsomboon, B., Eumkeb, G., Nakkiew, P. and Punopas, K. (2003). The study of antibacterial activity of some medicinal plants in Lamiaceae family**. Suranaree Journal Science Technology.** 11: 52-59.

Christensen, J., et al. (2002). Possible artifacts in the in vitro determination of antimalarial activity of natural products that incorporate into lipid bilayer: appareant antiplasmodial activity of dehydroabietinol, a constituent *of Hyptis suaveolens*. **Planta Med.** 68: 547-549.

Chui, V.W.D., Tsoi, K.W. and Wong, K.W. (1995). Control of mosquito larvae (Diptera: Culicidae) using BTI and teflubenzuron: labolatory evaluation and semi-field test. **Environment International.** 21 (4): 433-440.

Ciccia, G., Coussio, J. and Mongelli, E. (2000). Insecticidal activity *against Aedes aegypti* larvae of some medicinal South American plants. **Journal of Ethopharmacology.** 72: 185-189.

Dawra, R.K., Makkar, H.P.S. and Sharma, O.P. (1987). Isolation and partial purification of Lantana (*Laantana camara* L.) toxins. **Toxicology Letters.** 37 (2): 165-172.

Deena, M.J. and Thoppil, J.E. (2000). Antimicrobial activity of the essential oil of *Lantana camara*. **Fitoterapia.** 71: 453-455.

Dharmagadda, V.S., Mittal, P.K., Naik, S.N. and Vasudevan, P. (2005). Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. **Bioresource Technology**. 96: 1235-1240.

Dixit, O.P., Harshan, V. and Saxena, R.C. (1992). Insecticidal action *of Lantana camara* aginst *Callosobruchus chinensis* (Coleotera:Bruchidase**). Journal of Stored Products Research.** 28 (4): 279-281.

Donald, J. (2004). **Mosquitoes in Your Life.** Associate Research Professor in Entomology [On-line]. Availabl: http://www.rci.rutgers.edu/~insects/ moslife.htm

Ghisalberti, E.L. (2000). *Lantana camara* L. (Verbenaceae). **Fitoterapia.** 71: 467-486.

Goh, S.H., et al. (2003). Meliternatin: a feeding deterrent and larvicidal polyoxygenated flavone from *Melicope subunifoliolata*. **Phytochemistry.** 62: 1121-1124.

Jebanesan, A. and Venkatachalam, M.R. (2001). Repellent activity *of Ferronia elephantum* Corr. (Rutaceae) leaf extract against *Aedes aegypti* (L.). **Bioresource Thechnology.** 76: 287-288.

Jose, D.M. and Thoppil, J.E. (2000). Chemical composition of essential oil of *Lantana camara* L. **Acta Pharm.** 50: 259-262.

Khan, M., Naqvi, A.A., Singh, M., Srivastava, S.K.and Syamasundar, K.V. (2001). Chemical composition of leaf and flower essential oil of *Lantana camara* from India. **Flavour and Fragrance Journal.** 17 (1): 75-77.

Kuhnt, H., Rimpler, H. and Heinrich, M. (1994). Lignans and other compounds from the mixe Indian medicinal plant *Hyptis verticillata*. **Phytochemistry.** 36: 485-489.

Lee, H.K., et al. (2002). A piperidine amide extract from *Piper longum* L. Fruit shows activity against *Aedes aegypti* Mosquito Larvae. **Journal of Agricultural and Food Chemistry.** 50: 3765-3767.

Misra, L., Laatsch, H. (2000). Triterpenoids essential oil and photo-oxidative 28 → 13 – lactonization of oleanolic acid from from *Lantana camara*. **Phytochemistry.** 54: 969-974.

Ngassoum, M.B., et al. (1999). Chemical composition of essential oils *of Lantana camara* and flowers from Cameroon and Madagascar. **Flavour and Fragrance Journal.** 14 (4): 245-250.

Palsson, K. and Jaeson, T.G.T. (1999). Plant products used as mosquito repellents in Guinea Bissau, West Africa. **Acta Tropica.** 72: 39-57.

Peerzada, N. (1997). Chemical composition of the essential oil of Hyptis suaveolens. **Molecules.** 2: 165-168.

Pereda-Mianda, R. and Gascon-Figueroa, M. (1988). Chemistry *of Hyptis mutabilis*: new pentacyclic triterpenoids. **Journal of Natural Products.** 51: 996-998.

Pereda-Miranda, R. and Delgado, G. (1990). Triterpenoids and flavonoids from *Hyptis albida*. **Journal of Natural Products.** 53: 182-185.

Rathburn, C.B. (1990). **Adulticides in the '90s: the changing picture.** Wing Beat. 12-13.

Raja Rao, K.V., Rao, L.J.M. and Rao, N.S.P. (1990). An ring contracted triterpenoid from *Hyptis suaveolens*. **Phytochemistry.** 29: 1326-1329.

Pereda-Miranda, R., Garcia, M. and Delgado, G. (1990). Structure and stereochemistry of from *Hyptis oblonggifolia*. **Phytochemistry.** 29: 2971-2974.

Sakthivadivel, M. and Thilagavathy, D. (2003). Laraicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemony mexicana* L. seed. **Bioresource Thechnology.** 89: 213-216.

Sastri, B.N. (2000).The Wealth of Indian; Council of Scientific and Industrial Research. **New Delhi.** 6: 31.

Service, M.W. 1983. **Pest and Vectors Management in the Tropics.** pp 265-282.

Tripathi, V. and Yadav, S.B. (2003). A new triterpenoid from *Lantana camara*. **Fitoterapia.** 74: 320-321.

Singh, G. and Upadhyay, R.K. (1993). Biocontrol Potential and its Exploitation in Sustainable Agriculture. **J. Sci. Ind. Res.** 52: 676-683.

Soumare, M.L. and Ndiaye M., (2005). Ultrastructural studies of mosquito ovogenesis. **Tissue and Cell.** 37: 117-124.

APPENDICES

APPENDIX A

COMPOSED OF MULTIPLE VITAMINS IN SYRUP

COMPOSED OF MULTIPLE VITAMINS IN SYRUP

VITAMINS AND LYSINE

Male mosquitoes, *Ae. aegtpti*, were fed with vitamin syrup solution.

Each 5 ml. Contains:-

Vitamin A	1,000 I.U.
Vitamin D	200 I.U.
Vitamin B1 (Thaimine Hydrochloride)	5.0 mg.
Vitamin B2 (Riboflavin)	3.0 mg.
Vitamin B6 (Pyridoxine Hydrochloride)	1.0 mg.
Nicotinamide	20.0 mg.
Calcium Pantothenate	5.0 mg.
Vitamin B12	5.0 mg.
Lysine (L-Lysine Hydrochloride)	50.0 mg.

APPENDIX B

DATA ANALYSIS TESTS OF

BETWEEN-SUBJECTS EFFECTS

DATA ANALYSIS TESTS OF

BETWEEN-SUBJECTS EFFECTS

Showing data analysis comparison of larvicidal activity between *Hyptis suaveolens* and *Lantana camara* extracts on *Aedes aegypti* second instar larvae at 48 hours.

Between-Subjects Factors

		Value Label	N
CONCENT	1	0%	6
	2	10%	6
	3	20%	6
	4	30%	6
	5	40%	6
	6	50%	6
	7	60%	6
	8	70%	6
	9	80%	6
	10	90%	6
	11	100%	6

EXTRACT	1	Hyptis	33
	2	Lantana	33

Descriptive Statistics

Dependent Variable: MORTALITY

CONCENT	EXTRACT	Mean	Std. Deviation	N
0%	Hyptis	.0000	.0000	3
	Lantana	.0000	.0000	3
	Total	.0000	.0000	6
10%	Hyptis	20.6667	4.1633	3
	Lantana	8.0000	2.0000	3
	Total	14.3333	7.5277	6
20%	Hyptis	63.3333	1.1547	3
	Lantana	14.6667	3.0551	3
	Total	39.0000	26.7357	6
30%	Hyptis	74.6667	3.0551	3
	Lantana	18.6667	3.0551	3
	Total	46.6667	30.7939	6
40%	Hyptis	86.0000	4.0000	3
	Lantana	26.0000	3.4641	3
	Total	56.0000	33.0333	6

CONCENT	EXTRACT	Mean	Std. Deviation	N
50%	Hyptis	100.0000	.0000	3
	Lantana	29.3333	1.1547	3
	Total	64.6667	38.7126	6
60%	Hyptis	100.0000	.0000	3
	Lantana	32.6667	3.0551	3
	Total	66.3333	36.9306	6
70%	Hyptis	100.0000	.0000	3
	Lantana	44.0000	5.2915	3
	Total	72.0000	30.8545	6
80%	Hyptis	100.0000	.0000	3
	Lantana	58.6667	2.3094	3
	Total	79.3333	22.6863	6
90%	Hyptis	100.0000	.0000	3
	Lantana	65.3333	5.0332	3
	Total	82.6667	19.2527	6
100%	Hyptis	100.0000	.0000	3
	Lantana	68.0000	4.0000	3
	Total	84.0000	17.7088	6
Total	Hyptis	76.7879	34.3400	33
	Lantana	33.2121	22.5994	33
	Total	55.0000	36.2491	66

Tests of Between-Subjects Effects

Dependent Variable: MORTALITY

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power
Corrected Model	85082.000	21	4051.524	543.497	.000	11413.439	1.000
Intercept	199650.000	1	199650.000	26782.317	.000	26782.317	1.000
CONCENT	46288.000	10	4628.800	620.937	.000	6209.366	1.000
EXTRACT	31330.970	1	31330.970	4202.935	.000	4202.935	1.000
CONCENT * EXTRACT	7463.030	10	746.303	100.114	.000	1001.138	1.000
Error	328.000	44	7.455				
Total	285060.000	66					
Corrected Total	85410.000	65					

a Computed using alpha = .05

b R Squared = .996 (Adjusted R Squared = .994)

Showing data analysis comparison of larvicidal activity among *Hyptis suaveolens*, *Lantana camara* and their combinations on the second instar larvae of *Aedes aegypti*.

Between-Subjects Factors

		Value Label	N
CONCENT	1	0%	9
	2	10%	9
	3	20%	9
	4	30%	9
	5	40%	9
	6	50%	9
EXTRACT	1	Mixed	18
	2	Hyptis	18
	3	Lantana	18

Descriptive Statistics

Dependent Variable: MORTALITY

CONCENT	EXTRACT	Mean	Std. Deviation	N
0%	Mixed	.0000	.0000	3
	Hyptis	.0000	.0000	3
	Lantana	.0000	.0000	3
	Total	.0000	.0000	9
10%	Mixed	48.6667	1.1547	3
	Hyptis	20.6667	4.1633	3
	Lantana	8.0000	2.0000	3
	Total	25.7778	18.1781	9
20%	Mixed	68.0000	5.2915	3
	Hyptis	63.3333	1.1547	3
	Lantana	14.6667	3.0551	3
	Total	48.6667	25.7682	9
30%	Mixed	100.0000	.0000	3
	Hyptis	74.6667	3.0551	3
	Lantana	18.6667	3.0551	3
	Total	64.4444	36.1079	9
40%	Mixed	100.0000	.0000	3
	Hyptis	86.0000	4.0000	3
	Lantana	26.0000	3.4641	3
	Total	70.6667	34.1467	9

CONCENT	EXTRACT	Mean	Std. Deviation	N
50%	Mixed	100.0000	.0000	3
	Hyptis	100.0000	.0000	3
	Lantana	29.3333	1.1547	3
	Total	76.4444	35.3380	9
Total	Mixed	69.4444	37.7534	18
	Hyptis	57.4444	36.7171	18
	Lantana	16.1111	10.5490	18
	Total	47.6667	38.1709	54

Tests of Between-Subjects Effects

Dependent Variable: MORTALITY

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power
Corrected Model	77003.333	17	4529.608	745.728	.000	12677.378	1.000
Intercept	122694.000	1	122694.000	20199.622	.000	20199.622	1.000
CONCENT	39518.000	5	7903.600	1301.202	.000	6506.012	1.000
EXTRACT	28181.333	2	14090.667	2319.805	.000	4639.610	1.000
CONCENT * EXTRACT	9304.000	10	930.400	153.176	.000	1531.756	1.000
Error	218.667	36	6.074				
Total	199916.000	54					
Corrected Total	77222.000	53					

a Computed using alpha = .05

b R Squared = .997 (Adjusted R Squared = .996)

Showing data analysis comparison of the effect of *Hyptis suaveolens* and *Lantana camara* extracts at various concentrations on hatching of *Aedes aegypti* within 24 hours.

Between-Subjects Factors

		Value Label	N
CONCENT	1	0%	6
	2	10%	6
	3	20%	6
	4	30%	6
	5	40%	6
	6	50%	6
	7	60%	6
	8	70%	6
	9	80%	6
	10	90%	6
	11	100%	6
EXTRACT	1	Hyptis	33
	2	Lantana	33

Descriptive Statistics

Dependent Variable: MORTALITY

CONCENT	EXTRACT	Mean	Std. Deviation	N
0%	Hyptis	4.4767	1.528E-02	3
	Lantana	4.4553	.2014	3
	Total	4.4660	.1283	6
10%	Hyptis	4.3400	.6199	3
	Lantana	4.3350	.1525	3
	Total	4.3375	.4038	6
20%	Hyptis	4.5033	.4818	3
	Lantana	4.4097	.4360	3
	Total	4.4565	.4142	6
30%	Hyptis	4.6267	.2139	3
	Lantana	4.2843	.1433	3
	Total	4.4555	.2483	6
40%	Hyptis	5.1467	.2928	3
	Lantana	4.2607	.4199	3
	Total	4.7037	.5834	6
50%	Hyptis	5.2200	.3639	3
	Lantana	4.3260	.2059	3
	Total	4.7730	.5565	6

CONCENT	EXTRACT	Mean	Std. Deviation	N

60%	Hyptis	5.5667	.3926	3
	Lantana	4.3747	.4800	3
	Total	4.9707	.7616	6
70%	Hyptis	6.6733	.2996	3
	Lantana	4.4913	.2060	3
	Total	5.5823	1.2170	6
80%	Hyptis	7.2867	.5256	3
	Lantana	4.5710	.2210	3
	Total	5.9288	1.5305	6
90%	Hyptis	7.9800	.5012	3
	Lantana	4.8310	.3686	3
	Total	6.4055	1.7691	6
100%	Hyptis	8.4667	.2914	3
	Lantana	4.9967	.4441	3
	Total	6.7317	1.9301	6
Total	Hyptis	5.8442	1.4916	33
	Lantana	4.4851	.3506	33
	Total	5.1647	1.2747	66

Tests of Between-Subjects Effects

Dependent Variable: MORTALITY

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power
Corrected Model	99.840	21	4.754	36.259	.000	761.447	1.000
Intercept	1760.459	1	1760.459	13426.424	.000	13426.424	1.000
CONCENT	44.003	10	4.400	33.560	.000	335.598	1.000
EXTRACT	30.482	1	30.482	232.473	.000	232.473	1.000
CONCENT * EXTRACT	25.355	10	2.536	19.338	.000	193.376	1.000
Error	5.769	44	.131				
Total	1866.069	66					
Corrected Total	105.609	65					

a Computed using alpha = .05

b R Squared = .945 (Adjusted R Squared = .919)

Showing data analysis comparison of the effect of *Hyptis suaveolens*, *Lantana camara* and combination extract at various concentrations on hatching of *Aedes aegypti* within 24 hours.

Between-Subjects Factors

		Value Label	N
CONCENT	1	0%	9
	2	10%	9
	3	20%	9
	4	30%	9
	5	40%	9
	6	50%	9
EXTRACT	1	Mixed	18
	2	Hyptis	18
	3	Lantana	18

Descriptive Statistics

Dependent Variable: MORTALITY

CONCENT	EXTRACT	Mean	Std. Deviation	N
0%	Mixed	4.4617	.3049	3
	Hyptis	4.4767	1.528E-02	3
	Lantana	4.4553	.2014	3
	Total	4.4646	.1831	9
10%	Mixed	4.3543	.5846	3
	Hyptis	4.3400	.6199	3
	Lantana	4.3350	.1525	3
	Total	4.3431	.4329	9
20%	Mixed	4.7347	.4079	3
	Hyptis	4.5033	.4818	3
	Lantana	4.4097	.4360	3
	Total	4.5492	.4101	9
30%	Mixed	4.7560	.6837	3
	Hyptis	4.6267	.2139	3
	Lantana	4.2843	.1433	3
	Total	4.5557	.4219	9
40%	Mixed	5.2083	.3201	3
	Hyptis	5.1467	.2928	3
	Lantana	4.2607	.4199	3
	Total	4.8719	.5495	9

CONCENT	EXTRACT	Mean	Std. Deviation	N
50%	Mixed	6.2543	.4524	3
	Hyptis	5.2200	.3639	3
	Lantana	4.3260	.2059	3
	Total	5.2668	.8907	9
Total	Mixed	4.9616	.7702	18
	Hyptis	4.7189	.4750	18
	Lantana	4.3452	.2508	18
	Total	4.6752	.5902	54

Tests of Between-Subjects Effects

Dependent Variable: MORTALITY

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power
Corrected Model	12.961	17	.762	4.991	.000	84.854	1.000
Intercept	1180.307	1	1180.307	7727.168	.000	7727.168	1.000
CONCENT	5.161	5	1.032	6.758	.000	33.789	.994
EXTRACT	3.471	2	1.735	11.362	.000	22.723	.988
CONCENT * EXTRACT	4.329	10	.433	2.834	.011	28.342	.927
Error	5.499	36	.153				
Total	1198.767	54					
Corrected Total	18.460	53					

a Computed using alpha = .05

b R Squared = .702 (Adjusted R Squared = .561)

Showing data analysis comparison of percent mortality of adults between *Hyptis suaveolens* and *Lantana camara* extracts on *Aedes aegypti*.

Between-Subjects Factors

		Value Label	N
CONCENT	1	0%	6
	2	10%	6
	3	20%	6
	4	30%	6
	5	40%	6
	6	50%	6
	7	60%	6
	8	70%	6
	9	80%	6
	10	90%	6
	11	100%	6
EXTRACT	1	Hyptis	33
	2	Lantana	33

Descriptive Statistics

Dependent Variable: MORTALITY

CONCENT	EXTRACT	Mean	Std. Deviation	N
0%	Hyptis	.0000	.0000	3
	Lantana	.0000	.0000	3
	Total	.0000	.0000	6
10%	Hyptis	.0000	.0000	3
	Lantana	.0000	.0000	3
	Total	.0000	.0000	6
20%	Hyptis	2.6667	1.1547	3
	Lantana	.0000	.0000	3
	Total	1.3333	1.6330	6
30%	Hyptis	4.0000	.0000	3
	Lantana	.0000	.0000	3
	Total	2.0000	2.1909	6
40%	Hyptis	6.6667	1.1547	3
	Lantana	1.3333	1.1547	3
	Total	4.0000	3.0984	6
50%	Hyptis	10.6667	1.1547	3
	Lantana	2.6667	1.1547	3
	Total	6.6667	4.5019	6

CONCENT	EXTRACT	Mean	Std. Deviation	N
60%	Hyptis	15.3333	1.1547	3
	Lantana	4.0000	.0000	3
	Total	9.6667	6.2503	6
70%	Hyptis	19.3333	2.3094	3
	Lantana	6.6667	1.1547	3
	Total	13.0000	7.1274	6
80%	Hyptis	23.3333	1.1547	3
	Lantana	9.3333	1.1547	3
	Total	16.3333	7.7374	6
90%	Hyptis	27.3333	1.1547	3
	Lantana	12.6667	2.3094	3
	Total	20.0000	8.1976	6
100%	Hyptis	31.3333	3.0551	3
	Lantana	16.6667	1.1547	3
	Total	24.0000	8.2946	6
Total	Hyptis	12.7879	10.9538	33
	Lantana	4.8485	5.6796	33
	Total	8.8182	9.5368	66

Tests of Between-Subjects Effects

Dependent Variable: MORTALITY

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power
Corrected Model	5842.485	21	278.214	176.559	.000	3707.731	1.000
Intercept	5132.182	1	5132.182	3256.962	.000	3256.962	1.000
CONCENT	4296.485	10	429.648	272.662	.000	2726.615	1.000
EXTRACT	1040.061	1	1040.061	660.038	.000	660.038	1.000
CONCENT * EXTRACT	505.939	10	50.594	32.108	.000	321.077	1.000
Error	69.333	44	1.576				
Total	11044.000	66					
Corrected Total	5911.818	65					

a Computed using alpha = .05

b R Squared = .988 (Adjusted R Squared = .983)

Showing data analysis comparison of percent mortality of adults between *Hyptis suaveolens, Lantana camara* extracts and extract combination on *Aedes aegypti.*

Between-Subjects Factors

		Value Label	N
CONCENT	1	0%	9
	2	10%	9
	3	20%	9
	4	30%	9
	5	40%	9
	6	50%	9
EXTRACT	1	Mixed	18
	2	Hyptis	18
	3	Lantana	18

Descriptive Statistics

Dependent Variable: MORTALITY

CONCENT	EXTRACT	Mean	Std. Deviation	N
0%	Mixed	.0000	.0000	3
	Hyptis	.0000	.0000	3
	Lantana	.0000	.0000	3
	Total	.0000	.0000	9
10%	Mixed	.6667	1.1547	3
	Hyptis	.0000	.0000	3
	Lantana	.0000	.0000	3
	Total	.2222	.6667	9
20%	Mixed	3.3333	1.1547	3
	Hyptis	2.6667	1.1547	3
	Lantana	.0000	.0000	3
	Total	2.0000	1.7321	9
30%	Mixed	8.6667	1.1547	3
	Hyptis	4.0000	.0000	3
	Lantana	.0000	.0000	3
	Total	4.2222	3.8006	9
40%	Mixed	12.6667	1.1547	3
	Hyptis	6.6667	1.1547	3
	Lantana	1.3333	1.1547	3
	Total	6.8889	5.0111	9

CONCENT	EXTRACT	Mean	Std. Deviation	N
50%	Mixed	20.6667	2.3094	3
	Hyptis	10.6667	1.1547	3
	Lantana	2.6667	1.1547	3
	Total	11.3333	7.9373	9
Total	Mixed	7.6667	7.6158	18
	Hyptis	4.0000	3.9407	18
	Lantana	.6667	1.1882	18
	Total	4.1111	5.6890	54

Tests of Between-Subjects Effects

Dependent Variable: MORTALITY

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power
Corrected Model	1680.667	17	98.863	102.665	.000	1745.308	1.000
Intercept	912.667	1	912.667	947.769	.000	947.769	1.000
CONCENT	867.333	5	173.467	180.138	.000	900.692	1.000
EXTRACT	441.333	2	220.667	229.154	.000	458.308	1.000
CONCENT * EXTRACT	372.000	10	37.200	38.631	.000	386.308	1.000
Error	34.667	36	.963				
Total	2628.000	54					
Corrected Total	1715.333	53					

a Computed using alpha = .05

b R Squared = .980 (Adjusted R Squared = .970)

APPENDIX C

DATA ANALYSIS LC_{50} TESTS BY PROBIT ANALYSIS

DATA ANALYSIS LC_{50}

TESTS BY PROBIT ANALYSIS

Showing data analysis LC_{50} of *Hyptis suaveolens* on *Aedes aegypti* second instar larvae at 48 hours.

*** * * * * * * * * * * * P R O B I T A N A L Y S I S * * * * * * * * * * * ***

Confidence Limits for Effective CONCEN

95% Confidence Limits

Prob	CONCEN	Lower	Upper
.01	-8.47123	-16.89735	-2.96404
.02	-5.10719	-12.60974	-.14627
.03	-2.97281	-9.90107	1.65320
.04	-1.36720	-7.87110	3.01452
.05	-.06116	-6.22570	4.12768
.06	1.05048	-4.83001	5.07995
.07	2.02518	-3.61042	5.91906
.08	2.89790	-2.52212	6.67409

Prob	CONCEN	Lower	Upper
.09	3.69161	-1.53575	7.36415
.10	4.42221	-.63093	8.00249
.15	7.44713	3.07628	10.68436
.20	9.85123	5.96455	12.87393
.25	11.91374	8.38729	14.80753
.30	13.76594	10.50872	16.59822
.35	15.48227	12.42076	18.31134
.40	17.11091	14.18222	19.98981
.45	18.68663	15.83527	21.66493
.50	20.23737	17.41349	23.36211
.55	21.78811	18.94627	25.10473
.60	23.36383	20.46177	26.91740
.65	24.99247	21.98955	28.82955
.70	26.70881	23.56387	30.88039
.75	28.56100	25.22924	33.12715
.80	30.62351	27.05115	35.66158
.85	33.02761	29.14150	38.64906
.90	36.05253	31.73408	42.44556
.91	36.78314	32.35538	43.36742
.92	37.57684	33.02853	44.37070
.93	38.44957	33.76671	45.47584
.94	39.42426	34.58891	46.71235

Prob	CONCEN	Lower	Upper
.95	40.53591	35.52405	48.12518
.96	41.84194	36.61959	49.78820
.97	43.44755	37.96236	51.83672
.98	45.58193	39.74145	54.56576
.99	48.94598	42.53454	58.87805

Showing data analysis LC_{50} of *Lantana camara* on *Aedes aegypti* second instar larvae at 48 hours.

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Confidence Limits for Effective CONCEN

95% Confidence Limits

Prob	CONCEN	Lower	Upper
.01	-30.04249	-44.01484	-19.18765
.02	-17.79990	-30.00265	-8.28116
.03	-10.03237	-21.12754	-1.34618
.04	-4.18916	-14.46164	3.88124
.05	.56384	-9.04785	8.14175
.06	4.60939	-4.44711	11.77535

Prob	CONCEN	Lower	Upper
.07	8.15655	-.41968	14.96784
.08	11.33260	3.18032	17.83241
.09	14.22110	6.44860	20.44340
.10	16.87996	9.45147	22.85240
.15	27.88837	21.80843	32.90205
.20	36.63751	31.49984	41.01871
.25	44.14349	39.66863	48.12765
.30	50.88409	46.84129	54.67487
.35	57.13026	53.31455	60.91512
.40	63.05727	59.28814	67.00540
.45	68.79171	64.91750	73.04798
.50	74.43524	70.33365	79.11872
.55	80.07877	75.65193	85.28733
.60	85.81322	80.97974	91.63146
.65	91.74022	86.42672	98.24836
.70	97.98639	92.11898	105.26960
.75	104.72700	98.22172	112.88674
.80	112.23297	104.98219	121.40401
.85	120.98211	112.82911	131.36515
.90	131.99052	122.66730	143.93356
.91	134.64938	125.03915	146.97359
.92	137.53788	127.61426	150.27775

Prob	CONCEN	Lower	Upper
.93	140.71393	130.44402	153.91256
.94	144.26109	133.60251	157.97399
.95	148.30664	137.20257	162.60827
.96	153.05964	141.42955	168.05559
.97	158.90285	146.62270	174.75576
.98	166.67038	153.52122	183.66733
.99	178.91297	164.38513	197.72210

Showing data analysis LC_{50} of extract combination on *Aedes aegypti* second instar larvae at 48 hours.

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Confidence Limits for Effective CONCEN

95% Confidence Limits

Prob	CONCEN	Lower	Upper
.01	-7.38210	-29.61443	.86578
.02	-4.87230	-24.82126	2.65878
.03	-3.27991	-21.79348	3.80973

Prob	CONCEN	Lower	Upper
.04	-2.08201	-19.52439	4.68413
.05	-1.10762	-17.68514	5.40186
.06	-.27826	-16.12494	6.01805
.07	.44893	-14.76149	6.56288
.08	1.10004	-13.54473	7.05475
.09	1.69220	-12.44180	7.50576
.10	2.23728	-11.42997	7.92432
.15	4.49407	-7.28303	9.69960
.20	6.28769	-4.05109	11.17446
.25	7.82646	-1.34146	12.50285
.30	9.20833	1.02558	13.76206
.35	10.48883	3.14701	15.00090
.40	11.70390	5.08059	16.25589
.45	12.87949	6.86351	17.55796
.50	14.03645	8.52199	18.93554
.55	15.19341	10.07722	20.41637
.60	16.36900	11.54952	22.02905
.65	17.58407	12.96141	23.80573
.70	18.86458	14.34027	25.78715
.75	20.24644	15.72151	28.03216
.80	21.78521	17.15483	30.63685
.85	23.57883	18.71977	33.77871

Prob	CONCEN	Lower	Upper
.90	25.83562	20.57362	37.84709
.91	26.38070	21.00691	38.84419
.92	26.97286	21.47245	39.93259
.93	27.62397	21.97871	41.13496
.94	28.35116	22.53791	42.48403
.95	29.18052	23.16859	44.02975
.96	30.15492	23.90113	45.85419
.97	31.35281	24.79103	48.10778
.98	32.94520	25.95889	51.11865
.99	35.45500	27.77224	55.89148

CURRICULUM VITAE

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