بسم الله الرحمن الرحيم

University of Khartoum

Graduate College

Medical and Health Studies Board

Department of Epidemiology

TOXICITY OF *Ambrosia maritima* L. LEAVES AQUEOUS EXTRACT AND ABATE AQUEOUS SOLUTIONS, ON *CULEX*-SPECIES.

By

Alashary Adam Eisa Hamdoon

B.Sc. (Honours) University of Khartoum 2006.

A Dissertation Submitted In Partial Fulfillment for The Requirements of The Degree of M.Sc. In Public and Environmental Health (Medical Entomology).

2010

Supervisor

Dr. Tigani Mohamed Hassan Allam

Zoology Department, Faculty of Science,

University of Khartoum.

بسم الله الرحمن الرحيم

قال تعالي: ((إِنَّ اللَّهَ لاَ يَسْتَحْيِي أن يَضْرِبَ مَثَلاً مَّا بَعُوضَةً فَمَا فَوْقَهَا فَأَمَّا الَّذِينَ آمَنُواْ فَيَعْلَمُونَ أَنَّهُ الْحَقُّ مِن رَّبِّهِمْ وَأَمَّا الَّذِينَ كَفَرُواْ فَيَقُولُونَ مَاذَا أَرَادَ اللَّهُ بِهَذَا مَثَلاً يُضِلُّ بِهِ كَثِيرًا وَيَهْدِي بِهِ كَثِيرًا وَمَا يُضِلُّ بِهِ إِلاَّ الْفَاسِقِينَ)) (26)

صدق الله العظيم

سورة البقرة

DEDICATION

To my Family

To my Colleagues

To all Scientists in the Sudan

To all Staff of Khartoum University

Tables of contents

ACKNOWLDGEMENTS

Praise to Allah first and then, I would like to express my gratitude and sincere appreciation to my supervisor Dr. El Tigani M. H. Allam for his supervision, advice, cooperation and guidance. My great thanks to University of Khartoum for financing the M.Sc. study. I am greatly indebted to Dr. Mohammed Alhaj Alkhidir for his valuable suggestion and advice. Special thanks go to my colleague Modawy Elnour Modawy for his help in statistical analysis. I would like also to thank Ahmed Ibrahim for valuable assistance in field collection. My great thanks go to all those who shared and assisted me in this work.

ABSTRACT

Background: Efforts are being made to seek insecticides of natural origin as a safer alternative to synthetic insecticides. Many plant extracts have been studied for their efficiency in controlling larvae of different mosquito species the world over.

Design: Comparative study.

Setting: Khartoum locality of Khartoum State.

Study objectives: General objectives.

To compare the toxicity of *Ambrosia maritima* L (Damsesa) leaves aqueous extract and Abate aqueous solution on *Culex* larvae.

Specific objectives.

1- To draw the Regression line to the toxicity of *Ambrosia maritima* L leaves aqueous extract and Abate aqueous solutions on *Culex* larvae.

2- To determine the (LC_{50}) of *Ambrosia maritima* L leaves aqueous extract and Abate aqueous solution for *Culex* larvae.

3- To determine the (LC_{99}) of *Ambrosia maritima* L leaves aqueous extract and Abate aqueous solution for *Culex* larvae.

4- To find out the possibility of the use of *Ambrosia maritima* L leaves aqueous extract in the control of *Culex* larvae in the field.

Methodology: The susceptibility tests for *Culex* larvae were determined by following the World Health Organization standard procedures. Values of the lethal concentration for 50% of experimental larvae (LC_{50}) and for 99% of experimental larvae (LC_{99}) in part per million were calculated for all experiments and the average of the experiments for the extract of the *Ambrosia maritima* L and Abate by the computer excel 2007 program where regression lines were drawn and

finding it formula. The (pH) of the solutions for all concentrations was measured before and after adding the larvicide.

The percentage of death was transformed to probit units. Concentrations used in each experiment were set as part per million (ppm) logarithms of concentrations for (ppm) were calculated. After determining the mortality of larvae in this wide range of concentrations, the results were used to determine LC_{50} and LC_{99} values. All the experiments were performed according to WHO standard techniques.

Results: The Average percentage deaths for *Culex* larvae in the four experiments in Abate ranged between 5% and 100%. The LC_{50} for Abate was 0.008104 ppm and the LC_{99} for the same chemical was 0.045185 ppm.

The Average percentage deaths for *Culex* larvae in the four experiments of *Ambrosia maritima* L leaves aqueous extract range between 9% and 96%. The LC_{50} for *Ambrosia maritima* L was 13489 ppm and the LC_{99} for the same extract was 31088 ppm.

The study revealed that the aqueous, extract of *Ambrosia maritima* L was less toxic to *Culex* larvae than Abate.

Conclusion: Aqueous leaves extract of the *Ambrosia maritima* L results is toxic and can be used to control *Culex* larvae. On the other hand, Abate was more toxic than *Ambrosia maritima* L leaves aqueous extract. Finally, the following recommendations were made:

More studies are needed to isolate and identify the active ingredients in the extract of *Ambrosia maritima* L for use as a larvicide. Field trials should be carried out on the extracts.

المستخلص

الخلفية: تبذل الجهود للحصول علي المبيدات ذات الاصل الطبيعي باعتبارها بديلاً آمنا للمبيدات الحشرية الاصطناعية. وقد درست العديد من المستخلصات النباتية لكفاءتها في مكافحة يرقات البعوض من الانواع المختلفة في جميع انحاء العالم.

التصميم: دراسة مقارنة.

منطقة الدراسة: محلية الخرطوم من ولاية الخرطوم.

اهداف الدراسة: الاهداف العامة.

مقارنة سمية المستخلص المائي لأوراق *Ambrosia maritima* L (دمسيمة) و محلول Abate (الابيت) المائي علي يرقات الكيولكس.

الاهداف الخاصة.

1- رسم ميل خط سمية المستخلص المائي لأوراق *Ambrosia maritima* L (دمسيسة) و محلول Abate(الابيت) المائي علي يرقات الكيولكس.
2- تحديد LC_{50} للمستخلص المائي لأوراق *Ambrosia maritima* L (دمسيسة) و محلول Abate (الابيت) المائي علي يرقات الكيولكس.
3- تحديد LC_{99} للمستخلص المائي لأوراق *Ambrosia maritima* L (دمسيسة) و محلول Abate (الابيت) المائي علي يرقات الكيولكس.
4- اكتشاف امكانية أستخدام المستخلص المائي لأوراق *Ambrosia maritima* L (دمسيسة) في مكافحة يرقات الكيولكس في الحقل.

المنهجية: اختبارات الحساسية ليرقات الكيولكس تم تحديدها باتباع اجراءات و معايير منظمة الصحة العالمية. قيم التركيز القاتل لـ 50% ليرقات التجارب (LC_{50}) و 99% ليرقات التجارب (LC_{99}) في الجزء من المليون تم حسابها لكل التجارب و لمتوسط التجارب لمستخلص *Ambrosia maritima* L (دمسيسة) و Abate (الابيت) بالكمبيوتر برنامج اكسل 2007 كذلك تم رسم ميل خط السمية و ايجاد معادلته. pH المحلول لكل التراكيز تم قياسه قبل و بعد اضافة المبيد.

نسب الموت المئوية حولت لوحدات البروبيت. التراكيز المستخدمة في كل تجربة وضعت في الجزء من المليون (ppm) لوغريثمات التراكيز في الجزء من المليون حسبت. بعد تحديد موت اليرقات في مدي التراكيز الواسعة، استخدمت النتيجة لتحديد قيم LC_{50} و LC_{99}. كل التجارب اجريت طبقاً لمعايير وتقنيات منظمة الصحة العالمية.

النتائج: متوسط نسبة الموت المئوية ليرقات الكيولكس في تجارب Abate (الابيت) الاربعة تراوحت بين 5% و 100%. LC_{50} للابيت وجدت 0.008104 جزء في المليون و LC_{99} لنفس المادة الكيميائية 0.045185 جزء في المليون.

متوسط نسبة الموت المئوية ليرقات الكيولكس في تجارب المستخلص المائي لأوراق *Ambrosia maritima* L (دمسيسة) الاربعة تراوحت بين 9% و 96%. LC_{50} لمستخلص *Ambrosia maritima* L وجدت 13489 جزء في المليون و LC_{99} لنفس المستخلص 31088 جزء في المليون.

اظهرت الدراسة ان مستخلص *Ambrosia maritima* L (دمسيسة) اقل سمية علي يرقات الكيولكس من Abate (الابيت).

الخاتمة: المستخلص المائي لاوراق *Ambrosia maritima* L (دمسيسة) الناتج، سام ويمكن ان يستخدم لمكافحة يرقات الكيولكس. من ناحية اخري Abate (الابيت) اكثر سمية من المستخلص المائي لاوراق *Ambrosia maritima* L (دمسيسة). اخيراً، قدمت التوصيات التالية:

هنالك حاجة الي مزيد من الدراسات لعزل و تحديد المكونات الفعالة في المستخلص من *Ambrosia maritima* L (دمسيسة) للاستخدام كمبيد يرقات. وينبغي اجراء تجارب حقلية علي المستخلصات.

List of Figures

List of Tables

CHAPTER ONE

INTRODUCTION

1.1. General Introduction.

A vector is an insect or any living carrier that transports an infectious agent or a parasite from an infected individual or its wastes to a susceptible individual or its food or immediate surroundings. The parasite may or may not pass through a development cycle within the vector (WHO, 2003a).

Mosquitoes colonize rural and urban environments (WHO, 2003a). They do not generally fly far from the place where they breed unless swept by currents of wind. The range of flight varies with the species, and may reach up to eleven kilometers. Aircrafts and ships have increased the possibility of the dispersal of mosquitoes from one country another to country, and have created fresh problems of public health (Park, 2005). The male mosquito is a much weaker flyer than the female. Thus, the presence of a large number of adult male mosquitoes indicates that breeding places of that particular species are close by. In normal atmospheric circumstances, most individual mosquitoes of tropical species apparently fly within a range of one to three kilometers. However, there are records of a few species or occasional individual mosquitoes travelling much further. Certain temperate-zone species travel four to five kilometers and there are records of those that travelled up to ten kilometers (WHO, 1982).

Mosquitoes are major worldwide vectors of many diseases, including malaria, yellow fever, dengue, and filariasis. They may also be of significant nuisance through their bites, interfering with normal living, particularly outside home (Barnard, 2000). Men are compelled to fight them using available technical control measures. There was initial success in controlling mosquito vectors by using synthetic insecticides. The major drawback with these synthetic insecticides is that they are generally non-biodegradable, toxic to non-

targets, and vectors develop resistance against them (Sharma *et al*, 2004). Chemical control is the most important element in the integrated approach to control of vectors and pests of public health importance. It includes the use of vector control household and professional pest management pesticides. Diseases such as malaria, dengue and dengue hemorrhagic fever, affect the health and well-being of millions of people worldwide and are an impediment to social and economic development. Correctly used, insecticides play an important global role in the prevention and control of these diseases (WHO, 2003a). Four classes of chemical insecticides are recognized: the organochlorines, the organophosphates, the carbamates and the pyrethroids are still the mainstay of vector control programmers. Greater attention, has also been paid, to personal and household protection from insect vectors and intermediate rodent hosts and to community participation in eliminating vectors breeding sites. The provision of information on simple, effective, acceptable methods for reducing the sources of vectors and for personal protection at a reasonable cost is an important part of vector control programs (WHO, 2006). Control of mosquitoes has been largely achieved by means of synthetic insecticides. Their use on a vast and an increasing scale has led to the widespread development of insecticide resistance. Resistance has been defined as the development of the ability in a strain of insects to tolerate doses of toxicants, which would prove lethal to the majority of individuals in normal population of same species. Resistance has appeared in chlorinated hydrocarbons, organophosphates and carbamate insecticides. The physiological basis of resistance also varies with species and insecticide, and may arise through enhanced metabolism of the insect, or reduced nerve sensitivity (WHO, 1982). In the control of lymphatic filariasis, emphasis has recently shifted towards human drug treatment. However, it is unlikely that drug treatment alone will eliminate the disease, and vector control efforts need to be maintained. Mosquito management is an important challenge in countries in

which malaria is endemic and which often suffer from insufficient resources and limited sanitation infrastructures (WHO, 2002a).

1.2. Rationale of the Study.

The main method of larval control, in the Sudan, is the use of chemicals usually Temephos emulsifiable concentrate (EC) 50%. Areas targeted are mostly urban areas in big cities and riverine areas as appropriate. Environmental management is limited to agriculture-irrigated areas through drainage as well as intermittent irrigation. Biological control using larvivorous fish is also implemented on a small scale in the irrigated agricultural areas. Entomological monitoring including monitoring for insecticide resistance is important (NMCP, 2006).

Efforts are being made to seek insecticides of natural origin as a safer alternative to synthetic insecticides. Many plant extracts have been studied for their efficiency in controlling larvae of different mosquito species the all world over (Sharma *et al*, 2004).

In this study, it was decided to evaluate the larvacidal nature of aqueous extract of *Ambrosia maritima* L to control mosquito larvae.

1.3. Objectives

1.3.1. General objective

To compare the toxicity of *Ambrosia maritima* L. (Damsesa) leaves aqueous extract and Abate aqueous solutions on *Culex* larvae.

1.3.2. Specific objectives

1- To draw the Regression line to the toxicity of *Ambrosia maritima* L. leaves aqueous extract and Abate aqueous solutions on *Culex* larvae.

2- To determine the lethal concentration (LC) 50% of experimental larvae (LC_{50}) by *Ambrosia maritima* L. leaves aqueous extract and Abate aqueous solutions on *Culex* larvae.

3- To determine the lethal concentration (LC) (99%) of experimental larvae (LC_{99}) by *Ambrosia maritima* L. leaves aqueous extract and Abate aqueous solutions on *Culex* larvae.

4- To find out the possibility of the use of *Ambrosia maritima* L. leaves aqueous extract in the control of *Culex* larvae in the field level.

LITERATURE REVIEW

1.4.1. Classification of *Culex.*

One hundred and fifty six species, two subspecies and seven varieties of Culicidae are known to occur in the Sudan (Lewis, 1956a). The subfamily Culicinae contains 30 genera of mosquitoes, of which the medically important ones are *Culex*, *Aedes*, *Mansonia*, *Sabethes*, *Haemagogus* and *Psorophora*. The most important vector species include *Aedes aegypti*, *Aedes africanus*, *Aedes simpsoni*, *Aedes albopictus*, *Aedes scutellaris* group, *Culex quinquefaciatus*, *Culex tritaeniorhynchus*, *Mansonia uniformis*, *Mansonia dives*, *Mansonia bonneae* and *Haemagogus spegazzinii falco* (Service, 1980). About 550 species of *Culex* have been described mostly from tropical and subtropical regions of the world. Some species are important as vectors of bancroftian filariasis and arboviral diseases. In some areas, they are a considerable nuisance (Rozendaal, 1997). Forty-five species, two subspecies and one variety of *Culex* mosquitoes occur in the Sudan (Lewis, 1956a).

Four out of the six species listed by Edwards as being, common to the Ethiopian and Oriental Regions occur in the Sudan. *Culex tritaeniorhynchus*, which is widespread in India, has only a very limited distribution in the Sudan and has probably spread from East Africa. *Culex sitiens* is confined to the Red Sea Coast. *Culex theileri* is very localized in the Sudan, *Culex univittatus* is very widely distributed, and *Culex pipiens* has a rather wide distribution although subspecies *fatigans* is almost confined to the coast. *Culex arbiecni* is known from Jabal Marra and three areas outside the Sudan. *Culex univittatus* is unique in being abundant. *Culex tigripea*, *Culex poicilipes*, *Culex ethiopicus* and *Culex decens* are very widely distributed (Lewis, 1956b).

1.4.2. *Culex* Larvae.

The larva is free-swimming with an elongated body divisible into head, thorax and abdomen. It feeds on algae, bacteria and vegetable matter and passes through four instars. The *Culex* larvae are suspended in water with their heads downwards. They possess a siphon tube, which is situated on the eighth abdominal segment. The larval stage takes 5-7 days (Park, 2005). The first instar measures about 1.5 mm in length, the fourth about 8-10 mm. They have no legs and the body is covered with hairs (Rozendaal, 1997).

Larval habitats vary enormously, reflecting the evolutionary adaptability of mosquitoes. Potential water habitats include both permanent and temporary bodies of water, fresh and brackish water, standing water, canals and open streams, in bright sun light or deep shade. Larvae can live even in indoor containers, in shallow pools and deep wells, in clean drinking water and in water highly polluted with organic matter, in large open marshes and in the tiny pools of water that collect in plant axils, in tree holes, in rock or crab holes, in cattle footprints or in discarded tins or other artificial containers. Although some species have the ability to adapt to a wide range of breeding places, most are much less adaptable (Najera and Zaim, 2003).

1.4.3. Temperatures and Mosquitoes.

Temperature affects the developmental period related to different stages of a mosquito's lifecycle. They also affect blood-feeding rate, gonotrophic cycle and longevity. At increased temperatures, the rate of digestion of blood-meal increases, which in turn accelerates ovarian development and egg laying. A reduction in the duration of the gonotrophic cycle would make the vectors bite more frequently, thereby increasing the probability of transmission. At lower temperatures, the duration gets prolonged. However, at more than 40 °C, mortality occurs in adult mosquitoes. For larvae, 25 °C, was found to be the optimum temperature (Dhiman *et al*., 2008).

The range of environmental temperatures that allow survival would appear to be greater for *Anopheles arabiensis* than for *Culex quinquefasciatus*. Nevertheless, the latter is the more common species in the Khartoum (Sudan) area. This relative abundance is suggested to be attributed largely to the success of *Culex quinquefasciatus* in exploiting a wide range of breeding sites, even those which are obviously contaminated in various ways. Temperature, on the other hand, seems to be the major environmental factor, which affects, directly or indirectly, the survival and distribution of mosquitoes. So high temperature must check the number of emerging adults (a direct effect); and hasten the life span of larvae at the expense of size (an indirect effect) (El Rayah and Abu Groun, 1983).

1.4.4. Rainfall.

Rainfall is important for the availability of vector breeding sites. The effect of rainfall depends on the breeding habits of mosquitoes (WHO, 2007).

1.4.5. Water pH and the Effectiveness of Pesticides.

Some pesticides, particularly carbamates and organophosphates undergo a chemical reaction in the presence of alkaline water (water that has a pH value greater than 7). The reaction is known as alkaline hydrolysis, and it reduces the effectiveness of the pesticide's active ingredient. The speed with which the breakdown occurs depends on the specific chemical properties of the pesticide, the pH of the water mix and the length of time the pesticide is in contact with the water. Spray or a water -mix with a pH value between eight and nine can cause rapid hydrolysis to the point that the degree of pest control is greatly diminished or lost (Fishel and Ferrell, 2007).

In the case that your water mix has a pH of 7.5 or greater, consider lowering the pH, especially if applying a pesticide that is sensitive to a high pH. A pH of 4 to 7 is recommended for mixing most pesticides a value of 5.5 to 6.5 is ideal. If the spray rig is to left to stand for several hours before the contents are applied, adding an acidifying agent will be considered to prevent alkaline

hydrolysis. Some product labels give direct ions to avoid mixing the pesticide with alkaline water or other specific alkaline materials (Fishel and Ferrell, 2007).

1.4.6. Medical Importance.

1.4.6.1. Nuisance.

Culex is both a nuisance and the host/vectors of disease-causing parasitic organisms (WHO, 2002a).

1.4.6.2. Rift Valley Fever.

Rift Valley Fever is a virus disease, which was known to occur in south and East Africa. The disease is an important epizootic disease of livestock in Kenya where it was first discovered. The disease has been reported in Sudan, Egypt, Ethiopia and some other central Africa countries. In the Sudan, the occurrence was confined to irrigated and highly watered areas close to the rivers. The high population densities and high vector densities contribute to the high incidence. The reservoir is sheep and cattle. Goats and camels may also act as hosts. Irregular epidemics occur in man during and after epizootics in animals. Transmission in animals is by mosquitoes, e.g. *Culex pipiens*, *Culex theileri* and *Aedes caballus* (Ibrahim, 1990).

Rift Valley in sheep and other animal potential vectors include *Aedes* mosquitoes; *Aedes mcintoshi* may be infected transovarially and account for maintenance of Rift Valley Fever virus in epizootic foci. *Culex pipiens* was implicated in a 1977 epidemic of Rift Valley Fever in Egypt with at least 600 deaths. Mechanical transmission by hematophagous flies and transmission by aerosols or contact with highly infective blood may contribute to Rift Valley Fever outbreaks. Many human infections of Rift Valley Fever are associated with the handling of animal tissues during necropsy or butchering (Heymann, 2004).

Symptoms in man include diphasic fever and vomiting. Although recovery may occur, in severe cases haemorrhages, jaundice, hepatitis,

neurological sings, encephalitis and characteristic retinitis, may be observed. The disease may terminate fatally (Ibrahim, 1990). No Rift Valley Fever vaccine is available (WHO, 2003-2006).

1.4.6.3. West Nile Fever.

A virus of the flavivirus group of arboviruses causes West Nile fever. The virus has been isolated from mammals, birds and arthropods in Uganda, Sudan, Egypt, Zaire, Central African Republic and Nigeria. An antibody against the virus has been found in many species of animals practically in all African countries. The disease is endemic in the Nile delta where it primarily affects the infant population (Ibrahim, 1990). Chicken is the principal reservoir of the virus where a relatively long period of viraemia occurs; hence, the Chicken represents an important source of infection to other animals. Ornithophilic mosquitoes of the genus *Culex* transmit the virus. Although it has been isolated, from several species of *Culex* yet *Culex univittatus* played a primary role in the transmission cycle and maintenance of the virus in endemic areas. The disease occured during seasons of high mosquito activity. Infection varies from subclinical to mild temporary fever to serious encephalitis. The disease is mild in children, but is more serious in elderly people. It has a sudden onset with rise of temperature, cephalgia, lymphadenopathy and a cutaneous maculopapular eruption mainly on the trunk. Other symptoms include articular, muscular and ocular pain, and gastrointestinal disturbances. The mortality rate is low (Ibrahim, 1990).

1.4.6.4. Filariasis.

The most important vector-borne diseases harming human health are lymphatic filariasis transmitted by mosquitoes (WHO, 2004).

C. quinquefasciatus transmission of *Wuchereria bancrofti*. *Culex* complex' are responsible for most or all of the bancroftian filariasis transmission in Asian countries, Indonesia, Egypt, urban East Africa and the Americas (WHO, 2002b).

1.4.7 Control Directed at the Immature Stages.

1.4.7.1. Environmental Control.

A form of environmental management consisting in any physical transformation that is permanent or long lasting of land, water and vegetation, aimed at preventing, eliminating or reducing the habitats of vectors without causing unduly adverse effects on the quality of the human environment (WHO, 1982).

1.4.7.1.1. Environmental Modification.

Environmental modification includes drainage, filling, land leveling and transformation of impoundment margins. Although these works are usually of a permanent nature, proper operation and adequate maintenance are essential for their effective functioning (WHO, 1982).

1.4.7.1.1.1. Removal or Destruction of Breeding Sites.

Small containers, such as used cans, bottles, tyres and coconut husks used as breeding sites can be removed or destroyed (Rozendaal, 1997).

1.4.7.1.1.2. Filling.

The filling of mosquito breeding sites with soil, stones, ash or rubbish is the most permanent control measure available. It is most suitable for reducing breeding in small depressions, water holes, borrow-pits, abandoned ditches or pools, which do not require much filling material. The filling material should be obtained without creating new breeding sites. Waste materials can be used for most fillings. If refuse is used it should be compacted and covered with earth to prevent breeding by flies. All fills should be topped with clean earth and graded to make the areas attractive and suitable for use as building sites, playgrounds, etc (Rozendaal, 1997).

1.4.7.1.1.3. Drainage.

Construction of open ditches and dykes with tidal gates, subsoil drainage and pumping can accomplish the drainage of water. Proper drainage reduces mosquito breeding; however, the drainage systems used in agriculture or for the transportation of sewage and rainwater in cities are often an important source of breeding because of poor design and maintenance. Leakages, obstructions, and small pools or puddles of residual water in drainage ditches are often suitable breeding sites for mosquitoes. The planning and construction of drainage systems are complicated and require the expertise of engineers. However, less experienced people using simple equipment can carry out some small-scale drainage works intended to control mosquitoes (Rozendaal, 1997).

1.4.7.1.1.4. Closing, Screening or Covering Breeding Sites.

Potential breeding sites in relatively small-enclosed habitats, such as drinking water storage containers and wells, should be made inaccessible to adult mosquitoes. Removable covers, such as mosquito-proof lids or wire mesh screening, can be fitted in some cases. Wells can be made mosquito-proof by closing them with cement slabs and installing hand pumps. Latrines can be made insect-proof by improving their design. A less conventional approach is to cover the water surface completely with a material that is impenetrable to mosquitoes (Rozendaal, 1997).

1.4.7.1.2. Environmental Manipulation.

A form of environmental manipulation consisting in any planned recurrent activity aimed at producing temporary conditions unfavorable to breeding of vectors in their habitats." Water salinity changes, stream flushing, regulation of the water level in reservoirs, dewatering or flooding of swamps, vegetation removal, shading and exposure to sunlight are examples of environmental manipulation activities (WHO, 1982).

1.4.7.1.2.1. Flushing.

It is employed in small streams where there is a continuous and abundant supply of water flowing slowly enough to permit mosquitoes to breed in quiet places along the margins (Rozendaal, 1997).

1.4.7.1.2.2. Changes in Water Salinity.

Mosquitoes that breed in lagoons and coastal marshes can be controlled by letting in additional seawater. Most species will not be able to tolerate the increase in salt concentration (Rozendaal, 1997).

1.4.7.1.2.3. Clearing of Vegetation.

Clearing of vegetation may result in increased breeding by mosquito species that prefer sunlit water. However, some species need shaded water and may be effectively controlled. This method may also be effective in removing resting places for adult mosquitoes. In addition, it promotes evaporation and the drying up of small accumulations of water and makes breeding sites more visible for control purposes (Rozendaal, 1997).

1.4.7.1.2.4. Shading of Stream Banks.

Where mosquitoes prefer breeding sites that are partly or fully exposed to sunlight, they can be controlled by planting shrubs and trees along the banks of streams to provide dense shade (Rozendaal, 1997).

1.4.7.1.2.5. Straightening and Steepening Shorelines.

Shorelines of streams, ditches and ponds can be modified to reduce the availability of shallow places suitable for breeding of mosquitoes and to increase the flow of the water (Rozendaal, 1997).

1.4.7.2. Biological Control.

In biological control methods, pests are kept in check by natural enemies, including predators, parasites, and disease-causing bacteria or viruses. To be effective, predatory insects must be carefully chosen. Biological control is many advantages. In general, they destroy only the target species and are nontoxic to other species (Girard, 2005). This measure is costly because of the need for many repetitive applications during the season (WHO, 2007).

1.4.7.2.1. Predators.

Several attempts have been made to control mosquitoes by using predators. The most commonly used predators are fish, and species in several genera such as *Lebistes, Poecilia*, *Sarotherodon* (= *Tilapia*), *Panchax*, have been used, but two subspecies of *Gambusia* , namely *Gambusia affinis affinis* and *Gambusia affinis holbrooki*, commonly referred to as 'mosquito fish' have been employed more extensively than any others. However, some fish such as species of *Nothobranchius* and *Cynolebias*, which are called 'instant' or 'annual' fish, have drought resistant eggs and these are more suitable for introducing into small temporary habitats that repeatedly dry out. Polluted waters are also usually unsuitable habitats for most fish (Service, 1980).

A fish fauna survey in (1980) in the canals of the Gezira irrigation scheme showed the presence of *Oreochromis niloticus* and *Gambusia affinis* among the known larvivorous fish species. The food preferences of *Gambusia affinis* and *Oreochromis niloticus* in the Gezira irrigation canals were found carnivorous for mosquito larvae. *Oreochromis niloticus* were also markedly carnivorous and were reportedly useful for mosquito control (WHO, 2003b).

Distribution of larvivorous fish may be a good additional measure in subtropical and tropical environments where fish such as *Gambusia, Tilapia* and other mosquito predators are part of the local ecosystem (WHO, 2007).

A few mosquitoes have predacious larvae, for example, *Toxorhynchites* species, and these have been introduced into container habitats in certain areas to control larvae of other container-breeding mosquitoes (Service, 1980).

1.4.7.2.2 Pathogens.

There are numerous pathogens, such as viruses for example iridescent and cytoplasmic polyhedrosis viruses, bacteria for example *Bacillus thuringiensis* and *Bacillus sphaericus*, protozoa for example *Nosema*, *Thelohania* and fungi for example *Coelomomyces, Lagenidium, Culicinomyces* that cause larval mortality. There are several parasitic nematodes that kill mosquito larvae, and the most promising for control purposes are *Reesimermis nielseni* and more importantly *Romanomermis culicivorax*, which has in the past been misidentified as *Reesimermis nielseni*, which appear to be more specific in its host. These parasites and pathogens appear harmless to man (Service, 1980).

Bacillus thuringiensis israelensis is a biological control pesticide produced from bacteria; *Bacillus thuringiensis israelensis* serotype H-14. These bacteria produce spores containing crystals. When mosquito larvae eat the spores or the crystals, their digestive system is affected, killing the larvae. Used as a larvicide and applied directly to the water, *Bacillus thuringiensis israelensis* is not persistent. *Bacillus thuringiensis israelensis* is very specific, killing only the larvae of mosquitos, blackflies, and a few non-biting flies. *Bacillus thuringiensis israelensis* does not affect most other species of aquatic insects. The potential for toxicity to fish is so small as to be considered negligible. *Bacillus thuringiensis israelensis* does not accumulate in fish and wildlife (Paul and Sinnott, 2000).

Bacillus thuringiensis israelensis produces toxins, which are very effective in killing mosquito larvae after ingestion. It is harmless to fish, higher animals and humans at normal dosages and, depending on the formulation used, may be suitable for use in water used for drinking (with due attention to potential microbial contaminants in the formulated product) or for the irrigation

of food crops. It has the disadvantage that it is active only by ingestion and is rather heavy, sinking into the water (Najera and Zaim, 2003).

Bacillus sphaericus is a biological control pesticide produced from bacteria, *Bacillus sphaericus* serotype H5a5b, Strain 2362. These bacteria produce spores containing crystals, similar to *Bacillus thuringiensis israelensis*. When mosquito larvae eat the spores or the crystals, their digestive system is affected, killing the larvae. Used as a larvicide and applied directly to the water, *Bacillus sphaericus* is not persistent. It is particularly effective in murky, high organic water bodies. *Bacillus sphaericus* is very specific, and is labeled for only killing the larvae of mosquitoes. Most other species of aquatic insects are not affected by *Bacillus sphaericus*. The potential for toxicity to fish is so small as to be considered negligible. *Bacillus sphaericus* does not accumulate in fish and wildlife (Paul and Sinnott, 2000). *Bacillus sphaericus* is more effective in polluted water against *Culex* mosquitoes, while *Bacillus thurigniensis israelensis* is more effective in clean water (Najera and Zaim, 2003).

Microbial insecticides *Bacillus thuringiensis israelensis* (serotype H-14) and *Bacillus sphaericus* are possible alternatives to common chemicals for larviciding (WHO, 2006).

1.4.7.3. Chemical Control.

1.4.7.3.1. Petroleum Oils.

These were among the first larvicides to be used and are still used for stagnant waters, which are unsuitable both for animal drinking and for irrigation. Oils act mainly by forming a film on the water surface, which prevents larvae from breathing. The heavier the oil the less dispersible it will be and the more easily blocked by vegetation. Different grades of oil may be chosen, depending on the water temperature. Heavy oils, such as crude or used motor oil, may disperse adequately only at higher temperatures. Lighter oils, such as kerosene or diesel, are not only more easily dispersible but also less persistent, which may be desirable in cleaner waters. The dose of oil needed

varies with its dispersibility. This may be increased by the addition of detergents or vegetable oils, which would also reduce the cost of the application and improve its effectiveness by increasing its penetration of emerging vegetation (Najera and Zaim, 2003).

1.4.7.3.2. Monomolecular Surface Films.

These act in the same way as petroleum oils by denying access by the larvae to the surface to breathe. They have the advantage of being biodegradable, but the film that they form is so thin that a slight breeze may break it. As a result, they are used mainly for smaller bodies of water such as pools, ponds and containers, but also for larger aquatic habitats if these are sheltered from the effects of wind (Najera and Zaim, 2003).

1.4.7.3.3. Insect Growth Regulators.

These chemical compounds are highly toxic to mosquito larvae by preventing their development into adults. They have very low toxicity to mammals, birds, fish and adult insects, but are toxic to crustaceans and immature stages of aquatic Insects. Their use has generally been limited by their high cost and operational acceptability, but may be of particular interest where target species have developed resistance to organophosphate larvicides or where these compounds cannot be used because of their effect on the environment. Insect growth regulators can be divided into:

Juvenile hormone analogues, which prevent the development of larvae into viable pupae or of pupae into adults (they do not kill larvae) and

Chitin synthesis inhibitors, which interfere with the molting process killing the larvae when they molt (Najera and Zaim, 2003).

Insect growth regulators might, however, affect non-target organisms and should not be used in breeding sites with an abundance of arthropod species, unless an impact assessment has been carried out (WHO, 2006).

1.4.7.3.4. Polystyrene Beads.

These have been used in controlling culicinae by the treatment of abandoned wells and latrines (Najera and Zaim, 2003).

1.4.7.3.5. Abate (Temephos).

Temephos (Abate®) or 0,0,0',0'-tetramethyl, 0,0'-thiodi-p-phenylene phosphorothioate is an organophosphate compound characterized by a very low toxicity to warm blooded animals. The empirical formula is $C_{16}H_{20}O_6P_2S_3$. The molecular weight is 466.5 g. Its trademark is "Abate", "Abathion", "Abat", "Swebat", "Nimitex" and "Biothion"(Shian,2007).

Pure temephos has a low melting point about 30 ºC and the technical material is a viscous yellow to brown liquid at room temperature. It is a relatively high molecular weight organophosphorus compound, of low volatility and decomposing at about 100 ºC at atmospheric pressure. Temephos is of very low solubility in water 30 µg/l at 25 ºC but is soluble in many organic solvents. It has no acidic or basic characteristics, it is stable to hydrolysis half-life >30 days at pH 4-9 at 25 ºC and photolysis occurs only slowly half-life 15 days, continuous irradiation with artificial sunlight (WHO, 2005).

Abate (Temephos), because of its low toxicity, has been extensively used in India for the control of *Anopheles stephensi* in wells and in domestic water containers with good results at a dosage not greater than 1.0 ppm. Abate is less effective as adulticide (Park, 2005).

Organophosphate larvicides such as temephos in clean water, including drinking water; it is standard method of controlling culicine larvae (WHO, 2002a). Temephos is used primarily as a larvicide and is applied directly to the water. When applied at a concentration of 0.1 ppm, temephos will not harm most non-target aquatic insects, yet it is effective against mosquitoes. It does not pose a significant threat to fish. Temephos is not persistent. It is however a broad-spectrum pesticide and at higher concentrations it will kill target as well as non-target aquatic insects. It does not accumulate, so contamination of fish

flesh should not occur (Paul and Sinnott, 2000). Elshafie (2004) found the LC_{50} of Abate for *Culex* larvae 0.105 ppm (Yousif, 2007).

It kills insects by interfering with nervous system function, as do all members of the organophosphate chemical family. Normally, impulses are transmitted chemically from the end of one nerve cell to the beginning of another; one of the chemical transmitters used in animal nervous systems is called acetylcholine. After transmitting the nerve impulse, acetylcholine is destroyed by an enzyme called acetylcholinesterase (AChE) in order to clear the way for another transmission. Organophosphates attach to acetylcholinesterase and prevent it from destroying acetylcholine, causing overstimulation of the nerves. Mammal and insect nervous systems are similar enough that effects of organophosphates are similar (Cox, 2000).

1.4.8. *Ambrosia maritima* L.

Ambrosia maritima L. (Damsissa) is one of the wild plants present in Egypt and different African countries of the Nile valley. It belongs to the subfamily Tubuliflora, which is a branch of the family Compositae of flowering plants. It contains important sesquiterpene lactones and flavonoids, which showed molluscidal and cytotoxic activities (Abdel-Hamid and Tarabanko, 2004).

Latin Name *Ambrosia maritima* L., Synonym: *Ambrosia senegalensis* DC. Vernacular names: Arabic, Damseesa or Demsisa; English, Rag Weed (El Ghazali,*et al*., 2004). Sea ambrosia (Boulos, 1983).

1.4.8.1. Description.

Pilose erect branched herbs or under shrubs, up to two meters high. Odour aromatic herbs or under shrubs; herbs very strongly aromatic (El Ghazali, *et al*., 2004). It is a gray hairy herb with finely dissected, fragrant leaves found on muddy canal banks (Ammar, *et al*., 1993).

1.4.8.2. Distribution.

Northern and Central Sudan (El Ghazali, *et al*., 2004). *Ambrosia maritina* L, which is locally in Sudan known as Damsissa, is widely used in the folk medicine as antidiabetic. It has also been reported to have molluscicidal effects (Abuelgasim, *et al*., 2007).

1.4.8.3. Medical Uses.

Decoction of plant for rheumatic pains, asthma, bilharziasis, diabetes and to expel kidney stones. Flowering branches stimulant, stomachic, slightly astringent, emollient, vulnerary, diuretic, renal troubles (Boulos, 1983). Herb of *Ambrosia maritima* L was known to be used in folk medicine. Also that the alcoholic extract of *Ambrosia maritima* L showed on antibacterial activity (Ammar, *et al*., 1993). Drinking decoctions of Damsissa were the most commonly used remedy for schistosomiasis in Upper Egypt (Abdel-Hamid and Tarabanko, 2004).

1.4.8.4. Chemical Constituents.

Pseudoguaianolides, new chlorosesquiterpene lactone was isolated from the aerial parts (El Ghazali, *et al*., 2004). Chemically the aerial parts of this medicinal plant contained four pseudoguainolides, parthenin and neombrasin. Two new sesquiterpene lactones, characterized as 1'-noraltarnisin and (11R)-11, 13 dihydropsilostachyin, were isolated together with 7 known terpenoids from the leaves other lactones from this species have been reported to show molluscicidal activity against the intermediate hosts of *Schistosoma* species (Ammar, *et al*., 1993). The most active ingredients of this plant are ambrosin and damsin. Ambrosin belongs to a group of natural products known as pseudoguaianolides, it was totally synthesized and described by Grieco *et al.* (1982). Damsin is 2,3-dihydroambrosin (Abdel-Hamid and Tarabanko, 2004).

1.4.8.5. Toxicity.

Ambrosia maritima L has a very low toxicity to aquatic non-target organisms. It is not toxic when used at the mollucicidal concentration of 35to 70 mg/liter. A toxic principle of the plant has been found to contain the potentially allergenic sesquiterpene lactone: ambrosin and damsin (El Ghazali, *et al*., 2008). The toxic effect of the methanolic and water extracts of *Ambrosia maritima* on rats treated for three weeks with different doses was determined. The methanolic extract was injected intramuscularly and the water extract was given orally. The results revealed that a single intramuscular dose of 2000 mg kg-1 (Bwt) caused in appetence, deceased activity, lameness and even paralysis, although body weights were not affected. However, diarrhea was the most prominent sign in rats receiving the water extract at the doses 1000 and 500 mg kg-1 (Bwt) while at the dose 250 mg kg-1 (bwt) no clinical signs were observed. Lesions in both extracts consisted of generalized congestion, fatty change of the liver, degeneration of renal tubules and pancreatic hyperplasia (Abuelgasim, *et al*., 2007).

CHAPTER TWO
MATERIALS AND METHODS

2.1. Study Design.

The main objective of this study is to compare:

- the toxicity of *Ambrosia maritima* L. leaves aqueous extract against *Culex* larvae in Khartoum.
- the toxicity of Abate or Temephos which is an organophosphate aqueous solution against *Culex* larvae in Khartoum.

2.2. Study Area.

The study was carried out in Khartoum locality of Khartoum State. This area is bordered in the east by the Elgasr Avenue, the Blue Nile in the north, Elmogran district in the west and Khartoum Railway Station in the south. The main sources of the mosquito breeding sites in this area are drains, canals, broken water pipes and rain pools. Health and environmental vector control measures are important and there is a weekly program to treat these sites by larvicides mainly Abate.

2.3. Climate.

Climatically, the Khartoum State is considered as semi-desert with low rainfall and high evaporation potential. The short rainy season is confined to late summer and extends from July to October with an average annual rain of 160 mm. However, the maximum temperature (46 °C) is reached in May and the minimum (20 °C) in January. The average relative humidity is 36% - 64%. It reaches its maximum level in August and its minimum level in January (KMFP, 2008).

2.4. Larval Collection.

Culex larvae were collected from water polluted with organic matter, such as sewage water from Khartoum Railway Station, industrial waste water,

and drains near Khartoum Sunt Forest. In each field collection, the larvae were put in plastic containers in a large amount of water from the site of collection. The larvae were then, transported to and kept in the entomology laboratory of the Faculty of Public and Environmental Health.

2.5. Maintenance of the Larvae.

The *Culex* larvae were kept in containers in the laboratory at the temperature of 32 ºC and relative humidity of 50%. The larvae were kept in the laboratory for 24 hours to adapt to the laboratory conditions. Containers were covered with nets to stop newly emerging adults from escaping.

2.6. Plant Leaves Collection.

Ambrosia maritima L was collected from the riverbank of the Blue Nile in Khartoum and Tuti Island and identified in Medicinal and Aromatic Plants Research Institute (MAPRI) of the National Center for Research. The collected leaves were dried in shade, and powdered. Twenty grams of the leaf powder were added to 200 milliliters of distilled water and left for twenty four hours. The mixture was filtered and the filtrate was used as a stock solution and different test concentrations were prepared using this extract.

2.7. Laboratory Tests.

Susceptibility tests were performed according to WHO recommended procedure to determine the laboratory toxicity of *Ambrosia maritima* L and Abate 50% emulsifiable concentration against *Culex* larvae. The tests were carried out at a temperature of 32 ^{o}C and relative humidity 50%, using field larvae of *Culex*.

To prepare Abate stock solution one milliliter of the commercial product were added to 99 milliliters of distilled water to give a concentration of 1% (0.01). Subsequent serial dilutions of stock solutions, (0.001), and (0.0001) were made. When tested all these concentrations proved lethal to all larvae in. A suitable stock solution for doing the tests was found to be (0.00001).

Table 1. Preparation of concentrations of Abate in (parts per million) in distilled water and in water from the site of collection.

Serial	Milliliter				
	Stock solution	Distilled water	Water from site of collection	Whole volume	Concentration ppm
1	0.15	159.85	40	200	0.00375
2	0.25	159.75	40	200	0.00625
3	0.35	159.65	40	200	0.00875
4	0.45	159.55	40	200	0.01125
5	0.55	159.45	40	200	0.01375
6	0.65	159.35	40	200	0.01625
7	0.75	159.25	40	200	0.01875
8	0.85	159.15	40	200	0.02125
9	0.95	159.05	40	200	0.02375
10	1.05	158.95	40	200	0.02625

Table 2. Preparation of concentrations of *Ambrosia maritima* L. leaves aqueous extract in parts per million in distilled water and in water from the site of collection.

Serial	Milliliter				
	Stock solution	Distilled water	Water from site of collection	Whole volume	Concentration ppm
1	19	141	40	200	9500
2	22	138	40	200	11000
3	25	135	40	200	12500
4	28	132	40	200	14000
5	31	129	40	200	15500
6	34	126	40	200	17000
7	37	123	40	200	18500
8	40	120	40	200	20000
9	43	117	40	200	21500

Laboratory temperatures were measured using a mercury thermometer. pH value before treatment and after treatment was measured by the digital pH meter.

Batches of 25 third and fourth instars larvae were transferred by means of screen loops to small disposable test cup, each 300 milliliter. Small, unhealthy or damaged larvae were replaced. The depth of the water in the cups was kept between eight and ten centimeters. Deeper levels may cause undue mortality. The *Culex* larvae were exposed to ten concentrations for Abate and nine different concentrations for *Ambrosia maritima* L. leaves aqueous extract and the control was tap water.

Four replicates were set up for each concentrations and an equal number of controls were set up with tap water. The test containers were kept at 31 to 32 ℃. After 24 hours exposure, larval mortality in each concentration was recorded.

If more than 10% of the control larvae were pupates in the course of the experiment, the test was discarded and repeated. If the control mortality was between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott's formula:

$$\text{Mortality (\%)} = \frac{X - Y}{X} \times 100$$

Where:

X = percentage survival in the untreated control and

Y = percentage survival in the treated sample.

The percentage of death counting was as follow:

Larvae death $\times 100 \div$ total number of larvae

The percentage of death was transformed to the probit units. Concentrations used in each experiment were set as part per million (ppm) logarithms of concentration for (ppm) were calculated. After determining the mortality of larvae in this wide range of concentrations, the results were used to determine LC_{50} and LC_{99} values. All the experiments were performed according to WHO standard techniques.

2.8. Data Analysis.

The data were analyzed using Excel 2007 program and other computer soft ware.

The computer and the formula of the regression line were used to calculate the LC_{50} and LC_{99}

CHAPTER THREE

RESULTS

A series of laboratory experiments were conducted to examine the toxicity of *Ambrosia maritima* L leaves aqueous extract and Abate aqueous solution against *Culex* larvae.

The Average percentage deaths for *Culex* larvae in the four experiments in Abate ranged between 5% and 100% as has been shown in Tables 3, 4, 5, and 6 and summarized in Table 7. The LC_{50} for Abate was 0.008104 ppm and the LC_{99} for the same chemical was 0.045185 ppm (Fig. 5).

The Average percentage deaths for *Culex* larvae in the four experiments of *Ambrosia maritima* L leaves aqueous extract ranged between 9% and 96% as has been shown in Tables 8, 9, 10, and 11. These results have been summarized in Table 12. The LC_{50} for *Ambrosia maritima* L was 13489 ppm and the LC_{99} for the same extract was 31088 ppm (Fig. 10).

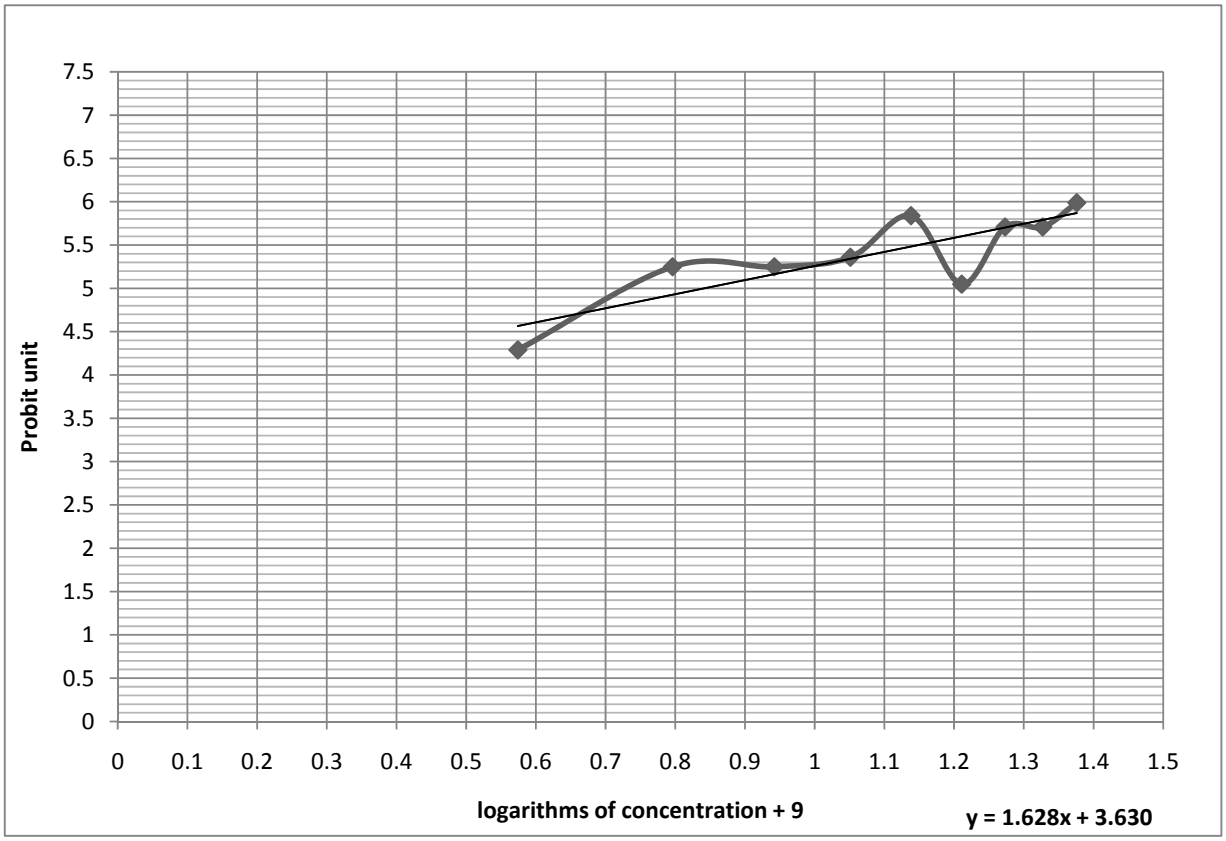
Thus, the *Ambrosia maritima* L leaves aqueous extract were far less toxic than Abate aqueous solutions. Higher concentrations of the *Ambrosia maritima* L leaves aqueous had to be used in these experiments to kill *Culex* larvae compared to Abate. The experimental results are shown in Tables 3,4, 5,6,7,8,9,10,11 and 12 and Fig. 1,2,3,4,5,6,7,8,9 and 10.

The hydrogen ion concentrations (pH) were nearly the same (below seven) before and after the execution of the experiments. This was possibly because the water brought from breeding sites was originally acidic.

Table 3: Percentage Death in *Culex* Larvae Treated with Different Concentrations of Abate.

Concentration ppm	Logarith-ms of concentr-ation	Logarith-ms of concentr-ation + 9	Death %	Corrected death %	Probit unit	pH measurement	
						Before treatment	After treatment
0.00375	-8.4260	0.5740	24%	24%	4.29	6.22	6.22
0.00625	-8.2041	0.7960	60%	60%	5.25	6.22	6.22
0.00875	-8.0580	0.9420	60%	60%	5.25	6.22	6.26
0.01125	-7.9490	1.0510	64%	64%	5.36	6.22	6.33
0.01375	-7.8620	1.1380	80%	80%	5.84	6.22	6.34
0.01625	-7.7891	1.2109	52%	52%	5.05	6.22	6.35
0.01875	-7.7270	1.2730	76%	76%	5.71	6.22	6.38
0.02125	-7.6730	1.3270	76%	76%	5.71	6.23	6.40
0.02375	-7.6243	1.3760	84%	84%	5.99	6.23	6.41
_____	_____	_____	_____	_____	_____	_____	_____

Fig.1: Abate Toxicity to *Culex* Larvae (Experiment 1).



When: y = 5.00

X = 0.8415

LC_{50} = 0.006942 ppm

When: y = 7.33

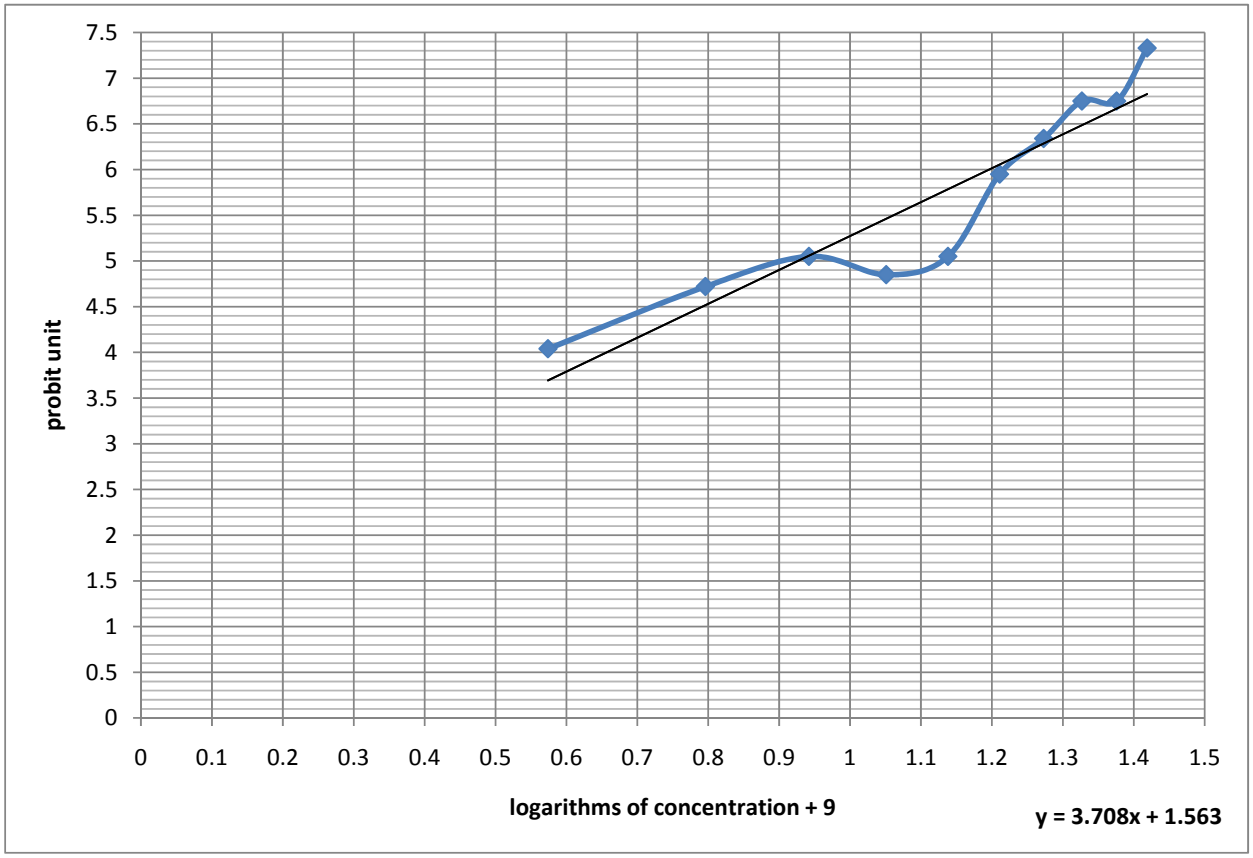
X = 2.2727

LC_{99} = 0.1874 ppm

Table 4: Percentage Death in *Culex* Larvae Treated with Different Concentrations of Abate.

Concentration ppm	Logarith-ms of concentr-ation	Logarith-ms of concentr-ation + 9	Death %	Corrected death %	Probit unit	pH measurement	
						Before treatment	After treatment
0.00375	-8.4260	0.5740	28%	17%	4.04	4.88	4.88
0.00625	-8.2041	0.7960	44%	39%	4.72	4.88	4.88
0.00875	-8.0580	0.9420	56%	52%	5.05	4.88	4.89
0.01125	-7.9490	1.0510	48%	44%	4.85	4.88	4.91
0.01375	-7.8620	1.1380	56%	52%	5.05	4.88	4.92
0.01625	-7.7891	1.2109	84%	83%	5.95	4.88	4.93
0.01875	-7.7270	1.2730	92%	91%	6.34	4.89	4.93
0.02125	-7.6730	1.3270	96%	96%	6.75	4.89	4.94
0.02375	-7.6243	1.3760	96%	96%	6.75	4.89	4.94
0.02625	-7.5810	1.4190	100%	100%	7.33	4.90	4.94

Fig. 2: The Abate Toxicity to *Culex* Larvae (Experiment 2).



When: y = 5.00

X = 0.9269

LC_{50} = 0.0084508 ppm

When: y = 7.33

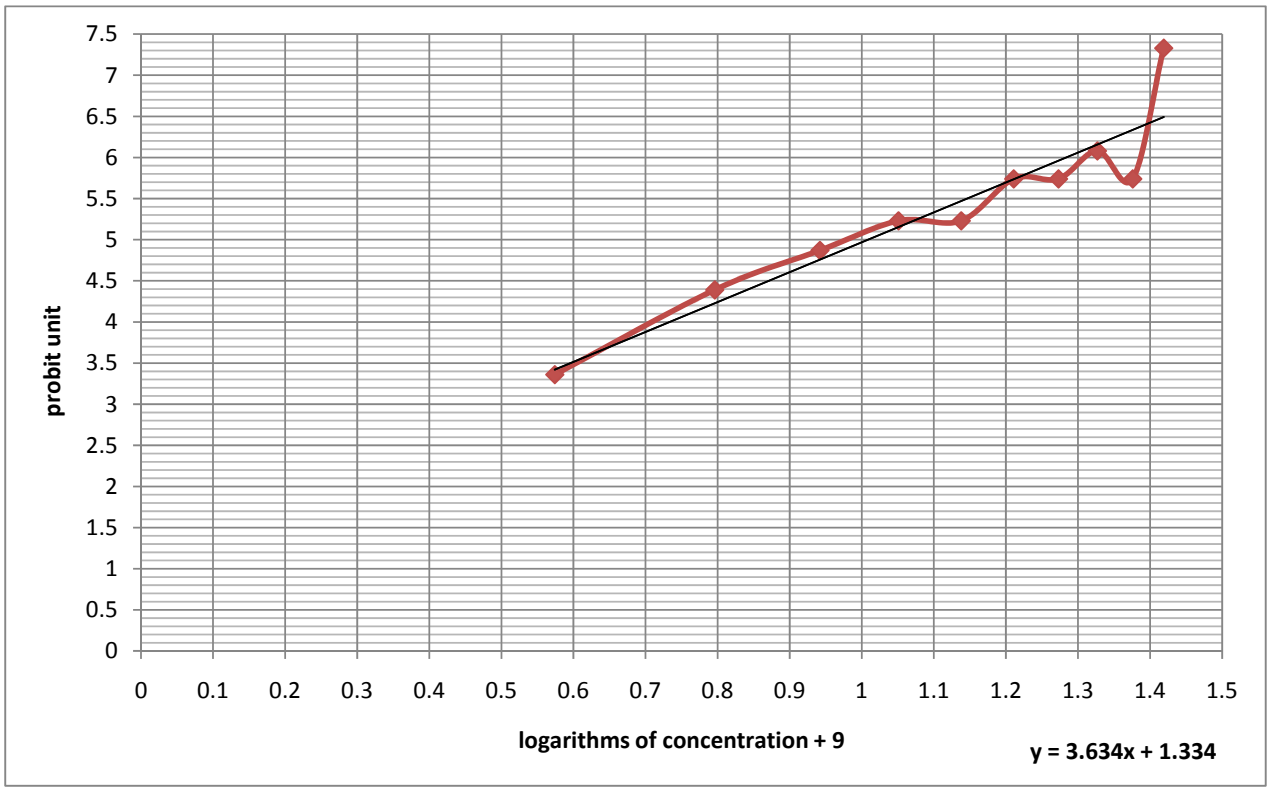
X = 1.5552

LC_{99} = 0.035908 ppm

Table 5: Percentage Death in *Culex* Larvae Treated with Different Concentrations of Abate.

Concentration ppm	Logarith-ms of concentr ation	Logarith-ms of concentr-ation + 9	Death %	Corrected death %	Probit unit	pH measurement	
						Before treatment	After treatment
0.00375	-8.4260	0.5740	16%	5%	3.36	4.93	5.00
0.00625	-8.2041	0.7960	36%	27%	4.39	4.93	5.00
0.00875	-8.0580	0.9420	52%	45%	4.87	4.93	5.01
0.01125	-7.9490	1.0510	64%	59%	5.23	4.93	5.03
0.01375	-7.8620	1.1380	64%	59%	5.23	4.93	5.04
0.01625	-7.7891	1.2109	80%	77%	5.74	4.93	5.04
0.01875	-7.7270	1.2730	80%	77%	5.74	4.93	5.04
0.02125	-7.6730	1.3270	88%	86%	6.08	4.93	5.04
0.02375	-7.6243	1.3760	80%	77%	5.74	4.94	5.06
0.02625	-7.5810	1.4190	100%	100%	7.33	4.94	5.08

Fig. 3: Abate Toxicity to *Culex* Larvae (Experiment 3).



When: y = 5.00

X = 1.0088

LC_{50} = 0.010205 ppm

When: y = 7.33

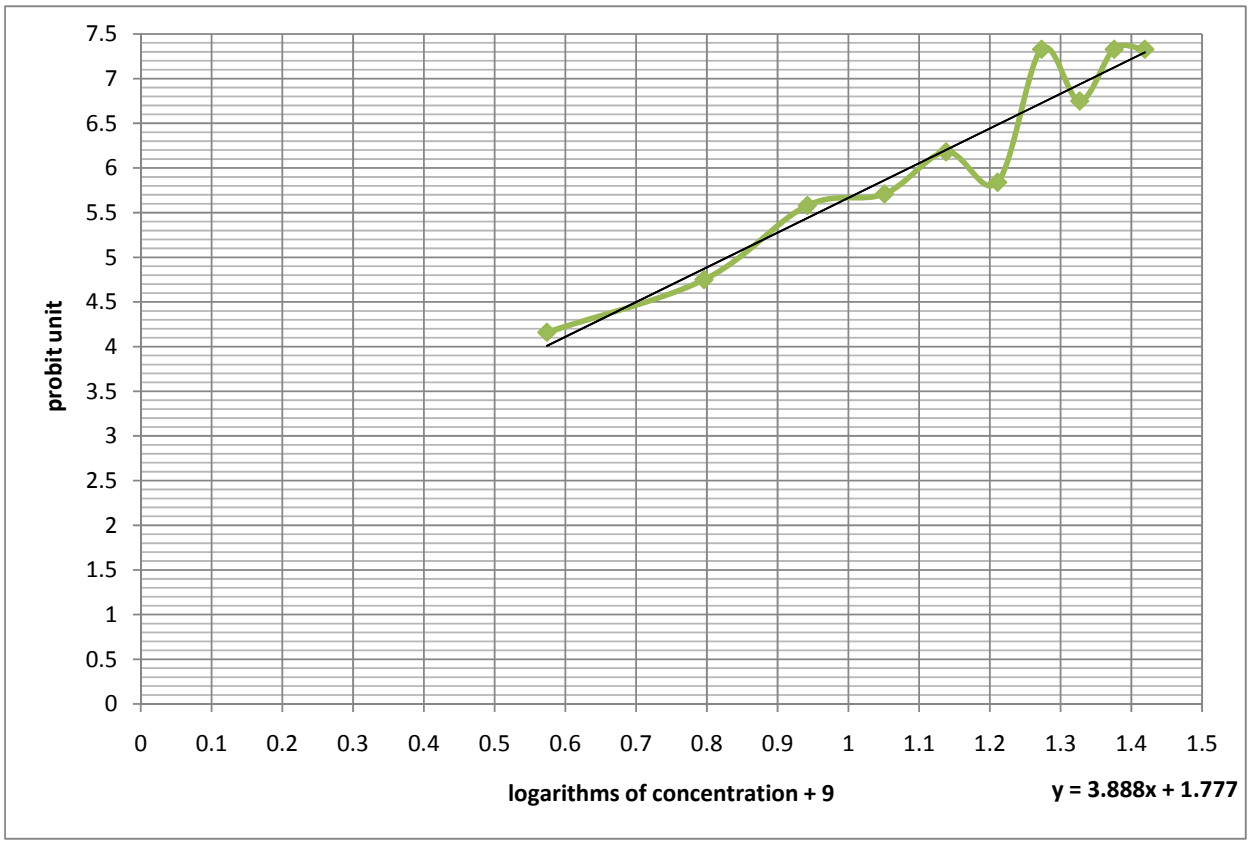
X = 1.6499

LC_{99} = 0.044658 ppm

Table 6: Percentage Death in *Culex* Larvae Treated with Different Concentrations of Abate.

Concentration ppm	Logarith-ms of concentr ation	Logarith-ms of concentr-ation + 9	Death %	Corrected death %	Probit unit	pH measurement	
						Before treatment	After treatment
0.00375	-8.4260	0.5740	20%	20%	4.16	4.45	4.69
0.00625	-8.2041	0.7960	40%	40%	4.75	4.49	4.70
0.00875	-8.0580	0.9420	72%	72%	5.58	4.50	4.70
0.01125	-7.9490	1.0510	76%	76%	5.71	4.50	4.83
0.01375	-7.8620	1.1380	88%	88%	6.18	4.51	4.83
0.01625	-7.7891	1.2109	80%	80%	5.84	4.52	4.83
0.01875	-7.7270	1.2730	100%	100%	7.33	4.53	4.84
0.02125	-7.6730	1.3270	96%	96%	6.75	4.54	4.84
0.02375	-7.6243	1.3757	100%	100%	7.33	4.54	4.84
0.02625	-7.5810	1.4190	100%	100%	7.33	4.54	4.84

Fig. 4: Abate Toxicity to *Culex* Larvae (Experiment 4).



When: y = 5.00

X = 0.8289

LC_{50} = 0.0067437 ppm

When: y = 7.33

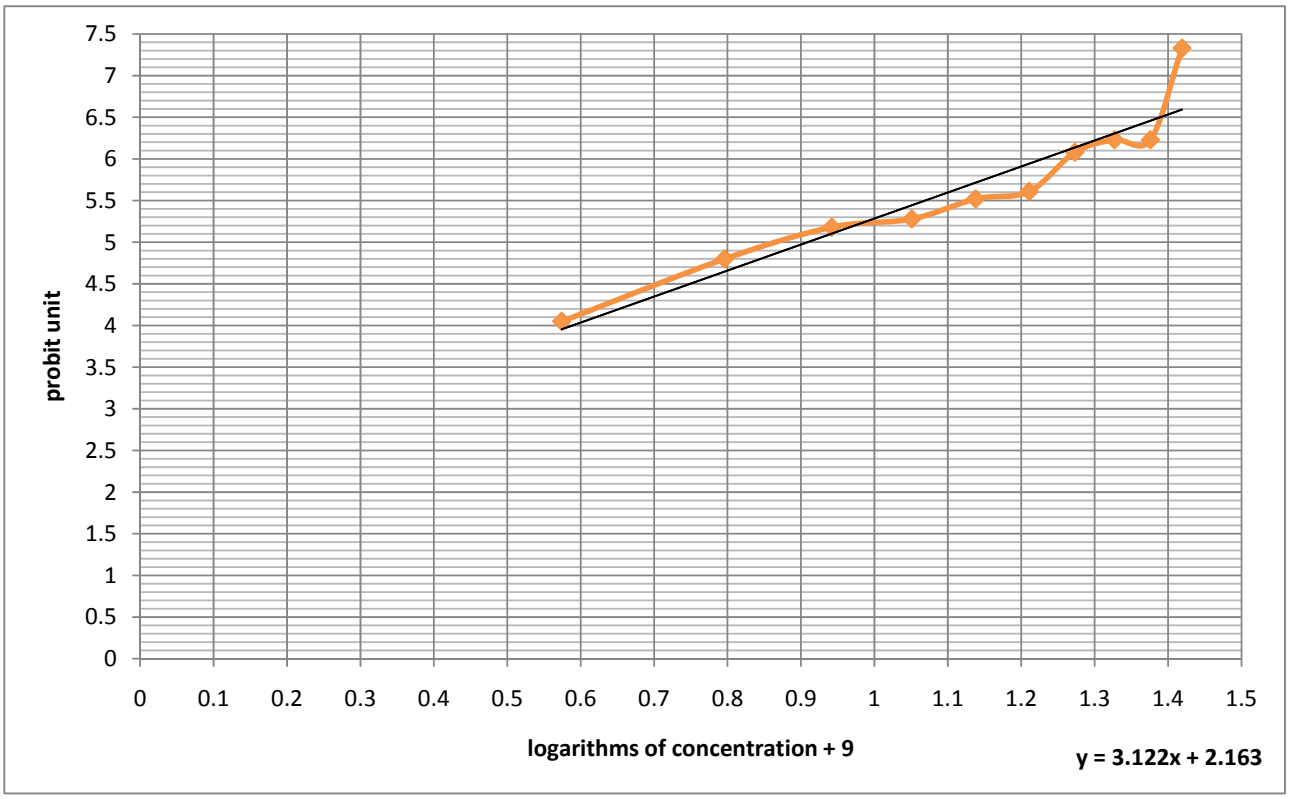
X =1.4282

LC_{99} = 0.026804 ppm

Table 7: The Average Percentage Deaths for *Culex* Larvae in the Four Experiments when Treated with Different Concentrations of Abate.

Concentration ppm	Logarithms of concentration	Logarithms of concentration + 9	Percentage of death				Average percentage of death	Probit unit	Average of the pH measurement	
			Ex-1	Ex-2	Ex-3	Ex-4			Before treatment	After treatment
0.00375	-8.4260	0.5740	24%	17%	5%	20%	17%	4.05	5.12	5.20
0.00625	-8.2041	0.7960	60%	39%	27%	40%	42%	4.80	5.13	5.20
0.00875	-8.0580	0.9420	60%	52%	45%	72%	57%	5.18	5.13	5.21
0.01125	-7.9490	1.0510	64%	44%	59%	76%	61%	5.28	5.13	5.28
0.01375	-7.8620	1.1380	80%	52%	59%	88%	70%	5.52	5.14	5.28
0.01625	-7.7891	1.2109	52%	83%	77%	80%	73%	5.61	5.14	5.29
0.01875	-7.7270	1.2730	76%	91%	77%	100%	86%	6.08	5.14	5.30
0.02125	-7.6730	1.3270	76%	96%	86%	96%	89%	6.23	5.15	5.31
0.02375	-7.6243	1.3760	84%	96%	77%	100%	89%	6.23	5.15	5.31
0.02625	-7.5810	1.4190	___	100%	100%	100%	100%	7.33	4.80	5.00

Fig. 5: Average Death Percentages for the Four Experiments of *Culex* Larvae Treated with Different Concentrations of Abate.



When: y = 5.00

X = 0.9087

LC_{50} = 0.0081040 ppm

When: y = 7.33

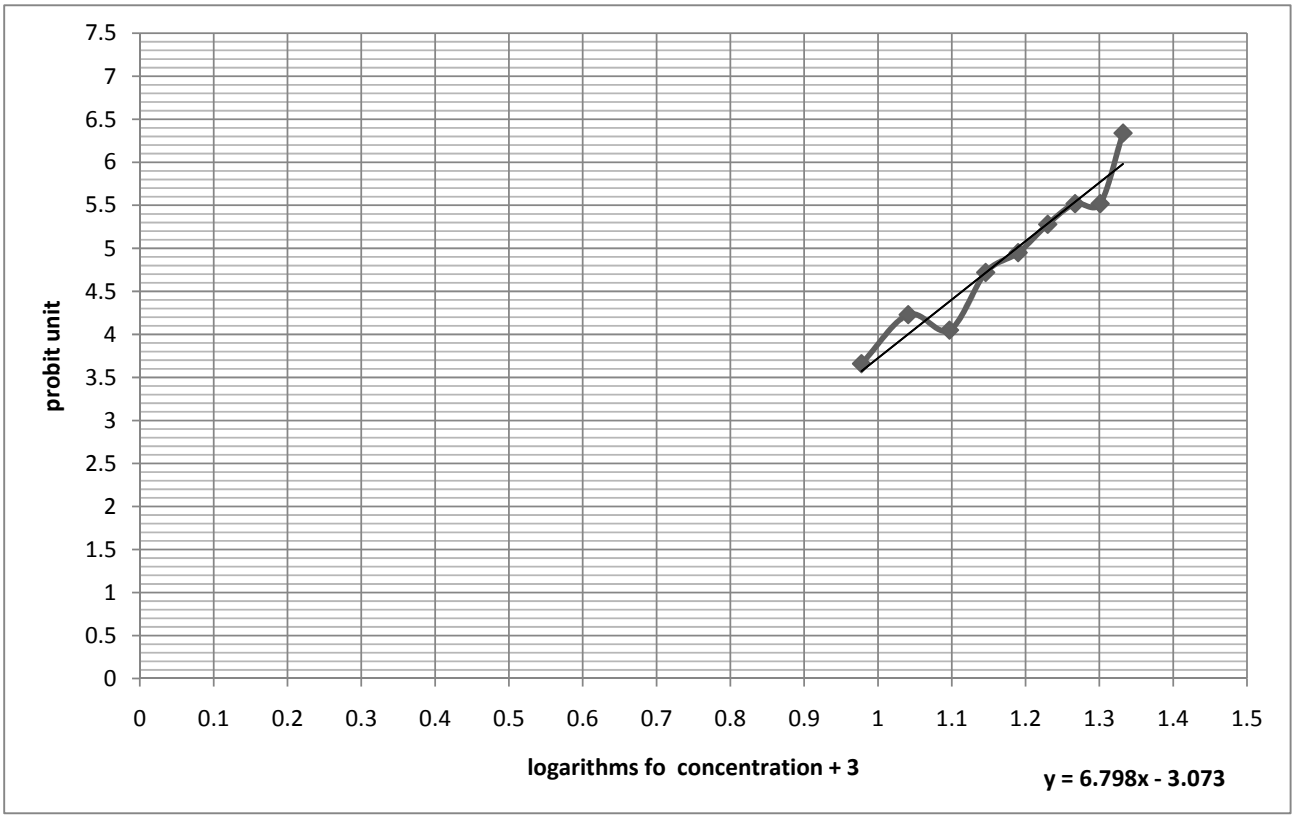
X = 1.6550

LC_{99} = 0.045185 ppm

Table 8: The Percentage Death in *Culex* Larvae Treated with Different Concentrations of *Ambrosia maritima* L. Leaves Aqueous Extract.

Concentration ppm	Logarith-ms of concentr ation	Logarith-ms of concentr-ation + 3	Death %	Corrected death %	Probit unit	pH measurement	
						Before treatment	After treatment
9500	-2.0222	0.9778	16%	9%	3.66	6.30	6.31
11000	-1.9590	1.0410	28%	22%	4.23	6.31	6.32
12500	-1.9030	1.0970	24%	17%	4.05	6.32	6.32
14000	-1.8540	1.1460	44%	39%	4.72	6.34	6.35
15500	-1.8100	1.1900	52%	48%	4.95	6.34	6.35
17000	-1.7700	1.2300	64%	61%	5.28	6.35	6.35
18500	-1.7330	1.2670	72%	70%	5.52	6.35	6.35
20000	-1.6990	1.3010	72%	70%	5.52	6.36	6.36
21500	-1.6680	1.3320	92%	91%	6.34	6.37	6.47

Fig. 6: Toxicity *Ambrosia maritima* L. Leaves Aqueous Extract to *Culex* Larvae (Experiment 1).



When: y = 5.00

X = 1.1876

LC_{50} = 15403 ppm

When: y = 7.33

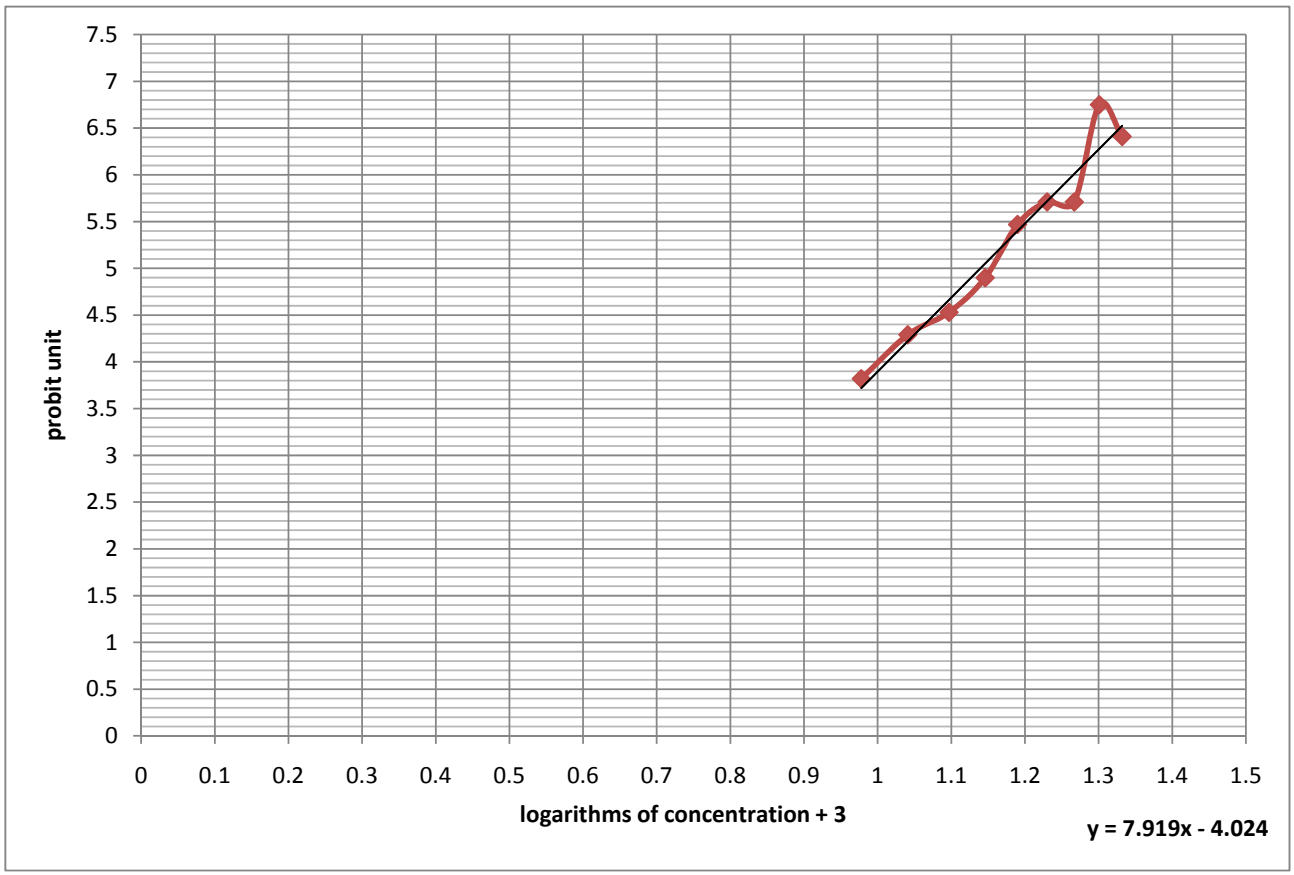
X = 1.53030

LC_{99} = 33910 ppm

Table 9: The Percentages Death of *Culex* Larvae Treated with Different Concentrations of *Ambrosia maritima* L. Leaves Aqueous Extract.

Concentration ppm	Logarith-ms of concentr ation	Logarith-ms of concentr-ation + 3	Death %	Corrected death %	Probit unit	pH measurement	
						Before treatment	After treatment
9500	-2.0222	0.9778	12%	12%	3.82	4.84	4.90
11000	-1.9590	1.0410	24%	24%	4.29	4.85	4.91
12500	-1.9030	1.0970	32%	32%	4.53	4.86	4.92
14000	-1.8540	1.1460	46%	46%	4.90	4.87	4.95
15500	-1.8100	1.1900	68%	68%	5.47	4.87	4.97
17000	-1.7700	1.2300	76%	76%	5.71	4.88	4.97
18500	-1.7330	1.2670	76%	76%	5.71	4.88	4.99
20000	-1.6990	1.3010	96%	96%	6.75	4.88	5.03
21500	-1.6680	1.3320	92%	92%	6.41	4.88	5.05

Fig. 7: Toxicity of *Ambrosia maritima* L. Leaves Aqueous Extract to *Culex* Larvae (Experiment 2).



When: y = 5.00

X = 1.1395

LC_{50} = 13788 ppm

When: y = 7.33

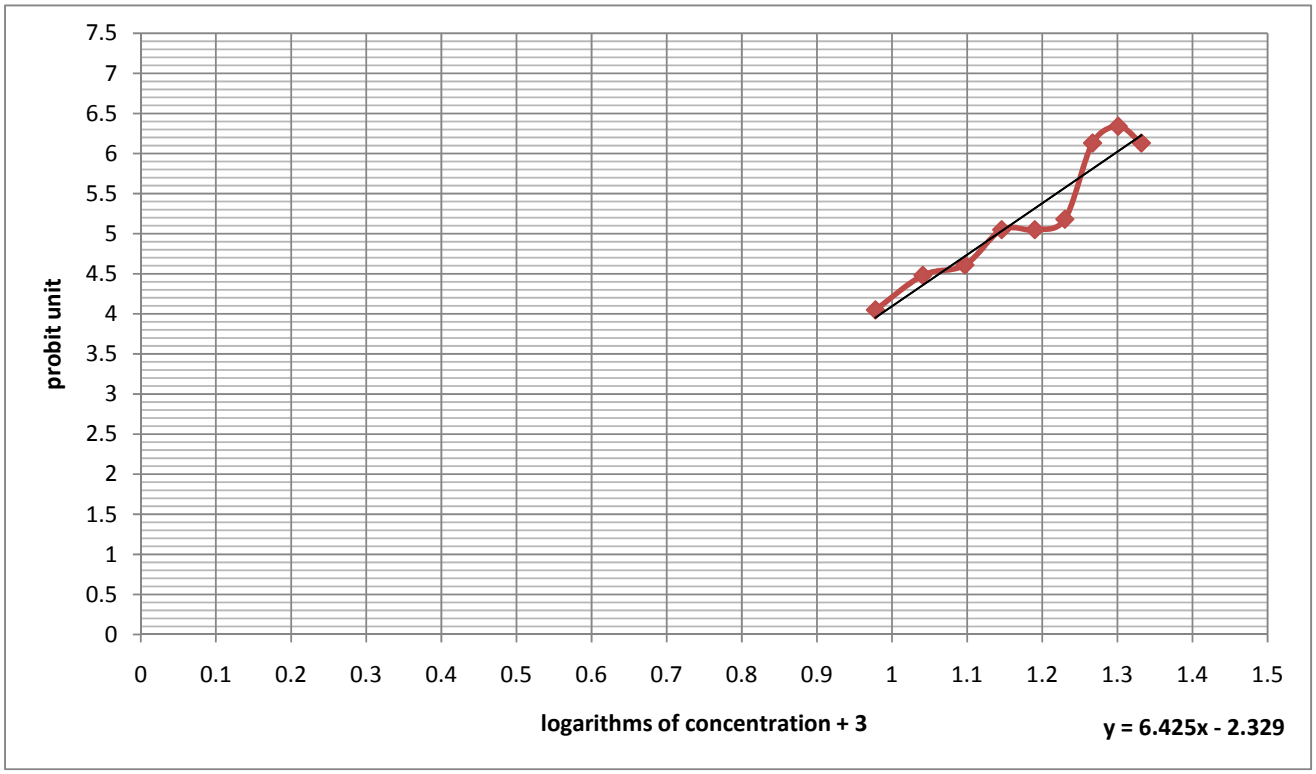
X = 1.4337

LC_{99} = 27146 ppm

Table 10: Percentage Death in *Culex* Larvae Treated with Different Concentrations of *Ambrosia maritima* L. Leaves Aqueous Extract.

Concentration ppm	Logarithms of concentration	Logarithms of concentration + 3	Death %	Corrected death %	Probit unit	pH measurement	
						Before treatment	After treatment
9500	-2.0222	0.9778	24%	17%	4.05	4.55	4.80
11000	-1.9590	1.0410	36%	30%	4.48	4.55	4.80
12500	-1.9030	1.0970	40%	35%	4.61	4.55	4.81
14000	-1.8540	1.1460	56%	52%	5.05	4.55	4.83
15500	-1.8100	1.1900	56%	52%	5.05	4.56	5.01
17000	-1.7700	1.2300	60%	57%	5.18	4.56	5.02
18500	-1.7330	1.2670	88%	87%	6.13	4.57	5.03
20000	-1.6990	1.3010	92%	91%	6.34	4.58	5.03
21500	-1.6680	1.3320	88%	87%	6.13	4.58	5.04

Fig. 8: Toxicity of *Ambrosia maritima* L. Leaves Aqueous Extract to the *Culex* Larvae (Experiment 3).



When: y = 5.00

X = 1.1407

LC_{50} = 13826 ppm

When: y = 7.33

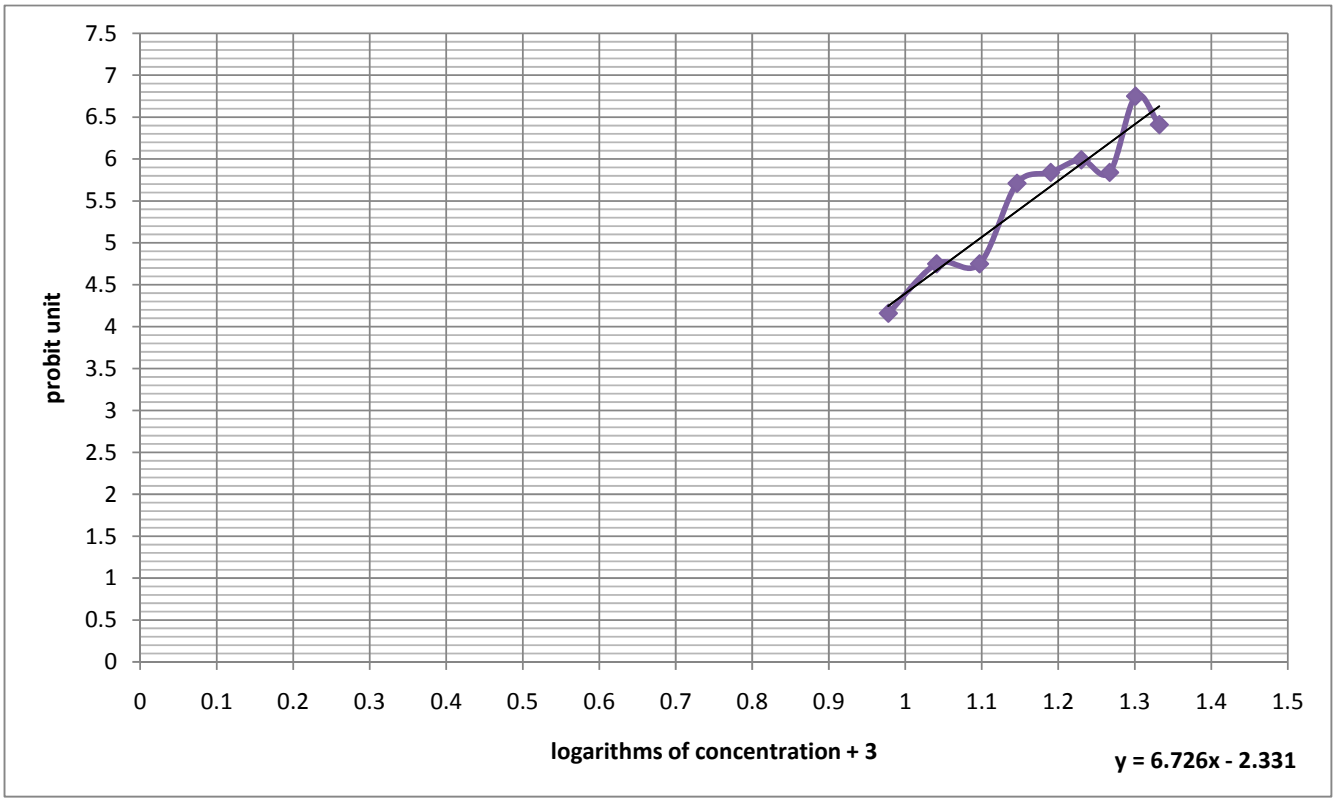
X = 1.5033

LC_{99} = 31864

Table 11: Percentage Death of *Culex* Larvae Treated with Different Concentrations of *Ambrosia maritima* L. Leaves Aqueous Extract.

Concentration ppm	Logarith-ms of concentr-ation	Logarith-ms of concentr-ation + 3	Death %	Corrected death %	Probit unit	pH measurement	
						Before treatment	After treatment
9500	-2.0222	0.9778	20%	20%	4.16	4.62	4.90
11000	-1.9590	1.0410	40%	40%	4.75	4.62	4.92
12500	-1.9030	1.0970	40%	40%	4.75	4.62	4.94
14000	-1.8540	1.1460	76%	76%	5.71	4.62	4.96
15500	-1.8100	1.1900	80%	80%	5.84	4.62	4.98
17000	-1.7700	1.2300	84%	84%	5.99	4.62	5.01
18500	-1.7330	1.2670	80%	80%	5.84	4.63	5.03
20000	-1.6990	1.3010	96%	96%	6.75	4.63	5.06
21500	-1.6680	1.3320	92%	92%	6.41	4.63	5.09

Fig. 9: Txicity of *Ambrosia maritima* L. Leaves Aqueous Extract to the *Culex* Larvae (Experiment 4).



When: y = 5.00

X = 1.0899

LC_{50} = 12299 ppm

When: y = 7.33

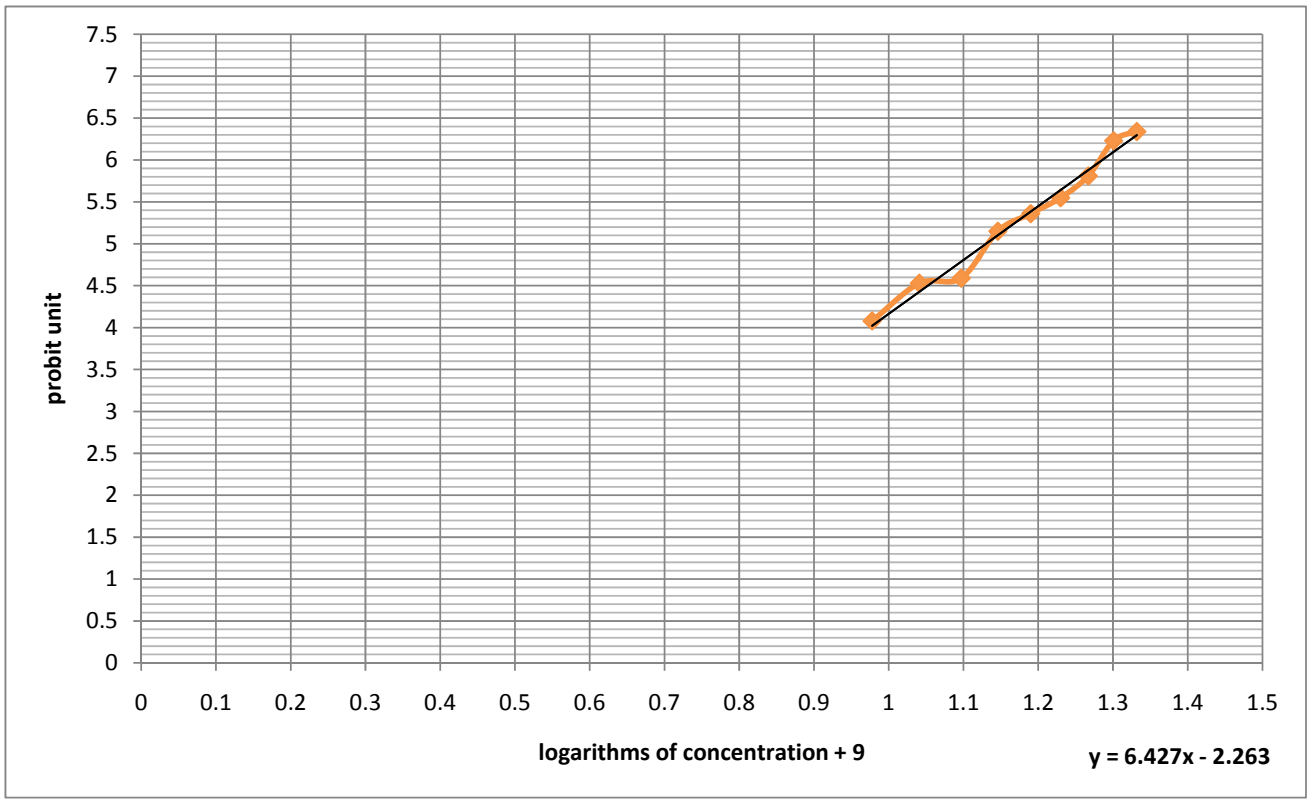
X = 1.4364

LC_{99} = 27315 ppm

Table 12: The Average Percentage Death for *Culex* Larvae in the Four Experiments when Treated with Different Concentrations of *Ambrosia maritima* L. Leaves Aqueous Extract.

Concentration ppm	Logarithms of concentration	Logarithms of concentration + 3	Percentage of death				Average percentage of death	Probit unit	Average of the pH measurement	
			Ex-1	Ex-2	Ex-3	Ex-4			Before treated	After treated
9500	-2.0222	0.9778	9%	12%	17%	20%	18%	4.08	5.08	5.23
11000	-1.9590	1.0410	22%	24%	30%	40%	32%	4.53	5.08	5.24
12500	-1.9030	1.0970	17%	32%	35%	40%	34%	4.59	5.09	5.25
14000	-1.8540	1.1460	39%	46%	52%	76%	56%	5.15	5.10	5.27
15500	-1.8100	1.1900	48%	68%	52%	80%	64%	5.36	5.10	5.33
17000	-1.7700	1.2300	61%	76%	57%	84%	71%	5.55	5.10	5.34
18500	-1.7330	1.2670	70%	76%	87%	80%	79%	5.81	5.11	5.35
20000	-1.6990	1.3010	70%	96%	91%	96%	89%	6.23	5.11	5.37
21500	-1.6680	1.3320	91%	92%	87%	92%	91%	6.34	5.12	5.41

Fig. 10: Average Percentage Deaths of *Culex* Larvae in Different Concentrations of *Ambrosia maritima* L. Leaves Aqueous Extract (In the Four Experiments).



When: y = 5.00

X = 1.1300

LC_{50} = 13489

When: y = 7.33

X = 1.4926

LC_{99} = 31088

CHAPTER FOUR

DISCUSSION

The present study was conducted in Khartoum locality of Khartoum State in 2009. The main objective of this study was to compare the toxicity of *Ambrosia maritima* L leaves aqueous extract and Abate aqueous solutions on *Culex* larvae. The study revealed that *Ambrosia maritima* L has the potential of controlling mosquito larvae. (Abdel-Hamid and Tarabanko, 2004), and (Boulos, 1983) stated, drinking boiled water extract of Damsissa was used as a remedy for schistosomiasis. (Ammar, *et al*., 1993) showed that the alcoholic extract of *Ambrosia maritima* L showed an antibacterial activity.

The LC_{50} and LC_{99} values for *Ambrosia maritima* L leaves aqueous extract were found to be 13489 ppm and 31088 ppm respectively. These LC_{50} and LC_{99} were calculated from the average percentage of deaths in the experiments carried out during this study. The toxicity of *Ambrosia maritima* L is due to the plant constituents (El Ghazali, *et al*., 2004) and (Ammar, *et al*., 1993). They mentioned that pseudoguaianolides and a new chlorosesquiterpene lactone were isolated from the aerial parts of the plant. Chemically the aerial parts of this medicinal plant contained four pseudoguainolides, parthenin and neombrasin. Two new sesquiterpene lactones, characterized as 1'-noraltarnisin and (11R)-11, 13 dihydropsilostachyin, were isolated together with seven known terpenoids from the leaves. Other lactones from this species have been found to have molluscicidal activity against the intermediate hosts of *Schistosoma* species. Abdel-Hamid and Tarabanko, (2004) mentioned that the most active ingredients of this plant are ambrosin and damsin. Ambrosin belongs to a group of natural products known as pseudoguaianolides and it was synthesized and described by Grieco *et al.*, (1982). Damsin is 2,3-dihydroambrosin.

The *Ambrosia maritima* L leaves aqueos extract was far less toxic than abate (the average of LC_{50} and CL_{99} for Abate were found to be 0.0081040 ppm, 0.045185 ppm respectively, while those of *Ambrosia maritima* were 13489 ppm and 31088 ppm. High concentrations of the *Ambrosia maritima* L leaves aqueous extract were used in this study contrary to Abate, which was used in low concentrations. This agreed with (El Ghazali, *et al*., 2008).

Ambrosia maritima L has a very low toxicity to aquatic non-target organisms and was not toxic when used at the mollucicidal concentration of 35 to 70 mg/liter. Abate is one of the important organophorus compounds, which have been used in the Sudan for the control of mosquito larvae. It kills insects by interfering with the functions of the nervous system causing overstimulation of the nerves (Cox, 2000). The LC_{50} 0.0081040 ppm of Abate in the present study is different from the LC_{50} (0.105 ppm) of Abate which was reported by Elshfie, (2004) as cited by (Yousif, 2007).

During this study, the water used was a mixture of distilled water and water from the site of collection. The pH of the mixed water showed no significant difference between the different concentration of *Ambrosia maritima* L leaves aqueous extract and Abate. The pH was found acidic. This agreeed with (Fishel and Ferrel, 2007) where a pH of 4 to 7 was recommended for mixing most pesticides. A value of 5.5 to 6.5 was ideal. Some pesticides, particularly carbamates and organophosphates insecticides, undergo a chemical reaction in the presence of alkaline water (water that has a pH value greater than 7). The reaction is known as alkaline hydrolysis, and it reduces the effectiveness of the pesticide's active ingredients.

There are many factors, which may have affected the experimental results since there were differences in mortality in the same concentration possibly because the sources of larvae were different and the age of the larval instars was not definite because they were mixed third and fourth instars taken randomly.

There were differences in the results in the various experiments possibly due to external factors since there was death in the controls. During the experiments, it was observed that the some fourth larval instars became pupae, which indicated that the larvicide was slow in action.

All experiments were conducted in a temperature range of 31°C to 32 °C, which may be different from temperatures of larvae habitats. (Dhiman *et al*., 2008) mentioned that 25 °C was found to be the optimum temperature for larval development. Increase temperatures may accelerate the number of emerging adults (El Rayah and Abu Groun, 1983).

CONCLUSIONS

The *Ambrosia maritima* L. leaves aqueous extract is toxic and can be used to control *Culex* larvae. LC_{50} and LC_{99} were determined and found 13489 ppm, 31088 ppm respectively. Abate aqueous solutions was significantly more toxic than the *Ambrosia maritima* L. leaves aqueous extract. The LC_{50} and LC_{99} of Abate aqueous solutions were calculated and found to be 0.0081040 ppm, 0.045185 ppm respectively.

RECOMMENDATIONS

In the light of the results, the following recommendations were suggested:-

* *Ambrosia maritima* L. leaves aqueous extract is toxic to *Culex* larvae and has a potential to be used for control. More careful investigations with lower doses are needed.

* Research on *Ambrosia maritima* L should concentrate on isolation of ingredients rather than whole extract in order to identify the active ingredients.

* Further field trials should be conducted to evaluate the toxicity of *Ambrosia maritima* L and Abate in polluted and tap water against mosquito larvae.

* Use of extractors other than water may lead to a better degree of extraction of the active ingredients.

* *Ambrosia maritima* L is less toxic than Abate therefore, its use by Khartoum Malaria Control Program as larvicide should be investigated.

REFERENCES

Abuelgasim, A.I, Fatih ELrahman, A.O, and Galal, M, (2007), Toxicopathological Effects of *Ambrosia maritima* L (Compositae) Extracts in Rats, Veterinary Research Vol.1, no. (3). pp 71-75.

Abdel-Hamid, N.M, and Tarabanko, V.E, (2004), Radioprotective Effect of an Egyptian Wild Herb *Ambrosia* maritima L, (Damsissa): Biochemical Study Neurotransmitters, ХИМИЯ РАСТИТЕЛЬНОГО СЫРЬЯ, no. (4) pp 53–58.

Ammar, N.M, Al Okbi, S.Y, and Badawy, I.H, (1993), The Hypoglycemic Effect of Different Extracts of Ambrosia maritima L Compositae. Journal of Islamic Academy of Sciences Vol. 6, no. (4) pp 298-301.

Barnard, D.R, (2000), Target Taxa for Personal Protection. In: Global Collaboration for Development of Pesticides for Public Health WHO, p. 5-8.

Boulos, L, (1983), Medicinal Plants of North Africa. United States of America. P. 52-53.

Cox, C, (2000), Diazinon Toxicology. Journal of Pesticide Reform, Vol. 20, no. (2). pp 15-20.

Dhiman, R.D, Pahwa, S, and Dash, A. P, (2008), Climate change and malaria in India, Interplay between temperatures and mosquitoes, Regional Health Forum,Vol. 12, no. (1). pp 27-31.

El Ghazali, G.E.B, Elegami, A. A/B, Ahmed, M.G.M, Abdalla, W.El, and Mohamed, H.A/G, (2008), Poisonous Plants of the Sudan. Sudan Currency Printing Presses.

El ghazali, G.E.B, Abdalla, W.E, Elegami, A.A/B, Almagboul, A.Z.I, and Hamad, A.A/B, (2004), Aromatic Plants of the Sudan. Medicinal and Aromatic Plants Research Institute, p. 29-30.

E1rayah, E, and Abugroun, N.A., (1983), Competition between *Culex quinquefasciatus* Say and *Anopheles arabiensis* Patton in the Khartoum Area Sudan, International Journal Biometeor, Vol. 27, no (1). pp. 63-64.

Fishel, F.M, and Ferrell, J A, (2007), Water pH and the Effectiveness of Pesticides.Pesticide Information Office, University of Florida series 156.

Girard, J.E, (2005), Principles of Environmental Chemistry. Jones and bartle H publishers, Canada.

Heymann, D.L, (2004), Arthropod Borne Viral Diseases. In: Control of Communicable Diseases Manual, 18th ed, p.30-58.

Ibrahim, A.EL/T, (1990), Epidemiology and Control of Some Important zoonoses. In: Human Health Hazards Created by Animal Diseases, Khartoum University Press, p.18-94

Khartoum Malaria Free Project, (2008), Entomological Survey Reports unpublished. Sudan.

Lewis, D.J, (1956a), Some Mosquitoes of the Sudan, Bulletin of Entomology Research, Vol. 47, no (4). pp 723-735.

Lewis, D.J, (1956b), The *Culex* Mosquitoes of The Sudan, Bulletin of Entomological Research, pp. 703-721.

National Malaria Control Programme, (2006), National Strategic Plan for Role Back Malaria, (2007-2012). Federal Ministry of Health.

Najera, J.A, and Zaim, M, (2003), Malaria Vector Control. decision making criteria and procedures for judicious use of insecticides, World Health Organization.

Park, K, (2005), Medical Entomology. In: Textbook of Preventive and Social Medicine, 18th ed, India, p.574-599.

Paul, A.E, and Sinnott, T.J, (2000), Bulletin of Fish and Wildlife Related Impacts of Pesticides Used for the Control of Mosquitoes and Black flies, New York State Department of Environmental Conservation.

Rozendaal, J.A, (1997), Mosquito and other biting Dipteral. In: Vector Control, World Health Organization Geneva. P.7-177.

Shian, L.C, (2007), The Effect of Sublethal Concentration of Abate on *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Says). Master Thesis. Universiti Sains Malaysia.

Sharma, P, Mohan, L, and Srivastava, C.N, (2004), Larval Susceptibility of *Ajuga Remota* Against Anopheline and Culicine Mosquitoes, Southeast Asian Journal of Tropic Medicine and Public Health, Vol. 35 no. (3). pp 608-610.

Service, M.W, (1980), A guide to Medical Entomology. the Macmillan presses.

World Health Organization, (2007), Guidelines on the Elimination of Residual Foci of Malaria Transmission. Series 33.

World Health Organization, (2006), Pesticides and their Application for the Control of Vectors and Pests of Public Health Importance. 6th ed.

World Health Organization, (2005), Specifications and Evaluation for Public Health Pesticides.Temephos.

World Health Organization, (2004), Integrated Vector Management Strategic Framework for the Eastern Mediterranean Region.

World Health Organization, (2003a), Guidelines on the Management of Public Health Pesticides.

World Health Organization, (2003b), Use of Fish for Mosquito Control.

World Health Organization, (2003-2006), Integrated Vector Management, Regional Strategic Framework, (2003-2006).

World Health Organization, (2002a), Report of insect vectors and human health.

World Health Organization, (2002b), Report of defining the roles of vector control and xenomonitoring in the global programme to eliminate lymphatic filariasis.

World Health Organization, (1982), Manual on environmental management for mosquito control. World Health Organization Geneva.

Yousif, Ab/R. Y, (2007), The Toxicity of Diazenon Insecticide on Mosquito Larvae, Khartoum, B.Sc. (honor) thesis. Department of Epidemiology, Faculty of Public and Environmental Health, University of Khartoum.

APPENDICES

Plate 1: Tools and equipments used to conduct the experiments in the laboratory of Entomology Faculty of Public Health 2009.

Plate 2: Scooping *Culex* Larvae from Khartoum Railway Station Drains 2009.

Plate 3: Maintenance of *Culex* larvae in the laboratory.

Plate 4: *Ambrosia maritima* L herb from Tuti Island 2009.