AN EVALUATION OF THE LARVICIDAL EFFICACY OF THE SEED OIL EXTRACTS OF SELECTED PLANTS AGAINST SOME MOSQUITO SPECIES

BY

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NIGERIA.

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BY

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DEPARTMENT OF BIOLOGICAL SCIENCES,
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MAY, 2015

DECLARATION

I declare that the work in this thesis entitled "**AN EVALUATION OF LARVICIDAL EFFICACY OF THE SEED OIL EXTRACTS OF SELECTED PLANTS AGAINST SOME MOSQUITO SPECIES**" has been carried out by me in the Department of Biological Sciences,Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and in a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any Institution.

Bayo KAMBA _____ _____

Signature Date

CERTIFICATION

This thesis entitled AN EVALUATION OF LARVICIDAL EFFICACY OF THE SEED OIL EXTRACTS OF SELECTED PLANTS AGAINST SOME MOSQUITO SPECIES by BAYO KAMBA meets the regulations governing the award of the degree of Master of Zoology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to Almighty God for seeing me throughout my studies, my family, Blessing and Godswill.

AKNOWLEGDENTS

I give glory, honour, praise and adoration to almighty God for seeing me throughout my study.

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ABSTRACT

The evaluation of the larvicidal potency of the plant seeds might lead to the development of safe and environmentally friendly insecticides. The oils of the seeds of *Adansoniadigitata*, *Parkiabiglobosa*and *Perseaamericana*wereevaluated for their chemical composition and theability to kill mosquito larvae of *Aedesaegypti*, *Aedesvittatus*and *Culexquinquefasciatus* under laboratory and outdoor conditions.The seeds of the three plants were extracted from 100g powdered kernel with 300mL petroleum ether using soxhlet extractor for 5-6hr. The solvent was evaporated under vacuum leaving behind oil seed.The extracted oils were quantified gravimetricallyto determine the presence of chemical constituents of larvicidal importance.Bioassay oflate third larval instar was tested using six concentrations of 0.01, 0.02, 0.04, 0.08, 0.16 and 0.32mL ofeach seed oil extract making up to 100mL with distilled water. A batch of 25 mosquito larvae were tested in three replicates for each concentration along with the respective control of distill water devoidof extracts. The chemical composition of the seed oil extracts revealed the presence of acid, free fatty acid, iodine, peroxide and saponification values in different quantities. Acid and free fatty acidvalues did not differ significantly ($p>0.05$) between the extracts of *A. digitata* (5.04 and 2.50mgKOH/g) and *P. biglobosa*(5.05 and 2.50mgKOH/g) except for*P. ameriacana* (19.63 and 9.90mgKOH/g) ($p<0.05$) where significance was recorded. Iodine and peroxide differ significantly ($p<0.05$) across all the three seed oil extracts. Saponins was very high in all the seed oil extracts compared to other phytochemical compositions (acid, free fatty and peroxide values), but was only significant ($p<0.05$)in *P. americana* (199.33mL/Kg). The cidal effect of the seed oil extracts was dose-dependent for *A. digitata, P. americana* and

P. biglobosa. The median lethal concentration (LC_{50})valuesof *Adansoniadigitata* seed oiltested against three mosquito species for laboratory and outdoor conditions were respectivelyfound to be as follows:*Ae. aegypti*0.1179mL/L and 0.4886mL/L;*Ae. vittatus*0.0998mL/L and 0.00055mL/L; and *Cx. quinquefasciatus*0.0519mL/L and 0.00397mL/L.For *Parkiabiglobosa*, theLC_{50}values determined for *Ae. aegypti*were 0.1084mL/L and 0.3327mL/L;*Ae. vittatus,* 0.1891ml/L and 0.0010ml/L; and *Cx. quinquefasciatus* 0.1058mL/L and 0.0134mL/L for laboratory and outdoor conditions respectively. *Perseaamericana*had the following LC_{50} values: *Ae. aegypti* 0.3026mL/L and 0.0561mL/L;*Ae. vittatus*0.1118mL/L and $7.0x10^{-6}$mL/L and *Cx. quinquefasciatus* 0.0858mL/L and 0.1862mL/L for laboratory and outdoor conditions,repectively.These results suggest that the seed oilsof *A. digitata, P. biglobosa*and *P. americana*can be harnessed for the development of bio-larvicide for the control of the mosquitoesspecies studied and invariably the diseases they transmit.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Mosquitoes represent a significant threat to human health because of their ability as vector pathogens which cause diseases which afflict millions of people worldwide (WHO, 2010). Several species belonging to the*Aedes, Anopheles* and *Culex* genera are vectors of the pathogens of various diseases like yellow fever, dengue fever, dengue hemorrhagic fever, malaria,yellow fever, Japanese encephalitis, West Nile virus and filariasis (Rahuman, 2009; Borah *et al.*, 2010; Samuel 2010).Mosquitoes have the potential to feed on more than one individual during a single gonotropic cycle and this enhances their vectorial roles (Mackenzie *et al*., 2004). Proper control of mosquitoes lies in personal protection and public awareness but the most economical method involves eradicating breeding sites and controlling these pests through application of environmental friendly larvicides (Corbel *et al*., 2004).

Among the thirteen genera of the family Culicidae, the genus *Aedes* is considered dangerous because of its significant public health threat all over the world (Rajesh *et al.*, 2013).*Aedes vittatus*and *Aedes aegypti*are potential vector speciesof chickungunya, dengue fever and yellow fever virus (Service, 1970; Ali*et al.*,2014).They can also transmit yellow fever virus from monkey to monkey in the laboratory (Bang *et al.*, 1981) and have been suspected as vectors in the Nubamountain epidemic in Sudan (Ali *et al.*, 2014). *Aedes* species are also important vectors of encephalitis and many other arboviruses, and in a few restricted areas, they are also vectors of *Wuchereria bancrofti* and *Brugiamalayi* (Kandaswamy *et al.*, 2012).

Anopheles speciesand *Culex quinquefasciatus* are responsible for the transmission of several species ofparasite that cause diseases such as malaria and filariasis respectively.*Anopheles* speciestransmit malaria that kills more people than HIV/AIDS with about 50% of the population experiencing atleast one episode of malaria each year (Greenwood *et al*., 2005).

Culex quinquefasciatus is one of the most widespread mosquitoes in the world. It is found throughout most of pan and subtropical Americas (Weinstein *et al*., 1997; Carver*et al*., 2009; Andreadis*et al*., 2010), the Neotropics, Afrotropics (White, 1975; Diaz-Badillo*et al*., 2011), Indomalayan, Australian (Lee *et al.*, 1989; David *et al*., 2012) and Eastern Asian regions of the world (Bram, 1967; Rios-Ibarra *et al*., 2010). It is also present in the United Kingdom and parts of the Middle East such as Pakistan, Iran among others.It is an important vector of periodic filariasis in parts of the world (Belkin, 1968; Rios-Ibarra*et al*., 2010) and is known to carry and transmit *Wuchereria bancrofti* to some degree in many regions of the globe.Of the estimated global 128 million lymphatic filarial cases, 91 per cent are caused by *Wuchereria bancrofti* Cobbold (Singh, 1967;Andreadis *et al*., 2010).

Currently, the main tool for mosquito control is the use of diverse synthetic chemicals as larvicides (Govindarajan and Rajeswary, 2014). The drastic effects of synthetic insecticides in the environment have received wide public apprehension (St. Lager *et al*., 1996; Morin andComrie, 2010). Indeed, synthetic insecticide misuse in agriculture and public health programmes has caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans and other non-target organisms (Sarwar *et al*., 2009). To alleviate these problems, major emphasis has now been shifted on the use of naturalderived

plant products as larvicides, which can serve as alternate to synthetic insecticides (Junwei *et al*., 2006).

One of the most effective alternative approaches under the biological based programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control (Shallan *et al.*, 2005). In addition, unlike conventional insecticides, which are based on a single active ingredient, plant-derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioural and physiological processes (Gokulakrishnan *et al*., 2013). Thus, there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Plant essential oilshave widespread insecticidal properties and will obviously work as a new weapon in the arsenal of insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases (Morais *et al*., 2007;Gokulakrishnan *et al*., 2013).

Botanicals are basically secondary metabolites that serve as part of the defence mechanism of plants to withstand the continuous selection pressure from herbivores, predators and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, saponins, essential oils and phenolics from different plants have beenreported for their insecticidal activities (Shallan *et al.*, 2005). Insecticidal activitiesof plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to the extraction methodology adopted and the polarity of the solvents used during extraction(Anupam*et al*., 2012). A wide selection of plants from herbs, shrubs and large trees

have been used for extraction of toxins using different methods for mosquito control(Anupam*et al*., 2012).

Phytochemicals have been extracted either from the whole body of little herbs or from various parts like fruits, seeds, leaves, stems, barks, roots, of larger plants or trees. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of the plant families- Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae and Rutaceae have various types of larvicidal, adulticidal or repellent activities against different species of mosquito (Shallan *et al.*, 2005).Some plants such as *Solanum villosum* have shown very strong insecticidal effect (Isman, 1999).

In the survey of literature on insecticidal properties of essential seed oil from the year 2004 onwards indicates that essential oils from about 90 plant genera belonging to 38 plant families were reported to have toxic properties against mosquito larvae (Mann and Kaufman, 2012) and pupae (Gokulakrishnan*et al*., 2013). In general, plant essential oils are important natural alternatives to insecticides (Gbolade *et al*., 2000). Many plant extracts and essential oils possess larvicidal activity against various mosquito species (Govindarajan *et al.,* 2013; Danga, *et al.,* 2014). Many plants contain chemicals which are helpful for the control of insects and are useful for field applications in mosquito control programmes (Kalyanasundaram and Das, 1985; Isman, 1999).

Adansonia digitata Linn. (Malvaceae) is commonly known as baobab, is a majestic tree revered in Africa for its medicinal and nutritional value(Buchmann, *et al*., 2010). The plant parts are used

to treat degrees of diseases such as diarrhoea, malaria and microbial infections. It has been reported that it has an excellent anti-oxidant due to the vitamin C content which is 6-10 times higher than the vitamin C content of oranges(Kamatou *et al*., 2011). Baobab has diverse biological properties such as antimicrobial, antiviral, anti-oxidant and anti-inflammatory activities. Phytochemical studies revealed the presence of flavonoids, saponins, phytosterols, amino acids, fatty acids, vitamins and minerals (Kamatou *et al*., 2011).

Parkia biglobosa Jacq., commonly known as the locust bean tree, is a perennial deciduous tree of the Fabaceae family (Teklehaimanot, 2004). It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Where the tree is grown, the crushing and fermenting of these seeds constitutes an important economic activity. Various parts of the locust bean tree are used for medicinal purposes (Abioye *et al*., 2013), such as treating hypertension (Karou *et al*., 2013), malaria (Traoré *et al*., 2013) and healing wounds (Adetutu *et al*., 2013).

*Persea americana*Mill., commonly known as pear or avocado tree belongs to the Lauraceae family has an edible fruit. It originated from Central America but is easily adaptable in tropical regions (Leite *et al*., 2009). The avocado fruit has an olive-green peel and thick pale yellow pulp that is rich in fatty acids such as linoleic, oleic, palmitic, stearic, capric and myristic acids (Dreher and Davenport, 2013). This fruit is normally used for human consumption, but it has also been used as a medicinal plant in Mexico and elsewhere in the world (Dreherand Davenport, 2013).The avocado seed represents 13–18% of the fruit, and it is a by-product that is generally not utilized. Normally, the seed is discarded during the processing of the pulp. The seed waste may present a severe ecological problem (Ortiz *et al.*, 2004). However, at the same time, it may be of interest to industry as a source of bioactive compounds. Chemicallyit is composed of

phytosterols, triterpenes, fatty acids and two new glucosides of abscisic acid (Ramos *et al*., 2004).

Several biological activities of the avocado seed have been reported such as antioxidant, antihypertensive, fungicidal, hypolipidemic and recently amoebicidal and giardicidal activities (Rodriguez-Carpena*et al*., 2011). From the perspective of the use of avocado seeds as sources of phytotherapeutic agents, they have been traditionally used to treat mycoses and parasitic infections. In addition, avocado seeds are known to have local anaesthetic effects that decrease muscle pain (Ozolua *et al*., 2009).

1.2 Statementofthe Research Problem

Several parts such as leaves, bark and stem of *Adansonia digitata* (baobab),*Parkia biglobosa*(locust bean) and*Persea americana*(avocado pear) have been investigated for their larvicidal action against mosquito larvae but the seed oil have not been fully explored in this respect such that their larvcidal potentials have not been investigated and therefore unknown.

1.3 Justification

By evaluatingthe larvicidal potency of the plant seeds might lead to the devolepment of safe and environmentally friendly insecticides.This will go a long way in controlling the vectors of the deadly diseases and eliminate the numerous ills that have been associated with synthetic pesticides such as high cost, environmental pollution, immune dysfunction, cancer,birth defects and resistance build up among others.

1.4 Aim

To evaluate the larvicidal prospects ofseed oilextratcs of*Adansonia digitata*(baobab),*Parkia biglobosa*(locust bean) and*Persea americana* (avocado)against the larvae of *Ae. aegypti, Ae. vittatus* and *Cx. quinquefasciatus* under laboratory and outdoor conditions.

1.5 Objectives

i. To determine the chemical composition of the seed oil extracts of baobab, locust bean and avocado.

ii. To determine the efficacy of the seed oil extracts of baobab, locust bean and avocado against the larvae of *Ae. aegypti*, *Ae. vittatus* and *Cx. quinquefasciatus* in both laboratory and outdoor settings.

1.6 Hypotheses

i. There is no significant difference in the chemical composition of baobab, locust bean and avocado seed oil extracts.

ii. The seed oil extracts of baobab, locust bean and avocado have no significance effects on the larvae of *Aedes aegypti*, *Aedes vittatus* and *Culex quinquefasciatus*in the laboratory and outdoor settings.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background onMosquitoes

Over 3,500 species of mosquito have already been described from various parts of the world (NCID, 2004). Some mosquitoes that bite humans routinely act as vectors for a number of infectious diseases affecting millions of people every year (Aina *et al.,*2009). Those that do not routinely bite humans but bite animmals, are vectors of animal diseases which could be carriers of zoonoticdiseases when their habitats have being disturbed due to human activities such as deforestation (Wilcox, 2006).

Vector-borne diseases, particularly mosquito-borne diseases, have been the most important worldwide health problems for many years and still represent a constant and serious risk to a large part of the world's population (Mgbemena and Ebe, 2012). Mosquitoes are vectors of *Plasmodium* that causes malaria; nematode worms (e.g *Wuchereria* species and *Brugia* species) that cause filariasis and a large number of arboviruses including two (i.e. yellow fever and dengue viruses) with great impact in the tropical and subtropical regions.(Mgbemena and Ebe, 2012).

2.2 Life Cycle of Mosquitoes

Mosquitoes go through four stages of development in their life cycles namelyegg, larva, pupa, and adult or imago. In most species, adult females lay their eggs in stagnant water (*Culex* species); some lay eggs near the edge of water; others attach their eggs to aquatic plants (Huang*et al.*, 2006). Each species selects the suitable habitat into which it lays its eggs and does so according to its own ecological adaptations. Mosquitoes breed in lakes, temporary puddles, marshes, some in salt-marshes (Kaufmann and Briegel, 2004). Among those that breed in salt water, some are equally at home in fresh and salt water up to about one-third the concentration

of seawater, whereas others must acclimatize themselves to the salinity. Such differences are important because certain ecological preferences keep mosquitoes away from most humans, whereas other preferences bring them right into houses at night (Kaufmann and Briegel, 2004).

The first three stages—egg, larva and pupa are largely aquatic. These stages typically last from five to fourteen days, depending on the species and the ambient temperature, but there are important exceptions. Eggs hatch to become larvae, which grow until they are able to change into pupae. The adult mosquito emerges from the mature pupa as it floats at the water surface. Bloodsucking mosquitoes, depending on species, gender, and weather conditions, have potential adult lifespans ranging from as short as a week to as long as several months (Devlin, 2010).

2.2.1Eggs and oviposition

Mosquito habits of oviposition, the ways in which they lay their eggs, vary considerably between species, and the morphologies of the eggs also vary accordingly (Sawabe and Moribayashi, 2000).In species that lay their eggs in rafts, rafts do not form adventitiously; the female *Culex* settles carefully on still water with her hind legs crossed, and as she lays the eggs one by one, she twitches to arrange them into a head-down array that sticks together to form the raft (Spielman and D'Antonio, 2001). *Aedes* females generally drop their eggs singly, much as *Anopheles* does, but not directly into water. Instead, they lay their eggs on damp mud or other surfaces near the edge of water. Such an oviposition site is commonly the wall of a cavity such as a hollow stump or a container such as a bucket or a discarded vehicle tyre (Adebote *et al*., 2011).

Clutches of eggs of the majority of mosquitoes species hatch as soon as possible, and all the eggs in the clutch hatch at much the same time. In contrast, a batch of *Aedes* eggs in diapause tends to hatch irregularly over an extended period of time (Grossman and James, 1993). This makes it much more difficult to control such species than those mosquitoes whose larvae can be killed all together as they hatch (Grossman and James, 1993).

2.2.2Larva

The mosquito larva has a well-developed head with mouth brushes used for feeding, a large thorax with no legs and a segmented abdomen (Wahid*et al.*, 2003). Larvae breathe through spiracles located on their eighth abdominal segments, or through a siphon, so must come to the surface frequently (Muslu*et al.*, 2011). The larvae spend most of their time feeding on algae, bacteria and other microbes in the surface microlayer. Larvae develop through four stages (instars) after which they metamorphose into pupae. At the end of each instar, the larvae moult, shedding their skins in which they emerge to pupae (Muslu*et al.*, 2011).

2.2.3Pupa

Mosquito pupa is comma-shaped. The head and thorax are merged into a cephalothorax, with the abdomen curving around underneath. The pupa can swim actively by flipping its abdomen, and it is commonly called a "tumbler" because of its swimming action. As with the larva, the pupa of most species must come to the surface frequently to breathe, which they do through a pair of respiratory trumpets on their cephalothoraxes (Bora *et al.,* 2010). However, pupae do not feed during this stage; typically they pass their time hanging from the surface of the water

by their respiratory trumpets. If their environment is disturbed, by a passing shadow, they nimbly swim downwards by flipping their abdomens. If their environment becomes undisturbed, they soon come up again. After a few days or longer, depending on the temperature and other circumstances, the pupa rises to the water surface, the dorsal surface of its cephalothorax splits, and the adult mosquito emerges. The lower activity of the pupa compared to the larva is quite clear because it does not feed, whereas the larva feeds constantly (Spielman and D'Antonio, 2001).

2.2.4 Adult

The period of development from egg to adult varies among species and is strongly influenced by ambient temperature. Some species of mosquito can develop from egg to adult in as few as five days, but a more typical period of development in tropical conditions would be some 40 days or more for most species. The variation of the body size in adult mosquitoes depends on the density of the larval population and food supply within the breeding water (Muslu *et al.*, 2011).

Adult mosquitoes usually mate within few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate (Muslu*et al*, 2011).Males typically live for about 5-7 days, using nectar and other sources of sugar as their food. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed (Shirai *et al*., 2004). This process depends on the temperature, but mostly takes two to three days in tropical conditions such as Nigeria. Once the eggs are fully developed, the female lays them into their appropriate habitat and resumes host-seeking (Mann and Kaufman, 2012).

The cycle repeats itself until the female dies. While females can live longer than a month in captivity, most do not live longer than one to two weeks in nature. The lifespan of mosquitoes depend on temperature, humidity and their ability to successfully obtain a blood meal while avoiding host defenses and predators (Huang *et al*., 2006).

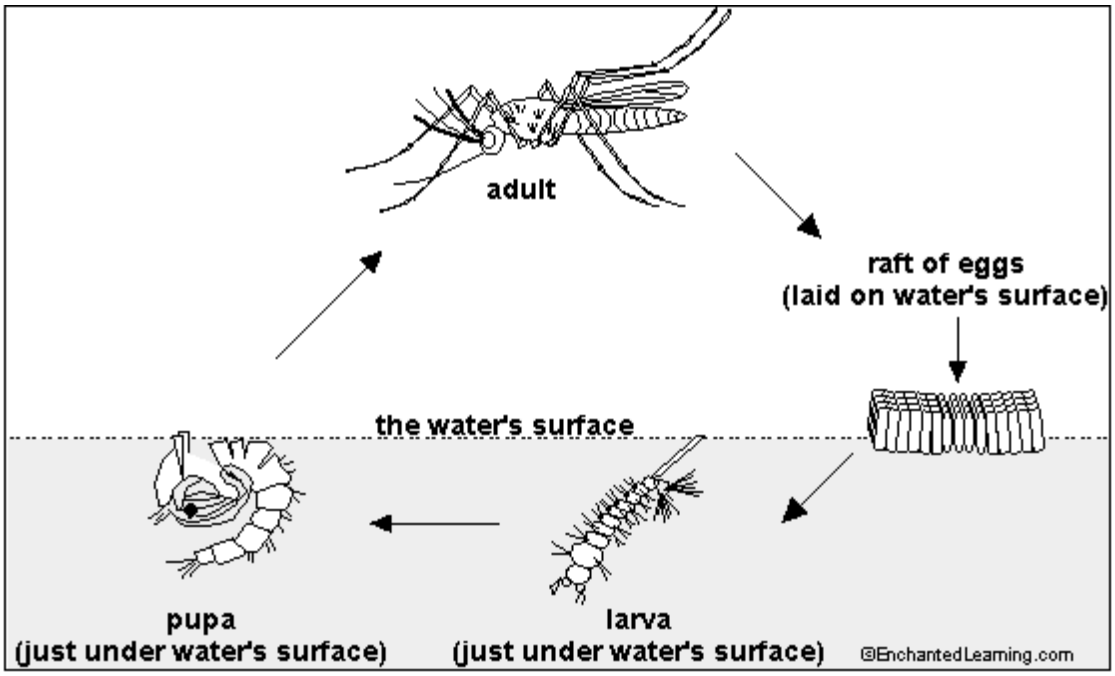

Figure 2.1: Generalised Life Cycle of Mosquito (CDC, 2012)

2.3General Morphological Features Used to Identify Some Mosquitoes Species

The following were the features used to distinguish *Aedes* mosquitoes from other mosquitoes.

i. Antennae of female is pilose with long and slender proboscis.

ii. Maxillary palps are short.

iii. Many, but not all adults have conspicuous patterns on the thorax formed by black, white or silvery scales, in some species yellow scales are present.

iv. The legs are often black and white rings.

v. Abdomen is often covered with black and white scales forming distinctive patterns.

vi. Abdomen of female is pointed at the tip and the cerci protrude.

2.3.1 Keys used for the identification of *Aedes aegypti*

Larvae with the following features were identified as *Aedes aegypti*

i. A lyre-shaped white markings present on scutum of thorax

ii. White knee spot present on all femora of fore, mid and hind limb.

iii. Mid femur with a narrow white longitudinal stripe on ventral half of anterior surface.

iv. Tibiae of fore, mid and hind limbs anteriorly dark, without any white band.

v. Wings with mainly narrow dark scales on all veins.

vi. Hind tarsus with a basal white band on tarsomeres 1-4.

2.3.2 Keys used for the identification of *Aedes vittatus*

Aedes vittatus were identified using the following features

i. Scutum with three pairs of distinct, small and white spots of narrow scales present.

ii. Tibiae of fore, mid and hind limb dark, each with a sub basal white spot and a white band present.

iii. Tarsomeres (1-4) of hind limb with a presence of white band.

iv. Wings with narrow dark scales on all veins with few white scales on the first vein.

2.3.3 Keys used for identification of *Culex quinquefasciatus*

i. Presence of head-seta ranging with 1-6 branches.

ii. Pecten-spines are usually less numerous which maybe upto 13 in number.

iii. The siphon index is wide and markedly less convex sides

2.4 Vectorial Importance of Mosquito

Mosquito-borne diseases remain a major problem in the world, particularly in tropical and subtropical regions. These diseases contribute significantly to disease burden, death, poverty and social weakness in tropical countries (Young-Su *et al.*, 2002). Mosquitoes are carriers of anumber of vector-borne diseases, such asmalaria, filariasis, yellow fever, brain fever,dengue fever, etc. (Jaswanth *et al.*, 2002).

Malaria, the most widespread mosquito-borne disease, affects 350-500 million people each year (WHO, 2013). Dengue fever infects between 50-100 million people, causing an estimated 25,000 deaths and an enormous economic lost in affected countries (WHO, 2013). Other serious illnesses transmitted by mosquitoes are on the rise. Chikungunya, yellow fever and eastern equine encephalitis are painful and debilitating diseases which can, in some cases prove fatal are all increasing in prevalence (Fulmali *et al*., 2008).

2.4.1 Chikungunya

Chikungunya virus infection is caused by an Alphavirus of the family *Togaviridae*. This RNA virus is transmitted to humans by mosquitoes of the genus *Aedes*, primarily *Aedes albopictus* and *Aedes aegypti(*Sissoko *et al.,* 2008*)*. According to the Centers for Disease Control and Prevention (CDC), symptoms of chikungunya infection include fever, headache, fatigue, nausea, vomiting, muscle pain, rash and joint pain (CDC, 2009). The joint pain can last for months. On rare occasions, infection may lead to viral encephalitis and death. Because there is no available vaccine or specific treatment, the best way to avoid chikungunya infection is to minimise contact with an infected mosquito (CDC, 2009).

There have been recent large outbreaks of chikungunya in India and South East Asia but the geographical spread is increasing and is of growing concern to health officials (Mawlouth *et al.,* 1999). On the island of La Réunion in 2006, over 20% of the population was estimated to have been infected. Similar outbreak also occurs in Europe in 2007 in Northern Italy (Soumahoro *et al*., 2011). The virus was believed to have been introduced into Italy by a vacationer from India and then transmitted by local mosquitoes. During the outbreak, there were 254 suspected and 78 laboratory-confirmed cases including one death (Soumahoro *et al*., 2011). This outbreak has forced global public health experts to re-evaluate the geographical threat of this mosquito-borne virus (Sissoko *et al.,* 2008).

2.4.2 Yellow fever

The yellow fever virus is an arbovirus of the *Flavivirus* genus with mosquito been the primary vector. Several species of the *Aedes* and Haematophagous mosquitoes transmit the virus. The mosquitoes either breed around houses (domestic), in the jungle (wild) or in both habitats

(semi-domestic). There are three types of transmission cycles (Barwick, 2004). It carries the virus from one host to another, primarily between monkeys, from monkeys to humans, and from person to person (Barwick, 2004).

Yellow fever is endemic to Africa and Latin America and used to be known as “Yellow Jack” among European sailors who visited the tropics. Once in a community it can spread rapidly. It is characterised by fever, muscle pain, headache, nausea and vomiting. Most patients improve and the symptoms will disappear but in some the disease becomes toxic. There is high mortality once the disease has entered the toxic phase (Barwick, 2004).According to WHO estimates, from the early 1990s, 200 000 cases of yellow fever, with 30 000 deaths, are expected globally each year, with 90% occurring in Africa (WHO, 2013). A recent analysis of African data sources estimates similar figures, but a slightly lower burden of 84 000–170 000 severe cases and 29 000–60 000 deaths due to yellow fever in Africa for the year 2013. Without vaccination, the burden figures would be much higher (WHO, 2013).

There is no cure for yellow fever but there is a vaccine, making monitoring and rapid vaccination critical in stopping the disease once an outbreak occurs. However it is expensive and availability is not sufficient to protect other parts of the world if the disease should extend beyond Latin America (CDC, 2011).

2.4.3 Dengue fever

Dengue fever is a disease caused by a family of viruses that are transmitted by mosquitoes. It is the fastest-growing mosquito-borne disease. The condition may be serious and sometimes fatal, which affects over 50 million people every year (Gubler, 2011). It is an acute illness of sudden

onset that usually follows a benign course with symptoms such as headache, fever, exhaustion, severe muscle and joint pains, swollen lymph nodes (lymphadenopathy) and rash. Other signs of dengue fever include bleeding gums, severe pain behind the eyes and red palms and soles (Kent *et al*., 2010). Dengue is a disease of all ages but tends to be more severe in people with compromised immune systems. Because it is caused by one of five serotypes of virus, it is possible to contract dengue fever multiple times. However, an attack of dengue produces immunity for a lifetime to that particular viral serotype to which the patient was exposed (Fulmali *et al*., 2008).

Dengue goes by other names, including "breakbone" or "dandy fever." Victims of dengue often have contortions due to the intense joint and muscle pain, hence the name breakbone fever (Fulmali *et al.,* 2008). Slaves in the West Indies who contracted dengue were said to have dandy fever because of their postures and gait (Dar *et al.,* 1999). Dengue haemorrhagic fever (DHF) is a specific syndrome that tends to affect children under 10 years of age. It is a more severe form of the viral illness. Symptoms include headache, fever, rash and evidence of haemorrhage in the body (Dar *et al.,* 1999; Baba and Muhammad, 2011). Petechiae (small red or purple splotches or blisters under the skin), bleeding in the nose or gums, black stools, or easy bruising are all possible signs of haemorrhage. This form of Dengue fever can be life-threatening and can progress to the most severe form of the illness, dengue shock syndrome (Baba and Muhammad, 2011).

The mortality rate with DHF is significant. With proper treatment, the World Health Organization estimates a 2.5% mortality rate. However, without proper treatment, the mortality

rate may rise to 20%. Most deaths occur in children. Thus, infants under a year of age are especially at risk of dying from DHF (Pandey *et al.*, 2012).

2.4.4 Filariasis

Filariasis is a parasitic infection caused by thread-like nematodes (filariae) that belong to the roundworm superfamily filarioidea. These infestations are endemic to tropical countries such as sub-Saharan Africa, Southern Asia, the Western Pacific islands, Brazil and Guyana (WHO, 2009). The World Health Organization estimates that worldwide there are more than 1 billion people at risk of infection, one third of this population live in Africa with population of over 120 million people are infected in which 40 million living in Africa (Ottesen *et al.*, 1997).

Eight types of filarial nematodes are known to use humans as their host and these are divided into three main groups according to the body area they tend to occupy, as follows:

Lymphatic filariasis: The lymphatic system is infected by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. In chronic cases, the host may develop elephantiasis, a condition where parts of the body swell to massive proportions (WHO, 2009).

Subcutaneous filariasis: This infection involves the subcutaneous tissue and is caused by the worms *Loa loa*, *Onchocerca volvulus*, *Mansonella streptocerca* and *Dracunculus medinensis* (WHO, 2009).

Serous cavity filariasis: It occurs in the serous cavity of the abdomen and is caused by *Mansonella perstans* and *Mansonella ozzardi* (WHO, 2009).

2.5 Mosquito Control Methods

As mosquitoes can transmit a number of diseases such as dengue fever, malaria, Japanese encephalitis, yellow fever, filariasis and so forth, efforts have been made to control them (Service, 1993). Control measures could be aimed at different stages of the insect in its life cycle. Good sanitation and elimination of stagnant water are generally good preventive strategies to keep mosquitoes from breeding in the first place (Knight *et al*., 2003).

Pesticides used for outdoor mosquito control can be broadly divided into larvicides and adulticides, depending on the stages of the mosquitoes being targeted.Larvicides are intended for killing immature mosquitoes in their aquatic habitats while adulticides are used for killing flying adult mosquitoes. Since larvicides are directed at the most concentrated developmental stage of mosquitoes, they can provide very effective control and reduce the need for adulticides later. To this end, when it is necessary to resort to chemical control, the use of larvicides should always be the backbone of the programme with adulticides as a supplementary only (Oshaghi *et al*., 2003).

2.5.1 Larvicides

When it is impossible to eliminate stagnant water that arebreeding grounds of mosquitoes, and there are no alternative methods of control, larvicides may be applied to the water bodies (Walton and Workman, 1998). The success of larval control depends upon many factors such as the chemical ingredients and formulation of the selected larvicides; the timing, rates and methods of application; and the physical and environmental conditions (Knight *et al*., 2003).

When mosquito problems necessitate the use of insecticides, it is generally best to employ larvicides and pupicides, as they are directed at the most concentrated developmental stages of the mosquito population and reduce the need for large-scale and expensive adulticiding (Knight *et al*., 2003). The purpose of mosquito control is to reduce their nuisance and disease tranmission potentials not to eradicate mosquitoes.

2.5.1.1 *S-Methoprene*

S-Methoprene is an insect growth regulator (IGR) that retards the development of larvae and prevents mosquitoes from developing into adults. Larvae treated with the chemical fail to moult successfully and simply die during the pupal stage. The target-specific S-Methoprene will not affect fish, waterfowl, mammals or the environment when applied at the recommended use rate. There are many different formulated S-Methropene products in the market; some only have little residual activity and are intended to target and control a single brood of mosquitoes while others are slow-release formulations offering an extended period of control which lasts from 30 up to 150 days. It is important to note that the chemical must be present in the water when the larvae are in their late developmental stage for it to be effective (FCCMC, 1998).

2.5.1.2 *Temephos*

Temephos is an organophosphate insecticide with a relative low toxicity of its kind. It is highly effective against mosquito larvae and may be used on any of the larval growth stages. With its neurotoxic mode of action different from other larvicides, it can be used in rotation with other larvicides to prevent the development of resistance. There are slow-release formulations that

can provide control for 30 days or more. Since some aquatic invertebrates may be highly susceptible to the chemical, it must be used carefully so that non-target fauna may not be adversely affected (FCCMC, 1998).

2.5.1.3 *Larvicidal Oil*

Larvicidal oil is a highly refined petroleum distillate designed especially for mosquito control. It can be applied to mosquito habitat where the water cannot be drained. As it spreads into a thin film over the water surface, only a small amount is needed. Larvicidal oil can provide effective control on both larvae and pupae as it basically kills by suffocation. As the oil can be degraded rapidly by light and microorganisms into harmless components, it should not pose much biological and environmental risks. When it is used in highly sensitive areas, it is necessary to exercise more care to avoid causing adverse impact on other non-target organisms (FCCMC, 1998).

2.5.1.4 *Monomolecular Film*

The active ingredient of monomolecular film (MMF) is Iso-alcohol ethoxylate. It is a degradable surfactant with a mode of action similar to that of larvicidal oil. It forms a very thin monomolecular film on the water surface to which it is applied and causes suffocation of larvae and pupae. Monomolecular film, an environmentally friendly pesticide, has very little negativeeffect on fish and plants (FCCMC, 1998).

2.5.1.5 *Bacillus thuringiensis varisraelensis*

Bacillus thuringiensis var*israelensis* (Bti) is a microbial insecticide made from naturally occurring soil bacteria (de Barjac and Southerland, 1990). The insecticide, which is proven to be effective against mosquito larvae, has a low level of toxicity to humans. It has high specific mode of action against a narrow host spectrum, with no risks to other non-target organisms or the environment when used properly (de Barjac and Southerland, 1990). However, it is not very effective in highly polluted water. *Bacillus thuringiensis* var*israelensis* is most effective when used against the early larval stages, when the larvae are actively feeding. When ingested by the larvae, it will disrupt their gut cells and cause death. If it is used for preventive control, repeated application at a 7-day interval is recommended in most situations. Some Bti products with a sustained release formulation may offer an extended larvicidal activity (Lacey and Undeen, 1986).

2.5.1.6*Bacillus sphaericus*

Bacillus sphaericus (Bs) is also a microbial insecticide with a nature and mode of action very similar to those of Bti.*Bacillussphaericus*, which is also highly specific to the larvae of mosquitoes, should be applied against the early larval stages when larvae are actively feeding (de Barjac and Southerland, 1990). In comparison with Bti, Bs is particularly effective against *Culex* species and it can also be used in water with high organic contents, such as lagoons or stagnant catch basins (de Barjac and Southerland, 1990).

2.5.2 Adulticides

The chemical control methods that target adult vectors are intended to have negative impact on mosquito densities, longevity and other transmission parameters. Adulticides are applied either as residual surface treatments or as space treatments (Service, 1993). Adulticiding may be

necessary for dispersed or migrating adult mosquitoes but should be avoided. Special attention should be given to the level of mosquito activity and the prevailing environmental conditions in order to ensure maximum efficiency of the application (Fang, 2010).

2.5.2.1 *Natural pyrethrins and pyrethroids*

Natural pyrethrins is a botanical insecticide made from extracts of *Chrysanthemum* flowers while Pyrethroids such as S-Bioallethrin, Cypermethrin, Permethrin, Etofenprox and Cyfluthrin are its synthetic chemical analogues (Cardarelli, 1976). These chemicals are broad-spectrum contact insecticides that provide rapid knockdown effects on insects but with only little residual activity. To increase their effectiveness, a synergist - Piperonyl butoxide (PBO) is often added to the formulations (Cardarelli, 1976). Pyrethrins and pyrethroids are usually applied as space spray i.e. by fogging to kill mosquitoes on contact. There are also some specially formulated pyrethroid products with extended residual activity that can be applied as residual sprays. As they are generally toxic to fish and other aquatic organisms, they must be used carefully so as not to contaminate water bodies (Knight *et al*., 2003).

2.5.2.2 *Organophosphates*

Organophosphates are broad-spectrum contact insecticides with relatively higher toxicity and longer residual activities (Cardarelli, 1976). Many organophosphates such as Chlorpyrifos and Malathion can be used as outdoor residual sprays for mosquito control (Robert, 2001). Organophosphates generally provide good residual effects from a few days up to several weeks depending on the formulated products. However, the ability may be reduced by environmental factors such as rain, high temperature or exposure to strong sunlight (Ekpereonne *et al.*, 2010).

Many organophosphates, in particular Chlorpyrifos, are highly toxic to fish. It is necessary to exercise extreme care and caution not to contaminate fish bearing water bodies (Knight *et al*., 2003).

2.5.2.3 *Residual treatment*

Perifocal treatment, involving the use of hand or power sprayers to deliver various formulations of insecticides, has both adulticiding and larviciding effects. Suitable insecticides can be applied with hand-operated compression sprayers. Care must be taken not to treat containers used to store potable water (Ekpereonne *et al.*, 2010). Indoor Residual Spraying (IRS) is the application of long-acting chemical insecticides on the walls and roofs of all houses and domestic animal shelters in a given area, in order to kill the adult vector mosquitoes that land and rest on these surfaces (Ekpereonne *et al.*, 2010). Residual surface treatments of insecticides are intended to reduce the density of vectors and their longevity. Although indoor residual spraying is frequently successful against malaria vectors, its effect on *Aedes*, which often do not rest on interior walls is uncertain. Further research is required on the effect of residual insecticides on curtains, container covers, screens and other materials which often have good community acceptability (WHO, 2012). In general, methods that improve the ability to deliver persistent treatments more rapidly and efficiently into large urban communities in a sustained way are urgently needed (WHO, 2012).

2.5.2.4 *Space sprays*

Space spraying is recommended for control only in emergency situations to suppress an ongoing epidemic or to prevent an incipient one. The objective of space spraying is the massive, rapid

destruction of adult vector population (Ekpereonne *et al.*, 2010). Any control method that reduces the number of infective adult mosquitoes, even for a short time, should reduce virus transmission during that time, but it remains unclear whether the transient impact of space treatments is epidemiologically significant in the long run (Ekpereonne *et al.*, 2010).If space spraying is used early in an epidemic and on a sufficiently large scale, the intensity of transmission may be reduced, which would give time for the application of other vector control measures that provide longer-term control, including larviciding and community-based source reduction (Fang, 2010).Thus, if disease surveillance is sensitive enough to detect cases in the early stages of an epidemic, and if the resources are available, emergency space spraying can be initiated at the same time as source reduction measures and larviciding are intensified (Ekpereonne *et al.*, 2010).

Space spraying efficiency is dependent on:

i. Method of release (aircraft, vehicle, hand-held equipment);
ii. Fog types (cold or thermal);
iii. Droplet size, application rate, climatic conditions;
iv. Building structures, configuration and penetration of space sprays;
v. Target area size;
vi. Terrain and accessibility;
vii. Peak flight times.

2.5.2.5 *Treatment cycle*

The treatment cycle of larvicide will depend on the species of mosquito, seasonality of transmission, patterns of rainfall, duration of efficacy of the larvicide and types of larval habitat.

Two or three application rounds carried out annually in a timely manner with proper monitoring of efficacy may suffice, especially in areas where the main transmission season is short. When a rapid reduction in vector density is essential, such as in emergencies, space treatment should ideally be carried out every 2–3 days for 10 days. Further applications should then be made once or twice a week to sustain suppression of the adult vector population. Continuous entomological and epidemiological surveillance should be conducted to determine the appropriate application schedule and the effectiveness of the control strategy (Gubler, 2011).

2.5.3 Previous control of mosquitoes using insecticide

In response to these potential disease carrying pests, communities organized the earliest mosquito control programmes in the eastern United States of America in the early 1900s. Eventually, other communities created similar programmes throughout the country in areas where mosquito problems occurred and where citizens demanded action by local officials. Modern mosquito control programmes in the United States of America are multifaceted and include surveillance, source reduction and a variety of larval and adult mosquito control strategies (WHO, 2012).

Effective vector control measures are critical to achieving and sustaining reduction of morbidity attributed to the mosquito related diseases. Preventive and vector control interventions aim to reduce disease transmission, thereby decreasing the incidence of infection and preventing outbreaks of diseases (Gubler, 2011).There are well-documented historical examples of both yellow fever and dengue being eliminated or significantly reduced through *Aedes aegypti* control. More recently, Singapore and Cuba greatly reduced Dengue transmission by enacting

anti-*Aedes* legislation and through sustained actions against the vector as reported by Gubler (2011).

The epidemic-prone countries initiate vector control measures after the onset of an epidemic which is too late to achieve significantresult. Endemic countries, on the other hand, too often carry out routine short-term interventions, which are neither sustained nor evaluated. Any successful control effort must be centered on the ability to sustain the intervention with sound monitoring and evaluation (WHO, 2012).Although insecticide space-spraying is recommended for vector control in epidemics, its efficacy in other situations has not been well documented (Ekpereonne *et al.*, 2010). Delivery of indoor space spraying is highly labour-intensive and, in most locations, it is impractical in the event of an outbreak (WHO, 2012).

The evolution and spread of resistance to insecticides are major concerns for the control of vectors. Bioassay data demonstrate that resistance to organophosphates (temephos) and pyrethroids is widespread in *Ae. aegypti* and resistance has also been reported in *Ae. albopictus* (Ranson *et al.*, 2009). Assessing the impact of insecticide resistance on vector control is complicated by variations in the methodology used to measure and report resistance and by the lack of studies on the epidemiological consequences of insecticide resistance (Ranson *et al.*, 2009). Monitoring resistance is necessary to ensure that effective insecticides are being used and that changes in insecticide policy are based on sound scientific data. Monitoring must be well coordinated at the local level with other vector-borne disease control programmes and usage of insecticides in agriculture. Innovative vector control tools are badly needed (Morisson *et al*., 2008).

The environmental hazards precautionary statements on many mosquito insecticide labels state that insecticides are toxic to birds, fish, wildlife, aquatic invertebrates and honeybees (Rappole, 2000). Detection of large numbers of immature mosquitoes in areas where source reduction or biological control is not feasible may require larvicide treatment to prevent the emergence of adult mosquitoes (Rawlings, 1998). Use of larvicides is less controversial than use of adulticides, although use of larvicides may lead to public concern about their effects on untargeted beneficial aquatic arthropods and vertebrates (Rappole, 2000). Some local mosquito control programmes would use an integrated programme if they had adequate resources, but may be so limited in funding and personnel that adulticiding trucks are the only means of mosquito intervention.

The first house-spraying campaigns after the Second World War, showed the capacity of this interventions to produce profound reductions in malaria transmission in a wide variety of circumstances (Aliyu, 2012). In Africa, the intervention was used in 1960s and 1970s but later abandoned except in some countries in southern and eastern Africa where insecticide residual spraying (IRS) remained the cornerstone of malaria control strategy (Aliyu and Alti-Mu'azu, 2009). Since 2005, however, there has been renewed interest in large scale IRS programmes. To date, 25 out of the 42 malaria endemic countries in the WHO Africa region have included IRS in their control strategy for malaria control (Aliyu, 2012). Of these, 17 countries routinely implement IRS as a major malaria control intervention, six including Nigeria are piloting the IRS in a few districts while 2 are planning to pilot. At the end of 2006, the National Malaria Control Programme (NMCP) and its partners initiated a pilot IRS project in 3 local government

areas/districts in 3 states (Lagos, Plateau and Borno states) using the WHOPES approved insecticides:

i. Lambdacyhalothrin CS10% (0.030g/m^2).
ii. Alphacypermethrin WP10% (0.030g/m^2)
iii. Bifenthrin WP10% (0.050g/m^2)

The evaluation of the local context of the 3 chemicals confirmed that residual effectiveness of the insecticides lasted for at least 4 months (Aliyu and Alti-Mu'azu, 2009).Despite various government policies, cost of the nets remains a significant barrier and a long obstacle to thegoal of Roll Back Malaria programme forthe universal coverage – defined as one long lasting insecticide-treated nets for every two people in the household with 80% usage. Insecticide Treated Nets development is a public good.

Nduka and Nwosu (2006) carried out study in Ishiagu community of Ebony State,Nigeria, they reported that spraying of insecticides was the preferred choice (43.0%) followed by the use of mosquito coils (25.2%) and the use of nettings (19.6%). The least preferred control method was the use of smoke from local herbs (5.6%). It was also observed that about 6.5% of the people in the two communities do not care about mosquito nuisance hence they do nothing to control them.

The preference of farmers to the use of insecticide is related to their knowledge and practice of using insecticides to control pest in their farms. This positive attitude influences their choice of mosquito control strategy. The availability of varieties of pesticides in the local market coupled with large number of clients may have helped to reduce the service cost charged by the local

pest control agents (Nduka and Nwosu, 2006).Biological controls (including birds and bats) may be present, but often not in sufficient numbers to provide satisfactory alternative control, particularly in coastal areas where salt-marsh mosquitoes are abundant or when human disease outbreaks occur (Robert, 2001).

2.5.4 Resistance

Vector resistance to certain larvicides and adulticides has occurred periodically. Failure of mosquito control indicating resistance must be verified by laboratory analysis or use of test kits because other factors (improper equipment calibration, dilution, timing and other application errors, off-specification products, climatic factors) can prevent insecticides from providing satisfactory control in the field. Resistance may occur between insecticides within a class or could be passed from immature to adult stages subject to the same insecticidal mode of action (Aliyu, 2012). Currently, pyrethroids are the only insecticides used for net treatment and are also increasingly used for spraying, so there is threat that if widespread resistance develops, the interventions will gradually become less cost-effective over time (Kara *et al.*, 2004). The development of insecticide resistance in the 1950s and recently by vectors has been a cause for concern. There have been reports of resistance to DDT in a wide range of sub-Saharan African countries, but this has not reached an operationally significant level (Alonso *et al.*, 1999).

2.6 Plant Materials

The three seed producing plants used in this study which include baobab (*Adansonia digitata*), locust bean (*Parkia biglobosa*) and avocado (*Persea americana*) have been reviewed.

2.6.1*Adansoniadigitata*

Adansonia digitata (Malvaceae Linn.) is a large, round canopied tree with a swollen trunk, about 10-25m in height, often with a bole of 3-10 m (giant individuals attain a girth of up to 28 m). Its bark is soft, smooth, fibrous, reddish-brown, greyish-brown or purplish-grey. Bark of leaf-bearing branches is normally ashy on the last node; a green layer below the outer, waxy layer of the bark, presumably to assist in photosynthesis when the tree has shed its leaves (Orwa *et al.*, 2009). The seeds are source of significant quantities of lysine, thiamine, calcium and iron (Kamatou *et al*., 2011). *Adansonia digitata* is an important commodity which is integral to the livelihood of rural communities. In addition, the global demand for baobab raw materials (e.g. seed oil, fruit pulp) by the food and beverage, nutraceutical and cosmetic industries has increased in recent years, thereby increasing the value and importance of baobab (Kamatou *et al*., 2011).

2.6.1.1*Uses ofAdansonia digitata*

Baobab tree is the desert grown plant that have several economical value, food source and medical importance.

Source of food:

The powdery fruit*A. digitata* is very rich in vitamins C and B_2 and makes a refreshing drink. Ripe fruits are collected and cracked to remove the 'flour', which is mixed with milk to prepare a flavoured fermented porridge (Proteggente *et al.*, 2002). The young leaves of baobab are also rich in Vitamin C, contain uronic acids and are high in demand in West Africa as a soup vegetable

(Sidibe *et al*., 1996). Ash from the shell, bark and seed is rich in potash and is widely used in making soap (Lockett *et al*., 2000).

Due to high water content, the wood is chewed by humans and animals in case of extreme water scarcity. The wood can be used as a salt substitute. The acid pith is used as a substitute for cream of tartar in baking, to curdle milk and smoke fish. It is also roasted and used as a coffee substitute (Saka *et al.*, 2007). The seeds contain appreciable quantities of tartaric acid and potassium bitar; they are refreshing to suck, and when soaked in water make a palatable drink (Saka *et al.*, 2007).

Economic value:

The wood of *A. digitata* contains some tannins and the acid pith is used to coagulate rubber. In East Africa, the roots produce a useful red dye of economic (Orwa *et al.*, 2007).

Medical importance:

Hyposensitive and antihistamine properties have been associated with the leaves of *A. digitata*, which are used to treat kidney and bladder diseases, asthma, general fatigue, diarrhoea, insect bites and Guinea worm (El-Kamali and El-Khalifa, 1999). Leaf and flower infusions are valued for respiratory problems, digestive disorders and eye inflammation(Tal-Dia *et al.*, 1997). The seed paste is used for curing tooth and gum diseases. The fruit pulp, seed and bark are reputedly an antidote to *Strophanthus* poisoning. Gum from the bark is used for cleansing sores. It is also used as an expectorant and a diaphoretic. The bark is used in steam baths for calming shivering

and high fever. A decoction of the roots is taken as a remedy for lassitude impotence and kwashiorkor (Tal-Dia *et al.*, 1997). The bark is boiled and taken as a cure for body pains. This infusion is also used to treat colds, fever and influenza. Seeds are used to cure gastric, kidney and joint diseases (Tal-Dia *et al.*, 1997).The bark is boiled for days to extract a substance poisonous to ants. Fruit pulp burns with an acrid, irritating smoke that can be used to deter insects troublesome to livestock (Szeto *et al.*, 2002).

2.6.2*Parkia biglobosa*

Parkia biglobosa, commonly known as the locust bean tree, African locust bean or néré, Dorowa, is a perennial deciduous tree of the Fabaceae family (Teklehaimanot, 2004). It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Locust bean is a perennial deciduous tree with heights ranging from 7 to 20 m, although it can reach 30 m under exceptional conditions. Its crown is large, spreads wide with branches low down on a stout bole; amber gum exudes from wounds; bark dark grey brown, thick and fissured. The leaves are alternate, dark green, bipinnate up to 30 cm long, pinnae up to 17 pairs with 13-60 pairs of leaflets, 8-30 mm x 1.5-8 mm, of distinctive shape and venation. Leaflets held on a long rachis (Orwa *et al*., 2009).

2.6.2.1*Uses ofParkia biglobosa*

Parkia bilobosa have various uses such as food source, economic benefits and medicinal value.

Food source:

The yellow pulp of *Parkia biglobosa* fruit, which contains the seeds, is naturally sweet "and is processed into a valuable carbohydrate food known as sikomu and daddawa among the Yoruba and Hausa people of Nigeria, respectively (Ntui *et al.*, 2012)." However the most valuable parts of the locust bean, and possibly the entire tree, are the seeds which are high in lipids (29%), protein (35%), carbohydrate (16%), and is a good source of fat and calcium for rural dwellers (Ntui *et al.*, 2012). The seed is first cooked to remove the seed coat and then fermented to produce the desired result. In a study conducted on the fermentation of dawadawa, it was found that *Gmelina arborea* as well as banana leaves accelerated fermentation of seeds, while also bringing "an increase in protein, crude fat and moisture contents with corresponding decrease in carbohydrate (Gernah *et al.*, 2007b).

Economic Value:

The cultivation of this tree can be seen as an important economic activity for many in Africa. Néré fruits are highly commercialized in Burkina Faso; "over 50% of respondents in a nation-wide survey said they participated in one way or the other in its trade'' (Teklehaimanot, 2004). In general, women are wholly responsible for the sale of fermented seeds (dawadawa) even though both men and women are equally responsible for the sale of dry seeds (Teklehaimanot, 2004).

Medical importance:

Indigenous healers in Africa use different parts of locust bean tree for health purposes. In a survey conducted on herbal plant used in Togo, *Parkia biglobosa* was one of the highest used for treating hypertension (Traoré *et al.*, 2013). The tree was also one of two

the plants "listed as having wound-healing properties in South-Western Nigeria, influencing the proliferation of dermal fibroblasts significantly (Adetutu *et al*., 2013). In a similar survey conducted in Guinea relating to their use of antimalarial plants, *Parkia biglobosa* was cited among those most often successfully used (Traoré *et al.*, 2013). In an analysis on the antibacterial properties of the plant, it revealed that "these properties compare favourably with those of streptomycin, making it a potential source of compounds used in the management of bacterial infections (Abioye *et al*., 2013). Indigenous healers in Africa use different parts of the locust bean tree for health benefits. In a survey conducted on healers in Togo, *Parkia biglobosa* was one of the highest cited plants used for treating hypertension(Karou *et al*., 2013).

2.6.3 *Persea americana*

The fruit of *Persea americana*, commonly known as avocado, is an edible fruit from Central America which is easily adaptable in tropical regions (Leite *et al*., 2009). The large, lustrous dark evergreen leaves and low-branching, open canopy of avocado makes it a wonderful shade tree but it is most often grown for the abundant production of its well-known, delicious, buttery fruits (Leite *et al*., 2009). Depending on cultivars and variety, the fruits may vary from smooth-skinned to rough and yellow-green to purple. Commonly seen at 35 to 40 feet in height but capable of growing much larger, avocado fits well into large residential landscapes in frost-protected locations (Edward and Dennis, 1994). It can be pruned to an open spreading form or left to grow tall forming a rather narrow oval. Older trees become more rounded. The somewhat showy, greenish flowers appear on terminal panicles in late winter to early spring and are followed by the large, pendulous, pear-shaped fruits, ripening late summer to early spring, depending upon variety (Arukwe *et al.,* 2012).

2.6.3.1 *Uses ofPersea americana*

Almost all part of *P. americana*have various activities such as hypertensive, wound healing ability, antioxidant and hyperglycemic functions.

Hypotensive activity:

The intravenous administration of doses of aqueous and methanol extracts of*P. americana* ranging from 6.25 to 50 mg/kg to normotensive anesthetized rats produced dose-related hypotensive effects. Doses above 12.5mg/kg showed hypotensive effects, which were significantly different from those of the control. Also, the duration of action appeared to be dose dependent (Adeboye *et al*., 1999)

Wound healing activity:

In the excision wound model, complete healing was observed on average on day 14 in rats, which received oral or topical treatment of *P. americana* fruit extract (300mg/kg/day) which differs from controls that took approximately 17 days to heal completely (Grant *et al*., 1991). The extract-treated wounds were found to epithelialize faster than the controls. Wet and dry granulation tissue weight and the hydroxyproline content of the tissue obtained from extract-treated animals used in the dead space wound model were significantly higher compared with those of the controls (Nayak, *et al*., 2008).

Furthermore, several studies have focused on the evaluation of acute toxicity of the fruit and leaves (Raharjo *et al*., 2008; Ozolua *et al*., 2009). Avocado leaves showed cardiotoxic effects in mammals and birds (Grant *et al*., 1991; Leite *et al*., 2009). Similarly, the mutagenicity of fruit and leaf extracts in human lymphocytes has been assessed (Kulkarni *et al*., 2010).

Antioxidant activity:

Presence of the compounds persenone A and B with unique antioxidant properties in avocado fruit. The antioxidant activity exhibited by the methanolic leaf extract of *P. americana* and its hepatoprotective action against acute paracetamol toxicity make it a potential agent against liver diseases and other pathologies associated with oxidative stress (Mohammad *et al*., 2010).

Hypoglycemic activity:

Aqueous leaf extract of *P. americana* possesses hypoglycemic effects in normal rats. The maximum antidiabetic activity was reached at 6 h after a single dose of 125mg/Kgof the extract was administered, producing 60.02 ± 6.83% reduction in blood glucose level (Anita *et al*., 2005; Brai *et al*., 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This research was conducted in the Department of Biological Sciences within the main campus of the Ahmadu Bello University (A.B.U.), Zaria. Some facilities at the National Research Institute for Chemical Technology (NARICT) Basawa, Zaria were used in the course of this study, especially the characterization of seed oil extracts.The main campus of Ahmadu Bello University is located in Samaru, a suburb of Zaria in Kaduna State, Nigeria. Samaru is situated on 11^{o} 12' N and 07^{o} 37' E, at an altitude of 550-700 metres above sea level (Laah *et al.*, 2011). Samaru evolved from a small colonial farming settlement to become a large community, referred to as "the University village". Zaria experiences a brief period of hot but dry weather in March and April, followed by a progressive incursion of tropical maritime air mass from the Atlantic Ocean which displaces the North East (Harmattan) winds. The rainy season lasts from May to September/October with long-term annual rainfall of 1040mm in about 90 rain days. The relatively deep tropical ferruginous soils and climate conditions of Zaria are suitable for and sustains a good cover of savanna woodland (Northern Guinea Savanna) with a variety of grasses, woody shrubs and short trees. The River Galma forms the main focus of the drainage systems in

Zaria. Although River Galma carries water throughout the year, most of its tributaries, such as the Samaru stream, dry up between January and June (Laah *et al*., 2011).

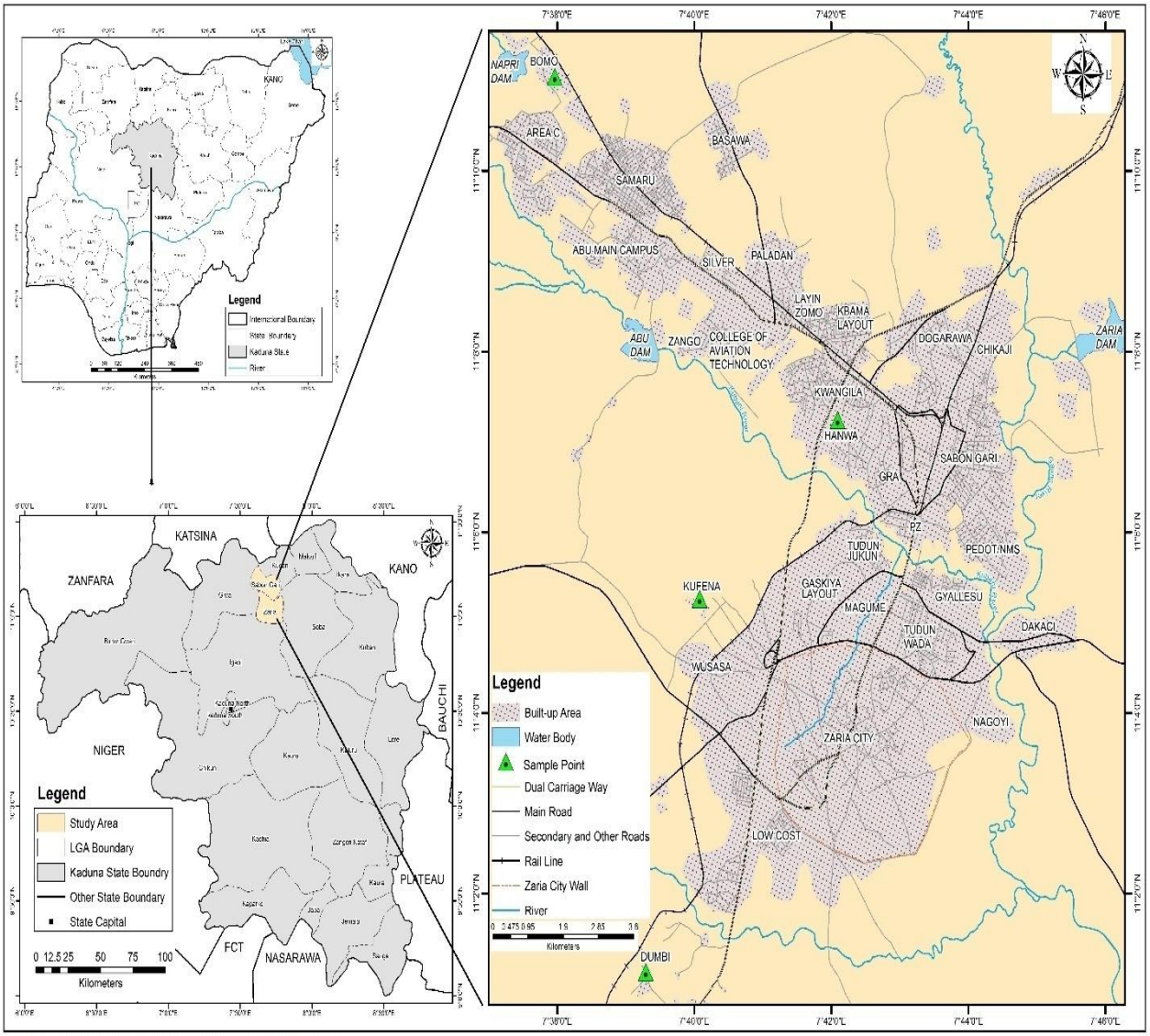

Figure 1: Map of the Study Area showing Sample Points
Source: Adapted and modified from the administrative map of Kaduna state

Figure 3.1: Map of Zaria

3.2 Collection and Identification of Plant Materials

Seedsof three plants,baobab (*Adansonia digitata*), locust bean (*Parkia biglobosa* and) avocado (*Persea americana*)were used in this study. Baobab and locust beanseeds were collected from fruiting trees within the main campus environment of the Ahmadu Bello University, Zaria, using sickle tied to a long wood after climbing the tree. Avocado fruitswere purchased from Sabon Gari Market, Zaria,Kaduna State.

The plants materials collected (fruits) were taken for identification in the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University Zaria, where voucher numbers were assigned(*A. digitata*-2512, *P. biglobosa*-2846 and *P. americana*-992) and also deposited.

3.3 Seed Processing

Adansonia digitata fruitswere sun-dried for five days.The pulp was manually separated from the seeds using a knife. Seeds were sun-dried for four days. It was pounded into fine powderusing mortar and pestle. The pulverized seedswas passed through a sieve of 40 mesh size and placed in an airtight plastic jar and stored at −20°C in refrigerator until used (Morais *et al*., 2007;Agomuo *et al.,* 2012).

The raw locust bean seeds of *Parkia biglobosa* were processed by dehusking, hydrating, depulping and dehulling using the methods adopted from Gernah *et al.* (2007a) as briefly outlined below. The outer covering (husk) was removed mechanically from the fruit to expose the dark-brown pulp. One kilogramme (1.0 kg)was soaked in 5 L of tap water for 12hr to soften the adhering pulpy materials. This was followed by the removal of pulp manually by rubbing the seeds between the palms and washingthem with tap water. The depulped cleaned seeds were sund-dried for four days and dehulled to free the seeds from the dark brown testae. The process was repeated until the required amount to measure (2.0 Kg) was obtained.

The riped fruits of *Persea americana*were cut open to obtain the seed portions. The seeds were sun-dried for several days to reduce their moisture contents. The dried samples were pulverized to fine powder using mortar and pestle, and stored in airtight bottles till extraction (Morais *et al*., 2007; Agomuo*et al.,*2012).

3.4 Seed Oil Extractions

The seed oil of *A. digitata*, *P. biglobosa* and *P. americana*were each extracted according to the method of Kyari (2008) as outlined below.Up to 300ml of petroleum ether (pet-ether) was poured into a round bottom flask (750cm^3) and 100g of the sample was placed in the filter paper thimble and inserted in the centre of a soxhletextractor. The soxhlet was heated at 60-65^{o}C. As the solvent boiled, the vapour rose through the verticaltube into the condenser at the top. The liquid condensate dripped into the filter paper thimble in the centre which contains the solid sample to be extracted. The extracts seeped through the pores of the thimble and filled the

siphon tube, flowing back down into the round bottom flask. This was allowed to continue for 5-6hrs. At the end of the extraction, the condenser was removed. The flask containing the resulting mixture was connected to aLiebig condenser and heated up to 65-85^{o}Cduring which the solvent wasevaporated off and recovered in a conical flask. Seed oil extracts were stored in refrigerator at -4^{o}Cfor further use.

3.5 Preparation of Test Solutions

Various volumes of the oil extracts were measured using syringe and introduced into the oil extracts mix with distilled water to make concentrations ranging from 0.1-3.2mL/L as 100mL/L solution according to the method of Murugan *et al*.(2007) with some modifications.

3.6 Phytochemical Characterization of Seed Oil Extracts

Seed oils of the three plants were characterized gravimetricallyfor their acid, free fatty acid, iodine, peroxide and saponification values following the standard protocols.

3.6.1 Acid value

Each2.0g of oil was weighed into a 250ml conical flask. Mixed solvent (25ml ethanol and 25ml diethylether) was added to the oil and swirled gently. The temperature of mixture was slightly raised to 30°C to ensure complete dissolution of the oil. Two to three drops of 1% phenolphthalein indicator was added and the mixture was titrated with 0.1N KOH until a pink

color which lasted for at least 30seconds appeared. This was repeated three times and the average titre value was recorded (Edcey, 1954).

Acid value of oil is calculated as;

Acid Value = [56.1 x N x V] / W

Where, N = Normality of KOH used

V = Volume (ml) of KOH used

W = Weight of sample used

3.6.2 Determination of the free fatty Acids of the oil samples

To 20ml solution of ethanol and diethyl ether (1:1 v/v), 2ml of 1% phenolphthalein solution was added and the mixture was neutralised using 0.10M NaOH solution. Five grams of each oil sample was added to the neutralised mixture and titrated against 0.10M NaOH solution with constant shaking until a pink colour developed and persisted for 15 minutes. The titre values were used to obtain the free fatty acid values (Admasu and Chandravanshi, 1984).

Percentage free fatty acid (% FFA) (as oleic) was determined by multiplying the acid value with the factors of 0.503. Thus % FFA = 0.503 x acid value.

3.6.3 Iodine value (Wij's method, 1942)

Iodine value was obtained using the method of Bailey (1951) in which 0.3g of the oil was weighed using Ohaus Precision Advanced Top Loading Balance (OPATLB) into a 250ml conical flask. The volume of 15ml of Carbon tetrachloride (CCl_4) was added to the oil and swirled. Wij's solution of 20ml was added, stoppered and kept in the dark for 30 minutes. Potassium iodide, 15ml of 10%,was added and rinsed out into the mixture with 100ml distilled water. Three drops of freshly prepared 1% starch indicator was added and titrated using 0.1N $Na_2S_2O_3.5H_2O$ (Sodium thiosulphate) untill a clear solution was obtained. The blank solution was back titrated;

Iodine of the oil was calculated as;

Iodine value = $12.69N (V_1–V_2) / W$

Where, N = Normality of thiosulphate

V_1 = Volume (ml) of thiosulphate solution used in test

V_2 = Volume (in ml) of thiosulphate solution used in blank

W = Weight of sample (0.20g)

3.6.4 Peroxide value

Peroxide value was determined according tothe method of Birnin-Yauri and Garba (2012) in which 1.0g each of seed oil was weighed into a 250ml conical flask, 1.0g of potassium iodide was added to the oil and a mixture of acetic acid and chloroform was added to the oil in the ratio of 2:1. The mixture was gently swirled and placed in a water bath for about 5minutes to ensure complete dissolution of the oil. It then followed by

20ml of 5% KI was added and the mixture was titrated with 0.002N $Na_2S_2O_3.5H_20$ using 1% starch indicator.

Peroxide value (PV) = [100(V_1–V_2) meg/Kg] / W

W = weight of sample

V_1 = Volume (ml) of thiosulphate used in test

V_2 = Volume (ml) of thiosulphate used in blank

N = Normality of thiosulphate ($Na_2S_2O_3$).

3.6.5 Saponification value

The seed oil extracts of 2.0g was weighed into 250ml round bottom flask, 50ml of ethanolic KOH was added and the mixture was gently swirled. The mixture was heated for an hour under reflux. Two to three drops of 1% phenolphthalein indicator was added, a pink coloration was formed, and the mixture was titrated with 0.5N HCl until a colourless solution was formed (Hildith and Williams, 1964). The same process was carried out for the blank.

Saponification value = 56.1N (V_1-V_2) / W

Where, N = Normality of Hydrocloric acid used

V_1 = Volume of Hydrochloric acid used in test 1

V_2 = Volume of Hydrochloric acid used in blank

W = Weight of oil used (1g)

3.7 Sampling,Identification and Rearingof Mosquitoes

Samples of mosquito species were collected fromdifferent breeding habitatsaround Zaria, using method developed by Adebote *et al*.(2008). Some mosquito sampleswere collected from discarded tyres on permission from vulcanizers along Samaru to Dan Magaji area around Zaria, Kaduna State. On the other hand,some larval species were obtained from Kufena inselbergaccording to the method described by Adebote *et al.*(2006) using a plastic scoup ladle dipper in rock pools.The collected larval samples were transported in jars containing water from their breeding sites to the Entomology and Parasitology Laboratory in the Department of Biological Sciences, A.B.U.,Zaria.The larvae were transferred to specimen containers (which was half - filled with water from the breeding sites) using pipette and subsequently taken to the laboratory for identification and rearing.

Both field collected larvae were identified to be *Aedes aegypti* and *Aedes vittatus*in the Laboratory according to Hopkins (1952) method.

Indoor resting blood fed adult of*Cx. quinquefasciatus* femaleswere trapped alive using test tube in the class rooms at early hours (Adebote *et al.,* 2011a).The gravid females were introduced into theentomological cagescontain dechlorinated water for oviposition(Oshaghi *et al*., 2003).Larvae of *Cx. quinquefasciatus* were identified microscopically using the keys of Hopkins (1952).

The larvaeof *Aedes* speciesand *Cx. quinquefasciatus*were mass reared and fed on yeast and dog biscuit in the ratio of 1:3. On every alternate day, the water from the culture bowl was changed carefully until pupation. The feeding was continued till the larvae transformed into the pupal

stage. The larvae and early pupae were maintained at 28 ± 2°C and65±5% relative humidity. Each adult that emerged from the pupa was allowed to mate in entomological cages. Females were carefully selected and fed with blood of white rats (*Rattus norvegicus*) and birds toproduce eggs (Shaalan *et al*., 2006).Eggs hatched into first larval instars in which they metamorphosed to second, third and fourth instars then becoming pupae. The pupae emerged as adults in which the females were blood-fed for egg productions. Second generation laboratory reared late third instar larvae were used for the study.

3.8 Laboratory Bioassay

The seed oil extracts of*Adansonia digitata*, *Parkia biglobosa* and *Persea americana*was diluted into sixconcentrations (0.01, 0.02, 0.04, 0.08, 0.16 and0.32ml/L) as 100mL volume. The control treatment consisted of 100mL of distilled water devoid of any of the extracts. Each concentration, in replica of three, of 100mL of each was placed in a 500mL plastic bowl. Twenty-five late instar larvae of the*Ae.aegypti,Ae. vittatus*and *Cx. quinquefasciatus* wereput in each plastic bowl containing the test concentrations according to Mohtar *et al.*(1999). The experiment was performedunder laboratory conditions of 28±2°C and 65±5% RH. No food was provided either to the test or controlorganisms during the experiments because the experiment lasted for 24hr. The number of dead larvae was counted after 24hr exposure.Larvae were counted as dead when they did not swim to the surface for respiration and probe insensitiveif they were not responsive to a gentle prodding with a fine needle (Sivagname and Kalyanasundaram, 2004).

3.9 OutdoorBioassay Trial

In thistrial, multiple plastic bowl containers of the same size (500mL) containing water were placed randomly in the open field of the forested garden of Biological Sciences Department. One hundred milliliter (100mL) of waterwas introduced into each experimental bowl. The level of water was marked and sustained in each bowl by addition of test solution for each dose throughout the experiment. The containers were covered with nylon mesh screen to prevent mosquitoes or other insects from laying eggs and to protect the water from falling debris. A batch of 25late third instar larvae of the three mosquito species were introduced separately into each bowl. After 2–3hrs of larval acclimatization, the bowls were treated with selected dosages of seed oilusing syringe. Three replicates for each dose and controls were used for sixdifferent concentrations (0.01, 0.02, 0.04, 0.08, 0.16, and 0.32mL/L making up to 100mL/L with distilled water). After 24 h,dead larvae were counted to score post-treatment larval mortality if they were not responsive to a gentle prodding with a fine needle(WHO, 2005).The dead larvae were expressed as percentage mortality for each concentration. In cases where bioassay tests showed more than 20% (negative) control mortality, these were discarded and repeated. However where negative control mortality ranged from 5-20%, the observed percentage mortality was corrected by Abbott's formula as follows:

% Mortality = (number of larvae dead / total number of larvae used) x 100.

Corrected Mortality (%) = [(% test mortality - % control mortality) / (75 - control mortality)] x 100 (Abbot, 1925).

3.10 Determination of Median Lethal Concentration (LC_{50}) of Seed Oil Extracts Against Mosquito Larvae

The median lethal concentration (LC_{50}) for each seed oil extracts under laboratory and outdoor conditions were computed by transforming the various concentrations of the seed oil extracts to natural logarithms, while mortalities were transformed to empirical probits of kill. Co-linear probit regression line was drawn by plotting the empirical probit values against logarithms of concentrations used. Regression equation and coefficient of determination were drawn from the graphs.

3.11 Data Analyses

The data obtained from the experiment was analysed as follows: Arithmetic Mean was calculated for each chemical composition value of seed oil and dead mosquito larvae.The corrected mortality was determined using the formula ofAbbott (1925) whenever required. Analysis of Variance (ANOVA) was used to test for significant differences in chemical composition of the seed oil extracts.Analysis of Variance was also used to determine the differences of the mortality of mosquito larvae between the controls and the experimental groups. Least significant difference (LSD) was used to separate differed means.Student's t-test was used to test for significant differences between larvicidal yield in laboratory and outdoor tests.Median lethal concentration (LC_{50}) was evaluated using probit analysis based on Statistical Analysis System (SAS) version 20 (Finney, 1971).

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Composition of *Adansonia digitata, Parkia biglobosa* and *Perseaamericana*Seed Oil Extracts.

Table 4.1 shows the physicochemical composition of oils extracted from the seeds of *A. digitata*, *P.biglobosa* and *P. americana*in terms of their acid, percentage free fatty acid, iodine and saponification values. The acid values of the oils ranged from 5.04mgKOH/g in *A.digitata* to 19.63mgKOH/g in *P. americana.* The acid value of the oil of *P. americana* (19.63mgKOH/g) was significantly($p<0.05$) higher than those of *A. digitata* (5.04mgkoh/g) and *P. biglobosa* (5.05mgKOH/g). The percentage free fatty acid (mgKOH/g) of the seed oils ranged from 2.50 in both *A. digitata* and *P.biglobosa* to 9.90 in *P. americana*. While there was no significant($p>0.05$) difference in the percentage free fatty acid values of the oils of *A. digitata* and *P.biglobosa*, the free fatty acid of *P. americana* oil was significantly($p<0.05$) highest. Peroxide values (meq/Kgfat) of seed oils ranged from 3.08 in *P. americana*to 10.15 in *A. digitata*. There was significant ($p<0.05$) differences in the peroxide values of the seed oils; with that of *A. digitata>P.biglobosa>P. americana*. Saponification values (mL/Kg) of the seed oils ranged from 186.00 in*A. digitata* to 199.33 in *P. americana*. While there was no significant ($p>0.05$) difference in the saponification values of the seed oils of *A. digitata* (186.00mL/Kg) and *P.biglobosa* (187.76mL/Kg), that of the seed oil of *P. americana* (199.33) was significantly ($p<0.05$) highest.

Table 4.1: Physicochemical composition (Mean±SE) of seed oil extracts of *Adansonia digitata, Parkia biglobosa* and *Persea americana*.

Physicochemical composition	Plants seed oils		
	Adansonia digitata	*Parkia biglobosa*	*Persea americana*
Acid value (mgKOH/g)	5.04^{a}±0.06	5.05^{a}±0.05	19.63^{b}±0.05
% free fatty acid (mgKOH/g)	2.50^{a}±0.06	2.50^{a}±0.07	9.90^{b}±0.15
Iodine value (meq/Kgfat)	3.80^{a}±0.01	67.26^{b}±0.03	99.00^{c}±0.29
Peroxide value (meq/Kgfat)	10.15^{c}±0.03	6.19^{b}±0.05	3.08^{a}±0.06
Saponification value (mL/Kg)	186.00^{a}±0.23	187.76^{a}±0.21	199.33^{b}±0.18

Means followed by the same superscript letter along rows are not significantly different (P>0.05).

SE = Standard Error

4.2 Larvicidal Effects of *Adansonia digitata* Seed Oil ExtractAgainst Mosquitoes Larvae

4.2.1 Effect on *Aedes aegypti*

The mortality of late third larval instar of *Aedes aegypti* on exposure for 24h to various concentrations of *Adansonia digitata* seed oil extract under laboratory and outdoor bioassays is shown in Table 4.2. Under laboratory condition, mortality of the larvae ranged from 0/75 (0%) in the control to 70/75 (93%) atthe dose of 3.2mL/L. Larval mortality increased in a dose-dependent fashion except for a slight reduction from 72% to 71% at 0.8mL/L. Mean larval mortality at the various experimental concentrations were significantly ($p<0.05$) higher than the control but increase in concentrations from 0.1mL/L to 3.2mL/L did not produce significant ($p>0.05$)increase in mean larval mortality. Under outdoorcondition,*Ae. aegypti* larval mortality varied from 0/75 (0%) in the control to 72/75 (96%) at 3.2mL/L. The percentage larval mortality decreased from 39% at 0.1mL/L to 23% at 0.4mL/L and then increased to 40% at 0.8mL/L to 96% at3.2mL/L. Mean larval mortality at various experimental concentrations were significantly($p<0.05$) higher than control, but increase in concentration from 0.1mL/L to 0.8mL/L did not produce significant($p>0.05$) increase in mean larval mortality. Mean larval mortality at dose 1.6mL/L (18.00) was significantly ($p<0.05$) higher than the mortality at lower concentrations of 0.1-0.8mL/L (5.67-10.00) but significantly ($p>0.05$) lower than the mean larval mortality at 3.2mL/L (24.00) as seen in Table 4.2. There was no significant($p>0.05$) difference in the susceptibility of *Ae. aegypti*larvae to *A. digitata* seed oil under laboratory and outdoor conditions.The LC_{50} under laboratory and outdoor conditions were 0.1179mL/L and 0.4887mL/L respectively in which it was higher in the outdoor (Table 4.3).

Table 4.2: Cidal effect of *Adansonia digitata*seed oil extract against *Aedes aegypti* larvae in laboratory and outdoor conditions after twenty-four hours exposure.

Study condition Conc. (mL/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
Laboratory				
Control (0)	0 (0.00[a]; 0.00-0.00±0.00)	0	10.8793	0.00013793
0.1	35(11.67[b]; -9.45-32.79±4.91)	47		
0.2	46 (15.33[b]; 10.16-20.50±1.20)	61		
0.4	54 (18.00[b]; 15.52-20.48±0.58)	72		
0.8	53 (17.67[b]; 5.42-29.92±2.85)	71		
1.6	61(20.33[b]; 14.07-26.59±1.45)	81		
3.2	70 (23.33[b]; 18.16-24.5±1.20)	93		
Outdoor				
Control (0)	0 (0.00[a]; 0.00-0.00±0.00)	0	11.5472	0.0009928
0.1	29 (9.67[b]; -8.99-28.33±4.34)	39		
0.2	22 (7.33[b]; -9.21-23.89±3.85)	29		
0.4	17 (5.67[b]; 2.81-8.53±0.66)	23		
0.8	30 (10.00[b]; 7.52-12.48±0.58)	40		
1.6	54 (18.00[c]; 9.41-26.60±1.20)	72		
3.2	72(24.00[d];21.52-26.48±0.58)	96		

Means followed by the same superscript letter per study condition are not significantly different (P>0.05).

N = 75 (Total number of larvae exposed in three replicates)

CI = Class Interval

SE = Standard Error

Table 4.3: Media lethal concentration (LC_{50}) of *Adansonia digitata* seed oil against larval *Ae.aegypti* in laboratory and outdoor conditions.

Study condition Conc.(mL/L)	Log-con	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Laboratory					
3.2	0.5051	93	6.4758	y = 0.9038x + 5.8393	0.1179
1.6	0.2041	81	5.8779	(0.9224)	
0.8	-0.0969	71	5.5534		
0.4	-0.3979	72	5.5828		
0.2	-0.6990	61	5.2793		
0.1	-1.0000	47	4.9247		
0 (Control)	-	0	-		
Outdoor					
3.2	0.5051	96	6.7507	y = 1.333x + 5.4146	0.4886
1.6	0.2041	72	5.5828	(0.6469)	
0.8	-0.0969	40	4.7467		
0.4	-0.3979	23	4.2612		
0.2	-0.6990	29	4.4466		
0.1	-1.0000	39	4.7207		
0 (Control)	-	0	-		

4.2.2 Effect on *Aedes vittatus*

Table 4.3 shows the mortality of *Ae. vittatus*larvae after 24hexposure to various concentrations of *A. digitata* seed oil extract under laboratory and outdoor conditions. Mortality of larvae under laboratory condition vary between 0% in the control to 100% at 1.6mL/L and 3.2mL/L. Larval mortality increasewas dose dependent from 61% at 0.1mL/L to 100% at 3.2mL/L. Mean larval mortality at the various concentrations were significantly($p<0.05$) higher than the control; but increase in concentration from 0.1mL/L to 0.2mL/L did not produce significant($p>0.05$) increase in mean larval mortality. Similarly, no significant($p>0.05$) difference in the mean larval mortality betweendoses 0.2mL/L (17.00) and 0.4mL/L (21.00). Increase in concentrations from 0.4mL/L up to 3.2mL/L did not produce significant($p>0.05$) increase in mean larval mortality. For the outdoor bioassay, mortality of *Ae.vittatus* ranged from 14/75 (19%) in the control to 75/75 (100%) as seen at 0.4mL/L and higher concentrations. Larval mortality increased in a dose dependent manner. Mean larval mortality at the various test concentrations were significantly($p<0.05$) higher than the control, but mean larval mortality amongst the test concentrations did not differ significantly ($p>0.05$).There was no significant($p>0.05$) difference between the two study conditions. The LC_{50} of the seed oil extractof *A. digitata* against *Ae. vittatus* larvae in the laboratory condition, 0.0998mL/L was higher than that of outdoor condition with 0.000549mL/L (Table 4.5).

Table 4.4: Cidal effect of *Adansonia digitata* seed oil extract against *Aedes vittatus* larvaein laboratory and outdoor conditions after twenty-four hours exposure.

Study condition Conc. (mL/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
Laboratory				
0 (Control)	0 (0.00[a];0.00-0.00±0.00)	0	70.4517	1.18×10^{-10}
0.1	46 (15.00[b]; 9.83-20.17±1.20)	61		
0.2	51 (17.00[bc]; 9.55-24.45±1.73)	68		
0.4	64(21.00[cd]; 13.40-28.60±1.77)	85		
0.8	72 (24.00[d]; 21.52-26.48±0.58)	96		
1.6	75 (25.00[d]; 25.00-25.00±0.00)	100		
3.2	75 (25.00[d]; 25.00-25.00±0.00)	100		
Outdoor				
0 (Control)	14 (4.67[a]; -22.48-31.82±1.20)	19	4.4435	0.0101121
0.1	72 (24.00[b]; 6.41-41.59±0.58)	96		
0.2	74(24.67[b]; -0.67-50.01±0.33)	99		
0.4	75 (25.00[b]; 25.00-25.00±0.00)	100		
0.8	75 (25.00[b]; 25.00-25.00±0.00)	100		
1.6	75 (25.00[b]; 25.00-25.00±0.00)	100		
3.2	75 (25.00[b]; 25.00-25.00±0.00)	100		

Means followed by the same superscript letter per study condition are not significantly different ($p>0.05$).

N = 75 (Total number of larvae exposed in three replicates)

CI = Class Interval

SE = Standard Error

Table 4.5: Median lethal concentration (LC_{50}) of *Adansonia digitata* seed oil against larval *Ae.vittatus*in laboratory and outdoor conditions.

Study condition Conc. (mL/L)	Log-conc.	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Laboratory					
3.2	0.5051	100	∞	y = 2.1485x + 7.1507	0.0998
1.6	0.2041	100	∞	(0.9377)	
0.8	-0.0969	96	6.7507		
0.4	-0.3979	85	6.0364		
0.2	-0.6990	68	5.4677		
0.1	-1.0000	61	5.2793		
0 (Control)	-	0	-		

Study condition Conc. (mL/L)	Log-conc.	% Mortality	Abbott's corrected mortality (%)	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Outdoor						
3.2	0.5051	100	100	∞	y = 0.9034x + 7.9455	0.00055
1.6	0.2041	100	100	∞	(0.6962)	
0.8	-0.0969	100	100	∞		
0.4	-0.3979	100	100	∞		
0.2	-0.6990	99	99	7.3263		

0.1	-1.0000	96	95	4.1221
0 (Control)	-	19	-	6.7507

4.2.3 Effect on *Culex quinquefasciatus*

The mortality of *Culex quinquefasciatus* larvae against *A. digitata* seed oil extract under laboratory and outdoor bioassays is shown in Table 4.4. The mortality under laboratory condition varied from 0/75 (0%) in the control to 75/75 (100%) for 1.6mL/L and 3.2mL/L. There was increase in larval mortality with increase in dose except for the decrease in percentage mortality from 96% at 0.4mL/L to 89% at 0.8mL/L. The lower doses showed that percentage mortality increased from 72% at 0.1mL/L to 96% at 0.4mL/L and then decreased to 89% at 0.8mL/L with 100% mortality recorded at 1.6mL/L and 3.2mL/L. Mean larval mortality for various experimental concentrations were significantly($p>0.05$) higher than the control. Mean larval mortality of lower doses of 0.1mL/L, 0.2mL/L and 0.8mL/L with their respective means 18.00, 21.67 and 22.33 did not differ significantly ($p>0.05$) but differ from the rest of concentrations. Similarly, doses with their corresponding means in bracket 0.4mL/L (24.00), 1.6mL/L (24.00) and 3.2mL/L (25.00) did not differ significantly ($p>0.05$) from each other. The outdoor bioassay larval mortality of *Cx. quinquefasciatus* ranged from 0/75 (0%) in the control to 75/75 (100%) in 1.6mL/L and 3.2mL/L. Percentage mortality increased with increase in the dose of oil extract but did not differ significantly ($p>0.05$). The Mean larval mortality forexperimental concentrations were significantly($p>0.05$) higher than the control. Although there was thin increase in mean larval mortality from 23.33 (0.1mL/L) to 25.00 (1.6mL/L and 3.2mL/L) but did not differ signifianlty ($p>0.05$). Seed oil etxracts of *A. digitata* did not significant ($p>0.05$) between laboratory and outdoorconditions. Laboratory and outdoor conditions LC_{50} of *A. digitata* seed oil against *Cx. quinquefasciatus* larvaewere 0.0519mL/L and 0.00397mL/L respectively (Table 4.7).

Table 4.6: Cidal effect of *Adansonia digitata* seed oil extract against *Culex quinquefasciatus* larvae in laboratory and outdoor conditions after twenty-four hours exposure.

Study condition Conc.(mL/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
Laboratory				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	57.5109	4.6×10^{-10}
0.1	54 (18.00[b]; 5.51-30.50±2.90)	72		
0.2	65 (21.67[b]; 17.87-25.47±0.88)	87		
0.4	72 (24.00[c]; 24.00-24.00±0.00)	96		
0.8	67 (22.33[b];19.47-25.19±0.66)	89		
1.6	75 (25.00[c]; 25.00-25.00±0.00)	100		
3.2	75 (25.00[c]; 25.00-25.00±0.00)	100		
Outdoor				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	537.978	1.0×10^{-15}
0.1	70 (23.33[b]; 20.47-26.19±0.66)	93		
0.2	73 (24.33[b]; 21.47-27.19±0.66)	97		
0.4	74 (24.67[b]; 23.23-26.11±0.33)	99		
0.8	71(23.67[b]; 22.23-25.11±0.33)	95		
1.6	75 (25.00[b]; 25.00-25.00±0.00)	100		
3.2	75 (25.00[b]; 25.00-25.00±0.00)	100		

Means followed by the same superscript letter per study condition are not significantly different ($p>0.05$).

N = 75 (Total number of larvae exposed in three replicates)

CI = Class Interval

SE = Standard Error

Table 4.7: Median lethal concentration (LC_{50}) of *Adansonia digitata* seed oil against larval *Cx. quinquefasciatus* in laboratory and outdoor conditions.

Study condition Conc. (mL/L)	Log-conc	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Laboratory					
3.2	0.5051	100	∞	y = 1.7165x + 7.2058	0.0519
1.6	0.2041	100	∞	(0.8278)	
0.8	-0.0969	89	6.2265		
0.4	-0.3979	96	6.5705		
0.2	-0.6990	87	6.1264		
0.1	-1.0000	72	5.5828		
0 (Control)	-	0	-		
Outdoor					
3.2	0.5051	100	∞	y = 1.0458x + 7.5101	0.00397
1.6	0.2041	100	∞	(0.6884)	
0.8	-0.0969	95	6.6449		
0.4	-0.3979	99	7.3263		
0.2	-0.6990	97	6.8808		
0.1	-1.0000	93	6.4758		
0 (Control)	-	0	-		

4.3 Larvicidal Effects of *Parkia biglobosa* Seed Oil ExtractAgainst Mosquito Larvae

4.3.1 Effect on *Aedes aegypti*

The mortality of *Ae.aegypti*larvae against the seed oil extractof*Parkia biglobosa* under laboratory and outdoor conditions is shown in Table 4.5. The larval mortality under laboratory bioassay ranged from 0/75 (0%) in the control to 73/75 (97%) in 3.2mL/L. There was increase in larval mortality progressively with increase in concentration. Mean larval mortality of the various experimental concentrations were significantly ($p<0.05$)different from the control. The mean larval mortalitiesof 19.00, 18.67 and 21.00 at doses 0.4mL/L, 0.8mL/L and 1.6mL/L correspondingly did not differ significantly ($p>0.05$) but differed from the first two lower means of 13.33 (0.1mL/L) and 15.00 (0.2mL/L) as well as from24.33 (3.2mL/L) which is at the highest level of significant ($p<0.05$). In the outdoor, the mortality varied from 0/75 (0%) in control to 73/75 (97%) at 3.2mL/L. Mortality in this study condition exhibited a dose-dependent activity with 37% recorded at 0.1mL/L and 97% at 3.2mL/L for the two extreme experimental doses. Mean larval mortality of various doses were significantly($p<0.05$) higher than the control. At lower concentrations (0.1-0.4mL/L), the oil extracts did not produce significant ($p<0.05$)kill except for dose 0.8mL/L with 14.67 mean in which it has a joint effect of non-significant from the first three lower concentrations and the dose after it 1.6mL/L (21.00), while concentration 3.2mL/L has 24.33 as mean was significantly($p<0.05$)highest. In this study, both laboratory and outdoor trial did not show significant($p>0.05$) difference. The median lethal concentrations (LC_{50}) of both study conditions of *P. biglobosa* seed oil extractagainst *Ae. aegypti* differed with laboratory bioassay having 0.1084mL/L and the outdoor value of 0.3327mL/L (Table 4.9).

Table 4.8: Cidal effects of *Parkia biglobosa* seed oil extract against *Aedes aegypti* larvaein laboratoryand outdoor conditions after twenty-four hours exposure.

Study condition Conc.(mL/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
Laboratory				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	93.5081	1.7×10^{-10}
0.1	40 (13.33[b]; 7.07-19.59±1.45)	53		
0.2	45 (15.00[b]; 12.52-17.48±0,58)	60		
0.4	57 (19.00[c]; 14.70-23.30±1.00)	76		
0.8	56 (18.67[c]; 14.87-22.47±0.88)	75		
1.6	63 (21.00[c]; 18.52-23.48±0.58)	84		
3.2	73 (24.33[d]; 22.89-25.77±0.33)	97		
Outdoor				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	13.3583	4.3×10^{-5}
0.1	28 (9.33[b]; -0.09-18.75±2.19)	37		
0.2	29 ((9.67[b]; 1.70-17.64±1.85)	39		
0.4	31(10.33[b]; 4.07-16.59±1.45)	41		
0.8	44 (14.67[bc]; 4.34-25.00±2.40)	59		
1.6	54 (18.00[c]; 1.70-1.70±3.79)	72		
3.2	73(24.33 [d]; 21.47-27.19±0.66)	97		

Means followed by the same superscript letter per study condition are not significantly different ($p>0.05$).

N = 75 (Total number of larvae exposed in three replicates)

CI = Class Interval

SE = Standard Error

Table 4.9: Median lethal concentration (LC_{50}) of *Parkia biglobosa* seed oil against larval *Ae.aegypti*in laboratory and outdoor conditions.

Study condition Conc.(mL/L)	Log-conc	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Laboratory					
3.2	0.5051	97	6.8808	y = 1.0649x + 6.0276	0.1084
1.6	0.2041	84	5.9945	(0.8785)	
0.8	-0.0969	75	5.6745		
0.4	-0.3979	76	5.7063		
0.2	-0.6990	60	5.2533		
0.1	-1.0000	53	5.0753		
0 (Control)	-	0	-		
Outdoor					
3.2	0.5051	97	6.8808	y = 1.3387x + 5.6399	0.3327
1.6	0.2041	72	5.5828	(0.7901)	
0.8	-0.0969	59	5.2275		
0.4	-0.3979	41	4.7725		
0.2	-0.6990	39	4.7202		
0.1	-1.0000	37	4.6684		
0(Control)	-	0	-		

4.3.2 Effect on *Aedes vittatus*

The result in Table 4.6 shows the larval mortality bioassay of *Ae vittatus* on exposure for 24h to seed oil extractof *P. biglobosa* under separate conditions of laboratory andoutdoor. The total number of dead larvae recorded in the laboratory condition per dose ranged from 0/75 (0%) in control to 74/75 (99) at 3.2mL/L. There was increase in larval percentage mortality as the concentration increased from 0.1mL/L having33% to the highest dose (3.2mL/L)with 99%. Experimental concentrations caused significant($p<0.05$) mortalitythan the control. The ranged of concentrations 0.2-1.6mL/L with their respective mean larval mortalities 18.00-20.67 did not differ significantly ($p>0.05$) but differed from the initial dose of 0.1mL/L with 8.33 as mean and the highest concentration 3.2mL/L (24.67). On the other hand, there was high mortality which ranged from 10/75 (13%) as observed in the control to 75/75 (100%) at doses of 0.4-3.2mL/L in the outdoor condition. The experimental mean larval mortality was significantly($p<0.05$) higher than the control. Amongst the concentrations, they did not differ significantly from each other as the lower doses recorded mean larval mortality of 23.33 at 0.1mL/L, 24.67 at 0.2mL/L and terminal mean death of 25.00 out of 25.00 at 0.4mL/L to 3.2mL/L. The larval mortality was significantly($p<0.05$) highest in the outdoor experiment than the laboratory condition.The susceptibility of *Ae.vittatus*larvae to *P. biglobosa* seed oil extract in the laboratory was higher than the outdoor condition with their $LC_{50}$of 0.1891mL/L and 0.2216mL/L respectively (Table 4.11).

Table 4.10: Cidal effect of *P. biglobosa* seed oilextract against *Aedes vittatus* larvaein laboratoryand outdoor conditions after twenty-four hours exposure.

Study condition Conc. (ml/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
Laboratory				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	28.4083	4.55×10^{-8}
0.1	25 (8.33[b]; -4.17-20.83±2.90)	33		
0.2	48 (18.00[c]; 13.03-22.97±1.15)	64		
0.4	52 (17.33[c]; 12.16-22.49±1.20)	69		
0.8	53 (17.67[c]; 11.09-24.25±1.50)	71		
1.6	62 (20.67[c]; 12.70-28.64±1.85)	83		
3.2	74 (24.67[d]; 23.23-26.11±0.33)	99		
Outdoor				
0 (Control)	10 (3.33[a]; 1.89+4.77±0.33)	13	455.775	3.17×10^{-15}
0.1	70 (23.33[b]; 19.53-27.13±0.88)	93		
0.2	74 (24.67[b]; 23.23-26.11±0.33)	99		
0.4	75 (25,00[b]; 25.00-25.00±0.00)	100		
0.8	75 (25.00[b]; 25.00-25.00±0.00)	100		
1.6	75 (25.00[b]; 25.00-25.00±0.00)	100		
3.2	75 (25.00[b]; 25.00-25.00±0.00)	100		

Means followed by the same superscript letter per study condition are not significantly different ($p>0.05$).

N = 75 (Total number of larvae exposed in three replicates)

CI = Class Interval

SE = Standard Error

Table 4.11: Median lethal concentration (LC_{50}) of *Parkia biglobosa* seed oil against larval *Ae.vittatus* in laboratory and outdoor conditions.

Study condition Conc. (mL/L)	Log-conc	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
3.2	0.5051	97	6.8808	y = 1.4878x + 6.0762	0.1891
1.6	0.2041	84	5.9945	(0.8381)	
0.8	-0.0969	75	5.6745		
0.4	-0.3979	76	5.7063		
0.2	-0.6990	60	5.2533		
0.1	-1.0000	53	5.0753		
0 (Control)	-	0	-		

Outdoor

Conc. (mL/L)	Log-conc	% Mortality	Abbot's corrected mortality (%)	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
3.2	0.5051	13	100	∞	y = 3.3026x + 7.1616	0.2216
1.6	0.2041	100	100	∞	(0.2798)	
0.8	-0.0969	100	100	∞		
0.4	-0.3979	100	100	∞		
0.2	-0.6990	100	99	7.3263		
0.1	-1.0000	99	92	6.4758		
0 (Control)	-	93	0	-		

4.3.3 Effect on *Culex quinquefasciatus*

Table 4.7 shows the larval mortality of *Cx quinquefasciatus* on exposure to various doses of *P. biglobosa* seed oil extract in both laboratory and outdoor conditions. The percentage mortality of larvae ranged from 0/75 (0%) in control to 75/75 (100%) at 3.2mL/L as recorded under laboratory bioassay. There was increase in mortality with increase in dose from 0.1-3.2mL/L except at 0.2mL/L and 0.4mL/L with 77% each where their percentage mortality did not change despite increase in dose. Mean larval mortality of experimental doses differed significantly($p<0.05$) from control. The mean mortality increased with increase in dose except for doses 0.2mL/L and 0.4mL/L having same mean each of 19.33,but statistically significant ($p<0.05$); while doses 0.4mL/L and 0.8mL/L with 19.33 and 21.330 means correspondingly did not differ. Similarly, there was no significant difference in mean mortalities of 24.33 and 25.00 at concenteations 1.6mL/L and 3.2mL/L accordingly. Under outdoor bioassay, mortality ranged from 0/75 (0%) in control to 74/75 (99%) at the highest dose (3.2mL/L). Percentage mortality increased as dose increases except for little changes in the trend in which there was slight decrease from 89% at 0.2mL/L to 88% at 0.4mL/L and 0.8mL/L. The mean larval mortality of experimental concentrations were significantly ($p<0.05$) higher than the control but did not differ significantly($p>0.05$)amongst the experimental doses.The laboratory LC_{50} (0.1058mL/L) of *P. biglobosa* seed oil was higher than the outdoor trial with 0.0134mL/L as LC_{50} (Table 4.13). The larvicidal prospects of *A. digitata* seed oil extracts against the three mosquioes species yielded high mortality at all experimental concentrations used. The larvicidal activity of *P. biglobosa* seed oil was significantly($p<0.05$) lethal in the outdoor than the laboratory settings.

Table 4.12: Cidal effect of *P. biglobosa* seed oil extract against *Culex quinquefasciatus*larvae in laboratory and outdoor conditions after twenty-four hours exposure.

Study condition Conc. (mL/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
Laboratory				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	125.695	2.32×10^{-11}
0.1	42 (14.00[b]; 9.03-18.97±1.15)	56		
0.2	58 (19.33[b]; 15.53-23.13±0.88)	77		
0.4	58 (19.33[c]; 16.47-22.19±0.66)	77		
0.8	64 (21.33[c]; 16.16-26.50±1.20)	85		
1.6	73 (24.33[d]; 22.89-25.77±0.33)	97		
3.2	75 (25.00[d]; 25.00-25.00±0.00)	100		
Outdoor				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	71.4515	1.08×10^{-9}
0.1	63 (21.00[b]; 16.70-25.30±1.00)	84		
0.2	67 (22.33[b]; 18.53-26.13±0.88)	89		
0.4	66 (22.00[b]; 13.41-30.60±1.20)	88		
0.8	66 (22.00[b]; 19.52-24.48±0.58)	88		
1.6	69 (23.00[b]; 18.70-27.30±1.00)	92		
3.2	74 (24.67[b]; 23.23-26.11±0.33)	99		

Means followed by the same superscript letter per study condition are not significantly different ($p>0.05$).

N = 75 (Total number of larvae exposed in three replicates)

CI = Class Interval

SE = Standard Error

Table 4.13: Median lethal concentration (LC_{50}) of *Parkia biglobosa* seed oil against larval *Cx. quinquefasciatus* in laboratory and outdoor conditions.

Study condition Conc. (mL/L)	Log-conc	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Laboratory					
3.2	0.5051	100	∞	y = 1.7483x + 6.7052	0.1058
1.6	0.2041	97	6.8808	(0.8721)	
0.8	-0.0969	85	6.0364		
0.4	-0.3979	77	5.7388		
0.2	-0.6990	77	5.7388		
0.1	-1.0000	56	5.1510		
0 (Control)	-	0	-		
Outdoor					
3.2	0.5051	99	7.7363	y = 0.9031x + 6.6905	0.0134
1.6	0.2041	92	6.4951	(0.6267)	
0.8	-0.0969	88	6.1750		
0.4	-0.3979	88	6.1750		
0.2	-0.6990	89	6.2265		
0.1	-1.0000	84	5.9945		
0 (Control)	-	0	-		

4.4 Larvicidal Effects of *Persea americana* Seed Oil ExtractAgainst Mosquitoes Larvae

4.4.1 Effect on *Aedes aegypti*

The mortality of *Aedes aegypti* larvae exposed to various concentrations of *Persea americana*under laboratory and outdoor conditions is shown in Table 4.8. The number of dead larvae per treatment dose ranged from 0/75 (0%) in control to 74/75 (99%) at 3.2mL/L. Larval percentage mortality increased in a dose-dependent fashion from 35% at 0.1mL/L to 99% at 3.2mL/L. Mean larval mortality at experimental conditions were significantly($p<0.05$) higher than the control, but increase in concentration from 0.1 to 0.4mL/L with their corresponding mean larval mortalities ranged from 8.67-11.00 did not produce significant($p>0.05$) increase. In the same way, there was no significant($p>0.05$) difference in mean larval mortality between 17.00 at 0.8mL/L and 19.67 at 1.6mL/L but differed significantly ($p<0.05$) from concentration 3.2mL/L with 24.67 as mean. For outdoor bioassay, larval death varied from 0/75 (0%) in control to 75/75 (100%) at 1.6mL/L and 3.2mL/L. Larval mortallity exhibited a dose-dependent manner which begins from 77% at 0.1mL/L to 100% at 1.6mL/L and 3.2mL/L. All the experimental concentrations of seed oil of *P. americana* were significantly($p>0.05$) higher than the control, but an increase in dose from 0.1mL/L to 0.2mL/L with 19.33 and 19.67 as their means respectively did not produce significant($p>0.05$) difference. Similarly, there was no significance($p>0.05$)increasein mean (22.67-25.00) larval mortality at concentrations 0.4mL/L up to 3.2mL/L.The larvicidal efficacy of *P. americana*produced mortalityat all doses in both study conditions which did not differ significantly ($p>0.05$). The LC_{50} of the two study conditions differed, with laboratory having higher value of 0.3026mL/L than the one recorded in outdoor of 0.0561mL/L (Taable 4.15).

Table 4.14: Cidal effect of *Persea americana* seed oil extract against *Aedes aegypti*larvae in laboratory and outdoor conditions after twenty-four hours post exposure.

Study condition Conc.(mL/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
Laboratory				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	37.6693	7.45×10^{-8}
0.1	26 (8.67[b]; 5.81-11.53±0.66)	35		
0.2	28 (9.33[b]; 4.16-15.00±1.20)	37		
0.4	33 (11.00[b]; 4.42-17.58±1.50)	44		
0.8	51 (17.00[c]; 17.58-23.58±1.50)	68		
1.6	59 (19.67[c]; 9.34-30.00±2.40)	79		
3.2	74 (24.67[d]; 23.23-26.11±0.33)	99		
Outdoor				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	225.506	4.18×10^{-13}
0.1	58 (19.33[b]; 15.53-23.13±0.88)	77		
0.2	59 (19.67[b]; 15.87-23.47±0.88)	79		
0.4	68 (22.67[c]; 18.87-26.47±0.88)	91		
0.8	73 (24.33[c]; 22.89-25.77±0.33	97		
1.6	75 (25.00[c]; 25.00-25.00±0.00)	100		
3.2	75 (25.00[c]; 25.00-25.00±0.00)	100		

Means followed by the same superscript letter per study condition are not significantly different ($p > 0.05$).

N = 75 (Total number of larvae exposed)

CI = Class Interval

SE = Standard Error

Table 4.15: Median lethal concentration (LC_{50}) of *Persea americana* seed oilin laboratory and outdoor condition bioassay of *Ae. aegypti.*

Study condition Conc. (mL/L)	Log-conc.	% Mortality	Empirical probit mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Laboratory					
3.2	0.5051	99	7.3263	y = 1.6705x + 5.8672	0.3026
1.6	0.2041	79	5.8064	(0.8304)	
0.8	-0.0969	68	5.4677		
0.4	-0.3979	44	4.8400		
0.2	-0.6990	37	4.6681		
0.1	-1.0000	35	4.6147		
0 (Control)	-	0	-		
Outdoor					
3.2	0.5051	100	∞	y = 1.8175x + 7.2742	0.0561
1.6	0.2041	100	∞	(0.9260)	
0.8	-0.0969	97	6.8808		
0.4	-0.3979	91	6.3408		
0.2	-0.6990	79	5.8064		

0.1	-1.0000	77	5.7388
0 (Control)	-	0	-

4.4.2 Effect on *Aedes vittatus*

The larval mortality of *Aedes vittatus* exposed to various concentrations of seed oil extractof *P. americana*under study conditions of laboratory and outdoor is shown in Table 4.9. Larval mortality varied from 0/75 (0%) in the control to 75/75 (100%) at 1.6mL/L and 3.2mL/L under laboratory bioassay. There was increase in larval percentage mortality progressively in a dose-dependent way from 37% observed at 0.1mL/L to total kill (100%) recorded at the two doses of 1.6mL/L and 3.2mL/L. The experimental mean larval mortality differed significantly($p<0.05$) from control. In the same vein, the first two corresponding means of 9.33 and 19.67 at lower doses of 0.1mL/L and 0.2mL/L differred significantly ($p<0.05$); while mortality at doses 0.4mL/L to 3.2mL/L with their respective means that rangd from 23.00 to25.00 did not produce significant($p>0.05$) difference. In the outdoor experiment, high larval mortality was recorded at lower doses in which the mortality was dose-independent. At concentration 0.1mL/L, 99% mortality was observed and reduced slightly at 0.2mL/L to 97%, then increased to 100% from 0.4mL/L up to 3.2mL/L. Mean larval mortality of experimental doses were significantly($p<0.05$) highestthan the control, but there was no significant($p>0.05$) difference among the mean larval mortality of various concentrations.Despite recording high mortality in the outdoor trial, but it did not differ significantly($p>0.05$) fromthe laboratory condition. There was high variation in susceptibility of *Ae. vittatus*larvae to seed oil of *P. americana* with outdoor median lethal concentration of 0.00007mL/L been lower than the laboratory bioassay value of 0.1118mL/L (Table 4.17).

Table 4.16:Cidal effect of *Persea americana* seed oil extract against *Aedes vittatus* larvaein laboratory and outdoor conditions after twenty-four hours exposure.

Study condition Conc.(mL/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
Laboratory				
0 (Control)	0 (0.00[a];0.00-0.00±0.00)	0	149.52	7.09×10^{-12}
0.1	28 (9.33[b]; 1.73-16.93±1.77)	37		
0.2	59 (19.67[c]; 18.23-21.11±0.33)	79		
0.4	69 (23.00[d]; 20.52-25.48±0.58)	92		
0.8	71(23.67[d]; 19.87-27.47±0.88)	95		
1.6	75 (25.00[d]; 25.00-25.00±0.00)	100		
3.2	75 (25.00[d]; 25.00-25.00±0.00)	100		
Outdoor				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	8500.7	4.22×10^{-24}
0.1	74 (24.67[b]; 23.85-25.49±0.33)	99		
0.2	73 (24.33[b]; 23.51-25.15±0.33)	97		
0.4	75 (25.00[b]; 25.00-25.00±0.00)	100		
0.8	75 (25.00[b]; 25.00-25.00±0.00)	100		
1.6	75 (25.00[b]; 25.00-25.00±0.00)	100		
3.2	75 (25.00[b]; 25.00-25.00±0.00)	100		

Means followed by the same superscript letter per study condition are not significantly different ($p>0.05$).

N = 75 (Total number of larvae exposed in three replicates)

CI = Class Interval

SE = Standard Error

Table 4.17: Median lethal concentration (LC_{50}) of *Persea americana*seed oil against larval *Ae. vittatus* in laboratory and outdoor condition.

Study condition Conc. (mL/L)	Log-conc.	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Laboratory					
3.2	0.5051	100	∞	y = 2.2971x + 7.1859	0.1118
1.6	0.2041	100	∞	(0.9464)	
0.8	-0.0969	95	6.6449		
0.4	-0.3979	92	6.4051		
0.2	-0.6990	79	5.8064		
0.1	-1.0000	37	4.6681		
0 (Control)	-	0	-		
Outdoor					
3.2	0.5051	100	∞	y = 0.7069x + 7.9362	7.0×10^{-6}
1.6	0.2041	100	∞	(0.5672)	
0.8	-0.0969	100	∞		
0.4	-0.3979	100	∞		
0.2	-0.6990	97	6.8808		
0.1	-1.0000	99	7.3263		

0 (Control)	-	0	-

4.4.3 Effect on *Culex quinquefasciatus*

The larval mortality of *Culex quinquefasciatus* on exposure to various doses of *P. americana* seed oil extractunder laboratory and outdoor conditions is shown in Table 4.10. There was increase in larval mortality as the dose increased which ranged from 0/75 (0%) in the control to 75/75 (100%) at the last two doses (1.6mL/L and 3.2mL/L). Mean larval mortality of all experimental doses of *P. americana* seed oil extracts were significantly($p<0.05$) higher than the control.For the experimental concentrations, the first two mean larval mortalities of 13.67 and 19.67 differed significantly ($p<0.05$) at their corresponding lower doses of 0.1mL/L and 0.2mL/L; but means at concentrations 0.4mL/L (23.00) up to 3.2mL/L (25.00) did not yield significant ($p>0.05$)difference. The outdoor bioassay larval mortality ranged from 0/75 (0%) in control to 74/75 (99%) at 3.2mL/L. Mortality in this study condition demonstrated a dose-dependent activity with the larval death ranged from 36% at 0.1mL/L to 99% at 3.2mL/L. The mean larval mortality of experimental doses were significantly($p<0.05$) higher than the control. The effects of concentrations 0.1mL/L and 0.2mL/L did not show significant($p>0.05$) mean (9.00 and 13.00 respectively) mortalities, but increase in mean death to 17.33 at dose 0.4mL/L did produce significant($p<0.05$)difference. While the effect of last three doses, 0.8mL/L (21.33), 1.6mL/L (23.00) and 3.2mL/L (24.67) with their corresponding mean mortalities in the brackets did not yield significant($p>0.05$) difference. Various mortalities were recorded at different concentrations in the two study conditions of laboratory and outdoor but did not differ significantly ($p>0.05$). The toxicity effect of *P. americana* was more in the laboratory (LC_{50} = 0.0858mL/L) than the outdoor (LC_{50} = 0.1862mL/L) conditions (Table 4.19).

Table 4.18:Cidal effect of *Persea americana*seed oilextract against *Culex quinquefasciatus* larvaein laboratory and outdoor conditions after twenty-four hours exposure.

Study condition Conc. (mL/L)	Larval mortality (Mean; 95% CI±SE) 24h post exposure	% Mortality	F-value	ANOVA probability

				(p-value)
Laboratory				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	104.9	8.0×10^{-11}
0.1	41 (13.67[b]; 7.41-19.93±1.45)	55		
0.2	59 (19.67[c]; 13.39-25.95±0.88)	79		
0.4	69 (23.00[d]; 20.52-25.48±0.58)	92		
0.8	72 (24.00 [d]; 19.70-28.30±1.00)	96		
1.6	75 (25.00 [d]; 25-25±0.00)	100		
3.2	75 (25.00 [d]; 25-25±0.00)	100		
Outdoor				
0 (Control)	0 (0.00[a]; 0.00-0.00±0.00)	0	85.892	3.11×10^{-10}
0.1	27 (9.00[b]; 6.52-11.48±0.58)	36		
0.2	39 (13.00[b]; 8.03-17.97±2.15)	52		
0.4	52 (17.33[c]; 12.16-22.50±1.20)	69		
0.8	64 (21.33[d]; 15.07-27.59±1.45)	85		
1.6	69 (23.00[d]; 18.70-27.30±1.00)	92		
3.2	74 (24.67[d]; 23.23-26.11±0.33)	99		

Means followed by the same superscript letter per study condition are not significantly different ($p>0.05$).

N = 75 (Total number of larvae exposed in three replicates)

CI = Class Interval

SE = Standard Error

Table 4.19: Median lethal concentration (LC_{50}) of *Persea americana* seed oil against larval *Cx. quinquefqsciatus* in laborarory and outdoor condition.

Conc. (mL/L)	Log-conc	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mL/L)
Laboratory					
3.2	0.5051	100	∞	y = 2.09x + 7.2285	0.0858
1.6	0.2041	100	∞	(0.9579)	
0.8	-0.0969	96	6.7507		
0.4	-0.3979	92	6.4051		
0.2	-0.6990	79	5.8064		
0.1	-1.0000	55	5.1257		
0 (Control)	-	0	-		
Outdoor					
3.2	0.5051	99	7.3263	y = 1.7112x + 6.2493	0.1862
1.6	0.2041	92	6.4051	(0.9787)	
0.8	-0.0969	85	6.0364		
0.4	-0.3979	69	5.4959		
0.2	-0.6990	52	5.0502		
0.1	-1.0000	36	4.6415		
0 (Control)	-	0	-		

CHAPTER FIVE

5.0 DISCUSSION

This study has demonstrated that petroleum ether extracted seed oils of *Adansonia digitata*, *Parkia biglobosa* and *Persea americana* have similar components of acid, free fatty acid, iodine, peroxide and saponification values. The proportions of acid and free fatty acids in extracts of *A. digitata*, and *P. biglobosa* seeds were similar but these properties were of approximately four folds higher values in *P. americana* (Table 4.1). This could be an indication that the seed oil of *P. americana*is richer than those of *A. digitata* and *P. biglobosa*. These differences could have affected their mosquito larvicidal prospects. Low value of seed oil acidity has been associated with high resistance to hydrolysis as reported by Gautam *et al.* (2013). Thus, the seed oils of both *A. digitata* and *P. biglobosa* could be more stable when applied in water against mosquito larvae, than the oil of *P. americana* with higher acid value. Hydrolysis is a double decomposition reaction with water and the implication of higher resistance to hydrolysis due to the low acid values of the oils could account for their persistence in water. This could portent positive larvicidal attributes for the oils of *A. digitata* and *P.biglobosa* as they are likely act for prolong time in aquatic habitats. The fatty acid profiles of the seed oils were not determinedin this study, however the percentage free fatty acid and iodine values determined for the oils were indicative of the presence of fatty acids. The high iodine values in *P. americana*and *P. biglobosa* oils over that of *A. digitata* are indication of the preponderance of unsaturated fatty acids as indicated by Akubugwo and Ugbogu(2007). Seed oil of *A. digitata* with lowest iodine value is saturated oil. Highly unsaturated oils with higher iodine values, as seen with *P. americana*, are

desentised as drying oil with the implication that they are more volatile. Thus, *P. americana*oil extract could evaporate faster when applied as mosquito larvicide and this can negatively affect its larvicidal prospect than the other two oils. The peroxide values of the three seed oils of *A. digitata*, *P. biglobosa* and *p. americana*were low, an indication of their freshness and high level of antioxidant in them. High peroxide valuesin the oil is an indication of high levels of oxidative rancidity as well as absence or low level of antioxidant. This reportsis in consonancewith the studies carried out byAkubugwo and Ugbogu (2007) and Tara *et al*. (2012) in which they recorded threshold between 30 and 40meq/Kgfat for oils that led to initiaterancidity. Thus, the three seed oils could be stored for long periods. Saponification value of the seed oils was higher in *P. americana*thanthe other two oils, which were also high on their own. Saponification number measure the average molecular weight or chain length of all fatty acids present in oils (Alabi *et al*., 2005). Saponins are heterosides (substances containing in their structure one or more sugar molecule) of plant origin (Chaieb, 2010).Oils with long chain fatty acids have low saponification values because they have a relatively fewer numbers of carboxylic functional groups (Alabi *et al*.,2005). It could then be deduced that the oils of *A. digitata*, *P. biglobosa* and *P. americana*have short chain fatty acids (Ajayi and Oderinde, 2002).

The seed oil extracts of *Adansonia digitata*, *Parkia biglobosa* and *Persea americana*have been shown to produce excellent larvicidal effects against*Aedes aegypti*, *Aedesvittatus* and *Culex quiquefasciatus* speciesin laboratory and outdoor conditions. The results of this study have established that all treatments of the oils tested against the larvae of the three mosquito species produced significant mortality over the controls. This was sufficiently enough to attribute the observed larval mortality to the seed oil etxracts treatments. This study has further proved the ability of the extracts of the seeds of tropical plants to elicit larvicidal effects against

noxious mosquitoes. To a large extent, observed mortality rates of the larvae were concentration (dose) dependent for all the seed oil extracts against all mosquito species. In some instances where slight anomaly against concentration dependency occurred, statistical analysis of mortality data proved otherwise. This study agreed with Rahuman *et al*. (2008) that linked dose dependent mortality as a result of homogeneous distribution of active compound in the extractof *Abutilon indicum* against larvae of *Aedes* species. Adebote *et al*., (2011a) also reported a dose dependent mortality response of *Cx. quinquefasciatus* larvae to treatments of *Bobgunia madagascariensis* stem bark extract. Saponins have been recorded with higher contents in all the seeds of the three plants than the other components of oil. Morrissey and Osbourn (1999) have suggested that the saponin molecules interact with the cuticle membrane of the larvae, ultimately disarranging the membrane could be the most probable reason for the larval death.Chaieb (2010) put forward thatsaponins have the ability offorming insoluble complexes withcholesterol, which results in cholesterol not absorbed in the digestive system of various animal species. The mechanism of formation of the cholesterol/saponin complexes is still unknown. Apart from the laboratory based bioassays, this study proceeded to conduct outdoor bioassays to subject the larvae to situations very close to conditions in their natural breeding habitats where practical controls would inevitably be done. It has been stated that only very few of the several plants from different families tested and confirmed for mosquitocidal activity were moved from the laboratory to field use (Samidurai, 2012; Samuel *et al*., 2012a). Modern mosquito control programmes have continuously been focused more on the elimination of mosquitoes at larval stage with plant extracts (Samuel *et al*., 2012b). The advantage of targeting mosquito larvae has been attributed to their inability to escape from their breeding locations until adult stage is attained and reduction in overall pesticide use in adult mosquito control by aerial application of adulticidal chemicals (Samuel *et al*.,

2012b).Several natural products, especially substances from *P. americana* with antifungal, antiviral, antibacterial and trypanosomicidal activity, have been tested through this assay, showing a significant relationship (Kulkarni *et al*., 2010). Essential oils are presumed to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects (Mann and Kaufman, 2012). The oils block the spiracles, which leads to the blocking of respiratory siphon and invariably respiration (asphyxiation) and death (Kaufmann and Briegel, 2004; Rotimi*et al*., 2011). Parra *et al*. (2001)showed that the use of essential oils is a fast, simple, practical and low cost assay.

It may suffice to comment on the performances of the seed oils against the target mosquito species under the two study conditions. *Adansonia digitata* seed oil extracts recorded up to 93% and 96% larval mortality of*Ae. aegypti* at the highest concentration under laboratory and outdoor conditions respectively (Table 4.2). Mortality recorded at the least concentration of the seed oil of *A. digitata* in the laboratory was comparable with and not significantly from mortality recorded at the highest extract concentration. This observation could be interpreted as very low concentrations of the seed oil of *A. digitata* were needed to cause appreciable larval mortality of *Ae. aegypti* in the laboratory. However, the larvae of *Ae.aegypti* only responded appreciably to higher concentrations (1.6mL/L and 3.2mL/L) of the seed oil of *A. digitata* under outdoor condition. This could explain why a higher LC_{50} value (0.4886mL/L) was obtained under outdoor condition than laboratory condition (0.1179mL/L) (Table 4.3). The larvae of *Ae.vittatus* responded better in terms of mortality to treatments of *A. digitata* seed oil extract under both study conditions than the *Ae. aegypti.* Up to 61% and 95% larval mortality of *Ae.vittatus* were obtained with the least seed oil concentration (0.1mL/L) of *A. digitata* under laboratory and outdoor conditions respectively (Table 4.4). Unlike the sub-lethal effects witnessed with units of

Ae. aegypti larvae, *A. digitata* seed oil extracts caused total (100%) mortality of *Ae. vittatus* larvae at 1.6mL/L and higher concentrations under laboratory condition and at 0.4mL/L and higher concentrations under outdoor condition. Mortality of *Ae.vittatus*, due to treatments of *A. digitata* seed oil, was better under outdoor than laboratory condition; which was responsible for lower LC_{50} (0.000549mL/L) under outdoor than (0.0998mL/L) laboratory condition (Table 4.5). The least concentration of *A. digitata* used against *Cx. quinquefasciatus* larvae produced 72% and 93% mortality under laboratory and outdoor conditions accordingly; higher concentrations (1.6mL/L and 3.2mL/L) produced total kill (100%) under both conditions. Larvae of *Cx. quinquefasciatus* showed a better response to *A. digitata* seed oil under outdoor (LC_{50} = 0.00397mL/L) than laboratory condition (LC_{50} = 0.0519mL/L) (Table 4.7).The increase in mortality with increase in efficiency of concentration in the two study conditions is probably due to increase in larvicidal ingredients of the oil extract and the anti-feeding (peroxide) components present that led to high mortality in higher doses. Adeyemi and Adebote (2010) reported that methanolic extracts of various parts of *Bobgunnia madagascariensis* have shown superior anti-feedant and contact toxicity effects on the rust red flour beetle, *Tribolium casteneum*. Almost all parts of the tree (*A.digitata*) are used in traditional medicine in Africa although this varies from one country to another. The various parts of the plant (leaves, bark, seeds) are used as a panacea, to treat diseases with specific documented uses that include malaria, tuberculosis, fever, microbial infections, diarrhoea, anaemia, dysentery, toothache among others (Nguta *et al*., 2010).*Parkia biglobosa* produced 97% larval mortality of *Ae. aegypti*at the highest treatments under both study conditions but the mortality was higher under laboratory (LC_{50} = 0.1084mL/L) than the outdoor (LC_{50} = 0.3327mL/L) condition (Tables 4.8 and 4.9). High susceptibility of *Ae.vittatus*to treatments of *P. biglobosa* seed oil was observed in this study to the extent that 99% and 100% mortality were recorded at the highest concentrations under

laboratory and outdoor conditions correspondingly (Table 4.10). *Aedes vittatus* larval response to *P. biglobosa* seed oil treatment was better under outdoor condition, where 100% kill occurred at 0.4mL/L and higher concentrations (LC_{50} = 0.0010mL/L), than laboratory condition (LC_{50} = 0.1890mL/L) (Table 4.11). Total (100%) kill of *Cx quinquefasciatus* occurred at the highest treatment (3.2mL/L) of *P. biglobosa* seed oil under laboratory condition while 99% kill was obtained at this concentration under outdoor condition (Table 4.12). Thus, the LC_{50} of the extract of *P. biglobosa* against *Cx. quinquefasciatus* (0.0134mL/L) under outdoor condition was lower than the LC_{50} of the extract (0.1058mL/L) under laboratory condition (Table 4.13). The larvicidal activity of *P. biglobosa* demonstrated in this study may be in consonance with the work of Bolarinwa (2012) in which the extract from raw seed of *P. biglobosa,* which appears to be relatively nontoxic to human, have therefore shown to be an effective candidate for the control of the termite (*Coptotermes intermedius*).*Aedes aegypti* larval response to *Persea americana* seed oil treatment was better under outdoor where 100% kill occurred at 1.6mL/L and higher concentration with LC_{50} value of 0.0561mL/L, than the laboratory condition with 99% mortality at the highest concentration and LC_{50} of 0.3026mL/L (Tables 4.14 and 4.15). Total (100%) kill of *Ae. vittatus* larvae occurred at 1.6mL/L and higher concentration of *P. americana* seed oil treatments under laboratory condition and at 0.4mL/L and higher concentrations under outdoor condition (Table 4.16). Thus, the LC_{50} of *P. american* seed oil etxrat against *Ae. vittatus* larvae was lower under outdoor condition (6.9977×10^{-5}mL/L) than laboratory condition (0.1118mL/L) (Table 4.17); an indication of better response of *Ae. vittatus*larvae under outdoor condition. This could be attributed to unstable environmental conditions (temperature, sun light, pH, among others) in the outdoor bioassay which differed from the one in the laboratory. The larval mortality of *Cx. quinquefasciatus*recorded 100%at 1.6mL/L and higher dose of *P. american*seed oil treatments under laboratory condition and 99% kill at 3.2mL/L under outdoor

condition (Table 4.18). Thus, the larvae of *Cx. quinquefasciatus* showed better susceptibility to extract of the seed of *P. americana*under laboratory condition with lower LC_{50} value of 0.0858mL/L than outdoor condition with LC_{50} of 0.1862mL/L (Table 4.19). This study agrees with George (2010) that recorded mortality of larvae and pupae of *Ae. albopictus*in all replications and concentrations of both extracts of seed and bark of *P. americana*. The lethal concentrations (LC_{50})of *P. americana*ethanolic seed and bark extracts for 3rd instar larvae and pupae of *Ae. albopictus*were 3.5 and7.4 mg/Lfor seed extract;4.2 and 8.2 mg/L for bark respectively.

The three species of mosquito whose larvae were observed to be highly susceptible to seed oils of *A. digitata*, *P. biglobosa* and *P. americana*in this study are among the medically important pest species with high anthropophilic nature and capability of human disease transmission. *Aedes aegypti* is a vector of arboviruses such as dengue, yellow fever and dirofilariasis. It breeds profusely in peridomestic environment in artificial containers of various sorts (Adebote *et al*., 2006; 2011b). The larvae of *Ae.aegypti* used in this study were obtained from discarded automobile tyres in several foci within Zaria. Thus, the seed oils of *A. digitata*, *P. biglobosa* and *P. americana*, that were tested and confirmed to possess excellent larvicidal prospects against *Ae. aegypti* in this study, could be used to reduce the population and disease transmission risk of *Ae. aegypti*domiciliated in discarded automobile tyres and allied peridomestic containers. *Aedesvittatus* is known to breed profusely in rock pools as their preferred habitats and a potential vector of arboviruses including yellow fever (Adebote *et al*., 2008). Larvae of the species were obtained from these habitats around Zaria. The seed oils of the three plants can also be harnessed to eliminate *Ae. vittatus* from rock pools and or reduce its pest status. *Culex quinquefasciatus* breeds in diverse aquatic habitats without and especially high organic contents and it rests characteristically in indoor environments close to humans where it constitutes biting

nuisance and has the potential of transmit disease such as filariasis. In the light of the excellent larvicidal prospects of the three seed oils against *Cx. quinquefasciatus* in supposedly clean water, it will be necessary to investigate the larvicidal capabilities of the three seed oils against *Cx. quinquefasciatus* in waters with high organic mater, so as to comprehend their ability to act against the mosquito species in preferred natural conditions.

The LC_{50} obtained for the three seed oil extracts against the three mosquito species in this study were lower than those obtained with other seed extracts against similar and or different species of mosquito by other authors. These could be interpreted that extracts of the seeds in this study were of better larvicidal prospects than those investigated by other authors. Elangovan *et al*. (2008) recorded LC_{50} values of 42.17mL/L, 30.32mL/L and 35.95mL/Lfor the seed oil against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* respectively. In another development, a study conducted by Abdelouaheb *et al*. (2009), they observed 0.35mg/L of LC_{50} value of Neem tree (azadirachtin) seed oil against some mosquito larvae in the Republic of Algeria. Waseem*et al*. (2010) also indicated thatout of the total citrus seed extracts tested, rough lemon and lemon had the lowest LC_{50} values (119.99 and 137.26mL/L, respectively), particularly after 24 hours of exposure, followed by red blood orange (295.63mL/L), chakutra (334.87mL/L), galgal (644.25mL/L), Brazilian sour (905.96mL/L) and kinnow (1022.67mL/L). Narangi had the highest LC_{50} value (2069.12mL/L) after 24h of exposure followed by grape fruit (1598.15mL/L) and musambi (1389.16mL/L). Zewdneh *et al.* (2011) reported LC_{50} value of crude methanol seed extract of *J.curcas* tested against *Anopheles arabiensis* to be 92.09mL/L.Pierre *et al*.(2014) reported essential oil of the two plants, *Plectranthus glandulosus* that caused

LC_{50} values of 2.66, 7.37 and 43.16mL/L; and *Callistemon rigidus* displayed LC_{50} values of 66.67, 99.61 and 176.81mLL against all the target mosquito species of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* larvae with significant larvicidal and pupicidal potentials.

In terms of practical sustainability of the prospects of the adoption of the seed oil extracts of *A. digitata*, *P. biglobosa* and *P. americana*inlarvalmosquito control, it will be necessary to discuss their economic values. The three plants are cultivated and also occur in the wild state in several regions of Nigeria and elsewhere in the tropics (Orwa *et al.*, 2009**)**. With the exception of the seed of *P. biglobosa* which form important human food additives and vital economic commodities in certain African cultures (Gernah *et al*., 2007a;Kamatou *et al*., 2011); the seeds of the other two plants are often discarded as waste by-products. None of the oils from the three plants are of high economic usage as vegetable oils of commerce or oils considered of biodiesel grade. In the light of their high mosquito larvicidal prospects as revealed by this study, it will not be out of place to project their ready availability, low cost of procurement and simple technology associated with their adoption in mosquito control.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The solvent extracted seed oil of *A. digitata, P. biglobosa* and *P. americana*showed potent larvicidal activities against *Ae. aegypti, Ae. vittatus*and *Cx. quinquefasciatus* species of mosquito under laboratory and outdoor conditions.The results suggested that the seedoil extracts havepotentials that can be exploited to develop larvicide that can supplement synthetic chemical pesticides for mosquito control programmes. The physicochemical properties of the

extracted seeds are similar but the components differ significantly in quantity among the species.Larvicidal efficacies of the seed oils did not differ significantly in both laboratory and simulated field conditions for two of the three extract except for *P. biglobosa* which was more effective under outdoor conditions. *Parkia biglobosa* performed significantly betterunder outdoor conditions against *Ae. aegypti*and *Cx. quinquefasciatus*.

6.2 Recommendations

Considering mosquito resistance and other problems being associated with synthetic insecticides, seed oils of *A. digitata*, *P. biglobosa* and *P. americana*could be good botanical substitutes to synthetic insecticide in view of their excellent efficacies demonstrated. It is therefore recommended that:

1. Adoption of the seed oils for mosquito control operations after safety tests.

2. Further work is needed to identify the potent active component(s) in the oils that areresponsible for the observed larvicidal effects.

3. There is need to test the *in situ* efficacies of these seed oils on larval mosquitoes in their breeding habitats

4. There is need to investigate the effects of these seed oils on mosquito-coinhabitant non-target aquatic organisms to ensure specificity.

5. Further studies are needed to unravel the mode(s) of action of the seed oils on mosquito larvae.

6. There is need to investigate the effects of the seed oils on other life stages (eggs, pupae and adults) ofmosquitospecies.

7. The seeds of *A. digitata, P. biglobosa* and *P. americana*can be harnessed for the control of noxious mosquito species andas replacement for synthetic pesticides.

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APENDICES

APPENDIX I:The Empirical Probit of Mortality Against Logarithm of Concentrations of *A. digitata* seed oil against *Ae. aegypti* in Laboratory Condition.

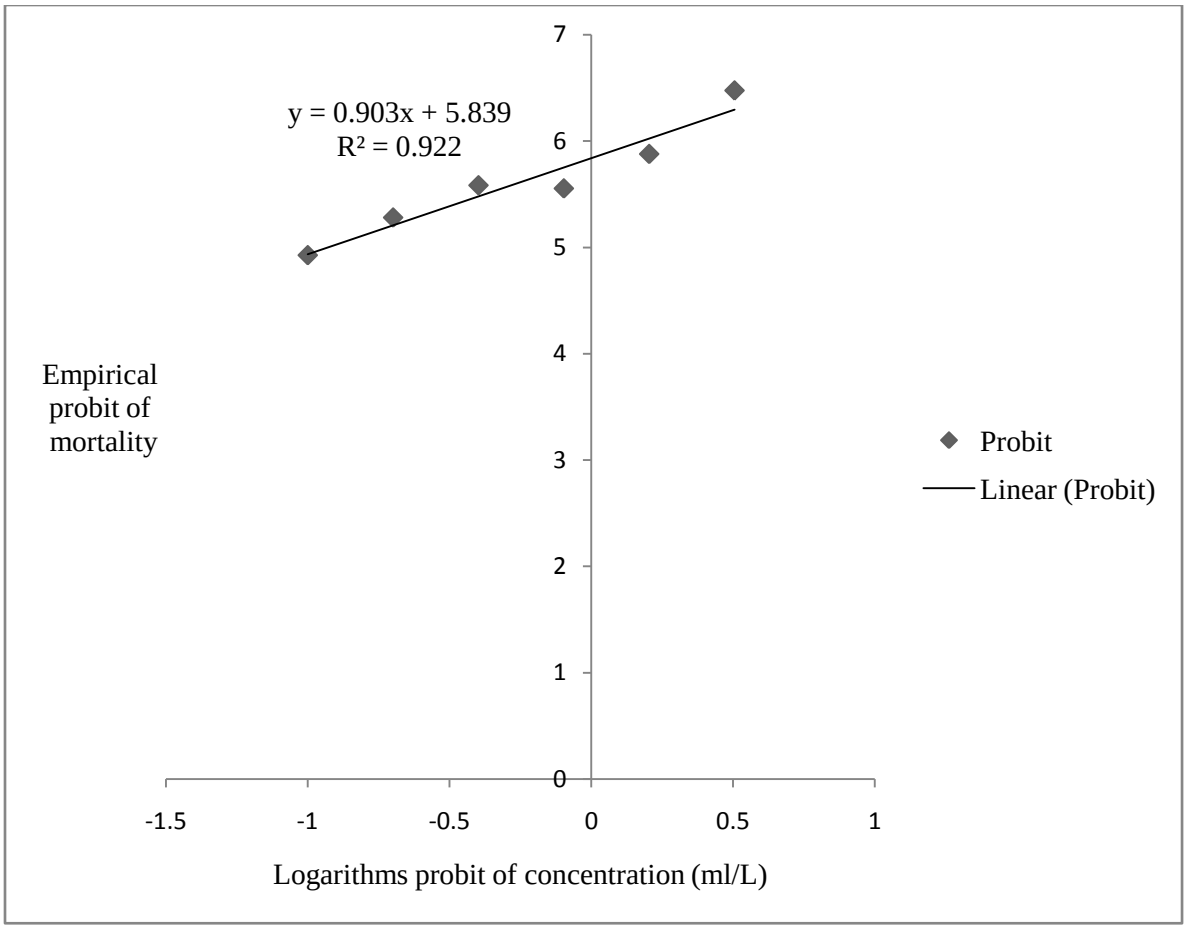

Figure 4.1: Logarithms of the concentrations of *Adansonia digitata* seed oil extractsagainst the empirical probit of mortality of *Aedes aegypti* larvae in the laboratory.

APPENDIXII: The Empirical Probit of Mortality Against Logarithm of Concentrations of *A. digitata* seed oil against *Ae. aegypti* in outdoor Condition.

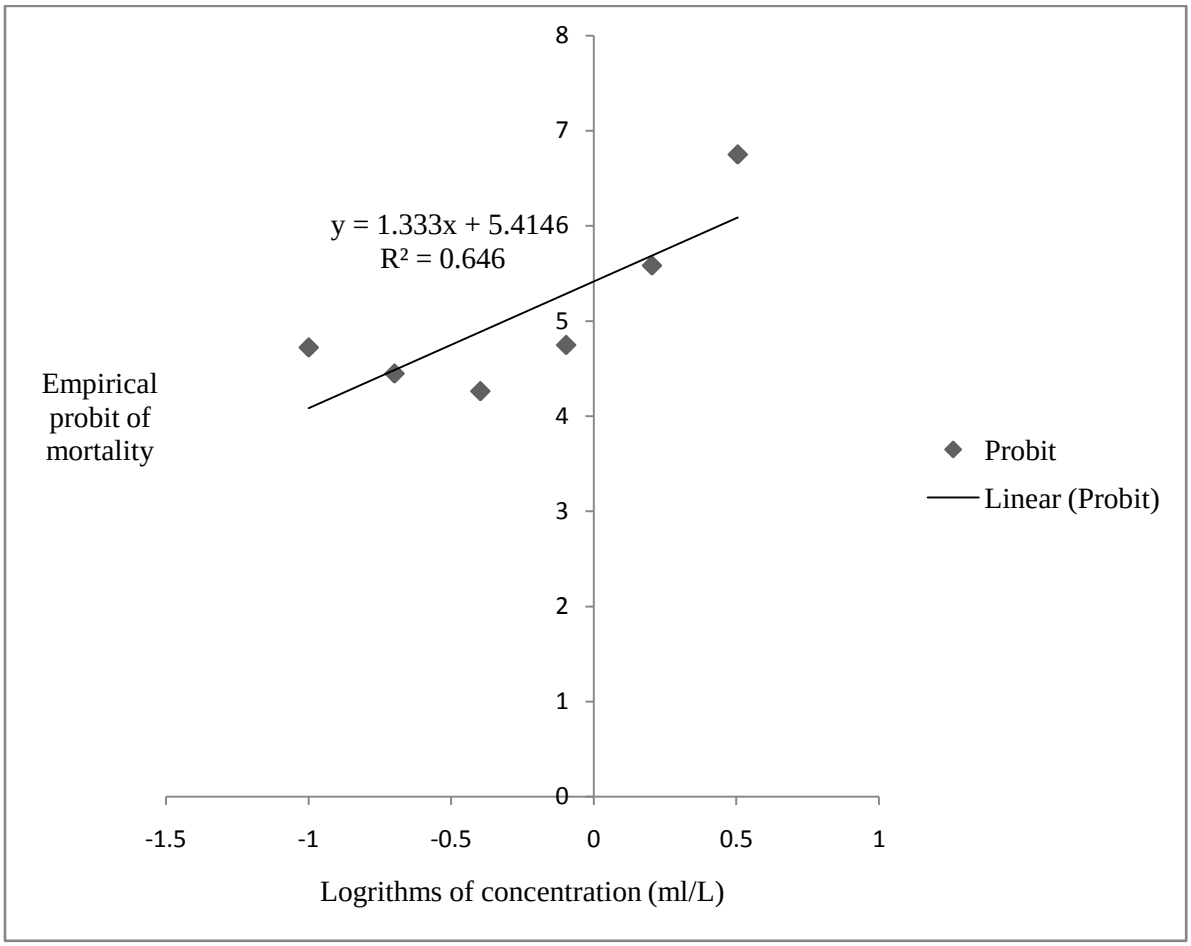

Figure 4.2: Logarithms of the concentrations of seed oil extractsof *Adansonia digitata* against the empirical probit of mortality of *Aedes aegypti* larvae in outdoor condition.

APPENDIX III: The Empirical Probit of Mortality Against Logarithm of Concentrations of *A. digitata* seed oil against *Ae.vittatus*larvae in Laboratory Condition.

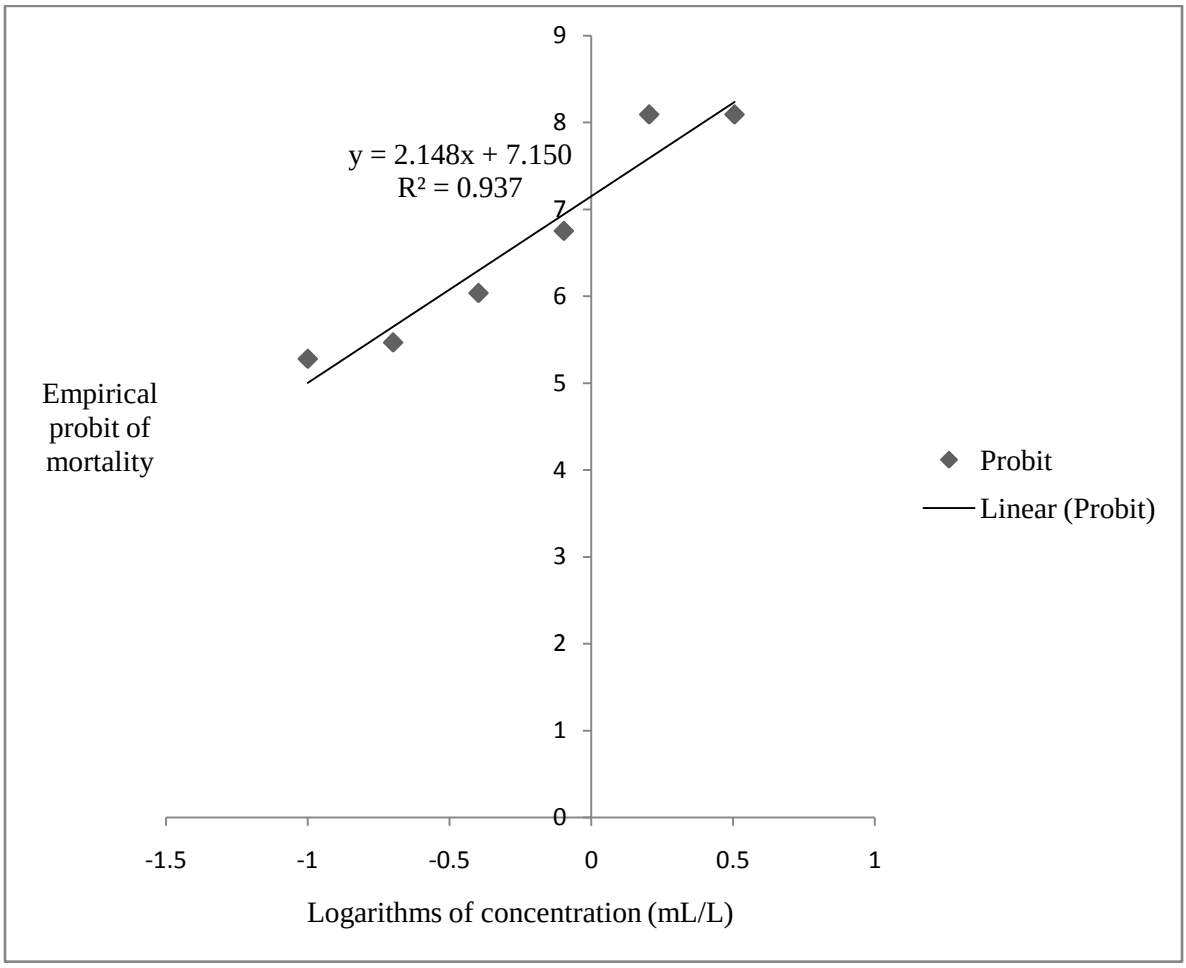

Figure 4.3: Logarithms of the concentrations of *Adansonia digitata* seed oil extracts against the empirical probit of mortality of *Aedes vittatus* larvae in laboratory

APPENDIX IV: The Empirical Probit of Mortality Against Logarithm of Concentrations of *A. digitata* seed oil against *Ae. vittatus*larvae in outdoor Condition.

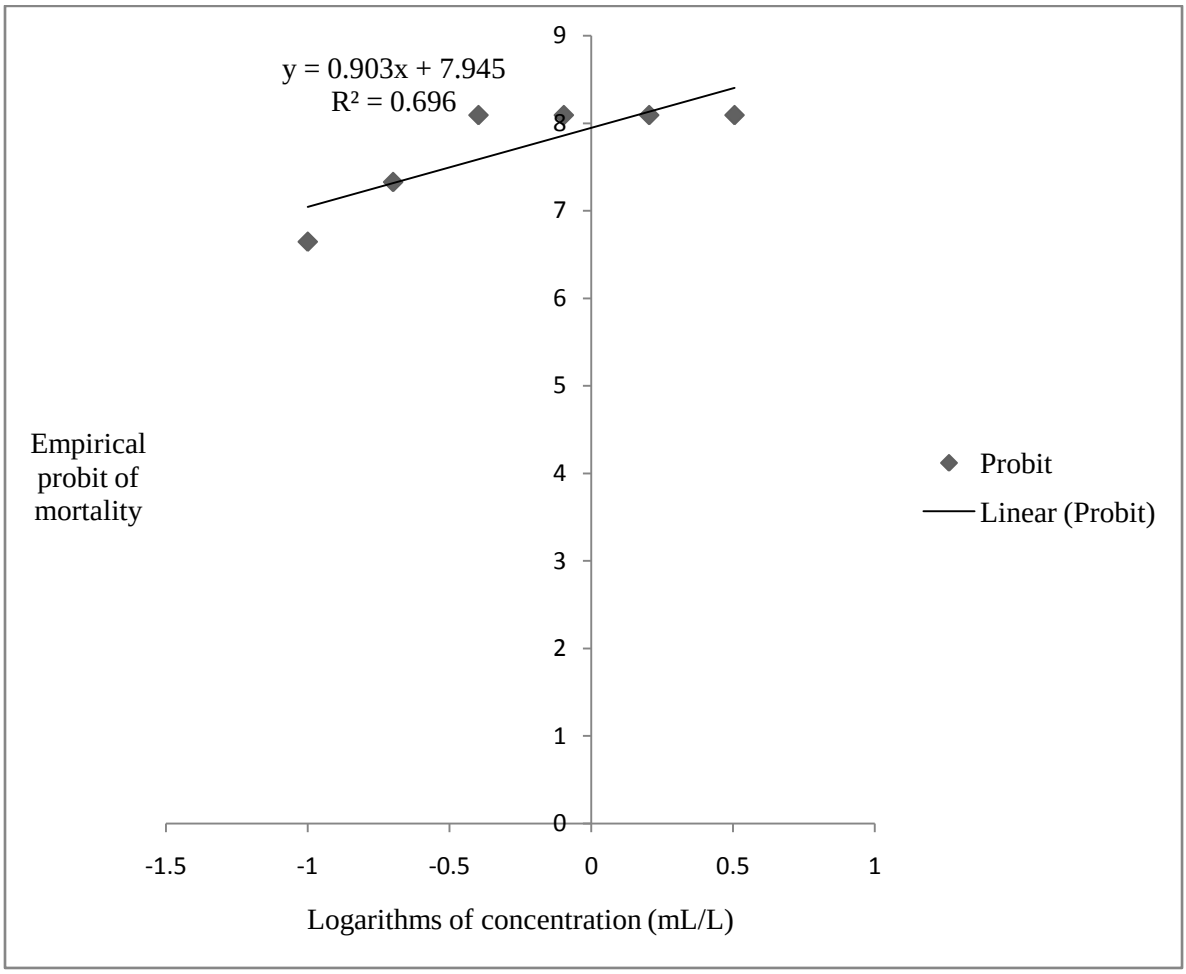

Figure 4.4: Logarithms of the concentrations of seed oil extractsof *Adansonia digitata* against the empirical probit of mortality of *Aedes vittatus* larvae inoutdoor condition.

APPENDIX V: The Empirical Probit of Mortality Against Logarithm of Concentrations of *A. digitata* seed oil against *Cx. quinquefasciatus* larvae in Laboratory Condition.

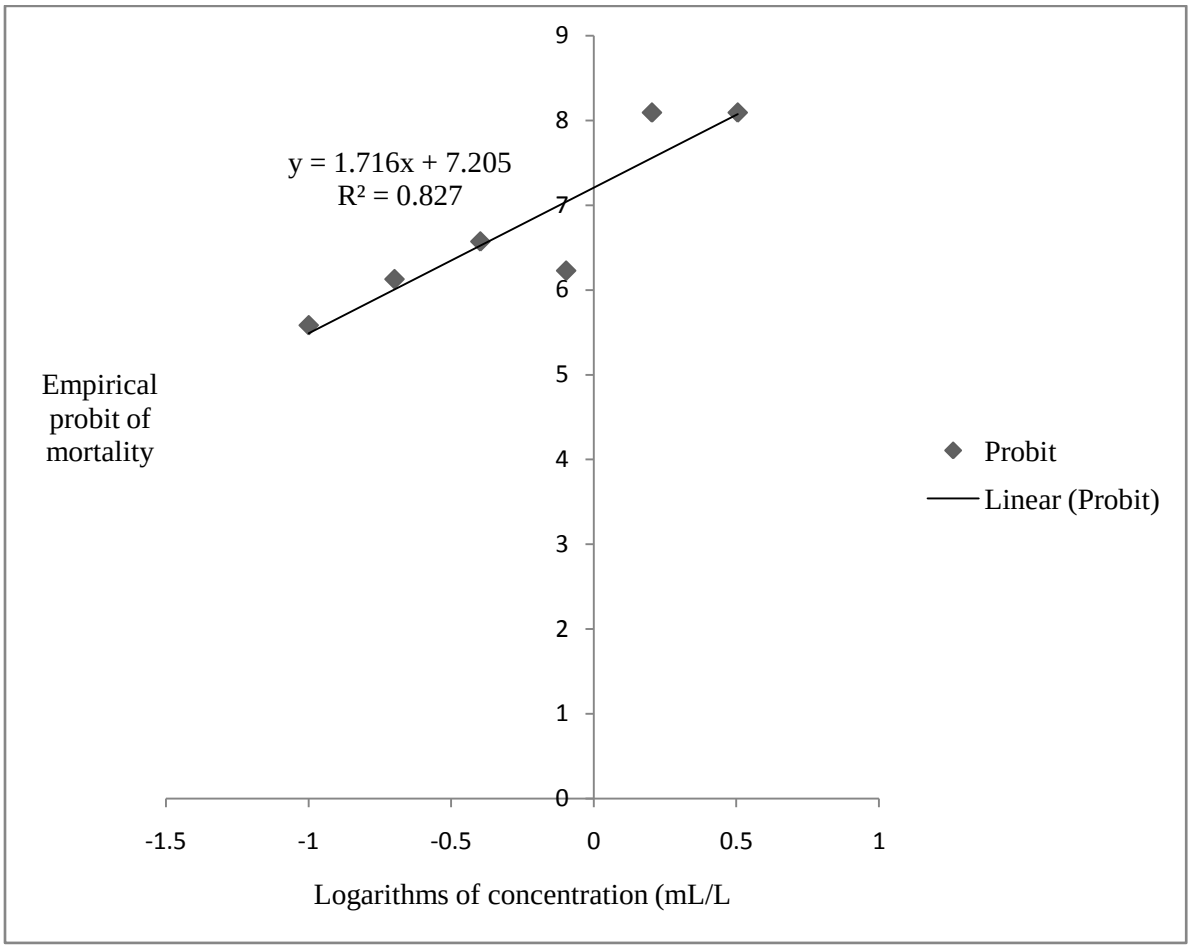

Figure 4.5: Logarithms of the concentrations of *Adansonia digitata* seed oil extractsagainst the empirical probit of mortality of *Culex quinquefasciatus* larvae in laboratory.

APPENDIXVI: The Empirical Probit of Mortality Against Logarithm of Concentrations of *A. digitata* seed oil against *Cx quinquefasciatus* larvae in Laboratory Condition.

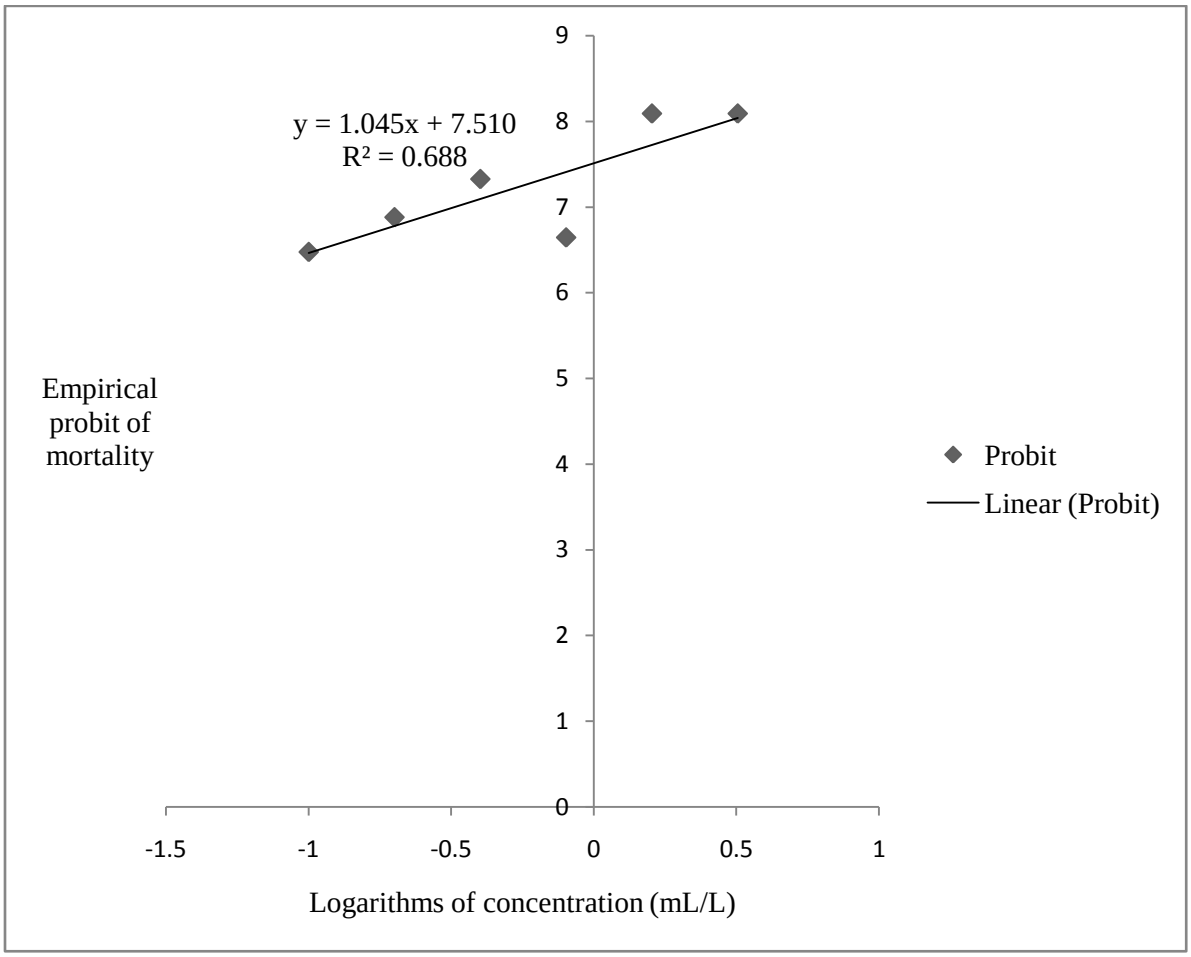

Figure 4.6: Logarithms of the concentrations of seed oil extractsof *Adansonia digitata* against the empirical probit of mortality of *Culex quinquefasciatus* larvae in outdoor condition.

APPENDIX VII: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. biglobosa* seed oil against *Ae. aegypti* larvae in Laboratory Condition.

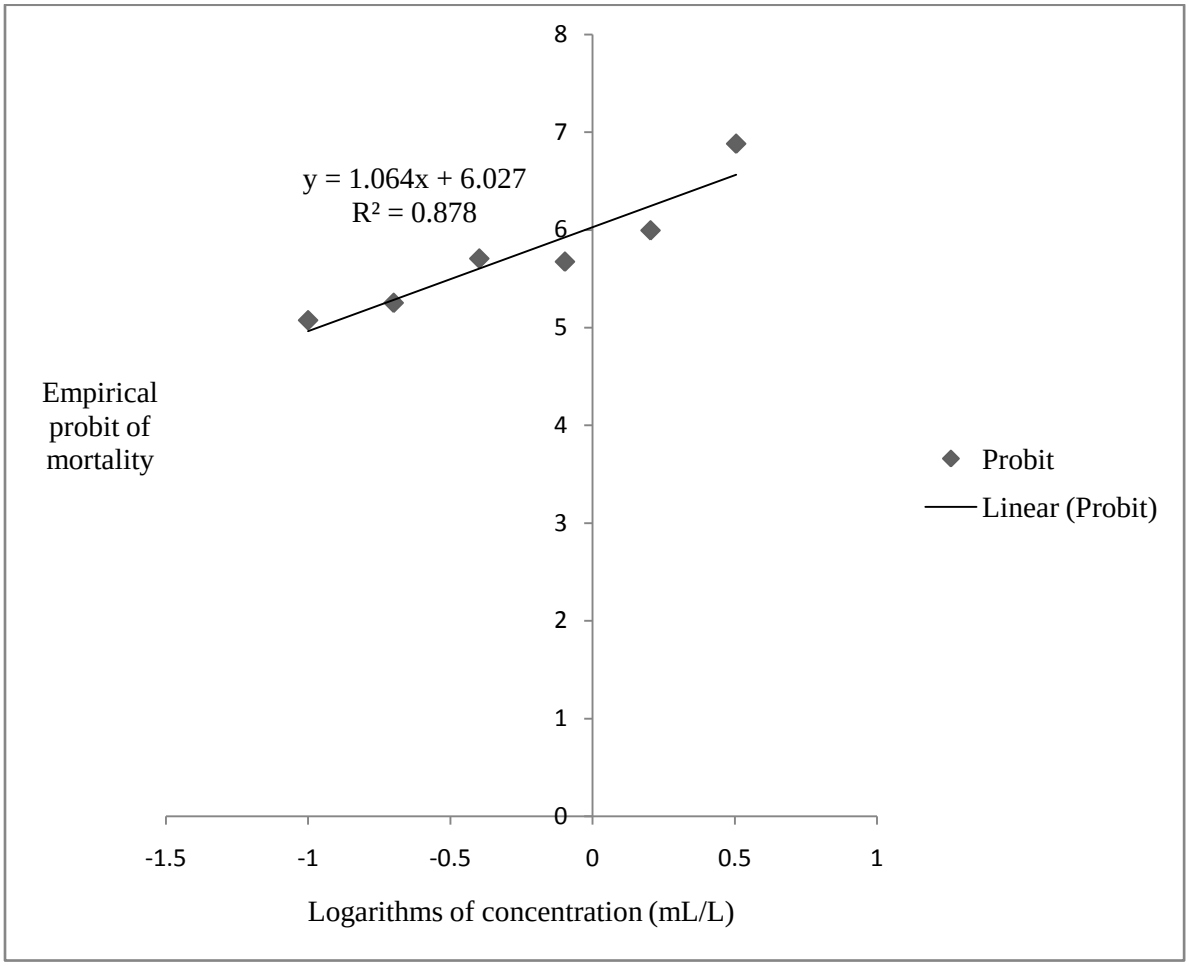

Figure 4.7: Logarithms of the concentrations of seed oil extractsof *Parkia biglobosa* against the empirical probit of mortality of *Aedes aegypti* larvae in laboratory.

APPENDIX VIII: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. biglobosa* seed oil against *Ae. aegypti* larvae in outdoor Condition.

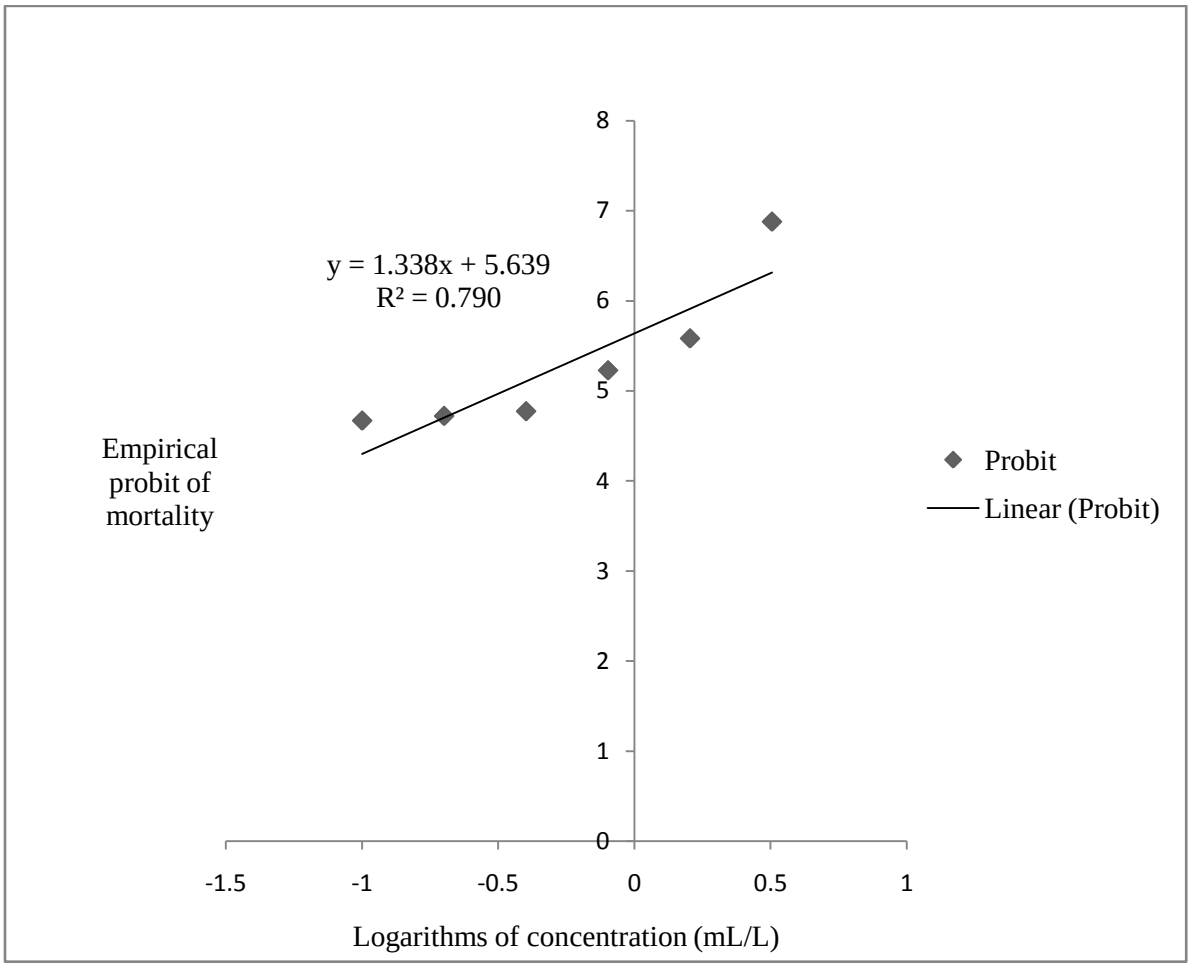

Figure 4.8: Logarithms of the concentrations of seed oil extractsof *Parkia biglobosa* against the empirical probit of mortality of *Aedse aegypti* larvae inoutdoor condition.

APPENDIXIX: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. biglobosa* seed oil against *Ae. vittatus*larvae in Laboratory Condition.

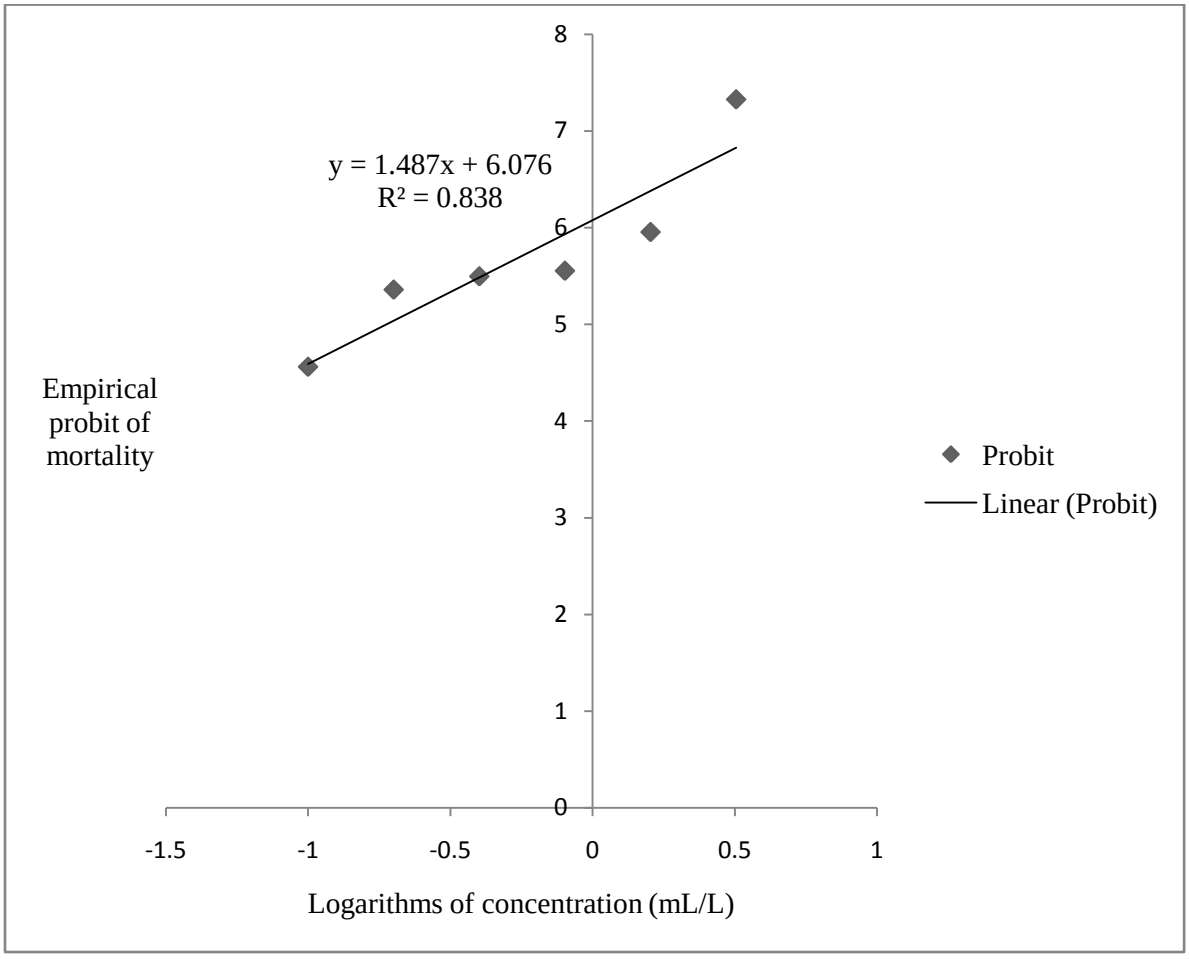

Figure 4.9: Logarithms of the concentrations of seed oil extractsof *Parkia biglobosa* against the empirical probit of mortality of *Aedes vittatus* larvae in laboratory.

APPENDIX X: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. biglobosa* seed oil against *Ae. vittatus*larvae in outdoor Condition.

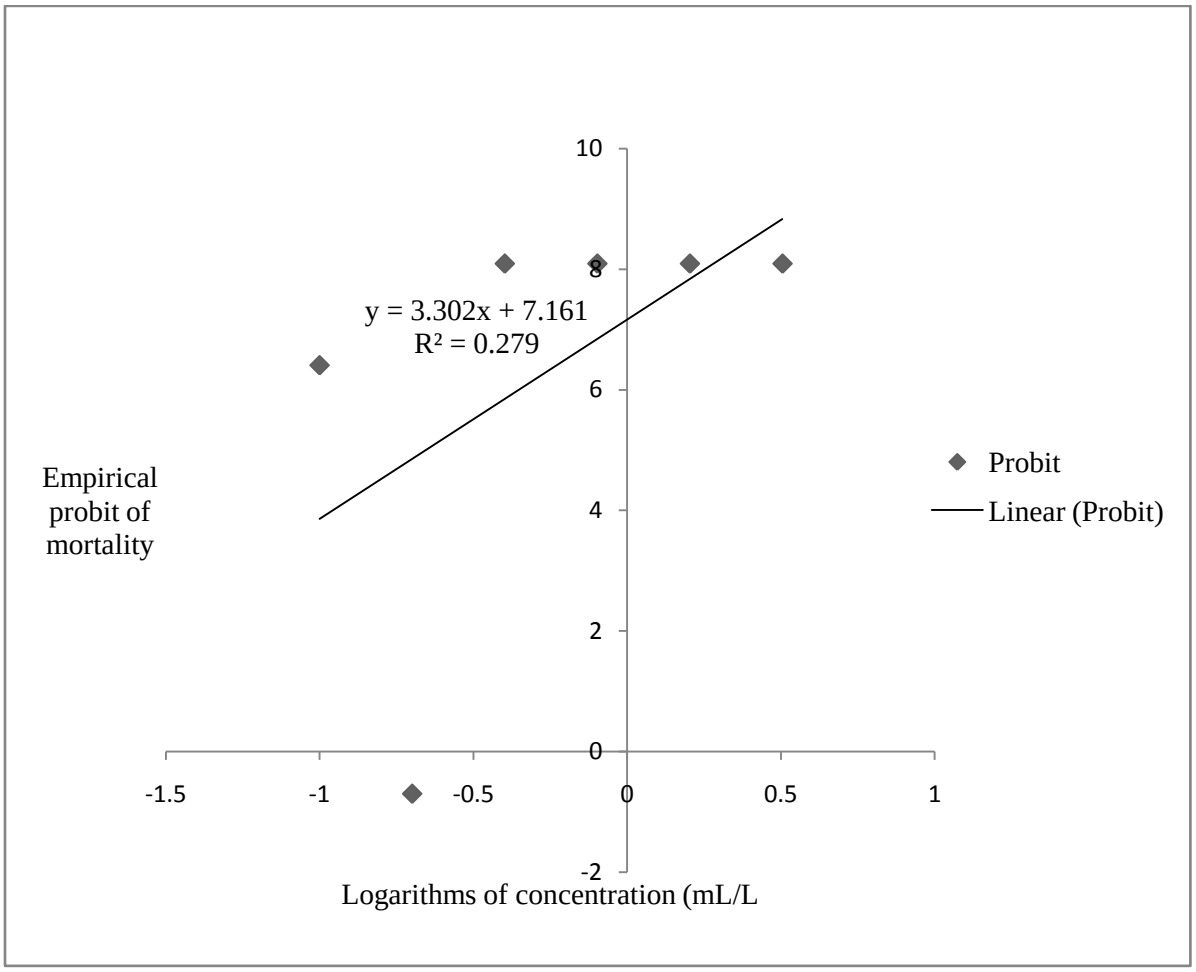

Figure 4.10: Logarithms of the concentrations of seed oil extractsof *Parkia biglobosa* against the empirical probit of mortality of *Aedes vittatus* larvae inoutdoor condition.

APPENDIX XI: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. biglobosa* seed oil against *Cx. quinquefasciatus* larvae in Laboratory Condition.

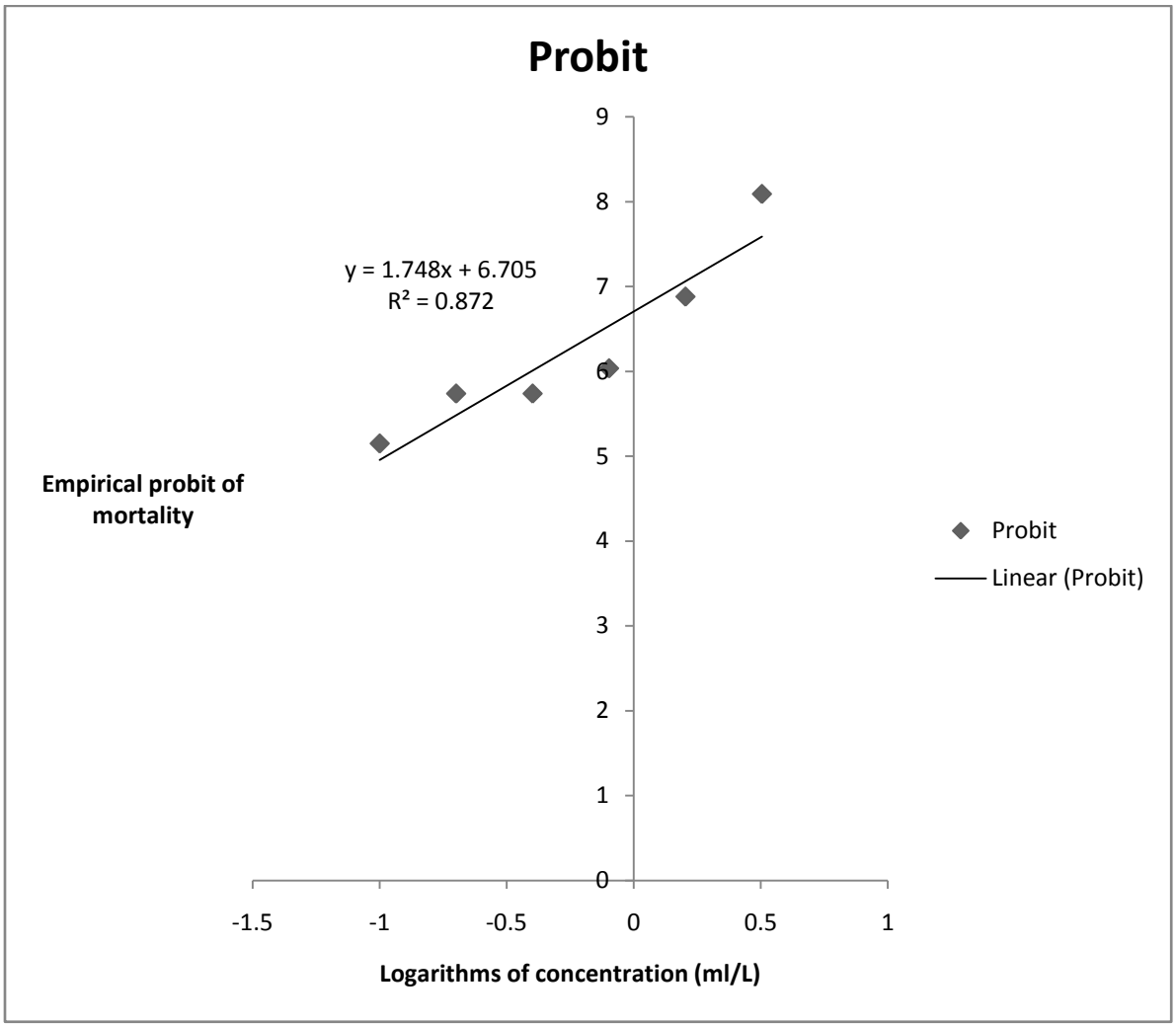

Figure 4.11: Logarithms of the concentrations of seed oil extractsof *Parkia biglobosa* against the empirical probit of mortality of *Culex quinquefasciatus* larvae in laboratory.

APPENDIX XII: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. biglobosa* seed oil against *Cx. quinquefasciatus* larvae in outdoor Condition.

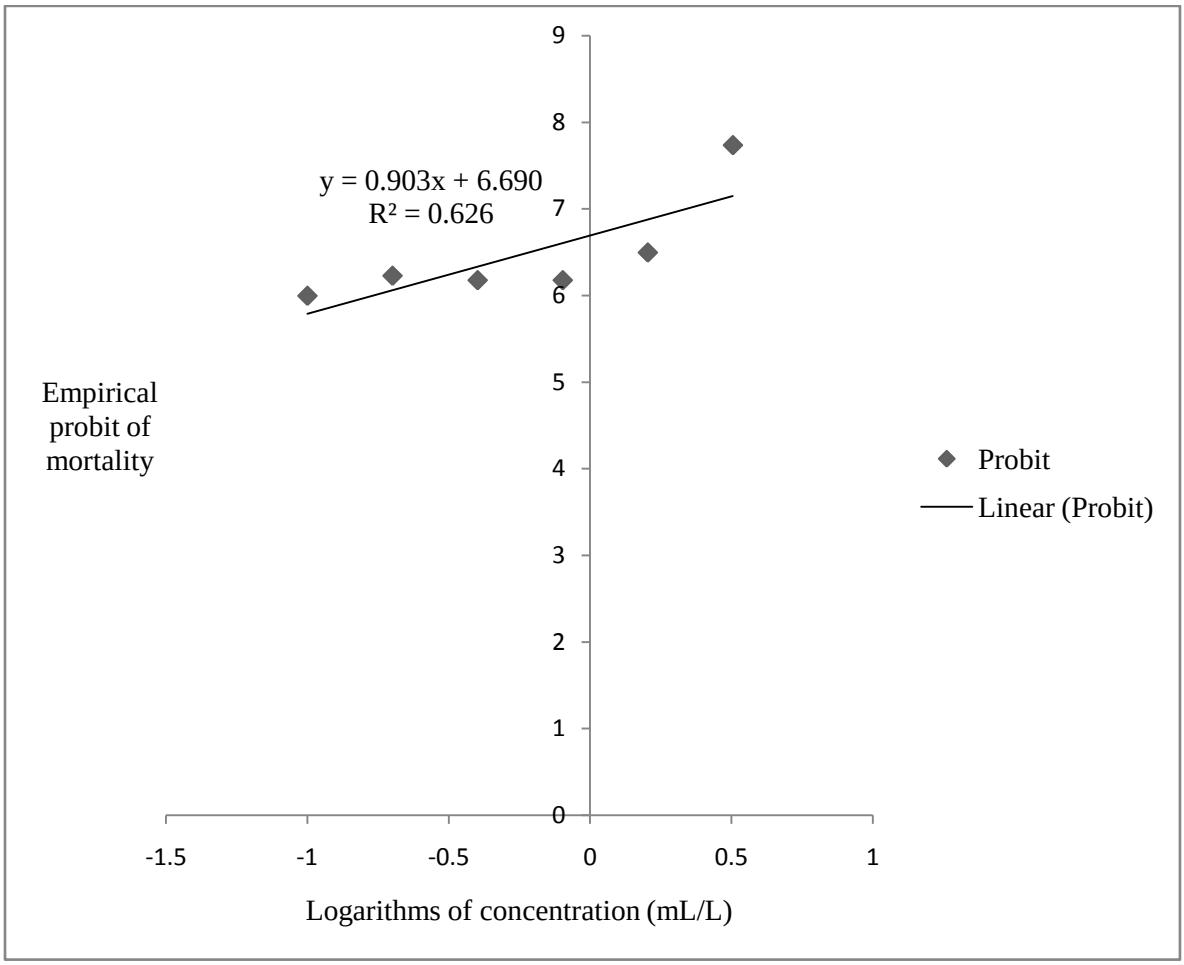

Figure 4.12: Logarithms of the concentrations of seed oil extractsof *Parkia biglobosa* against the empirical probit of mortality of *Culex quinquefasciatus* larvae inoutdoor condition.

APPENDIX XIII: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. americana* seed oil against *Ae. aegypti* larvae in Laboratory Condition.

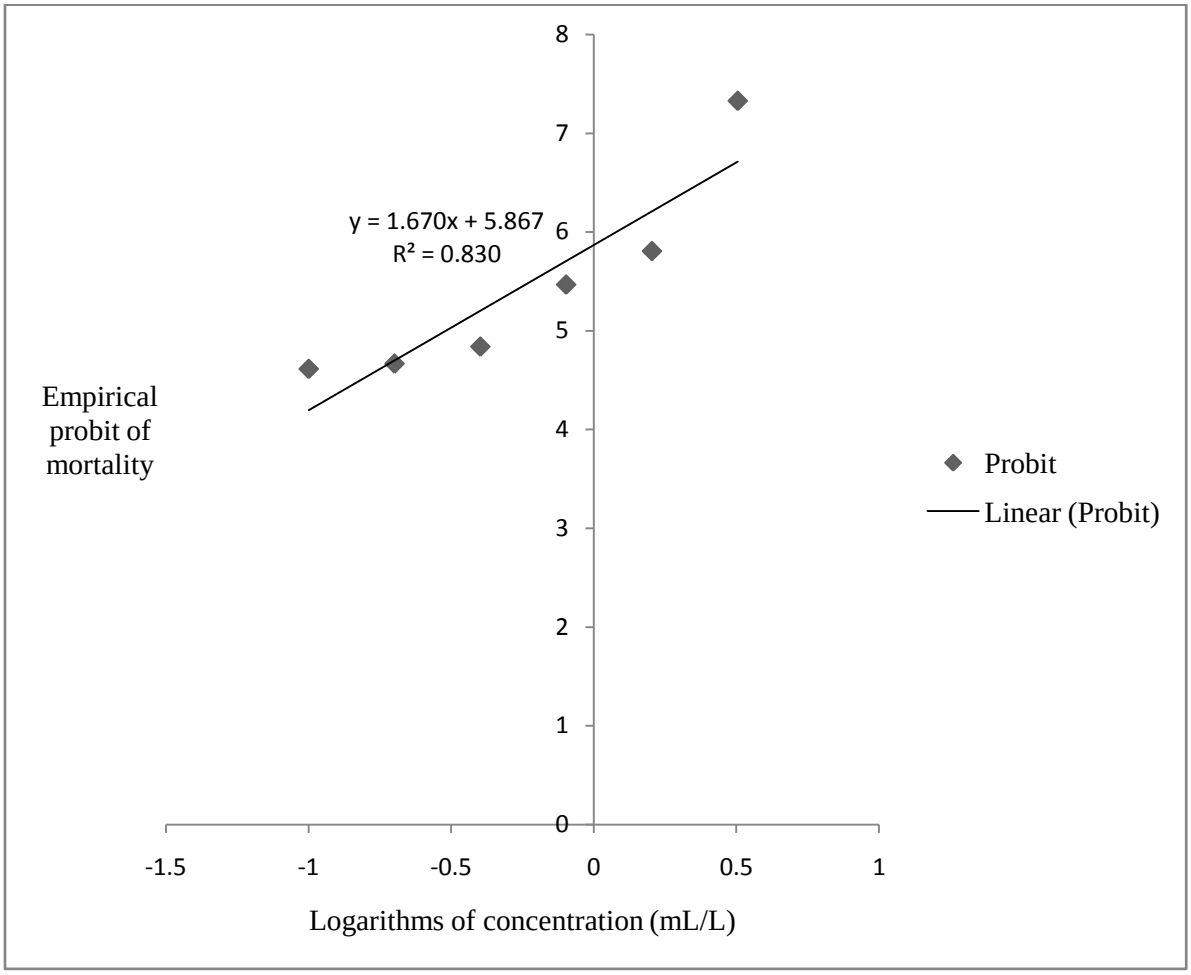

Figure 4.13: Logarithms of the concentrations of seed oil extractsof *Persea americana*against the empirical probit of mortality of *Aedes aegypti* larvae in laboratory.

APPENDIX XIV: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. americana* seed oil against *Ae. aegypti* larvae in outdoor Condition.

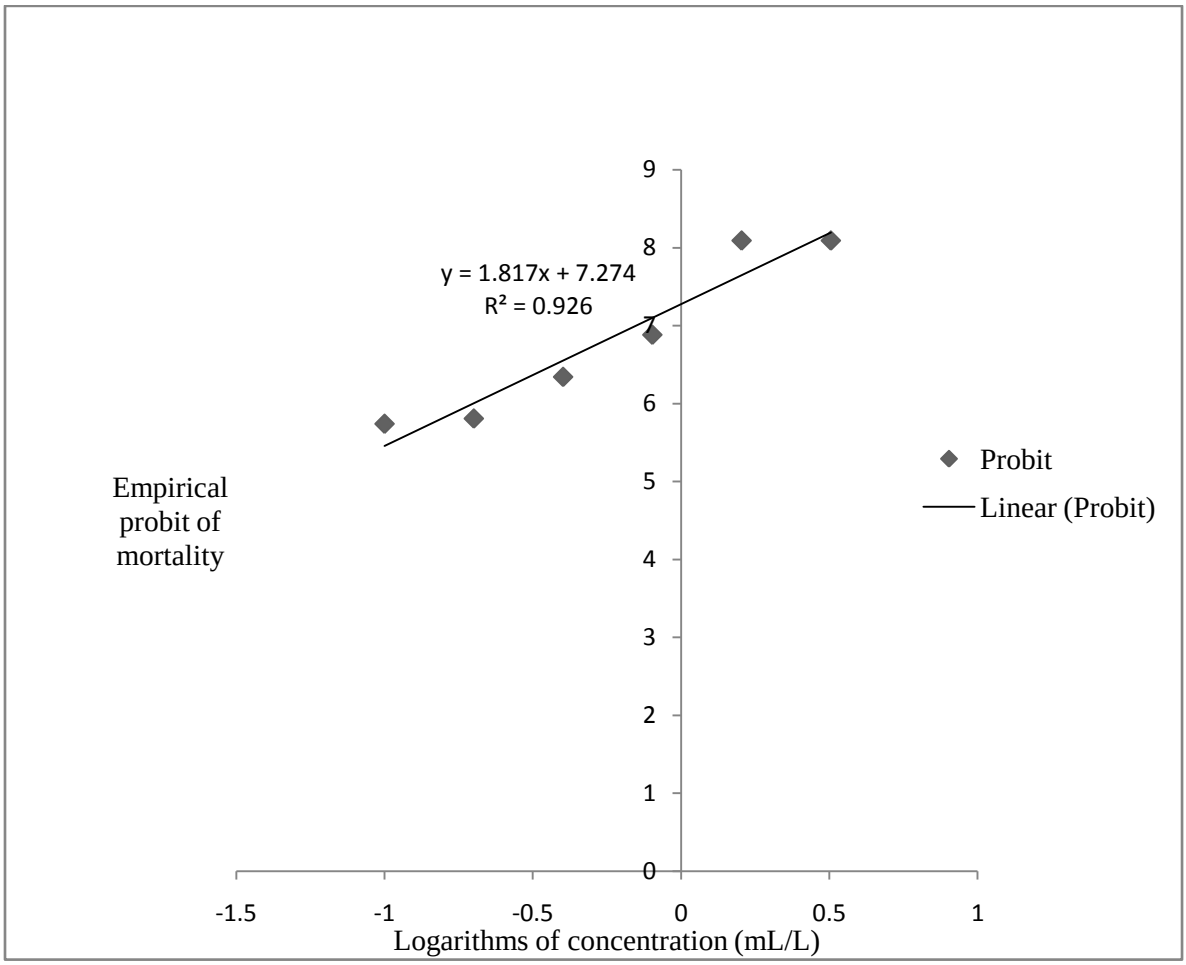

Figure 4.14: Logarithms of the concentrations of seed oil extractsof *Persea american*against the empirical probit of mortality of *Aedes aegypti* larvae inoutdoor condition.

APPENDIX XV: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. americana* seed oil against *Ae. vittatus*larvae in Laboratory Condition.

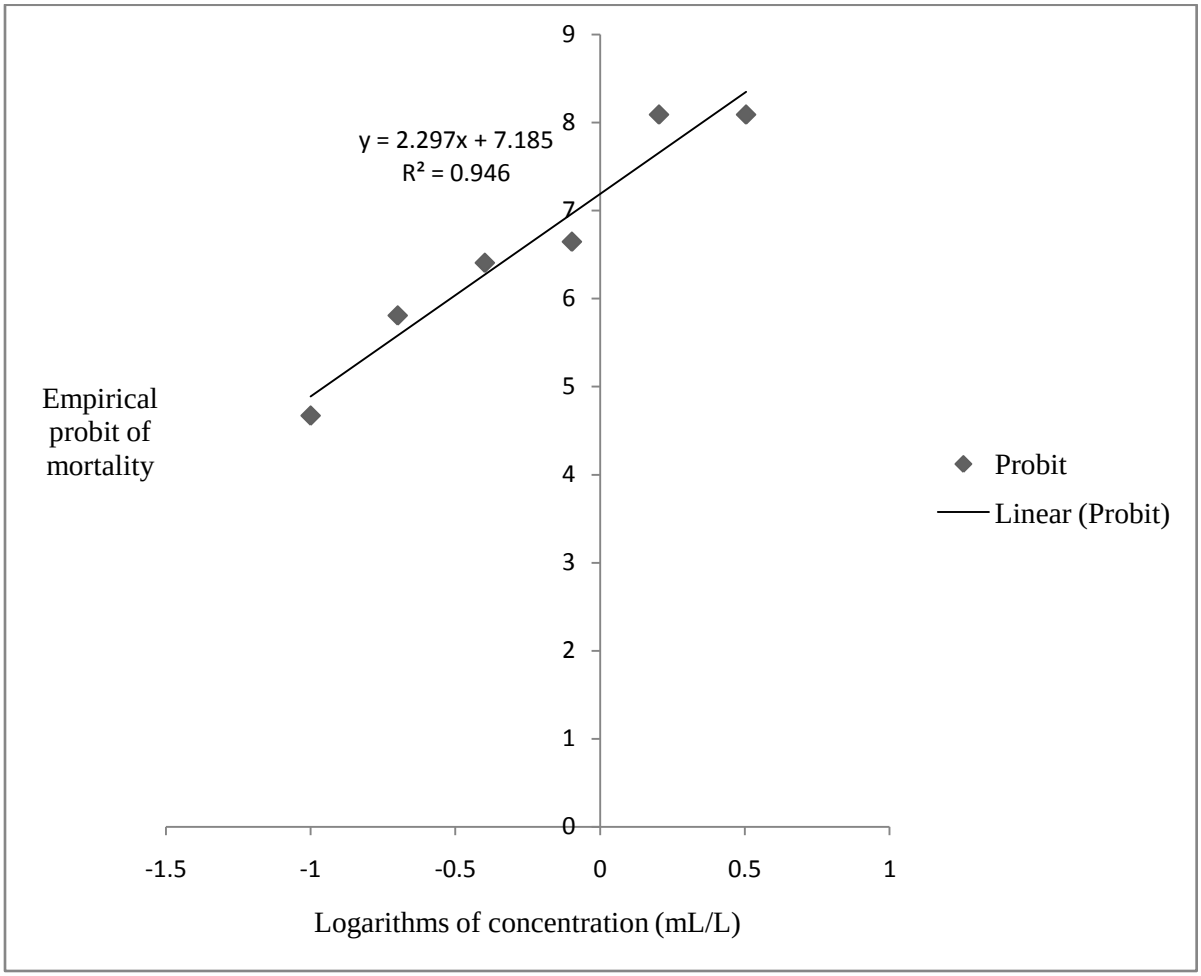

Figure 4.15: Logarithms of the concentrations of seed oil extractsof *Persea americana*against the empirical probit of mortality of *Aedes vittatus* larvae in laboratory.

APPENDIX XVI: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. americana* seed oil against *Ae. vittatus*larvae in outdoor Condition.

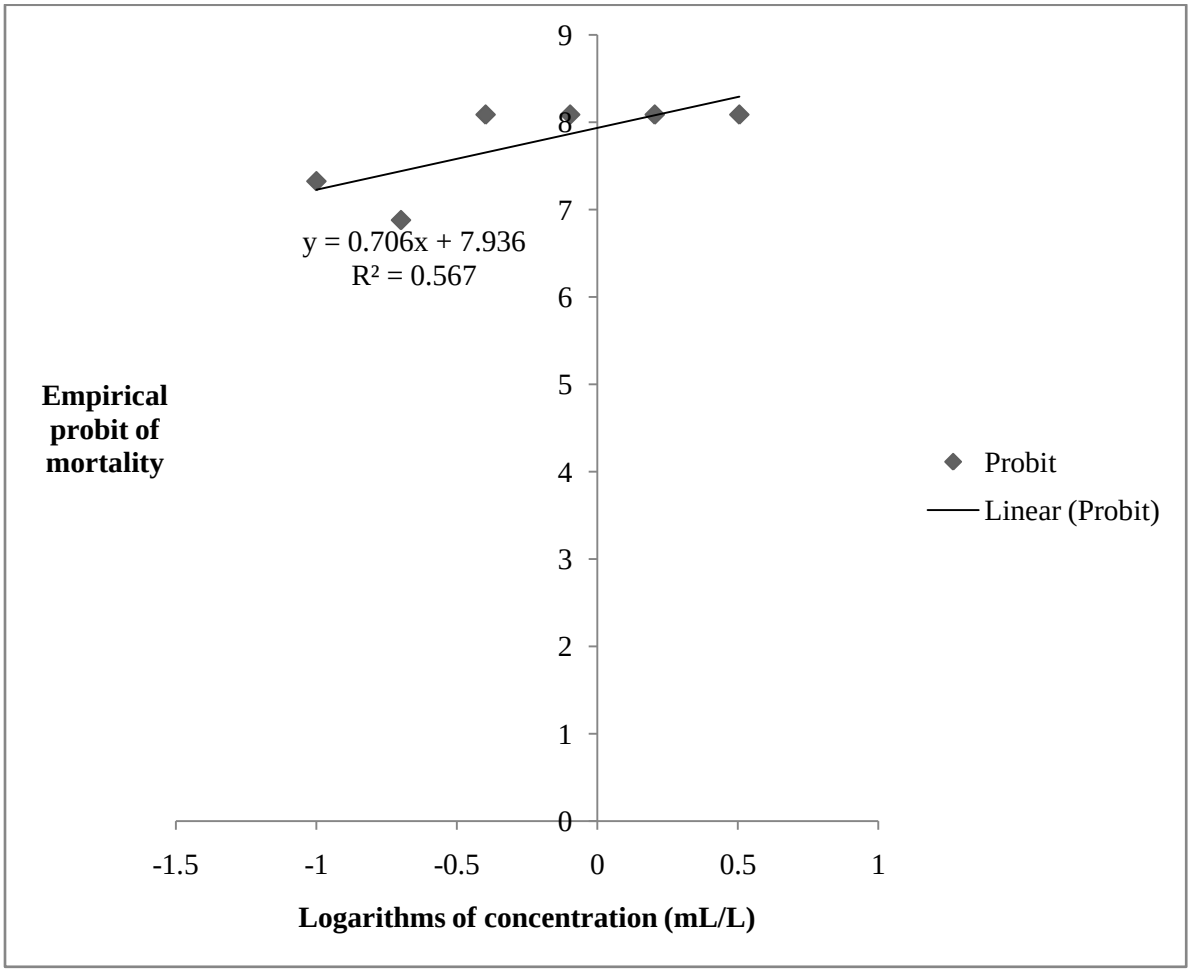

Figure 4.16: Logarithms of the concentrations of seed oil extractsof *Persea american*against the empirical probit of mortality of *Aedes vittatus* larvae inoutdoor condition.

APPENDIXXVII: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. americana* seed oil against *Cx. quinquefasciatus* larvae in Laboratory Condition.

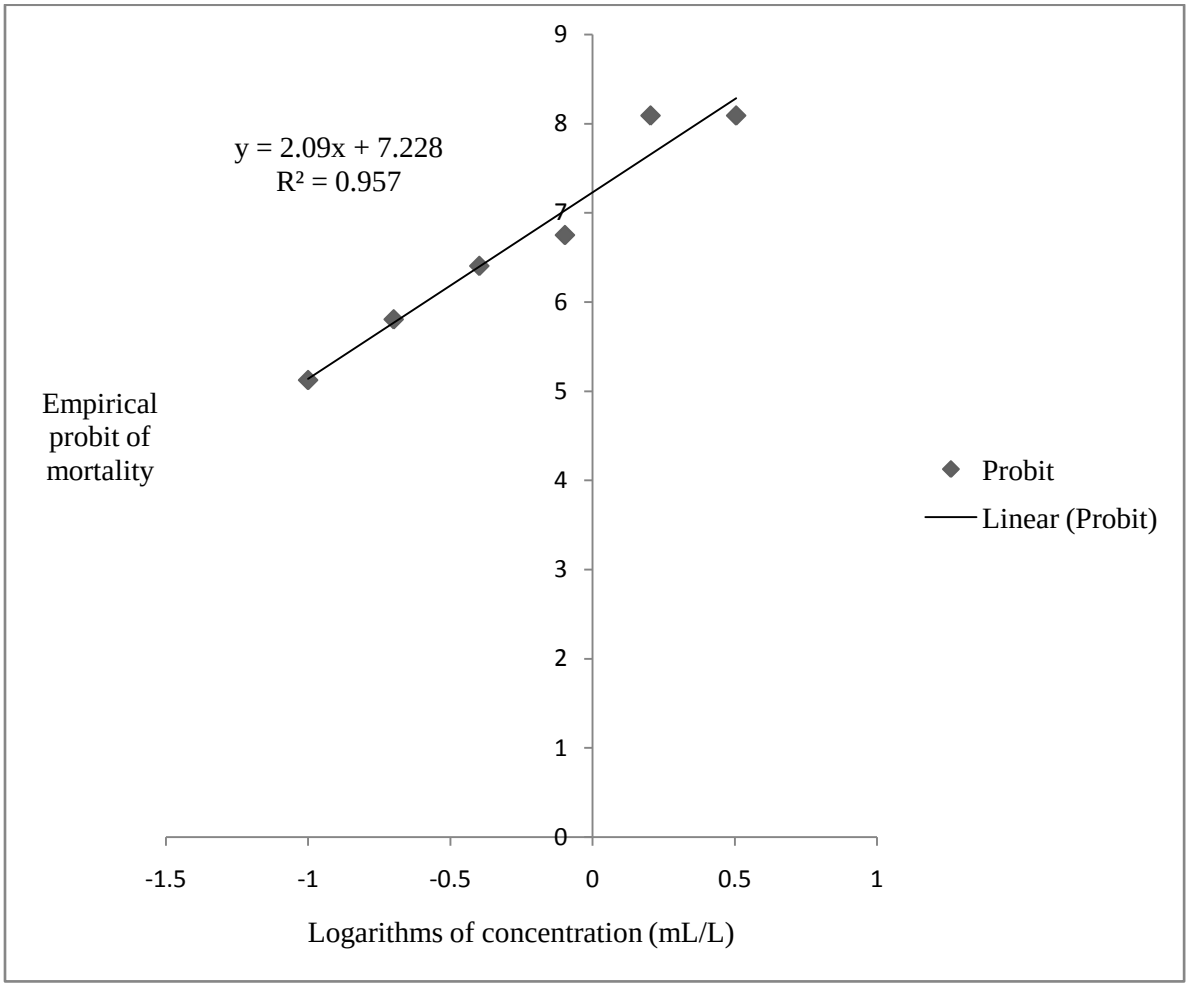

Figure 4.17: Logarithms of the concentrations of seed oil extractsof *Persea americana*against the empirical probit of mortality of *Culex quinquefasciatus* larvae in laboratory.

APPENDIX XVIII: The Empirical Probit of Mortality Against Logarithm of Concentrations of *P. americana* seed oil against *Cx. quinquefasciatus* larvae in outdoor Condition.

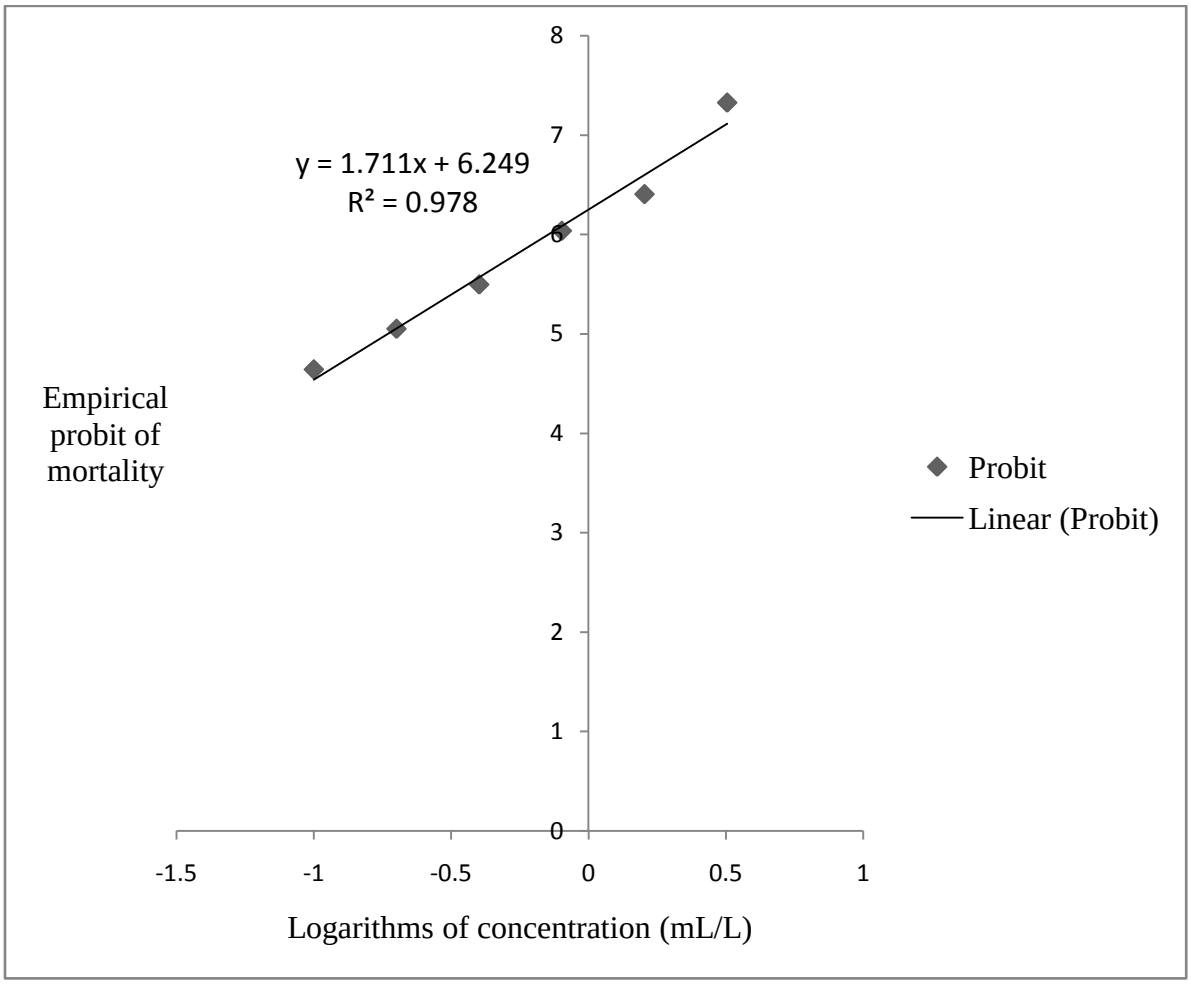

Figure 4.18: Logarithms of the concentrations of seed oil extractsof *Persea americana*against the empirical probit of mortality of *Culex quinquefasciatus* larvae in outdoor condition.