Design and Evaluation of an Insect Gage for Studying the Potentiality of Commercial Mosquito-Repellent Products

AbdAlkhalig Hassan Mohamed Siddig
B.Sc. (Hon.) in Pharmacy, Faculty of Pharmacy, University of Gezira, 2005

A Dissertation

Submitted to University of Gezira in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in

Biosciences and Biotechnology (Biotechnology)
Center of Biosciences and Biotechnology
Faculty of Engineering and Technology

June 2015
Design and Evaluation of an Insect Gage for Studying the Potentiality of Commercial Mosquito-Repellent Products

AbdAlkhalig Hassan Mohamed Siddig

Supervision Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Mutaman Ali Kehail</td>
<td>Supervisor</td>
<td>……………..</td>
</tr>
<tr>
<td>Dr. YasirMohamedAbdelrahim</td>
<td>Co-Supervisor</td>
<td>……………..</td>
</tr>
</tbody>
</table>

Date: June, 2015
Design and Evaluation of an Insect Gage for Studying the Potentiality of Commercial Mosquito-Repellent Products

AbdAlkhalig Hassan Mohamed Siddig

Examination Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Mutaman Ali Kehail</td>
<td>Chairperson</td>
<td>……………..</td>
</tr>
<tr>
<td>Prof. Elnaeim Abd Allah Ali</td>
<td>External Examiner</td>
<td>……………..</td>
</tr>
<tr>
<td>Dr. Abdalla Ibrahim Abdalla</td>
<td>Internal Examiner</td>
<td>……………..</td>
</tr>
</tbody>
</table>

Date: 25, June, 2015
Design and evaluation of insect cage for studying the commercial mosquito-repellent products

AbdAlkhalig Hassan Mohamed Siddig

Abstract

The mosquito repellent products are those products which has the ability to orienting the movement of the adult mosquito away from the source. These products are usually used for protection and not for killing mosquitoes. The aim of this study was to design and evaluation an insect cage prepared for studying the repellency of the commercial adult-insect-repellent products. Two commercial mosquito-products (Active and Lavanda-herbal) were brought from the popular pharmacy, Wad Medani Town, also the metal experimental cage was design as an upside down T-shape (1.5 m for each part from the cage center) and it was covered with a thick cloth from the back and a clear cloth from the front, so as to facilitate the measurements of horizontal and vertical repells of the adult mosquitoes. A thin layer chromatography test was run in this study, in addition to measuring the time required for one gram of the repellent product dispersed in one square inch to dissipate completely. Some of the laboratory reared Anopheles and Culex adult mosquitoes were put in the center of the cage with a dish containing one gram of the repellent product so as to measure its repellent efficiency. The distance of the orientation of the adult mosquito at the horizontal and vertical levels after the repellent product was put was measured for 6 hours with interval of 30 minutes. The results of volatility rate test revealed that, the Active product has a volatility rate make it efficient for 7:30 hours, with 2.8 mg volatility in one square inch/minute, while the least distance for the horizontal orientation was 24 cm and 42 cm for the vertical repellent, whereas the efficiency of the violet Lavanda product lasted for 6:40 hours and also with 3.2 mg volatility in one square inch/minute, with 81 cm for the horizontal orientation and 53 cm for the vertical one.

The TLC test showed a clear difference in the number and types of the active ingredients for the two products. The design and the tests helped to understand the behavior of mosquitoes toward the repellent products. This study recommend to applied the required and the constructive modifications on the design and the tests so as to facilitate running similar studies on all commercial mosquito-repellent products available at the local markets.
تصميم وتقييم قفص حشرات لدراسة المنتجات التجارية الطاردة للبعوض

عبد الخالق حسن محمد صديق

المتخصصة

المنتجات الطاردة للبعوض هي تلك المركبات التي لها القدرة على توجيه حركة بالغات البعوض بعيداً عن المكان الذي وضعت فيه. تستخدم هذه المنتجات عادة للحماية وليس لقتل البعوض. هدف البحث لتصميم وتقييم قفص حشرات معتمد للبحث لدراسة المقدرة الطاردة للمنتجات التجارية للحشرات البالغة. تم إحضار عينتين تجارية من طاردات البعوض (أكتيف ولافاندا أعشاب) من الصيدلية الشعبية، مدينة ود مدني. كما تم تصميم قفص التجربة المعدني والمكسو بقماش سميك من الخلف وشفاف من الأمام وعلى شكل حرف T الإنجليزية مقوب (مقياس 1.5 متر لكل ضلع من مركز القفص) ليسهل عملية حساب الطرد الأفقي والرأسي للبعوض البالغ. تم في هذه الدراسة إجراء اختبارات إختيار كرومتوغرافيا الطبقة الريخية، حساب الزمن الذي يأخذه واحد جرام موزع على مساحة واحدة وبوصة الممتدة من هذه المادة تبتكر مادتها النفاذة كلياً. تم تربية بالغات من بعوض الأنزولس وأخري من جنس الكيولكس عملياً وتم وضع عدد من هذه البالغات في مركز القفص مع طبق به واحد جرام من المادة المراد قياس فعاليتها الطاردة. تم حساب المسافة التي ابتعدتها بالغات البعوض على المستوى الأفقي والرأسي بعد وضع المادة الفعالة لمدة 5 ساعات يفصل 30 دقيقة لكل قراءة. أوضحت نتائج تحليل معدل طباطس المواد الطاردة أن معدل تعاطي منتج أكتيف يجعله فعالاً لمدة 5:55 ساعة، مع نسبة تعاطية 2.8 ملغ في البوصة المربعة/ دقيقة، بينما كانت أقل مسافة للإحالة الأفقية هي 24 سم و 42 سم على المستوى الرئيسي بينما استمرت فعالية منتج بنفسج لافتانا إلى 5:12 ساعة. وأيضاً مع نسبة تعاطية 3.2 ملغ في البوصة المربعة/ دقيقة. بينما كانت أقل مسافة للإحالة الأفقية هي 81 سم و 53 سم على المستوى الرئيسي. أوضح اختبار كرومتوغرافيا الطبقة الريخية اختلاف واضح في عدد وتنوع الكمونات الفعالة للمنتجين. ساعد التصميم والإختيارات لفهم سلوك البعوض تجاه المنتجات الطاردة. أوصى البحث بإجراء التعديلات اللازمة والبناء على التصميم والاختيارات لتسهيل أجراء دراسات مماثلة على كل المنتجات التجارية للبعوض الموجودة بالسوق المحلي.
<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>Iii</td>
</tr>
<tr>
<td>Abstract</td>
<td>V</td>
</tr>
<tr>
<td>Arabic Abstract</td>
<td>Vi</td>
</tr>
<tr>
<td>List of Contents</td>
<td>Vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>IX</td>
</tr>
<tr>
<td>List of Plates</td>
<td>X</td>
</tr>
<tr>
<td><strong>Chapter One: Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Chapter Two: Literature Review</strong></td>
<td>3</td>
</tr>
<tr>
<td>2.1 Mosquitoes</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2 Life Cycle</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2.1 Eggs</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2.2 Larvae</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2.3 Pupae</td>
<td>4</td>
</tr>
<tr>
<td>2.1.2.4 Adults</td>
<td>4</td>
</tr>
<tr>
<td>2.1.4. Control</td>
<td>5</td>
</tr>
<tr>
<td>2.1.4.1 Larval Stage:</td>
<td>5</td>
</tr>
<tr>
<td>2.1.4.2. Adult Stage:</td>
<td>5</td>
</tr>
<tr>
<td>2.1.4.3. Natural Products</td>
<td>6</td>
</tr>
<tr>
<td>2.1.4.4. Other Methods</td>
<td>6</td>
</tr>
<tr>
<td>2.2. Mosquito repellents</td>
<td>7</td>
</tr>
<tr>
<td>2.2.1. DEET product</td>
<td>7</td>
</tr>
<tr>
<td>2.2.2. The mosquito-repellent products used in this study</td>
<td>8</td>
</tr>
<tr>
<td>2.2.2.1. Lavenda:</td>
<td>8</td>
</tr>
<tr>
<td>2.2.2.2. Active product</td>
<td>8</td>
</tr>
<tr>
<td>2.3. Repellent test cages:</td>
<td>9</td>
</tr>
<tr>
<td><strong>Chapter Three: Materials and Methods</strong></td>
<td>13</td>
</tr>
<tr>
<td>3.1 Materials</td>
<td>13</td>
</tr>
<tr>
<td>3.2 Methods</td>
<td>13</td>
</tr>
<tr>
<td>3.2.1 Repellency potentiality</td>
<td>13</td>
</tr>
<tr>
<td>3.2.2. The repellent products characteristics</td>
<td>13</td>
</tr>
</tbody>
</table>
3.2.2.1. pH
3.2.2.2 Percentage moisture and volatile matters
3.2.2.4 volatility rate
3.2.2.5 Polar and apolar constituents
3.2.2.6 Thin layer chromatography (TLC) test

3.3. Statistical analysis

Chapter Four: Results and Discussion
4.1 The volatility rate of the mosquito-repellent products
4.2 The moisture and volatile contents of mosquito-repellent products
4.3 The Apolar contents in Active and Lavenda repellent products
4.4 The repellency potentiality of Active and Lavender Products
4.5 Thin layer chromatography of Active and Lavenda products

Chapter Five: Conclusions and Recommendations
5.1 Conclusions
5.2 Recommendations

References

List of Tables

<table>
<thead>
<tr>
<th>Table No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>The rate of volatility (g) of Active and Lavenda products per minutes per onesquare inch</td>
<td>17</td>
</tr>
<tr>
<td>4.2</td>
<td>The moisture and volatile contents (%) of Active and Lavenda products</td>
<td>19</td>
</tr>
<tr>
<td>4.3</td>
<td>Apolar (lipophilic) contents in Active and Lavenda products</td>
<td>21</td>
</tr>
<tr>
<td>4.4</td>
<td>The least vertical and horizontal orientation distance (cm) of Anopheles and Culex mosquito in respect to Active and Lavenda products</td>
<td>23</td>
</tr>
<tr>
<td>4.5</td>
<td>Active ingredients in the Active and Lavenda products</td>
<td>25</td>
</tr>
</tbody>
</table>
## List of Plates

<table>
<thead>
<tr>
<th>Plate No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Some repellent cage designs</td>
<td>10</td>
</tr>
</tbody>
</table>
Chapter One
Introduction

There are about 3,500 species of mosquitoes found throughout the world. About 380 species of Anopheles were recorded. In some species of mosquito, the females feed on humans, and are therefore vectors for a number of infectious diseases affecting millions of people per year. Some scientists believe that eradicating mosquitoes would not have serious consequences (Mda.state, 2008).

Males live for about a week, feeding on nectar and other sources of sugar. Females will also feed on sugar sources for energy but usually require a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed (usually takes 2-3 days in tropical conditions). Once the eggs are fully developed, the female lays them and resumes host seeking. The cycle repeats itself until the female dies; while females can live longer than a month in captivity, most do not live longer than 1-2 weeks in nature. Their lifespan depends on temperature, humidity, and also their ability to successfully obtain a blood meal while avoiding host defenses (CDC, 2004).

Mosquitoes transmit the most serious diseases in the Sudan, special in Gezira, because the habitats in Gezira are suitable for breeding of mosquitoes all around the year. Mosquitoes also were suggested to be involved in some diseases other than yellow fever and malaria, therefore the control of mosquitoes means more than the control of malaria and nuisances. In Gezira, insecticides were applied for many years for controlling mosquitoes and other agricultural insects. Insecticides are very expensive but effective and can be easily applied (Wayne, 1989).

Adult mosquito control may be undertaken through adulticides, genetic control, natural products, natural predators, sterility of mosquitoes by radiation, environmental...
management (reduction of the breeding sites), bed nets (impregnated mosquito bed-nets), traps (chemical or physical traps) and repellents, e.g. DEET.

Mosquito repellents usually do not kill mosquitoes. If it applied on the body, they prevent mosquitoes from biting for a period of time. How long repellents stay effective depends on the nature of the chemicals present and how humid the weather is. Repellents could be made from chemicals, such as DEET and N,N-Diethylbenzamide, made from plants, such as citronella, neem, peppermint or eucalyptus, or made with plants such as marigold, Thai lemongrass and catnip, which ward off mosquitoes (Baby Center, 2013).

**The objective of this study**

The aim of this study was to design and evaluation an insect cage prepared for studying the repellency of the commercial adult insect-repellent products (Active and Lavanda-herbal).
CHAPTER TWO
LITERATURE REVIEW

2.1 Mosquitoes

Mosquito and mosquita (from the Spanish meaning little fly) is a common insect in the family culicidae. Mosquitoes resemble crane flies (family:Tipulidae) and chironomid flies (family:chironomidae), with which they are sometimes confused by the casual observer (Molavi, 2003).

Many species are native to tropical and subtropical regions, such as Aedes have successfully adapted to cooler regions. In the warm and humid tropical regions, they are active the entire year long; however, in temperate regions they hibernate over winter. In addition, adults can survive throughout winter in suitable microhabitats (Romiet al., 2006).

2.1.2 Life Cycle

2.1.2.1 Eggs

The eggs are about 0.5 mm long and typically boat-shaped, in nearly all species, the egg is provided with a pair of air-filled, lateral floats that allow it to float on the water surface. The egg is covered with a grey-black exochorion, the patterning of which is sometimes useful for identification of species. The collections of water in which adult lay their eggs, and which provide the larval and pupal habitat, are commonly known as breeding sites (Daivid and Herbert, 2002).

Adult females lay 50-200 eggs per oviposition. Eggs are laid single or in cluster on water and are unique in having floats on either side. Eggs are not resistant to drying and hatch within 2-3 days (CDC, 2004).

2.1.2.2 Larvae

Eggs hatch within 48 hours in tropical conditions. The emerging larvae feed on bacteria, yeast, protozoa and other microorganisms as well as on particulate organic matter in the water, all of which were filtered out with their mouth brushes. Larvae move actively with a wriggling motion. Growth takes place through a series of moults, during which the larvae pass through four larval instars.

The main body of the larva is white to pale brown in colour, contrasting with the dark brown head capsule, which bears a pair of eyes and the conspicuous mouth brushes used to sweep food particles into the mouth. Thorax has several groups of long hairs that are useful in species identification. Abdomen composed of 10 segments, but only nine are visible. The first six to seven segments usually have a pair of palmate
hairs or float-hairs on the dorsal surface; segments one to eight may also carry a dark, oval tergal plate and in some species, small accessory tergal plates and featherlike, lateral hairs. The penultimate visible segment has a pair of round spiracular openings to the respiratory system, allowing air to be breathed when the larva at the water surface. This is the most obvious diagnostic characteristic, distinguishing anophlelines from other mosquitoes, which carry their spiracular openings at the tip of a prominent respiratory tube, the siphon (Daivid and Herbert, 2002). Prior to pupation, the fourth instar larva ceases to feed, becomes quiescent and then moults to become the pupa.

2.1.2.3 Pupae

The pupa is a comma-shaped and differs in appearance from the larvae. The anterior part consists of fused head on thorax, the cephalothorax, on the dorsal surface of which is a pair of short, epically flared respiratory trumpets. The abdomen has eight freely moveable, visible segments, which terminate in a pair of oval, flattened paddles. Anopheline pupae differ from those of other mosquitoes by having, in addition to various fine hairs, a short peg-like hair at the posterior corners of most abdominal segments, but their identification to species is usually impossible.

Pupae do not feed during their brief life of some 2-4 days (in the tropics). They remain at the water surface, taking in air through their trumpets, unless disturbed, when they swim down to bottom with characteristic jerky movements, just prior to adult emergence, the pupa becomes quiescent at the water surface, the upper part of the integument then splits open and the adult emerges to rest briefly at the water surface before taking to flight. After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges (CDC, 2004).

2.1.2.4 Adults

The adults are the stages most frequently encountered and carry a number of morphological features useful in diagnosis. The head bears a pair of prominent compound eyes and a pair of filamentous, segmented antennae, which are plumose in the males but pilose in the females. Antennae are also involved in sound detection and probably assist males in locating females. The thorax has three pairs of legs, there is a single pair of wings, and the hind wings being represented by a small pair of drumstick-shaped halters. The wings of all mosquitoes have a characteristic arrangement of veins covered with small pale and dark scales.
The abdomen has eight visible segments, which unlike those of culicine mosquitoes, are mostly devoid of scales. The last segment terminates in a pair of small finger-like cerci in females, and a pair of prominent claspers in males that are used to seize females during mating. Most anopheline adults rest with an inclined angle to the surface on which they stand, whereas culicine mosquitoes stand with the abdomen more or less parallel to the surface (Daivid and Herbert, 2002).

The duration from egg to adult varies considerably among species and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as short as 5 days but usually take 10-14 days in tropical conditions. Like all mosquitoes, adult *Anopheles* have slender bodies with 3 sections: head, thorax and abdomen (CDC, 2004).

Most medically important species of mosquitoes have close similarities to other species, which do not bite man. Specific identification may depend on minute features of the male, the larvae, or even the egg, often making it unreliable to identify individual female specimens. DNA barcoding is a technique for assigning specimens to species or other taxonomic units based on their DNA sequence in standard marker (Gesmallah, 2009).

2.1.4. Control

2.1.4.1 Larval Stage:

Mosquito larvae can be controlled by using: bacterial insecticides, e.g.: *Bacillus thuringiensis* (Eliass, 2010) and *B. sphaericus*; insect growth inhibitor, e.g.: methoprene; aquatic predators, e.g. *Gambusia* fish (Mohammed Nor, 2010); temperature and electric shocks (Nour, 2009; Abdallatif, 2009; and Ali, 2009); radiation, e.g. x-ray (Abo Agarib, 2009), and chemical insecticides, e.g.: temephos (Kehail, 1996). Other materials, e.g.: mineral oils, monomolecular films oils and films dispersed as a thin layer on the surface of the water, which cause larvae and pupae to drown.

2.1.4.2. Adult Stage:

Adult mosquito control may be undertaken to combat an outbreak of mosquito-borne disease or a very heavy nuisance infestation of mosquitoes in a community. Insecticides registered for this purposes are known as adulticides and are applied either by aircraft or on the ground employing truck-mounted sprayers. Adulticides, e.g. fenitrothion, that were used for public health mosquito control
programs in the Gezira area, were well reviewed by, El Safi (1994) and El Safi and Haridi (1986).

Genetic control (manipulation) appears, in theory at least, to be boundless with large protective potential exhibited by mosquitoes. Successful genetic control will be difficult on a large scale, but possible in isolated populations. More information was reviewed by Abo Agarib (2009).

2.2. Natural Products

A natural product is a chemical compound or substance produced by a living organism – found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. A natural product can be considered as such even if it can be prepared by total synthesis.

Not all natural products can be fully synthesized and many natural products have very complex structures that are too difficult and expensive to synthesize on an industrial scale. These include drugs such as penicillin, morphine, and paclitaxel (taxol). Such compounds can only be harvested from their natural source – a process which can be tedious, time consuming, and expensive, as well as being wasteful on the natural resource. The number of structural analogues that can be obtained from harvesting is severely limited (Meenakshiet al., 2005).

Efforts are directed towards finding some natural products alternatives than the use of conventional insecticides. The uses of some natural products against mosquitoes have been discussed by several scientists (e.g. AbdAldafae, 2009; Kehail, 2004; Elsayed, 1992; Zarrough, et al., 1990, and Koul, 1988).

2.1.4.3. Other Methods

1- Sterility of mosquitoes by radiation (Abo Agarib, 2009).
2- Environmental management (reduction of the breeding sites).
3- Bed nets (impregnated mosquito bed-nets).
4- Traps (chemical or physical traps).
5. Natural predators (CDC, 2004 and Mohammed Nor, 2010).
7- Repellents, e.g. DEET (Najerar and Zaim, 2008).

2.2. Mosquito repellents

Mosquito repellents usually do not kill mosquitoes. If it applied on the body, they prevent mosquitoes from biting for a period of time. How long repellents stay
effective depends on the nature of the chemicals present and how humid the weather is. Repellents could be made from chemicals, such as DEET and N,N,Diethylbenzamide, made from plants, such as citronella, neem, peppermint or eucalyptus, or made with plants such as marigold, Thai lemongrass and catnip, which ward off mosquitoes.

Repellents made from chemicals and plants are available in the form of creams, lotions, sprays, wipes, roll-on sticks, foams, liquid vapourisers, sprays, coils and mats. Electronic repellents are also widely available. These use ultrasound and electromagnetic waves to repel mosquitoes and other insects. Baby Center Expert advises that it is safer to use mosquito repellent creams and lotions only on babies over six months old. The chemicals in the repellents could be harmful for younger babies. They may cause an allergic reaction on your baby's delicate skin. For babies younger than six months, it's better to use baby mosquito nets and natural or herbal remedies (Baby Center, 2013).

In many Indian homes, neem leaves are burnt as the smoke that is produced helps to drive away mosquitoes. Essential oils of neem, citronella, peppermint, and eucalyptus are all known to be effective mosquito repellents. They are strong and known to cause allergies on sensitive skin. A few drops of essential oils on the bedding will be sufficient. Other plants were also tested (Achim, 2010; Akhtar, 2008; Akhtar and Yazan, 2008).

2.2.1. DEET product

N,N-Diethyl-m-toluamide, also known as DEET or m-DET, is an aromatic amide that is an effective insect repellent for control of biting flies, biting midges, black flies, chiggers, deer flies, fleas, gnats, horse flies, mosquitoes, no-see-ums, sand flies, small flying insects, stable flies, and ticks. DEET was first developed by the U.S. Department of Agriculture for military use in 1946 and was first registered in the United States in 1957. It has been estimated that approximately 38 percent of the U.S. population uses DEET-containing repellents annually (Veltri and Osimitz, 1994). In 1998, 225 DEET products were registered with the EPA; and have DEET concentrations ranging from approximately 4 % to 100 % (USEPA, 1998).

Technical DEET is composed of more than 95 percent m-DET isomers. Ortho (o-DET) and para (p-DET) isomers are slightly more and less toxic than m-DET, respectively (Ambrose and Yost, 1965).

2.2.2. The mosquito-repellent products used in this study
2.2.2.1. Lavenda:

Lavender oil is one of the most popular and most used oils in the practice of aromatherapy. It is popular because it is believed to have a number of healing properties and is gentle in its actions. A number of lavender oils are available, so make sure that you know which type of lavender oil you are using. Lavender oil can be used as a bug repellent and applied in a couple of ways. Consult a qualified health professional before using lavender oil as a bug repellent. Lavender oil is believed to possess a number of therapeutic properties. Lawless writes that lavender is analgesic, insecticidal, antiseptic and calming. These particular healing properties of lavender are suitable in both preventing insect bites and soothing insect bites and stings. Lavender oil has been used as a bug repellent for many centuries. Lavender was used in the past to protect clothes and linens from the infestation of moths and other insects. Davis advises to use lavender oil to prevent bites from mosquitoes and other biting insects. Lavender oil also prevents the spread of infection caused by a bug bite and controls the itching and inflammation often associated with bug bites. Lavender oil is used in a lotion base. Although lavender oil is one of the few essential oils that can be applied to the skin undiluted, it is not advisable to do so without consulting a qualified health professional. It is advises mixing lavender oil with grapefruit or eucalyptus oil to use as a bug repellent.

Lavender oil is, in general, gentle, nontoxic and non sensitizing oil. It is suitable for use with children and the elderly. It can also be used in pregnancy. Before using lavender oil as a bug repellent, however, consult a qualified health care professional, as individual circumstances may differ (Sharon, 2013).

2.2.2.2. Active product

Appearance is white cream and it hasa pleasantodor. It can cause eye irritation (Avoid contact with eyes and lips). The routes of exposure are the eyes, skin, inhalation, ingestion. In the eyes it may cause moderate eye irritation, while it may cause skin reactions in rare cases. If this product is ingested it may cause abdominal discomfort, or cause irritation to mouth, throat and stomach. The aggravated medical condition is not known. The chemical composition of this product is deionized water, N,N-diethyl-m-toluamide and distearyldimethylammonium (SC Johnson and Son, 2014).
2.3. Repellent test cages:

Several cage designs for testing repellency were proposed. The variations between these cages depended on the study purposes, the repellent product, available materials, target organism and its size (Plate, 2.1).
Plate (2.1): Some repellent cage designs

Pest stop fox cage

Deet repellent cage

Comed-brossegrande pinkies
Cage for mosquitoes

Plate (2.1): Continue ...

For Screening Resistant

Rat-mosquito cage
Biogents AG: WHO Tube Tests

Plate (2.1): Continue ..
Chapter Three
Materials and Methods

3.1 Materials:

Two commercial mosquito-repellent products (Lavenda-herb and Active) were brought from some super markets of Wad Medani Town. All these products were supplied as small sac-containers of 20 g. Some of these samples were used for the physical tests and the repellency potentiality. Other samples were used to detect the volatility rate/inch²/minute and to detect the active ingredients in each polar and apolar extract of each sample.

The experimental cage was designed of a metal skeleton of three arms (150x20x20 cm for each arm) as an upside down T-shape. It was covered with a thick white cloth from the back, upper and lower parts and with a clear mosquito-net from the front, so as to facilitate monitoring and measurements of horizontal and vertical orientation of the adult mosquitoes. At the cage center, a sleeve was made so as to facilitate the move of adult mosquitoes and the tested product in and out.

3.2 Methods

3.2.1 Repellency potentiality

About 50 adults of the laboratory reared Anopheles and Culex mosquitoes were put in the center of the cage with a dish containing one gram of the repellent product. The adult mosquitoes were randomly distributed at the experimental cage whether a repellent product was put inside the cage or not, but the least distance recorded for each mosquito species from the cage center at the horizontal or vertical levels during the first 30 minutes from putting the repellent product in this test were recorded. The recorded "least distance" was monitored for 6 hours. The whole tests was run at the room temperature. The room-fan was put on the medium level so as to make an average model. The proposed cage was never put directly under the fan.

3.2.2 The repellent products characteristics

3.2.2.1 pH

pH was determined by using a pH-meter in which the sensors of the device were immersed into the cream sample for seconds. The pH-meter was immediately recalibrated by using a set of buffers before the second sample was read.

3.2.2.2 Percentage moisture and volatile matters
For moisture and volatiles content determination, a certain weight of each cream samples were taken in a flat-bottom dish, kept for 2 hours in a hot air oven at 105°C and weighed. The loss in weight was regarded as the moisture and volatile content which is usually expressed in percentage (AOAC, 1990), according to the equation:

\[
\text{Moisture and volatile content} \% = \frac{(W_1 - W_2) \times 100}{S}
\]

Where:

- \(W_1\): Weight of crucible and sample before drying
- \(W_2\): Weight of crucible and sample after drying
- \(S\): Original weight of the sample

### 3.2.2.4 Volatility rate

A set of plastic sheets of four inch\(^2\) were prepared. The weight of each plastic sheet was measured. Certain weight (the primary weight) of each mosquito-repellent product was smeared on these sheets. A sensitive digital balance was used in this test. The weight of each mosquito-repellent product in each pre-weighed sheet was measured each 30 minutes at the room temperature (27°C), for continuous 5.5 hours (330 minutes). The difference in weight reflected the volatility rate for each product. The data of weight obtained for each product in respect to time were used to estimate the volatility rate for each product per one square inch.

### 3.2.2.5 Polar and apolar constituents

Each of the semi-dried product (moisture and volatile-free) was used in this test. The polar and apolar percentages were done through two successive extractions (Kehail, 2004); the first by using hexane and the second by using ethanol. The detection of the polar and apolar contents in each sample will help to estimate the lipophilic contents. Considering the original weight of each product sample was \((W_1)\) and the dried weight of the filter paper that used to enrolled the sample was \((x)\), the sample weight before the first extraction was \((W_1 + x)\), and the dried weight after the first extraction was \((W_2 + x)\). Considering also the dried weight of the sample after the second extraction was \((W_3 + x)\). The polar and apolar were calculated from the following formulas:

\[
\% \text{ Apolar} = \frac{[(W_1 + x) - (W_2 + x)] \times 100}{(W_1)}
\]

\[
\% \text{ Polar} = \frac{[(W_2 + x) - (W_3 + x)] \times 100}{(W_1)}
\]

### 3.2.2.6 Thin layer chromatography (TLC) test
The TLC plates were made by mixing the adsorbent (silica gel), containing small amount of calcium sulphate (gypsum) as an inert binder, with twice the volume of distilled water. The mixture was spread as slurry on previously cleaned glass micro-plates (20×20 cm dimension) using a TLC spreader of 0.5 mm thick layer. The plates were set to be air dried at room temperature for 30 minutes and activated at 110°C in an oven for 30 minutes.

Samples of polar and apolar extracts of each repellent product were drawn with capillary tubes and spotted on stationary phase (pre-coated silica-gel plate) in a line at about 2 cm from the bottom. The TLC plates were developed in saturation chambers using in mobile phase solvent system composed of hexane: acetone in a ratio of 80:20 (by volume), respectively. Prior to development, the solvent was poured into chromatography tank, covered and allowed to saturate at room temperature (28°C ± 2°C) for 30 minutes. The plate loaded with the samples was then carefully placed into the chromatography tank. At the end of the chromatography development, the plate was removed out and left to dry at room temperature (Kotze and Eloff, 2002). The separated spots were visualized under Ultra-Violet light (UV265 nm). The plates were then exposed to iodine vapour. Distances between the spots and the end of the mobile phase were measured, and the retention factor ($R_f$) values were calculated and recorded according to the distance that the spot moves in respect to point that the solvent reached.

3.3. Statistical analysis

Microsoft Office, Excel 2007, was used to analyze the data obtained. Simple regression analysis was run to calculate the R-square (correlation coefficient), the regression coefficient (the rate of volatility (g)/minute) and the standard error for X variable (SE-X) and the standard error for Y variable (SE-Y).
Chapter Four
Results and Discussion

4.1 The volatility rate of the mosquito-repellent products

Table (4.1) showed the rate of volatility (g) of the tested mosquito-repellent products (Active and Lavenda products) in respect to time (minutes) per one square inch. The original weight of Active product was 2.06 g at the beginning of the first minute of the volatility rate test, and with the progress of the time the weight decreased respectively. The weight decreased to 1.22 g after 330 minutes (five and half hours), with 0.84 g (40.78%) loss from the original weight. The statistical analysis revealed that, the constant rate (X-coefficient) of weight loss (as a result of volatility) was 2.7 mg/minute/inch$^2$.

The original weight of Lavenda herbal product was 2.13g at the beginning of the first minute of the test. The weight decreased to 0.88 g after 330 minutes, with 1.25 g (58.69%) loss from the original weight. The statistical analysis revealed that, the constant rate of weight loss was 3.6 mg/minute/inch$^2$. It was clear that, Lavenda product has a high volatility rate (3.6 mg/minute) than Active product (2.7 mg/minute). The volatility rate affected greatly the time the product takes to protect persons from mosquito contact, if the effective constituents was the aroma.

Lavender was used in the past to protect clothes and linens from the infestation of moths and other insects. Lavender oil is, in general, gentle, nontoxic and non sensitizing oil. Before using lavender, however, consult a qualified health care professional, as individual circumstances may differ (Sharon, 2013).

The chemical composition of Active product is deionized water, N,N-diethyl-m-toluamide and distearyldimethylammonium, which are responsible for their volatility and repellent potentiality (SC Johnson and Son, 2014).
Table (4.1) The rate of volatility (g) of Active and Lavenda products per minutes per onesquare inch

<table>
<thead>
<tr>
<th>Time (minute)</th>
<th>Lavenda</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.13</td>
<td>2.06</td>
</tr>
<tr>
<td>30</td>
<td>1.99</td>
<td>1.97</td>
</tr>
<tr>
<td>60</td>
<td>1.89</td>
<td>1.90</td>
</tr>
<tr>
<td>90</td>
<td>1.80</td>
<td>1.83</td>
</tr>
<tr>
<td>120</td>
<td>1.68</td>
<td>1.73</td>
</tr>
<tr>
<td>150</td>
<td>1.60</td>
<td>1.65</td>
</tr>
<tr>
<td>230</td>
<td>1.37</td>
<td>1.44</td>
</tr>
<tr>
<td>260</td>
<td>1.27</td>
<td>1.35</td>
</tr>
<tr>
<td>330</td>
<td>0.88</td>
<td>1.22</td>
</tr>
</tbody>
</table>

\[
R^2 = 0.982 \quad 0.998
\]

Intercept

<table>
<thead>
<tr>
<th>X-coff.</th>
<th>SE-Y</th>
<th>SE-X</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.0036</td>
<td>0.031</td>
<td>0.0001</td>
</tr>
<tr>
<td>-0.0027</td>
<td>0.006</td>
<td>0.0004</td>
</tr>
</tbody>
</table>
4.2 The moisture and volatile contents of mosquito-repellent products

Table (4.2) showed the moisture and volatile contents (g) of the tested mosquito-repellent products (Active and Lavenda products). The original weight of Active product was 19.34 g. After 2 hours in a hot air oven at 105°C the weight was found to be 9.81 g. The difference in weight was 9.53 g (49.28%) from the original weight. This value can be taken as an estimation moisture and volatile contents in Active product.

By using some calculations, the sample of 2 g of Active product, possessed about 0.99 g (990 mg) of a volatile and moisture content. And with a rate of volatility of 3.6 mg/minute, at the room temperature, this product will last for 366.67 minutes (6 hours and 6 minutes) to loss its volatility and its moisture contents, assuming that, the loss of moisture and volatile contents was consistency, during this period.

The original weight of Lavenda herbal product was 20.24 g. the weight found to be 8.32 g after the sample was taken out of the oven. The weight loss was 11.92 g (58.89%) which estimated the moisture and volatile contents in Lavenda product.

By using the same calculations, the sample of 2 g of Lavenda product, possessed about 1.18 g (1180 mg) of a volatile and moisture content. And with a rate of volatility of 3.6 mg/minute, at the room temperature, this product will last for 327.78 minutes (5 hours and 27 minutes) to loss its volatility and its moisture contents, also, assuming that, the loss of moisture and volatile contents was consistency, during this period. Lavenda product possessed relatively high percentage of moisture and volatile contents than Active products, but, Lavenda product can last for a relatively less period the Active before they loss their moisture and volatile contents in the room temperature.

Omsaham.com (2014), estimated an effective period for Dee dee product as long as 6 – 8 hours.
Table (4.2) The moisture and volatile contents (%) of Active and Lavenda products

<table>
<thead>
<tr>
<th>Type</th>
<th>Lavenda</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Original weight (g)</strong></td>
<td>20.24</td>
<td>19.34</td>
</tr>
<tr>
<td><strong>Final weight (g)</strong></td>
<td>8.32</td>
<td>9.81</td>
</tr>
<tr>
<td><strong>Moisture and volatile weight</strong></td>
<td>11.92</td>
<td>9.53</td>
</tr>
<tr>
<td><strong>% volatile and moisture contents</strong></td>
<td><strong>58.89</strong></td>
<td><strong>49.28</strong></td>
</tr>
</tbody>
</table>
4.3 The Apolar contents in Active and Lavenda repellent products

Apolar (lipophilic) and polar (in combination to other undissolved materials) of Active and Lavenda products were presented in Table (4.3). The original weight of Active product was 19.34 g. The weight was 9.81 g after the moisture and volatile contents test. The moisture and volatile free product was subjected to cool extract by using hexane for 24 hours. After filtration, the dried filtrates was 3.67 g with a differences of 5.05 g (26.11 %), which reflected the estimated apolar content in this product. By adding the moisture and volatile content percentage to the apolar contents, the polar and others (undissolved and non-volatile constituents) was 24.61% for the product of Active.

Concerning Lavenda product, The original weight was 20.24g and it decreased to 8.32 g after the moisture and volatile contents test. The product weight was 4.15 g after it was subjected to cool extract by using hexane for 24 hours. The differences was 5.17 g (25.54 %), which reflected the estimated apolar content in this product. By adding the moisture and volatile content percentage to the apolar contents, the polar and other contents was 15.57% for this product.

It was clear that, Active and Lavenda products were relatively similar in their apolar (lipophilic) contents, although that there was a difference of about 0.5 g for Lavenda, but Active product possessed more polar and other undissolved materials (24.61%) than Lavenda (15.57%).
Table (4.3) Apolar (lipophilic) contents in Active and Lavenda products

<table>
<thead>
<tr>
<th>Type</th>
<th>Lavenda</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original weight (g)</td>
<td>20.24</td>
<td>19.34</td>
</tr>
<tr>
<td>Moisture-free weight (g)</td>
<td>8.32</td>
<td>9.81</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>4.15</td>
<td>3.76</td>
</tr>
<tr>
<td>Difference</td>
<td>5.17</td>
<td>5.05</td>
</tr>
<tr>
<td>% apolar</td>
<td>25.54</td>
<td>26.11</td>
</tr>
<tr>
<td>% polar and others</td>
<td>15.57</td>
<td>24.61</td>
</tr>
</tbody>
</table>
4.4 The repellency potentiality of Active and Lavender Products

The repellency potentiality of Active and Lavender products, in term of the least vertical and horizontal distances (cm) that the adult *Anopheles* and *Culex* mosquitoes were oriented away from the product center source, were presented in Table (4.4).

The results showed that, the least distance that *Anopheles* mosquito was oriented away from the cage center was 26 cm at the vertical level, when Active product was used, as relatively similar to that of the horizontal level (24 cm). *Culex* mosquito showed a relatively far distance than *Anopheles* (it scored 45 cm distance from the center at the vertical level and 42 cm at the horizontal level).

Concerning Lavender product, the least distance that *Anopheles* mosquito was oriented away from the cage center was 85 cm at the vertical level, which was relatively similar to that of the horizontal level (81 cm). *Culex* mosquito showed a different behavior against this product, it scored 58 cm distance from the center at the vertical level and 53 cm at the horizontal level.

The obtained data reflected the dispersal behavior of volatile constituents of each of these product, assuming that, the distribution of adult mosquito was occur in respect to the repellent products (not random). The action of some repellent products in hiding the human scent cannot be ignored and cannot be proved in this work.

Two finding were cleared obviously, Lavender product was more potent than Active product as were showed by the measured least vertical and horizontal levels for both products. Also, *Anopheles* mosquito was more sensitive to Lavender product than *Culex*, but *Culex* mosquito was more sensitive to Active product than *Anopheles*. 
Table (4.4) The least vertical and horizontal orientation distance (cm) of *Anopheles* and *Culex* mosquito in respect to Active and Lavenda products

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Target species</th>
<th>Lavenda</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vertical level</strong></td>
<td><em>Anopheles</em></td>
<td>85</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td><em>Culex</em></td>
<td>58</td>
<td>45</td>
</tr>
<tr>
<td><strong>Horizontal level</strong></td>
<td><em>Anopheles</em></td>
<td>81</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td><em>Culex</em></td>
<td>53</td>
<td>42</td>
</tr>
</tbody>
</table>

4.5 Thin layer chromatography of Active and Lavenda products

Table (4.5) showed number and *Rf* values of the spots separated by the TLC test of the apolar extract of each of Lavenda and Active mosquito-repellent products. It was clear that, two different spots were separated from each of Active and Lavenda products. No evidence that, the active ingredients of both products were similar.

Lavenda mosquito-repellent product was made mainly of Lavender oil which is, in general, gentle, nontoxic and non sensitizing oil (Sharon, 2013), while that, the chemical composition of Active product is deionized water, N,N-diethyl-m-toluamide and distearyldimethylammonium (SC Johnson and Son, 2014).

Some scientists pointed out that, the insect repellent products should be safe and containing no traces of toxic materials (Zadikoff, 1979; Yong and Evans, 1998)

Table (4.5) Active ingredients in the Active and Lavenda products

<table>
<thead>
<tr>
<th>Spot</th>
<th>Lavenda</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>0.58</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Chapter Five
Conclusions and Recommendations

5.1 Conclusions

1- The constant rate of weight loss (as a result of volatility) in Active product was 2.7 mg/minute/inch$^2$, while it was 3.6 mg/minute/inch$^2$ in Lavenda product.

2- Lavenda product possessed relatively high estimated percentage (58.89%) of moisture and volatile contents than Active products (49.28%), but, Lavenda product can last for a relatively less period than Active before they lose their moisture and volatile contents in the room temperature.

3- Active and Lavenda products were relatively similar in their apolar (lipophilic) contents, although that there was a difference of about 0.5 g for Lavenda.

4- The least distance that Anopheles mosquito was oriented away 26 cm at the vertical level and 24 cm at the horizontal level, when Active product was used. Culex mosquito showed a relatively far distance than Anopheles (45 cm at the vertical level and 42 cm at the horizontal level).

5- The least distance that Anopheles mosquito was oriented away from the cage center was 85 cm at the vertical level and 81 at the horizontal level, but Culex mosquito showed a different behavior against this product, it oriented 58 cm at the vertical level and 53 cm at the horizontal level.

6- The TLC techniques separated two different spots from each, and hence, no evidence that, the active ingredients of both products were similar.

5.2 Recommendations

1- The designed cage was useful to evaluate the repellency potentiality of aroma produce repellent product, with aid of some physical and chemical tests.

2- The required modification should be applied on the design and the tests, so as to facilitate running similar studies on all commercial mosquito-repellent products available at the local markets including the human scent hider products.
References


Baby Center (2013). How do mosquito-repellent work. Available at: http://www.babycenter.in/a1011642/how-do-mosquito-repellents-work#ixzz5PtLDvYoo


SC Johnson and Son (2014). OFF!® ACTIVE LOTION INSECT REPELLENT. Available at: http://www.scjohnson.ca/msds/06-08_Off_Active_Lotion.pdf


