بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Insecticidal Effects of Clove (*Syzygium aromaticum*) and Basil (*Ocimum basilicum*) againstLarvae of Mosquito (*Culex quinquefasciatus* Say)

فاعلية القرنفل و الريحان كمبيدات حشرية ضد يرقات بعوض الكيولكس (*Culex quinquefasciatus Say*)

A thesis submitted in partial fulfillment of the requirements for the M. Sc. degree in Plant Protection

By:

Roaa Idrees Hamza Khashmalmous

B.Sc. Agric. (Honors), 2014.

Department of Crop Protection

University of Nile Valley

Supervisor: Dr. Loai Mohamed Elamin Ahmed

Department of Plant Protection

College of Agricultural Studies -Shambat

Sudan University of Science and Technology

April , 2021

Approval Page

Name of Candidate:

Roaa Idrees Hamza Khashmalmous

Thesistitle:

Insecticidal Activity of Clove (Syzygium aromaticum) and Basil (Ocimum basilicum) Against Larvae of Mosquito (Culex quinquefasciatus Say - Diptera - Culicidae)

Approved by:

1. External Examiner

Name: Dr. Hamid Ahmed Hamid

Signature: .... Date: 29/4/2021

2. Internal Examiner

Name: Dr. Saif Eldin Mohamed Kheir

Signature: .... Date: 29.4.2021

3. Supervisor

Name: Dr. Loai Mohamed Elamin Ahmed

Signature: .... Date: 29/4/2021

الآيــة

قال تعالى:

إِنَّ اللَّهَ لَا يَسْتَحْيِي أَنْ يَضْرِبَ مَثَلًا مَا بَعُوضَةً فَمَا فَوْقَهَا فَأَمَّا الَّذِينَ آمَنُوا فَيَعْلَمُونَ أَنَّهُ الْحَقُّ مِنْ رَبِّهِمْ وَأَمَّا الَّذِينَ كَفَرُوا فَيَقُولُونَ مَاذَا أَرَادَ اللَّهُ بِهَذَا مَثَلًا يُضِلُّ بِهِ كَثِيرًا وَيَهْدِي بِهِ كَثِيرًا وَمَا يُضِلُّ بِهِ إِلَّا الْفَاسِقِينَ (البقرة/26)

صدق الله العظيم

DEDICATION

I dedicate this research to my father's soul

To my Mother, husband, sisters and brother

Finally, to all teaching staff in the Department of Plant Protection.

ACKNOWLEDGEMENT

All thanks are due to Almighty Allah who gave me health and strength to produce this work. Also thanks to my supervisor Dr. **Loai Mohamed Elamin** for his guidance, patience, keen interest and continuous participation throughout this study. Thanks to my family for their continuous support. Thanks extended to everyone who helps me during this study.

List of Contents

Title	Page No.
الايه	I
Dedication	II
Acknowledgment	III
List of contents	IV
List of Tables	VII
List of Figures	VIII
List of Plates	X
Abstract	XI
ملخص البحث	XII
CHAPTER ONE	1
INTRODUCTION	1
CHAPTER TWO	3
LITERATUREREVIEW	3
2.1. Mosquitoes (*Culex quinquefasciatus)*	3
2.1.1. Classification	3
2.1.2. Description and Morphology	3
2.1.2.1. Eggs	3
2.1.2.2. Larvae	4
2.1.2.3. Pupae	4
2.1.2.4. Adults	5
2.1.3. Distribution	5
2.1.4. Biology and Life Cycle	6
2.1.5. Medical importance	8
2.1.5.1. Nuisance	8
2.1.5.2. Lymphatic filariasis	8
2.1.5.3. St. Louis encephalitis viru	9
2.1.5.4. Western equine encephalitis virus	7
2.1.5.5. West Nile virus	10

2.1.5.6. Rift valley virus	10
2.1.6. Mosquitoes control	10
2.1.6.1. Environmental control	11
2.1.6.1.1. Source reduction	11
2.1.6.1.2. Environmental manipulation and modification	12
2.1.6.1.3. Personal protection	12
2.1.6.2. Physical control	13
2.1.6.3.Genetic Control	15
2.1.6.4. Biological control	16
2.1.6.5. Chemical control	18
2.1.6.4.1. Chemical control directed against aquatic stages	18
2.1.6.4.2. Chemical control directed against adults	19
2.1.6.4 .3. Insecticides resistance	21
2.1.6. 6. Integrated Mosquito Management (IMM)	21
2.2 Clove plant (*Syzygium aromaticum*)	23
2.2.1 Classification	23
2.2.2. Morphology and description	23
2.2.3. Ecology and distribution	24
2.2.4. Economic importance and uses	24
2.2.5. Chemical constituents	25
2.2.6. Pesticidal properties	26
2.3. Sweet basil (*Ocimum basilicum* L.)	29
2.3.1. Classification	29
2.3.2. Description	29
2.3.3. Ecology and distribution	30
2.3.4. Economic importance and uses	30

2.3.5. Chemical constituents	31
2.3.6. Pesticidal properties	32
CHAPTER THREE	35
MATERIALS AND METHODS	35
3.1. Study location	35
3.2. Insect collection and rearing	35
3.3. Experiment materials	35
3.4. Preparation and extraction method	35
3.6. Bioassay tests	38
3.7. Statistical analysis	38
CHAPTER FOUR	40
RESULTS	40
4.1. Mortality (%) of clove (*Syzygium aromaticum)* water extracts against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito	41
4.2. Mortality (%) of clove oil against 2^{ed} in star larvae of *Culex quinquefasciatus* mosquito	41
4.3. Mortality (%) of clove oil against 4th in star larvae of *Culex quinquefasciatus* mosquito	41
4.4. Mortality (%) of Basil (*Ocimum basilicum*) oil against 2^{ed}in star larvae of *Culex quinquefasciatus* mosquito	44
4.5. Mortality (%) of Basil (*Ocimum basilicum*) oil against 4th instar larvae of *Culex quinquefasciatus* mosquito	44
CHAPTER FIVE	47
DISCUTION	47
CONCLUSION	49
RECOMMENDATIONS	50
References	51

List of Tables

Title of tables	**Page No.**
Table 1. Mortality (%) of clove water extract against 2^{ed} in star larvae of *Culex quinquefasciatus* mosquito	41
Table 2. Mortality (%) of cloves oil against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito	42
Table 3. Mortality (%) of clove oil against 4th instar larvae of *Culex quinquefasciatus* mosquito	43
Table 4. Mortality (%) of Basil oil against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito	45
Table 5. Mortality (%) of Basil oil against 4thin star larvae of *Culex quinquefasciatus* mosquito	46

List of Figures

Figures title	**Page No.**
Figure 1. Mortality (%) of clove water extracts against 2ed instar larvae of *Culex quinquefasciatus* mosquito	41
Figure 2. Mortality (%) of clove oil against 2ed instar larvae of *Culex quinquefasciatus* mosquito	42
Figure 3. Mortality (%) of clove oil against 4th instar larvae of *Culex quinquefasciatus* mosquito	43
Figure 4. Mortality (%) of Basil oil against 2ed instar larvae of *Culex quinquefasciatus* mosquito	45
Figure 5. Mortality (%) of Basil oil against 4thin star larvae of *Culex quinquefasciatus* mosquito	46

List of Plates

Plates title	Page No.
Plate 1. Larvae collecting site.	36
Plate 2. Larvae rearing cage	36
Plate 3. Clove and basil oils	37
Plate 4. Buds of clove	37
Plate 5. Clove Powder	39
Plate 6. The Treatments	39

Abstract

The experiments were conducted in the research Laboratory, of College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST), during May, 2019.The average temperature is between 30-35°C and relative humidity (RH) is between 30 – 35 C %.

The aim of this study is to verify the effectiveness of the clove plant, *Syzygium aromaticum* (water and oil extract), and basil plant *Ocimum basilicum*, an (oil extract) , a commercial botanical oil, branded al hawag, was used. Against larvae of culex (*Culex quinquefasciatus)* under laboratory condition. Four different concentrations were used for each single experiment(2%, 1%, 0.50, and 0.25). in the first experiment, using clove as water extract, and in the second, using clove and basil as oil extract, plus one drop of liquid soap was added as a surfactant, and the treatment was done on the second and fourth stages of the larva of the mosquito (*Culex quinquefasciatus),*except for the water extract of clove in the second star only. Each treatment was replicated three times in addition to the control, and mortality % was recorded after (1, 6, 12, 24, 48, and 72 hour) respectively.

The result showed different effects between extracts as well as between different stages of larva as insecticides. The water extract of clove gave mortality% (98.3%, 43.3%) the clove oil in 2^{th} and 4^{th} start gave (96.6%, 58.3% and 96.6%, 66.6%) respectively. And basil oil in 2^{th} and 4^{th} start gave (85.0%, 41.6% and 58.3%, 46.7) respectively after 72h of treatment at the highest and lowest concentration.

These results confirmed that the oil extract of clove has a greater effect than the water extract, and that clove oil has a greater effect on Culex larvae than basil oil, which can be used as a source of plant insecticides in the future.

ملخص البحث

أجريت التجارب بالمختبر البحثي ، كلية الدراسات الزراعية (شمبات)، جامعة السودان للعلوم والتكنولوجيا (SUST) خلال شهر مايو 2019 , كانت درجة الحرارة تتراوح ما بين **30 – 35** درجة مئوية. الهدف من هذه الدراسة هو التحقق من فاعلية نبات القرنفل ***Syzygium aromaticum*** (مستخلص مائي وزيتي) ونبات الريحـــــــان *Ocimum basilicum*(مستخلص زيتي) ضد يرقات العمر الثاني والرابع لبعوض الكيولكس تحت ظروف المختبر , ثم تصميم التجربة بواسطة التصميم العشوائي الكامل وتم تحليل النتائج بواسطة Statistic – 8 , تم استخدام أربعة تركيزات مختلفة (2% , 1 , 0.50 , 0.25) لكل معاملة , تم تكرار المعاملة ثلاث مرات بالإضافة الي الشاهد وتم تسجيل نسب الموت بعد (72 , 48 , 24 , 12 , 6 ,1) ساعة علي التوالي , أظهرت النتائج ان القرنفل (المستخلص المائي والزيت) والريحان(الزيت) فاعلية كمبيدات حشرية ضد يرقات بعوض الكيولكس أعطي المستخلص المائي للقرنفل تركيز (2%) نسبة موت *98.3% علي يرقات العمر الثاني , بينما* أعطي المستخلص الزيتي للقرنفل نسبة موت 96.6% ضد كل من يرقات العمر الثاني والرابع بعد 72 ساعة من المعاملة , أظهرت هذه التجربة ان زيت الريحان اقل فاعلية مقارنة بالقرنفل خاصة ضد العمر اليرقي الرابع لبعوض الكيولكس حيث أعطي نسبة موت 85% و 58.3% في اعلي تركيز (2%) لكل من العمر الثاني والرابع علي التوالي بعد 72 ساعة من المعاملة , خلصت الدراسة ان القرنفل والريحان تأثير كمبيدات حيوية ضد يرقات البعوض ويمكن ان تستخدم كمبيدات يرقيه في المستقبل .

CHAPTER ONE

INTRODUCTION

Mosquitoes are among the most serious bloodsucking arthropods and insect pests of medical importance worldwide. They are vectors of various disease agents some of which cause millions of cases of illnesses and deaths in humans and animals each year. Among these diseases malaria, yellow fever, dengue, filariasis, and Rift Valley fever at endemic and epidemic areas in many countries (WHO, 1991 and Lerdthusnee, *et al*., 1995). There are approximately 3200 known mosquito species, which vary widely in their habit and host range. Some mosquitoes are anthropophilic, feeding only on human beings, some are zoophilic, preferring animals, and some are opportunistic or generalist and feed on many different species (Service, 2000). Lymphatic filariasis (LF) is an important public health and socio-economic problem worldwide. It affects 120 million people in over 80 countries (WHO, 2000). Lymphatic filariasis is endemic in Sudan, based on published and unpublished data from scattered spot surveys and hospital records (WHO, 2003). Different strategies have been developed to reduce the prevalence of mosquito– borne disease in many regions of the world. Mosquito control by continued applications of organophosphates, such as temephos, and fenthion, and insect growth regulator such as diflubenzuron, and methoprene is most common, and widely used (Gosh, 2008) Synthetic insecticides have created a number of ecological problems, such as the development of resistant in some insect species, ecological imbalance, and also are highly toxic to human, animals and environment (Abdul Rahuman and Venkatesan, 2008 and Karunamoorthi *et al*., 2008a). Consequently, researchers and scientists are currently investigating natural substances to use as insecticides for controlling larvae of mosquitoes, as they are considered to be more environmentally biodegradable and considered safer than synthetic insecticides (Cetin *et al*., 2006).

The main objectives of this study was to investigated through laboratory experiments the activity of two botanical extracts clove (*Syzygium aromaticum*), and Rehan (*Ocimum basilicum*) against mosquitoes (*Culex quinquefasciatus)*.

CHAPTER TWO

LITERATURE REVIEW

2.1. Mosquitoes (*Culex quinquefasciatus)*

2.1.1.Classification

Kingdom : Animalia

Phylum : Arthropoda

Class : Insecta

Order : Diptera

Family : Culicidae

Genus : *Culex*

Species : *quinquefasciatus*

S.N.: Culex quinquefasciatus Say

2.1.2. Description and Morphology

2.1.2.1. Eggs

The *Culex* females lay their eggs in rafts comprising several hundred eggs locked together in a boat-shaped structure. During oviposition, the females stand on the water surface with the hind-legs in a V-shaped position.The eggs are released through the genital opening and grouped together between the hind-legs, forming a raft where the eggs stand vertically on their anterior poles attached together by chorionic protrusions (Clements, 1992). Their egg numbers per egg-raft depend upon the species and the quality and quantity of blood meal taken by them. A single gravid female may lay up to 5 egg rafts in its life time. This normally hatches at the optimum temperature of 250C to 300C within 24 to 30 hours after being oviposited (Barik et *al.,* 2012).The egg-raft in position and when rafts drift to their aquatic boundaries they tend to remain there. Immediately following oviposition, the eggs are soft and white, but they sclerotize and darken within1–2h. (Norbert *et al.,* 2010)

2.1.2.2. Larvae

The legless (apodous) larval body is divided into three distinct parts: the head with mouth-parts, eyes and antennae; the broader thorax and abdomen which is composed of seven almost identical segments and three modified posterior segments. These posterior segments bear four anal papillae to regulate the electrolyte levels. At the abdominal segment VIII, a siphon in culicines, or only spiracular lobes in anophelines, are developed where the tracheal trunks open at spiracles for the intake of oxygen (Norbert *et al.,* 2010).The larval head is short and stout, becoming darker toward the base. The mouth brushes have long yellow filaments that are used for filtering organic materials. The abdomen consists of eight segments, the siphon, and the saddle. Each segment has a unique setae pattern (Sirivanakarn and White, 1978). The siphon is on the dorsal side of the abdomen, and in *Cx. quinquefasciatus* the siphon is four times longer than it is wide with multiple setae tufts (Darsie and Morris, 2000). The saddle is barrel shaped and located on the ventral side of the abdomen with four long anal papillae protruding from the posterior end (Sirivanakarn and White, 1978).

2.1.2.3. Pupae

After the 4th larval instar completes its development, it moults into a non-feeding but highly mobile stage called the pupa. Similar to other mosquito species, *Cx. quinquefasciatus* pupae are comma shaped and consist of a fused head and thorax (cephalothorax and an abdomen). The cephalothorax color varies with habitat and darkens on the posterior side. The abdomen has eight segments. The first four segments are the darkest, and the color lightens towards the posterior. The paddle, at the apex of the abdomen, is translucent and robust with two small setae on the posterior end (Sirivanakarn and White, 1978).

The pupa breathes through a pair of tube-like organs (trumpet) situated at the 'head' end of the comma-shaped body. The duration of the pupal stage again is dependent on temperature but is generally of the order of 2-3 days for Anopheles, Aedes and Culex species. Once the adult tissues have developed and it is time for emergence, the pupa swims to the water surface and stretches itself out to full length and the pupal skins splits along the back and the teneral adult mosquito emerges above the water surface After emerging from the pupal casing, the adult mosquito rests on the water surface for a short time, to allow its wings and body to dry, before flying off in search of nourishment and a mate. Male mosquitoes develop faster than females, and are usually the first to emerge. (Barik *et al.*, 2012)

2.1.2.4. Adult

Adult *Cx. quinquefasciatus* vary from 3.96 to 4.25 mm in length (Lima *et al*., 2003). The mosquito is brown with the proboscis, thorax, wings, and tarsi darker than the rest of the body. The head is light brown with the lightest portion in the center. The antennae and the proboscis are about the same length, but in some cases the antennae are slightly shorter than the proboscis. The flagellum has thirteen segments that have few to no scales (Sirivanakarn *et al*., 1978). The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of each tergite. The bands barely touch the basolateral spots taking on a half-moon shape (Darsie and Ward, 2005).

The wings are long and narrow, number and arrangement of wing veins is the same for all species .The veins are covered with scales. Scales also project as a fringe along the posterior border of the wings .The legs of Mosquitoes are long and slender also covered with scales .The tarsus usually terminates in a pair of toothed or simple claws. Culex has a pair of small fleshy pulvilli between the claws (Service, 2004).

2.1.3. Distribution

Mosquitoes have a worldwide distribution; they spread throughout the tropical and temperate region. There are some of 3300 species of mosquitoes belonging to 41 genera, all contained in the Family: Culicidae and about 100 species are vectors of some human disease (Rozendaal, 1997 and Service, 2004).

Culex quinquefasciatus is a sub-tropical species usually found within the latitudes 36° N and 36° S. However, in the United States between 36° N and 39° N there is a broad hybrid zone where *Cx.quinquefasciatus* freely mates with *Culex pipiens* Linnaeus, which is usually not found south of 39° N. Mating between these two members of the *Culex pipiens* complex produces viable offspring within the hybrid zone. *Culex quinquefasciatus* is found in North America, South America, Australia, Asia, Africa, the Middle East, and New Zealand.

In the Sudan distribution of about thirty species of the genus Culex have been reported by Lewis (1954). *Cx. pipiens* has been collected from Malakal and from Jebel- Marra. *Cx*. Molestus Forskal occurs between Khartoum and Egyptian border. *Cx. p. fatigans* (*Cx. quenquifasciatus* Say) are recorded from coastal regions, 13parts of the Red sea hills and the neighborhoods of Kassala, Khartoum and various places in Upper Nile (Abu shama, 1974). But Mobozy (1977) notice that the species has spread thought out the Sudan closely following urbanization, in Khartoum province, Nile province (Atbara and Shendi), Kassala province, White Nile province (Duem and Kosti), Gezira province (Wad Medeni) and the Blue Nile province (Sennar).

2.1.4. Biology and Life Cycle

Mostly mating occurs after emergency (Service, 2004 and Harwood and James, 1979). After mating, sperm passed into the female spermatheca. These usually serve to fertilize all eggs laid during life time, thus only one insemination are required to produce eggs, but at intervals. *Cx. quinquefasciatus* females fly during the night to nutrient-rich standing water where they will lay their eggs. They will oviposit in waters ranging from waste water areas to bird baths, old tires, or any container that holds water. If the water evaporates before the eggs hatch or the larvae complete their life cycle, they die. the eggs are soft and white, but they sclerotize and darken brown to blackish ≤1mm or less long within1–2h. (Service, 2004, Rozendaal, 1997 and Norbert *et al.,* 2010). Usually the culicine larvae hang head downwards from the water surface. Larvae moult four times at intervals, before reaching the pupal stage. At each moult, the head capsule is increased to the full size characteristic of the next instar, whereas the body grows continuously. Thus the size of the head capsule is a fairly good morphometric indicator for the larval instar. Each moult is coordinated by the relative concentrations and interactions of juvenile hormone and ecdysone, a molting hormone.The development of larvae is temperature dependent (Norbert, *et al.,* 2010) .The larvae feed on biotic material in the water and require between five to eight days to complete their development at 30°C. The larvae progress through four larval instars, and towards the end of the fourth instar they stop eating and molt to the pupal stage. Larvae feed on yeasts, bacteria, protozoans and numerous other micro-organisms, and decaying organic material found in the water. *Culex quinqueasciatus* can breed prolifically in water contaminated with excreta or rotting vegetation (Service, 2004). Pupae do not feed and spends most of its time at the water surface, if disturbed it dives swiftly to the bottom. when mature the pupal skin splits at one end and a fully developed adult mosquito emerges. In the tropic the pupal period lasts 1-3 days Following 36 hours at 27°C the adults emerge from the pupal stage. The time of development under natural conditions for all stages is variable and dependent on temperature. Both males and females take sugar meals from plants. Following mating, the female seeks a blood meal. *Culex quinquefasciatus* are opportunistic feeders, feeding on mammals and/or birds throughout the night. Males survive only on sugar meals, while the female will take multiple blood meals. After a female mosquito digests the blood meal and the eggs develop, she finds a suitable place to lay her eggs, and the cycle begins again.

A single female can lay up to five rafts of eggs in a lifetime (Gerberg *et al*., 1994) the entire period from egg to adult take about 7-13 days. (Service, 2004 and Rozendaal, 1997).

2.1.5. Medical importance

Mosquitoes are a serious threat to public health, as they transmit many vector-borne diseases and cause millions of deaths every year (Rozendaal, 1997and Service, 2004). Three genera of medically important mosquitoes are found in the Sudan (*Anopheles, Culex* and *Aedes*).

Culex quinquefasciatus is a vector of many pathogens of humans, birds and both domestic and wild animals. It is the primary vector of lymphatic filariasis (LF), St. Louis encephalitis virus (SLEv), Western equine encephalitis virus (WEEv) , West Nile virus (WNv) , Rift valley virus and several protozoa like *Plasmodium relictum* that causes bird malaria (Dusek,1995 and Lanciotti *et al*.,1999) .

2.1.5.1. Nuisance

In the northern areas of temperate region; outdoor activities can be made impossible by swarms of biting Mosquitoes. Some Mosquitoes, cause painful bites some time followed by localized swelling and inflammation. Irritation may last for weeks (Rozendaal, 1997). Although *Anopheles* Mosquitoes may not be disease vectors in an area they may nevertheless constitute a biting nuisance. Usually, however, it is the Culicine mosquitoes, especially *Aedes*, *Ochlerolatus* and *Psorophora* species that cause biting problems (Service, 2004).

2.1.5.2. Lymphatic filariasis

Lymphatic filariasis (LF) is a widely distributed tropical disease with around 120 million people infected worldwide and 44 million people have lymph edema of the upper or lower limb, breast, scrotum or genitals or hydrocele (Abdul Rahuman *et al*., 2008a).In the Sudan the disease was restricted to Kordufan, Darfur and the

Blue Nile states, but recently it is widely distributed to include most of the states (El-Rayah, 2007).

Lymphatic filariasis caused by three species of parasitic worm *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* which occur in the lymph vessels and may cause huge swellings of the limbs and genital organs. Lymphatic filariasis is one of the major public health problems over 90% of these infections are due to *W. bancrofti* (Ottesen, 2006). Although the disease causes much suffering and disability it is rarely life – threating (Rozendaal, 1997and Schmidt and Roberts, 2005). Bancroftian filariasis is affecting people living in many tropical regions in old and new world. It transmitted mainly by *Culex quinquefasciatus,* and by some *Anopheles* and *Aedes* species. In contrast *Brugian filariasis*, caused by *Brugia malayi* and *Brugia timori*, is more rural disease and has a much more restricted distribution, occurring in Asian countries, it is absent from Africa and the Americas. It is the main vectors are the *Mansonia species* (Rozendaal, 1997; Service, 2004 and Schmidt and Robert, 2005). Both bancroftian and brugian filariasis occurs in two basic forms: nocturnal periodic and nocturnal sub-periodic. Anopheline together with certain culicine such as *Culex quinquefasciatus*, various species of *Mansonia,* and a few *Ochlerotatus* species, are the vector of the nocturnal periodic form. Culicine are vectors of sub periodic form in the nocturnal form most of the microfilaria-during the day- is in the blood vessels supplying the lungs (Ottesen, 2006).

2.1.5.3. St. Louis encephalitis virus

Saint Louis encephalitis virus (SLEV) is an arthropod-borne virus and a member of the Japanese encephalitis virus complex within the genus *Flavivirus*, family *Flaviviridae* (Calisher *at el.,* 1989). SLEV prototype strain (Parton) was isolated in 1933, in Saint Louis, Missouri, USA, from suspensions of brain specimens from deceased patients, by intra-cerebral inoculation of rhesus monkeys and white mice.

In 1941/42, Hammon *et al*. established the involvement of wild birds as reservoir hosts and *Culex* mosquitoes as vectors of the virus (Reisen, 2003).

2.1.5.4. Western equine encephalitis virus

Western equine encephalitis virus (WEEV) is a mosquito borne arbovirus and the causative agent of western equine encephalitis (WEE). Infections of humans and horses can be fatal, and survivors often suffer permanent neurological sequelae (Whitley and Gnann, 2002). WEEV is found in North and South America. In North America, it circulates enzootically among passerine birds and is transmitted by its primary mosquito vector, *Culex* (*Culex*) *tarsalis*. Mammals can participate in a secondary cycle (Hardy *et al.*, 1983).

2.1.5.5. West Nile virus

West Nile virus was first isolated from a human in the West Nile district of Uganda in 1937 and later from many other vertebrate hosts including horses, dogs, rodents and bats (Eldridge and Edman, 2000). West Nile Virus is a mosquito-borne virus contracted through mosquito bites. All six species of mosquitoes mentioned above are potential vectors for West Nile virus. The bites generally happen at dusk and dawn, indoors, in shady areas, or when the weather is cloudy. People of all ages (including children) can contract the virus. About 20% of those who contract WNV will come down with what is called "West Nile fever"; the other 80% of those infected show none or only mild symptoms of the virus. Symptoms of West Nile fever can include: fever, headache, body aches, swollen lymph glands, tiredness and rash on the trunk of the body (Gouge, 2012).

2.1.5.6. Rift valley virus

Rift Valley fever virus (RVFV) was first isolated in Kenya as a virus with the capacity to infect livestock herds of sheep and cattle, as well as humans (Elysse , and Desiree,.2020). Since its initial discovery, RVFV has been primarily contained within the African continent, with the exception of movement of the eastern coast of African to the island of Madagascar in 1990 (Morvan *et al.,* 2000). Significant emergence into neighboring regions occurred in the early 2000s when outbreaks were reported in Saudi Arabia and Yemen (Ahmad, 2000). To this day, much of sub-Saharan Africa and Egypt is endemic for RVFV or has been detected by sporadic outbreaks (Davies, 2010). Transmission of RVFV utilizes mechanisms described by the "One Health" framework, wherein the health and conditions of the environment, animals, and humans intersect and influence each other. Animal transmission is driven by mosquito vectors, primarily *Culex* spp. and floodwater-breeding Aedes spp. (Turell *et al.,* 2007). Wild animals have been suspected to contribute to maintenance of RVFV, yet evidence driving such speculation is limited to the presence of antibodies in certain wildlife species. Amplification of the virus in mosquitoes, is linked to mosquito abundance and breeding behaviors that are expanded by periods of heavy rainfall following extreme drought (Gerdes, 2004).Of the many competent vector species (Rostal *et al.,* 2010), infected females of some mosquito species may transmit the virus to their offspring during oviposition, or transovarial transmission (TOT), readily allowing future generations of mosquitoes to transmit RVFV (Lumley *et al.,* 2017).

2.1.6. Mosquitoes control

2.1.6.1. Environmental control

2.1.6.1.1. Source reduction

A simple by eliminating larval breeding sites such as water-filled tree-holes, septic tanks, ponds, small marshes can be filled in with rubble, earth or sand. Filling in tree –holes can be difficult because many can be at great heights and difficult to locate. Various containers such as abandoned tin cans, metal drums, diffused water a storage pots and old tire can be removed. Elimination of breeding sites can lead to permanent control, but this approach is not always feasible (Service, 2004 and Rozendaal, 1997).). Denying Mosquito Access to Urban Areas and into Homes Construction sites will often host a variety of water bodies, including water storage containers, water-filled excavations, flooded basements, wheel ruts, and temporary drainage systems. Such habitats will host a range of mosquitoes, including *Aedes*, *Culex* and *Anopheles* species (Norbert *et al.,* 2010).

2.1.6.1.2. Environmental manipulation and modification

If it is not feasible to eliminate mosquito breeding places it may be possible to alter them to make them unsuitable as larval habitats. For example increase flow to prevent the buildup of static pockets of water. Other environmental modifications include the removable of overhanging vegetation to reduce breeding by shade – preference mosquitoes. Intermittent flooding of fields to allow drying out every 3-5 days can substantially reduce population of several important vectors (Service, 2004and Rozendaal, 1997). Larval habitats can also be altered such as drainage operations, intermittent irrigation, and change in river discharge or configuration, removal water plants or algae, making habitats unsuitable. Few interesting results against Anopheles mosquitoes have been reported but they require massive engineering efforts (Mouchet *et al*., 2004)

2.1.6.1.3. Personal protection

Insecticide-treated bed nets (ITNs) are a form of personal protection that has repeatedly been shown to reduce severe disease and mortality due to malaria in endemic regions. In community-wide trials in several African settings, ITNs have been shown to reduce all-cause mortality by about 20%. Untreated bed nets form a protective barrier around persons using them. However, mosquitoes can feed on people through the nets, and nets with even a few small holes provide little, if any, protection. The application of a residual insecticide greatly enhances the protective efficacy of bed nets. The insecticides used for treatment kill mosquitoes and other insects. The insecticides also have repellent properties that reduce the number of mosquitoes that enter the house and attempt to feed. There are several types of nets available. Nets may vary by size, material, and/or treatment. Most nets are made of polyester but nets are also available in cotton, polyethylene, or polypropylene. Currently, only pyrethroid insecticides are approved for use on ITNs. These insecticides have very low mammalian toxicity but are highly toxic to insects and have a rapid knock-down effect , even at very low doses. Pyrethroids have a high residual effect: they do not rapidly break down unless washed or exposed to sunlight. Previously, nets had to be retreated at intervals of 6-12 months, more frequently if the nets were washed. Nets were retreated by simply dipping them in a mixture of water 23 and insecticide and allowing them to dry in a shady place (Chavasse *et al*., 1999 and WHO , 2001).Repellents are the most common means of personal protection against blood seeking arthropods and for the prevention of arthropod-borne disease transmission. Previous work concentrated mainly on simple solutions of topical repellents and the chemical treatment of clothing to prevent blood-sucking arthropods (Rutledge *et al*., 1978). Current protection is based on controlled release personal use of controlled-release arthropod repellent formulations, and the impregnation of fabrics with permethrin. Repellents may be applied to the skin, clothing or in some cases, to screens to prevent or deter arthropods from attacking humans (Norbert *et al.,* 2010).

2.1.6.2. **Physical control**

Physical control may be defined as suppression of insects by physical or mechanical means. This contrasts with the use of insecticides, which kill insects by chemical or biochemical means, and with the use of environmental measures which do not kill insects directly, but prevent their establishment, reproduction or impact by, for example, eliminating their larval habitat, or preventing their access to the host, for example, by better designed housing. In practice, there is some overlap between these various terms. Physical control techniques exist for control of both mosquito larvae (*e.g.* polystyrene beads) and adults (*e.g.* mass-trapping). Physical control may be used in isolation, but is often seen as a component of integrated vector management (IVM). In general, mosquitoes are considered unlikely to develop resistance to physical control techniques (Norbert *et al.*, 2010). The more paraffinic oils have a largely physical interaction with the insect's siphon, preventing effective oxygen uptake which resulting in drowning or suffocation. Oils with a higher aromatic content, parti cularly those with a higher volatility are considered to be toxic to mosquito larvae Several studies have determined the impact of oil treatments on non target aquatic life. When surfactants and monomolecular organic surface films are used against mosquitoes they exert their control by physicochemical modification of the air–water interface (Garrett and White, 1977). Larvae and pupae cannot penetrate the film at the water surface to obtain atmospheric oxygen, and newly emerged adults will drown on the treated water surface. Their effectiveness in controlling mosquito larvae and pupae is attributed to a reduction in water surface tension, with a subsequent wetting of tracheal structures resulting in anoxia, rather than chemical toxicity. Large numbers of film forming surface active agents were evaluated as mosquito larvicides ,and some of these were found to have potential for practical mosquito control programmes(Norbert *et al.*, 2010). Kline (2007) reviewed progress and development in mass-trapping technology, and lists a number of commercially available mosquito trapping devices, using various combinations of light, carbon dioxide, water vapour, heat, and octenol as attractants. The mosquitoes are typically killed either by desiccation within the device, or by high voltage grids. He describes operational use of mosquito traps on an isolated island surrounded by salt-marsh, in which *Oc. taeniorhynchus* was very abundant. Mosquito Magnet Pro devices were placed around the island at a rate of one device/0.44ha. After about 2 weeks trapping, mosquito numbers were reduced by >80%, and these results were repeated over several consecutive years. Other evaluations of mass trapping have not been so successful (Henderson *et al*. 2006).

There have been recent reports of the development of a laser device for mosquito control (Guth, 2009). The device is claimed to be able to detect individual flying female mosquitoes and destroy them with a burst of laser energy. It has been proposed that the devices could be mounted as a "fence" to protect residential areas against incoming mosquitoes.

2.1.6.3. Genetic Control

Population Elimination via the Sterile Insect Technique (SIT) The potential success of the SIT hinges around the fact that female mosquitoes are monogamous, *i.e.* they mate once only, and use the sperm stored in their spermatheca to fertilize each successive batch of eggs. If that mating happens to be with a sterile male, then that female will lay only sterile eggs, and will not contribute to the next generation of mosquitoes. If sterilized male mosquitoes can be released in sufficient numbers and over a sufficiently extended period, so that they out-compete the indigenous males in terms of mating with the females, then the population should decline to extinction(Norbert *et al.,* 2010). Sterilization of the male pupae has been achieved by a variety of techniques: Chemosterilization is typically carried out by immersion of the pupae for a fixed period of time in a standard solution of an alkylating aziridinyl compound, such as thiotepa or bisazir. High levels of sterility may be achieved, with minimal loss of fitness (Seawright *et al.,* 1977). However use of large-scale chemosterilization has largely ceased now, partly due to concerns about safety to staff working with these potentially mutagenic chemicals.

Irradiation is now the most commonly used technique. It is typically the pupal stage that is exposed to the gamma rays, but work has also been carried out on adults. The gamma source used is typically cobalt-60 with a dose in the range 70–120Gy. The exposure induces dominant lethal mutations in the sperm of the male mosquitoes. The lower doses cause partial sterilization, while higher doses induce a more complete level of sterilization but are associated with damage to other cells within the male mosquito, causing a in fitness and competitiveness (Helinski and Knols, 2009). It has been suggested that when using lower radiation doses, the benefits of higher fitness may outweigh the drawbacks of partial sterilization (Helinski *et al*., 2006).

2.1.6.4. Biological control

Presence of Insecticide resistance and greater awareness of environmental contamination leads to interest in biological control methods. They are however, usually more difficult to implement and maintain than insecticidal methods. Moreover, biological control dose not lead to rapid control, it takes some days, or weeks. Larvivorous fish are the most widely used biological control agents, the most common being the mosquito fish *Gambusia affinis*. This is a warm- water fish originally native to the southern USA but which has been introduced to over 60 countries worldwide. (Service, 2004 and Walton, 2007). Essential to study the biology of predacious organisms with special reference to prey selectivity, reproductive potential, and their benefit as predators in relation to costs and the environmental damage they might cause, before they are actually released. The ecosystem must also be carefully evaluated to determine the potential effect of the released organisms on the existing biota. This process is even more important if non-indigenous species are to be released (Hurst *et al*., 2006 and Chandra *et al*., 2008). There are numerous pathogens, such as viruses, bacteria, protozoa and fungi that cause larval mortality, bacteria, *Bacillus thuringiensis* var *israelensis* is the most useful pathogen as it can be easily mass-produced, is toxicologically safe to humans and, and is more or less specific in killing mosquito larvae, moreover it can also kill Simuliids larvae. It is commonly formulated as a powder that after mixing with water is sprayed on breeding places, but because of no multiplication of the bacterial there to be repeated application, as with most chemical larvicides. Other species of bacteria, *Bacillus sphaericus*, differ in that in some situations it can recycle in larval habitats. This species is also more effective in organically polluted waters and is especially effective against Culex species. Resistance to both B.t.i and *B. sphaericus* has been observed in laboratory colonies of a few mosquitos' species (Service, 2004 and Rozendaal, 1997). Genetic control is considered as a form of biological control. The most common methods of genetic control involve the release in the field of sterile male mosquitoes that have been reared in the laboratory. Sterilization can be achieved by ionization radiation, crossing closely related species to produce infertile hybrid males, or more usually by introducing chemosterilant into larval breeding which makes the emerging adults sterile. The aim is to introduce large numbers of healthy and sterile males into field population that will compete with natural fertile males for female mates, resulting in large number of infertile insemination. (Service, 2004).

2.1.6.5. Chemical control

Chemical insecticides are commonly considered to be the most effective control strategy against mosquitoes. However, public concern has increased significantly regarding their negative effects, such as potential health hazards, water contamination, environmental pollution, toxicity to non-target organisms, the development of resistance in insects, and residual effects (Lee *et al*., 2001; Azmi *et al*., 2009; Zacharia, 2011; Bilal and Hassan, 2012 and Ndakidemi *et al*., 2016).

2.1.6.5.1. Chemical control directed against aquatic stages

Most control directed against mosquitoes, consists of applying larvicides. Adults are highly mobile flying insects that can readily detect and avoid many intervention measures whereas mosquito eggs, larvae and pupae are confined within relatively small aquatic habitats and cannot readily escape control measure (Killen *et al*., 2002).

Oils is the one of the oldest control methods consists of spraying mineral oils onto the water surface of breeding places to kill mosquitoes larvae by poisoning and suffocation. Diesel oil and kerosene have been used for over 100 years, but because of environmental concerns smaller dosage of lighter mineral oils are recommended. Such oils are still used, mainly in tropical countries (Service, 2004)

Paris- green another old control method is application of a fine dust of Paris- green (Copper acetoarsenite) to breeding places. It acts as a stomach poison, when ingested by surface feeding larvae, such as *Anopheles* species. Although mosquito larvae have not developed resistance to Paris- green, this insecticide has largely been replaced by the organophosphates and carbamate insecticides. Recommended larvicides include organophosphates (Malathion, pirimiphos methyl, fenitrothion and temephos), carbamates (e.g. propoxur) and pyrethroids (permethrin and deltamethrin) but they tend to kill great numbers of other aquatic insects. In organically polluted waters insecticides are less effective and so either higher dosage rates must be used or the more effective organophosphates, such as, fenthion or chlorpyrifos applied. All insecticides usually have to be sprayed on breeding places in tropical areas every 10-14 days, and more frequently on highly polluted waters. Temphos had very low mammalian toxicity and briquettes, granules or microencapsulated formulations which slowly release the insecticide over days or even weeks, can be placed in containers holding potable water to control *Aedes aegypti* (Rozendaal, 1997 and Service, 2004). Insect growth regulators (IGRs) are compounds, such as, methoprene and pyriproxyfen, that arrest larval development of insects, or compounds, like diflubenzuron, which inhibit chitin formation in the immature stages. These chemicals have the benefit of being environment friendly because they are more or less specific in killing mosquitoes and possess extremely low toxicity to humans. Methoprene is considered by the WHO to be safe for use in drinking water. It is the most commonly used IGR for mosquito's control. However, the relatively high cost of IGRs limits their use in poorer countries (Service, 2004).

2.1.6.5.2. Chemical control directed against adults

Some mosquitoes, such as many malaria vectors and *Culex quinquefasciatus* rest in houses before and/or after blood-feeding. Their population can be reduced by insecticidal spraying of houses. Residual house –spraying is the spraying of water dispersible (wettable) powders of residual insecticides to the interior surfaces of walls, ceiling and roofs of houses. Even in highly endemic areas houses need to be sprayed only at six-monthly intervals, and where malaria transmission is very seasonal, a single spraying before the rains may be sufficient to give good control. If mosquitoes are resistant to DDT, then organophosphate such as Malathion, fenthion or fenitrothion or the carbamate propoxure can be used, usually at rates of 1.5-2 g/m2- Bendiocarb, another carbamate, has a lower dosage rate of just 0.4 g/m2. Alternatively, lambda-cyhalothrin at 0.02-0.03g/m2 or permethrin at 0.5g/m2 can be used. However, the effectiveness of spraying houses in malaria control programmes depends on the mosquitoes resting indoors. There is also evidence that house-spraying may promote the selection of exophilic population of a species, that is population that before spraying rested in houses now rest out of doors. Such population changes have been recorded in the *An. gambiae* complex in Zimbabwe, *An. nuneztovai* in Venezuela, *An.minimus* in Thailand and in a few other species (Rozendaal, 1997 and Service, 2004). Nets that are impregnated with insecticides such as permethrin (200-500 mg/m2), deltamethrin (15-25mg/m2) or lambda –cyhalothrin (10-20mg/m2), all of which repel and kill mosquitoes, will protect people against bites even if they are torn or people sleep up against them. Nest impregnated by dipping in insecticide remain effective only for 4-6 months before they need re-impregnated. The latest development involves impregnating the fibers used to make nets with permethrin during their manufacture. Net made with insecticide- impregnated fibres apparently remain effective for 4 years. In West Africa and Kenya child mortality in large-scale trials has been reduced by 17-36%. It appears that if used on a large scale impregnated nets can have a mass-killing effect on vector population which can benefit the whole community, even those without nets. It also give protection against filariasis vectors, but will not be effective against any vector that bites out of doors in the early evening before people have gone to bed (Service, 2004 and Rozendaal, 1997).

Repellent are among the most commonly used methods to prevent mosquitoes and other blood-sucking pest from biting. They are applied directly to the skin or to clothing and other fabrics such as bed nets and anti-mosquito's screens. Repellents act by preventing human-insect contact and do not knockdown or kill. The duration of protection by a repellent applied to skin may range from 15 minutes to 10 hours; on clothing and other fabrics the effect lasts much longer. In the first half of this century, were dimethyl phthalate, indalone and ethyl hexanediol, these substances are still among the active ingredients of some commercial repellent. A breakthrough came in 1954 with the discovery of N, N-diethyl 3- toluamide or Deet, acolour less, oily liquid with a slight odour. It is still the best available product, repelling a wide variety of insects and generally lasting longer than the other repellents Data from India suggest that N,N- diethylphenyl acetamide (DEPA) is as effective as Deet but less expensive. Citronella is often used because it is inexpensive and some people think that it has a more pleasant smell (Service, 2004). Motorized knapsack mist-blowers, carried thermal fogger or vehicle – mounted machines that generate insecticidal aerosols <50μm, or mists 50-100μm can be used to kill outdoor resting (exophilic) adult mosquitoes. Indoor resting (endophilic) adults are also occasionally killed by mist-blower or thermal foggers. Several insecticides can be used, including malathion, fenitrothion, pirimiphos-methyl, bendiocarb and pyrethroids. In emergency situations aerial spraying gives the fastest and most effective control of vectors, and has been used to stop transmission of haemorrhagic dengue, Japanese encephalitis and North America encephalitis viruses (Service, 2004 and Takken, 2002).

2.1.6.5.3. Insecticides resistance

Resistance is a common result of insecticide use and selection pressure on the insect population. Resistance to insecticides has appeared in the major insect vectors from every genus. As of 1992, the list of insecticide – resistant vector species includes 56 Anopheline and 39 Culicine mosquitoes. Resistance has developed to every chemical class of insecticide including microbial drugs and insect growth regulators, insecticide is expected to directly and profoundly affect the reemergence of vector –borne diseases, and it is expected to threaten disease control. The six different toxin types in the *Bacillus thuringiensis-israelensis* strain used for vector control were expected to retard or prevent development of comprehensive resistance mechanism; however, multitoxin resistance to *B.tisrealensis* has already appeared (Brogdon and Mc Allister, 1998).

2.1.6.6. Integrated Mosquito Management (IMM)

The most successful approach for controlling mosquitoes is when an Integrated Mosquito Management (IMM) strategy is implemented, in which all appropriate technological and management techniques are utilized, to bring about an effective decline of target-species populations in a cost-effective manner (WHO, 2004). An IMM strategy could include biological, chemical, genetic, physical, environmental management, and educational components However, the utilization of a range of control and management methods within an IVM approach may be more cost effective, cause less environmental damage, be effective for a longer period of time, and be better suited at the community level (Rafatjah ,1982). For this reason, the IVM concept was developed, which incorporates the application of sound ecological principles to maintain mosquito populations below the nuisance and health injury threshold. It is more precisely defined as follows: “Integrated vector control is the rational combination of all available control methods in the most effective, economical, and safe manner to maintain mosquito vector populations at acceptable levels” (Norbert *et al.,* 2010).

2.2 Clove plant (*Syzygium aromaticum*)

2.2.1 classification

Kingdom : Plantae

Phylum : Angiosperms

Clade : Eudicots

Clade : Rosids

Order : Myrtales

Family : Myrtaceae

Genus : *Syzygium*

Species : *S. aromaticum*

Binomial name *Syzygium aromaticum* (L.)

2.2.2. Morphology and description

Clove is an aromatic spice tree. The term clove is taken from French word 'clove' and 'clou' which means 'nail'. Clove is conical myrtle, medium sized tree with straight trunk which grows up to 10 to 12 m in height. The branches are semi erect, grayish in color and dense. Leaves are large oblong to elliptic, simple obovate opposite, glabrous and possess plenty of oil glands on the lower surface. Tree begins flowering in about 7 years and continues flowering for 80 years or more. Flowers are small, crimson in color and are hermaphrodite (bisexual) borne at the terminal ends of small branches. Each peduncle carries 3 to 4 stalked flowers and inflorescence length remains between 4 to 5 cm. Initially flower buds are pale yellow in color with glossy appearance and turn green to bright red at maturity. (Kamatou *et al*. 2012) These are 1-2 cm long with cylindrical thick ovary consisting of four fleshy sepals. Buds are divided into elongated stem and a globose bulbous head which stimulates into nail. Commercially cloves used are air-dried unopened flower buds, 2.5 cm in length and 1.25 cm wide. Fruit matures nine months after flowering and the red ovary gradually turns to reddish purple.

The fruit nearly contains one or two seeds known as ‘mother of clove’. The cultivated trees are rarely allowed to reach fruit stage. These are harvested when they develop dark red ellipsoid berry (Ortes-Rojas *et al.*, 2014).

2.2.3. Ecology and distribution

Syzygium aromaticum (Clove) belongs to family *Myrtaceace*, a taxon of dicotyledon plants is one of most valuable and second most important spice in the world trade. Various synonymes used for the clove are *Caryophyllus aromaticus, Caryophyllus silvestris, Eugenia caryophyllus, Jambosa caryophyllus* and *Myrtus caryophyllus* (Soh and Parnell, 2015). Clove is commonly used in cultivation and indigenous to North Maluku Islands in Indonasia. Major cultivator countries of clove are ,Zanzibar, Indonesia, Madagascar and some of wild clove varieties are found in Iran. In India cultivation of clove is restricted to three states Karnataka, Tamil Nadu and Kerala. India becomes second largest consumer of clove after the Indonesia (Board, 2010) Cloves are available throughout the year due to different harvest seasons in different countries. The different varieties of clove tree vary in canopy shape from pyramidal to cylindrical. The clove tree can live up to 100 years and above. The tree prefers to grow in well drained soil with sufficient soil moisture. Clove tree requires heavy sunlight with high atmospheric temperature (25 to 35°C), well-distributed rainfall above 150 cm and high humidity above 70% (Danthu *et al.*, 2014). The crop cannot withstand water logged conditions. In India clove grows well in deep black loamy soil of humid tropics and successfully grows in the red soils of midlands of Kerala and in the hilly terrain of Western Ghats in Karnataka and Tamil Nadu (Byng, 2016).

2.2.4. Economic importance and uses

Cloves have many uses ranging from culinary to medicine. Clove is a valuable kitchen spice which can be used for studding onions, tomatoes, salads, herbal teas, and soups. It is also used to flavor meat products, cookies, chewing gums, spiced fruits, pickles, chocolates, soft drinks, puddings, sandwiches, pastries, and candies. Volatile oil is used to impart essence to perfumes soaps, toothpastes, and pharmaceuticals. In Indonesia, mixture of clove and tobacco in a ratio of 1:2 is used to make a special cigarette “Kretek”. Clove possesses antibacterial potential and is used in a variety of mouth washes, dental creams, throat sprays, and tooth pastes to kill pathogens. It is also used to relive sore gums. Mixture of eugenol (major bioactive constituent of clove) and zinc oxide is used for short-term filling of dental cavities (Cai and Wu1, 996).Clove and its main constituents possess antimicrobial, antioxidant, anti-inflammatory, analgesic, anticancer & anesthetic effects. Moreover, they showed insecticidal, mosquito repellant, aphrodisiac, and antipyretic activities (Hussain *et al.,* 2017).

2.2.5. Chemical constituents

Clove oil is a powerful agent against oxidation, with inhibition of per oxidation and hydroxylation equal to or greater than the commonly used synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), as well as isolated eugenol and quercetin (Jirovetz and Buchbauer, 2006). It consists of 82-88% eugenol, little amount of eugenyl acetate, and other minor constituents. A major component of clove taste is imparted by the chemical eugenol . Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material (Yadav *et al.*, 2020). The chemical composition of the essential oil from the bud of clove (*Syzygium aromaticum*) are as follows: eugenol (87.00%), eugenyl acetate (8.01%) and β-Caryophyllene (3.56%).This essential oil comprises in total 23 identified constituents, among them eugenol (76.8%), followed by β-caryophyllene (17.4%), α-humulene (2.1%), and eugenyl acetate (1.2%) as the main components (Alfikri and Pujiarti ,2020).

2.2.6. Pesticidal properties

Clove oil is effective against a broad range of acid-fast, gram-positive and -negative bacteria, as well as against yeasts and fungi (Khan and Ehab, 2010). Various aflatoxin producing fungi are susceptible to clove oil (Hussain *et al*., 2012).

The insecticidal properties of clove oil and its derivatives have been studied since the 1940s (West, 1944). Clove oil toxicity varies among different insects because of different insect susceptibilities, variability in the concentration of eugenol and other active substances in the clove oil, and feeding behaviors. Susceptible arthropods include mites, termites and mosquitoes. A set of experiments involving 38 essential oils found that clove oil gave the longest duration of 100% repellency (2–4 hr.) against three species of mosquitoes: *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles dirus* (Trongtokit *et al*., 2005). Clove oil (50%) combined with geranium oil (50%) or with thyme oil (50%) prevented biting by *Anopheles albimanus* mosquitoes for 1¼ to 2½ hours (Barnard, 1999).

Clove oil (0.3%) combined with the other active ingredients 1% peppermint oil and 1.3% sodium lauryl sulfate caused over 90% mortality of bed bugs (*Cimex lectularius*) (Singh *et al*., 2014). Commercially available Rescue® traps with two known stink bug pheromone attractants were used to test the potential repellency of essential oils to the brown marmorated stink bug (*Halyomorpha halys*). Traps treated with 14 mg/day of clove oil repelled over 95% of the insects (Zhang *et al*., 2014).. Clove oil reduced oviposition and adult emergence of the cowpea weevil (*Callosobruchus maculatus*) on a variety of seeds (Ajayi and Lale 2001). Common bean (*Phaseolus vulgaris*) treated with clove oil grew less and resulted in increased mortality of the bean weevil, (*Acanthoscelides obtectus*. Applications of clove oil at 43.6 μL/kg beans resulted in a 50% mortality of bean weevil (Viteri *et al*., 2014). Clove oil is generally more effective when delivered orally than as a contact insecticide.Clove bud oil killed 100% of Formosan termites (*C. formosanus*) in 2 days at 50 μg/cm2 (2 kg/ha) (Zhu *et al*., 2001). And also produced 100% mortality in Japanese termites (*Reticulitermes speratus*) at 0.5 μL/L of air (Park and sang, 2005).

Clove oil can be effective against soft-bodied insects. Pear psyllid (*Cacopsylla chinensis*) treated with clove oil had a contact LD50 of 0.730 μg/adult and 1.795 μg/nymph (Tian *et al*., 2015). An experiment that compared the efficacy of 92 essential oils for the control of sweet potato whitefly (*Bemisia tabaci*) found clove oil to be one of the more effective, with an LC50 of 0.22 mL/cm3 for clove bud oil, 0.26 mL/cm3 for clove leaf oil and 100% mortality at a rate of 2.4 mL/cm3 for the oil from both species (Kim *et al*., 2011).Experiments in Korea showed that clove oil is effective as an acaricide as well. A formulation of 20% clove oil, 40% cottonseed oil and 10% garlic oil (GC-Mite) was the most effective of 12 natural treatments for two spotted spider mite (*Tetranychus urticae*) with nearly 100% mortality after 21 days (Cloyd *et al*., 2009). In a test with adults of two species of dust mites, *Dermatophagoides farinae* and *D. pteronyssinus* adults, the clove oil resulted in 100% mortality at 12.7 μg/cm2 against both species (Kim *et al*., 2003). The copra mite (*Tyrophagus putrescentiae*) was also 100% susceptible to clove bud oil at a dose of 12.7 μg/cm2 (Kim *et al*., 2003).

Greenhouse trials showed that clove oil drenches had inconsistent results in reducing southern root knot nematode (*Meloidogyne incognita*) populations before transplant of cucumber (*Cucumis sativus*), muskmelon (*Cucumis melo*), pepper (*Capsicum annuum*), and tomato (*Solanum lycopersicum*) seedlings (Meyer *et al*., 2008).

Clove oil exhibits phytotoxicity, which means it has herbicidal activity that can also make it undesirable as a foliar insecticide or fungicide for some plants at certain growth stages (Bainard *et al*., 2006; Boyd and Brennan ,2006; Cloyd *et al*., 2009 and Meyer *et al.*, 2008).

Because of the relatively high application rate, clove oil-based 25 exempt herbicides such as Matran® 2 and Burnout II® EC are often not seen as cost-effective herbicides, even in high value vegetable systems (Dayan *et al*., 2009). At the lowest recommended rate, clove-based herbicides were estimated to cost about $600/ha (Brainard *et al*., 2013)

2.3. Sweet basil (*Ocimum basilicum* L.)

2.3.1. Classification

Kingdom : Plantae

Unranked : Asterids

Order : Lamiales

Family : Lamiaceae

Genus *: Ocimum*

Species *: basilicum*

S.N. *Ocimum basilicum L.*

2.3.2. Description

Sweet basil is indigenous to Persia and Sindh and lower hills of Punjab in India. (. Nadkarni, 2005). The plant is widely grown as an ornamental and field crop throughout the greater part of India, Burma, Cylone (Kirtikar and Basu , 2003) and several Mediterranean countries including Turkey.Basil species are lower growing annual herbs or shrubby plants, forming dense tuft of 20-30cm in height. Leaves are pale green oval and slightly toothed, and the flowers are white or purple, lanceolate, located in the axils of the leaves. Upper lobe of calyx is rounded and spreading (Bailey and Bailey, 1976). An erect branching herb, 0.6 to 0.9 m high, glabrous, more or less hispidly pubescent. Stems and branches are green or sometimes purplish. Leaves of *Ocimum basilicum* are simple, opposite,(Jayaweera ,1981). 2.5-5 cm or more long, ovate, acute, entire or more or less toothed or lobed with a cuneate and entire base. The petiole is 1.3-2.5 cm long. The leaves have numerous dot like oil glands which secrete strongly scented volatile oil. Whorls densely racemose, where the terminal receme is usually much longer than the lateral ones. The bracts are stalked, shorter than the calyx, ovate and acute. Calyx is five mm long, enlarging in fruit and very shortly pedicelled. Its lower lip with the two central teeth is longer than the rounded upper lip. Corolla being 8-13 mm long are white, pink or purplish in colour, glabrous or variously pubescent. The upper filaments of slightly exerted stamen are toothed at the base. Nutlets are about two mm long, ellipsoid, black and pitted. .(Kirtikar and Basu , 2003). Sepals of flower are five and remain fused into a 2-lipped calyx. Ovary is superior and there is a 2-carpellary, 4-locular and a 4-partite fruit of four achenes. (Jayaweera , 1981).

2.3.3. Ecology and distribution

The plant originated in Central America, but it has wide distribution throughout the world, from central Africa through Asia, America, and part of Australia, it grows to a height of approximately 50cm.. *Ocimum basilicum* commonly known in the Sudan as ‘’Rehan’’ is one of the commonest weeds in Gezira area. However it is cultivated in some parts of the tropics. It looks very similar to the previous sp., but grows little bit taller (50-80cm) (Cobley, 1956) The crop was brought from India to Europe through the Middle East in the sixteenth century, and subsequently to America in the seventeenth century (Muenscher *et al.,* 1978).

The plant prefers light (sandy) and medium (loamy) soils, and requires well-drained soil. It requires moist soil and can grow well in acid, neutral and alkaline soils with pH ranges between5-8, but it can’t grow in the shade. The plant commonly grows as an aromatic and medicinal her in worm temperate and tropical climates. In tropics the plant is perennial, but it is frost tender and needs to be grown as half-hardy annual in temperate zones (Bown, 1995).

2.3.4. Economic importance and uses

Among the aromatic herbs, basil has economic importance, and its consumption occurs both in nature and added for industrial processing. The essential oil is valued in the international market and widely used in condiments, cosmetics and medicinal industries of (Carvalho, 2006). The Basil oil has high economic value due to the presence of specific substances such as estragol, lineol, linalool, eugenol, methyl cinamato, limonene and geraniol (Sifola and Barbieri, 2006). Studies of the oils from aromatic and medicinal plants is growing because these species are known specifically for possessing biological activity, such as antibacterial, antifungal and antioxidant properties (Politeo *etal.,* 2007). The antioxidants can be classified as any substance, present in low concentration, compared with a oxidizable substrate, delays or inhibits oxidation of the substrate effectively (Babovic, 2010)

Dalzeil (1937) revealed that *O.americanum* was used for rselieving fever, dysentery, and as mouth wash to release toothache. Basil is useful in diseases of heart and blood, biliousness *kapha* and *Vata*, leucoderma etc. The juice relieves joints pain, gives luster to eyes, is good for toothache, earache and cures epistaxis when used with camphor. .(Kirtikar and Basu ,2003) The warm leaves juice of this plant along with honey is used to treat croup. It also forms an excellent nostrum for the cure of ringworm. It is used as a lotion for sore eyes too. In Guinea, the decoction of leaves and stem are given to treat fever, neuralgia, catarrh, renal troubles and burning micturation.(Chopra *etal.*,2002) The seeds washed and pounded are used in poultices for unhealthy sores and sinuses. An infusion of seeds is given in fever. The seeds are chewed in case of snake-bite, one portion is swallowed and the other portion is applied to the bitten part. A cold infusion of it is said to relieve the after pain of parturition. They are also given internally to treat cystitis, nephritis and in internal piles. (Nadkarni , 2005).

2.3.5.Chemical constitutes

The chemical composition of essential oil from *Ocimum basilicum* was studied by Brophy and Jogia (1986). Chemical analysis indicated the presence of two chemo types, one rich in methyl cinnamate (80%) and the other containing significant quantities of linalool and methyl eugenol. Guenther (1961) and Gildemeister and Hoffmann (1961) reviewed that *Ocimum basilicum* oils contain methyl chavicol, anethole, methyl cinnamate, linalool, cincole, champhor, terpenes, and sesquiterpenes. AI-Magboul (1981) reviewed that Sudanese *Ocimum basilicum* var. Lthyrsifolum essential oil contains linalool, cineol and 'methyl chavicol. Deshpandae and Tipins (1977) obtained by TLC, the fractions of ocimene, cineol, linalool, methyl cinnamate and methyl chavicol from essential oil of *Occimum basilicum.*

2.3.6. Pesticidal properties

The efficacy of certain *Ocimum basillicum* extracts against aphid (*Aphis gossypii*) was investigated at different concentrations i.e. 0.1, 0.5 and 1%. Depending on the concentration, the extract of mature seeds of the plant was shown to give 45.4-56.22 mortality (Pandey *et al*., 1983). Malaka (1972) mentioned that preparation from *O. basilicum* L. leaves had been used among other many methods to protect against termites. Deshpande and Tipnis (1977) tested the activity of different Rehan fractions against stored grain insects *Tribolium castaneum*, *Sitophillus oryzae*, and *Bruchus chinensis*. Methyl chavicol and methyl cinnamate were found to be the most effective components. Bower and Nishida (1980) isolated two compounds with highly potent juvenile hormone activity from *0. basilicum* L. oil. The two identified fractions were named juvocimene I, and juvocimene IL respectively. The later was found to induce nymph-adult intermediates at concentration as low as 10 picograms in the milkweed bug. Hassan (1988) and Gubara (1983) reported that *0.basilicum* had an anti-feeding and growth retarding effects against the African melon ladybird beetle, migratory locust adults, khapra beetle, and red flour beetle.

Dalzeil in (1937) reported that *Ocimum vividae* Wild, has been named the mosquito plant and it was used to repel mosquito in West Africa. The herbivorous animals refused to eat *O.canumsims*, a perennial which had not suffered from any disease. Malaka (1972) stated that a preparation of the leaves of *O. basilicum* L. Has been used against termites.

Shah and Patel (1976) found that O. *sanctum* contained methyl eugenol and had been used to attract the mango fruit fly. Deshpande and Tiphis (1977) obtained by TLC eight fractions from the essential oil of *Ocimum basilicum* .They tested the activity of each fraction against stored grains insect pests, namely *Tribolium castaneum ,Sitophilus oryzae ,Stagobium paniceum and Bruchidiuus chinensis*. From bioassay tests methylcinnamate and methylcharicol were found to be the components mainly responsible for the insecticidal activity. Rajendran and Copalan (1978) found that *O. sanctum* showed no clear juvenile- hormone like activity. The same authors, Rajendran and Copalan (1979) reported that, the extracts of four plant species including *Ocimum sanctum* Linn. caused mortality in *Dysedercus cinqulatus* Fabr. *Spodopter liturales* Fabr and *Pericalla ricini*. They concluded that, these plants have not only juvenile-hormone mimicking substance but also have insecticidal properties. Kamararj *et al*,(2008) reported that *Ocimum canum*, *Ocimun sanctum* have larvicidal activity against fourth instar larvae of *Spodoptera liturales* (Lepidoptera: Noctudiae).

Acetone extract of *Ocimum basillicum* essential oil killed 95 % of the larvae of *Culex quinquefasciatus* at concentrations 50 and 100 ppm (Hartzell and Wilcixon, 1941). Essential oils of *Ocimum basillicum* and two sub-species of *Ocimum sanctum* possess some insecticidal action against flies and mosquitoes, but are much less effective than pyrethrum (Chopra *et al*., 1942). Guenther (1961) reported that *Ocimum viridae* was used as mosquito repellant. Watt and Breyer (1962) revealed that there is a practice of burning *Ocimum americanum* in rooms to repel mosquitoes in West Africa.

Jain and Jain (1973) found that steam distilled volatile oil of *O. basilicum* exhibited remarkable anti-wormal properties. The active principle from *O. canum* obtained by steam distillation inhibited the growth of *mycobacterium tuberculosis* at dilutions of 1:5000, and 1:125000, (Gubta and Wiswanathan, 1956). Suri and Sind (1978), reported that the essential oil extracted from O. *kilimandscharicum* exhibited potent anti-bacterial activity against *Staphylocoecus aureus, and Escherichia coli,* it has also antiseptic activity against *Proteus vulgaris and Bacillus subtilis,* and it has antifungal activity against *Candida albicans*, *Penicillum notatum and Microsporcum gyseum. (*Wiswanathan, 1956). Essential oil from *O. basilicum* and their components showed different inhibition effect against *Fusarum oxysporum*, and *Rhizopus nigricans* (Rewni *et al*., 1984).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study location

The experiments were conducted in the Research Laboratory, College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST), during May, 2019.The average temperature is between 35-40°C and relative humidity (RH) is between 30 to 35 %.

3.2. Insect collection and rearing

The Mosquitoes **(***Culex quinquefasciatus)* was collected from Alamab Naser North Khartoum, Sudan. Larva of Culex mosquitoes collected from different water drainage places(Plate 1) and kept in plastic containers(Plate 2) and then taken to the laboratory and left for 24 hours at normal room temperature till experiment.

3.3. Experiment materials

The commercial clove oil, basil oil (Al hawaj® products - Plate 3) and clove buds were brought from local market. Clove buds (Plate 4) was washed and dried for 24 hours under room conditions, and then powdered by electric blinder and preserved in plastic container till used (Plate 5).

3.4. Preparation and extraction method

Forty grams of clove powder were mixed with 100 ml of water to obtain 40% concentration as stock solution, the mixture placed in a shaker for one hour, then left to settle for 30 minutes, after which the solution was filtered using filter paper in a conical flask .Four concentrations (2%, 1%, 0.50% and 0.25%) were prepared by diluting the stock solution.

Two ml of each clove and basil oil were added to 100ml of water plus one drop of liquid soap (0.01 ml) to obtain 2% concentration, and other concentrations (1%, 0.50% and 0.25%) were prepared.

3.6. Bioassay tests

A total of 20 Culex larvae introduced into 100 ml dechlorinated tap water in each plastic cup. Four concentrations (2 %, 1 %, 0.50% and 0.25 %) of each clove water extract, clove oil and basil oil were tested against the 2nd and 4th larval instars of Culex mosquito. All treatments were replicated three times; each replicate set contained one control. The numbers of dead larvae were counted and recorded 6 hrs, 12 hrs, 24 hrs, 48 hrs, and 72 hrs after exposure. The mortality percentage values were calculated from the average of four replicates. The experiments were conducted under Lab conditions.

3.7. Statistical analysis

The experiment was designed in a Complete Randomized Design (CRD) and the data was statistically analyzed according to analysis of variance (ANOVA) using Statistic 8.0 program. LSD test was used for means separation.

Plate 1. Larvae collecting site

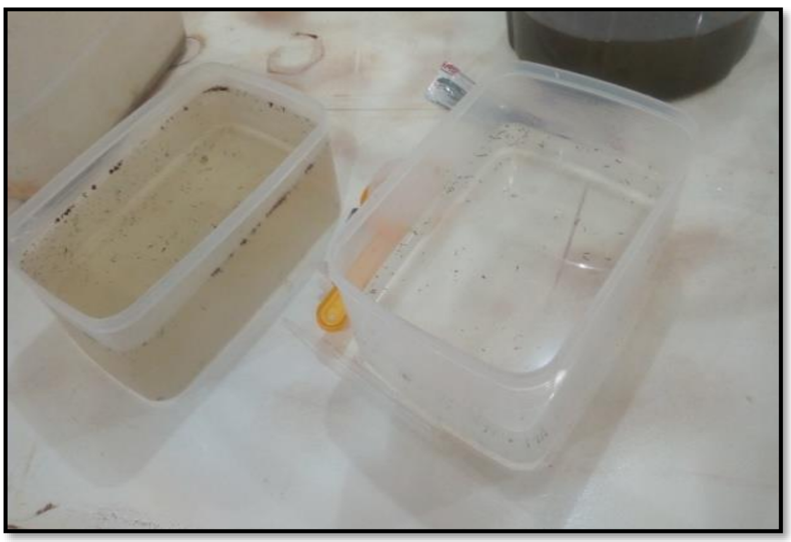

Plate 2. Larvae rearing cage

Plate 3. Clove and basil oils

Plate 4. Buds of clove

Plate 5. Clove Powder

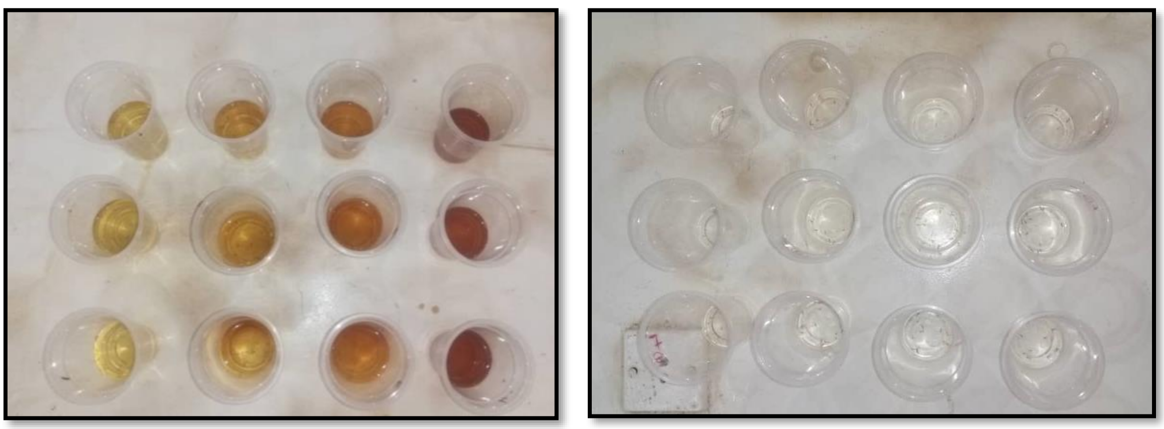

Plate 6. The Treatments

CHAPTER FOUR

RESULTS

4.1. Mortality (%) of clove (*Syzygium aromaticum)* water extracts against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito

Results in table (1) and figure (1) showed that clove water extract was highly effective against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito at highest concentration (2%) which caused mortality of 98.3% after 72 hrs post treatment, while the other concentrations (1%, 0.5% and 0.25%) showed moderate effect (61.6, 51.6 and 43.3 %), respectively. The statistical analysis showed that there are a significant difference between all concentrations and control and also between the highest concentration and other concentrations.

4.2. Mortality (%) of clove (*Syzygium aromaticum)* oil against 2^{ed} in star larvae of *Culex quinquefasciatus* mosquito

Results in table (2) and figure (2) showed that clove oil was very effective against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito. The highest two concentrations (2% and 1%) caused mortality% of (96.6 and 91.6) after 72 hrs post treatment. The statistical analysis showed that there is a significant difference between all concentrations and control.

4.3. Mortality (%) of clove (*Syzygium aromaticum)* oil against 4th in star larvae of *Culex quinquefasciatus* mosquito

Results in table (3) and figure (3) showed that clove oil was more effective against 4th instar larvae of mosquito *Culex quinquefasciatus* when compared to 2nd instar larvae, which they gave mortality % of (88.3 and 81.3) at two highest concentrations (2% and 1%) after only 12 hrs post treatment. The highest concentration (2%) caused mortality of 96.6% after 72 hrs post treatment, while the third concentration (0.5%) achieved mortality of 83.3%. after the same period of exposure. The statistical analysis showed that there are a significant difference between all concentrations and control but there are no significant difference between concentrations them self.

4.4. Mortality (%) of Basil (*Ocimum basilicum*) oil against 2^{ed}in star larvae of *Culex quinquefasciatus* mosquito

Results in table (4) and figure (4) showed that basil oil was effective against 2^{ed} instar larvae of *Culex* mosquito. The highest concentrations (2% and 1%) caused mortality of (85% and 76.6%) respectively after 72 hrs post treatment. The mortality (%) was gradually increased by increasing of concentration and exposure time. The statistical analysis showed that there are a significant difference between all concentrations and control.

4.5. Mortality (%) of Basil (*Ocimum basilicum*) oil against 4^{th} instar larvae of *Culex quinquefasciatus* mosquito

Results in table (5) and figure (5) showed that basil oil was less effective against 4^{th} instar larvae when compared to 2^{nd} instar larvae of *Culex* mosquito. The four concentrations (2%, 1%, 0.5% and 0.25%) gave mortality ranged between (58.3 % and 46.7%) after 72 hours post treatment. The statistical analysis showed that there are no significant differences between all concentrations but a significant difference was observed between concentrations and control.

Table 1. Mortality (%) of clove (*Syzygium aromaticum)* water against 2ed instar larvae of *Culex quinquefasciatus* mosquito

Concentrations%	Exposure Period (hours)						Means
	1	6	12	24	48	72	
2	3.3 a	35.0 a	55.0 a	80.0 a	86.6 a	98.3 a	70.4 a
1	0.0 a	6.6 b	11.6 b	30.0 b	43.3 b	61.6 b	25.5 b
0.50	0.0 a	8.3 ab	15.0 b	18.3 bc	31.6 c	51.6 bc	20.5 bc
0.25	1.6 a	8.3 ab	11.6 b	11.6 cd	18.3 d	43.3 c	15.8 bc
Control	0.0 a	0.0 b	3.3 b	3.3 d	3.3 e	10.0 d	3.3 c
C.V%	16.9	13.3	8.85	4.83	4.10	3.84	8.2
L.S.D	2.4	26.9	27.6	12.2	11.5	14.3	21.7
S.E±	5.3	12.1	12.4	5.4	5.0	6.4	10.5

Means followed by the same letter (s) are not significantly different at (P<0.05).

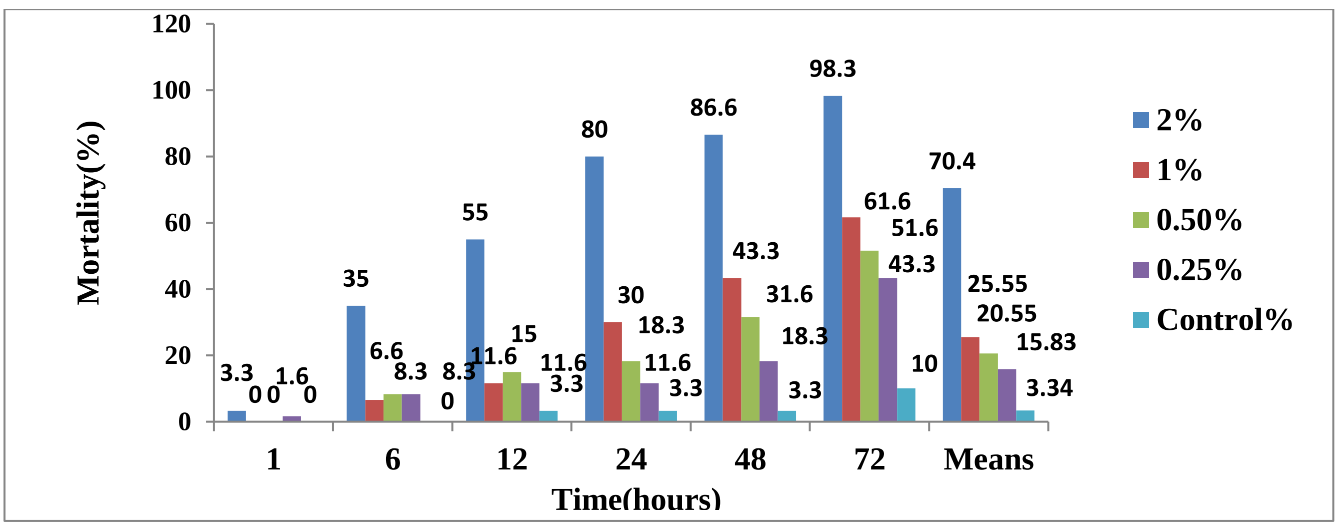

Figure 1. Mortality (%) of clove water extracts against 2ed instar larvae of *Culex quinquefasciatus* mosquito.

Table 2. Mortality (%) of clove (*Syzygium aromaticum*) oil against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito

Concentrations%	Exposure Period (hours)						
	1	6	12	24	48	72	Means
2	18.3^{a}	38.3^{a}	48.3^{a}	61.6^{a}	90.0^{a}	96.6^{a}	58.89^{a}
1	3.3^{b}	11.6^{b}	31.6^{b}	40.0^{b}	75.0^{b}	91.6^{a}	42.21^{ab}
0.50	1.6^{b}	6.6^{bc}	21.6^{c}	33.3^{b}	65.0^{b}	78.3^{b}	34.4^{abc}
0.25	1.6^{b}	1.6^{c}	13.3^{c}	18.3^{c}	41.6^{c}	58.3^{c}	22.5^{bc}
Control	0.0^{b}	0.0^{c}	3.3^{d}	3.3^{d}	3.3^{d}	10.0^{d}	3.34^{c}
C.V%	9.3	6.65	4.74	4.23	3.64	3.10	9.1
L.S.D	7.4	9.4	9.6	10.2	13.3	11.7	31.7
S.E±	3.3	4.2	4.3	4.6	5.9	5.3	15.4

Means followed by the same letter (s) are not significantly different at ($P<0.05$).

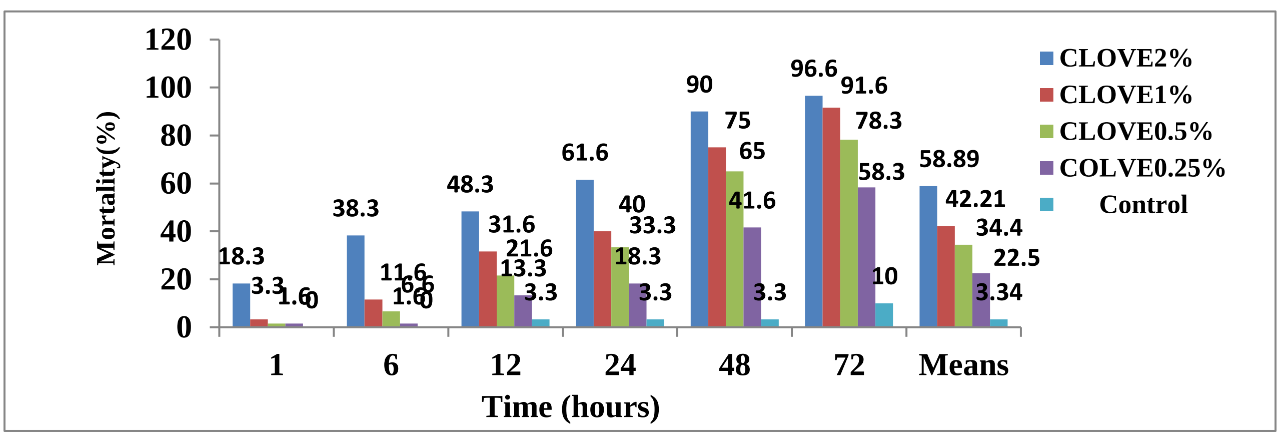

Figure 2. Mortality (%) of clove oil against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito

Table 3. Mortality (%) of clove oil against 4th instar larvae of *Culex quinquefasciatus* mosquito

Concentrations%	Exposure Period (hours)						Means
	1	6	12	24	48	72	
2	48.3 [a]	80.0 [a]	88.3 [a]	90.0 [a]	95.0 [a]	96.6 [a]	83.1 [a]
1	6.6 [b]	60.0[ab]	81.6 [a]	81.6 [a]	85.0[ab]	90.0 [a]	68.8 [ab]
0.50	3.3 [b]	33.3 [b]	70.0 [a]	73.3 [a]	78.3[ab]	83.3 [a]	59.73 [ab]
0.25	3.3 [b]	31.6[bc]	60.0 [a]	60.0 [a]	61.6 [b]	66.6 [a]	47.2 [b]
Control	0.0 [b]	0.0 [c]	0.0 [b]	3.3 [b]	5.0 [c]	5.0 [b]	2.225 [c]
C.V%	7.33	6.5	5.47	5.2	5.2	5.0	6.7
L.S.D	12.4	32.4	32.7	31.2	32.4	32.0	28.4
S.E±	5.5	14.5	14.6	14.0	14.5	14.3	13.8

Means followed by the same letter (s) are not significantly different at ($P<0.05$).

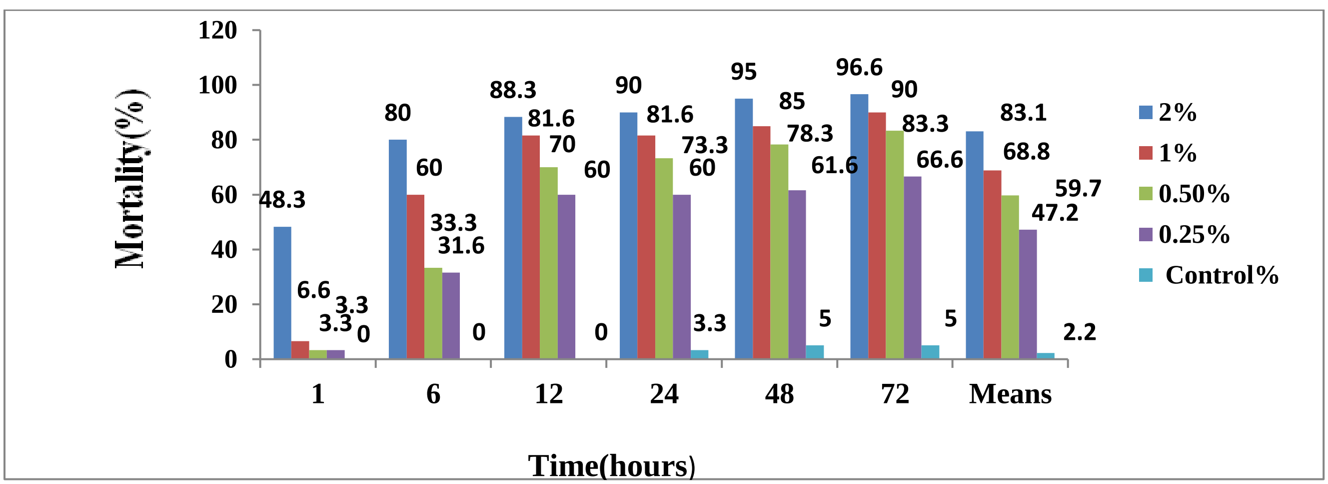

Figure 3. Mortality (%) of clove oil against 4th instar larvae of *Culex quinquefasciatus* mosquito

Table 4. Mortality (%) of Basil (*Ocimum basilicum*) oil against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito

Concentrations%	Exposure Period (hours)						Means
	1	6	12	24	48	72	
2	3.3 ^{a}	31.6 ^{a}	66.6 ^{a}	73.3 ^{a}	81.6 ^{a}	85.0 ^{a}	56.9 ^{a}
1	0.0 ^{a}	20.0 ^{b}	51.6 ^{b}	58.3^{ab}	68.3 ^{ab}	76.6 ^{ab}	45.8 ^{a}
0.50	0.0 ^{a}	15.0 ^{b}	38.3 ^{b}	43.3 ^{b}	55.0 ^{b}	61.6 ^{b}	35.5 ^{ab}
0.25	0.0 ^{a}	3.3 ^{c}	11.6 ^{c}	16.6 ^{c}	23.3 ^{c}	41.6 ^{c}	16.3 ^{bc}
Control	0.0 ^{a}	0.0 ^{c}	3.3 ^{c}	3.3 ^{c}	3.3 ^{c}	10.0 ^{d}	3.34 ^{c}
C.V%	19.6	6.4	4.9	5.2	5.3	4.3	8.6
L.S.D	4.7	10.5	13.9	19.4	24.2	18.6	27.7
S.E±	2.1	4.7	6.3	8.7	10.8	8.4	13.5

Means followed by the same letter (s) are significantly different at (P<0.05).

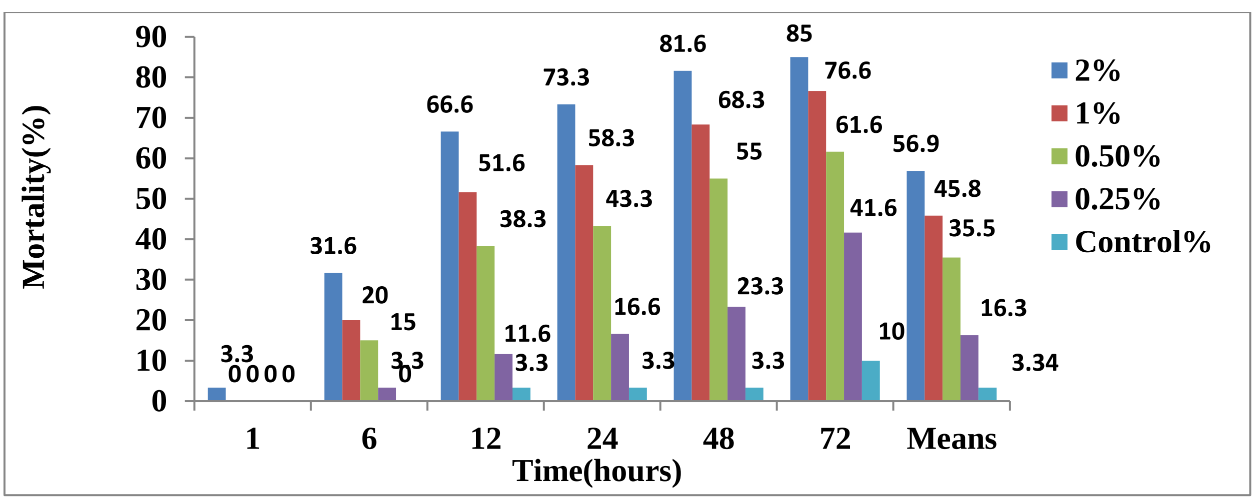

Figure 4. Mortality (%) of Basil oil against 2^{ed} instar larvae of *Culex quinquefasciatus* mosquito

Table 5. Mortality (%) of Basil (*Ocimum basilicum*) oil against 4th in star larvae of *Culex quinquefasciatus* mosquito

Concentration%	Exposure Period (hours)						Means
	1	6	12	24	48	72	
2	18.3 a	38.3 a	43.3 a	50.0 a	51.67 a	58.3 a	43.4 a
1	11.6 a	33.3 a	45.0 a	46.6 a	48.33 a	51.6 a	40.5 a
0.50	8.3 a	28.3 ab	23.3 ab	43.3 a	45.0 a	46.7 a	35.3a
0.25	1.6 a	23.3 ab	30.0 ab	33.3 a	40.0 a	46.7 a	29.2 a
Control	0.0 a	0.0 b	0.0 b	3.33 b	5.00 b	5.0 b	2.2 b
C.V%	11.4	8.2	7.8	6.8	6.8	6.7	6.6
L.S.D	18.7	31.7	30.8	29.4	32.4	33.6	15.8
S.E±	8.4	14.3	13.8	13.2	14.5	15.0	7.7

Means followed by the same letter (s) are not significantly different at (P<0.05).

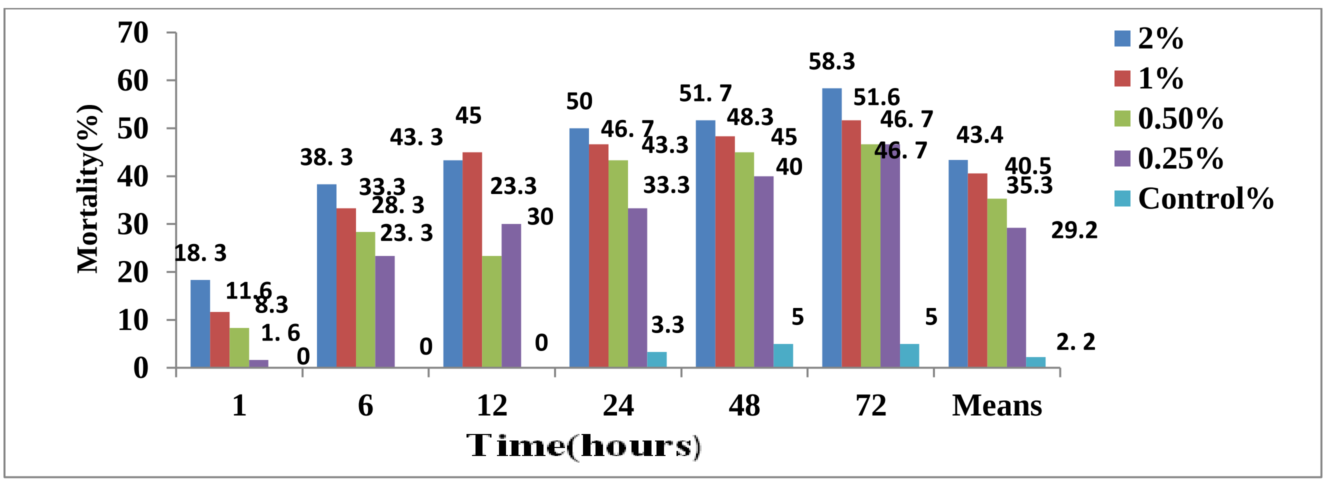

Figure 5. Mortality (%) of Basil oil against 4th in star larvae of *Culex quinquefasciatus* mosquito

CHAPTER FIVE

DISCUTION

Phytochemicals derived from many different plant species are important source for safe and biodegradable chemicals (Mittal and Subbarao, 2003).More than 2,000 plant species contain different compounds used in preventing or killing insects, or known to possess some insecticidal properties. Those compounds fall into several groups, including toxins, anti-feedants, repellents, and growth regulators (Maia and Moore, 2011).

This study was carried out to investigate the activity of two botanicals, Clove (water and oil) and Basil oil against the larvae of *Culex quinquefasciatus* mosquito under laboratory condition.

The mentioned results showed that, Clove and Basil oils have insecticidal activity against larvae *Culex quinquefasciatus*.

The clove water extract at highest concentration (2%) posed mortality of 98.3% against 2nd instar larvae, while clove oil gave mortality of 96.6% against both 2nd and 4th instar larvae after 72 hrs post treatment. These results agreed with Tian *et al*. (2015) how found that clove have insecticidal properties against psyllid (*Cacopsylla chinensis*). Also Trongtokit *et al*. (2005) found that clove oil have potent repellency effect (100%) against three species of mosquitoes: *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles dirus*. Clove oil (50%) combined with geranium oil (50%) prevented biting by *Anopheles albimanus* mosquitoes for 1¼ to 2½ hours (Barnard, 1999). Kim *et al.* (2011) reported that clove oil was very effective against sweet potato whitefly (*Bemisia tabaci*) at LC_{50} of 0.22 mL/cm3 for clove bud oil and 0.26 mL/cm3 for clove leaf oil.

The basil oil at highest concentration (2%) caused mortality of (85% and 58.3%) against 2nd and 4th instar larvae respectively after 72 hrs post treatment. That means

2nd instar larvae was more susceptible to basil oil when compared to 4th instar larvae. These results agreed with Hartzell and Wilcixon (1941) how reported that acetone extract of *Ocimum basillicum* essential oil killed 95 % of the larvae of *Culex quinquefasciatus* at concentrations 50 and 100 ppm. Chopra *et al.* (1942) found that essential oils of *Ocimum basillicum* and two sub-species of *Ocimum sanctum* possess some insecticidal action against flies and mosquitoes, but are much less effective than pyrethrum. Dalzeid (1937) and Guenther (1961) reported that *Ocimum viridae* was used as mosquito repellant. Watt and Breyer (1962) revealed that there is a practice of burning *Ocimum americanum* in rooms to repel mosquitoes in West Africa.

CONCLUSION

- Based on the above mentioned results, buds powder water extract and oil of clove (*Syzygium aromaticum)* was very effective against 2nd and 4th instars larvae of *Culex quinquefasciatus* mosquito especially at concentration of 2%.
- Basil (*Ocimum basilicum*) oil was effective for controlling larvae of *Culex quinquefasciatus* especially against 2nd instar larvae.
- Basil oil (*Ocimum basilicum*) extract was less effective when compared to clove water and clove oil (*Syzygium aromaticum).*

RECOMMENDATIONS

- Clove water extract and clove oil is a promising botanical insecticides and it can be considered as alternative biochemical used to control mosquito larvae.
- Basil oil is the one of effective bio-insecticide and it can be used against mosquito larvae
- Further comparative studies should be conducted to evaluate the effects of these botanicals against other insect pests.
- Field experiments are needed to confirm the activity of clove and basil oils.
- Future studies and efforts should be made for identifying the active ingredients and formulation of these botanicals pesticides.

References

Abdul Rahuman, A. and Venkatesan, P. (2008). Larvicidal activity of five cucurbitaceous plant leaf extracts against mosquito species. J. Parasitol. Res. 103:133−139.

Abdul Rahuman, A. Vekatesan, P., Geetha, K., Gopalakrishnan, G. Bagavan, A. and Kamaraj, C. (2008 a). Mosquito larvicidal activity of gluanol acta atetra cyclic triterpenes derived from *Ficus racemosa* Linn. J.Parasitol. Res. 103:333−339.

Abdul Rahuman, A. Gopalakrishnan, G. Venkatesan, P.Geetha, k. and Bagavan, A. (2008 b). Mosquito larvicidal activity of isolated compound from the rhizome of *Zingiber officinale*. Phytother. Res. 22, 1035- 1039.

Abu shama, F.T. (1974). *Flies, Mosquitoes, and Disease in the Sudan.*pp.20- 38. Khartoum University Press.

Ajayi, F. and Lale, N.(2001). Seed Coat Texture, Host Species and Time of Application Affect the Efficacy of Essential Oils Applied for the Control of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in Stored Pulses. International Journal of Pest Management, 47 (3): 161–166.

Ahmad, K. (2000). More deaths from Rift Valley fever in Saudi Arabia and Yemen. Lancet, 356, 1422.

Alfikri, F. and Pujiarti,R.(2020). Yield, Quality, and Antioxidant activity of clove (*Syzygium aromaticum* L.) Bud oil at the Different Phenological Stages in Young and Mature Trees. ISSN: 2320-2882 .

Almagboul, A . (1981). Antimicrobial Activity of certain Sudanese plants used in Folkloric Medicine. Screening for Antimicrobial Activity (IV). Fitoterapia; 56: 331.

Azmi, M. Naqvi ,S. Akhtar, K. Parveen, R.and Aslam, M. (2009). Effect of pesticide residues on health and blood parameters of farm workers from rural Gadap Karachi Pakistan. J Environ Bio 30: 7747-756.

Bailey, L. and Bailey, E. (1976). Hortus third: Aconcise dictionary of plants cultivated in the United States and Canada. Macmillan, New York.

Barik, T. Raghavendra, K. and Goswami ,A. (2012). Silica nanoparticle: a potential new insecticide for mosquito vector control. Parasitol Res 111:1075–1083.

Barnard, D. and Donald, R. (1999). Repellency of Essential Oils to Mosquitoes (Diptera: Culicidae). Journal of Medical Entomology, 36 (5): 625–629.

Bainard, Luke, D.Isman, M. and Upadhayaya, M. (2006). Phtyotoxicity of clove oil and its primary constituent eugenol and the role of leaf epicuticular wax in the susceptibility to these essential oils. Weed Science, 54:833-837.

Babovic, N. (2010) . Supercritical carbon dioxide extraction of antioxidant fractions from selected Lamiaceae herbs and their antioxidant capacity, Innovative Food Science and Emerging Technologies 11 98–107.

Bilal, H. and Hassan,S. (2012). Plants secondary metabolites for mosquito control. Asian Pac J Trop Dis 2: 168-168.

Board, N. (2010). Hand book on Spices. Asia Pacific Business Press Inc, Delhi, 199-213.

Bown, D. (1995). Encyclopedia of herbs and their uses. Dorling Kindersley press London.

Boyd, N. and Brennan, E. (2006). Burning Nettle, Common Purslane, and Rye Response to a Clove Oil Herbicide. Weed Technology, 20 (3): 646– 650.

Brogdon, W., and Mc Allister, J. (1998). Insecticide resistance and vector conrol. Emergine infectious disease,4: (4).

Brophy , J. and Jogia, M.(1986). Essential oils from Figian *Ocimmum basilicum* L. Flavour and Fragrance Journal, 53-55.

Byng,W.(2016) .Revision of Eugenia and *Syzygium* (Myrtaceae) from Comoro archipelago. Phytotaxa, 252:162-184.

Brainard, C. William, S. Curran, Robin, R., and John, B. (2013). Temperature and Relative Humidity Affect Weed Response to Vinegar and Clove Oil. Weed Technology, 27 (1): 156–64. Doi:10.1614/WT-D-12- 00073.1.

Cai, L. and Wu, C. (1996). Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. Journal of natural products. 59(10): 987-990.

Calisher, C. Beaty, B. and Shope, R. (1989). Diagnostic procedures for viral, rickettsial and chlamydial infections. 6. ed. Washington, American Public Health Association, p. 797-855.

Carvalho Filho, S. Blank, A. Alves, P. Ehlert, D. and Melo, A. (2006). Influence of the harvesting time, temperature and drying period on basil (*Ocimum basilicum* L.) essential oil, Brazilian Journal of Pharmacognosy 16 24-30.

Cetin, H. Cimbligel, I. Yanikoglu, A. and Gokceoglu, M. (2006). Larvici activity of some labiatae (Lamiaceae) plant extract from Turkey. Phytother .Res.20.1088−1090.

Chandra, G. Bhattacharjee ,I. Chatterjee ,S. and Gosh ,A.(2008). Mosquito control by larvivorous fish. Indian J Med Res 127:13–27

Chopra, R. Nayar ,S. and Chopra, I. (2002). Glossary of Indian Medicinal Plants. 6th ed. New Delhi: NISCIR.178-79.

Clements, A. (1992). The biology of mosquitoes. Vol. 1. Development, nutritio and reproduction. Chapman and Hall, New York.

Chavasse, D.Reed, C. and Attawell ,K.(1999). Insecticide Treated Net Projects. A Handbook for Managers. Malaria Consortium , London and Liverpool.

Chopra, R. Badhawar, R. and Ghosh, S. (1942). Poisonous Plants of India, vol. 1. 2nd Ed. New Delhi: Indian Council of Agricultural Research.

Cobley, I. (1956). An introduction to the botany of tropical crops .4th edition, western printing services LTD., Bristol, 336-337pp.

Cloyd, Raymond A.Cindy L. Galle, Stephen R. Keith, Nanette A. Kalscheur, and Kenneth ,E. (2009). Effect of Commercially Available Plant-Derived Essential Oil Products on Arthropod Pests. Journal of Economic Entomology, 102 (Copyright (C) 2015 U.S. National Library of Medicine.): 1567–79.

Danthu, P. Penot, E. Ranoarisoa, K. Rakotondravelo, J. Michel, I. Darsie, R. and Ward, R.(2005). Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. Gainesville, FL: University of Florida Press.

Darsie Jr, R. and Morris, C. (2000). Keys to the adult females and fourth-instar larvae of the mosquitoes of Florida (Diptera: Culicidae). Technical Bulletin of the Florida Mosquito Control Association, 1: 148–155.

Dalzeil, J. (1937). The useful plants of west tropical Africa .The crown agent for colonies 4, Millbark, Westminster ,London, 72-75pp.

Dayan, Franck, E. Charles, L. Cantrell, and Stephen, O.(2009). Natural Products in Crop Protection. Bioorganic & Medicinal Chemistry, 17 (12): 4022–4034.

Davies, F. (2010). The historical and recent impact of Rift Valley fever in Africa. Am. J. Trop. Med. Hyg., 83(Suppl. 2), 73–74.

Deshpandae, R. and Tiphis, H. (1977). Insecticidal activity of *Ocimum basilicum* Linn.Pesticides 11(5):11-12.

Dusek ,R. (1995). Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (Vestiaria coccinea). Parasitology, 111:S59-S69.

El-Rayah, E. (2007). Mosquitoes of the Sudan. Sudan Notes &Records, 6:153-187.

Eldridge, B. and Edman, J. (2000) Medical Entomology: a Textbook on Public Health and Veterinary Problems Caused by Arthropods.

Dordrecht: Kluwer.

Elysse,N. and Desiree,A. (2020). Rift Valley Fever: Important Considerations for Risk Mitigation and Future Outbreaks Department of Pediatrics, Division of Infectious Disease, Stanford University School of Medicine, Stanford, CA 94305, USA

Garret ,W. and White, S. (1977). Mosquito control with monomolecular organic surface films: I-selection of optimum filmforming agent. Mosq News 37:344–348

Gerberg, E. Barnard, D. and Ward, R.(1994). Manual for Mosquito Rearing and Experimental Techniques. American Mosquito Control Association Bulletin, 5: 61–62.

Gerdes, G. (2004). Rift Valley fever. Rev. Sci. Tech., 23, 613–623.

Gildemeister, E. and Hofmann, F. (1961). Die Aetherischen Oele Vol.7, 5th edi., Akademische Verlag, Berlin, Germany. 478-516 pp.

Goddard, J. (2007). Physician's Guide to Arthropods of Medical Importance- 5th edition. CRC Press, 480 pp.

Gosh, A. Chowdhury, N. Chandra, G. (2008). Laboratory evaluation of phytosteroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of *Culex quiquefasciatus* (Diptera: Culicidae) and nontarget organisms. Parasitol. Res.103:271−277.

Gouge, D.(2012). From Dusk to Dawn Mosquitoes Suck Cooperative Extension, College of Agriculture & Life Sciences, University of Arizona, http://cals.arizona.edu/apmc/docs/AugustMozzieMadnessArticle2012.

Gupta, K. and Wiswanathan, R. (1956). Anti-tubarcuulous substances from plants. Antibiotics chemotherapy, 6:194-205.

Guenther, E. (1961). The essential oils Van Nostrand Company Inc., 3:339-433.

Gubara, A. (1983). A comparative study on the insecticidal potentialities of Neem *Azadirachta inica* and Rehan *Ocimum ssp*. M.Sc.Thesis.U.of K.

Guth,R. (2009). Rocket Scientists Shoot Down Mosquitoes with Lasers. Wall Street J, March 14th

Hardy, J. Houk, E. Kramer, L. and Reeves, W. (1983). Intrinsic factors affecting vector competence of mosquitoes for arboviruses. Annual Review of Entomology 28:229-262.

Hartzell, A. and Wilcoxon, F. (1941). A survey of plant products for insecticidal properties. Contrib. BoyceThomp Inst., 12: 127-141.

Harwood, P. and James, M. (1979). Entomology in Human and Animal Health, [7]th edition. Macmillan Publishing Co. INC. New York.

Hassan, M.(1988). Screening of the potential active chemical control agents extracted from *Ocimum basilicum* L.(Rehan) on selected crop pests M.Sc. thesis, U of K.

Helinski, M. and Knols,B. (2009). Sperm quantity and size variation in un-irradiated and irradiated males of the malaria mosquito *Anopheles arabiensis* Patton. Acta Tropica 109(1):64–69

Helinski, M. Parker,A. and Knols, B. (2006) Radiation-induced sterility for pupal and adult stages of the malaria mosquito *Anopheles arabiensis*. Malariol J 5(41).

Henderson, J. Westwood ,R.and Galloway ,T. (2006). An assessment of the effectiveness of the Mosquito Magnet Pro Model for suppression of nuisance mosquitoes. J Am Mosq Control Assoc 22(3):401–407.

Hubalek, Z. and Halouzka, J.(1999).West Nile fever- A reemerging mosquito-borne viral disease in Europe. Emerg. Infect Dis., 5(5):643-650.

Hurst ,T. Kay ,B. Brown ,M. and Ryan, P. (2006). Laboratory evaluation of the effect of alternative prey and vegetation on predation of *Culex annulirostris* immatures by Australian native fish species. J Am Mosq Control Assoc 22:412–417.

Hussain ,S. Rahman ,R.and Mushtaq ,A.(2017). : Clove: A Review of a precious with multiple uses: Int J. of Che & Bio Sci, *IJCBS, 11:129-133*.

Hussain, Arshad, Shafqatullah, Javed Ali, and Zia-ur-Rehman. (2012). Inhibition of Aflatoxin Producing Fungus Growth Using Chemical, Herbal Compounds/Spices and Plants. Pure and Applied Biology, 1: 8–13, 6 pp. doi:10.19045/bspab.2012.11002.

Jain, S. and Jain, M. (1973). Investigation on the essential oil of *Ocimum basilicum* , Plant med., 24(3): 286-289.

Jayaweera, D.(1981). Medicinal Plants, (Indigenous and Exotic) Used in Ceylon. Part III. Colombo: The National Science Foundation of Sri Lanka;. 101-3.

Jirovetz, L. and Buchbauer, G. (2006). Chemical Composition and Antioxidant Properties of Clove Leaf Essential Oils. Journal of Agricultural and Food Chemistry, 54:6303-6307.

Kamararj, C. Abdul Rahuman, A. and Begavan, A. (2008) . Antifeedant and larvicidal effects of plants extract against *Spodoptera litura* (F.), *Aedes aegypty* L. and *Culex quiquefasciatus* Say. J. Parasitol Res. 103:325−331.

Kamatou, G. Vermaak, I. and Viljoen, A. (2012). Eugenol from Maluka Islands to the international market place: A review of remarkable and versatile molecules. Molecules, 17:6953-6981.

Kanzok, S. and Zheng, L. (2003). The mosquito genome-a turning point? TRENDS in Parasitology, 19 (8):329-331.

Kline, D. (2007). Semiochemicals, Traps/Targets and Mass- Trapping Technology for Mosquito Management. J Am Mosq Control Associ 23(2):241–251

Kim, Soon-Il, Song-Hwa, Hee-Sung, Seong-Hum, and Young-Joon. (2011). Contact and Fumigant Toxicity of Plant Essential Oils and Efficacy of Spray Formulations Containing the Oils against B- and Q-Biotypes of *Bemisia tabaci*. *Pest Management Science* 67 (Copyright (C) 2015 American Chemical Society (ACS). All Rights Reserved.): 1093–99. doi:10.1002/ps.2152.

Kim, E. Kim, H. Choi, D. and Ahn, Y. (2003). Acaricidal activity of clove oil compounds against *Tyrophagus putrescentiae* (Acari: Acaridae). Applied Entomology and Zoology,38:261-266.

Killen, G. Fillinger, U. and Knols, B. (2002). Advantages of larval conrol for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malaria Journal 1:8.

Kirtikar, K. and Basu, B.(2003). Indian Medicinal Plants with Illustrations. 2nd ed. Vol VIII. Uttaranchal: Oriental Enterprises,2701-2705.

Khan, I. and Ehab, A.(2010). Leung's Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics /. 3rd ed. Hoboken, N.J.: JohnWiley & Sons.

Lanciotti, R. Roehrig, J. and Deubal, V. (1999). Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science, 286:2333-7.

Lee, S. Kim ,J. and Lee ,H. (2001). Insecticide resistance in increasing interest. J Appl Biol Chem 44: 105-112.

Lerdthusnee, K. Romoser, W. Faran , M. and Dohm, D. (1995). Rift Valley Fever virus in the cardia of *Culex pipiens* an immunocy to chemical and Ultra structural study. Am. J. Trop. Hyg. , 53 (4): 331-7.

Lewis, D. (1954). *Culex* mosquitoes of subgenera other than *Culex* in the Anglo-Egyptian Sudan. The Annals and Magazine of Natural History,Ser., 12(7) : 7

Lima, C. Almeida, W. Hurd, H. and Albuquerque, C. (2003). Reproductive aspects of the mosquito *Culex quinquefasciatus* (Diptera: Culicidae) infected with *Wuchereria bancrofti* (Spirurida: Onchocercidae)." *Memórias do Instituto Oswaldo Cruz.* 98: 217–222.

Lumley, S. Horton, D. Johnson, N. Fooks, A. and Hewson, R. (2017) Rift Valley fever virus: Strategies for maintenance, survival and vertical transmission in mosquitoes. J. Gen. Virol., 98, 875–887.

Malaka,S.(1972). Some measures applied in control of termites in parts of Nigeria .Nigerian Entomologist Magazine,2(4):137-141.

Maia ,M.F. and Moore, S.J.(2011). Plant based insect repellents: a review of their efficacy , development and testing . Malaria J., 10(1):11.

Mansour, F. Ravid,U. and Putievky ,E.(1986). Studies of the effect of essential oils isolated from 14 species of Labiatate on the carmine spidermites, *Tetranychus cinnabarinus*.Phytoparasitica,14(2):137-142.

Meyer, Susan, L. Dilip, K. Lakshman, Inga A. Zasada, Bryan, T. Vinyard, and David, J. (2008). Phytotoxicity of Clove Oil to Vegetable Crop Seedlings and Nematotoxicity to Root-Knot Nematodes. Hort.Technology. 18: 631–38.

Miltal, P.K. and Subbarao , S.K.(2003). Prospect of using herbal products in mosquito control. ICMR Bull,33 (1) :1-10.

Mobozy, M. (1977). Studies of the susceptibility of *Culex pipiens fatigans* to insecticides for various Sudanese population .M.Sc. thesis, Faculty of Science, University of Khartoum. Sudan.

Mouchet, J. Carnevale, P. Coosemans, M. Julvez, J. Manguin, S. Richard-Lenoble, D. and Sircoulon, J. (2004). *Biodiversité du paludisme dans le monde*. John Libbey Eurotext, Paris, France.

Morvan, J. Rollin, P. Laventure, S. Rakotoarivony, I.and Roux, J. (2000). Rift Valley fever epizootic in the central highlands of Madagascar. Res. Virol. 1992, 143, 407–415.4. Ahmad, K. More deaths from Rift Valley fever in Saudi Arabia and Yemen. Lancet, 356, 1422.

Muenscher ,W .Walter ,C. and Myron, A. (1978). Garden Spice and Wild Pot-Herbs. Ithaca, NY: Cornell University Press, 1978.

Nadkarni, K.(2005). The Indian Plants and Drugs. New Delhi: Shrishti Book Distributors, 263.

Ndakidemi ,B. Mtei ,K. Ndakidemi ,P. (2016). Impacts of synthetic and botanical pesticides on beneficial insects. Agric Sci 7: 364- 372.

Norbert ,B. Marija, Z. Minoo,M. and Achim ,K.(2010). Mosquitoes and Their Control DOI 10.1007/978-3-540-92874-4 Springer Heidelberg Dordrecht London New York .Library of Congress Control Number: 2009931710.

Ortes-Rojas,D.Femandes,C.and Oliveria,W.(2014). Clove (*Syzygium aromaticum*): A precious spice. Asian Pacific Journal of Tropical Biomedical. 4:90-96.

Ottesen, E. (2006). Lymphatic filariasis : Treatment, control and elimination.

Park, I- Kwon and Sang-Chul Shin.(2005). Fumigant Activity of Plant Essential Oils and Components from Garlic (*Allium sativum*) and Clove Bud (*Eugenia caryophyllata*) Oils against the Japanese Termite (*Reticulitermes speratus* Kolbe). Journal of Agricultural and Food Chemistry, 53 (11): 4388–4392.

Park, W. Choi, S. Ahn, J. Sohn, Y. and Kim, C. (2011). Herbicidial action of the clove oil on cucumber seedlings. Weed Biology and Management, 11:235-240.

Pandey ,U. Srivastava, C. Lekha, and Singh, A. (1983). Efficacy of certain plant extracts against brinjal aphid (*Aphis gosypii*) glover. Indian j. Entomol., 45(3):313-314.

Politeo,O. Jukic, M.and Milos, M.(2007) .Chemical composition and antioxidant capacity of free volatile aglycones from basil (Ocimum basilicum L.) compared with its essential oil, Food Chemistry 101 379–385.

Rafatjah, H. (1982) Prospects and progress on IPM in worldwide malaria control. Mosq News 42:41–97

Rajendran ,B. and Copalan ,M.(1979). Note on the insecticidal properties of certain plant extract.Indian J. Agric .Sci.49:295-297.

Rajendran ,B. and Copalan ,M.(1978). Note on juvenomimetic activity of some plants .Indian J .agric .Sci.,48(5) :306-308.

Reisen, W. (2003). - Epidemiology of St. Louis encephalitis virus. Advanc. Virus Res., 61:139-183.

Rewni , R. Fleischer, and Putievsky, E.(1984). Fungi static activity of essential oil from *Ocimum basilicum* chemo types .J. phytopath, 110:20-22.

Rostal, M. Evans, A. Sang, R.and Gikundi, S. (2010).Identification of potential vectors of and detection of antibodies against Rift Valley fever virus in livestock during interepizootic periods. Am. J. Vet. Res., 71, 522–526.

Rozendaal , J. (1997). Vector Control: Methods For Use By Individuals and Communities .World Health Organization-Geneva.

Rutledge, L. Sofield ,R. and Moussa ,M. (1978). A bibliography of diethyl toluamide. Bull Ent Soc Am 24:431–439.

Schmidt,G. and Roberts,L. (2005). Foundation of parasitology, 7th edition. McGraw - Hill companies.

Seawright, J. Kaiser, P.and Dame, D. (1977). Mating competitiveness of chemosterilised hybrid males of *Aedes aegypti* (L.) in field tests. Mosq News 37(4):615–619.

Service, M. (2004). Medical Entomology for Students- Third edition - Chapter 1, 2, and 3. pp 2-73. Cambridge University Press.

Service, M. (2000). Medical Entomology for Students.2nd edition pp.x 283. Cambridge, Cambridge University Press.

Shah ,A. and Patel, R. (1976). Role of the tulsi plants (*Ocimmum sanctum*) in the control of mango fruit fly *Daucus correctus* Bezzi (Tephritidae:Diptera).Curr.Sci.45:313-314.

Shalaby, Shehata, E. Mona , Sherif, A. Mettwally, and Zakaria, A. (2011). Toxicological Affects of Essential Oils from *Eucalyptus globules* and Clove *Eugenia caryophyllus* on Albino Rats. Polish Journal of Environmental Studies, 20 (2).

Sirivanakarn, S. and White, G. (1978). Neotype designation of *Culex quinquefasciatus* Say (Diptera: Culicidae)." Proceedings of the Entomological Society of Washington, 80: 360–372.

Singh, N. Wang, C. and Cooper, R.(2014). Potential of essential oil-based pesticides and detergents for bed bug control. Journal of Economic Entomology, 107:2163-2170.

Sifola, M.and Barbieri, G. (2006). Yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field, Scientia Horticulturae 108 408–413.

Soh, W. and Parnell, J. (2015). Revision of *Syzygium gaerth* (Myrtaceace) in Indoch (Cambodia, Laos and Vietnam). Adansonia., 37:179-275.

Suri, R. and Sind, T. (1978). Antibacterial acivities of some essential oil Indian drugs pharmaceuticals, 13(6):25-38.

Takken , W. (2002). Do insecticide-treated bed nets have an effect on malaria vectors? Trop Med Int Hlth 7(12):1022–1030.

Tian, Bao-Liang, Zhi-Long Liu, Peng Li, Jie-Wen Wang, and Qi-Zhi Liu (2015). Insecticidal Potential of Clove Essential Oil and Its Constituents on *Cacopsylla chinensis* (Hemiptera: Psyllidae) in Laboratory and Field. Journal of Economic Entomology, 108 (Copyright (C) 2015 U.S. National Library of Medicine.): 957-61.

Tiollier, M. (2014). The clove tree of Madagascar: a success story with an unpredictable future. Bois et Forets des Tropiques, 320:83-96.

Turell, M. Lee, J. Richardson, J. Sang, R. Kioko, E. Agawo, M. Pecor, J.and O'Guinn, M. (2007). Vector competence of Kenyan Culex zombaensis and Culex quinquefasciatus mosquitoes for Rift Valley fever virus. J. Am. Mosq. Control Assoc., 23, 378–382.

Trongtokit, Y. Rongsriyam, Y. Komalamisra, N. and Apiwathnasorn, C. (2005). Comparative repellency of 38 essential oils against mosquito bites. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 19(4): 303-309.

Viteri, J. Luis, O. Leda, R. Marco, A. Pimentel, and Gutierres, N.(2014). "Potential Use of Clove and Cinnamon Essential Oils to Control the Bean Weevil, *Acanthoscelides obtectus* Say, in Small Storage Units." *Industrial Crops and Products* 56: 27–34. doi:10.1016/j.indcrop.2014.02.038.

Walton ,W.(2007) .Larvivorous fish including Gambusia. J Am Mosq Control Assoc 23:184–220

Watt, J. and Breyer, M. (1962). The medicinal and poisonous plants of Southern and Eastern Africa.

West, T. (1944). Toxicity to Flies of Derivatives of Eugenol and Cis-and Trans-Lsoeugenol. Nature, 154: 488–488.

Whitley. R,and Gnann, J. (2002). Viral encephalitis: familiar infections and emerging pathogens. Lancet 359:507–513. http://dx.doi.org/10.1016 /S0140-6736(02)07681-X.

WHO (2004). Global strategic framework for integrated vector management. Geneva WHO/CDS/CPE/PVC/2004.10

WHO (2003). Global Programme to Eliminate Lymphatic Filariasis , Annual Report on Lymphatic Filariasis, 154 pp.

WHO (2000). Preparing and Implementing National Plan to Eliminate Lymphatic Filariasis. In WHO/CDS/CPE/CEEE/2000.15. WHO, Geneva.

WHO (2001). WHO Recommended Strategies for Prevention & Control Communicable Diseases. WHO/CDS/CPE/SMT/ 13.188 P. WHO by UNAIDS .WHO/ CDS /CPE / SMT/ 2001. 13- 188 P.

WHO (1991). Tropical diseases: Progress in research,1989–1990.Tenth programme report. UNDP/World Bank/who special programme for research and training in tropical diseases (TDR). Geneva. Switzerland.

Yadav, Gupta, Bharti and Yogi,B.(2020). *Syzygium aromaticum* (clove): Review on Various phytochemicals and pharmacological activities in medicinal plant World J. of Pharmaceutical Research, 9(11).

Zacharia , J. (2011). Ecological effects of pesticides . In : Stoytcheva M, editor. Pesticides in the Modern World - Risks and Benefits. Rijeka, Croatia: InTech Open Publisher, pp. 129-142.

Zhang, Schneidmiller, Hoover, Zhou, G. Margaryan, A. and Bryant,P.(2014). Essential Oils as Spatial Repellents for the Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Journal of Applied Entomology, 138 (7): 490–499. doi:10.1111/jen.12101.

Zhu, Betty ,C. Gregg ,H.Feng, C.Huixin ,F.and Roger,A. (2001). "Evaluation of Vetiver Oil and Seven Insect-Active Essential Oils against the Formosan Subterranean Termite." *Journal of Chemical Ecology* 27 (8): 1617–1625.