CHAPTER ONE

INTRODUCTION

Mosquitoes serve as vectors for several diseases including malaria, dengue fever, yellow fever, encephalitis, filariasis, West Nile fever and chikungunya. These diseases are a global problem, represent a significant threat to human health and cause enormous impact on economics and social human communities, despite considerable national and international control efforts *i.e.* malaria alone kills annually around one million people (WHO, 2010). Malaria is a mosquito-borne infectious disease caused by a eukaryotic protist of the genus *Plasmodium*. It is widespread in tropical and subtropical regions, including parts of the Americas (22 countries), Asia, and Africa. Each year, there are approximately 350–500 million cases of malaria (Malaria Facts, 2011), killing between one and three million people, the majority are young children in sub-Saharan Africa (Snow *et al*., 2005). Malaria is commonly associated with poverty, but is also a cause of poverty (Douglas and Christian, 2007) and a major hindrance to economic development.

The World Health Organization (1995) suggests that there are three essential elements of malaria control. First element is the selective application of vector control by, reducing the numbers of vector mosquitoes either by eliminating, where feasible, or reducing mosquito breeding sites; destroying larval, pupal, and adult mosquitoes and reducing human-mosquito contact. Second element is early diagnosis and effective and prompt treatments of malaria disease. The third element is early detection or forecasting of

epidemics and rapid application of control measures (Fong and Karl Drlica ,1995).

Mosquito vector control is the most effective means of reducing risk of these diseases. It targets both the aquatic immature stages and adult mosquitoes. It has been well documented by the World Health Organisation (WHO) and in numerous scientific investigations and reports that vector control by use of synthetic insecticides can dramatically reduce the risk of insect-borne diseases, particularly in the case of malaria (Hemingway and Bates, 2003; WHO, 2006) . Current vector control strategies rely heavily on use of insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS). Space spraying constitute the first line of defence in case of epidemics. Although, larval control by using insecticides still did not receive much interest in the current strategies, it has been documented that such targeting of larval control at focal of vectors breeding sites from around human habitations would result in a substantial reduction in transmission of these diseases, particularly in the case of malaria (Carter *et al*., 2000; Killeen *et al*., 2002; Fillinger *et al*., 2003). There has been evidence that larval controls have been successfully reducing or eradicating malaria in several parts of the world (Bang *et al*., 1975 and 1977; Kitron and Spielman, 1989).

The advantages of targeting the larval stages are that mosquitoes are killed before they disperse to human habitations, and that mosquito larvae, unlike adults, cannot change their behaviour to avoid control activities targeted at the larval habitat (Charlwood & Graves, 1987; Killeen *et al*., 2002). At present, mosquito larvicides that are used under field programme include mainly organophosphate insecticides. However, the problem of resistance in mosquito species to several synthetic insecticides coupled with the hazards

of environmental pollution, have revived interest in exploiting the pest control potential of plants (Grainge and Ahamed , 1988). Current research focuses on microbials such as *Bacillus thuringiensis* var. *israelensis (Bti)*, *Bacillus sphaericus* (Shililu *et al*., 2003; Fillinger *et al.*, 2003), and on botanicals with larvicidal, oviposition inhibiting , repellent or insect growth regulatory effects (Lucantoni *et al*., 2006; Kamsuk *et al*., 2007).

Phytochemicals may also have potential uses including growth and reproduction inhibitors, repellents, and oviposition deterrents. Plant products, pending polarity, can be obtained by extraction with different solvents including water, petroleum ether, chloroform, methanol , and ethanol. Mosquitoes do not only cause nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries (Jang *et al*., 2002). The mosquito *Culex quinquefasciatus* acts as a vector for *Wuchereria bancrofti* responsible for filariasis in India. However, control of such diseases are becoming increasingly difficult because the over production of detoxifying mechanisms of chemical insecticides has been reported for *Culex* species (Severini *et al*., 1993). On the other hand, some mosquito species have developed high levels of resistance to microbial control agents (Rao *et al*., 1995). One alternative approach is the use of natural products from plant origin (Consoli and Oliveira, 1994).

The present study is designed to investigate the effects of extracts of a variety of potential plant species against larval development of the major malaria vector *Anopheles arabiensis*, filaria vector *Culex quinquefasciatus*, and dengue vector *Aedes aegypti* (Diptera: Culicidae). While malaria is a

well known threat to human health throughout the country, dengue fever has emerged recently in Red Sea and Kassala States of Eastern Sudan (Hyamas *et al.*, 1986; Malik *et al.*, 2011; and Seidahmed *et al.*, 2012). Similarly, filaria cases were reported recently from Blue Nile and Khartoum States (Mc Carthy Mc, *et al.*, 1996; Blue Nile Health Project ,1989).

Phytochemical activity of four varieties of plant species will investigated against development of immature stages of important mosquito vectors, i.e *Anopheles arabiensis*.(Patton) , *Culex quinquefasciatus*.(Say) , and *Aedes aegypti* L. (Diptera: Culicidae). Also , a 24 hr-bioassays of plant extracts on vector mosquito stages were undertaken. Treatment effects are to be assessed by determining larval mortality, and further deleterious effects of extracts upon developmental processes of immature stages.

Little information is available on botanicals and botanical biocides and their application, usage and efficacy on malaria, filariasis and dengue fever vector control. By keeping the above points in view, the present study proposed with the following objectives.

1. General cbjective :

To improve human health by using eco-friendly potential botanical insecticides for control of vectors of malaria, filariasis, and dengue fever disease vectors.

1.2. Specific Objectives :

1.To evaluate the efficacy of four plant species (*Balanites aegyptiaca* (Higleeg) (root), *Azadirachta indica* (Neem) (seed) , *Solenostemma argel* Hargal (shoot) and *Eclipta prostrata* (Tamr Al- Ghanam) (leaves) extracts,

as potential larvicidal agent against the major mosquito vectors *Anopheles arabiensis*, *Culex quinquefasciatus*, and *Aedes aegypti* in Sudan.

2. To determine tentative structures of active ingredients of *Balanites aegyptiaca* (root) , *Solenostemma argel* (shoots), *Eclipta prostrata* (leaves) and *Azadirachta indica* (seed).

3. outputs:

To innovate , effective , safe and environmentally friendly botanical potential insecticides for control of vectors of malaria, filariasis , and dengue fever diseases.

CHAPTER TWO

LITERATURE REVIEW

2. Mosquitoes

2.1. Mosquito species

The true mosquitoes are included in the Family Culicidae which furtherly sub-divided into sub-family Anophelinae, sub-family Culicinae and subfamily Toxorhynchitinae.

The Sub-family Anophelinae includes about 430 identified anopheles mosquito species in the world. From which, only 30-40 species that transmit the four different *plasmodium* species that cause human malaria in endemic areas. Several species of Anopheles are primarily involved in malaria transmission (MacDonald, 1957; Davidson *et al*., 1967; Gillies and De Meillon, 1968; Bryan, 1979). These included mainly *Anopheles gambiae***,** *Anopheles funestus***,** *Anopheles nili***,** and *Anopheles moucheti* (Lindsay *et al*.,1998, Coetzee *et al*., 2000). Some 40 species of these anopheline mosquitoes are of major importance in human malaria transmission (Bruce-Chwatt, 1993; Service, 1993; Service and Townson, 2002). Each vector has its own geographical and ecological distribution area. In addition, mosquites are also incriminated in transmission of filariasis and some arbovirus diseases. From peer reviewed Scientific literature, Kiszewski and Teklehaimanot , (2004) identified a total of 33 dominant malaria vectors distributed over 260 regions in the world. The most efficient vectors were *Anopheles funestus*, *Anopheles gambiae s.s*, *Anopheles arabiensis*, *Anopheles punctulatus s1*, *Anopheles melas*, *Anopheles farauti*, *Anopheles*

sundaicus, *Anopheles pharaoensis* , *Anopheles pseudopunctipennis*, *Anopheles darlingi*, *Anopheles minimus*, *Anopheles dirus*, and *Anopheles flavirostris*. Of these *Anopheles funestus*, *Anopheles gambiae s.s* and *Anopheles arabiensis* are considerd the most effective vectors of malaria parasites (Coetzee *et al*., 2000), because they are responsible for 85-90% of all malaria cases that occurred over the World. Some species of *Anopheles* also can serve as the vectors for canine heartworm *Dirofilaria immitis*, the Filariidae *Wuchereria bancrofti* and *Brugia malayi*, and viruses like the one that is the cause of O'nyong'nyong fever. Mosquitoes in other genera (*Aedes*, *Culex*) can also serve as vectors of disease agents (Douglas and Christian, 2007).

In the Sudan about 31 Anopheles species have been identified, but only few of them are known to be malaria vectors (Nugud *et al*., 1997). The chief malaria vector is *Anopheles arabiensis* (*Anopheles gambiae* (sp B*) is regarded as the most important vector. It is a robust long–lived and widely distributed species. It feeds preferably on Man and animals (Bruce-Chwatt , 1985), besides these characters *Anopheles arabiensis* breeds and survives in different kinds of fresh water sites and frequently rest inside houses. It has a great susceptibility rate to infection (as high as 30%) by plasmodium which is not matched by any other malaria vector in the World (Akood, 1980). This vector was reported from many localities all over the country (Haridi,1995; Petrarca *et al*., 1986; and El-Safi, 1992) . In Southern part of Sudan *Anopheles funestus*, and may be also *Anopheles gambiae s.s* (sp.B) are the main malaria vectors. *Anopheles pharaoensis* co-exist with *Anopheles arabiensis* but has no role in transmitting malaria in Sudan although it is the main vector of malaria in Egypt (Akood, 1980). *Anopheles nili* in the

Southern region with affinity to bite animal and rest out side , can be ignored as malaria vector in Sudan.

Anopheles funestus which co-exist with *Anopheles arabiensis* in extreme South, plays a role in malaria transmission, particularly during the drier months of the year , and thus contributes to perennial intensive malaria transmission and holoendemicity. Other Anopheles mosquitoes present in Sudan such as *Anopheles dthali* and *Anopheles rufipes* are of no medical importance due to their predominant zoophilic tendencies and their extremely low densities even during the rainy season (Nugud *et al*.,1997).

Sub-family Culicinae includes *Aedes* and *Culex* mosquito species that are most important vectors of several human and animal diseases.

a. *Aedes* mosquitoes

This genus of mosquitoes belongs to the sub-family Culicinae which includes about 1500 species. It is moderately sized, strictly marked, reddish to dark in colour, with broad scales and broad thorax. *Aedes aegypti* is predominantly a domestic species and diurnal in habit and its activity and development are greatly affected by temperature. It is widely distributed through the tropics and between latitudes 45º N to 35º S in the World. *Aedes aegypti* is the most important vector of yellow fever and many other arboviral diseases. The virus of yellow fever has a simple man-mosquito-man transmission cycle.

b. *Culex* mosquitoes

Culex mosquitoes are increasing in Africa as urbanization provides favourable habitats and sanitary measures are discarded. *Culex* are birds feeders. They are important vectors of arboviruses and avian malaria. *Culex pipiens* L and *Culex fatigans* Weis, are occurred widely in Africa and bite Man and suck his blood. These mosquitoes invade houses in large numbers and become a considerable menace. In addition, *Culex pipiens* and *Culex quinquefasciatus* may serve as intermediate host of human filarial worm *Wuchereria bancrofti* , *Culex fatigans* is also a potential vector of yellow fever.

In Sudan , there are about thirty species of the genus culex has been described by Lewis (1955). *Culex pipiens molestus* Forskol occurs between Khartoum and Egyptian frontier. It has been found breeding in wells, in belge –water of barges, in pit latcrines and in pools. Female bites Man indoors by day and night. *Culex fatigans* appears to be restricted to coastal regions, parts of the Red Sea Hills in Sudan and neighbourhood of Kassala . It has also been recorded from Khartoum and various places in Upper Nile . In Sudan , Lewis, (1947) has discussed the relationship of mosquitoes and yellow fever in Sudan. It appears that most of the southern half of Sudan is a yellow fever area in the sense that people immune to the disease have been found in many parts of that territory. The chance of the disease spreading to parts hitherto free is liable to be increased by such outbreaks as the great Nuba Mountains epidemic of 1941. Some 163 species of mosquitoes are known from Sudan, and, of these, twelve species are known to be capable of transmitting the yellow fever virus, while several other species are suspected in this connexion. Two further species are able to retain this virus throughout

life without apparently possessing the ability to transmit the disease by their blood-sucking habits.

2. 2 Malaria

2.2.1. Malaria burden

2.2.1.1. Malaria burden in Africa

Malaria is a major public health problem in Africa. The World Health Organization (WHO) estimates that there are about 300–500 million clinical malaria cases annually and that 1–1.5 million people die of malaria each year. *Anopheles arabiensis* and *Anopheles gambiae* are the principal vectors of malaria in sub-Saharan Africa, but in some areas, such as the Great Rift Valley in East Africa, *A. arabiensis* is the predominant malaria vector species (Minakawa *et al.*, 2002). The alarming spread of mosquito resistance to pesticides, with the limited numbers of effective drugs against malaria now available, underline how important it is to find new control agents for both mosquitoes and their transmission of diseases. Malaria continues to place a large social and economic burden on African communities. Programs to control malaria transmission typically target the adult primary vectors, using techniques such as imprignated bed nets and indoor residual spraying that have a high impact on vectorial capacity. However, these methods are vulnerable to development of vector resistance to insecticides, (e.g., Hargreaves *et al.*, 2003), and vulnerable to development of vector behavioural adaptation, such as changing preferences for feeding and resting outdoors (e.g., Killeen *et al.*, 2002) and logistics and funding problems in reaching the poor, who are most at risk (e.g., Barat *et al.*, 2004). Historically, environmental management methods that targeted the

larval stages of malaria vectors were effective in substantially reducing malaria transmission (e.g., Soper and Wilson , 1943).These methods fell out of favour with the widespread introduction of synthetic insecticides and bed nets, which reduce biting rates and are not dependent on such site specific knowledge as is required for larval control methods (Keiser *et al*., 2005). Integrated vector management programmes, employing a variety of tools including larval control, may provide the greatest chance for success in reducing malaria transmission rates (Carter *et al.,* 2000) . Methods that target the larval stages of mosquitoes have the potential to be effective, low-cost and with low environmental impact, (Killeen *et al*., 2002; Gu and Novak, 2005; WHO, 2006). If modern-day larval control is to be a useful addition to the toolbox of malaria abatement methods, it will need to be both low-cost and sustainable.

2.2.1.2. Malaria in Sudan

Malaria in Sudan continues to be a major public health problem. There are an estimated 7.5 million cases and 35 000 deaths every year (Malik, *et al.*, 2003; WHO, 2002). Sudan has been stratified into 5 operational strata: riverine, seasonal, urban, irrigated and high perennial transmission areas (Malik and Khalafalla , 2004). *Plasmodium falciparum* is the predominant species, accounting for more than 95% of all malaria cases, with *Anopheles arabiensis*, *An. gambiae* and *An. funestus* as the main disease vectors. Each year, more than 1.2 million women become pregnant in Sudan (Dafalla *et al*., 2003), of those 750,000 are in areas of intense perennial, high seasonal transmission or in areas of irrigation. It is well documented that the frequency and severity of malaria is greater in pregnant compared with non-pregnant women (Chandramohan and Greenwood, 1998), 94% of clinicians

interviewed in a situational analysis of malaria in pregnancy in Sudan , stated that it was a problem . Brabin (2001) estimates that , 1% - 23% of African hospital maternal deaths are due to malaria related complications. Maternal mortality in Sudan is currently 509/100,000 (Safe Motherhood Survey, 1999; Dafalla *et al*., 2003). Mosquito-borne diseases cause a high level of morbidity and mortality, but they are also responsible for great socioeconomic loss. In 2002, WHO reported that 273 million people in 100 countries were annually exposed to malarial infections that caused an estimated 1.09 million deaths, with more than 90% in Africa alone. Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throwaway society, the increasing resistance of mosquitoes to current commercial insecticides (Ciccia *et al*., 2000), and toxicity in the public use (Zadikoff, 1979). These problems indicate a need for new and improved larvicides and strategies for protection from mosquito attack. Researchers are now looking for natural larvicides that do not have ill effects on non target populations and are easily degradable.

2.2.1.3. Malaria parasites and malaria vectors

Malaria parasites are members of the genus *plasmodium* (phylum Apicomplexa). In humans malaria is caused by *p.falciparum, P.malariae P.ovale, P.vivax* and *P. knowlesi*. (Mueller *et al*., 2007; Singh *et al*., 2004). *P. falciparum* is the most common cause of infection and is responsible for about 80% of all malaria cases, and is also responsible for about 90% of the deaths from malaria (Beadle *et al* ., 1995; Adam and Elbashir, 2004 and Adam *et al*., 2000). Parasitic *Plasmodium* species also infect birds, reptiles, monkeys, chimpanzees and rodents. (Escalant and Ayala , 1994) . There have been documented human infections with several

simian species of malaria parasites , namely , *P. knowlesi*, *P. inui*, *P. cynomolgi* (Garnham, 1966). *P. Simiovale*, *P. brazilianum*, *P. schwetzi* and *P. simium*; however, with the exception of *P. knowlesi*, these are mostly of limited public health importance (Collins and Barnwell, 2009). Malaria parasites are transmitted by several species of Anopheles mosquito, the most important being *Anopheles arabiensis*, *Anopheles gambiae*, *Anopheles stephensi*, *Anopheles culicifacies* and *Anopheles funestus*. Of all the insects that transmit diseases , mosquitoes represent, the greatest menace. WHO has declared the mosquito " public enemy number one" because mosquitoes are responsible for the transmission of various dreadful diseases (WHO, 1995). Civil strife in the South, leading to successive waves of population movements, coupled with drought and desertification, major floods in the North and a severe loss of human resources, especially in the health sector. These have resulted in an extremely fractured and under-resourced health infrastructure (WHO Sudan Country Cooperation Strategy, 2004-2007).

2.2.1.4. Life cycle of Malaria Vectors

Adult female mosquito lay 50-200 eggs per oviposition . Eggs are oval and are about 0.5-0.635 mm long. They laid in free water or on moist surfaces. Generally eggs are not resistant to drying and hatch within 2-3 days. However, in cooler climates hatching may take up to 2-3 weeks. The larvae of all mosquitoes live in water and have four developmental stages (Caroline, 2004). Mosquito larvae have well developed heads, a large thorax and a segmented abdomen. They have no legs. Mosquito larvae, which survive in a wide range of habitats, feed on algae, bacteria, organic particulates and other microorganisms in the surface micro layer. Mosquito's

pupae are very active and like the larvae they live in water. They differ greatly from larvae in shape and appearance. Pupae have a comma-shaped body divisible into two distinct regions the cephalothorax, which includes the greatly enlarged head and thorax, and the abdomen. The duration from egg to adult, which is usually influenced by ambient temperature, lasts for 10-14days under tropical conditions. Mosquito adults have slender bodies with 3 sections head, thorax and abdomen. Adult mosquitoes usually mate within a few days after emerging from the pupal stage. Males live for about a week. Once the eggs are fully developed, the female lays them and resumes host seeking. The cycle repeats itself until the female dies. Females can survive up to one month in insectaries, but do not live longer than 1-2 weeks in nature, (Clements, 1992; CDC, 2008).

2.2.1.5. Malaria transmission

Malaria is transmitted exclusively through the bites of *Anopheles* mosquitoes. The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment. About 20 different *Anopheles* species are locally important around the world. All of the important vector species bite at night. *Anopheles* mosquitoes breed in water and each species has its own breeding preference; for example some prefer shallow collections of fresh water, such as puddles, rice fields, and hoof prints. Transmission is more intense in places where the mosquito life -span is longer (so that the parasite has time to complete its development inside the mosquito) and where it prefers to bite humans rather than other animals. For example, the long life - span and strong human-biting habit of the African vector species is the main reason why more than 90% of the world's malaria deaths are in Africa. Transmission also depends on climatic conditions that

may affect the number and survival of mosquitoes, such as rainfall patterns, temperature and humidity. In many places, transmission is seasonal, with the peak during and just after the rainy season. Malaria epidemics can occur when climate and other conditions suddenly favour transmission in areas where people have little or no immunity to malaria. They can also occur when people with low immunity move into areas with intense malaria transmission, for instance to find work, or as refugees. Human immunity is another important factor, especially among adults in areas of moderate or intense transmission conditions. Partial immunity is developed over years of exposure, and while it never provides complete protection , it does reduce the risk that malaria infection will cause severe disease. For this reason, most malaria deaths in Africa occur in young children , whereas in areas with less transmission and low immunity, all age groups are at risk (WHO, 2011).

2.2.1.6. Diagnosis of malaria

Malaria is diagnosed by the clinical symptoms and microscopic examination of the blood. It can normally be cured by antimalalial drugs. The symptoms, fever, shivering, pain in the joints and headache, quickly disappear once the parasite is killed. In certain regions, however, the parasites have developed resistance to certain antimalarial drugs, particularly chloroquine. Patients in these areas require treatment with other more expensive drugs. Cases of severe disease including cerebral malaria require hospital care. In endemic regions, where transmission is high, people are continuously infected so that they gradually develop immunity to the disease. Until they have acquired such immunity, children remain highly vulnerable. Pregnant women are also

highly susceptible since the natural defence mechanisms are reduced during pregnancy (Microbiology Notes: Malaria, 2009).

2.2.1.7. Malaria control

2.2.1.7.1. Mosquito control

Mosquito control depends on: surveillance programmes, source reduction , environmental management, larvicides, biological and physical control means , i.e., parasites and predators, repellents, traps (light, pheromone lure , carbon dioxide and Suction traps), bacteria, fungi and viruses, trypsine. Adulticides, using Insecticide Residual Spraying (IRS), space spray with convential, ULV sprays and fogging with insecticides/petroleum oil mixtures. According to the World malaria report 2011, there were about 216 million cases of malaria and an estimated 655 000 deaths in 2010. Malaria mortality rates have fallen by more than 25% globally since 2000, and by 33% in the WHO African Region. Most deaths occur among children living in Africa where a child dies every minute from malaria.

2.2.1.7.2. Source Reduction

Source reduction is removal or permanent destruction of mosquito breeding sites. The larval habitats may be destroyed by filling depressions that collect water, by draining swamps, or by ditching marshy areas to remove standing water. Container-breeding mosquitoes are particularly susceptible to source reduction as people can be educated to remove or cover standing water in cans, cups, and rain barrels around houses. Mosquitoes that breed in irrigation water can be controlled through careful water management. For some mosquito species, habitat elimination is not possible. For these species, chemical insecticides can be

applied directly to the larval habitats. Other methods, which are less disruptive to the environment, are usually preferred: Oils may be applied to the water surface , suffocating the larvae and pupae. Most oils in use today are rapidly biodegraded .

source reduction is supposed to be an ideal approach to mosquito control. However, larval habitats may be small, widely dispersed, and transient. *Anopheles gambiae*, one of the primary vectors of malaria in Africa, breeds in numerous small pools of water that form due to rainfall. The larvae develop within a few days, escaping their aquatic environment before it dries out. It is difficult, if not impossible, to predict when and where the breeding sites will form , and treat them before the adults emerge. Therefore, larval mosquito control for the prevention of malaria in Africa has not been attempted on a large scale (CDC, 2008).

2.2.1.7.3. Prevention

Vector control is the main way to reduce malaria transmission at the community level. It is the only intervention that can reduce malaria transmission from very high levels to close to zero. For individuals, personal protection against mosquito bites represents the first line of defence for malaria prevention. Two forms of vector control are effective in a wide range of circumstances.

2.2.1.7.4 Long-lasting insecticides-treated nets (LLINs)

More recently, several companies have developed long-lasting insecticide-treated nets (LLINs) that retain lethal concentrations of insecticide for at least 3 years. The WHO Pesticide Evaluation Scheme has recommended five of these LLINs for use in the prevention of malaria. CDC is currently testing

these and other LLINs in Atlanta and Kenya . The WHO Pesticide Evaluation Scheme recommends these LLINs: Dura Net (Clarke Mosquito Control). Interceptor Net (BASF). Net Protect (Intelligent Insect Control) (also marketed as ICONLife by Syngenta). Olyset Net (Sumitomo Chemical) and PermaNet (Vestergaard-Frandsen), (Chavasse *et al.*, 1999; WHO (2001). Insecticide-treated mosquito nets (ITMN) have limited utility in dengue control programmes, since the vector species bites during the day. However, treated nets can be effectively utilized to protect infants and night workers who sleep by day (WHO, 2006).

2.2.1.7.5 Elimination

Malaria elimination is defined as interrupting local mosquito-borne malaria transmission in a defined geographical area, *i.e.* zero incidence of locally contracted cases . Also, it is defined as the permanent reduction to zero of the worldwide incidence of malaria infection caused by a specific agent; *i.e.* applies to a particular malaria parasite species. Many countries – especially in temperate and sub-tropical zones – have been successful in eliminating malaria. The global malaria eradication campaign, launched by WHO in 1955, was successful in eliminating the disease in some countries, but ultimately failed to achieve its overall goal, thus being abandoned less than two decades later in favour of the less ambitious goal of malaria control.

Since the early 1960s , WHO has maintained an official Register of areas where malaria elimination has been achieved. WHO also maintains a Supplementary list to the official register, listing countries where malaria never existed or disappeared years or decades ago, and where full WHO certification of malaria elimination would not be needed. WHO published

the first supplementary list in 1963 including 23 countries. The most recent list was published in the (World Malaria Report 2012) and included 62 countries.The path towards malaria-free status is characterized by four distinct programme phases: control , pre-elimination , elimination and prevention of reintroduction . Each phase is defined by a set of specific programme interventions needed for prevention , treatment , surveillance, monitoring and evaluation and health systems strengthening . Every year , the *World Malaria Report* includes an updated classification of countries by elimination programme stage . Progression between stages is determined by a set of programmatic and epidemiological criteria, and countries may elect to adopt a phased elimination strategy, either by parasite species or by geographical area . To minimize the period of intensive field operations, malaria elimination is usually undertaken as a time-limited programme. Even in the most ideal operational environments, a minimum period of 8-10 years is required to achieve elimination in any given area with ongoing transmission. Importation of malaria by international travellers carries the risk of resumption of transmission, as does the frequent decline in political support for malaria efforts when a programme is successful. It is critical to ensure political and financial commitment to maintaining malaria-free status; failure to do so can have not only public health but economic consequences, linked to decreases in tourism and business investments.

In recent years, however, interest in malaria eradication as a long-term goal has re-emerged. Large-scale use of WHO-recommended strategies, currently available tools, strong national commitments, and coordinated efforts with partners, will enable more countries particularly those where malaria transmission is low and unstable to progress towards malaria elimination. In

recent years, 4 countries have been certified by the WHO Director-General as having eliminated malaria: United Arab Emirates (2007), Morocco (2010), Turkmenistan (2010), and Armenia (2011),(WHO , 2007) ; (World Malaria Report, 2012).

2.2.1.7.6. Chemical Larvicides

The timing of larvicide application is dependent on the nature of the control agent. Conventional insecticides, for example, kill larvae at all stages and thus can be applied when convenient. Bacterial toxins must be consumed by the larvae and are usually applied well before the 4^{th} instar to ensure that consumption occurs. IGRs mimic an essential hormone normally secreted in high concentrations in early instar larvae, and declined later to low concentrations in late (4^{th}) instar larvae . Exposure of 4^{th} instar larvae to the IGR upsets the physiological molting process and kills mosquitoes in the subsequent pupal stage. IGRs can be formulated as slow release insecticides and application should be in the 2^{nd} or 3^{rd} instar to obtain adequate amounts during exposure of the 4^{th} instar to it. Chitin synthesis inhibitors affect the ability of the larvae to reattach their muscles to the exoskeleton during the molting process and thus are effective throughout the entire larval life. Monomolecular films prevent the insect from remaining at the surface of the water by reducing surface tension. Under these conditions larvae and pupae deplete their energy reserves trying to stay at the surface and succumb to exhaustion.

There are three insecticides that can be used for treating containers that hold drinking water. The most normally used is temephos with its formulation 1% sand granules**:** One per cent temephos sand granules are applied to

containers. This dosage has been found to be effective for 8-12 weeks, especially in porous earthen jars, under normal water use patterns. Although resistance to temephos in *Ae. aegypti* and *Ae. albopictus* populations has not been reported from the South-East Asia Region, the susceptibility level of Aedes mosquitoes should be monitored regularly in order to ensure the effective use of the insecticide.

2.2.1.7.7 Adulticides

i. Aqua- Reslin

Aqua-Reslin is a pyrethroid insecticide applied as ULV cold or hot fogs through foggers during evening and early morning to control adult mosquitoes . Aqua- Reslin can be diluted in water or oil. It provides quick knock down and effective control of adult mosquitoes. It is formulated as 20% permethrin, 20% piperonyl butoxide, Technical and 60% other inert ingredients. The product is diluted and dispersed at a rate of 0.0035 lbs of active ingredient per acre.

ii. Pyrethrin

Pyrethrins are a natural mosquito control products. There are several different products, all containing extracts from the chrysanthemum flower. These products are widely available, and are popular concentrates for use in controlling insects. It is used by the Public Health teams in Sudan as indoor residual sprays for controlling mosquitoes and other household flying insects of medical importance .

iii. Space spray

generated by portable ULV equipment, often used to provide indoor mosquito control in houses, tents, trailers, warehouses, etc.

2.2.1.7.8 Personal protection

2.2.1.7.8.1 Insecticide-treated mosquito nets (ITNs)

Insecticide-treated bed nets (ITNs) are a form of personal protection that has repeatedly been shown to reduce severe disease and mortality due to malaria in endemic regions. In community-wide trials in several African settings, ITNs have been shown to reduce all-cause mortality by about 20%. Untreated bed nets form a protective barrier around persons using them. However, mosquitoes can feed on people through the nets, and nets with even a few small holes provide little, if any, protection. The application of a residual insecticide greatly enhances the protective efficacy of bed nets. The insecticides used for treatment kill mosquitoes and other insects. The insecticides also have repellent properties that reduce the number of mosquitoes that enter the house and attempt to feed. There are several types of nets available. Nets may vary by size, material, and/or treatment. Most nets are made of polyester but nets are also available in cotton, polyethylene, or polypropylene. Currently, only pyrethroid insecticides are approved for use on ITNs. These insecticides have very low mammalian toxicity but are highly toxic to insects and have a rapid knock-down effect , even at very low doses. Pyrethroids have a high residual effect: they do not rapidly break down unless washed or exposed to sunlight. Previously, nets had to be retreated at intervals of 6-12 months, more frequently if the nets were washed. Nets were retreated by simply dipping them in a mixture of water

and insecticide and allowing them to dry in a shady place (Chavasse *et al*., 1999; WHO (2001).

2.2.1.7.8.2 Vaccine against Malaria

There are currently no licensed vaccines against malaria or any other human parasite. One research vaccine against *P. falciparum*, known as RTS,S/AS01, is most advanced. This vaccine is currently being evaluated in a large clinical trial in 7 countries in Africa. Final results and a recommendation as to whether or not this vaccine should be added to existing malaria control tools is expected in 2015. The malaria parasite develops both in humans and in the female Anopheles mosquitoes. The size and genetic complexity of the parasite mean that each infection presents thousands of antigens to the human immune system. The parasite also changes through several life stages even while in the human host, presenting different antigens (proteins) at different stages of its life cycle. Understanding which of these can be a useful target for vaccine development has been complicated. In addition, the parasite has developed a series of strategies that allow it to confuse, hide, and misdirect the human immune system, (National Institute of Allergy and Infectious Diseases, 2010).

2.3 Sterile Insect Techniques

The emergence of problems associated with malaria treatment and control, such as drug and insecticide resistance, have led to a search for alternative control measures. Therefore the integration of the sterile insect technique (SIT) in an area-wide integrated pest management (AW-IPM) programme for malaria vectors is being considered. The Sudan Government has requested support from the International Atomic Energy Agency (IAEA) and

the Islamic Development Bank (IDB) for the application of the SIT in Northern State to help eliminate the population of *An. arabiensis* from this region. This region fulfils certain criteria that make it suitable for an AW-IPM programme incorporating the release of sterile males (Malcom *et al*., 2009). In spite of several SIT trials carried out on mosquitoes (Benedict , and Robinson, 2003; Laven , 1967 ; Lofgren *et al.*, 1974 ; Pal, R : WHO/ICMR , 1974 ; Patterson *et al*., 1970 and Dame *et al*., 2009) , this is the first time that a malaria vector is being targeted on an area-wide basis using the SIT in sub-Saharan Africa. Sterile insect technique is emerging as a potential mosquito control method. In addition Progress towards transgenic, or genetically modified, insects suggest that wild mosquito populations could be made malaria-resistant. Ito *et al*., (2002) have created the world first transgenic malaria mosquito, with the first plasmodium resistant Anopheles species. Also, an even more futuristic method of vector control is the idea that lasers could be used to kill flying mosquitoes (Robert Guth, 2009).

2.4 Genetic control

Different genetic markers have been used to study the population genetic structure of *A. gambiae* and *A. arabiensis*, including chromosomal inversions, allozymes, random amplified polymorphic DNA (RAPD), mitochondrial DNA (mtDNA) sequences, and microsatellite markers (Coluzzi *et al*., 1979 and 1985; Lanzaro *et al*., 1995; Lehmann *et al*., 1996a, Lehmann *et a*l., 1996b and 1999, 2003, and Toure *et al.*, 1998). Microsatellites are tandem repeats of two to five nucleotides and have been regarded as excellent molecular markers for studying population genetic structure because they are very abundant throughout the genome,

codominantly inherited, usually neutral, and relatively easy to score at low cost (Bowcock *et al*., 1994; Estoup *et al*., 1995; Goldstein and Schlotterer, 1999). Previous studies suggest that *A. arabiensis* populations generally show a lower level of genetic differentiation than *A. gambiae*. For example, significant genetic differentiation was found for *A. gambiae* populations between western Kenya and coastal Kenya using microsatellite markers; hence it was inferred that the Great Rift Valley is a major gene flow barrier for *A. gambiae* (Lehmann *et al*., 1999 and 2003), but no significant genetic differentiation was detected for *A. arabiensis* populations from the two regions using the same loci (Kamau *et al*., 1999). Similarly Donnelly and Townson (2000) did not detect significant genetic structure for *A. arabiensis* populations within Malawi and Sudan; however, a high level of genetic differentiation was found for *A. arabiensis* populations from two islands that are 240 km apart in the Indian Ocean (Simard *et al*., 1999). Some species are poor vectors of malaria, as the parasites do not develop well (or at all) within them. There is also variation within species. In the laboratory, it has been possible to select strains of *A. gambiae* that are refractory to infection by malaria parasites. These refractory strains have an immune response that encapsulates and kills the parasites after they have invaded the mosquito's stomach wall. Scientists are studying the genetic mechanism for this response. It is hoped that some day, genetically modified mosquitoes that are refractory to malaria can replace wild mosquitoes, thereby limiting or eliminating malaria transmission.(CDC, 2012).

New genetic approaches are also being considered as ways to control mosquito populations. Being flightless is a huge genetic disadvantage. The flightless female mosquitoes are unable to "sing" and court with male

mosquitoes using their wing oscillation "song." Predators can more easily prey on flightless female mosquitoes. The researchers theorize that these genetically engineered mosquitoes could be used to control mosquito populations and reduce dengue transmission. The same group of researchers recently genetically modified male mosquitoes to be sterile, and they released these mosquitoes in a trial in Grand Cayman , a Caribbean island, to wipe out dengue fever (Fu *et al*., 2007).

2.5 Biological control of mosquito

Biological approaches are also being considered as alternatives to control mosquito populations. For example, predatory crustaceans called copepods (Choi , 2005 ; WHO, 2003) and many varieties of fish, including mosquitofish and goldfish, eat mosquito larvae. When these organisms are placed in container habitats, decorative ponds, and pools, they prey on mosquito larvae, effectively preventing mosquito development. The addition of copepods into large water-storage tanks was successful in limiting dengue transmission in Vietnam (Nature Education , 2011). Other live predators such as dragonflies, small aquatic turtles, and beetle larvae have also been shown to be effective in killing *Aedes aegypti*. Scientists in Australia infected mosquitoes with bacteria called *Wolbachia pipientis* (MC Meniman *et al*., 2009) a parasite that shortens the lives of fruit flies. They hypothesized that *Wolbachia* could also shorten the lives of dengue-infected mosquitoes. Instead of eradicating mosquitoes, the scientists aimed to use the bacteria to shift the age of the mosquito population. Although mosquitoes would still be around, they would have shorter life spans than they do now. Also, Mc Menimman *et al*., (2009) tested another *Wolbachia*

strain that seems to kill the dengue virus inside *Aedes aegypti*, which could serve as a dengue vaccine for mosquitoes (Nature Education , 2011).

2.6 Application of traps

Another method of reducing *Aedes aegypti* is to use ovitraps (Nature Education , 2011). These devices are black, cylindrical containers filled with water. The top of the ovitrap is fitted with a circular wire mesh and a floatation ring that floats on the surface of the water in the container. Above the mesh, two paddles are mounted. To *Aedes aegypti*, ovitraps, appear to be ideal location to lay their eggs. The female lays its eggs on the paddles. The eggs then fall beneath the mesh, where the mosquitoes develop through the immature larval and pupal stages. When the adult mosquitoes emerge, however, they are trapped beneath the mesh and are unable to escape from the ovitrap. Some ovitraps have been adapted to include sticky surfaces that entrap adult mosquitoes. Others include the use of a pesticide on the device. Ovitraps can also be used for mosquito surveillance. When sufficient numbers of ovitraps are used and frequently maintained, the vector population can be diminished.

2.7 Chemical control

2.7.1 Indoor spraying with residual insecticides

Indoor residual spraying (IRS) with insecticides is a powerful way to rapidly reduce malaria transmission (Najera and Zaim, 2001). Its full potential is realized when at least 80% of houses in targeted areas are sprayed. Indoor spraying is effective for 3–6 months, depending on the insecticide used and the type of surface on which it is sprayed. IRS involves coating the walls and other surfaces of a house with a residual insecticide and long - lasting for

several months. The insecticide will kill mosquitoes and other insects that come in contact with these surfaces. IRS with DDT and dieldrin was the primary malaria control method used during the Global Malaria Eradication Campaign (1955-1969). DDT can be effective for 9–12 months in some cases. Longer-lasting forms of existing IRS insecticides programmes, are under development. Antimalarial medicines can also be used to prevent malaria , through chemoprophylaxis, which suppresses the blood stage of malaria infections and preventing its occurance.

2.7.2 Larvicides

i. Altosid Pellets: are insect growth regulators in powder form , applied by hand at a rate of 2.5lbs/acre of water to sites that require frequent treatments such as areas of standing water with larval populations.

ii. Altosid Briquettes: also an insect growth regulator in form of briquet placed in sites subject to heavy flow of water , dispersed by hand at a rate of 1 briquette per every 75 gallons of water or 100 sq.ft of area.

iii. Bti Briquettes and granules: This is a larviciding agent that floats and has a sustained release of bacteria for long-term control of mosquito and psychodid fly larvae.

iv. Temephos 50%EC.(Abate 50% EC).

It is an organophosphorus compound registered in Sudan as a standard mosquito larvicide. It is sprayed weekly as a Public Health programme to control mosquito larvae at their breeding sites, in pools, ponds, drainage systems, and path rooms. Abate is considered an important chemical used in source reduction method of mosquito vector control.

2.7.3 Bioinsecticides.

Bioinsecticides are normally used as a combination of biological controls and insecticides. One example of a bioinsecticide is *Bacillus thuringiensis israelensis* (*Bti*), which is a naturally occurring soil bacterium that can effectively kill mosquito larvae present in water. There are many strains of *Bacillus thuringiensis*, each having unique toxicity characteristics, and *Bti* is very specific for mosquitoes. *Bti* is available in small, slow-release bricks called "mosquito dunks" that float on the water surface and are effective in treating deep water. Other bioinsecticides, such as pyriproxyfen and methoprene, act as juvenile hormone analogues that prevent mosquito larvae from changing into adults.

The use of chemicals is considered the last resort and least efficient mosquito control technique which is usually taken for managing mosquito population that have reached the adult stage in spite of efforts to intervene in the larval stage or when such treatment has not been conducted. Ground or aerially applied, cold or thermal application of adulticides is also used in some areas but to a much lesser degree.

2.7.4 Adult Fungicides (microbial control)

Many biological control agents have been evaluated against larval stages of mosquitoes, of which the most successful ones comprise bacteria such as *Bacillus thuringiensis israelensis* and *B. sphaericus* (Becker and Margalit, 1993); (Fillinger *et al*., 2003), mermithid nematodes such as *Romanomermis culicivorax* (Zaim *et al.*, 1988), microsporidia such as *Nosema algerae* (Undeen and Dame, 1987) and several entomopathogenic fungi (Lacey and Undeen, 1986). Other mosquito-pathogenic fungi that

target larval instars include the chytidriomycetes *Coelomomyces* **(** Shoulkamy; Lucarotti, 1998 and Lucarotti, 1992) and the deuteromycetes *Culicinomyces* (Sweeney, 1981, Debenham and Russell, 1977), *Beauveria* (Clark *et al.*,1968) and *Metarhizium* (Roberts , 1970). Only a handful of studies have control, evaluated biological control agents/methodologies to adult stages of tropical disease vectors (Soarés , 1982) , infected adult *Ochlerotatus sierrensis* with the deuteromycete *Tolypocladium cylindrosporum*, resulting in 100% mortality after 10 days.

2.8 Diseases

2.8.1 Bancroftian filariasis

Lymphatic filariasis (LF) caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* is one of the major public health problems in Southeast Asia. It is estimated that there are 120 million cases of this disease worldwide, and over 90% of these infections are due to *W. bancrofti* (Ottesen, 2006) . The disease is found throughout sub- Saharan Africa, Southeast Asia, India, South America, and various Pacific islands and has been associated with significant morbidity in these regions (WHO, 1992). Lymphatic filariasis is caused by parasitic worms of nematodes family of *Filariodidae* that are transmitted to humans by mosquitoes of the genera *Culex*, *Anopheles* and *Aedes*.The African continent has a large burden of LF, which is caused by the parasite *W. bancrofti* and predominantly transmitted by Anopheles mosquitoes in rural areas and by Culex in urban and coastal areas of East Africa (Sasa , 1976, Van den Berg *et al*., 2013, de Souza *et al*., 2012, Monguin *et al*., 2009 and Raghavan, 1961). An estimated 120 million people from 81 countries are infected with lymphatic filariasis. An estimated 1.43 billion live in areas where filariasis is endemic and are at risk of infection. Approximately 65% of those at risk reside in South-East Asia Region, 30% in the African Region and the remainder in other parts of the tropical world . The most common clinical manifestations of lymphatic filariasis include lymphoedema and scrotal hydrocoele. These two manifestations adversely affect personal and social life, and limit occupational activities, making lymphatic filariasis the second leading cause of chronic disability worldwide.

2.8.2 Lymphatic filariasis in the Eastern Mediterranean Region

Eastern Mediterranean Region (EMRO) has three main endemic countries: Sudan, Egypt and Yemen. .It is the main challenge in the EMRO. More than 90% of the filariasis cases of the EMRO are living in Sudan. Sudan was unable to finish the mapping and to start the Mass Drug Administration (MDA) programme for elimination of lymphatic filariasis because of the on-going unrest. Despite the war, mapping has been done in many areas in Sudan. None of the examined areas were negative. This would raise the suggestion that all Sudanese are living in filariasis endemic areas. WHO is now supporting one office in Sudan in Khartoum . In 1998 , a pharmaceutical company announced its commitment to collaborate with WHO by providing albendazole free of charge for as long as needed to eliminate the disease. The donating pharmaceutical company expanded its donation programme for Onchocerciasis to provide ivermectin for lymphatic filariasis elimination in all countries where lymphatic filariasis and onchocerciasis were co-endemic. Limited susceptibility and rapid build-up of resistance to synthetic pyrethroids by culicine filariasis vectors (Chandre *et al*.,1999) combined with the availability of effective anti-filarial drugs (Ramaiah *et al.,* 2002) is causing a gradual shift from vector control to mass-chemotherapy, though resurgence of transmission in the absence of vector control remains problematic (Burkot *et al*., 2002) and Sunish *et al*., (2002).

2.8.3 Filariasis in Sudan :

Kirk (1954) cited that there are four species of human filaria in the Sudan, namely : *Loa loa*, *Acanthocheilonema perstans*, *Wucherera bancrofti* and *Onchocerca volvulus* .

2.8.4 Loa loa :

Concerning the vectors of *Loa loa*, Gordon (1955), stated that no evidence has been produced to incriminate any genus of Diptera other than *Chrysops* in the transmission of the worm of Loa loa in the Sudan.

Acanthocheilonema perstan*s*:

This parasite has been known for longer than any other human blood filaria in the Sudan.The distribution of the worm is wider than that of *Loa loa* , but less precisely known. Archibald (1914), found *A.perstans* in a case of kala-azar, a boy from Kurmuk on the Abassinian frontier.

Sharp (1928), demonstrated that *A.perstans* will undergo development up to the infective stage in *Culicoides austeni* which he considered to be the main vector in the Cameron . Macfie .(1947) , cited that , the known distribution of Culicoides in the Sudan can be summarized as follows:

Culicoides austeni: Erkoit.

Culicoides pycnostictus: Wad Medani.

Culicoides schultzei: Lagawa, Merebea, Wad Medani.

Culicoides similis: Katena, Wad Medani.

Culicoides fuscicandae: Wad Medani.

Culicoides bedfordi: Wad Medani.

Culicoides milnei: Lagawa.

Wucherera bancrofti

In 1944, Dr Mohi Din Mahdi found that *W.bancrofti* was common in the Nuba mountains, in the area round Kadugli. Although the existence of occasional cases of elephantiasis in the Nuba mountains had been known for years, this was the first identification of the causal filarial. Bloss (1949) cited that, *Onchocerciasis* and *Simulium damnosum* have recently been found, and there are the old records of Sir Robert Archibald which indicate that *A.perstans* and probably also *W.bancrofti* are present, but nothing is known about the prevalence of these infections.

No studies have been made to determine the vectors of *W.banctrofti* in the Sudan. It is known that this parasite can be transmitted by many different mosquitoes, including species of *Anopheles*, *Culex* and *Aedes*. In all places in the Sudan where *W.bancrofti* has been found, several species of mosquitoes, proved vectors in other places, were known to be present.

2.8.5 Dengue fever

Dengue is the most common mosquito-borne viral infection, with an estimated 100 million infections worldwide per year (Gibbons and Vaughn, 2002; Wilder-Smith and Schwartz, 2005 and Deen *et al*., 2006). Many factors like urbanization, increased population density, air travel, and limited resources for dengue prevention has led to dengue becoming a major public health problem in the tropics (Gibbons and Vaughn, 2002). Of the 100 million annual infections, 250-500 thousand persons manifest severe

disease, with the remainder being mild, non- specific, or even asymptomatic (Deen *et al*., 2006). In the WHO Eastern Mediterranean Region, dengue is an emerging health problem in several countries such as Djibouti, Pakistan, Saudi Arabia, Somalia, Sudan and Yemen (DCD News Letter, 2005; and Seidahmed, *et al*., 2012; Abdalla *et al*., 2012 and Adam *et al*., 2010). Dengue have been reported in different regions of the Sudan especially Port Sudan in the Red Sea State (McCarthy *et al*., 1996 and Ageep *et al*., 2006; Ahmed ; Karsany and Adam, 2008). Despite the prevalence of dengue in Sudan, there are no data on the maternal and fatal consequences of dengue during pregnancy. Abdalla *et al*., (2012). Cited that, about (71.7%) out of 113 patients 81 had confirmed dengue infection at Kassala, Eastern Sudan, during the period of August throughout November 2010.

The coastal area of the Red Sea State of Sudan has been subject to repetitive outbreaks of dengue fever (DF) and dengue haemorrhagic fever (DHF) in the past decade, particularly the main port of the country Port Sudan (19°37' N; 37°13' E) (Kay, Vu, 2005) [National programme for Epidemics Control and Zoonotic Diseases, Sudan, (unpublished data, 2010)] . Three serotypes of the virus (DEN V1, DEN V2 and DEN V3) are known to circulate (Hayms *et al*., 1986 and Malik *et al*., 2011) and infestations of *Aedes aegypti*, the mosquito vector, have been documented in the area (Seidahmed , *et al*., 2012) , this is linked to the limited drinking-water and consequent water storage habits of the local community (Lewis, 1955). A lack of an efficient surveillance system has resulted in delayed response to these outbreaks. Moreover, the vector control approach during these outbreaks was the same as that for a malaria outbreak. Interventions were been selected and implemented without consideration to differences in bionomics and

ecology between the malaria and dengue vectors (Seidahmed, unpublished report, 2010). An unprecedented dengue outbreak started in January 2010 in Port Sudan (Seidahmed *et al* ., 2012).

2.8.5.1 Control of Dengue fever out breaks :-

Because there is currently no effective vaccine against dengue and no specific treatment for the disease, controlling and preventing dengue fever outbreaks are essential steps for keeping people healthy.

2.8.5.2 Environmental management of Dengue mosquito populations

Conrol of *Aedes aegypti*:

Mosquito habitats :-

The primary preventative measure to reduce dengue infections is the control of mosquito populations. Because the transmission of dengue requires mosquitoes as vectors, the spread of dengue can be limited by reducing mosquito populations. One practical and recommended environmental management strategy is to eliminate unnecessary container habitats that collect water (such as plastic jars, bottles, cans, tires, and buckets) in which *Aedes aegypti* can lay their eggs. When container habitats are removed and water storage containers are covered with a fine mesh to prevent mosquitoes from getting inside them, mosquitoes have fewer opportunities to lay eggs and cannot develop through their aquatic life stages. Source reduction can be effective when performed regularly, especially when members of a community are mobilized and educated about vector control.

Environmental management initiatives can also include major changes in a community, such as installing water systems with direct connections to

residences and replacing wells and other water-storage containers, which can be mosquito-breeding habitats**.** Community-based approaches must go hand in hand with educational initiatives that teach people about mosquito vectors and the risks of having mosquito-breeding habitats near their homes.

2.9 Botanical larvicides as new alternatives to chemical pesticides

The plant world comprises a rich untapped pool of phytochemicals that may be widely used in place of synthetic insecticides in mosquito control programmes. The botanical insecticides are generally pest specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment. One plant species may possess substances with a wide range of activities , for example extracts from the neem tree *Azadirachta indica* showed antifeedant, antioviposition, repellent and growth–regulating activity (Farzana , 2005). Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of the plant families:- Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various types of larval, adulticidal or repellent activities against different species of mosquitoes (Shaalan *et al.,* 2005). Roark (1947) described approximately 1,200 plant species having potential insecticidal value, while Sukumar *et al*.,(1991), listed and discussed 344 plant species that only exhibited mosquitocidal activity.

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. Generally the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components (Rattan, 2010). Of these, the most important activity is the inhibition of acetylcholinerase activity (AChE) as it is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway; AChE has been observed to be organophosphorus and carbamate resistant, and it is well- known that the alteration in AChE is one of the main resistance mechanisms in insect pests (Senthilnathan, *et al* ., 2008).

2.9.1 *Azadirachta indica* A.Juss. (**Neem Tree).**

Neem seeds contain approximately 99 biologically active compounds of which azadirachtin, nimbin, nimbidin and nimbolides are major molecules. The bioactive compounds in the neem tree were found throughout the tree, but those in the seed kernel were the most concentrated and accessible, specially Azadirachtin which is the main bioactive constituent (Grunewald *et al*., 1992). Many of these Neem products have been shown to exhibit a wide range of effects that are potentially useful for malaria control and include antifeedancy (Lucantoni *et al*.,2006), ovicidal activity, fecundity suppression (Su and Mulla, 1998 b) , insect growth regulation (Mordue (Luntz), and Blackwell ,1993; Mordue (Luntz) and Nisbet, 2000) and repellency (Singh *et al*., 1996 ; Sharma and Dhiman, 1993). The neem tree, *Azadirachta*

indica A. Jussieu (Sapindales: Meliaceae) , has well-known insecticidal (Wandscheer *et al*., 2004) and insect growth regulatory (IGR) constituents (Sukumar *et al*. , 1991; Batra *et al*. 1998; Copping and Menn 2000) , and has been used for centuries in India (Schmutterer, 1990). Various neem products have been studied extensively for their phytochemistry and exploitation in pest control programs (Mulla and Su 1999).

Numerous studies investigating mosquitocidal properties of the neem leaves, fruits, and bark have been carried out (Awad and Shimaila 2003, Wandscheer *et al*., 2004; Howard *et al*., 2009; Martínez-Tomás *et al*., 2009). However, most neem extracts require expertise to prepare, making it difficult for local people to adopt and use neem-based insecticides on their own.

Aliero (2003) suggested that, the seed oil and leaf extracts of neem (*Azadirachta indica*) contains properties that could be developed and used in the control of mosquitoes in the tropics as a result of his studies on the larviciding effect of neem on *Anopheles* mosquitoes. These effects are frequently attributed to the azadirachtin contents of the products (Isman, 2006, Mordue(Luntz) and Blackwell, 1993). Also, Aliero (2003) found that, seed oil appeared as the most lethal among the various parts tested against *Anopheles* species and he attributed this to the deficiency of dissolved oxygen in the water. Recent studies have also demonstrated neem-induced effects on vitellogenesis and severe degeneration of follicle cells during oogenesis in mosquitoes (Lucantoni *et al*., 2006). It has been argued that the pesticidal efficacy, environmental safety, and public acceptability of neem and its products for control of crop pests would ensure its adoption into mosquito control programmes (Su and Mulla, 1998a; 1998b).

2.9.2 *Eclipta prostrata* L. (Tamr AL-ghanam)

Is an annual herb from the family *Asteraceae*. It is distributed throughout Asia (China, Japan, Korea), South and Southeast Asia (Bangladesh, India, Indonesia, Cambodia, Lao PDR, Malaysia, Nepal, Pakistan, Philippines, Sri Lanka, Thailand, Vietnam) and in some other parts of the world (Angola, Arabian Peninsula, Argentina, Australia, Brazil, Colombia, Costa Rica, Cote d'Ivoire, Cuba, Egypt, Fiji, Ghana, Iraq , Mexico, Peru, Portugal, Puerto Rico, Rhodesia, Sudan, Surinam, Trinidad, United States of America, Zambia, Zimbabwe).(Sandhya *et al*., 2006). *E. protstrata* is an erect or prostrate, branching herb, with rooting at the nodes, stem and branches. The leaves measuring 2.5 to 7.5 cm long are opposite, sessile and lanceolate). (Sandhya *et al*., 2006) and (Himalayahealthcare, 2007).The flower-heads measuring 6-8 mm in diameter are solitary or with two on unequal axillary stalks. The involucral bracts are 8 to 10 in number. They are white, strigose and ligulate. The disk flower is tubular, often with 4-tubed corollas (Himalayahealthcare, 2007). In a study by Gopiesh Khana and Kannabiran (2007) , leaves water extract of *Eclipta prostrata* have been tested for its activity against mosquito larvae , and the results showed that , more than 50% were killed at the lowest concentration(500 ppm) and gave 100% mortality of the larvae at the highest concentration (2500 ppm). The study recommended that *Eclipta prostrata* can be used as environment friendly and sustainable insecticide to control mosquito (Gopiesh Khanna and Kannabiran , 2007). Govindarajan and Karuppannan (2011) found that, the LC_{50} values of benzene, hexane, ethyl acetate, methanol and chloroform extract of *E. alba* against early third instar larvae of *Ae. aegypti* were 151.38, 165.10, 154.88, 127.64 and 146.28 ppm, respectively. Maximum larvicidal activity was observed in the methanol extract followed by chloroform, benzene, ethyl acetate and hexane extract. No mortality was observed in control. Among five

solvent tested the methanol extract was found to be most effective for ovicidal activity against *Ae. aegypti*. The methanol extracts exerted 100% mortality (zero hatchability) at 300 ppm.

2.9.3 *Solenostemma argel* (Del. Hayne) , (Hargal) (Asclepiadaceae)

Is a perennial shrub widely distributed in the deserts of Arabia, Egypt, Libya, Chad, Sudan and Palestine, where it is known as "Argal" or "El Hargal". *Solenostemma argel* was used in folk medicine as a remedy for bronchitis, gastrointestinal cramps, stomach ache, colic, cold and urinary tract infections (Hegazi *et al*., 1994). In Libya and Chad, a decoction of leaves is used to treat neuralgia and sciatica (Jabeen *et al*., 1984). The white fleshy leaves are also used as adulterant of Senna (Jabeen *et al* ., 1984). Phytochemical studies of the plant showed the presence of kaempferol glycosides (Khaled *et al.*, 1974), monoterpene and pregnane glycosides (Kamel *et al*., 2000 , Hamed , 2001 and Plaza *et al.*, 2003) as well as pregnene, triterpene and acylated phenolic derivatives (Hassan *et al.*, 2001 and Kamel, 2003). Furthermore, the few pharmacological studies on *Solenostemma argel* evidenced spasmolytic (El Tahir *et al.*, 1987) and anti-microbial activity (Tharib and El-Migirab , 1986 and Hegazi *et al.*, 1994).

2.9.4 *Balanites aegyptiaca* (L.) Del. (Higleeg) .

Belongs to the family Balanitaceae. It is a multibranched, evergreen tree distributed throughout the drier parts of India (Anon , 1986). It is widely grown in the Sudano-Sahielian region of Africa , the Middle East and South Asia (Hall and Walker, 1991). It is known by various names, e.g. Arabic names: Heglig (tree), lalob (fruit); trade name: zaccone, zachun, desert date (dried fruit); (Hardman and Sofowora, 1972) in India: Hindi name is Hingot and English name is thorn tree/desert date. *B.aegyptiaca* is a rich source of saponins. Saponins are glycosides consisting of

sugar residues (one or more units of glucose, galactose, etc.) linked through oxygen with complex multi-ring compounds usually containing 27-31 carbon atoms. The aglycone part, which is also called sapogenin, is either a steroid (C27) or a triterpene (C30). (Hostemann and Marston, 1995). Saponin containing plants are used in folk medicine, especially in Asia, and are intensively used in food, veterinary and medical industries. *B. aegyptiaca* contains different types of saponins, namely, balanitin -1, 2, 3, 4, 5, 6 and7. *Balanites aegyptiaca* (Higleeg) acts as a potential natural larvicidal agent against mosquito larvae due to the larvicidal activities present in the saponin rich extracts in the various tissues such as fruit pulp, kernel, root, bark and leaf (Zarroug *et al*., 1990 ; Wiesman and Chapagain 2003 ; Chapagain and Wiesman, 2005 ; Wiesman and Chapagain, 2006 and Wiesman, 2006). The water extracts of fruit kernel of *B. aegyptiaca* were found to be effective against the larvae of *Anopheles arabiensis, Culex quinquefasciatus* and *Aedes aegypti* and it was concluded that *A. arabiensis* larvae were the most susceptible, followed by *C. quinquefasciatus* and then *A. aegypti* (Zarroug , *et al*., 1990). The root extract of the plant was found to be most lethal (Chapagain, and Wiesman, 2005), (Wiesman and Chapagain, 2006). On the basis the very low adult emergence (only 18%) on using only 50 ppm of the root derived callus , saponins of *B. aegyptiaca* showed a great possibility for drastically reducing the *A. aegypti* population in the concerned areas. (Chapagain, Saharan, Wiesman, 2008). The main reason behind the larvicidal activity of plant extract may be the interaction of saponin molecules with cuticle membrane of the larvae, ultimately disarranging these (Morrissey and Osboun, 1999).

2.5.5. Application of Botanical Insecticides against important medical and veterinary pests in Sudan

Interest in exploiting the pest control potential of plants in Sudan have revived as early as the 1930s (Zarroug, 1984), when Archibad (1933) advocated planting the desert palm *Balanites aegyptiaca* (Higleeg) (L.) Del., along water courses of the River Nile. The problem of resistance build-up in vectors of malaria, filaria and dengue fever (WHO, 1992) and other vector-borne diseases to several synthetic insecticides; coupled with hazard of pollution to the environment have revived the interest in exploiting the pest control potential of plants (Grainge and Ahmed, 1988).

Hassan (1988) , tested the potential effects of *Ocimum basilicum* (Basil) essential oils as a control agent against five insects of economic importance , namely *Trogoderma granarium* Everts, *Tribolium castanium* Herbst, *Locusta migratoria* L., *Henosepilachna elaterii* Ross, and ,beside Basil essential oils extract, he tested Basil leaves water extract against *culex* species. Kehail and Bashir (2003), have used water extracts of twenty indigenous plant species including *Ocimum basilicum* (L.) against mosquitoes . In search of a natural larvicide, *Ricinus communis* (Castor) was tested for its larvicidal activity against *Culex quinquefasciatus*; the castor was observed to be potent; its extract exhibited potential results and can be exploited as a preferred natural larvicide for the control of mosquitoes (Batabyal *et al*., 2009). The *R. communis* crude extract was found to be active on larvae of *Anopheles arabiensis*. Results revealed that the crude extract of *R.communis* possesses remarkable larvicidal, adult emergence inhibition and oviposition deterrent properties against *Anopheles arabiensis* and can be used as biological control means (Elimam *et al*., 2009).

Eltayeb *et al*., (2009), tested leaves water extracts of *Solenstemma argel* Del Hyne (Hargal), and *Calotropis procera* Ail. (Usher), against the larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes.Results showed that , water extract of the two plants caused a significant increase in mortality percentage of the of the two mosquito larvae, providing that the highest concentrations of the two plant extracts were as effective as that of the standard larvicide, Temephos, which caused 100% mortality of mosquito larvae after 24 hours. Results also showed that, both Hargal and Usher water extracts were effective against both mosquito larvae and may represent a good friendly alternative to synthetic larvicides in Sudan.

Taha *et al*., (2011), evaluated the larvicidal activity of ethanolic extracts from leaves of three plants: castor bean (*Ricinus communis*), vinca (*Vinca rosea*) and lantana (*Lantana camara*) at different concentrations against the 3rd instar larvae of *Anopheles arabiensis*. The concentrations of the three tested plant extracts were compared with the standard larvicide Temephos (Abate®). The result showed that the three plant extracts have larvicidal effects on *Anopheles arabiensis*, and the castor extract had the greatest larvicidal effect on *Anopheles arabiensis*. The statistical analysis revealed no significant differences between the higher concentration of each of the three plants tested and the standard larvicide (Temephos). The study recommend to consider the three plants as promising larvicides against *Anopheles arabiensis*.

Isam *et al*., (1988), screened water extract of eight plant species for their larvicidal activity against 2nd and 4th instar larvae of the three local mosquito species: *Anopheles arab*iensis, *Culex quinquefasciatus* and *Aedes aegypti*. Three out of the eight plant water extracts tested against mosquito larvae, produced high larval mortality. These three potent plant extract were from: *Randia nilotica*, *Gardenia lutea* and *Balanites aegyptiaca.*

Results showed that *Balanites aegyptiaca* (L.) Del, (Higleeg , was the most potent. In addition, analysis of the results revealed that , second instar were more susceptible than the fourth instar larvae in all cases, and that the larvae of *Anopheles arabiensis* were the most susceptible.

Taha *et al*., (2011), have carried out a field study to investigate the effects of the powder of *Solenostemma argel* Del Hyne (Hargal), and *Calotropis procera* Ail. (Ushar), against the green pit scale insect (*Asterolicanium phoenicis* Rao.), at different treatment regimes as (1) 100g powder/tree; (2) 100 g powder /10 litre tap water/tree as spray; and (3) 100 g powder as soil dressing + 100 g powder/10 litre tap water for spraying/ tree. Besides, the synthetic insecticide Actara 25 W.G. Thimethoxam was used as a standard insecticide. The results showed that , the plant treatments were effective against the immature stages of the green pit scale insect, and their effectiveness lasted for 8- 10 weeks after treatment. Most of the plant treatments were comparable to those of Actara in efficiency. The cost of protection as dose/plant with the plant treatments compared with the insecticide Actara treatment, was significantly lower as (1:25). Accordingly, Hargal and Ushar soil treatment (100g/tree), were recommended as an effective treatment for control of the green pit scale insect immature stages.

CHAPTER THREE

MATERIALS AND METHODS

Part I. Larvicidal activity of the plant extracts against vector mosquito species

3.1 Preparation of mosquito colonies culture

3.1.1 Anopheles ***Anopheles arabiensis*** **Patton** .

Batches of *An. arabiensis* eggs kept in filter papers were kindly provided by Malaria Control Department-Directorate General of Preventive Medicine Ministry of Health Khartoum State. Also, additional collections for different stages of *Anopheles arabiensis* were made from ditches and rain water collections at Abu Seid area - Omdurman (plate No.1). (Occasionally, more collections were also made from other sites in Khartoum State, e.g., Appendix plates: 4,5,6,7,8). The eggs were placed in white enamel dishes filled with water up to 4 inches and incubated until hatching in the laboratory of Entomology of the Faculty of Agricultural Studies - Shambat , to start the rearing and mass culturing , following the procedure of the WHO (1975 and 1992). After hatching, the first instar larvae were reared in a glass container (40x40x40 cm) with tap water and fed with fish food and wheat flour until reaching pupation. The obtained pupae were collected and transferred to an open petri dish containing water inside a glass cage (40x40x40 cm), covered with muslin cloth to prevent the escape of the adults, and left under conditions suitable for development. Emerging males and females were fed with Sugar dissolved in tap water placed in small vials, each provided with a thin cotton robe extending from the top to the base

side, to allow adult feeding on the sugar solution by sucking on the tip of the wet robe. Also adult females were provided with a 1-week-old chick for blood feeding. In addition, an electric bulb put on for 12 hours light, 12 hours dark. Five days after blood feeding ,gravid females used for egg laying of different ages at 3 - hours intervals were provided with 70 ml petri dishes lined with filter papers inside the rearing cage for egg laying. After 2 days, gravid females started to lay eggs on the surface of the wet filter papers. Eggs laid by females were collected every day, and transferred in white enamel dishes to the adult rearing cage to start new culture of *Anopheles arabiensis*. By following to do so, early 4^{th} instar larvae of the second generation were provided for the different bioassay tests.

Plate No. 1. Immature stages of *Anopheles arabiensis* in running rain water and river side at Abu Seid area - Omdurman.

3.1.2. ***Culex quinquefasciatus*** **(** Say).

Rafts of *Cx. quinquefasciatus* eggs kept in filter papers were kindly provided by Malaria Training Centre in Sennar State, beside some egg rafts and egg batches which were collected regularly from several ditches and stagnant water habitat around Shambat area (plates No. 2 & 3). (Occasionally, more collections were also made from other sites in Khartoum State, e.g., Appendix plates: 4,5,6,7,8). Collected eggs were poured in big plastic buckets, and the materials collected were taken to the laboratory and transferred to white metal dishes containing dechlorinared tap water. After hatching, first instar larvae were fed on small amounts of wheat flour until reaching the third instar. After pupation the start of new culture of the second generation of *Culex quinquefasciatus* to provide larvae for different bioassay tests was followed as previously described for *Anopheles arabiensis*.

Plate No. 2. Collection of immature stages of *Culex quinquefasciatus* around Shambat area .

Plate No. 3. Collection of mosquito stages (*Culex quinquefasciatus*) - around Shambat area

3.1.3. ***Aedes aegypti*** L.

Regular stock of egg batches of *A. aegypti* kept in filter papers were also kindly provided by malaria training Centre in Sennar State, and Malaria Control Programme at Kassala. Also, some collections of immature stage of *Aedes aegypti* were made from water storage containers in Kassala (plates No. 4 , 5 & 6). (Occasionally, more collections were also made from other sites in Kassala, e.g., Appendix plates: 1,2 &3). Eggs were poured in plastic buckets , and transferred to white metal dishes containing dechlorinated tap water up to 4 inches and incubated until hatching in the laboratory of Entomology, to start the rearing and mass culturing according to the WHO (1975 and 1992). After hatching, the first instar larvae were reared in a glass container (40x40x40 cm) with tap water and fed with fish food and wheat flour until reaching pupation. The obtained pupae were collected and transferred to an open petri dish containing water inside a glass cage (40x40x40 cm), covered with muslin cloth to prevent the escape of the adults, and left under conditions suitable for development. Emerging males and females were fed with Sugar dissolved in tap water placed in small vials , each provided with a thin cotton robe extending from the top to the base side, to allow adult feeding on the sugar solution by sucking on the tip of the wet robe.Also adult females were provided with a 1-week-old chick for blood feeding. In addition, an electric bulb put on for 12 hours light, 12 hours dark. Five days after blood feeding ,gravids females used for egg laying of different ages at 3 - hours intervals were provided with 70 ml petri dishes lined with filter papers inside the rearing cage for egg laying. After 2 days, gravid females started to lay eggs on the surface of the wet filter papers. Eggs laid by females were collected every day, and transferred in

white enamel dishes to the adult rearing cage to start new culture of *Aedes aegypti*. By doing so, early 4th instar larvae of the second generation were provided for the different bioassay tests.

Plate No. 4. water storage containers at (Khatmeya North –Kassala) (keeping large numbers of *Aedes aegypti* immature stages) .

Plate No. 5. Limited drinking water and habit of water storage (Banat-Kassala) - (perminant breeding habitats for *Aedes aegypti*)

Plate No. 6. Sucking out all immature stages of *Ae. aegypti* from water pots (Banat – Kassala) .

Also parallel colonies of the eggs and larvae of *Anopheles arabiensis, C. quinquefasciatus* and *Aedes aegypti* were maintained in the insectary at the Faculty of Agricultural Studies for regular supply of eggs and larvae for the experiment , in accordance with the procedure of WHO (1975 and 1992).

3 .2. Preparation of the plant materials :-

Solenostemma argel Del Hyne, "Argel", (Hargel) (leaves) and *Eclipta prostrata* L. " False daisy, swamp daisy, eclipta,white eclipta", (Tamr Al-ghanam). Family: (Asteraceae) leaves, *Balanites aegyptiaca* (L) Del. " desert date", (Higleeg). Family: (Zygophyllaceae), (root), and fruits of *Azadirachta indica* A. Juss "Neem", (Al- neem). Family: (Meliaceae), were tested for their larvicidal activities against the larvae of the *Aedes aegypti*, *Anopheles arabiensis* (patton), and *Culex quinquefasciatus* (Say), mosquito species under laboratory condition.

Aerial parts of Hargal were collected during Autumn season from Shendi area , River Nile State. Tamr Al-ghanam aerial parts and Neem seeds, were collected from Shambat area and also kindly provided by the Environmental and Natural Resources and Desertification Research Institute (ENRDRI) at Khartoum , and Higleeg roots were collected from Rufa,a forest , Gezira State. All samples collected were washed thoroughly with tap water and shade dried under room temperature. Dry samples were ground into fine powder with an electric blender or spice mill. Each experiment was carried directly after extraction, whether it was water or organic extract.

According to the recommended test concentration of each plant extract, five concentrations were serially prepared by adding distilled water.

The plant organic chemicals were prepared through the soxhlet apparatus. Methanol and hexane solvents were used to separate a polar and polar components, respectively, according to the procedure of Harbone (1983). So, 200 gms from each plant was extracted separately for 8 hours in the soxhlet. The extracts later were dried from the solvents using the rotary evaporator, then kept in black bottles and stored in a refrigerator (at 5° C) until furtherly being used . If were needed to be used, 10 grams of , the organic extract were firstly dissolved in 1 ml (ethanol) , then the volumes completed to 200 ml with distilled water to prepare the stock solution. Five concentrations of each methanol or hexane extracts, were prepared serially from their stock solutions according to the recommended test concentration for each plant extract.

3.2.1 Preparation of plant extracts and concentrations

Two extraction methods were used for each plant species, an aqueous and organic solvent extract .

3.2.2. Aqueous extract of the plant materials

3.2.2.1. Aqueous extract of *Balanites aegyptiaca* (Higleeg) roots

The obtained powder material was passed through a 1 mm mesh sieve and stored in glass jars covered with plastic covers and left at room conditions in the laborotary. Twenty grams of the ground powder were placed in Erlenmeyer flask , 100 ml of tap water were added , and mixed vigorously. The mixture was kept for 24 h with occasional shaking. After twenty-four hours, the mixture was filtered using a very fine muslin cloth and the final volume was adjusted to 100 ml. A series of dilutions (40 ppm, 100 ppm, 200

ppm, 400 ppm, and 500 ppm %) were prepared from the stock solution using tap water.

3.2.2.2. Aqueous extract of *Solenostemma argel* (Hargal) shoots.

The obtained powder of Hargel was passed through a 1 mm mesh sieve and stored in glass jars covered with plastic covers, and left at room conditions for further use.

Twenty grams of the powder were placed in a conical flask, macerated in distilled water and shaked by a magnetic stirrer for 6 hrs.The extract was left for 24 hrs and then filtered through a cotton cloth. The volume of the filterate was adjusted to 400 ml with distilled water to give a stock solution of 50 mg/L., then series of dilutions were prepared from the stock solution (62.5 ppm , 125 ppm , 250 ppm , 500 ppm , and 750 ppm).

3.2.2.3 Leaves aqueous extract of *Eclipta prostrata* (Tamr Al-ghanam)

The powder obtained was passed through a 1 mm mesh sieve and stored in glass jars covered with plastic covers and left at room conditions for further use. For preparation of the aqueous extract, twenty grams of the powder were placed in a conical flask, macerated in distilled water and shacked by a magnetic stirrer for 6 hrs. The extract was filtered after 24 hrs through a cotton cloth. The volume was adjusted to 400 ml with distilled water for a stock concentration of 50 mg/L., and a series of dilutions were prepared from this stock solution (500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, and 2500 ppm).

3.2.2.4.Aqueous extract of *Azadirachta indica* (Neem) seeds.

One kg of neem fruit were obtained from the Environment Natural Resources and Desertification Research Institute (ENRDRI), Khartoum . Fruits were washed thoroughly with water, shade dried . Then the whole fruits were oven dried at 35- 40 °C for 3-5 mintues . After the fruits were depulped , seeds were crushed using a morter and pistile to extract the oil by placing the whole content to dry on papers under shade in the laboratory to remove the excess oil and lastly pulverised into powder . 20 grams of the seed powder were placed in 200 ml distilled water , liquidified with an electric blender for 3-5 minutes and placed in a conical flask of 500 ml (Mya *et al.*,2002 ; Perez *et al* ., 2004) . After 24 hours , the mixture was strained through a fine muslin cloth and the remained residues were discarded . This 1000 ppm aqueous exract , which represented the stock solution , was used to prepare the different concentrations for testing in the experiment . To prepare a 100 ppm concentration , 1ml of the stock solution , was mixed with 9 ml of distilled water . A series of dilutions were prepared (62.5 ppm, 125 ppm , 250 ppm , 500 ppm , and 1000 ppm).

3.2.3. Preparation of plant solvent extracts:

3.2.3.1. Preparation of methanolic extract of *Balanites aegyptiaca* (Higleeg) roots.

One kilogram of the finely ground root powder was subjected to extraction in a Soxhlet apparatus (Borosil, Mumbai, India) in methanol , for 72 h for complete extraction (Saxena *et al.*, 1994 ; Mohan *et al.*, 2006). The extract was concentrated under reduced pressure in a vaccum rotary evaporator (Biocraft Scientific Industries, India) until the solvent was completely

evaporated and a crude extract was left. The crude extract was diluted with distilled water to series of concentrations (40 ppm 100 ppm, 200 ppm, 400 ppm and 500 ppm).

3.2.3.2 Preparation of methanolic extract of *Solenostemma argel* (Hargal) shoots.

One kilogram of the finely ground powder of Hargal leaves was subjected to extraction in a Soxhlet apparatus (Borosil, Mumbai, India) in methanol, for 72 h for complete extraction (Saxena *et al.,* 1994; Mohan *et al.*,2006). The extract was concentrated under reduced pressure in a vaccum rotary evaporator (Biocraft Scientific Industries, India) until the solvent was completely evaporated and a crude extract was obtained. The crude extract was diluted with distilled water to a series of concentrations (62.5 ppm , 125 ppm , 250 ppm , 500 ppm and 750 ppm).

3.2.3.3 Preparation of methanolic extract of *E. prostrata* (Tamr Al-ghanam) leaves .

Two hundred gm of the finely ground powder of *E. prostrata* leaves was subjected to extraction in a Soxhlet apparatus (Borosil, Mumbai, India) in methanol, for 72 h for complete extraction (Saxena *et al.,* 1994; Mohan *et al.,* 2006). The extract was concentrated under reduced pressure in a vaccum rotary evaporator (Biocraft Scientific Industries , India) until the solvent was completely evaporated and a crude extract was obtained. The crude extracts was diluted with distilled water to a series of concentrations (500 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm).

3.2.3.4. Preparation of *Azadirachta indica* neem seed hexane extract.

Two hundred kgs of dry neem fruits were soaked in a bucket overnight , the fruit outer cover and seed kernels were removed manually,the neem seeds were left to dry and pressed using a mortar and pistile then , the crushed neem seeds placed in a cloth sac and hanged on a porter to collect the oil in a glass bottle , which kept for further use .The content then shade dried on papers in the laboratory and pulverized into powder.The dried neem seed powder was weighed and subjected to extraction in a Soxhlet apparatus (Borosil, Mumbai, India) in hexane subsequently for 72 h for complete extraction (Saxena *et al*., 1994; Mohan *et al*., 2006). The extract was concentrated under reduced pressure in a vaccum rotary evaporator until the solvent was completely evaporated and a crude extract was obtained. Neem oil was emulsified with a cattleś bile , with few drops of liquid soap , or "Bushra Soap" , used to solubilize the crude extract in water and furtherly diluted with distilled water to prepare the recommended concentrations of (50, 100, 500, 750 and 1000 ppm).

3.2.4. The standard Larvicide Abate.

The larvicide Abate 50% EC (Temephos Super-50 % EC) is the standard insecticide registered for mosquito larval control in Sudan. The commercial formulation "Temephos Super 50% EC", (Abate 50% EC) is used to control insects of public health importance, was used at (1 ml/L) to compare its efficacy with the efficacy of plant extracts tested in the experiments of the present study . Abate was kindly provided by the Malaria Control Administration at Khartoum.

3.2.5. Bioassays tests

3.2.5.1. Larvicidal bioassay treastments

Knock down effects on target species:

Larvicidal activities of water and solvent extract concentrations of leaves of *Solenostemma argel*, *Eclipta prostrata*, root of *Balanites aegyptiaca* and fruit of *Azadirachta indica*, respectively, were subjected to biological assays against the early 4th instar larvae of *Anopheles arabiensis*, *Culex quinquefasciatus* and *Aedes aegypti* under laboratory condition. The larvicide Abate 50 EC (1 ml/L) was included for comparison , in addition to untreated water controls. Plastic cups containing 100 ml of each concentration of the plant extracts were used in each treatment . Twenty larvae of each of the three mosquito species were added to each cup of the plant extracts, in addition to the cups containing larvicide Abate and the untreated controls. All treatments were replicated 4 times in a Completely Randomized Design (Gomez and Gomez, 1984).

Higleeg root aqueous extract at concentrations of 40 ppm, 100 ppm, 200 ppm, 400 ppm and 500 ppm, were applied for testing the knock down effect of the larvae of the three mosquito species. Extracts of *Solenostemma argel* at concentrations of 62.5 ppm, 125 ppm, 250 ppm, 500 ppm and 750 ppm, *Eclipta prostrata* at 500 ppm , 1000 ppm , 1500 ppm, 2000 ppm and 2500 ppm, and *Azadirachta indica* , at 62.5 ppm, 125 ppm , 250 ppm, 500 ppm and 1000 ppm , and 50 ppm , 100 ppm , 500 ppm , 750 ppm and 1000 ppm , were transferred to 100 ml of distilled water taken in 500 ml cups. The bioassay was undertaken at ambient temperature (27 ± 2 º C), and at 85% (RH), (standard procedure of WHO, 1975).

Mortality in larvae was recorded after 24 , 48 , 72 hrs post – exposure , to evaluate the knock down effect of the extract treatments.

Criteria of mortality **:** If the larvae were probed with a needle and failed to swim to the surface or unable to go to the bottom of the cup, this was considered as an indicator for larval mortality.

The larval mortality percentages were calculared from the average of the 4 replicates , in addition to the analysis of varience and mean separation using Duncan Multiple Range Test. The larvicidal efficacy of the plant extracts was compared with Abate 50 EC at the recommended dose (1ml/L) in 100 ml tap water as a standard commercially available larvicide. (Amalraj and Dass, 1996). The 50% lethal concentration (LC_{50}) and 95% confidence limits (LC 95) , were estimated using mortality regression probit analysis (Finney, 1971). Abbott's formula was applied to correct the observed mortality (Abbott, 1925).

3.2.6. Experimental Design

All experiments were conducted as factorial experiments, arranged in a Completely Randamized Design (CRD) with 4 replicates.

For application of aqueous plant extract experiments, each experiment included: one plant extract, 3 mosquito species, 5 extract concentrations, untreated control and Abate (50% EC) as standard larvicide.

For application of solvent plant extract experiments , each experiment included: one solvet extract, 3 mosquito species, 5 extract concentrations, untreated control and Abate (50% EC) as standard insecticide.

3.2.7. Data analysis

Data were transformed using Arc Sine and ANOVA was applied according to the experimental design of the study Completely Randomized Design (CRD). Duncan's Multiple Range Test was used for mean separation; and

the probit analysis was applied (Finney, 1971; Busvine, 1971; and Sokal & Rohlf, 1973) to calculate the LD 50 for the different extracts.

Part II. Trials for testing the tentative structure of the active ingredients of the botanical extracts:-

This part of the study was carried out at the ENRDRI Laboratories in Khartoum with the generous help provided by the Staff and Technicians of the Institute.

3.3 Preparation of botanical extracts

Two extracts (*viz*., water and methanol), were obtained from the prepared powder of Higleeg, Hargal and Tamr Al-ghanam or (water and Hexane) from powder of Neem fruit. For water extraction, 200 gm each of the powders from Higleeg root , leaves of Hargal , Tamr Al-ghanam and Neem fruit , were mixed with 1 litre of distilled water in a conical flask, agitated thoroughly with a magnetic stirrer for 6 hrs and left overnight. The second day, the solution was agitated for 3 minutes with a glass rod and filtered through a fine muslin cloth to obtain the stock solution, which coinciding with the bioassay experiment. On the other hand , a soxhlet apparatus was used to obtain the methanol (polar) or hexane (a polar) extracts of the samples . Upon completion of each extraction round, the filtrate was air-dried and re-extracted successively by the next solvent in the same way (Harborne,1984). Extracts were dried from solvents using a rotary evaporator, moreover, the plant samples were re-weighed at the end of each extraction step, so as to determine the percentage of extracted materials in relation to sample weight and then kept in black bottles and stored in the

refrigerator at (5° C) until used. Lastly the extract obtained was also weighed for more confirmation.

3.4 Phytochemical analysis

Phytochemical screenings of all the prepared extracts for eight major secondary metabolites, *i.e.*, alkaloids, saponins, flavonoids, flavones, tannins, amino acids, steroids and triterpenoids, were carried out through chemical analytical means as described by (e.g., Harborne, 1973, Harborne 1983, Harborne, 1988). So, the presence or absence of such chemical groups in each extract was tested according to these methods. The required reagents: Mayer, Ninhydrin, potassium hydroxide, Ferric chloride and Vanillin specific for testing alkaloids, amino acids, flavonoids, tannins and sterols/triterpenoids, respectively, were kindly provided by (ENRDRI), prepared and used as described in the standard procedures to identify the constituents.

3.4.1 Test for alkaloids

Mayer's test (Evans, 1997) . Mayer's reagent was firstly prepared by mixing 7.36 g of mercuric chloride and 5.00g potassium iodide in 60 ml and 100 ml distilled water, respectively, then each completed with water to 100 ml volume. A volume of 30 ml aliquots of each prepared extract was evaporated to dryness in evaporation dish placed in a water bath , 5 ml of 2N of HCL was added to the dried extracts and stirred while heating on a water bath for 10 minutes. The extracts were then cooled at room temperature and transferred to test tubes. 2-3 ml of Mayer's reagent were added. Formation of slight creamy or white precipitate was taken as evidence for the presence of alkaloids.

3.4.2 Test for amino acids

Ninhydrin reagent was prepared by dissolving about 0.2g of ninhydrin in 100 ml of ethanol to give 0.2% solution. Two 2 ml of ninhydrin (0.2%) were added to 5 ml of each extract; after few minutes a purple –blue colour was appeared indicating the presence of amino acids.

3.4.3 Test for carbohydrates

Benedicts test (means of Harborne**).**

To 0.5 ml of each filtrate, 0.5 ml of Benedicts reagent was added. The mixture was heated on boiling water bath for 2 min. A characteristic red coloured pericipitate indicates the presence of sugar.

3.4.4 Test for saponins (means of Harborne).

The test was simply done by putting 10 ml of aliquots from each extract in a test tube, where 5 ml of water were added . The tubes were closed with a cork and shaken vigorously. Formation of foam layer, honey comb in shapes, which remains for a minimum of 30 minutes, indicated the possible presence of saponins (Kokate, 1999).

3.4.5 Test for sterols and triterpenes (Finar test, 1986) .

Libermann – Buchards test (means of Harborne).

Vanillin reagent is specifically used for testing triterpenoids. It is prepared by dissolving 0.5g of vanillin in 100 ml of H_2SO_4 (sulphuric acid). 20 ml of the prepared extracts were evaporated to dryness in a water bath and cooled. The residues were collected in 10 ml chloroform and dehydrated over anhydrous sodium sulphate. 2.5 ml of this solution was mixed with 0.25 ml

of acetic anhydride acid, followed by one drop of vanillin dissolved in concentrated sulphuric acid. The gradual appearance of green – blue colour was taken as an indication of the sterols, while the pink to purple colour was taken as an evidence for the presence of triterpenes.

3.4.6 Test for phenolic compounds and tannins (Mace, 1963)

Ferric chloride test

Each extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

3.4.7 Test for tannins (means of Harborne)

Ferric chloride was prepared by putting 5g of anhydrous ferric chloride in 100 ml volumetric flask, where 100 ml of distilled water were added to give 5% (w/v) ferric chloride solution. 25 ml of the prepared extracts were evaporated to dryness on a water bath . The insoluble residues were stirred with 10 ml of hot saline solution, then the mixture was cooled and 2-3 ml of ferric test reagent were added to each extract. Formation of a blue- black or a green colour indicated the presence of tannins.

3.4.8 Test for flavonoids and flavones (means of Harborne).

Potassium hydroxide solution was prepared by adding 10 ml of distilled water to 5.0g potassium hydroxide in 50 ml volumetric flask to give 50% (w/v) potassium hydroxide solution. 20 ml aliquot of each extract was evaporated to dryness on a water bath. The residue was dissolved in 30 ml of the same solvent used in the preparation of the extract and filtered. The filtrate was used for both flavonoid and flavones. In the flavonoid case, 1 ml

of potassium hydroxide solution was added to 3 ml of the filtrate in a test tube. A dark yellow colour indicates the presence of flavonoids. Regarding the flavones test, 0.5 ml of concentrated hydrochloric acid and few pieces of magnesium turning (as metal) were added to 3 ml of the filtrate. The appearance of pink or red colour indicates the presence of flavones. Accordingly, the different classes of chemicals present in each extract were tentatively identified.

CHAPTER FOUR

RESULTS

Part 1. Results of the larvicidal activity of the plant extracts :

The data on larvicidal activity of aqueous and organic extracts of *Balanites aegyptiaca* (root), *Solenostemma argel* (shoots) , *Eclipta prostrata* (leaves), and neem *Azadirachta indica* seeds against three larval mosquito stages (*Aedes aegypti*, *Anopheles arabiensis* and *Culex quinquefasciatus*) were given in Tables (4.1a – 4.8a and 4.1b – 4.8b).

4.1 Larvicidal effect of Higleeg *Balanites aegyptiaca* root extract on the mosquito species

4.1.1 Larvicidal effects of root aqueous extracts on the three mosquito species

Larvicidal effects (mortality percentage and the LC_{50}) of *Balanites aegyptiaca* root aqueous extract are shown in Table 4.1a and 4.1b which showed a significant increase in larval mortality percentage of all three mosquito species ($p \leq 0.0125$), compared to the untreated control. The root aqueous extract of *Balanites aegyptiaca* resulted in 89.9% larval mortality percentage of *Anopheles arabiensis* ($p = 0.671$), 51.0% mortality percentage of *Culex quinquefasciatus* ($p = 0.045$) and 48.48% mortality of the larvae of *Aedes aegypti* ($p = 0.00$), when using the root aqueous extract at (40 ppm), 24 hours post exposure, respectively. In Table 4.1a : Application of the probit analysis showed LC_{50} of 0.000357 ml/L for *Anopheles arabiensis* larvae (Figure 1) , LC_{50} of 0.001335 ml/L for *Culex quinquefasciatus* larvae (Figure 2) and LC_{50} of 0.001542 ml /L for *Aedes aegypti* larvae (Figure 3). It is clear that the LC_{50} for the *Anopheles arabiensis* larvae to Higleeg root aqueous extract is 0.000357ml/L , which equals to (3-4) time folds lower than that for the *Culex quinquefasciatus* or the *Aedes aegypti* larvae,

respectively. This means that, the activity of the root aqueous extract of Higleeg on the larvae of *Anopheles arabiensis* is (3-4) time folds higher than that on the larvae of *Culex quinquefasciatus* or *Aedes aegypti*, after 24 hours. The highest concentration (500 ppm) of the root aqueous extract caused (94.9%), larval mortality of the *Aedes aegypti*, (99.9%) of the *Anopheles arabiensis* and (99.9%) of the *Culex quinquefasciatus*, 24 hours post exposure, respectively, which was as effective as that of the standard larvicide Temephos, that caused 100% mortality of the larvae of the three mosquito species after 24 hours.

Table 4.1a Effect of different concentrations of Higleeg aqueous root extract on the mortality (%) of the three mosquito species.

	Species					
	Aedes aegypti		*Anopheles arabiensis*		*Culex quinquefasciatus*	
	Mortality (%)					
Concentrations	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
00.00	00.00	00.00	00.00	00.00	00.00	00.00
40 ppm	48.48 (43.2)	58.7 (50.0)d	89.95(73.9) c	96.27 (81.8)c	51.0 (43.7)d	73.7 (59.4)d
100 ppm	57.4 (49.4)d	72.4 (58.5)c	95.0 (78.9)b	97.5 (83.3)b	76.3 (60.9)c	84.9 (67.7)c
200 ppm	73.7 (59.3)c	77.5 (61.9)b	99.99(89.4) a	99.99 (89.3)a	84.9(67.4)b	96.3 (80.3)b
400 ppm	89.4 (78.7)b	99.9 (89.4)a	99.99(89.4)a	99.99 (89.3)a	99.9 (89.3)a	99.00 (89.3)a
500 ppm	94.9 (89.4)a	99.9 (89.4)a	99.9 (89.4)a	99.99 (89.3)a	99.99(89.3)a	99.90 (89.3)a
Temephos	99.99 (89.4)	99.99(89.4)	99.99(89.4)	99.99(89.4)	99.99 (89.4)	99.99 (89.4)
Control	0.012 (0.64)	0.013 (0.64)	0.0125 (0.64)	0.013 (0.64)	0.0125(0.64)	0.0125 (0.64)
SE±	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)
C.V(%)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Table 4.1b Effect of Higleeg root aqueous extract on the LC_{50} of three mosquito species larval phases.

Plant tested	Aqueous extracts		
	Species	LC_0 (CI)	Test of Significance
Higleeg	*Anopheles*	0.357(0.076 - 0.662)	^{2} = 1.745 df = 3, p = 0.671
	Culex	1.335(0.400 - 1.965)	^{2} = 8.051, df = 3, p = 0.0450
	Aedes	1.542 (0.00 - 0.00)	^{2} = 18.602, df = 3, p = 0.000

*CI=confidence interval with lower and upper bound

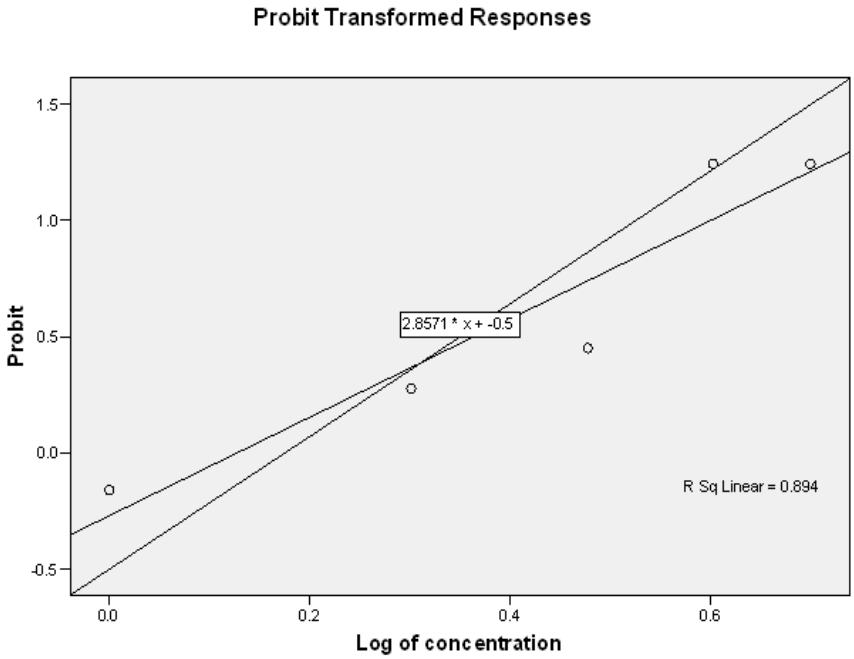

Fig. 4.1. Probit transformed responses of *Anopheles arabiensis* larvae against Higleeg aqueous root extract.

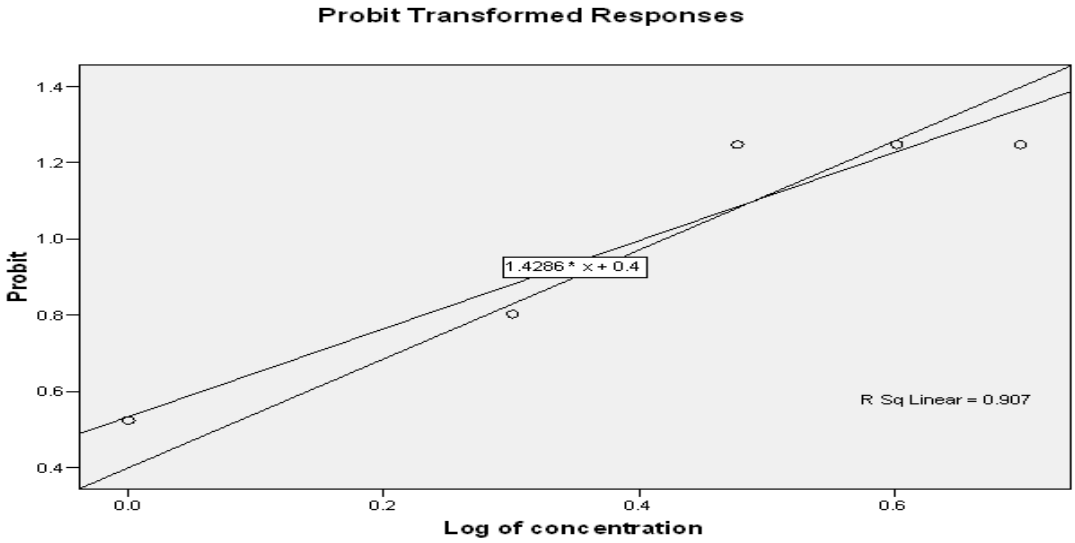

Fig. 4.2. Probit transformed responses of *Culex quinquefasciatus* larvae against Higleeg aqueous extract.

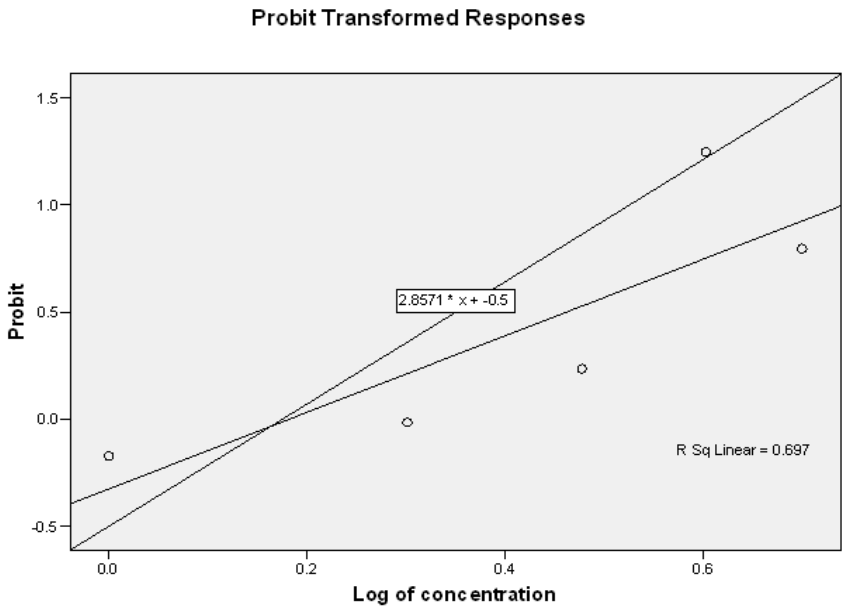

Fig.4.3. Probit transformed responses of *Aedes aegypti* larvae against Higleeg aqueous extract .

4.1.2. Larvicidal effects of Higleeg root methanolic extract on three mosquito species

The results of the effect of the root methanolic extract of Higleeg *B. aegyptiaca* on the larvae of the three mosquito species are presented in Table 4.2a as mortality percentage and 4.2b represent the LC_{50} **,** however**,** when using the root methanolic extract at (40 ppm) dosage rate, it achieved 96.2% mortality on the larvae of *Anopheles arabiensis* (p = 0.862), 77.4% of *Culex quinquefasciatus* (p = 0.709) and showed 51.2% mortality of *Aedes aegypti* (p = 0.058), respectively, after 24 hours. The highest concentration (500 ppm) of Higleeg root methanolic extract caused 99.9%, 99.9% and 94.9% larval mortality of the *Anopheles arabiensis*, the *Culex quinquefasciatus* and the *Aedes aegypti* mosquito species, which was as effective as that of the standard larvicide Temephos, that caused 100% of the larvae of the three mosquito species after 24 hours. Application of the probit analysis for the results of the bioassay of *Balanites* methanolic root extract, showed LC_{50} of 0.000313 ml /L, 0.000028 ml/L, and 0.001227 ml/L for the *Aedes* (Table 4.2b), and (Figure 4.4 *Aedes aegypti*), (Figure 4.5 *Anopheles*) and (Figure 4.6 *Culex*) larvae, respectively. This mean that the methanolic extract has high significant effect on the larvae of the *Anopheles arabiensis* (96.2%), while of moderate effects on *Culex quinquefasciatus* and *Aedes aegypti* larvae at a concentration of (40 ppm), 24 hours post exposure. Application of the Chi square test, showed no significant differences between the Culex and Aedes mosquito larvae at the concentration (40 ppm), after 24 hours Table 4.2a, and Table 4.2 b. The highest concentration (500 ppm), of Higleeg root extract caused 99.9% larval mortality of the three mosquito species. More over the activity of the root methanolic extract is gradually

increasing with increasing of the concentration and time period exposure on the larvae of the three mosquito species, respectively Table 4.2a.

Table 4.2a Effect of different concentrations of Higleeg methanolic extract on the mortality (%) of three mosquito species.

Concentrations ppm	Species					
	Aedes aegypti		*Anopheles arabiensis*		*Culex quinquefasciatus*	
	Mortality (%)					
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
00.00	00.00	00.00	00.00	00.00	00.00	00.00
40 ppm	5 1.2 (48.7)	91.3 (75.4)c	96.2 (79.89)c	96.2 (81.89)c	77.4 (61.8)d	97.5 (83.3)c
100 ppm	7 3.8 (59.3)d	94.9 (80.4)c	97.5 (83.3)b	97.5 (83.34)b	99.9(89.3)a	98.7 (86.3)b
200 ppm	84.9 (68.1)c	96.3 (81.9)b	99.9 (89.4)a	99.9 (89.36)a	99.9(80.3)c	99.9 (89.3)a
400 ppm	98.8 (86.3)b	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.36)a	99.9(86.3)b	99.9 (89.3)a
500 ppm	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.36)a	99.9(89.3)a	99.9(89.3)a
Temephos	99.99(89.4)	99.99(89.4)	99.99(89.4)	99.99(89.4)	99.99(89.4)	99.99(89.4)
Control	0.0125(0.64)	0 .0125(0.64)	0.0125(0.64)	0.0125(0.64)	0.0125(0.6	0.0125(0.64)
SE±	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)
Lsd (0.05)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent

Table 4.2b Effect of Higleeg solvents extract on the LC_{50} of three mosquito species larval stages .

Plant tested	Methanolic extract		
	Species	LC_{50} (CI)	Test of Significance
Higleeg	*Anopheles*	0.028 (0.00-0.260)	^{2} = 0.747, df = 3, p = 0.862
	Aedes	0.313 (0.053-0.313)	^{2} = 1.385, df = 3, p = 0.709
	Culex	1.227 (0.904-1.499)	^{2} = 7.464, df = 3, p = 0.058

*CI=confidence interval with lower and upper bound.

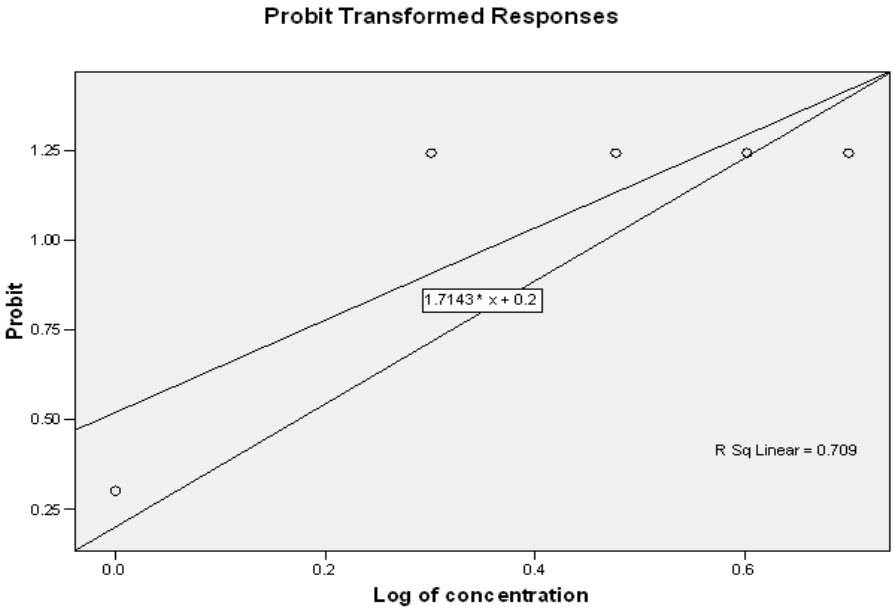

Fig.4.4. Probit transformed responses of *Aedes aegypti* larvae against Higleeg methanolic extract.

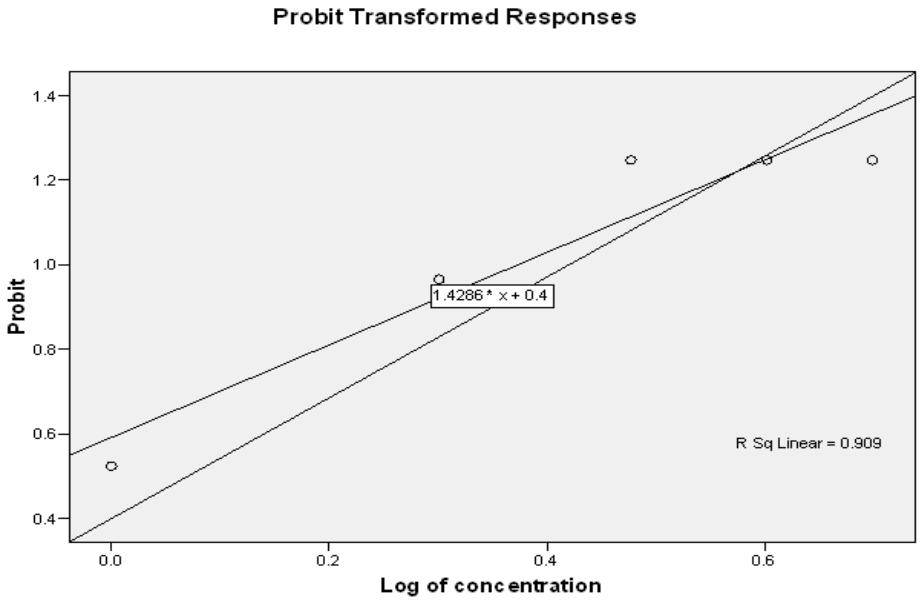

Fig. 4.5. Probit transformed responses of *Anopheles arabiensis larvae* against Higleeg Methanolic extract.

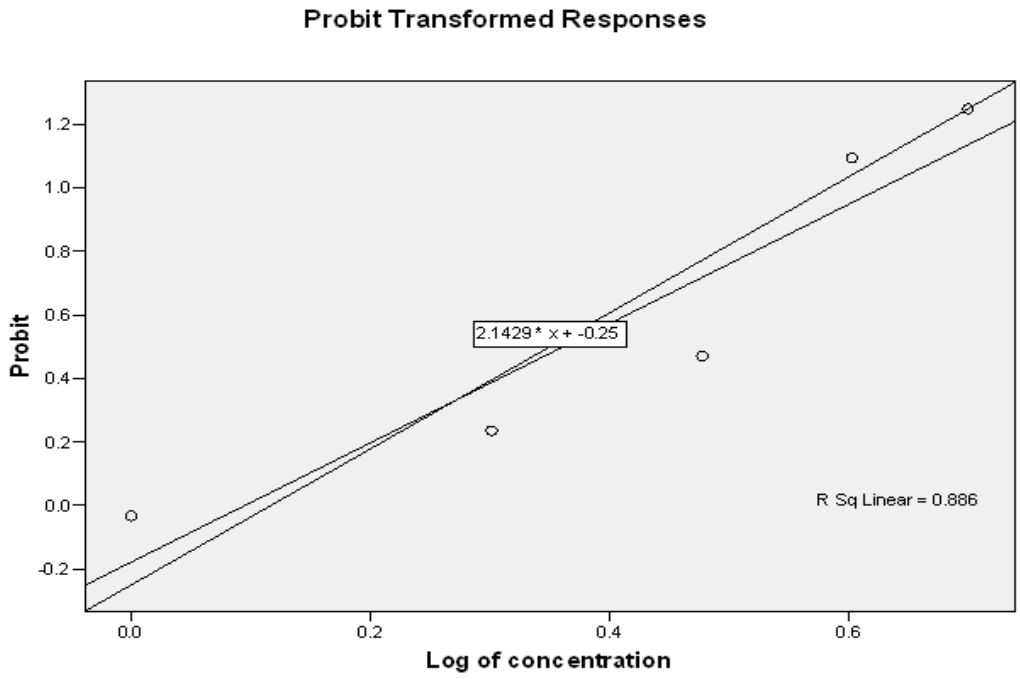

Fig. 4.6 Probit transformed responses of *Culex quinquefasciatus* larvae against Higleeg Methanolic extract .

4.2 Larvicidal effect of neem seeds (*Azadirachta indica*) extracts on three mosquito species

4.2.1 Larvicidal effects of neem seed aqueous extracts

The larvicidal activity of Neem seeds caused a significant increase in percentage mortality of the larvae of the three mosquito species ($p \leq 0.0125$), compared to the untreated control (Table 4.3a and 4.3b). The aqueous extract of Neem causd 40.75%, 62.25% and 72.25% larval mortality of *Aedes aegpti* ($p = 0.181$), and showed 64.75%, 72.75% and 84.25% death of the larvae of *Anopheles arab*iensis ($p = 0.443$), and caused 67.75%,74.5% and 85.25% larval mortality of *Culex quinquefasciatus* ($p = 0.775$), when used at 62.5 ppm, 125 ppm% and 250 ppm, respectively, after 24 hours. The highest concentration of the fruit aqueous extract (1000 ppm), caused 84.75 % larval death of *Aedes*. However, in the case of the two other mosquito species, it caused 97.4%, 98.75% larval mortality of *Anopheles* and *Culex* mosquito species, respectively, after 24 hours, which was approximately as that of the standard larvicide Temephos, which caused 100% mortality of the larvae of the three mosquito species after 24 hours. Application of the probit analysis for the results of the bioassay of neem seeds aqueous extract, showed LC_{50} of 0.008732 ml/L , LC_{50} of 0.003942 ml/L, and 0.008699 ml/L, for the *Aedes* Table 4.3b and (Figure 4.7 *Aedes aegypti*), (Figure 4.8 *Anopheles arabiensis*) and (Figure 4.9 *Culex quinquefasciatus*) larvae, respectively.

Table 4.3a Effect of Neem seed aqueous extract on three mosquito species larval stage.

Concentration (g/ml)	Species					
	Aedes aegypti		*Anopheles arabiensis*		*Culex*	
	Mortality (%)					
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
62.5 ppm	40.75(39.67)	51.75(46.0)	64.75(53.59)	66.5(54.49)	67.75(55.41)	65.75(54.19
125 ppm	62.25(52.10)d	63.25(52.71)d	72.75(56.1)d	72.75(58.55)d	74.5 (61.0)d	78.75(62.56
250 ppm	72.25(58.23)c	67.75(55.56)c	84.25(66.72)c	86.25(68.29)c	85.25(74.0)c	86.0(68.09)c
500 ppm	76.75(61.18)b	76.0(62.89)b	95.75(78.69)b	93.5(73.63)b	96.5(78.70)b	96.0(78.19)b
1000 ppm	84.75(67.19)a	88.0(69.77)a	97.4(85.10)a	97.75(84.04)a	98.75(84.34)a	99.0(84.94)a
Temephos	99.99(89.4)	99.99(89.4)	99.99(89.4)	99.99(89.4)	99.99(89.4)	99.99(89.4)
Control	0.0125(0.64)	0.0125(0.64)	0.0125(0.64)	0.0125(0.64)	0.0125(0.64)	0.0125(0.64)
SE ±	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)
Lsd (0.05)	4.556	4.556	4.556	4.556	4.556	4.556
C.V%	(6.4)	(6.4)	(6.4)	(6.4)	(6.4)	(6.4)

*Figures between brackets are arcsine transformation for percent.

Table 4.3b. Effect of neem seed aqueous extract on the LC_{50} of three mosquito species larval stage.

Plant tested	Aqueous extracts		
	Species	LC_{50} (CI)	Test of Significance
Neem	*Anopheles*	3.942(3.409-4.787)	^{2} = 2.648 df =3, p = 0.443
	Aedes	8.732(6.050-19.591)	^{2} = 4.871 df = 3, p = 0.181
	Culex	8.699(5.801-23.050)	^{2} = 1.109 df = 3, p = 0.775

*CI=confidence interval with lower and upper bound

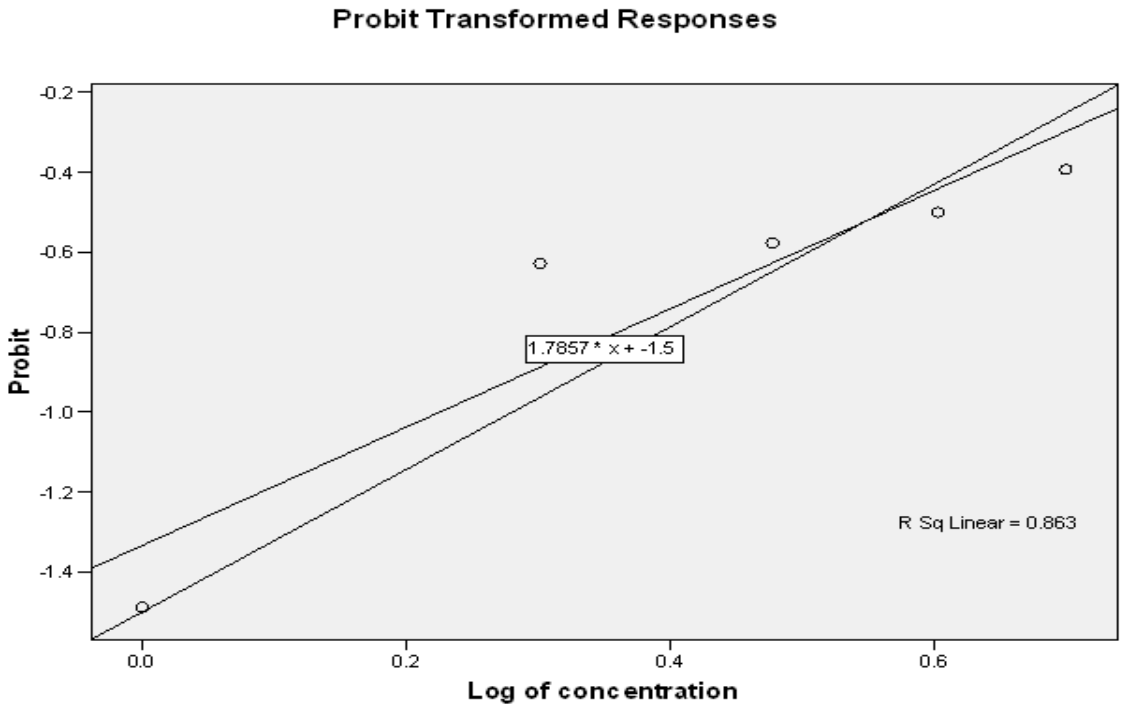

Fig. 4.7 Probit transformed responses of *Aedes aegypti* larvae against Neem seed Aqueous extract.

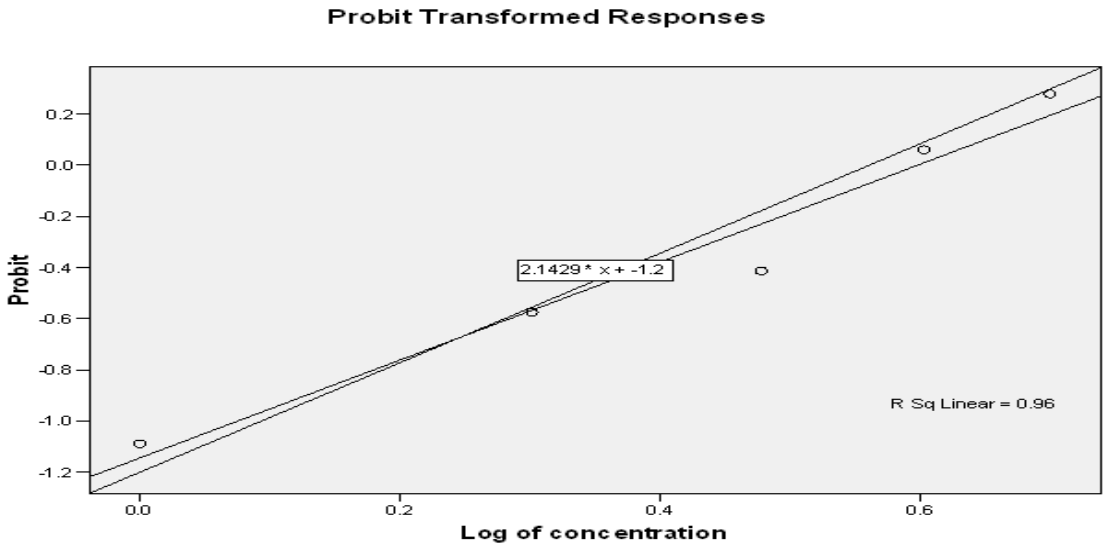

Fig. 4.8 Probit transformed responses of *Anopheles arabiensis larvae* against neem seed aqueous extract.

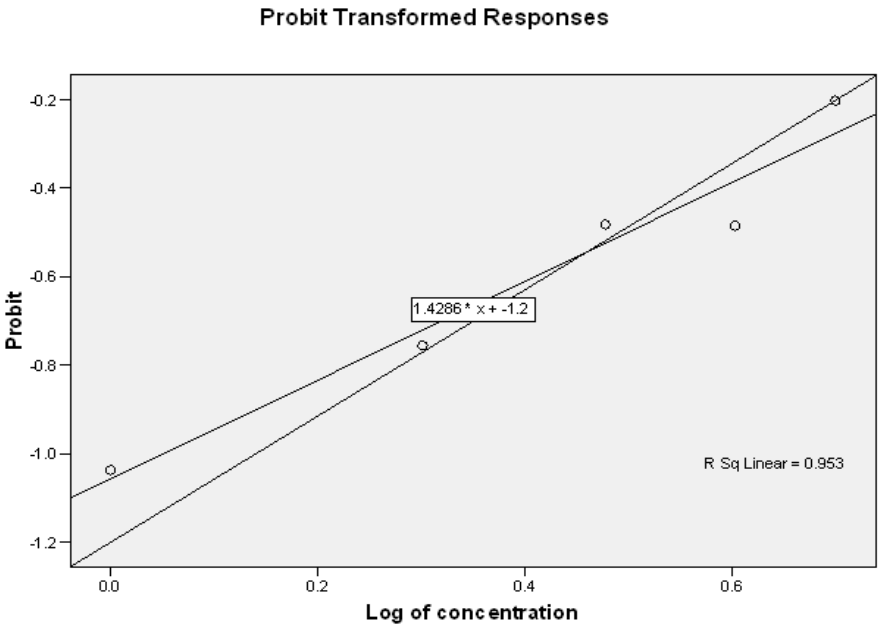

Fig. 4.9 Probit transformed responses of *Culex quinquefasciatus* larvae against neem seed aqueous extract .

4.2.2 Effects of neem seed Hexane extract on the larvae of three mosquito species.

Treatment of the Larvae of the three mosquito species with hexane extract at the testing concentrations of 50 ppm, 100 ppm and 500 ppm, shown in Table 4.4a and 4.4b caused a significant increase in mortality percentage of the larvae of the mosquito species($p \leq 0.0125$), compared to the untreated control Table 4.4. The hexane extract at the concentrations (50, 100 and 500 ppm), showed 34.25% , 58.0% and 72.0% larval death of *Aedes aegypti* ($p = 0.640$) , and caused 72.5%, 85% and 91.0% mortality of the larvae of *Anopheles arabiensis* ($p = 0.154$), and effected 71.0%, 88.0 % and 96% mortality on the larvae of *Culex quinquefasciatus* ($p = 0.620$), respectively, after 24 hours. The highest concentration (1000 ppm) of neem seed hexane extract caused 87.5% larval mortality of *Aedes aegypti*, 99.8% of the *Anopheles* and 100% of the *Culex* mosquito species, respectively, which in the case of the *Anopheles arabiensis* and *Culex quinquefasciatus* was approximately as that of the standard larvicide Temephos, that caused 100% mortality of the larvae of the three mosquito species after 24 hours. Application of the probit analysis for the results of the bioassay of neem seed hexane extract, showed LC_{50} of 0.004932 , LC_{50} of 0.002641, $_0$ and LC_{50} of 0.003323 ml/L , for the *Aedes* (Table 4.4b) and (Figure 4.10), the *Anopheles* (Figure 4.11) and the *Culex* (Figure 4.12) larvae, respectively .

Table 4.4a Effect of Neem seed hexane extract on three mosquito species larval instar.

concentrations	Species					
	Aedes larvae		*Anopheles*		*Culex*	
	Mortality (%)					
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
50 ppm	34.25 (35.81)	41.5 (40.10)	72.5 (58.38)	83.5(66.09)	71.0(58.55)	87 (65.16)
100 ppm	58.0 (49.61)d	56.75(48.88)d	85.0(67.22)d	88.25(70.05)d	86.3 (68.26)d	89 (68.48)d
500 ppm	72.0 (58.06)c	73.25(58.87)c	91.0(72.61)c	96.0(78.87)c	87.25(69.11)c	98 (76.93)c
750 ppm	83.25(65.24)b	77.0 (60.40)b	98.5(83.07)b	98.75(85.70)b	95.25(77.46)b	100 (83.88)b
1000 ppm	87.5 (69.86)a	89 (68.48)a	99.8(88.57)a	99.8(90.00) a	98.25(83.60)a	100 (87.66)a
Temephos	99.99(89.4)	99.99(89.4)	99.99(89.4	99.99(89.4)	99.99(89.4)	99.99(89.4
Control	0.0125(0.64	0.0125(0.64	0.0125(0.64)	0.0125(0.64	0.0125(0.64	0.0125(0.64
SE ±	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)
Lsd (0.05)	4.556	4.556	4.556	4.556	4.556	4.556
C.V%	(6.4)	(6.4)	(6.4)	(6.4)	(6.4)	(6.4)

*Figures between brackets are arcsine transformation for percent.

Table 4.4b Effect of Neem seed hexane extract on the LC_{50} of three mosquito species larval stage.

Plant tested	Hexane extract		
	Species	LD_{50} (CI)	Test of Significance
Neem	*Aedes*	4.932 (3.892-7.512)	^{2} = 1.139, df = 3, p = 0.768
	Anopheles	2.641(1.555-4.200)	^{2} = 14.361, df = 3, p = 0.002
	Culex	3.323 (0.00-0.00)	^{2} = 15.858, df = 3, p = 0.001

**CI=confidence interval with lower and upper bound.*

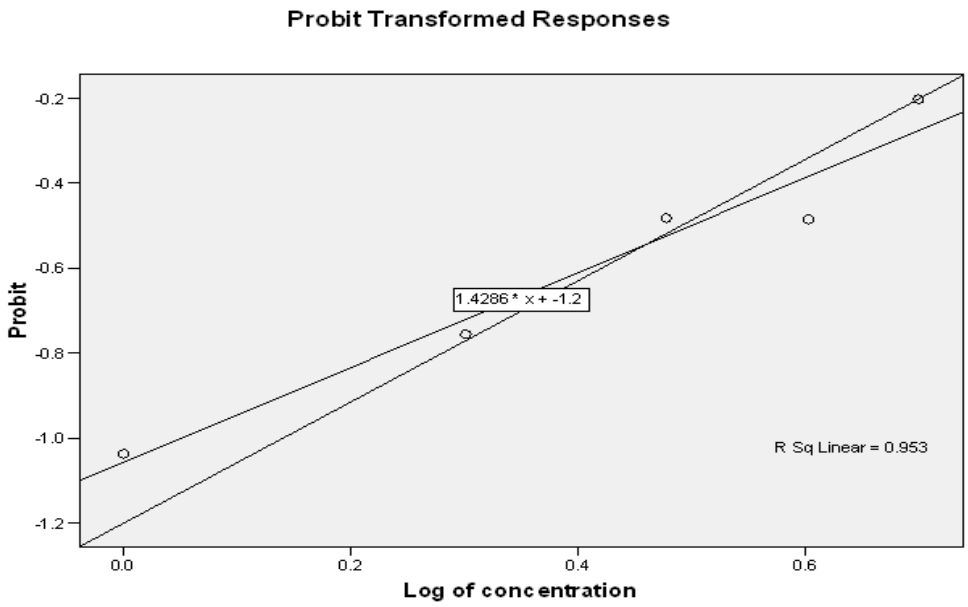

Fig. 4.10. Probit transformed responses of *Aedes aegypti* larvae against Neem seeds Hexane extract.

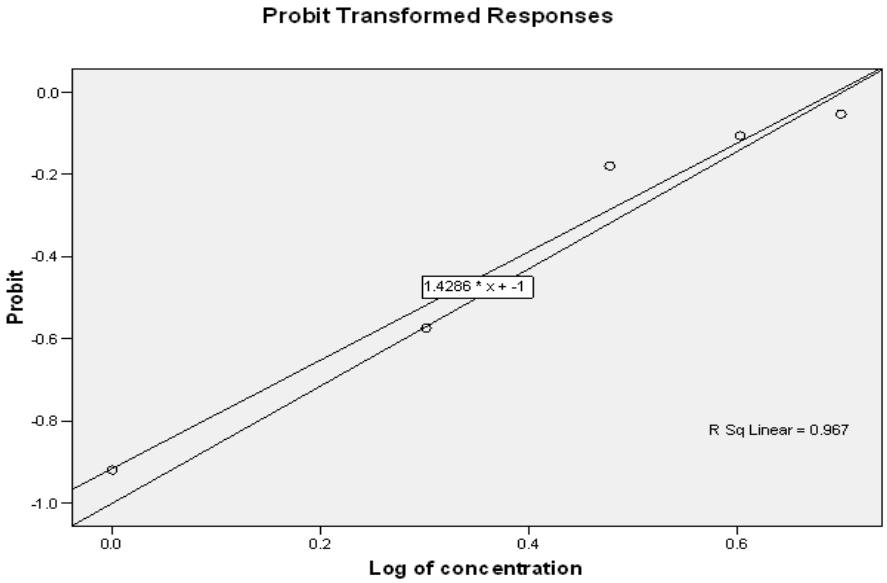

Fig. 4.11. Probit transformed responses of *Anopheles arabiensis larvae* against Neem seed hexane extract.

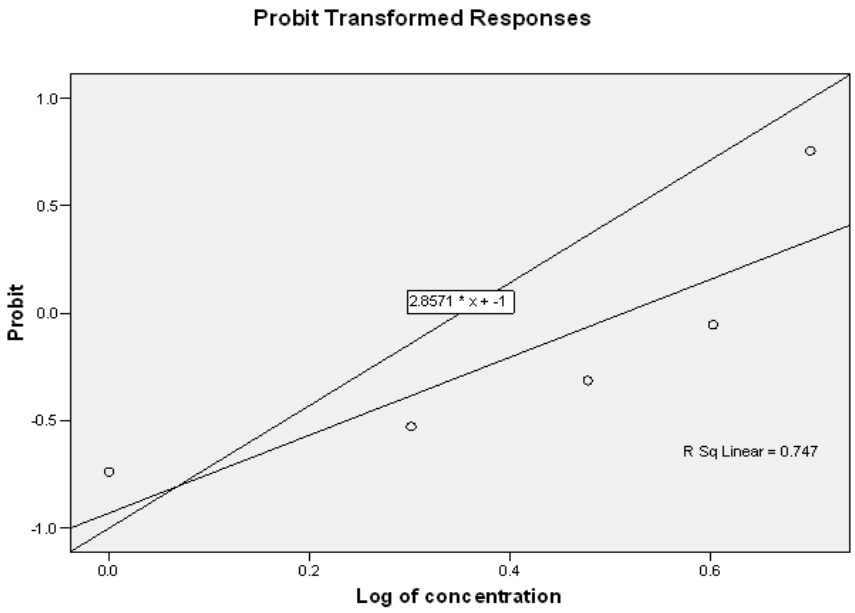

Fig. 4.12. Probit transformed responses of *Culex quinquefasciatus* larvae against Neem Hexane extract.

4.3. Larvicidal effects of *Solenostemma argel* (Hargal) shoots extract on the three mosquito species.

4.3.1. Effect of shoots aqueous extract of Hargal on the three mosquito species.

Aqueous leaves extracts of Hargal caused a significant increase in mortality percentage of the larvae of the three mosquito species ($p \leq 0.0125$), compared to the untreated control Table 4.5a It caused 25.05%, 58.7% and 73.8% larval mortality of *Aedes aegypti* ($p = 0.185$), and effected 10.2%, 41.2% and 68.8% mortality on the larvae of the *Anophels* ($p = 0.650$) , and caused 22.5% , 42.4% and 56.2% larval mortality of the *Culex* ($p = 0.326$) mosquito species , when used at the concentrations of 62.5 ppm, 125 ppm % and 250 ppm, respectively, after 24 hours. The highest concentration (750 ppm) of Hargal shoot aqueous extract, caused 98.7% larval mortality of the *Aedes aegypti*, 91.1% the *Anopheles arabiensis* and 93.8% the *Culex quinquefasciatus* mosquito species. Application of the probit analysis for the result of the bioassay of Hargal aqueous extract showed LC_{50} of 0.008748 ml / L , for the *Aedes aegypti* Table 4.5b and (Fig. 4.13), LC_{50} of 0.002706 ml/L, (Fig. 4.14) for the *Anopheles arabiensis* , LC_{50} of 0.002568 ml/L and (Fig. 4.15) for the *Culex quinquefasciatus* larvae, respectively. It is observed that Hargal leaves aqueous extract caused over 90% mortality of the larvae of the three mosquito species, and caused (98.7%) reduction in mortality percentage of *Aedes aegypti* larvae, but when applying the Chi square tests, there is no significant differences in mortality of the larvae of the three mosquito species.

Table 4.5a Effect of Argel shoots aqueous extract on three mosquito species larval stage.

Concentration (ppm)	Species					
	Aedes aegypti		*Anopheles arabiensis*		*Culex quinquefasciatus*	
	Mortality (%)					
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
00.00	00.00	00.00	00.00	00.00	00.00	00.00
62.5 ppm	25.05 (29.98)d	54.9 (47.8)d	10.2 (9.0)	36.2 (36.5)	22.5(22.0)d	49.9(45.0)
125 ppm	58.7 (50.09)c	67.6 (55.4)c	41.2 (39.9)d	53.8 (47.2)d	42.4 (40.6)c	58.8(50.1)d
250 ppm	73.8 (59.2)b	82.4 (65.2)b	68.8 (56.2)c	71.3 (57.7)c	56.2 (48.6)c	72.5(58.5)c
500 ppm	97.6 (86.3)a	99.9 (89.4)a	75.0 (67.8)b	95.0 (78.9)b	82.5 (65.5)b	87.5(72.0)b
750 ppm	98.7 (85.4)a	99.9 (89.4)a	91.1 (75.1)a	99.9 (89.4)a	93.8 (77.4)a	99.9(89.4)a
Control	0.0125 (0.64)	0.0125 (0.64)	0.013 (0.64)	0.0125 (0.64)	0.013 (0.64)	0.0125 (0.64
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)	99.9 (89.4)	99.99 (89.4)	99.9 (89.4)
SE±	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent .

Table 4.5b Effect of Argel shoots aqueous extract on the LC_{50} of three mosquito species larval instar .

Plant tested	Species	LC_{50} (ml/l)	95% CI (ml/l)		Test of Significance
		Aqueous extracts	lower	Upper	
Argel	*Anopheles*	2.706	2.446	2.987	^{2}=1.642, df=3,p=0.650
	Aedes	8.748	6.058	19.657	^{2}=4.821, df=3,p=0.185
	Culex	2.568	2.247	2.923	^{2}=3.456, df=3,p=0.326

*CI=confidence interval with lower and upper bound .

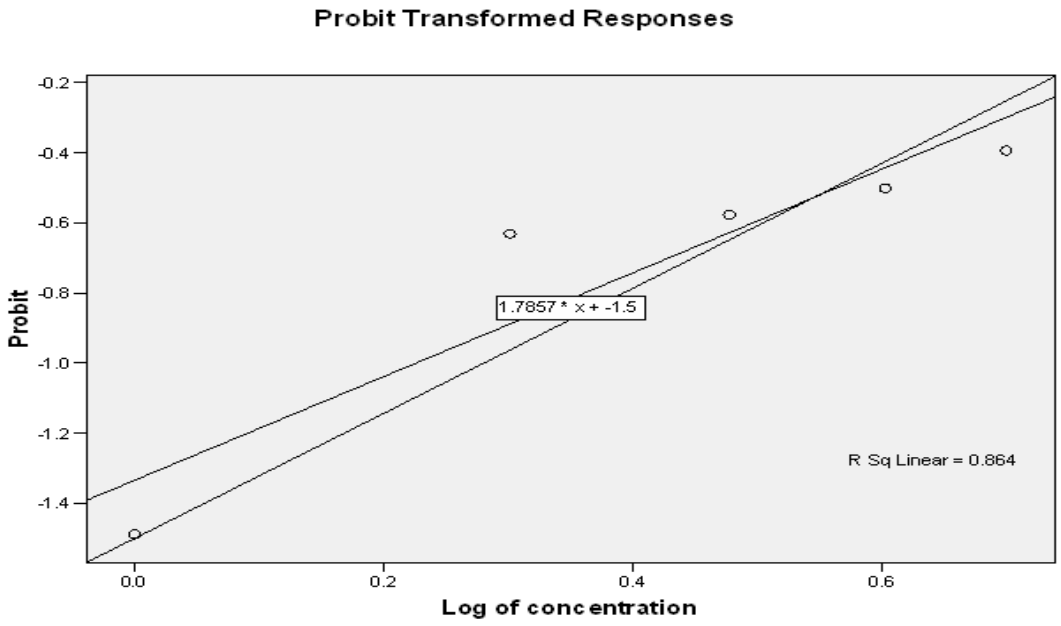

Fig. 4.13 Probit transformed responses of *Aedes aegypti* larvae against Hargal shoots water extract.

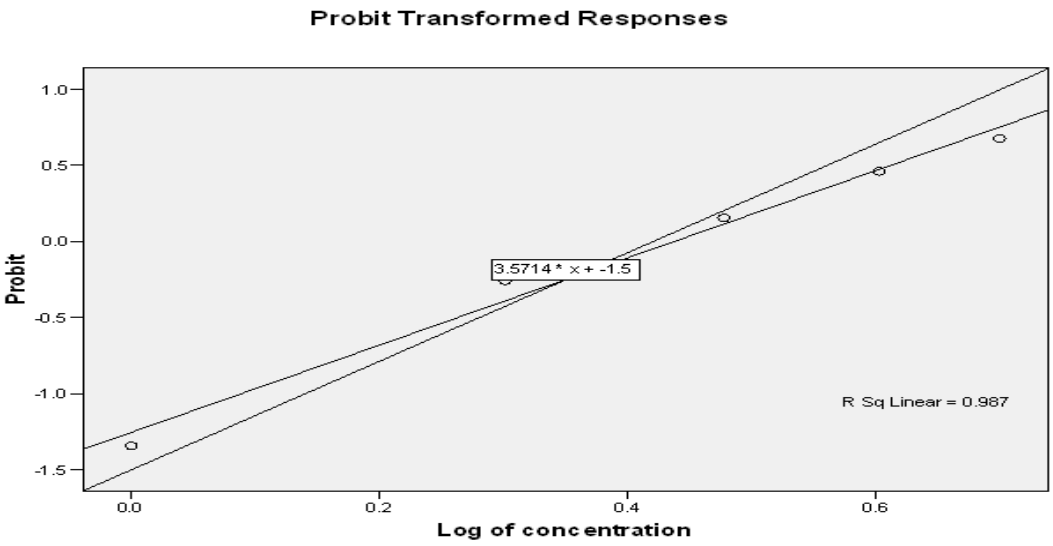

Fig. 4.14 Probit transformed responses of *Anopheles arabiensis* larvae against Hargal shoots water extracts .

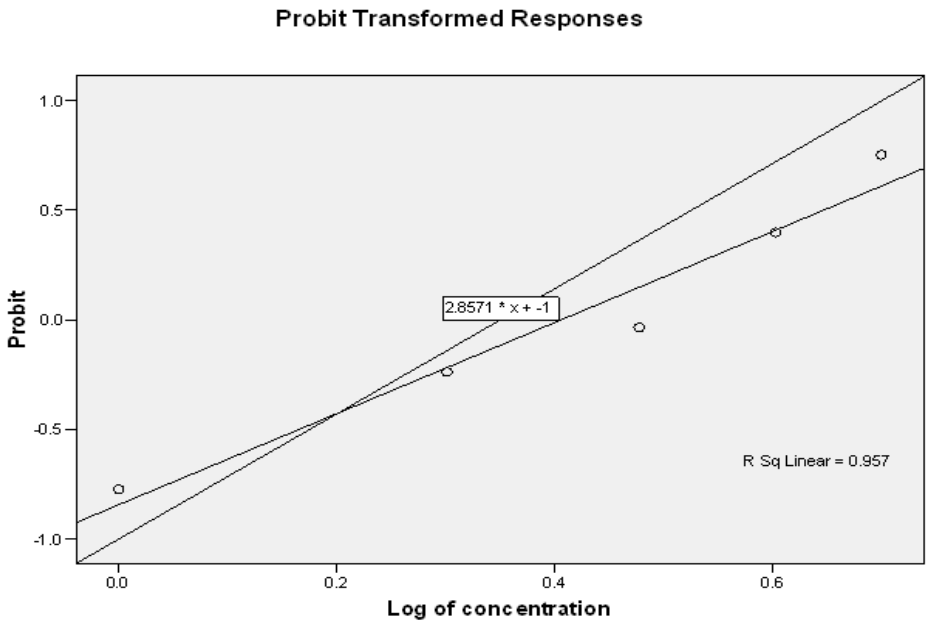

Fig. 4.15 Probit transformed responses of *Culex quinquefasciatus* larvae against Hargal shoots water extract.

4.3.2. Effect of methanol extract of Hrgal shoots on the larvae of the 3 mosquito species.

The result of the effect of methanolic Hargal leaves extract when used at the same dosage rates on the larvae of the three mosquito species, is shown in (Table 4.6a), in which 18.8%, 41.2% and 90.0% achieved as larval mortality of the *Aedes*(p = 0.012), 12.7% , 37.5% and 87.6% larval mortality of the *Anopheles* (p = 0.004), 35.04%, 57.6% and 96.4% mortality of the *Culex* (p = 0.030), respectively, when applied at 62.5 ppm 125 ppm and 250 ppm after 24 hours. The highest concentration (750ppm) of the leaves methanolic extract, showed 99.9% mortality of the three mosquito larvae after 24 hours, however, treating by Hargal shoot methanolic extract at (500 ppm) and most upper concentrations, caused 99.9% larval mortality percentages of the three mosquito species comparable to that of the standard larvicide Temephos, after 24 hours. It is obeviously observed that, the activity of the extract is very slow on the larvae and increasing gradually with increase of concentrations and time span of exposure. The highest concentration (750 ppm), showed 99.9% larval mortality of the three mosquito species which as that of the standard larvicide Abate. Application of the probit analysis for the results of the bioassay of the methanolic extract of the leaves of *Solenostemma argel*, showed LC_{50} of 0.001906 ml/L (Table 4.6b) and Fig.(4.16), for the *Aedes* , LC_{50} of 0.001463 ml/L, Fig.(4.17) for the *Anopheles* and LC_{50} of 0.001479 ml/L and (Figure 4.18) for the *Culex* larvae, respectively.

Table 4.6a Effect of Hargal shoots methanolic extract on the larvae of the three mosquito species .

Concentration (ppm)	*Species*					
	Aedes aegypti		*Anopheles arabiensis*		*Culex quinquefasciatus*	
	Mortality (%)					
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
00.00	00.00	00.00	00.00	00.00	00.00	00.00
62.5 ppm	18.8(25.6)d	42.6 (40.7)d	12.7(12.0)	47.5(43.5)d	35.0 (39.2)	70.0 (56.9)c
125 ppm	41.2(40.0)c	62.5 (52.3)c	37.5 (37.7)d	60.1 (50.8)c	57.6(49.35)d	60.1(50.9)d
250 ppm	90.0(72.3)b	95.1 (78.9)bc	87.6(70.5)c	84.9 (67.5)b	96.4(80.33)c	96.2 (81.9)b
500 ppm	99.9(89.4)a	97.6 (83.3)b	97.5 (83.3)b	99.9 (89.4)a	98.8 (86.4)b	99.9 (89.4)a
750 ppm	99.9(89.4)a	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.4)a
Temephos	99.99(89.4)	99.99(89.4)	99.9(89.4)	99.99(89.4)	99.99 (89.4)	99.99 (89.4)
control	0.0125(0.64)	0.0125(0.64	0.012(0.64	0.0125(0.64	0.0125(0.64	0.0125 (0.64
SE±	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent

Table 4.6b. LC_{50} of Argel shoots methanolic extract on the larvae of the three mosquito species

Plant tested	Species	LC_{50} (CI)	95% CI (ml/l)		Test of Significance
		Methanol extract	lower	Upper	
Argel	*Anopheles*	1.463	0.168	2.302	= 13.481, df = 3, p = 0.004
	Aedes	1.906	1.160	2.560	= 10.903, df = 3, p = 0.012
	Culex	1.479	0.651	2.108	= 8.926, df = 3,p = 0.030

*CI=confidence interval with lower and upper bound.

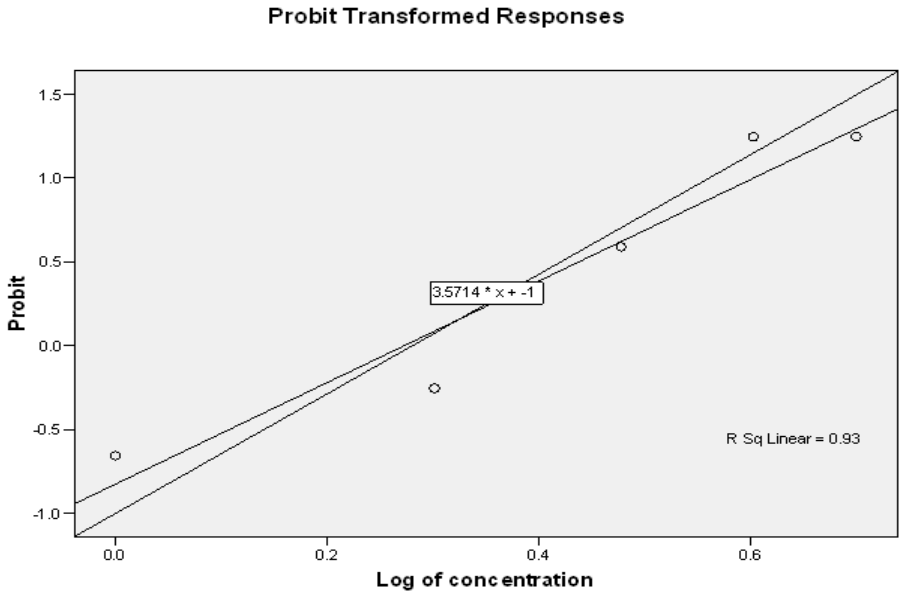

Fig. 4.16 Probit transformed responses of *Aedes aegypti* larvae against argel shoots methanol extract.

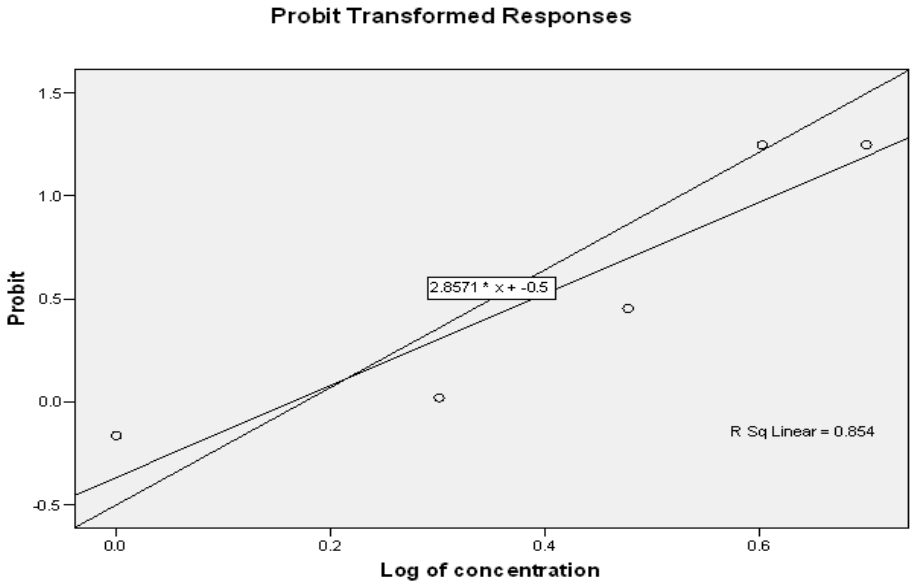

Fig. 4.17 Probit transformed responses of *Anopheles arabiensis* larvae against Hargal shoots methanol extract.

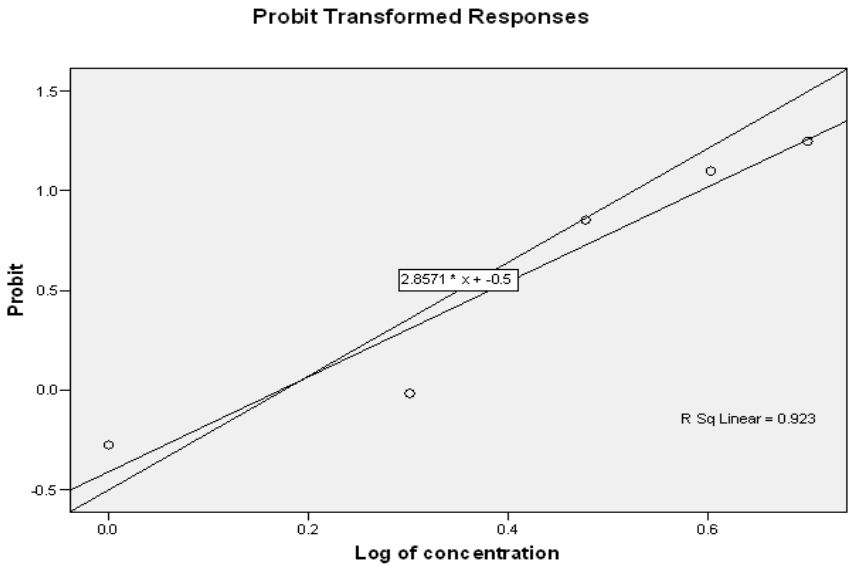

Fig. 4.18 Probit transformed responses of *Culex quinquefasciatus* larvae against argel shoots methanol extract.

4.4. Larvicidal effect of *Eclipta prostrata* leaves extract on the larvae of the three mosquito species.

4.4.1. Effect of aqueous extract of *E. prostrata* leaves on the larvae of the three mosquito species.

Results of the effect of water leaves extracts of *Eclipta prostrata* on the larvae of the three mosquito species are presented in (Table 4.7a), which showed a significant increase in mortality percentage of the larvae of the three mosquito species ($p \leq 0.0125$), compared to the untreated control . It resulted in 11.3%, 27.5% and 40.0% larval mortality of the *Aedes aegypti* ($p = 0.671$), and 10.0%, 23.8% and 30.1% of *Anopheles arabiensis* ($p = 0.405$), and 7.5%, 19.9% and 32.5% of *Culex quinquefasciatus* ($p = 0.393$), when treated with aqueous extract at concentrations of 500 ppm, 1000 ppm and 1500 ppm, respectively, after 24 hours. The highest concentration (2500 ppm), caused over 75% larval mortality of the three mosquito species, after 24 hours, less than that of the standard larvicide Temephos, which caused 99.9% mortality of the mosquito species after 24 hours. It is observed that the activity of the aqueous extract of the leaves is weak at the lower three concentrations, with slow increase in activity with increasing time of exposure. Application of the probit analysis for the results of the bioassay of the aqueous extract, showed LC_{50} of 0.003862 ml / L (Table 4.7b) and (Figure 4.19) for the *Aedes aegypti* , LC_{50} of 0.0041510 ml/L (Figure 4.20) for the *Anopheles arabiensis* and LC_{50} of 0.004343 ml/L and (Figure 4.21) for the *Culex quinquefasciatus* larvae, respectively.

Table 4.7a. Effect of *Eclipta prostrata* aqueous extract on the larvae of the three mosquito species

Concentratin (ppm)	Species					
	Aedes aegypti		*Anopheles arabiensis*		*Culex quinquefasciatus*	
	Mortality (%)					
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
00.00	00.00	00.00	00.00	00.00	00.00	00.00
500 ppm	11.3(19.5)	9.99(18.42)	10.0(12.5)	10.0(18.15)	7.5(13.8)	12.47(20.1)
1000 ppm	27.5(31.5)d	30.0 (33.20)d	23.8(29.1)d	28.8(32.42d	19.9(26.3)d	28.75 (32.3)d
1500 ppm	40.0(39.2)c	44.99(42.09)c	30.1(33.06)c	46.3(42.85c	32.5(34.4)c	48.8 (44.4)c
2000 ppm	58.8(50.1)b	84.90(67.27)b	53.8(47.16)b	78.8(62.66b	47.4(43.5)b	77.5 (62.2)a
2500 ppm	76.3(60.92a	86.13(68.21)a	76.3(60.92)a	87.4(69.31a	75.0(60.6)a	68.8 (55.6)b
Temephos	99.99(89.4	99.99 (89.4)	99.99(89.4)	99.99(89.4)	99.99(89.4	99.99(89.4)
control	0.0125(0.64	0.0125(0.64)	0.0125(0.64)	0.0125(0.64	0.0125(0.64)	0.0125(0.64)
SE±	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent

Table 4.7b LC_{50} of *E. prostrata* leaves aqueous extract on the larvae of the three mosquito species.

Plant tested	Species	LC_{50} (CI)	95% CI (ml/l)		Test of Significance	Upper
		Water extracts	Lower	Upper		
Tamr Al-ghanam	*Anoph..sarabiensis*	4.1510 (-)	3.566	5.122	^{2}=2.917, df=3,p=0.405	1.543
	Aedes aegypti	3.862	3.246	4.935	^{2}=1.550, df=3,p=0.671	3.092
	Culex quinquefasciatus	4.343	3.688	5.495	^{2}=2.991 df=3,p=0.393	1.604

*CI=confidence interval with lower and upper bound

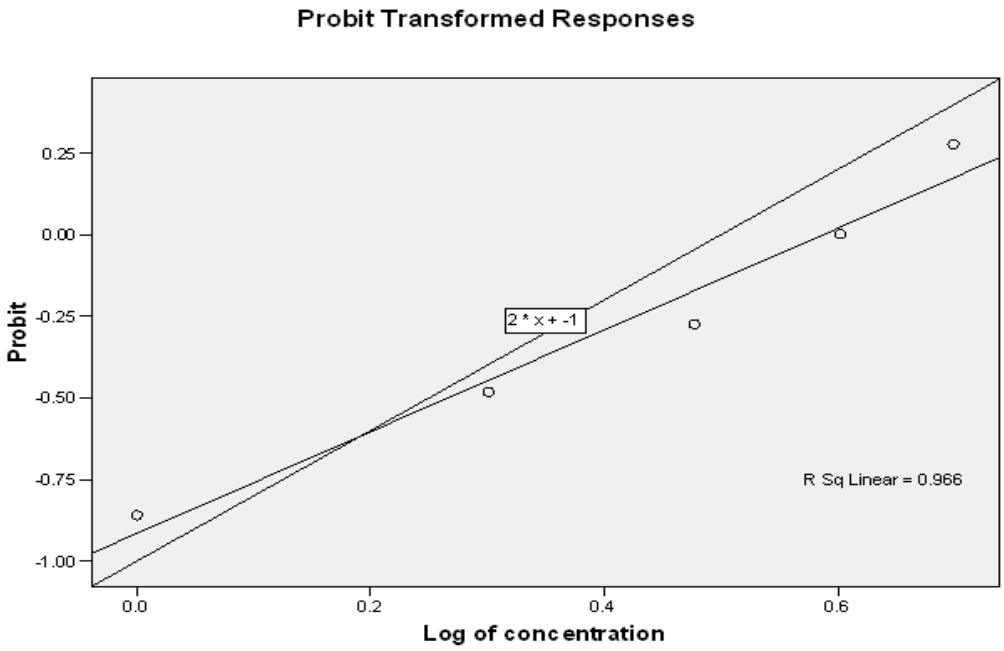

Fig. (19) : Probit transformed responses of *Aedes aegypti* larvae against *Eclipta prostrata* leaves water extracts .

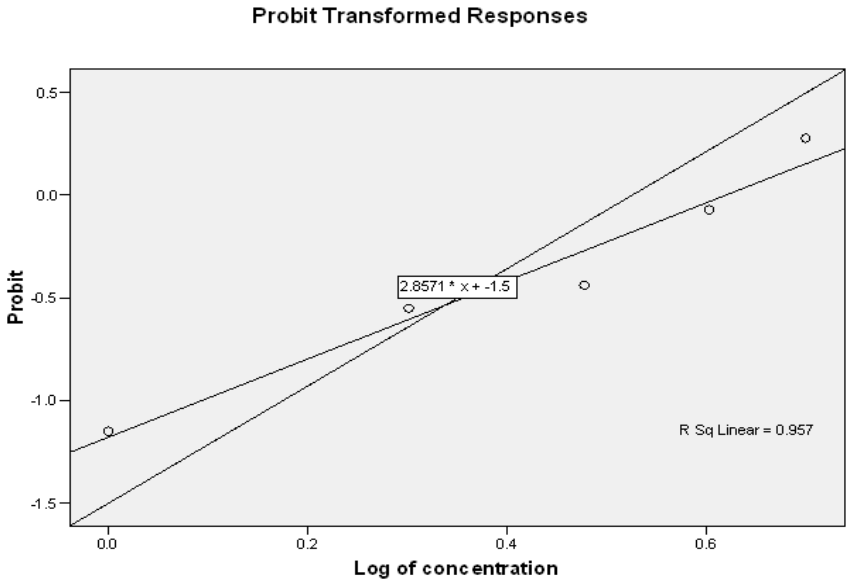

Fig. (20) : Probit transformed responses of *Anopheles arabiensis* larvae against *Eclipta prostrata* leaves water extract.

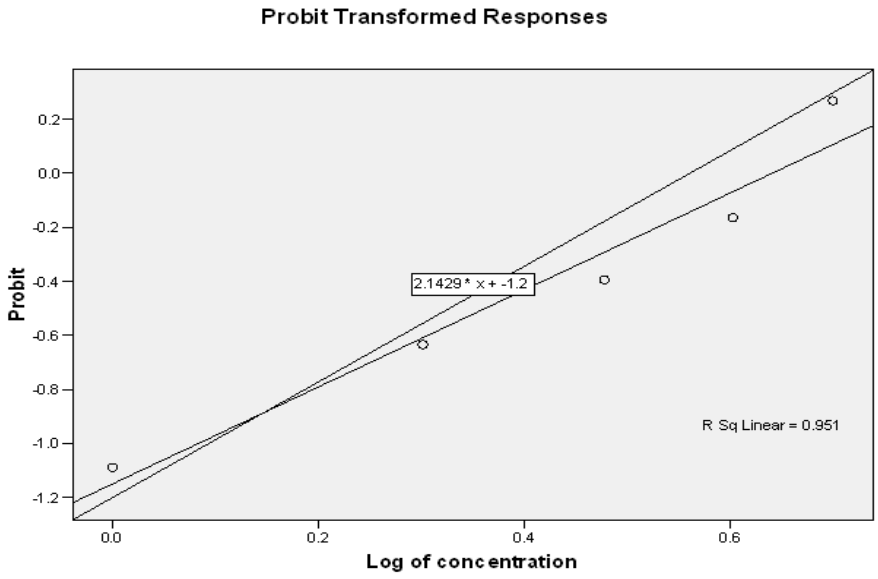

Fig. (21) : Probit transformed responses of *Culex quinquefasciatus* larvae against *Eclipta prostrata* leaves water extract .

4.4.2 Effect of methanol extract of the leaves of *E. prostrata* on the larvae of the 3 mosquito species.

Larvicidal effect of methanol extract of the leaves of *Eclipta prostrata* is presented in (Table 4. 8) , showed 48.7% , 52.5% and 63.8% larval mortality of the *Aedes*(p = 0.943) , 52.6% , 74,9% and 89.9% of the *Anopheles*(p = 0.282) , 41.3% , 60.4% and 76.3% of the *Culex* (p = 0.351) , when used at the same three lower concentrations (500 , 1000 and 1500 ppm) on the larvae of the three mosquito species , respectively , after 24 hours . The highest concentration (2500 ppm) , showed 78.8% larval death of the *Aedes* mosquito , and caused 99.9% mortality of the *Anopheles* and *Culex* larvae which was approximately as that of the standard larvicide Temephos , which caused 99.9% larval mortality of the mosquito species after 24 hours . Application of the probit analysis for the results of the bioassay of the methanolic extract of *Eclipta prostrata* , showed LC_{50} of 0.002241ml /L (Table 4.8a) , and (Figure 22) for the *Aedes aegypti* , LC_{50} of 0.001251 ml /L (Table 8a) and (Figure 23) for the *Anopheles arabiensis* and LC_{50} of 0.001345 ml /L for *Culex quinquefasciatus* larvae , respectively . From the result of the methanolic leaves extract of the *Eclipta prostrata* , it is noticed that the mosquito larvae were more affected even at the lower concentrations and mortality increased with increase of concentration and time of exposure , moreover , *Anopheles arabi*ensis and *Culex quinquefasciatus* were observed to be more susceptible than the *Aedes aegypti* , so, it can be concluded that leaves methanolic extract of *Eclipta prostrata* is more toxic to mosquito larvae than the shoot water extract.

Table 4.8a Mortality of the larvae of *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* at different concentrations of *Eclipta prostrata* methanolic extract during 24 and 48 hours periods

concentratin (%)	Species					
	Aedes aegypti		*Anopheles arabiensis*		*Culex quinquefasciatus*	
	Mortality (%)					
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
00.00	00.00	00.00	00.00	00.00	00.00	00.00
500 ppm	48.7(44.3)	91.29(73.3)c	52.6 (46.5)	74.9 (60.8)	41.3(43.9)d	78.7 (69.3)b
1000 ppm	52.5(46.4)d	93.80(77.8)b	74.9 (60.1)d	88.8 (70.9)d	60.4 (58.7)c	84.9(50.8)d
1500 ppm	63.8(53.3)c	91.26(73.0)c	89.9 (71.9)c	96.2 (81.9)c	76.3 (70.5)b	91.3 (67.5)c
2000 ppm	71.1(58.0)b	97.58(83.3)a	92.5 (76.3)b	98.8 (86.3)b	96.5 (83.3)a	99.9 (89.4)a
2500 ppm	78.8 (63.0)a	98.70(85.7)a	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.4)a
Temephos	99.99(89.4)	99.99 (89.4	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
control	0.0125(0.64)	0.0125(0.64	0.012 (0.64)	0.012 (0.64)	0.0125(0.64)	0.0125(0.64)
SE±	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent

Table (4.8 b) : LC_{50} of *Anopheles arabiensis*, *Culex quinquefasciatus* and *Aedes aegypti* larvae exposed to ECL Methanol extract .

Plant tested	Species	Test of Significance	LC_{50}(CI)	95% CI (ml/l)		Test of Significance
			Methanolic extracts	**Lower**	**Upper**	
Tamr Al-ghanam	*Anopheles*	^{2} = 2.917, df = 3, p = 0.405	1.251	0.901	1.543	^{2}=3.819, df=3,p=0.282
	Aedes	^{2} = 1.550, df = 3, p = 0.671	2.241	1.431	3.092	^{2}=0.387, df=3,p=0.943
	Culex	^{2}=2.991 df=3,p=0.393	1.345	1.042	1.604	^{2}=3.276 df=3,p=0.351

*CI=confidence interval with lower and upper bound.

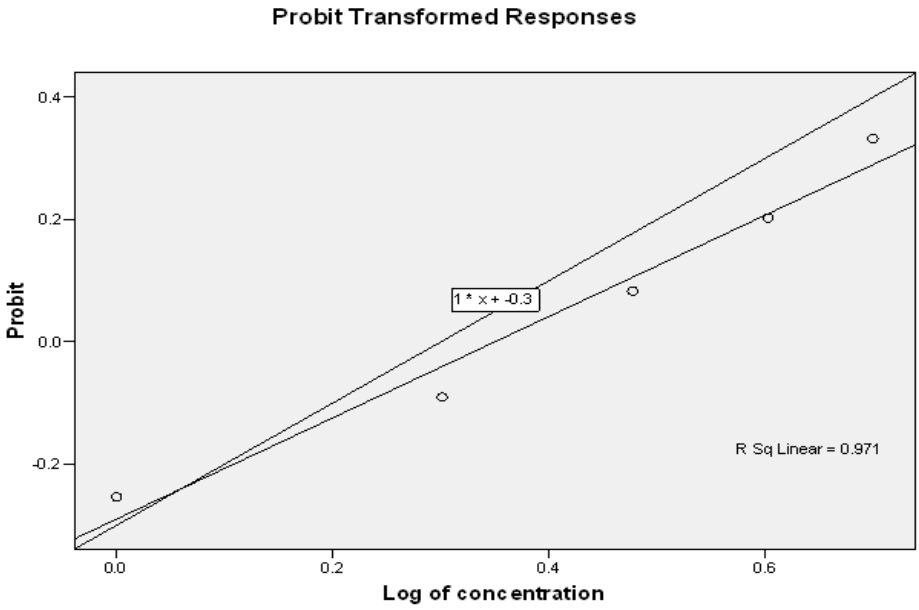

Fig. (22) : Probit transformed responses of *Aedes aegypti* larvae against *Eclipta prostrata* Methanol leaves extract

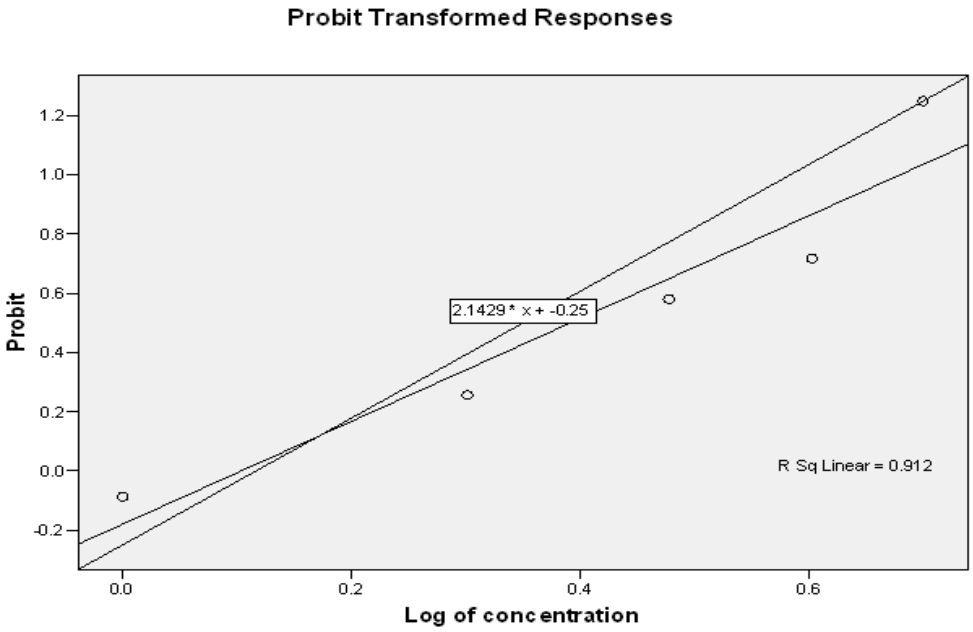

Fig. (23) : Probit transformed responses of *Anopheles arabiensis* larvae against *Eclipta prostrata* Methanol leaves extract

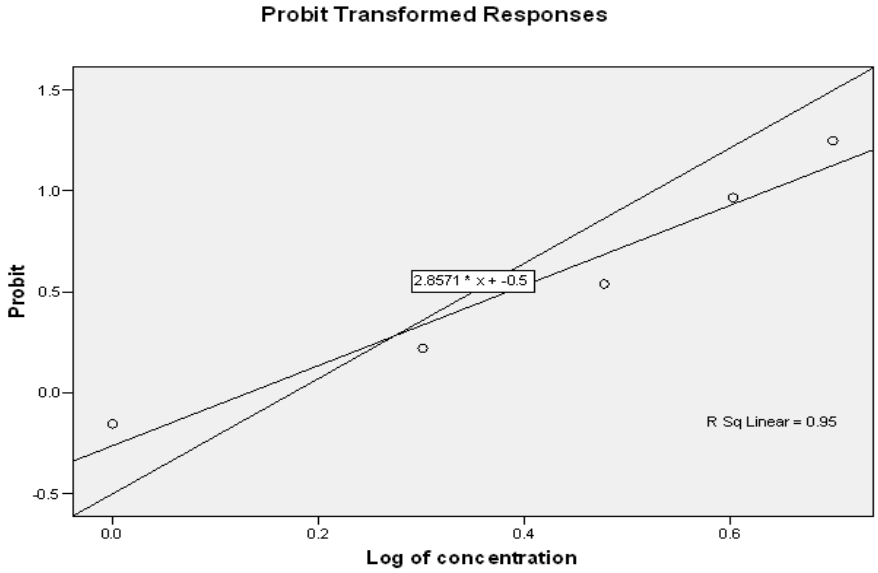

Fig. (24) : Probit transformed responses of *Culex quinquefasciatus* larvae against *Eclipta prostrata* Methanol leaves extract

Part 11. Trials of the screening the tested plant materials:

Results and Discussion

4.5 Phytochemical constituents of plant extracts

Higher plants constitute different types of secondary active metabolites which have profound effects on living organisms.Various authors stated their role in combating pest and disease vectors of human , animal and agriculture. Therefore , the current phytochemical study have shown various groups of active compounds in the different studied parts of all the tested plants (viz., *Balanites aegyptiaca ,Solenostemma argel* , *Eclipta prostrata* , and *Azadirachta indica*) . Results of presence or absence of the eight tested secondary compounds in all plant samples were presented in three separate tables for each extract. The result of water extracts were shown in Table (5) . The main compounds in the four plants were alkaloids , saponins , flavonoids and flavones . Besides , tannins , amino acids and triterpenoids in fruit , root and bark of *Balanites aegyptiaca* .

The results of methanol extracts were presented in Table (6) . Methanol extracts of *Balanites aegyptiaca* and *Eclipta prostrata* revealed more or less similar to those obtained by water extracts , but *S. argel* showed amino acids , saponin , alkaloids and flavones . The phytochemical constituents of Hexane extracts of *Azadirachta indica* presented in Table (7) , revealed all the tested groups of phytochemicals, amino acids , saponins , alkaloids , flavonoids , flavones , tannins , sterols and triterpenes , in addition to different intermediates traces.

Table (5). Phytochemical constituents of water extracts of different botanical parts of four plant species

Plant species	Parts	Chemical groups							
		Am	Sa	Al	Fl	Fn	Tn	St	Tr
B.aegyptiaca	Root	+	+	+	+	+	+	+	+
	Leaves	+	+	+	+	+	+	+	+
	Fruit	+	+	+	+	+	+	+	+
	Bark	+	+	+	+	+	-	+	+
S. argel	Leaves	+	+	+	-	+	-	-	-
E.prostrata	Leaves	-	+	+	+	-	+	+	-
Aza. Indica	Fruit	+	+	+	+	-	-	+	+

(+) present , (-) non present . Amino acids (Am) , Saponins (Sa), alkaloids (Al) , flavonoids (Fl) , flavones (Fn) , Tnnins (Tn) , sterols (St) and triterpenes (Tt).

Table (6) . Phytochemical constituents of methanol extracts of different botanical parts of three plant species

Plant species	Parts	Chemical groups							
		Am.	Sa	Al	Fl	Fn	Tn	St	Tr
B.aegyptiaca	Root	+	+	+	+	+	-	+	+
	Leaves	+	+	+	+	+	-	-	+
	Fruit	+	+	+	+	+	-	+	+
	Bark	+	+	+	+	+	-	+	+
S. argel	Leaves	+	+	+	-	+	-	-	-
E.prostrata	Leaves	-	+	+	+	-	+	+	+

(+) present , (-) non present .

Amino acids (Am) , Saponins (Sa), alkaloids (Al) , flavonoids (Fl) , flavones (Fn) , Tnnins (Tn) , sterols (St) and triterpenes (Tt).

Table (7). Phytochemical constituents of Hexane extract of Neem seed.

Plant species	Parts	Chemical groups							
	Parts	Am.	Sa.	Al.	Fl.	Fn.	Tn.	St.	Tr.
Aza. indica	Fruit	+	+	+	+	+	-	+	+

(+) present , (-) non present .

Amino acids (Am) , Saponins (Sa), alkaloids (Al) , flavonoids (Fl) , flavones (Fn) , Tnnins (Tn) , sterols (St) and triterpenes (Tt).

4.5.1 Phytochemical constituents of the tested extracts of Hargal :-

The chemical test revealed the presence of various chemical groups at variable levels . Polar and intermediately polar ingredients were shown in wide range . Alkaloids , saponins , flavones and amino acids were the major chemicals detected. Methanol extracts of the plants revealed more or less the same previous compounds , plus some trace amounts of tannins , sterols and triterpenes .

Table (8) showed the phytochemical constituents of *Solenostemma argel* , it is clear that the detected groups were different according to the type of the extracts(viz., water , methanol). In general , water extract revealed the presence of various chemical groups at variable ranges of polar and intermediately polar ingredients. Those , were Alkaloids , saponins , flavons and amino acids which were the major chemical groups detected . While methanol extract revealed more or less the same of the water extract chemical compounds , plus some embedded traces amounts of tannins , sterols and triterpenes .

Table (8). Phytochemical constituents of two extracts Prepared from leaves of *Solenostemma argel* .

Extracts	Chemical groups detected in different extracts							
	Am	Sa	Al	Fl	Fn	Tn	St	Tr
Leaves water extract	+	+	+	+	-	-	-	+
Leaves methanol extract	+	+	+	+	-	+	-	+

NOTE : Am = Amino Acids ; Sa = Saponins ; Al = Alkaloids ; Fl = Flavonoids ; Fn = Flavones ; Ta = Tannins ; St = Steroids ; Tr = Triterpenes ; (-) = Non present ; (+) = present .

Harbone and Turner (1984) proved the role of ethanol and hexane solvents in extraction of non polar compounds (e.g . , triterpenes and sterols) and alcohol (ethanol) for polar compounds (Alkaloids and flavonoids) . Investigations elsewhere also showed certain biologically active chemicals in *Solenostemma argel*. Debella *et al*., (2008) , showed the presence of saponins , alkaloids , glycosides and polyphenols as the major components of *Solenostemma arge*l aqueous extract.

Despite the fact that studies on phytochemicals analysis were very scanty in Sudan , more or less similar active principles were indicated in *Solenostemma argel* by some authors (Al kamali , 1991) . However , according to Satti *et al* .(2010) , sclepiadceae was placed among the important flora families showing several bioactive plant species in the country.

Literature on phytochemical screening of active plant ingredients , is scanty, especially when concerning the indigenous flora. Generally , Harborne and Turner (1984) stated that a polar solvents like Hexane and petroleum ether are involved in the extraction of non polar compounds (e.g., triterpenes and sterols) , while alcohol solvents for polar components such as alkaloid and polar flavonoids . Investigators elsewhere also showed certain biologically active chemicals in *S. argel*. For instance , Saponins are structurally diverse class of secondary metabolites that consist of sugar moeities linked to triterpene or sterol aglycone (Price *et al* . , 1987). Therefore, the triterpenoids and sterols appeared in water and methanol extracts of the present study may contain such hydrophilic sugar part. In contrast to what have been found else where in India by (Upsathani *et al*., 2003) , who obtained flavonoids from leaf aqueous extract of *R.communis* , this class of

chemicals (sugar moieties linked to triterpene or sterol aglycone), was not detected in the studied samples (Upsathani *et al*.,2003). The reasons for such difference were not clear, may be due to geographical effects. However, some authors attributed such variability in plant phytochemicals and their consequent bioactivities to differences in several factors including climatic , genetic and soil conditions, besides, the differences in morphological parts used (Satti , 2010 and Schmutterer , 1990). The present results were also in agreement with previous studies on other plants which revealed similar trends of polar and a polar compounds from different extracts , and showed the richness of Hargal plant over other botanical parts (El-Kamali , 2001, Eltayeb , *et al*.,(2009) and Edriss *et al*., 2013).

4.5.2 Phytochemical constituents of *Balanites aegyptiaca* Del. (Higleeg)

The phytochemical screening (Table 9) revealed different types of secondary bioactive compounds in the various parts of *Balanites aegyptiaca*, including saponins, alkaloids , flavonoids , carbohydrates , coumarin , and steroids.

Table (9). Phytochemical constituents of three extracts Prepared from different parts of *Balanites aegyptiaca.*

Extracts	Chemical groups detected in different extracts								
	Pr	Sa	Al	Fl	CHO	sterol	O.Acid	Tr	Coum
Leaves water extract	+	+	-	+	+	-	-	+	+
Leaves methanol ext.	+	+	+	+	+	-	+	+	+
Fruit water, ethanol ext.	+	+	-	+	+	+	+	+	+
Barkwater,methanol	+	+	-	+	+	+	+	+	+
Root water,methanol ex	-	+	-	+	+	+	+	+	-

NOTE : Or = Organic Acids ; Sa = Saponins ; Al = Alkaloids ; Fl = Flavonoids ; Pr = Protein ; Lip = Lipid ; St = Steroids ; Tr = Triterpenes ;CHO = carbohydrates ;coum.=coumarin ; (-) = Non.

The detected superior insecticidal activities of the Higleeg root water and methanol extracsts on mosquito larvae may suggests the presence of potent active compounds in these extracsts with toxicant effects on the larvae of mosquito species which also need to be ascertained. Anyhow, the results partially confirmed what has been recorded by Mohamed (2003) who verified the insecticidal effect of desert date seed hexane extract against *Trigoderma granarium* **.** Secondary metabolites like rotenone , coumarin , bergopin, steroids yamogenin and the flavonoids isorhamntetin-3-rutinoside and 3-rhamnoglactoside were detected in different parts of the tree (El Ghazali, *et al*., 1997). Also, *B. aegyptiaca* is named as an African-Asian saponin-producing plant due to its high constituent of saponin compounds. These multiple chemicals have proved different biological activities including molluscicidal, mosquitocidal and insect antifeedant properties, besides other industrial uses (Hall and Walker, 1991; Osman *et al*., 2003; Wiesman and Chapagain, 2003; Chapagain and Wiesman, 2005). Generally, *B. aegyptiaca* was reported to contain various classes of secondary metabolites with different biocidal effects (Hall and Walker, 1991; El Ghazali, *et al*.,1997). Also, Chothani DL, Vaghasiya HU , 2011), reported that , *Balanites aegyptiaca* contains : protein , lipid , carbohydrate , alkaloid, saponin , flavonoid , and organic acid.

4.5.3 Phytochemical constituents of *Eclipta prostrata* (Tamr Alghanam)

Table (10). The qualitative analysis of the phytochemicals of aqueous extracts of *Eclipta prostrata* leaves.

Chemicals constituents detected in the aqueous extract of the leaves of *Eclipta prostrata*	
Alkaloids	-
Carbohydrates	+
Saponins	+
Phytosterols	+
Phenols	+
Flavonoids	+
Terpenoids	+
Tannins	+
Coumarin	+

(+) presence of the compound , **(-**) Absence of the compound.

The qualitative study carried out on the aqueous extract of the leaves of *Eclipta prostrata* (Table 10) , revealed the presence of biologically active constituents such as saponins , phytosterols , phenols , flavonoids , tannins, terpenoids and carbohydrates. Also, many authors reported that, crude saponin was the major constituent in the plant resembling (4.5%) and crude tannins was the second constituent in the plant and resembling (1.0%) . Besides , there were other phytochemicals estimated but were present only in very low concentrations.

Generally , also some authors else where , reported more other phytochemical constituents in the plant extracts such as triterpenoid saponin (eclalbasaponins I-VI, XI and XII, ecliptasaponin C and D, eclalbatin), flavonoids (apegenin and luteolin), coumestans (wedelolactone, dimethyldelolactone and strychnolactone) and alkaloids (25-β-hydroxyverazine, ecliptalbine, ecliptine and traces of nicotine). (Coldecott and Bhringaraja , 2006 ; Himalaya herb,2007; Zhao *et al* .,2001; Zhang and Chen, 1996; Zhao *et al.*,1997; Zhang and Guo ,2001; Kumari *et al*.,2006). *E. prostrata* also contains α-formylterthienyl, α-terthienyl, sixteen related polyacetylanic thiophenes, dithienenyacetyline esters I, II, and III, β-sitosterol, stigmasterol, daucoterol, stigmasterol-3-0-glucoside, nonacosanol, stearic acid, lacceroic acid, 3,4-dihydroxy benzoic acid, α-amyrin, ursolic acid, oleanolic acid and ascorbic acid.(Coldecott and Bhringaraja ,2006) , (Herbfinder, 2007 ; Zhang and Chen , 1996 ; Zhang and Guo , 2001; Kumari , *et al* . , 2006) . When preparing leaves extract with different solvents (viz., water , methanol , hexane , ethylacetate , petroleum ether etc.,) . *Eclipta prostrata* also was reported by some authors to contain a large amount of resin and an alkaloid ecliptine. Studies else where reported that the plant extract yielded coumestans, polypeptides, polyacetones, triterpenes and flavonoids. Also, it was stated that methanolic extract yielded tannins , flavonoids, coumestans, saponins, alkaloids, etc.

Dried leaves have been reported to contain Coumarins , and many Hydrocarbons. Whole plant is said to have many triterpenene like saponin.

4.5.4 Phytochemical constituents of *Azadirachta indica* A.Juss (Neem):-

The chemical test (Table 11) revealed various bioactive compounds diversified in their qualities and availabilities in the water and hexane extracts. These included: alkaloid , saponin , phenols , triterpene , flavons , sterols and traces of phenol and esters.

Table (11). Chemical constituents of *Azadirachta indica* A.Juss (Neem).

Extract	Alkaloid	Saponin	Flavonoid	Flavons	Sterols	Phenols	Amino acid	Triterpene
Water	+	+	-	+	+	+	-	+
Hexane	+	+	+	+	+	+	+	+

The biochemical compounds belong to a general class of natural products

called "triterpenes", more specifically, "limonoids." Nine neem limonoids have demonstrated an ability to block insect growth in a wide variety of most deadly pests of agriculture and human health. the most significant of the so far discovered limonoids are azadirachtin, salannin, meliantriol, and nimbin.

Azadirachtin is mainly responsible for the insecticidal properties of the neem. Azadirachtin disrupts the growth and reproduction in most of the pest. It is one of the most potent growth regulators. It will repel or reduce the feeding of many species of pest insects as well as some nematodes. In fact, it is so potent that a mere trace of its presence prevents some insects from even touching plants.

Azadirachtin is structurally similar to insect hormones called "ecdysones". Ecdysones which controls metamorphosis in the insects as they pass from larva to pupa to adult. It affects the corpus cardiacum, an organ similar to the human pituitary gland that controls the secretion of hormones. Metamorphosis requires a careful synchrony of many hormones and other physiological changes to be successful. Azadirachtin because of its structural similarity to ecdysone will block the ecdysone’s action in metamorphosis and the release of these vital hormones. Insects then will not molt. This of course breaks their life cycle.

Meliantriol in extremely low concentrations causes insects to cease eating.The demonstration of its ability to prevent locusts chewing on crops was the first scientific proof for neem's traditional use for insect control on India's crops.

Salannin, the third triterpenoid isolated from neem inhibits feeding, but not the molting of insects. Laboratory and field tests indicated that Salannin deterred the feeding habit of migratory locust, California red scale, striped cucumber beetle, houseflies, and the Japanese beetle.

Two more neem components, nimbin and nimbidin, have been found to have antiviral activity. They affect potato virus X , vaccinia virus, and fowl pox virus. They could perhaps open a way to control these and other viral diseases of crops and livestock. Nimbidin is the primary component of the bitter principles obtained when neem seeds are extracted with alcohol. It occurs in sizable quantities-about 2 percent of the kernel.

Newly identified limonoids from neem include deacetylazadirachtinol function as antihormones. This ingredient, isolated from fresh fruits, appears to be as effective as azadirachtin in assays against the tobacco budworm, but it has not yet been widely tested in field practice. Research has shown that some of these minor neem chemicals even paralyze the "swallowing mechanism" and so prevent insects from eating. Two other compounds related to salannin, 3-deacetylsalannin and salannol, recently isolated from neem, also act as anti-feedants.

Each and every part of the tree contains the Bioactive compounds. However, the seed kernels contain the most concentrated and accessible compounds. Extraction of the compounds is mostly from the kernels and, to a lesser extent, from the pressed cake. Although the active ingredients are only slightly soluble in water, they are freely soluble in organic solvents such as hydrocarbons, alcohols, ketones, or ethers. (Lakshmi Sridharan,2009).

Various non-terpenoidal constituents have also been identified by different groups.These included hydrocarbons , aromatics , phenolics , coumarins, isocoumarins , flavones , fatty acids and their esters, sulfides, *etc.* (Akhila and Rani, 1999; Ali *et al.*, 1996; Kaushik and Vir, 2000; Sharma *et al.*, 1998; Siddiqui *et al.*, 1988, 1992). The pesticidal activity of neem botanicals (Chemical preparations of plant origin) , including that of triterpenoids , neem

oil and fractions containing volatiles against a variety of house and crop insects has remained the subject of interest since decades. Mosquitoes, carriers for certain diseases are among these (Ascher, 1997; Khan *et al.*, 1999; Tariq *et al.*, 2001, 2002; Naqvi *et al.*, 1994; Dhar *et al.*, 1996; Siddiqui *et al.*, 1999, 2000a,b, 2002; Mulla and Su, 1999; Vietmeyer, 1992) . Aliero , 2003, reported that the potency of *A. indica* in the control of mosquito larvae seemed to be for the reason that , seed oil appeared as the most lethal among the various parts tested , moreover , the high mortality recorded for seed oil extract might be attributed to deficiency of dissolved oxygen in the water . The bioactive compounds in the neem tree were found throughout the tree , but those in the seed kernel were the most concentrated and accessible , specially Azadirachtin which is the main bioactive constituent (Grunewald *et al*., 1992) . Isman ,2006, Mordue *et al* .,1993) attributed these effects of activity of *Azadirachta indica* against mosquito larvae are frequently due to the azadirachtin contents of the products which agreed with the findings of neem seed extracts screening in the current study. Recent studies have also demonstrated neem-induced effects on vitellogenesis and severe degeneration of follicle cells during oogenesis in mosquitoes (Lucantoni *et al* ., 2006). It has been argued that the pesticidal efficacy, environmental safety, and public acceptability of neem and its products for control of crop pests would ensure its adoption into mosquito control programmes (Su and Mulla ,1998, Su and Mulla ,1998).

Finaally, in the present study , it can be concluded that , the results of the tested plant extracts against the larvae of the 3 mosquito species showed that , root extracts of *Balanites aegyptiacawere* found to be most toxic thus have the highest larvicidal potency on the larvae of the three mosquito species , even when tested at the lowest concentration (40 ppm) , which credited *Balanites*

aegyptiaca root extracts to be superior upon all the other tested plant extracts , moreover , the order of larvicidal potencies among the tested plant extracts were, roots of *Balanites aegyptiaca* > shoots of Hargal > neem seed > leaves of Tamr Al-ghanam.

The following tables (A , B, C, D, E, F , G, H, I, J, K and L) , presented the larvicidal potencies of these plant extracts at their lowest and highest concentrations against the three mosquito larvae.

Table A .effects of Higleeg water and organic solvent extracts at the lowest and highest concentrations on tested larvae of *Aedes aegypti* mosquito species.

Tested conc.	Extraction Methods					
(ppm)	water			Solvent(methanol)		
	Aedes aegypti larval mortality			*Aedes aegypti* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest (40)	48.48(42.2)	58.7(50)d	74.9(60.1)	51.2(48.7)	91.3(75.4)c	99.9(89.3)a
Highest (500)	94.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.3)a
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)a	99.9(89.4)	99.9(89.4)a

*Figures between brackets are arcsine transformation for percent.

Table B . Effect of Higleeg water and organic solvent extracts at the lowest and highest concentrations on tested larvae of *Anopheles arabiensis* mosquitospecies

Test conc.	Extraction Methods					
(ppm)	water			Solvent(methanol)		
	An. arabiensis larval mortality			*An. Arabiensis* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest (40 ppm)	89.95(74)a	96.27(81.8a)	99.9(89.3)a	92.6(79.89)a	96.2(81.89)a	99.9(89.3)a
Highest (500)	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

Table C. Effect of Higleeg water and organic solvent extracts at the lowest and highest concentrations on tested larvae of *Culex quinquefasciatus* mosquito species.

	Extraction Methods					
	water			Solvent (methanol)		
Concentration	*Culex quinquefasciatus* larval			*Culex quinquefasciatus* larval		
(ppm)	mortality			mortality		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Lowest (40 ppm)	51.0(43.7)	73.7(59.4)	91.2(72.9)	77.4(61.8)	97.5(83.3)	99.9(89.3)
Highest (500ppm)	99.9(89.3)	99.9(89.3)	99.9(89.3)	99.9(89.3)	99.9(89.3)	99.9(89.3)
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

Table D . Effects Hargal water and solvent extracts at the lowest and highest concentrations on tested larvae of *Aedes aegypti* mosquito species .

Concentration	Extraction Methods					
(ppm)	*water*			Solvent(methanol)		
	Aedes aegypti larval mortality			*Aedes aegypti* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest(62.5ppm)	25.05(29.9)	54.9(47.8)d	70.0(57.0)c	18.8(25.6)d	42.6(40.7)d	83.9(67.9)c
Highest (750ppm)	98.7(85.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

Table E . Effects Hargal water and solvent extracts at the lowest and highest concentrations on tested larvae of *Anopheles arabiensis* mosquito species .

Concentration	Extraction Methods					
(ppm)	water			Solvent(methanol)		
	An. Arabiensis larval mortality			*An. Arabiensis* larval mortality		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Lowest (62.5ppm)	10.2(9.0)	36.2(36.5)	36.2(36.5)	12.5(12.0)	47.5(43.5)	72.4(68.5)
Highest (750ppm)	91.1(75.1)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)	99.9(89.4)a	99.9(89.4
Temephos	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a	99.9(89.4)a

*Figures between brackets are arcsine transformation for percent.

Table F. Effects of Hargal water and solvent extracts at the lowest and highest concentrations on tested larvae of *Culex quinquefasciatus* mosquito species .

Concentration	Extraction Methods					
(ppm)	water			Solvent (methanol)		
	Culex quinquefasciatus larval mortality			*Culex quinquefasciatus* larval mortality		
	24hrs	48 hrs	72 hrs	24hrs	48 hrs	72 hrs
Lowest (62.5ppm)	22.5(22.0)	49.9(45.0)	68.8(56.21)	35.04(39.2)	70.0(56.9)c	72.4(58.5)
Highest(750ppm)	93.8(77.4)a	99.9(89.4)a	99.9(89.4)a	99.9 (89.4)a	99.9 (89.4)a	99.9 (89.4)a
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

Table G. Effects Neem water and solvent extracts at the lowest and highest concentrations on tested larvae of *Aedes aegypti* mosquito species .

Concentrations (ppm)	Extraction Methods					
	water			Solvent (Hexane)		
	Aedes aegypti larval mortality			*Aedes aegypti* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest(62.5 ppm)	40.75(39.7)	51.8(46.0)	60.3(50.95)	34.25(35.8	41.5(40.10)	66.25(54.49
Highest(1000ppm	84.75(67.2)	88.0(69.77)	94.25(76.4)	87.5(69.86	89(68.48)	99.5(87.13)
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

Table H . Effects of Neem water and solvent extracts at the lowest and highest concentrations on tested larvae of *Anopheles arabiensis* mosquito species

concentrations	Extraction Methods					
(ppm)	water			Solvent (Hexane)		
	An. Arabiensis larval mortality			*An. Arabiensis* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest (62.5 ppm)	64.75(53.59	66.5(54.49)	67.8(55.4)	72.5(58.4)	83.5(66.1	91.8(73.4)
Highest (1000ppm)	99.5(85.10)	97.75(84.04)	98.8(85.47	99.8(88.5)	99.8(90)	99.95(88.6
Temephos	99.9 (89.4)	99.9 (89.4)	99.9 (89.4)	99.9(89.4)	99.9 (89.4)	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

Table I. Effects of Neem water and solvent extracts at the lowest and highest concentrations on tested larvae of *Culex quinquefasciatus* mosquito species.

Concentrations	Extraction Methods					
(ppm)	water			Solvent (Hexane)		
	Culex quinquefasciatus larval mortality			*Culex quinquefasciatus* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest (62.5ppm)	67.7(55.41)	65.75(54.19)	72.75(57.1	72.8(58.55)	82.3(65.2	94.8(71.4)
Highest(1000ppm)	98.8(84.34)	99.0(84.94)	98.75(84.3	98.3(83.60)	99.8(87.7	100 (89.36
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

Table J . Effects of Tamr Al-ghanam water and solvent extracts at the lowest and highest concentrations on tested larvae of *Aedes aegypti* mosquito species

Concentrations (ppm)	Extraction Methods					
	water			Solvent(methanol)		
	Aedes agypti larval mortality			*Aedes agypti* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest(500ppm)	11.3(19.5)	9.98(18.42)	35.0(36.2)	48.7(44.3)	91.29(73.3)	99.9(89.4)
Highest (2500ppm)	76.3(60.92)	86.13(68.21)	99.9(89.4)	78.8(63)	98.70(85.7)	99.9(89.4)
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

Table K. Effects of Tamr Al-ghanam water and solvent extracts at the lowest and highest concentrations on tested larvae of *Anopheles arabiensis* mosquito species

Concentrations (ppm)	Extraction Methods					
	water			Solvent (methanol)		
	An.arabiensis larval mortality			*An.arabiensis* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest(500ppm)	10.0(12.5)	10.0(18.15)	30.0(33.2)	52.6(46.5)	74.9 (60.8)	92.5 (76.3)
Highest(2500ppm)	76.3(60.92)	87.4(69.31)	93.7(79.3)	99.9(89.4)	99.9(89.4)	99.9(89.4)
Temephos	99.9 (89.4)	99.9 (89.4)	99.9 (89.4)	99.9 (89.4)	99.9 (89.4)	99.9 (89.4)

*Figures between brackets are arcsine transformation for percent.

Table L. Effects of Tamr Al-ghanam water and methanolic extracts at the lowest and highest concentrations on tested larvae *Culex quinquefasciatus* mosquito species .

Concentrations (ppm)	Extraction Methods					
	water			Solvent (methanol)		
	Culex quinquefasciatus larval mortality			*Culex quinquefasciatus* larval mortality		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Lowest(500ppm)	7.5(13.8)	12.47(20.1)	36.8(47.2)	41.3(43.9)	78.7(69.3)	92.4(76.3)
Highest(2500ppm)	75.0(60.6)	68.8(55.6)	95.0(80.8)	99.9(89.4)	99.9(89.4)	99.9(89.4)
Temephos	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)	99.9(89.4)

*Figures between brackets are arcsine transformation for percent.

CHAPTER FIVE

DISCUSSION

The use of botanicals as an alternative to the chemical compounds is gaining tremendous momentum because of its multivarious advantages. In view of its increasing importance in controlling insect pests , it represents the best solution for the standing-today problems that appeared recently from the extensive application of the chemical insecticides in the field of crop pests and diseases , pests of public health and veterinary concern control in the World . The extensive uses of chemical pesticides , have led to serious draw backs , the most important are the evolution of mosquito resistant strains as well as toxicity hazards to man , livestock and wildlife (Bay. 1976) . Accordingly , these passive effects , revived the interest in exploiting the pest control potentials of plants for control of agricultural pests and insect-borne diseases of man and animal in different parts of the world (Grainge and Ahmed , 1988). Roark.(1947) described approximately 1,200 plant species having potential insecticidal value , while Sukumar *et al*.,1991 listed and discussed 344 plant species that only exhibited mosquitocidal activity. The sounded examples of these botanical insecticides were the products derived from the Neem tree (*Azadirachta indica*), which were found to be potent against more than 400 species of pests (Schmutterer *et al* .,1995) , and the Higleeg tree (*Balanites aegyptiaca*) ,) which acts as a potential natural larvicidal agent against mosquito larvae due to its larvicidal activities that present in the saponin rich extracts in its various tissues such as fruit pulp, kernel, root, bark and leaf. (Zarroug *et al*.,1990 ; Wiesman , and Chapagain , 2003 ; Chapagain and Wiesman , 2005 ; Wiesman , Chapagain , 2006 and

Wiesman , 2006). In addition , several studies were carried out in Sudan exploiting plant extracts against a number of insect pests of agricultural , medical or veterinary importance (Siddig , 1991 ; Ali , 2004 ; Asaad , 2010, Satti , 2008 and Satti , *et al* ., 2013) .

5.1.1 Effects of Higleeg aqueous extracts on mosquito larvae:

Effect of root water extract of Higleeg is presented in (Table 1). The lowest concentration (40 ppm) caused 48.48% , 89.9% and 51.0% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively , after 24 hours . The results showed that, the highest concentration (500 ppm) of Higleeg root water extract , gave the best significant treatment for the control of the three mosquito species , and caused 94.9% , 99.9% and 99.9% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quiquefasciatus* mosquito species respectively . The results agreed with Zarroug *et al*. (1988) who had tested some Sudanese plant extracts in which the best results he obtained , were in the case of water extract of *Balanites aegyptiaca* at 1000 ppm , the percentages of mortality recorded were 97.5 and 90% with *A. arabiensis* second and fourth larval instars, respectively. Also the results agreed with the findings of (Zarroug *et al* ., 1990 ; Wiesman and Chapagain ,2003 ; Chapagain and Wiesman , 2005 ; Wiesman and Chapagain , 2006 and Wiesman ,2006) , who stated that , mosquito larvicidal effect of saponin extract of *Balanites aegyptiaca* was more active than water extract of *Balanites aegyptiaca* fruit kernel, against second and fourth instar larvae of *Anopheles arabiensis*, *Culex quinquefasciatus*, and *Aedes aegypti* . They also stated that second instar larvae were more susceptible than the fourth instar larvae in all cases. Moreover , the larvae of *Anopheles arabiensis* ,

were more susceptible than the *Culex quinquefasciatus* , and *Aedes aegypti* to its larvicidal effects . The probit analysis showed that, Higleeg water extract showed LC_{50} of 0.000357 ml/L for *Anopheles* , LC_{50} of 0.001335 mL/L for *Culex* , and LC_{50} of 0.001542 ml/L for *Aedes* larvae . Also Chapagain and Wiesman (2005) ; Wiesman and Chapagain (2006) , stated that , the root extract of the plant was found to be most lethal , followed by the bark among the various parts tested (fruit pulp, seed kernel, root, bark and leaves). Also , Chapagain *et al* ., (2008) cited that , on the basis the very low adult emergence (only 18%) on using only 50 ppm of the root derived callus , saponins of *B. aegyptiaca* showed a great possibility for drastically reducing the *A. aegypti* population in the concerned areas.Since the larvicidal activities of *Balanites aegyptiaca* are present in the saponin rich extracts in the various tissues as fruit pulp , kernel , root , bark and leaf , it was found that , the saponin interact with the cuticle membrane of the larvae in a way that causing damage to the membrane structure , which could be suspected as the possible reason for death of the larvae (Lee , 2000 ; Wiesman and Chapagain , 2005 ; Khanna and Kannabiran , 2007) .Wiesman and Chapagain (2006) reported that saponin extracted from the fruit of *Balanites aegyptiaca* showed 100% larvicidal activity against *A. aegypti* mosquito larvae.

As presented in (Table .1) , the positive relationship between the larvicidal mortalities of the aqueous root extract with increase in exposure time (24 -48 hrs) and concentration , confirmed what has been cited by several investigators that , the gradual building up of the extract activity against mosquito larvae is in relation to time of exposure and concentration (Abdu

Zahir *et al*.,2009 ; Mulla *et al* ., 2008 ; Abdulrahman *et al* ., 2008 and Tonk *et al* ., 2006).

The variation among the extract activities were mainly due to the plant species and their parts used in current test .This revealed that the plants constitute different quantities of biologically active ingredients depending on plant species , their habitats and environments (Satti *et al* ., 2010) .The superior mortality of *Anopheles arabiensis* caused by *Balanites aegyptiaca* root aqueous extract , may be due to larvicidal activities present in the saponin rich extracts in the various tissues of *Balanites aegyptiaca* or, may be due to the hyper sensitivity of the mosquito which is found to be the most susceptible followed by Culex and then Aedes (Zarroug *et al*.,1990 ; Wiesman and Chapagain , 2003 ; Chapagain and Wiesman , 2005 ; Wiesman and Chapagain ,2006 and Wiesman,2006).

5.1.2. Effects of root methanolic extract of Higleeg on mosquito larvae

Effects of methanolic root extract of Higleeg on mosquito larvae are presented in (Table 3). The lowest concentration (40 ppm) , caused 51.2% , 96.2% and 77.4% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* ,respectively after 24 hours. The probit analysis showed LC_{50} of 0.001227 ml / L , 0.00028 ml /L and 0.000313 ml / L , for the *Aedes aegypti* , *Anopheles arabiensis and Culex quinquefasciatus* mosquito species , respectively. The highest concentration (500 ppm) caused 99.9% death to the larvae of the three mosquito species , respectively, after 24 hours.The results of root methanolic extract fell in line with the findings of Chapagain (2008) who cited that , on the basis the very low adult emergence (only 18%) on using only 50 ppm of the root derived callus , saponins of *B. aegyptiaca* showed a great

possibility for drastically reducing the *A. aegypti* population in the concerned areas . This demonstrates the activity of saponin extracted from *Balanites aegyptiaca* even when used at very low concentration (50 ppm) which equals approximately the lowest concentration percent of root water or methanolic extract (40 ppm) of the present study.

5.2. *Azadirachta indica* A.Juss (Neem)

The larvicidal effect of *Azadirachta indica* on the larvae of the three mosquito species is presented in Tables (3 - 4).

5.2.1. Effect of the aqueous extract of neem seeds on mosquito larvae

As shown in Table 4.3 the Neem fruit aqueous extract at the lowest concentration (62.5 ppm) , had caused 40.75% , 64.75% and 67.75% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively after 24 hours . The highest concentration (1000 ppm) , caused 84.75% , 97.4% and 98.75% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively after 24 hours.These results fell in line with Aliero (2003),who investigated the effect of crude aqueous extracts of *Azadirachta indica* (neem) against the larvae of *Anopheles* mosquito . Exposure of the larvae to undiluted extracts of seed oil, leaf and bark for 12 hours led to 100% , 98% , and 48% mortality, respectively. Dilution of these extracts also resulted in mortality of the larvae .

Results of probit regression analysis (Table 3a) and figures (7 - 9) showed the different trends of toxicity of the neem fruit aqueous extract on the different mosquito species which indicate the potency of neem on mosquito

.This result confirmed what has been reported by several researchers that neem bioactive compounds were found throughout the tree and used for mosquito and malaria control (Howard *et al*. 2009; Martínez-Tomás *et al.* 2009 ; Lucantoni *et al*., 2006; Wandscheer *et al.,* 2004 ; Aliero , 2003 ; Awad and Shimaila , 2003 ; Copping and Menn 2000 ; Mordue (Luntz) and Nisbet , 2000 ; Mulla and Su 1999 ; Batra *et al*. 1998 ; Su and Mulla , 1998 b ; Singh *et al*., 1996 ; Mordue (Luntz) , and Blackwell , 1993 Sharma and Dhiman , 1993 ; Grunewald *et al*., 1992 ; Ruskin , 1992 ; Sukumar *et al*., 1991 and Schmutterer 1990) .

5.2.2. Effects of Neem seed Hexane extract on mosquito larvae

The results as presented in (Table 4) showed that the neem seed hexane extract at the lowest concentration (50 ppm) caused 34.25% , 72.5% and 71.0% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively , after 24 hours.The highest concentration(1000 ppm) caused 87.5% , 99.8% and 98.25% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively , after 24 hours. This fell in line with Ruskin (1992) who stated that compound extracted from *Azadirachta indica* caused mortality to the fourth instars of *Anopheles arabiensis* ; however , Aliero (2003) suggested that the seed oil and leaf extract of neem (*Azadirachta indica*) , contain properties that could be used in the control of mosquitoes in the tropics as a result of his studies on the larviciding effect of neem on Anopheles mosquitoes . Neem seed could be justified by the fact mentioned by Grune wald *et al*.,(1992) that , the bioactive compounds in the neem tree were found throughout the tree , but these in the seed kernel were the most concentrated and accessible , specially Azadirachtin which is the main

bioactive constituent. Hexane solvent was well known to remove the oil from the leaves and seeds(non-polar). The larvicidal mortalities in these results could be attributed to the deficiency of dissolved oxygen in the water as a result of the presence of the oil. Also , Aliero (2003) stated that , seed oil appeared as the most lethal among the various parts tested against Anopheles species and attributed this to the deficiency of dissolved oxygen in the water. The result also agreed with the findings of Batra *et al* .,(1998) who reported that, neem oil emulsion in water was found to control breeding of *Culex quinquefasciatus Anopheles stephensi* , and *Aedes aegypti* in pools , basement tanks , and desert coolers.The same result was found by Howard *et al*.,*(*2009) ; Martinez- Tomas *et al* .,(2009) who demonstrated that crude extracts from different parts of neem are potent against different mosquitoes, including *Culex quinquefasciatus* and *Anopheles gambiae* . Neem products have been shown to exhibit a wide range of effects that are potentially useful for malaria control and include antifeedancy (Lucantoni *et al*., 2006), ovicidal activity, fecundity suppression (Su and Mulla,1998), insect growth regulation (Mordue *et al* .,1993,Mordue *et al*., 2000) and repellency (Singh *et al*.,1996; Sharma *et al*.,1993 and Sharma *et al*,1993). These effects are frequently attributed to the azadirachtin contents of the products (Isman, 2006., Mordue *et al*.,1993). Recent studies have also demonstrated neem-induced effects on vitellogenesis and severe degeneration of follicle cells during oogenesis in mosquitoes (Lucantoni *et al*., 2006). It has been argued that the pesticidal efficacy, environmental safety, and public acceptability of neem and its products for control of crop pests would ensure its adoption into mosquito control programmes (Su and Mulla ,1998,Su and Mulla ,1998).

5.3. Effects of Hargal extracts on mosquito larvae

The results presented in (Table 5) , showed that , the lowest concentration (62.5 ppm) of Hargal aqueous leaves extract gave the best significant mortality percntage of *Aedes aegypti* , *Culex quinquefasciatus* and *Anopheles arabiensis* mosquito larvae , and caused 25.05% , 10.2% and 22.5% larval death of the three species , respectively , after 24 hours. The probit analysis revealed that , Hargal water extract showed an LC_{50} of 0.002706 ml/L for *Anopheles*, 0.002568 ml / L for *Culex* and 0.008748 ml/L for *Aedes* larvae .The result agreed with Feiha *et al*., (2009) , who stated that Hargal plant at a concentration of (1.0%) , (which = 500 ppm) water extract had caused 100% larval death for both the *Culex quinquefasciatus* and *Anopheles arabiensis* , respectively , after 24 hours. The uppermost concentration (1.5% =750 ppm) in this study agreed with her findings. El – Kamali(2001) applied water extracts from the stems , roots , fruits and flowers of Hargal against *Culex* larvae , and obtained LC_{50} of 0.0010 , 0.0008 , 0.0005 and 0.00025 ml / L , respectively .It seems from these different contrasting figures that , application of Hargal extract as each part alone gave different figures as he obtained , the present study agreed with his findings . Also agreed with Al-Kamali (2008) who found that , when crude aqueous extracts of dried fruit pericarp, flower, root, and stem of *Solenostemma argel* (Del.) Hayne were tested for larvicidal activity against the third instar larvae of the mosquito *Culex quinquefasciatus* Say, the extract of the fruit pericarp was most effective with LC_{50} of 0.00049 ml/L after 24 h. The LC_{50} of crude aqueous extracts of flower, root and stem for mosquito larvae were 0.00174 ml /L, 0.00302 ml / L , and 0.00398 ml/ml , respectively . The highest concentration (750 ppm) , caused 98.7% , 91.1%

and 93.8% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively , after 24 hours . Results of Hargal shoot methanolic extract is presented in (Table 5),which revealed that , the lowest concentration (62.5 ppm) of Hargal leaves methanolic extract caused 18.8% , 12.7% and 35.04% larval mortality of the *Aedes* , *Anopheles* and *Culex* mosquito species, however , the highest concentration (750 ppm) of Hargal leaves methanolic extract gave 100% larval mortality of the three mosquito species after 24 hours (Table 6). The probit analysis showed that , Hargal methanolic extract gave an LC_{50} of 0.001463 ml /L for *Anopheles arabiensis* , LC_{50} of 0.001479 ml/L for *Culex quinquefasciatus*and LC_{50} of 0.001906 ml/L for *Aedes aegypti* mosquito larvae , respectively. It seems from the result that , Hargal leaves when extracted with methanol is highly effective against mosquito larvae . This result agreed with the finding of (Edriss , *et al* ., 2012) , when tested the water and organic solvent extracts against the mosquito species *Anopheles arabiensis* , and found that the ethanol and petroleum ether leaves extract of the plant , caused 90% larval mortality of the *Anopheles arabiensis* mosquito species. Also , the study revealed that the methanolic leaves extract of Hargal , induced significant best control of the larvae of *Anopheles arabiensis* , *Culex quinquefasciatus* and *Aedes aegypti*, when used at 125 ppm and upper concentrations, which is comparable to that of the standard larvicide Abate.

5.4. Effects of *Eclipta prostrata* (Tamr Al-ghanam) extracts on mosquito larvae

The results presented in (Table 7), showed the effect of leaves water extract of *Eclipta prostrata* on the larvae of the three mosquito species . The leaves water extract of the plant at the lowest concentration(500 ppm) , caused

11.3% , 10.0% and 7.5% larval mortality of the *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* , respectively , after 24 hours . The probit analysis , showed LC_{50} of 0.03862 ml /L , 0.0041510 ml /L and 0.004343 ml /L for the *Aedes aegypti* , *Anopheles arabien*sis and *Culex quinquefasciatus* mosquito species .respectively. The highest concentration (2500 ppm) , caused 76.3% , 76.3% and 75.0% larval mortality of the *Aedes* , *Anopheles* and *Culex* mosquito species . respectively , after 24 hours .

Gopieshkanna and Kannabiran (2007) reported the larvicidal effect of aqueous extracts of *Hemidesmus indicus* roots, *Gymnema sylvestre* and *Eclipta prostrata* leaves against *Culex quinquefasciatus* larvae at the concentration of 1, 2, 3, 4 and 5 percent upto three days. All the extracts showed larval mortality. Larval mortality was 100 percent with the use of 5 percent concentration of root extract of *H. indicus*, leaf extracts of *G. sylvestre* and *E. prostrata* after two days. Qualitative analysis of the phytochemicals of aqueous extract revealed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in all plants. Moreover , they added that , among these, the crude saponin was the major phytochemical constituent present in highest percentage followed by crude tannin in all the three plants. It is suggested that all the three plants possess larvicidal properties that could be developed and used as natural insecticides for mosquito control.

The results agreed with the findings of Gopiesh Khanna and Kannabiran (2007) who found that , the larvicidal activity of aqueous extract from leaves of *Eclipta prostrata* caused greater than 50% mortality of *Culex* larvae when used at 1000 ppm and higher concentrations , respectively after 3 days. Only the highest concentration (2500 ppm) showed 100% larval mortality of *Culex*

quinquefasciatus . He also reported that , on the third day and at the concentration (2000 ppm) , it killed more than 95% of the larvae. Moreover , Gopiesh Khanna and Kannabiran (2007) stated that , the aqueous leaf extract from *Eclipta prostrata* contains active phytochemicals of which the crude saponin was the major constituent (4.5%) , followed by crude tannins , moreover, they stated the presence of other medicinally active constituents including carbohydrates , phytosterol , phenols, and flavonoids. Also , Gopiesh Khanna and Kannabiran (2011) stated that , *Eclipta prostrata* contains a large amount of resin and alkaloid ecliptine.

The result presented in (Table 8) , showed the effect of leaves methanolic extract of *Eclipta prostrata* on the larvae of *Aedes aegypti , Anopheles arabiensis* and *Culex quinquefasciatus* mosquito species .The lowest concentration (500ppm) caused 48.7% , 52.6% and 41.3% larval mortality of the *Aedes aegypti , Anopheles arabiensis* and *Culex quinquefasciatus* ,respectively , after 24 hours .The probit analysis showed LC_{50} of 0.002241ml/L , 0.001251ml / L and 0.001345 ml / L , for the *Aedes aegypti , Anopheles arabiensis* and *Culex quinquefasciatus* . respectively . The highest concentration (2500 ppm) , caused 78.8% , 99.9% and 99.9% larval mortality of the *Aedes aegypti , Anopheles arabiensis* and *Culex quinquefasciatus* mosquito species , respectively after 24 hours . It seems that the methanolic leaves extract of *Eclipta prostrata* induced the best significant control of the larvae of the three mosquito species than the water extract , moreover , it caused 100% larval death of the *Anopheles arabiensis* and *Culex quin*quefasciatus mosquito species . The results agreed with Govindarajan and Karuppan (2011) who found that , maximum larvicidal activity of *Eclipta alba* against early third instar larvae of *Aedes aegypti* was

observed in methanol extract , followed by chloroform , benzene , ethyl acetate and hexane extract.

Methanolic leave extract of *Eclipta prostrata* yielded tannins , flavonoids , coumestans, saponnins , alkaloid , etc. (Gopieshkanna and Kannabiran , 2011) . In addition they were also , stated that *Eclipta prostrata* leaves extract yielded coumestans , polypeptides, polyacetones, triterpenes and flavonoids .

The mosquito larvicidal properties of the biologically acive secondary metabolites of the tested plants , particularly the saponins alkaloids , tannins , steroids , terpenoids , in addition to azadirachtin , limenoids .etc.,were reported in different studies stated their activities against mosquito species . Although , the actual modes of action of all ingredients were not fully elaborated, it is likely that these chemicals interfere mainly with certain biological , ecological and physiological aspects of the insect larvae . As for instance , saponin was found to interact with the cuticle membrane in a way causing its disarrangement , which was considered as the most probable reason for larval death (Lee , 2000 ; Wiesman and Chapagain , 2005 ; Khanna and Kannabiran , 2007 ; Chowdhury *et al* ., 2008) . Therefore, with little intreset , such vast repositories of diversified biologically active compounds in plants can provide untapped sources for environmentally safe mosquitocides with manifold activities.

CONCLUSION

Higleeg

The results of the study showed that *Balanites aegyptiaca* root aqueous extract induced the best significant control of the larvae of *Anopheles arabiensis* at the lowest concentration (40 ppm) as compared with the lowest concentrations of all other tested plant species after 24 hours . However , the same concentration of the root aqueous extract , showed moderate effect on the larvae of *Aedes aegypti* and *Culex quinquefasciatus* , respectively , 24 hours post treatment. In addition , it is observed that the activity of the extract was increased progressively with time of exposure up to three days. Moreover, the highest concentration (500 ppm) of the extract , gave the best significant control of the larvae of the three mosquito species , respectively, after 24 hours , which was comparable to that of the standard larvicide Abate. Results also showed that , the methanolic root extract of *Balanites aegyptiaca* affected the best significant control of the larvae of *Anopheles arabiensis* at the lowest concentration (40 ppm) after 24 hours , while showed moderate to high(51.2% and 77.4%) larval mortality of *Aedes aegypti* and *Culex quinquefasciatus* , respectively , after 24 hours. It is observed that the potency of the extract against the larvae of the three mosquito species also , has the tendency to increase with time of exposure up to 3 days at the end of which it caused 100% death of the larvae of the three mosquito species . This may explain the extendable residual effect of the methanolic extract for up to 3 days post treatment. The highest concentration of the methanolic extract (500 ppm) induced the best significant mortality of the larvae of the three mosquito species which was comparable to that of the standard larvicide Abate after 24 hours.

As presented in (Table .1, Table 2) ; the positive relationship between the larvicidal mortalities of the aqueous root extract with increase in exposure time (24 -48 hrs) and concentration , confirmed what has been cited by several investigators that , the gradual building up of the extract activity against mosquito larvae is in relation to time of exposure and concentration (Zahir *et al*.,2009 ; Mulla *et al* ., 2008 ; Abdulrahman *et al* ., 2008 and Tonk *et al* ., 2006).

The variation among the extract activities were mainly due to the plant species and their parts used in current test .This revealed that the plants constitute different quantities of biologically active ingredients depending on plant species , their habitats and environments (Satti *et al* ., 2010) .The superior mortality of *Anopheles arabiensis* caused by *Balanites aegyptiaca* root aqueous extract , may be due to hyper sensitivity of *Anopheles arabiensis* (Zarroug ,1990). The result showed that *Balanites aegyptiaca* root aqueous extract at (500 ppm) induced the best significant control of the *Anopheles arabiensis* , *Culex quinquefasciatus* and *Aedes aegypti* larvae , respectively,under laboratory condition , so *Anopheles arabiensis* came the first in ranking.

Neem

The results of the study showed that , the neem fruit aqueous extract at the lowest concentration (62.5 ppm) caused moderate mortality percentage on the larvae of the three mosquito species after 24 hours. The activity of the extract was observed initially to affect very slow and gradually increased with increase of time of exposure after 3 days. The highest concentration (1000 ppm) , induced the best significant control of the larvae of *Anopheles*

arabiensis and *Culex quinquefasciatus* which was comparable to that of the standard larvicide Abate , and effected (84.75%) larval mortality of *Aedes aegypti* after 24 hours.

The results of the neem seed hexane extract (Table 4) showed initially weak mortalrty percentage on the larvae of *Aedes aegypti* at the lowest concentration (50 ppm) , while showed best significant larval control of *Anopheles arabiensis* and *Culex quinquefasciatus* respectively after 24 hours , with extract activity increased gradually with time of exposure .The highest concentration (1000 ppm) showed significantly high mortality of the larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* , which was comparable to that of the standard larvicide Abate, and caused(87%) larval mortality of *Aedes aegypti* , respectively, after 24 hours . It is observed that the potency of the extract was increasing with increasing time of exposure .

Hargal

The study showed that results of aqueous extract of Hargal at the lowest concentration (62.5 ppm) induced considerable mortality of *Anopheles arabiensis* **,** *Aedes aegypti* and *Culex quinquefasciatus* , respectively, after 24 hours . However , the activity of the extract was observed to increase with time of exposure . The highest concentration (750 ppm) showed the best significant control of the larvae of the three mosquito species after 24 hours , which was comparable to that of the standard larvicide Abate.

The results of Hargal leaves methanolic extract at the lowest concentration (62.5 ppm) , showed weak mortality percentage on the larvae of the three mosquito species after 24 hours. The highest concentration (750 ppm) induced the best significant control of the larvae of the 3 mosquito species ,

respectively after 24 hours . It is observed that the potency of the extract increased with increase of concentration and time of exposure.

Tamr Al-ghanam

Results of the study showed that *Eclipta prostrata* leaves aqueous extract at the lowest concentration (500 ppm) caused weak to moderate mortality percentage to the larvae of *Culex quinquefasciatus* , *Anopheles arabiensis* and *Aedes aegypti* mosquito species , respectively, after 24 hours . The highest concentration (2500 ppm) gave the best significant control of the larvae of *Aedes aegypti* , *Anopheles arabiensis* and *Culex quinquefasciatus* after 24 hours. It is observed that larval mortality percentage increased with concentration and time of exposure which resulted the best significant control of the larvae of the three mosquito species , comparable to that of the standard larvicide Abate after 3 days.

Results of the leaves methanolic extract of *Eclipta pro*strata at the lowest (500ppm) ,caused moderate mortality percentage on the larvae of *Anopheles arabiensis* and showed less than (50%) larval mortality of the *Aedes aegypti* and *Culex quinquefasciatus* mosquito species, respectively, after 24 hours. The highest concentration (2500 ppm) , caused the best significant control of the larvae of *Anophele arabiensis* and *Culex quinquefasciatus* comparable to that of the standard larvicide Abate and caused (78.8%) larval mortality of *Aedes aegypti* , respectively after 24 hours. It is observed that mortality percentage increased with concentration and time of exposure causing 100% death of the larvae of the three mosquito species after 3 days.

RECOMMENDATIONS

(1) The study revealed that aqueous extract of the root of *Balanites aegyptiaca* showed best significant activities against mosquito larvae which considered an important vectors of serious human and animal diseases . Since *Balanites aegyptiaca* acts as potential natural larvicidal agent against mosquito larvae due to the larvicidal activities present in the biologically active ingredient " the saponin" rich extracts in the various tissues such as fruit pulp , kernel , root , bark , and leaf , it can be recommended that this plant could be utilized for control of mosquito vectors of human and animal diseases , particularly , extraction of all the plant parts could be carried out using simple method as possible which can be done in any remote or urban areas of the World where Balanites plants grow naturally.

(2) As many investigators confirmed that , neem products have been shown to exhibit a wide range of effects that are potentially useful for malaria control and include antifeedancy (Lucantoni *et al* ., 2006), ovicidal activity, fecundity suppression (Su and Mulla,1998) , insect growth regulation (Mordue *et al* .,1993,Mordue *et al* .,2000) and repellency (Singh *et al.*,1996; Sharma *et al*.,1993 and Sharma *et al* ,1993) . These effects are frequently attributed to the azadirachtin contents of the products (Isman ,2006, Mordue *et al* .,1993) . Recent studies have also demonstrated neem-induced effects on vitellogenesis and severe degeneration of follicle cells during oogenesis in mosquitoes (Lucantoni *et al* ., 2006) . It has been argued that the pesticidal efficacy, environmental safety, and public acceptability of neem and its products for control of crop pests would ensure its adoption into mosquito control programmes (Su and Mulla ,1998, Su and Mulla ,1998) . So , neem products can be recommended as an important natural toolbox for

mosquito vector control combined with stressed research studies to develop alternative biocides to chemical pesticides in this field. Also , neem products could be recommended for control of pests and diseases of agricultural crops.

(3) Results of the study of Hargal plant showed best significant control of the larvae of the three mosquito species when used as aqueous or solvent extracts comparable to that of the standard larvicide Abate , so ,it can be recommended for control of mosquito larvae as a naturally botanical compound and best alternative to chemical pesticides, since it is cheap and available in the country in vast areas . Also , it could be recommended for control of pest and diseases of agricultural crops, human and animal diseases as many studies were carried by investigators in the World.

(4) Results of the study of Tamr Al-Ghanam demonstrated the larvicidal activity of the plant on mosquito larvae giving comparable results to that of the standard larvicide Abate at higher doses , so the plant products can be recommended for mosquito larval control as it contains high percentage of biologically active compounds against mosquito larvae , also it is available all over the country hence it was considered to be cheapest alternative to synthetic chemical pesticides used in this purpose .

(5) Since all the tested plant extracts in this study proved to be potent against pests and diseases of Man ,animal and agriculture , it is recommended to encourage governmental and private sectors to adopt manufacturing of these natural plant products in the country and stop importing chemical pesticides used in these purposes .

(6) Scientests and Technicians are able to innovate , manufacture what we need from the untapped plant materials of Sudan , but the advanced technical

laboratory equipments and chemical materials are not available , the striking examples are as simple as chromotographic means (High power chromatography (HPLC ,TLC , MSD , for chemical analysis and equipments for separation of natural chemical compounds found in plants.

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Appendix Table Table 1

Table 1. Mortality of *Aedes aegypti* larvae at different concentrations of Higleeg root aqueous extract.

Dose concentration (%)	*Aedes aegypti* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
40 ppm	48.48 (43.2)	58.7 (50.0)	74.9 (60.1)
100 ppm	57.4 (49.4)	72.4 (58.5)	86.1 (68.2)
200 ppm	73.7 (59.3)	77.5 (61.9)	98.8 (86.4)
400 ppm	89.4 (78.7)	99.9 (89.4)	99.9 (89.4)
500 ppm	94.9 (89.4)	99.9 (89.4)	99.9 (89.4)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix (2).

Table 2. Mortality of *Anopheles arabiensis* larvae at different concentrations of Higleeg root aqueous extract.

Dose concentration (%)	*Anopheles arabiensis* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
40 ppm	89.95 (73.9)	96.27 (81.8)	99.9 (89.3)
100 ppm	95.0 (78.9)	97.5 (83.3)	99.9 (80.3)
200 ppm	99.9 (89.4)	99.9 (89.3)	99.9 (86.3)
400 ppm	99.9 (89.4)	99.9 (89.3)	99.9 (89.3)
500 ppm	99.9 (89.4)	99.9 (89.3)	99.9 (89.3)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix (3).

Table 3. Mortality of *Culex quinquefasciatus* larvae at different concentrations of Higleeg root aqueous extract.

Dose concentration (%)	*Culex quinquefasciatus larvae* (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
40 ppm	51.0 (43.7)	73.7 (59.4)	91.2 (72.9)
100 ppm	76.3 (60.9)	84.9 (67.7)	96.3 (80.3)
200 ppm	84.9 (67.4)	96.3 (80.3)	98.7 (86.3)
400 ppm	99.9 (89.3)	99.9 (89.3)	99.9 (89.3)
500 ppm	99.9 (89.3)	99.9 (89.3)	99.9 (89.3)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(4).

Table 4. Mortality of *Aedes aegypti* larvae at different concentrations of Higleeg methanolic extract.

Dose concentration (%)	*Aedes aegypti* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
40 ppm	51.2 (48.7)	91.3 (75.4)	99.9 (89.3)
100 ppm	73.8 (59.3)	94.9 (80.4)	99.9 (89.3)
200 ppm	84.9 (68.1)	96.3 (81.9)	99.9 (89.3)
400 ppm	98.8 (86.3)	99.9 (89.4)	99.9 (89.3)
500 ppm	99.9 (89.4)	99.9 (89.4)	99.9 89.3)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(5).

Table 5.Mortality of *Anopheles arabiensis* larvae at different Higleeg methanolic extract conc.s.

Dose concentration (%)	*Anopheles arabiensis* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
40 ppm	96.2 (79.89)	96.2 (81.89)	99.9 (89.3)
100 ppm	97.5 (83.3)	97.5 (83.34)	99.9 (89.3)
200 ppm	99.9 (89.4)	99.9 (89.36)	99.9 (86.3)
400 ppm	99.9 (89.4)	99.9 (89.36)	99.9 (89.3)
500 ppm	99.9 (89.4)	99.9 (89.36)	99.9 (89.3)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(6).

Table 6. Mortality of *Culex quinquefasciatus* larvae at different concentrations of Higleeg methanolic extract.

Dose concentration (%)	*Culex quinquefasciatus* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
40 ppm	77.4 (61.8)	97.5 (83.3)	99.9 (89.3)
100 ppm	99.9 (89.3)	98.7 (86.3)	99.9 (89.3)
200 ppm	99.9 (80.3)	99.9 (89.3)	99.9 (86.3)
400 ppm	99.9 (86.3)	99.9 (89.3)	99.9 (89.3)
500 ppm	99.9 (89.3)	99.9 (89.3)	99.9 (89.3)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix (7).

Table 7 .Mortality of *Aedes aegypti* larvae at different concentrations of Aqueous extract of neem seed.

Dose concentration 100gm /L	*Aedes aegypti* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
62.5 ppm	40.75(39.67)	51.75 (46.0)	60.25 (50.95)
125 ppm	62.25 (52.10)	63.25 (52.71)	71.0 (57.47)
250 ppm	72.25 (58.23)	67.75 (55.56)	86.5 (68.46)
500 ppm	76.8 (61.18)	76.0 (62.89)	91.0 (72.77)
1000 ppm	84.75 (67.19)	88.0 (69.77)	94.25 (76.44)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brakets are arcsine transformation for percent.

Appendix (8).

Table 8.Mortality of *Anophele arabiensis* larvae at different concentrations of neem seed aqueous extract.

Dose concentration 100gm /L	*Anopheles arabiensis larvae* (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
62.5 ppm	64.75 (53.59)	66.5 (54.49)	67.75 (55.40)
125 ppm	72.75 (58.56)	72.75 (58.55)	74.5 (59.68)
250 ppm	84.25 (66.72)	86.25 (68.29)	85.25 (67.47)
500 ppm	95.75 (78.69)	93.5 (73.63)	93.25 (75.28)
1000 ppm	99.5 (85.10)	97.75 (84.04)	98.75 (85.47)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix (9).

Table 9. Mortality of *Culex quinquefasciatus* larvae at different concentrations of neem seed Aqueous extract.

Dose concentration 100gm /L	*Culex quinquefasciatus* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
62.5 ppm	67.7 (55.41)	65.75 (54.19)	72.75 (57.11)
125 ppm	74.5 (59.69)	78.75 (62.56)	82.25 (65.14)
250 ppm	85.25 (67.47)	86.0 (68.09)	88.5 (70.18)
500 ppm	96.5 (78.70)	96.0 (78.19)	96.0 (78.51)
1000 ppm	98.75 (84.34)	99.0 (84.94)	98.75 (84.34)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

* Figures between brakets are arcsine transformation for percent .

Appendix(10).

Table 10. Mortality of *Aedes aegypti* larvae at different concentrations of hexane extract of neem seed.

Dose concentration ppm /100 ml	*Aedes aegypti* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
50 ppm	34.25 (35.81)	41.5 (40.10)	66.25 (54.49)
100 ppm	58.0 (49.61)	56.75 (48.88)	85.0 (67.22)
500 ppm	72.0 (58.06)	73.25 (58.87)	89.75 (71.58)
750 ppm	83.25 (65.24)	77.0 (60.40)	97.75 (81.75)
1000 ppm	87.0 (69.86)	83.0 (65.84)	99.5 (87.13)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brakets are arcsine transformation for percent.

Appendix(11).

Table 11. Mortality of *Anophele arabiensis* larvae at different concentrations of neem seed Hexane extract.

Dose concentration ppm /100ml	*Anopheles arabiensis larvae* (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
50 ppm	72.5 (58.38)	83.5 (66.09)	91.75 (73.44)
100 ppm	85.0 (67.22)	88.25 (70.05)	97.0 (80.10)
500 ppm	91.0 (72.61)	96.0 (78.87)	98.25 (82.47)
750 ppm	98.5 (83.07)	98.75 (85.70)	99.5 (88.57)
1000 ppm	99.75 (88.5)	99.8 (90.00)	99.95 (88.57)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(12).

Table 12.Mortality of *Culex quinquefasciatus* larvae at different concentrations of hexane extract of neem fruit .

Dose concentration ppm/100ml	*Culex quinquefasciatus* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
50 ppm	72.75 (58.55)	82.3 (65.16)	94.75 (71.37)
100 ppm	86.25 (68.26)	86.5 (68.48)	93.3 (75.15)
500 ppm	87.25 (69.11)	94.5 (76.93)	97.5 (81.20)
750 ppm	95.25 (77.46)	98.5 (83.88)	99.0 (84.94)
1000 ppm	98.25 (83.60)	99.8 (87.7)	100.0 (89.36)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brakets are arcsine transformation for percent.

Appendix(13).

Table 13. Mortality of *Aedes aegypti* larvae at different concentrations of Hargal aqueous extract.

Dose concentration (%)	*Aedes aegypti* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
62.5 ppm	25.05 (29.98)	54.9 (47.8)	70.0 (57.0)
125 ppm	58.7 (50.09)	67.6 (55.4)	90.2 (72.0)
250 ppm	73.8 (59.2)	82.4 (65.2)	99.9 (86.4)
500 ppm	97.6 (86.3)	99.9 (89.4)	99.9 (89.4)
750 ppm	98.7 (85.4)	99.9 (89.4)	99.9 (89.4)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix (14).

Table 14. Mortality of *Anopheles arabiensis* larvae at different concentrations of Hargal aqueous extract.

Dose concentration (%)	*Anopheles arabiensis* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
62.5 ppm	10.2 (9.0)	36.2 (36.5)	36.2 (36.5)
125 ppm	41.2 (39.9)	53.8 (47.2)	53.8 (47.2)
250 ppm	68.8 (56.2)	71.3 (57.7)	74.3 (57.9)
500 ppm	75.0 (67.8)	95.0 (78.9)	95.4 (78.9)
750 ppm	91.1 (75.1)	99.9 (89.4)	99.9 (89.4)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)		(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(15).

Table 15. Mortality of *Culex quinquefasciatus* larvae at different concentrations of Hargal aqueous extract.

Dose concentration (%)	*Culex quinquefasciatus* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
62.5 ppm	22.5 (22.0)	49.9 (45.0)	68.8 (56.21)
125 ppm	42.4 (40.6)	58.8 (50.1)	91.3 (73.32)
250 ppm	56.2 (48.6)	72.5 (58.5)	99.9 (89.4)
500 ppm	82.5 (65.5)	87.5 (72.0)	99.9 (89.4)
750 ppm	93.8 (77.4)	99.9 (89.4)	99.9 (89.4)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent .

Appendix(16).

Table 16 . Mortality of *Aedes aegypti* larvae at different concentrations of Hargal methanolic extract.

Dose concentration (%)	*Aedes aegypti* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
62.5 ppm	18.8 (25.6)	42.6 (40.7)	83.9 (67.9)
125 ppm	41.2 (40.0)	62.5 (52.3)	92.6 (74.8)
250 ppm	90.0 (72.3)	95.1 (78.9)	99.9 (89.4)
500 ppm	99.9 (89.4)	97.6 (83.3)	99.9 (89.4)
750 ppm	99.9 (89.4)	99.9 (89.4)	99.9 (89.4)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent

Appendix(17).

Table 17 . Mortality of *Anopheles arabiensis* larvae at different concentrations of Hargal methanolic extract.

Dose concentration (%)	*Anopheles arabiensis* larvae (Mortality %)					
	24 hours		48 hours		72 hours	
0.0	0.0		0.0		0.0	
62.5 ppm	12.7	(12.0)	47.5	(43.5)	72.4	(68.5)
125 ppm	37.5	(37.7)	60.1	(50.8)	78.6	(69.1)
250 ppm	87.6	(70.5)	84.9	(67.5)	97.6	(87.5)
500 ppm	97.5	(83.3)	99.9	(89.4)	99.9	(97.8)
750 ppm	99.9	(89.4)	99.9	(89.4)	99.9	(99.9)
Untreated control	0.0125	(0.64)	0.0125	(0.64)	0.0125	(0.64)
Temephos	99.99	(89.4)	99.99	(89.4)	99.99	(89.4)
SE±		(1.06)		(1.06)		(1.06)
Lsd(0.05)		(3.14)		(3.14)		(3.14)
CV (%)		(3.6)		(3.6)		(3.6)

*Figures between brackets are arcsine transformation for percent

Appendix(18).

Table 18. Mortality of *Culex quinquefasciatus* larvae at different concentrations of Hargal methanolic extract.

Dose concentration (%)	*Culex quinquefasciatus* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
62.5 ppm	35.04 (39.2)	70.0 (56.9)	72.4 (58.5)
125 ppm	57.6 (49.35)	60.1 (50.9)	78.6 (62.9)
250 ppm	96.4 (80.33)	96.2 (81.9)	98.8 (86.4)
500 ppm	98.8 (86.4)	99.9 (89.4)	99.9 (89.4)
750 ppm	99.9 (89.4)	99.9 (89.4)	99.9 (89.4)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(19).

Table1 9.Mortality of *Aedes aegypti* larvae at different concentrations of *Eclipta prostrata* aqueous extract.

Dose concentration (%)	*Aedes aegypti* larvae (Mortality %)					
	24 hours		48 hours		72 hours	
0.0	0.0		0.0		0.0	
500 ppm	11.3	(19.5)	9.98	(18.42)	35.0	(36.2)
1000 ppm	27.5	(31.5)	30.0	(33.20)	48.7	(44.3)
1500 ppm	40.0	(39.2)	44.99	(42.09)	72.6	(59.7)
2000 ppm	58.8	(50.1)	84.90	(67.27)	96.3	(81.9)
2500 ppm	76.3	(60.92)	86.13	(68.21)	99.9	(89.4)
Untreated control	0.0125	(0.64)	0.0125	(0.64)	0.0125	(0.64)
Temephos	99.99	(89.4)	99.99	(89.4)	99.99	(89.4)
SE±		(1.06)		(1.06)		(1.06)
Lsd(0.05)		(3.14)		(3.14)		(3.14)
CV (%)		(3.6)		(3.6)		(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(20).

Table 20 .Mortality of *Anopheles arabiensis* larvae at different concentrations of *Eclipta prostrata* aqueous extract.

Dose concentration (%)	*Anopheles arabiensis* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
500 ppm	10.0 (12.5)	10.0 (18.15)	30.0 (33.2)
1000 ppm	23.8 (29.1)	28.8 (32.42)	45.0 (42.1)
1500 ppm	30.1 (33.06)	46.3 (42.85)	61.2 (51.5)
2000 ppm	53.8 (47.16)	78.8 (62.66)	90.0 (72.2)
2500 ppm	76.3 (60.92)	87.4 (69.31)	93.7 (79.3)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(21).

Table 21. Mortality of *Culex quinquefasciatus* larvae at different concentrations of *Eclipta prostrata* aqueous extract.

Dose concentration (%)	*Culex quinquefasciatus* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
500 ppm	7.5 (13.8)	12.47 (20.1)	36.8 (47.2)
1000 ppm	19.9 (26.3)	28.75 (32.3)	52.3 (57.7)
1500 ppm	32.5 (34.4)	48.8 (44.4)	74.9 (67.2)
2000 ppm	47.4 (43.5)	77.5 (62.2)	81.0 (78.9)
2500 ppm	75.0 (60.6)	68.8 (55.6)	95.0 (80.8)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix (22).

Table 22. Mortality of *Aedes aegypti* larvae at different concentrations of *Eclipta prostrata* methanolic extract.

Dose concentration (%)	*Aedes aegypti* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
500 ppm	48.7 (44.3)	91.29 (73.3)	99.99 (89.4)
1000 ppm	52.5 (46.4)	93.80 (77.8)	99.99 (89.4)
1500 ppm	63.8 (53.3)	91.26 (73.0)	99.9 (89.4)
2000 ppm	71.1 (58.0)	97.58 (83.3)	99.9 (89.4)
2500 ppm	78.8 (63.0)	98.70 (85.7)	99.9 (89.4)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent.

Appendix(23).

Table 23. Mortality of *Anopheles arabiensis* larvae at different concentrations of *Eclipta prostrata* methanolic extract.

Dose concentration (%)	*Anopheles arabiensis* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
500 ppm	52.6 (46.5)	74.9 (60.8)	92.5 (76.3)
1000 ppm	74.9 (60.1)	88.8 (70.9)	95.0 (78.9)
1500 ppm	89.9 (71.9)	96.2 (81.9)	98.8 (86.3)
2000 ppm	92.5 (76.3)	98.8 (86.3)	99.9 (89.4)
2500 ppm	99.9 (89.4)	99.9 (89.4)	99.9 (89.4)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent

Appendix (24).

Table 24.Mortality of *Culex quinquefasciatus* larvae at different concentrations of *Eclipta prostrata* methanolic extract.

Dose concentration (%)	*Culex quinquefasciatus* larvae (Mortality %)		
	24 hours	48 hours	72 hours
0.0	0.0	0.0	0.0
500 ppm	41.3 (43.9)	78.7 (69.3)	92.4 (76.3)
1000 ppm	60.4 (58.7)	84.9 (50.8)	78.6 (67.5)
1500 ppm	76.3 (70.5)	91.3 (67.5)	97.6 (87.5)
2000 ppm	96.5 (83.3)	99.9 (89.4)	99.9 (97.6)
2500 ppm	99.9 (89.4)	99.9 (89.4)	99.9 (99.9)
Untreated control	0.0125 (0.64)	0.0125 (0.64)	0.0125 (0.64)
Temephos	99.99 (89.4)	99.99 (89.4)	99.99 (89.4)
SE±	(1.06)	(1.06)	(1.06)
Lsd(0.05)	(3.14)	(3.14)	(3.14)
CV (%)	(3.6)	(3.6)	(3.6)

*Figures between brackets are arcsine transformation for percent

Appendix (1).

Plate (1). Water pots are preferable breeding habitats for *Aedes* . Gash west – Kassala.

Appendix (2).

Plate (2).Out side water pots are normally good breeding sites for *Aedes aegypti* .

Appendix (3).

Plate (3).All immature stages of *Anopheles arabiensis* and *Culex quinquefasciatus* are found in running and stagnant water of the area .(Remaila River side and ditches of bricks making area).

Appendix (4)

Plate (4).Gadwal Al-Fetaih at Shegailab – Perminant breeding site of *Anopheles arabiensis* and *Culex quinquefasciatus* – Gabel Awleya Municipality ,Khartoum.

Appendix (5).

Plate (5).Rain water in a field is good breeding sites for mosquito vectors

(Gammoyia Scheme)

Appendix (6).

Plate (6). Rain water ponding represents suitable breeding site for *Anopheles arabiensis* and *Culex quinquefasciatus* . (Fetaihab Bridge side – Omdurman).

Appendix (7).

Plate (7).Fresh water body attracts *Anopheles arabiensis* to breed –Al-Ghamayer –Omdurman.

Appendix (8).

Plate (8). Flower pots also considered (preferable habitats of all immature stages of *Aedes aegypti*) –Gash –West , Kassala.

Plate (9). Water pots normally containing eggs and other immature stages of *Aedes aegypti*.

(Beer Yayei – Kassala).