MOSQUITO LARVICIDAL PROSPECTS OF *TERMINALIA CATAPPA* (L.) AND *TAMARINDUS INDICA* (L.) SEED EXTRACTS IN LABORATORY AND FIELD BIOASSAYS

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

MARCH, 2016

DECLARATION

I declare this dissertation entitled "MOSQUITO LARVICIDAL PROSPECTS OF *TERMINALIA CATAPPA* (L.) AND *TAMARINDUS INDICA* (L.) SEED EXTRACTS IN LABORATORY AND FIELD BIOASSAYS**"** has been carried out by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The information extracted from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree in this or any other institution.

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CERTIFICATION

This dissertation entitled "MOSQUITO LARVICIDAL PROSPECTS OF *TERMINALIA CATAPPA* (L.) AND *TAMARINDUS INDICA* (L.) SEED EXTRACTS IN LABORATORY AND FIELD BIOASSAYS**"** by Kabadiya Diana DAWUDA meets the regulations governing the award of the degree Masters of Science in Biology by Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this dissertation to God Almighty, the Author, Protector and Guardian of my life, for every good and perfect gift I have ever and will ever receive.

ACKNOWLEDGEMENTS

My appreciation goes to God Almighty for His gifts of life, wellbeing and provision of resources during this study. I sincerely appreciate my supervisors, Prof. M. L. Balarabe and Mr. D. A. Adebote for their efforts, comments, time, advice, tolerance, guidance and constructive criticism to see that this study was completed successfully.

I thank my parents and siblings especially; Bakut, Koshi, John and Kasham for their encouragement, prayers and support to ensure the successful completion of this programme. I also thank my extended family members for their support directly and indirectly.

My appreciation also goes to my friends and coursemates especially Patrick, O., Badugu, E., Joseph, G., Ugochukwu, Kamba, B. and Gauji, B. for the help and support they rendered to me in plants and larval collection during this study. Finally, I also thank Mallam Kabir of the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences for the support shown to me during the extraction of the oils; and technical staff of National Research Institute of Chemical Technology (NARICT), Zaria for helping me during physicochemical, fatty acids and allied metabolites determination of the oils.

May God Almighty bless you all. Absolutely, there are no words that can sufficiently express my profound appreciation.

ABSTRACT

Phytochemical characteristics of petroleum ether extracted seeds of the Indian almond, *Terminalia catappa* (L.) (Combretaceae) and the tamarind, *Tamarindus indica* (L.) (Fabaceae) for their larvicidal prospects against third instar larvae of *Aedes aegypti*, *Aedes vittatus* and *Culex quinquefasciatus* species of mosquito were evaluated under laboratory and simulated field conditions. Dried seeds of both trees were pulverised and extracted with petroleum ether (60 - 80°C) in a Soxhlet apparatus. Physicochemical characteristics of the seed oils were determined using standard protocols. Gas Chromatography - Mass Spectroscopy (GC-MS) was used to characterise the phytochemicals contained in the oils. Distilled water diluted extracts of the seeds at separate triplicate treatment concentrations of 0mL/L (control), 0.5mL/L, 1mL/L, 2mL/L, 4mL/L and 8mL/L were each tested on 25 triplicates (n=75) of each larval species for 24 hours under both conditions. Larval mortality was determined thereafter and the data was subjected to ANOVA to test for treatment based significant differences in mean larval mortality, and to determine species specific median lethal concentrations (LC_{50}). Seed oils' acid values, percentage free fatty acids, peroxide values and refractive indices of 16.60mgKOH/g, 8.41mgKOH/g, 12.88meq/kg fat and 1.48 respectively in *T. indica* were significantly ($p < 0.05$) higher than the 10.66mgKOH/g, 5.33mgKOH/g, 9.27meq/kg fat and 1.46 respectively in *T. catappa.* However, the iodine value (39.35meq/kg fat) of *T. catappa* seed oil was significantly ($p < 0.05$) higher than the 16.18meq/kg fat value of *T indica* seed oil. There was no significant ($p > 0.05$) difference between the saponification values of 162.75mL/kg and 187.94mL/kg for *T. catappa* and *T. indica* seed oils respectively. Sixteen metabolites of mainly saturated and unsaturated fatty acids

including pentadecanoic acid (8.57%), linolelaidic acid (15.30%), elaidic acid (9.15%), stearic acid (5.09%), ricinoleic acid (1.52%), eicosenoic acid (2.83%), eicosanoic acid (2.82%) and heneicosanoic acid (8.77%) were detected in the seed oil of *T. indica*. Fourteen metabolites of mainly saturated and unsaturated fatty acids including hexadecanoic (palmitic) acid (13.36%), linolelaidic acid (9.97%), oleic acid (11.02%), stearic acid (5.83%) and eicosanoic (arachidic) acid (0.66%) were detected in the seed oil of *T. catappa*. *Terminalia. catappa* seed oil caused significantly ($p < 0.05$) highest larval *Ae. aegypti* mortality of 81% and 55% and LC_{50} values of 4.840mL/L and 10.884mL/L; highest larval *Ae. vittatus* mortality of 53% and 45% and LC_{50} of 11.143mL/L and 9.099mL/L; highest larval *Cx. quinquefasciatus* mortality of 91% and 80% and LC_{50} of 2.275mL/L and 3.055mL/L, under laboratory and simulated field conditions respectively. *Tamarindus indica* seed oil caused significantly ($p < 0.05$) highest larval *Ae. aegypti* mortality of 99% and 95% and LC_{50} values of 1.248mL/L and 1.359mL/L; highest larval *Ae. vittatus* mortality of 60% and 76% and LC_{50} of 4.842mL/L and 1.191mL/L; highest larval *Cx. quinquefasciatus* mortality of 95% and 100% and LC_{50} of 0.690mL/L and 0.625mL/L, under laboratory and simulated field conditions respectively. *Tamarindus. indica* extract was a better larvicidal agent against the three mosquito species than *T. catappa*. Seed extracts of both trees could thus be adopted in mosquito control operations.

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LIST OF ABBREVIATION

WHO - World Health Organization

cm - centimeter

m - meter

DDT - dichloro diphenyl trichloroethane

LC_{50} - lethal concentration of the seed extracts at 50%

ml/m^2 - mills per square meter

mL/L - mills per litre

ppm - parts per millions

^oC - Degrees centigrade

g - Grams

M - Moles

N - North

E - East

CI - Confidence interval

SE - Standard error

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background of the Study

Mosquitoes are the most important single group of insects well-known for their public health importance. Since they act as vectors for many tropical and subtropical diseases, they cause nuisance by their bites and also transmit deadly diseases like malaria, filariasis, yellow fever, dengue and Japanese encephalitis, which contribute significantly to poverty and social debility in tropical countries (Jang *et al*., 2002; Tiwary *et al*., 2007). They can transmit more diseases than any group of arthropods and affect millions of people throughout the world. World Health Organization (WHO) has declared mosquitoes as "public enemy number one" (WHO, 1996).

Aedes species are important vectors of yellow fever, dengue, encephalitis viruses and many other arboviruses, and in a few restricted areas they are also vectors of *Wuchereria bancrofti* and *Brugia malayi*, (Kandaswamy *et al*., 2012). *Aedes aegypti* is the primary vector of dengue and chikungunya. *Aedes vitattus* is a vector of chikungunya and yellow fever (Misvar *et al*., 2014).

Culex quinquefasciatus (Say), commonly known as the southern house mosquito is a medium-sized brown mosquito that exists throughout the tropics and the lower latitudes of temperate regions. *Culex quinquefasciatus* is the primary vector of St. Louis encephalitis virus and also transmits West Nile virus (Hill and Connelly, 2013). Despite progress in vaccine development, no effective and acceptable multivalent vaccines are currently available against mosquito-borne diseases (Klempner *et al*., 2007).

Owing to poor drainage system, the presence of many abandon fish ponds, irrigation ditches, rock pools and rice fields, abundant mosquito breeding places are available. According to Singha *et al*. (2011), to reduce the incidence of different mosquito borne diseases, regulation of mosquito population is very important. The approach for control of these mosquito-borne diseases largely relies on the interruption of disease transmission cycle by preventing mosquitoes from biting humans using insecticides and causing larval mortality in a large scale at breeding sites using larvicides (Rajmohan and Ramaswamy, 2007). Proper control of mosquitoes is based on personal protection and public awareness as the most economical method in eradicating breeding sites through environmentally friendly larvicides (Corbel *et al*., 2004).

Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides-organophosphates and insect growth regulators. Although effective, their repeated use has disrupted natural biological control systems and sometimes resulting in undesirable effects on non-target organisms (Tiwary *et al*., 2007). This also fostered environmental and human health concern that initiates a search for alternative control measures (Prabhu *et al*., 2011).

Many plants produce secondary components that have insect growth inhibitory activities. Besides the use of such compounds as agricultural insect pest control agents, their use in mosquito larvae control is an interesting perspective (Markouk *et al*., 2000). Plant derived products are being investigated for their larvicidal prospects to the extent that more than 2000 plant species are already known to have insecticidal properties (Nazar *et al*., 2009; Pankaj and Anita, 2010; Kamaraj *et al*., 2011; Rajasekaran and Duraikannan, 2012).

Terminalia catappa (L.) belongs to the family Combretaceae and is widely grown in tropical regions of the world as an ornamental tree for the shade its large leaves provide. It is commonly known as 'tropical almond'. The fruit is edible and turns pinkish-reddish or yellow-brown on ripen before falling to the ground. Fruit is almond-shaped, green, turning brown with fibrous shell surrounding an edible nut. The seeds are nutritive, sweet, astringent and edible (Unnikrishnan, 2014).

Tamarindus indica (Linn.) belongs to the family Fabaceae and almost every of its parts can be used. It is indigenous to tropical Africa, north and south America and is also cultivated in subtropical China, Pakistan, Java and Spain, (Alkofahi and Atta, 1999). Its fruit is dessert, often eaten directly from the pod but its seeds are very hard and not edible. It is commonly known as 'tamarind' (Yusue *et al*., 2007)

1.2 Statement of Research Problem

Diseases such as malaria, yellow fever and dengue which are responsible for millions of cases of sickness and death among people living in developing countries of the tropical regions continue to be of great concern to health authorities. Efforts to control mosquito transmitted diseases have primarily been concentrated on the use of synthetic insecticides. Unfortunately, these have resulted in the disruption of natural biological control systems, appearance of physiological mosquito resistance, toxicity problems to humans resulting in immune dysfunction, cancer and birth defects; environmental contamination, ecological imbalance and economic burden (Nigam and Venkatakrishna, 2001; Vahitha *et al*., 2002; Dua *et al*., 2009). These problems have created the need to look for alternative, environmentally friendly control measures based on those found in nature. Many plants have been found to contain chemicals which are helpful for the

control of insects (Markouk *et al*., 2000). These include the essential oils from plants, some of which have been used for years by people for medicinal purposes without knowing their active components (Vargas, 2012). These have received much attention as potentially useful bioactive compounds against insects, due to their low mammalian toxicity and rapid degradation in the environment (Cheng *et al*., 2003). However, the mosquitocidal prospects of several plant species remain largely unexplored creating large gaps in knowledge and applicability.

1.3 Justification

Plants offer an alternative source of insect control agents because they contain a range of bioactive chemicals many of which are selective and have little or no harmful effect on non-target organism and the environment (Hedlin *et al*., 1997). Plants are nature's chemical factories providing rich source of organic chemicals on earth (Agbofor and Nwachukwu, 2011). According to WHO, more than 80% of the world's population relies on herbal therapy of traditional medicine for primary health care needs due to the broader degree of chemical diversity and novelty found than any other source (Sukanya *et al*., 2009). Botanical phytochemicals and essential oils with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal and adulticidal properties (Samidurai, 2012).

Currently, plant-based essential oils have received renewed attention as potent bioactive compounds against various species of mosquitoes (El-Hag *et al*., 2001) having low mammalian toxicity and degrading rapidly in the environment (Tiwary *et al*., 2007). Larval control has been identified as one of the most effective methods for the control of

mosquito-borne diseases (Singh *et al*., 2006). The control of mosquito at the immature stage is necessary and efficient in integrated mosquito management because during the immature stages, mosquitoes are relatively immobile (Elimam *et al.*, 2009).

Indeed, source reduction by targeting the larval stage of mosquitoes with potent larvicides is one of the most appropriate mosquito control strategy, as the target is exceptionally specific unlike adult control, (Amer and Mehlkorn, 2006). Many plant-based products are widely used for their insecticidal/repellent properties for control of mosquitoes/protection from mosquito bites (Sukumar *et al*., 1991). This study has the potential of promoting new agents for mosquito control from indigenous plant sources.

1.4 Aim of the Study

The aim of this study was to evaluate mosquito larvicidal prospects of the seed extracts of *Terminalia catappa* and *Tamarindus indica* in laboratory and field bioassays.

1.5 Research Objectives

I. To identify the physicochemical characteristics of *T. catappa* and *T. indica* seed extracts.

II. To determine and compare the larvicidal prospects of the seed extracts of *T. catappa* and *T. indica* against *Aedes aegypti, Aedes vittatus* and *Culex quinquefasciatus* third instar larvae in laboratory and field bioassays.

1.6 Research Hypotheses

I. Seed extracts of *T. catappa* and *T. indica* have no physicochemical characteristics.

II. Seed extracts of *T. catappa* and *T. indica* have neither larvicidal prospects nor significant differences in toxicities against *Aedes aegypti, Aedes vittatus* and *Culex quinquefasciatus* in laboratory and field bioassays.

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 Classification of Mosquitoes

Kingdom: Animalia; Phylum: Arthropoda; Class: Insecta; Order: Diptera; Suborder: Nematocera; Infraorder: Culicomorpha; Superfamily: Culicoidea; Family: Culicidae; Subfamilies: Anophelinae, Culicinae and Toxorhynchitinae.

Mosquitoes belongs to the family Culicidae, order Diptera (the two-winged flies). The family is a large and abundant group which occurs throughout temperate and tropical regions of the world, and well beyond the Arctic Circle. The family includes 3,540 species, classified in three subfamilies and 112 genera. The subfamily Anophelinae has three genera and Culicinae has 109 genera. Several species belonging to genera *Aedes*, *Anopheles* and *Culex* are vectors. The most common, and most dangerous, are the various species in the *Culex, Anopheles*, and *Aedes* genera (Darsie-Jr *et al*., 2002; Tennyson *et al*., 2012).

2.2 Characteristics of Mosquitoes

Adult mosquitoes are slender, long-legged insects that are easily recognised by their long proboscis and the presence of scales on most parts of their bodies. Their larvae are distinguished from other aquatic insects by the absence of legs, the presence of a distinct head bearing mouth brushes and antennae, a bulbous thorax that is wider than the head and abdomen, posterior anal papillae and either a pair of respiratory openings (subfamily Anophelinae) or an elongated siphon (subfamily Culicinae) borne near the end of the abdomen. Mosquitoes are usually and most reliably identified at third and fourth instars

larvae; and adults. In Europe, mosquitoes were called "gnats" by the English, meaning ‘the true flies’. Female mosquitoes' mouthparts form a long piercing-sucking proboscis. Males differ from females by having feathery antennae and mouthparts not suitable for piercing skin (American Mosquito Control Association, 2014). Male and female adult mosquitoes feed on nectar and plant juices but only females feed on blood because a blood meal is usually required for development of eggs. Females can feed on the blood of amphibians, birds, reptiles and mammals - including humans (Oleg, 2007).

2.3 Life Cycle of Mosquitoes

Life cycle of mosquitoes takes place in four distinct stages, from egg stage through to adult stage. According to Centers for Disease Control and Prevention (CDC) the entire life cycle of mosquitoes lasts approximately 8-10 days at room temperature, depending on the level of their feeding. There is an aquatic phase (exhibited by the eggs, larvae and pupae) and a terrestrial phase (exhibited by the adults) in the life-cycle.

Eggs: Female mosquitoes lay eggs about every third day during their lifespan, usually in clumps of 100 to 300 eggs. The eggs are deposited either as “rafts” or singly (based on genera) floating on the surface of standing water and or on the ground in areas that regularly flood. Mosquitoes can lay eggs in as little as one inch of water. The eggs, generally white and hardy when laid, cannot hatch unless they are in water, usually for two to three days. Eggs of *Aedes* can survive desiccation for months and hatch once submerged in water (American Mosquito Control Association, 2014).

Larvae: When the eggs hatch, the larvae emerge. The mosquito larva has a well-developed head with mouth brushes used for feeding, a large thorax with no legs, and a segmented abdomen.They are called “wigglers” because of their mode of swimming. The

larvae of most mosquitoes obtain oxygen from the atmosphere by coming to the water surface. Most of the time, they hang from the surface of the water, breathing through tubes (siphons). The wigglers (larvae) pass through four instar stages before pupae emerge. Majority of larvae feed on suspended particulate and organic matter in the water and microorganisms which they extract from the water with filamentous mouth brushes. Some larvae resort to scavenging or cannibalism when food is scarce (www.mosquitoworld.net. 2014).

Pupae: The pupae are called "tumblers" because of the way they fall into the deepest part of the water when threatened by predators. They are comma-shaped, partially encased in cocoons, with the head at one end and tiny flippers at the other. The pupae do not feed while developing, but breathe through tubes (siphons) like the larvae. It takes about four days for the adult mosquito to emerge out of it (www.mosquitoworld.net. 2014).

Adults: The newly emerged adults climb out of the water to rest and wait for their bodies to dry out. The males will take a day or two to fully develop their reproductive organs, and then seek out a female, by the sound of her wingbeats, for mating. They will live about three to five days after that, feed on fruit and plant nectar. The females mate once, but continue laying eggs after every blood meal. Under the best conditions, they can live up to a month or two (American Mosquito Control Association, 2014).

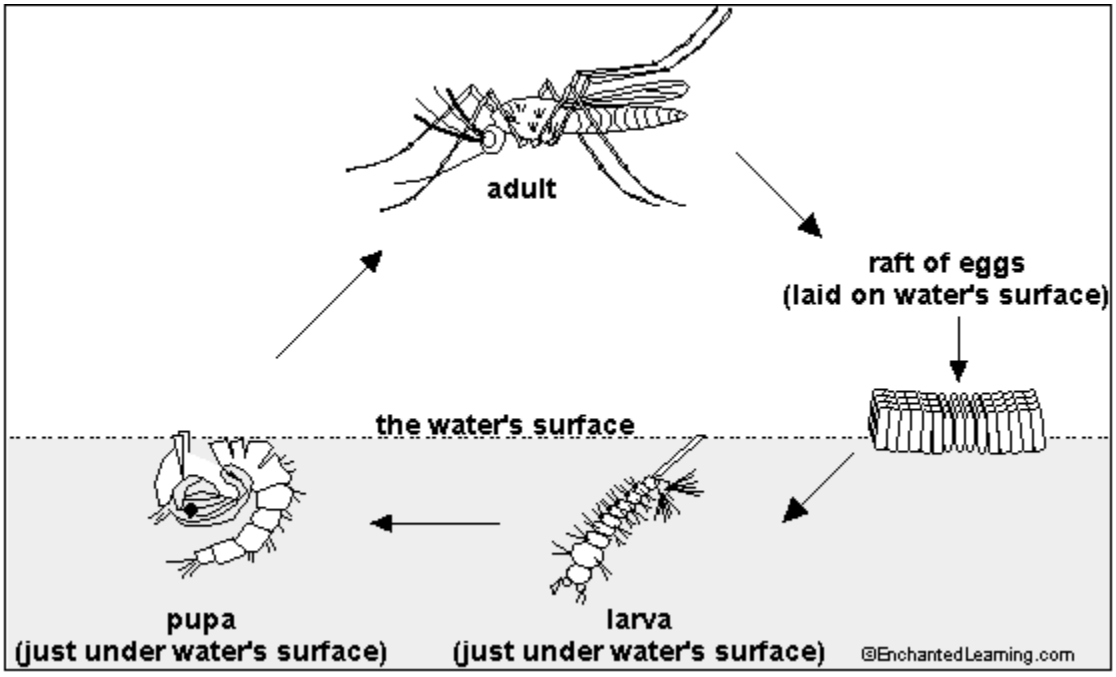


Figure 2.1: Diagram of a generalized life cycle of mosquito (Source: www.enchantedlearning.com)

2.4 Description of Larvae and Adults of Some Mosquito Species

2.4.1 *Culex quinquefasciatus* (Say)

The larval head is short, stout and darker toward the base. The mouth brushes have long yellow filaments that are used for filtering organic materials. The abdomen consists of eight segments, siphons, and saddle. Each segment has a unique setae pattern (Hill and Connelly, 2013). The siphon is on the dorsal side of the abdomen and is four times longer than its width with multiple setae tufts (Darsie-Jr and Morris, 2000). The saddle is barrel shaped and located on the ventral side of the abdomen with four long anal papillae protruding from the posterior end (Hill and Connelly, 2013).It possesses comb scales in a patch of 30-40,10-12 teeth on each side of the mental plate (New Zealand Biosecure Entomology Laboratory, 2008).

Adult *Culex quinquefasciatus* vary from 3.96 to 4.25 mm in length (Lima *et al*., 2003). The mosquito is brown with the proboscis, thorax, wings and tarsi darker than the rest of the body. The antennae and the proboscis are about the same length, but in some cases the antennae are slightly shorter than the proboscis (Hill and Connelly, 2013). The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of each tergite (Darsie-Jr and Ward, 2005).



Figure 2.2: Photomicrograph of *Culex quinquefasciatus* larva (Source: Cutwa-Francis and O'meara, 2007)

2.4.2 *Aedes aegypti* (L)

Adult *Ae. aegypti* is a small to medium-sized mosquito approximately 4-7mm in length and dark in colour. It has white scales on the dorsal (top) surface of the thorax that form the shape of a violin or lyre. Each tarsal segment of the hind legs possesses white basal

bands, forming what appear to be stripes. The abdomen is generally dark brown to black in colour, but may also possess white scales (Zettel and Kaufman, 2013). Females are larger than males and possess small palps tipped with white scales. Males have plumose antennae while females have sparse short hairs (Cutwa-Francis and O'meara, 2007). *Aedes aegypti* larvae have short siphon which is held above the water surface during breathing while the rest of the body hangs vertically. The larvae spend short time in the first three instar stages and up to three days in the fourth instar stage (Foster and Walker, 2002).

According to Christophers (1960), the main distinguishing characteristics of the larvae of *Aedes aegypti* are its rather cylindrical and elongate form and the dead white colour of the body due to the almost entire absence of sclerotised parts other than the head and siphon, which latter being almost black offers a marked contrast to the rest of the insect. The head is rather globular and, at least in the later stages, small in relation to the thorax. Anteriorly, are the structures carrying the feeding brushes used for feeding. The antennae are much reduced in size compared with many culicine larvae, small, cylindrical and smooth (Christophers, 1960).

The head hairs are small and inconspicuous. Laterally are the developing compound eyes and the larval ocelli. The thorax is roughly globular in shape consisting of the fused three thoracic segments except just after hatching when these are distinct. The segments are, however, indicated by their hair series. Each of the segments, besides many small hairs, carries long lateral hairs arising from small chitinised plates, those of the meso- and metathorax being produced into sharp, thorn-like points very characteristic of the species (Christophers, 1960). Though they are movable, the lateral hairs are not supplied with any muscles and if used in swimming must take their movements secondarily from those

of the body; most probably their function is sensory and protective. The abdomen, apart from the small terminal anal segment, consists of eight roughly equal segments increasing in size somewhat to the third and then decreasing to the last or eighth (Christophers, 1960). Each of these segments carries a number of hairs. The anal segment carries dorsally a saddle-shaped plate, which increases in proportionate size with each successive instar, until in the fourth instar it forms a cuff-like plate nearly surrounding the segment. On the ventral side of the segment towards its posterior end is the ventral fan. This is not present in the first instar, but becomes more and more developed at each successive instar. In front of the fan are some hairs (usually two) without thickened bases (Christophers, 1960).



Figure 2.3: Photomicrograph of *Aedes aegypti* larva (Source: Cutwa-Francis and O'meara, 2007).

2.4.3 *Aedes vittatus* (Bigot)

Aedes vittatus (Bigot) was formerly placed in the subgenus *Stegomyia*. The species has long been recognized to not fully conform to any recognsed subgenus of *Aedes* (Becker *et al*., 2003). Huang (1977) transferred *Ae. vittatus* from subgenus *Stegomyia* Theobald to subgenus *Aedimorphus* Theobald, mainly based on the structures of the male hypopygium (Becker *et al*.,2003). Reinert (2000) included *Aedes vittatus* in the subgenus *Stegomyia* Theobald and placed it in a monotypic group, apart from other species of the subgenus. After a comparism of *Ae. vittatus* with all currently recognized subgenera and genera in the Tribe Aedini, Reinert (2000) found the species to share some characteristics with the subgenus *Stegomyia* and *Aedimorphus*, but possess unique unusual features, that are of sub generic rank, therefore established the new subgenus *Fredwardsius*, with *Aedes vittatus* the type species (Snow and Ramsdale, 2003), laying its eggs out of water on damp surfaces in natural rock hollows. When rains occur, the rock hollows fill with water and the now submerged eggs are stimulated to hatch (Derek, 2004).

The larva is reddish-brown in colour, having its antenna about 0.5mm the length of its head with few fine spicules. The thorax has single seta which is long and barbed. Its siphon is short, sclerotised along its entire length, except for a very small area at the extreme base with indistinct anus and setae very short (Yiau-Min, 1977; Becker *et al*., 2003). The comb of segment VIII composed of 5-10 thorn-like scales in an irregular row, ventral brush with lateral grid bars and pectin with one or more spines widely spaced (Reinert *et al*., 2004; Rattanarithikul *et al*., 2010).

Adult *Ae. vittatus* have narrow preapical, white scaled bands on the fore, mid and hind femora. The vertex has decumbent curved blackish brown scales and black erect forked

scales and there are three pairs of distinct white spots on the scutum (Rattanarithikul *et al*., 2010). The antenna is slightly more than half as long as the head but shorter than the proboscis (Becker *et al*., 2003).

2.5 Distribution of Mosquitoes

Culex quinquefasciatus is a domesticated species which is often found living in close proximity to humans (New Zealand Biosecure Entomology Laboratory, 2008). Their larvae are found in freshwater habitats such as pools, ditches, ponds, and even in effluents of sewage treatment plants (Derraik and Slaney, 2005). These mosquitoes tend to hibernate over the winter and breed during the warmer months, laying rafts of eggs at night on the surface of standing water anywhere it can be found. Over a period of about two weeks, the eggs hatch, larvae emerge, develop into pupae, and then into adult mosquitoes. They normally do not travel more than one kilometer from a hatching point (New Zealand Biosecure Entomology Laboratory, 2008). Adults feed primarily from dusk until a few hours after dark (are most active at dusk, active daytime biters) and are considered aggressive and persistent biters, although they prefer birds to people. Females are anautogenous; need the protein in blood to develop eggs, before laying the first batch of eggs (Oda *et al*., 2002). They can live up to a month.

Aedes aegypti immature stages are found in temporary floodwater pools, fresh and brackish marshes containers. Adults are abundant and bite readily outdoors at all hours of the day so it is not uncommon for them to enter homes to feed on human blood (Public Health Pest Control, 2001). Adult mosquito in this genus prefers to breed in treeholes, overflow ditches, and old discarded tyres. As a predominantly tropical and subtropical group, *Aedes* mosquitoes tend to breed in warm weather, although some species can survive in colder environments. The adults feed day and night, and several of the species

are considered particularly troublesome. Its lifespan range from two weeks to a month, depending on environmental conditions (Maricopa, 2006). *Aedes aegypti* survives in three polytypic forms; domestic, sylvan and peridomestic forms. The domestic form breeds in urban areas, often around or inside houses. The sylvan form breeds in tree holes, generally in forest and the peridomestic form thrives in environmentally modified areas such as coconut groves and farms (Foster and Walker, 2002).

Aedes vittatus (Bigot) immature stages prefer to breed in rock pools, coconut shell, latex collecting containers, mud pot, plastic container, tank and sometimes treehole/ tree stump (Jomon *et al*., 2009). Their eggs can survive drying (desiccation) and hatch once flooded by water in the natural rock hollows. When rains occur, the rock hollows fill with water and the submerged eggs are stimulated to hatch. Thus, in countries with a distinct dry season, the eggs must be resistant to prolonged dehydration, while awaiting the rains. This ability is common among *Aedes* species (Derek, 2004). *Aedes vittatus* is the dominant species of mosquito that breeds in rock pools. Research shows that the species is catholic in its choice of breeding microhabitat in rock pools and the least affected by physicochemical conditions of the rock pools (Adebote *et al*., 2008).

2.6 Public Health Importance of Mosquito

Mosquitoes can be annoying, causing serious problems in human domain. They interfere with human work and spoil hours of human leisure time. They can attack farm animals which result in loss of weight and decreased production. Some mosquitoes are capable of transmitting diseases such as malaria, yellow fever, dengue, filariasis and encephalitis to humans and animals (American Mosquito Control Association, 2014).

Culex quinquefasciatus is vector of many pathogens of humans, as well as both domestic and wild animals. Viruses transmitted by this species include West Nile virus, St. Louis encephalitis and Western equine encephalitis viruses. The species is the principal vector of St. Louis encephalitis in the southern U.S.A. Although *Culex quinquefasciatus* is not considered the likely primary vector of West Nile virus in Florida, it likely plays an important role in maintaining the virus within bird populations, and is capable of transmitting it to humans (Hill and Connelly, 2013). Certain species of *Aedes* are also important in the transmission of yellow fever and dengue, for example *Aedes albopictus*, (the Asian tiger mosquito), transmits dengue fever and eastern equine encephalitis, while *Aedes aegypti* (the yellow fever mosquito) transmits dengue and yellow fever also. *Aedes aegypti* bites cause minor localized itching and irritation to the human skin, this makes outdoor adventure very unpleasant (Foster and Walker, 2002). Due to their small size and the limited probing abilities of the proboscis, mosquitoes are limited to feeding on nectar sources within flowers that have shallow or flat corollas. Unlike the relatively large pollinators like bees and butterflies, mosquitoes can feed on nectar efficiently without coming into contact with pollen-coated stamens. Thus, although they may transfer some pollen during the course of acquiring meal of nectar, mosquitoes are not important pollinators in general (Foster, 1995). Mosquitoes are natural component of many aquatic and terrestrial ecosystems. Like other aquatic insects with terrestrial adult stages, mosquitoes provide a link between aquatic and terrestrial habitats (USFWS, 2004).

2.7 Phytochemicals

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources (Shahi *et al*., 2010). Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous

selection pressure from predators and other environmental factors. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction (Ghosh *et al*., 2012). A wide selection of plants from herbs, shrubs and large trees are used for extraction of mosquito toxins. Phytochemicals can be extracted either from the whole body of herbs, shrubs or from various parts like fruits, leaves, stems, barks, roots and or seeds of larger plants. In all cases where the most toxic substances were concentrated upon, are used for mosquito control (Ghosh *et al*., 2012). Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Shaalan, *et al*., 2005).

At present, phytochemicals make up to one percent of world's pesticide market (Isman, 1997). The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species (Ghosh *et al*., 2012). Sukumar *et al*. (1991) described the existence of variations in the level of effectiveness of phytochemical compounds on target mosquito species vis-à-vis plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction.

It has been shown that the extraction of active biochemical compounds from plants depends upon the polarity of the solvents used. Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules. This was achieved by using solvent

systems ranging from petroleum ether, the most nonpolar (polarity index of 0.1 that mainly extracts essential oil) to that of water, the most polar (polarity index of 10.2) that extracts biochemical with higher molecular weights such as proteins and glycans (Ghosh *et al.*, 2012).

2.8 Description of Plants Used

Terminalia catappa (L.) is widely grown in tropical regions of the world as an ornamental tree, have been sighted in various university campuses in Nigeria where they form excellent shade trees its large leaves provide. The fruit is edible, tasting slightly acidic. It grows to about 35m in height, with an upright, symmetrical crown and horizontal branches. As the tree gets older, its crown becomes more flattened to form a spreading, vase shape. The leaves are large about 15-25cm long and 10-14cm wide, ovoid, glossy dark green and leathery. They are dry-season deciduous; before falling they turn pinkish-reddish or yellow-brown, due to pigments such as violoxanthin, lustein and zeaxanthin (Unnikrishnan, 2014). It is commonly known as umbrella tree in Nigeria. Different languages in Nigeria have different names for it, such as 'ebelebo' in Edo State (Egharevba and Ikhatua, 2008); 'odan' in Ekiti State (Kayode and Kayode, 2008) and 'igi furutu' in Ogun State (Erinoso and Aworinde, 2012). It is generally called 'tropical almond' or Indian almond originally from India (Untwal and Kondawar, 2006).

Up to 700,000 tons of *T. catappa* fruits are produced annually worldwide and it can be adopted as livestock feed ingredients (Nwosu *et al*., 2008), in ethnomedicine for the management of several human health disorders (Muhammad and Mudi, 2011; Akharaiyi *et al*., 2011) and several medicinal organic compounds have been isolated from its fruits (Ololade *et al*., 2014).The leaves have many medicinal uses which include diaphoretic,

anti-indigestion and anti-dysentery. The bark, leaves and unripe fruits are used as astringent, haemostatic, digestive, antiseptic purposes. The seeds are nutritive, sweet, bitter, astringent and edible as substitute for real almond. It is also used to prepare ointment for the treatment of scabies, leprosy and other skin diseases. An infusion of the young leaves or scraped bark is occasionally taken as a potion for treating mouth infections in Tonga and Samoa (Unnikrishnan, 2014). Young leaves are used in the Philippines to cure headache and colic (Unnikrishnan, 2014). The bark is mild diuretic, cardiotonic and antidysenteric. Leaf is sudorific, antirheumatic, antileprotic and anticephalalgic. Fish breeders use the leaves in an aquarium to lower the pH and heavy metal content of the water (Unnikrishnan, 2014). This tree has a characteristic pagoda shape because it sends out a single stem from the top center (Untwal and Kondawar, 2006). When the single stem reaches a good height, it sends out several horizontal branches. It can get very large, reaching 30m in ideal conditions (Untwal and Kondawar, 2006). The fruits are almond-shaped and green turning brown to purple when ripe. The fibrous shell surrounds an edible nut which helps the fruit to float as the seed is dispersed by water. The nuts are edible, taste very much like almonds although it can be a challenge to remove the flesh from the hard stone. Unlike the commercial almond, this almond can be eaten raw or preserved by smoking (Untwal and Kondawar, 2006). Oil extracted from the dried nuts can be edible and can be used in cooking. (Monnet *et al.*, 2012).

Tamarindus indica (L.) is an evergreen tree plant, which is indigenous to tropical Africa, north and south America, Florida, Brazil and is also cultivated in subtropical China, Pakistan, Indochina, Philippines, Java and Spain (Valle, 2005). In Nigeria, it is commonly known as ‘Tsamiya’ in the north especially among the Hausa (Ajayi *et al*., 2006); ‘Icheku oyibo’ among the Igbo in the east and ‘Ajagbon’ among the Yoruba in the

southwest (Nigeria National Medicine Development Agency, 2006). It grows to about 33m in height and is a multipurpose tree of which almost every part finds at least some use (Kumar and Bhattacharya, 2008). *Tamarindus indica* fruit pulp, a dessert fruit, is often eaten directly from the pod (Khanzada *et al*., 2008). The fruit is brown, straight or curved pod which is about 5-18cm long to 25cm wide, containing a succulent mesocarp (pulp) with an endocarp of hard cells which surrounds each seed individually, (Marmara,1989). *Tamarindus indica* seed is a byproduct of the tamarind pulp industry, an underutilized product often considered as waste material (Bhattacharya *et al*., 1997). *Tamarindus indica* seeds contain a crude lipid of 6.94-11.43% (Yusue *et al*., 2007). Tamarind fruit is regarded as a digestive, carminative, laxative, expectorant and blood tonic (Komutarin *et al*., 2004). In traditional practice, the pulp is applied on inflammations, is used in a gargle for sore throat, used for seasoning and mixed with salt, as a cream for rheumatism (Jimoh and Onabanjo, 2012). It is, further, administered to alleviate sunstroke, *Datura* poisoning, and alcoholic intoxication. The pulp is said to aid the restoration of sensation in cases of paralysis (Caluwé *et al.*, 2010).

In Nigeria, both plants are not amongst the deliberately cultivated economic trees of commerce and as such their adoption for mosquito pest management could encourage their mass cultivation especially if found to constitute vital industrial raw material sources for mosquito pesticide formulations.

2.9 Control of Mosquito

Applications of phytochemicals in mosquito control were in use since the 1920s, but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programmes. After facing several problems due to

injudicious and over application of synthetic insecticides in nature, there is the need to develop natural insecticides from plant origin for their control because they are rich in bioactive chemicals (Soummane *et al*., 2011).

There is a very important adaptation of dengue vectors that makes controlling their populations a difficult task because their eggs can withstand desiccation for several months, which means that even if all larvae, pupae, and adults were eliminated at some point in time, repopulation will occur as soon as the eggs in the containers are flooded with water.(www.cdc.org).Larvae are the easiest to kill, using oils that block their breathing tubes. There are different methods of controlling mosquitoes which include the following:

Source reduction; is the alteration or elimination of mosquito larval habitat. This remains the most effective and economical method of providing long-term mosquito control. Source reduction can include activities as simple as the removal of used tires, the cleaning of rain gutters and bird baths by individual property owners, to extensive regional water management projects conducted by mosquito control agencies on state and/or federal levels. All of these activities eliminate or substantially reduce mosquito breeding sites and the need for repeated applications of insecticides in the affected habitat (Brattsten and Hamilton, 2012).

Sanitation: the by-products of the activities of people have been a major contributor to the creation of mosquito larval habitats. An item as small as a bottle cap or as large as the foundation of a demolished building can serve as a mosquito larval habitat. Sanitation is a major part of all mosquito control programs exemplified by tire removal, de-snagging waterways, catch basin cleaning, and container removal. Mosquito control agencies

should have statutory police powers that allow for due process and summary abatement of mosquito-related public health nuisances created on both public and private property (Brattsten and Hamilton, 2012). Educational information including videos, slide shows and fact sheets distributed at press briefings, fairs, schools and other public areas should have information regarding the importance of sanitation. Public education designed to teach the general public mosquito biology and to encourage residents to use simple preventive sanitation techniques such as; fact sheets and brochures, classroom lectures at schools, slide shows, films and videos on mosquitoes and their control, and exhibits at fairs should be encouraged. The role of sanitation as an effective mosquito control modality that is a cost effective and high result method of preventing disease potential and mosquito interference with our ability to enjoy outdoors activities should continually be emphasized (Brattsten and Hamilton, 2012). Adult mosquitoes prefer to rest on weeds and other vegetations, so the number of areas where adult mosquitoes can find shelters should be reduced (American mosquito control association, 2014).

Surveillance: species-specific records should be kept on the composition of mosquito populations prior to enacting control of any kind. Records on the composition of mosquito populations after management to determine the effectiveness of control operations should also be kept. Surveillance can be larval or adult surveillance. Larval surveillance involves sampling a wide range of aquatic habitats for the presence of pest species during their developmental stages. Most counties have a team of inspectors to collect larval specimens on a regular basis. The services of a mosquito identification specialist is needed, who is schedule with the task of identifying the larvae to species level, separating mosquito species that cause nuisance and disease from those that are non-pests or beneficial species (Brattsten and Hamilton, 2012). Adult Surveillance

measures mosquito populations that have successfully developed and emerged from aquatic habitats. Light trap has been the standard for collecting adult mosquitoes and most county agencies operate light traps from early May through October. Portable traps baited with carbon dioxide are useful in areas where electricity is not available. Not all mosquito species are attracted to light and other forms of adult surveillance are frequently employed, such as, gravid traps. In coastal areas of New Jersey, 1-minute landing rates are used to assess the comparative size of host seeking salt marsh mosquitoes during daylight hours. Resting boxes are frequently used to measure populations of *Culiseta melanura*, a bird-feeding mosquito that functions in the amplification of eastern equine encephalitis (EEE) virus. Bird-baited traps are sometimes employed to measure *Culex* mosquitoes that amplify St. Louis encephalitis virus (Brattsten and Hamilton, 2012).

Biological control: This is the manipulation of natural agents and their by-products to control pest and vector species. Biological control is advantageous because it is generally host-specific with limited non-target effects. Fish are the primary biological control agent used to suppress mosquito populations. Predacious fish, typically *Gambusia* species, are reared and stocked in mosquito larval habitats. Other species of fish for mosquito control include the fathead minnow (*Pimephales promelas*), the freshwater killifish (*Fundulus heteroclitus*) and sunfish (*Centrarchus macropterus*) (Brattsten and Hamilton, 2012). .

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 (WHO 2014). This remains the most widely used malaria vector control method. Its application has been thoroughly standardized and there are clear specifications for suitable equipment and insecticides. Field guidelines on technical and operational issues are available in almost

all languages of malaria-endemic countries. As the main effect of indoor residual spraying is the killing of mosquitoes entering houses and resting on sprayed surfaces; it is not useful for the control of mosquitoes which tend to rest outdoors, although it may be effective against outdoor biting mosquitoes which enter houses for resting after feeding. It is a method for community protection and, to achieve its full effect, it requires a high level of coverage, in space and time, of all the surfaces where the mosquito is likely to rest, with an effective dose of insecticide. The selection of the insecticide has to take into account the susceptibility status of local vectors and duration of the residual effect in relation with the length of the transmission season (Lagos State Ministry of Health, 2014). 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 (WHO, 2012).

2.10 Previous Studies on the Larvicidal Efficacies of Some Plant Extracts on Mosquito Larvae

Larviciding activities of plant extracts against *Aedes vittatus* larvae appears not to have been documented. Studies of mosquito larvicidal prospects of essential oils obtained from such plants as *Cymbopogon citratus, Tagetes minuta, Mentha piperita, Dalbergia sissoo, Lippia sidoides* and *Hyptis martiusii* have demonstrated their promising larvicidal activities against mosquito vectors (Sukumar *et al*., 1991; Perich *et al*., 1995; Ansari *et al*., 2000a; Ansari *et al*., 2000b; Carvalho *et al*., 2003). In *Dalbergia sissoo* oil experiment, larvicidal activity was proportional to dosage and lower dosages (0.4ml/m^2 to 0.8ml/m^2) did not produce more than 50% mortality after 72h of exposure; but 90-100% mortality was obtained within 24h of larval exposures to 4-5ml/m^2 against *Ae. aegypti* and *Culex quinquefasciatus.* Up to 60-88% larval mortality of *Anopheles*

stephensi was obtained (Ansari *et al*., 2000b). With the use of *Mentha piperita* oil, 100%, 90% and 85% mortality of *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* larvae respectively were obtained within 24hours at 3ml/m^2 (Ansari *et al*., 2000b).

Adoum (2009) observed that eleven out of 20 plant species used in his study showed significant toxicity against brine shrimp larvae of *Artemia salina* at lower LC_{50} values. The activity of *Acacia nilotica* and *Kigelia africana* on Brine Shrimp Test (BST) was found to be consistent with the larvacidal effects of the two plants on *Culex* mosquito. Amusan and Oke (2004) revealed that the acute LC_{50} of *Xylopia aethiopica* oil extract against *Culex quinquefasciatus* was 257ppm and that very few deaths were recorded in the first 6 hours but percentage mortality increased with increase in time. The highest concentration of 400ppm killed only 5% of the population within 1hour test period, 3% within 6 hours and 75% within 24 hours test period. Toxicity of *X. aethiopica* was observed to be gradual and lasted throughout the test periods. The toxicity and persistence of the hexanolic extract of *X. aethiopica* were very reassuring in the control of *Culex quinquefasciatus* in the field since it lay its eggs on a daily basis when and where breeding exist.

Waseem *et al*. (2010) observed that oils from several *Citrus* varieties showed strong larvicidal effects against *Aedes albopictus* species of mosquito. Out of the total citrus seed extracts tested, rough lemon and lemon had the lowest LC_{50} values of 119.993 and 137.258ppm respectively; particularly after 24 hours of exposure, followed by red blood orange (295.630 ppm), chakutra (334.874ppm), galgal (644.250ppm), brazilian sour (905.96ppm) and kinnow (1022.674ppm). Narangi variety of *Citrus* had the highest LC_{50} value (2069.117ppm) after 24h of exposure followed by grape fruit (1598.15ppm) and

musambi (1389.162ppm). After 48 hours of treatment, rough lemon and lemon had the lowest LC_{50} values (108.85 and 119.853ppm respectively), followed by narangi (166.598ppm), galgal (177.441ppm), red blood orange (293.225ppm), brazilian sour (322.36ppm) and chakutra (342.086ppm). Musambi had the highest LC_{50} values (587.093ppm), followed by kinnow (532.159ppm) and grape fruit (506.775ppm). The χ^2 values for larval mortality tests showed no heterogeneity in all the experiments. Rough lemon and lemon showed highest percent mortalities (95.6 and 88.9% respectively), after 24 hours of exposure, followed by chakutra (84.4%) and red blood orange (82.8%) while narangi gave lowest percent mortality (11.7%). Rough lemon and lemon also showed highest percent mortalities (99.6 and 97.7%, respectively) after 48 hours of exposure followed by chakutra (91.6%) while musambi and grape fruit gave lowest percent mortalities (46.7 and 47.2%, respectively). After 72 hours of exposure chakutra, galgal, lemon, narangi, red blood orange and rough lemon showed almost 100% mortalities (Waseem *et al*., 2010).

Ravikumar *et al*. (2011) showed that the extracts from seaweeds had such varieties of phytochemicals as saponin, steroids, terpenoid, phenols and proteinsaponins which can also serve as natural larvicidal compounds, with minimum LC_{50} values of 21.7 and 63ppm against the dengue vector *Aedes aegypti*. It has been revealed that extract of the seaweed *Dictyota dichotoma* was highly effective in causing mortality of mosquito larvae with the minimum lethal concentration (LC_{50}) of 0.0683±0.0084µg/mL (Ravikumar *et al*., 2011). Cetin *et al*. (2010) reported maximum larvicidal activity of *Dictyota dichotoma* which caused 50 percent mortality of the larvae of *Anopheles stephensi* and *Culex quinquefasciatus* at the concentrations of 22 and 34ppm respectively, as well as total mortality at 80 and 100ppm respectively. The extracts of *Targetes erecta* also

showed larvicidal activity against *Culex quinquefasciatus* and the nematode, *Meloidogyne incognita* (Farjana *et al*., 2011). According to Rathi, *et al*., (2008), all the plants tested possess at least three or more than three secondary metabolites so they can be utilized as pesticidal plants.

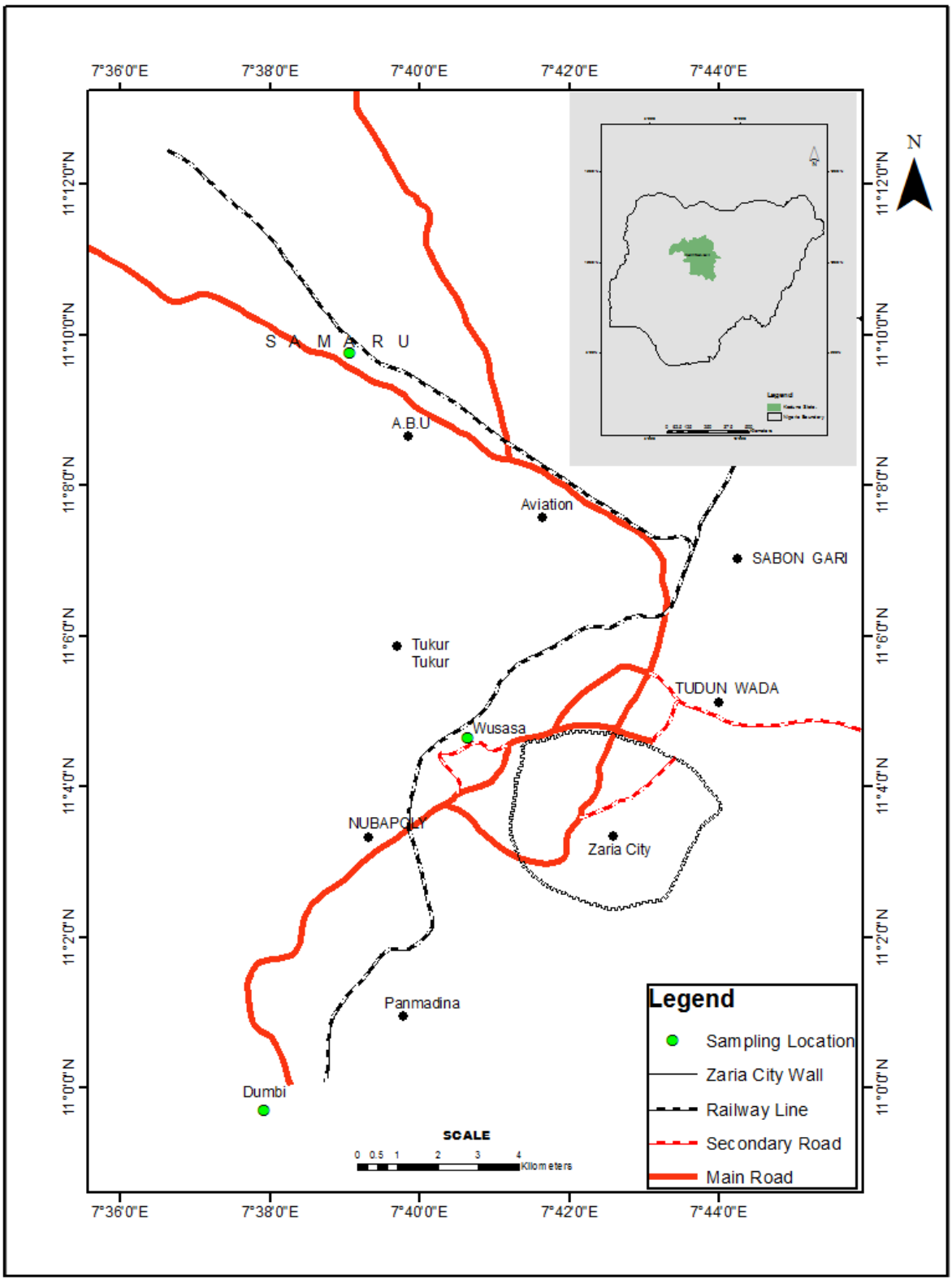
CHAPTER THREE
3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in the Department of Biological Sciences at the Ahmadu Bello University (A.B.U), Samaru, Zaria, Nigeria (Figure 3.1). Some facilities at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences of the University and at the National Research Institute for Chemical Technology (NARICT), Bassawa, Zaria, were also used during the study. Zaria is located between longitude 7^{o} 36´ and 7^{o} 42´ E and latitude 11^{o} 00´ and 11^{o} 10´ N of the equator. It is on the high plains of northern Nigeria, in sub Saharan Africa (Ubogu *et al*., 2011). Zaria experiences a tropical savanna climate with distinct rainy (wet) and dry seasons. It experiences six months of rainy and six months of dry season. The rainy season begins in May and last till late October, while the dry season is from early November to April. The area falls within the guinea savannah vegetation of the country (Yusuf and Shuaib, 2012).

3.2 Collection and Identification of Plant Materials

Fruits of *Terminalia catappa* and *Tamarindus indica* were collected from fruiting trees within the main campus of Ahmadu Bello University, Zaria, Nigeria during the months of December to February. The fruits were taken to the herbarium unit of the Department of Biological Sciences of the University, for identification and were assigned voucher numbers of 1556 for *T. catappa* and 900265 for *T. indica*.



(Source: adopted and modified from Zaria topographic map)

Figure 3.1**:** Mosquito sampling locations within the study area

3.3 Preparation of Plant Materials

The fruits were sun dried for 14days and carefully cracked using hammer to remove the seeds. The seeds were then shade dried for 10days allowing them to dry properly. The dried seeds were pulverized using mortar and pestle. The pulverized seeds were stored in separate labeled polythene bags prior to extraction.

3.4 Extraction of Plant Materials

Extraction of the seeds was by the Soxhlet extraction method using petroleum ether (60^{o}C-80^{o}C) solvent. The Soxhlet apparatus consists of a solvent flask connected to an extracting chamber (quick fit) with a reflux condenser. Procedurally, the pulverized seeds of the plant were held separately in a thimble (a porous tube) and placed in the extracting chamber. Petroleum ether solvent was then introduced into the holding flask. The content was boiled on heating mantle for hours continuously until the pulverized seeds in the quick-fit turned colourless indicating that the extraction was complete. On cooling, the unwanted residue in the thimble was discarded while the filtrate was allowed to recondense making the solvent to evaporate (Anyanwu *et al*., 2001). Extracted seed oils were exposed in a rotary evaporator to expel excess solvent. Extracted seed oils were stored in labeled glass bottles with lids under room conditions until used for bioassay.

3.5 Physicochemical Characterization of Seed Extracts

Characterization of the seed extracts of both plants was carried out at the National Research Institute for Chemical Technology (NARICT), Zaria. The following parameters were determined;

3.5.1 Acid Value

Two grams of each seed oil was weighed separately into a 250ml conical flask. Mixed solvents (25ml ethanol and 25ml diethylether) were added to the oil and swirled gently. The mixture was slightly heated to ensure complete dissolution of the oil. Two drops of 1% phenolphthalein indicator was added and the mixture titrated with 0.1M Potassium hydroxide (KOH) until a pink colour which lasted for at least 30 seconds appeared. This was repeated twice and the average titre value was recorded. (AOAC, 2001).

Acid value of oil was calculated as;

$$A.V = \frac{titre\ value\ of\ oil\ test \times molarity\ of\ KOH \times 56.1}{weight\ of\ the\ oil\ sample\ used}$$

3.5.2 Iodine Value

Exactly 0.3g of each seed oil was weighed using an OHAUS Precision Advanced Top Loading Balance, into a 250ml conical flask. Exactly 15ml of Carbon tetrachloride (CCl_4) was added to the oil to dissolve it and the mixture was swirled. Twenty millilitre (20ml) of Wij's solution (Iodine monochloride in glacial acetic acid solution) was added, stoppered and the content was vigorously swirled and then kept in the dark for 30 minutes. Fifteen millilitre (15ml) of 10% Potassium iodide was added and rinsed out into the mixture with 100ml distilled water. Three drops of freshly prepared 1% starch solution was added as indicator and titrated using 0.1M $Na_2S_2O_3.\ 5H_20$ (Sodium thiosulphate solution) till a clear solution was obtained. The blank solution was back titrated; (Wij's method as outlined by Oderinde and Ajayi, (2000).

Iodine value (I.V) of the seed oil was calculated as;

$$I.V = \frac{(titre\ value\ of\ blank - titre\ value\ of\ oil\ test) \times molarity\ used \times 12.69}{weight\ of\ oil\ sample\ used}$$

3.5.3 Saponification Value

Two gramms (2g) of the oil was weighed into a 250ml round bottom flask and 50ml of ethanolic Potassium hydroxide (KOH) (with molecular weight of 56.1) was added and the mixture was gently swirled. The mixture was heated for 1hour under reflux and allowed to cool. Two drops of 1% phenolphthalein indicator was added to form a pink coloration, and the mixture was titrated with 0.5M Hydrochloric acid (HCl) until a colourless solution was formed. The same process was carried out for the blank. The saponification value (S.V) was then calculated as reflected (AOAC, 2001):

$$S.V = \frac{(titre\ value\ of\ blank\ test - titre\ value\ of\ oil\ test) \times molarity\ of\ acid\ used \times 56.1}{weight\ of\ oil\ sample\ used}$$

3.5.4 Peroxide Value

One gramm of the seed oil was weighed into a 250ml conical flask and 1g of Potassium iodide was added to the oil. A mixture of acetic acid and chloroform (ratio 2:1) was added to the oil mixture. The mixture was gently swirled and placed in a water bath for about 5 minutes to ensure complete dissolution of the oil. Exactly 20ml of 5% Potassium iodide (KI) was added and the mixture was titrated with 0.002M Sodium thiosulphate ($Na_2S_2O_3.5H_20$) using 1% starch indicator to colourless at end point. This was also done for the blank. (AOAC, 2001). The peroxide value was calculated as indicated here in.

$$P.V = \frac{(titre\ value\ of\ blank\ test - titre\ value\ of\ oil\ test) \times molarity\ used \times 1000}{weight\ of\ oil\ sample\ used}$$

3.5.5 Colour and State of oil

The colour and state of the seed extracts at room temperature were assessed by visual observation in day light (Ajayi *et al.*, 2006).

3.5.6 Refractive Index Determination

The refractive indices of the oils were determined using Abbe's Refractometer 300918. The sample chamber containing the lens was opened and cleaned with acetone,it was then plugged to source of light the equipment was calibrated with a drop of water,then a drop a drop of the samplewas added into the sample chamber and closed. The adjustment knob was turned until the light and dark field crossed the cross bar then the readings were recorded (AOAC, 2001).

3.5.7 Fatty acids and Allied Metabolites Compositions

The fatty acids and allied metabolites composition of the oils were determined using Gas Chromatography-Mass Spectrometry (GC-MS) QP2010 PLUS SHIMADZU, JAPAN model at injection temperature of 250^0C, pressure of 116.9kPa, solvent cut time of 2.50mins, threshold of 2000 and column oven temperature of 70^0C. Percentage areas of the detected compounds were recorded (Shimadzu, 2010).

3.6 Collection and Identification of Mosquito Larvae for Bioassays

Indoor resting blood fed adult female *Culex quinquefasciatus* mosquito were collected within the Ahmadu Bello University main campus using test tubes and released to lay eggs into entomological cages containing bowls of borehole water in the Entomology and Parasitology Laboratory of the Department of Biological Sciences of the University. The eggs laid were maintained to hatch and develop into larvae under 27 ± 2^oC, 70±10% relative humidity and approximately 13:11 light and dark photoperiodic conditions during the rainy season (May-October). The larvae were fed with biscuits and baker's yeast

(ratio 3:1) until they developed into third instar and then used for bioassay (Tiwary *et al*., 2007).

Larvae of *Aedes aegypti* were collected from piles of discarded used tyres within Samaru (latitude $11^0$10´N and longitude $07^0$37´E). *Aedes vittatus* larvae were obtained from rock pools on the Dumbi inselberg (latitude 10° 57.7´ N and longitude 7° 39.3´ E at an elevation of 111.56m above the surrounding) and on the Wusasa inselberg (latitude 11° 04.597' N and longitude 7° 40.475´ E at an elevation of 32m above the surrounding), all around Zaria (Figure 3.1) (Adebote *et al*., 2008; Yusuf and Shuaib, 2012).

All larvae collected were identified based on observable characteristics of their siphon, antenna, seta, thorn-like scales and colour with the aid of a dissecting microscope using the keys of Hopkins (1952).

3.7 Mosquito Bioassays

Bioassays of the larval mosquito species with the seed oils were performed using the standard WHO methods in both laboratory and simulated field conditions (WHO, 2005) during the months of May to October.

3.7.1 Laboratory bioassay

In the laboratory (Entomological and Parasitology Laboratory, Department of Biological Sciences, A.B.U, Zaria), 500mL-sized experimental bowls were filled with 100mL of distilled water. Twenty-five third instar larvae of each of the three mosquito species were isolated from breeding media using dropping pipette into the 100mL distilled water in the 500mL sized experimental bowls under average temperature of 26^{o}C and 70% relative humidity. The seed extracts of *T. catappa and T. indica* were each administered into each

experimental bowl at volumes of 0.05mL, 0.10mL, 0.20mL, 0.40mL, and 0.80mL respectively per 100mL distilled water using syringe to give respective concentrations of 0.5mL/L, 1.0mL/L, 2.0mL/L, 4.0mL/L and 8.0mL/L. A control test that consisted of twenty-five third instar mosquito larvae in 100mL distilled water was also set up. Treatments and controls were replicated thrice for each species of mosquito larvae. The contents of the experimental and control bowls were covered with mesh screen to prevent other mosquitoes/insects from laying eggs. Treatment and control experimental bowls were labelled according to the mosquito species, concentration of seed oil and time of inoculation of seed oil. Mortality of larvae in each experimental bowl was determined and recorded after 24hours post treatments. The larvae were considered dead when they failed to move when prodded with a needle (IL-Kwon *et al*., 2002; WHO, 2005).

3.7.2 Simulated-field bioassay

Twenty-five third instar larvae of each of the three mosquito species were isolated from the breeding media using dropping pipette and introduced into 100mL of distilled water in 500mL-sized experimental bowls. They were placed at strategic areas within the forested garden of the Department of Biological Sciences at an average temperature of 27^{o}C, rainfall of 10.54mm, sunshine of 7hours, relative humidity of 71% and wind direction of southwest. The larvae were allowed to acclimatize for 2-3hours.The bowls contents were then treated with the seed extracts of *T. catappa* and *T. indica* at different concentrations of 0.05mL, 0.1mL, 0.2mL, 0.4mL and 0.8mL respectively per 100mL distilled water. The contents of the experimental bowls were covered with mesh screen to prevent other mosquitoes/insects from laying eggs and debris from falling into the bowls. The control test also contained twenty-five larvae of each mosquito species in 100ml distilled water devoid of seed extracts. Treatments and control bowls were replicated

thrice for each larval mosquito species at the different concentrations. Treatment and control experimental bowls were labeled according to the mosquito species, concentration of seed oil and time of inoculation of seed oil. Mortality of larvae in each bowl was recorded 24hours post treatments. The larvae were considered dead if they failed to move when prodded with a needle (IL-Kwon *et al*., 2002; WHO, 2005).

3.8 Statistical Analysis of Data

Percentage mortality of the larvae in the different doses of the seed oil extracts was determined. Treatment mortality means were compared using Analysis of Variance (ANOVA). Probit analysis of mortality data was done to determine the median lethal concentrations (LC_{50}) of the extracts against the three mosquito species (Finney, 1971). Abbott's formula (Abbott, 1925), was used to correct for mortality encountered in the control test. Logarithms of the concentrations of extracts used were plotted against the empirical probit of kill to obtain regression equations and coefficients of determination (R^2) for each extract type against each mosquito species. Student's t-test was used to determine significant differences between physicochemical compositions of both seed extracts and discriminating between laboratory and simulated field bioassays.

CHAPTER FOUR
4.0 RESULTS

4.1 Physicochemical Composition of *Terminalia catappa* and *Tamarindus indica* Seed Extracts

Table 4.1 shows the physicochemical composition of the oils extracted from the seeds of *Terminalia catappa* and *Tamarindus indica* in terms of their acid, percentage free fatty acid, iodine, peroxide and saponification values as well as their refractive indices, colour and physical nature at room temperature. The acid values of the oils ranged from 10.66 mgKOH/g in *T. catappa* to 16.60 mgKOH/g in *T. indica*. The acid value of the seed oil of *T. indica* was significantly higher (p□ 0.05) than that of *T. catappa*. The percentage free fatty acid (mgKOH/g) of *T. indica* (8.41) was significantly higher (p □ 0.05) than that of *T. catappa* (5.33). The iodine value (meq/Kg fat) of the seed of *T. indica* (16.18) was significantly lower (p□0.05) than that of *T. catappa* (39.35). The peroxide values (meq/Kg fat) of the seed oils ranged insignificantly (p □ 0.05) from 9.27 in *T. catappa* to 12.88 in *T. indica*. Saponification values (mL/Kg) of the seed oils did not differ significantly (p□0.05) between 162.75 in *T. catappa* and 187.94 in *T. indica*. The refractive index of the oil in *T. catappa* (1.46) was significantly lower (p□ 0.05)than that in *T. indica* oil (1.48). While both oils were in the liquid state at room temperatures, the colour of *T. catappa* oil was yellow and that of *T. indica* was a darker yellowish brown (Table 4.1).

Table 4.2 shows the percentage composition of fatty acids and allied metabolites in the seed oils of *Terminalia catappa* and *Tamarindus indica* obtained via GC-MS analyses. In all, fourteen compounds were identified in the seed oil of *T. catappa* while sixteen compounds were identified in the seed oil of *T. indica* (Table 4.2).

Table 4.1: Physicochemical composition of *Terminalia catappa* and *Tamarindus indica* seed extracts

Parameters	*Terminalia catappa*	*Tamarindus indica*	t-test(p-value)
Acid value (mgKOH/g)	10.66^{b}±0.56	16.60^{a}±0.28	0.01093
% Free Fatty Acid (mgKOH/g)	5.33^{b}±0.28	8.41^{a}±0.14	0.01038
Iodine value(meq/Kg fat)	39.35^{a}±0.65	16.18^{b}±0.32	0.0009755
Peroxide value(meq/Kg fat)	9.27^{a}±1.04	12.88^{a}±0.52	0.08882
Saponification value (mL/Kg)	162.75^{a}±2.76	187.94^{a}±5.62	0.05633
Refractive index	1.46^{b}±0.003	1.48^{a}±0.0014	0.02698
Colour	Yellow	Yellowish-brown	
State of seed extract at room temp.	Liquid	Liquid	

Means followed by the same superscript in the same row are not significantly different ($p>0.05$).

Table 4.2: Fatty acids and allied metabolites composition of *T. catappa* and *T. indica* seed extracts

Parameters (Compounds)	Molecular formula	% area
Terminalia catappa seed extract		
Cyclopentane,1-methyl-2-(2-propenyl)	C_9H_{16}	5.46
Cyclohexane,2-propenyl	C_9H_{16}	8.28
Benzene,1-ethyl-2-methyl	C_9H_{12}	7.13
Decane	$C_{10}H_{22}$	9.80
Decane,4-methyl	$C_{11}H_{24}$	3.27
Benzene,1,2,3-trimethyl	C_9H_{12}	2.49
Undecane	$C_{10}H_{24}$	2.47
Hexadecanoic acid,methylester (Palmitic acid, methylester)	$C_{17}H_{34}O_2$	13.36
9,12-Octadecadienoic acid,methyl ester (Linolelaidic acid,methyl ester)	$C_{19}H_{34}O_2$	9.97
9-Octadecenoic acid(Z)-,methylester (Oleic acid,methyl ester)	$C_{19}H_{36}O_2$	11.02
Octadecenoic acid,methyl ester (Stearic acd, methylester)	$C_{19}H_{38}O_2$	5.83
Eicosanoic acid, methylester (Arachidic acid methyl ester)	$C_{21}H_{42}O_2$	0.66
9-Octadecenal (Octadecenyl aldehyde)	$C_{18}H_{34}O$	3.46
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,,8a,9,19,11,12,12a,14,14a-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	16.79
Tamarindus indica seed extract		
Cyclohexane,1 (-propenyl)	C_9H_{16}	6.14
Cyclohexane,2-propenyl (Cyclohexane, allyl)	C_9H_{16}	9.29
Benzene,(1-methyethyl)	C_9H_{12}	7.81
Decane	$C_{10}H_{22}$	9.81
Decane, 4-methyl	$C_{11}H_{24}$	3.75
Benzene, 1,2,4-trimethyl	C_9H_{12}	2.85
Undecane	$C_{11}H_{24}$	2.58
Pentadecanoic acid, 14-methyl, methyl ester	$C_{17}H_{34}O_2$	8.57
9,12-Octadecadienoic acid,methyl ester (Linolelaidic acid,methyl ester)	$C_{19}H_{34}O_2$	15.30
9-Octadecenoic acid, methyl ester,(E) (Elaidic acid,methyl ester)	$C_{19}H_{36}O_2$	9.15
Octadecenoic acid,methyl ester (Stearic acd, methylester)	$C_{19}H_{38}O_2$	5.09
Methyl ricinoleate (Ricinoleic acid methyl ester)	$C_{19}H_{36}O_3$	1.52
Eicosenoic acid, methyl ester (Methyl (11E)-11-icosenoate)	$C_{21}H_{40}O_2$	2.83
Eicosanoic acid, methylester (Methyl arachisate)	$C_{21}H_{42}O_2$	2.82
Heneicosanoic acid, methyl ester (Methyl heneicosanoate)	$C_{22}H_{44}O_2$	3.73
Heneicosanoic acid, methyl ester (Methyl heneicosanoate)	$C_{22}H_{44}O_2$	8.77

For *T. catappa*, percentage composition of the identified compounds ranged from 0.66% of archidic acid to 16.79% of the major compound is octadecahydro-2H-picen-3-one ($C_{30}H_{48}O$). Percentage composition of identified compounds in the seed oil of *T. indica* ranged from 1.52% of ricinoleic acid ($C_{19}H_{36}O_3$) to 15.30% of the major compound is linolelaidic acid. Seven compounds, including Cyclohexane, 2-propenyl (C_9H_{16}), Decane ($C_{10}H_{22}$), Decane,4-methyl ($C_{11}H_{24}$), Undecane ($C_{10}H_{24}$), Linolelaidic acid ($C_{19}H_{34}O_2$), Stearic acid ($C_{19}H_{38}O_2$) and Arachidic acid ($C_{21}H_{42}O_2$), were identified in varying degrees in both seed oils (Table 4.2).

4.2 **Larvicidal Effects of *Terminalia catappa* Seed Extract Against Mosquitoes**

4.2.1 Effect on *Aedes aegypti*

The mortality of late third larval instar of *Aedes aegypti* on exposure for 24 hours to various concentrations of *Terminalia catappa* seed extract under laboratory and simulated field bioassay conditions is shown on Table 4.3. Under laboratory condition, mortality of *Ae. aegypti* larvae ranged from 0/75 (0%) in the control to 61/75 (81.33%) at concentration of 8.0mL/L. Larval mortality increased in a concentration-dependent fashion. Mean larval mortalities at the various experimental concentrations were significantly (p□0.05) higher than the control but increase in concentrations from 0.5mL/L to 2.0mL/L did not produce significant (p□0.05) increase in mean larval mortality. Further increase in concentration to 4.0mL/L and 8.0mL/L produced significant (p □ 0.05) increase in mean larval mortality; with significant (p□ 0.05) highest mean mortality at 8.0mL/L (Table 4.3). Under simulated field condition, *Ae. aegypti* larval mortality varied from 0/75 (0%) in the control to 41/75 (54.67%) at

8.0mL/L. The percentage larval mortality increased from 20% at 0.5mL/L and increased with increase

Table 4.3: Mortality of *Aedes aegypti* larvae on exposure for 24 hours to various concentrations of *Terminalia catappa* seed extract in distilled water under laboratory and simulated field conditions

		Laboratory condition				Simulated field condition			
Concen. of seed extract (mL/L)	Number of larvae exposed	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	% mortality	F- value	ANOVA (p-value)	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	% mortality	F- value	ANOVA (p-value)
0.0	75	0(0.00^{d};0.00-0.00±0.00)	0.00	182.587	$6.876x10^{-11}$	0(0.00^{b};0.00-0.00±0.00)	0.00	25.832	$4.940x10^{-6}$
0.5	75	4(1.33^{c};-0.11-2.77±0.33)	5.33			15(5.00^{c};0.03-9.97±1.15)	20.00		
1.0	75	5(1.67^{c};0.23-3.11±0.33)	6.67			16(5.33^{c};0.16-10.50±1.20)	21.33		
2.0	75	11(3.67^{c};-0.13-7.47±0.88)	14.67			16(5.33^{c};1.53-9.13±0.88)	21.33		
4.0	75	22(7.33^{b};5.89-8.77±0.33)	29.33			24(8.00^{c};3.70-12.30±1.00)	32.00		
8.0	75	61(20.33^{a};16.53-24.13±0.88)	81.33			41(13.67^{a};12.23-15.11±0.33)	54.67		

Means followed by the same superscript letter, under the same study condition are not significantly different (p□ 0.05)

in concentration to 21.33% at 1.0mL/L and 2.0mL/L and then increased to 32% and 54.67% at 4.0mL/L and 8.0mL/L respectively. Mean larval mortalities at various experimental concentrations were significantly (p□0.05) higher than control, but increase in concentration from 0.5mL/L to 4.0mL/L did not produce significant (p□0.05) increase in mean larval mortalities. Mean larval mortality at 8.0mL/L (13.67) was significantly (p□0.05) higher than the mortalities at lower concentrations of 0.5 □ 4.0mL/L (5.00 □ 8.00) as shown on Table 4.3.

Table 4.4 shows the results of the probit analyses of mortalities of *Ae. aegypti* larvae on exposure to various concentrations of *T. catappa* seed extract under laboratory and simulated field conditions. On conversions of the extract concentrations to natural logarithms, and percentage mortalities to empirical probits of kill, a plot of probits of kill against logarithms of concentrations (Figure 4.1) gave a regression equation of Y=1.99X + 3.637 and a coefficient of determination (R^2) of 0.868; and a median lethal concentration (LC_{50}) of 4.840mL/L for the seed extract of *T. catappa* against *Ae. aegypti* larvae under laboratory condition. Similarly, a plot of probits of kill against logarithms of concentrations (Figure 4.2) gave a regression equation of Y= 0.76X + 4.212 and a coefficient of determination (R^2) of 0.756 and a median lethal concentration (LC_{50}) of 10.884mL/L for the seed extract of *T. catappa* against *Ae. aegypti* larvae under simulated field condition (Table 4.4). LC_{50} value obtained under simulated field condition {10.884mL/L) was higer than that under laboratory condition (4.840mL/L).

4.2.2 Effect on *Aedes vittatus*

Table 4.5 shows the mortality of *Ae. vittatus* larvae after 24 hours exposure to various concentrations of *Terminalia catappa* seed extract under laboratory and simulated field conditions.

Table 4.4: Median lethal concentrations (LC_{50}) of *Terminalia catappa* seed extract against third instar larvae of *Aedes aegypti* under laboratory and simulated field conditions by probit analyses

		Laboratory condition				Simulated field			
Concentration of seed extract(mL/L)	Logarithm of concentration	Percentage mortality (%)	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)	Percentage mortality (%)	Empirical probit of mortality	Regression equation (R^2 value	LC_{50} (mL/L)
8.0	0.90	81	5.88	Y=1.99X+3.637	4.840	55	5.13	Y=0.76X+4.212	10.884
4.0	0.60	29	4.45	(R^2=0.868)		32	4.53	(R^2=0.756)	
2.0	0.30	15	3.96			21	4.19		
1.0	0.00	7	3.52			21	4.19		
0.5	-0.30	5	3.36			20	4.16		
0.0	-	0	-			0	-		

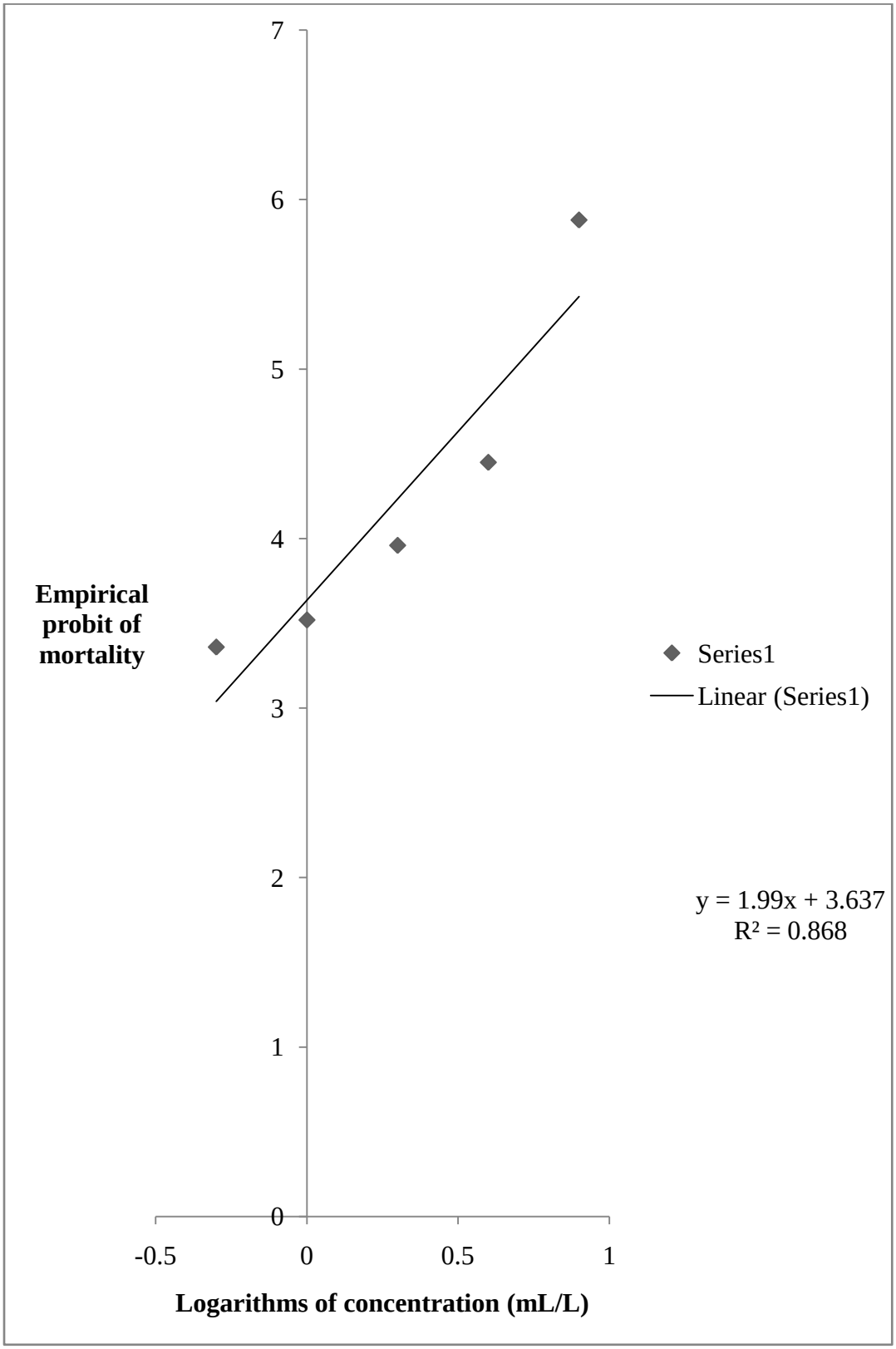


Figure 4.1: Empirical probit of mortality of *Aedes aegypti* larvae in laboratory bioassay against the logarithms of the concentrations of *Terminalia catappa* seed extract.

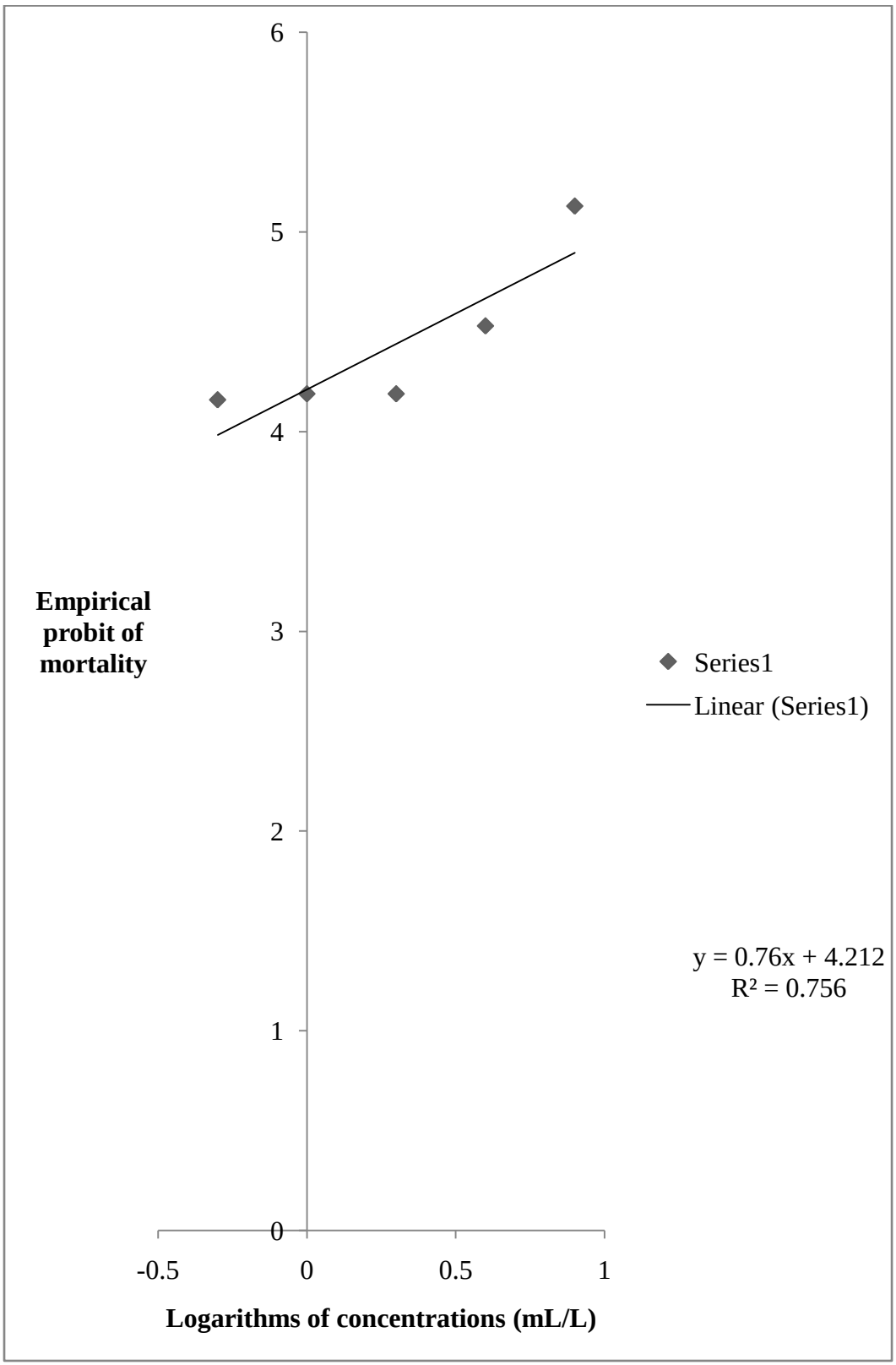


Figure 4.2: Empirical probit of mortality of *Aedes aegypti* larvae in simulated field bioassay against the logarithms of the concentrations of *Terminalia catappa* seed extract.

Table 4.5: Mortality of *Aedes vittatus* larvae on exposure for 24 hours to various concentrations of *Terminalia catappa* seed extract in distilled water under laboratory and siimulated field conditions

		Laboratory condition				Simulated field			
Concen. of seed extract (mL/L)	Number of larvae exposed	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	Percentage mortality (%)	F-value	ANOVA (p-value)	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	Percentage mortality (%)	F-value	ANOVA (p-value)
0.0	75	0(0.00^{d};0.00-0.00±0.00)	0.00	72.454	$1.527x10^{-8}$	1(0.33^{c};1.11-1.77±0.33)	1.33	72.365	$1.538x10^{-8}$
0.5	75	12(4.00^{c};1.52-6.48±0.58)	16.00			15(4.67^{b};3.23-6.11±0.33)	18.67		
1.0	75	19(6.33^{c};2.53-10.13±0.88)	25.33			16(5.33^{b};3.89-6.77±0.33)	21.33		
2.0	75	29(9.67^{b};6.81-12.53±0.66)	38.67			27(9.00^{a};6.52-11.48±0.58)	36.00		
4.0	75	40(13.33^{a};11.89-14.77±0.33)	53.33			34(11.33^{a};9.89-12.77±0.33)	45.33		
8.0	75	25(8.33^{b};6.89-9.77±0.33)	33.33			34(11.33^{a};7.53-15.13±0.88)	45.33		

Means followed by the same superscript letter, under the same study condition are not significantly different (p□ 0.05).

Mortality of *Ae. vittatus* larvae under laboratory condition varied between 0% in the control to 53.33% at 4.0mL/L and then dropped to 33.33% at the maximum 8.0mL/L. Larval mortality was essentially dose dependent between 0.5mL/L (16.0%) and 4.0mL/L (53.33%) but for the observed reduction of mortality at 8.0mL/L (33.33%). Mean larval mortalities at the various concentrations were significantly ($p=1.527\times10^{-8}$; i.e. P□ 0.05) higher than the control; but increase in concentration from 0.5mL/L to 1.0mL/L did not produce significant (p □ 0.05) increase in mean larval mortality. Further increase in concentration from 2.0mL/L to 8.0mL/L produced a significant (p □ 0.05) increase in mean larval mortality, but 4.0mL/L produced significantly (p □ 0.05) highest mean (13.33) larval mortality (Table 4.5). For the simulated field bioassay, mortality of *Ae. vittatus* ranged from 1/75 (1.33%) in the control to 34/75 (45.33%) at 4.0mL/L and 8.0mL/L. Larval mortality was dose (concentration) dependent. Mean larval mortality at the various test concentrations were significantly ($p=1.538\times10^{-8}$; i.e. p□0.05) higher than the control, but increase in concentration from 0.5mL/L to 1.0mL/L did not produce significant (p□0.05) incremental mean larval mortality. Further increase in concentrations of *T. catappa* from 2.0mL/L to 8.0mL/L produced significant (p□0.05) incremental mean mortality of *Ae. vittatus* larvae over and above those recorded at lower concentrations but the incremental mean larval mortality so produced did not differ significantly (p□0.05) amongst 2.0mL/L (9.00), 4.0mL/L(11.33) and 8.0mL/L (11.33) (Table 4.5).

Table 4.6 shows the results of the probit analyses of mortality of *Aedes vittatus* larvae on exposure to various concentrations of *T. catappa* seed extract under laboratory and simulated field conditions. On conversions of the extract concentrations to natural

logrithims; and percentage mortality to empirical probits of kill, a plot of probits of kill against logarithms of concentration (Figure 4.3) gave a regression equation

Table 4.6: Median lethal concentrations (LC_{50}) of *Terminalia catappa* seed extract against third instar larvae of *Aedes vittatus* under laboratory and simulated field conditions by probit analyses

	Laboratory condition					Simulated field				
Concentration of seed extract(mL/L)	Logarithm of concentration	Percentage mortality (%)	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)	Percentage mortality (%)	Abbott's corrected mortality	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)
8.0	0.90	33	4.56	Y=0.616X+4.355	11.143	45	44	4.85	Y=0.743X+4.287	9.099
4.0	0.60	53	5.08	R^2=0.527		45	44	4.85	(R^2=0.906)	
2.0	0.30	39	4.72			36	35	4.61		
1.0	0.00	25	4.33			21	20	4.16		
0.5	-0.30	16	4.01			19	18	4.08		
0.0	-	0	-			1	0	-		

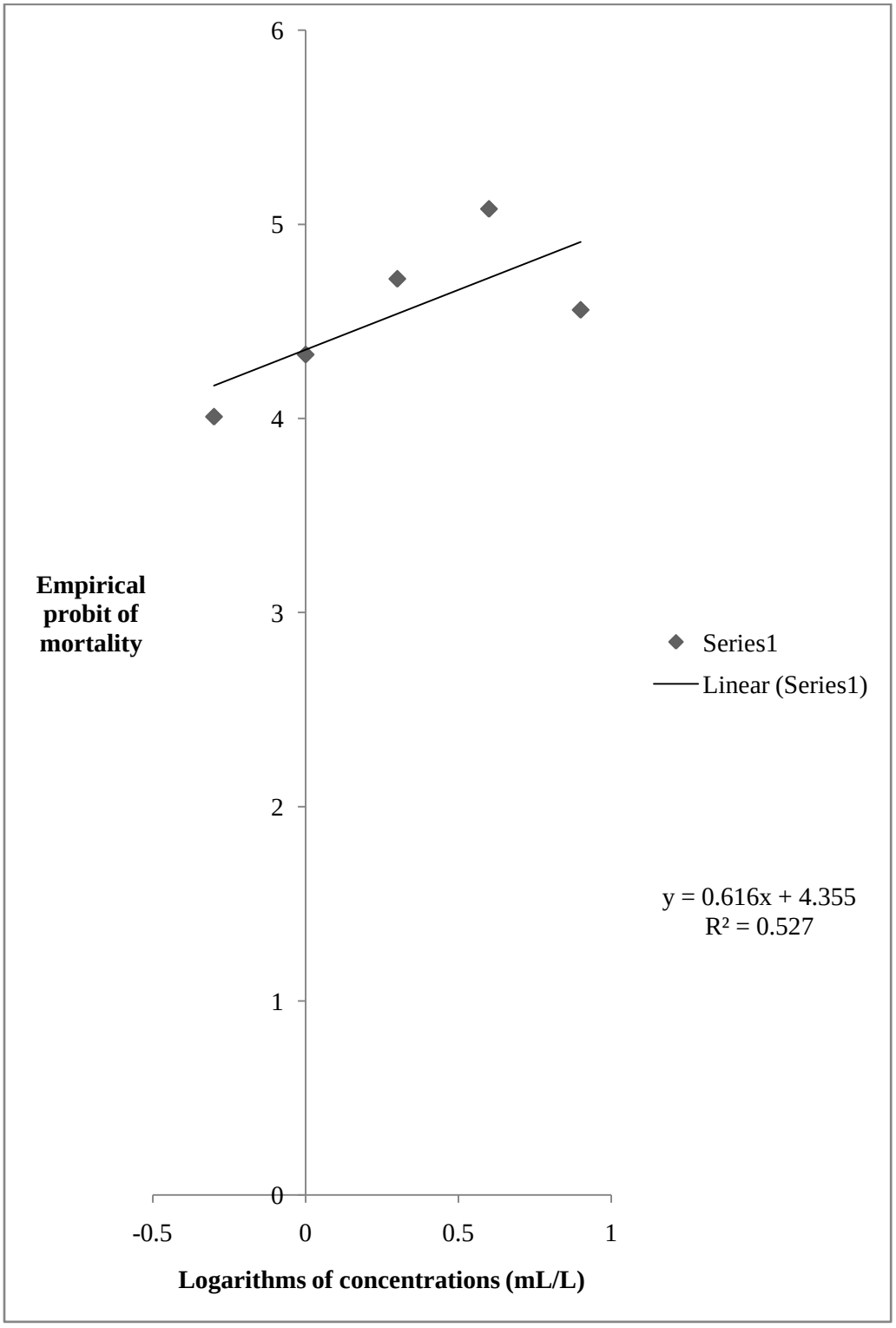


Figure 4.3: Empirical probit of mortality of *Aedes vittatus* larvae in laboratory bioassay against logarithms of the concentrations of *Terminalia catappa* seed extract.

of Y=0.6167X+4.355 and R^2 of 0.527; and an LC_{50} of 11.143mL/L for the seed extract of *T. catappa* against *Ae. vittatus* larvae under laboratory condition (Table 4.6). Similarly, a plot of empirical probits of kill against logarithms of concentrations (Figure 4.4) gave a regression equation of Y=0.7433X+4.287, R^2 of 0.9065 and an LC_{50} of 9.099mL/L for the seed extract of *T. catappa* against *Ae. vittatus* larvae under simulated field condition (Table 4.6). LC_{50} value produced under laboratory condition (11.143mL/L) was higher than that obtained under simulated field condition (9.099mL/L).

4.2.3 Effect on *Culex quinquefasciatus*

The mortality of *Culex quinquefasciatus* larvae after 24 hours exposure to various concentrations of *Terminalia catappa* seed extract under laboratory and simulated field conditions is shown on Table 4.7. The mortality under laboratory condition varied from 0/75 (0%) in the control to 68/75 (90.67%) at 8.0mL/L. There was increase in larval mortality with increase in concentration. Mean larval mortality for the various experimental concentrations were significantly ($p=3.294\times10^{-9}$; i.e. p□0.05) higher than the control. Mean larval mortality at lower concentrations of 0.5mL/L and 1.0mL/L, with respective means of 3.33 and 4.67 did not differ significantly (p□0.05) but were significantly lower (p□0.05) than the higher concentrations (2.0mL/L, 4.0mL/L, and 8.0mL/L). Mean larval mortality at 2.0mL/L (9.00), 4.0mL/L (16.67) and 8.0mL/L (22.67) differ significantly (p□0.05) from each other (Table 4.7). Larval mortality of *Culex quinquefasciatus* against various concentrations of *T. catappa* seed extract, under simulated field condition, ranged from 6/75 (8.0%) in the control to 60/75 (80.0%) at 8.0mL/L. Mortality increased as concentration increases. Mean larval mortality for the various experimental concentrations except 0.5mL/L, were significantly ($p=6.841\times10^{-8}$; i.e. p□0.05) higher than the control. Thus 0.5mL/L did not produce significantly

(p□0.05) incremental mean larval mortality over the control. Similarly, there was no significant

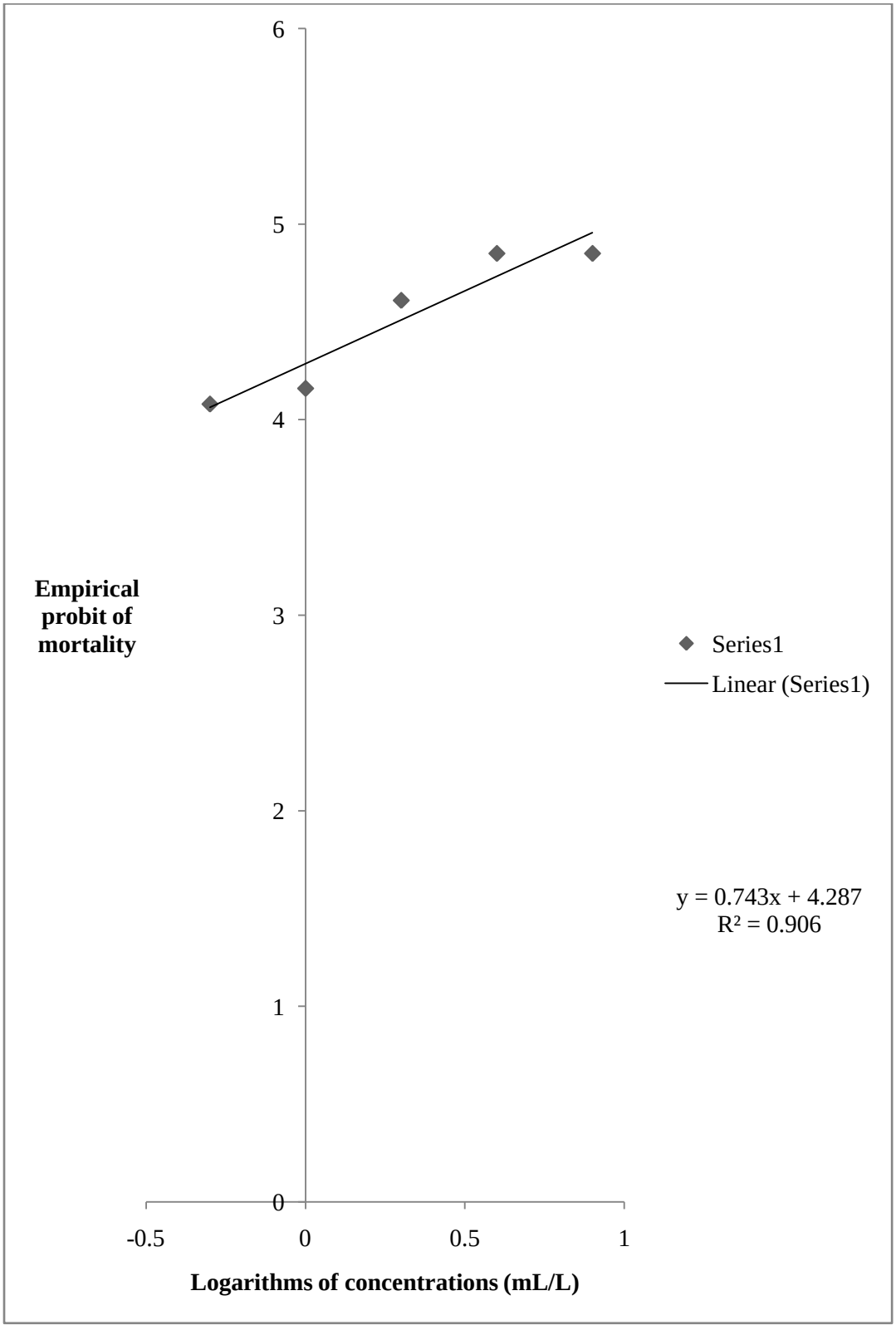


Figure 4.4: Empirical probit of mortality of *Aedes vittatus* larvae in simulated field bioassay against the logarithms of the concentrations of *Terminalia catappa* seed extract.

Table 4.7: Mortality of *Culex quinquefasciatus* larvae on exposure for 24 hours to various concentrations of *Terminalia catappa* seed extract in distilled water under laboratory and simulated field conditions

		Laboratory condition				Simulated field			
Concen. of seed extract (mL/L)	Number of larvae exposed	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	Percentage mortality (%)	F-value	ANOVA (p-value)	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	Percentage mortality (%)	F-value	ANOVA (p-value)
0.0	75	$0(0.00^{e};0.00\text{-}0.00\pm0.00)$	0.00	94.416	$3.294\text{x}10^{-9}$	$6(2.00^{d};4.58\text{-}8.52\pm1.53)$	8.00	55.786	$6.841\text{x}10^{-8}$
0.5	75	$10(3.33^{d};1.89\text{-}4.77\pm0.33)$	13.33			$13(4.33^{d};2.89\text{-}5.77\pm0.33)$	17.33		
1.0	75	$14(4.67^{d};-0.50\text{-}9.84\pm1.20)$	18.67			$22(7.33^{c};5.89\text{-}8.77\pm0.33)$	29.33		
2.0	75	$27(9.00^{c};2.42\text{-}15.58\pm1.53)$	36.00			$33(11.00^{b};8.52\text{-}13.48\pm0.58)$	44.00		
4.0	75	$50(16.67^{b};12.87\text{-}20.47\pm0.88)$	66.67			$41(13.67^{b};8.50\text{-}18.84\pm1.20)$	54.67		
8.0	75	$68(22.67^{a};21.23\text{-}24.11\pm0.33)$	90.67			$60(20.00^{a};17.52\text{-}22.48\pm0.58)$	80.00		

Means followed by the same superscript letter, under the same study condition are not significantly different ($p \square 0.05$).

($p \square 0.05$) incremental mean larval mortality when the seed extract concentration was increased from 2.0mL/L (11.00) to 4.0mL/L (13.67). However, mean larval mortality at these two concentrations were significantly ($p \square 0.05$) higher than those obtained at lower concentrations (0.5mL/L and 1.0mL/L) on one hand and significantly lower ($p \square 0.05$) than the mean larval mortality obtained at the highest concentration of 8.0mL/L on the other hand (Table 4.7).

Table 4.8 shows the results of the probit analyses of the mortality of *Culex quinquefasciatus* larvae on exposure to various concentrations of *T. catappa* seed extract under laboratory and simulated field conditions. On conversions of seed extract concentrations to natural logarithms; and percentage mortality to empirical probits of kill, a plot of the probits of kill against the logarithms of concentration (Figure 4.5) gave a regression equation of $Y=2.0867X+4.256$, R^2 of 0.955 and an LC_{50} of 2.275mL/L for the seed extract of *T. catappa* against *Cx. quinquefaisciatus* larvae under laboratory condition. Similarly, a plot of empirical probits of kill against the logarithms of concentration (Figure 4.6) gave a regression equation of $Y=1.623X+4.213$, R^2 of 0.984 and an LC_{50} of 3.055mL/L for the seed extract of *T. catappa* against *Cx. quinquefasciatus* larvae under simulated field condition (Table 4.8). LC_{50} value obtained under laboratory condition (2.275mL/L) was lower than that obtained unber simulated field condition (3.055mL/L).

4.3 Larvicidal Effects of *Tamarindus indica* Seed Extract Against Mosquitoes

4.3.1 Effect on *Aedes aegypti*

The mortality of *Aedes aegypti* larvae on exposure to various concentrations of *Tamarindus indica* seed extract under laboratory and simulated field conditions is shown

Table 4.8: Median lethal concentrations (LC_{50}) of *Terminalia catappa* seed extract against third instar larvae of *Culex quinquefasciatus* under laboratory and simulated field conditions by probit analyses

	Laboratory condition					Simulated field				
Concentration of seed extract(mL/L)	Logarithm of concentration	Percentage mortality of larvae (%)	Empirical probit of mortality	Regression equation(R^2 value)	LC_{50} (mL/L)	Abbott's corrected mortality (%)	Percentage mortality of larvae (%)	Empirical probit of mortality	Regression equation(R^2 value)	LC_{50} (mL/L)
8.0	0.90	91	6.34	Y=2.086X+4.256	2.275	78	80	5.77	Y=1.623X+4.213	3.055
4.0	0.60	67	5.44	(R^2=0.955)		51	55	5.03	(R^2=0.984)	
2.0	0.30	36	4.64			39	44	4.72		
1.0	0.00	19	4.12			23	29	4.26		
0.5	-0.30	13	3.87			10	17	3.72		
0.0	-	0	-			0	8	-		

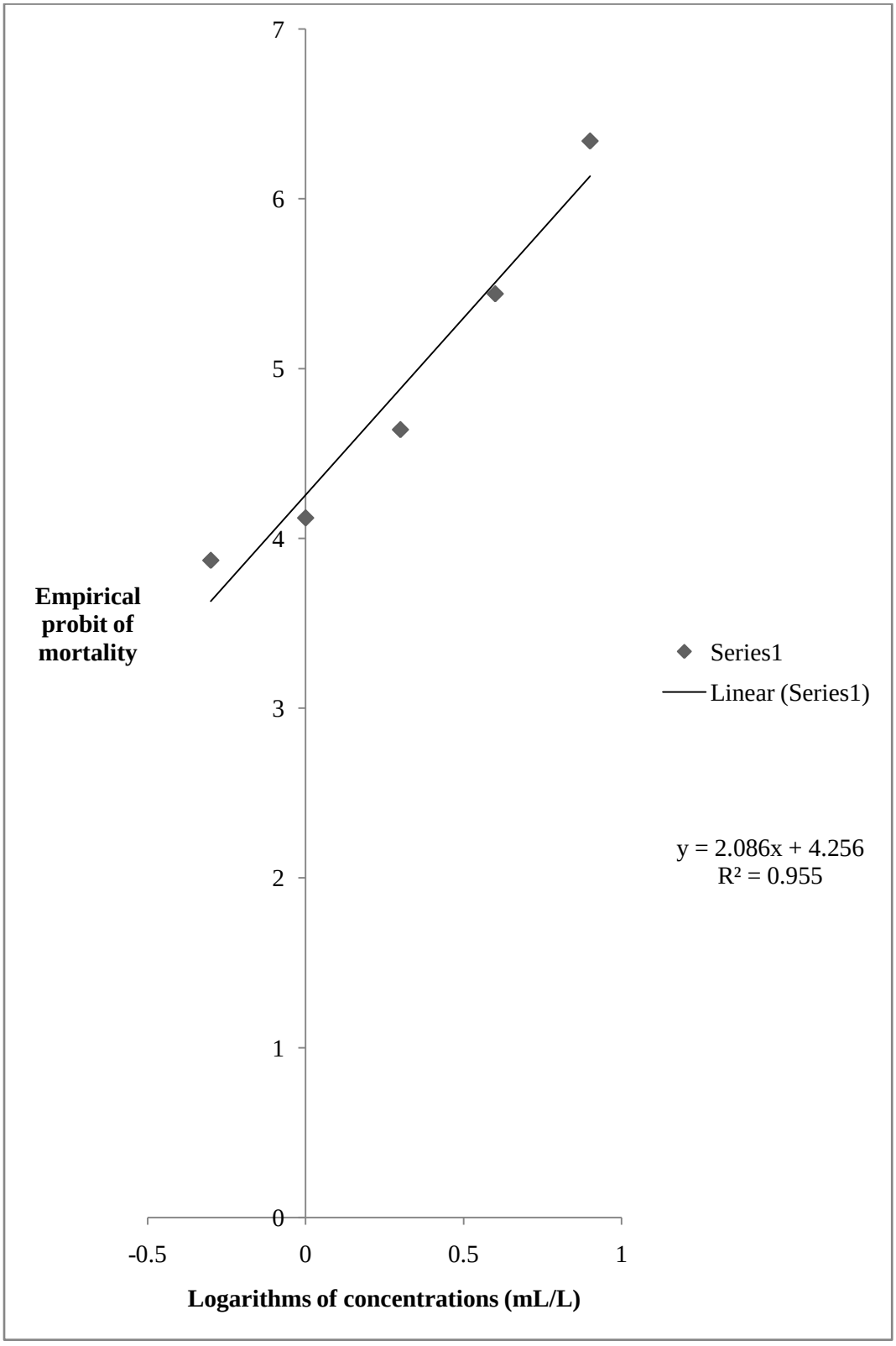


Figure 4.5: Empirical probit of mortality of *Culex quinquefasciatus* larvae in laboratory bioassay against the logarithms of the concentrations of *Terminalia catappa* seed extract.

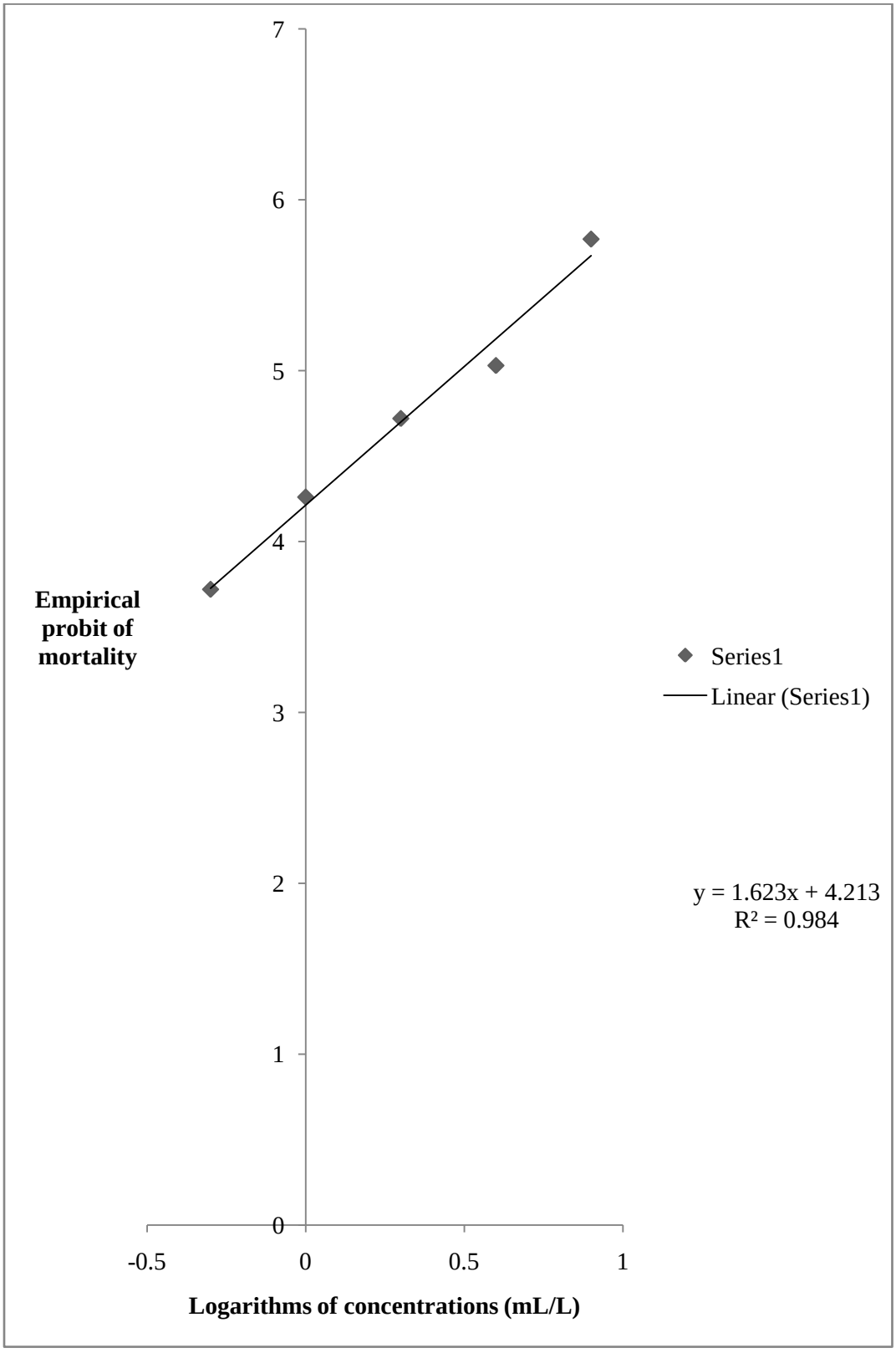


Figure 4.6: Empirical probit of mortality of *Culex quinquefasciatus* larvae in simulated field bioassay against the logarithms of the concentrations of *Terminalia catappa* seed extract.

on Table 4.9. The larval mortality under laboratory bioassay condition ranged from 2/75 (2.67%) in the control to 74/75 (98.67%) at 8.0mL/L.Larval mortality increased progressively with increase in seed extract concentrations. Mean larval mortality at the various experimental concentrations were significantly ($p=4.707\times10^{-6}$; i.e. $p\leq0.05$) higher than control mortality. However, increase in seed extract concentration from 0.5mL/L through 1.0mL/L, 2.0mL/L to 4.0mL/L did not produce significant ($p\leq0.05$) increase in mean larval mortality of *Ae. aegypti*. Mean larval mortality of *Ae. aegypti* at the highest seed extract concentration of 8.0mL/L (24.67) was significantly ($p\leq0.05$) higher than those of the other lower concentration (Table 4.9). Under the simulated field bioassay condition, larval mortality of *Ae. aegypti* ranged from 5/75 (6.67%) in the control to 71/75 (94.67%) at 8.0mL/L of *T. indica* seed extract. Larval mortality increased progressively with increase in seed extract concentrations. The mean larval mortality at the various experimental treatments were significantly ($p=1.331\times10^{-5}$; i.e. $p\leq0.05$) higher than the control mortality; except that 0.5mL/L did not yield significantly ($p\leq0.05$) higher mean larval mortality over the control. While the mean larval mortality of *Ae. aegypti* at 1.0mL/L (12.33) was significantly ($p\leq0.05$) higher than those of the lower concentrations, the mean larval mortality at 1.0mL/L was not significantly ($p\leq0.05$) lower than those of 2.0mL/L (18.00) and 4.0mL/L (18.33). But mean larval mortality (23.67) at the highest concentration (8.0mL/L) was significantly higher than that of 1.0mL/L. Increase in concentrations of seed extract from 2.0mL/L through 4.0mL/L to 8.0mL/L did not produce significant ($P\leq 0.05$) increment in mean larval mortality under simulated field condition (Table 4.9).

Table 4.10 shows the results of probit analyses of the mortality of *Ae. aegypti* larvae on exposure to various concentrations of *T. indica* seed extract under laboratory and

Table 4.9: Mortality of *Aedes aegypti* larvae on exposure for 24 hours to various concentrations of *Tamarindus indica* seed extract in distilled water under laboratory and simulated field conditions

		Laboratory condition				Simulated field			
Concen. of seed extract (mL/L)	Number of larvae exposed	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	Percentage mortality (%)	F- value	ANOVA (p-value)	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	(%) mortality	F-value	ANOVA (p-value)
0.0	75	2(0.67^{c};-0.77-2.11±0.33)	2.67	26.0644	$4.707x10^{-6}$	5(1.67^{b};-2.13-5.47±0.88)	6.67	21.444	1.331×10^{-5}
0.5	75	29(9.67^{b};3.07-22.41±2.96)	38.67			17(5.67^{b};-0.07-11.41±1.33)	22.67		
1.0	75	31(10.33^{b};6.53-14.13±0.88)	41.33			37(12.33^{c};6.59-18.07±1.33)	49.33		
2.0	75	41(13.67^{b};5.70-21.64±1.85)	54.67			54(18.00^{ab};7.17-28.83±2.52)	72.00		
4.0	75	43(14.33^{b};10.41-18.25±0.91)	57.33			55(18.33^{ab};5.83-30.83±2.90)	73.33		
8.0	75	74(24.67^{a};23.23-26.11±0.33)	98.67			71(23.67^{a};20,81-26.53±1.15)	94.67		

Means followed by the same superscript letter, under the same study condition are not significantly different (p□ 0.05).

Table 4.10: Median lethal concentrations (LC_{50}) of *Tamarindus indica* seed extract against third instar larvae of *Aedes aegypti* under laboratory and simulated field conditions by probit analyses.

		Laboratory condition					Simulated field				
Concen. of seed extract (mL/L)	Logarithm of conc.	% mortality	Abbott's corrected mortality (%)	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)	% mortality	Abbott's corrected mortality (%)	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)
8.0	0.90	99	99	7.33	Y=1.923X+4.815	1.248	95	95	6.64	Y=1.953X+4.74	1.359
4.0	0.60	57	56	5.15	R^2=0.681		73	71	5.55	(R^2=0.940)	
2.0	0.30	55	54	5.10			72	70	5.52		
1.0	0.00	41	39	4.72			49	45	4.87		
0.5	-0.30	39	37	4.66			23	17	4.05		
0.0	-	3	0	-			7	0	-		

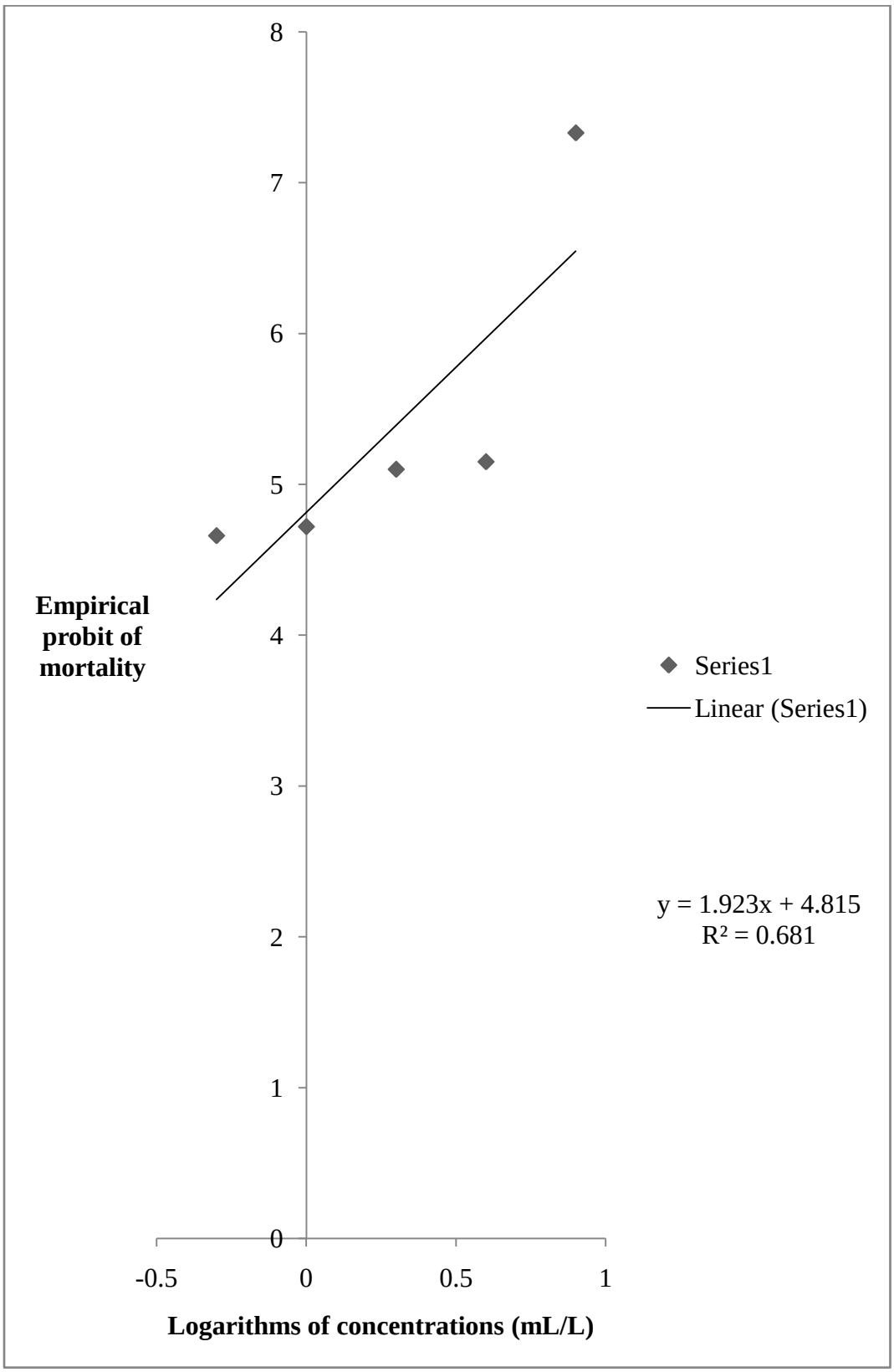


Figure 4.7: Empirical probit of mortality of *Aedes aegypti* larvae in laboratory bioassay against the logarithms of the concentrations of *Tamarindus indica* seed extract.

simulated field conditions. On conversion of *T. indica* seed extract concentrations to natural logarithms; and percentage mortality to empirical probits of kill, a plot of the probits of kill against the logarithms of concentration (Figure 4.7) gave a regression equation of Y=1.9233X+4.815, R^2 of 0.6812 and an LC_{50} of 1.248mL/L for the seed extract of *T. indica* against *Ae. aegypti* larvae under laboratory condition. Similarly, a plot of empirical probits of kill against the logarithms of concentrations (Figure 4.8) gave a regression equation of Y=1.9533X+4.74, R^2 of 0.9407 and an LC_{50} of 1.359mL/L for the seed extract of *T. indica* against *Ae. aegypti* larvae under simulated field condition (Table 4.10). LC_{50} value obtained under laboratory condition (1.248mL/L) was lower than that obtained under simulated field condition (1.359mL/L).

4.3.2 Effect on *Aedes vittatus*

Table 4.11 shows the mortality of *Ae. vittatus* larvae after 24 hours exposure to various concentrations of *Tamarindus indica* seed extract under laboratory and simulated field conditions. Mortality of *Ae. vittatus* larvae under laboratory condition varied from 2/75 (2.67%) in the control to 45/75 (60.0%) at 4.0mL/L and then decreased to 41/75(54.67%) at 8.0mL/L. There was progressive increase in larval mortality of *Ae. vittatus* with increase in concentration of *T. indica* seed extract from 0.5mL/L (8.0%) to 4.0mL/L (60.0%). Generally, extract concentrations at and above 2.0mL/L produced significant ($p=1.785\times10^{-7}$; i.e. p □ 0.05) increase in mean larval mortality over the control. However, the mean larval mortality at 0.5mL/L (2.00) and 1.0mL/L (3.33) did not differ significantly (p□0.05) from mean larval mortality for the control (0.67). While the mean larval mortality of *Ae. vittatus* at 2.0mL/L (7.33) was significantly (p □ 0.05) higher than mortality at the lower concentrations, mean larval mortality at 2.0mL/L was significantly

(p□ 0.05) lower than the values at higher concentrations of 4.0mL/L (15.00) and 8.0mL/L

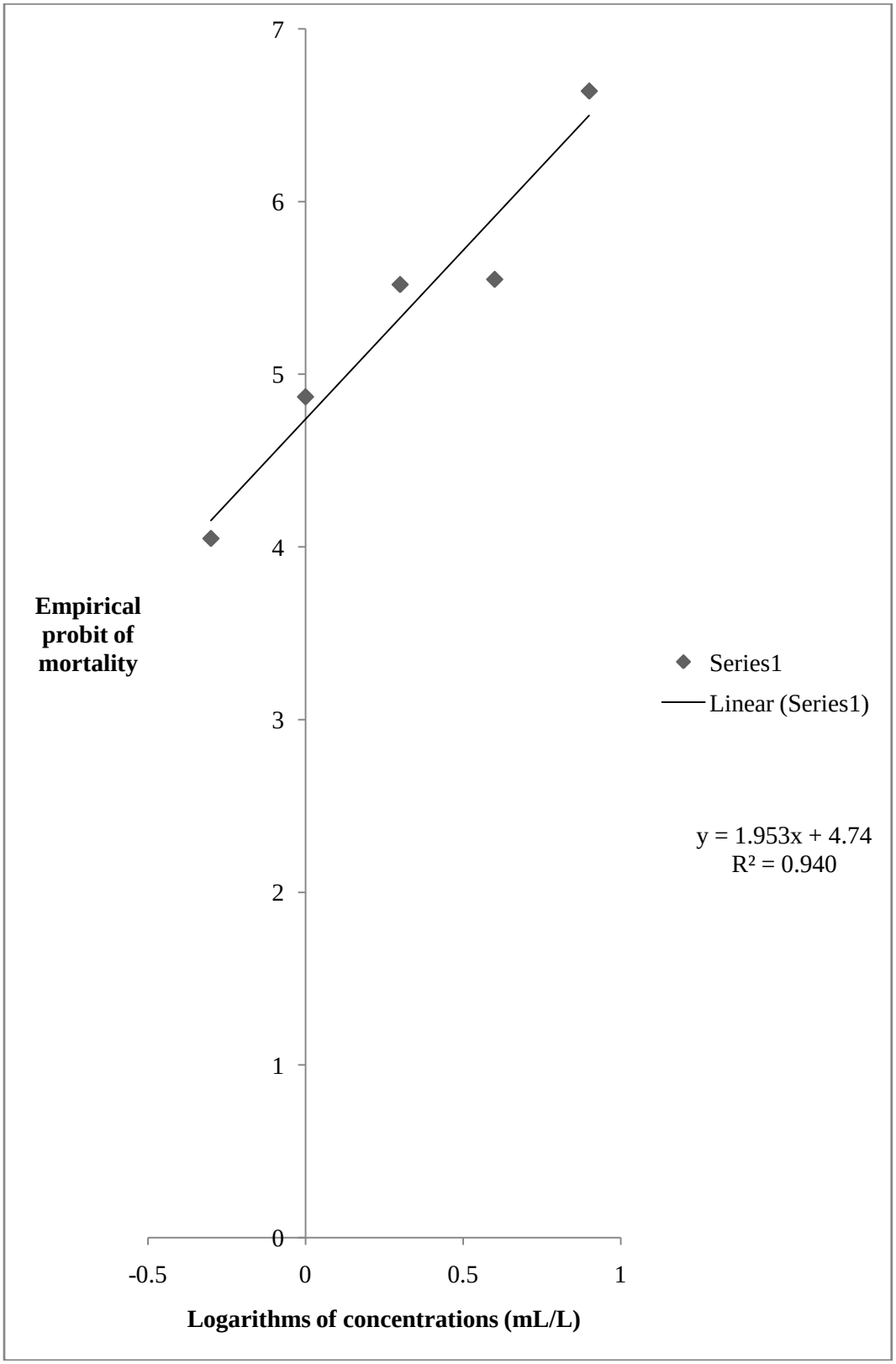


Figure 4.8: Empirical probit of mortality of *Aedes aegypti* larvae in simulated field bioassay against the logarithms of the concentrations of *Tamarindus indica* seed extract

Table 4.11: Mortality of *Aedes vittatus* larvae on exposure for 24 hours to various concentrations of *Tamarindus indica* seed extract in distilled water under laboratory and simulated field conditions

		Laboratory condition				Simulated field			
Concen. of seed extract (mL/L)	Number of larvae exposed	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	% mortality	F- value	ANOVA (p-value)	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	% mortality	F- value	ANOVA (p-value)
0.0	75	2(0.67^{c};-2.19-3.53±0.66)	2.67	47.1088	$1.785x10^{-7}$	0(0.00^{d};0.00-0.00±0.00)	0.00	87.647	5.077×10^{-9}
0.5	75	6(2.00^{c};-2.30-6.30±1.00)	8.00			22(7.33^{c};5.89-8.77±0.33)	29.33		
1.0	75	10(3.33^{c};1.89-4.77±0.33)	13.33			37(12.33^{b};6.07-18.59±1.45)	49.33		
2.0	75	22(7.33^{b};2.16-12.50±1.20)	29.33			50(16.67^{ab};12.87-20.47±0.88)	66.67		
4.0	75	45(15.00^{a};10.03-19.97±1.15)	60.00			55(18.33^{a};15.47-21.19±0.66)	73.33		
8.0	75	41(13.67^{a};10.81-16.53±0.66)	54.67			57(19.00^{a};16.52-21.48±0.58)	76.00		

Means followed by the same superscript letter, under the same study condition are not significantly different (p□ 0.05).

(13.67). There was no significant ($p \square 0.05$) difference betweenthe mean larval mortality of these latter concentrations (Table 4.11). Under the simulated field bioassays condition, mortality of *Ae. vittatus* larvae ranged from 0/75 (0%) in the control to 57/75 (76.0%) at 8.0mL/L. Mortality of larvae was concentration dependent and the experimental mortality was significantly ($p=5.077\times10^{-9}$;i.e. $p \square 0.05$) higher than the control mortality. Increase in seed extract concentration above 0.5mL/L produced significant ($p \square 0.05$) increase in mean larval *Ae. vittatus* mortality under the simulated field condition. However, there was no significant ($p \square 0.05$) increase in mean larval *Ae. vittatus* mortality when seed extract concentration was increased from 1.0mL/L to 2.0mL/L on one hand and when it was increased from 2.0mL/L through 4.0mL/L to 8.0mL/L. Mean larval mortality at 4.0mL/L and 8.0mL/L were significantly ($p \square 0.05$) higher than at 1.0mL/L and below (Table 4.11).

Table 4.12 shows the results of the probit analyses of mortality of *Ae. vittatus* larvae on exposure to various concentrations of *T. indica* seed extract under laboratory and simulated field condition. On conversions of the extract concentrations to natural logarithms and percentage mortality to empirical probits of kill, a plot of the probits of kill against logarithms of concentrations (Figure 4.9), gave a regression equation of $Y=1.6633X+3.861$, R^2 of 0.9171 and LC_{50} of 4.842mL/L for the seed extract of *T. indica* against *Ae. vittatus* larvae under laboratory condition. Similarly, a plot of the probits of kill against logarithms of concentrations (Figure 4.10), gave a regression equation of $Y=1.0533X+4.92$, R^2 of 0.9122 and an LC_{50} of 1.191mL/L for the seed extract of *T. indica* against *Ae. vittatus* larvae under simulated field condition (Table 4.12). LC_{50} value produced under simulated field condition (4.842mL/L) was lower than that produced under laboratory condition (1.191mL/L).

Table 4.12: Median lethal concentrations (LC_{50}) of *Tamarindus indica* seed extract against third instar larvae of *Aedes vittatus* under laboratory and simulated field conditions by probit analyses

		Laboratory condition					Simulated field			
Concentration of seed extract(mL/L)	Logarithm of concentration	Percentage mortality of larvae (%)	Abbott's corrected mortality (%)	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)	Percentage mortality of larvae (%)	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)
8.0	0.90	55	54	5.10	Y=1.663X+3.861	4.842	76	5.71	Y=1.053X+4.92	1.191
4.0	0.60	60	59	5.23	R^2=0.917		73	5.61	(R^2=0.912)	
2.0	0.30	29	27	4.39			67	5.44		
1.0	0.00	13	10	3.72			49	4.97		
0.5	-0.30	8	5	3.36			29	4.45		
0.0	-	3	0	-			0	-		

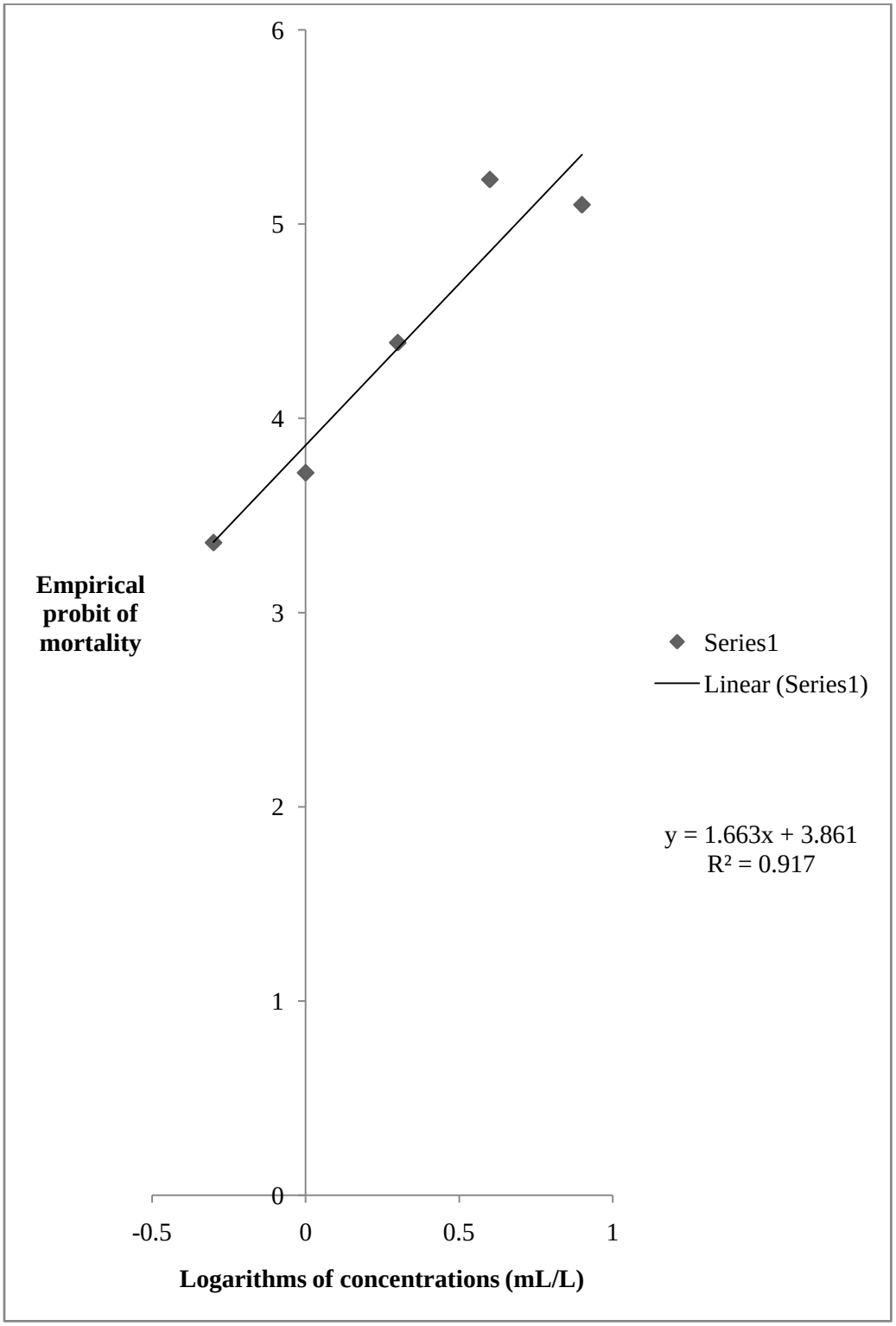


Figure 4.9: Empirical probit of mortality of *Aedes vittatus* larvae in laboratory bioassay against the logarithms of the concentrations of *Tamarindus indica* seed extract

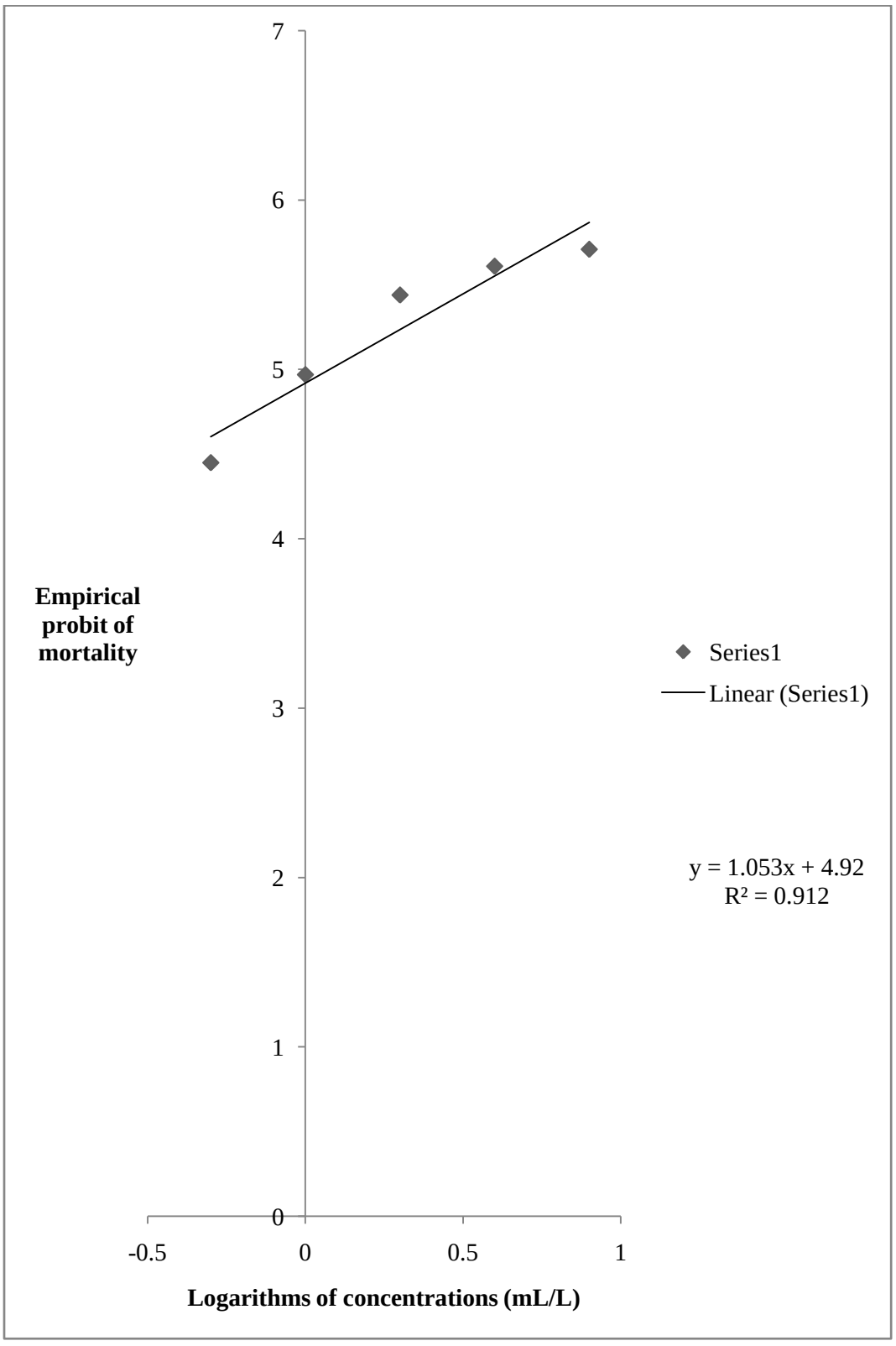


Figure 4.10: Empirical probit of mortality of *Aedes vittatus* larvae in simulated field bioassay against the logarithms of the concentrations of *Tamarindus indica* seed extract

4.3.3 Effect on *Culex quinquefasciatus*

Table 4.13 shows the mortality of *Cx. quinquefasciatus* larvae after 24 hours exposure to various concentrations of *Tamarindus indica* seed extract under laboratory and simulated field conditions. Mortality of *Cx. quinqeufasciatus* larvae under laboratory condition varied from 0/75 (0.0%) in the control to 71/75 (94.67%) at 8.0mL/L. Mortality of larval *Cx. quinquefasciatus* increased in a concentration dependant fashion and progressively from 0.5mL/L (50.67%) to 8.0mL/L (94.67%). Generally, there was significant ($p=5.204\times10^{-7}$; i.e. p□0.05) increase in mean larval mortality amongst the treatments over the control. However, increase in *T. indica* seed extract concentrations from 0.5mL/L through 1.0mL/L to 2.0mL/L did not produce significant (p □ 0.05) increase in mean larval mortality. Mean larval mortality of *Cx. quinquefasciatus* under laboratory condition at 4.0mL/L (21.67) and 8.0mL/L (23.67) did not differ significantly (p □ 0.05); but these were significantly (p □ 0.05) higher than mean larval mortality at lower concentrations (Table 4.13). Under the simulated field bioassays condition, mortality of *Culex quinquefasciatus* larvae on expose to various concentrations of *T. indica* seed extract varied from 9/75 (12.0%) in the control to 75/75 (100%) at 8.0mL/L. Mortality of larval *Cx. quinquefasciatus* increased progressively in a concentration dependent fashion. Generally, there was significant ($p=7.865\times10^{-10}$; i.e. p□ 0.05) increase in mean larval mortality amongst the treatment over the control. Increase in concentration of *T. indica* seed extract above 0.5mL/L produced significant (p□0.05) increase in mean larval mortality of *Cx. quinquefasciatus*. However, the mean larval mortality produced at 1.0mL/L (17.33) was not significantly (p□0.05) higher than the mean larval mortality at 2.0mL/L (17.67). Mean larval mortality of *Cx. quinquefasciatus* at 4.0mL/L (22.33) was significantly (p□0.05) higher than lower concentrations and significantly (p□0.05) lower than 8.0mL/L concentration (25.00) (Table 4.13).

Table 4.13: Mortality of *Culex quinquefasciatus* larvae on exposure for 24 hours to various concentrations of *Tamarindus indica* seed extract in distilled water under laboratory and simulated field conditions

		Laboratory condition				Simulated field			
Concen. of seed extract (mL/L)	Number of larvae exposed	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	% mortality	F- value	ANOVA (p-value)	Number(mean;95%CI± SE) of dead larvae at 24hours post exposure	% mortality	F-value	ANOVA (p-value)
0.0	75	0(0.00^{c};0.00-0.00±0.00)	0.00	38.9343	$5.204x10^{-7}$	9(3.00^{e};0.52-5.48±0.58)	12.00	120.66	$7.865x10^{-10}$
0.5	75	38(12.67^{b};7.50-17.84±1.20)	50.67			39(13.00^{d};6.42-19.58±1.53)	52.00		
1.0	75	42(14.00^{b};3.17-24.83±2.52)	56.00			52(17.33^{c};15.89-18.77±0.33)	69.33		
2.0	75	46(15.33^{b};9.07-21.59±1.45)	61.33			53(17.67^{c};16.23-19.11±0.33)	70.67		
4.0	75	65(21.67^{a};17.87-25.47±0.88)	86.67			67(22.33^{b};20.89-23.77±0.58)	89.33		
8.0	75	71(23.67^{a};22.23-25.11±0.33)	94.67			75(25.00^{a};25.00-25.00±0.00)	100.00		

Means followed by the same superscript letter, under the same study condition are not significantly different (p□ 0.05).

Table 4.14 shows the results of the probit analyses of mortality of *Cx quinquefasciatus* larvae on exposure to various concentrations of *T. indica* seed extract under laboratory and simulated field conditions. On conversions of the extract concentrations to natural logarithms and percentage mortality to empirical probits of kill, a plot of the probits of kill against logarithms of concentrations (Figure 4.11), gave a regression equation of Y=1.4X+5.226, R^2 of 0.890 and an LC_{50} of 0.690mL/L for the extract of *T. indica* against *Cx. quinquefasciatus* larvae under laboratory condition. Similarly, a plot of the probits of kill against logarithms of concentrations (Figure 4.12), gave a regression equation of Y=1.326X+5.271, R^2 of 0.908 and an LC_{50} of 0.625mL/L for the seed extract of *T. indica* against *Cx. quinquefasciatus* larvae under simulated field condition (Table 4.14), with simulated field condition (0.625mL/L) having a lower LC_{50} value than laboratory condition (0.690mL/L).

4.4 Comparative Larvicidal Efficacies of *Terminalia catappa* and *Tamarindus indica* Seed Extracts Against the Three Mosquito Species

4.4.1 Under laboratory conditions

The mortality of each of the three mosquito species at the various concentrations of *T. catappa* and *T. indica* under the two study conditions are shown on Table 4.15. Under the laboratory condition, there was no significant ($p > 0.05$) difference in the mean control mortality of the three mosquito species. However, the mean mortality of *Ae. aegypti* larvae on exposure to 0.5mL/L to 4.0mL/L of *T. catappa* was significantly ($p < 0.05$) lower than the mean mortality of both *Ae. vittatus* and *Cx. quinquefasciatus* at these concentrations; mean mortality of larval *Ae. vittatus* was not significantly ($p > 0.05$) different from those of larval *Cx. quinquefasciatus* at the concentrations. At the maximum 8.0mL/L concentration of *T.catappa*, mortality of larval *Ae. vittatus* (8.33) was significantly ($p < 0.05$) lower than those of*Ae. aegypti* (20.33) and *Cx. quinquefasciatus*

Table 4.14: Median lethal concentrations (LC_{50}) of *Tamarindus indica* seed extract against third instar larvae of *Culex quinquefasciatus* under laboratory and Simulated field conditions by probit analyses

	Laboratory condition					Simulated field				
Concentration of seed extract(mL/L)	Logarithm of concentration	Percentage mortality of larvae (%)	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)	Percentage mortality of larvae (%)	Abbott's corrected mortality (%)	Empirical probit of mortality	Regression equation (R^2 value)	LC_{50} (mL/L)
8.0	0.90	55	6.64	Y=1.4X+5.226	0.690	76	100	∞	Y=1.326X+5.271	0.625
4.0	0.60	60	6.13	(R^2=0.890)		73	88	6.18	(R^2=0.908)	
2.0	0.30	29	5.28			67	67	5.44		
1.0	0.00	13	5.15			49	65	5.39		
0.5	-0.30	8	5.03			29	45	4.87		
0.0	-	3	-			0	0	-		

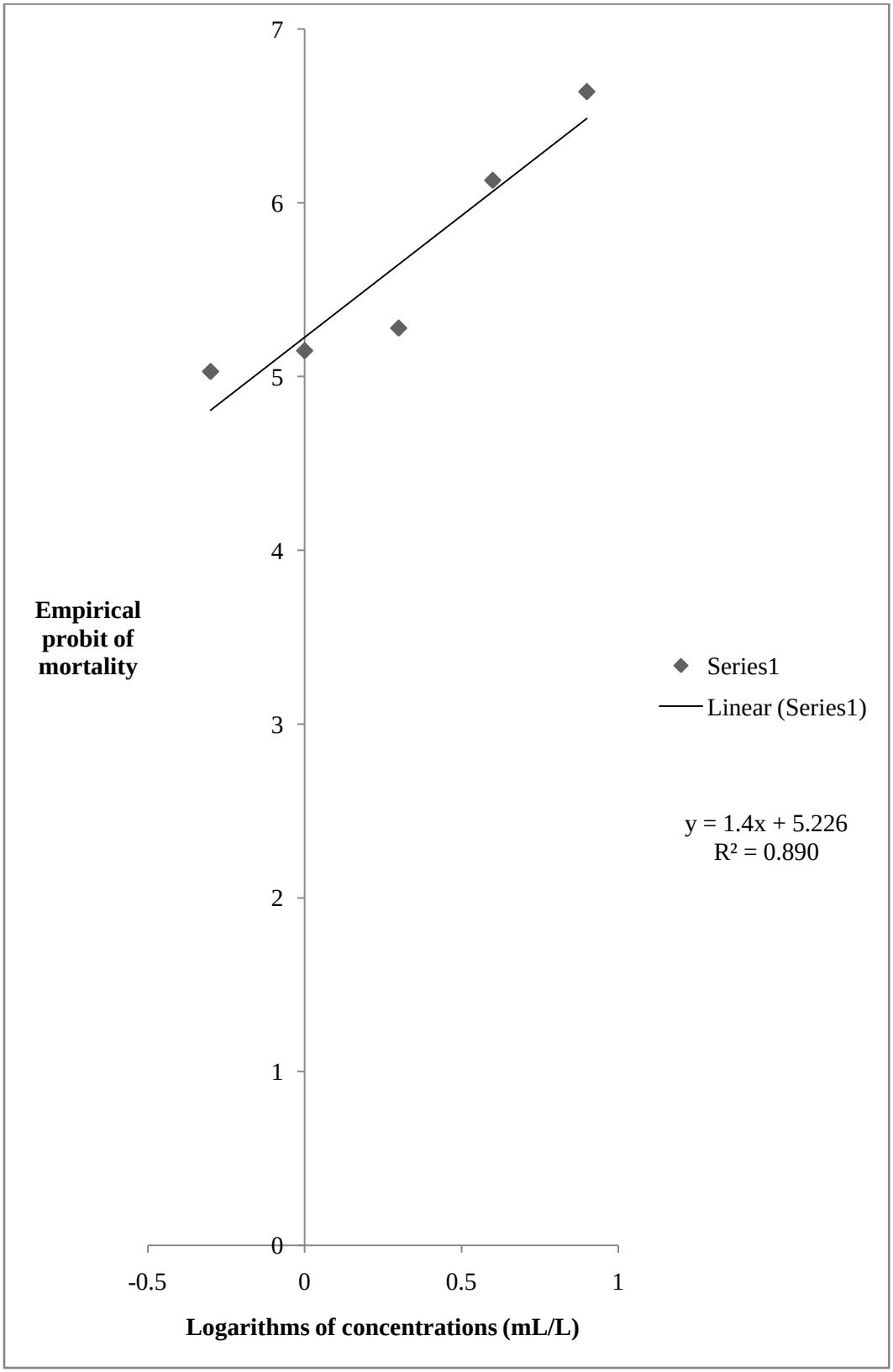


Figure 4.11: Empirical probit of mortality of *Culex quinquefasciatus* larvae in laboratory bioassay against the logarithms of the concentration of *Tamarindus indica* seed extract.

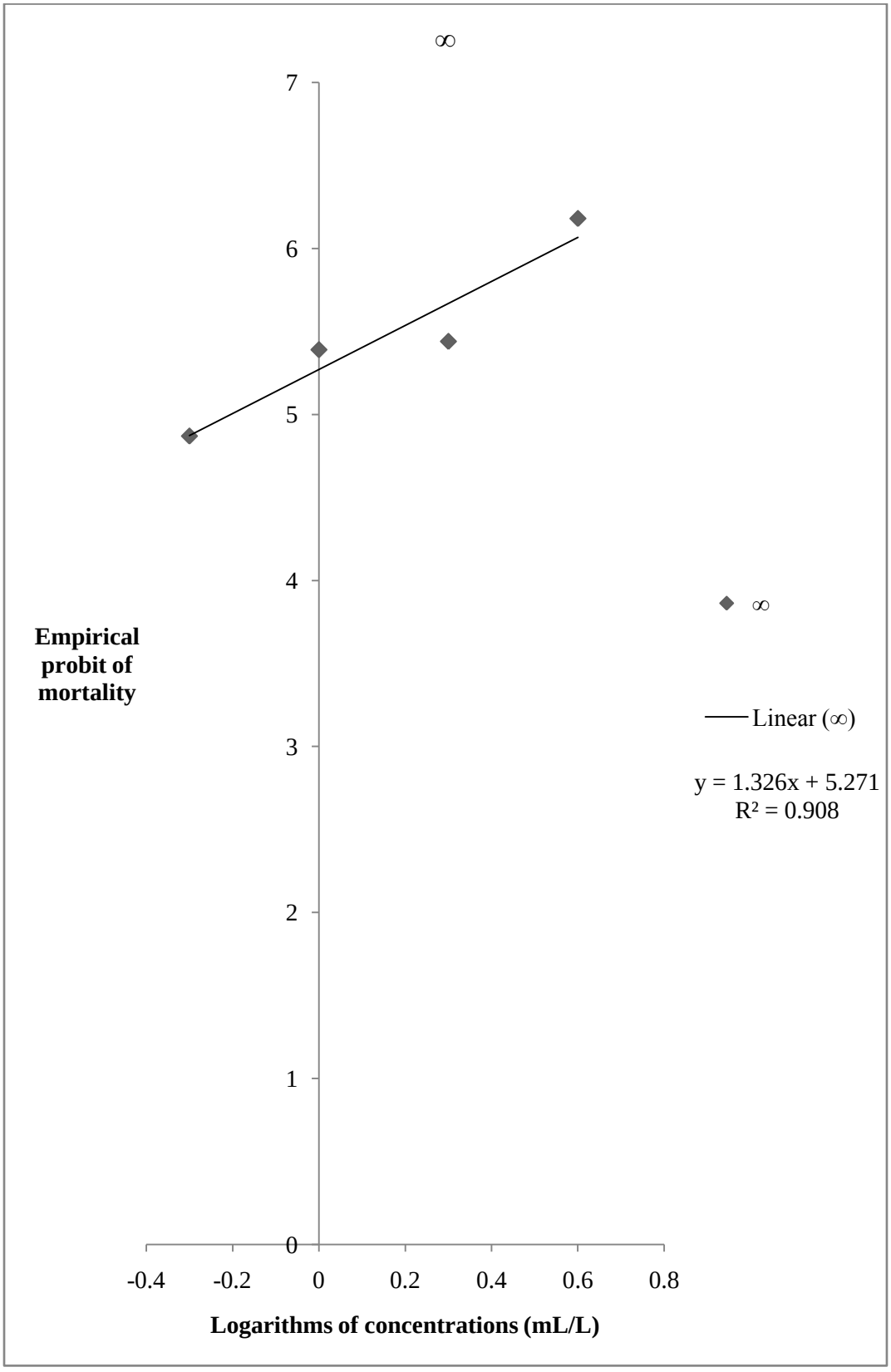


Figure 4.12: Empirical probit of mortality of *Culex quinquefasciatus* larvae in simulated field bioassay against logarithms of the concentrations of *Tamarindus indica* seed extract.

Table 4.15: Comparative larvicidal efficacies of *Terminalia catappa* and *Tamarindus indica* seed extracts against the three mosquito species

		Laboratory				Simulated field			
		Mean mortality±SE			p- value	Mean mortality±SE			p- value
Seed extract	Conc (mL/L)	*Ae. aegypti*	*Ae. vittatus*	*Cx. quinquefasciatus*		*Ae. aegypti*	*Ae. vittatus*	*Cx. quinquefasciatus*	
T. catappa	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.00	0.00 ± 0.00^{a}	0.33 ± 0.33^{a}	0.00 ± 0.00^{a}	0.421
	0.5	1.33 ± 0.33^{b}	4.00 ± 0.58^{a}	3.33 ± 0.33^{a}	0.011	5.00 ± 1.15^{a}	4.67 ± 0.33^{a}	5.00 ± 1.15^{a}	0.961
	1	1.67 ± 0.33^{b}	6.33 ± 0.88^{a}	4.67 ± 1.20^{a}	0.025	5.33 ± 1.20^{a}	5.33 ± 0.33^{a}	5.33 ± 1.20^{a}	1.00
	2	3.67 ± 0.88^{b}	9.67 ± 0.67^{a}	9.00 ± 1.53^{a}	0.015	5.33 ± 0.88^{b}	9.00 ± 0.58^{a}	5.33 ± 0.88^{b}	0.025
	4	7.33 ± 0.33^{b}	13.33 ± 0.33^{a}	16.67 ± 0.88^{a}	$0.000(7.632\times10^{-5})$	8.00 ± 1.00^{b}	11.33 ± 0.33^{a}	8.00 ± 1.00^{b}	0.047
	8	20.33 ± 0.88^{a}	8.33 ± 0.33^{b}	22.67 ± 0.33^{a}	$0.000(4.482\times10^{-6})$	13.67 ± 0.33^{a}	11.33 ± 0.88^{b}	13.67 ± 0.33^{a}	0.043
	Total	5.72 ± 1.69^{a}	6.94 ± 1.04^{a}	9.39 ± 1.95^{a}	0.267	6.22 ± 1.04^{a}	7.00 ± 0.98^{a}	6.22 ± 1.04^{a}	0.823
T. indica	0	0.67 ± 0.33^{a}	0.67 ± 0.67^{a}	0.00 ± 0.00^{a}	0.490	1.67 ± 0.88^{a}	0.00 ± 0.00^{a}	1.67 ± 0.88^{a}	0.244
	0.5	9.67 ± 2.96^{a}	2.00 ± 1.00^{b}	12.67 ± 1.20^{a}	0.019	5.67 ± 1.33^{a}	7.33 ± 0.33^{a}	5.67 ± 1.33^{a}	0.509
	1	10.33 ± 0.88^{a}	3.33 ± 0.33^{b}	14.00 ± 2.52^{a}	0.007	12.33 ± 1.33^{a}	12.33 ± 1.45^{a}	12.33 ± 1.33^{a}	1.000
	2	13.67 ± 1.86^{a}	7.33 ± 1.20^{b}	15.33 ± 1.45^{a}	0.022	18.00 ± 2.52^{a}	16.67 ± 0.88^{a}	18.00 ± 2.52^{a}	0.879
	4	14.33 ± 0.88^{b}	15.00 ± 1.15^{b}	21.67 ± 0.88^{a}	0.003	18.33 ± 2.91^{a}	18.33 ± 0.67^{a}	18.33 ± 2.91^{a}	1.000
	8	24.67 ± 0.33^{a}	13.67 ± 0.67^{b}	23.67 ± 0.33^{a}	$0.000(5.545\times10^{-6})$	23.67 ± 0.67^{a}	19.00 ± 0.58^{b}	23.67 ± 0.67^{a}	0.003
	Total	12.22 ± 1.81^{a}	7.00 ± 1.39^{b}	14.56 ± 1.91^{a}	0.009	13.28 ± 1.95^{a}	12.28 ± 1.67^{a}	13.28 ± 1.95^{a}	0.908

Means followed by the same superscript in the same row are not significantly different ($p>0.05$).

(22.67); the mean mortality of the latter two species were not different significantly (p□ 0.05). Under the laboratory condition, total (overall) mean mortality of the larvae of the three species of mosquito against *T. catappa* seed extract did not differ significantly (p=0.267; i.e. p□0.05). On Table 4.15, *T. indica* seed extract under laboratory condition at 0.5mL/L to 2.0mL/L and 8.0mL/L produced significantly (p□0.05) lower mean larval mortality of *Ae. vittatus* than either of *Ae. aegypti* and *Cx. quinquefasciatus*; mean larval mortality of the latter two mosquito species were not different significantly (p□0.05) at these ranges of concentrations. At 4.0mL/L of *T. indica* seed extract, under laboratory condition, mean larval mortality of *Cx. quinquefasciatus* (21.67) was significantly (p□0.05) higher than those of *Ae. aegypti* (14.33) and *Ae. vittatus* (15.00); those of these latter two species not different significantly (P□ 0.05). The total (overall) mean larval mortality of *Ae. vittatus* (7.00) in *T. indica* seed extract was significantly (p□0.05) lower than those of *Ae. aegypti* (12.22) and *Cx. quinquefasciatus* (14.56); total mean mortality of the latter two species did not differ significantly (p□0.05) (Table 4.15).

4.4.2 Under simulated field conditions

The mean control mortality of the three species of mosquito did not differ significantly (p□0.05) for experiments with both *T. catappa* and *T. indica* under the simulated field condition (Table 4.15). Mean larval mortality of the three species of mosquito did not differ significantly (p□0.05) when treated with 0.5mL/L and 1.0mL/L of *T. catappa* seed extract. At 2.0mL/L and 4.0mL/L of *T. catappa* seed extract treatment, mean larval mortality of *Ae. vittatus* were significantly (p□0.05) higher than those of *Ae. aegypti* and *Cx. quinquefasciatus*; those of the latter two species did not differ significantly (p□0.05). At 8.0mL/L treatment of *T. catappa*, mean larval mortality of *Ae. vittatus* (11.33) was significantly (P□ 0.05) lower than those (13.67) of*Ae. aegypti* and *Cx. quinquefasciatus*.

However, the overall mean larval mortality of the three species of mosquito did not differ significantly (p= 0.823; i.e. p□0.05) (Table 4.15). Exposures of the three larval species of mosquito to seed extract of *T. indica* at concentrations of 0.5mL/L to 4.0mL/L did not produce significant (p□0.05) difference in their mean larval mortality. However, the mean larval mortality of *Ae. vittatus* at 8.0mL/L (19.00) was significantly (p= 0.003; i.e. p□0.05) lower than those (23.67) of *Ae. aegypti* and *Cx. quinquefasciatus*. The overall mean larval mortality of the three species of mosquito under simulated field exposures to *T. indica* seed extract did not differ significantly (p=0.908; i.e. p□0.05) (Table 4.15).

4.5 Comparative Larvicidal Efficacies of *Terminalia catappa* and *Tamarindus indica* Seed Extracts Against the three Mosquito Species Under Laboratory and Simulated field Study Conditions

Comparison of the larvicidal efficacies of the seed extracts of *Terminalia catappa* and *Tamarindus indica* against the three mosquito species under laboratory and simulated field conditions are shown on Table 4.16. Under laboratory condition, mean larval mortality with *T. catappa* ranged insignificantly (p=0.267; i.e. p□0.05) from 5.72 for *Ae. aegypti* to 9.39 for *Cx. quinquefasciatus*. The mean larval mortality under simulated field condition with *T. catappa* ranged insignificantly (p=0.823; i.e. p□0.05) from 6.22 for both *Ae. aegypti* and *Cx. quinquefasciatus* to 7.00 for *Ae. vittatus*. Mean mortality of *Ae. aegypti* larvae on exposure to extract of *T. catappa* seeds did not differ significantly (p= 0.802; i.e p□0.05) between both study conditions. Mean mortality of *Ae. vittatus* larvae due to exposure to seed extract of *T. catappa* between both study conditions did not differ significantly (p=0.967; i.e. p□0.05). Similarly, the mean mortality of *Cx. quinquefasciatus* larvae on exposure to seed extract of *T. catappa* between both study conditions did not differ significantly (p=0.161; i.e. p□0.05) (Table 4.16). On this table, the mean mortality of *Ae. vittatus* (7.00) due to exposure to *T. indica* seed extract under

Table 4.16: Comparison of larvicidal efficacies of *Terminalia catappa* and *Tamarindus indica* seed extracts against the three mosquito species under laboratory and simulated field bioassay conditions.

Condition	Seed extract	Mean larval mortality ± SE			p-value
		Ae. aegypti	*Ae. vittatus*	*Cx. quinquefasciatus*	
Laboratory	*T. catappa*	5.72±1.69[a]	6.94±1.04[a]	9.39±1.95[a]	0.267
Simulated field	*T. catappa*	6.22±1.04[a]	7.00±0.98[a]	6.22±1.04[a]	0.823
	p- value	0.802ns	0.967ns	0.161ns	
Laboratory	*T. indica*	12.22±1.81[a]	7.00±1.39[b]	14.56±1.91[a]	0.009
Simulated field	*T. indica*	13.28±1.95[a]	12.28±1.67[a]	13.28±1.95[a]	0.908
	p- value	0.693 ns	0.021*	0.642ns	
Laboratory	*T. catappa*	5.72±1.69	6.94±1.04	9.39±1.95	
	T. indica	12.22±1.81	7.00±1.39	14.56±1.91	
	p-value	0.013*	0.973ns	0.067ns	
Simulated field	*T. catappa*	6.22±1.04	7.00±0.98	6.22±1.04	
	T. indica	13.28±1.95	12.28±1.67	13.28±1.95	
	p -value	0.003**	0.010**	0.003**	

Means followed by the same alphabet along same row are not significantly (P□ 0.05) different; ns=notsignificantly different (P□ 0.05); *=significantly different (p□ 0.05);**=highly significantly different (p≤0.01).

laboratory condition was significantly (P=0.009; i.e. p□0.05) lower than those of *Ae. aegypti* (12.22) and *Cx. quinquefasciatus* (14.56); mean mortality of the latter two species did not differ significantly (p□0.05). Under the simulated field condition, the mean mortality of the larvae on exposure to *T. indica* seed extract ranged insignificantly (p=0.908; i.e. p□0.05) from 12.28 for *Ae. vittatus* to 13.28 for both *Ae. aegypti* and *Cx. quinquefasciatus*. There were no significant (p=0.693 and p=0.642) difference in mean larval mortality of *Ae. aegypti* and *Cx. quinquefasciatus* respectively due to exposure to *T. indica* seed extract between laboratory and simulated field study conditions. The mean larval mortality of *Ae. vittatus* due to exposure to *T. indica* seed extract was significantly (p=0.021; i.e. p□0.05) higher under simulated field condition (12.28) than under the laboratory condition (7.00) (Table 4.16).

Comparison of susceptibility of each of the three mosquito species to extracts of both plants under similar (either laboratory or simulated field) study condition are shown on the lower half of Table 4.16. In laboratory bioassays, mean mortality of *Ae. aegypti* larvae was significantly (p=0.013; i.e p□0.05) higher on exposure to *T. indica* seed extract (12.22) than on exposure to *T. catappa* extract (5.72). Exposure of *Ae. vittatus* larvae to seed extracts of *T. catappa* and *T. indica* in the laboratory did not produce significant (p=0.973; i.e. p□0.05) difference in mean larval mortality. Similarly, exposure of *Cx. quinquefasciatus* larvae to seed extracts of *T. catappa* and *T. indica* in the laboratory did not produce significant (p=0.067; i.e. p□0.05) difference in mean larval mortality (Table 4.16). Under the simulated field conditions, mean larval mortality of each of the three mosquito species was significantly (p□0.05) higher on exposure to *T. indica* than when exposed to *T. catappa* seed extract.

CHAPTER FIVE
5.0 DISCUSSION

This study has demonstrated that petroleum ether extracted seeds of *Terminalia catappa* and *Tamarindus indica* yielded oils with such characteristics as acid, percentage free fatty acid, iodine, peroxide and saponification values as well as refractive indices. The proportions of acid and free fatty acids were significantly higher in *T. indica* seed oil than *T. catappa* seed oil; but the proportion of iodine value of *T. catappa* seed oil was more than twice that of *T. indica* seed oil. However, the peroxide and saponification values of *T. indica* seed oil were higher, but not significantly, than those of *T. catappa* seed oil (Table 4.1). These were indications of innate differences in the characteristics of both seed oils which could have affected their biological activities as mosquito larvicides. Oils have been described as naturally occurring esters of long straight-chain carboxylic acids that belong to the saponifiable group of lipids (Atinafu and Bedemo, 2011).

Acid value is a measure of the free fatty acids in oil and the higher the acid value, the higher the level of free fatty acid which translates into decreased oil quality due to higher tendency to become rancid, that is off-flavour (Tamzid *et al*., 2007). Low value of seed oil acidity has been associated with high resistance to hydrolysis as reported by Gautam *et al*. (2013). Thus the seed oil of *T. catappa*, with significantly (P□0.05) lower acid value than the seed oil of *T. indica*, could be more stable when applied in water against mosquito larvae.

Hydrolysis is a double decomposition reaction with water, and the implication of a higher resistance to hydrolysis by *T. catappa* seed oil due to its lower acid value could make it persistent in water. This could portend positive larvicidal attributes for the seed oil of *T. catappa*, as it may likely act for prolonged period in aquatic habitats (Aremu *et al*. 2006).

Higher percentage free fatty acids in the seed oil of *T. indica* than seed oil of *T. catappa* could be a reflection of the total fatty acid profiles in both oils. While five fatty acids were detected in the seed oil of *T. catappa*, nine were detected in the seed oil of *T. indica* (Table 4.2). Oils are constituted of triacylglcerol molecules, mainly formed by unsaturated (oleic, linoleic, linolenic acids etc.) and saturated (myristic, palmitic, stearic acids etc) fatty acids esterified to glycerol unit (Barison *et al*., 2010). High degree of saturated fatty acids enables oil to be stable against oxidative rancidity and confers a longer shelf-life (Nwinuke and Barine, 2009).

Evidence are emerging that the fatty acid contents of plant oils constitute the active compounds responsible for their lethal effects on mosquito larvae. Herada *et al*., (2000) reported the isolation of a mixture of unsaturated fatty acid (oleic, linoleic and linolenic acids) and saturated fatty acids (myristic, palmitic and stearic acids) from *Oscillatoria agardhii* which were tested aganist the larvae of *Aedes albopictus* mosquito. Of these hydrophobic compounds, authentic unsaturated fatty acids were showed to be toxic whereas saturated ones were not active.

In a similar observation, Ramsewak *et al*. (2001) showed that linoleic and oleic acids compounds isolated from seed hexane extract of *Dirca palustris* were insecticidal against fourth instar larvae of *Aedes aegypti*. Similarly, oleic and linoleic fatty acids from *Citrullus colocynthis* have been identified as mosquito larvicidal compounds against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*; with oleic acid having lower LC_{50} values than linoleic acid (Rahuman *et al*., 2000). The hexadecanoic acid (palmitic acid) identified in *T. catappa* seed extract in this study has been found by Rahuman *et al.,* (2000) to be an effective larvicide from the leaves of *Feronia limonia,* against fourth instar larvae of *Cx. quinquefascitus, An. stephensi* and *Ae. aegypti*. It was

also demonstrated that oil extract of the salt marsh mangrove plant, *Sesuvium portulacastum*, contained fatty acid such as oleic acid and hexadecanoic acid which produced high larval mortality of *Anopheles stephensi* and *Aedes aegypti* (Syed Ali *et al*., 2013). Recently, Perumalsamy *et al*., (2015) showed that oleic acid and palmitic acid isolated from *Millettia pinnata* seed were effective against *Culex pipiens palens*, *Aedes aegypti* and *Aedes albopictus* larvae. They observed that the unsaturated fatty acids (oleic acid, linoleic acid, linolenic acid and elaidic acid) were more pronounced in toxicity to the three mosquito species larvae than the saturated fatty acids (arachidic acid, behenic acid, palmitic acid and stearic acid). The toxicity of oleic, linoleic and linolenic acids did not differ significantly (Perumalsamy *et al*., 2015). These was observed in the seed oil extract of *T. indica* which had unsaturated fatty acids (linolelaidic acid, elaidic acid, ricinoleic acid, eicosenoic acid and heneicosanoic acid) in this study than *T. catappa* seed oil extract of unsaturated fatty acids (linolelaidic acid and oleic acid). Perumalsamy *et al*., (2015) also suggested the possible use of unsaturated fatty acids as environmentally safe and effective mosquito larvicides, and that structural characteristics, such as the degree of saturation, the side chain length of fatty acid and geometric isomerism could play a role in determining fatty acids toxicity to mosquito larvae.

The acid values of the seed oil of *T. catappa* (10.66mgKOH/g) and *T. indica* (16.60mgKOH/g) were comparable to those of rapeseed K-16 (10.38) and Niger seed K-7 (12.06) but higher than those of cottonseed oil (7.57), rapeseed (7.01), Niger seed K-16 (8.69), olive oil (7.29), palm oil (4.77) and sunflower oil (5.05) (Atinafu and Bedemo, 2011). Unrefined vegetable oils were known to have higher acid values than refined oils (Rajko *et al*., 2010). The higher iodine value of *T. catappa* seed oil over that of *T. indica* was suggestive of the preponderance of unsaturated fatty acids in the flower as indicated by Akubugwo and Ugbogu (2007). Highly unsaturated oils with higher iodine values, as

seen with *T. catappa*, are drying oils with the implication that they are more volatile. Thus, *T. catappa* oil could evaporate faster when applied as mosquito larvicide and this can negatively affect its larvicidal prospect than the seed oil of *T. indica*.

Atinafu and Bedemo (2011) reported that peroxide value is a measure of oxidation during storage and the freshness of lipid matrix. It is also a useful indicator of early stages of rancidity occurring under wild condition; a measure of the primary lipid oxidation products and maximum of 10 meq/kg fat is the permissibility level for vegetable oil deterioration. The peroxide values of the seed oil of *T. catappa* (9.27 meq/kg fat) and *T. indica* (12.88 meq/kg fat) were comparable but high; especially for the latter which was above the maximum 10 meq/kg fat permissible level for vegetable oil deterioration. This is an indication that the seed oil of *T. indica* could deteriorate with storage. One of the most important parameters that influence lipid oxidation is the degree of unsaturation of its fatty acids. When double bonds of unsaturated fats are oxidized, peroxides are among the oxidation products formed. High peroxide value is an indication of oxidation level and the greater the peroxide value, the more oxidized the oil (http:www.bioriginal.com). Peroxide values of the seed oils of *T. catappa* and *T. indica* used in this study were higher than those of cottonseed oil (0.60), rapeseed (2.10), rapeseed K-16 (3.50), Niger seed K-16 (3.80), Niger seed K-7 (3.10), olive oil (1.70), palm oil (2.20) and sunflower oil (2.07) which indicates that the seed oil extracts used in this study (*T. catappa* and *T. indica*) have high tendency of oxidizing with storage.

The saponification values obtained for both *T. catappa* and *T. indica* were lower than the standard permissibility level of 190-209 mgKOH/g; which suggest that they contained high molecular weight long chain fatty acids and are therefore not suitable for soap making and unsuitable for human nutrition (Akinhanmi and Atasie, 2008). Saponification

value is an indication of the molecular weights of triglycerides in oils. Higher saponification value indicates high proportion of lower fatty acids, since saponification value is inversely proportional to average molecular weight or chain length of the fatty acids (Muhammad *et al*., 2011). Therefore the shorter the average chain length (C_4-C_{12}) the higher is the saponification number (Tamzid *et al*., 2007). From the multitude of compounds detected in the seed extracts of *T. catappa* and *T. indica* (Table 4.2), it is evident that these crude extracts possess several potential active ingredients that could have been responsible for observed larvicidal effects against the three mosquito species. Crude plant extracts often consist of complex mixture of active compounds and the use of complete mixture may act synergistically to show greater overall bioactivity compared to the individual constituents (Sumroiphon *et al*., 2006). It has been advocated that insect resistance to crude plant extract is much less likely to develop with mixtures of active compounds; and these reasons support the use of crude chemically unrefined plant extracts, containing mixtures of bioactive plant compounds with different modes of action rather than the use of pure individuals (Mandal, 2012; Samidurai, 2012). Rajasekaran and Duraikannan (2012) also attributed observed differences of different formulations of plant extracts to inhibit developmental stages of *Ae. aegypti* to synergistic effect of phytochemical constituents of the extracts.

Refractive indices of the oils shows there is significant difference between *T. catappa* (1.46) and *T. indica* (1.48). This is comparable with the refractive indices obtained in the study conducted by Akpan *et al*. (2006) in which refractive index of crude castor oil is 1.4686 and refined castor oil is 1.467. Refractive index is the ratio of speed of light at a defined wavelength to its speed in the oil itself. This value varies with wavelength and temperature, the degree and type of unsaturation, the type of substitutions of component

fatty acids and with accompanying substances. It is also used in quality c0ntrol to check for purity of the oil (Aremu *et al*. 2015).

The seed oils of *T. catappa* and *T. indica* have been shown to produce excellent larvicidal effects against *Ae. aegypti*, *Ae. vittatus* and *Cx. quinquefasciatus* species of mosquito in laboratory and simulated field conditions. The results of this study have established that almost all treatments of the oils against the larvae of the three mosquito species produced significant mortality over the controls. These were sufficient to attribute unequivocally the observed larval mortality to the seed extracts treatments. The study has further demonstrated the ability of the seed extracts of tropical plants to elicit larvicidal effects against noxious mosquito species and their potential to be adopted for mosquito control operations. Oils block the respiratory siphon of the larvae resulting in their suffocation and eventual death (Rotimi *et al*., 2011).

In this study it was observed that, mortality rates of larvae in almost the three species of mosquito were concentration (dose) dependent for the two seed extracts. Okumu *et al.* (2007) reported correspondingly high larvicidal activity at high dosage of neem oil against *Anopheles gambiae* vector of malaria. Adeleke *et al.* (2009) reported increase in percentage mean larval mortality of *Ae. aegypti* with increased concentration of the oils of *Pterocarpus santalinoides* and tropical *Manihot* species. Fayemiwo *et al.* (2014) also observed dose dependent effects of the oils of *Syzygium aromaticum* and *Pinus sylvestris* against fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus.* Similar observations of dependence of mortality of larval *Aedes aegypti* on increasing concentration of extract of *Abutilon indicum* was attributed to homogenous distribution of active compound in the extract. Adebote *et al*. (2011a) also reported a dose dependent mortality response of *Cx. quinquefasciatus* larvae to treatments of *Bobgunnia madagascariensis* (snake bean plant)

stem bark extract. This study proceeded to conduct bioassays beyond the usual laboratory confines in order to subject the larvae to extracts under situations very close to conditions in natural breeding habitats where practical controls would inevitably be done.

In general, comparison of larvicidal efficacies of both *T. catappa* and *T. indica* against each of the three mosquito species between laboratory and simulated field conditions did not show appreciable differences on mean larval mortality. These observations could be interpreted that both plant extracts acted on the mosquito larvae independent of the environmental conditions that could have operated at both study situations, it has however been stated that only very few of the several plants from different families tested and confirmed for insecticidal 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 mosquito at larval stage with plant extracts (Samuel *et al*., 2012b). Targeting mosquitoes as larvae has the advantage of dealing with a relatively immobile pest without the need to use added pesticides for adult mosquito control (Samuel *et al*., 2012b).

Dose - mortality relationships of the seed extracts to the larvae are typically expressed as LC_{50}; values which define the toxin concentrations required to kill 50% of the test larval populations in a specified period. According to Jyoti and Brewer (1999), the lower the LC_{50} value, the greater the toxicity and larvicidal activity of the larvicide used. The LC_{50} obtained for seed oils of *T. catappa* (range: 2.275 - 11.143mL/L) and *T. indica* (range: 0.625 - 4.842mL/L) against the three mosquito species in this study were indications of the larvicidal superiority of the latter over the former. These values were essentially lower than those obtained and reported for seed extracts of other plants tested against similar and or different species of mosquito by other workers. These are suggestive that

seed extracts of *T. catappa* and especially *T. indica* could possess better mosquito larvicidal attributes than seed extracts of some other plants that have been investigated.

The LC_{50} values of 42.17mL/L, 30.32mL/L and 35.95mL/L reported by Elangovan *et al.* (2008) for plant oil formulation against *Ae. aegypti, Anopheles stephensi* and *Cx. quinquefasciatus* respectively, were higher than those obtained in this study. However, Abdelouaheb *et al.* (2009) obtained comparative LC_{50} of 0.35mL/L for the seed oil of neem tree against some mosquito larvae in Algeria. Of the several varieties of *Citrus* seed extracts tested for mosquito larvicidal effects by Waseem *et al.* (2010), the least and highest LC_{50} values of 119.99mL/L and 2069.12mL/L obtained for rough lemon and narangi varieties respectively were higher than the values obtained for both seed extracts in this study.

Souza *et al.* (2011) reported LC_{50} values of 9.8µg/mL and 15.2µg/mL for the seed extract of *Myracrodruon urundeuva,* and 580.9µg/mL and 661.6µg/mL for the seed extract of *Schinopsis brasiliensis,* against field collected and insecticide - susceptible strains of *Ae. aegypti* respectively. Zewdneh *et al.* (2011) reported LC_{50} of 92.09mL/L for crude methanol extract of the seeds of *Jatropha curcas* against *Anopheles arabiensis. T. catappa* seed oil extract in this study produced LC_{50} of 4.84mL/L and 10.884mL/L against *Ae. aegypti*; 11.43mL/L and 9.099mL/L against *Ae. vittatus*; 2.275mL/L and 3.055mL/L against *Cx. quinquefasciatus* under laboratory and simulated field condition respectively which were higher than the LC_{50} of the essential oil of *Plectranthus glandulosus* which had 2.66 mL/L and 7.37 mL/L against *Ae. aegypti* and *An. gambiae* but lower than the LC_{50} of 43.16mL/L against *Cx. quinquefasciatus* respectively as reported by Pierre *et al.*(2014). Essential oil of *Calistemon rigidus* was also reported by Pierre *et al.* (2014) to give respective LC_{50} of 66.67, 99.61 and 176.81 mL/L against *Ae.*

aegypti, An. gambiae and *Cx. quinquefasciatus.* Perumalsamy *et al.* (2015) reported LC_{50} values of 24.19, 27.70 and 61.30mg/L for the methanol extract of the seeds of *Millettia pinnata* against insecticide-susceptible *Culex pipiens pallens, Ae. aegypti* and *Ae. albopictus* respectively. The extract of *T. indica* which showed a lower LC_{50} value after 24hours, suggest its high efficacy against the test organisms.

The trio of *Ae. aegypti, Ae. vittatus* and *Cx. quinquefasciatus* whose larvae were tested and found to be highly susceptible to the seed oils of *T. catappa* and *T. indica* in this study are among the medically important pest species with high anthropophilic nature and capability to transmit human diseases. *Aedes aegypti* is vector of several arboviruses, especially those responsible for yellow fever and dengue; it is also capable of transmitting the zoonotic parasitic nematode causing human and canine dirofilariasis. *Aedes aegypti* has been shown to breed profusely in human peridomestic environments and in several artificial containers within and around Zaria, Nigeria (Adebote *et al.*, 2006; 2011b). The seed oils of *T. catappa* and *T. indica,* with the excellent larvicidal prospect displayed, could be used in population management and disease transmission reduction strategies against *Ae. aegypti* breeding in discarded automobile tyres and allied peridomestic receptacles. Likewise, the oils could be used as larvicides to stem the breeding and pest status of *Ae. vittatus*, a potential vector of arboviruses including that for yellow fever; known to breed extensively in discrete rock pool habitats around Zaria (Adebote *et al.,* 2008). *Culex quinquefasciatus* breeds in diverse aquatic habitats especially with high organic contents and rests indoors in close proximity to humans, where it constitutes biting nuisance and possesses the potential of transmitting filariasis and other human and animal ailments (Adebote *et al.*, 2011a). The larvicidal prospects of the seed oils of *T. catappa* and *T. indica* against *Cx. quinquefasciatus* could be extended to practically eliminate its breeding in the diverse aquatic habitats. It will be necessary to

investigate the larvicidal prospects of these oils in organically polluted waters, such as street side gutters and septic tanks, which *Cx. quinquefasciatus* has been preferentially associated with.

Both *T. catappa* and *T. indica* have been found to possess mosquito larvicidal prospects in this study. The sustainability of their mosquito control potentials needs to be discussed in line with other known ethnobotanical applications of both plants. The seed oils of both plants are not amongst the vegetable oils of commerce and they are not considered to be of biodiesel grade. These could reduce demand driven competition if they are eventually adopted for the manufacture of pesticides. Other complementary uses of both plants may not preclude their adoption for mosquito control due to their availability and prospect for mass cultivation, low cost of procurement and simple technology associated with their extraction for mosquito larvicidal uses.the use of essential oils is a fast, simple and practical assy (Parra *et al*., 2001).

CHAPTER SIX
6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY AND CONCLUSIONS

Extracts of the seeds of *Terminalia catappa* and *Tamarindus indica* yielded oils with respective acid values of 10.66mg KOH/g and 16.60mg KOH/g; respective percentage free fatty acid (mg KOH/g) of 5.33 and 8.41; respective iodine values (meq/kg fat) of 39.35 and 16.18; respective peroxide values (meq/kg fat) of 9.27 and 12.88; respective saponification values (mL/kg) of 162.75 and 187.94 and respective refractive index of 1.46 and 1.48. A total of fourteen compounds, consisting of fatty acids and allied metabolites were detected, via GC-MS analyses, in the seed oil of *T. catappa*; ranging from 0.66% arachidic acid to 16.79% of the major compound- octadecahydro-2H-picen-3-one. A total of sixteen compounds were detected in the seed oil of *T. indica* and these ranged from 1.52% of ricinoleic acid to 15.30% of major compound- linolelaidic acid. Seven compounds, including cyclohexane,2-propenyl, decane, decane,-4-methyl, undecane, linolelaidic acid, stearic acid and arachidic acid were detected in varying degrees in the seed oil of both plants. Overt larvicidal activities of both seed extracts against the three species of mosquito could be attributed to their respective fatty acids profiles.

The seed extract of *T. catappa* showed very good larvicidal effects on the third instar larvae of *Aedes aegypti*, *Aedes vittatus* and *Culex quinquefasciatus* species of mosquito in both laboratory and simulated field bioassays. Extract of *T. catappa* seeds produced 81.33% and 54.67% mortality of *Ae. aegypti* larvae in laboratory and simulated field conditions respectively; and respective LC_{50} of 4.840mL/L and 10.884mL/L. extract of *T. catappa* seeds also produced 53.33% and 45.33% mortality of larval *Ae. vittatus* in

laboratory and simulated field conditions respectively; and respective LC_{50} of 11.143mL/L and 9.099mL/L. The seed extract of *T. catappa* also produced 90.67% and 80.00% mortality of larval *Cx. quinquefasciatus* under laboratory and simulated field conditions respectively; and respective LC_{50} of 2.27mL/L and 3.055mL/L.

Extract of the seeds of *Tamarindus indica* showed excellent larvicidal effects on third instar larvae of *Ae. Aegypti*, *Ae. vittatus* and *Cx. quinquefasciatus* species of mosquito under both laboratory and simulated field conditions. Extract of *T. indica* seeds produced 98.67% and 94.67% mortality of *Ae. aegypti* larvae in laboratory and simulated field conditions respectively; and respective LC_{50} of 1.248mL/L and 1.359mL/L. In addition, *T. indica* seed extract produced 60% and 76% mortality of *Ae. vittatus* larvae in laboratory and simulated field conditions respectively; and respective LC_{50} of 4.842mL/L and 1.191mL/L. Mortality of *Cx. quinquefasciatus* larvae due to *T. indica* seed extract were 94.67% and 100% under laboratory and simulated field conditions respectively; and respective LC_{50} of 0.690mL/L and 0.625mL/L. *Tamarindus indica* seed extract produced higher percentage larval mortality of two of the three (except *Ae. aegypti*) mosquito species under the simulated field than laboratory conditions.

In conclusion, *T. catappa* seed extract produced higher percentage larval mortality of the three mosquito species under the laboratory than the simulated field conditions. *Tamarindus indica* seed extract produced higher percentage larval mortality and had lower LC_{50} values against each of the three mosquito species under both study conditions and was therefore a more potent mosquito larvicidal agent than *T. catappa* seed extract. Via GC-MS analyses, a total of fourteen compounds were detected in the seed oil of *T. catappa* while a total of sixteen compounds were detected in the seed oil of *T. indica*.

6.2 Recommendations

- Seed extracts of *T. catappa* and *T. indica* showed excellent larvicidal effects on noxious mosquito species and could be adopted in mosquito control operations.
- The active fatty acids and other metabolites detected in both seed extracts need to be partitioned and tested for their individual effects on mosquito larvae in order to identify potent active compound(s).
- The mode(s) of action of the active principle(s) in the seed extract against the mosquito larvae needs to be ascertained.
- There is the need to test the larvicidal effect of the seed extracts against other vector mosquito species including *Anopheles* vector of malaria.
- The safety and activities of the seed extracts in natural aquatic breeding habitats of mosquitoes and their effects on non-target aquatic fauna need to be determined.
- The seed extracts, after scaling necessary environmental tests, could be adopted for use in mosquito control operations and as a replacement for synthetic chemicals so as to stem the several ills associated with their use in pest management.

REFERENCES

Abbott, W.S. (1925). A method for computing effectiveness of an insecticide. *Journal of Economic Entomology*, **18**: 265-267.

Abdelouaheb, A., Nassima, R. and Noureddine, S. (2009). Larvicidal activity of a neem tree extract (Azadirachtin) against mosquito larvae in the republic of Nigeria. *Jordan Journal of Biological Sciences*, **2**(1):15-22.

Adebote, D. A., Hassan-Adeyemi, M. M. and Atsukwei, B. T. (2011a). Larvicidal efficacy of solvent-extracted stem bark of *Bobgunnia madagascariensis* (Desv.) J.H. Kirkbr and Wiersema (Caesalpiniaceae) against *Culex quinquefasciatus* mosquito. *Journal of Applied Environmental and Biological Sciences*, **1**(7):101-106.

Adebote, D. A., Kogi, E., Oniye, S. J. and Akoje, F. (2011b). Epidemiological significance of breeding of mosquitoes in discarded automobile tyres in Zaria, northern Nigeria. *Journal of Communicable Diseases*, **43**(3): 183-192.

Adebote, D. A., Oniye, S. J. and Muhammed, Y. A. (2008). Studies on mosquitoes breeding in rock pools on inselbergs around Zaria, northern Nigeria. *Journal of Vector Borne Diseases*, **45**: 21–28.

Adebote, D. A., Oniye, S. J. and Ndams, I. S. and Nache, K. M. (2006). The breeding of mosquitoes (Diptera: Culicidae) in peridomestic containers and implication in yellow fever transmission in villages around Zaria, northern Nigeria. *Journal of Entomology*, **3**(2): 180-188.

Adeleke, M.A., Popoola, S.A., Agbaje, W.B., Adewale, B., Adeoye, M.D. and Jimoh, W.A. (2009*).* Larvicidal efficacy of seed oils of *Pterocarpus santalinoides* and Tropical Manihot species against *Aedes aegypti* and effects on aquatic fauna. *Tanzania Journal of Health Research,* **11**(4): 250-253.

Adoum, O.A. (2009). Determination of toxicity levels of some savannah plants using brine shrimp (BST). *Bayero Journal of Pure and Applied Sciences*, **2**(1):135-138.

Agbofor, K. N. and Nwachukwu, N. (2011). Phytochemical analysis and antioxidant property of leaf extract of *Vitex doniana* and *Mucuna pruriens*. *Biochemical Research International,* pp.4.

Ajayi, L.A., Oderinde, R.A., Kajogbola, D.O. and Uponi, J.I. (2006). Oil content and fatty acid composition of some underutilized legume from Nigeria. *Food Chemistry Journal*, **99**:115-120.

Akharaiyi, F.C., Ilori,R. M. and Adesida, J.A. (2011). Antibacterial effect of *Terminalia catappa* on some selected pathogenic bacteria. *International Journal of Pharmaceutical and Biomedical Research,* **2**(2): 64-67.

Akinhanmi, T.F. and Atasie, V.N. (2008). Chemical composition and physicochemical properties of cashew nut (*Anacardium occidentale*) oil and cashew nut shell liquid. *Journal of Agriculture Food and Environmental Science,* **2**:1-10.

Akpan, U.G., Jimoh, A. and Mohammed, A.D. (2006). Extraction, characterization and modification of castor seed oil. *Leonardo Journal of Sciences* **8**(8): 43-52.

Akubugwo, I.E. and Ugbogu, A.E. (2007). Physicochemical studies on oils from five selected Nigerian plant seeds. *Pakistan Journal of Nutrition*, **6**(1): 75-78.

Alkofahi, A. and Atta, A. H. (1999). Pharmacological screening of anti-ulcerogenic effects of some Jordanian medicinal plants in rats. .*Journal of Ethnopharmacology*, **67**: 341-348.

Amer, A. and Mehlkorn, H. (2006). Repellency effects of forty-one essential oils against *Aedes*, *Anopheles* and *Culex* mosquitoes. *Parasitology Research*,**199**: 478-490.

American Mosquito Control Association (2014). www.mosquitoworld.net/mosquitospecies 1120 Route 73, Suite 200 Mount Laurel, NJ amca@mosquito.org.

Amusan, A.A.S. and Oke, O.A. (2004). The toxicity of hexanolic extract of *Xylopia aethiopica* to laboratory reared larvae of *Culex quinquefasciatus*. Proceedings of the International Conference on Science and National Development. 109-112.

Ansari, M.A., Razdan, R.K., Tandon, M. and Vasudevan, P. (2000a). Larvicidal and repellent actions of *Dalbergia sissoo* Roxb. (F. Leguminosae) oil against mosquitoes. *Bioresource Technology*, **73**: 207-211.

Ansari, M.A., Vasudevan, P., Tandon, M. and Razdan, R.K. (2000b). Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. *Bioresource Technology*.**71**: 267-271.

Anyanwu, G. I., Amaefule, E. C. and Ngurukwem, C. (2001). Larvicidal effects of Lemon peels on mosquito larvae. *Journal of Aquatic Sciences*. **20**(2):111-114.

Association of Official Analytical Chemists. AOAC. (2001). Official methods of analysis, 17th ed., *Association of Official Analytical Chemists*. U.S.A.

Atinafu, D.G. and Bedemo, B. (2011). Estimation of total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir DAR. *Journal of Cereals and Oil Seeds*, **2**(6): 71-76.

Aremu, M.O., Ibrahim, H. and Bamidele, T.O. (2015). Physiocochemical characteristics of the oils extracted from some Nigerian plant foods- A review. *Chemical and Process Engineering Research*, **32**: 36-52.

Aremu, M.O., Olonisakin, A., Bako, D.A. and Madu, P. C. (2006). Compositional studies and physicochemical characteristics of cashew nut (*Anarcadium occidentale*) flour. *Pakistan Journal of Nutrition*, **5**(4): 328-333.

Barison, A., Pereira da Silva, C. W., Campos, F. R., Simonelli, F., Lenz, C. A. and Ferreira, A. G. (2010). A simple methodology for the determination of fatty acid composition in edible oils through HNMR spectroscopy. *Magnetic Resonance in Chemistry*, **48**(8): 642-650.

Barku, V.Y.A., Nyarko, H.D. and Dordumu, P. (2012). Studies on the physicochemical characteristics, microbial load and storage stability of oil from indian almond nut (*Terminalia catappa* L.). *Food Science and Quality Management*. **8**: 2224-6088.

Becker, N., Petric, D., Zgomba, M., Boase, C., Dahi, C., Lane, J. and Kaiser, A. (2003). Mosquitoes and their control, *Springer Science and Business Media*, New York. 205-206.

Bhattacharya, S., Bal, S., Mukherjee, R.K. and Bhattacharya, S. (1997). Kinetics of tamarind seed hydration. *Journal of Food Engineering*. **33**: 29-138.

Brattsten, L. B. and Hamilton, G. C. (2012). Insecticides recommended for mosquito control in new Jersey. Department of Entomology, Rutgers, New Jersey Agricultural Experiment Station, Publication No. P-08001-01-12. 1-18.

Caluwé, E.D., Halamová, K. and Damme, V. P. (2010). *Tamarindus indica* L. – A review of traditional uses, phytochemistry and pharmacology, *AFRIKA* focus, **23**(1): 53-83.

Carvalho, A.F.U., Melo, V.M.M., Craveiro, A,A,. Machado, M.I.L., Bantaim, M.B., Rabelo, E.F. 2003). Larvicidal activity of the essential oil from *Lippia sidoides* Cham, against *Aedes aegypti* L. *Memorias Institute Oswaldo Cruz*, **98**; 569-571. ISSN 1678-8060.

Centers for Disease Control and Prevention, Dengue Branch,1324 CalleCañada, San Juan, Puerto Rico.

Cetin, H., Gokoglu, M. and Emre, O. (2010). Larvicidal activity of the extract of seaweed, *Caulerpa scalpelliformis*, against *Culex pipiens*. *Journal of the American Mosquito Control Association.* **26**(4): 433-435.

Cheng, S. S., Chang, H. T., Chang, S. T, Isai, K. H. and Chen, W. J. (2003). Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aeypgti* larvae. *Bioresource Technology*, **89**: 99-102.

Christophers, S. R. (1960). *Aedes aegypti* (L) '*The yellow fever mosquito': its life history, bionomics and structure.* Cambridge University Press, London. pp.195- 200

Corbel, V., Duchon, S., Zainm, M. and Hougand, J.M. (2004). Dinotefuran: A potential neonicotinoid insecticide against resistant mosquitoes. *Journal of Medical Entomology*, **41**: 712-717.

Cutwa-Francis, M.M. and O'meara, G.F. (2007). *An identification guide to the common mosquitoes of Florida*. Florida Medical Entomology Laboratory http://fmel.ifas.ufl.edu

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

Darsie-Jr, R.F., Vlach, J.J. and Fussell, E.M. (2002). New addition to the mosquito fauna of the United States, *Anopheles grabhamii* (Diptera; Culicidae). *Journal of Medical Entomology*, **39**: 430-431.

Darise-Jr, R.F. and Ward, R.A. (2005). *Identification and geographical distribution of the mosquitoes of north America, north of Mexico*. University of Florida Press, Gainesville. 300 pp.

Derek, R. (2004). Prolonged survival of eggs of the rock-pool mosquito, *Aedes vittatus*, in the extreme heat of the Arabian peninsula. *Journal of Arid Environment,* **57**(2): 203-210.

Derraik, J.G.B. and Slaney, D. (2005). Container aperture size and nutrient preferences of mosquitoes (Diptera: Culicidae) in the Auckland region, New Zealand. *Journal of Vector Ecology*, **30**(1): 73-82.

Dua, V. K., Pandey, A. C., Raghavendra, K., Gupta, A., Sharma, T. and Dash, A.P. (2009). Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. *Malaria Journal*, **8**:124.

Egharevba, R. K. A. and Ikhatua, M. I. (2008). Ethno- medical uses of plants in the treatment of various skin diseases in Ovia north east, Edo State. *Nigeria Research Journal of Agriculture and Biological Sciences,* **4**(1): 58-64.

Elangovan, A., Veeraiyan, G., Elumalai, K. and Prakash, M. (2008). Larvicidal activity of plant oil formulation against three important vector mosquito species. *The Internet Journal of Veterinary Medicine*, **6**(1).

El-Hag, E.A., El-Rahman, A., El-Nadi, H. and Zaitoon, A. A. (2001). Effects of methanolic extracts of neem seeds on egg hatchability and larval development of *Culex pipiens* mosquitoes. *Indian Veterinary Journal,* **78**: 199-201.

Elimam, A.M., Elmalik, K.H. and Ali, F.S. (2009). Efficacy of leaves extract of *Calotropis procera* (Asclepiadaceae) in controlling *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes. *Saudi Journal of Biological Sciences,* **16**: 95-100.

Erinoso, S. M. and Aworinde, D. O. (2012). Ethnobotanical survey of some medicinal plants used in traditional health care in Abeokuta areas of Ogun State, Nigeria. *African Journal of Pharmacy and Pharmacology*. **6**(18): 1352-1362.

Farjana, N., Rowshanul, M.H., Zahangir, A., Saud, M. and Rezaul, M.K. (2011).*Targets erecta* L. and it's mosquitocidal potency against *Culex quinquefasciatus*. *Asian Pacific Journal of Tropical Biomedicine,* **1**: 186-188.

Fayemiwo, K. A., Adeleke, M. A., Okoro, O. P., Awojide, S. H. and Awoniy, I. O. (2014). Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes. *Asian Pacific Journal of Tropical Biomedicine*, **4(1)**: 30–34.

Finney, D.J. (1971). Probit Analysis. (3rd ed.). Cambridge University press, London.

Foster, W. A. (1995). Mosquito sugar feeding and reproductive energetics. *Annual Review of Entomology*. **40**: 443-474.

Foster, W. A. and Walker, E. D. (2002). Mosquitoes (Culicidae). In: *Medical and Veterinary Entomology.* Mullen,G. and Durden,L. (eds). Academic Press, San Diego. pp. 263-262.

Gautam, K., Padma, K. and Poonia, S. (2013). Larvicidal acticity and GC-MS analysis of flavanoids of *Vitex negundo* and *Androgrphis paniculata* against two vector mosquitoes *Anopheles stephensi* and *Aedes aegypti*. *Journal of Vector Borne Diseases*, **50**:171-178.

Ghosh, A., Chowdhury, N. and Chandra, G. (2012). Plant extract as potential mosquito larvicides, *Indian Journal of Medical Research.* **135**(5): 581-598.

Hedlin, P. A., Holing-Warth, R. M., Master, E. P., Miyamoto, J. and Thopson, D. G. Eds.(1997). *Phytochemicals for pest control*. American Chemical Society (ASC). Symp Services, Washington D. C., 372 pp.

Herada, K., Soumalainen, M., Uchida, H., Masui, H., Ohmura, K., Kiviranta, J., Niku-Paavola, M.L. and Ikemoto, T. (2000). Insecticidal compounds against mosquito larvae from *Oscillatoria agardhii* strain 27. *Environmental Toxicology*, **15**(2): 14-119.

Hill, S. and Connelly, C. R. (2013). Southern House Mosquito *Culex quinquefasciatus* Say. University of Florida. UF/ IFAS Extension. EENY457. 1-6.

Hopkins, G.H.E. (1952). *Mosquitoes of the Ethiopian Region I.—Larval bionomics of mosquitoes and taxonomy of culicine larvae*. 2nd ed.: British Museum, London.

http: www.bioriginal.com

Huang, Y. (1977) Notes on the Taxonomic Status of *Aedes vittatus* (Diptera: Culicidae). *Contributions of the American Entomological Institute*, **14**: 113-132.

IL-Kwon, P., Sang-Gil, L., Sang-Chul, S., It-doo, P. and Young-Joon, A. (2002). Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. *Journal of Agricultural and Food Chemistry*, **50**: 1866-1870.

Isman, M.B. (1997). Neem and other botanical insecticides: Barriers to commercialization. *Phytoparasitica*. **25**: 339-344.

Jang, Y.S., Kim, M.K., Ahn, Y.J. and Lee, H.S. (2002). Larvicidal activity of Brazilian plants against *Aedes aegypti* and *Culex pipiens pallens* (Diptera: Culicidae). *Agricultural Chemistry and Biotechnology*, **45**(3): 131-134.

Jimoh, S.O. and Onabanjo, O.O. (2012). Potential of *Tamarindus indica* (Linn) in jam production. *Journal of Agriculture and Social Research*, **12**(2): 29-43.

Jomon, K.V., Sudharamini, S. and Thomas, T. (2009). *Aedes* mosquitoes in arboviral endemic prone area of Kottayam District, Kerala, India. *Academic Review*, **9**(1 and 2):171-178.

Jyoti, J.L., and Brewer, G.J., (1999). Median lethal concentration and efficacy of *Bacillus thuringiensis* against banded sunflower moth (Lepidoptera: *Tortricidae*) biological and microbial control. *Journal of Economic Entomology*, **92** (6): pp. 1289–1291.

Kamaraj, C., Bagavan, A., Elango, G., AbduzZahir, A., Rajakumar, G., Marimuthu, S., Santhoshkumar, T. and Abdul-Rahuman, A. (2011). Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Indian Journal of Medical Research*, **134**:101-106.

Kandaswamy, K., Sengottayan, S. and Arunachalam, G.M. (2012). Biological activity of selected Lamiaceae and Zingiberaceae plant essential oils against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Parasitology Research*, **110**:1261- 1268.

Kayode, J. and Kayode, G.M. (2008). Ethnomedicinal survey of botanicals used in treating sexually transmitted diseases in Ekiti State, Nigeria. *Ethnobotanical Leaflets*, **12**: 44-55.

Khanzada, S.K., Shaikh, W., Sofia, S., Kazi, T.G., Usmanghani, K., Kabir, A. and Sheerazi, T.H. (2008).Chemical constituents of *Tamarindus indica* L. medicinal plant in Sindii. *Pakistan Journal of Botany*, **40**: 2553-2559.

Klempner, M.S., Unnash, T.R. and Hu, L.T., (2007). Taking a bite out of vector transmitted infectious diseases. *New England Journal of Medicine*, **356**: 2567-2569.

Komutarin, T., Azadi, S., Butterworth L., Keil, D., Chitsomboon, B., Suttajit, M. and Meade, B.J. (2004). Extract of seedcoat of *Tamarindus indica* inhibits nitric oxide production by marine macrophages in vitro and in vivo. *Journal of Food and Chemical Toxicology*, **42**(4): 649-658.

Kumar, C.S. and Bhattacharya, S. (2008) Tamarind seed: properties, processing and utilization. *Critical Reviews in Food Science and Nutrition*, **48**: 1-20.

Lagos State Ministry of Health. (2014). Malaria Control Program site developed by Thgirb Company Limited.

Lima, C. A., Almeida, W.R., Hurd, H. and Albuquerque, C.M. (2003). Reproductive aspect of the mosquito *Culex quinquefasciatus* (Diptera: Culicidae) infected with *Wuchereria bancrofti* (Spirurida: Onchocercidae). *Memorias do Instituto Oswalde Cruz*, **98**: 217-222.

Mandal, S. (2012). Mosquito vector management with botanicals- the most effective weapons in controlling mosquito-borne diseases. *Asian Pacific Journal of Tropical Biomedicine*, **2**:336-336.

Maricopa Country Environmental Services (2006). Life cycle and Information on *Aedes aegypti* Mosquitoes. Maricopa County, AZ www.maricopa.gov/EnvSvc/Vector

Markouk, M., Bekkouche, K., Larhsini, M., Bousaid, M., Lazrek, H. B. and Jana, M. (2000). Evaluation of some Moroccan medicinal plant extracts for larvicidal Activity. *Journal of Ethnopharmacology*, **73**: 293-297.

Marmara, A.H. (1989). The Physical Parameters of *Tamarindus indica* and *Palcia* fruits. Dissertation for B.Sc. Hons., Department of Biological Sciences, A.B.U., Zaria. pp. 8-20.

Misvar K. A., Asha, A.V. and Aneesh, E. M. (2014). Bioecology and vectorial capacity of *Aedes* mosquitoes (Diptera: Culicidae) in Irinjalakuda municipality, Kerala, India in relation to disease transmission. *International Journal of Current Research and Academic Review*, **2**(4): 43-49.

Monnet, Y.T., Gbogouri, A.M., Koffi, P.K.B. and Kouame, P. (2012). Chemical Characterization of Seeds and Seed Oils from Matured *Terminalia catappa* Fruits Harversted in Cote d'Ivoire. *International Journal of Biosciences*. **10**(1):110-124.

Muhammad, N., Bamishaiye, E., Bamishaiye, O., Usman, L., Salawu, M., Nafiu, M. and Oloyede, O. (2011). Physicochemical properties and fatty acid composition of *Cyperus esculentus* (Tiger nut) tuber oil. *Bioresources Bulletin*, **5**: 51-54.

Muhammad, A. and Mudi, S.Y. (2011). Phytochemical screening and antimicrobial activities of *Terminalia catappa* leaf extracts. *Biokemistri*, **23**(1):35-39.

Nazar, S., Ravikumar, S., Prakash, W. G., Syed, A. M. and Suganthi, P. (2009). Screening of Indian Coastal Plant Extracts for Larvicidal Activity of *Culex quinquefasciatus*. *Indian Journal of Science and Technology*, **2** (3): 24-27.

New Zealand Biosecure Entomology Laboratory (2008). *Culex (Culex) quinquefasciatus* (Say) southern or brown mosquito. Division of Southern Monitoring Services Limited (SMS) version **2**. 1-5.

Nigam, S. K. and Venkatakrishna, B. H. (2001). Occupational cancer: Introduction and Intervention. *Indian Journal of Occupational Health*, **89**: 49.

Nigeria National Medicine Development Agency (2006). *Medicinal plants of Nigeria*. Federal Ministry of Science and Technology, Lagos. 72 pp.

Nwinuke, N.M. and Barine, I. N. (2009). Physicochemical properties and fatty acid composition of *Dennettia tripetala* fruit oil (pepper fruit). *Nigerian Journal of Biochemistry and Molecular Biology*, **24**: 42-46.

Nwosu, F.O., Dosumu, O.O. and Okocha, J.O.C. (2008). The potential of *Terminalia catappa* (Almond) and *Hyphaene thebaica* (Dum palm) fruits as raw materials for livestock feed. *African Journal of Biotechnology*, **7**(24): 4576-4580.

Oda, T., Eshita, Y., Uchida, K., Mine, M., Kurokawa, K., Ogawa, Y., Kato, K., and Tahara, H., (2002). Reproductive activity and survival of *Culex pipiens pallens* and *Culex quinquefasciatus* (Diptera: Culicidae) in Japan at high temperature. *Journal of Medical Entomology*, **39**(1): 185-190.

Oderinde, R.A. and Ajayi, L.A. (2000). Physicochemical and metal composition of *Calophyllumino phyllum* seed and seed oil. *Pakistan Journal of Industrial Research*, **43**(6): 357-358.

Okumu, F.O., Knols, B.G.J. and Fillinger, U. (2007). Larvicidal effects of a neem (*Azadirachta indica*) oil formulation the malaria vector *Anopheles gambiae*. *Malaria Journal*, **1**:1-8.

Ololade, Z.S., Olatunde, O.Z., Oyelese, O.J., Olaye, O.O. and Odewande, R.A. (2014). Total phenolic content, free radical inhibition, antioxidant and antibacterial potentials of the medicinal organic compounds in the fruit of *Terminalia catappa* Linn. *Nature and Science*, **12**(2): 46-50.

Oleg K. (2007). *Mosquito*. Microsoft @Encarta @online encyclopedia

Parra, A.L., Yhebra, R.S., Sardinas, I.G. and Buela, L. I. (2001). Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50}) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine*, **8**: 395-400.

Pankaj, T. and Anita, S. (2010). Assessment of larvicidal properties of aqueous extracts of Four plants against *Culex quinquefasciatus* larvae. *Jordan Journal of Biological Sciences*, **3**(1):1 - 6.

Perich, M.J., Wells, C., Bertsch, W. and Tredway, K.E. (1995). Isolation of the insecticidal components of *Tagetes minuta* (Compostitae) against mosquito larvae and adults. *Journal of American Mosquito Control Association*, **11**: 307-310.

Perumalsamy, H., Jin Jang, M., Kim, J-R., Kadarkarai, M. and Ahn, Y-J. (2015). Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Milletta pinnata* seed toward three mosquito species. *Parasite and Vectors*, **8**: 237.

Pierre, D.Y. S., Nchiwan, N. E., Okechukwu, E. C., Lame, Y. (2014). Larvicidal and pupicidal toxicities of *Plectranthus glandulosus* and *Callistemon rigidus* Leaf essential oils against three mosquito species. J*ournal of Mosquito Research*, **4**(2): 5-14.

Prabhu, K., Murugan, K., Nareshkumar, A., Ramasubramanian, N. and Bragadeeswaran, S. (2011). Larvicidal and repellent potential of *Moringa oleifera* against dengue vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, **1**:127-132.

Public Health Pest Control (2001). Applicator Training Manual. Bureau of Entomology. Jacksonville, FL DACS.

Rahuman, A.A., Gopalakrishnan, G., Ghouse, B. S., Arumugam, S. and Himalayan, B. (2000). Effect of *Feronia limonia* on mosquito larvae. *Fitotetapia*, **71**(5): 553-555.

Rajasekaran A. and Duraikannan, G. (2012). Larvicidal activity of plant extracts on *Aedes aegypti* L. *Asian Pacific Journal of Tropical Biomedicine*, **2**: S1578-S1582.

Rajko, V., Sergeja, V. and Helena, A. (2010). Biochemical parameters and oxidative resistance to thermal treatment of refined and unrefined vegetable edible oils. *Czech Journal of Food Science*, **28**: 376-384.

Rajmohan, D. and Ramaswamy, M. (2007). Evaluation of larvicidal activity of the leaf extract of a weed plant *Ageratina adenophora* against two important species of mosquito *Aedes aegypti* and *Culex quinquefasciatus*. *African Journal of Biotechnology*, **6**: 631-638.

Ramsewak, R.S., Nair, M.S., Marugesan, S., Mattson, W. J. and Zasada, J. (2001). Insecticidal fatty acids and triglycerides from *Dirca palustris* (Thymelaeaceae). *Journal of Agricultural and Food Chemistry*.

Rathi, J. M., Absara, S., Priyadharshini, K. and Jegathambika, V. (2008). Qualitative phytochemical screening of some locally available insecticidal plants. *Journal of Biopesticides*, 52-54.

Rattanarithikul, R., Harbach, R. E., Harrison, A. B., Panthusiri, P., Coleman, R. E. and Richardson, J. H. (2010). Illustrated Keys to the mosquitoes of Thailand VI. Tribe Aedini. *Southeast Asian Journal of Tropical Medicine of Public Health*, **41**(1): 16.

Ravikumar, S., Ali, M. S. and Beula, J. M. (2011). Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. *Asian Pacific Journal of Tropical Biomedicine*, **1**(2): 143-146.

Reinert, J.F. (2000). Description of *Fredwardsius*, a new subgenus of *Aedes* (Diptera: Culicidae). *European Mosquito Bulletin* **6**: 1-7.

Reinert, J.F., Harbach, R.E. and Kitching, I.J. (2004). Phylogeny and classification of Aedini (Diptera: Culicidae) based on morphological characters of all life stages. *Zoological Journal of the Linnean Society*, **142**: 289–368.

Rotimi, O.A., Chris, O.A., Olusola, O.O., Joshua, R. and Josiah, A.O. (2011). Bioefficacy of the extracts of some indigenous Nigerian plants on the developmental stages of mosquito (*Anopheles gambiae*), *Jordan Journal of Biological Sciences*, **4**(4): 836-841.

Samidurai, K. (2012). Mosquito larvicidal and ovicidal properties of *Pemphis acidula* Frost (Lythraceae) against *Culex tritaeriorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, **2**: S1862-S1866.

Samuel, T., Ravindran, K. J. and Arivoli, S. (2012a). Screening of Twenty Five Plant Extracts for Larvicidal Activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, **2**: S1130-S1134.

Samuel, T., Ravindran, K. J. and Arivoli, S. (2012b). Bioefficacy of botanical insecticides against the dengue and chikungunya vector *Aedes aegypti* (L.) (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, **2**: S1842-S1844.

Shaalan, E.A.S., Canyonb, D., Younesc, M.W.F., Abdel-Wahaba, H., Mansoura, A.H. (2005). A Review of Botanical Phytochemicals with Mosquitocidal Potential. *Environment International* **3**:1149-66.

Shahi, M., Hanafi-Bojd, A. A., Iranshahi, M., Vataandoost, H. and Hanafi-Bojd, M.Y. (2010). Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (diptera: Culicidae). *Journal of Vector Borne Diseases*, **47**: 185-188.

Shimadzu QP (2010). Gas chromatography mass spectrophotometer, QP- Plus, Japan.

Singh, R. K., Dhiman, R. C. and Mittal, P. K. (2006). Mosquito Larvicidal Properties of *Mormordica charantia* Linn (Family: Cucurbitaceae). *Journal of Vector Borne Diseases*, **43**: 88-91.

Singha, S., Siddharthasankar, B. and Goutam, C. (2011). Synergistic effect of *Croton caudatus* (fruits) and *Tiliacora acuminate* (flowers) extracts against filarial vector *Culex quinquefastciatus*. *Asian Pacific Journal of Tropical Biomedicine*, **1**: 159-164.

Snow, K. R. and Ramsdale, C. D. (2003). A reviewed checklist of European mosquitoes, *Journal of the European Mosquito Control Association*, **15**: 1-5.

Soummane, H., Larhsini, M., Naamani, K. and Coll, J. (2011). Studies of larvicidal and adulticidal activities of some halophyte plant extracts against *Ceratitis capitata* (Wiedemann). *Journal of Entomology*, 8:548-556.

Souza, T.M., Farias, D.F., Soares, B.M., Viana, M.P., Lima, G.P.G., Machado, L.K.A., Morais, S.M. and Carvalho, A.F.U. (2011). Toxicity of brazilian plant seed extracts of two strains of *Aedes aegypti* (Diptera: Culicidae) and nontarget animals. *Journal of Medical Entomology*, **48**(4): 846-851.

Sukanya, S. I., Sudisha, J., Hariprasad, P., Niranjana, S.R., Prakash, H.S. and Fathimsa, S. K. (2009). Antimicrobial activity of leaf extracts in Indian medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology*, **8**(23): 6677-6682.

Sukumar, K., Perich, M.J. and Boobar, L.R. (1991). Botanical derivatives in mosquito control: A Review. *Journal of the American Mosquito Control Association*,**7**: 210-237.

Sumroiphon, S., Yuwaree, C., Arunlertaree, C., Komalamisra, N. and Rongsriyany, Y. (2006). Bioactivity of citrus seed for mosquito-borne diseases larval control. *Southeast Asian Journal of Tropical Medicine and Public Health*, **37**(3): 123-127.

Syed Ali, M.Y., Anuradha, V., Sirajudeen, S.A., Vijaya, P., Yogananth, N., Rajan, R. and Parveen, P.M.K. (2013). Mosquito larvicidal properties of volatile oil from salt marsh mangrove plant of *Sesuvium portulacastrum* against *Anopheles stephensi* and *Aedes aegypti*. *Journal of Coastal Life Medicine*, **1**(1): 65-70.

Tamzid, H. M., Alam, M.T. and Islam, M. A.U. (2007). Physicochemical and nutritional studies of *Terminalia belerica* Roxb. Seed oil and kernel. *Journal of Bio-Science*, **15**:117-126.

Tennyson, S., Ravindran, K.J. and Arivoli, S. (2012). Bioefficacy of botanical insecticides against the Dengue and Chikungunya Vector *Aedes aegypti* (L.) (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, S1842-S1844.

Tiwary, M., Naik, S.N., Dhananjay, K. T., Mittal, P.K. and Yadav, S. (2007). Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum aramatum* DC (Rutaceace) against three mosquito vectors. *Journal of Vector Borne Disease*. **44**: 198-204.

Ubogu, A.E. (2011). Determination of the locational decisions of informal sector entrepreneurs in urban Zaria. *Journal of Geography and Geology*, **3**(1): 216.

United States Fish and Wildlife Services (USFWS). (2004). Environmental effects of mosquito control, paper.

Unnikrishnan, G. (2014). Larvicidal and pupicidal activity of *Terminalia catappa* leaf extracts on *Aedes aegypti* mosquito: A vector Intervention. *Journal of Pharmacy and Biological Sciences*, **9**(2): 58-63.

Untwal, L.S and Kondawar, M.S. (2006). Use of *Terminalia catappa* fruit extract as an indicator in acid-base titrations. *Indian Journal of Pharmaceutical Science*, **68**: 399-401.

Vahitha, R., Venkatachalam, M.R., Murugan, K. and Jebanesan, A. (2002). Larvicidal efficacy of *Pavonia zeylanica* L. and *Acacia ferruginea* D.C. against *Culex quinquefasciatus* Say. *Bioresouce Technology*, **82**: 203-204.

Valle, D.I. (2005). Peptic ulcer disease and related disorder. In: Brawn, W. C., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L., Jameson, J.L., Editors; *Harrison's Principles of Internal Medicine* 16thed.McGraw Hill 17 NewYork. Pp. 46-62.

Vargas, M. V. (2012). An Update on Published Literature (Period 1992-2010) and Botanical Catergories of Plant Essential Oils with Effects on Mosquitoes (Diptera: Culicidae). *Boletin de Malariologia Y Salud Ambiental*. **52** (2): 143-193.

Waseem, A., Khan, H.A.A., Hafeez, F., Bilal, H., Yeon, K.K. and Lee, J. (2010). Potential of citrus seed extracts against dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pakistan Journal of Botany*, **42**(4): 3343-3348.

World Health Organization (1996). Report of the W.H.O. Informal consultation on the evaluation on the testing of insecticides, CTD/WHO PES/IC/96.1. Geneva. p. 69. In: Anupam, G., Nandita, C. and Goutam, C. (2012). Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*, **135**: 581-598.

World Health Organization (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization Communicable Disease Control, Prevention and

Eradication, Geneva, W.H.O. *Pesticide Evaluation Scheme*. WHO/CDS/WHOPES/GCDPP/2005.**13**: 7-29.

World Health Organization (2012). Global Strategy for Dengue Prevention and Control 2012-2020,W.H.O Library Cataloguing-in-Publication Data, France. http://www.who.int. 3-25.

World malaria report. (2014). Malaria, fact sheet. www.who.int. 4/10/2014.

www.mosquitoworld.net 10/10/2014. http://www.mosquitoworld.net/mosquitolifecycle.php#

www.enchantedlearning.com 8/12/2014

www.cdc.gov 8/12/2014. http://www.cdc.gov/Dengue/entomologyEcology/m_lifecycle.html

Yiau-Min, H. (1977).The Subgenus *Stegomyia* of *Aedes* in Southeast Asia: the *Edwarsi* and *Walbus* group of Species, and totes on the Taxonomic status of *Aedes vittatus*. (Diptera: Culicidae).Contributions of the American Entomological Institute, *Medical Entomological Studies.***14** (1): 114-121.

Yusue, A.A., Mofio, B. M. and Ahmed, A. B. (2007). Proximate and mineral composition of *T. indica* Linn 1753 Seeds. *Science World Journal*. **2**:1-5.

Yusuf, Y.O. and Shuaib, M.I. (2012). The effects of wastes discharge on the quality of Samaru stream, Zaria, Nigeria, *Ecological Water Quality- Water Treatment and Reuse*, Dr. Voudouris (Ed.), 377-386.

Zettel, C. and Kaufman, P. (2013). Yellow fever mosquito *Aedes aegypti* (Linnaeus) (Insecta: Diptera: Culicidae).EENY-434. UF/IFAS extension, University of Florida. pp. 1-6.

Zewdneh, T., Mamuye, H., Asegid, T., Yalemtsehay, M. and Beyene, P. (2011). Larvicidal effects of *Jatropha curcas* L. against *Anopheles arabiensis* (Diptera: Culicidae). *Momona Ethiopian Journal of Science*, **3**(1): 52-64.

APPENDICES
APPENDIX I

ANOVA tables of *T. catappa* seed extract against *Aedes aegypti, Aedes vittatus* and *Culex quiuquefasciatus* larvae under laboratory and simulated field conditions.

Table Ia: ANOVA of *T. catappa* seed extract against *Aedes aegypti* under laboratory condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	865.919	5	173.184	182.587	$6.876x10^{-11}$
Within groups	11.382	12	0.9485		
Total	877.301	17			

Table Ib: ANOVA of *T. catappa* seed extract against *Aedes aegypti* under simulated field condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	301.296	5	60.2591	25.8316	$4.940x10^{-6}$
Within groups	27.9932	12	2.33277		
Total	329.289	17			

Table IIa: ANOVA of *T. catappa* seed extract against *Aedes vittatus* under laboratory condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	322.189	5	64.4378	72.4536	$1.527x10^{-8}$
Within groups	10.6724	12	0.889367		
Total	332.861	17			

Table IIb: ANOVA of *T. catappa* seed extract against *Aedes vittatus* under simulated field condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	282.613	5	56.5227	72.3645	$1.538x10^{-8}$
Within groups	9.373	12	0.781083		
Total	291.986	17			

Table IIIa: ANOVA of *T. catappa* seed extract against *Culex quinquefasciatus* under laboratory condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	1130.05	5	226.01	94.416	$3.294x10^{-9}$
Within groups	28.7252	12	2.39377		
Total	1158.77	17			

Table IIIb: ANOVA of T. *catappa* seed extract against *Culex quinquefasciatus* under simulated field condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	651.846	5	130.369	55.786	$6.841x10^{-8}$
Within groups	28.0434	12	2.33695		
Total	679.889	17			

APPENDIX II

ANOVA tables of *T. indica* seed extract against *Aedes aegypti, Aedes vittatus* and *Culex quiuquefasciatus* larvae under laboratory and simulated field conditions

Table Ia: ANOVA of *T. indica* seed extract against *Aedes aegypti* under laboratory condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	915.102	5	183.02	26.0644	4.707×10^{-6}
Within groups	84.2622	12	7.02185		
Total	999.365	17			

Table Ib: ANOVA of *T. indica* seed extract against *Aedes aegypti* under simulated field condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	1048.02	5	209.604	21.4443	1.331×10^{-5}
Within groups	117.292	12	9.77435		
Total	1165.31	17			

Table IIa: ANOVA of *T. indica* seed extract against *Aedes vittatus* under laboratory condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	561.407	5	112.281	47.1088	1.785×10^{-7}
Within groups	28.6014	12	2.38345		
Total	590.008	17			

Table IIb: ANOVA of *T. indica* seed extract against *Aedes vittatus* under simulated field condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	829.009	5	165.802	87.647	$5.077x10^{-9}$
Within groups	22.7004	12	1.8917		
Total	851.709	17			

Table IIIa: ANOVA of *T. indica* seed extract against *Culex quinquifasciatus* larvae under laboratoy condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	1050.05	5	210.01	38.9343	$5.204x10^{-7}$
Within groups	64.7274	12	5.39395		
Total	1114.78	17			

Table IIIb: ANOVA of *T. indica* seed extract against *Culex quinquifasciatus* larvae under simulated field condition

Source of variation	Sum of squares	Df	Mean square	F-statistic	P-value
Between groups	908.166	5	181.633	120.664	$7.865x10^{-10}$
Within groups	18.0634	12	1.50528		
Total	926.229	17			

APPENDIX III

LC_{50} Calculations from regression equations of *Terminalia catappa* seed extracts under laboratory and simulated field conditions.

LC_{50}*Terminalia catappa* seed extract against *Aedes aegypti* larvae in laboratory condition

y=1.99x+3.637

5=1.99x+3.637

1.99x=5-3.637

x=1.363/1.99=0.6849

Antilog x=4.840mL/L

LC_{50}*Terminalia catappa* seed extract against *Aedes aegypti* larvae in simulated field condition

y=0.76x+4.212

5=0.76x+4.212

5-4.212=0.76x

0.76x=0.788

x=0.788/0.76=1.0368

Antilog x=10.884mL/L

LC_{50}*Terminalia catappa* seed extract against *Aedes vittatus* larvae in laboratory condition

y=0.616x+4.355

5=0.6167x+4.355

5-4.355=0.616x

0.645=0.616x

x=0.645/0.616=1.047

Antilog x=11.143mL/L

LC_{50}*Terminalia catappa* seed extract against *Aedes vittatus* larvae in simulated field condition

y=0.743x+4.287

5=0.743x+4.287

5-4.287=0.743x

0.713=0.743x

x=0.713/0.743=0.959

Antilog x=9.099mL/L

LC_{50}*Terminalia catappa* seed extract against *Culex quinquefasciatus* larvae in laboratory condition

y=2.086x+4.256

5=2.086x+4.256

5-4.256=2.086x

0.744=2.086x

x=0.744/2.086=0.357

Antilog x=2.275mL/L

LC_{50}*Terminalia catappa* seed extract against *Culex quinquefasciatus* larvae in simulated field condition

y=1.623x+4.213

5=1.623x+4.213

5-4.213=1.623x

0.787=1.623x

x=0.787/1.623=0.485

Antilog x=3.055mL/L

APPENDIX IV

LC_{50} Calculations from regression equations of *Tamarindus indica* seed extracts under laboratory and simulated field conditions.

LC_{50} *Tamarindus indica* seed extract against *Aedes aegypti* larvae in laboratory condition

y=1.923x+4.815

5=1.923x+4.815

5-4.815=1.923x

0.185=1.923x

x=0.185/1.923=0.0962

Antilog x=1.248ml/l

LC_{50} *Tamarindus indica* seed extract against *Aedes aegypti* larvae in simulated field condition

y=1.953x+4.74

5=1.953x+4.74

5-4.74=1.953x

0.26=1.953x

x=0.26/1.953=0.1331

Antilog x=1.35mL/L

LC_{50} *Tamarindus indica* seed extract against *Aedes vittatus* larvae in laboratory condition

y=1.663x=3.861

5=1.663x+3.861

5-3.861=1.663x

1.139=1.663x

x=1.139/1.663=0.685

Antilog x=4.842mL/L

LC_{50} *Tamarindus indica* seed extract against *Aedes vittatus* larvae in simulated field condition

y=1.053x+4.92

5=1.053x+4.92

5-4.92=1.053x

0.08=1.053x

x=0.08/1.053=0.076

Antilog x=1.191mL/L

LC_{50} *Tamarindus indica* seed extract against *Culex quinquefasciatus* larvae in laboratory condition

y=1.4x+5.226

5=1.4x+5.226

5-5.226=1.4x

-0.226=1.4x

x=-0.226/1.4=-0.161

Antilog x=0.690mL/L

LC_{50} *Tamarindus indica* seed extract against *Culex quinquefasciatus* larvae in simulated field condition

y=1.326x+5.271

5=1.326x+5.271

5-5.271=1.326x

-0.271=1.326x

x=-0.271/1.326=-0.204

Antilog x=0.625mL/L

APPENDIX V

Abbott's correction for control mortality of the seed extracts exposed mosquito larvae

Abbott (1925) = $\frac{\text{\% mortality in experimental -\% mortality in control}}{\text{100 - \% mortality in control}} \times 100$

Abbott's calculation for *T. catappa* against *Aedes vittatus* under simulated field condition

At 8.0mL/L = $\frac{45-1}{100-199} \times 100 = \frac{44}{} \times 100 = 44\%$

At 4.0mL/L = $\frac{45-1}{100-199} \times 100 = \frac{44}{} \times 100 = 44\%$

At 2.0mL/L = $\frac{36-1}{100-1} \times 100 = \frac{35}{99} \times 100 = 35\%$

At 1.0mL/L = $\frac{21-1}{100-1} \times 100 = \frac{20}{99} \times 100 = 20\%$

At 0.5mL/L = $\frac{19-1}{100-1} \times 100 = \frac{18}{99} \times 100 = 18\%$

At 0.00mL/L = $\frac{1-1}{100-1} \times 100 = 0\%$

Abbott's calculation for *T. catappa* against *Culex quinquefasciatus* larvae under simulated field condition

At 8.0mL/L = $\frac{80-8}{100-8} \times 100 = \frac{72}{92} \times 100 = 78\%$

At 4.0mL/L = $\frac{55-8}{100-8} \times 100 = \frac{47}{92} \times 100 = 51\%$

At 2.0mL/L = $\frac{44-8}{100-8} \times 100 = \frac{36}{92} \times 100 = 39\%$

At 1.0mL/L = $\frac{29-8}{100-8} \times 100 = \frac{21}{92} \times 100 = 23\%$

At 0.5mL/L = $\frac{17-8}{100-8} \times 100 = \frac{9}{92} \times 100 = 10\%$

At 0.00mL/L = $\frac{8-8}{100-8} \times 100 = \frac{0}{92} \times 100 = 0\%$

Abbott's calculation for *T. indica* against *Aedes aegypti* larvae under laboratory condition

At 8.0mL/L = $\frac{99-3}{100-3} \times 100 = \frac{96}{97} \times 100 = 99\%$

At 4.0mL/L = $\frac{57-3}{100-3} \times 100 = \frac{54}{97} \times 100 = 56\%$

At 2.0mL/L = $\frac{55-3}{100-3} \times 100 = \frac{52}{97} \times 100 = 54\%$

At 1.0mL/L = $\frac{41-3}{100-3} \times 100 = \frac{38}{97} \times 100 = 39\%$

At 0.5mL/L = $\frac{39-3}{100-3} \times 100 = \frac{36}{97} \times 100 = 37\%$

At 0.00mL/L = $\frac{3-3}{100-3} \times 100 = \frac{0}{97} \times 100 = 0\%$

Abbott's calculation for *T. indica* against *Aedes aegypti* larvae under simulated field condition

At 8.0mL/L = $\frac{95-7}{100-7} \times 100 = \frac{88}{93} \times 100 = 95\%$

At 4.0mL/L = $\frac{73-7}{100-7} \times 100 = \frac{66}{93} \times 100 = 71\%$

At 2.0mL/L = $\frac{72-7}{100-7} \times 100 = \frac{65}{93} \times 100 = 70\%$

At 1.0mL/L = $\frac{49-7}{100-7} \times 100 = \frac{42}{93} \times 100 = 45\%$

At 0.5mL/L = $\frac{23-7}{100-7} \times 100 = \frac{16}{93} \times 100 = 17\%$

At 0.00mL/L = $\frac{7-7}{100-7} \times 100 = \frac{0}{93} \times 100 = 0\%$

Abbott's calculation for *T. indica* against *Aedes vittatus* larvae under laboratory condition

At 8.0mL/L = $\frac{55-3}{100-3} \times 100 = \frac{52}{97} \times 100 = 54\%$

At 4.0mL/L = $\frac{60-3}{100-3} \times 100 = \frac{57}{97} \times 100 = 59\%$

At 2.0mL/L = $\frac{24-3}{100-3} \times 100 = \frac{26}{97} \times 100 = 27\%$

At 1.0mL/L = $\frac{13-3}{100-3} \times 100 = \frac{10}{97} \times 100 = 10\%$

At 0.5mL/L = $\frac{8-3}{100-3} \times 100 = \frac{5}{97} \times 100 = 5\%$

At 0.00mL/L = $\frac{3-3}{100-3} \times 100 = \frac{0}{97} \times 100 = 0\%$

Abbott's calculation for *T. indica* against *Aedes aegypti* larvae under simulated field condition

At 8.0mL/L = $\frac{100-12}{100-12} \times 100 = \frac{88}{88} \times 100 = 100\%$

At 4.0mL/L = $\frac{89-12}{100-12} \times 100 = \frac{77}{88} \times 100 = 88\%$

At 2.0mL/L = $\frac{71-12}{100-12} \times 100 = \frac{59}{88} \times 100 = 67\%$

At 1.0mL/L = $\frac{69-12}{100-12} \times 100 = \frac{57}{88} \times 100 = 65\%$

At 0.5mL/L = $\frac{52-12}{100-12} \times 100 = \frac{40}{88} \times 100 = 45\%$

At 0.00mL/L = $\frac{12-12}{100-12} \times 100 = \frac{0}{88} \times 100 = 0\%$