LARVICIDAL POTENTIAL OF *CITRUS SINENSIS* AND SE*SAMUM INDICUM* LEAF N-HEXANE EXTRACTS AGAINST *CULEX QUINQUEFASCIATUS* MOSQUITO SPECIES

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AUGUST, 2016

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DEPARTMENT OF BIOCHEMISTRY
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AUGUST, 2016

DECLARATION

I declare that the work in this dissertation "Larvicidal Potential of *Citrus sinensis* and Se*samum indicum* Leaf n-Hexane Extracts against *Culex quinquefasciatus* Mosquito Species" has been carried out by me in the Department of Biochemistry, under the supervision of Prof. H.C Nzelibe and Dr. M.A. Musa. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Buzu, Sim Blessing

.....	.....	.....
Name of Student	Signature	Date

CERTIFICATION

This project titled "Larvicidal Potential of *Citrus sinensis* and Se*samum indicum* Leaf n-Hexane Extracts against *Culex quinquefasciatus* Mosquito Species" meets the regulation governing the award of the degree of Masters in Biochemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to Almighty God for His divine favour, grace, mercy, love and provision.

ACKNOWLEGEMENT

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ABSRACT

The present study was carried out to evaluate the larvicidal potential of crude and partially purified fractions of *Citrus sinensis* and *Sesamum indicum* leaf extracts against third instar larvae of *Culex quinquefasciatus* mosquito species at various concentrations (6.25, 12.5, 25, 50, 100, 200 and 400ppm). Aqueous, methanolic, ethyl acetate and n-hexane extracts of these two plants were tested for larvicidal activity. The Highest larval mortality in *Citrus sinensis* was observed in the n- hexane extract with a percentage mortality of 90% and LC_{5O} & LC_{90} values of 0.210 and 6.498ppm respectively. Highest larval mortality in *Sesamum indicum* was also observed in the n-hexane extract with a percentage mortality of 47% and LC_{50} & LC_{90} values of 3.179 and 10.344ppm respectively. Phytochemical screening of these extracts revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, sterols, tannins and terpenoids. After partial purification using chromatographic techniques, seven different fractions were collected for each of the two n-hexane extracts and were tested for larvicidal activity. The fractions with the highest larvicidal activity were fraction 3 of *Citrus sinensis* (CSHE-F_3) with percentage mortality of 93% at 400ppm after 24hrs and fraction 6 *of Sesamum indicum* (SIHE-F_6) with percentage mortality of 52% at 400ppm after 24hrs.The two potent fractions were further subjected to Fourier Transform Infra Red Spectroscopy. *Citrus sinensis* fraction was found to contain alkyl halides, alcohols, alkanes, carboxylic acid functional groups while *Sesamum indicum* fraction revealed alkyl halides, alkenes, amides, nitro compounds, aldehydes and carboxylic acids functional groups which may be implicated in the larvicidal activity. The n-hexane fractions of *Citrus sinensis* and *Sesamum indicum* exhibited larvicidal activity and these results indicate promising approaches against *Culex quinquefasciatus* mosquito species. Hence, leaf n-hexane extracts of *Citrus sinensis* and *Sesamum indicum* can be considered as mosquito control agents without pollution of the environment.

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ABBREVATIONS, GLOSSARIES AND SYMBOLS

ANOVA	Analysis of Variance
Bt	*Bacillus thuringiensis*
CSHE	*Citrus sinensis* n-Hexane Extract
Cx	*Culex quinquefasciatus*
DDT	DichloroDiphenylTrichloroethane
FTIR	Fourier Transform Infrared Spectroscopy
GABA	Gamma-Amino Butyric Acid
IRS	Indoor Residual Spray
ITNs	Insecticide Treated Mosquito Nets
LC_{50}	Lethal Concentration for Killing 50% of the Treated Larvae
LC_{90}	Lethal Concentration for Killing 90% of the Treated Larvae
LLINs	Long Lasting Insecticide Nets
Op	Organophosphate
TLC	Thin Layer Chromatography
WHO	World Health Organization
SIHE	*Sesamum indicum* n-Hexane Extract

CHAPTER ONE

1.0 INTRODUCTION

Mosquitoes are the most important of the bloodsucking arthropods, causing diseases in humans, other mammals, and birds. About 3500 species of mosquitoes have been described worldwide and relatively few of them are significant vectors of human diseases however, the mosquito- transmitted disease problem world-wide is quite severe (Goddard, 2007). Mosquitoes are important vectors of malaria, various forms of filariasis and numerous arboviruses, the best known being dengue, yellow fever and West Nile virus (Service, 2008). Early efforts to control arthropod pests and disease vectors relied primarily on the application of broad-spectrum pesticides. This approach often caused undesirable side effects, including environmental pollution, the development of insecticide resistance and sometimes sudden increases in old, or even new and worse, pests (Youdeowei and Service, 1993).

Filariasis is an endemic, disabling, disfiguring disease and the filarial worm, *Wuchereria bancrofti* (W. bancrofti) responsible for human filariasis is carried by *Culex quinquefasciatus* which is a pantropical pest and probably the most abundant house mosquito in towns and cities of tropical countries (Samuel and Williams, 2007). Interest in the control of *Culex quinquefasciatus* lies in the fact that it acts as a vector of filarial fever, a serious public health problem in many developing countries. One of the strategies of the WHO in combating tropical diseases is to destroy their vectors (Wilcox and Ellis, 2006). Since no effective vaccine is available for filarial fever, the only efficacious approach to minimizing the incidence of this disease is to eradicate and control mosquito vectors mainly

by application of insecticides to larval habitats. The control of mosquito at the immature stage is necessary and efficient in integrated mosquito management because during the immature stages, mosquitoes are immobile (Elimam *et al.,* 2009). Ethnobotanical search reveals use of many traditional herbs in treatment of various diseases, which are usually free from side effects, economical and also accessible to humans and provide significant potential for the development of novel biomolecules (Prabakaran *et al.,* 2011) However, 80% of the world's population use plant as their primary source of medication (Abubakar *et al.*,2011).

Many studies on plant extracts against mosquito larvae have been conducted around the world. Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, as they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for use in control of mosquito larvae (Chakraborthy *et al.*, 2008). Many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Amer and Mehlhorn, 2006; Sharma *et al.,* 2006).

Citrus sinensis belonging to the Rutaceae family, is the most widely grown and commercialized citrus species. It has been cultivated for thousands of years, and probably a hybrid between other citrus species in Southern and East Asia (Zohary and Hopt, 1993). Although its evolutionary history is hard to pinpoint, it can be found literally all over the world, widely cultivated in tropics and subtropics. It is called '**ọsàn'** in Yoruba, 'oloma' in igbo and 'lemu' in hausa. Leaf extracts of *C. sinensis* have been used in Nigerian local folk medicine to treat neurological disorders and help facilitate the digestion of food (Kanaze *et al* 2008). It has also been used as an antidiabetic, antibacterial, antifungal, hypotensive,

antioxidant, insect repellent, larvicidal, antiviral, uricosuric, anti-yeast, antihepatotoxic and antimutagenic agent (Ozcan *et al* 2006; Hemandra *et al* 2007)

Sesamum indicum is a flowering plant in the genus *Sesamum*. It is a member of family Pedaliaceae. *Sesamum indicum* is commonly called "sesame" internationally, while it is called "benniseed" in West Africa; "simsim" in East Africa and "Till" in India. In Nigeria, it is called different names in different localities. It is called "ridi" in the Northern States, the Igalas, Idomas and Tivs of Benue State call it "Igogo", "Ocha" and "Ishwa" respectively. The Ibos call it "isisa" and Yorubas call it "Ekuku" or "Eeku" in parts of Ogun, Ondo and Oyo states and Ilorin in Kwara State (Aboje, 2011). Numerous wild species occur in Africa with smaller number in India. It is widely naturalized in tropical regions around the world and cultivated for its edible seeds, which grow in pods. Sesame seed is one of the oldest oilseed crops known, domesticated well over 3000 years ago (Morebise *et al*., 2002). Sesame oil obtained from the seeds of the plant is highly nutritive as it is a rich source of natural oxidant such as sesamin and sesamol (Rajeswari *et al*., 2010). Sesame is a reputed plant in folk medicine in Africa and Asia. *Sesamum indicum* seeds among other species are implicated as having certain properties such as anti- tumorigenic, antioxidant (Kang *et al*., 2005) and anti- estrogenic (Shittu *et al*., 2007). All parts of the plant are useful and consumed locally as a staple food especially the seed, oil and leaves by subsistence farmers in South-West and Middle Belt areas of Nigeria (Akpan-iwo *et al*., 2006).

1.1 Statement of Research Problem

One of the strategies recommended by World Health Organization to control mosquito-borne diseases is the use of organochlorines (DDT, endosulfan), organophosphates

(parathion, temephos) and carbamates. However, these chemical interventions are severely compromised by the development of insecticide resistance in some mosquito vectors and environmental concerns (Ruikar *et al*, 2012). Also in many African countries the most widely tested interventions based on bed nets treated with pyrethroid, have been difficult to implement correctly because of problems related to cost and acceptability (Promsiri *et al.*, 2006).

A drawback with the use of insecticides is that they are non-selective and could be harmful to non-target organisms in the environment. It has also provoked undesirable effects and fostered environmental and human health concerns (Lee *et al*., 2001). The toxicity problem, together with the growing incidence of insect resistance, has prompted the need for novel insecticides (Macedo *et al.*, 1997; Ansari *et al.*, 2000).

1.2 Justification

Although, there are proven strategies to control mosquito-borne diseases, mosquitoes still cause a huge public health problem in Africa. Across Africa, people are exposed to mosquito bites because the larval habitats are widely distributed in humid areas such as flood areas, rice farms, drainages, tree trunks, empty cans, etc. These habitats with larvae might be disturbed to decrease the emergence of adult mosquito population for the interruption of disease transmission. These diseases have impacted negatively on human health and well being, causing economic losses and death. In view of these, there is a need to search for new, cheap, safe and effective larvicides. Plant products have been considered to be environmentally safe and biodegradable as alternative sources in the control of insects of public health importance. Natural products contain a range of bioactive compounds and

related commercial insecticides commonly perceived as "safe" in comparison to synthetic repellents (Maia, 2011).

1.3 General Aim

The aim of this study was to investigate the larvicidal potential of *Citrus sinensis* and *Sesamum indicum* on *Culex quinquefasciatus* mosquito species

1.4 Specific Objectives

i. Evaluation of the phytochemical constituents of *Citrus sinensis* and *Sesamum indicum*.
ii. Determination of the larvicidal effect of n-hexane, ethyl acetate, methanolic and aqueous, crude extracts of *Citrus sinensis* and *Sesamum indicum.*
iii. Isolation of the fractions with the highest larvicidal effect using TLC and column chromatographic techniques.
iv. Characterization of the larvicidal compounds in the active fractions by Fourier transform infra red spectroscopy (FTIR).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquitoes

Mosquitoes are general vectors that carry disease-causing viruses and parasites from person to person. Some of these diseases can be life threatening, such as malaria, yellow fever, filariasis, dengue hemorrhagic fever and Japanese encephalitis. For instance, yellow fever, haemorrhagic fever and dengue fever are transmitted by the *Aedes aegyptia* mosquito. These diseases exist worldwide in tropical and subtropical stagnant water. To minimize and eradicate the occurrence of mosquito-borne diseases, many steps have been taken to prevent their spread to different extents. For example;

- Mosquito eradication at early or late stages,
- Disease prevention via prophylactic drugs and vaccines and
- The prevention of mosquito bite using repellents.

In all cases, larval mosquito control should be considered as the first option for abatement. By controlling larval mosquitoes, adults may never become a problem. Larviciding has the greatest control impact on mosquito populations because the larvae are immobile and accessible. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water and are thus easy to deal with (Syed and Leal, 2008).

There are over 3,500 species of mosquito. However, most of these do not transmit diseases to humans. Mosquitoes are usually divided into two sub-families, the *Anophelinae* and the *Culicinae.* The latter group consists of about 40 genera, including *Culex* and *Aedes*, which

contain some species that transmit diseases to humans (such as yellow fever, dengue fever and West Nile virus). The former contains the genus *Anopheles*, which are the mosquitoes that transmit malaria. There are about 460 described species of *Anopheles* mosquito, of which about 100 can transmit malaria, though the vast bulk of transmission is usually limited to about 30 species (Ohkuma *et al.*, 2003; Syed and Leal, 2008).

2.2 The Scientific Classification of *Culex quinquefasciatus*

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Culicidae

Genus: Culex

Species*:* ***C. quinquefasciatus*** Say, 1823

2.3 Life Cycle of *Culex quinquefasciatus*

The mosquito goes through four separate and distinct stages of its life cycle and they are as follows: Egg, Larva, pupa, and adult. Each of these stages can be easily recognized by their special appearance. They complete their life cycle in both aquatic and terrestrial habitats, but different species have different ecological adaptations with respect to where they lay their eggs.

2.3.1 Egg

Eggs are laid one at a time or attached together to form "rafts." They float on the surface of the water. In the case of *Culex* and *Culiseta* species, the eggs are stuck together in rafts of up to 200. *Anopheles*, *Ochlerotatus* and *Aedes*, as well as many other genera, do not make egg rafts, but lay their eggs singly. *Culex*, *Culiseta*, and *Anopheles* lay their eggs on the water surface while many *Aedes* and *Ochlerotatus* lay their eggs on damp soil that will be flooded by water. Most eggs hatch into larvae within 48 hours. Water is a necessary part of their habitat.

2.3.2 Larva

The larva has a short and stout head. The mouth brushes have long yellow filaments used for filtering organic materials. The abdomen consists of eight segments, the siphon, and the saddle. Each segment has a unique setae pattern. The siphon is on the dorsal side of the abdomen, and is four times longer than its breadth. The siphon has multiple setae tufts. The saddle is barrel-shaped and located on the ventral side of the abdomen with four long anal papillae protruding from the posterior end (Hill *et al*., 2009)

The larva (plural - larvae) lives in the water and comes to the surface to breathe. Larvae shed (molt) their skins four times, growing larger after each molt. Most larvae have siphon tubes for breathing and hang upside down from the water surface. Anopheles larvae do not have a siphon and lie parallel to the water surface to get a supply of oxygen through a breathing opening. The larvae feed on microorganisms and organic matter in the water. During the fourth molt, the larva changes into a pupa.

2.3.3 Pupa

The pupal stage is a resting, non-feeding stage of development, but pupae are mobile, responding to light changes and moving (tumble) with a flip of their tails towards the bottom or protective areas. This is th e time the mosquito changes into an adult. This process is similar to the metamorphosis seen in butterflies when the butterfly develops - while in the cocoon stage - from a caterpillar into an adult butterfly. When development is complete, the pupal skin splits and the adult mosquito (imago) emerges.

2.3.4 Adult

The newly emerged adult rests on the surface of the water for a short time to allow itself to dry and all its body parts to harden. The wings have to spread out and dry properly before it can fly. The period of development from egg to adult varies among species and is strongly influenced by ambient temperature. Some species of mosquitoes can develop from egg to adult in as little as five days but a more typical period of development in tropical conditions could be 40 days or more for most species. The variation of the body size in adult mosquitoes depends on the density of the larval population and food supply within the breeding water. Adult mosquitoes usually mate within a few days after emerging from the pupal stage. Males typically live for about a week, feeding on nectar and other sources of sugar while the female needs a blood meal. After mating, the female will rest for a few days while the blood digests and eggs are developed. The blood digested serves as a source of protein for the production of eggs. This process depends on the temperature but usually takes two to three days in tropical conditions. Once the eggs are fully developed, the female lays them and resumes host-seeking (Spielman *et al*., 2001).

The cycle repeats itself until the female dies. Female mosquitoes can live longer than a month in captivity; most do not live longer than one to two weeks in nature. Their lifespan depends on temperature, humidity and their ability to successfully obtain a blood meal while avoiding host defenses and predators (Zhu, 2006).

2.4 Mosquito Saliva

For the mosquito to obtain a blood meal, it must circumvent the vertebrate's physiological responses. The mosquito, as with all blood-feeding arthropods, has mechanisms to effectively block the homeostasis system with their saliva, which contains a mixture of secreted proteins. Mosquito saliva negatively affects vascular constriction, blood clotting, platelet aggregation, angiogenesis and immunity, and creates inflammation (Ribeiro and Francischetti, 2003). It contains enzymes that aid in sugar feeding (Grossman and James, 1993) and antimicrobial agents to control bacterial growth in the sugar meal (Rossignol and Lueders, 1986). The composition of mosquito saliva is relatively simple, as it usually contains fewer than 20 dominant proteins (Valenzuela *et al*., 2002). Despite the great strides in knowledge of these molecules and their role in blood feeding achieved recently, scientists still cannot ascribe functions to more than half of the molecules found in arthropod saliva (Valenzuela *et al*., 2002). It is now well recognized that mosquitoes, have an ability to modulate the immune response of the animals (hosts) on which they feed (Ribeiro and Francischetti, 2003). The mechanism for mosquito saliva-induced alteration of the host immune response is unclear, but the data have become increasingly convi ncing that such an effect occurs and recent research suggests mosquito saliva exacerbates West Nile virus infection as well as other mosquito-transmitted viruses (Schneider *et al*., 2006).

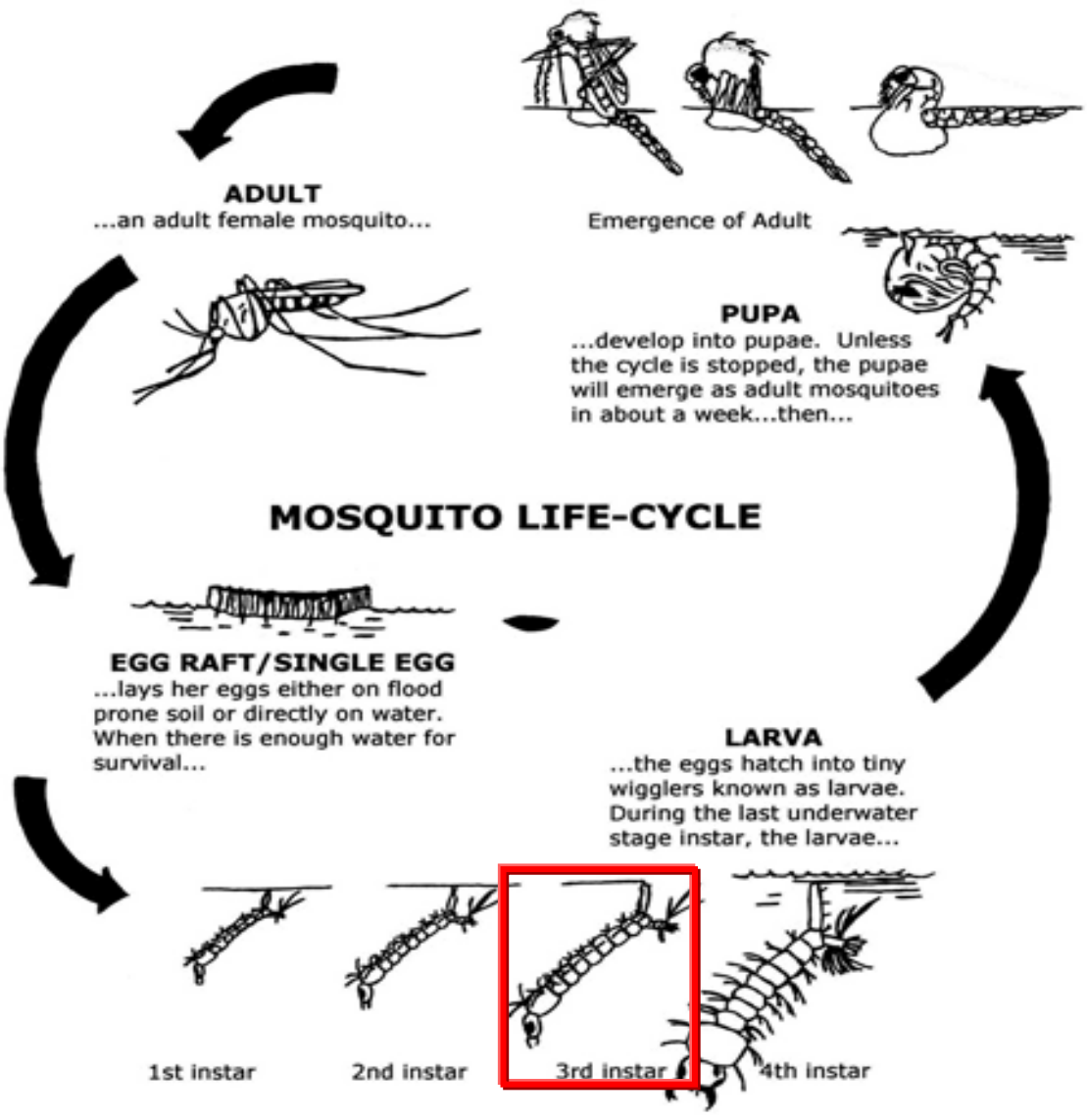


Figure 2.3: Life Cycle of *Culex quinquefasciatus* Mosquito

2.5 Mosquito Morphology and Feeding Habits

While male mosquitoes are harmless, feeding only on nectar and water, the females of the species are out for blood. Female mosquitoes use two very different food sources. They need sugar for energy, which is taken from sources such as nectar, and they need blood as a source of protein for egg development. Because biting is risky and hosts may be difficult to find, mosquitoes take as much blood as possible when they have the opportunity. Most bites occur at either dusk or dawn, when mosquitoes are most active. They choose their targets through a combination of smell, heat and visual cues, and continue seeking blood meals until their abdomens are full. The females can live up to about a month and feed every two or three nights during that time. Mosquitoes locate blood meals first by using sensors on their antennae to detect trace amounts of carbon dioxide released in human breath. That CO_2 rises in plumes that mosquitoes can sense from hundreds of feet away. A mosquito seeking prey follows the plume toward its source. Once it's close enough, it begins to pick up other chemical odors that signal the presence of people. Certain people will smell better to the mosquito than others, and scientists are not exactly sure why. Larger people and pregnant women tend to attract mosquitoes, possibly because they release more CO_2. Cholesterol and folic acid also act as attractants, as do perfumes, colognes, and lotions (Balenghien *et al.*, 2006).

The mosquito also depends on its large compound eyes, designed for spotting motion, to help guide it. Two simple eyes, called ocelli, are photosensitive, so the mosquito keys on lights and bright colors as well. Finally, thermal sensors on the insect's antennae and around its mouth detect heat emanating from warm-blooded bodies, allowing it to land on exposed skin and find capillaries closest to the surface. Then, the mosquito pierces the skin with a

serrated proboscis containing two tubes. Through one, it injects saliva that numbs the area and keeps the blood from clotting. Through the other, it begins to suck blood into its abdomen. The mosquito will suck until it is full, withdraw the proboscis and fly away. Often, the person bitten doesn't even realize it until a minor allergic reaction causes the site to swell and itch. The common symptoms, a tell tale red bump and itching, aren't caused by the bite itself, but by a reaction of the body's immune system to proteins in the mosquito's saliva.

2.6 Means of Mosquito Dispersal

The introduction of various mosquito species over large distances into regions where they are not indigenous has occurred through human agencies, primarily on sea routes, in which the eggs, larvae, and pupae inhabiting water-filled used tires and cut flowers are transported. However, apart from sea transport, mosquitoes have been effectively carried by personal vehicles, delivery trucks, trains, and aircraft. Man-made areas such as storm water retention basins, or storm drains also provide sprawling sanctuaries. Sufficient quarantine measures have proven difficult to implement.

2.7 Vector-Borne Diseases

Despite several decades of control effort, arthropod-borne diseases are still regarded as a major public health problem in the tropical and subtropical regions of the world. They are more common in the developing as well as resource-poor countries and inflict enormous burden in terms of morbidity and mortality. Besides the heightened health impact, they also contribute to extensive poverty, under-development and school absenteeism and dropout etc. These diseases are also responsible for huge economic losses both in terms of health-

care costs and loss of productivity, mostly in countries that can least afford them (Jacobs and James, 2002). The uncontrolled population growth in many areas has led to extensive deforestation, irrigation, and unplanned urbanization. These high population densities and associated environmental modifications have created conditions that favor the proliferation of certain arthropod vectors (Service, 1991). Vector-borne diseases are responsible for 17% of the global burden of parasitic and infectious diseases. They result in preventable ill-health and death, economic hardship for affected communities, and are a serious impediment to economic development (WHO, 2008). More than a billion people, primarily in developing countries, are now at the risk of contracting such diseases like malaria, filariasis, leishmaniasis, onchocerciasis, trypanosomiasis, dengue, yellow fever, *Japanese encephalitis*, Plague, the relapsing fevers, and various rickettsial diseases (Pant, 1987).

In the 21st century, the emergence and resurgence of vector-borne diseases still constitute an important threat to human health, causing over a million death and considerable mortality and morbidity worldwide. Indeed global warming is a major environmental driving force, which directly as well as indirectly attributes to entomological and epidemiological issues for disease transmission. It also influences the frequency and transmission dynamics of infectious diseases. In addition, it may play a pivotal role in sustaining the transmission cycle between vectors and human hosts by the re-emergence of vectors, increased man-vector contact and parasite exposure to diverse strains. Therefore at the moment, control of vector-borne diseases is becoming a great challenge, demanding for novel as well as innovative approaches (Karunamoorthi, 2012).

2.7.1 Mosquito as a disease vector

Mosquitoes can act as disease vectors for many disease-causing viruses and parasites. Infected mosquitoes carry these organisms from person to person without exhibiting symptoms themselves. Mosquito-borne diseases include;

(a) The parasitic diseases collectively called malaria, caused by various species of Plasmodium. It is carried by mosquitoes of the genus *Anopheles* and some members of *Culicidae.*

(b) Viral diseases such as yellow fever, dengue fever and chikungunya, transmitted mostly by *Aedes aegypti*

(c) Lymphatic filariasis which is the main cause of elephantiasis can be spread by *culex quinquefasciatus* mosquito species and also a vector of avian malaria in birds (Muslu *et al.*, 2011)

2.7.2 Malaria

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan (a type of single cell microorganism) of the *Plasmodium* type. Malaria causes symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases it can cause yellow skin, seizures, coma or death). In those who have recently survived an infection, re-infection typically causes milder symptoms. This partial resistance disappears over months to years if there is no ongoing exposure to malaria (Caraballo, 2014). Commonly, the disease is transmitted by the bite of an infected female *Anopheles* mosquito. This bite introduces the parasites from the mosquito's saliva into a person's blood. The parasites then travel to the liver where they mature and reproduce. Five species of

Plasmodium can infect and be spread by humans (Caraballo, 2014). Most deaths are caused by *P. falciparum* because *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria. The species *P. knowlesi* rarely causes disease in humans (Malaria Fact Sheet, 2014).

Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests. Methods that use the polymerase chain reaction to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity (Nadjm and Behrens, 2012).The disease is widespread in tropical and subtropical regions that are present in a broad band around the equator. This includes much of Sub-Saharan Africa, Asia, and Latin America. The World Health Organization estimates that in 2012, there were 207 million cases of malaria. That year, the disease is estimated to have killed between 473,000 and 789,000 people, many of whom were children in Africa (Malaria Fact sheet, 2014).

2.7.3 Lymphatic filariasis

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. Infection occurs when filarial parasites are transmitted to humans through mosquitoes. Infection is usually acquired in childhood causing hidden damage to the lymphatic system. The painful and profoundly disfiguring visible manifestations of the disease, lymphoedema, elephantiasis and scrotal swelling occur later in life and lead to permanent disability. These patients are not only physically disabled, but suffer mental, social and financial losses contributing to stigma and poverty (Ottesen *et al*., 1997).

Currently, more than 1.4 billion people in 73 countries are living in areas where lymphatic filariasis is transmitted and are at risk of being infected. Globally, an estimated 25 million men suffer with genital disease and over 15 million people are afflicted with lymphoedema. Eliminating lymphatic filariasis can prevent unnecessary suffering and contribute to the reduction of poverty (Addiss *et al*., 2004).

Over 120 million people are currently infected; with about 40 million disfigured and incapacitated by the disease. Lymphatic filariasis can result in an altered lymphatic system and the abnormal enlargement of body parts, causing pain, severe disability and social stigma. It is caused by infection with parasites classified as nematodes (roundworms) of the family Filariodidea. There are 3 types of these thread-like filarial worms:

- *Wuchereria bancrofti*, which is responsible for 90% of the cases
- *Brugia malayi*, which causes most of the remainder of the cases
- *B. timori*, which also causes the diseases.

Adult worms lodge in the lymphatic system and disrupt the immune system. The worms can live for an average of 6-8 years and, during their life time, produce millions of microfilariae (immature larvae) that circulate in the blood. Mosquitoes are infected with microfilariae by ingesting blood when biting an infected host. Microfilariae mature into infective larvae within the mosquito. When infected mosquitoes bite people, mature parasite larvae are deposited on the skin from where they can enter the body. The larvae then migrate to the lymphatic vessels where they develop into adult worms, thus continuing a cycle of transmission.

Lymphatic filariasis is transmitted by different types of mosquitoes for example by the *Culex* mosquito, widespread across urban and semi-urban areas; Anopheles mainly in rural areas, and Aedes, mainly in endemic islands in the Pacific. This infection involves asymptomatic, acute, and chronic conditions. The majority of infections are asymptomatic, showing no external signs of infection. These asymptomatic infections still cause damage to the lymphatic system and the kidneys as well as alter the body's immune system (Ottesen *et al*., 1997).

Acute episodes of local inflammation involving skin, lymph nodes and lymphatic vessels often accompany the chronic lymphoedema or elephantiasis. Some of these episodes are caused by the body's immune response to the parasite. However most are the result of bacterial skin infection where normal defenses have been partially lost due to underlying lymphatic damage.

When lymphatic filariasis develops into chronic conditions, it leads to lymphoedema (tissue swelling) or elephantiasis (skin/tissue thickening) of limbs and hydrocele (scrotal swelling). Involvement of breasts and genital organs is common. Such body deformities lead to social stigma, as well as financial hardship from loss of income and increased medical expenses. The socioeconomic burdens of isolation and poverty are immense.

2.8 Vector Control

Vector control is defined as measures of any kind, directed against vectors of diseases and intended to limit their ability to transmit diseases. Over the past several decades, v ector control has remained as one of the most powerful weapons in the battle against vector-borne diseases. Even today, it is considered as a corner stone in the malaria control and

elimination campaigns, under the specific set of environmental conditions. It is indeed extremely helpful to manage vector populations to reduce/interrupt disease transmission cycle (Karunamoorthi, 2011).

Insecticides are considered to be a powerful weapon in the developing countries in order to enhance the agriculture productivity and considerably to improve the major public health indices too (Karunamoorthi *et al*., 2012). They remain as the mainstay in most of the vector control programs and are commonly applied against adult insects through indoor residual sprays, fumigants, space sprays and treated bed nets. They are highly effective when optimally implemented (Lengeler, 2004, Mabaso *et al*., 2004). Nevertheless the compounding factors such as inadequate resources and operational capability (Kleinschmidt *et al*., 2007), insecticide resistance (Cohen *et al.,* 1991), and the use of adulterated or poor-quality insecticides (WHO, 2001) may all combine to reduce their efficacy.

There is overwhelming evidence that insecticide treated nets (ITNs) have enormous potential to alleviate the unbearable burden of malaria in Africa and rest of the world. Furthermore, the efficacy of ITNs can be improved by retreatment with insecticides (Karunamoorthi *et al*., 2010). Indeed, insecticides are one of the most potent arsenals in the fight against many vector-borne diseases, particularly malaria. Over the past several decades, indoor residual spraying (IRS) has been serving as an important component in the National Malaria Control Programmes (NMCPs) in most of the malaria endemic countries (Karunamoorthi *et al*., 2012). IRS is playing a vital role to reduce the prevalence of vector, by minimizing the population/life span and disease incidence considerably. However, it is important to note that the real success of an IRS programme heavily depends upon the quality of the spray. Indeed, the current malaria control heavily relies on one class of

insecticides, the pyrethroids. The accumulating evidence clearly suggests that genes responsible for resistance to pyrethroid insecticides have been spreading rapidly and are now widespread in African malaria vector mosquitoes. There is ample evidence that at least some of these genes have the potential to threaten the effectiveness of current malaria vector control interventions (Ranson *et al*., 2011). Insecticide-based vector control interventions are serving as the mainstay to minimize the vector-borne disease burden throughout the world (Karunamoorthi *et al*., 2012). Almost all malaria vector control programs overly rely on either IRS or LLINs. At the moment, insecticides belonging to different group viz., organochlorines, organophosphates, carbamates and synthetic pyrethroids are widely used for various vector control programmes.

2.8.1 Mosquito control methods

Mosquito control manages the population of mosquitoes to reduce their damage to human health, economies, and enjoyment. Mosquito control is a vital public-health practice throughout the world and especially in the tropics because mosquitoes spread many diseases, such as malaria, yellow fever, dengue fever, etc. Many methods are employed for the control of mosquitoes but the most important methods are;

(1) Source reduction/Water Management

(2) Biological control methods

(3) Chemical control methods

2.8.2 Source reduction

Anti-larval operations causing the reduction or permanent elimination of mosquito breeding places or sites are defined as source reduction methods. Source reduction primarily aims to prevent development of aquatic stages of mosquito larvae by reducing breeding source. These methods are environment friendly, economical in the long run with minimum maintenance and surveillance. Source reduction methods are further classified into: Elimination or reduction of breeding sites primarily involving engineering methods and Environmental manipulation.

Elimination or reduction of breeding sites include filling and draining of breeding sites by draining away the water collections or reducing vector breeding by channeling water to a few places which can be easily controlled. Environmental manipulation on the other hand are measures by which changes are effected in the natural conditions under which mosquitoes exist, rendering them unfavorable to the life and activities of these insects either in their aquatic or adult stages. The examples are pollution of water, changing the salt content of water, silting, flooding, fluctuating water levels, agitating of the water surface, mudding, shading, etc

2.8.3 Biological control methods

Biological control methods take advantage of natural enemies to reduce mosquito populations. These natural enemies can fall into one of three categories: they prey on mosquitoes, parasitize them, or act against their hormonal systems to preclude complete development. These mosquito eating fish are stocked seasonally in semi-permanent and permanent water sites known to produce mosquitoes. They are very adaptable to most water

conditions, bear live young which begin feeding immediately, and their gestation period is quite short so that they are able to produce several generations per season. Plus, one full grown adult can devour over 100 mosquito larvae per day.

Effective biocontrol agents include predatory fish that feed on mosquito larvae such as mosquitofish (*Gambusia affinis*) and some cyprinids (carps and minnows) and killifish. Tilapia also consumes mosquito larvae. Direct introduction of tilapia and mosquitofish into ecosystems around the world have had disastrous consequences (Kenneth, 1995).

Bti is an environmentally responsible bio-larvicide derived from *Bacillus thuringiensis sub. israeliensis* (Bti), a naturally occurring spore and crystal forming soil bacterium. The active ingredient of Bti is a crystalline (δ) delta-endotoxin. Mosquito larvae are killed through the ingestion of the delta-endotoxin. When larvae ingest the Bti, the delta-endotoxin reacts with the stomach secretions and causes gut paralysis and death. Bti is the most widely used mosquito larvicide in the United States. The safety of Bti was determined by extensive toxicological testing before approval for use. Research and field trials have shown that Bti has no toxic effects on beneficial and predacious arthropods and insects such as honeybees, beetles, mayflies, dragonflies, damselflies, stoneflies, caddisflies and true bugs (Hock *et al.*, 2000).

2.8.4 Chemical control methods

Aside from source reduction, using pesticides to reduce mosquito populations is the oldest form of control. It is the application of natural or synthetic compounds (insecticides) to reduce mosquito populations to tolerable levels. Chemical mosquito control has the considerable advantage of succeeding where the physical and biological methods are

infeasible, uneconomical or only partially successful. Chemical insecticides will usually give immediate control of either larvae or adult mosquitoes. Several insecticide formulations and application options exist in modern mosquito control, but each has their drawbacks. In the long run chemical insecticides can be expensive, may cause environmental damage and are only a temporary means of control.

Larvicides are applied to water where mosquito larvae develop or where it may provide a habitat for mosquitoes. Currently, light mineral oils and insect-growth regulators and some organophosphates such as temephos and malathion are used as mosquito larvicides in many countries. As mineral oils and organophosphates can also kill other aquatic animals such as fish and crustaceans in the water body, these chemicals should be applied only when absolutely necessary. Larvicides have very little residual effect. As the life cycles for mosquitoes are about 7 days, larvicides have to be applied on larval habitats at about every 7 days. Most insecticides will not kill mosquito eggs; they are mainly directed at killing larvae. They are not very efficient at killing the pupae though mineral oils can also kill pupae. However, a product that can form a monomolecular film (MMF) over water surfaces when applied to water bodies is commercially available. The film will suffocate mosquito larvae and pupae. It can kill the eggs and emerging adults as well (O'Neil, 1997).

Adulticides are applied to surfaces where adult mosquito will rest or in the air where it flies. Insecticides with residual effect are applied to the interior surfaces of walls for killing indoors resting adult mosquitoes such as the endophagic malaria vectors.

2.9 Mode of Action of Insecticides/Classification

Mode of action is the most fundamental property of an insecticide more so even than chemical structure itself, as compounds of widely different chemical structure can bind at the same target site and have exactly the same mode of action. Furthermore, heritable changes that hamper action at the target site can confer resistance to entire classes of insecticides. The Insecticide Resistance Action Committee (IRAC) is a specialist technical group of the industry association Crop Life, organized to provide a coordinated industry response to prevent or delay the development of resistance in insect and mite pests. IRAC classifies insecticides into groups with a common mode of action and then into chemical subgroups within those groups.

While consolidating chemical insecticide classes into mode of action groups is very useful, further grouping the modes of action groups into four categories provides a broader understanding of this relationship. The four categories are:

- Neuromuscular toxins, which attack the nervous system or muscles;
- Insect growth regulators (IGRs), which affect growth and development;
- Respiratory poisons, also called metabolic poisons, which affect energy metabolism; and
- Gut disruptors, which destroy the integrity of the gut lining.

2.9.1 Neuromuscular disruptor insecticides

(a) Acetyl cholinesterase (AChE) inhibitors: Acetylcholinesterase (AChE), a critical enzyme in the function of the insect central nervous system, is the target of inhibition by

organophosphate (OP) and carbamate insecticides. Their use has been in slow decline, due largely to regulatory action and also resistance, Organophosphates and carbamates are broad-spectrum insecticides with a large variety of crop and non-crop uses. The group includes several of the most important soil-applied and foliar insecticides for row crops and vegetables. Some members of these classes also control nematodes and mites (Hock *et al.*, 2000).

Mode of action: Acetyl cholinesterase inhibitors bind to and inhibit the enzyme that's normally responsible for breaking down ACh after it has carried its message across the synapse. This allows the ACh to continue stimulating the postsynaptic neuron, leading to overstimulation of the nervous system and eventual death of the insect. AChE is one of the fastest enzymes known, each molecule being able to degrade 25,000 ACh molecules per second. Carbamates and organophospates are suicide inhibitors of AChE (Hock *et al.*, 2000).

(b) GABA-gated chloride channel antagonists: Cyclodiene, Organochlorines and Cyclodienes were introduced in the 1940s. Their stability in soil and the environment, broad spectrum of activity, high level of performance and relatively low cost gained the group extensive use globally. However, due to wide spread insect resistance, persistence, long-range transport and bio-magnification in wildlife food chains, the use of cyclodienes, once a mainstay of the insecticide market, has seen a period of rapid decline.

Mode of action: Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter used to transmit signals that inhibit the activity of postsynaptic cells. A certain amount of inhibition in the nervous system is essential for normal function and blocking of the GABA-

gated chloride channels by cyclodienes and phenylpyrazoles leads to overstimulation and convulsions.

(c) Sodium channel modulators: Pyrethroids, Pyrethrum is the powdered dried flower head of the pyrethrum daisy, a species of chrysanthemum that has been used as an insecticide. Pyrethrins are the insecticidal compounds that occur naturally in this material. They have been specifically designed to be more environmentally stable than Pyrethrins, whose activity is measured in hours. They provide long-lasting control and improved mammalian safety relative to other products in use at the time they were developed. These compounds are generally effective against caterpillars, beetles, certain aphids and mites in crops, and are used for mosquito, termite and cockroach control in non-crop segments. In addition, certain members of the class are used to control ecto-parasites on pets and humans.

Mode of action: Sodium channel modulators are neurotoxins that act on the action potential sodium channel. They slow the closing and inactivation of the channel, causing it to remain open longer than normal, which has the effect of prolonging the action potential. When the action potential is not promptly terminated, it can re-excite the same area of membrane, leading to repetitive firing (Rattan, 2010).

Because nerve axons occur throughout the insect's body, even near the surface of the cuticle in sensory organs and motor nerve terminals, pyrethroids and DDT cause symptoms as soon as they enter the body and are considered extremely fast-acting, causing immediate "knockdown".

(d) Nicotinic acetylcholine receptor (nAChR) agonists: Neonicotinoids provide excellent acute and residual control of sucking insects, including aphids, leafhoppers, planthoppers

and whiteflies, as well as certain chewing insects. Possessing high water solubility and robust plant systemicity**,** neonicotinoids can be applied by foliar spray, soil treatment, seed treatment, trunk injection or painting onto plant tissue. The application rates for neonicotinoids are low compared to most insecticide groups (Hock *et al.,* 2000).

Mode of action: This group of insecticides acts as agonists of acetylcholine receptors, meaning that they mimic the action of the neurotransmitter acetylcholine (ACh). The similarity between these chemicals is shown by the alignment of their structures with key areas on the receptor that interact with certain parts of the molecules (ellipses). The popular view is that neonicotinoids and indeed nicotine itself continually stimulate the receptors and in so doing cause nerve overstimulation, but it is becoming clear that the action is more complicated. Most receptors do not remain activated indefinitely when an agonist is bound, but instead become desensitized. Desensitization involves a conformational transition of the nicotinic acetylcholine receptor (nAChR)-insecticide complex to a very stable desensitized state that binds the insecticide 500-fold more strongly than the activated state. Desensitized nAChR-neonicotinoid complexes no longer conduct ions and are essentially inhibited. While most receptors undergo desensitization, some insects have been found to also possess non-desensitizing receptors, which, lacking a high-affinity desensitized state, are relatively insensitive to neonicotinoids.

2.9.2 Growth and development disruptor insecticides

(a) Juvenile hormone mimics: Juvenile hormone (JH) mimics do not control adult insects or early larval stages, but because of their anti-metamorphic effect, they prevent transformation to the adult stage. They also have an ovicidal effect, which is very important

for many uses. They are used as mosquito larvicides, for controlling fleas on domestic animals, and as an additive to cattle feed to prevent fly breeding in the dung. Pyriproxyfen is also used to control whiteflies in cotton.

Mode of action: Methoprene is a very close chemical analog, and in fact all of the JH mimics work by binding to and mimicking the action of JH at its receptor. Activation of JH receptors during molting inhibits the expression of genes needed to form larval or adult structures. In immature molts this has little or no effect, but it can severely disrupt metamorphosis, leading to incomplete molting, or in some cases, a successful molt to a supernumerary immature stage.

(b) Mite growth inhibitors: These compounds interfere with mite development and are active against eggs, larvae and nymphs, but not adults.

Clofentezine, Hexythiazox and Diflovidazin Clofentezine, hexythiazox and diflovidazin are acaricides. Clofentezine is used for the control of spider mites on a wide range of crops, including fruits and nuts. It acts primarily as an ovicide, but has some activity against early motile stages of mites. Hexythiazox is used to control mites on a variety of crops. It has ovicidal, larvicidal and nymphicidal activities and can be applied at any stage of plant growth from budding to fruiting. It has very long residual activity of up to four months.

Mode of action: Clofentezine and Diflovidazin are close analogs and are grouped with Hexythiazox because they commonly exhibit cross-resistance, despite being structurally distinct. The target site of these compounds is considered unknown, but recent investigations of resistance mechanisms indicate that these compounds may be chitin synthase inhibitors (Rattan, 2010)

(c) Inhibitors of chitin biosynthesis: Chitin is an essential structural component of insect cuticle. These insecticides interfere with the biosynthesis of chitin in Lepidoptera and some other orders. When an insect cannot make chitin, it dies during the molt. Insecticides in this group are used to control certain major pests in a variety of crops, including boll weevils and Lepidoptera (moths and butterflies) in cotton and Lepidoptera and Coleoptera (beetles) in citrus, rice, tea, soybeans, vegetables and ornamentals. These insecticides generally enter the insect orally through the consumption of treated foliage or insecticide baits.

Mode of action: Chitin biosynthesis inhibitors interfere with the formation of chitin during molting, resulting in a weak, soft exoskeleton and deformed appendages and sexual organs. The molecular target of the chitin biosynthesis inhibitors is not known.

2.9.3 Energy production disruptors

All organisms generate energy from the intake of food. As the nutrients consumed in the food are digested, they store energy from those nutrients in the form of ATP and this stored energy is used to do work. Some insecticides inhibit energy production. When electron transport is disrupted, oxidative phosphorylation is inhibited and energy cannot be stored for later use. Some insecticides disrupt energy production through uncoupling oxidative phosphorylation from electron transport chain, hence no ATP is produced (Larson, 2001).

2.9.4 Gut disruptors

Some insecticides block feeding. Various classes of insecticides work through different mechanisms.

(a) Bacillus thuringiensis (bt) - is a microbe that produces a crystal with a toxic effect against some insects. When Bt crystals are ingested by an insect, they dissolve under the alkaline conditions of the gut to liberate one or more protein toxins. Of those, only the crystal, or Cry toxins, has been engineered into crops. More than 500 Cry proteins in 67 classes were identified in the various known Bt strains. Cry toxins liberated from Bt crystals are actually protoxins, requiring two successive transformations in the host before they become active. The first step is the cleavage by gut protein-digesting enzymes to yield an activated toxin monomer. The activated monomer passes through the peritrophic membrane and binds to a specific cadherin on the brush border membrane of epithelial cells. Cadherins are proteins on the surface of cells that serve a variety of functions, including binding cells together into tissue. Binding to a cadherin triggers removal of the toxin fragment's N-terminal end, known as helix α-1. With helix α-1 gone, toxin monomers are able to self-assemble into tetramers, which bind to secondary receptors on the epithelial surface. These receptors can be either aminopeptidase N or alkaline phosphatase, both of which are abundant. After binding, the toxin tetramer partially inserts into the membrane to make pores that lyse the cells and destroy the integrity of the gut. This gut destruction by the action of the toxin is sufficient to kill the insect by fluid loss and septicemia. In bacterially infected hosts, the actively replicating bacteria can also invade and replicate in the host tissues. Although a few days may elapse before the insect dies, it stops feeding soon after ingesting *Bt* (Hock *et al.*, 2000).

2.10 Insecticide Resistance

According to WHO (1957), insecticide resistance has been defined as "the developed ability in a strain of insects to tolerate doses of toxicant which would prove lethal to majority of

individuals in a normal population of the same species". It is the major threat to effective prevention and control of malaria (USAID, 2007). More than 40 years of intensive organic synthetic insecticide use to control arthropod pests and disease vectors have resulted in pesticide resistance among over 450 species. The indiscriminate and injudicious use of pesticides has led to the widespread development of resistance. Some insects' species have shown multiple resistances to all classes of insecticide, making their control by means of chemical methods to be extremely difficult and expensive.

The resistance usually occurs due to insecticide detoxification by mutant enzymes (isozymes) engendered by resistant gene alleles, but some resistance may also be conferred by the reduced toxicant uptake. The rapid development of resistance to dichlorodiphenyltrichloroethane (DDT) and organophosphates (OPs) is well documented (Rawlins *et al*., 1997). Resistance also arises through the over-use and/or misuse of a pesticide against a pest species and results from the selection of resistant forms of the pest and the resulting evolution of populations become resistant to that pesticide and its mode of action (Bellinger, 2011).

2.10.1 Insect resistance development (IRD)

The natural selection by an insecticide allows some initially rare naturally occurring pre-adapted insects with resistance genes, to survive and pass the resistance trait on to their progenies. The continuous application of insecticides with similar mode of action, results in the selection of the resistant individuals and hence resistance gradually increases among insect population which eliminates the susceptible ones. The resistant insects may multiply

more rapidly than the susceptible ones, when the selection pressure continues to be constant; it directly indicates that the insecticide is no longer effective.

Mosquito control has focused on the use of insecticides (initially organochlorines, followed by organophosphates and carbamates) through indoor residual spraying. By 1990, more than 500 species of insects and mites had developed resistance to one or more classes of insecticides (Georghiou *et al*., 1990). The use of ITNs for both individual and collective protection against malaria has shown potential in reducing childhood malaria morbidity by 50% and global mortality by 20–30% in the Gambia, Ghana, and Kenya (Alonso *et al*., 1991, Binka *et al.*, 1996., Nevill *et al.*1996). The insecticides of choice for bed net impregnation are pyrethroids because of their high efficacy, rapid rate of knockdown, strong mosquito excito-repellent properties, and low mammalian toxicity (Diabate *et al*., 2002). The recommended doses of pyrethroids for mosquito net treatment pose little or no hazard to people treating the nets or to users of the treated nets. Even the frequent exposure to lower concentrations of pyrethroids, the risk of toxicity is remote (Zaim *et al*., 2000).

2.10.2 The mechanisms of insecticide resistance

There are four major forms of resistance mechanisms described on the biochemical basis;

(i) Altered target-site resistance, which occurs when the insecticide no longer binds with its targets.

(ii) Metabolic resistance that occurs due to the modified activities of three major enzyme groups such as esterases, oxidases, or glutathione S-transferases (GST) which inhibit the insecticides from reaching their potential target sites. The resistant insects may possess

overproduction of detoxification enzymes that break-down the toxic insecticides into non-toxic compounds.

(iii) Behavioral resistance occurs when the resistant insects avoid the insecticide treated surface area by changing their customary behavior. For instance the *An. arabiensis* mosquito has changed its resting behavior from endophilic (resting indoor) nature to exophilic (resting outdoor) in order to avoid the contact or exposure to indoor residual spray in Africa.

(iv) Penetration resistance occurs when the cuticle (outer layer of insects) absorb the insecticides molecules much more slowly than the susceptible insects. In addition, the mechanism on thermal stress response has also been proposed by (Patil *et al*.1996), but its significance has not been well-established.

2.11 Larviciding

A larvicide is an insecticide that is specifically targeted against the larval life stage of an insect. Larviciding is an ecologically safe preventive method used to interrupt the development of larvae or pupa into adult mosquitoes. Larviciding program emphasizes targeting mosquitoes in their habitats where they reproduce. This gives us the advantage of controlling the mosquitoes before they are biting adults. Larvacides may be contact poisons, stomach poisons, growth regulators, or (increasingly) biological control agents.

In most settings, insecticide treated nets (ITNs), which include long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the most powerful, reliable and practicable tools for malaria vector control; however these two interventions are not perfect, and they

cannot serve all vector control purposes in all settings. For example, it has often been observed in Africa that indoor transmission can be greatly reduced by careful indoor residual spraying (IRS) (Kouznetsov, 1977) but outdoor transmission may persist and prevent the complete interruption of transmission. However, it is important to note that major African malaria vectors prefer to rest indoors, where they are exposed to insecticides, even if they sometimes bite outdoors. Larviciding has the potential to overcome this problem, because it is expected to affect indoor and outdoor biting vectors equally.

2.12 Phytochemicals

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920 (Shahi *et al*., 2010), but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme.After facing several problems due to injudicious and overapplication of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure andcommercial production has been initiated. At present, phytochemicals make up to 1% of world's pesticide market (Isman, 1997). Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Shaalan *et al*., 2007).

Insecticidal effects of plant extracts vary not only according to plant species,mosquito species, geographical varieties and parts used, but also due to extraction methodology

adopted and the polarity of the solvents used during extraction. A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks,roots, etc, of larger plants or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control. Members of the plant families- Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various types of larval, adulticidal or repellent activities against different species of mosquitoes (Shaalan *et al*., 2007).

2.12.1 Application of phytochemicals as mosquito larvicide

Human beings have used plant parts, products and secondary metabolites of plant origin in pest control since early historical times. Vector control has been practiced since the early 20th century. During the pre-DDT era, reduction of vector mosquitoes mainly depended on environmental management of breeding habitats, i.e., source reduction. From the early 1950s, DDT and other synthetic organochloride and organophosphate insecticides were extensively used to interrupt transmission of vector borne diseases by reducing densities, human-vector contact and, in particular, the longevity of vector mosquitoes. In the mid-1970s, the resurgence of vector borne diseases, along with development of insecticide resistance in vector population, poor human acceptance of indoor house spraying and environmental concerns against the use of insecticides led to a rethinking in vector control strategies (WHO, 2005). As a result, emphasis was given on the application of alternative methods in mosquito control as part of the Integrated Mosquito Management (IMM) (Rose, 2001).

Integrated Mosquito Management (IMM) is a decision-making process for the management of mosquito populations, involving a combination of methods and strategies for long-term maintenance of low levels of vectors. The purpose of IMM is to protect public health from diseases transmitted by mosquitoes, maintain healthy environment through proper use and disposal of pesticides and improve the overall quality of life through practical and effective pest control strategies.

The main approaches of IMM include:

- Source reduction and habitat management by proper sanitation, water management in temporary and permanent water bodies, and channelirrigation. Vegetation management is also necessary to eliminate protection and food for mosquito larvae,
- Larviciding by application of dipteran specific bacteria, insect growth regulators, surface films and oils, expanded polystyrene beads, phytochemicals, organophosphates and organochlorides,
- Adulticiding by application of synthetic pyrethroids, organophosphates and synthetic or plant derived repellents, insecticide impregnated bed nets, genetic manipulations of vector species, etc,
- Use of mosquito density assessment in adult and larval condition and disease surveillance; and
- Application of biological control methods by using entomophagous bacteria, fungi, microsporidians, predators and parasites.

Of the above avenues of IMM, larviciding approach is the more proactive, proenvironment, target specific and safer approach than controlling adult mosquitoes (Shaalan *et al*., 2007). Application of larvicide from botanical origin was extensively studied as an essential part of IMM, and various mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin were developed (Shaalan *et al*., 2007).

2.12.2 Scope for isolation of toxic larvicidal active ingredients

Several studies have documented the efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito larvae but only a few have been commercially produced and extensively used in vector control programmes. The main reasons behind the failure in laboratory to land movements of bioactive toxic phytochemicals are poor characterization and inefficiency in determining the structure of active toxicingredients responsible for larvicidal activity (Foseca *et al*., 2001; Ghosh, 2012). For theproduction of a green biopesticide, the following steps can be recommended during any research design with phytochemicals:

- ✓ Screening of floral biodiversity in search of crude plant extracts having mosquito larvicidal potentiality
- ✓ Preparation of plant solvent extracts starting from non-polar to polar chemicals and determination of the most effective solvent extract
- ✓ Evaporation of the liquid solvent to obtain solid residue and determination of the lethal concentration (LC_{50}/LC_{90} values)

- ✓ Phytochemical analysis of the solid residue and application of column chromatography and thin layer chromatography to purify and isolate toxic phytochemical with larvicidal potentiality
- ✓ Determination of the structure of active principle by infra-red (IR) spectroscopy, nuclear magnetic resonance (NMR) and gas chromatography and mass spectroscopy (GCMS) analysis
- ✓ Study of the effect of active ingredient on non target organisms
- ✓ Field evaluation of the active principle before its recommendation in vector control programme and commercial production (Ghosh *et al*., 2012).

2.13 *CITRUS SINENSIS*

2.13.1 Scientific classification of *Citrus sinensis*

Kingdom: Plantae

Unranked: Angiosperms

Unranked: Eudicots

Unranked: Rosids

Order: Sapindales

Family: Rutaceae

Genus: Citrus

Species: ***C. sinensis***

2.13.2 Description

Sweet orange (*Citrus sinensis* L. Osbeck) (to distinguish it from closely related species like sour orange, *C. aurantium, C. reticulata* and mandarin orange), is a small evergreen tree

7.5m high and in some cases up to 15 m. It originated from southern China where it has been cultivated for many years, but is today grown commercially worldwide in tropical, semi-tropical and some warm temperate regions to become the most widely planted fruit tree in the world (Nicolosi *et al*., 2000; Ehler, 2011). Orange produces leathery and ever green leaves of different shapes, ranging from elliptical to oblong to oval, 6.5-15 cm long and 2.5-9.5 cm wide, often bearing narrow wings on the petioles. It bears fragrant white flowers either singly or in whorls of 6, about 5 cm wide, with 5 petals and 20-25 yellow stamens. The small, white or purple scented hermaphroditic flowers produce nectar for pollination by insects. The fruit, which may be globose to oval is 6.5 to 9.5 cm wide, and ripens to orange or yellow.

Anatomically, the fruit consists of two distinct regions: the pericarp also called the peel, skin or rind, and the endocarp, or pulp and juice sacs. The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell. The quantity of wax is dependent on the variety, climatic conditions and growth rate

The flesh or pulp of the fruit is typically juicy and sweet, divided into 10 to 14 segments (although there are seedless varieties) and ranges in color from yellow to orange to red. The ripe fruit is classified as a hesperidium which is a type of berry with multiple seeds and is fleshy. Fleshy juice sacs accumulate sugars, organic acids and large amount of water, causing difficulties in the extraction of nucleic acids and proteins. The centre of origin of most citrus cultivars is perhaps unknown, but the ancient relatives of citrus are native to China, the Southeast Asia, the Malay Archipelago, New Caledonia and Australia (Atta *et al*,

2012). The genetic origin of sweet orange is not clear, although it is believed to be derived from the interspecific hybridization of some primitive citrus species (Xu *et al*, 2013).

2.13.3 Distribution

Oranges probably originated from south East Asia, and were cultivated in China by 2500 BC (Nicolosi et al, 2008), where it was referred to as ‘‘Chinese’’ apple (Ehler, 2011). Today, it is grown almost all over the world as a source of food for humans because of its high nutritional values, source of vitamins and other uses (Mortton, 1987).

Citrus is widely grown in Nigeria and many other tropical and subtropical regions (Piccinelli et al, 2008). In terms of volume in production, citrus ranks after banana as the world second fruit crop with more than 108 million tons (FAO Statistics 2006). Sweet orange (*Citrus sinensis* L. Osbeck) is a major source of vitamins, especially vitamin C, sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium (Angew, 2007).



Plate 1: Image of *Citrus sinensis* leaves

2.13.4 Medicinal benefits of *Citrus sinensis*

The human diet contains important micronutrients namely vitamins C and E, carotenoids and flavonoids, essential for maintenance of human health. Multiple dietary sources of these compounds are present virtually in all plant material (Di Majo *et al.,* 2005). The nutritional importance of foods is due to the presence of these functional food ingredients and antioxidant nutraceuticals or phytochemicals. Phytochemicals are present in edible fruits and vegetables and when eaten potentially modulate human metabolism in a favourable manner, thereby prevent chronic and degenerative diseases (Tripoli *et al*., 2007). Increase in fruits and vegetables consumption protects against degenerative pathologies such as cancer and atherosclerosis (Keys, 1995); as epidemiological surveys had shown an inverse relationship between dietary flavonoid intake from citrus and cardiovascular diseases (Hertog *et al*., 1993; Di Majo *et al*, 2005). Citrus fruits are the main source of important phytochemical nutrients and for long have been valued for their wholesome nutritious and antioxidant properties. It is scientifically proven that oranges being rich in vitamins and minerals have many health benefits. Moreover, it is now appreciated that other biologically active, non-nutrient compounds found in citrus fruits such as phytochemical antioxidants, soluble and insoluble dietary fibres are known to be helpful in reducing the risk for cancers, many chronic diseases like arthritis, obesity and coronary heart diseases (Crowell, 1999).

Antioxidant

The biological activity and the health effects of citrus flavonoids as antioxidants have been reported (Tripoli *et al*, 2007). These group of pigments as found in plants and together with anthocyanin play a role in flower and fruit colouration. Studies indicate that flavonoids are

excellent radical-scavengers of the hydroxyl radical (Cillard and Cillard, 1988; Darmon *et al*., 1990), due to their ability to inhibit the hydroxyl radical and donate hydrogen atom (Di Majo *et al*., 2005, Tripoli *et al*. 2007). Oranges as excellent source of vitamin C, contain powerful natural antioxidant, folate, dietary fibre and other bioactive components, like carotenoids and flavonoids that prevent cancer and degenerative diseases (Ejaz *et al*., 2006).

Anti-inflamation

Citrus flavonoids contain compounds with anti-inflammatory activity due to the presence of regulatory enzymes (protein kinase C, phosphodiesterase, phospholipase, lipoxygenase, and cyclooxygenase) that control the formation of the biological mediators, responsible for the activation of endothelial cells and specialized cells involved in inflammation. Flavonoid inhibition of the immune and inflammation responses can be associated with their inhibition of these enzymes (Tripoli *et al*., 2007). Indeed, citrus flavonoids are able to inhibit the kinases and phosphodiesterases essential for cellular signal transduction and activation. They also affect the activation of a number of cells involved in the immune response, including T and B lymphocytes (Manthey *et al*., 2001). Citrus flavonoids also prevent atherosclerosis, inhibiting the formation of atheroma (Hertog *et al*., 1993). Tripoli *et al*., (2007) reported that hesperidin obtained from citrus cultures may have a potential therapeutical use as a mild anti-inflammatory agent, being also useful as a precursor of new flavonoids endowed with this activity (Da -Silva *et al*., 1994).

Anti-cancer and anti-arteriosclerosis

Citrus flavonoids can prevent câncer through selective cytotoxicity, antiproliferative actions and apoptosis (Elangovan *et a.l*, 1994; Hirano *et al*., 1994). Flavonoids are

antimutagenic, thus protects the DNA from damage by their ability to absorb ultraviolet light (Stapleton and Walbot, 1994). They neutralize free radicals that promote mutations when they are generated near DNA. This hás been shown in mice body irradiated with c-ray (Shimoi *et al*., 1994). Flavonoids can also protect the DNA by interacting directly with the tumoral agents, as in the induced chromosomal aberrations by bleomycin (Heo *et al*., 1994). The inhibitory effects of citrus flavonoids on tumoral development and cell proliferation by rat malignant cells, in cardiac and hepatic tissue of syngenetic rats have been reported. The ability to function as such by citrus flavonoids are based on cell mobility inhibition (Bracke *et al*., 1989).

Anti-obesity

Sweet oranges contain low calories and no saturated fats or cholesterol, but are rich in dietary fibre, pectin which is very effective in persons with obesity. Pectin as bulk laxative protects the mucous membrane from exposure to toxic substances, as well as by binding to cancer causing chemicals in the colon. Pectin has also been shown to reduce blood cholesterol levels by decreasing its re-absorption in the colon by binding to bile acids in the colon (Walton *et al*., 1945). Orange peels contain the alkaloid synephrine, which reduces the production of cholesterol in the liver. The antioxidant elements in oranges combat oxidative stress that oxidizes the LDL (low-density lipoprotein) in the blood.

2.14 *SESAMUM INDICUM*

2.14.1 Scientific classification of *Sesamum indicum*

Kingdom: Plantae

(Unranked): Angiosperm

(Unranked): Eudicots

(Unranked): Asterids

Order: Lamiales

Family: Pedaliaceae

Genus: Sesamum

Species*:* ***S.indicum***

2.14.2 Description

It is an annual plant growing 50 to 100 cm (1.6 to 3.3 ft) tall, with opposite leaves 4 to 14 cm (1.6 to 5.5 in) long with an entire margin; they are broad lanceolate, to 5 cm (2 in) broad, at the base of the plant, narrowing to just 1 cm (0.4 in) broad on the flowering stem. The flowers are yellow, tubular, 3 to 5 cm (1.2 to 2.0 in) long, with a four-lobed mouth. The flowers may vary in colour with some being white, blue or purple.

Sesame fruit is a capsule, normally pubescent, rectangular in section and typically grooved with a short triangular beak. The length of the fruit capsule varies from 2 to 8cm; its width varies between 0.5 to 2 cm, and the number of loculi from 4 to 12. The fruit naturally splits open (dehisces) to release the seeds by splitting along the septa from top to bottom or by means of two apical pores, depending on the varietal cultivar. The degree of dehiscence is of importance in breeding for mechanized harvesting as is the insertion height of the first capsule.

Sesame seeds are small. The size, form and colors vary with the thousands of varieties now known. Typically, the seeds are about 3 to 4 millimeters long by 2 millimeters wide and 1 millimeter thick. The seeds are ovate, slightly flattened and somewhat thinner at the eye of

the seed (hilum) than at the opposite end. The weight of the seeds is between 20 and 40 milligrams (Oplinger *et al.,* 1990).The seed coat (testa) may be smooth or ribbed. Sesame seeds come in many colours depending on the cultivar harvested. The most traded variety of sesame is off-white coloured. Other common colours are buff, tan, gold, brown, reddish, gray and black.

Sesame seed is sometimes sold with its seed coat removed (decorticated). This is the variety often present on top of buns in developed economies (Ray-Langham, 2008).

2.14.3 Distribution

Sesame (*Sesamum indicum L*.) is an important oil seed crop being cultivated in the tropics and the temperate zone of the world. (Biabani and Pakniyat , 2008). It is one of the oldest oil crops and is widely cultivated in Asia and Africa. (Ali *et al*., 2007).It was a highly prized oil crop of Babylon and Assyria at least 4000 years ago (Roos, 2005). Sesame oil, otherwise also referred to as gingelly oil, is one of the major sources of edible oil in India and is culturally associated from the Vedic period. Natural sesame oil derived from good quality seed has a very pleasant flavour and can be consumed without further purification. The natural oil has excellent stability due to the presence of high levels of natural antioxidants (Lyon, 1972).



Plate II: Image of *Sesamum indicum* leaves

2.14.4 Medicinal uses

In the southwest and middle belt areas of Nigeria, *Sesamum indicum* is widely used as herbal remedies for the treatment of various ailments like diarrhea and urinary infections (Chakraborthy *et al.*, 2008). Some studies have also shown that sesame leaves and seeds are used for medicinal purposes as it been found that the leaves are rich in a gummy matter and when mixed with water, they form rich blend mucilage which is used in the treatment of infant cholera, dysentery, catarrh and bladder problems (Beckstrom-Sternberg *et al*., 1994; Moerman, 1998; Brown, 1995).

Sesame lignans have anti-oxidants and health promoting activities. High amounts of both sesamin and sesamolin have been identified in sesame (Sirato-Yasumoto *et al*., 2001). Both sesamin and sesamolin were reported to increase both the hepatic mitochondrial and the peroxisomal fatty acid oxidation rate. Sesame seed consumption appears to increase plasma gamma- tocopherol and enhanced vitamin E activities which are believed to prevent cancer and heart diseases (Cooney *et al.,* 2001). Africans use sesame to prepare perfumes, and colognes have been made from sesame flowers. Mystyric acid from sesame oil is used as an ingredient in cosmetics. Sesamin has bacteriocide and insecticide activities, sesamolin also has insecticidal properties and is used as a synergist for pyrethrum insecticides (Morris, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Beakers, Cotton wool, Filter paper, Foil paper, Micro-haematocrit capillary tubes, Pipettes, Sample bottles, Silica gel for column, Silica gel 60 F_{254} plates for TLC, Spatula, Strainer for transferring larvae into test bowls, Measuring cylinder, Nylon netting cage, Wire loops,100 ml disposable bowls.

3.1.1 Chemicals and reagents

All chemicals and reagents used were of analytical grade and were purchased from Sigma Aldrich Company Limited, U.S.A except otherwise stated.

3.1.2 Plants collection and identification

Leaves *of Citrus sinensis and Sesamum indicum* were collected in Dumbi, along Kaduna road and Hanwa low cost Zaria, respectively. The plants were identified at the herbarium unit of Department of Biological Science, Ahmadu Bello University Zaria and given voucher numbers *Citrus sinensis* (990) and *Sesamum indicum* (04).

3.2 Methods

3.2.1 Preparation of extracts

Plants collected were washed with clean water, shade dried under room temperature and ground into fine powder using mortar and pestle. The dried powdered plant material was used to prepare crude extracts of n-hexane, ethyl acetate, methanol and distilled water.

Exactly 100g of the dried material was weighed and mixed with 1000ml of each solvent (n-hexane, ethyl acetate, methanol and distilled water) with periodic shaking for 24hrs. It was then filtered using Whatman No. 1 filter paper and the filtrates were concentrated using a rotary evaporator and then stored in air-tight bottles at room temperature until when needed.

3.2.2 Mosquito larvae culture

Larvae were collected in open drains and transported to the laboratory in plastic containers. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the mosquitoes were identified and authenticated at the Entymology research laboratory of Department of Biological Science, A.B.U. Zaria before rearing. The larvae were reared in plastic trays in tap water. They were maintained at room temperature and fed with larval food (dog biscuits and yeast in the ratio 3:1). Pupae was transferred from the trays to a cup containing tap water and placed in screened cages where adults emerged. Adult mosquitoes were fed on 10% sucrose solution soaked on a cotton wool and placed in the cage.

After mating, the adult female mosquitoes were blood-fed with laboratory reared albino mice placed in a restrained position. A petri dish with moisten filter paper was provided in the cage for oviposition and was maintained at the same environmental condition. The third instar larvae of the second generation were used for the bioassay (SenthilNathan, 2007).

3.3 EXPERIMENTAL DESIGN

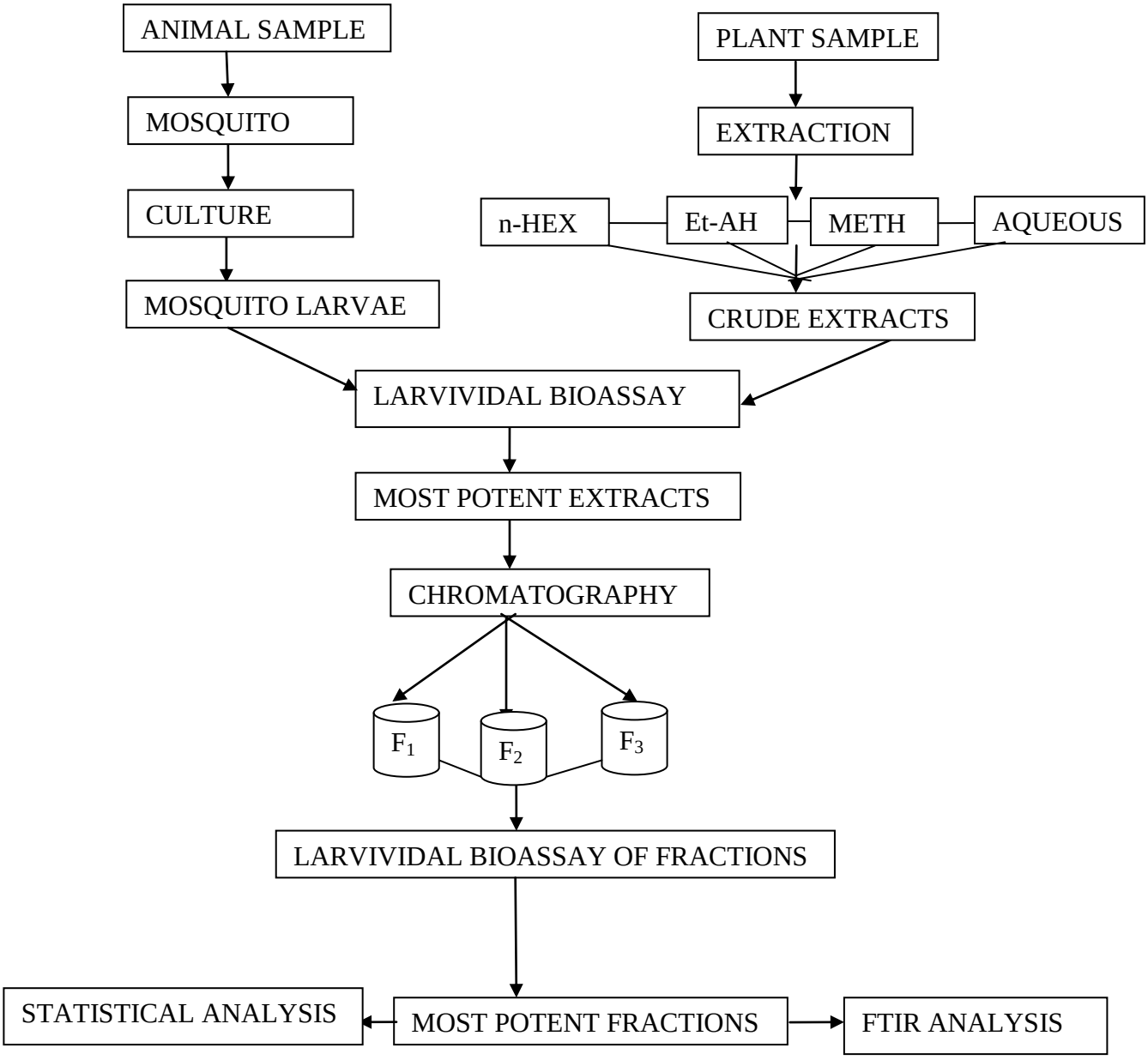


Figure 3.3: Experimental Design

3.4 Qualitative Phytochemical Analysis

Chemical tests were carried out on the two plant extracts using standard procedures to identify the constituents as described by Harbone (1973), Sofowora (1993) and Trease and Evans (2002).

3.4.1 Test for alkaloids

Exactly1ml of the aqueous plant extract was treated with 2ml of picric acid solution. Formation of orange colour indicates the presence of alkaloids.

Principle: Dragondorff's reagent (potassium iodide-bismuth nitrate) is a widely used reagent to detect alkaloids. The heavy metal atom (BiI_4) combines with the nitrogen in the alkaloid to form ion pairs. This ion pair forms insoluble orange red colored complex.

3.4.2 Test for cardiac glycosides

The test for cardiac glycosides was carried out using the Keller- Kiliani test. About 5ml of the extract was treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A violet ring may appear above and below the brown ring, formed gradually throughout the thin layer. This indicates the presence of cardiac glycosides.

Principle: *Keller- Kiliani test* (for de-oxy sugars in cardiac glycosides): This is specific for deoxy sugars. Brown ring at interphase is characteristic of deoxysugars in cardenolides.

3.4.3 Test for flavonoids

Dilute ammonia solution (5ml) was added to a portion of aqueous filtrate followed by addition of concentrated sulphuric acid. A yellow colouration observed indicates the presence of flavonoids.

Principle: Flavonoids are hydroxylated phenolic compounds which occur as aglycones, glycosides and methylated derivatives but the basic structure is aglycone. They form oxonium salts in acidic medium to give a yellow colour.

3.4.4 Test for saponins

Exactly 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. About 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsification.

Principle: Saponins could be steroidal, triterpenoidal or alkaloidal depending on the nature of the aglycone. The aglycone part of saponin is sapogenin while the glycone parts are generally oligossacharides. They react with distilled water to form a persistent froth lasing for at least 15 minutes.

3.4.5 Test for sterols

Exactly 2ml of acetic anhydride was added to 0.5g of the extract with 2ml sulfuric acid. The colour change from violet to blue or green indicates the presence of sterols.

Principle: Sterols are a subgroup of steroids with a hydroxyl group at the 3-position of the A-ring system. Treatment with strong acids through dehydration and subsequent salt formation gives numerous colour reactions.

3.4.6 Test for tannins

About 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube then filtered and 1ml of 0.1% ferric chloride was added and observed for brownish-green or blue-black coloration.

Principle: Tannins are polyphenolic compounds which bind and precipitate proteins; they form insoluble complexes which react by forming phenolic rings with $FeCl_3$.

3.4.7 Test for terpenoids

Using Salkowski's test, about 5ml of the extract was mixed in 2ml of chloroform and 3ml of concentrated sulfuric acid and was carefully added to form a layer. A reddish-brown colouration of the interface formed shows a positive result for the presence of terpenoids.

Principle: Terpenoids are modified terpenes where methyl groups are moved or oxygen atoms are added.

3.5 Preparation of Test Concentration for Bioassay

The stock solution prepared was serially diluted according to WHO (2005) guidelines. Triplicates of test volumes were prepared into final concentration of 100ml; seven concentrations of aqueous, methanolic, ethyl acetate and n-hexane extracts at 6.25, 12.5, 25, 50, 100, 200 and 400ppm were prepared in chlorine-free water.

3.6 Larvicidal Bioassay

Bioassay was performed according to WHO (2005) guidelines. After preparing the test concentrations, twenty five third instar larvae were introduced into each plastic bowl (100ml capacity). Healthy mosquito larvae were exposed to a wide range of test concentrations and a control to check the larval mortality. The number of dead larvae was recorded 12, 24 and 48hrs after treatment and the percentage mortality was calculated. A standard drug Dichlorvos, was also used to check the mortality rate

Batches of 25 third instar larvae were transferred by means of strainers and a dropper into small disposable bowls each containing 100ml of water. The depth of the water in the bowls was maintained between 5cm and 10cm to avoid undue mortality due to lack of oxygen.

The experiment was set up in triplicates for each concentration with an equal number of controls simultaneously with dechlorinated water, to which 1drop of their respective solvents was added. The test containers were held at 25–28 ^{o} C and preferably a photoperiod of 12 h light followed by 12 h dark (12L:12D). For long exposures, larval food should be added to each test cup, particularly if high mortality is recorded in the control.

3.6.1 Determination of lethal concentration

The number of dead larvae at various concentrations recorded from the triplicate readings were used to generate dose response plots. The lethal concentrations (LC_{50} and LC_{90}) were calculated using probit analysis (Finney, 1971).The percentage mortalities and corrections where necessary were done by using Abbot's (1925) formula; (A method of computing the effectiveness of an insecticide).

Moribund larvae were counted and added to dead larvae for calculating percentage mortality.

- Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region.
- Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed.

The mortalities of treated groups were corrected according to Abbot's formula;

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Number of larvae exposed}} \times 100$$

$$\text{Corrected \% mortality} = \left(1 - \frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}}\right) \times 100$$

Where; n= Insect population, T= treated, Co= Control.

3.7 Thin Layer Chromatography (TLC)

TLC was carried out to determine the number of components in the extract and the best solvent system for column chromatography. A chromatographic silica gel 60 F_{254} plate was used. The sample was dissolved in n-hexane and then applied to the plate using a micro haematocrit capillary tube until the quantity loaded was adjudged sufficient for the experiment. The plate was placed in a chromatographic tank and eluted with a mixture of hexane/ethyl acetate (9:1 to1:9) ratio. The plate was removed from the TLC tank, air dried and developed with 20% sulphuric acid in alcohol (Ejele *et al*., 2012).

3.8 Column Chromatography

The chromatographic column was packed with silica gel 60-120 (the stationary phase). The crude n-hexane extracts of *Citrus sinensis* and *Sesamum indicum* were mixed with activated sand and loaded on the column. The components of the crude n –hexane extracts were separated by elusion on silica packed column using an elution mixture obtained from TLC.

Fractions of 20ml aliquots were collected into different beakers and solvents were allowed to evaporate and their contents were profiled on TLC plates. The TLC carried out enabled the fractions to be pulled together into 7 main fractions each (Ejele *et al*., 2012).

Larvicidal activities of the fractions were tested against *Culex quinquefasciatus* mosquito larvae. The fractions with the highest larvicidal activities for the two plants were selected for spectroscopic analysis.

3.9 Characterization of Larvicidal Compounds in the Bioactive Fractions by FTIR.

The FTIR analysis of the fraction with the highest larvicidal activity was carried out to determine the functional group(s) present using FTIR spectrophotometer (Shidmazu model), available at the National Research Institute for Chemical Technology (NARICT) laboratory, Zaria.

The standard method to prepare solid sample for FTIR spectrometer is to use KBr. About 2 mg of sample and 200 mg KBr were dried and ground. The mixture was squeezed to form transparent pellets which could be measured directly. For liquids with high boiling point or viscous solution, it can be added in between two NaCl pellets. Then the sample was fixed in

the cell by skews and measured. For volatile liquid sample, it is dissolved in CS_2 or CCl_4 to form 10% solution. Then the solution was injected into a liquid cell for measurement.

A background spectrum was gotten by collecting an interferogram and its subsequent conversion to frequency data by inverse Fourier transform. The background spectrum was gotten because the solvent in which we placed our samples had traces of dissolved gases as well as solvent molecules that contribute information that are not the sample. The background spectrum also takes into account several other factors related to the instrument performance, which includes information about the source, interferometer, detector, and the contribution of ambient water.

Next, a single-beam spectrum of the sample was collected which contained absorption bands from the sample as well as the background (gaseous or solvent). The ratio between the single-beam sample spectrum and the single beam background spectrum gives the spectrum of the sample. Data analysis was done by assigning the observed absorption frequency bands in the sample spectrum to appropriate normal modes of vibrations in the molecules.

3.10 Statistical Analysis

The lethal concentrations (LC_{50} and LC_{90}) were calculated using SPSS version 20 (Probit analysis). Data was analyzed by ANOVA. The differences between various parameters were compared using the Duncan Multiple Range Test. Differences between means ± SD was considered significant at $P < 0.05$. P-values of less than 0.05 were statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Qualitative and Quantitative Determination of Phytochemicals in *Citrus sinensis* and *Sesamum indicum* Leaf Extracts

4.1.1 Qualitative determination of phytochemicals in *C.sinensis* and *S.indicum* crude extracts

The qualitative determination of phytochemicals present in the four different extracts of *Citrus sinensis* and *Sesamum indicum* are presented in Table 4.1.1. For *Citrus sinensis*, C. glycosides, sterols, tannins and terpenoids were present in all the extracts while anthracene was absent in all. Saponins were present only in the methanolic and aqueous extracts while alkaloids were present only in the aqueous extracts and flavonoids were minimal only in n-hexane extract.

For *Sesamum indicum*, C.glycosides, flavonoids, sterols and terpenoids were present in all extracts while anthracene was absent in all extracts. Alkaloids and saponins were present in the aqueous and methanolic extracts. Tannins were present in all extracts except the hexane extract.

Table 4.1.1 Qualitative determination of phytochemicals in aqueous, methanol, ethyl acetate and n-hexane extracts of *Citrus sinensis* and *Sesamum indicum*

		Phytochemicals							
Plants	**Extracts**	**Anthracene**	**Alkaloids**	**C.gly**	**Flavonoid**	**Saponin**	**Sterol**	**Tannin**	**Triterpene**
	Aqueous	-	+	+	+	+	+	+	+
C.s	**Methanol**	-	-	+	+	+	+	+	+
	E.Acetate	-	-	+	+	-	+	+	+
	N-hexane	-	-	+	-	-	+	+	+
	Aqueous	-	+	+	+	+	+	+	+
S.i	**Methanol**	-	+	+	+	+	+	+	+
	E.Acetate	-	-	+	+	-	+	+	+
	N-hexane	-	-	+	+	-	+	-	+

+ = Positive - = Negative, *C.s = Citrus sinensis, S.i = Sesamum indicum,* E. Acetate=Ethyl acetatae, C.Gly = Cardiac Glycosides

4.1.2 Quantitative determination of phytochemicals in *Citrus sinensis* and *Sesamum indicum* leaf n-hexane extracts

The quantitative determination of phytochemicals present in the n-hexane extracts of *Citrus sinensis* and *Sesamum indicum* are presented in Table 4.1.2. *Citrus sinensis* showed significantly ($p<0.05$) high amounts of sterols and triterpenes and relatively low amounts of cardiac glycosides and saponins. *Sesamum indicum*, showed significantly high amounts of alkaloids, sterols and triterpenes with relatively low quantity of saponins and cardiac glycosides.

Table 4.1.2 Quantitative determination of phytochemicals in *C.sinensis* and *S.indicum* leaf n-hexane extracts

Phytochemical (mg/100g)	***Citrus sinensis***	***Sesamum indicum***
Alkaloids	1.00 ± 0.04^{d}	1.24 ± 0.02^{e}
Flavonoids	0.62 ± 0.03^{c}	0.99 ± 0.06^{d}
Saponins	0.11 ± 0.03^{a}	0.05 ± 0.02^{a}
Tannins	0.42 ± 0.02^{b}	0.36 ± 0.02^{c}
Steroids	1.97 ± 0.03^{f}	1.73 ± 0.04^{g}
Cardiac glycosides	0.06 ± 0.03^{a}	0.11 ± 0.02^{b}
Triterpenes	1.52 ± 0.02^{e}	1.48 ± 0.06^{f}

Values are Mean ± SD of n=3. Values with different superscript down the column are significantly ($p<0.05$) different.

4.2 Larvicidal Bioassay

Crude extracts and partially purified fractions of *Citrus sinensis* and *Sesamum indicum* were assayed for toxicity against early third instar larvae of *Culex quinquefasciatus* mosquito.

4.2.1 Lethal concentration of aqueous, methanol, ethyl acetate and n-hexane leaf extracts of *Citrus sinensis* against *Culex quinquefasciatus* larvae

The Lethal concentration of the crude extracts is shown in Figure 4.2.1. The LC_{50} and LC_{90} of the n-hexane extract was the lowest with values of 0.21 and 6.498ppm respectively. The n-hexane leaf extract had a percentage mortality of 90% while the aqueous, methanol and ethyl acetate extracts had mortalities of 24, 36 and 60% respectively. This implies that the n-hexane extract had a better mortality compared to other solvent extracts.

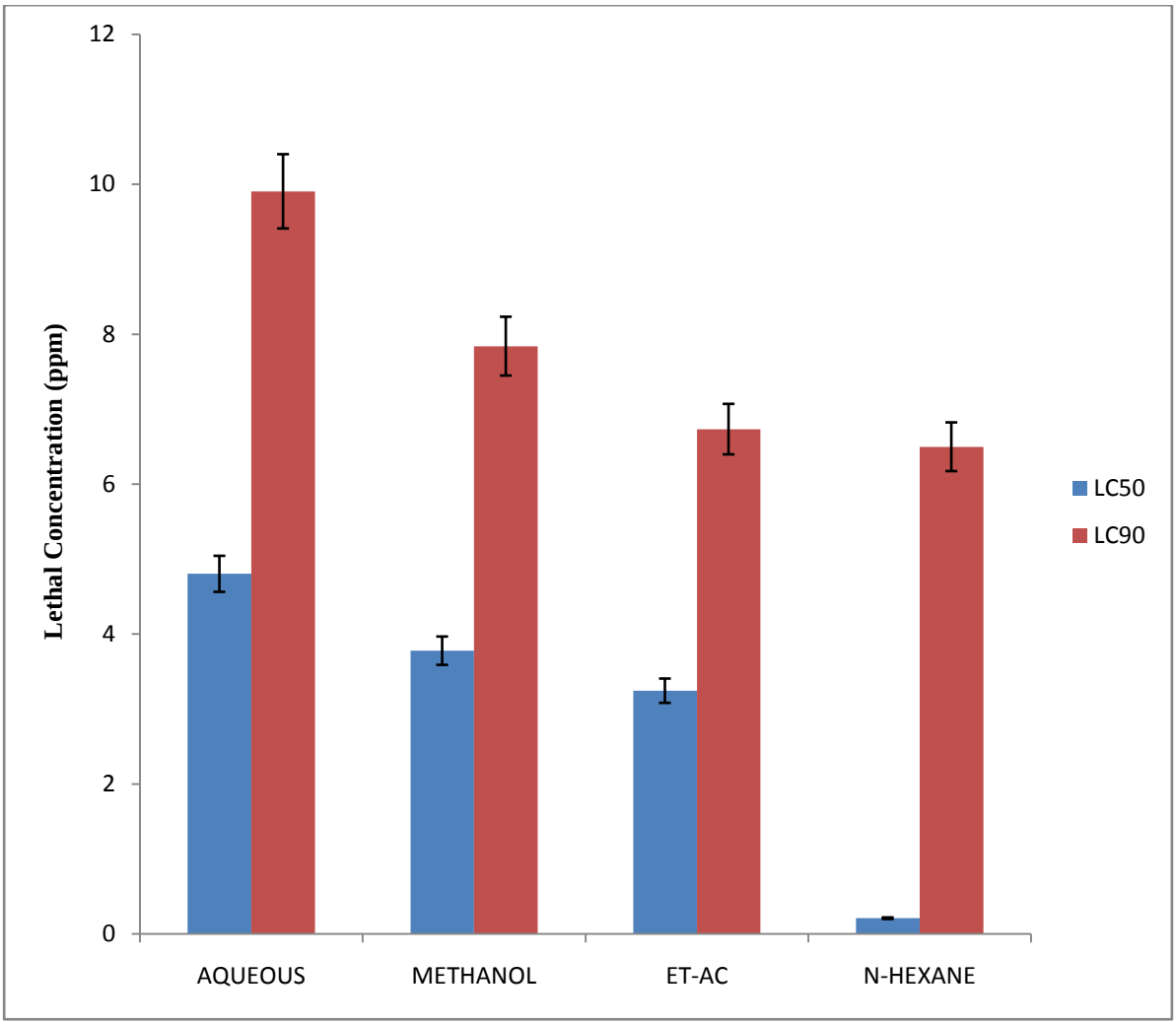


Figure 4.2.1: LC_{50} and LC_{90} values of *C. sinensis* aqueous, methanol, ethyl acetate and n-hexane extracts

4.2.2 Lethal concentration of aqueous, methanol, ethyl acetate and n-hexane leaf extracts of *Sesamum indicum* against *Culex quinquefasciatus* larvae

The Lethal concentration of crude extracts is shown in Figure 4.2.2. The LC_{50} and LC_{90} of the n-hexane extract though quite high, was the lowest with values of 3.179 and 10.344ppm respectively. The n-hexane leaf extract had a percentage mortality of 47% while the aqueous, methanol and ethyl acetate extracts had mortalities of 20, 15 and 32% respectively. This implies that the n-hexane extract had a better mortality compared to other solvent extracts.

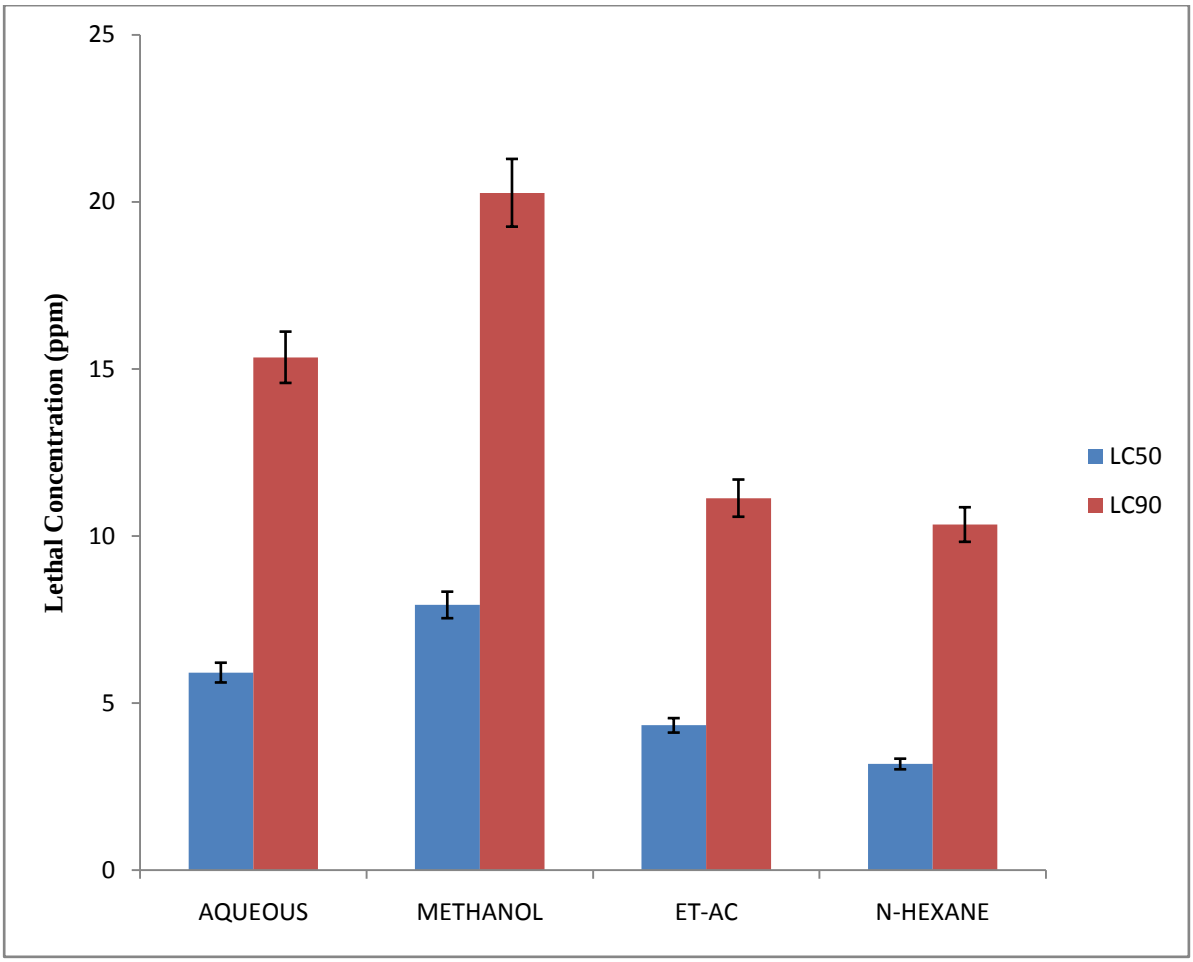


Figure 4.2.2: LC_{50} and LC_{90} values of *S.indicum* aqueous, methanol, ethyl acetate and n-hexane extracts

4.3 Partial Purification of the n-Hexane Extracts of *Citrus sinensis* and *Sesamum indicum*

4.3.1 Thin layer chromatography

Figure 4.3 shows that the two extracts expressed seven different spots each.

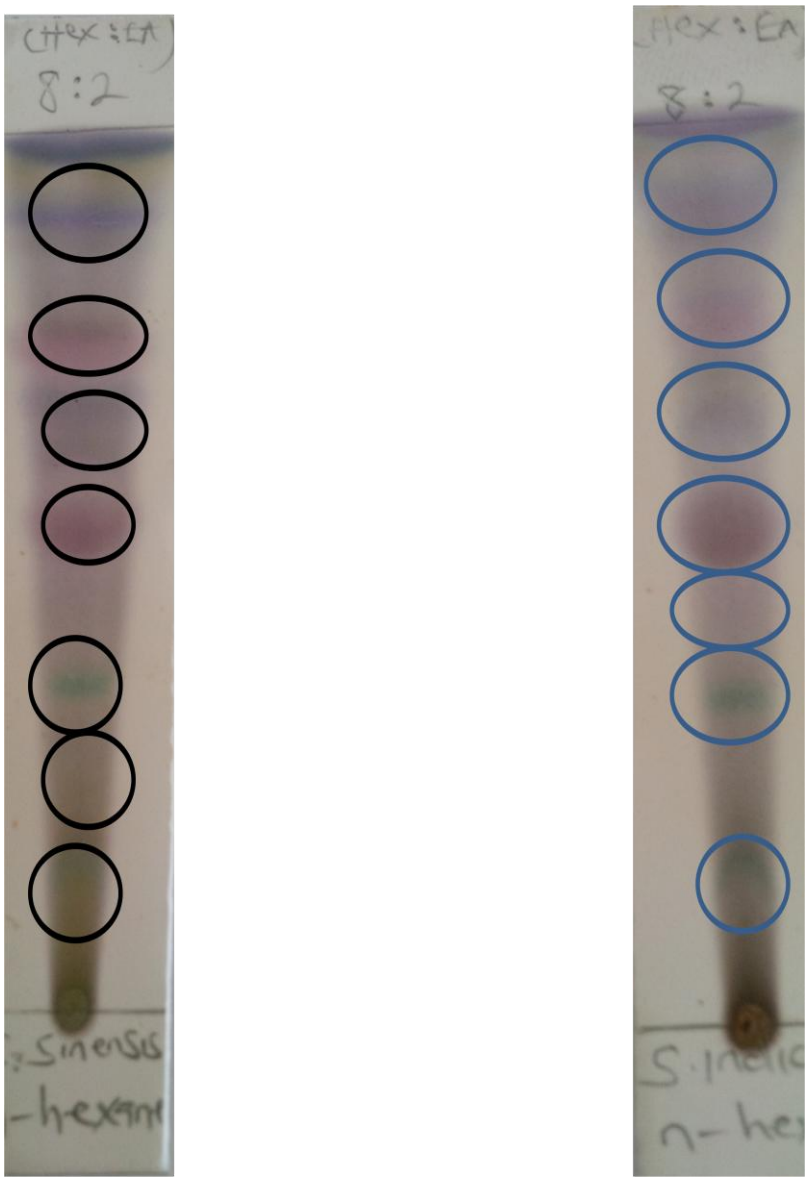


Figure 4.3: TLC plates showing the various spots of the *C.sinensis* and *S.indicum* n-hexane extracts using n-Hexane: Ethyl acetate (8:2) as mobile phase.

4.3.2 Column chromatography

The various purified fractions obtained after column chromatography was then tested for their larvicidal activity against the *Culex quinquefasciatus* mosquito larvae.

4.4 Larvicidal Bioassay of Different Fractions from *Citrus sinensis* and *Sesamum indicum* n-Hexane Leaf Extracts

Partially purified fractions of *C.sinensis* n-hexane and *S.indicum* n-hexane extracts were assayed for toxicity against early third instar larvae of *Culex quinquefasciatus* mosquito.

4.4.1 Larvicidal activity of *Citrus sinensis* n-hexane fractions against *Culex quinquefasciatus* mosquito larvae

The toxicity of the *C.sinensis* n-hexane fractions showed that fraction 3 (CSHE-F_3) had the highest larvicidal activity with a significant mortality of 93% and LC_{50} and LC_{90} values of 0.54 and 2.089ppm respectively. Fraction 1(CSHE-F_1) had the lowest mortality of 21% and LC_{50} and LC_{90} values of 5.741 and 9.322ppm respectively.

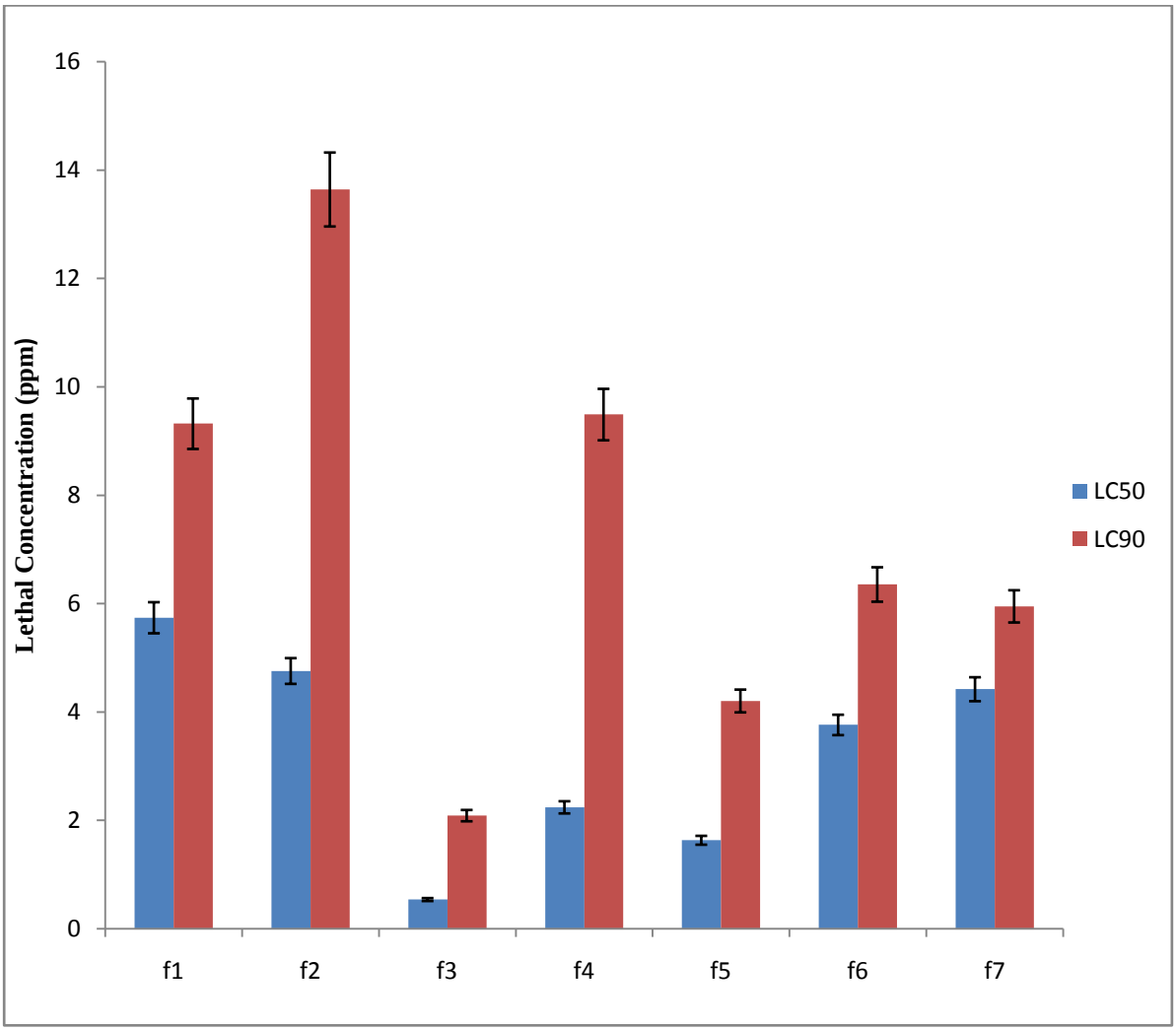


Figure 4.4.1: LC_{50} and LC_{90} of *C. sinensis* n-hexane extract fractions

4.4.2 Larvicidal activity of *Sesamum indicum* n-hexane fractions against *Culex quinquefasciatus* mosquito larvae

The toxicity of the *Sesamum indicum* n-hexane fractions showed that fraction 6 (SIHE-F_6) had the highest larvicidal activity with a percentage mortality of 52% and LC_{50} and LC_{90} values of 0.965 and 2.233ppm respectively. Fraction 1(SIHE-F_1) had the lowest mortality of 19% and LC_{50} and LC_{90} values of 6.391ppm and 9.488ppm respectively.

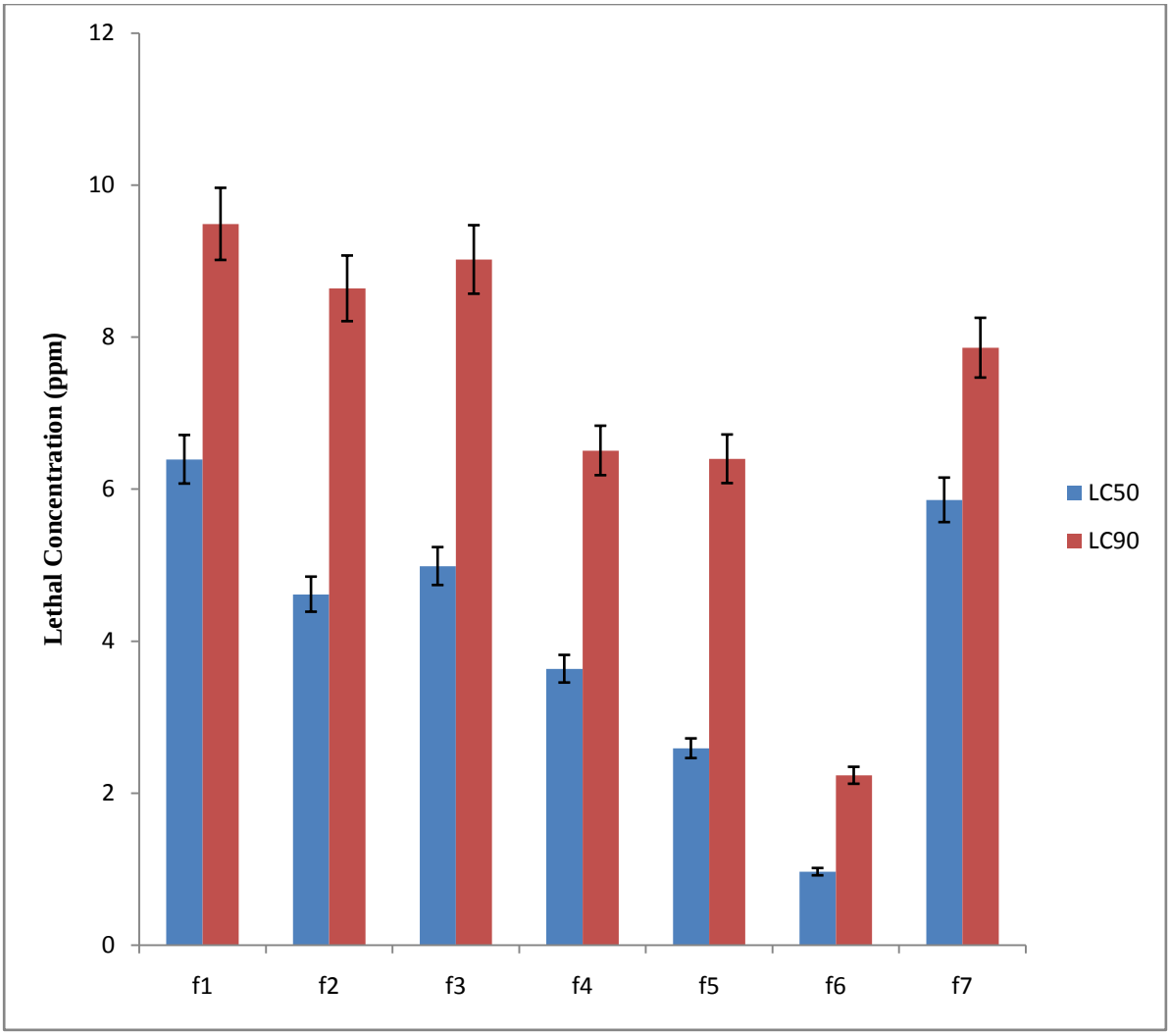
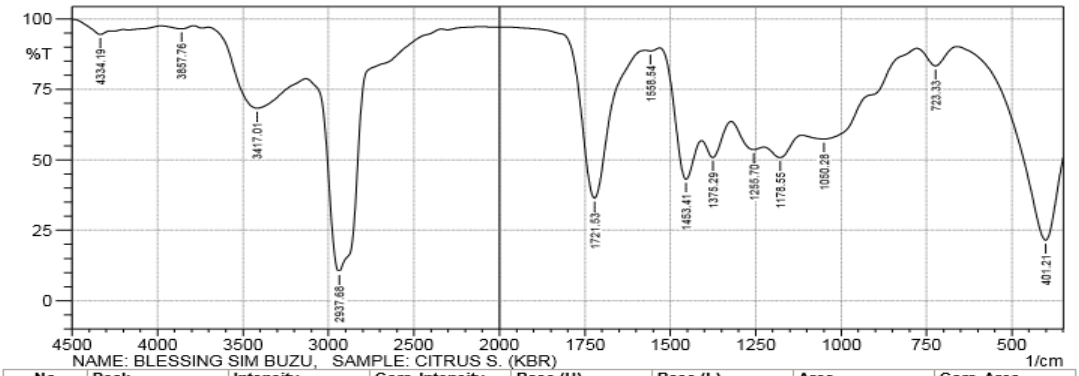


Figure 4.4.2: LC_{50} and LC_{90} of *S.indicum* n-hexane extract fractions

4.5 Fourier Transform Infra Red Spectroscopy

FTIR analysis was carried out at National Research Institute for Chemical Technology (NARICT), Zaria on *Citrus sinensis* and *Sesamum indicum* n-hexane extracts to determine the type of functional groups present. The result for *C.sinensis* revealed the presence of some functional groups which include; Alkyl halides, Alkanes, Alkenes, Alcohols, Amides, Ethers, Aldehydes and Carboxylic acids. For *S.indicum*, it revealed Alkyl halides, Alkenes, Amides, Nitro compounds, Aldehydes and Carboxylic acids functional groups.

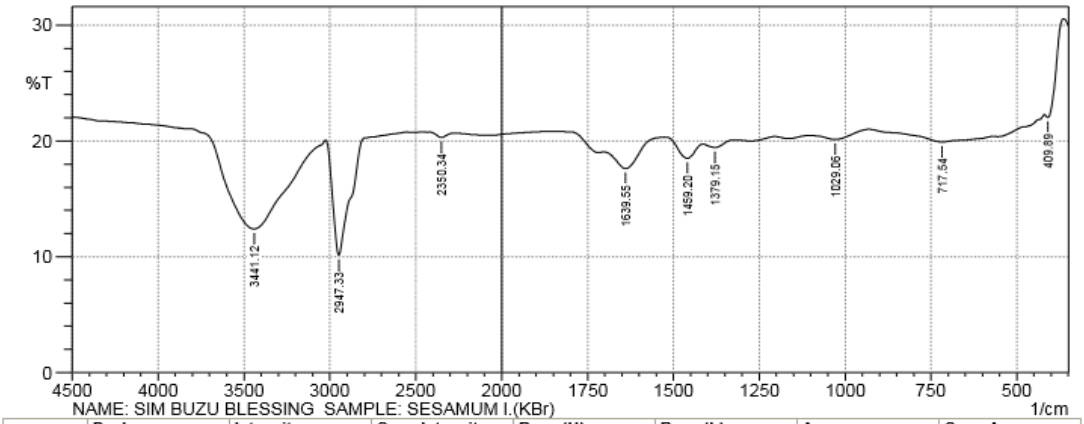


No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	401.21	21.497	36.053	659.68	349.13	78.983	29.867
2	723.33	83.331	6.537	778.3	660.64	7.205	1.758
3	1050.28	57.386	7.193	1113.93	778.3	53.876	7.057
4	1178.55	50.782	5.521	1225.8	1113.93	29.934	2.272
5	1255.7	53.613	3.734	1322.25	1225.8	23.784	1.614
6	1375.29	50.823	8.612	1408.08	1322.25	21.67	2.714
7	1235.45	43.113	25.739	1530.57	1409.05	26.991	9.373
8	1558.54	88.637	0.523	1573.97	1530.57	2.198	0.063
9	1721.53	36.503	55.314	1984.82	1574.93	42.964	30.621
10	2937.68	10.333	72.783	3130.57	2338.77	174.624	127.559
11	3417.01	68.353	19.616	3701.52	3131.54	62.051	28.942
12	3857.76	96.493	1.086	3981.21	3788.32	2.529	0.466
13	4334.19	94.515	2.26	4500.08	4278.26	2.943	0.792

Figure 4.5.1: FTIR spectrum of *C.sinensis* n-hexane fraction 3 (CSHE-F_3) showing the different peaks.

Table 4.5.1 Fourier Transform Infra Red Spectroscopy of *Citrus sinensis* n-hexane extracts

Peak S/No.	Absorption peak(cm^{-})	Intensity	Bond	Functional group
i.	401.21	strong	C-I	Alkyl halides
ii.	723.33	m-s, broad	C-H	Alkenes
iii.	1050.28	Very strong, m-s	C-F, C-O	Alkyl halide , Alcohols
		m-s	C-O	Alcohols
iv.		m-s	C-O	Alcohols
v.	1255.7	Very strong	C-F	Alkyl halide
		m-s	C-O	Alcohols
		m-s	=C-O-C	Ethers
vi.	1375.29	m	CH_3CH	Alkanes and Alkyls
vii.		Very strong	C-F	Alkyl halides
		m-s	=C-O-C	Ethers
viii.	1558.54	m-s	N=H	Amides
ix.	1721.53	s	C-O	Aldehydes
x.	2937.68	s	C-H	Alkanes and Alkyls
		s, broad	O-H	Carboxylic acid
xi.	3417.01	w-m	N-H	Amides



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	409.89	22.045	1.674	419.53	363.59	33.421	0.798
2	717.54	19.921	0.717	930.68	570.95	248.66	2.545
3	1029.06	20.141	0.568	1106.21	930.68	120.92	0.998
4	1379.15	19.446	0.424	1413.87	1323.21	63.952	0.411
5	1459.2	18.504	1.473	1524.78	1413.87	79.125	1.672
6	1639.55	17.62	1.883	1702.24	1524.78	128.057	2.779
7	2350.34	20.294	0.431	2418.82	2279.94	95.489	0.564
8	2947.33	10.131	10.064	3023.52	2552.87	353.702	29.018
9	3441.12	12.402	8.2	3801.82	3023.52	611.45	76.792

Figure 4.5.2: FTIR spectrum of *S.indicum* n-hexane fraction 6 (SIHE-F_6) showing the different peaks.

Table 4.5.2 Fourier Transform Infra Red Spectroscopy of *S.indicum* n-hexane extracts

Peak S/No.	Absorption peak(cm^{-})	Intensity	Bond	Functional group
i.	409.89	22.045	R-I	Alkyl halides
ii.	717.54	19.921	R-I	Alkyl halides
iii.	1029.06	20.141	C-F	Alkyl halides
iv.	1379.15	19.446	$R-NO_2$	Nitro compounds
v.	1459.2	18.504	$R-NO_2$	Alkanes and Alkyls
vi.	1639.55	17.620	C=O	Amide
vii.	2350.34	20.294	X=C=Y	Isocyanates, isothiocyanates
viii.	2947.33	10.131	C=C	Alkyne
ix.	3441.12	12.402	O-H	Carboxylic Acids

CHAPTER FIVE

5.0 DISCUSSION

The use of plant derived compounds for mosquito control is appealing. Unlike synthetic insecticides or pesticides, they are biodegradable, economically cheap and environmentally sound alternative control measures (Gbolade, 1999). There are reports of medicinal plants with insecticidal activity including ovicidal, anti feedant, larvicidal and repellence from plants belonging to *Annonaceae, Papilionaceae, Meliaceae, Mimosaceae and Lamiaceae.* Many plants contain chemical compounds which are toxic to insects, the presence of such bioactive compounds appear to be a natural endowment (Mehrotra and Aggrawal, 2003). Some of these compounds have been known for a very long time, but few are commercially developed and processed into insecticidal products used for insect pest control (Nathanson, 1993). Phytochemicals have more specific effects and could be usefully integrated with other control measures to design comprehensive, appropriate and effective management protocols with less collateral harm to the environment and non-target species (Silva *et al.*, 2010). Different parts of the *Citrus* plant which includes fruits, seeds, roots and leaves have been tested for their use as mosquitocidal components (Traboulsi *et al*., 2005; Akram *et al*., 2010). A number of studies have also been carried out on the larvicidal potential of essential oil extracted from *Citrus* leaves and peels (Lee, 2006; Melliou *et al*., 2009).

The phytochemical analysis of the leaves of *C.sinensis* and *S.indicum* using different solvents revealed that the extracts contained alkaloids, cardiac glycosides, flavonoids, saponins, sterols, tannins and terpenoids. According to Pedro *et al.,* (2014) phytochemicals of plants serve as storage of compounds that have biological action. Alkaloids, flavonoid,

tannins and saponins are known to possess medicinal and pesticidal properties and are also responsible for insecticidal effects and toxicity to other animals.

The screening of locally available medicinal plants for mosquito control would reduce dependence on expensive imported products (Aziz *et al*., 2013). Alkaloids primarilly act as feeding deterrents to insects and other herbivores (Harborne, 1993) in many cases by directly interacting with molecular targets within the nervous system (Wink, 2000). Terpenoids also exhibit a wide range of effects within the insect central nervous system. The neurotoxic deterrent properties of many monoterpenes in insects have been shown to include interactions with the octopamenergic system, cholinesterase inhibition and multiple direct interactions with the GABA(Gamma-Amino Butyric Acid) system including blockade of GABA-gated chloride channels (Rattan, 2010). The ecological roles of phenolic compounds include constitutive and induced roles in toxicity and feeding deterrence in insects (Treutter, 2006). These phytochemicals have been shown to have insecticidal property against several eggs, larvae, pupa, nymph and adult of insects (Egunyomi *et al*., 2009).The *C.sinensis* n-hexane extract had significantly high amounts of sterols and terpenoids while the *S.indicum* n-hexane extract had significantly higher amounts of sterols, terpenoids and alkaloids which could be responsible for the oily active ethnobotanicals for topical application as mosquito larvicide and repellent property (Egunyomi *et al*; 2009).

Evaluation of the mosquito larvicidal potential of the crude aqueous, methanolic, ethyl acetate and n-hexane extracts of *C.sinensis* against the third-instar mosquito larvae showed that the n-hexane extract had a percentage mortality of 90% at the concentration of 400ppm after 48 hours while the aqueous, methanolic and ethyl acetate extracts registered 24, 36 and 60% respectively. On the other hand, it was also observed that the n-hexane extract of

S.indicum caused a percentage mortality of 47% at 400ppm after 48hours. The aqueous, methanolic and ethyl acetate extracts had percentage mortalities of 20, 15 and 32% respectively.

The LC_{50} and LC_{90} values of the extracts were also determined using the log-probit regression analysis. For *C. sinensis*, the n-hexane extract showed an excellent toxicity with an LC_{50} value of 0.210 with corresponding LC_{90} of 6.498ppm. The aqueous, methanolic and ethyl acetate extracts had LC_{50} values of 4.803, 3.778 and 3.244ppm and LC_{90} values of 9.905, 7.840 and 6.733ppm respectively. The n-hexane extract had the lowest LC_{50} value and hence, was selected for further analysis. For *S.indicum*, the n-hexane extract also had the lowest LC_{50} value of 3.179 and LC_{90} of 10.344ppm.The aqueous, methanolic and ethyl acetate extracts had LC_{50} values of 5.914, 7.938 and 4.335ppm and LC_{90} values of 15.349, 20.271 and 11.134ppm respectively. The n-hexane extract was also selected for further studies. Numerous authors have observed a converse relationship between extract effectiveness and solvent polarity where the efficacy increases with decreasing polarity. (Aivazi and Vijayan, 2009; Prathibha *et al.*, 2010; Sharma *et al.*, 2014). *Singh et al.*, 2007, reported the LC_{50} values of the hexane extract of *Eucalyptus citriodora* against the fourth instar larvae of *Anopheles stephensi* (69.86 ppm) and *Aedes aegypti* (91.76 ppm). Hexane extracts of other plants have also shown similar satisfactory larvicidal potency. LC_{50} of hexane leaf extract of *Citrus sinensis* (L.) against early fourth instars of *Aedes aegypti* was found to be 446.84 ppm (Warikoo, *et al.,* 2012). The LC_{50} of hexane leaf extract of *Murraya koenigii* (L.) against third instars *Culex quinquefasciatus* was found to be 963.53 ppm (Kovendan *et al*., 2012). Therefore, the present study suggests that the compounds majorly responsible for the larvicidal activity are most probably in the non-polar and intermediate

range and are not polar. Even though this study shows that methanol extract of *S. indicum* produced the least mortality, other plants such as *Morinda citrifolia* and *Erythrina indica* have been shown to produce greater susceptibility for their methanol extracts when compared to their hexane extracts (Kovendan *et al*., 2012; Govindarajan, 2014)

The thin layer chromatography of the n-hexane extracts of *C.sinensis* and *S.indicum* both revealed seven different spots which suggest that they both contain several bioactive compounds (Figure 4.3).

Larvicidal bioassay of the various fractions obtained after column chromatography of n-hexane extracts of *Citrus sinensis* (CSHE) and *Sesamum indicum* (SIHE) revealed that CSHE-F_3 (*Citrus sinensis* n-hexane extract, fraction 3) and SIHE-F_6 (*Sesamum indicum* n-hexane extract, fraction 6) had the best larvicidal activity with percentage mortalities of 93% and 52% respectively at 400ppm after 24hrs. The LC_{50} and LC_{90} values for CSHE-F_3 were 0.540 and 2.089ppm respectively while those of SIHE-F_6 were 0.965 and 2.233ppm. However, larvicidal activities of the partially purified fractions were slightly higher when compared with the crude extracts. This depicts that purification enhanced activity. It was observed that the percentage mortalities of the mosquitoes increased with increasing concentration of extracts. It was also increased in relation to time of exposure i.e. higher concentrations of the plant extracts had higher mortality.

The clear difference in mortality between the control group and test groups of the n-hexane fractions imply that the efficacy of the extracts as larvicides is not due to the use of n-hexane as the extracting solvent, but is attributed to the various phytochemicals present in

the extracts. Hence, the two most potent fractions were taken for Fourier Transform Infra Red Spectroscopy.

The FTIR result of CSHE-F3 revealed eleven peaks with different intensities and different functional groups including alkyl halides, alkenes, amides, aldehydes, alcohols, ethers and carboxylic acids while that of SIHE-F6 revealed 9 peaks with alkane, alkenes, alkyl halides, nitro compounds and carboxylic acids functional groups.

The mortalities observed in the crude and partially purified fractions of *C.sinensis* and *S.indicum* is an indication that these two plants are potential larvicides .The low larvicidal mortality observed in *S.indicum* could imply that it is a larvicide that might need another plant extract to boost its activity and hence, it's mortality rate. Spreading these extracts over water surfaces obstruct the breathing of the larvae and thereby suffocates them. At the end of this study, it was clear that CSHE and SIHE have potentials as sources of mosquito control agents.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the present study, the phytochemical screening of aqueous, methanol, ethyl acetate and n-hexane leaf extracts of *C. sinensis* and *S. indicum* showed the presence of alkaloids, saponins, tannins, flavonoids, cardiac glycosides, steroids and terpenoids. The quantitative analysis for the two n-hexane extracts showed significantly higher amounts of steroids, terpenoids and alkaloids compared to the other extracts.

Larvicidal bioassay for the aqueous, methanol, ethyl acetate and n- hexane leaf extracts of *C. sinensis* and *S. indicum* showed that the n-hexane extracts of the two plants had the highest percentage mortalities of 90% and 47% respectively and both were selected for chromatrography.

Thin layer chromatography was carried out on the n-hexane extracts of *C. sinensis* and *S. indicum* to determine the best solvent system for column chromatography and it was found to be n-hexane:ethyl acetate system (8:2). Colum chromatography was also carried out and various fractions were collected.

Larvicidal bioassay for the seven (7) different fractions collected for each extract after column chromatography showed that *C. sinensis* fraction 3 (CSHE-F_3) and *S. indicum* fraction 6 (SIHE-F_6) had the highest percentage mortalities.

FTIR analysis was carried out on the two fractions and various functional groups were observed.

Both crude extracts and partially purified fractions of *Citrus sinensis* and *Sesamum indicum* showed larvicidal activity. Hexane extracts showed better mosquito larvicidal efficacy than those of other extracts which indicate that the compounds responsible for the larvicidal activity of both plants are most probably in the non-polar and intermediate range. These results indicate promising approaches against *Culex quinquefasciatus* mosquito species and hence, the n-hexane extracts of *Citrus sinensis* and *Sesamum indicum* can be considered as

mosquito control agents, effective for comprehensive control of disease vectors without pollution of the environment.

6.2 Recommendations

i. There is a need to fully identify the compounds present in the active fractions that might be responsible for the larvicidal activity.

ii. More advanced purification techniques such as HPLC and NMR should be employed to maximize the yield.

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Appendix 1

Percentage Yield of Aqueous, Methanol, Ethyl acetate and n-Hexane Extracts of *C.sinensis* and *S.indicum*

	Solvents	Weight of extracts (g)	Percentage yield (%)
Citrus sinensis	Aqueous	10.25	20.50
	Methanol	11.78	23.56
	Ethyl acetate	10.88	21.76
	N-hexane	4.38	8.76
Sesamum indicum	Aqueous	2.0	2.86
	Methanol	1.23	1.76
	Ethyl acetate	1.17	1.67
	N-hexane	1.22	1.74

Appendix 2

Percentage Mortality and Lethal Concentration of Aqueous, Methanol, Ethyl Acetate and n-Hexane Leaf Extract of *Citrus sinensis* against *Culex quinquefasciatus* Larvae

Extracts	**Concentration in parts per million (ppm)**									
	Time (hrs)	**Percentage Mortality (%)**							**Lethal Conc.(ppm)**	
		Control	6.25	12.5	25	50	100	200	400	LC_{50}	LC_{90}
	12	---	---	---	4	5	11	12	16		
Aqueous	24	---	---	---	4	7	12	15	20	4.803	9.905
	48	---	---	6	9	11	16	20	24		
	12	---	---	---	4	8	11	15	25		
Methanol	24	---	---	4	11	12	15	21	27	3.778	7.840
	48	---	---	8	15	16	20	24	36		
	12	---	---	4	8	9	16	20	39		
Ethyl	24	---	---	4	12	20	28	35	49	3.244	6.733
Acetate	48	---	---	8	16	23	32	41	60		
	12	**---**	**12**	**20**	**29**	**36**	**41**	**54**	**60**		
n-	**24**	**---**	**18**	**27**	**36**	**46**	**64**	**71**	**75**	**0.210**	**6.498**
Hexane	**48**	**---**	**24**	**32**	**39**	**60**	**70**	**80**	**90**		

*(r=3), No. of replicates @ 25 larvae/replicate at each concentration; LC_{50}, lethal concentration for killing 50 percent of the treated larvae; LC_{90}, concentration for killing 90 percent of the treated larvae.

Appendix 3

Percentage Mortality and Lethal Concentration of Aqueous, Methanol, Ethyl acetate and n-Hexane Leaf Extract of *Sesamum indicum* against *Culex quinquefasciatus* Larvae

Extracts	**Concentration in parts per million (ppm)**									
	Time (hrs)	**Percentage Mortality (%)**							**Lethal Concentration**	
		Control	6.25	12.5	25	50	100	200	400	LC_{50}	LC_{90}
	12	---	---	---	3	3	5	10	12		
Aqueous	24	---	---	3	4	5	8	11	15	5.914	15.349
	48	---	---	5	7	12	15	17	20		
	12	---	---	---	3	4	4	5	7		
Methanol	24	---	---	---	3	5	5	8	9	7.938	20.271
	48	---	---	---	5	5	11	12	15		
	12	---	---	3	5	7	12	15	17		
Ethyl	24	---	---	4	5	11	15	19	21	4.335	11.134
Acetate	48	---	3	5	9	12	17	20	32		
	12	**---**	**---**	**4**	**5**	**11**	**15**	**16**	**23**		
n-hexane	**24**	**---**	**4**	**7**	**8**	**13**	**20**	**27**	**36**	**3.179**	**10.344**
	48	**---**	**7**	**12**	**12**	**20**	**24**	**29**	**47**		

*(r=3), No. of replicates @ 25 larvae/replicate at each concentration; LC_{50}, lethal concentration for killing 50 percent of the treated larvae; LC_{90}, concentration for killing 90 percent of the treated larvae.

Appendix 4

Percentage Mortality and Lethal Concentration of the n-Hexane Leaf Extract Fractions of *Citrus sinensis* against *Culex quinquefasciatus* Larvae

Extracts	**Concentration in parts per million (ppm)**										
	Time (hrs)	**Percentage Mortality (%)**								**Lethal Concentration**	
		Control	6.25	12.5	25	50	100	200	400	LC_{50}	LC_{90}
	6	---	---	---	---	3	7	15	15		
CSHE-	12	---	---	---	3	3	7	16	16	5.741	9.322
F1	24	---	---	---	3	4	7	16	21		
	6	---	---	5	8	8	11	12	12		
CSHE-	12	---	---	5	8	9	12	16	20	4.758	13.645
F2	24	---	---	**7**	8	9	12	16	24		
	6	---	**16**	**28**	**36**	**40**	**48**	**48**	**56**		
CSHE-	**12**	---	**16**	**30**	**39**	**41**	**51**	**60**	**81**	**0.540**	**2.089**
F3	**24**	---	**23**	**30**	**39**	**44**	**60**	**75**	**93**		
	6h	---	**7**	8	12	17	20	32	36		
CSHE-	12	---	8	8	12	20	27	39	44	2.242	9.491
F4	24	---	8	11	13	24	32	40	51		
	6	---	---	11	16	23	24	31	37		
CSHE-	12	---	11	12	19	24	27	32	40	1.634	4.205
F5	24	---	13	13	23	24	29	36	47		
	6	---	---	---	8	12	16	21	28		
CSHE-	12	---	---	3	8	12	19	24	32	3.763	6.355
F6	24	---	---	3	11	16	19	28	37		
	6	---	---	---	---	8	16	20	22		
CSHE-	12	---	---	---	---	12	17	24	25	4.422	5.951
F7	24	---	---	---	---	12	20	24	29		

*(r=3), No. of replicates @ 25 larvae/replicate at each concentration; LC_{50}, lethal concentration for killing 50 per cent of the treated larvae; LC_{90}, concentration for killing 90 per cent of the treated larvae; CSHE-F *Citrus sinensis* hexane extract fraction

Appendix 5

Percentage Mortality and Lethal Concentration of the n-Hexane Leaf Extract Fractions of *Sesamum indicum* against *Culex quinquefasciatus* Larvae

Extracts	**Concentration in parts per million (ppm)**										
	Time (hrs)	**Percentage Mortality (%)**								**Lethal Concentration**	
		Control	6.25	12.5	25	50	100	200	400	LC_{50}	LC_{90}
	6	---	---	---	---	---	4	8	12		
SIHE-	12	---	---	---	---	3	4	11	15	6.391	9.488
F1	24	---	---	---	---	3	8	12	19		
	6	---	---	---	7	8	12	16	20		
SIHE-	12	---	---	---	8	9	12	19	27	4.616	8.640
F2	24	---	---	---	8	11	12	20	27		
	6	---	---	---	4	8	12	15	16		
SIHE-	12	---	---	---	4	9	12	15	16	4.986	9.020
F3	24	---	---	---	4	9	16	17	24		
	6	---	---	---	8	12	16	20	28		
SIHE-	12	---	---	4	8	12	20	25	29	3.635	6.507
F4	24	---	---	4	11	15	21	28	29		
	6	---	---	4	8	12	20	25	28		
SIHE-	12	---	---	12	16	19	20	25	31	2.589	6.397
F5	24	---	8	12	17	19	20	28	32		
	6	**---**	**8**	**16**	**24**	**27**	**36**	**44**	**48**		
SIHE-	**12**	**---**	**12**	**23**	**25**	**28**	**36**	**48**	**52**	**0.965**	**2.233**
F6	**24**	**---**	**20**	**24**	**28**	**33**	**43**	**52**	**52**		
	6	---	---	---	---	---	4	12	16		
SIHE-	12	---	---	---	---	---	8	16	20	5.857	7.859
F7	24	---	---	---	---	---	8	17	20		

*(r=3), No. of replicates @ 25 larvae/replicate at each concentration; LC_{50}, lethal concentration for killing 50 percent of the treated larvae; LC_{90}, concentration for killing 90 per cent of the treated larvae; SIHE-F *Sesamum indicum* hexane extract fraction.

Appendix 6

Percentage Mortality of Standard Drug (Dichlorvos)

Time	Concentration	Av. Larval death	Percentage mortality	Total no. of larvae
6hrs 12hrs 24hrs	6.25ppm	5.7 7 8	23 28 32	25
6hrs 12hrs 24hrs	12.50ppm	9.7 13 16.3	39 52 65	25
6hrs 12hrs 24hrs	25ppm	13 20 21	52 80 84	25
6hrs 12hrs 24hrs	50pppm	17.7 23.3 25	71 93 100	25
6hrs 12hrs 24hrs	100ppm	25 25 25	100 100 100	25
6hrs 12hrs 24hrs	200ppm	25 25 25	100 100 100	25

Appendix 7

Preparation of Stock Solution

A solution of 20ml of 1% was prepared by weighing 200mg of the crude extract, adding a few drops of Tween 80 as an emulsifier and then making it up to 20ml with distilled water which is referred to as the stock solution. The solution was kept in a screw cap vial and covered with aluminum foil prior to use.