Formulation of Mosquito-Repellent Products Based on some Natural Products and Testing their Physical, Chemical and Repellency Characteristics

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Center of Biosciences and Biotechnology
Faculty of Engineering and Technology

November 2015
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Date: 21 November, 2015
بسم الله الرحمن الرحيم

قال الله تعالى:

(وقل اعملوا فسيري الله عملكم ورسله والمؤمنون وستردون إلي عالم الغيب والشهادة فيبنكم بما كنتم تعملون)

صدق الله العظيم

النبوة 105
DEDICATION

To all my pleasure and love . . .

My father

To whom I could not live without . . .

My family

To whom I am very pleased with their association . . .

My colleges

To whom who learned me (knowledge is power) . . .

My teachers

To all of you I dedicate all the fragrance . . .

Haifa
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Firstly … I am greatly thankful to ALLAH for the journey of success following me in all steps of my life.

I would like to express my deepest gratitude and appreciation to my supervisor Dr. Mutaman Ali A. Kehail for his helpful, guidance, encouragement and logical criticism during this study. Thanks to Dr. Yasir Mohamed Abdelrahim for his generosity in providing help and encouragement throughout this work.

My deep thankful and appreciation go to my husband Safwan for his support this work to become a reality. Also I want to thank the Staff of pharmacy department of Wad Medani Emergency Hospital for their support.
Formulation of Mosquito Repellent Products Based on some Natural Products and Testing their Physical, Chemical and Repellency Characteristics

Haifa Osman Mohamed Ahmed
M.Sc. in Biosciences and Biotechnology (Biotechnology) November 2015
Center of Biosciences and biotechnology
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Abstract

Mosquito borne diseases are major human and animals health problem in all tropical and subtropical countries. The diseases transmitted include Malaria, Yellow fever, Filariasis, Japanese encephalitis, Dengue fever and others. In Sudan, Malaria is the most problematic disease, that transmitted by female Anopheles mosquito. Some techniques were developed to decrease the exposure to mosquito bite. This research is focusing on formulation of mosquito-repellent products based on some natural sources and to study the physical and chemical characteristics of these products and to determine their repellency activity on adult mosquito. Three natural sources were used as basal materials: Clove (dry), Ginger (fresh and dry), and garlic (fresh and dry) to formulate the repellent products. 25 gram of the fresh and 12.5 gram of the dry were weighed and mixed with 250 ml of olive oil as a vehicle, then the mixture was heated and cooled five times. 25 gram of bees wax was added to make semi solid products to ease the use. Some physical and chemical tests were run to the formulations. The pH for the product of the dry clove was 6, dry garlic 6.4, fresh garlic 6.2, dry ginger 6.3, and fresh ginger 6.5. The moisture and volatile matter percentage for the product of dry clove was 6%, dry garlic 2.5%, fresh garlic 4.5%, dry ginger 3%, and fresh ginger 3.5%. The refractive index for the product of dry clove was 1.4699, dry garlic 1.4660, fresh garlic 1.4681, dry ginger 1.4690, and fresh ginger 1.4697. The density for the product of dry clove was 0.99, dry garlic 0.96, fresh garlic 0.91, dry ginger 0.92, and fresh ginger 0.94. The volatility rate for the product of dry clove was 15 mg/min/inch², dry garlic 19 mg/min/inch², fresh garlic 14 mg/min/inch², dry ginger 11 mg/min/inch² and fresh ginger 6 mg/min/inch². The lipophilic content percentage (a polar) for the product of dry clove was 76.5%, dry garlic 86%, fresh garlic 82.5%, dry ginger 79.5%, and fresh ginger 85%. The effect of each product as a mosquito repellent was measured at the vertical and horizontal levels. All mosquitoes were oriented away from these products in different distances according to the material used. This study recommends using of such natural products as mosquitoes-repellent instead of chemical one like DEET that may cause skin irritation, hot sensation, rashes or allergy, in addition to economical feasibility.
تحضير منتجات طاردة للبعوض من بعض المنتجات الطبيعية واستكبار خواصها الفيزيائية والكيميائية

هفاء عثمان محمد أحمد

ماجستير العلوم في العلوم والتقنية البيولوجية (تقنية بيولوجية) نوفمبر 2015
مركز العلوم والتقنية البيولوجية
كلية الهندسة والتكنولوجيا

ملخص الدراسة

تعتبر الأمراض المكتولة بالبعوض من أكبر المشاكل الصحية التي تواجه الإنسان والحيوان في كل المناطق الإستوائية وشبه الاستوائية. الأمراض المكتولة هي الملاريا، الحمى الصفراء، داء القيافات، انتفاخ الدماغ البشري، حمى الضنك وغيرها. تعتبر الملاريا أكثر الأمراض التي تسبب المشاكل الصحية في السودان، وتنتقل عن طريق أنثى الأنوفليس. لقد تم تطوير وسائل مكافحة البعوض لتقليص احتمالات تعرض الدغات البعوض. يهدف هذا البحث إلى تحضير منتجات طاردة للبعوض من بعض المصادر الطبيعية ودراسة خواصها الفيزيائية والكيميائية وفعاليتها كمواد طاردة للبعوض. تم استخدام ثلاثة مصادر طبيعية أساسية وهي القرنفل المجفف والزنجبيل (طازج ومجفف)، الثوم (طازج ومجفف) لتكون منتجات طاردة للبعوض. تم تحضيرها بوزان محددة، 25 جرام للطازج و 12.5 للمجفف، وتم مزجها مع زيت زيتون 250 مل كمادة ناقلة وتسخينها، وتربيدها لخمسة مرات. تم إضافة 25 جرام من شمع النحل حتى يتم صنع مستحضر شبه صلب يسهل التعامل معه. تم دراسة بعض الخواص الكيميائية والفيزيائية للمنتجات. درجة حموضة/ قلوية منتج القرنفل المجفف 6، الثوم المجمف 6.4، الزنجبيل المجفف 6.2، الزنجبيل الطازج 6.5، نسبة الرطوبة لمنتج القرنفل المجفف 6%، الثوم المجمف 2.5%، الزنجبيل المجفف 3%، الزنجبيل الطازج 3.5%.

نتيجة معامل انكسار الضوء لمنتج القرنفل المجفف 1.4699، الثوم المجفف 1.4660، الزنجبيل المجفف 1.4690، الزنجبيل الطازج 1.46679. نتيجة قياس الكثافة لمنتج القرنفل المجفف 0.99، الثوم المجفف 0.95، الزنجبيل مجفف 1.4660، الزنجبيل الطازج 1.4681. نتائج قياس الكثافة لمنتج القرنفل المجفف 0.99، الثوم المجفف 0.96، الزنجبيل المجفف 0.91، الزنجبيل الطازج 0.94. معدل التظليل لمنتج القرنفل المجفف 15ملجم/دقيقة/انش^2، الثوم المجفف 19ملجم/دقيقة/انش^2، الزنجبيل المجفف 11ملجم/دقيقة/انش^2، الزنجبيل الطازج 6ملجم/دقيقة/انش^2. نتائج اختبار القطبية لمنتج القرنفل المجفف 76.5%، الثوم المجفف 86%، الزنجبيل المجفف 85.5%، الزنجبيل الطازج 85%.

تم قياس فعالية كل منتج كمادة طاردة للبعوض على المحرر الأفقي والراسي. ابتعد البعوض عن هذه المنتجات بمسافات مختلفة حسب المادة المستخدمة. توصى هذه الدراسة باستخدام هذه المواد الطبيعية كمواد طاردة للبعوض للحد من أخطار المواد الكيميائية المستخدمة مثل ال DEET التي يمكن أن تسبب تهيجات في الجلد، إحساس بالسعون وطفح جلدي، بالإضافة للجدوى الاقتصادية.
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CHAPTER ONE

INTRODUCTION

Mosquito borne diseases are major human and animals health problem in all tropical and subtropical countries. The diseases transmitted include Malaria, Yellow fever, Filariasis, Japanese encephalitis and Dengue fever and others. There has been exploration of various methods over the centuries to combat threats from mosquito borne diseases. With the beginning of the 20th century there grew an interest for use of biological controls agents but this was declined with the discovery of insecticidal properties of DDT in 1939. However its deleterious impact on non-target population and the development of resistance prompted for the search of alternative, simple and sustainable methods of mosquito control (Siri and Thavara, 2006; Kumar and Hwang, 2006; Shaalan and Canyon, 2009).

Considerable research efforts have proved that essential oil compounds and their derivatives are an effective and alternative means of controlling nuisance mosquitoes and their property of rapid degradation in the environment has favoured for its increased specificity (Tripathi et al., 2009).

The justification of essential oils as green pesticides lies in the fact that the constituents of all essentials oils are moderately toxic or mostly found to be non toxic to mammals, birds and the aquatic ecosystem (Koul et al., 2008).

Although there exist several advancement in the field of synthetic drug chemistry and antibiotics but plants still continue to be one of the major raw materials for drugs treating various ailments of human. In fact clinical and pharmaceutical investigations have helped in elevating the status of various medicinal plants by identifying the role of active principles present in them and exploring their mode of action in human and animal systems (Vishwanathan and Basavaraju, 2010). However essential oils due to their volatile nature demand for frequent re-application to maintain its potency. They evaporate completely and thereby their effectiveness is short lived and so complete protection cannot be achieved (Patel et al., 2012).

Objective
The objective of this study was to formulate mosquito repellent products based on some natural plants and testing their physical, chemical, and repellency characteristics.
CHAPTER TWO
LITERATURE REVIEW

2.1. Mosquito Borne Diseases

Since ancient times, mosquitoes have been reported as the source of various ailments affecting human. Comprising approximately 3500 species, mosquitoes are found beyond the tropical and subtropical regions of the world. The chief genera which vector human disease causing pathogens are Anopheles (malaria, filariasis), Aedes (yellow fever, dengue, chikungunya) and Culex (west Nile virus, Japanese encephalitis, filariasis). Over its life span a female mosquito repeatedly takes a blood meal as protein source to complete egg development. By injecting the saliva which may contain pathogens into the host animal, the pathogens thus complete an obligatory life cycle phase and multiply in the mosquito's salivary glands. This thereby makes female mosquito an ideal transmitter of diverse blood borne pathogens and agents of devastating human diseases (Tolle, 2009; Michel and Kafatos, 2005).

2.1.1 Malaria

Over the Years malaria have been considered as one of the leading cause of death in Sudan. Malaria is caused by the protozoal parasites Plasmodium vivax, P. malariae, P. ovale, and P. falciprum, which are transmitted by female Anopheles mosquitoes. Studies suggest that for any form of malaria to be endemic in a certain area a number of requirements must be fulfilled, it needs the presence of a large number of competent anopheline mosquitoes with sufficient preference for human blood and an exposed human population with enough number of malaria carriers and susceptible individuals for the chain of infection to persist (Adams et al., 2011).

2.1.2 Dengue Fever

100 million cases of dengue fever and half million cases of dengue hemorrhagic fever (DHF) occur globally, with an average case of fatality rate around 5%. Potential reasons for the global resurgence and spread of dengue fever and DHF epidemics are assumed due to population growth, uncontrolled urbanization in tropical and sub tropical countries, proliferation of breeding sites for Aedes mosquitoes and the lack of effective mosquito control (Gurugama et al., 2010).
2.1.3 Japanese Encephalitis

One of the leading causes of acute encephalopathy is encephalitis affecting children and adolescents particularly in the tropics. Mosquitoes proliferate in close association with pigs and other animal reservoirs and are found to spread virus of Japanese encephalitis basically in malnourished children of poor families from rural area (Kumar et al., 2012).

2.2 Control of Mosquito

The efficient way to control these diseases is to control mosquito vector populations and prevent mosquito bites. Studies reflect that insect repellents play an important role in preventing the mosquito vector, deterring an insect from flying to, landing on or biting human and animal skin. Generally the widely used compounds as insect repellents are synthetic chemical repellents but they bear the disadvantage of being not safe for human, especially children, domestic animals because they may cause skin irritation, hot sensation, rashes or allergy (Sritabutra et al., 2011).

2.2.1 Definition of Mosquito Repellent

A mosquito repellent is a substance applied to skin, clothing, or other surfaces which discourages insects (and arthropods in general) from landing or climbing on that surface (Sah et al., 2010).

2.2.2 Mechanism of Action of Mosquito Repellents

Carbon dioxide, excretory products and lactic acid present in sweat in warm blooded animals act as an attractive substance for female mosquitoes. The perception of the odor is through chemo receptors present in the antennae of mosquitoes. The repellents block the lactic acid receptors thus destroying upwind flight and as a result the mosquitoes loses its contact with the host (Elissa et al., 2004; Sah et al., 2010).

Usually insect repellents work by masking human scent, or by using a scent which insects naturally avoid. Permethrin is different in that it is actually a contact insecticide.

2.3 Methods of Mosquito Control

2.3.1 Chemical Method

There are a number of natural and chemical mosquito repellents that work to repel mosquitoes. The synthetic chemical repellent, DEET, is the most effective. It is essentially a poison that masks the natural odor and carbon monoxide that is released from the human body (Patel et al., 2012).
2.3.1.1 Synthetic Repellents

More effective and longer lasting than "natural" repellents in comparative studies, IR3535 (3-[N-Butyl-N-acetyl]- aminopropionic acid, ethyl ester) was as effective as DEET in protection against mosquitoes. However, some plant-based repellents may provide effective relief as well. Essential oil repellents can be short lived in their effectiveness, since essential oils can evaporate completely DEET exposure were more likely to have insomnia, mood disturbances and impaired cognitive function. Examples are:

- DEET (N,N-diethyl-m-toluamide)
- Icaridin, also known as picaridin, Bayrepel, and KBR 3023
- Nepetalactone, also known as "catnipoil"
- Permethrin
- Bog Myrtle
- IR3535 (3-[N-Butyl-N-acetyl]- aminopropionic acid, ethyl ester) (Patel et al., 2012).

2.3.1.1.1. Advantages of Synthetic Repellents

- Synthetic repellents containing DEET or picaridin are more effective than repellents with "natural" active ingredients.
- All the synthetics gave almost100 % repellency for the first 2 hours, where the natural repellent products were most effective for the first 30 to 60 minutes, and required reapplication to be effective over several hours (Patel et al., 2012).

2.3.1.1.2. Disadvantages of Synthetic Repellents

- Cause rashes, swelling, eye irritation, and worse problems, though they're unusual including brain swelling in children, anaphylactic shock, low blood pressure, and one report of death.
- DEET must be used with caution, especially with children.
- It has been known to cause dizziness and can severely irritate the skin. DEET may even cause cancer and defect in child birth. For these reasons, many people choose to use a natural mosquito repellent like a citronella spray (Sah et al., 2010).

2.3.1.2 Natural Repellents

Many repellents are nowadays available which can easily fend off the mosquitoes but are not good for the health as it contain a harmful chemical called DEET. It is fine to use a natural repellent which can make human unattractive in the
eyes of mosquitoes. It is non-sticky; non-toxic and environmentally friendly; safer on sensitive skins and some can be used on children as young as 3 months; reduced irritation and harmless to most plastics and fabrics, but it is more expensive; may need more frequent re-application to maintain full protection; Essential oil repellents can be short-lived in their effectiveness, since essential oils can evaporate completely may need more frequent re-application to maintain full protection and cannot apply directly on the skin, if applied can cause rashes on skin (Patel et al., 2012).

2.3.2 Non Chemical Methods

2.3.2.1 Physical Methods

Emptying the stagnant water in rain gutters, old tires, buckets, plastic covers, etc.; mosquito net, medicated net; non medicated net; mosquito traps, mechanical methods; electric mosquito zapper and mosquito magnet (Enayati et al., 2007).

2.3.3 Biological Methods

By growing some fish species that feeds on mosquito larvae in water bodies. (Patel et al., 2012).

2.3.4 Other Methods

Fogging is a temporary method of controlling mosquitoes and such other pests but is particularly necessary in the context of health threats from severe bug populations and for an outdoor activity where these pests are unwanted. The method employs a thermal fogger which produces a pesticide fog or smoke by heating the fogging solution with a coil inside the unit. It is ready to use fogging solution; each gallon contains 0.5% pyrethrins and 5% piperonyl butoxide. Another revolutionary approach is the transdermal technology to deliver a natural mosquito repellent into the blood stream for a complete 24-hours mosquito protection. The active ingredient in the patch is Vitamin B1 or Thiamine as it is known to be the most effective natural mosquito repellent discovered to date. It was found that female mosquitoes are repulsive to the scent of Thiamine, therefore the patch works by inducing a controlled amount of vitamin B1 into the blood stream (Patel et al., 2012).

2.4. Insect Repellents from Natural Sources

There are many preparations from naturally occurring sources that are repellent to certain insects. Some of these act as insecticides while others are only repellent.

Basil (Ocimum basilicum), Castor oil (Ricinus communis), Catnip oil (Nepeta species) (nepetalactone against mosquitoes), Cedar oil (mosquitos, moths), Celery
extract (*Apium graveolens*), Cinnamon oil (leaf oil kills mosquito larvae), Citronella oil (repels mosquitoes), Clove oil (mosquitoes), Eucalyptus oil (70%+ eucalyptol), (cineol is a synonym), (mosquitoes,), Fennel oil (*Foeniculum vulgare*) (mosquitoes), Garlic (*Allium sativum*) (rice weevil, wheat flour beetle), Geranium oil (also known as *Pelargonium graveolens* Lavender (repels insects), Lemon eucalyptus (*Corymbia citriodora*) essential oil and its active ingredient p-menthane-3,8-diol (PMD), Lemongrass oil (*Cymbopogon* species) (mosquitoes), Neem oil (*Azadirachta indica*) (Repels or kills mosquitoes, their larvae and a plethora of other insects including those in agriculture), Peppermint oil (*Mentha piperita*) (mosquitoes), Rosemary (*Rosmarinus officinalis*) (mosquitoes), Solanum villosum berry juice (against *Stegomyia aegypti* (mosquitoes), Nepetalactone, also known as "catnip oil" (*Sah et al.*, 2010; *Enayati et al.*, 2007).

2.5 Natural Plants used as Mosquito Repellent in this Study

2.5.1 Clove

Clove are the aromatic flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum*. Cloves are commercially harvested primarily in Indonesia, India, Madagascar, Zanzibar, Pakistan, Sri Lanka and Tanzania. Clove oil has been studied for its antibacterial, antimicrobial, and antifungal properties against cutaneous infectious manifestations and has been shown to be environmentally safe and nontoxic to humans for use in medicine, perfume, and food flavoring. *Trongtokit et al.*, 2005; *Chaieb et al.*, 2007, describe clove oil to encompass antibacterial, antiviral, antimicrobial, antifungal, anti-inflammatory, cytotoxic, insect repellency, anesthetic, and antitumor properties.

2.5.2 Garlic

Garlic (*Allium sativum*) is ageophytic perennial plant belonging to liliaceae family. Allicin, responsible of the characteristic smell in freshly crushed garlic, is one of the most active compounds of garlic.

It is well known that garlic extract shows antibacterial, antihelminthic and antiprotozoal properties. It has also been documented that garlic components have toxic and/or repellent effect against several groups of arthropods of agricultural and medical importance (*Amonkar and Banerji*, 1971; *Jarial*, 2001; *Mc Hug et al.*, 2001; *Stjemberg and Berglund*, 2000).
Of a particular interest is the hypothesis that the daily consumption of garlic oil as food integrator in the diet components can protect dogs from the bite of phlebotomine sand flies, the proven vectors of *Leishmania* sp. A similar effect has been observed in Sweden for human protection against tick attack (Stjemberg and Berglund, 2000). Based on the above hypothesis Garlic oil could represent a new tool as mosquito repellant.

2.5.3 Ginger

Ginger (*Zingiber officinale* Roscoe) is a flowering plant in the family Zingiberaceae whose rhizome. Ginger grows in China, India, Africa, the Caribbean, and other warm climate. Ginger has a long history of medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds (Grant and Lutz, 2000). *Z. officinale* is a well-known and widely used herb, especially in Asia, where it has been widely used as a spice and condiment in different societies. Besides its food-additive functions, ginger has a long history of medicinal use for the treatment of a variety of human ailments including common colds, fever, rheumatic disorders, gastrointestinal complications, motion sickness, diabetes, cancer, etc. Ginger contains several nonvolatile pungent principles viz. gingerols, shogaols, paradols, and zingerone, which account for many of its health beneficial effects (Kundu et al., 2009).

The Larvicidal activity of a petroleum ether extract of *Z. officinale* was evaluated against *A. aegypti* and *C. quinquefasciatus*; bioassay-guided fractionation led to the isolation of (a) 4-gingerol, (b) (6)-dehydrogingerdione, and (c) (6) dihydrogingerdione which were tested against mosquitoes (Rahuman et al., 2008). There are several reports of the insect activity of *Z. officinale* extracts (Sahayaraj 1998; Shelly et al., 2003; Shelly and McInnis 2001). This was observed earlier for other isolated compounds from *Z. officinale*, for example [6]-gingerol and [6]-dehydroshogaol exhibited maximum insect growth regulatory (IGR) and antifeedant activity against *Spilosoma oblique*. (Agarwal et al., 2001). Prajapati et al., (2005) reported that the essential oils of *Z. officinale* and *Rosmarinus officinalis* were found to be ovicidal and repellent against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. Ginger contains a number of pungent constituents and active ingredients. Steam distillation of powdered ginger produces ginger oil, which contains a high proportion of sesquiterpene hydrocarbons, predominantly zingiberene (Govindarajan, 1982a).
The major pungent compounds in ginger, from studies of the lipophilic rhizome extracts, have yielded potentially active gingerols, which can be converted to shogaols, zingerone, and paradol (Govindarajan, 1982b).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

Three natural materials were brought from the local markets of Wad Medani City, which were: Clove, Ginger, and Garlic to be used to prepare the mosquito's repellent products as basal materials. Olive oil as a vehicle (Tunisian Product from Algalkhi Company), and bees wax were also purchased from the same local market.

Anopheles and Culex adult mosquitoes were brought from the Blue Nile Institute for Communicable Diseases, Wad Medani City.

3.2 Methods

3.2.1 Preparation of Repellent Products

3.2.1.1. From the Dry Clove, Ginger and Garlic Materials:

Sufficient amount of each of fresh Clove, Ginger and Garlic was cut to small pieces by using small knife and then dried under shade for twenty four hours. 25 g of clove, 12.5 g of ginger and 12.5 g of garlic were weighed separately and each of which was mixed with 250 ml olive oil. Each mixture was heated for five minutes, then cooled in room temperature for two minutes. Such heating and cooling was repeated five times. After the last heating, 25 g of bees wax was added to the mixture before cooling. The mixture was filtered by using a plastic filter mesh, before it get solidified, two times and then divided to five equal amounts in small containers.

3.2.1.2. From the Fresh Ginger and Garlic Materials:

A weight of 25 g of fresh ginger or garlic was cut to small pieces by using small knife and mixed with 250 ml olive oil. Each mixture was heated for five minutes, and then cooled in room temperature for two minutes. Such heating and cooling was repeated five times. After the last heating, 25 g of bees wax was added to the mixture before cooling. The mixture was filtered by using a plastic filter mesh, before it get solidified, two times and then divided to five equal amounts in small containers.

3.2.2 The Physical Properties of the Prepared Products

3.2.2.1 Volatility Rate

A set of plastic sheets (2 x 2 inch) were prepared and weighed. Certain amount of each prepared repellent product was smeared as a thin layer over each plastic sheet (primary weight), and then weighed again after adding of samples. This test was
depended on weighting the plastic sheets each an hour at the room temperature (27°C) for continuous 5 hours (300 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.

3.2.2.2 Moisture and Volatile Matter Percentage

This test was done to measure the percentage of moisture and volatile matters in each sample. Five Petri dishes were weighed. Small amount of each sample was added to these Petri dishes and reweighed. All samples were left under room temperature for twenty four hours, after which all Petri dishes were weighed for last time. The difference in weight for each prepared product was recorded and used to calculate the percentage of moisture and volatile matters of each sample.

Moisture and volatile matter % = loss weight/original weight*100

3.2.2.3 pH

pH was determined by using a pH-meter in which the sensors of the device were immersed into the product sample for seconds. The pH-meter was immediately recalibrated by using a set of buffers before the second sample was read, so as to offer necessary physical information when applied on those suffering of sensitive skins.

3.2.2.4 Refractive Index

The refractive index of each sample was measured by using (Bellingham + Stanley limited 60/70 ABBE) Refractometer. The ABBE60 direct reading models, are available with three measuring range. They can be used with a white light source. The working temperature range for these instruments is from +5 and 75°C. Instruments come complete with thermometer, standardization plate and contact liquid, scale illuminator, spare illuminator lamp, power supply, plastic dust cover and instruction for use.

3.2.2.5 Density

The product density was determined according to AOAC , (1990) methods, using pycnometer. An empty stoppered was pycnometer weighed, filled with water and kept at constant temperature of 27°C in a water bath for 30 minutes. The weight of water at 27°C was determined by subtracting weight of empty pycnometer from its weight when filled with water. The end of time stoppered pycnometer was adjusted to proper level dried with a cloth and weighted. In the same manner, the weight of the product at 27°C was determined. The density was calculated as follows:
The density at 27°C = \( W_1/W_2 \)

Where:

\( W_1 = \) Weight of oil at 27°C

\( W_2 = \) weight of water at 27°C

### 3.2.3 The Chemical Properties of the Prepared Products

#### 3.2.3.1 Lipophilic Content (a polar)

The percentage of lipophilic content of each sample was measured by using oil content determining methods (AOAC, 1993). 2 g of each sample was foiled by tissue and filter papers. The weight of each sample was recorded before extraction (using hexane solvent). The final weight of each sample was measured after extraction. This test was used to determine the amount of lipophilic contents in each sample.

#### 3.3 Repellency Test

The repellency effect of each of the prepared product against adult mosquitoes \((Anopheles Culex)\) was measured at vertical and horizontal levels by using simple T-shape repellency cage (locally manufactured from iron skeleton and clothed with white fabric that did not allow pass out of mosquito from it). Each product sample was put at the center of this cage. The repellency orientation against each product was measured and recorded for the vertical and horizontal levels.

#### 3.4 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 (mg/minute).
CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1 Some Physical Characteristics of the Formulated Repellent Products

The physical characteristics (color, odor, ph, density and refractive index) for five formulated products (dry clove, dry garlic, fresh garlic, dry ginger, fresh ginger), were presented in Table (4.1).

The color was pale yellow for all products, while the odor was sharp+ for both dry and fresh garlic products, pleasant for both dry and fresh ginger product and sharp for dry clove product.

Concerning pH, the products of dry clove, dry garlic, fresh garlic, dry ginger, fresh ginger has a pH of 6, 6.4, 6.2, 6.3, and 6 respectively, while there has a density of 0.99, 0.96, 0.91, 0.92, 0.94, following the same order.

Concerning refractive index, the products of dry clove, dry garlic, fresh garlic, dry ginger, fresh ginger has refractive index of 1.4699, 1.4660, 1.4681, 1.4690, and 1.4697, respectively.

Normal skin pH ranges from 4.5 - 6.5, which means it is always on the slightly acidic side. This acidity of the skin is maintained by sebaceous glands, sweat glands, normal skin flora and among others. It serves many protective functions to the skin, one of which is killing unwanted bacteria (Joachim et al., 2002).

The pH results of formulated mosquito products indicate that, these products are safe when applied to the skin.

Density measurement is very important because it help in determining the characteristic of materials. The density of water is 1g/ml. The density measured results of five formulated products were less than water density.

Refractive index is a physical property of a substance, like density, melting point, boiling point, etc.; therefore, it useful as a means of assisting in identifying a substance and in establishing its purity. Even though it is a nonspecific property, relatively few substances have identical RIs at a given temperature and wavelength. It is a physical constant for many substances, such as fats, fatty oils, waxes, sugars, organic solvents, etc.
Table (4.1) Some physical characteristics of formulated repellent products

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dry clove</th>
<th>Dry garlic</th>
<th>Fresh garlic</th>
<th>Dry ginger</th>
<th>Fresh ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Pale yellow</td>
<td>Pale yellow</td>
<td>Pale yellow</td>
<td>Pale yellow</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Odor</td>
<td>Sharp</td>
<td>Sharp+</td>
<td>Sharp+</td>
<td>Pleasant</td>
<td>Pleasant</td>
</tr>
<tr>
<td>Density</td>
<td>0.99</td>
<td>0.96</td>
<td>0.91</td>
<td>0.92</td>
<td>0.94</td>
</tr>
<tr>
<td>Ref. index</td>
<td>1.4699</td>
<td>1.4660</td>
<td>1.4681</td>
<td>1.4690</td>
<td>1.4697</td>
</tr>
<tr>
<td>pH</td>
<td>6</td>
<td>6.4</td>
<td>6.2</td>
<td>6.3</td>
<td>6.5</td>
</tr>
</tbody>
</table>
4.2 Moisture and Volatile Matter Percentage

The moisture and volatile matter percentage of five formulated products were showed in Table (4.2). The original weight of dry clove product, dry garlic product, fresh garlic product, dry ginger product, fresh ginger product was 0.54, 1.01, 0.86, 0.93, and 0.74 respectively. After twenty four hours being in room temperature (27°C), the weight of these products were decreased to 0.51, 0.97, 0.82, 0.88 and 0.7. The loss weight was 0.03, 0.04, 0.04, 0.05 and 0.04 following the same order. The estimated moisture and volatile matter was 5.5% for dry clove, 3.9% for dry garlic, 4.6% for fresh garlic, 5.3% for dry ginger, and 5.4% for fresh ginger.

It was clear that, dry clove product has higher moisture and volatile content followed by fresh ginger, dry ginger, fresh garlic and dry garlic which has lowest moisture and volatile contents.
Table (4.2) Percentage of moisture and volatile matter of formulated mosquito repellent products

<table>
<thead>
<tr>
<th>Product</th>
<th>Dry clove</th>
<th>Dry garlic</th>
<th>Fresh garlic</th>
<th>Dry ginger</th>
<th>Fresh ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original wt/g</td>
<td>0.54</td>
<td>1.01</td>
<td>0.86</td>
<td>0.93</td>
<td>0.74</td>
</tr>
<tr>
<td>Final wt/g</td>
<td>0.51</td>
<td>0.97</td>
<td>0.82</td>
<td>0.88</td>
<td>0.70</td>
</tr>
<tr>
<td>Lost wt/g</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>5.5</td>
<td>3.9</td>
<td>4.6</td>
<td>5.3</td>
<td>5.4</td>
</tr>
</tbody>
</table>
4.3 Volatility Rate

The volatility rates (g) at room temperature (27 ± 2 °C) of five formulated repellent products in respect to time (minutes) per one square inch, were presented in Table (4.3). The original weight (in gram) of dry clove product, dry garlic, fresh garlic, dry ginger and fresh ginger, were 1.16 g, 1.01 g, 1.11 g, 1.06 g, 1.04 g, respectively, and after 300 minutes (5 hours), the weight being 1.09 g, 0.91 g, 1.04 g, 1.01 g and 1.01 g, following the same order. With respect to time (per minutes), the regression analysis revealed that, the constant rate of losing weight (as a result of evaporation), were 15 mg/min/inch² for Dry clove product, 19 mg/min/inch² for dry garlic product, 14mg/min/inch² for fresh garlic product, 11mg/min/inch² for dry ginger product and 6 mg/min/inch² for fresh ginger product.

It was clear that, dry garlic product has the highest volatility rate, followed by dry clove product, then fresh garlic product, dry ginger and lastly fresh ginger which has the far lowest volatility rate.

By using a mathematical calculations, a sample of 2 g of dry clove product, spread on one inch² plastic sheet (instead of the absorbent skin) could possessed about 1.1 g (1100 mg) of a volatile and moisture content, resulted from multiplying the percentage moisture and volatile content (5.5%) by the sample weight (2 g). and by considering its volatility rate (15mg/minute/inch²), at the room temperature, this product will last for 73 minutes (1 hour and 13 minutes) before all of its moisture and volatile contents were dissipated, assuming that, there was a constant rate of loss of moisture and volatile contents, during this period.

And by applying the same consumptions, it can be estimated that, for dry garlic product, the volatile and moisture content from 2 g was 0.78 g (780 mg). The expected time for this amount to dissipate (with a volatility rate of 19 mg/minute/inch²), was 41 minutes.

Concerning fresh garlic product, the volatile and moisture content from 2 g was 0.92g (920 mg). The expected time for this amount to dissipate (with a volatility rate of 14 mg/minute/inch²), was 65 minutes (1 hour and 5 minute).
Table (4.3) Volatility rates of the formulated mosquito repellent products

<table>
<thead>
<tr>
<th>Product weight (g)</th>
<th>Dry Clove</th>
<th>Dry garlic</th>
<th>Fresh garlic</th>
<th>Dry ginger</th>
<th>Fresh ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary wt/g</td>
<td>1.16</td>
<td>1.01</td>
<td>1.11</td>
<td>1.06</td>
<td>1.04</td>
</tr>
<tr>
<td>After 60 min</td>
<td>1.14</td>
<td>0.96</td>
<td>1.08</td>
<td>1.05</td>
<td>1.03</td>
</tr>
<tr>
<td>After 120 min</td>
<td>1.12</td>
<td>0.93</td>
<td>1.06</td>
<td>1.03</td>
<td>1.01</td>
</tr>
<tr>
<td>After 180 min</td>
<td>1.10</td>
<td>0.92</td>
<td>1.05</td>
<td>1.02</td>
<td>1.01</td>
</tr>
<tr>
<td>After 240 min</td>
<td>1.09</td>
<td>0.91</td>
<td>1.04</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>After 300 min</td>
<td>1.09</td>
<td>0.91</td>
<td>1.04</td>
<td>1.01</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Regression analysis

<table>
<thead>
<tr>
<th>R²</th>
<th>0.93</th>
<th>0.82</th>
<th>0.88</th>
<th>0.94</th>
<th>0.71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.15</td>
<td>0.99</td>
<td>1.10</td>
<td>1.06</td>
<td>1.03</td>
</tr>
<tr>
<td>x-coefficient</td>
<td>-0.015</td>
<td>-0.019</td>
<td>-0.014</td>
<td>-0.011</td>
<td>-0.006</td>
</tr>
</tbody>
</table>
For dry ginger product, the volatile and moisture content from 2 g was 1.06 g (1060 mg). The expected time for this amount to dissipate completely (with a volatility rate of 11 mg/minute/inch\(^2\)), was 96 minutes (1 hour and 36 minutes).

For fresh ginger product, the volatile and moisture content from 2 g was 1.08 g (1080 mg), the expected time for this amount to dissipate completely (with a volatility rate of 6 mg/minute/inch\(^2\)), was 180 minutes (3 hours).

According to the calculated estimations, fresh ginger product could last for longer time before it get without scent, followed by dry ginger product, then dry clove product, fresh garlic and Lastly dry garlic product. Also, the more amount used of this product at the same surface area, the additional protection period will be gained.

According to the above results, these products may need more frequent re-application to maintain full protection. The natural repellent products were most effective for the first 30 to 60 minutes, and required reapplication to be effective over several hours (Patel et al., 2012).

### 4.4 Lipophilic Content

The percentage of lipophilic content of five formulated mosquito repellent products was showed in Table (4.4). The original weights before the cool extract was made were 3.93 g of dry clove product, 3.32 g of dry garlic, 3.3 g of fresh garlic, 4.14 g of dry ginger and 3.2 of fresh ginger. The weight reduced to 2.4, 1.6, 1.65, 2.55, and 1.5 g, respectively. The differences in weights were 1.53, 1.72, 1.65, 1.59, and 1.7 g following the same order. The calculated percentage a polar contents were 76.5% for dry clove product, 86% for dry garlic, 82.5% for fresh garlic, 80% for dry ginger and 85% for fresh ginger. The most important finding from this results was that, dry garlic product, which possessed a very low moisture and volatile contents (3.9%), has a higher percentage of a polar contents (86%).

The lipophilic (oils and lipids) belong to the most important ingredients in cosmetic products. When applied, they help to regenerate damaged skin (they support the lipid layer of the skin), which acts as the major barrier against external influences. Products based on oils with low solidification point spread easily and evenly on the skin (Hans, 2004). All the above formulated products have high lipophilic contents (>75%), that reflect the safety and efficacy of them when applied on the skin.
Table (4.4) The percentage of lipophilic contents of formulated mosquito repellent products

<table>
<thead>
<tr>
<th>Product weight (g)</th>
<th>Dry Clove</th>
<th>Dry garlic</th>
<th>Fresh garlic</th>
<th>Dry ginger</th>
<th>Fresh ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original wt/g</td>
<td>3.93</td>
<td>3.32</td>
<td>3.30</td>
<td>4.14</td>
<td>3.20</td>
</tr>
<tr>
<td>Final wt/g</td>
<td>2.40</td>
<td>1.60</td>
<td>1.65</td>
<td>2.55</td>
<td>1.50</td>
</tr>
<tr>
<td>Lost wt/g</td>
<td>1.53</td>
<td>1.72</td>
<td>1.65</td>
<td>1.59</td>
<td>1.70</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>76.5</td>
<td>86.0</td>
<td>82.5</td>
<td>80.0</td>
<td>85.0</td>
</tr>
</tbody>
</table>
4.5 Repellency Effects

Table (4.5) show the least vertical and horizontal orientation distance (cm) of *Anopheles* and *Culex* mosquitoes in respect to the formulated products. The orientation on vertical level of *Anopheles* mosquito were 36 cm, 18 cm, 58 cm, 34 cm and 65 cm for the dry clove, dry garlic, fresh garlic, dry ginger and fresh ginger products respectively, While the orientation on the same level of *Culex* mosquito were 27 cm, 14 cm, 53 cm, 31 cm and 61 cm following the same order.

The orientation on horizontal level of *Anopheles* mosquito were 42 cm, 21 cm, 52 cm, 44 cm and 54 cm for the products of dry clove, dry garlic, fresh garlic, dry ginger and fresh ginger respectively, While the orientation on the same level of *Culex* mosquito were 36 cm, 16 cm, 52 cm, 33 cm and 56 cm following the same order.

No significant difference between *Anopheles* and *Culex* toward the formulated products (f=1.82; f-crit=3.49), means both adult mosquitoes behave relatively similar to these products, but there were a significant difference between the repellency effect of the formulated products (f=56.77; f-crit=3.26), means some are better than the others.

Fresh ginger product was the best (average orientation distance was 59 cm, followed by fresh garlic product (53 cm), dry ginger product (35.5 cm), dry clove product (35 cm), and lastly dry garlic product (17 cm).
Table (4.5) The least vertical and horizontal orientation distance (cm) of *Anopheles* and *Culex* mosquitoes in respect to the formulated products.

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Species</th>
<th>Dry clove</th>
<th>Dry ginger</th>
<th>Fresh ginger</th>
<th>Dry Garlic</th>
<th>Fresh garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical</td>
<td>Anopheles</td>
<td>36</td>
<td>34</td>
<td>65</td>
<td>18</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Culex</td>
<td>27</td>
<td>31</td>
<td>61</td>
<td>14</td>
<td>53</td>
</tr>
<tr>
<td>Horizontal</td>
<td>Anopheles</td>
<td>42</td>
<td>44</td>
<td>54</td>
<td>21</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Culex</td>
<td>36</td>
<td>33</td>
<td>56</td>
<td>16</td>
<td>52</td>
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</tbody>
</table>

### SUMMARY

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<thead>
<tr>
<th></th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles-vertical</td>
<td>5</td>
<td>211</td>
<td>42.2</td>
<td>365.2</td>
</tr>
<tr>
<td>Culex-vertical</td>
<td>5</td>
<td>186</td>
<td>37.2</td>
<td>374.2</td>
</tr>
<tr>
<td>Anopheles-horizontal</td>
<td>5</td>
<td>213</td>
<td>42.6</td>
<td>171.8</td>
</tr>
<tr>
<td>Culex-horizontal</td>
<td>5</td>
<td>193</td>
<td>38.6</td>
<td>257.8</td>
</tr>
<tr>
<td>Fresh garlic</td>
<td>4</td>
<td>215</td>
<td>53.75</td>
<td>8.25</td>
</tr>
<tr>
<td>Dry garlic</td>
<td>4</td>
<td>69</td>
<td>17.25</td>
<td>8.92</td>
</tr>
<tr>
<td>Fresh ginger</td>
<td>4</td>
<td>236</td>
<td>59</td>
<td>24.67</td>
</tr>
<tr>
<td>Dry ginger</td>
<td>4</td>
<td>142</td>
<td>35.5</td>
<td>33.67</td>
</tr>
<tr>
<td>Clove</td>
<td>4</td>
<td>141</td>
<td>35.25</td>
<td>38.25</td>
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</table>

### ANOVA

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<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
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<th>P-value</th>
<th>F-crit</th>
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<td>3</td>
<td>35.52</td>
<td>1.82</td>
<td>0.197866</td>
<td>3.49</td>
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<td>Columns</td>
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<td>4</td>
<td>1110.33</td>
<td>56.77</td>
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<td>3.26</td>
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<tr>
<td>Error</td>
<td>234.7</td>
<td>12</td>
<td>19.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4782.55</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1- The color was pale yellow for all products, while the odor was sharp for both dry and fresh garlic products, pleasant for both dry and fresh ginger product and sharp for dry clove product.

2- The pH of the products of dry clove DC, dry garlic DG, fresh garlic FG, dry ginger DGn and fresh ginger FGn were 6, 6.4, 6.2, 6.3 and 6.5, respectively, while they have a density of 0.99, 0.96, 0.91, 0.92 and 0.94, following the same order.

3- The refractive index values were 1.4699, 1.4660, 1.4681, 1.4690 and 1.4696 for the formulated products of DC, DG, FG, DGn and FGn, respectively.

4- DG product has the highest volatility rate (19 mg/minute), followed by DC product, then FG product, DGn product and lastly FGn which has the far lowest volatility rate (6 mg/minute).

5- DC product has highest moisture and volatile contents (5.5%), followed by FGn, DGn, FG and Lastly DG (3.9%).

6- DG product has the highest apolar contents (86%), followed by FGn product (85%), FG product (82.5%), then DGn product (80%) and lastly DC product (76.5%).

7- FGn product has the highest repellency effect on adult mosquitoes, followed by FG product, DGn product, DC product, and lastly DG product.

5.2 Recommendations

1. Great attention, due to the easy availability, low budget and less adverse environmental impact, must be given for developing plant origin insecticides as an alternative to chemical insecticide.

2. There is widespread use of plants as repellents, scientific understanding of these plants is, however, largely unexplored and therefore there is a need to collect ethno-botanical information on these plants as a first step prior to evaluation of their efficacy and safety as repellents.

3. Mosquito repellent from natural sources that may applied directly on the skin need more study to decrease the incidence of skin rashes specially in those who have sensitive skin. To avoids this problem, automatic devices should be developed to pump the repellent contents every specific time.
REFERENCES


