CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of The Study

Vector borne diseases impose a massive burden on the world's populace in terms of indisposition and mortality contributing 17% of total infectious diseases affecting mankind with more than 700,000 deaths yearly (WHO, 2019a; Jacob-lorena and James, 2002). WestNile Virus (WNV), Malaria, Encephalitis, Filariasis, Dengue, Zika and Yellow Fever (YF) are the most transmitted vector borne diseases with debilitating outcomes and the vectors are mosquitoes (Becker *et al*., 2003). Malaria; the foremost of these diseases poses a serious threat to man-kind affecting mostly pregnant women and children under age five (WHO, 2018) accounting for ninety-four percent (94%) of global deaths (380,700) reported from the African region in 2018 with Nigeria (24%) and The Democratic Republic of Congo (11%) accounting for the largest percentage (35%) (WHO, 2019a). In Nigeria, the North-Central zone has the second-highest malaria prevalence rate (50.7% Rapid Diagnostic Test and 32.0% microscopy test) accounting for 60% outpatient hospital visit and 30% hospitalization among children under age five (NMIS, 2015). The vectors incriminated for this high malaria morbidity and mortality statistics in North-central Nigeria is the *Anopheles gambiae* complex which contains sibling species like *Anopheles gambiae, An. arabiensis* and *An. coluzzii* (Oduola *et al*., 2016, Okorie *et al*., 2011, Olayemi *et al*., 2011) and have been identified as the major vectors of *Plasmodium falciparum* that causes malaria (WHO, 2019a).

Vector control (i.e. mosquito control) is a major pillar for malaria management and elimination (WHO, 2017) and the use of synthetic insecticides remains a major component of control programmes (WHO, 2016). Indoor Residual Spray (IRS) and Long-Lasting Insecticide Treated Nets (LLIN) are two key vector control interventions (WHO, 2016) which has been efficient in reducing Mosquito-borne disease burden over the years (WHO, 2019a). IRS and LLIN have been effectual in reducing the adult population and preventing vector-human contact (WHO, 2016), therefore preventing disease transmission by reducing the number of cases and deaths from 251 million cases and 585,000 deaths in 2010 to 228 million cases and 405,000 deaths in 2018 globally (WHO, 2019a). Increasing ownership of LLIN in Nigeria from forty-four percent in 2010 (NDHS, 2015) to sixty-five percent in 2018 (NDHS, 2018) attest to reliance on this tool as a major vector control intervention. In recent times, the high operational costs of implementing these interventions, insecticide resistance development, toxicity on non-target organisms, environmental pollution have challenged the effectiveness and sustainability of these tools (Ranson and Lissenden, 2016). Also, behavioural changes by vectors; in response to the deployment of these interventions (LLIN and IRS) as reported in The Republic of Benin, Equatorial Guinea, Tanzania and Kenya (Killeen and Chitnis, 2014; Russel *et al*., 2013; Corbel *et al*., 2012; Reddy *et al*., 2011) and zoophagic tendencies of some vectors which are known to be opportunistic i.e. *An. arabiensis* (Gilles and Coetzee, 1987, Kweka *et al*., 2009, Oduola *et al*., 2016) have also been reported as challenges. There is, therefore, an urgent need to effectively manage these insect vectors by seeking alternatives that can effectively reduce the burden they impose on the populace.

In many rural communities in Africa, plants are being used for the management of haematophagous insects and agricultural pests and the treatment of different medical disease conditions (Karunamoorthi *et al*., 2009a). This has given rise to ethnobotanical survey which is a tool which has been immensely deployed in different parts of Africa and the world to identify insecticidal and medicinal plants (Karunamoorthi, 2012). The high usage of these plants is mostly due to numerous bioactive components present in them hence their effectiveness and also their accessibility, availability, affordability and biodegradability (Kamaraj *et al*., 2010). Local plants which possess insecticidal potentials against haematophagus insects can again play a significant role in the effective management of mosquitoes in place of synthetic interventions (Karunamoorthi *et al*., 2009b). It has been documented that different ethnic groups use different plants in various forms to prevent bites from mosquitoes (Hebbalkar *et al*., 1992). Most of these ethnic groups utilise these plants by burning them to produce smoke and hanging them fresh in places of abode to prevent haematophagous insects from entering and resting indoors (Waka *et al*., 2004).

Of recent, substantial research has been steered in search of novel strategies for the management of vector borne diseases and diverse plants have been reported as potential sources of both toxicity and repellency proficiencies against mosquitoes (Muema *et al*., 2016). This research work plays a pivotal role in the conservation of traditional knowledge about insecticidal plants in North-central Nigeria and also elucidate their bio-efficacy against *Anopheles* mosquitoes.

1.2 Statement of Research Problem

Available interventions (IRS and LLIN) though successful are only able to protect humans from mosquito borne diseases indoors. Literature have shown that mosquitoes bite outdoors in the late evenings and early mornings therefore ensuring parasite or pathogen transmission and have developed resistance to insecticides used in these interventions which are synthetic based. This study investigates insecticidal plants that could provide a broad scope of available and effective insecticides that can inhibit human vector contact both indoors and outdoors.

1.3 Justification

Changes in biting and resting behaviours and resistance development by insect vectors especially mosquitoes in response to interventions (LLIN and IRS) used has constituted a challenge to vector control efforts necessitating the need to source for other insecticides that can protect humans from infectious bites. Research activities and cultural practices over the years have shown that plants are rich sources of active components that have been used to develop drugs and treatment remedies. Plants are therefore a potential source of identifying new insecticidal components which are cost-effective, available, accessible and sustainable and can act as alternatives in the management of mosquito borne disease. The results from this study would play a vital part in the conservation of traditional knowledge of insecticidal plants in North-Central Nigeria and the discovery of natural insecticides that could be used to manage mosquitoes.

1.4 Aim

This study was designed to survey and evaluate the bio-efficacy of selected insecticidal plants in North-Central, Nigeria in the management of *Anopheles gambiae*, a major vector of malaria.

1.5 Specific Objectives

The study was guided by the following specific objectives which include:

- To determine the knowledge and usage customs of plants with insecticidal potential in North-central, Nigeria;
- To determine the repellent activity of shortlisted and identified plant materials against *Anopheles gambiae* s.l.;

- To determine the repellent activity of oils from selected plants against *Anopheles gambiae* s.l.;
- To determine the contact toxicity of oils from selected plants against *Anopheles gambiae* s.l.;
- To determine the vapour toxicity of oils from selected plants against *Anopheles gambiae* and;
- To identify the major chemical components in oils from *Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacum* and *Ageratum conyzoides.*

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 Roles of Insects in Vector Borne Diseases

Most disease vectors are haematophagus insects and are responsible for the transmission of disease-causing organisms (i.e. parasites and pathogens) to mankind that causes infectious diseases (WHO, 2019a). Hundreds of millions of people in Africa suffer from vector-borne diseases annually and these diseases include malaria, yellow fever, dengue, trypanosomiasis, and leishmaniasis, which are transmitted by vectors likes sand-flies, mosquitoes, tsetse flies, blackflies etc. (Carey and Carlson, 2011) Table 1. Vector-borne diseases are human ailments that are cause by parasites, bacteria and viruses that are transmitted by vectors like tsetse flies, blackflies, mosquitoes, lice etc (WHO, 2019b). Vector-borne diseases are highest in subtropical and tropical areas of the world affecting the poor (WHO, 2019b). The distribution of Vector-borne diseases is determined by demography, environmental and social factors (WHO, 2019b). In recent times, global warming, trade, unplanned urbanization and unchecked anthropogenic activities have contributed to the emergence and resurgence of many vector-borne diseases (Karunamoorthi, 2013). These diseases are a major impediment to socio-economic development accounting for 17% of all infectious diseases and causing 700,000 deaths yearly (WHO, 2019a; Karunamoorthi and Yirgalem, 2013) with mosquito-borne diseases been the most responsible.

2.2 Mosquito-borne Disease Burden in Sub-Saharan Africa

Mosquitoes have become the most important single group of insects well-known for their public health importance since they act as the vector for many parasites and pathogens in the tropics (Egunyomi *et al*., 2010). Mosquitoes belong to the family Culicidae which is composed of 42 genera (Akinkurolere and Zhang, 2007). The genus *Aedes*, *Culex* and *Anopheles* are responsible for the transmission of the most common and important disease-causing organisms resulting in grave losses annually (WHO, 2019b). For example, in 2018, there was 218 million reported malaria cases and 405,000 mortality globally making malaria the greatest killer of humans so far and mosquitoes the deadliest animal on the planet (WHO, 2019a). In addition to transmitting parasitic organisms (*Plasmodium falciparum*) that causes malaria, they are also vectors of filaria worm (*Wuchereria bancrofti, Brugia malayi* and *Brugia timori*) that causes filariasis and a few viruses that causes arboviruses (yellow fever, dengue fever, zika, Japanese encephalitis, West Nile virus) (WHO, 2019b). Below are details on each of these mosquito-borne diseases;

2.2.1 Burden of Malaria Disease in Sub-Saharan Africa

Malaria is a disease caused by single-celled protozoan parasites of the genus *Plasmodium* and there are four (4) species of Plasmodium that infect humans: *Plasmodiumfalciparum, P. malariae, P. ovale*, and *P. vivax* (White, 2009). Among these, *P. falciparum* is the most dangerous, it is associated with almost all of the serious and fatal cases of malaria in Africa (Gkrania-Klotsas and Lever, 2007). *Plasmodium* parasites are transmitted from person to person by the bite of infected female mosquitoes of the genus *Anopheles* (White, 2009). Malaria is widespread in the tropics and also occurs in subtropical and temperate regions being endemic in 109 countries (WHO, 2018). In 2018, the World Health Organization (WHO) estimated that most of the malaria cases and deaths in the world occurred in sub-Saharan Africa with Nigeria toping the rank with twenty-four percent (WHO, 2019a). Malaria is the second leading cause of death from infectious diseases in Africa after Human Immunodeficiency Virus (HIV) and it is the highest cause of deaths among children under age five in Africa (WHO, 2019a).

Malaria is a major public health concern in Nigeria accounting for the highest reported cases as well as deaths compared to other countries with all of the population at risk, and seventy-six percent of the population living in malaria hotspots (NDHS, 2018). It is responsible for eleven percent maternal mortality and it is the leading cause of deaths in children under age five years (NMIS, 2015). Transmission is all year round in the Southern part to three months or less in the Northern part and the South-West zone of Nigeria has the highest malaria prevalence (50.3%) followed by North-Central (49.4%) and North-West (48.2%) zones (NDHS, 2018). The parasite responsible for the transmissions in Nigeria is *Plasmodium falciparum* and the major vector across is *Anopheles gambiae* (Okorie *et al*., 2011; Malaria Operational Plan FY, 2019).

2.2.2 Burden of Yellow Fever Disease in Sub-Saharan Africa

Yellow fever (YF) is an acute viral haemorrhagic disease caused by a single-stranded RNA virus that belongs to the genus *Flavivirus* which is transmitted between humans primarily by the bite of an infected *Aedes aegypti* mosquitoes (Amaku *et al*., 2011). Yellow fever is prevalent in the tropical regions of Africa (Monath, 2001), with more than 600 million people living in endemic areas (Tolle, 2009). The World Health Organisation estimates that there are 200,000 cases of YF and 30,000 deaths yearly globally (WHO, 2018) with majority (90%) of these deaths occurring in the African continent (Tolle, 2009).

In Nigeria, there has been recent outbreaks of yellow fever diseases in different part of the country with 3,742 suspected cases and 106 confirmed cases and 20 confirmed deaths (WHO, 2019a). The largest and concentrated outbreak was found in Edo state in which 71 confirmed cases was reported (WHO, 2019b).

Table1: Burden of vector borne diseasesglobally.

Disease transmitted	**Insect Vectors**	**Cases**	**Burden**	**Geographical Distribution**
Malaria	Anopheles mosquitoes	228 million	405,000 deaths annually worldwide	Africa, Asia, America
Lymphatic filariasis	Culex, Anopheles and Aedes mosquitoes	120 million infected	40 million of them are disfigured and incapacitated	Africa
Yellow fever	Aedes Mosquitoes	200,000	30,000 deaths annually worldwide	Africa, America
Human African trypanosomiasis	Tsetse Fly	20,000	65 million people at risk	Africa
Onchocerciasis	Black Flies	99% of infected people live in 31 African countries	NA	Africa
Leishmaniasis	Sand Flies	1.3 million	30,000 deaths annually worldwide	Africa
Zika	Aedes mosquitoes	NA	62 countries and territories	Africa, the America, Asia and the Pacific
Dengue	Aedes mosquitoes	50 million asymptotic	40% of the world is at risk	Africa, America
Chikungunya	Aedes mosquitoes	NA	NA	Africa, Asia

Source: WHO, 2018, NA = Not Applicable

2.2.3 Burden of Dengue Fever Disease in Sub-Saharan Africa

Dengue fever (DF) is caused by the dengue virus (DENV) that belongs to the genus *Flavivirus*. There are four closely related DENVs that can cause DF; DENV-1, DENV-2, DENV-3 and DENV-4 (Gubler, 2004) and these viruses are transmitted between people mainly by the bite of infected *Aedes aegypti* mosquitoes; *Ae. albopictus* can also act as a vector (Rozendaal, 1997). Dengue fever is endemic in more than 100 countries in Africa, the Americas, the western Mediterranean, South and East Asia, and the western Pacific. The incidence of DF has increased dramatically over the last decade and over 2.5 billion people are at risk (Gubler, 2004). It is estimated that there are between 50 and 100 million cases of DF and 22, 000 deaths each year (WHO, 2018). Recently, dengue viruses are a major cause of acute fevers in Nigeria with a report of dengue sero-prevalence among children to be 30.8% (Otu *et al*., 2019) indicating an endemicity.

2.2.4 Burden of Lymphatic Filariasis in Sub-Saharan Africa

Lymphatic filariasis (LF), commonly known as elephantiasis is caused by nematode parasites of the family Filariodidea with three species responsible for the disease, namely, *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* (Bandyopadhyay, 1996). *W. bancrofti* is responsible for 90% of infections and the parasites are transmitted from person to person by the bite of an infected mosquitoes i.e. *Culex quinquefasciatus* and by some *Anopheles* and *Aedes* species while *B. mulayi* and *B. timori* are mainly transmitted by *Mansonia* species (Rozendaal, 1997). Lymphatic filariasis is endemic in 73 countries throughout the tropical and subtropical regions of Asia, Africa, the western Pacific and some parts of the Americas, with more than 120 million people estimated to be infected and 40 million disfigured as a result of the disease (WHO, 2018). Nigeria has a high burden of lymphatic filariasis with 106 million people at risk of the disease (WHO, 2019a).

2.3 Vector Control Interventions Performance Over Years

One of the most effective strategies used to reduce vector-borne disease has been vector control. Vector control interventions are designed to capture the behaviour of both people and the insect vector to minimize human-vector contact (Chen *et al*., 2005). Vector control relies on the use of insecticides and offers the best prospect for control of insect vectors especially mosquitoes by decreasing human-vector contacts (NMIS, 2015). For mosquitoes, this methodincludes adulticide which focuses on the adult mosquitoes e.g. indoor residual spraying (IRS), use of insecticide-treated bed nets (ITNs) and larviciding which focuses on the larval stage of the mosquitoes (Lengeler, 2004). However, the world's prime choice for mosquito control in Africa still remains the selective application of residual synthetic insecticides through either IRS or LLINs (WHO, 2003). These approaches have been highly effective in reducing mosquito-borne disease's burden and results can be obtained on a large scale at an affordable cost (WHO, 2012).

2.3.1 Impact of Insecticide Treated Nets (ITNs) in Disease Burden Reduction

The widespread use of Insecticide-treated bed nets aims to prevent malaria infection in malarious areas that is why ITNs are widely promoted by international agencies and governments with a bid to reduce malaria burden (RBM, 2011). ITNs possess the ability to repel and kill mosquitoes that comes into contact with the impregnated insecticide on the netting material (WHO, 2019a). ITNs have been found to be highly effective in reducing childhood mortality and morbidity from malaria by providing 15 to 20% protective efficacy compared to no nets and up to 23% protective efficacy compared to untreated nets (Lengeler, 2004). Shifting from conventional nets which require insecticide reimpregnation every 6 to 12 months, several companies have recently developed long-lasting insecticide-treated nets (LLINs) capable of retaining lethal concentrations of insecticide for 3 to 5 years and the World Health Organization is strongly recommending the use of these LLINs for malaria prevention in Africa (WHO, 2008). The use of ITNs still remains the most powerful tool for personal protection currently available for effective malaria parasite transmission reduction globally (WHO, 2019a; Choi *et al*., 1995). In Nigeria, sixty-one percent of households own at least one ITN and there are campaigns to increase ITN-ownership in several part of the country (NMIS, 2018).

2.3.2 Contribution of Indoor Residual Spray (IRS) to Disease Burden Reduction

Indoor residual spray (IRS) is the application of lethal doses of insecticide to a potential mosquito resting surface with the hope that the mosquito will come into contact with the insecticide (WHO, 2019a). The basic principle behind IRS is that, after a female mosquito takes blood meals, it looks for a place to rest indoors which if sprayed makes it to pick-up a lethal dose of insecticide that harms the mosquito preventing parasite transmission (Pluess *et al*., 2010) hence IRS is most effective against indoor resting i.e. endophilic mosquito species. IRS is a powerful tool if properly implemented to reduce adult mosquito density and longevity and therefore reduce parasite transmission (WHO, 2019a). In Nigeria, IRS was first used in the Garki project in the North-West zone of Nigeria in 1973 (Malaria Operational Plan FY, 2019). From 2011 to 2013, Presidential Malaria Initiative (PMI) supported IRS in two local government areas in Nassarawa state while the National Malaria Elimination Program (NMEP) conducted IRS in 14 local governments in 6 states in Nigeria from 2011 to

2014. In 2016, a public private partnership implemented IRS in selected local governments in 6 states (Malaria Operational Plan FY, 2019).

2.3.3 Use of Repellents in Disease Burden Reduction

Repellents are substances that act locally or at a distance, deterring insects from flying to, landing on, or biting human or animal (Blackwell *et al*., 2003). Usually, insect repellents work by providing a vapour barrier deterring insects from coming into contact with the surface (Brown and Hebert, 1997). Repellents are widely used to prevent the transmission of mosquito-borne diseases by minimizing the contact between humans and vectors both indoors and outdoors making it an effective strategy in mosquito control (Das *et al*., 2003; Pitasawat *et al*., 2003). They can be applied directly to the skin or to clothing and other fabrics such as bed-nets and anti-mosquito screens (Rozendaal, 1997). There is a wide range of mosquito repellent formulations on the market, with varying levels of effectiveness and which last for different lengths of time. The majority of these repellents are prepared using synthetic chemicals such as DEET (N, N-diethyl-3-methylbenzanmide), KBR 3023/Picardin [2- (2-hyroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester] and IR3535 (2[N-hydroxy-N-acetyl] amino proprionic acid ethyl ester) (Qiu *et al*., 1998). Among these synthetic chemicals, DEET is the most widely used and most effective repellent currently on the market (Das *et al*., 2003). There are concerns with DEET use because of its unpleasant smell, oily feel, high skin penetration, ability to dissolve plastic (Qiu *et al*., 1998) and it is not safe for public use because of toxicological issues (side effects) (Das *et al*., 2003) hence a renewed clamour for the development or discovery of repellents with less side effects.

2.3.4 Larval Source Management for Disease Burden Reduction

Larviciding is a successful strategy for controlling mosquito-borne diseases by targeting the larval stages of mosquitoes and this method dates back to 1899 when Ronald Ross applied kerosene on anopheline larval breeding sites in Sierra Leone (Bockarie *et al*., 1999). The advantages of targeting the larval stages are that mosquitoes are killed before they emerge to adult and disperse to human habitations, and that mosquito larva, unlike adults cannot change their behaviour to avoid targeted control activities (Fillinger and Lindsay, 2006). Larvicides may act as stomach poisons, which must be ingested by the larvae when feeding, or as contact poisons, which penetrate the body wall or the respiratory tract but at present, mosquito larviciding depends primarily on the use of synthetic larvicides (Fillinger and Lindsay, 2006). The most commonly used synthetic larvicides are organophosphorus components such as temephos, fenthion, chlorpyrifos, fenitrothion, jodfenphos, malathion and primiphos methyl and among these components, temephos is the most widely used larvicide, because it has low toxicity to fish, birds, mammals and humans (Rozendaal, 1997).

2.3.5 Successes with Synthetic Insecticide-based Vector Control Interventions

In the last decade, the global fight against mosquito borne diseases has recorded an appreciable success with the decline in the global burden for instance malaria mortality rate has dropped by 70 percent between 2000 and 2018 (WHO, 2019a). Similarly, the reduction in malaria mortality due to malaria in children under the age of five is even more with about 69 percent drop between 2000 and 2015 and a further 35 percent between 2010 and 2015 (WHO, 2016). Half of the people at risk of malaria in Sub-Saharan Africa presently sleep under an ITN (WHO, 2019a). In Nigeria, the story is not different as the record shows a decline in the national malaria prevalence from 42 percent in 2010 to 23 percent in 2018 (NMIS, 2018). Thus, the global reduction in malaria burden which is the result of direct scale-up of the core malaria interventions using the insecticide-treated nets (ITNs), has prevented around 663 million cases of malaria in the sub-Saharan Africa (WHO, 2019a).

2.3.6 Issues Associated with Synthetic Insecticide-based Vector Control Interventions

Despite the long history of use of synthetic insecticide-based vector control methods, total eradication has still not been achieved and this is partly due to the rapid development of insecticide resistance over the last five decades because of an over-reliance on synthetic chemicals for IRS and ITNs (Hargreaves *et al*., 2000; Awolola *et al*., 2002; WHO, 2003). Also, over-reliance on synthetic insecticide-based vector control methods could have its long-term effect on the environment and humans which also is of great concern (Diabate *et al*., 2004; Matambo *et al*., 2006). Insecticide resistance poses a serious threat to sustainable insecticide-based vector control in many African countries (Youmsi *et al*., 2017) because resistance to insecticides has spread to all classes of insecticides currently used in public health and is widespread geographically (Chandre *et al*., 1998; Hemingway and Ranson, 2000; Etang *et al*., 2003; Coetzee *et al*., 2006; Abdalla *et al*., 2007; Munhenga *et al*., 2008). Furthermore, due to the extensive use of ITNs, mosquitoes may develop resistance to the insecticide been used on the ITN due to prolonged exposure of the mosquito to an insecticide over several generations (Coetzee *et al*., 2006). Several researchers have reported on the increasing occurrence of insecticide resistance in *Anopheles* mosquito populations to commonly used insecticides for ITNs (Hargreaves *et al*., 2000; Awolola *et al*., 2002; Coetzee *et al*., 2006; N'Guessan *et al*. 2007).

Although widespread access to ITNs is currently being advocated by the Roll Back Malaria initiative, Lengeler (2004) quoted that universal deployment will require major financial, technical, and operational inputs and, moreover, ITN coverage does not guarantee usage.

However, the cost of an ITN is a major barrier to ownership and usage for large proportion of Africans who are amongst the poorest in the world and are also the most affected by mosquito-borne diseases (N'Guessan *et al*. 2007). Also, ITNs have their limitations; primarily, that they do not protect against exophagic vectors i.e. those vectors that bite at times when people are not sleeping under their bed nets (Gonzalez *et al*., 2002).

It is currently recommended that an integrated approach be used which involves the utilization of all appropriate technological and management techniques to bring about an effective degree of vector suppression in a cost-effective manner (WHO, 2003). This, in fact, is not a new concept has it was developed in the early part of the twentieth century but was overshadowed by the development of the long-lasting residual insecticides during the 1940s which presented a single method of vector control with an extremely high, but incalculable benefit and became the major component in many vector control efforts (Collins and Paskewitz, 1995).

2.4 Ethnobotanical Survey of Insecticidal Plants in Africa

Most African people still rely heavily on traditional based medicine and personal protection against biting insect because this remains the popular health care option among most communities in Africa due to its ready availability, affordability, accessibility and more importantly being an integral part of their cultural beliefs and practices (WHO, 2002). Since the beginning of the millennial, there has been an increasing interest in the study of medicinal and insecticidal plants and their traditional use (Muthu *et al*., 2006; David, 2010; Uprety *et al*., 2010). Plant products have been used in many parts of the world for killing or repelling haematophagous insects e.g. mosquitoes either as whole plants, extracts or oils (Peterson and Coats, 2001) and our ancestors had exclusively depended on them for their personal protection. The knowledge and custom usage of insecticidal plants are pivotal to the discovery of effective plants and bioactive components that can be used in the management of haematophagous insects in Africa.

2.4.1 Evidence and Prospects of Extensive Use of Insecticidal Plants in Africa

Plants with insecticidal properties are well known before the advent of synthetic chemicals (Karunamoorthi *et al*., 2008) because our fore-fathers completely depended on the use of plants to manage blood-sucking insects (Kidane *et al*., 2013). Chemical components extracted from plants have potential use as; insecticides, larvicides, repellents, oviposition deterrents or attractants, insect growth hormone regulator and deterrent agents (Okumu *et al*., 2007). Because of this, plant with insecticidal potential have been receiving attention as an alternative green measure for personal protection against blood-sucking insects because they don't pose hazards of toxicity to humans and domestic animals at low doses, possess environmentally friendly nature and are easily biodegradable (Das *et al*, 2003).

Plant-based products have been used in various parts of the world as mosquitocides either as extracts or as whole plants (Peterson and Coats, 2001). Local plants with repellent or insecticidal activity may play an important role in regions (especially developing countries like Nigeria) where mosquitoes are developing behaviours to evade interventions use and LLIN distribution and coverage is low (Waka *et al*, 2004). There are numerous plants in Africa reported to having effective insecticidal and repellent activity against insects of vector-borne diseases (Maia and Moore, 2011). Plants that are commonly used to repel or kill blood-sucking insects mostly have insecticidal properties because they mostly contain essential oils so that when crushed or brushed against leaves release strong odours that are not pleasant or toxic to some insects (Youmsi *et al*., 2017). Mosquitocidal plants are a viable source of material for use in protection against mosquitoes and mosquito-transmitted diseases (Maia and Moore, 2011) and have some advantages over the current gold-standard synthetic repellent, N, N-diethyl-m-toluamide (DEET) and insecticide, deltamethrin (Moore, 2004). Common plant-based insecticides are Pyrethrum from *Chrysanthemum cineraritolium* (Curtis, 1989), neem oil from *Azadirachta indica* (Sharma *et al*., 1993) or repellent such as quwenling from *Eucalyptus maculadon citrodion* (Trigg, 1996), citronella from *Cymbopogon nardus* (Osmani *et al*., 1972).

Worldwide, it is estimated that the number of individuals using medicinal and insecticidal plants is now increasing with increasing population in developing countries (David, 2010) and this, in turn, has raised doubts concerning the sustainable use of medicinal and insecticidal plants; the conservation of their biodiversity, appropriate forms of local cultivation and production of safe and effective natural medicine and insecticides (Balick and Cox, 1996). It is evident that many medicinal and insecticidal plants face extinction or severe genetic loss due to misuse and over-exploitation (WHO, 2002) there is therefore, a need to conserve traditional knowledge of plant species which is important for the utilization of herbal medicines and insecticides of plant origin, either traditionally, through trade or through the pharmaceutical industry (Carlson *et al*., 2001; Dhillion *et al*., 2002; Tabuti *et al*., 2003). Traditional knowledge is built upon the long-term experience and close observation by local communities (Karunamoorthi and Hailu, 2014). However, over the past decade, a steady decline of traditional knowledge has been reported worldwide (Brockman *et al*., 1997; Twang and Kapoor, 2004; Karunamoorthi, 2013) hence the importance of this study.

Ethnobotanical studies are mainly for two purposes which is to discover new plants and their products and to document the traditional use of plants (Waka *et al*., 2004). During ethnobotanical studies in Ethiopia, it was discovered that most of the respondents were amply aware of plants with insecticidal potential (Kidane *et al*., 2013; Karunamoorthi *et al*, 2009a) and twenty-two insecticidal plants were identified and shortlisted (Karunamoorthi and Hailu, 2014). This trend was also reported in Cameroon were sixteen insecticidal plants were identified and shortlisted (Youmsi *et al*., 2017) and South Africa where thirteen plant species were identified and shortlisted (Mavundza *et al*., 2011). Also, an ethnobotanical study was carried out in the Eastern part of Nigeria, where twenty-four insecticidal plant species were identified and shortlisted (Edwin-Wosu *et al*., 2013). This research work will present the first ethnobotanical study carried out in the North-Central zone of Nigeria.

Insecticidal plant usage custom is a result of thousands of years of experience and this knowledge has been passed down many generations majorly through word of mouth which may result in misrepresentation or loss of indigenous knowledge (Karunamoorthi *et al*., 2009a). In South Africa, most of the respondents (70%) traced back the source of knowledge of plants with insecticidal potential to family members (Mavundza *et al*., 2011). Also, the non-sustenance of knowledge of insecticidal plants was attributed to the habitual culture of passing down information to the eldest son in the family alone and the lack of interest shown by the younger generation to cultures and traditions in Ethiopia (Karunamoorthi and Hailu, 2014).

In Tanzania, it was discovered that females were more aware about plants with insecticidal potential than males (Innocent *et al*., 2016). In Ethiopia and Tanzania, it was reported that there was no relationship between knowledge about insecticidal plants and respondent's sex, educational status and age while there was a relationship between usage customs of insecticidal plants and sex and monthly income but not with educational status (Karunamoorthi and Hailu, 2014; Karunamoorthi *et al*., 2009a). All this information is possibly attributed to the widespread use of insecticidal plants in poor communities in Africa as well as culture (Karunamoorthi and Hailu, 2014).

Also, the farming of insecticidal plants should be encouraged which could be a source of income for small scale farmers in Nigeria and Africa (Maia and Moore, 2011).

2.4.2 Methods of Application of Insecticidal Plants for Personal Protection Purposes in Africa

Plants have been used for centuries in the crude form where they are burnt to drive away mosquitoes and other haematophagous insects and also as formulations that are applied on skins or to clothes as firstly recorded by ancient Greeks, Roman and Indian scholars (Karunamoorthi *et al*., 2009b). The burning and/or hanging of fresh and dried leaves from insecticidal plants around and within people's abode to provide protection against the bites of mosquitoes and other blood sucking insects is widely practised in Ethiopia (Karunamoorthi *et al*., 2009b) as well as other parts of Africa (Seyoum *et al*., 2002; 2003; Moore *et al*., 2006). In Guatemala and Papua New Guinea, up to ninety percent of rural households interviewed burned dried waste plant materials i.e. coconut husk and woods to produce smoke in other to protect themselves from mosquito bites (Klein *et al*., 1995; Vernede *et al*., 1994).

It has been reported that smouldering (burning of dried plant material to produce smoke) was the most used method of application of insecticidal plants for the control of mosquitoes in Ethiopia (Karunamoorthi and Hailu, 2014; Kidane *et al*., 2013; Karunamoorthi *et al*., 2009a; Karunamoorthi *et al*., 2008), Tanzania (Kweka *et al*., 2008;), Kenya (Seyoum *et al*., 2002), South Africa (Mavundza *et al*., 2011), Guinea Bissau (Pålsson and Jaenson, 1999) and Cameroon (Youmsi *et al*., 2017). It was postulated that insecticidal activity of burned plant's parts appears to be due to the release of specific volatile components, either already present in the plant or created during combustion process (Fitsum *et al*., 2011).

Hanging of insecticidal plants in the house or spreading the leaves on the floor is also a method of personal protection employed against haematophagus insects by locals (Sangat-Roenatyo, 1990). In Cameroon, it was reported that some of the respondents mashed insecticidal plants and thereafter applied it on their body while others hanged them in their houses in a bid to protect themselves from mosquito bites (Youmsi *et al*., 2017). In Tanzania, insecticidal plants were applied by spreading fresh plant parts on the ground within inhabited houses and by soaking the plant products in water and then spraying them in and around habited houses (Innocent *et al*., 2016).

Leaves were the most used insecticidal plant parts in local communities in Cameroon (Youmsi *et al*., 2017), Tanzania (Kweka *et al*., 2008) and South Africa (Mavundza *et al*., 2011) followed by bark and the whole plant for the control of haematophagous insects; because leaf's volatile component has the desired activity and is readily available. In Nigeria, various parts of the insecticidal plants were reportedly used (i.e. Leaf, fruit, bark, seed and whole plants) for the management of blood-sucking insects with leaves being the most used part (Edwin-Wosu *et al*., 2013). The act of harvesting roots and barks, as well as whole plants, can threaten local plant population unless a sustainable harvesting strategy is developed. The use of leaves for insecticidal purposes is a sustainable way of preserving or conserving insecticidal plants against the use of other parts; roots, barks and whole plants which is not sustainable (Youmsi *et al*., 2017) and this need to be encouraged.

Plant products can either be obtained from the whole plant or from a specific part by extraction and evaporation with different types of solvent such as aqueous, methanol, acetone, petroleum ether, chloroform, hexane etc. depending on the polarity of the phytochemicals (Sukumar *et al*., 1991). The efficacy of phytochemicals against the different life forms of haematophagous insects vary significantly depending on plant species, plant part used, age of plant parts, solvent use during extraction as well as upon available vector species (Sukumar *et al*., 1991).

2.4.3 Preference for Use of Plant for Personal Protection Purposes against Haematophagus Insects

Most vector control interventions used in Africa are produced outside Africa making it very expensive because of cost of production and importation (Waka *et al*., 2004). Insecticidal plants sourced locally will remove totally cost of importation (Maia and Moore, 2011). It has been reported that respondents in Ethiopia (Karunamoorthi and Hailu, 2014; Karunamoorthi *et al*., 2009a), Tanzania (Innocent *et al*, 2016), Cameroon (Youmsi *et al*., 2017) and South Africa (Mavundza *et al*., 2011) perceived insecticidal plants to be potentially useful, cost effective in haematophagous insects management and easily accessible since Africa is blessed with a rich diversity of flora. It has also been perceived that insecticidal plants are used by locals in Africa because it is easily accessible and available year-round (Karunamoorthi and Hailu, 2014). In a study carried out in Tanzania, it was reported that most members of the rural communities evaluated had to travel short distances of less than one kilometre in other to harvest insecticidal plants and most were collected in the wild (Innocent *et al*., 2016). Oils extracted from plant families like Lamiaceae (mint family), Poaceae (aromatic grass family) and Pinaceae (Pine and cedar family) are the most commonly used insecticidal plants around the world (Maia and Moore, 2011). Based on the accessibility and affordability of insecticidal plants, it will be important to proffer ways of incorporating these plants in mosquito management in poor communities in Nigeria and Africa.

In Ethiopia (Karunamoorthi and Hailu, 2014; Karunamoorthi *et al*., 2009a), Tanzania (Innocent *et al*., 2016), Eritrea (Waka *et al*., 2004) and Kenya (Odalo *et al*., 2005), local residents of rural communities evaluated acknowledged that they preferred insecticidal plants for personal protection purposes against synthetic based interventions owing to its affordability, accessibility and availability.

Table 2. An overview of insecticidal plants from ethnobotanical studies in Africa(database sources: ISI Web of Science, SCOPUS and Google Scholar, accessed September, 2018).

Botanical Name	**Family**	**Part Used**	**Area of traditional Use**	**Method of Application**	**References**
Allium sativum Linn.	Alliaceae	Bulb	Ethiopia	C	3
Aloe ferox Mill.	Xanthorrhoeaceae	Leaves	South Africa	C, T	6
Aloe pulcherrima M.G. Gilbert & Sebsebe.	Xanthorrhoeaceae	Leaves	Ethiopia	C, T	3, 8
Atalaya alata (Sim) H.H.L. Forbes	Sapindaceae	Roots	South Africa	C, T	6
Azadirachta indica A. Juss	Meliaceae	Leaves, Flowers, seeds	Nigeria, Kenya, Ethiopia	C	4, 7, 8, 10
Azolla pinnata R. Br. var. *africana*	Azollaceae	Whole plant	Nigeria	C	7
Balanites maughamii Sprague.	Balanitaceae	Bark	South Africa	T	6
Boerhaavia diffusa Linn.	Nyctaginaceae	Whole plant	Nigeria	C	7
Boswellia papyrifera (Del.) Hochst.	Burseraceae	Bark	Ethiopia	C	3, 9
Brassica nigra Linn. Koch	Brassicaceae	Seeds	Ethiopia	C, T	3
Buddleja polystachya Fresen.	Buddlejaceae	Roots	Ethiopia	C, T	3
Calpurnia aurea (Ait.) Benth.	Fabaceae	Leaves	Ethiopia	C, T	8
Carapa procera DC.	Meliaceae	Seeds	Nigeria	C	7
Carica papaya Linn.	Caricaceae	Leaves	Ethiopia	T	3
Citrus aurantifolia (Christm.)	Rutaceae	Peels of fruits	Ethiopia, Nigeria	C	3, 7
Citrus sinensis (L.) Osb.	Rutaceae	Peels of fruits	Ethiopia	C, T	3
Clausena anisata (Willd.) Hook. f.	Rutaceae	Leaves	South Africa	T	6
Colchicum autumnale Linn.	Colchicaceae	Barks, Roots	Ethiopia	T	3
Corymbia citriodora Hook.	Myrtaceae	Leaves	Ethiopia, Kenya	T	2

Croton macrostachyus Hochst. ex Del.	Euphorbiaceae	Leaves	Ethiopia	C, T	3, 9
Croton menyarthii Pax	Euphorbiaceae	Leaves	South Africa	T	6
Cupressus lusitanica Mill.	Cupressaceae	Leaves, Roots, barks	Ethiopia	T	3, 9
Cymbopogon citratus L.	Poaceae	Stems	Ethiopia	C, T	9
Duranta plumeri Linn.	Verbenaceae	Whole plant	Nigeria	C	7
Duranta rapens Linn.	Verbenaceae	The whole plant, seeds	Nigeria	C	7
Echinops kebericho Mesfin.	Asteraceae	Root	Ethiopia	C, T	3
Eucalyptus camadulensis Dehnh.	Myrtaceae	Leaves	Ethiopia, Nigeria	C	2, 7
Eucalyptus citriodora Hook.	Myrtaceae	Leaves	Ethiopia	T	3
Eucalyptus globulus Labill	Myrtaceae	The whole plant, Leaves	Ethiopia	C, T	3, 8
Hensia crinita (Afzel.) G. Tayl.	Rubiaceae	Leaves, bark	Nigeria	C	7
Hyptis suaveolens (L.) Poit.	Lamiaceae	Whole plant, Leaves	Kenya, Nigeria	T	5, 7
Justicia schimperiana T.	Acanthaceae	Leaves	Ethiopia	T	3
Khaya senegalensis (Desr.) A. Juss	Meliaceae	Wood ash, Seeds	Nigeria	C, T	7
Lantana camara L.	Verbenaceae	Leaves	Tanzania, Nigeria, Kenya	C, T	1, 4, 7
Lepidium sativum Linn	Brassicaceae	Seeds	Ethiopia	C	3
Lippia javanica (Brum.f) Spreng.	Verbenaceae	Leaves	Kenya, Tanzania, Ghana, Zimbabwe, South Africa	C, T	4, 6
Ludwigia leptocarpa (Nutt.) Hara.	Onagraceae	Whole plant	Nigeria	C, T	7
Mangifera indica L.	Anacardiaceae	Seeds	South Africa	T	6
Melia azedarach L.	Meliaceae	Leaves, Flowers, Seeds	South Africa	C, T	6, 9
Microsorium spp. Link.	Polypodiaceae	Whole plant	Nigeria	C	7
Napoleonaea imperalis P. Beauv.	Lecythidaceae	Leaves	Nigeria	C	7
Nerium oleander Linn.	Apocynaceae	Leaves	Nigeria	C	7
Ocimum basilicum L.	Lamiaceae	The whole plant, Leaves	Ethiopia, Kenya	C, T	2, 5
Ocimum gratissimum Linn.	Lamiaceae	Whole plant	Nigeria	C	7
Ocimum kilimandscharicum Guerke	Lamiaceae	Leaves	East Africa	C	1
Ocimum lamiifolium Hochst. ex Benth.	Lamiaceae	Leaves	Ethiopia	T	3
Ocimum suave Willd.	Lamiaceae	whole plant, Leaves	Ethiopia	T	2, 3
Olax dissitiflora Oliver CEP	Olacaceae AC	Bark	South Africa	T	6
Olea europaea Linn.	Oleaceae	Leaves, Barks	Ethiopia	T	3, 9, 10
Otostegia integrifolia (Forssk.)	Lamiaceae	Whole plant	Ethiopia	C	8, 10
Paullina pinnata Linn.	Sapindaceae	Whole plant	Nigeria	C	7
Pavonia urens Cav.	Malvaceae	Leaves	Ethiopia	C	3
Piper guineense Schum. & Thonn.	Piperaceae	Fruits	Nigeria	C	7
Ricinus communis Linn.	Euphorbiaceae	Seeds	Ethiopia	T	3

Sclerocarya birrea (A.Rich.) Hochst.	Anacardiaceae	Seeds	South Africa	T	6
Sesamum indicum Linn.	Pedaliaceae	Leaves	Nigeria	C	7
Sida acuta Burn. F.	Malvaceae	Leaves	Nigeria	C	7
Sida cordifolia Linn.	Malvaceae	Leaves, flowers, fruits	Nigeria	C	7
Silene macroserene L.	Caryophyllaceae	Whole plant	Ethiopia	C, T	8, 10
Stachytapheta cayennensis (LC. Rich) Schau.	Verbenaceae	Whole plant	Nigeria	C	7
Stachytapheta jameaicensis Vahl.	Verbenaceae	Whole plant	Nigeria	C	7
Tagetes minuta L.	Asteraceae	Whole plant, leaves	Kenya	T	5
Trichilia emetica Vahl	Meliaceae	Seeds	South Africa	C, T	6
Vernonia amygdalina Del.	Asteraceae	Leaves, barks	Ethiopia	C	3

C = Crushing the plant parts, juice applies on the exposed parts of the body T= Thermal expulsion and direct burning to generate smoke **References:** 1. Weiss 1979; 2. Dugassa *et al*. 2009; 3. Karunamoorthi and Hailu 2014; 4. Maia and Moore 2011; 5. Seyoum *et al*. 2002; 6. Mavundza *et al*. 2011; 7. Edwin-Wosu *et al*. 2013; 8. Kidane *et al*. 2013; 9. Karunamoorthi *et al*. 2009a; 10. Karunamoorthi *et al*. 2009b. **Modified from Benelli and Pavela, 2018**

2.5 Shortlisted Insecticidal Plants Used for Mosquito Control in Nigeria.

Extracts (crude or oils) from plants may be alternative sources for the management of the life forms of mosquitoes, as they constitute a rich source of bioactive components that are biodegradable into non-toxic products (Sarkar and Kshirsagar, 2014). Studies carried out so far have shown that some phytochemicals act as general contact toxicant against both adult (adulticidal) as well as larval (larvicidal) stages of mosquitoes while produce olfactory stimuli (repellent) (ICMR, 2003). The use of identified and shortlisted insecticidal plants in this present study for mosquito control in Nigeria are reviewed below:

2.5.1 Use of *Ageratum conyzoides* for Mosquito Control in Nigeria

Ageratum conyzoides is an annual herbaceous plant that grows to about 60cm high found in both tropical and sub-tropical regions and widely utilized in traditional medicine as anti-fungal, anti-bacterial, nematocidal, anti-helminthic and insecticidal agent (Musa *et al*., 2015). It has high variability of secondary metabolites such as; alkaloids, flavonoids, coumarins, and tannins and these components have been shown to possess many biological activities affecting insect development, used as an anti-juvenile hormone and resulting in sterile adults (Borthakur and Baraah, 1987).

Methanolic and aqueous extracts from the leaves of *Ageratum conyzoides* has been reported to display excellent larval toxicity activity against *Culex quinquefasciatus* (LC_{50} = 47.11), *Aedes aegypti* (LC_{50} = 55.87) and *An. gambiae* s.l. (LC_{50} = 55.87) after 24hrs of exposure (Musa *et al*., 2015).

2.5.2 Use of *Annona senegalensis*for Mosquito Control in Nigeria

Annona senegalensis is a shrub of 2 to 6 metre tall but may reach 11 m under favourable conditions, commonly known as Wild Custard Apple or Soursop found growing throughout Nigeria especially in Northern Nigeria, primarily in Nasarawa, Kaduna, Kano, Plateau, and Niger States and in the Federal Capital Territory, Abuja and usually known as Gwándàn dààjìì (Hausa) or dukuu-hi (Fulani) (Orwa *et al*., 2009).

Excellent larvicidal activity was reported on different extracts of *A. senegalensis* against the larvae of *Cx. quinquefasciatus* (Magadula *et al*. 2009), *Aedes aegypti* (Lame *et al*., 2015) and *Anopheles gambiae* (Mukungulu *et al*., 2015).

2.5.3 Use of *Cassia mimosoides* for Mosquito Control in Nigeria

Cassia mimosoides is a medicinal herb native to China but now grows wild in many parts of the world including Nigeria which is traditionally used for urine voiding, anti-inflammatory and as a cure for diarrhoea with the whole or plant leaves and roots being used (Sugimoto, 2001).

Extracts of *cassia mimosoides* were reported to display hundred percent mortality against larval stages of *Anopheles gambiae* after 24hrs post exposure (Alayo *et al*., 2015).

2.5.4 Use of *Hyptis suaveolens* for Mosquito Control in Nigeria

Hyptis suaveolens (L.) Poit a stocky tropical aromatic herb with the common name wild hops; it is an annual shrub of the mint family Lamiaceae distributed evenly in the tropic of West Africa (Oliver-Bever, 1986). In Nigeria, *H. suaveolens* is of high economic and medicinal value and is employed widely for personal protection because of its mosquito-repellent property hence the name mosquito plant (Okigbo *et al*., 2010; Hemen *et al.*, 2013). The plant oils from the leaves has demonstrated antifertility and irritant properties (Oliver-Bever, 1986).

Extracts of *H. suaveolens* have been reported to significantly reduced the viability of the eggs of *An. gambiae* (Ivoke *et al*., 2009) and displayed average larvicidal, pupacidal and adulticidal activity against *Culex quinquefasciatus* (Ayange-Kaa *et al*., 2015; Okigbo *et al*., 2010).

2.5.5 Use of *Ocimum gratissimum* for Mosquito Control in Nigeria

Ocimum gratissimum commonly called Wild basil(also known as scent plant) is popularly seen and known in Nigeria as Efinrin ajase in Yoruba, Aai doya to gida in Hausa and Nchonwu in Igbo (Owulade *et al.*, 2004) belongs to the genus *Ocimum* of the family Lamiaceae. There are many species in the *Ocimum* genus, *O. canum, O. gratissimum, O. basilicum O. urticifolium, O. trichodon, and O. americanum* amongst these *O. basilicum* and *O. gratissimum* are believed to have insecticidal propertiesagainst haematophagous insects (Iwu, 1993). Farmers in northern Nigeria indigenously use various parts of the plants to protect cereals and legumes against pest damage during storage (Mann and Gbate, 2003). In India whole plant preparations are used to treat stomach ache, sunstroke, headache and influenza while in Nigeria, the seeds have been reported to have laxative properties as well as been used in the treatment of gonorrhoea while the plant oils is used to treat fever, inflammations of the throat, ears, eyes, stomach pain, diarrhoea and skin diseases (Orwa, 2012).

Extracts from the leaves of *Ocimum gratissimum* possess excellent adulticidal activity against *Cx. quinquefasciatus* (Okigbo *et al*., 2010) and larvicidal activity against *Aedes aegypti* (LC_{50} = 19.50mg/ml)(Mgbemena, 2010) and *Cx. quinquefasciatus* (Nzelibe and Chintem, 2015) after 24 and 48 hours post treatment indicating their potential as excellent bio larvicides. Also, Ointments formulated with *O. gratissimum* oil has been reported to possess excellent repellent properties against *Ae. aegypti* and *Culex quinquefasciatus* (Esimone *et al*., 2011).

2.5.6 Use of *Eucalyptus globulus* for Mosquito Control in Nigeria

Eucalyptus globulus commonly referred to as Tasmanian Blue Gum belongs to the family Myrtaceae is native to Tasmania and South-East Australia (Akolade *et al*., 2012). This species that has pendant bearing leaves is the most widely introduced plant species and has been established in many countries including Nigeria because it is fast growing (Akolade *et al*., 2012). Apart from its extensive use in the pulp industry, it also produces an Oleum eucalyptus that is extracted on a commercial scale in many countries as raw materials in perfumery, beverages, food, cosmetics, phytotherapy and aromatherapy (Akolade *et al*., 2012).

Nganjiwa *et al*. (2015) reported that *E. globulus* extracts can induce fifty-five percent mortality on the larvae of *Culex quinquefasciatus*.

2.5.7 Use of *Cymbopogon citratus* for Mosquito Control in Nigeria

Cymbopogon citratus commonly known as lemongrass is a native herb from India and is cultivated in other tropical and subtropical countries including Nigeria (Gore *et al*., 2010) locally called “Kooko-oba” by the Yoruba, “Isauri” by the Hausa, “Achara ehi” by the Igbos (Ukpong *et al*., 2016). It is used as a traditional medicine in the treatment of gastrointestinal, nervous disturbances, malaria, fever and hypertension, etc. and also considered as an insect repellent (Gore *et al*., 2010).

Extract from *C. citratus* have been reported to have excellent larvicidal activity against *Aedes aegypti* (LC_{50} = 34.67mg/ml) (Mgbemena *et al*., 2010) and *Cx. quinquefasciatus* (LC_{50} = 109.65mg/l) (Suleiman *et al*., 2014).

2.5.8 Use of *Nicotiana tabacum* for Mosquito Control in Nigeria

Nicotiana tabacum commonly known as Tobacco is one of the annual herbs chiefly available in Nigeria and it is about 9 to 12cm tall (Owoeye *et al*., 2016).

Owoeye *et al*. (2016) reported that extracts from *Nicotiana tabacum* produced hundred percent mortality on adult of *A. gambiae* within 1 hour while same displayedsignificant larvicidal and pupaecidal activity. Also, Ileke *et al*. (2015) reported that extracts of *N. tabacum* elicited hundred percent mortality on larvae and pupae of *An. gambiae*.

2.5.9 Use of *Latanna camara* for Mosquito Control in Nigeria

Lantana camara L. is a flowering ornamental plant belonging to the family Verbenaceae with local names “Ewonadele” in Yoruba, “Kimbamahalba” in Hausa, and “Anya nnunu” in Igbo in Nigeria (Gabi *et al*., 2011). According to Sanjeeb *et al*. (2012), the plant is said to have antibacterial, antifungal, wound healing, anti-inflammatory, antimotility, antiulcerogenic, haemolytic, antihyperglycemic, anti-filarial, anti-urolithiatic, anticancer, antiproliferative, antimutagenic, embryo toxicity, antioxidant, and mosquitoes controlling activities.

Suleiman *et al*. (2014) reported that *L. camara* caused high larval mortality on *An. gambiae* s.l. (LC_{50} = 56.23mg/ml).

2.5.10 Use of *Citrus sinensis* for Mosquito Control in Nigeria

Plant oils from *Citrus sinensis* fruit peels have been identified as very important natural resource of either insecticides (Raguraman and Singh, 1997; Gbolade, 2001) or repellent (Oyedele *et al*., 2000; Govere *et al*., 2000) against mosquitoes. They have been used as both topical preparations and combustible products like incense sticks (Govere *et al*., 2000). In some places in Africa, dried *Citrus sinensis* fruit peels are burnt on a charcoal fire to repel and/or kill mosquitoes in homes (Gbolade, 2001).

2.5.11 Use of *Moringa oleifera* for Mosquito Control in Nigeria

Moringa oleifera (Lam), a multi-purpose tree native to North-West India has so many names in Nigeria depending on ethnicity, including Zogalle (Hausa), Ewé ilé (Yoruba) and Okochi egbu (Ibo) (Ohia and Ana, 2017). It is a widely cultivated, fast growing edible plant that is naturalized in the tropics and grown in settled areas as a backyard vegetable and oftentimes utilized as border plants (Isman, 1993). *Moringa* tree is a deciduous perennial tree that is regarded as one of the world's most useful trees since almost every part of it is useful (Isman, 1993).

Ohia and Ana (2017) reported that extracts of *Moringa oleifera* seed displayed excellent larvicidal activity against *An. gambiae* s.l. after 24 hours of treatment and the rate of larval mortality increased as the concentration increased across the different treatment levels.

2.5.12 Gaps to Knowledge in the Use of Shortlisted Insecticidal Plants for Mosquito Control in Nigeria.

The review showed there was no information on the testing of the toxic and repellent activity of extracts from *Allium cepa*, *Capsicum annuum*, *Ertyphleum suaveolens*, *Hibiscus rosa sinensis*, *Parkia biglobosa* and *Thymus vulgaries* against all life stage of mosquitoes. There was a lot of testing of other shortlisted insecticidal plants on the larvae stages of mosquitoes while the testing of the adulticidal activity of extracts from insecticidal plants against different species of mosquitoes was only done for *Hyptis suaveolens, Ocimum gratissimum* and *Nicotiana tabacum*. The repellency activity of extracts from insecticidal plants was only tested for *Ocimum gratissimum* on *Anopheles* mosquitoes. There is therefore a need to fill in these gaps so that the bioactivity of most of the shortlisted insecticidal plants can become public knowledge which can inform their use and development for personal protection against haematophagus insects hence the purpose of this study.

S/No.	Scientific name	Activity	Test Organisms	Form Tested	References
1.	*Ageratum conyzoides*	Larvicidal	Anopheles, Culex, Aedes	Plant Extract	Musa *et al*, 2015
2.	*Annona senegalensis*	Larvicidal	Anopheles, Culex, Aedes	Plant Extract	Lame *et al*, 2015,
3.	*Cassia mimosoides*	Larvicidal	Anopheles	Plant Extract	Alayo *et al*, 2015
4.	*Hyptis suaveolens*	Insecticidal, Larvicidal	Anopheles, Culex	Plant Extract, Essential oil	Ayange-Kaa *et al*, 2015, Ivoke *et al*, 2009, Okigbo *et al*, 2010, Suleiman *et al*, 2014, Nzelibe and Chintem, 2015, Amusan *et al*., 2005
5.	*Ocimum gratissimum*	Insecticidal, Larvicidal	Culex, Aedes	Plant Extract, Essential oil	Afolabi, 2016, Mgbemena, 2010, Nzelibe and Chintem, 2015, Adefolalu *et al*, 2015, Okigbo *et al*, 2010, Keziah *et al*, 2015
6.	*Eucalyptus globulus*	Larvicidal	Culex	Plant Extract	Nganjiwa *et al*, 2015
7.	*Cymbpogon citratus*	Larvicidal, Repellency	Culex, Aedes	Plant Extract, Essential oil	George *et al*, 2014; De, Mgbemena, 2010, Suleiman *et al*, 2014
8.	*Nicotiana tabacum*	Insecticidal, Larvicidal	Anopheles	Plant Extract, Essential oil	Owoeye *et al*, 2016, Ileke *et al*, 2015
9.	*Latanna camara*	Insecticidal, Larvicidal	Culex, Aedes	Plant Extract, Essential oil	Keziah *et al*, 2015, Suleiman *et al*, 2014
10.	*Citrus sinensis*	Repellency	Anopheles	Plant Extract	Effiom *et al*, 2012, Amusan *et al*., 2005
11.	*Moringa oleifera*	Insecticidal, Larvicidal	Anopheles	Plant Extract, Essential oil	Ohia and Ana, 2017

Table 3: Review of literature from the rest of Nigeria on the mosquitocidal effect of insecticidal plants found in North-Central, Nigeria.

Absent: *Capsicum annuum, Hibiscus rosasinensis, Ertyphleum suaveolens, Thymus vulgaries, Allium cepa, Parkia biglobosa*

2.6 Vapour Toxicity Activities of Botanicals against Mosquitoes in Africa

The health risks associated with vector-borne disease vectors have long encouraged research into methods for protection in endemic areas, in both the grassroots and scientific communities (Moore, 2004). Diligent investigations into personal protection methods using plants by the scientific community are providing new bio-rational, effective and affordable products, increasing knowledge and confidence in traditional protection methods and inherently reducing vector-borne diseases (Dube *et al*., 2011). Plant oils possess a wide spectrum of biological activities including anti-microbial, fungicidal, insecticidal, insect repellent, herbicidal, acaricidal, and nematocidal (Noutcha *et al*., 2016). Mosquito behaviour elicited in response to airborne components which involves avoidance of a chemical stimulus, loss of host detection, knockdown and mortality is collectively referred to as vapour toxicity (Ogoma *et al*., 2014). Vapour toxicity do not require physical contact of the mosquito with treated surfaces because they act in the vapour state at a distance (Ogoma *et al*., 2014). Example of vapour toxicants products include; Mosquito coils, candles and emanators impregnated with volatile pyrethroids and plant oils.

An example of where plants are used as vapour toxicants is displayed in Eritrea where fresh leaves and shoots of *Ocimum forskolei* where hanged on the wall and put around bed frames in inhabited houses and it was reported that there was 53% reduction in a mean number of *An. arabiensis* per house (Waka *et al*., 2004). Also, Keizah *et al*. (2015) showed that the lowest concentration of ethyl acetate extract of *O. gratissimum* displayed 82% protection against female *Ae. Aegypti* while Hexane and Methanolic extracts displayed 63% and 47% protection in comparison with DEET that displayed 100% protection. Ukpong *et al*. (2016)showed that oils from *C. citratus* prevented mosquito landing attempts and bites for up to three hours post treatment. In same experiment, it was also discovered that mosquitoes were observed to fly restlessly exhibiting frantic attempts to escape from the oil-treated section into the untreated section of the insect box within the first minute of exposure meanwhile in another direct contact toxicity experiment, 100% knockdown was observed after 18mins of mosquito exposure (Ukpong *et al*., 2016). Complete mortality was observed when the fumigant effect of extract of *N. tabacum* was carried out on adult *An. gambiae*(Ileke *et al*., 2015). Keziah *et al*. (2015) reported that ethyl acetate extracts of *L. camara* exhibited 78% protection, whereas Hexane and Methanolic Extract exhibited 64% and 63% protection from mosquito bites at the lowest concentration (Keziah *et al*., 2015). Effiom *et al*. (2012) reported that extracts from the peels of all *Citrus* species repelled mosquitoes for varying times at different concentrations. Similarly, extracts from all *Citrus* species except *C. vitis* showed long-lasting repellent effect of more than 5 hours (Effiom *et al*., 2012). This shows that some insecticidal plant extracts and oils possess excellent vapour toxicity activities.

2.7 Chemical Components of Plant Oils as Raw Materials for Industries

Phytochemicals produced by plants are basically secondary metabolites that serve as a mechanism of defence for plants to withstand the continuous selection pressure of herbivore predators and other environmental factors (Sarkar and Kshirsagar, 2014). Over the past century, the phytochemicals found present in plants have been a very important pipeline for pharmaceutical discovery (Kidane *et al*., 2013). The diversity of phytochemicals in botanical extracts is also useful which is redundancy in the presence of numerous analogues of one component and is known to increase the efficacy of extractives in the metabolism of components and prevent the evolution of insecticide resistance when selection occurs over several generations (Arnason *et al*., 2015). Plant oils are produced commercially from several plant sources, many of which are of the mint family, Lamiaceae which are generally composed of complex mixtures of monoterpenes biogenetically related phenols and sesquiterpenes (Isman, 2006).

Oils of *Citrus sinensis* peels are medicinally very important and show a variety of biological effects because they are rich in flavonoids, carotenes, terpenes and coumarins which have been reported to possess antimicrobial activity (Tepe *et al*., 2005). Consequently, *Citrus sinensis* plant oils are extensively used in pharmaceutics as an antimicrobial, anti-diabetic, antioxidant, antiviral, insect repellent, larvicidal, carminative, antihepatotoxic and antimutagenic agent (Kanaze *et al*., 2008). D-Limonene (Dextrorotatory-Limonene) has been identified as the major chemical component in oilsfrom *Citrus sinensis* peels while other major chemical components identified were *α*-terpineol, carvone, carveol, eugenol, spathulenol and caryophyllene (Ahmad *et al*., 2006). Also, Espina *et al*. (2011) and Njoku and Evbuomwan, (2014) reported that D-Limonene among other components as the major chemical component found in oils from *Citrus sinensis* fruit peels.

The major chemical components identified in oils from *Ageratum conyzoides* in Nigeria are; β-caryophyllene, β-cubebene, germacreneD, α-caryopyllene, trans-β-farnesene, Germacrene B, cis-β-fernesene, α-cubebene, eugenol, Car-4-ene, camphene and D-Limonene in order of composition (Usman *et al*., 2013).

The major chemical component identified in the oils from *Ocimum gratissimum* are; eugenol, α-bulnesene, and β-caryophyllene (Joshi, 2017). Similarly, Saliu *et al*. (2011) and Dambolena *et al*. (2010) identified eugenol as the major chemical component found in volatile oil from *Ocimum gratissimum*.

Noudogbessi *et al*. (2013) reported the major chemical components in oils from *Hyptis suaveolens* from different African countries and it was reported that in Nigeria it contained sabinene, trans-α-bergamortene, terpinen-4-ol, α-pinene, β-caryophyllene and caryophyllene oxide while in Cameroon and Senegal sabinene and β-caryophyllene were the major chemical components identified.

Kidah (2018) reported that the major chemical components in the extracts from the leave of *Nicotiana tabacum* was composed of 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, P-xylene, Cyclohexane, Farnesol and 1- Naphthalenepropanol.

D-Limonene (Dextrorotatory-Limonene) is a colourless liquid hydrocarbon classified as a cyclic terpene occurring naturally in citrus and other fruits, vegetables, meats and spices (Almeida *et al*., 2015). D-Limonene is used in many food products, soaps and perfumes for its lemon-like flavour and odour (Lee *et al*., 2003). D-Limonene is also a registered active ingredient in different insecticide products used as insect repellent, dog and cat repellents (Prevention, Pesticides and Toxic Substances, 1994). D-Limonene is of relatively low acute toxicity when taken orally (Prevention, Pesticides and Toxic Substances, 1994). D-Limonene has insecticidal effects toward stored grain insects (*Sitophilus oryzae, Tribolium castaneum, Oryzaephilus surinamensis*)(Lee *et al*., 2003), German cockroach (Karr and Coats, 1988) and termites (*Heterotermes sulcatus*)(Almeida *et al*., 2015). D-Limonene has also been reported to destroy the wax coating of the insect’s respiratory system (EPA, 1993).

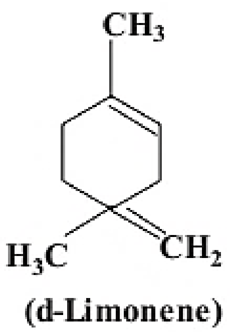


Figure 1: Structural formula of D-Limonene (Almeida *et al*., 2015)

β-caryophyllene oxide is a bicyclic sesquiterpene molecule, occurring naturally in plant oils from various medicinal and edible plants and used as a food flavouring agent and a fragrance and cosmetic ingredient (Tabanca *et al.*, 2015). Di Sotto *et al.* (2013) reported that β-caryophyllene oxide could pass through the cell membrane without inducing genotoxic effects at gene or chromosomal level by using a bio-membrane model, suggesting that it may be safe for use in repellent formulations. Moreover, β-caryophyllene oxide is approved as a flavouring agent by the Food and Drug Administration (FDA) and by the European Food Safety Authority (EFSA) (Fidyt et al., 2016). β-caryophyllene oxide has the ability to repel mosquitoes (Tabanca *et al.*, 2015).

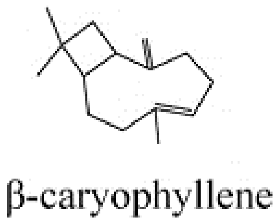


Figure 2: Structural formula of β-caryophyllene (Tabanca *et al.*, 2015)

Eugenol a phenylpropene, an allyl chain-substituted guaiacol is colourless to pale yellow oily liquid (Ajayi *et al.*, 2014). Eugenol has been shown to exhibit insecticidal property toward *Sitophilus zeamais* (Huang *et al.*, 2002), beetle (*Dinoderus bifloveatus*)(Ojimelukwe and Adler, 2000) and tick (*Ixodes ricinus*) (Bissinger and Roe, 2010), and *Callosobruchus maculatus* (Ajayi *et al.*, 2014).

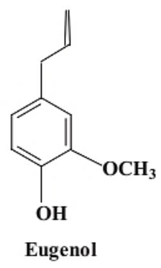


Figure 3: Structural formula of Eugenol (Ajayi *et al.*, 2014)

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study Area for Ethnobotanical Study

The study area for the ethnobotanical survey was in the North-central geo-political zone of Nigeria which is composed of six states, namely; Benue, Plateau, Kogi, Nasarawa, Kwara and Niger, with a total land mass of 296,898 km^2 and a total population of 20.36 million people with 48% living in rural communities. Situated between latitudes $6^0 30''$ N and $11^0 20''$ N and longitudes 7^0E and 10^0E, the region has average annual rainfall that ranges from 1,500 mm to 1,800 mm, with average annual temperature varying between 20^0C and 35^0C (NPC, 2006). These states are found around the river Niger and Benue and some of the states are richly endowed with natural mineral resources. The major ethnic groups in this zone are; Tiv, Yoruba, Idoma, Igala, Ebira, Nupe, Gbayi, Berom and Mangu. Farming is the mainstay of the rural communities' economy found in North-Central Nigeria.

3.2.1 Experimental Design and Procedure for Ethnobotanical Survey

The study was undertaken as a descriptive cross-sectional survey between January and July 2017. Several communities were selected based on ethnicity in the states found in the North-Central zone. Because of the unrest in Nassarawa state during the time of the survey, communities could not be selected and surveyed from the ethnic groups present in the state. The geographical positioning system (GPS) for the surveyed communities are shown in figure 4. Before the survey was conducted, community leaders were consulted to gain their trust and help identify possible contact person and respondents. People conversant with plants used for health care were the target of this survey which were grouped into community leaders, elderly villagers, herb sellers and herbalists adopting Dike *et al*. (2012) groupings.

With the aid of a local contact person conversant with the language and culture of the locality, targeted respondents were identified, oral interviews were carried out and data was collected using semi-structured questionnaires. The number of respondents to be interviewed in each ethnic group was based on the sample size calculation which was determined using the formula below:

$$\text{Sample Size Calculation} = \frac{Z_1 - \alpha/2^2 \, P\,(1-P)}{d^2}$$

Where $Z_1 - \alpha/2^2 = 1.96$, $d = 0.05$ and P = expected proportion in a population in percentages. (Yamane, 1967).

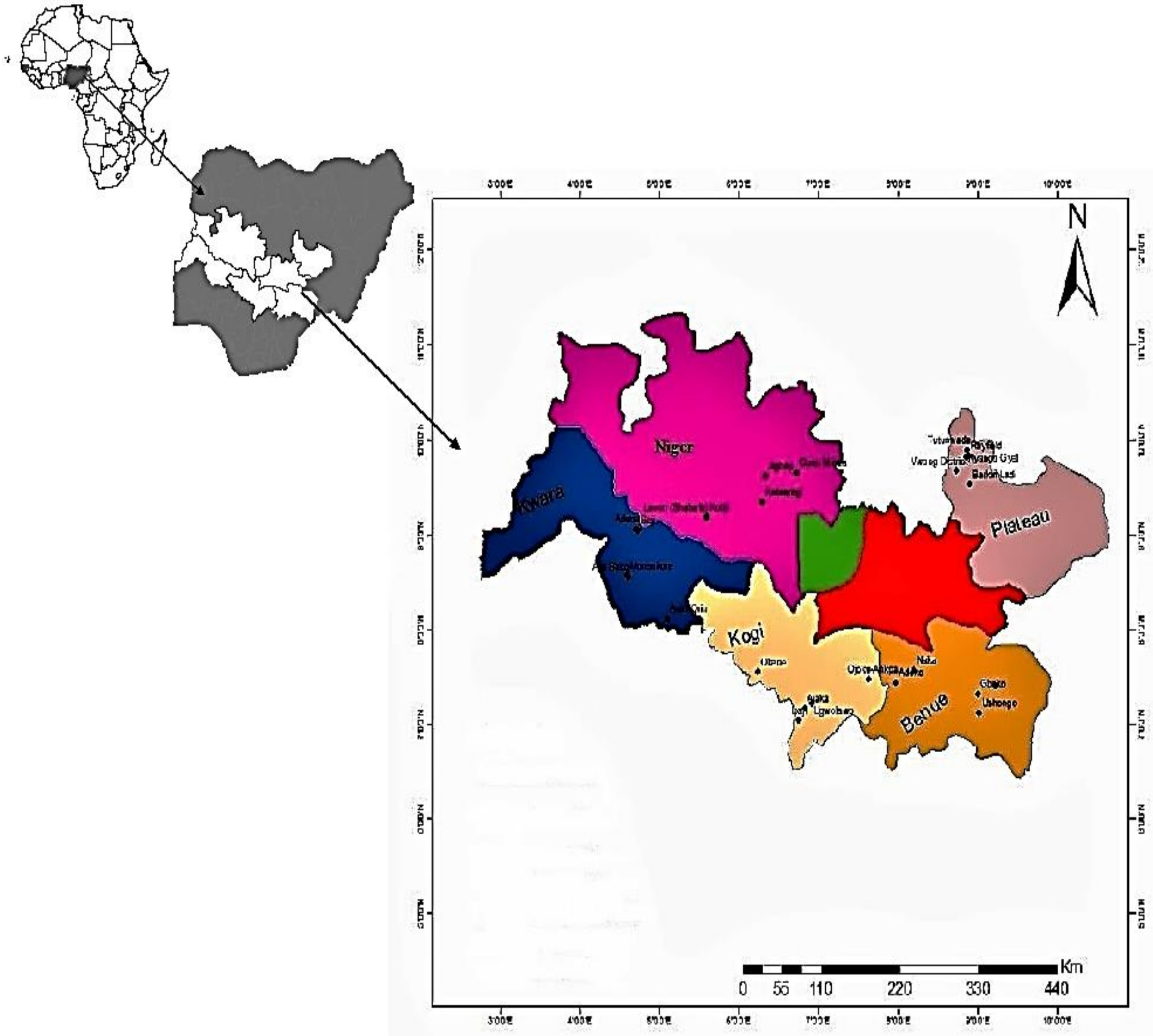


Figure 4: Map of the selected communities in the study area within the North-Central Zone, Nigeria.

3.1.2 Data Collection and Analysis

The questionnaires (appendix 5.5.1) were filled out using oral interviews with translation done by the contact person where necessary. The questionnaire gathered information on locality, sociodemographic data, insects of public health importance around them, plants used for control of these insects, vernacular names of these plant, plant parts used and habit, the condition of the plant material (dried or fresh), methods and frequency of application, accessibility, availability, source of knowledge and efficacy of insecticidal plants. All plant species mentioned were collected with the help of the villagers and identified at the Herbarium of the Department of Plant Biology, University of Ilorin.

The local importance of each plant species was calculated based on the Relative Frequency of Citation (RFC)(Iyamah and Idu, 2015). The RFC was calculated as follows:

RFC= UR/N

Where UR = number of respondents who claim the use of plants and N = total number of respondents interviewed.

ArcGis 10.1 was used to locate the GPS coordinate of questionnaire survey points and generate spatial map. Summary statistics were performed using SPSS ver. 20 (IBM) and Microsoft Office Excel 2016. The range and means of information gotten from administered questionnaire were analysed and appropriate graphs, tables, charts and percentage details were displayed. The chi-square analysis was performed to test the hypothesis and the level of significance was also determined by using a 95% confidence interval and *P*-value.

3.2 Extraction and Isolation of Plant Oils

The leaves of *Hyptis suaveolens, Ocimum gratissimum, Nicotiana tabacum, Ageratum conyzoides* and fruit peel of *Citrus sinensis*were air dried under shade for one week before hydro-distillation. The plant oils were isolated by steam-distillation using a Clevenger apparatus. The isolated oil was dried over anhydrous sodium sulphate and stored in amber coloured vials at 4^0C until required for assays.

3.2.1 Procedure

Two hundred and fifty grams (250 grams) of the air-dried leaves each of *Hyptis suaveolens, Ocimum gratissimum, Nicotiana tabacum, Ageratum conyzoides* and fruit peel of *Citrus sinensis* was subjected to hydro-distillation for 3 hours using a Clevenger type apparatus (Soham Scientific, UK). Sodium chloride (1 gram) and 20 mL of dichloromethane were added with the aqueous distillate in a separating funnel and shaking were continued for 40 minutes and this was allowed to stand for 15 minutes. The organic layer was separated and concentrated under reduced pressure. The oils dissolved in the organic layer was dried over anhydrous sodium sulphate and preserved in a sealed vial at refrigerated temperature.

3.2.2 Concentration Preparation

The five plant oils (*Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacum, Ageratum conyzoides*) to be evaluated were prepared in three concentrations: 5% (vol:vol) – 0.5mg/ml, 10% (vol:vol) – 1.0mg/ml, 15% (vol:vol) – 1.5 mg/ml, 20% (vol:vol) – 2.0mg/ml, 25% (vol:vol) – 2.5 mg/ml, 30% (vol:vol) – 3.0 mg/ml, 40% (vol:vol) – 4.0 mg/ml and 50% (vol:vol) – 5.0mg/ml. The oils were diluted in technical grade acetone (vol:vol) and applied to Whatman filter papers prior to bioassays.

From the plant oils (*Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacum, Ageratum conyzoides*), one millilitre was measured and dissolved in one hundred millilitre of acetone solvent to make the stock solution. Then five millilitres of the stock solution were mixed with ninety-five millilitres of acetone solvent to give 5% (0.5 mg/ml) of plant oil. For 10% (1.5 mg/ml) concentration, ten millilitres were drawn from the stock solution and mixed with ninety millilitres of acetone solvent. For 15% (1.5 mg/ml) concentration, fifteen millilitres of the stock solution were mixed with eighty-five millilitres of acetone solvent. For 20% (2.0 mg/ml) concentration, twenty millilitres of the stock solution were mixed with eighty millilitres of acetone solvent. For 25% (2.5 mg/ml) concentration, twenty-five millilitres of the stock solution were mixed with seventy-five millilitres of acetone solvent. For 30% (3.0 mg/ml) concentration, thirty millilitres of the stock solution were mixed with seventy millilitres of acetone solvent. For 40% (4.0 mg/ml) concentration, forty millilitres of the stock solution were mixed with sixty millilitres of acetone solvent. For 50% (5.0 mg/ml) concentration, fifty millilitres of the stock solution were mixed with fifty millilitres of acetone solvent.

3.3 Mosquito Rearing

The larvae of *Anopheles gambiae* s.l. mosquitoes were collected from the study area and reared in the Entomological Research Laboratory, University of Ilorin. The colony was maintained in a climatic controlled room at 27 ± 2 ^{0}C and 80 ± 10 RH. Mosquitoes were fed a diet of non-fatty biscuit mixed with yeast. Emerged adults were aspirated mechanically into holding cages and provided with 10% sugar solution. Adult mosquitoes were identified and Males and females were separated using morphological keys by Gillies and DeMeillon (1968; 1987).

3.4 Repellent Activity Assays

The repellent activity assays were performed on*Anopheles gambiae* s.l. mosquitoes collected from the study area. The apparatus used for this assay consisted of two connected tubes used in the WHO test kit divided into two chambers, one treated and one untreated (Deletre *et al*., 2015 modified) as shown in Figure 5.

3.4.1 Experimental Design and Procedure for Screened Collected Plants

Plant parts mentioned and shortlisted in the ethnobotanical survey as being used to manage haematophagous insects (test product) were put into the treatment chamber while the inner surface of the untreated chamber was left bare (Figure 5a). Ten non-blood-fed female *Anopheles* mosquitoes aged 4 to 7 days old were introduced into the treated chamber containing collected insecticidal plant part and after 30 seconds of the acclimation period, the sliding door separating the two chambers was opened for 10 minutes. At the end of the test period (10 minutes), the chamber was closed and the number of female mosquitoes in each chamber was recorded (Deletre *et al*., 2015 modified). This test was replicated five times for each test product.

3.4.2 Experimental Design and Procedure for Selected Plant Oils

Five plant oils were selected based on the relative frequency of citation and percentage repellency after screening. The different concentrations of plant oils to be evaluated were (Test product) applied on Whatman paper (1.0mg/ml, 3.0mg/ml and 5.0mg/ml) and put into the inner surface of the treatment chamber while the inner surface of the untreated chamber was left bare (Figure 5b). The concentrations used were selected based on their efficacy and range from the screening test. Twenty non-blood-fed female *Anopheles* mosquitoes aged 4 to 7 days old were introduced into the neutral chamber and after 30 seconds of the acclimation period, the sliding doors separating the three chambers was opened for 30 minutes and readings were taken at time intervals. At the end of the test period (30 minutes), the chambers were closed and the number of female mosquitoes in each chamber was recorded. This test was replicated four times for each test product.

The experimental designs were as follow:

1. Treated chamber - worn socks (mimicking human odour) vs untreated chamber – blank.
2. Treated chamber - solvent vs untreated chamber – blank.
3. Treated chamber - plant oils + worn socks (mimicking human odour) vs untreated chamber – blank.
4. Treated chamber – DEET + worn socks (mimicking human odour) vs untreated chamber – blank.

3.4.3 Data Analysis for Repellent Activity Assays

The proportion of *Anopheles* mosquitoes in treated and control were analysed using Student's t-test, Two-way Analysis of Variance and Tukey's post-hoc tests with the aid of Graph Pad Prism 6. The Preference Index was calculated using the formula below;

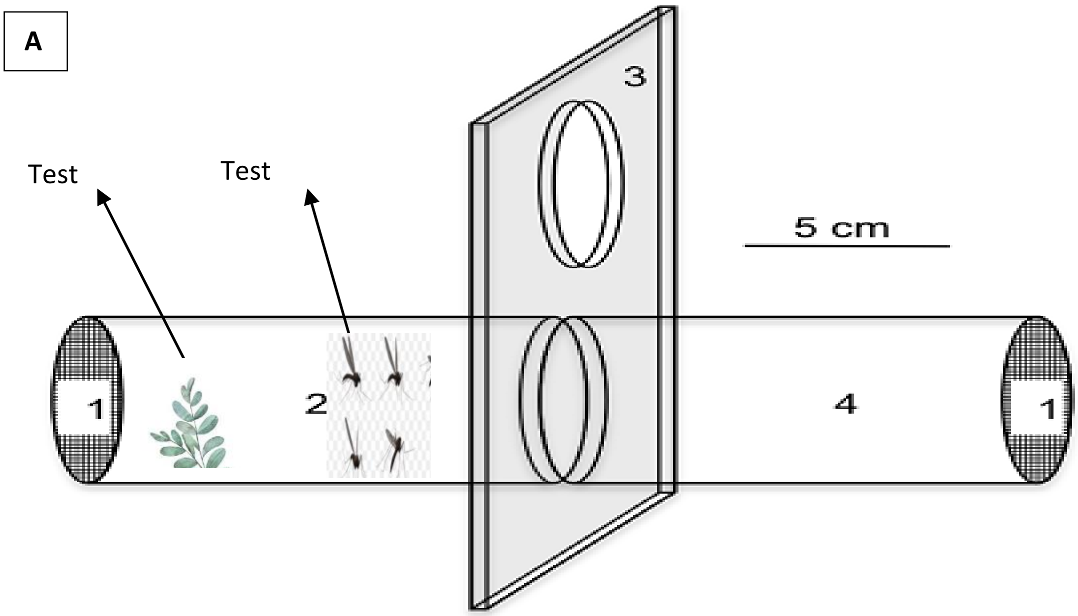
$$\text{Preference Index} = \frac{\text{Number of mosquitoes in Treated} - \text{Number of mosquitoes in Control}}{\text{Number of mosquitoes in Treated} + \text{Number of mosquitoes in Control}}$$

(Kain *et al.*, 2013)

The percentage repellency will be calculated using the formula below.

$$\text{Percentage Repellency} = \frac{\text{Number in Treated} - \text{Number in Control}}{\text{Number in Treated} + \text{Number in Control}} \times 100$$

(Kain *et al*., 2013)



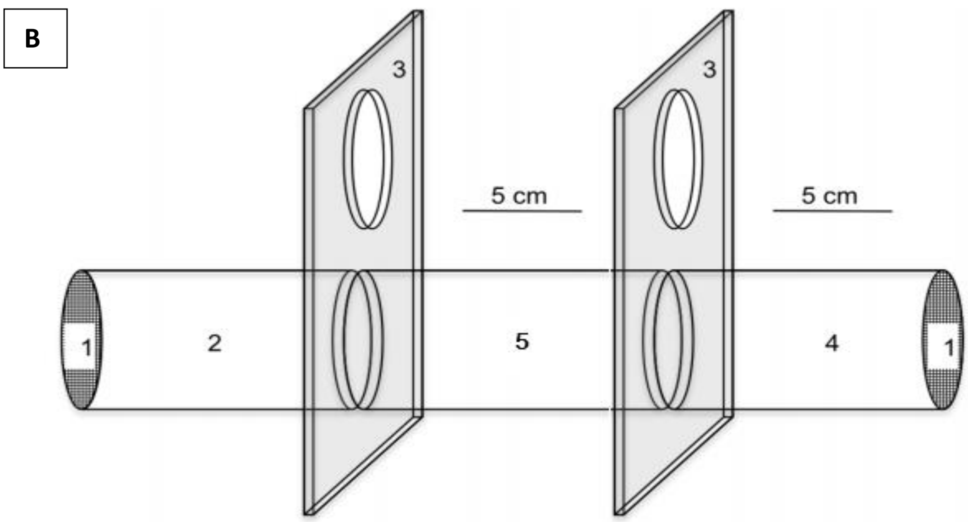


Figure 5: Schematic drawing of a simplified WHO diagnostic test kit adapted and used for the repellent activity assays: (a) 1-end cap covered by the net; 2- treated chamber; 3-Sliding

door; 4-untreated chamber. (b) 1-end cap covered by net; 2- treated chamber; 3-Sliding door; 4-untreated chamber; 5-neutral chamber. Adapted from Deletre *et al*. (2013).

3.5 Contact Toxicity Assays for Oils from Selected Plants

3.5.1 Bioassay for Assessing Larvicidal Potential of Plant Oils

The larvicidal activity of the plant oils were evaluated according to the protocol of the WHO with slight modifications (WHO, 2005). Twenty-five third instar larvae of *Anopheles* mosquitoes were introduced into test cups of 5 to 10cm depth containing 100ml of distilled water and appropriate dilution of different concentrations of the plant oils (0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml, 2.5mg/ml, 3.0mg/ml, 4.0mg/ml, 5.0mg/ml) were added. Distilled water was used as control. The number of dead larvae was recorded after 24 and 48 hours of exposure and the percentage mean mortality was calculated. The larvae were considered dead if they did not move when prodded with a needle in the syphon or cervical region. Each treatment was tested in four replicates. If the control mortality was between 5% and 20%, the mortalities of treated groups were corrected according to Abbott's formula (Abbott, 1925).

3.5.2 Bioassay for Assessing Adulticidal Potentials of Plant Oils

The adulticidal activity of the plant oils was evaluated following the WHO standard method with slight modifications (WHO, 2013). Two and a half millilitres (2.5 ml) of concentrations to be tested (0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml, 2.5mg/ml, 3.0mg/ml, 4.0mg/ml, 5.0mg/ml) were impregnated on Whatman papers (12 × 15 cm). Deltamethrin (0.05%) was used as positive control while acetone solvent was used as negative control. The impregnated papers were air-dried for 5 min and then inserted into an exposure tube in the WHO testing kit. Twenty, 2-5-day-old, blood-starved female mosquitoes were introduced into the holding tube and held for 1 h to acclimatize. The mosquitoes were transferred gently into the exposure tube and knockdown mosquitoes were determined for a 60minutes period and percentage knockdown was calculated afterwards. After 1 hr in the exposure tube, mosquitoes were transferred back to the holding tube for recovery. A pad of cotton soaked with 10% glucose solution were placed on the mesh screen to support recovering mosquitoes. At the end of the 24 hrs recovery period, the number of dead mosquitoes were recorded and the percentage mortality calculated. Each plant oil test concentrations were replicated four times.

3.5.3 Data Analysis for Contact Toxicity Assessment

Percentage knockdown was calculated using the formula below:

$$\% \text{ Knock Down} = \frac{\text{Total number of knockdown mosquitoes after 60 minutes}}{\text{Total number of exposed mosquitoes}} \times 100$$

Analysis of Variance (ANOVA) and the Tukey test was used to compare the mortality rates and percentage Knockdown in test treatments and control. The KdT_{50}, KdT_{95}, LC_{50} and LC_{90} were determined using probit analysis (Finney, 1971) with the aid of GraphPad Prism 6.

3.6 Vapour Toxicity Bioassay for Oils from Selected Plants

The toxicity assay was performed using female *Anopheles gambiae* originating from the insecticide susceptible reference Kisumu strain which was maintained in a climatic controlled room and reared following standard procedures. This test was conducted in a Peet Grady Chamber measuring 180 x 180 x 180 cm (Figure 6). A total of 100 laboratory-cultured sucrose-fed adult female mosquitoes age 2-5 days were released into the chamber from the release ports. The following concentrations 1.0mg/ml, 3.0mg/ml and 5.0mg/ml of the plant oils were applied to 200cm^2 Whatman paper using a pipette. The concentrations were selected based on screening results. The filter paper with a concentration of the treatment was hung in the centre of the chamber 50cm from the ceiling and the fan circulating air for the chamber was turned on. Knockdown mosquitoes were observed every 10 minutes for 60 minutes. After 60 minutes, all mosquitoes were then collected and placed in a clean well-labelled paper cups and the mosquitoes were provided with 10% sugar solution on cotton wool. Mortality was observed after 24 hours post-treatment. All tests were conducted at a temperature of 27±2º C and relative humidity of 80±10%. The test was replicated four times.

3.6.1 Data Analysis for Vapour Toxicity Assessment

Percentage knockdown was calculated using the formula below:

$$\% \text{ Knock Down} = \frac{\text{Total number of knockdown mosquitoes after 60minutes}}{\text{Total number of exposed mosquitoes}} \text{ X } 100$$

Analysis of Variance (ANOVA) and the Tukey test was used to compare the mortality rates and KdT in test treatments and control. The KdT_{50}, KdT_{95} was determined using probit analysis (Finney, 1971) with the aid of GraphPad Prism 6.

Figure 6: A diagram showing a Peet Grady Chamber used for Vapour toxicity assessment

3.7 Analysis of Selected Plant Oils to Identify Major Chemical Components

Coupled gas chromatography-mass spectrometry analysis (GC-MS) for five plant oils (*Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacum, Ageratum conyzoides*) were performed on a Hewlett Packard 5890 II gas chromatograph, interfaced to a single quadrupole mass selective detector (Model 5972). The column was an HP-5 MS capillary column (30 × 0.25 mm, film thickness 0.25 mm). Helium was the carrier gas, set at a flow rate of 0.6 ml/min. Injector and MS transfer line temperatures were set at 220 and 250 °C, respectively. The oven programme temperature was held at 35 °C for 5 mins and then 4 °C/min to 150 °C for 2 mins and then finally 20 °C/min to 250 °C for 5 mins. Diluted samples (10:100 in CH_2C_{l2}, v/v) of 1 µL were injected manually and in a split mode (1:100 split ratio). The identification of the components was accomplished by comparison of their relative retention indices as well as comparison of mass spectra with those of standards (for main components), those found in the literature and those supplemented by National Institute of Standards and Technology (NIST) provided by Hewlett Packard with the GC/ MS control and data processing software.

3.8 Ethical Consideration

Ethical clearance for this study was gotten from the Ethical Committee, Faculty of Life Science, University of Ilorin (UERC/ASN/2017/898). Before the commencement of the study, the aims and objective of the study were clearly explained and informed consent was obtained from each respondent during the ethnobotanical survey.

CHAPTER FOUR

4.0 RESULTS

4.1 Ethnobotanical Survey

4.1.1 Socio-Demographic Characteristics of Respondents

A total of 388 respondents were interviewed from nine ethnic groups in North-Central Nigeria. The communities were selected based on their ethnicity and the possibility of getting the required information as suggested by contact persons who are natives of these regions. The ethnic groups include; Tiv (12.4%), Nupe (24.7%), Yoruba (9.8%), Berom (13.9%), Igala (11.9%), Ebira (8.2%), Gbayi (2.6%), Mangu (4.1%) and Idoma (12.4%) with the ethnic group with the most respondents being Nupe which is found in both Kwara and Niger state followed by Berom and Tiv in Plateau state and Idoma in Benue state. The demographic data collected showed that 54.1% of the respondents were males and 45.9% were females while with regards to religious affiliation, 52.6% of the respondents were Christians, 40.7% were Islam and 6.7% were Traditional. It was also noticed that most of the respondents had a form of education with the highest (33.5%) having secondary education followed by those who had primary education (30.9%), no education (20.1%), adult education (8.2%) and tertiary education (7.2%). The predominant age group was between 41 to 60 years (48.5%) with most of the respondents being between 41 to 50 years (25.8%) followed by above 71 years (24.7%), 51 to 60 years (22.7%), 61 to 70 years (12.4%), 31 to 40 years (9.8%), less than 20 years (3.1%) and 21 to 30 years (1.5%). Majority of the respondents were married (85.1%) followed by those who are widowed (7.7%) and single (7.2%) (Table 4). A total of 388 respondents were interviewed which was based on their knowledge of plants used for health care in their communities. The respondents were grouped into herb sellers (31.96%), traditional healers (15.98%), community heads (4.64%) and elders

(47.42%) with the most interviewed been community elders followed by herb sellers (Figure 5).

Table4:Socio-demographic Characteristics of Respondents in North-Central Nigeria

Socio-demographic Characteristics of Variables		Frequency (n=388)	Percent
Gender	Male	210	54.1
	Female	178	45.9
Ethnicity	Tiv	48	12.4
	Nupe	96	24.7
	Yoruba	38	9.8
	Berom	54	13.9
	Igala	46	11.9
	Ebira	32	8.2
	Gbayi	10	2.6
	Mangu	16	4.1
	Idoma	48	12.4
Educational Status	None	78	20.1
	Primary	120	30.9
	Secondary	130	33.5
	Tertiary	28	7.2
	Adult Education	32	8.2
Religion	Christianity	204	52.6
	Islam	158	40.7
	Traditional	26	6.7
Age	Less than 20 years	12	3.1
	21-30 years	6	1.5
	31-40 years	38	9.8
	41-50 years	100	25.8
	51-60 years	88	22.7
	61-70 years	48	12.4
	Above 71 years	96	24.7
Marital Status	Single	28	7.2
	Married	330	85.1
	Widowed	30	7.7

n = total number of respondents

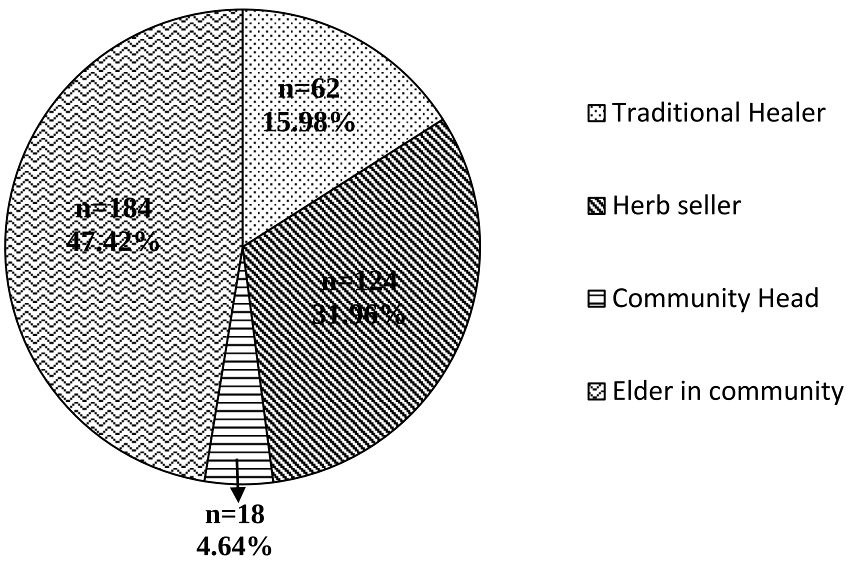


Figure 5:Target respondents interviewed during the ethnobotanical survey**.**

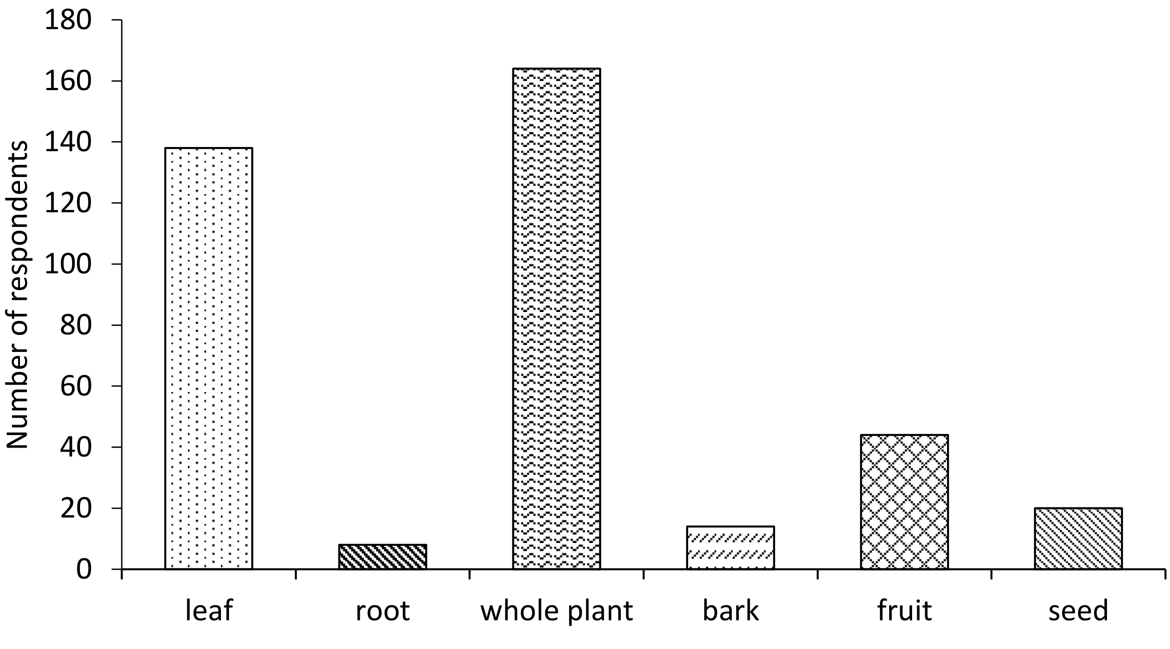


Figure 6:Insecticidal plant materials used by respondents for the management of haematophagous insects.

4.1.2 Information on Identified Insecticidal Plants in The Study Areas

The ethnobotanical survey documented seventeen plant species which were used by local inhabitants in the study area for personal protection against haematophagous insects. The identified and shortlisted plants belonged to 12 families among which Lamiaceae was the most represented with three species (*Hyptis suaveolens*, *Ocimum gratissimum* and *Thymus vulgaries*) followed by Solanaceae (*Capsicum annuum* and *Nicotiana tabacum*) and Fabaceae (*Cassia mimosoides* and *Parkia biglobosa*) with two species each and Asteraceae, Amaryllidaceae, Annonaceae, Rutaceae, Poaceae, Myrtaceae, Malvaceae, Verbenancae, Moringaceae with one species each. The most frequently mentioned plants were *Hyptis suaveolens* (19.6%, UR = 76/388, RFC= 0.196) followed by *Ocimum gratissimum*(18.7%, UR = 73/388, RFC= 0.187), *Citrus sinensis* (10.8% UR = 42/388, RFC=0.108), *Ageratumconyzoides*, *Cymbpogon citratus*and *Thymus vulgaries* (6.2%, UR = 24/388, RFC= 0.062 each) and *Nicotiana tabacum* (5.6%, UR=22/388, RFC=0.057). Other plants identified are; *Capsicum annuum* and *Cassia mimosoides* (4.1%, UR=16/388, RFC=0.041), *Annona senegalensis, Eucalyptus globulus and Parkia biglobosa*(3.1%, UR=12/388, RFC=0.031), *Hibiscus rosasinensis* (2.3%, UR=9/388, RFC=0.023),*Allium cepa* and *Ertyphleum suaveolens* (2.1%, UR=8/388, RFC=0.021), *Latanna camara* (1.5%, UR=6/388, RFC=0.015) and *Moringa oleifera* (1.0%, UR=4/388, RFC=0.010) (Table 5).

Allium cepa was classified by respondents as herb while *Ageratumconyzoides*,*Capsicum annuum*, *Cymbpogon citratus*,*Hibiscus rosasinensis*,*Hyptis suaveolens*,*Latanna camara*, *Nicotiana tabacum*,*Ocimum gratissimum* and *Thymus vulgaries*were classified by respondents as shrubs and *Annona senegalensis, Eucalyptus globulus, Moringa oleifera, Ertyphleum suaveolens, Parkia biglobosa* and *Citrus sinensis* were classified as trees.

4.1.3 Plant Parts Used in The Study Sites and Their Modes of Administration

Most of the plants identified were classified as shrubs (58.8%) while the others were trees (35.3%). Whole plants (42.3%) were the most used plant parts followed by leaves (35.6%). Other plant parts used were fruits (11%), seeds (5%) bark (4%) and roots (2%) (Figure 6). Most of the respondents reported that they used plants in their fresh states (52.9%) by hanging them inside their abode while the rest of the respondents reported that the plants were used dried (47.1%) by smouldering the plant materials to make smoke (Table 5). Pictures of collected, shortlisted and identified plants parts are shown in plates 1-17.

Table 5:Information on Insecticidal plants used for management of haematophagus insects in North-central Nigeria.

S/No	Scientific name	Family name	Local name	Voucher number	UR (%)	RFC (n=388)	State of use	Habit	Method of Application
1.	*Ageratum conyzoides*	Asteraceae	Itanajuwe(I), Hurhur(T)	UILH/013/140	24(6.2)	0.062	Fresh	Shrub	Hanging
2.	*Allium cepa*	Amaryllidaceae	Lubasakuchi(B)	UILH/008/1332	8(2.1)	0.021	Fresh	Herb	Hanging
3.	*Annona senegalensis*	Annonaceae	Abobo(Y), Gwandardaji(N)	UILH/004/499	12(3.1)	0.031	Fresh	Tree	Hanging
4.	*Capsicum annuum*	Solanaceae	Akpoko(I)	UILH/015/532	16(4.1)	0.041	Fresh	Shrub	Smoke
5.	*Cassia mimosoides*	Fabaceae	Gabaruwankasa(B)	UILH/017/534	16(4.1)	0.041	Dried	Tree	Smoke
6.	*Citrus sinensis*	Rutaceae	Ekpo Osan(Y)	UILH/010/159	42(10.8)	0.108	Dried	Tree	Smoke
7.	*Cymbpogon citratus*	Poaceae	Ile(I), Ganyenti(B)	UILH/011/949	24(6.2)	0.062	Fresh	Shrub	Hanging
8.	*Ertyphleum suaveolens*	Leguminae	Goska(B) Gwaska (N), Obo (Y)	UILH/016/221	8(2.1)	0.021	Dried	Tree	Smoke
9.	*Eucalyptus globulus*	Myrtaceae	Turare (N) Raskata (B)	UILH/006/1073	12(3.1)	0.031	Fresh	Tree	Hanging
10.	*Hibiscus rosasinensis*	Malvaceae	Roasemosquita(B)	UILH/007/710	9(2.3)	0.023	Dried	Shrub	Hanging
11.	*Hyptis suaveolens*	Lamiaceae	Tamwotswagi(N), Jogbo(Y), Olufofo(I), Daddoya tadaji(B), Hurhur(T)	UILH/003/931	76(19.6)	0.196	Fresh	Shrub	Hanging
12.	*Latanna camara*	Verbenacae	Latana(B)	UILH/009/509	6(1.5)	0.015	Dried	Shrub	Smoke
13.	*Moringa oleifera*	Moringaceae	Zogole(B)	UILH/002/559	4(1.0)	0.010	Dried	Tree	Smoke
14.	*Nicotiana tabacum*	Solanaceae	Taba (N)	UILH/005/504	22(5.6)	0.057	Dried	Shrub	Smoke
15.	*Ocimum gratissimum*	Lamiaceae	Efirin(Y), Anyeba(I), Daidoya(T), KunguraKu-u-tamen(N)	UILH/012/984	73(18.7)	0.188	Fresh	Shrub	Hanging
16.	*Parkia biglobosa*	Fabaceae	Nungoro(N), Ekunigba(Y)	UILH/001/948	12(3.1)	0.031	Dried	Tree	Smoke
17.	*Thymus vulgaries*	Lamiaceae	Tamwotswagi(N) Efrin wewe (Y)	UILH/012/851	24(6.2)	0.062	Fresh	Shrub	Hanging

UR= *Number of respondents who claim the use of plants, RFC= Relative Frequency of Citation, N=Nupe, Y=Yoruba, B=Berom, T=Tiv, I=Igala*

Plate 1: *Moringa olifera*

Plate 2: *Hyptis suaveolens*

Plate 3: *Annona senegalensis*

Plate 4: *Parkia biglobosa*

Plate 5: *Nicotiana tabacum*

Plate 6: *Eucalyptus globulus*

Plate 7: *Hibiscus rosa sinensis*

Plate 8: *Allium cepa*

Plate 9: *Latanna camara*

Plate 10: *Citrus sinensis*

Plate 11: *Cymbpogon citratus*

Plate 12: *Ocimum gratissium*

Plate 13: *Ageratum conyzoides*

Plate 14: *Thymus vulgaries*

Plate 15: *Capsicum annuum*

Plate 16: *Ertyphleum suaveolens*

Plate 17: *Cassia mimosoides*

4.1.4 Distribution of Shortlisted Plants by Ethnicity and Target Respondents in North-Central Nigeria

Hyptis suaveolens and *Ocimum gratissimum* were the most represented insecticidal plant shortlisted by all of the nine ethnic groups for personal protection against haematophagus insects followed by *Citrus sinensis* which was shortlisted in 6 ethnic groups namely; Berom, Idoma, Mangu, Nupe, Tiv and Yoruba and *Nicotiana tabacum* shortlisted in 4 ethnic groups (Berom, Ebira, Idoma and Yoruba). *Thymus vulgaries* was shortlisted in 3 ethnic groups (Idoma, Nupe and Tiv) while *Ageratum conyzoides,Annona senegalensis, Capsicum Annuum, Cymbpogon citratus, Eucalyptus globulus, Hibiscus rosa sinensis* and *Parkia biglobosa* were shortlisted in two ethnic groups and the others in one ethnic group (Table 6).

Hyptis suaveolens and *Thymus vulgaries* were the only plants shortlisted by all the target respondents as perceived to having insecticidal potential followed by *Ageratum conyzoides, Annona senegalensis, Capsicum annuum, Citrus sinensis, Cymbpogon citratus, Eucalyptus globulus, Nicotiana tabacum* and *Ocimum gratissimum* were only shortlisted by three target respondents as having insecticidal potential. Majority of the insecticidal plants were shortlisted by Herb sellers and Elders in communities (Table 7).

Table 6: Distribution of Shortlisted plants by Ethnicity in North-central Nigeria

Plants	**Berom**	**Ebira**	**Gbayi**	**Idoma**	**Igala**	**Mangu**	**Nupe**	**Tiv**	**Yoruba**	**Total**
Ageratum conyzoides	NR	NR	NR	11	NR	NR	NR	13	NR	24
Allium cepa	NR	NR	NR	NR	NR	NR	8	NR	NR	8
Annona senegalensis	8	NR	NR	NR	NR	4	NR	NR	NR	12
Capsicum annuum	NR	4	NR	NR	12	NR	NR	NR	NR	16
Cassia mimosoides	NR	NR	NR	NR	NR	NR	16	NR	NR	16
Citrus sinensis	6	NR	NR	8	NR	2	14	8	4	42
Cymbpogon citratus	16	NR	NR	NR	NR	NR	NR	8	NR	24
Ertyphleum suaveolens	8	NR	NR	NR	NR	NR	NR	NR	NR	8
Eucalyptus globulus	8	NR	NR	NR	NR	4	NR	NR	NR	12
Hibiscus rosa sinensis	6	NR	NR	NR	NR	NR	NR	3	NR	9
Hyptis suaveolens	3	24	4	8	4	4	2	9	18	76
Latanna camara	6	NR	NR	NR	NR	NR	NR	NR	NR	6
Moringa oleifera	4	NR	NR	NR	NR	NR	NR	NR	NR	4
Nicotiana tabacum	4	4	NR	8	NR	NR	NR	NR	6	22
Ocimum gratissimum	3	23	2	8	4	4	2	9	18	73
Parkia biglobosa	NR	NR	NR	NR	NR	NR	5	NR	7	12
Thymus vulgaries	NR	NR	NR	6	NR	NR	12	6	NR	24
Total	72	55	6	49	20	18	59	56	53	388

NR = No Record

Table 7: Distribution of Shortlisted plants by target respondents in North-central Nigeria

Plants	**Community Head**	**Elder in community**	**Herb seller**	**Traditional Healer**	**Total**
Ageratum conyzoides	NR	15	3	6	24
Allium cepa	NR	8	NR	NR	8
Annona senegalensis	NR	3	3	6	12
Capsicum annuum	2	6	8	NR	16
Cassia mimosoides	4	12	NR	NR	16
Citrus sinensis	NR	23	5	14	42
Cymbpogon citratus	NR	12	11	1	24
Ertyphleum suaveolens	NR	2	NR	6	8
Eucalyptus globulus	4	4	4	NR	12
Hibiscus rosa sinensis	NR	5	4	NR	9
Hyptis suaveolens	5	37	21	13	76
Latanna camara	NR	NR	4	2	6
Moringa oleifera	NR	NR	4	NR	4
Nicotiana tabacum	NR	6	8	8	22
Ocimum gratissimum	NR	38	31	4	73
Parkia biglobosa	NR	NR	12	NR	12
Thymus vulgaries	4	12	4	4	24
Total	19	183	122	64	388

NR = No Record

4.1.5 Investigation of Source of Knowledge on the Use of Plant, its Availability and Accessibility among the Respondents

Majority of the respondents acknowledged that the shortlisted insecticidal plants were available around the community (88.4%) while the other respondents reported that they collected the plants from the forest (8.8%) and bought it from the market (2.8%). Assessing the accessibility of insecticidal plant among respondents, majority of the respondents had to travel less than 1km to harvest plant materials used for personal protection (77%) while the other respondents had to travel between 1 to 2 km (20.4%) and greater than 5km (2.6%) to harvest these plants. Information on the source of knowledge on plant use among respondents indicated that 83.8% of them obtained the information from family members while 12.6% of the respondents obtained the information from friends and neighbours and 3.6% through divination.

4.1.6 Assessment of Respondents Knowledge on how Plants are Applied and its Effectiveness.

The assessment of knowledge on how plants are applied showed that majority of the respondents (99%) applied the plants inside their houses while 1% of the respondents applied it in sewage systems found around their houses. The frequency of application of these plants was mostly once a day (71.6%) followed by once a week (21.7%), month (4.6%) and year (2.1%) as shown in Table 9. The respondents (76.3%) ascertained that the identified insecticidal plants were percived to be very effective against haematophagus insects while 23.2% of the respondents felt that the insecticidal plants were effective and 0.5% of the respondents considered insecticidal plants as not so effective in controlling haematophagous insects.

Table 8:Perception of respondents to insecticidal plant accessibility, effectiveness and source of information

S/No.	Scientific name	Respondents		Availability			Distance travelled to harvest plant			Source of Knowledge		
		UR (n=388)	%	Forest	Around community	Market	< 1Km	1-2 Km	>5 Km	Family Members	Divination	Friend/ Neighbour
1.	*Parkia biglobosa*	12	3.0	2(16.7)	10(83.3)	NR	10(83.3)	2(16.7)	NR	10(83.3)	NR	2(16.7)
2.	*Moringa oleifera*	4	1.0	NR	4(100)	NR	4(100)	NR	NR	4(100)	NR	NR
3.	*Hyptis suaveolens*	76	19.6	NR	76(100)	NR	76(100)	NR	NR	72(94.7)	NR	4(5.3)
4.	*Annona senegalensis*	12	3.3	NR	12(100)	NR	12(100)	NR	NR	12(100)	NR	NR
5.	*Nicotiana tabacum*	22	5.6	NR	22(100)	NR	22(100)	NR	NR	10(45.5)	NR	12(54.5)
6.	*Eucalyptus globulus*	12	3.3	2(16.7)	6(50)	4(33.3)	8(66.7)	4(33.3)	NR	12(100)	NR	NR
7.	*Hibiscus rosa sinensis*	9	2.3	2(22.2)	4(44.5)	3(33.3)	4(44.5)	5(55.5)	NR	9(100)	NR	NR
8.	*Allium cepa*	8	2.1	8(100)	NR	NR	NR	8(100)	NR	8(100)	NR	NR
9.	*Latanna camara*	6	1.5	4(66.7)	2(33.3)	NR	NR	2(33.3)	4(66.7)	4(66.7)	NR	2(33.3)
10.	*Citrus sinensis*	42	10.8	2(4.8)	40(95.2)	NR	42(100)	NR	NR	20(47.6)	2(4.8)	20(47.6)
11.	*Cymbpogon citratus*	24	6.1	NR	24(100)	NR	22(91.7)	2(8.3)	NR	24(100)	NR	NR
12.	*Ocimum gratissimum*	73	18.7	2(2.7)	71(97.3)	NR	47(64.4)	20(27.4)	6(8.2)	69(94.5)	4(5.5)	NR
13.	*Ageratum conyzoides*	24	6.2	NR	24(100)	NR	24(100)	NR	NR	24(100)	NR	NR
14.	*Thymus vulgaries*	24	6.2	NR	24(100)	NR	14(58.3)	10(41.7)	NR	15(62.5)	4(16.7)	5(20.8)
15.	*Capsicum annuum*	16	4.1	NR	12(75)	4(25)	14(87.5)	2(12.5)	NR	12(75)	4(25)	NR
16.	*Ertyphleum suaveolens*	8	2.1	8(100)	NR	NR	NR	8(100)	NR	8(100)	NR	NR
17.	*Cassia mimosoides*	16	4.1	4(25)	12(75)	NR	NR	16(100)	NR	12(75)	NR	4(25)
	Total			**34**	**343**	**11**	**299**	**79**	**10**	**325**	**14**	**49**
	Percent			**8.8**	**88.4**	**2.8**	**77**	**20.4**	**2.6**	**83.8**	**3.6**	**12.6**

UR= *Number of respondents who claim the use of plants, the percentage in parenthesis*, NR = *No Record of information from respondents*, % = Percentage

Table 9:Method and Frequency of Application of Insecticidal plants

S/No.	Scientific name	Respondents		Method of Application		Frequency of Application				Efficacy		
		UR	%	Inside Houses	Sewage Systems	Once a day	Once a week	Once a month	Once a Year	Very Effective	Effective	Not So Effective
1.	*Parkia biglobosa*	12	3.0	12(100)	NR	12(100)	NR	NR	NR	12(100)	NR	NR
2.	*Moringa oleifera*	4	1.0	4(100)	NR	NR	4(100)	NR	NR	4(100)	NR	NR
3.	*Hyptis suaveolens*	76	19.6	76(100)	NR	52(68.4)	16(21.1)	8(10.5)	NR	46(60.5)	30(39.5)	NR
4.	*Annona senegalensis*	12	3.3	12(100)	NR	12(100)	NR	NR	NR	12(100)	NR	NR
5.	*Nicotiana tabacum*	22	5.6	22(100)	NR	18(81.8)	4(18.2)	NR	NR	16(72.7)	6(27.3)	NR
6.	*Eucalyptus globulus*	12	3.3	12(100)	NR	8(66.7)	NR	4(33.3)	NR	10(83.3)	2(16.7)	NR
7.	*Hibiscus rosa sinensis*	9	2.3	9(100)	NR	NR	9(100)	NR	NR	9(100)	NR	NR
8.	*Allium cepa*	8	2.1	8(100)	NR	4(50)	NR	4(50)	NR	4(50)	4(50)	NR
9.	*Latanna camara*	6	1.5	6(100)	NR	2(33.3)	NR	NR	4(66.7)	4(66.7)	2(33.3)	NR
10.	*Citrus sinensis*	42	10.8	42(100)	NR	38(90.5)	4(9.5)	NR	NR	36(85.7)	4(9.5)	2(4.8)
11.	*Cymbpogon citratus*	24	6.1	22(91.7)	2(8.3)	16(66.7)	6(25)	NR	2(8.3)	22(91.7)	2(8.3)	NR
12.	*Ocimum gratissimum*	73	18.7	73(100)	NR	46(63)	25(34.3)	2(27)	NR	61(83.6)	12(16.4)	NR
13.	*Ageratum conyzoides*	24	6.2	24(100)	NR	22(91.7)	2(8.3)	NR	NR	24(100)	NR	NR
14.	*Thymus vulgaries*	24	6.2	24(100)	NR	20(83.3)	4(16.7)	NR	NR	24(100)	NR	NR
15.	*Capsicum annuum*	16	4.1	16(100)	NR	12(75)	4(25)	NR	NR	4(25)	12(75)	NR
16.	*Ertyphleum suaveolens*	8	2.1	6(75)	2(25)	NR	6(75)	NR	2(25)	8(100)	NR	NR
17.	*Cassia mimosoides*	16	4.1	16(100)	NR	16(100)	NR	NR	NR	NR	16(100)	NR
	Total			**384**	**4**	**27.8**	**84**	**18**	**8**	**296**	**90**	**2**
	Percent			**99**	**1**	**71.6**	**21.7**	**4.6**	**2.1**	**76.3**	**23.2**	**0.5**

UR= *Number of respondents who claim the use of plants, the percentage in parenthesis,* NR = *No Record of information from respondents*

Table 10:Knowledge and effectiveness of insecticidal plants in relation to gender, age, Educational Status and Ethnicity

Variables		**Respondents**		**Knowledge of plants**	**Efficacy of Plant**			**P-Values**
		UR	**%**		**VEF**	**EF**	**NSEF**	
Gender	Male	210	54.1	210	126	78	NR	p=0.000
	Female	178	45.9	178	152	24	2	df=2
								χ^2=31.396
Age	Less than 20 years	12	3.1	12	12	NR	NR	
	21-30 years	6	1.5	6	4	2	NR	
	31-40 years	38	9.8	38	18	20	NR	
	41-50 years	100	25.8	100	80	20	NR	p=0.007
	51-60 years	88	22.7	88	44	42	2	df=12
	61-70 years	48	12.4	48	32	10	NR	χ^2=64.445
	Above 71 years	96	24.7	96	88	8	NR	
Educational Status	None	78	20.1	78	70	8	NR	
	Primary	120	30.9	120	88	24	2	p=0.000
	Secondary	130	33.5	130	106	24	NR	df=8
	Tertiary	28	7.2	28	10	18	NR	χ^2=102.405
	Adult Education	32	8.2	32	4	28	NR	
Ethnicity	Tiv	48	12.4	48	48	NR	NR	
	Nupe	96	24.7	96	40	56	NR	
	Yoruba	38	9.8	38	26	6	NR	
	Berom	54	13.9	54	48	4	2	p=0.000
	Igala	46	11.9	46	28	18	NR	df=16
	Ebira	32	8.2	32	20	12	NR	χ^2=116.865
	Gbayi	10	2.6	10	10	NR	NR	
	Mangu	16	4.1	16	10	6	NR	
	Idoma	48	12.4	48	48	NR	NR	

UR= *Number of respondents who claim the use of plants, the percentage in parenthesis, VEF-Very Effective, EF-Effective, NSEF-Not So Effective, NEF-Not Effective,* NR = *No Record of information from respondents*

4.1.7 Relationship Between Perceived Insecticidal Plant Efficacy and Gender, Age, Educational Status and Ethnicity of Respondents.

Complete knowledge of insecticidal plants used for personal protection purposes against haematophagus insects was recorded in thid study by respondents. Out of the male respondents, 126 perceived the plants to be very effective and 78 perceived the plants to be effective while out of the female counterparts, 152 perceived the plants to be very effective and 24 perceived them as effective while 2 perceived them as not so effective. Within the age group 41 to 50 years, 80 perceived them as very effective and 20 perceived them as effective while within the age group 51 to 60 years, 44 perceived them as very effective, 42 perceived them as effective and 2 perceived them as not effective. Within the age group that is greater than 71 years, 88 perceived it as very effective and 8 perceived them as effective. Based on their educational status, within those with no education, 70 perceived them to be very effective and 8 perceived them to be effective while within those with primary education, 88 perceived them to be very effective, 24 perceived them to be effective and 2 perceived them as not so effective. Within those with secondary school education, 106 of them perceived them to be very effective and 24 of them perceived them to be effective.

However, there exist a significant relationship in the perceived efficacy of these insecticidal plants for personal protection against haematophagus insects and gender (P value=0.000, df=2, χ^2 =31.396), age (P value=0.007, df = 112, χ^2=64.445), educational status (P value=0.000, df=8, χ^2 = 102.405) and ethnicity (P value = 0.000, df = 16, χ^2 = 116.865) of respondents (Table 10).

4.2 Repellency Activity of Shortlisted Plants against *Anopheles gambiae* s.l.

4.2.1 Repellency Effect of *Ageratum conyzoides* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Ageratum conyzoides* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (9.33±1.15) was significantly more (P=0.0007) in comparison to those in the treated chamber (0.67±1.15). After analysis of the difference between *Ageratum conyzoides* leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Ageratum conyzoides* (L) (ANOVA, $F_{(1, 72)}$ = 359.5, P>0.9999) (Figure 7). The preference index is -0.87 while the percentage repellency is 86.67% which is same as obtainable in DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

4.2.2 Repellency Effect of *Allium cepa* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Allium cepa* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (7.33±1.53) was not significantly more (P=0.0202) in comparison to those in the treated chamber (2.67±1.53). After analysis of the difference between *Allium cepa*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Allium cepa*(L) (ANOVA, $F_{(1, 72)}$ = 359.5, P=0.3662) (Figure 8). The preference index is -0.47 while the percentage repellency is 46.67% which was significantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

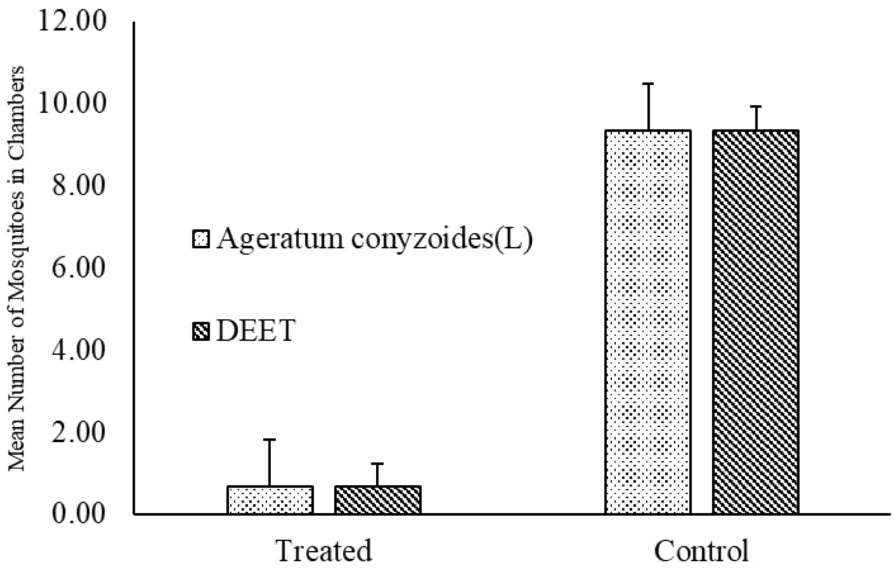


Figure 7: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Ageratum conyzoides* Leave and DEET

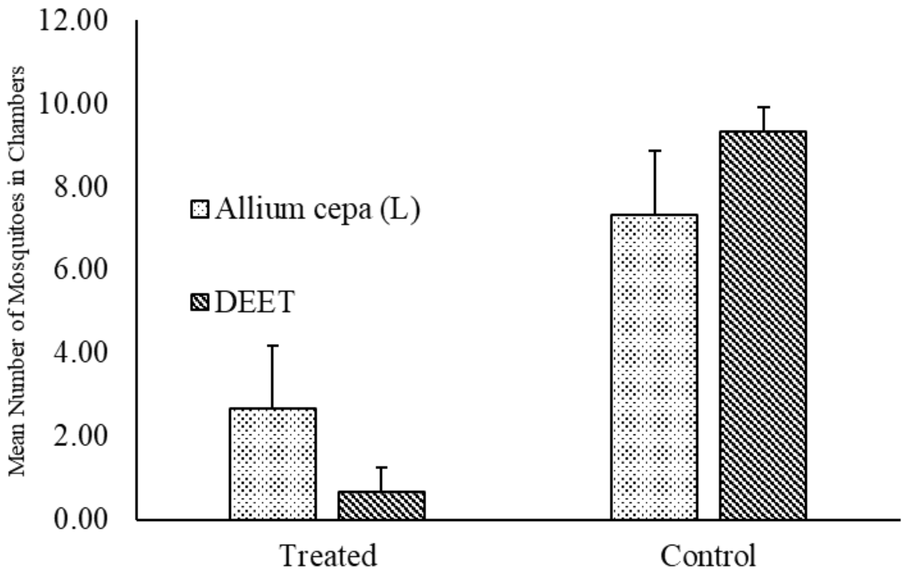


Figure 8: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Allium cepa*Leave and DEET

4.2.3 Repellency Effect of *Annona senegalensis* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Annona senegalensis* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (7.00±1.00) was not significantly more (P=0.0080) in comparison to those in the treated chamber (3.00±1.00). After analysis of the difference between *Annona senegalensis*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Annona senegalensis* (L) (ANOVA, $F_{(1, 72)} = 359.5$, P=0.1978) (Figure 9). The preference index is -0.40 while the percentage repellency is 40.00% which was significantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

4.2.4 Repellency Effect of *Capsicum annuum* Fruit on *Anopheles gambiae* s.l.

Repellency activity of *Capsicum annuum* Fruit on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (7.67±1.53) was not significantly more (P=0.0129) in comparison to those in the treated chamber (2.33±1.53). After analysis of the difference between *Capsicum annuum*Fruit and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Capsicum annuum*(F) (ANOVA, $F_{(1, 72)} = 359.5$, P=0.6074) (Figure 10). The preference index is -0.53 while the percentage repellency is 53.33% significantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

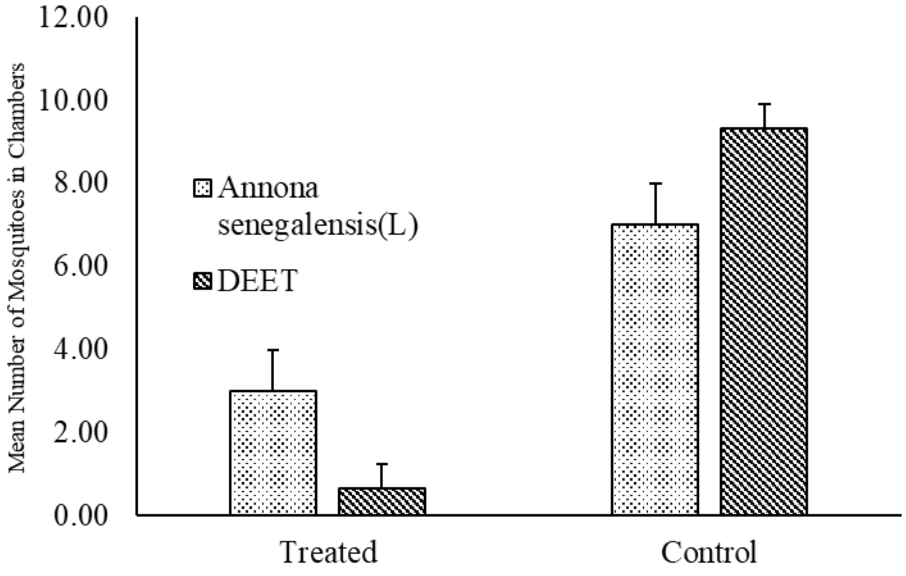


Figure 9: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Annona senegalensis* Leave and DEET

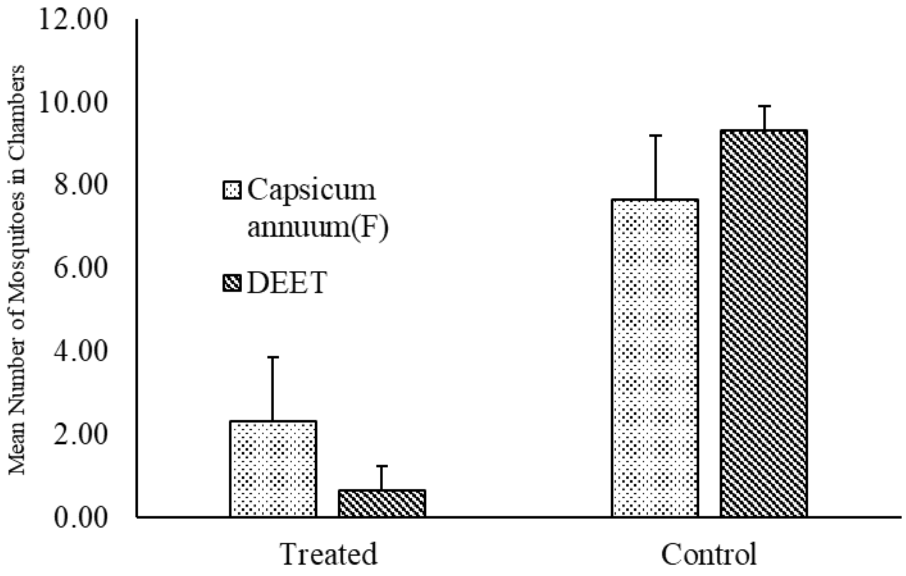


Figure 10: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Capsicum annuum*Fruit and DEET

4.2.5 Repellency Effect of *Cassia mimosoides* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Cassia mimosoides* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (4.33±1.53) was not significantly more (P=0.3438) in comparison to those in the treated chamber (5.67±1.53). After analysis of the difference between *Cassia mimosoides*Leave and DEET (a standard repellent), it was observed that there was significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Ageratum conyzoides* (L) (ANOVA, $F_{(1, 72)} = 359.5$, P< 0.0001) (Figure 11). The preference index is 0.13 while the percentage repellency is -13.33% which was significantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

4.2.6 Repellency Effect of *Citrus sinensis* Fruit peel on *Anopheles gambiae* s.l.

Repellency activity of *Citrus sinensis* Fruit peels on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (1.00±1.00) was significantly more (P=0.0006) in comparison to those in the treated chamber (9.00±1.00). After analysis of the difference between *Citrus sinensis* Fruit peeland DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Citrus sinensis*(F) (ANOVA, $F_{(1, 72)} = 359.5$, P=0.9996) (Figure 12). The preference index is -0.80 while the percentage repellency is 80.00% which was close to what was obtainable in DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

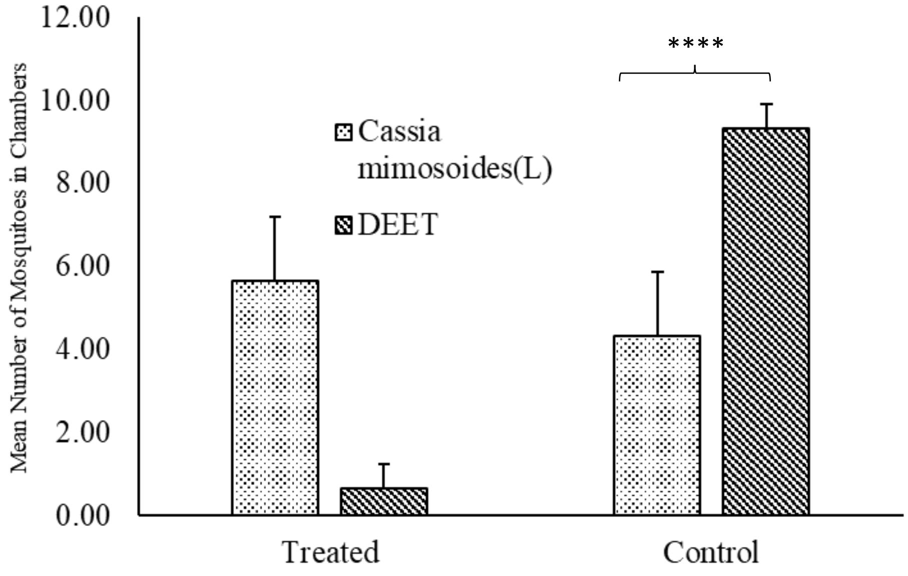


Figure 11: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Cassia mimosoides*Leave and DEET

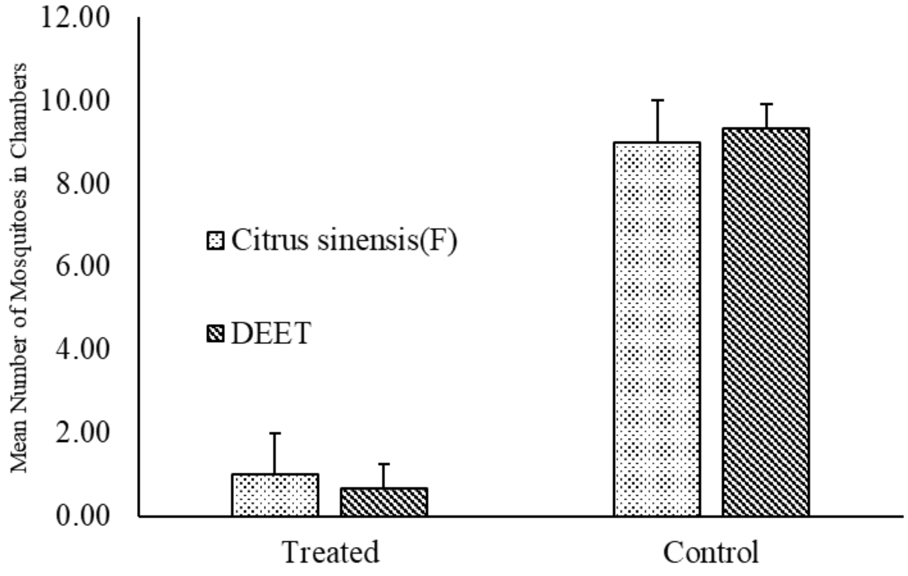


Figure 12: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Citrus sinensis* Fruit peel and DEET

4.2.7 Repellency Effect of *Cymbpogon citratus* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Cymbpogon citratus* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (7.00±2.00) was not significantly more (P=0.0704) in comparison to those in the treated chamber (3.00±2.00). After analysis of the difference between *Cymbpogon citratus*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Cymbpogon citratus*(L) (ANOVA, $F_{(1, 72)} = 359.5$, P=1978) (Figure 13). The preference index is -0.40 while the percentage repellency is 40.00% which was signifiantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

4.2.8 Repellency Effect of *Ertyphleum suaveolens* Bark on *Anopheles gambiae* s.l.

Repellency activity of *Ertyphleum suaveolens* bark on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (7.33±1.53) was not significantly more (P=0.0704) in comparison to those in the treated chamber (2.67±1.53). After analysis of the difference between *Ertyphleum suaveolens* Barkand DEET (a standard repellent), it was observed that there was significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Ertyphleum suaveolens*(B) (ANOVA, $F_{(1, 72)} = 359.5$, $P < 0.0001$) (Figure 14). The preference index is -0.20 while the percentage repellency is 20.00% which was significantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

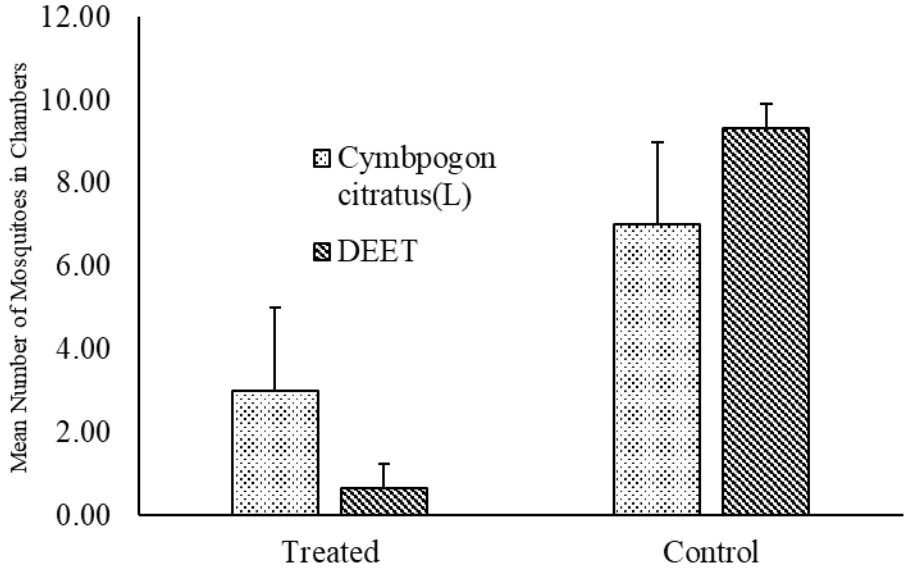


Figure 13: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Cymbpogon citratus*Leave and DEET

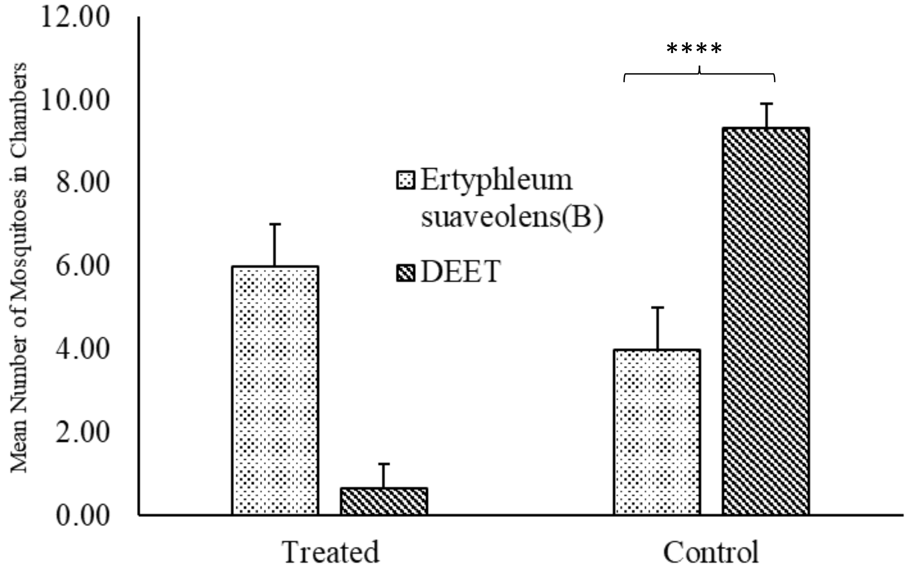


Figure 14: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Ertyphleum suaveolens* Barkand DEET

4.2.9 Repellency Effect of *Eucalyptus globulus* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Eucalyptus globulus* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (7.67±1.53) was not significantly more (P=0.0129) in comparison to those in the treated chamber (2.33±1.53). After analysis of the difference between *Eucalyptus globulus*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Eucalyptus globulus*(L) (ANOVA, $F_{(1, 72)} = 359.5$, P= 0.6074) (Figure 15). The preference index is -0.53 while the percentage repellency is 53.33% which was significantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

4.2.10 Repellency Effect of *Hibiscus rosa sinensis* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Hibiscus rosa sinensis* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (7.67±0.58) was significantly more (P=0.0003) in comparison to those in the treated chamber (2.33±0.58). After analysis of the difference between *Hibiscus rosa sinensis*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Hibiscus rosa sinensis*(L) (ANOVA, $F_{(1, 72)} = 359.5$, P=0.6074) (Figure 16). The preference index is -0.53 while the percentage repellency is 53.33% which was significantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

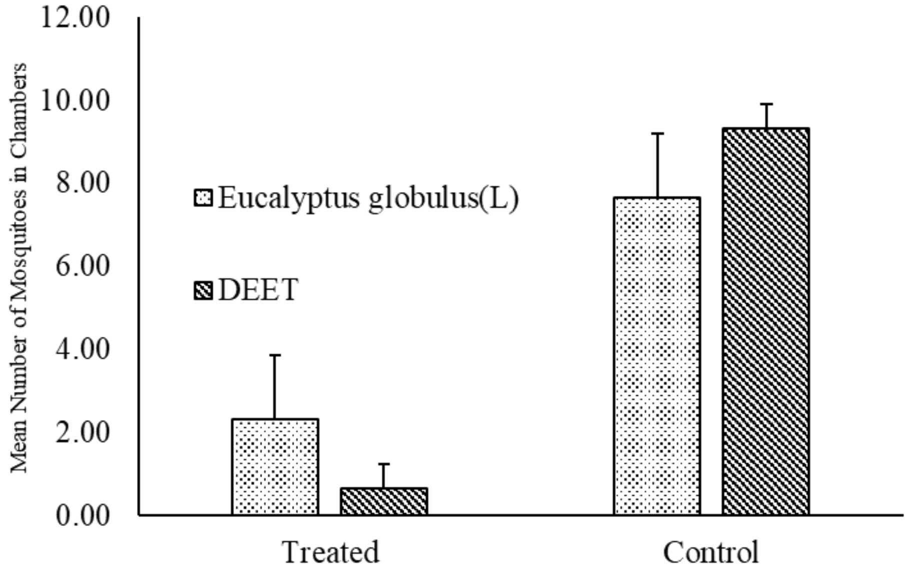


Figure 15: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Eucalyptus globulus*Leave and DEET

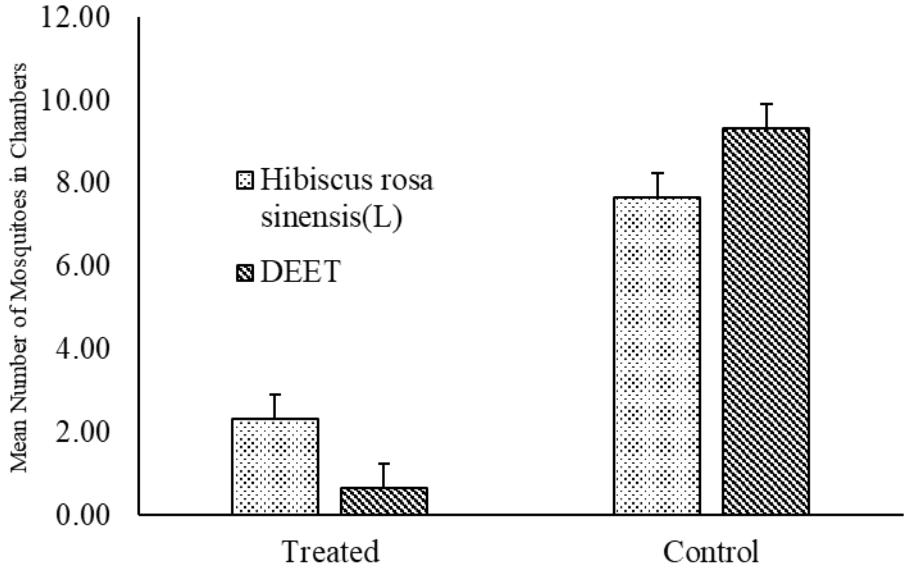


Figure 16: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Hibiscus rosa sinensis*Leave and DEET

4.2.11 Repellency Effect of *Hyptis suaveolens* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Hyptis suaveolens* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (9.33±1.15) was significantly more (P=0.0007) in comparison to those in the treated chamber (0.67±1.15). After analysis of the difference between *Hyptis suaveolens*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Hyptis suaveolens*(L) (ANOVA, $F_{(1, 72)} = 359.5$, $P > 0.9999$) (Figure 17). The preference index is -0.87 while the percentage repellency is 86.67% which was same with that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

4.2.12 Repellency Effect of *Latanna camara* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Latanna camara* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (8.00±2.00) was not significantly more (P=0.0213) in comparison to those in the treated chamber (2.00±2.00). After analysis of the difference between *Latanna camara*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Latanna camara*(L) (ANOVA, $F_{(1, 72)} = 359.5$, P=0.8467) (Figure 18). The preference index is -0.60 while the percentage repellency is 60.00% which was significantly lower than what was obtainable in DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

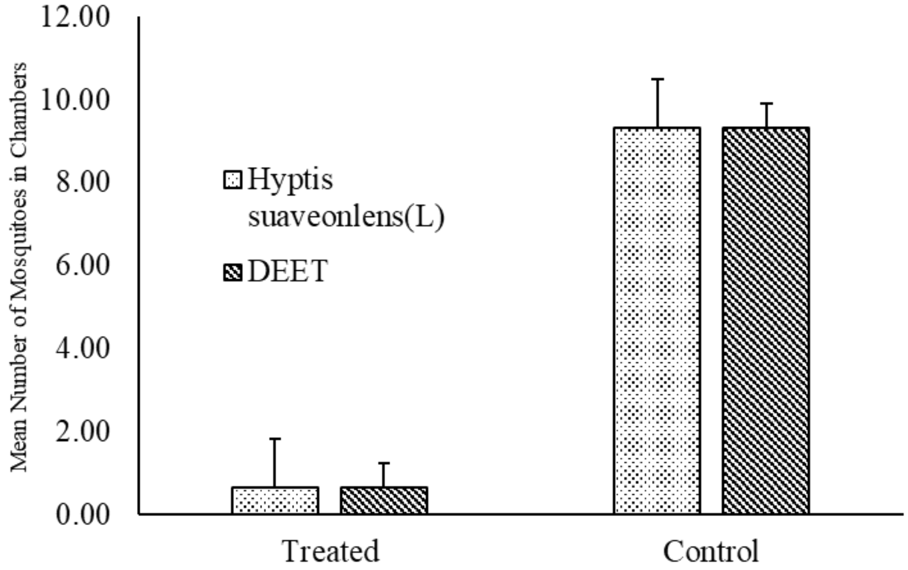


Figure 17: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Hyptis suaveolens*Leave and DEET

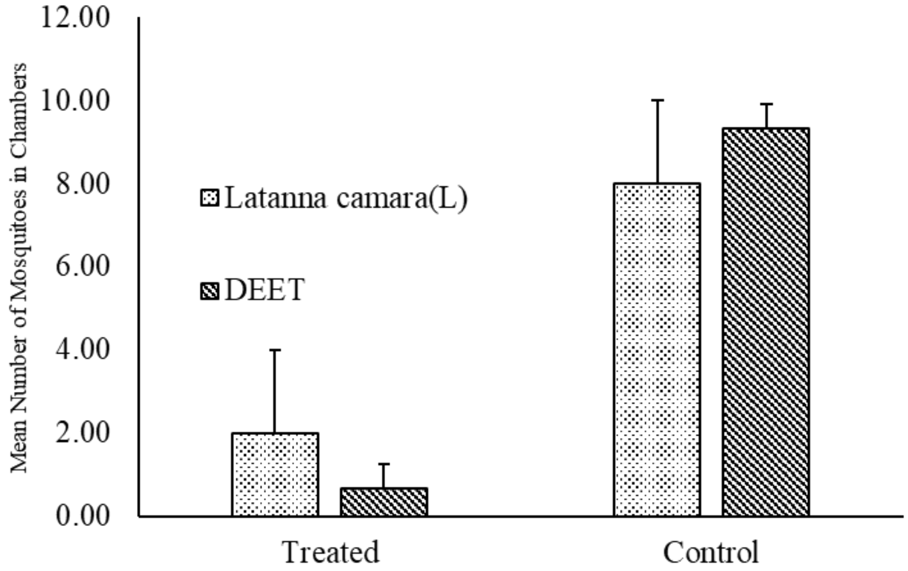


Figure 18: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Latanna camara*Leave and DEET

4.2.13 Repellency Effect of *Moringa oleifera* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Moringa oleifera* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (6.33±0.58) was not significantly more (P=0.0049) in comparison to those in the treated chamber (3.67±0.58). After analysis of the difference between *Moringa oleifera*leave and DEET (a standard repellent), it was observed that there was significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Moringa oleifera*(L) (ANOVA, $F_{(1, 72)}$ = 359.5, P= 0.0411) (Figure 19). The preference index is -0.27 while the percentage repellency is 26.67% which was significantly lower than that of DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

4.2.14 Repellency Effect of *Nicotiana tabacum* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Nicotiana tabacum* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (9.00±1.00) was significantly more (P=0.0006) in comparison to those in the treated chamber (1.00±1.00). After analysis of the difference between *Nicotiana tabacum*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Nicotiana tabacum*(L) (ANOVA, $F_{(1, 72)}$ = 359.5, P=0.9996) (Figure 20). The preference index is -0.80 while the percentage repellency is 80.00% which was close to what was obtainable in DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

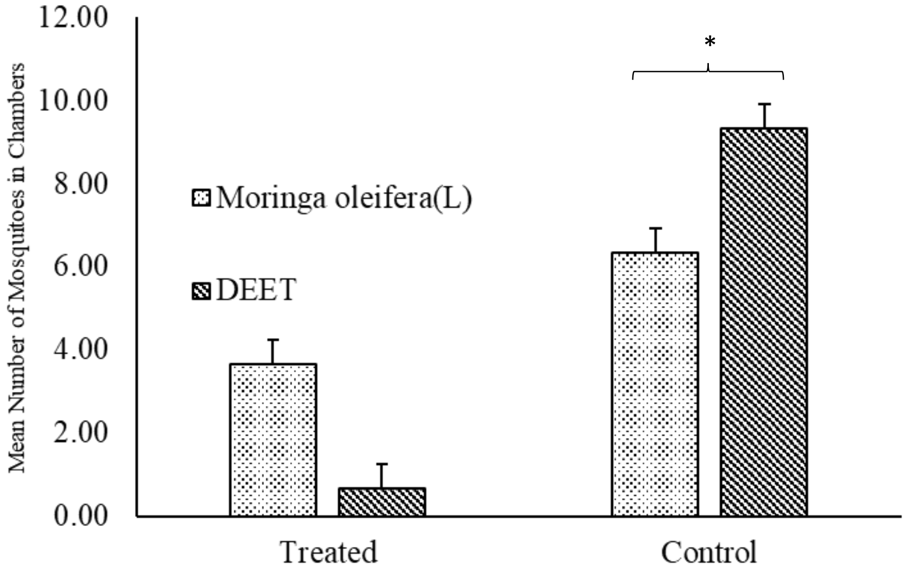


Figure 19: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Moringa oleifera*Leave and DEET

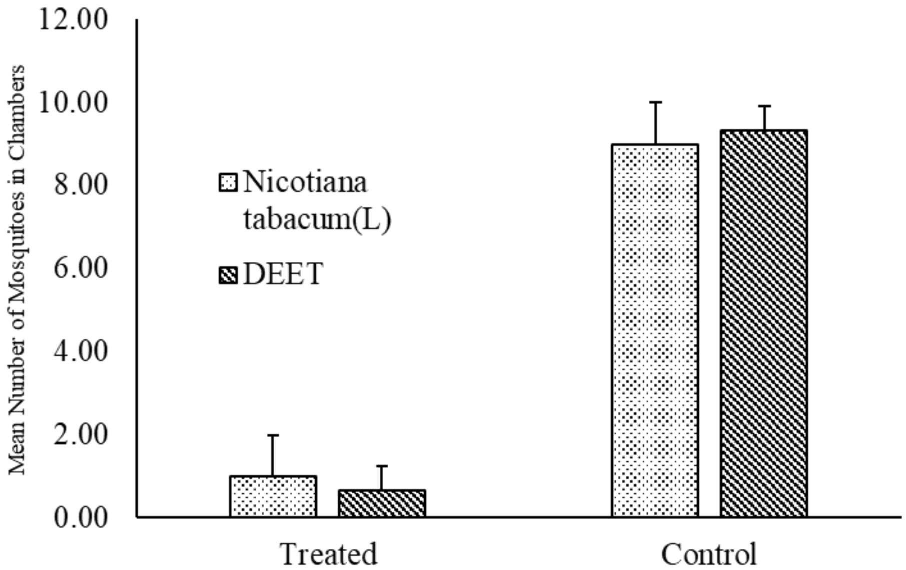


Figure 20: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Nicotiana tabacum*Leave and DEET

4.2.15 Repellency Effect of *Ocimum gratissimum* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Ocimum gratissimum* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (8.67±0.58) was significantly more (P=0.0001) in comparison to those in the treated chamber (1.33±0.58). After analysis of the difference between *Ocimum gratissimum*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Ocimum gratissimum*(L) (ANOVA, $F_{(1, 72)} = 359.5$, P= 0.9990) (Figure 21). The preference index is -0.73 while the percentage repellency is 73.33% which was close to what was obtainable in DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

4.2.16 Repellency Effect of *Parkia biglobosa* Fruit on *Anopheles gambiae* s.l.

Repellency activity of *Parkia biglobosa* Fruit on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (1.00±1.00) was significantly less (P=0.0006) in comparison to those in the treated chamber (9.00±1.00). After analysis of the difference between *Parkia biglobosa*Fruit and DEET (a standard repellent), it was observed that there was significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Parkia biglobosa*(F) (ANOVA, $F_{(1, 72)} = 359.5$, P< 0.0001) (Figure 22). The preference index is 0.80 while the percentage repellency is -80.00% which was significantly lower than what was obtainable in DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 9).

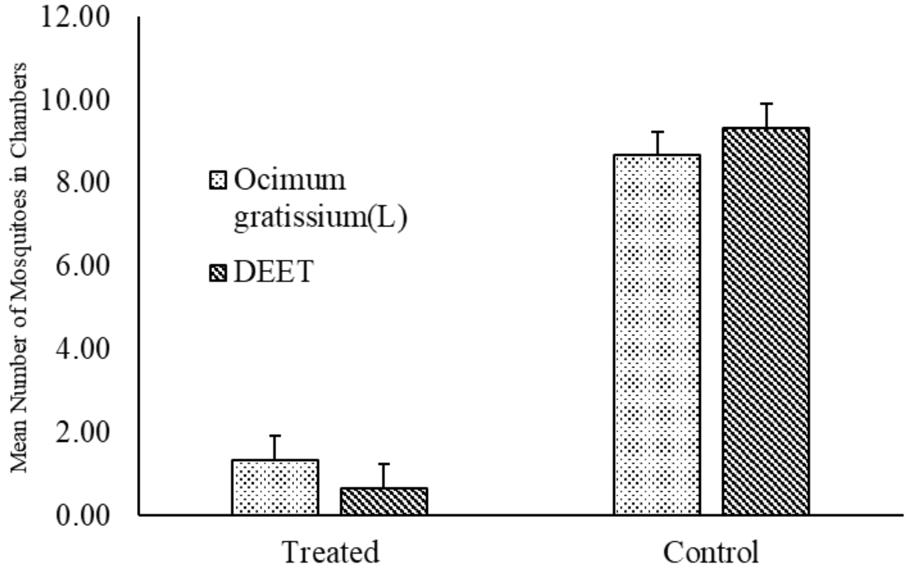


Figure 21: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Ocimum gratissimum*Leave and DEET

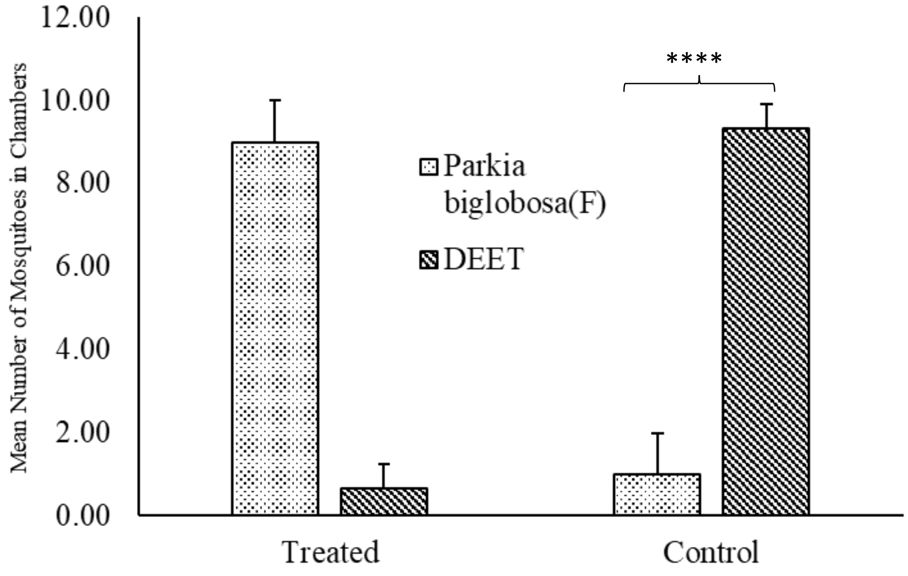


Figure 22: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Parkia biglobosa*Fruit and DEET

4.2.17 Repellency Effect of *Thymus vulgaries* Leaves on *Anopheles gambiae* s.l.

Repellency activity of *Thymus vulgaries* leave on *Anopheles gambiae* s.l. was evaluated, it was observed that the mean number of mosquitoes in the control chamber (7.33±0.58) was significantly more (P=0.0107) in comparison to those in the treated chamber (2.67±0.58). After analysis of the difference between *Thymus vulgaries*leave and DEET (a standard repellent), it was observed that there was no significant difference between the mean number of *Anopheles* mosquitoes in chamber containing DEET and chamber containing *Thymus vulgaries*(L) (ANOVA, $F_{(1, 72)}$ = 359.5, P= 0.3662) (Figure 23). The preference index is -0.47 while the percentage repellency is 46.67% which was significantly lower than what was obtainable in DEET with preference index of -0.87 and percentage repellency of 86.67% (Table 11).

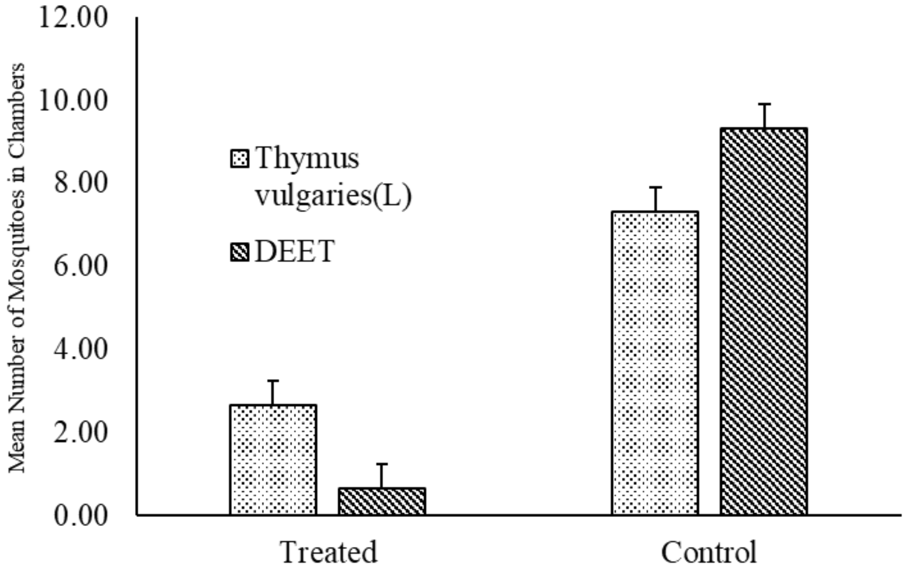


Figure 23: Mean number of *Anopheles gambiae* s.l. mosquitoes both treated and control chambers for *Thymus vulgaries*Leave and DEET

Table 11: Percentage Repellency and Preference Index of mention plant materials in North-central Nigeria on *Anopheles gambiae* s.l.

Mentioned Plant Material	Treated (Mean±SD)	Preference Index	Percentage repellency
***Ageratum conyzoides*(L)**	0.67±1.15	-0.87	86.67
***Allium cepa* (L)**	2.67±1.53	-0.47	46.67
Annona senegalensis(L)	3.00±1.00	-0.40	40.00
Capsicum annuum(F)	2.33±1.53	-0.53	53.33
Cassia mimosoides(L)	5.67±1.53	0.13	-13.33
Citrus sinensis(F)	1.00±1.00	-0.80	80.00
Cymbpogon citratus(L)	3.00±2.00	-0.40	40.00
Ertyphleum suaveolens(B)	6.00±1.00	0.20	-20.00
Eucalyptus globulus(L)	2.33±1.53	-0.53	53.33
Hibiscus rosa sinensis(L)	2.33±0.58	-0.53	53.33
Hyptis suaveonlens(L)	0.67±1.15	-0.87	86.67
Latanna camara(L)	2.00±2.00	-0.60	60.00
Moringa oleifera(L)	3.67±0.58	-0.27	26.67
Nicotiana tabacum(L)	1.00±1.00	-0.80	80.00
Ocimum gratissium(L)	1.33±0.58	-0.73	73.33
Parkia biglobosa(F)	9.00±1.00	0.80	-80.00
Thymus vulgaries(L)	2.67±0.58	-0.47	46.67
DEET (Positive Control)	0.67±0.58	-0.87	86.67
Solvent (Negative Control)	0.00±0.00	1.00	-100.00

SD = standard deviation, L = leave, F=fruit and B=bark

4.3 Repellency Activity of Oils from Selected Plants on *Anopheles gambiae* s.l.

Five plants were selected from the seventeen plants mentioned based on high relative frequency of citation (RFC) and percentage repellency (PR) which was close to that of the standard (DEET) after the screening of shortlisted insecticidal plants. Thetop five selected insecticidal plants in which their oils were extracted and evaluated are; *Ageratum conyzoides* (RFC=0.062, PR=86.67%), *Hyptis suaveolens* (RFC=0.196, PR=86.67%), *Citrus sinensis* (RFC=0.108, PR=80.00%), *Nicotiana tabacum* (RFC=0.057, PR=80.00%) and *Ocimum gratissimum* (RFC=0.188, PR=73.33%).

4.3.1 Repellency Activity of Oils from *Hyptis suaveolens* on *Anopheles gambiae* s.l.

It was observed that the treated chamber had significantly ($P<0.05$) more mean numbers of *Anopheles* mosquitoes compared to the control chamber when human odour was tested and the solvent did not significantly ($P>0.05$) alter this activity after 30 mins of the experimental procedure. Meanwhile when different concentrations of *Hyptis suaveolens* plant oil (1.0mg/ml, 3.0mg/ml and 5.0mg/ml) and DEET where tested, after 30 mins there was significantly ($P<0.05$) more *Anopheles* mosquitoes in the control chamber compared to the treated chamber even in the presence of human odour (figure 24). However, there was no significant difference ($P>0.05$) between the activity elicited by the different plant oil concentrations but there was a significant difference ($P<0.05$) between all the concentrations evaluated and DEET. The concentrations 1.0mg/ml, 3.0mg/ml and 5.0mg/ml of plant oil from *Hyptis suaveolens* recorded hundred percent repellency against *Anopheles gambiae* s.l. which was the same like what was obtainable for DEET even in the presence of human odour (Table 12).

4.3.2 Repellency Activity of Oils from *Ocimum gratissimum*on *Anopheles gambiae* s.l.

It was observed that the treated chamber had significantly ($P<0.05$) more mean numbers of *Anopheles* mosquitoes compared to the control chamber when human odour was tested and the solvent did not significantly ($P>0.05$) alter this activity after 30 mins of the experimental procedure. Meanwhile when different concentrations of *Ocimum gratissimum* plant oil (1.0mg/ml, 3.0mg/ml and 5.0mg/ml) and DEET where tested, after 30 mins there was significantly ($P<0.05$) more *Anopheles* mosquitoes in the control chamber compared to the treated chamber in the presence of human odour (figure 25). However, there was no significant difference ($P>0.05$) between the activity elicited by the different plant oil concentrations but there was a significant difference ($P<0.05$) between all the concentrations and DEET. The concentrations 1.0mg/ml, 3.0mg/ml and 5.0mg/ml of plant oil from *Ocimum gratissimum* recorded 100 percent repellency against *Anopheles gambiae* s.l. which was the same like what was obtainable for DEET even in the presence of human odour (Table 12).

4.3.3 Repellency Activity of Oils from *Ocimum gratissimum*on *Anopheles gambiae* s.l.

It was observed that the treated chamber had significantly ($P<0.05$) more mean numbers of *Anopheles* mosquitoes compared to the control chamber when human odour was tested and the solvent did not significantly ($P>0.05$) alter this activity after 30 mins of the experimental procedure. Meanwhile when different concentrations of *Ageratum conyzoides* plant oil (1.0mg/ml, 3.0mg/ml and 5.0mg/ml) and DEET where tested, after 30 mins there was significantly ($P<0.05$) more *Anopheles* mosquitoes in the control chamber compared to the treated chamber in the presence of human odour (figure 26). However, there was no significant difference ($P>0.05$) between the activity elicited by the different plant oil concentrations but there was a significant difference (P<0.05) between all the concentrations and DEET. The concentrations 1.0mg/ml, 3.0mg/ml and 5.0mg/ml of plant oil from *Ageratum conyzoides* recorded 100 percent repellency against *Anopheles gambiae* s.l. which was the same like what was obtainable for DEET even in the presence of human odour (Table 12).

4.3.4 Repellency Activity of Oils from *Citrus sinensis* on *Anopheles gambiae* s.l.

It was observed that the treated chamber had significantly (P<0.05) more mean numbers of *Anopheles* mosquitoes compared to the control chamber when human odour was tested and the solvent did not significantly (P>0.05) alter this activity after 30 mins of the experimental procedure. Meanwhile when different concentrations of *Citrus sinensis* plant oil (1.0mg/ml, 3.0mg/ml and 5.0mg/ml) and DEET where tested, after 30 mins there was significantly (P<0.05) more *Anopheles* mosquitoes in the control chamber compared to the treated chamber in the presence of human odour (figure 28). However, there was no significant difference (P>0.05) between the activity elicited by the different plant oil concentrations but there was a significant difference (P<0.05) between all the concentrations and DEET. The concentrations 1.0mg/ml, 3.0mg/ml and 5.0mg/ml of plant oil from *Citrus sinensis* recorded 100 percent repellency against *Anopheles gambiae* s.l. which was better than what was obtainable for DEET (73.6%) even in the presence of human odour (Table 12).

4.3.5 Repellency Activity of Oils from *Nicotiana tabacum*on *Anopheles gambiae* s.l.

It was observed that the treated chamber had significantly (P<0.05) more mean numbers of *Anopheles* mosquitoes compared to the control chamber when human odour was tested and the solvent did not significantly (P>0.05) alter this activity after 30 mins of the experimental procedure. Meanwhile when different concentrations of *Nicotiana tabacum* plant oil (1.0mg/ml, 3.0mg/ml and 5.0mg/ml) and DEET where tested, after 30 mins there was significantly ($P<0.05$) more *Anopheles* mosquitoes in the control chamber compared to the treated chamber in the presence of human odour (figure 29). However, there was no significant difference ($P>0.05$) between the activity elicited by the different plant oil concentrations but there was a significant difference ($P<0.05$) between all the concentrations and DEET. The concentrations 1.0mg/ml, 3.0mg/ml and 5.0mg/ml of plant oil from *Nicotiana tabacum* recorded 100 percent repellency against *Anopheles gambiae* s.l. which was better than what was obtainable for DEET (62.7%) even in the presence of human odour (Table 12).

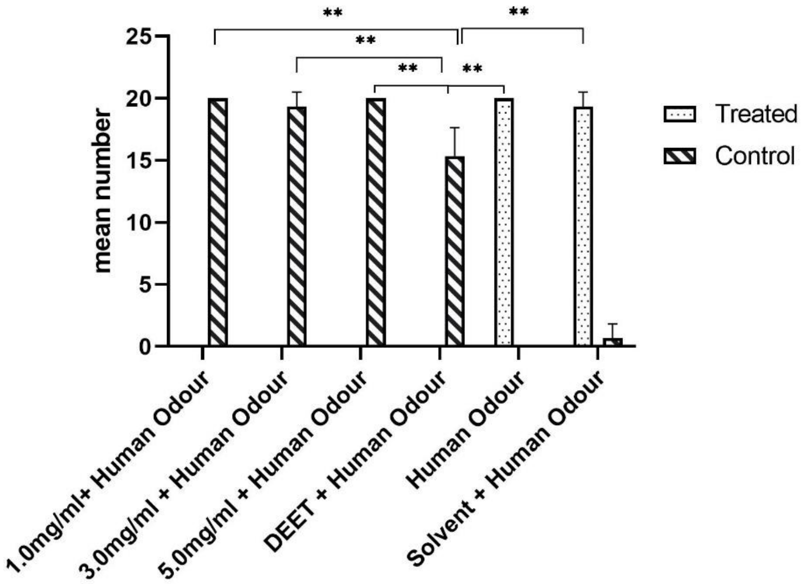


Figure 24: Mean number of *Anopheles* mosquitoes in both treated and control chamber when *Hyptis suaveolens* plant oil was tested.

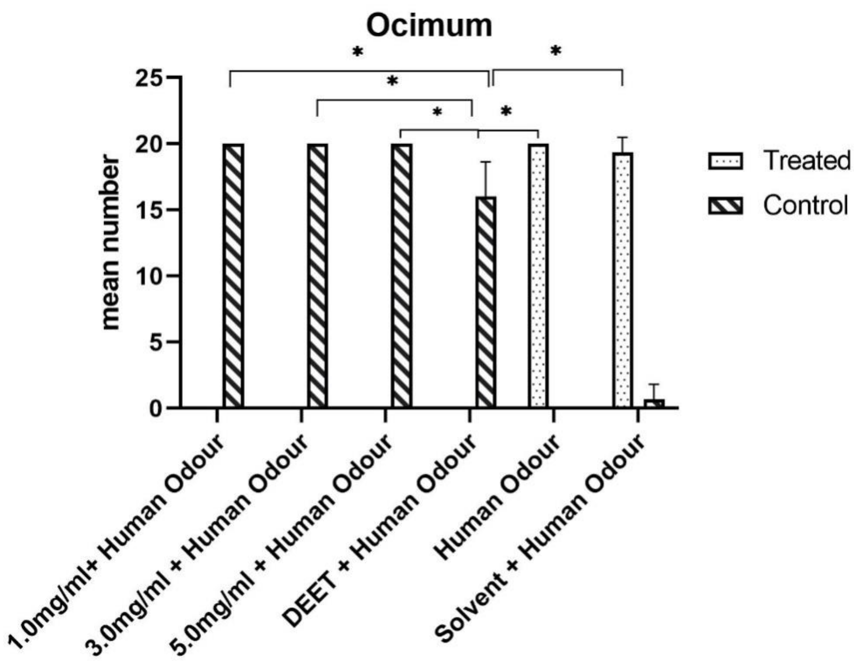


Figure 25: Mean number of *Anopheles* mosquitoes in both treated and control chamber when *Ocimum gratissimum* plant oil was tested.

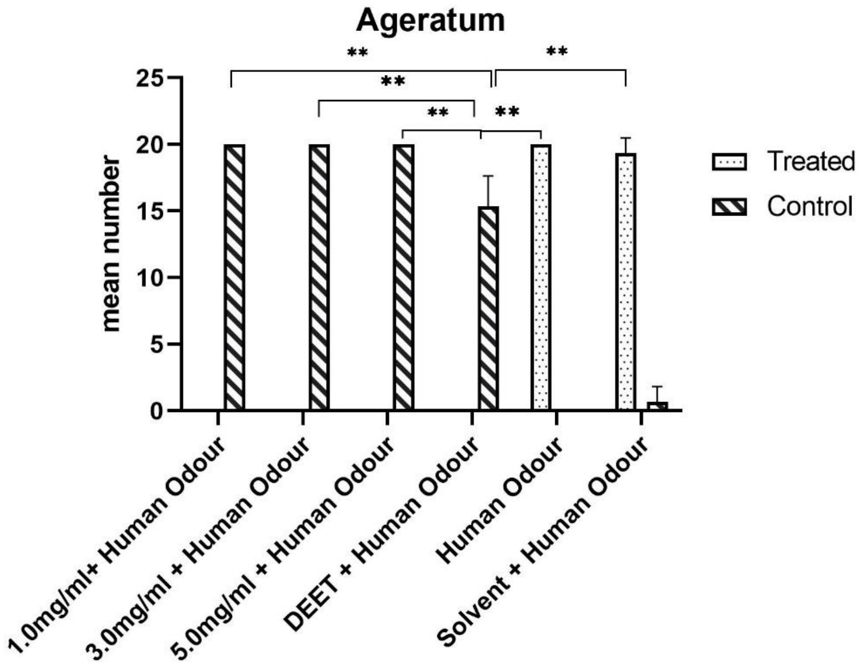


Figure 26: Mean number of *Anopheles* mosquitoes in both treated and control chamber when *Ageratum conyzoides* plant oil was tested.

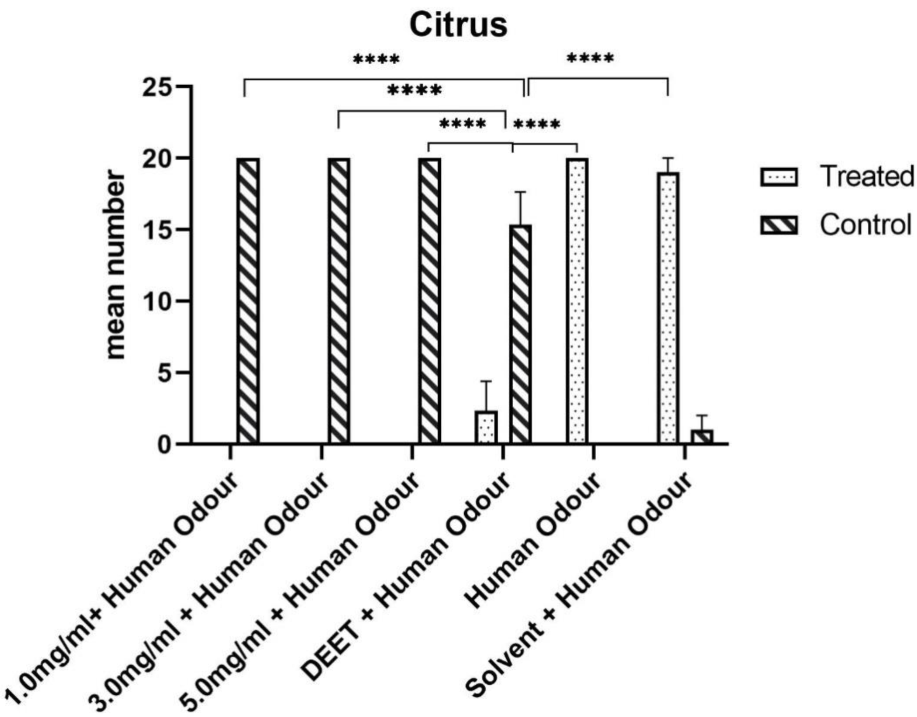


Figure 27:Mean number of *Anopheles* mosquitoes in both treated and control chamber when *Citrus sinensis* plant oil was tested.

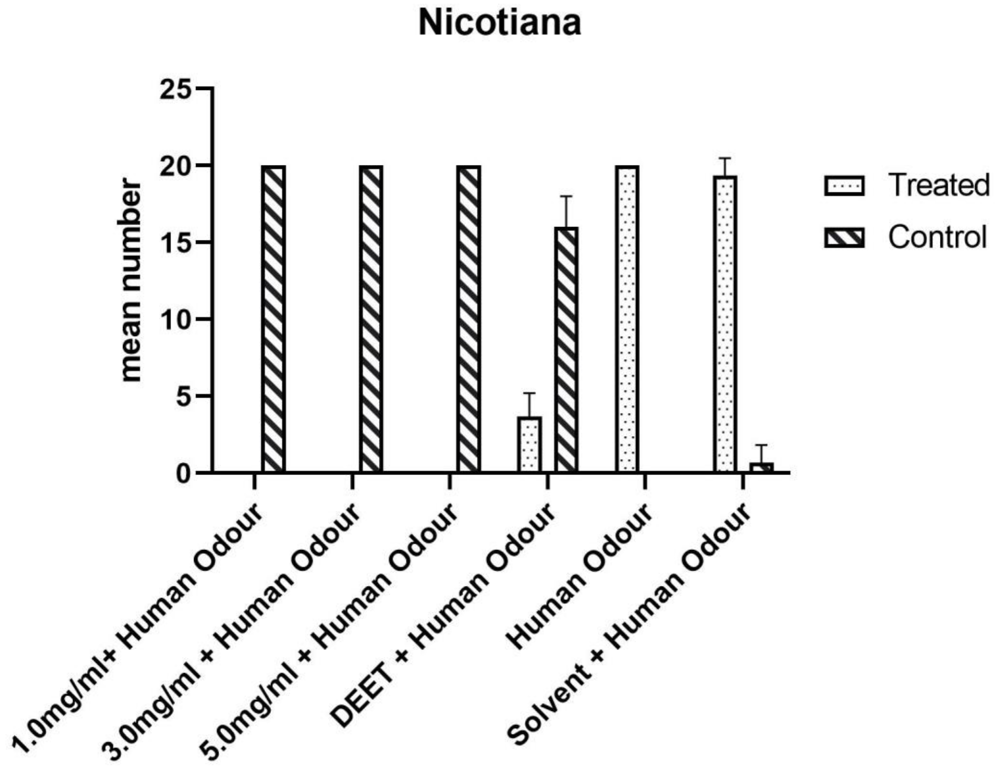


Figure 28: Mean number of *Anopheles* mosquitoes in both treated and control chamber when *Nicotiana tabacum* plant oil was tested.

Table 12: Percentage Repellency of Oils from Selected Plants on *Anopheles gambiae* s.l.

Treatments	***Nicotiana tabacum***	***Citrus sinensis***	***Ageratum conyzoides***	***Ocimum gratissimum***	***Hyptis suaveolens***
1.0mg/ml+ Human Odour	100.00	100.00	100.00	100.00	100.00
3.0mg/ml + Human Odour	100.00	100.00	100.00	100.00	100.00
5.0mg/ml + Human Odour	100.00	100.00	100.00	100.00	100.00
DEET + Human Odour	62.71	73.58	100.00	100.00	100.00
Human Odour	-100.00	-100.00	-100.00	-100.00	-100.00
Solvent + Human Odour	-93.33	-90.00	-93.33	-93.33	-93.33

4.4 Contact Toxicity of Oils from Selected Plants on *Anopheles gambiae* s.l.

4.4.1 Larvicidal Activity of Oils from Selected Plants on *Anopheles gambiae* s.l.

Plant oils from leaves of *Hyptis suaveolens, Ocimum gratissimum, Nicotiana tabacum,Ageratum conyzoides* andFruit peels of *Citrus sinensis* were assayed for their toxicity against the early third and fourth instar larvae stages of *Anopheles gambiae* s.l. and the mortality rates were observed and recorded after both 24 and 48 hours. The percentage mean mortality and lethal concentrations of the different concentrations from *Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacum* and *Ageratum conyzoides* against *Anopheles gambiae* s.l. mosquito larvae are presented in table 13.

After 24 hours exposure, 100 percent mortality was observed at 3.0mg/ml for *Hyptis suaveolens,* 4.0mg/ml for *Citrus sinensis* and 5.0mg/ml for both *Nicotiana tabacum* and *Ageratum conyzoides* respectively while after 48 hours exposure, 100 percent mortality was observed in 2.0mg/ml for *Hyptis suaveolens* and 3.0mg/ml for *Citrus sinensis*, *Nicotiana tabacum* and *Ageratum conyzoides*. Meanwhile *Ocimum gratissimum* did not achieve 100% mortality in all of the concentration at both 24 hours and 48 hours exposure.

After 24 hours exposure, at 0.5, 1.0, 1.5 and 2.0mg/ml, there was a significant difference ($P<0.05$) in the mean larval mortalities between *Hyptis suaveolens* and *Citrus sinensis*, *Ocimum gratissimum* and *Nicotiana tabacum* but there was no significant difference ($P>0.05$) between *Hyptis suaveolens* and *Ageratum conyzoides* and between *Citrus sinensis* and *Nicotiana tabacum* while at 2.5mg/ml, there was significant difference ($P<0.05$) in the mean larval mortalities between *Hyptis suaveolens* and *Ocimum gratissimum* and *Nicotiana tabacum* but there was no significant difference ($P>0.05$) between *Hyptis suaveolens* and *Ageratum conyzoides* and *Citrus sinensis*. At 3.0 and 4.0mg/ml, there was significant difference ($P<0.005$) in the mean larval mortalities between *Hyptis suaveolens* and *Ocimum* *gratissimum*, *Nicotiana tabacum* and *Ageratum conyzoides* but there was no significant difference (P>0.05) between *Hyptis suaveolens* and *Citrus sinensis* and also between *Nicotiana tabacum* and *Ageratum conyzoides* while at 5.0mg/ml, there was significant difference (P<0.05) in the mean larval mortalities between *Hyptis suaveolens* and *Ocimum gratissimum* but there was no significant difference (P>0.05) between *Hyptis suaveolens* and *Citrus sinensis* and also between *Nicotiana tabacum* and *Ageratum conyzoides*.

After 48 hours exposure, at 0.5 and 1.0, mg/ml, there was significant difference (P<0.05) in the mean larval mortalities between *Hyptis suaveolens* and *Citrus sinensis*, *Ocimum gratissimum* and *Ageratum conyzoides* but there was no significant difference (P>0.05) between *Hyptis suaveolens* and *Nicotiana tabacum* while at 1.5mg/ml, there was significant difference (P<0.05) in the mean larval mortalities between *Hyptis suaveolens* and *Citrus sinensis*, *Ocimum gratissimum*, *Nicotiana tabacum* and *Ageratum conyzoides*. At 2.0 and 2.5mg/ml, there was significant difference (P<0.05) in the mean larval mortalities between *Hyptis suaveolens* and *Citrus sinensis*, *Ocimum gratissimum*, *Nicotiana tabacum* and *Ageratum conyzoides* but there was no significant difference (P>0.05) between *Citrus sinensis* and *Ageratum conyzoides* at concentration of 2.0mg/ml and also between *Citrus sinensis* and *Nicotiana tabacum* and *Ageratum conyzoides* at concentration of 2.5mg/ml while at 3.0, 4.0 and 5.0mg/ml, there was significant difference (P<0.05) in the mean larval mortalities between *Hyptis suaveolens* and *Ocimum gratissimum* but there was no significant difference (P>0.05) between *Hyptis suaveolens* and *Citrus sinensis*, *Nicotiana tabacum* and *Ageratum conyzoides*.

Table 13: Mean larval mortality induced selected plant oils at different concentrations against 3rd instar larvae of *An. gambiae* s.l.

Essential oils	**% Mean Mortality ± SD (mg/ml)**									**Lethal Conc. (mg/ml)**		
	0.5	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**4.0**	**5.0**	**Control**	**LC_{50} (95% CL)**	**LC_{90} (95% CL)**	**R^2**
24hrs Exposure												
Hyptis suaveolens(L)	45.3±1.5$_a$	60.0±1.0$_a$	62.7±0.6$_a$	65.3±0.6$_a$	77.3±1.5$_a$	100.0±0.0$_a$	100.0±0.0$_a$	100.0±0.0$_a$	0.0±0.0	2.43 (2.22 to 2.64)	3.19 (2.63 to 3.87)	0.914
Citrus sinensis(F)	10.7±0.6$_b$	40.0±1.0$_b$	54.7±0.6$_b$	80.0±1.0$_b$	82.7±0.6$_a$	94.7±0.6$_a$	100.0±0.0$_a$	100.0±0.0$_a$	0.0±0.0	1.36 (1.19 to 1.54)	3.69 (2.74 to 4.95)	0.984
Ocimum gratissimum(L)	2.7±0.6$_c$	6.7±0.6$_c$	8.0±0.0$_c$	29.3±0.6$_c$	42.7±0.6$_b$	58.7±0.6$_b$	60.0±0.0$_b$	65.3±0.6$_b$	0.0±0.0	2.17 (2.08 to 2.26)	3.20 (2.90 to 3.53)	0.987
Nicotiana tabacum(L)	17.3±0.6$_b$	29.3±0.6$_d$	42.7±0.6$_d$	48.0±1.0$_d$	68.0±1.0$_c$	78.7±1.5$_c$	89.3±2.5$_c$	100.0±0.0$_a$	0.0±0.0	2.62 (1.94 to 3.53)	7.55 (3.40 to 16.77)	0.971
***Ageratum conyzoides*(L)**	40.0±1.0$_a$	61.3±0.6$_a$	66.7±0.6$_a$	70.7±0.6$_a$	78.7±0.6$_a$	81.3±1.2$_c$	90.7±0.6$_c$	100.0±0.0$_a$	0.0±0.0	NA	NA	0.965
48 hrs Exposure												
Hyptis suaveolens(L)	65.3±1.5$_a$	84.0±1.7$_a$	86.7±1.2$_a$	100.0±0.0$_a$	100.0±0.0$_a$	100.0±0.0$_a$	100.0±0.0$_a$	100.0±0.0$_a$	0.0±0.0	0.91 (0.52 to 1.60)	2.21 (1.37 to 3.56)	0.900
Citrus sinensis(F)	65.3±1.5$_b$	46.7±1.5$_b$	61.3±1.5$_b$	85.3±1.5$_b$	89.3±1.5$_b$	100.0±0.0$_a$	100.0±0.0$_a$	100.0±0.0$_a$	0.0±0.0	1.46 (1.29 to 1.65)	2.92 (2.26 to 3.78)	0.966
Ocimum gratissimum(L)	6.7±0.6$_c$	12.0±1.0$_c$	17.3±1.5$_c$	41.3±0.6$_c$	48.0±1.0$_c$	61.3±0.6$_b$	77.3±0.6$_b$	84.0±1.0$_b$	0.0±0.0	2.47 (2.19 to 2.76)	5.07 (3.69 to 6.95)	0.980
Nicotiana tabacum(L)	24.0±1.0$_a$	40.0±1.0$_a$	48.0±1.0$_d$	69.3±1.2$_d$	86.7±0.6$_b$	100.0±0.0$_a$	100.0±0.0$_a$	100.0±0.0$_a$	0.0±0.0	1.87 (1.73 to 2.01)	3.14 (2.64 to 3.72)	0.976
***Ageratum conyzoides*(L)**	54.7±0.6$_d$	68.0±1.0$_d$	72.0±1.0$_e$	78.7±0.6$_b$	90.7±1.5$_b$	100.0±0.0$_a$	100.0±0.0$_a$	100.0±0.0$_a$	0.0±0.0	1.86 (1.54 to 2.24)	4.16 (2.48 to 6.96)	0.938

NA = Not Applicable, SD = Standard Deviation, CL = Confidence Level, *Values along column with different subsets differs significantly* ($P>0.05$)

The LC_{50} for 24 hours exposure was lowest in *Citrus sinensis* (1.36mg/ml) followed by *Ocimum gratissimum* (2.17mg/ml), *Hyptis suaveolens* (2.43mg/ml) and the highest in *Nicotiana tabacum* (2.62mg/ml). The LC_{50} for 48 hours exposure, it was lowest in *Hyptis suaveolens* (0.91mg/ml) followed by *Citrus sinensis* (1.46mg/ml), *Ageratum conyzoides* (1.86mg/ml), *Nicotiana tabacum* (1.87mg/ml) and the highest in *Ocimum gratissimum* (2.47mg/ml). Meanwhile, the LC_{90} for 24 hours exposure was lowest in *Hyptis suaveolens* (3.19mg/ml) followed by *Ocimum gratissimum* (3.20mg/ml), *Citrus sinensis* (3.69mg/ml) and the highest in *Nicotiana tabacum* (7.55mg/ml). The LC_{90} for 48 hours exposure, it was lowest in *Hyptis suaveolens* (2.21mg/ml) followed by *Citrus sinensis* (2.92mg/ml), *Nicotiana tabacum* (3.14mg/ml), *Ageratum conyzoides* (4.16mg/ml) and the highest in *Ocimum gratissimum* (5.07mg/ml).

A dose-dependent reduction in survival rates of different plant oils against *Anopheles gambiae* s.l. larvae were demonstrated in figure 29 for 24 hours exposure period and in figure 30 for 48 hours exposure period. This revealed that mortality was concentration dependent. At 0.5mg/ml after 24hrs exposure time, the highest percentage mean mortality was recorded in *Hyptis suaveolens* followed by *Citrus sinensis* and lowest in *Ocimum gratissimum*. At 0.5mg/ml after 48hrs exposure time, the highest percentage mean mortality was recorded in *Hyptis suaveolens* and lowest in *Ocimum gratissimum*.

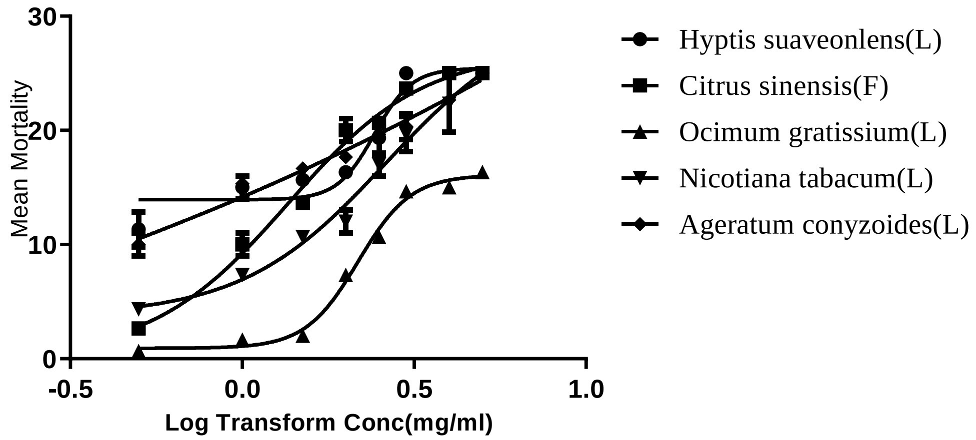


Figure 29:Dose-response curves for *An. gambiae* s.l. larvae to selected plant oils for 24 hrs post exposure.

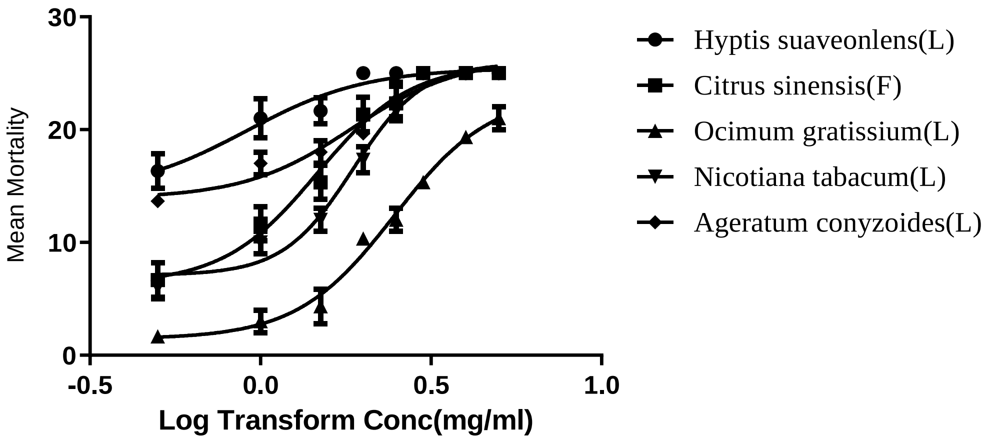


Figure 30: Dose-response curves for *An. gambiae* s.l. larvae to selected plant oils for 48 hrs post exposure.

4.4.2 Mortality Effect of Oils from Selected Plants on Adult *Anopheles gambiae* s.l.

At 0.1mg/ml, there was significant difference (P<0.05) in the mean adult mortalities between *Hyptis suaveolens* and *Ocimum gratissimum*, *Nicotiana tabacum* and *Ageratum conyzoides* while at 1.5mg/ml, 2.0mg/ml, 2.5mg/ml, 4.0mg/ml and 5.0mg/ml, there was significant difference (P<0.05) in the mean adult mortalities between *Hyptis suaveolens* and *Citrus sinensis*, and *Ocimum gratissimum*, *Nicotiana tabacum* and *Ageratum conyzoides*. At 3.0mg/ml, there was a significant difference (P<0.05) in the mean adult mortalities between *Hyptis suaveolens* and *Citrus sinensis,* and *Ocimum gratissimum, Nicotiana tabacum* and *Ageratum conyzoides* but there was no significant difference (P>0.05) between *Citrus sinensis* and *Ageratum conyzoides* (Table 14).

Nicotiana tabacum had the highest percentage mortality (100%) recorded at 5.0mg/ml against *Anopheles gambiae* s.l. and compares favourably with Deltamethrin with 100% mortality. Also, exposure of *Anopheles gambiae* s.l. to *Ocimum gratissimum* (5.0mg/ml and 4.0mg/ml) and *Nicotiana tabacum* (4.0mg/ml) resulted in 85%, 75% and 80% mortalities respectively which is comparable to what is obtainable in Deltamethrin. The lowest percentage mortality was recorded in 0.5mg/ml of *Ageratum conyzoides* (3.3%). At 0.5mg/ml, there was a significant difference (P<0.05) in the mean adult mortalities between *Hyptis suaveolens* and *Ocimum gratissimum,* and *Ageratum conyzoides* but there was no significant difference (P>0.05) between *Hyptis suaveolens* and *Citrus sinensis,* and *Nicotiana tabacum*. Within the plant oils, there was a significant difference (P<0.05) in the adult percentage mortalities between *Hyptis suaveolens* and *Ocimum gratissimum* (Figure 31).

Essential oil	**Control**	**Concentrations (mg/ml)**							
		0.5	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**4.0**	**5.0**
Hyptis suaveolens	0.00±0.00	1.33±0.58$_{a}$	2.33±0.58$_{a}$	2.67±0.58$_{a}$	3.33±0.58$_{a}$	3.67±0.58$_{a}$	5.00±1.00$_{a}$	6.00±1.00$_{a}$	7.33±0.58$_{a}$
Citrus sinensis	0.00±0.00	1.67±0.58$_{a}$	2.67±0.58$_{a}$	4.00±1.00$_{b}$	6.67±0.58$_{b}$	8.33±0.58$_{b}$	10.00±1.00$_{b}$	11.67±1.53$_{b}$	14.00±2.65$_{b}$
Ocimum gratissimum	0.00±0.00	4.33±0.58$_{b}$	7.33±0.58$_{b}$	7.67±1.53$_{c}$	11.00±1.00$_{c}$	10.33±0.58$_{c}$	10.67±1.53$_{c}$	15.00±1.00$_{c}$	17.00±1.00$_{c}$
Nicotiana tabacum	0.00±0.00	1.67±0.58$_{a}$	3.67±0.58$_{c}$	5.67±0.58$_{d}$	8.67±0.58$_{d}$	9.00±1.00$_{d}$	9.67±0.58$_{d}$	16.00±1.00$_{d}$	20.00±0.00$_{d}$
Ageratum conyzoides	0.00±0.00	0.67±0.58$_{c}$	1.67±0.58$_{d}$	3.33±0.58$_{e}$	5.33±0.58$_{e}$	6.00±1.00$_{e}$	7.67±0.58$_{b}$	8.67±0.58$_{e}$	11.33±0.58$_{e}$
Deltamethrin	20.00±0.00								

Table 14: Mean mortality of different concentrations of selected plant oils on Adult *Anopheles gambiae* s.l.

Values along column with different subsets differs significantly (P>0.005)

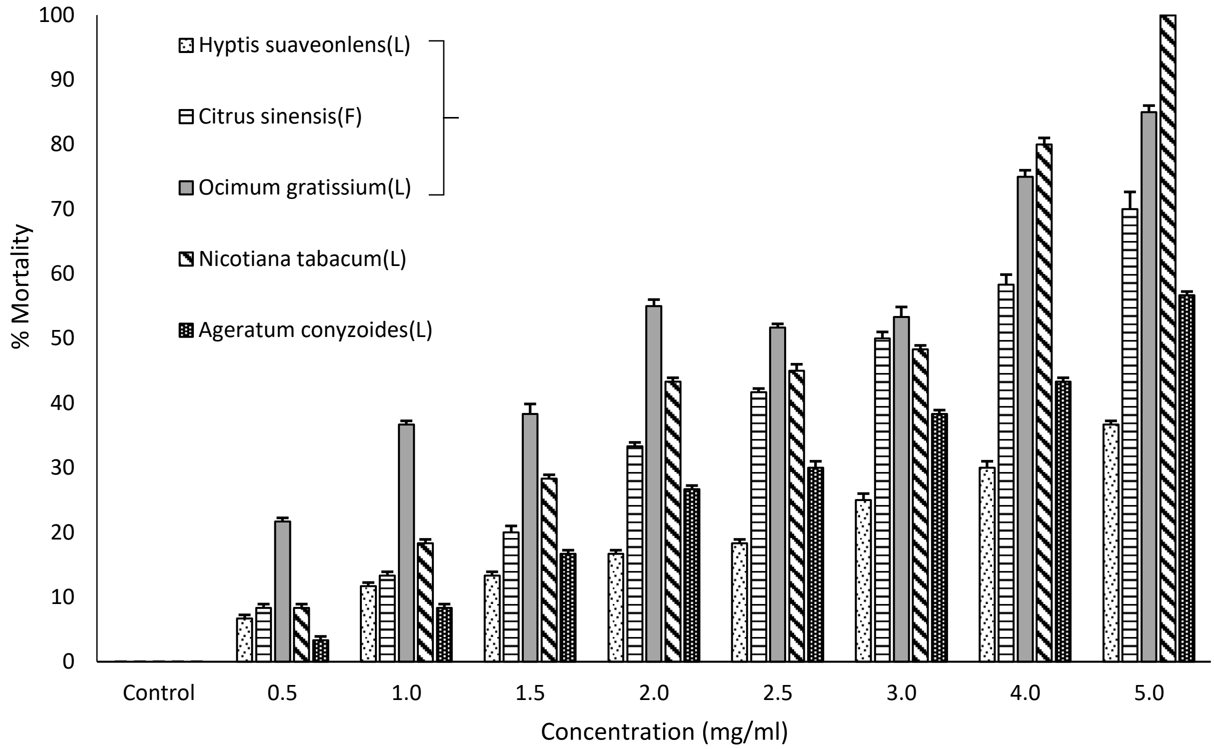


Figure 31: Adulticidal activity (Dose-response assay) of selected plant oils on *Anopheles gambiae* s.l.

The lowest KdT_{50} was recorded in *Nicotiana tabacum* at 2.5mg/ml (20.3mins) followed by *Ocimum gratissimum* at 3.0mg/ml (22.93mins). Also, *Ageratum conyzoides* (1.0mg/ml), *Latanna camara* (5.0mg/ml) and *Citrus sinensis* (4.0mg/ml) recorded low KdT_{50} which are 26.29mins, 27.89mins and 29.06mins respectively. The highest KdT_{50} was recorded in *Citrus sinensis* at 0.5mg/ml (75.56 mins) followed by *Hyptis suaveolens* at 3.0mg/ml (73.25mins).

The lowest KdT_{95} was recorded in *Ageratum conyzoides*at 1.0mg/ml (35.97mins) and 0.5mg/ml (45.27mins) followed by *Ocimum gratissimum* at 3.0mg/ml (48.11mins), *Citrus sinensis* at 4.0mg/ml (48.23mins), Hyptis suaveolens at 0.5mg/ml (53.88mins). The highest was recorded in *Nicotiana tabacum* at 2.0mg/ml (313.1mins) followed by *Citrus sinensis* at 0.5mg/ml (288.9 mins) and 3.0mg/ml (267.5 mins).

The lowest LC_{50} was recorded in *Citrus sinensis* at 2.745mg/ml while the highest was recorded in *Ageratum conyzoides* at 4.751 mg/ml. The lowest LC_{95} was recorded in *Citrus sinensis* at 9.870 mg/ml while the highest was recorded in *Ageratum conyzoides* at 43.96 mg/ml. For *Hyptis suaveolens*, *Ocimum gratissimum* and *Nicotiana tabacum* the LC_{50} and LC_{95} were too ambiguous hence not applicable.

Plant oils	**Conc. (mg/ml)**	**N**	**% Kd after 1hr exposure**	**KdT_{50} (95%CL)**	**KdT_{95} (95%CL)**	**LC_{50} (95%CL)**	**LC_{95} (95%CL)**

Hyptis suaveolens (L)	0.5	60	6.67	31.05 (22.80 to 42.29)	53.88 (19.30 to 150.4)	NA	NA
	1.0	60	11.67	49.86 (24.19 to 102.8)	102.3 (19.65 to 532.4)		
	1.5	60	13.33	NA	NA		
	2.0	60	16.67	NA	NA		
	2.5	60	18.33	NA	NA		
	3.0	60	25.00	73.25 (0.002 to 2.827e+006)	NA		
	4.0	60	30.00	NA	NA		
	5.0	60	36.67	NA	NA		
Citrus sinensis (F)	0.5	60	8.33	75.56 (0.06 to 93831)	288.9 (0.002 to 4.243e+007)	2.75 (2.497 to 3.018)	9.87 (7.363 to 13.23)
	1.0	60	13.33	NA	NA		
	1.5	60	20.00	NA	NA		
	2.0	60	33.33	NA	NA		
	2.5	60	41.67	NA	NA		
	3.0	60	50.00	48.19 (0.9433 to 2462)	267.5 (0.012 to 5.760e+006)		
	4.0	60	58.33	29.06 (25.49 to 33.13)	48.23 (32.33 to 71.93)		
	5.0	60	70.00	36.21 (20.93 to 62.63)	84.95 (14.79 to 488.1)		
Ocimum gratissimum (L)	0.5	60	21.67	NA	NA	NA	NA
	1.0	60	36.67	NA	NA		
	1.5	60	38.33	37.08 (23.97 to 37.22)	121 (15.01 to 975.0)		
	2.0	60	55.00	40.1 (18.90 to 85.05)	144.5 (16.65 to 1254)		
	2.5	60	51.67	34.19 (20.35 to 57.41)	152.5 (22.11 to 1053)		
	3.0	60	53.33	22.93 (18.84 to 27.91)	48.11 (25.77 to 89.83)		
	4.0	60	75.00	NA	NA		
	5.0	60	85.00	NA	NA		
Nicotiana tabacum (L)	0.5	60	8.33	30.90 (20.51 to 46.56)	69.21 (16.84 to 284.5)	NA	NA
	1.0	60	18.33	NA	NA		
	1.5	60	28.33	29.87 (19.01 to 72.31)	77.19 (34.32 to 173.6)		
	2.0	60	43.33	60.4 (4.634 to 787.4)	313.1 (1.547 to 63336)		
	2.5	60	45.00	20.3 (8.496 to 48.49)	98.22 (4.662 to 2069)		
	3.0	60	48.33	32.96 (25.57 to 42.47)	78.26 (32.88 to 186.3)		
	4.0	60	80.00	NA	NA		
	5.0	60	100.00	27.89 (23.15 to 33.60)	69.74 (35.07 to 138.7)		
Ageratum conyzoides **(L)**	0.5	60	3.33	44.68 (0.0 to +infinity)	45.27 (0.0 to +infinity)	4.75 (3.142 to 7.185)	43.96 (17.80 to 108.6)
	1.0	60	8.33	26.29 (19.79 to 34.93)	35.97 (23.43 to 55.22)		
	1.5	60	16.67	NA	NA		
	2.0	60	26.67	NA	NA		
	2.5	60	30.00	64.02 (13.25 to 309.4)	129.1 (9.150 to 1821)		
	3.0	60	38.33	NA	NA		
	4.0	60	43.33	30.25 (25.76 to 35.52)	69.59 (39.76 to 121.8)		
	5.0	60	56.67	NA	NA		

Table 15:Knock-down Effect and Lethal Concentration of selected plant oils *Anopheles gambiae* s.l.

Conc. =Concentration, % Kd = Percentage Knock Down, NA = Not Applicable, KdT = Knock Down Time, LC= Lethal Concentration

4.5 Vapour Toxicity of Oils from Selected Plants on *Anopheles gambiae*

4.5.1 Vapour Toxicity of Oils from *Hyptis suaveolens*on *Anopheles gambiae*

It was observed that the percentage knock down from 1.0mg/ml, 3.0mg/ml and 5.0mg/ml of oils from *Hyptis suaveolens* on*An. gambiae* was lower than what was observed in DEET even after 60 minutes of exposure (Figure 32). However, 5.0mg/ml of the plant oil achieved 98.33% knockdown which was close to what was observed in DEET (100%) after 60 minutes post exposure while 1.0mg/ml and 3.0mg/ml of oils from *Hyptis suaveolens* achieved 58.33% and 90% knockdown respectively. There was no significant difference (P>0.05) between the percentage knockdown on *An. gambiae* observed in 5.0mg/ml of oil from *Hyptis suaveolens* and DEET while there was a significant difference (P<0.05) between the percentage knockdown observed in DEET and 1.0mg/ml and 3.0mg/ml of oil from *Hyptis suaveolens*.

4.5.2 Vapour Toxicity of Oils from *Citrus Sinensis* on *Anopheles gambiae*

It was observed that after 20 minutes of exposure, 5.0mg/ml of oils from *Citrus sinensis* (83.3%) had a higher percentage knock down than DEET (70%) on *An. gambiae* and this trend was same until they both achieved 100% knock down after 60 minutes of exposure while 1.0mg/ml and 3.0mg/ml of same plant oil (68.3% and 90% respectively) had lower percentage knock down on *An. gambiae* compared to DEET after 60 minutes of exposure (Figure 33). However, there was no significant difference (P>0.05) between the percentage knock down recorded by 5.0mg/ml of oils from *Citrus sinensis* on *An. gambiae* in comparison to DEET while there was a significant difference (P<0.05) between the percentage knockdown observed in DEET and 1.0mg/ml and 3.0mg/ml of same plant oil.

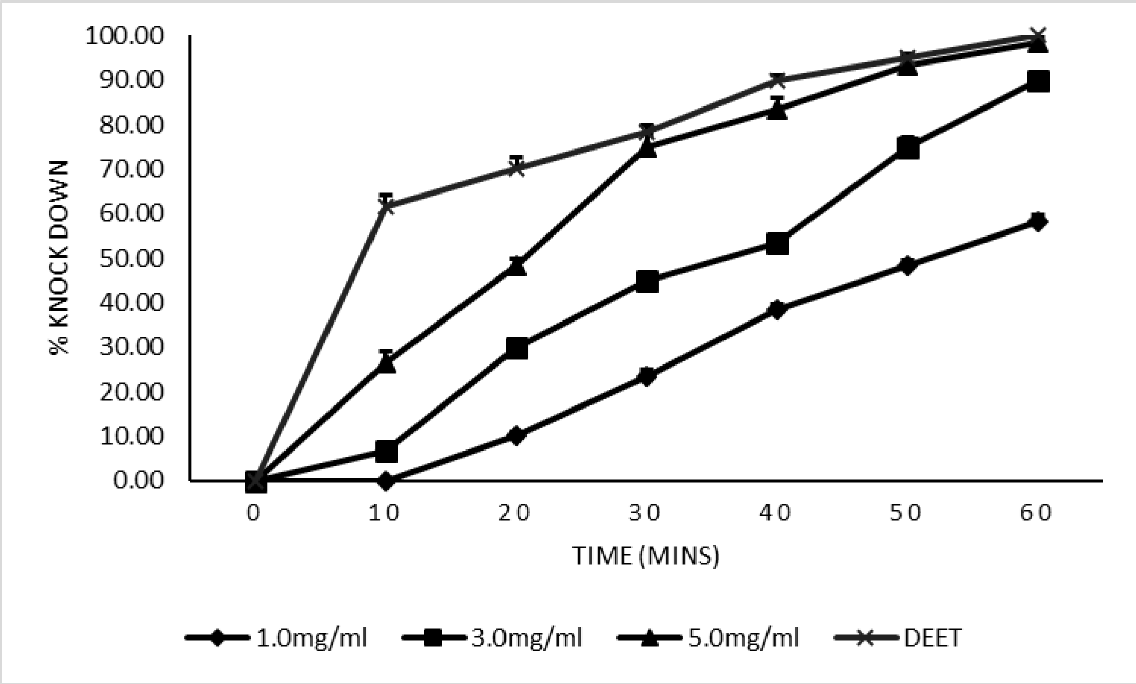


Figure 32: Percentage knockdown of *Anopheles gambiae* exposed to oils from *Hyptis suaveolens*leaves

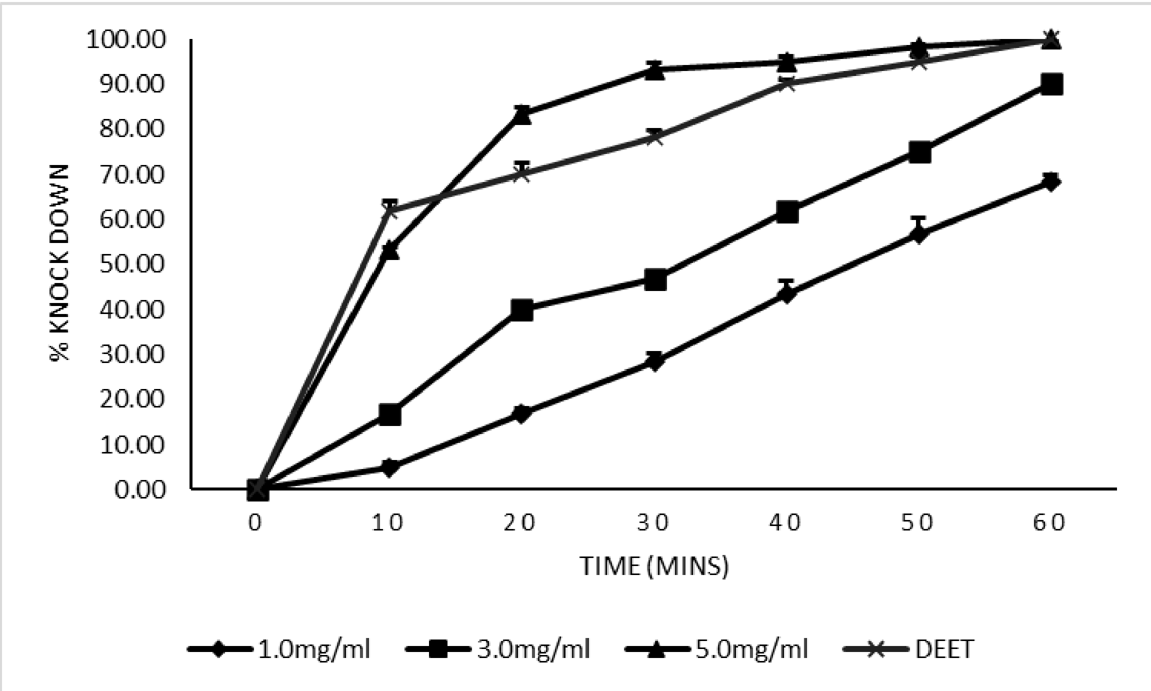


Figure 33: Percentage knockdown of *Anopheles gambiae* exposed to oils from *Citrus sinensis* fruit peels

4.5.3 Vapour Toxicity of Oils from *Ocimum gratissimum*on *Anopheles gambiae*

It was observed that after 30 minutes of exposure, 5.0mg/ml of oils from *Ocimum gratissimum* had a higher percentage knockdown (90%) on *An. gambiae* than DEET (78.3%) while after 60 minutes, 3.0mg/ml and 5.0mg/ml achieved a hundred percent knockdown which was same as DEET. However, 1.0mg/ml of same plant oil did not achieve one hundred percent knockdown on *An. gambiae* after 60 minutes of exposure (Figure 34). There was no significant difference ($P>0.05$) between the percentage knockdown observed in 3.0mg/ml and 5.0mg/ml of oils from *Ocimum gratissimum* on *Anopheles gambiae* and DEET while there was a significant difference ($P<0.05$) between the percentage knockdown observed in 1.0mg/ml of same plant oil and DEET.

4.5.4 Vapour Toxicity of Oils from *Nicotiana tabacum*on *Anopheles gambiae*

It was observed that after 20 minutes of exposure, 5.0mg/ml of oils from *Nicotiana tabacum* had same percentage knockdown on *An. gambiae* as DEET (70%) while after 30 minutes of exposure, 5.0mg/ml of same plant oil (85%) had a higher percentage knockdown that DEET (78.3%) and this trend continued until 60 minutes where both achieved one hundred percent knock down. However, 1.0mg/ml and 3.0mg/ml of same plant oil (71.7% and 96.7% respectively) had lower percentage knock down on *An. gambiae* compared to DEET after 60 minutes of exposure but the percentage knockdown observed in 3.0mg/ml was close to that of DEET (Figure 35). There was no significant difference ($P>0.05$) between the percentage knockdown of 3.0mg/ml and 5.0mg/ml of oils from *Nicotiana tabacum* on *An. gambiae* and DEET while there was a significant difference ($P<0.05$) between the percentage knockdown observed in DEET and 1.0mg/ml.

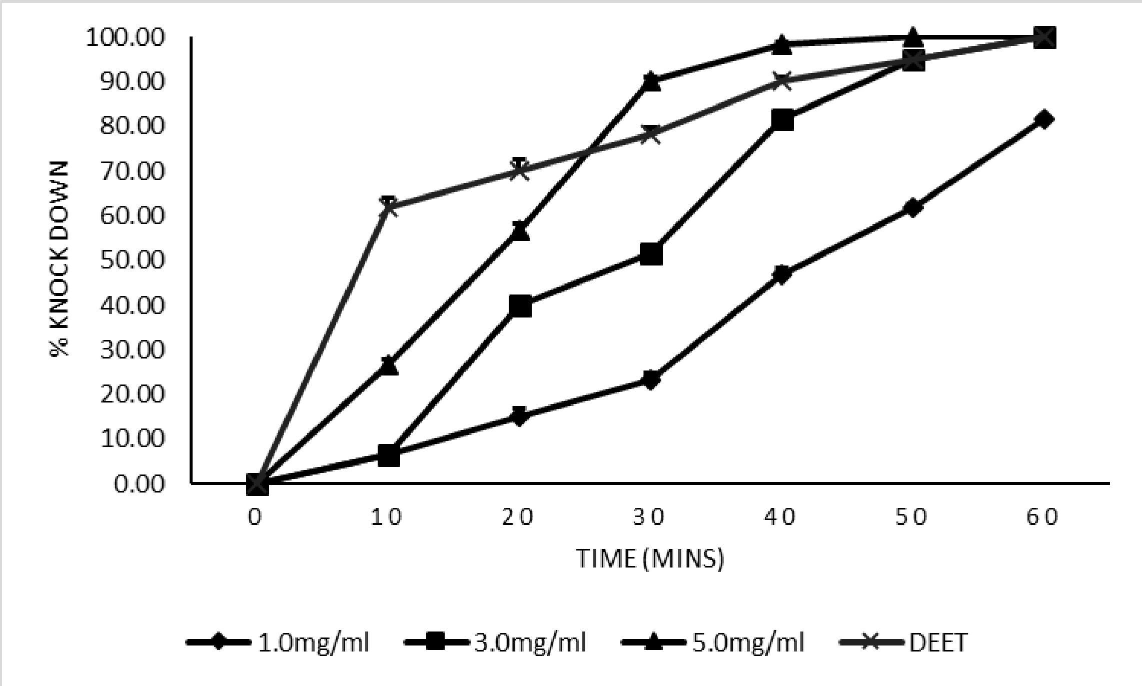


Figure 34: Percentage knockdown of *Anopheles gambiae* exposed to oils from *Ocimum gratissimum*leaves

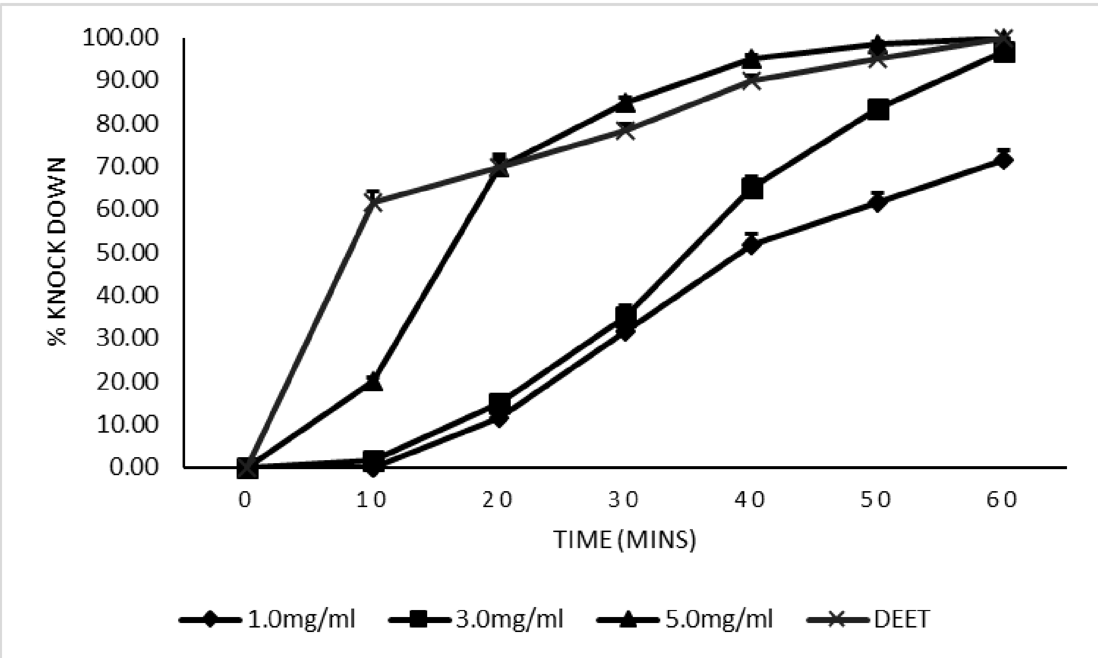


Figure 35: Percentage knockdown of *Anopheles gambiae* exposed to oils from *Nicotiana tabacum*leaves

4.5.5 Vapour Toxicity of Oils from *Ageratum conyzoides*on *Anopheles gambiae*

It was observed that the percentage knock down from 1.0mg/ml, 3.0mg/ml and 5.0mg/ml of oils from *Ageratum conyzoides*on*An. gambiae* (66.7%, 96.7% and 95% respectively) was lower than what was observed in DEET (100%) even after 60 minutes of exposure (Figure 36). Interestingly 3.0mg/ml achieved a higher percentage knockdown than 5.0mg/ml after 60 minutes of exposure and both achieved percentage knockdowns that were close to what was observed in DEET. There was no significant difference (P>0.05) between the percentage knockdown on *An. gambiae* observed in 3.0mg/ml and 5.0mg/ml of oil from *Ageratum conyzoides* and DEET while there was a significant difference (P<0.05) between the percentage knockdown observed in DEET and 1.0mg/ml of oil from *Ageratum conyzoides*.

4.5.6 Percentage Mortality and Knock Down Values of Oils from Selected Plants on *Anopheles gambiae*

It was observed that 3.0mg/ml and 5.0mg/ml achieved the same percentage mortality as DEET which was hundred percent for all the five plant oils (*Citrus sinensis*, *Hyptis suaveolens*, *Nicotiana tabacum*, *Ageratum conyzoides* and *Ocimum gratissimum*) evaluated on *Anopheles gambiae* after 24 hours and there was no significant difference (P>0.05) between DEET and 3.0mg/ml and 5.0mg/ml concentrations of the plant oils. However, it was observed that 1.0mg/ml concentration of all the five plant oils evaluated did not achieve one hundred percent mortality and there was a significant difference (P<0.05) between the concentration of the five plant oils and DEET (Table 16).

It was observed that the KdT_{50} and KdT_{95} for 5.0mg/ml of *Ocimum gratissimum* (21.32 mins and 37.05 mins respectively)was the lowest followed by 5.0mg/ml of *Hyptis suaveolens* (25.18 mins and 73.49 mins respectively) and 1.0mg/ml (34.80 mins and 92.51 mins respectively) and 3.0mg/ml (38.70 mins and 95.58 mins respectively) of *Nicotiana tabacum* and these concentrations and plant oils were lower than what was obtainable in DEET (36.10 mins and 114.9 mins respectively) (Table 14). Interestingly, it was observed that *Nicotiana tabacum* had KdT_{50} and KdT_{95} that were lower than what was observed in DEET on *An. gambiae* at the lowest concentration of 1.0mg/ml while the lowest concentration of other plant oils had KdT_{50} and KdT_{95} that was higher than what was obtainable in DEET. However, it was observed that 1.0mg/ml of *Citrus sinensis* and *Ageratum conyzoides* had the highest KdT_{50} (73.08 mins and 66.27 mins respectively) and KdT_{95} (403.2 mins and 372.6 mins respectively) values. The overlap between the 95% confidence level of KdT_{50} and KdT_{95} for oils from 1.0mg/ml and 3.0mg/ml of *Nicotiana tabacum* and 5.0mg/ml of *Ocimum gratissimum*and *Hyptis suaveolens* shows that there is no significant difference ($P>0.05$) between these knockdown values.

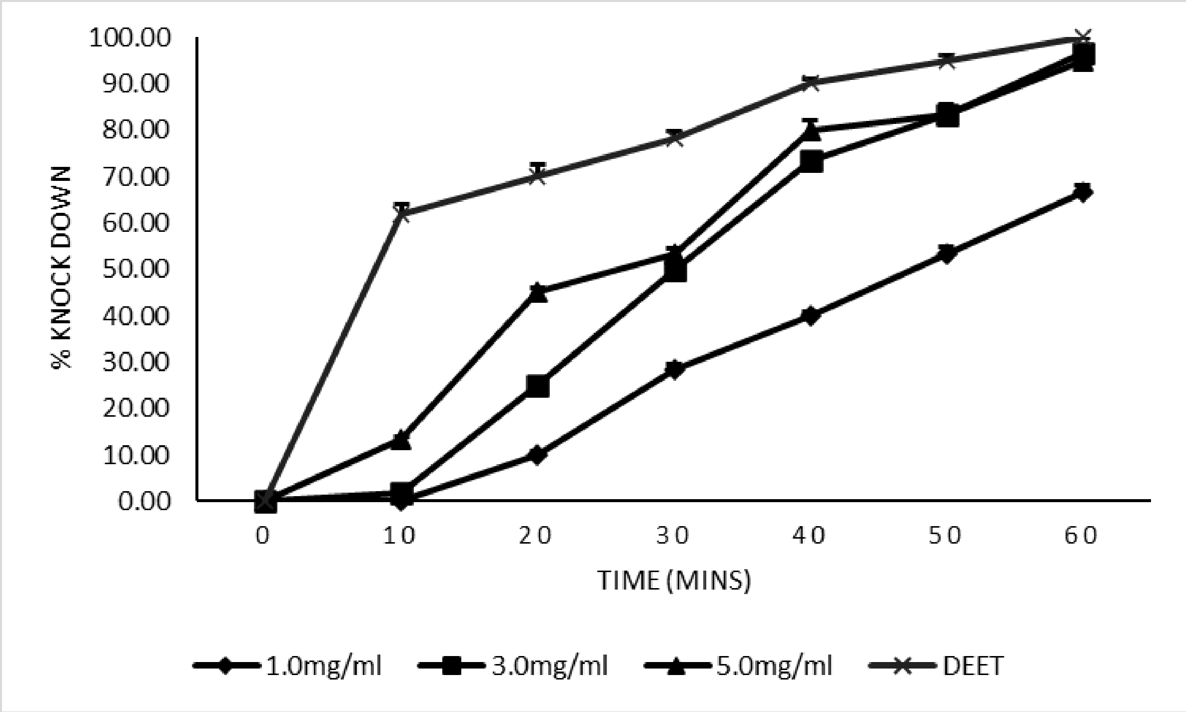


Figure 36: Percentage knockdown of *Anopheles gambiae* exposed to oils from *Ageratum conyzoides*leaves

Table 16: Percentage Mortality and knock down values of selected plant oils on *An. gambiae*

Plant oils	Conc. (mg/ml)	No.	KdT_{50} (95% CL)	KdT_{95} (95% CL)	% Mortality
Hyptis suaveolens	1.0	100	44.48 (18.73-105.6)	163.2 (17.78-1498)	68
	3.0	100	NA	NA	100
	5.0	100	25.18 (17.89-35.43)	73.49 (17.27-312.8)	100
Citrus sinensis	1.0	100	73.08 (0.218-24497)	403.2 (0.008-NA)	85
	3.0	100	NA	NA	100
	5.0	100	NA	NA	100
Ocimum gratissimum	1.0	100	58.72 (18.33-188.1)	172.2 (18.42-1610)	83
	3.0	100	36.68 (13.25-101.5)	271.7 (5.693-12970)	100
	5.0	100	21.32 (19.99-22.74)	37.05 (29.94-45.85)	100
Nicotiana tabacum	1.0	100	34.80 (21.83-55.48)	92.51 (19.84-431.4)	82
	3.0	100	38.70 (28.29-52.95)	95.58 (37.71-242.3)	100
	5.0	100	NA	NA	100
Ageratum conyzoides	1.0	100	66.27 (5.297-829.2)	372.6 (2.568-54055)	77
	3.0	100	33.47 (24.30-46.12)	127.6 (39.08-416.5)	100
	5.0	100	NA	NA	100
DEET		100	36.10 (13.55-96.19)	114.9 (4.899-2697)	100

Conc.- Concentration, KdT – knockdown time, NA – Not Applicable, % Mortality-Percentage mortality after 24hrs

4.6 Major Chemical Components found in Selected Plant Oils

4.6.1 Major Chemical Components found in Oil from *Citrus Sinensis* fruit peels

The GC-MS analysis of oil from *Citrus sinensis* fruit peels revealed the presence of 50 components. D-Limonene (92.59%), 3-Octen-1-ol, (Z) (0.97%), Acetonitrile, 2-(2H-tetrazol-2-yl) (0.78%), Bicyclo[2.2.0]hexane-1-carboxaldehyde (0.64%) and 2-Butyne, 1-methoxy- (0.57%) were the major chemical components present. D-Limonene is the most abundant and predominant chemical component with 92.59% composition (Table 17).

4.6.2 Major Chemical Components found in Oil from *Ageratum conyzoides*leaves

The analysis of the major chemical components identified in the oil of *Ageratum conyzoides* leaves revealed the presence of 50 components. D-Limonene (63.96%), 1,3-Cyclobutanedicarbonitrile, trans- (1.55%), 1,6-Octadiene, 3,7-dimethyl- (1.28%), Bicyclo[10.1.0]trideca-4,8-diene-1 3-carboxamide, N-(3-chlorophenyl)- (1.23%), Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-(12.39%) and 29-Oxabicyclo[6.1.0]nonane, cis- (2.12%) were the major chemical components present in the. D-Limonene is the most abundant chemical component with 92.59% followed by Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)- with 12.39% (Table 18).

Table 17: Major chemical components identified in the oil from *Citrus sinensis*fruit peels

s/no.	**Chemical Component**	**RT (min)**	**Area (%)**	**Molecular Formula**	**Molecular Weight (g/mol)**
1	D-Limonene	11.331	92.59	$C_{10}H_{16}$	136.238
2	2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, (2R,5S)	13.089	0.11	$C_{10}H_{16}O_2$	170.252
3	1,5-Heptadiyne	20.673	0.24	C_7H_8	92.141
4	Acetonitrile, 2-(2H-tetrazol-2-yl)	21.076	0.78	$C_{10}H_7N_5O_2$	229.199
5	Cyclobutane, 1,2-diethyl-	13.199	0.15	C_8H_{16}	112.216
6	2-Butyne, 1-methoxy-	14.481	0.57	C_5H_8O	84.118
7	Bicyclo[2.2.0]hexane-1-carboxaldehyde	16.02	0.64	$C_7H_{10}O_2$	126.155
8	5-Hexenal, 4-methylene-	25.985	0.32	$C_7H_{10}O$	110.156
9	Butanedinitrile	28.696	0.11	$C_6H_8N_2$	108.144
10	3-Octen-1-ol, (Z)-	56.318	0.97	$C_8H_{16}O$	128.215
11	Cyclohexanebutanoic acid	56.941	0.25	$C_{10}H_{18}O_2$	170.252
12	1-Trifluoroacetoxy-10-undecene	71.009	0.17	$C_{13}H_{21}F_3O_2$	266.304

RT – Retention time

Table 18: Major chemical component identified in the oil from *Ageratum conyzoides*leaves

s/no.	Chemical Constituent	RT (min)	Area (%)	Molecular Formula	Molecular Weight (g/mol)
1	D-Limonene	11.221	63.96	$C_{10}H_{16}$	136.238
2	2,3-Dimethylamphetamine	14.848	0.22	$C_{11}H_{17}N$	163.264
3	1,5-Decadiyne	18.694	0.31	$C_{10}H_{14}$	134.222
4	Borane, diethylmethyl	20.636	0.43	$C_5H_{13}B$	83.969
5	1,3-Cyclobutanedicarbonitrile, trans-	21.076	1.55	$C_6H_6N_2$	106.128
6	1,3-Butadiene, 2-ethyl-	26.388	0.29	C_6H_{10}	82.146
7	1,6-Octadiene, 3,7-dimethyl-	25.985	1.28	$C_{10}H_{18}$	138.254
8	Bicyclo[10.1.0]trideca-4,8-diene-1 3-carboxamide, N-(3-chlorophenyl)-	23.091	1.23	$C_{20}H_{24}ClNO$	329.868
9	1-(3,5-Dimethyl-1-adamantanoyl)sem icarbazide	28.256	0.63	$C_{14}H_{23}N_3O_2$	265.357
10	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano	28.586	0.58	$C_{20}H_{27}NO_2$	313.4339
11	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	31.443	12.39	$C_{15}H_{24}$	204.3511
12	Cyclopropane, 1-ethyl-2-methyl-, cis-	13.163	0.20	C_6H_{12}	84.162
13	Geranyl formate	14.445	0.55	$C_{11}H_{18}O_2$	182.263
14	2,3-Dimethylamphetamine	14.848	0.22	$C_{11}H_{17}N$	163.264
15	Pyridine, 4-methyl-, 1-oxide	15.324	0.29	C_6H_7NO	109.128
16	3-Octyn-2-one	16.02	0.87	$C_8H_{12}O$	124.183
17	1,5-Heptadiyne	20.087	0.76	C_7H_8	92.141
18	2,6-Bis(diazo)adamantane	28.916	0.21	$C_{10}H_{12}N_4$	188.234
19	Spiro[cyclopropane-1,2'-[6.7]diazabicyclo[3.2.2]non-6-ene]	29.245	0.32	$C_9H_{14}N_2$	150.221
20	7-Chlorobicyclo[4.1.0]hept-3-ene	29.868	0.78	C_7H_9CL	128.599
21	2,8-Decadiyne	31.81	0.34	$C_{10}H_{14}$	134.222
22	11-Bromoundecanoic acid	50.457	0.53	$C_{11}H_{21}BrO_2$	265.191
23	9-Oxabicyclo[6.1.0]nonane, cis-	56.282	2.12	$C_8H_{14}O$	126.199
24	4-Aminocyclohexanone, N-acetyl-	56.831	0.23	$C_8H_{13}NO_2$	155.197
25	11-(2-Cyclopenten-1-yl)undecanoic	71.009	0.36	$C_{16}H_{28}O_2$	252.398

RT – Retention time

4.6.3 Major Chemical Components found in Oil from *Ocimum gratissimum*leaves

The analysis of the major chemical components identified in the oil of *Ocimum gratissimum* leaves revealed the presence of 50 components. D-Limonene (2.62%), Precocene I (95.63%), Caryophyllene (0.92%), 2,5-Octadiene (0.06%), 3-Methyl-1-hexyne (0.06%), 1,3,6-Octatriene, 3,7-dimethyl-, Z)-(0.05%), Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano- (0.05%) and (E,Z)-.alpha.-Farnesene (0.05%) were the major chemical components present. Precocene I was the most abundant chemical component with 95.63% followed by D-Limonene with 2.62% (Table 19).

4.6.4 Major Chemical Components found in Oil from *Hyptis suaveolens* leaves

The analysis of the major chemical components identified in the oil of *Hyptis suaveolens* leaves revealed the presence of 50 components. 2-Butanamine (4.45%), Cyclopentene (2.10%), cis-1-Methyl-2-(2'-propenyl)cyclopropane (26.77%), 1-Octyn-3-ol (9.81%), 4-Cyclopentene-1,3-diol, trans- (1.43%), 5-Hexyn-1-ol (3.82%), Hexane, 1,6-dichloro- (1.98%) and 1-Trifluoroacetoxy-10-undecene (6.08%) were the major chemical components present. Cis-1-Methyl-2-(2'-propenyl) cyclopropane is the most abundant chemical component with 26.77% followed by 1-Octyn-3-ol with 9.81% (Table 20).

Table 19: Major chemical components identified in oil from *Ocimum gratissimum*leaves

s/no.	Chemical Constituent	RT (min)	Area (%)	Molecular Formula	Molecular Weight (g/mol)
1	D-Limonene	11.184	2.62	$C_{10}H_{16}$	136.238
2	Precocene I	31.553	95.63	$C_{12}H_{14}O_2$	190.242
3	Caryophyllene	28.989	0.92	$C_{15}H_{24}$	204.357
4	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	49.797	0.04	$C_{20}H_{30}O_4$	334.456
5	2-Hexene, 6-nitro-	56.062	0.04	$C_6H_{11}NO_2$	129.159
6	Ethanol, 2-[(1-methylene-2-propenyl)oxy]-	14.408	0.03	$C_6H_{10}NO_2$	114.144
7	1-Ethynylcyclopentanol	16.02	0.04	$C_7H_{10}O$	110.156
8	2,5-Octadiene	20.05	0.06	C_8H_{14}	110.2
9	3-Methyl-1-hexyne	21.039	0.06	C_7H_{12}	96.173
10	2-Methyl-1,5-heptadiene (c,t)	22.871	0.03	C_8H_{14}	110.2
11	1,6-Octadiene, 3,7-dimethyl-	26.131	0.03	$C_{10}H_{18}$	138.254
12	1,3,6-Octatriene, 3,7-dimethyl-, Z)-	30.381	0.05	$C_{10}H_{16}$	136.234
13	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-	32.433	0.05	$C_{20}H_{27}NO_2$	313.4339
14	(E,Z)-.alpha.-Farnesene	33.532	0.05	$C_{15}H_{24}$	204.357

RT – Retention time

Table 20: Major chemical component identified in oil from *Hyptis suaveolens*leaves

s/no.	Chemical Constituent	RT (min)	Area (%)	Molecular Formula	Molecular Weight (g/mol)
1	2-Butanamine	50.31	4.45	$C_4H_{11}N$	73.139
2	Cyclopentene	55.659	2.10	C_5H_8	68.119
3	cis-1-Methyl-2-(2'-ropenyl) cyclopropane	56.208	26.77	C_6H_{12}	84.162
4	1-Octyn-3-ol	56.795	9.81	$C_8H_{14}O$	126.199
5	4-Cyclopentene-1,3-diol, trans-	57.088	1.43	$C_5H_8O_2$	100.117
6	5-Hexyn-1-ol	57.381	3.82	$C_6H_{10}O$	98.145
7	1,2:4,5:9,10-Triepoxydecane	58.553	1.32	$C_{10}H_{16}O_3$	184.235
8	1-Propene, 3,3'-oxybis-	60.898	1.08	$C_6H_{10}O$	98.143
9	3-Hexen-1-ol, acetate, (Z)-	63.755	1.21	$C_8H_{14}O_2$	142.195
10	1-Heptyn-4-ol	67.675	1.06	$C_7H_{12}O$	112.172
11	Hexane, 1,6-dichloro-	69.873	1.98	$C_6H_{12}CL_2$	155.062
12	1-Trifluoroacetoxy-10-undecene	71.082	6.08	$C_{13}H_{21}F_3O_2$	266.304
13	Z-1,9-Hexadecadiene	80.278	1.18	$C_{16}H_{30}$	222.416
14	3-Octen-1-ol, (Z)	85.077	1.09	$C_8H_{16}O$	128.215
15	Paromomycin	85.883	1.20	$C_{23}H_{45}N_5O_{14}$	615.634

RT – Retention time

4.6.4 Major Chemical Components found in Oil from *Nicotiana tabacum*leaves

The analysis of the major chemical components identified in the oil of *Nicotiana tabacum* leaves revealed the presence of 50 components. Cyclohexane (8.43%), P-Xylene (12.37%), Nonane (4.35%), 1, 2, 3-trimethyl- Benzene (2.10%), Decane (3.60%), 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (16.37%), Farnesol (3.08%) and 1-Naphthalenepropanol (3.08%) were the major chemical components present. 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol is the most abundant chemical component with 16.37% followed by Xylene with 12.37% (Table 21).

Table 21: Major chemical components identified in oil from *Nicotiana tabacum*leaves

s/no.	Chemical Constituent	RT (min)	Area (%)	Molecular Formula	Molecular Weight (g/mol)
1	Cyclohexane	73.60	8.43	C_6H_{12}	84.162
2	P-Xylene	74.00	12.37	C_8H_{10}	106.168
3	1-Ethyl-3-methylcyclohexane	74.40	2.59	C_9H_{18}	126.243
4	Nonane	74.65	4.35	C_9H_{18}	126.243
5	Propyl-Cyclohexane	75.20	2.20	C_9H_{12}	120.191
6	1-ethyl-3-methyl- Benzene,	75.80	2.19	C_9H_{12}	120.195
7	1, 2, 3-trimethyl- Benzene	76.70	8.73	C_9H_{12}	120.195
8	Decane	77.30	3.60	$C_{10}H_{22}$	142.286
9	1, 2, 5-trimethyl- Benzene	77.70	2.10	C_9H_{12}	120.195
10	Undecane	82.40	2.49	$C_{11}H_{24}$	156.313
11	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	163.70	16.37	$C_{20}H_{40}O$	296.539
12	Farnesol	187.80	3.08	$C_{15}H_{26}O$	222.372
13	1-Naphthalenepropanol	156.50	3.08	$C_{20}H_{36}O_2$	308.498
14	9-methyl- Nonadecane	218.0	2.97	$C_{20}H_{42}$	282.556

RT – Retention time

CHAPTER FIVE

5.0 DISCUSSION

5.1 Ethnobotanical Survey

The ethnobotanical survey carried out to evaluate the knowledge associated with the traditional use of insecticidal plants against haematophagous insects in North-Central Nigeria showed that most of the targeted respondents interviewed from the nine tribes had adequate knowledge of the use of plants as protectants against these insects. A similar study conducted in Ethiopia showed that 97.2% of the targeted respondents had adequate knowledge as well as usage customs concerning traditional insecticidal plants used to repel haematophagous insects (Karunamoorthi *et al*., 2009a). This shows that wealth of knowledge of plants used as protectant against haematophagous insect dwells with certain groups of people in rural communities in North-Central Nigeria. This knowledge needs to be preserved through documentation hence the purpose of this research work.

This study focused more on respondents that were perceived to be endowed with knowledge of plants used for health care purposes which consisted of community leaders and elders, herb sellers and traditional healers among which elders from communities were the most interviewed respondents during the survey which is an indication that wealth of knowledge dwells with the elderly in communities in North-Central Nigeria. The second most reached and engaged target respondents were the herb sellers and they are reported by Iyamah and Idu (2015) to play a vital role in making herbs and they are open to sharing their knowledge of these herbs and their uses with researchers. This might be the reason why they are the second most interviewed group of respondents. Also, herb sellers are helpful as sources of knowledge of plants because that is their trade making them very familiar with plants around them. Iyamah and Edu (2015), also reported that herb sellers acknowledged obtaining regular feedback from their customers on the effectiveness of plants used for the treatment of malaria and personal protection against blood-sucking insects hence the authenticity of the information they present.

Most respondents in this study acknowledged that the source of their knowledge of insecticidal plants were passed to them from close family members. Similar observations of respondents obtaining knowledge of insecticidal plants used from family elders was also reported in South-Africa (Mavundza *et al*., 2011). This therefore evidently suggests that the elderly in communities in North-Central Nigeria possess sound knowledge of plants used for personal protection against haematophagous insects and this knowledge is sustained from one generation to another verbally and can be lost or distorted in the process. There is therefore, a need to conserve this knowledge by document it for future generations which this research work was able to achieve.

In this present research work, seventeen plant species used for protection against haematophagousinsects were shortlisted and identified which belonged to twelve families. Similarly, Mavundza *et al.* (2011) reported thirteen plant species used for personal protection against mosquito in South Africa belonging to nine families, Kweka *et al.* (2008) reported five insect repellent plant species belonging to four families in Tanzania, Karunamoorthi *et al*. (2009), reported nine mosquito repellent plants belonging to eight families in Ethiopia while Pålsson and Jaenson (1999) reported eight plant species which had the ability to keep away mosquitoes from human dwellings in Guinea Bissau.

Among the families of insecticidal plants collected, Lamiaceae was the most represented plant family used for personal protection against haematophagous insects. Similarly, Karunamoorthi *et al*. (2009b) reported that Lamiaceae formerly known as Meliaceae has the most represented family in an ethnobotanical study carried out in Ethiopia. The family Lamiaceae is important in the management of mosquito and other haematophagous insects because of the perceivedinsecticidal potential of the plant species members. In this study, members of the family Lamiaceae; *Hyptissuaveolens* and *Ocimumgratissimum* were the most frequently used plant species for personal protection purposes against haematophagous insects as mentioned by respondents from all the ethnic groups in North-Central Nigeria. Okigbo *et al*. (2016) reported that *Hyptissuaveolens* commonly known as mosquito plant because of its insecticidal potential against mosquitoes is widely used for personal protection against haematophagous insects in several communities in Nigeria. There are reports of the insecticidal potentials (repellency and toxicity bioactivities) of extracts from *Hyptissuaveolens* and *Ocimumgratissimum* in different parts of Nigeria against all life stages of mosquitoes (Afolabi, 2016; Adefolalu *et al*., 2015; Ayange-Kaa *et al*., 2015; Nzelibe and Chintem, 2015; Sulieman *et al*., 2014; Okigbo *et al*., 2010; Ivoke *et al*., 2009; Gbolade, 2001; Oyedele *et al*., 2000; Iwu, 1993).

The use of traditional medicines is rampant in regions where western medicines are inaccessible due to their unavailability and high cost (Light *et al.*, 2005). The main reason why locals depend on plants that have insecticidal potential for personal protection against blood-sucking insects is because they are accessible and available (Oyedele *et al*., 2000). Majority of the respondents from the study area indicated that they could easily access the insecticidal plants shortlisted and they were readily available. The accessibility of insecticidal plants to locals of the study area was estimated by the distance they had to travel to harvest these plants which was less than one kilometre and majorly around the community while the availability was estimated by the source of the plants. It has been speculated that most people living in rural communities in Africa rely on traditional medicine and insecticidal plants due to their availability and affordability in comparison with modern medicine and insecticides (WHO, 2002).

In the study area, most of the respondents affirmed the use of whole plant for personal protection against haematophagous insects followed by leaves. Similarly, Karunamoorthi *et al*. (2009a) reported that leaves were the most used plant's parts for personal protection against mosquitoes in Ethiopia, Kweka *et al*. (2008) and Mavundza *et al*. (2011) also reported same trend in Tanzania and South Africa. Karunamoorthi and Husen (2012) emphasized the use of leaves of insecticidal plants for personal protection against haematophagous insects against whole plants, roots and barks of insecticidal plants because it is a more sustainable option since the natural plant growth would not be disrupted. Hence, the use of the whole plant in North-Central Nigeria is not a sustainable option and should be discouraged. Apart from sustainability, another reason why the use of the leaves of insecticidal plants is more important than other parts might be due to the ready availability of their active components that are more probable volatile in nature (Mavundza *et al*., 2011). Indeed, plants that are usually used for personal protection because of their insecticidal properties are habitually those that contain volatile oils so that when the leaves are crushed, it releases strong odour which are unpleasant to blood-sucking insects (Youmsi *et al*., 2017).

Usage of plants in their fresh state by hanging inside habited houses as well as smouldering of dried plants were frequent ways in which respondents from this study applied plants for personal protection purposes against mosquitoes and other blood-sucking insects. This is comparable to other reports where plants were hanged or spread inside habited houses (Kweka *et al*., 2008) and where plant’s part where smouldered to manage blood-sucking insects (Karunamoorthi *et al*., 2009b; Karunamoorthi *et al*., 2008; Klein *et al*., 1995; Vernede *et al*., 1994; Sukumar *et al*., 1991).

The observation of the present study shows that there was no significant difference (P>0.05) in the knowledge of the insecticidal plants among gender, educational status and ethnicity of respondents suggesting that plants with insecticidal potential are well known by all classes and status of the communities in North-Central Nigeria. This is in consistency with earlier study which established that there was no significant relationship (P>0.05) between the gender, educational status and age of respondents and knowledge and usage custom concerning insect repellent plants (Kidane *et al*., 2013). In contrast, a strong association (P<0.05) was observed between the perceived efficacy of insecticidal plants used for personal protection and gender, educational status and ethnicity of the respondents. This suggests that gender, educational status and ethnicity had a part to play in the perception of how effective these insecticidal plants are in the management of blood sucking insects.

5.2 Screening of Collected Plant Materials for Repellency against *Anopheles gambiae* s.l.

In this study, seventeen insecticidal plants were identified by locals to possess insecticidal potential against haematophagous insects. The parts mentioned were collected with the help of the locals and screened for possible repellent bioactivity against *Anopheles gambiae* s.l. Out of all the collected and identified insecticidal plants that were screened, the leaves of *Cassia mimosoides*, *Ertyphleum suaveolens*, *Moringa oleifera* and the fruits of *Parkia biglobosa* showed no significant (P>0.05) repellent activity against *Anopheles gambiae* s.l. while the leaves of *Ageratum conyzoides*, *Allium cepa*, *Annona senegalensis*, *Cymbopogon citratus*, *Eucalyptus globulus*, *Hibiscus rosa sinensis*, *Hyptis suaveolens*, *Latanna camara*, *Nicotiana tabacum*, *Ocimum gratissimumThymus vulgaries* and the fruits of *Capsicum annuum* and fruit peels of *Citrus sinensis* showed significant (P<0.05) repellent activity against *Anopheles gambiae* s.l.

Laboratory evaluation of *Ageratum conyzoides* and *Hyptis suaveolens* showed that they both compared favourably in terms of repellency when compared with DEET which is a standard repellent against *Anopheles gambiae* s.l. *Hyptis suaveolens* is of high economic and medical value as it is being employed widely for personal protection against mosquito because of its repellent properties (Hemen *et al*., 2013). The leaves of *Hyptis suaveolens* has been reported to induce a ten-fold reduction in mosquito bites (Curtis *et al*., 1991) and a seventy-three percent repellent activity against mosquitoes for 2 hours in a field study (Trongtokit *et al*., 2004). *Ageratum conyzoides* have been reported to have a detrimental effect on survival, developmental and adult emergency of mosquitoes (Hemen *et al*., 2013). Also, *Citrus sinensis*, *Nicotiana tabacum* and *Ocimum gratissimum* displayed similar level of repellent activity that was observed in DEET against *Anopheles gambiae* s.l. The above-mentioned plants could be used as alternatives for personal protection in place of DEET in highly malarious areas in North-Central Nigeria where vectors outdoor biting behaviour has been established.

5.3 Repellency Activity of Oils from selected Plant against *Anopheles gambiae* s.l.

Reports from ethnobotanical survey and repellency activity on plants used as protectant against biting insects from this study showed that *Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacum* and *Ageratum conyzoides* had the highest frequency of citation by respondents in North-Central Nigeria as well as the highest repellency activity against *Anopheles gambiae* s.l. which was comparable with what was observed in DEET hence the need to encourage further research to develop these plants into products.

Plants possess varying parts which have complex chemicals with unique bioactivity which is due to toxins and secondary metabolites that act as either toxicant or repellents to haematophagous insects. In this study, all the concentration of oils from *Hyptis suaveolens*, *Ocimum gratissimum*, *Ageratum conyzoides*, *Citrus sinensis* and *Nicotiana tabacum* displayed a hundred percent repellency against *Anopheles gambiae* s.l. in the presence of human odour which is a natural *Anopheles* mosquito attractant. Similarly, it has been reported that oils from *Hyptis suaveolens* (Benelli *et al*., 2012), *Ocimum gratissimum* (Afolabi, 2016) and *Citrus sinensis* (Gbolade, 2001) displayed hundred percent repellency against *Anopheles* mosquitoes. Also, plants oils from *Hyptis suaveolens* (Benelli *et al*., 2012), *Ocimum gratissimum* (Afolabi, 2016) and *Citrus sinensis* (Gbolade, 2001) have been reported to exhibit 120 to 150 mins protection activity against *Anopheles* mosquitoes.

In the present study, DEET displayed excellent repellent activity against *Anopheles gambiae* s.l. in the presence of human odour affirming its efficacy as a brilliant mosquito repellent both locally and international. However, despite this feat, there are concerns in relation to skin irritation and toxicological with the use of DEET for personal protection (Benelli *et al*., 2012) hence the need for alternatives. The plant oils tested displayed better repellent activities against *Anopheles* mosquitoes when compared to DEET hence the need to consider them as alternatives for personal protection against *Anopheles* mosquitoes. Similar studies have reported that plant oils display repellent activities that are higher than those displayed by synthetic repellents (Moore *et al*., 2006). Consequently, these plant oils can be used to manage *Anopheles* mosquitoes that bite during the day and outdoors in malarious areas. They can be employed for personal protection in communities were locals spend more time outdoors in the early evening.

5.4 Contact Toxicity of Oils from Selected Plants against *Anopheles gambiae* s.l.

5.4.1 Contact Toxicity of Oils from Selected Plants against *Anopheles gambiae* s.l. Larvae

Mosquito larvicidal efficacy of plant oils and extracts vary according to plant species, the part of the plant, the geographical location where the plant is grown and application methods (Das *et al*., 2003; Ghosh and Chowdhury, 2012). In the current study, 3.0mg/ml concentration of *Hyptis suaveolens*, 4.0mg/ml concentration of *Citrus sinensis* and 5.0mg/ml concentrations of *Nicotiana tabacum* and *Ageratum conyzoides* evoked hundred percent mortality on *An. gambiae* s.l. larvae after 24 hours of treatment. Also, it was observed that 2.0mg/ml concentration of *Hyptis suaveolens* and 3.0mg/ml concentrations of *Citrus sinensis, Nicotiana tabacum* and *Ageratum conyzoides* elicited hundred percent mortality on *An. gambiae* s.l. larvae after 48 hours of treatment. This compares with results from Kavendan *et al*. (2014) and Bobbo *et al*. (2016) who demonstrated that *Hyptis suaveolens* caused high mortalities against *An. gambiae* mosquito larvae after 24 hours post-exposure. Similarly, Musa *et al*. (2015) reported that methanol and aqueous extract of *Ageratum conyzoides* evoked 92.0% and 87.5% mortality respectively against *An. gambiae* s.l. after 24hrs of exposure while Ileke *et al*. (2015) demonstrated that*N. tabacum* elicited high mortality to the larvae and pupae of *An. gambiae*. Hence, oils from *Hyptis suaveolens*,*Citrus sinensis*, *Nicotiana tabacum* and *Ageratum conyzoides* could be adopted as natural larvicides for the management of *Anopheles* mosquitoes in malarious areas where breeding sites can easily be identified and accessed. However, it was observed that all the concentrations of oils from *Ocimum gratissimum* tested elicited an average larvicidal activity against *An. gambiae* larvae meaning it is not a good larvicidal agent. It was also observed that the toxicity of all the plant oils evaluated against *An. gambiae* larvae was concentration dependent.

The lowest LC_{50} after 24 and 48 hours of treatments was observed in oils from *Citrus sinensis* against *An. gambiae* s.l. larvae while the highest was observed in *Nicotiana tabacum*. Also, the lowest LC_{90} after 24 and 48 hours of treatments was observed in oils from *Hyptis suaveolens* against *An. gambiae* s.l. while the highest was observed in oils from *Nicotiana tabacum* after 24 hours post-treatment and *Ocimum gratissimum*after 48 hours post-treatment. Similarly, Musa *et al*., (2015) reported that extracts of *Ageratum conyzoides* elicited a low LC_{50} and LC_{90} against *An. gambiae* s.l. larvae. Oils from *Citrus sinensis* and *Hyptis suaveolens* displayed highest mortality at lowest concentrations echoing their potential as good larvicides and should be considered for the management of *Anopheles* mosquitoes while *Nicotiana tabacum* should not be considered because of its poor activity.

5.4.2 Contact Toxicity of Oils from Selected Plants against Adult *Anopheles gambiae* s.l.

Significant efforts have been put into the search for novel strategies to disrupt disease transmission cycle by targeting insect vectors in their adult stages and different plant oils and extracts have been reported to have adulticidal capabilities (Ileke *et al*., 2015; Owoeye *et al*., 2016). The current study was successful in investigating the adulticidal potential of *Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacum* and *Ageratum conyzoides* against a major malaria vector *Anopheles gambiae* s.l. in North-Central Nigeria. In this current study, it was observed that 5.0mg/ml concentration of *Nicotiana tabacum* recorded hundred percent mortality on adult *Anopheles gambiae* s.l. within one-hour test period which was same result that was observed in Deltamethrin the positive control. Similarly, Owoeye *et al*. (2016) and Ileke *et al*. (2015) reported that *Nicotiana tabacum* elicited hundred percent mortality on adult *A. gambiae* within one-hour test period. *Anopheles* mosquitoes have been reported to have developed resistance to Deltamethrin based interventions in so many parts of Nigeria (Awolola *et al*., 2002). Therefore, oils from

Nicotiana tabacum can be turned to as alternative to the use of Deltamethrin for vector control programmes especially in the treatment of insecticide treated bed nets because of its efficacy and biodegradability. Also, in the study, it was interesting to note that 5.0mg/ml of *Ocimum gratissimum* recorded high mortalities which were close to the mortalities obtained in Deltamethrin. This shows that they could also be turned to as alternatives for *Anopheles* mosquito control in places where they naturally grow. However, the mortalities observed for oils from *Ageratum conyzoides* against *Anopheles gambiae* s.l. showed that this plant oil possessed poor toxicity activity against adult *Anopheles* mosquitoes.

The lowest KdT_{50} against *Anopheles gambiae* s.l. was observed in 2.5mg/ml of *Nicotiana tabacum* (20.3 minutes) while the highest was observed in the lowest concentration of *Citrus sinensis*. Also, the lowest KdT_{95} against *Anopheles gambiae* s.l. was observed in 3.0mg/ml of *Ocimum gratissimum* (48.11 mins) while the highest was observed in the lowest concentration of *Citrus sinensis*. This shows that *Nicotiana tabacum* and *Ocimum gratissimum* plant oils has the potential to be used for the management of adult malaria vectors in Nigerian communities where they are available and can easily be accessed against the use of pyrethroid-based synthetic insecticides as is the norm today. These plants can be incorporated for use in Insecticide Treated Nets and Indoor Rsidual Sprays.

5.5 Vapour Toxicity of Selected Plant Oils Against Adult *Anopheles gambiae*

One of the behaviours elicited by mosquitoes in response to airborne components are knockdown and mortality which reiterates the vapour toxicity of these components (Ogoma *et al*., 2014). Interestingly, all five plant oils evaluated elicited a knockdown activity on *An. gambiae*. This study revealed that 5.0mg/ml of oils from *Citrus sinensis* displayed a better knockdown activity compared to DEET on *An. gambiae* after 20 minutes of exposure and one hundred percent knockdown activity after 60 minutes of exposure. Similarly, Manimaran *et al*. (2012) and Gbolade (2001) reported that *Citrus sinensis* was burnt by locals in some rural communities in Africa and it displayed a capability of killing and repelling mosquitoes. Also, it was observed that after 30 minutes of exposure time, 5.0mg/ml of oils from *Nicotiana tabacum* and *Ocimum gratissimum* achieved a better knockdown activity on *An. gambiae* compared to DEET. These plant oils achieved one hundred percent knockdown activity which is the same as the effect observed in DEET after 60 minutes of exposure suggests that *Nicotiana tabacum* and *Ocimum gratissimum* have potential to be used as repellents if further developed. Similarly, Ileke *et al*. (2015) investigated the fumigant effect of *Nicotiana tabacum* on *An. gambiae* and recorded 100% mortality. Also, 100% knockdown activity was observed in 3.0mg/ml of oils from *Ocimum gratissimum* after 60 minutes exposure time. This shows that oils from *Citrus sinensis*, *Nicotiana tabacum* and *Ocimum gratissimum* could be employed as repellent for personal protection purposes against malaria vectors both indoors and outdoors in place of DEET. The plant oils can also be used by tourists who travel to malarious zone for personal protection and for military personals among others.

Interestingly, hundred percent mortality was observed in 3.0mg/ml and 5.0mg/ml concentrations of all the plant oils evaluated on *Anopheles gambiae* after 24 hours displaying their potential to be incorporated into indoor residual spray or house paints instead of the presently used synthetic chemicals.

Lower KdT_{50} and KdT_{95} was observed in *An. gambiae*exposed to oils from *Ocimum gratissimum*and *Hyptis suaveolens* showed these plant oils have better knockdown effect compared to DEET (Ogoma *et al*., 2014). *Ocimum gratissimum*and *Hyptis suaveolens* could be used for the management of outdoor biting *Anopheles* mosquitoes in places where they have been reported to have developed resistance to commonly used insecticides for vector control interventions and there are concerns with the use of DEET.

5.6 Major Chemical Components Identified in Oils from *Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacumand Ageratum conyzoides*

In this study, D-limonene has been identified by GC-MS analysis as the major chemical component in oils from *Citrus sinensis*, *Ageratum conyzoides* and *Ocimum gratissimum*. Ahmad *et al*. (2006) and Espina *et al*., (2011) reported D-Limonene as a key chemical component in oils from *Citrus sinensisAgeratum conyzoides* and *Ocimum gratissimum*. In contrast, D-Limonene was identified as least abundant chemical component in oils from *Ageratum conyzoides* while Caryophyllene was identified as the most abundant (Usman *et al*., 2013).

Also, Precocene I and Caryophyllene were identified as major chemical components in oil from *Ocimum gratissimum*. Similarly, Caryophyllene was identified as a major chemical component in oils of *Ocimum gratissimum* however Eugenol was identified as the most abundant (Joshi, 201; Saliu *et al*., 2011; Dambolena *et al*., 2010).

In the present study, P-Xylene was identified as a major chemical component in oil from *Nicotiana tabacum*. This is similar to a report from Kidah (2018) where P-Xylene was identified as the key chemical component in oils from *Nicotiana tabacum*.

Studies on insecticidal plants have led to the isolation and characterization of the active components for example p-menthane-3-8-diol (PMD) was characterized and isolated from oils of citronella and lemon (Okwute, 2012). This study successfully identified D-Limonene as a major chemical component present in oils from *Citrus sinensis*, *Ageratum conyzoides* and *Ocimum gratissimum* and the isolated form has been reported to have insecticidal potential against stored grain insects (e.g. *Sitophilus oryzae, Tribolium castaneum, Oryzaephilus surinamensis*)(Lee *et al*., 2003), cockroaches (Karr and Coats, 1988) and termites(Almeida *et al*., 2015). D-Limonene has also been reported to act has contact poison against some insects e.g. fleas, mites and wasp (Okwute, 2012). There is need to isolate and evaluate the insecticidal potential of D-Limonene on mosquitoes.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The ethnobotanical knowledge, bio-efficacy and characterization of plants with insecticidal potential against *Anopheles gambiae* in North-Central Nigeria was evaluated and the following are summaries from the study;

- Elders from communities evaluated and herb sellers were the most interviewed respondents in the study area because of their knowledge of plants used for health care purposes.
- Insecticidal plants were perceived by respondents to be available and easily accessible.

- Whole plants were the most used plant parts by locals of North-Central Nigeria for personal protection against biting insects followed by leaves.
- Collected *Ageratum conyzoides* and *Hyptis suaveolens* parts (leaves) produced the same repellent activity as DEET against adult *Anopheles gambiae* s.l.
- *Hyptis suaveolens*, *Ocimum gratissimum*, *Ageratum conyzoides*, *Citrus sinensis* and *Nicotiana tabacum* oils displayed hundred percent repellency against *Anopheles gambiae* s.l. compared to DEET in the presence of human odour.
- Excellent toxicity on *Anopheles gambiae* s.l. was observed when oils from *Hyptis suaveolens*, *Citrus sinensis* and *Nicotiana tabacum* were evaluated on larvae stage and oils from *Nicotiana tabacum* were evaluated on adult stage which compared favourably with Deltamethrin.
- *Hyptis suaveolens*, *Ocimum gratissimum*, *Ageratum conyzoides*, *Citrus sinensis* and *Nicotiana tabacum* oils at 0.3mg/ml and 0.5mg/ml concentrations caused 100% mortality to adult *An. gambiae* after 24 hours.
- D-Limonene was identified as a major component in oils from *Citrus sinensis, Ageratum conyzoides* and *Ocimum gratissimum* and could be responsible for insecticidal potential of these plants.

6.2 Conclusion

This study was able to document and evaluate plants with insecticidal potential against *Anopheles gambiae* in North-Central Nigeria. These plants were perceived to be available and accessible and there is need to conserve this knowledge hence the goal of this study. Mentioned and shortlisted insecticidal plants displayed excellent repellent, contact and vapour toxicity properties against *Anophelesgambiae* elucidating their insecticidal potential hence the need to develop and use them in the management of *Anopheles* mosquitoes in Nigeria in place of synthetic insecticides presently used in vector control interventions especially with the recent rise in reports of resistance to these synthetic insecticides and toxicological concerns with the use of DEET. Also, the study was able to identify D-Limonene an insect contact toxicant as a major component in three insecticidal plants evaluated.

6.3 Recommendation

- There is the need to promote the cultivation and conservation of the plants documented in this study because of their availability, accessibility and potency against *Anopheles* mosquitoes and also to discourage the use of whole plant and encourage the use of the leaves for personal protection purposes which is sustainable.
- For personal protection in places where there is residual malaria transmission, *Ageratum conyzoides* and *Hyptis suaveolens* could be turned to as repellents in place of DEET. Also, Oils from *Citrus sinensis, Nicotiana tabacum* and *Ocimum gratissimum* could be formulated into forms that can be applied topical or sprayed on

clothing for personal protection against *Anopheles* mosquitoes in places where these vectors have been established to bite during the day and outdoors.

- Oils from *Hyptis suaveolens*, *Citrus sinensis* and *Nicotiana tabacum* have good larvicidal properties and could be used in areas where the larvae stage of *Anopheles* mosquitoes are easily accessible while *Nicotiana tabacum* and *Ocimum gratissimum* could be used for the treatment of bed nets in areas where resistance to pyrethroid based interventions have been established.
- All of the five plant oils tested can be used as vaporisers indoors in place of pyrethroid based aerosols used for personal protection in places where vectors have been identified to be anthropophilic and endophilic but resistant to the commonly used aerosols and also be incorporated in IRS.

Suggestions for Further Study

- There is also a need to evaluate the mode of action of these plant oils against *Anopheles* mosquitoes since they have been established as excellent repellent and toxicant.
- There is need to isolate D-Limonene and evaluate its bioactivity against *Anopheles* mosquitoes as well as understand its mechanism of action.
- There is also a need to investigate the human toxicological impact of all the plant oils evaluated to validate their safety.