1. INTRODUCTION

Mosquitoes are ideal transmitters of a number of disease causing agents, including *Plasmodium falciparum*, filarial nematodes, yellow fever virus, dengue virus, and West Nile virus. Mosquito-borne diseases are the number one killers worldwide, with an average death toll in the millions each year. Among these, malaria is the most important and serious disease transmitted mainly by *Anopheles* species (Marshall, 2000; Jacob, 2001 cited in Liu *et al.,* 2006). Recent estimates indicate that there are 300-500 million cases per year of malaria alone, leading to more than one million deaths annually. About 90% of these occur in Africa, south of the Sahara and *Plasmodium falciparum* is known to be the major cause of the disease (WHO, 1995; 2000). There are also 50-100 million human cases of dengue fever every year (Monath, 1994; Gubler & Meltzer, 1999 cited in Liu *et al.,* 2006), which is mainly transmitted by *Aedes aegypti*. There are also, fast spreading West Nile virus, mainly transmitted by *Culex* mosquitoes (Granwehr *et al.*, 2004 cited in Liu *et al.,* 2006), outbreaks of Venezuelan equine encephalitis virus in South America, of Rift Valley fever virus in the Middle East. The prevalence of mosquito-borne diseases is projected to double in the next 20 years if no new actions are taken (Breman, 2001).

Malaria is widespread in about l00 countries. Africa takes the largest and the regions of southern Sahara appear to be the most affected (WHO, 2000). Mosquito borne diseases contribute significantly to disease burden, death, poverty, and social debility in tropical countries. Ghai and Gupta (2000) described that more than 40% of the world's populations live in malarious areas. Malaria is a highly complex disease vectored by many different species of *Anopheles* mosquitoes.

The prevention and control of several important vector-transmitted, communicable diseases of man heavily depend on measures for the control of populations of the vectors (WHO 1982). Even though many vector control methods have been developed in the attempt to control diseases transmitting agents, man couldn't completely control vectors and pests. One of the most important reasons could be some of the weaknesses of the control methods developed so far. Especially, the exclusive use of some synthetic organic

insecticides caused hazards to the environment, physiological resistance towards many of these compounds in many disease vectors and pest species, and influences on other beneficial organisms and dangers to animal life including man. Consequently, there is a growing interest in phyto-chemicals which can act as repellents, insecticides, growth regulators, antifeedants, and antiovipositions.

1.1. Malaria situation in the world and in Ethiopia

There is an estimate of 300-500 million annual clinical cases of malaria worldwide with one to three million deaths per year. Most of these are children under the age of five (MOH, 2002; Liu *et al.,* 2006). Pregnant women are also more subject to severe attacks of malaria. Of malaria cases that occur throughout the world, 90% are estimated to occur in Africa and 90-95% of malaria and malaria related deaths are also estimated to occur in the continent (Samba, 2000).

It is believed that malaria remains one of the major determinants of ill health in many countries of Africa, Asia and Latin America. In Africa south of Sahara, malaria is the leading health problem, with almost the entire population being at risk (MOH, 2002).

Regardless of the current control measures, various reports show that the malaria situation is worsening. According to MOH (2002), malaria has spread into areas that previously had low or no transmission. For example, in 1970s and 1980s the resurgence of imported malaria in some of the developed countries, such as the U.S.A., Europe and the U.K. was reported (Gilles and Warrel, 1993).

The epidemic of malaria is increasing because of the widespread of resistant malaria parasites to antimalarial drugs and the widespread of resistant vectors to insecticides. This made malaria control complex (WHO, 1996). For instance, the number of *Anopheles* species resistant to insecticides is on the increasing momentum. In 1946, only two species of *Anopheles* were resistant to DDT. However, from the two subsequent reports by 1991 and 1992 a total of 55 resistant *Anopheles* species have been recorded. Out of these 21 were very important vectors of malaria (WHO, 1996). Almost all members of *Anopheles*

gambiae complex that are potent vectors of malaria in Tropical Africa have shown various degrees of resistance to DDT and other organo-chlorine insecticides (Gilles and Warrel, 1993). For example, *Anopheles gambiae* s. s. has been reported as resistant to permethrin with delayed knockdown (decreased susceptibility) to deltamethrin and lambdacyhalothrin in several West African countries. In Indonesia, *Aedes aegypti* is reported as highly resistant to propoxur, bendiocarb and pyrethroids (WHO, 1996). Frequent climate changes and environmental modifications are also the main reasons for an increasing trend of malaria (Tulu, 1993).

Since it imposes very significant economic costs on some of the poorest nations, malaria is also an important obstacle to economic and social development in most developing countries like Ethiopia. In 1997, for instance, it is estimated that the African region lost more than $ 2 billion as a result of malaria and malaria-related diseases (MOH, 2002). Hence, malaria remains one of the major health problems in developing countries. Interest in undertaking the malaria problem increased towards the end of the 1980s, with the call for the African countries to evaluate their malaria control situation and reallocate adequate resources towards malaria control efforts. World Health Organization has strengthened research on malaria and partners sensitized to participate in malaria control efforts in Africa. The Roll Back Malaria movement will further efforts towards cooperation of all stakeholders for better planning and implementation of malaria control programs (MOH, 2002).

Malaria is a major cause of health problem and mortality in Ethiopia like other Sub-Saharan countries. Three quarters (75%) of the whole landmass is thought to be malarious and about two thirds (≈68%) of the country's population live in the malaria risk areas (Tulu, 1993; Adhanom *et al.,* 2006). It is the major obstacle to social and economic developments in the country. Different reports show that there are more than 4 million malaria cases every year in Ethiopia. Clinical malaria is accountable for 10%-40% of the whole outpatient clinical visits. Morbidity among children under 5 years is expected to be 10%-20%. It is also responsible for 13%-26% of the total inpatient admissions in the various health centers. Malaria accounted for13%-35% of death in

health centers. Generally, malaria accounts for 30% of the disease burden in all age classes in the country (MOH, 2001).

Malaria is uneven in Ethiopia primarily due to the country's topographical and climatic characteristics. Since the country is situated in the tropical zone which comprises mass of central high lands, belted by low lying, hot and arid regions, the distribution and transmission dynamics of malaria is related to the diversity of the physical geography, variations in climate, socio-economic patterns of the population (MOH, 2002). The transmission season of malaria differs highly from season to season and from place to place. The probability of acquiring malaria is higher following the rainy seasons. There are two major transmission seasons. The first and most intense transmission period is between September and December following the long rainy season while the second is between April and May following the short rainy season (MOH, 2000; Tullu, 1993). A serious out break of malaria in the highlands of Ethiopia in 1958 resulted in 3 million cases and 150,000 deaths (Fontaine *et al.,* 1961).

Of the four species of *Plasmodium*, two species are epidemiologically important- *P. falciparum* which takes about 60% malaria transmission followed by *P. vivax* which takes about 40% (Abose *et al.,* 1998; Adhanom *et al.,* 2006).
Currently, the high population increase, frequent drought, famine, and agro-industrial developments in the highlands make people more interested to resettle in the lowlands. This resettlement and agro industrial expansion undertaken in lowland areas changed the pattern of malaria transmission in the country especially in Gambella (Nigatu *et al.,* 1992).

1.2. The major malaria vectors in Africa and Ethiopia

The malaria-vector system is very complex. Worldwide 422 species of *Anopheles* mosquitoes exist, of which only 70 are so far recognized as potential vectors of malaria (Service, 1993). They are a widespread species exhibiting a capacity to adapt to an increasing ecological and environmental change rapidly. These adaptations are also

accompanied with an increasing number of species complexes, thus indicating a high level of genetic diversity and flexibility (Molyneux, 1998).

The members of *An. gambiae* complex are the most important vectors of malaria in sub-Sahara Africa. Two species of the complex: *An. gambiae* s.s. and *An. gambiae* s.l., are both most broadly distributed and the most efficient vectors of malaria (Tulu, 1993; Fontenille and Lochouarn, 1999; Coetzee *et al.,* 2000).

According to Lindsay *et al.* (1998), the range and relative abundance of *An. gambiae* s.l. and *An. gambiae* s.s are strongly influenced by climatological factors, especially total annual rain falls. In general, *An. gambiae* s.l. tends to dominate in arid savannas where as *An. gambiae* s.s. is the dominant species in humid forest zones. Nevertheless, the two species occur in sypmpatry in most African countries (Coetzee *et al.,* 2000). *An. funestus*, *An. bwamnbe*, *An. merus* and *An. melas* are also important species in malaria transmission in localized areas (Coetzee *et al.,* 2000). *An. funestus* is particularly important at the end of the rainy season (Depiny *et al.,* 2004).

There are 42 *Anopheles* species in Ethiopia with distribution varying by altitudinal zone and microhabitats (Tulu, 1993; Adhanom *et al.,* 2006). Till now there are only two members of *An. gambiae* complex found in Ethiopia, *An. gambiae* s.l. and *An. qaudriannulatus* species B (White *et al.,* 1980; Hunt *et al.,* 1998). *Anopheles qaudriannulatus* sp. B is not a malaria vector as it feeds mainly on cattle (White *et al.,* 1980; Gilles and Warrell, 1993). *Anopheles qaudriannulatus* species A is found in South Africa (Hunt *et al.,* 1998). This complex comprises seven species of anopheline mosquitoes.

An. gambiae s.l. is the most important vector of malaria in Ethiopia (White *et al*., 1980; Abose *et al*., 1998). This species is concentrated in the lower rainfall zones in distribution. These areas represent the drier savannah areas, in collection sites where annual rainfall <1000 mm (Coetzee *et al*., 2000). Their larvae prefer small, temporary, and sunlit water bodies with emergent vegetation and are a very important vector where malaria is unstable (Fontenille and Lochouarn, 1999). It also primarily feeds outdoors and rests indoors. However, this could vary from place to place.

An. pharoensis and *An. funestus* are considered to be secondary vectors of malaria next to *An. gambiae* s.l. in Ethiopia (Tulu, 1993). They breed in large, permanent and shaded water found in swamps, along lake shores and in irrigation ditches. *Anopheles pharoensis* bites man or animals both indoors and outdoors, but rests predominantly outdoors (Abose *et al.,* 1998). *An. nili* is the least common species and more localized to southwestern, western and northwestern parts of the country (Tulu, 1993). It bites man predominantly, but also bites domestic animals and feeds both indoors and outdoors. It breeds in the edges of rivers, and in some areas of high population density, along rivers, and they exhibit good vectoral capacity (Fontenille and Lochouarn, 1999).

1.3. Malaria vector control methods

Several vector control methods have been developed in the attempt to control and get rid of diseases transmitting arthropods. There has been a continuous war between man and arthropod vectors and other pests. However, man couldn't completely control vectors and pests. One of the most important reasons might be some of the weaknesses of the control methods developed so far.

The prevention and control of several important vector-transmitted, communicable diseases of man heavily depend on measures for the control of populations of the vectors. Such measures often represent the most successful and the most practical and economical way of disease prevention and control (WHO, 1982). The control measures are usually applied in combination, in a balanced mix of various methods. They are integrated to suit local conditions and needs and resources and to ensure the maximum cost-effectiveness and benefit (WHO, 1982). The commonly used methods of mosquito control are usually classified into chemical methods, biological methods, environmental methods, personal protection methods and genetic methods.

1.3.1. Chemical methods

Chemical control of mosquitoes usually involves two major operations. The first one is larviciding and the second one is adulticiding. Adulticiding is used strictly for supplemental or emergency purposes (Glogoza *et al.,* 2000). Recognition of active transmission of a mosquito-borne disease is an instance of such an emergency case. For example, Glogoza *et al*. (2000) stated that in the North Dakota State Health Department consistently examine levels of arbovirus transmission all over the state and may be contacted for information on the status of disease transmission so as to apply chemicals. A number of pesticides have been registered for use in arthropod vector control.

1.3.1.1. Residual insecticides

In Ethiopia and elsewhere, application of residual insecticides is the major mosquito control method regardless of many problems with their uses. According to Abose *et al.* (1998), indoor residual insecticides have been applied on a selective basis. Yearly assessment is conducted to stratify the localities into three groups based on available epidemiological data. Hence, moderately malarious and highly malarious groups of localities are respectively sprayed once and twice yearly. The third group that are with negligible transmission are exempted from receiving insecticidal application. They also stated that DDT has been used in Ethiopia for more than three decades. In some areas, where the major vector has developed resistance to DDT, malathion is being applied as an alternative insecticide.

Although the development of residual insecticides over the past 40 years provided a relatively simple and inexpensive tool of controlling many of disease vectors, their use has the following limitations:

i) emergence and spread of insecticide resistance in many species,

ii) concern of environmental pollution from inappropriate use of insecticides and

iii) high prices of new chemical insecticides (Ismail and Phinichpongse 1980 and Filnt and den Bosch 1981 cited in Wongsiri and A'ndre 1984).

1.3.1.2. Botanical insecticides

Botanical insecticides are derived from plant products, such as nicotine (from tobacco), rotenone (from the derris plant and cube), and pyrethrins (from certain species of *Chrysanthemum*). These substances have both larvicidal and adulticidal values. The mode of action of these insecticides varies according to their active ingredients (Melman, 1985).

As stated above, the exclusive use of some synthetic organic insecticides is hazardous to the environment. Also, disease vectors and pest species have become physiologically resistant to many of these compounds. As well, these pesticides may affect other beneficial organisms and dangerous to animals including man. Thus, there is an attempt for the development and use of appropriate cost effective and environmentally safe alternative natural insecticides. Since these chemicals are preferably based on renewable resources, they will reduce the economic burden particularly on the third world developing countries. Consequently, there is a growing interest in phyto-chemicals which can act as insecticides. These chemicals do not have any side effect on non-target organisms and are easily degradable as well as they are locally available. As described by Sukumar *et al.* (1991), the search for such compounds has been directed extensively to the plant kingdom certain phyto-chemicals have photo-activated toxins effective against all insect stages while others interfere with the growth and reproduction of disease vectors. The most commonly used botanical insecticides are the pyrethrins, synthetic pyrethrins, rotenone, and d-limonene. These are frequently used in combinations with residual insecticides; the botanical insecticides degrade rapidly but generally give quicker knockdown than residual resources (Melman, 1985).

Now days, many plant-derived products are widely used for their insecticidal or repellent properties for control of mosquitoes or protection from mosquito bites (Sukumar *et al.*, 1991). Phyto-chemicals that are obtained from the enormous diversity of plant species are believed to be important sources for safe and biodegradable chemicals, which can be screened for mosquito repulsive and insecticidal activities and tested for mammalian toxicity (Mittal and Subbarao, 2003 cited in Ansari *et al.*, 2005). A large number of plant products have been reported to have mosquito larvicidal and/or repellent activity against

adult mosquitoes (Ansari *et al.*, 2005). The larvicidal activity of oil extracts of 10 species of plants in 8 genera against *An. stephensi* were tested (Kumar and Dutta, 1987 cited in Mulla and Su, 1999). According to their work *Melia azadirachta* ranked 4th with a LC_{50} of 88.5 ppm, with the range of 63.2-113.0 ppm for all tested plants. Mortality was high in treated larvae, especially during the molting and melanization processes. The LC_{50} for larval mortality was 50 µg/ml in 48 h. The active compound was not identical to AZ, and had greater acute toxic and growth inhibiting effects against *Ae. aegypti* than the AZ-containing fraction (Mwangi and Rembold 1987, 1988 cited in Mulla and Su, 1999).

The essential oils from the Brazilian plants *O. americanum* and *O. gratissimum* have been reported as new sources for larvicidal activity. Essential oils of *Lippia sidoides* Cham. (Carvalho *et al.*, 2003) and *Cymbopogon citratus* were shown to have good potential as larvicides in the later developmental stages of *Ae. aegypti* (Sukumar *et al.*, 1991). Essential oil components such as E-nerolidol and E,E-farnesol; sesquiterpene alcohols; the phenylpropanoids cinnamic aldehyde, eugenol, and safrole; and the monoterpenes 3- and e-pinene are considered as important tools for control of dengue (Simas *et al.,* 2004 cited in Selene *et al.*, 2006).

1.3.2. Environmental management methods

According to Bruce-Chwatt (1985), environmental management is one components of the integrated vector control which in long run is cost effective, environmentally safe and community oriented control strategy. It has been defined as the planning, organization, carrying out and monitoring of activities for the modification and/or manipulation of environmental factors on their interaction with man, with the view of preventing or minimizing vector propagation and reducing man-vector contact (Bruce-Chwatt, 1985).

Communities can decrease the number of mosquitoes in their surroundings by eliminating mosquito-breeding sites on their property. Not only the property but also surrounding areas should be checked reporting any trouble spots to relevant civic authorities. To control mosquitoes that can migrate greater than one-half mile distances,

community action is indispensable (Pitts *et al.*, 2000). The following actions can be taken in environmental management:

i) disposing anything outside that can hold water such as tin, cans, containers, ceramic materials.
ii) drilling holes in the bottoms of recycling containers left outdoors.
iii) cleaning clogged roof gutters every year if leaves plug the drains.
iv) not allowing water to stagnate in birdbaths, ornamental pools, water gardens, and swimming pools or their covers.
v) empty accumulated water from boats and cargo trailers.
vi) alter the landscape of their properties to eliminate standing water.

During warm weather, mosquitoes can breed in any puddle of water. Therefore, eradication of their breeding habitats is important in reducing their reproduction (Pitts *et al.*, 2000).

In Ethiopia, vector control is carried out mainly by means of environmental and chemical measures, either applied singly or in an integrated manner, based on local epidemiological conditions (Abose *et al.,* 1998). In some areas of the country, the community is actively participating in source reduction, with the technical guidance provided by malaria control and other health workers. These efforts have shown satisfactory results particularly in urban centres, settlement villages, army camps and agro-industrial centres.

1.3.3. Biological control

Biological control is defined, as the action of predators, parasites or pathogens in maintaining another organism's population density at a lower average than would occur in their absence (Wongsiri and A'ndre, 1984). According to Wongsiri and A'ndre (1984), a significant result of the support for IPM in Thailand has been the use of the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) as a biological control agent.

Different larval or adult predators are also used to control arthropod vectors. Examples: fish (*Gambusia affinis*, *Poecilia reticulata*, *Oreochromis niloticus*, *Oryzius* spp., *Dermogenys* spp. *Panchax* spp., and *Trichopsis* spp. etc), reptiles (gecko -*Hemidactylus*

frenatus), copepods (*Mesocyclops* spp., *Macrocyclops* spp.), *Toxorhynchites,* and a number of other organisms.

Although biological control is not commonly practiced in Ethiopia, there are some promising activities. Indigenous larvivorous fish have been identified and screened for their feeding efficiency on aquatic stages of Anopheline mosquitoes under laboratory and field settings (Fletcher *et al.,* 1993 cited in Abose *et al*., 1998). Adequate results have been obtained using *Aphanius dispar* in water cisterns and wells (Fletcher *et al.,* 1992 cited in Abose *et al.*, 1998).

Concerning the use of biological method, research has shown that entomopathogens and predators successfully decrease mosquito population densities. However, the practical value of biological control was questioned (Service, 1983 cited in Beidler, 1995) because reductions of mosquito larvae and pupae are usually not immediate (Legner and Sjogren, 1984). The main problems will also be commercial development and registration. For example, biological mosquito control is tiny market so any product must appeal to a lot of us or have other uses (Service 1983 cited in Beidler, 1995).

1.3.4. Genetic methods

Coleman and Alphey (2004) described that genetic control is not a single strategy rather it is a variety of potential techniques that may be subdivided into two main categories: population suppression and population replacement. The common factor to both categories is that the controlling mechanisms are genetic traits introduced into the wild population by mating. According to (Curtis, 2002 cited in Balázs, 2002), the problems of insecticide resistance in some mosquito populations and the difficulties of implementing traditional control methods justify the development of improved control methods like potentially the use of transgenic insects to eradicate wild populations of these vectors of disease.

Sterile Insect Technique (SIT) is probably the most familiar genetic control strategy. It is a species-specific and environmentally non-polluting means of controlling insect

populations. It has a proven track record in eliminating a range of agricultural pests and disease vectors (including the Mediterranean fruit fly, the screwworm fly and the tsetse fly) over large areas (Coleman and Alphey, 2004). SIT involves mass rearing, sterilization and release of large numbers of the sterile males over the target area. The released sterile males then mate with wild females, reducing the reproductive potential of the wild population and so cause a reduction in the wild population in the following generation. If enough sterile males are released continuously, it is obvious that the target population will collapse or even totally eliminates over the release area. The use of transgenesis to improve sterile insect release programs might be accomplished by improving methods for separating the sexes in mass rearing factories so that only males are produced (Curtis, 2002 cited in Balázs, 2002).

Based on the above principles, there was a great optimism in the late 1960s and the early 1970s regarding the development of SIT as a control strategy against mosquito vectors of malaria and other diseases (Curtis, 2002 cited in Coleman and Alphey, 2004). According to Dame (1984), mosquitoes offer a wide variety of challenges as targets for control by sterility techniques, e.g. mass production, product quality, competitiveness and behavioral integrity, assortative mating, isolation, and pre-release suppression of the indigenous population. However, they can be propagated in large numbers, are easily sterilized with chemical sterilants, and can be distributed to natural breeding sites readily. Nevertheless, effective mosquito control will require the cumulative effect of a number of complementary methods.

1.3.5. Insect growth regulators (IGRs)

Mosquitoes' resistance to conventional insecticides is gradually increasing. Thus requirement of higher dosage and unsystematic use of insecticides causes irreversible environmental pollution and also affects the non-target organisms (Baruah and Das, 1996). The search for alternative methods have been tried by several workers, which resulted in selection of IGRs as fourth generation insecticides due to their specificity and selective action (Baruah and Das, 1996).

Siddall (1976) defined an IGRs in terms of its mechanism of action, as a substance which acts within an insect to accelerate or inhibit a physiological regulatory process essential to the normal development of the insect or its progeny, in such a way that the action of the substance is necessarily dependent on the life stage of the insect. IGRs are products or materials that interrupt or inhibit the life cycle of a pest or a vector. If an animal can't reach adulthood, obviously it is not capable of reproducing. Although there are numerous other physiological processes which are essential for the survival of an insect, chemicals such as organophosphates or carbamates which interfere with these other processes are not included. The reason is that they interfere with processes which accompany but do not regulate normal development of the vector.

Insect growth hormones are natural chemicals in insects that control various stages of insect metabolism, morphogenesis and reproduction. They are important in organ development and maturation and the development and growth of larvae in general. Chemical compounds mimic/analogue to IGRs are now available and found quite effective for mosquito control (Amalraj *et al.*, 1988 cited in Baruah and Das, 1996). Baruah and Das (1996) also stated that the biological and environmental dynamics of IGRs had been reviewed and most were found safer than conventional insecticides.

1.3.6. Personal protection methods

In personal protection method, using bed nets and repellents can help to reduce man-vector contact. They create a barrier between a host and the vectors.

1.3.6. 1. Insecticide treated bed nets (ITNs)

Use of bed nets is a very old method of reducing contact between man and vector. It may have been used in the Middle East as early as the 6th century BC (Lindsay & Gibson, 1988). Nevertheless, the investigation of the killing effect of pyrethroid ITNs and materials started in the early eighties (Curtis 1992 in Binka and Adongo, 1997) and has become a center of attention for most malaria control programmes. The World Health

Organization was prompted to fund a trial in northern Ghana. This was due to initial reports of the reduction in malaria-related morbidity in several ITNs studies and other reports in the Gambia of a 63% reduction in child mortality (Choi *et al.*, 1995). The impact of permethrin-impregnated bed nets on child mortality was evaluated in the trial, which was a part of a 4-country multi-centre study under different malaria transmission settings and different intensities of local use of bed nets. Three of these studies which were carried out in different epidemiological and cultural settings have been published, verifying the effectiveness of ITNs with 17, 25 and 33% protective efficacy in Ghana, Kenya and Gambia respectively (Binka *et al.,* 1996; D'Alessandro *et al.*, 1995; Nevill *et al.*, 1996).

In many countries, particularly the developing ones, bed nets are not easily available and under certain circumstances people couldn't afford it. For example in northern Ghana bed nets were not easily available in the local markets, except in a few cases where 'used bed nets' were on sale in bales of second-hand clothing. Even these used bed nets were still found costly to the people (Binka and Adongo, 1997). This and many more can be stated as drawbacks of use of bed nets.

1.3.6.2. Repellents

Repellents are one of the most effective tools that play an important role for protecting humans from vector attack and from vector-borne diseases, such as dengue hemorrhagic fever, malaria, encephalitis, and filariasis etc by reducing man-vector contact (Das *et al.*, 2003; Curtis *et al.*, 1990; Yang *et al.*, 2004). Now days, natural and synthetic insect repellent are well established as means of personal protection against biting arthropods and the different pathogens they may carry (Gupta *et al.*, 1994 and Braverman *et al.*, 2000 cited in Seyoum *et al.*, 2002a).

1.3.6.2.1. Synthetic repellents

According to Yang *et al.* (2004), the most effective and commonly used synthetic mosquito repellent is N, N-diethyl-m-toluamide (DEET). The majority of commercial repellents are prepared by using chemicals like allethrin, DEET, dimethyl phthalate (DMP) and N, N-diethyl mendelic acid amide (DEM) (Das *et al.*, 2003).

According to Barnard (2005), the most desirable characteristics of a chemical repellent intended for use on skin are: non-toxic to humans, non-irritating to skin, activity against a wide range of blood sucking arthropod species, protection from bites for several hours despite arthropod biting pressure, cost and user acceptability. For fabric impregnants, there are additional desirable characteristics: resistance to weathering, washing/laundering, and photodegradation, and long persistence of biological activity.

However, some repellents of synthetic origin may cause skin irritation and affect the dermis. For example, the compound DEET has got some side effects such as an unpleasant odor, damaging plastics and synthetic rubber, and possessing high skin penetration characteristics (Yang *et al.*, 2004).

Rowland *et al.* (2004) described that regardless of the intuitive appeal of synthetic repellents, there had been surprisingly few attempts at demonstrating protection against malaria and no trial has been fully successful. An early study in which DEET was distributed to one member of a pair of Indian villages produced no evidence for a reduction in transmission (Vittal & Limaye 1984 cited in Rowland *et al*., 2004). A study on school children in Tanzania also did not show a significant effect (Curtis *et al.*, 1994 cited in Rowland *et al.,* 2004).

1.3.6.2.2. Mosquito coils and other vaporizers

Mosquito coils are composed of formulations of different insecticides and repellents and are often employed for personal protection to reduce human/vector contact. According to Krieger *et al.* (2003), the annual global consumption of the four major types of residual insecticide products (aerosols, mosquito coils, liquid vaporizers, and vaporizing mats) for

protection from nuisance and disease bearing mosquitoes are in the billions of units. Coils consist of an insecticide/repellent, organic fillers capable of burning with smoldering, binder, and additives such as synergists, dyes, and fungicide.

Mosha *et al.* (1992) tested biological efficacy of new formulations of mosquito coils namely "Tembo-Tox" containing 0.21 w/w pyrethrins and "African Flowers" which contain variable amounts of pyrethrins and plant material from Tephrosia spp. and reported that all were found to be effective, as determined from higher mortality, feeding inhibition and egress rates in treated huts when compared to control huts. Mosha *et al.* (1992) carried out field trials with mosquito coils containing 0.15% Esbiothrin (the registered trademark for the allethrin series pyrethroid with 72% *D-trans* chrysanthemate of *d*-allethrolone as the active ingredient) and reported that a 52% reduction in biting rates of *An. arabiensis* Patton and a 73% reduction of *Cx quinquefasciatus* Say mosquitoes was achieved. They also reported that no mosquito biting activity occurred during the coil burning period which started at 20.00 hours daily and lasted for an average of 8.6h.

On the other hand, this method of vector protection has its own problems like other methods of mosquito control. According to Sharma (2001), researchers are now providing data on the harmful effects of repellents used against mosquitoes. The main site of action of the pyrethroids is the sodium channel. This channel is kept open for long periods of time causing prolonged sodium current to flow that leads to hyper-excitation of the nervous system (Narahashi *et al.*, 1992 cited in Sharma., 2001).

1.3.6.2.3. Plant-based repellents

As described above, synthetic repellents that are used for control of vectors have some side effects. The problems necessitate a search for new and improved repellents and strategies for protection from attacks of mosquitoes and other arthropod vectors. As a result of this, much interest has focused on plant extracts and plant essential oils as potential mosquito and other vector repellent agents. Plants could be alternative sources for mosquito repellents because they constitute a potential source of bioactive chemicals (Yang *et al*., 2004). They are also typically free from harmful effects (Isman 1995 cited

in Yang *et al*., 2004). To avoid the adverse effects of the synthetic repellents, researches on repellents that are derived from plant extracts to replace them have been conducted in many laboratories.

In recent times, extracts of several plants, including neem (*A. indica* A. Juss), basil (*Ocimum basilicum* L., *O. basilicum* L. fa. *Citratum* Bach, *Ocimum gratissimum* L., *Ocimum americanum* L., *Ocimum tenuiflorum* L.), citronella grass (*Cymbopogon nardus* Rendle), galingale (*Alpinia galangal* L.), clove (*Syzygium aromaticum* L.) and thyme (*Thymus vulgaris* L.), have been studied as possible mosquito repellents (Tawatsin *et al.*, 2001). According to Tawatsin *et al.* (2001) the essential oils of citronella grass (*Cymbopogon nardus* Randle) and hairy basil (*Ocimum americanum* L.) are potential repellents of mosquitoes (both oils provided 100% repellency 6 hours post application against *Culex quinquefasciatus*) that could potentially replace DEET.

Trigg and Hili (1996) reported that *p*- menthane-3; 8-diol (PMD) from lemon eucalyptus (*C. citriodora*) which is similar to quwenling was almost as effective as DEET against *An. gambiae* s.l., Gilles and effective against midges, ticks and the stable flies. Quwenling (meaning effective repeller of mosquitoes) is made from the waste distillate after extraction of oil from lemon eucalyptus plant and PMD is its principal active component (Curtis *et al.,* 1990). Trigg (1996) evaluated three formulations of PMD (50% a.i.) (a pump spray, a stick and a gel) and found no significant difference between PMD and DEET both in terms of efficacy ($p > 0.05$) and longevity ($p > 0.05$) of repellency against total anopheline biting for each of the formulations tested. All the repellents tested at the chosen dose rate provided greater than 6 hr protection from biting. Citronella oil is popular oil in India and is one of the few natural products which are used in commercial repellents in Europe and North America (Curtis *et al.,* 1990).

1.3.6.2.4. Traditional use of repellents

People used acaricidal and insecticidal plants traditionally to protect from blood-feeding arthropods (Duke, 1987; Moore and Lenglet, 2004 cited in Jaenson *et al.*, 2006). The repellent properties of plants to mosquitoes and other insect pests were well known before the beginning of synthetic chemicals. Traditional repellents are used widely among different societies and very common in many communities (Curtis *et al.*, 1990). The plant products have been used to repel or kill mosquitoes in many parts of the world by tradition. Emphasis was given to the urgent need for the investigation of phyto-chemicals as repellents for mosquito control (Novak, 1985 cited in Das *et al.*, 2003).

Herbs of the basil family (Labiatae) are used as mosquito repellents and traditional medicines in East and West Africa (Kokwaro 1976; Curtis *et al.,* 1990). Branches of *Ocimum suave* are often placed around the windows and doors in Tanzania to repel mosquitoes (Chogo and Crank, 1981). In northern Ghana, there is a wide use of traditional methods of mosquito control such as burning green or dried leaves of special plants and burning by-products from the production of shear butter oil to generate smoke, and using fresh strong-scented leaves arranged around beds to repel mosquitoes (Binka and Adongo, 1997). Plants such as *Ocimum forskolei, Nicotiana glauca, Salvia shimperi, Otostegia integrifolia, Neorautanenia mitis,* and *Calpurnea aurea* were traditionally used against vectors by the societies in the highland eastern escarpment and wet lowland of Eritrea. The first two were used against mosquitoes, the next two against fleas and the last two against animal lice (Waka *et al.,* 2004). However, the people didn't differentiate whether their effect was repellency or insecticidal, because their primary interest was the absence of biting, whether the insect was killed or driven away as a secondary matter. Of course, as Waka *et al.,* (2004) described, it is very difficult to differentiate without laboratory cages and field facilities to exclude scavenging ants etc. As described by Palsson and Jaenson (1999), two plants, i.e. *Hyptis suaueolens* Poit. (Lamiaceae) and *Daniellia oliveri* Roife (Caesalpiniaceae) are traditionally used to reduce numbers of mosquitoes indoors at night. In Guinea Bissau and Gambia many people utilize the tree *D. oliveri* by burning its bark and resins in order to reduce biting activity indoors.

There is also a wide spread belief in the lay community in the ability of garlic, a member of lily family, to repel insects. Regardless of these popular beliefs, there are few publications

documenting the efficacy of garlic (Rajan *et al.,* 2005). Rajan *et al.* (2005) also reported that the data in their study did not provide evidence of systemic mosquito repellence of garlic.

Simple and highly adaptable traditional methods are also being practiced to repel the house entry mosquitoes in different rural communities of Africa. These traditional methods include direct burning of plant materials and bruising the leaves of the plants and hanging them around the bed. In Kenya, plants such as *Ocinum americanum*, *Lantana camara* and *Lippia ukambensis* and *Corymbia citriodora* (Lemon eucalyptus) and leaves and seeds of *Ocimum kilimandscharicum* are traditionally used by these methods (Seyoum *et al.,* 2003). Seyoum *et al.* (2002a) tested different local plants against *An. gambiae* s.s. in Kenya and reported that thermal expulsion of leaves and seeds of *O. suave* and *Ocimum kilimandsharicum* and leaves of *C. citriodora* were effective repellents. Direct burning of leaves of *C. citrodora* also resulted in repellency against the test mosquito. They also reported that when the possible effects due to other factors such as the arrangement of huts, collectors, time of collections and replicates were taken into account, *0. americanum*, *L. camara*, and *L. uckambensis* were all found to be effective repellents and reduced domestic exposure by 30-40 %. Combining *0. americanum* with either *L. camara* or *L. uckambensis* was similarly effective in semi-field experimental huts (Seyoum *et al.*, 2002b). The whole potted plants of *O. americanum,*and *L. camara* showed considerable repellency against *An. gambiae* s.s. under natural field condition (Seyoum *et al.*, 2003). Waka *et al.* (2004) reported 53 % reduction in mean number of mosquitoes per house by testing the effect of the fresh leaves and shoots of *O. forskolei* by hanging them on walls at the head and foot of beds. They also found that the appropriate doses in $\mu l/cm^2$ for 50 % repellency with the extracts of *S. schimperi, N. glauca,* and *O. integrifolia* were 0.97, 1.72 and 2.44 respectively.

According to Curtis *et al.* (1990), traditional practices and repellents can give real protection against biting and even less malaria incidence was indicated by Philip *et al.* (1947) (cited in curtis *et al.,* 1990) in parts of India. They associated lower spleen indices among women who practice smearing their bodies with turmeric and gingili or mustard oil before bathing without soap. Men who did not do this were found to be more frequently bitten by *An.*

fluviatilis than women sleeping nearby. Likewise, a hand smeared in this way was not bitten by caged mosquitoes which fed intensely on an untreated hand.

Consequently, assessment and knowledge of traditional repellents is desirable. Moreover, these are the methods that people use without persuasion from scientific experts. Freely available method, but of smaller benefit may be more useful than the one which is more effective, but unaffordable (Curtis *et al.,* 1990). The other reason given is that an expert's advice about a new method will be far more convincing if he describes his tests as compared to a traditional method with which his audience is familiar.

1.3.7. Integrated vector management

According to WHO (1983a), integrated vector control has been defined as "the utilization of all appropriate technological and management techniques to bring about an effective degree of vector suppression in a cost-effective manner". The control methods depend on local problems and have to be worked out on an area-to-area basis. It reduces reliance on insecticides emphasizing on other vector control measures in an integrated manner (WHO, 1983b). Even though a combination of two or more methods is more complicated both operationally and technically as compared to a single method, it is necessary and may provide the desired degree of control at a lower cost or with a greater long-term benefit (Bang, 1985). The word "integrated" was stated to mean to form into a whole, to unite, to incorporate into a large unit. It conveys the idea of a judicious interconnection of parts into a whole, not simply the use of several parts. The idea of the integrated vector management is based on ecological principles and integrates multi-disciplinary methods in developing ecosystem management strategies that are practical, effective, economical and protective of both public health and the environment. A sound knowledge of vector population dynamics, natural mortality factors and risk factors for disease transmission are essential for implementation of integrated disease vector control method.

Current IPM has developed primarily in line with an agricultural model (Wongsiri and A'ndre, 1984). According to Bang (1985), although the concept has been there for a long

time, it has been ignored because of the use of DDT as the basis of operational programs. The requirement for integrated vector control is the availability of more than one method of control that should be applied simultaneously or consecutively (WHO, 1983a; 1983b). The operational unit for each method will depend on level of stratification and selectivity in the use of the methods (WHO, 1995).

According to WHO (1979), there is general agreement that insecticides are now and will continue to be primary tools of anti-malaria programs for the predictable future and regulation and rational use is essential within the context of integrated control. Phyto-chemicals that are obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Plant products can be used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites depending on the type of activity they possess (Mittal and Subbarao, 2003 cited in Ansari *et al.*, 2005).

As discussed above, chemical insecticides (mainly DDT and sometimes malathion) and environmental management are the main vector control measures in Ethiopia. Currently, ITNs are also being popularized and used as anti-malarial activities. Ethiopia is now aiming at 100% ITNs coverage at the end of 2007. Plant-based products which are believed to be safer than conventional insecticides are not widely used. Of course, it is an active research area now days. This research is intended to contribute to fill the gap of utilizing the natural products in integrated vector control in the country and else where.

2. OBJECTIVES OF THE STUDY

❖ General objectives

To evaluate the impact of traditional application of selected mosquito repellent plants (*C. citriodora, Eucalyptus camaldulensis*, *O. suave*, and *O. basilicum*) in the reduction of the human-vector contact of malaria vectors and culicine nuisance mosquitoes in Ethiopia.

❖ **Specific objectives**

i) To evaluate the repellent action of thermal expulsion of *C. citriodora, E. camaldulensis*, *O. suave*, and *O. basilicum* against *An. gambiae* s.l., *An. pharoensis* and *Culex* spp. in Ethiopia.

ii) To evaluate the repellent action of direct burning of *C. citriodora, E. camaldulensis*, *O. suave*, and *O. basilicum* against *An. gambiae* s.l., *An. pharoensis* and *Culex* spp in Ethiopia.

iii) To compare the effectiveness of the two application methods (thermal expulsion and direct burning) in reducing man-vector contact.

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Koka Negewo kebele (Shulki village, 8° 25' N 39° 01' E) which is located in Oromia (East Shewa Zone). It is about 94 km south of Addis Ababa where the Awash river crosses the main road to Awassa at an altitude of 1700 m above sea level. Awash river over floods and retreats during the rainy season leaving temporary accumulated water that remains for much of the dry season. This and irrigation agricultural activities give rise to the breeding sites of mosquitoes in the area. The annual rainfall varies from 72 to 85 mm and falls in two main seasons (National Meteorological Service Agency, 2006). The long rains occur in June to August while short rains are experienced in February/ March with the mean annual temperature of about 21 ^{o}C.

The area is one of the lowland malarious areas where irrigation farming is carried out and mostly tomatoes, onions, peppers and other vegetables are produced. Farming of crops such as maize, millet and teff are the main economic activities. These crops are grown for local consumption. Fishing is also widely practiced as a source of income. Livestock keeping is carried out on small-scale basis and the animals reared include sheep, goats, and cattle. Currently, there are vast flower farming activities and flower farming enterprises in the area. Some inhabitants are employed in the enterprises running the activities of flower farming. These economic activities (Plate 1a) attract many people from the local and surrounding areas hence are at risk of malaria infection.

The village was selected due to its proximity to Addis Ababa, availability of the test plants in the surrounding areas and the presence of mosquitoes that involve in malaria transmission. The experiments were undertaken from the first week of January to the second week of June 2007. This study is part of a bigger project on malaria and its control by traditional means which is supported by WHO/AFRO region.

(b)

(a)

Plate 1. Some economic activities of Koka area (a) and one of the study houses (b)

3.2. Description of test plants

***Eucalyptus camaldulensis* Dehnh. (Myrtaceae; Red river gum, Key bahirzaf (Amh.), Barzafi dima (Orom.))**

This is a tree originally cultivated at Camalduli, Naples, Italy (Friis, 1995). It is usually 20 m high, sometimes reaching 40 m. Its bark is smooth throughout, white, grey, and brown or red. This tree is one of the early introduced species of *Eucalyptus* and recorded by Breitenbach (1961) (cited in Friis 1995) as widely cultivated throughout Ethiopia and Eritrea in the Italian colonial period. This plant was collected from shulki village (the study area). 10 g of its leaves were weighed at a time during day time and kept in six different plastic bags for the treatments that were going to be done during the night time.

In this study evaluation of its repellent activity against the major mosquito vectors has two main reasons. Firstly, its essential oils were found to be efficient repellents. Secondly,

related plants exhibit similar properties as in the case of a member of the family Lamiaceae (http://www.ansci.cornell.edu/plants/medicinal /basil.html). Since *C. citriodora* was reported to be good repellent and *E. saligna* is traditionally used (Seyoum *et al.,* 2002a) it was suspected to have similar effect. Thus, it was selected for evaluation though the traditional use of this plant as a repellent against biting insects was not recorded.

***Corymbia citriodora* Hook. (Myrtaceae; Lemon eucalyptus, Shito bahirzaf (Amh.))**

This is a tree 25-40m high. Its bark is mostly smooth throughout, often mottled, pale-grey, powdery, sometimes blue-grey and somewhat thinly flaky on older trunks (Friis, 1995). It was successfully planted at Alemaya (Adugna Zerihun, 1981 and Ibrahim, 1986 cited in Friis, 1995) and at Wendo Genet (Michelson, 1992 cited in Friis, 1995). It was widely planted but succeeded only in places with deep soil and rainfall over 1000mm (Friis, 1995). This plant was collected from Wendo Genet Essential Oil Research Institute. 10 g of its leaves were weighed at a time during day time and kept in six different plastic bags for the treatments that were going to be done during the night time.

Various works indicated its efficacy against different mosquito species. Since it has repellence effects against biting insects and its effect may vary depending on differences in geographical areas (places) like some other plants (Curtis *et al.,* 1990), evaluating its effectiveness in this area was found to be necessary.

***Ocimum basilicum* L. (Lamiaceae; Basil, Besobila (Amh.))**

O. basilicum is a dense woody shrub, reaching heights of 1-2 meters. Its leaves are pale green, lanceolate and flowers are white or pink in whorls in the axils of leaves, upper lobe of calyx rounded and spreading. It is distributed in India, Southeast Asia and Northeast Africa; Sennegal to Southern Nigeria in habitat (http://www.fao.org/docrep/x2230e /x2230e09.htm). This plant was obtained from Shashamane area. 10 g of its leaves were weighed at a time during day time and kept in six different plastic bags for the treatments that were going to be done during the night time.

O. basilicum was chosen for evaluation due to its wide use as a traditional repellent and medicine. This and related plants in the genus *Ocimum*, as well as their oils, have attracted lots of attention for their potential medicinal properties.

***Ocimum suave* L. (Lamiaceae; Dama-Kasseh (Amh.), Anchabi (Orom.))**

As it is already stated, this family consists of many medicinal herbs. *O.* suave is a branched, erect shrub to 1 m in height. It is widely distributed in habitat including Guinea to West Camaroons and generally across Africa to East Africa into tropical Asia and also widespread on open waste areas (http://www.fao.org/docrep/x2230e/x2230e09.htm). It is common in the highland forest areas of East Africa.
This plant was collected from Wendo Genet Essential Oil Research Institute. It is widely used as traditional repellent and medicine. 10 g of its leaves were weighed at a time during day time and kept in six different plastic bags for the treatments that were going to be done during the night time.

This plant was chosen due to various reports on its wide use as traditional repellent and medicine. Even though it has been tested in different areas, it was selected in this study for the chances of variation depending on geographical areas as described by Curtis *et al.* (1990).

Mosquito coils (Positive control)

Mosquito coils are composed of formulations of different insecticides and repellents and are often employed for personal protection and to reduce human/vector contact. Different researchers reported that different formulations of mosquito coils are effective in reducing human exposure to endophilic mosquitoes (Mosha *et al.,* 1992). In this research the coil was used as a positive control so as to compare its effects with that of the test plants. In fact it was not used in the first two blocks of the experiment because it was not

available on market during the initial studies. The coil was used to widen the possibility of comparing the effects of the test plants with its effects as a standard fumigant repellent. Later in the study, it was available in one of the local stores in Addis Ababa. It contains allethrin as active ingredient (Greek product).

Plate 2. A picture of mosquito coil taken at Shulki village

3.3. Small-scale repellency tests by direct burning and thermal expulsion

Ten homesteads were selected for the field tests of the candidate plants by thermal expulsion and direct burning. Later on, after two blocks of the experiment, one other house was added for mosquito coil treatment. The mosquito coil was not available in the market during the initial studies. All experimental houses were mud-walled with grass-thatched roofs in which open eaves, unscreened small holes and doors allow ready access to mosquitoes. An open eave was one of the architectural features that enable vectors of malaria to enter houses. The distances between huts were varied from approximately 50 to 100 meters. Ten huts, which are at least 50 m far apart, were selected from Shulki village of Koka Negewo Kebele in the first two blocks. Five huts were used for direct burning test and the rest five were used for thermal expulsion test. As discussed earlier,

one hut was added for mosquito coil application as a positive control in the third and fifth block of thermal expulsion and in the fourth block of direct burning. Untreated bed nets were distributed to the hut owners.

The plant leaves were placed on the top of thin metal plates that were placed directly above the charcoal in a traditional stove (thermal expulsion) and the plant leaves were placed directly on the charcoal (direct burning) according to the work of Seyoum *et al.* (2002a) the houses were fumigated by applying fresh plant material every hour from 18:00 – 24:00 hrs (mid-night) local time. Each hour, 10 g of pre-weighed plant materials were placed on the thin metal plate on the traditional stove in thermal expulsion (Plate 3a) and directly on the burning charcoal in direct burning (Plate 3b) for fumigation of individual huts. Three hundred gram of charcoal was used to light the traditional stoves followed by additional 150 g of charcoal every hour. A five by five Latin square design was used for each thermal expulsion and direct burning of plant materials in five treatment huts, one hut with only burning charcoal stove without plant material (negative control) in the first two blocks. One hut was added on the thermal expulsion method in third and fifth blocks and on the direct burning method in the fourth block of the study for mosquito coil treatment as a positive control. Therefore, three six by six Latin square designs were done. The huts were randomly assigned for the candidate plants and the control on the first sampling night. The treatments and the control were then assigned by rotation in consecutive sampling nights in different huts to compensate potential spatial variations of mosquito density in individual huts selected for experimentation.

(a) (b)

Plate 3 .Pictures showing thermal expulsion (a) and direct burning (b) with battery operated CDC light traps and untreated bed nets with persons inside.

CDC miniature light traps (model 512, John W. Hock Company, USA) that were set close to occupied untreated bed nets inside bedrooms were used for sampling mosquitoes. Each light trap was operated on a 6 V 10 Ah battery and fitted with a 150 mA bulb (6.3 V) and a lid. The traps were positioned at the foot end of the bed (Fig. 3) (Mboera *et al.,* 1998). Traps were operated between 18:00 hr and 24:00 hr (midnight). The collection bags were then collected at 24:00 hr (midnight) for the experiments by direct burning and thermal expulsion, to evaluate the impact on mosquito density during fumigation of individual huts. The mosquitoes were identified to species level using morphological characteristics (Verrone, 1962; Gillies and Coetzee, 1987). The tests were carried out with an intervening period of one night to avoid potential residual effect of the plants by periodic thermal expulsion and direct burning. The tests were replicated for 27 nights (five completed blocks) for thermal expulsion and 26 nights (five completed blocks) direct burning. Blocks of five or six nights run during different seasons. Thus, each treatment or control was assigned five or six times to all experimental huts for both methods.

3.3. Data analysis

Percentage repellency (%R) was calculated based on the formula described by Sharma and Ansari (1994) for each test in each blocks of the study.

%R= C-T/C*100 , where

-C = the number of mosquitoes collected in control hut and

-T = the number of mosquitoes collected in treatment hut

A three way analysis of variance (ANOVA) was done using stata/SE 8.2 for all blocks of the study to observe the over all effects of the treatments in all seasons (blocks).The over all effects of repellent plants were analyzed by allowing for the differences between experimental units by generalized linear modeling of the relationship between mosquito collections in control (C) and in treatment huts (T). The effects of treatment repellency (R) and the effects of different household experimental units (E) were modeled as follows as described by Seyoum *et al.* (2003):

$T = (1-R)\ E.C$…… (1)

Since mosquito collections are usually highly aggregated and vary over wide ranges (Smith 1995 cited in Seyoum *et al.,* 2003) they are best expressed in logarithmic form to minimize heterogeneity of variances for generalized linear modeling. The data were standardized using ln (x+1) to enable the inclusion of nights with zero collections. This was done to minimize differences in the population density of mosquitoes in time. When significant differences were observed, the means were separated using Tukey's test. Therefore, to all counts one was added to enable the inclusion of nights with zero collections, assume T + 1 = T and C + 1 = C and ln transform both sides of equation (1).

$\ln T = \ln(1 - R)\ E.\ C = \ln(1 - R) + \ln E + \ln C$……(2)

which can be fitted to a generalized linear model of the form:

$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$ …… (3)

Where, β_1 represents the effects of different repellent plants, β_2 represents the effects of different experimental units (huts and residents), and β_3 represents the equivalence of collections where treatment and experimental unit effects are negligible. Therefore, replicate or sampling night was treated as a randomly varying factor determining overall collection size and estimated values for β_1 and β_2 as fixed factors that influence this outcome. Substituting β_1 into the first term of equation 2 and rearranging allows calculation of proportional repellency as follows:

$$\mathbf{R = 1 - e^{\beta_1}}$$, where R= proportion repellency

e = base of natural logarithm

β_1= Coefficient of the treatments

4. RESULTS

4.1. Mosquito collection

A total of 17,895 mosquitoes were collected by CDC light traps which were set close to occupied untreated bed nets from all blocks of the experiment. Of these 13,824 (77.25 %) were *An. gambiae* s.l., 1,973 (11.03 %) were *An. pharoensis* and 2,098 (11.72 %) were culicine mosquitoes.

4.2. Repellency status of the test plants in the five by five Latin square experimental design of the study without the positive control (coil)

There were four blocks of direct burning and three blocks of thermal expulsion experiments that were done by five by five Latin square design without coil. Records in all blocks were pooled together for analysis to see the over all effects of the treatment plants. The numbers of mosquitoes collected in each block and the corresponding percent repellencies are presented in the appendices 2-10.

4.2.1. Repellency status of the test plants in the first (January), second (March), third (April) and fifth (June) blocks of the study by direct burning

In the five by five Latin square experimental set ups of the direct burning methods which was analyzed for blocks one, two, three and five, all the test plants significantly reduced the number of *An. gambiae* s.l, *An. pharoensis,* and culicines ($p<0.05$). *O. basilicum* can be ranked as the most effective repellent plant of all the test plants against *An. gambiae* s.l. when they are compared based on percent repellency (73.11%, $p<0.001$) followed by *O. suave* (71.51%, $p<0.001$), *C. citriodora* (70.59%, $p<0.001$) and *E. camaldulensis* (65.29%, $p<0.001$) (Table 1). However, *C. citriodora* was found to show the highest repellency (72.87%, $p< 0.001$) against *An. pharoensis*. Nevertheless, these plants did not exhibit significant differences among themselves regardless of variations in their percent repellencies.

Table 1. Repellency status of the test plants by direct burning (blocks- one (January), two (March), three (May) and five (June) of the experiment)

	Test plant species	The mosquito species	Number collected*	Parameter estimate of treatments (β**± SE)	Repellency percentage (95% CI)	p- value***
1	*C. citriodora*	*An. gambiae* s.l.	485	-1.224 ± 0.1845	70.59 (57.55, 79.62)	<0.001
		An. pharoensis	94	-0.752 ± 0.2049	72.87 (59.23, 81.95)	<0.001
		Culicines	99	-0.291 ± 0.2554	55.91 (26.74, 73.47)	0.002
2	*O. suave*	*An. gambiae* s.l.	498	-1.257 ± 0.1845	71.51 (58.95, 80.30)	<0.001
		An. pharoensis	111	-1.193 ± 0.2049	69.66 (54.40, 79.81)	<0.001
		Culicines	86	-0.742 ± 0.2554	52.36 (20.83, 71.33)	0.005
3	*O. basilicum*	*An. gambiae* s.l.	514	-1.313 ± 0.1845	73.11 (61.11, 81.37)	<0.001
		An. pharoensis	112	-1.205 ± 0.2049	70.03 (54.96, 80.06)	<0.001
		Culicines	105	-0.903 ± 0.2554	59.46 (32.63, 75.61)	0.001
4	*E. camaldulensis*	*An. gambiae* s.l.	605	-1.058 ± 0.1845	65.29 (49.90, 75.95)	<0.001
		An. pharoensis	118	-1.097 ± 0.2049	66.60 (59.23, 81.95)	<0.001
		Culicines	86	-0.785 + 0.2554	54.37 (24.17, 72.54)	0.003
5	Control (charcoal)	*An. gambiae* s.l.	1318	-	-	-
		An. pharoensis	428	-	-	-
		Culicines	231	-	-	-

SE= Standard error
*= Total number of mosquitoes collected during the twenty test nights
**= Coefficient of the treatments
***= P-value obtained from Tukey's studentised range test at α= 0.05

4.2.2. Repellency status of the test plants by thermal expulsion

In five by five experimental set ups of thermal expulsion (blocks one, two and four) similar results were obtained except that *C. citriodora* and *O. basilicum* showed similar repellency effects (78.69%, $p<0.001$; 78.66%, $p<0.001$, respectively) followed by *O. suave* (73.55%, $p<0.001$) and *E. camaldulensis* (71.91%, $p<0.001$) against *An. gambiae* s.l. as indicated in Table 2. Similar trend was observed in *An. pharoensis,* and culicines except that *E. camaldulensis* was found to exhibit the highest repellency (55.92%, $p=0.004$) next to *C. citriodora* (56.52%, $p = 0.003$) against culicines.

The differences among all the treatment plants were not statistically significant when compared for repellency against all the test mosquitoes ($p>0.05$)

Table 2. Repellency status of the test plants in the total of blocks one (January), two (March) and four (May) of the experiment by thermal expulsion

	Test plant species	The mosquito species	Number collected*	Parameter estimate of treatments (β**± SE)	Repellency percentage (95% CI)	p- value***
1	*C. citriodora*	*An. gambiae* s.l.	244	-1.546 ± 6.782	78.69 (66.83, 86.30)	<0.001
		An. pharoensis	39	-1.460 ± 0.735	76.76 (60.76, 86.24)	<0.001
		Culicines	41	-0.833 ± 1.333	56.52 (25.41, 74.65)	0.003
2	*O. suave*	*An. gambiae* s.l.	294	-1.330 ± 7.905	73.55 (58.84, 83)	<0.001
		An. pharoensis	43	-1.389 ± 0.850	75.06 (57.88, 85.23)	<0.001
		Culicines	42	-0.398 ± 0.846	32.81 (-15.26, 60.83)	0.146
3	*O. basilicum*	*An. gambiae* s.l.	219	-1.544 ± 5.880	78.66 (66.79, 86)	<0.001
		An. pharoensis	29	-1.568 ± 0.492	79.15 (64.78, 87.66)	<0.001
		Culicines	43	-0.706 ± 0.951	50.61 (15.28, 71.21)	0.011
4	*E. camaldulensis*	*An. gambiae* s.l.	330	-1.270 ± 9.091	71.91 (56.29, 81.95)	<0.001
		An. pharoensis	45	-1.281 ± 0.697	72.22 (53.07, 83.55)	<0.001
		Culicines	37	-0.819 ± 1.055	55.92 (24.39, 74.31)	0.004
5	Control (charcoal)	*An. gambiae* s.l.	1220	-	-	-
		An. pharoensis	199	-	-	-
		Culicines	109	-	-	-

SE= Standard error
*= Total number of mosquitoes collected during the fifteen test nights
**= Coefficient of the treatments
***= P-value obtained from Tukey's studentised range test at α= 0.05

4.3. Repellency status of the test plants in the six by six Latin square experimental design of the study with positive control (coil)

There is one block (block four) for direct burning and two blocks (blocks three and five) for thermal expulsion which were done with a six by six design. The results obtained are presented below.

4.3.1. Repellency status of the test plants in the third (April) and fifth (June) blocks of the experiment by thermal expulsion

In the six by six Latin square experimental design of thermal expulsion (blocks three and five), mosquito coil was found to be the most effective repellent (81.72%, $p < 0.001$) of all the treatments against all the test mosquitoes as indicated in Table 3. Although the differences in percentage repellencies obtained by the coil and the treatment plants were high, they were not statistically significant ($p > 0.05$).

Table 3. Repellency status of the test plants in the total of blocks three (April) and five (June) of the experiment by thermal expulsion

	Test plant species	The mosquito species	Number collected*	Parameter estimate of treatments (β**± SE)	Repellency percentage (95% CI)	p- value***
1	*C. citriodora*	*An. gambiae* s.l.	431	-1.188 ± 0.2708	69.53 (47.55, 82.29)	<0.001
		An. pharoensis	59	-1.198 ± 0.3062_	69.82 (44.26, 83.66)	<0.001
		Culicines	164	-1.021 ± 0.3745	63.97 (19.66,82.99)	0.009
2	*O. suave*	*An. gambiae* s.l.	468	-1.016 ± 0.2708	63.80 (37.67, 78.95)	<0.001
		An. pharoensis	69	-1.162 ± 0.3062	68.71 (42.21,83.06)	<0.001
		Culicines	202	-0.867 ± 0.3745	57.96 (10.95,80.15)	0.024
3	*O. basilicum*	*An. gambiae* s.l.	508	-1.014 ± 0.2708	63.71 (37.56,78.91)	<0.001
		An. pharoensis	72	-1.023 ± 0.3062	64.05 (33.57,80.53)	0.002
		Culicines	210	-0.714 ± 0.3745	51.05 (-3.68,76.89)	0.062
4	*E. camaldulensis*	*An. gambiae* s.l.	595	-0.802 ± 0.2708	55.14 (22.83,73.93)	0.005
		An. pharoensis	80	-1.070 ± 0.3062	65.70 (36.64, 81.43)	<0.001
		Culicines	187	-0.969 ± 0.3745	62.07 (19.66,82.09)	0.049
5	Coil (+ve control)	*An. gambiae* s.l.	376	-1.700 ± 0.2708	81.72 (68.56,89.35)	<0.001
		An. pharoensis	30	-1.781 ± 0.3062	83.14 (68.86,90.87)	<0.001
		Culicines	64	-1.572 ± 0.3745	79.24 (56.02,90.20)	<0.001
6	Control (charcoal)	*An. gambiae* s.l.	1097	-	-	-
		An. pharoensis	185	-	-	-
		Culicines	298	-	-	-

SE= Standard error
*= Total number of mosquitoes collected during the twelve test nights
**= Coefficient of the treatments
***= P-value obtained from Tukey's studentised range test at α= 0.05

4.3.2. Repellency status of the test plants in the fourth (May) blocks of the experiment by direct burning

In the six by six Latin square design of direct burning (block 4), all the test plants exhibited significant repellency. Mosquito coil was found to be the most effective repellent (82.09%, $p = 0.001$) of all the treatments against all the test mosquitoes as indicated in Table 4. The differences between the effects of the coil and that of the test plants were not statistically significant ($p>0.05$) though there were high differences in percentage repellency.

Table 4. Repellency status of the test plants in the fourth block (May) of the experiment by direct burning

	Test plant species	The mosquito species	Number collected*	Parameter estimate of treatments (β**± SE)	Repellency percentage (95% CI)	p- value***
1	*C. citriodora*	*An. gambiae* s.l.	651	-1.022 ± 0.2640	64.01 (37.58, 79.25)	0.001
		An. pharoensis	31	-0.967 ± 0.4546	61.98 (1.80, 85.26)	0.046
		Culicines	11	-0.831 ± 0.350	56.46 (9.64, 79.01)	0.028
2	*O. suave*	*An. gambiae* s.l.	660	-0.808 ± 0.2640	55.44 (22.71, 74.31)	0.006
		An. pharoensis	33	-1.361 ± 0.4546	74.36 (33.81, 90)	0.007
		Culicines	11	-0.929 ± 0.350	60.52 (18.07, 80.97)	0.015
3	*O. basilicum*	*An. gambiae* s.l.	660	-0.967 ± 0.2640	61.99 (34.06, 78.08)	0.002
		An. pharoensis	31	-0.8650 ± 0.4546	57.89 (-8.69, 83.68)	0.072
		Culicines	10	-0.947 ± 0.350	74.36 (33.34, 77.84)	0.014
4	*E. camaldulensis*	*An. gambiae* s.l.	660	-0.956 ± 0.2640	61.21 (19.50, 81.30)	0.002
		An. pharoensis	35	-1.182 ± 0.4546	69.34 (20.86, 88.12)	0.017
		Culicines	13	-0.851 ± 0.350	57.30 (11.40, 79.42)	0.025
5	Coil (+ve control)	*An. gambiae* s.l.	441	-1.320 ± 0.2640	73.30 (53.68, 84.60)	<0.001
		An. pharoensis	20	-1.720 ± 0.2640	82.09 (53.76, 93.06)	0.001
		Culicines	10	-0.929 ± 0.350	60.52 (18.07, 80.97)	0.015
6	Control (charcoal)	*An. gambiae* s.l.	1550	-	-	-
		An. pharoensis	110	-	-	-
		Culicines	39	-	-	-

SE= Standard error
*= Total number of mosquitoes collected during the six test nights
**= Coefficient of the treatments
***= P-value obtained from Tukey's studentised range test at α= 0.05

4.4. Comparison of the two test methods

The application methods, direct burning and thermal expulsion were compared in this study by using the two blocks (one and two) which were done in the same seasons. No significant difference was observed between them ($p>0.05$ for all the test mosquitoes). However, it can be said that thermal expulsion is better than direct burning when they are compared with each other in terms of percent repellencies (Fig. 1).

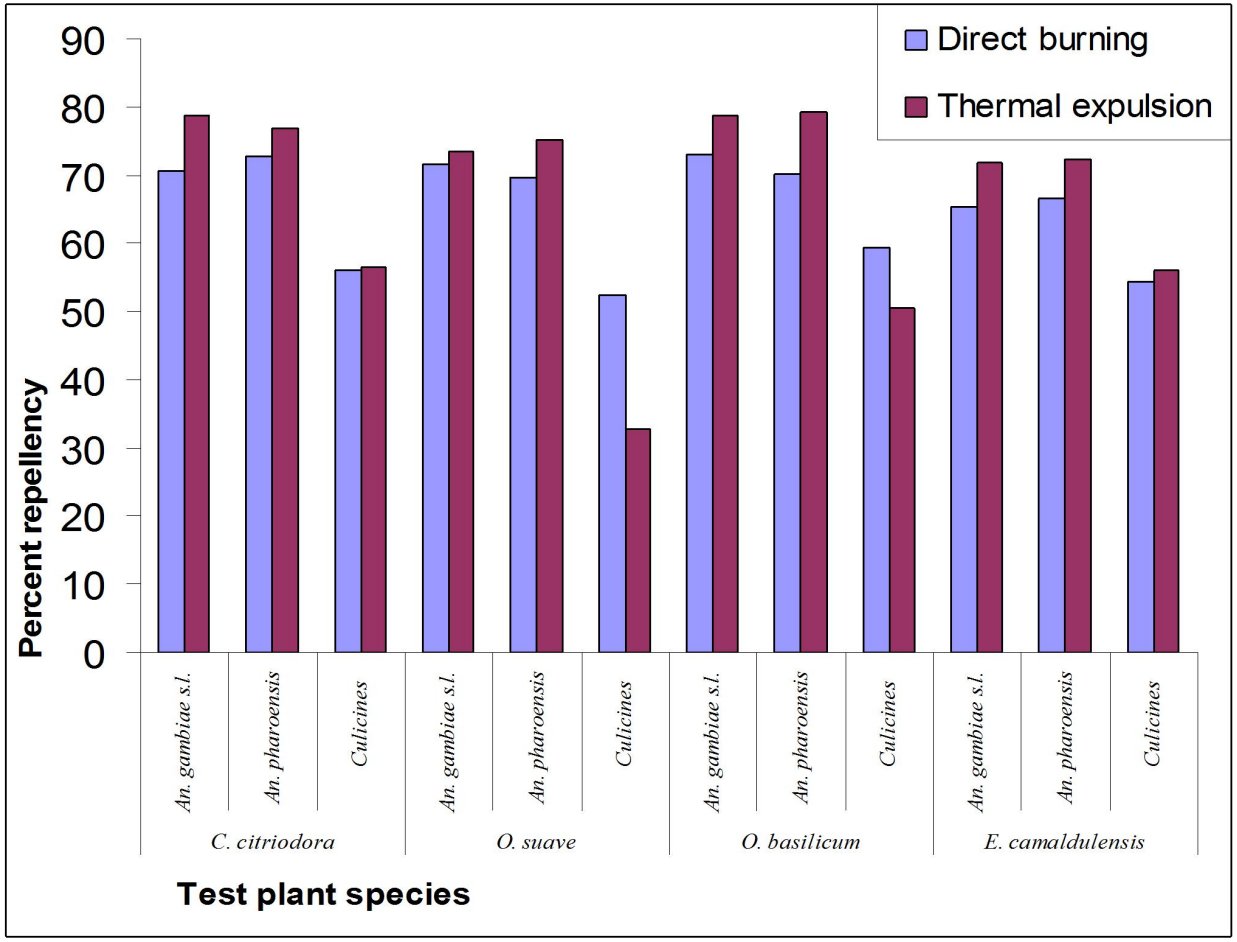

Fig. 1. Comparisons of direct burning and thermal expulsion

5. DISCUSSION

In this study, the repellent efficacies of the selected plants against *An. gambiae* s.l., *An. pharoensis* and culicines were evaluated by direct burning and thermal expulsion under natural field conditions. The results of this study obviously indicate that the test plants have different levels of protection against the different mosquito species though they did not show significant level of difference among themselves. In most of the treatments, the plants exhibited significant repellent effects against the test mosquitoes (*An. gambiae* s.l, *An. pharoensis* and culicines).

O. basilicum was found to be the most efficacious of all the test plant species followed by *O. suave, C. citriodora* and *E. camaldulensis* against *An. gambiae* s.l. by direct burning method (five by five Latin square design). *C. citriodora* followed *O. basilicum* in repelling culicines and followed by *E. camaldulensis*. *C. citriodora* was found to have higher percentage repellency than *O. basilicum* against *An. pharoensis.*.

O. basilicum was also found to exhibit the highest percentage repellency with more or less similar result with *C. citriodora* followed by *O. suave* and *E. camaldulensis* against *An. gambiae* s.l. by thermal expulsion method. *O. basilicum* also exhibited the highest percentage repellency against *An. pharoensis* when thermally expelled followed by *C. citriodora, O. suave* and *E. camaldulensis*. *C. citriodora* exhibited the highest efficacy against culicines in the thermal expulsion experiment. *E. camaldulensis* and *O. basilicum* were found to be the next effective plants. *O. suave* did not show significant result against culicines when thermally expelled. Even though *E. camaldulensis* was found to significantly reduce the number of *An. gambiae* s.l. and *An. pharoensis* that would enter the treatment houses in its absence, it was found to be the least effective of all the test plants when ranked by percent repellencies and levels of significances. Although there are reports that thermal expulsion is a better application method (Seyoum *et al.*, 2002a; Atsbeha, 2005), it did not show significant difference from direct burning in the present study. But it can

generally be said that thermal expulsion was better than direct burning when they are compared depending on percent repellencies. Thermal expulsion of the leaves of the test plants were found to be better than their direct burning, because the leaves smoke slowly, and the smoke remain longer than direct burning.

In the tests that were carried out along with mosquito coil (blocks three and five for thermal expulsion and block four for direct burning), the mosquito coil was found to exhibit the best percentage repellencies of all the local plants against all the test mosquitoes even if their differences were not statistically significant. Since the objective of conducting the experiments with six by six Latin square set up was to simply observe the efficacies of our local plants as compared to mosquito coil (standard repellent) and the coil was not available on time on market, they were not replicated sufficiently unlike the five by five Latin square design done earlier. Nevertheless, the records were expected to be similar to the present result.

In general, the treatments did not show significant differences among themselves when compared ($p > 0.05$) though they can be ranked using their percentage protections against the test mosquitoes. Likewise, the two application methods did not show significant differences. However, the test plants generally showed better percentage repellencies when thermally expelled.

The impacts of seasonal variations of mosquito densities, seasonal variations of plant species and many other complex interacting environmental factors were well observed among the different blocks of the study. The total numbers of mosquitoes collected in the later blocks (seasons) were extremely larger than those collected in the earlier blocks (seasons). Especially, *An. gambiae* s.l. and culicines were found to considerably vary among seasons, test nights and houses.

From literatures, it can be inferred that the effectiveness of a repellent plant depends on the method of application, species of the plant and species of the mosquitoes. Application of fresh plant parts, direct burning, thermal expulsion and topical application of repellent oils are some of the application methods (Seyoum *et al.,* 2002b; 2002a; Tawatsin *et al.,* 2001).

In addition, seasonal variation of the test plants may be other influencing factor of repellency. In this study, variations in seasons, location of houses and test nights were tried to be corrected by replication.

Seyoum *et al.* (2003) reported that none of their test plants (*C. citriodora*, *O. suave* and *O. kilimandischaricum*) except thermal expulsion of *O. kilimandischaricum* showed significant protection against *An. funestus*. However, all showed high repellency against *An. gambiae* s. l. both during and after application. This shows that the efficacy of a repellent plant varies with mosquito species.

Live potted plants of *O. suave* and *C. citriodora* and the other test plants were not even mildly repellent to *An. gambiae* (Seyoum *et al.,* 2002b). However both were found to be quite potent when directly burned or thermally expelled (Seyoum *et al.,* 2002a). Counts were made of the numbers of *Cx quinquefaciatus* biting two people inside a hut with a charcoal fire on a series of nights (Nicholson *et al.,* 1987 cited in Curtis *et al.,* 1990). *H. suaveolens* did not show any significant reduction in biting when it was bruised freshly and hang in the house (Lines and Curtis, 1986 cited in Curtis *et al.,* 1990). However, three series of experiments indicated up to ten fold reduction in biting in the presence of *H. suaveolens* smoke and lesser reductions when other plant materials such as grasses, coconut husks or neem was burned. On the other hand, *H. suaveolens* did not show any significant reduction in a fourth series some months later. A possible explanation of this disappointing result is that this experiment was done at a season at which the plants had become senescent and lost much of their ingredients, as happens with senescent Artemisia (Blust *et al.,* 1987 cited in Curtis *et al.,* 1990). According to the work of Seyoum *et al*. (2002b) on live potted plants, *C. citriodora* was found to be less efficacious than *O. suave*. However, Seyoum *et al.* (2002a) reported that *C. citriodora* showed higher repellency effect than *O. suave* by direct burning and thermal expulsion methods.

There are slight non-continuities among the different results obtained in different blocks. For example, *O. basilicum* did not show significant level of repellency against *An. pharoensis* in the fourth block (direct burning) of this study. In addition, *O. suave* showed

greatest percentage repellency of all the test plants against *An. pharoensis* in this block. These slight non-continuities may be due to the differences among the different blocks, test nights, test plants and location of the experimental houses (Seyoum *et al.,* 2002a). In fact, there are many other complex factors that affect experiments that are undertaken under natural field conditions. According to Curtis *et al.* (1990), there may be wide variations in the biting densities of natural insect populations, seasonal changes in plants and unpredictable weather over both time and space. However, these challenges shouldn't be undefeatable. According to them, the solution may often be no more- or less- than greatly increased replication. It is not totally necessary to understand all the complex and intricating variables controlling the attraction of biting insects to hosts. An effective repellent should reduce biting for everyone in a range of conditions. On the other hand, standardized laboratory tests of traditional methods that are desirable may miss out important features of the field conditions.

Lemon eucalyptus has been evaluated for its repellency by applications as intact live potted plant, direct burning, thermal expulsion and its essential oil reported to be effective by various researchers. Seyoum *et al.* (2002b) evaluated repellency of some traditionally used mosquito repellent plants by similar methods in semi-field experimental huts in western Kenya. The result of our study is in agreement with their report that *C. citriodora* (lemon eucalyptus) was found to be effective repellent plant by both methods (74.5%, $p < 0.0001$- thermal expulsion and 53.1%- direct burning). A preliminary laboratory evaluation of *p*-menthane-3, 8-diol (PMD) from lemon eucalyptus which is similar to quwenling was reported to be almost as effective as DEET aginst *An. gambiae* s.l., Gilles and also to be effective against midges, ticks and the stable flies (Trigg and Hili, 1996). Quwenling (meaning effective repeller of mosquitoes) is made from the waste distillate after extraction of oil from lemon eucalyptus plant and PMD is its principal active component (Curtis *et al.,* 1990). Trigg (1996) evaluated three formulations of PMD (50% a.i.) (a pump spray, a stick and a gel) and found no significant difference between PMD and DEET both interms of efficacy ($p > 0.05$) and longevity ($p > 0.05$) of repellency against total anopheline biting for each of the formulations tested. All the repellents tested at the chosen dose rate provided greater than 6 hr protection from biting. Citronella oil is popular oil in India and is one of

the few natural products which are still used in commercial repellents in Europe and North America (Curtis *et al.,* 1990). In April 2005, the U.S. Centers for Disease Control and Prevention (CDC) approved two non-DEET mosquito repellents, including PMD (Carrol and Loye, 2006). They reported that almost all of the subjects treated with the PMD repellents and the 20% deet repellent were completely protected throughout the field exposure of their study.

It has been reported that several species and varieties of plants of the genus *Ocimum* yield oil of diverse nature, commonly known as basilic oils. It was also reported that some essential chemical components such as eugenol, linalool, methyl cinnamate, camphor and thymol are usually found in these plants (Craveiro *et al*., 1981 cited in Holetz *et al.,* 2003). *Ocimum* spp have already been shown to be effective mosquito repellents (Pathak *et al.*, 2000). A member of this genus, which consists of several repellent and medicinally important herbs, *O. suave* was also reported as effective mosquito repellent plant by various researchers.

The effectiveness of *O. suave* is also in agreement with the similar work of Seyoum *et al.* (2002a). They reported that it was effective against *An. gambiae* by both methods (53.1%, $p < 0.0001$- thermal expulsion and 28.0%, $p<0.0255$ - direct burning). Even though the intact live potted *O. suave* didn't show significant repellency (Seyoum *et al.,* 2002b), it has reduced the feeding rates of *An. gambiae* mosquitoes from Tanznia by 45% (White, 1973 in Seyoum *et al.,* 2002b.)

There are various reports about traditional uses and repellency effects of *O. basilicum* against diseases vectors. Being a member of the genus *Ocimum*, it possesses medicinal and repellency uses. Extracts from *O. basilicum* and some other plants have been studied as possible mosquito repellents. Seyoum *et al.* (2002a) reported from their ethnobotanical study that *O. basilicum* is among the most commonly known repellent plants (30.4%) in Rambira community (west Kenya). Ntonifor *et al.* (2006) carried out ethnobotanical surveys and field evaluation following the same method described by the respondents and reported that it is one of the most frequently used plants as a traditional repellent in Cameroon. They also reported that *O. basilicum* significantly reduced the number of mosquitoes as compared to control although it was not better than DEET. There is a report

that essential oil of *O. basilicum* repelled *Ae. aegypti* for 75 minutes (Tawatsin *et al.,* 2001). The essential oils of *Ocimum basilicum*, *Pogostemon heyneanus* and *Eucalyptus* showed insecticidal activity against *Sitophilus oryzae*, *Stegobium paniceum* (L.), *Tribolium castaneum* and *Callosobruchus chinensis* (Desphande *et al.,* 1974, Desphande and Tipnis, 1977 cited in Shaaya *et al.,* 1997). The compounds derived from the plant *Ocimum basilicum* possess exceedingly high juvenile hormone activity and contain ocimene as a portion of their chemical structure and thus named as juvocimene (Bowers and Nishida, 1980).

Efficacy of *E. camaldulensis* has not been previously evaluated by any of the traditional methods. There is also no report concerning its traditional use as a mosquito repellent plant. However, different reports are available about repellency of its essential oil. A new compound named eucamalol and 4-isopropylbenzyl alcohol were isolated as mosquito repellents from the essential oil of *E. camaldulensis* (Watanabe *et al.,* 1993). According to their report, all the test compounds exhibited potent mosquito repelling activities against *Ae. aegypti* immediately after application. Eucamalol showed 75% repellency even 3hour after treatment. This was better than DEET which exhibited 2 hour duration of effectiveness after treatment in their test method. The components (cymol and limonene) of the essential oil of *E. camaldulensis* have significant insecticidal action, being lethal to the stored product pests (Ibrahim *et al.,* 2001).

There are many research results about the repellent activity of mosquito coils. It was used as a positive control (standard) in this study and showed the highest efficacy of all the treatment plants. Of course, there is no statistically significant difference between the test plants and the coil. Mosha *et al.* (1992) reported that field trials with mosquito coils containing 0.15% Esbiothrin at Usa River settlement, northern Tanzania, during 1986 achieved 52% reduction in biting rates of *An. gambiae* s.l. Patton and 73% reduction of *Cx quinquefasciatus* Say mosquitoes. Moreover, no mosquito biting activity occurred during the coil burning period which started at 20.00 hours daily and lasted for average of 8.6 hours. Their sociological survey also indicated that the coils favorably accepted and produced no serious side-effects.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

- The efficacy of four local plant species (*C. citriodora*, *O. suave*, *O. basilicum* and *E. camaldulensis*) were evaluated and compared against *An. gambiae* s.l., *An. pharoensis*, and culicines under natural field conditions. Although many studies on traditional mosquito repellent plants are conducted in different parts of the world, only few studies are conducted in Ethiopia. *An. gambiae* s.l. were found to be dominant of the three mosquito species collected from the experimental houses with CDC light traps followed by culicines and *An. pharoensis*.

- *O. basilicum* was found to be the most efficacious of all the test plant species followed by *O. suave*, *C. citriodora* and *E. camaldulensis* against *An. gambiae* s.l. by direct burning method. *O. basilicum* also showed the highest repellency aginst culicines followed by *C. citriodora* , *E. camaldulensis* and *O. suave*. However, *C. citriodora* was found to have higher percentage repellency than *O. basilicum* against *An. pharoensis*.

- *O. basilicum* was also found to exhibit the highest percentage repellency with more or less similar result with *C. citriodora* followed by *O. suave* and *E. camaldulensis* against *An. gambiae* s.l. by thermal expulsion method. *O. basilicum* also exhibited the highest repellency percentage against *An. pharoensis*. *C. citriodora* was found to be the second effective repellent plant followed by *O. suave* and *E. camaldulensis*. *C. citriodora* exhibited the highest efficacy against culicines followed by *E. camaldulensis* and *O. basilicum*. *O. suave* did not show significant difference as compared to the negative control against culicines when thermally expelled.

- Generally, the test plants were found to be effective against those mosquito vectors. They were comparably as effective as mosquito coil used in the present study.

6.2. Recommendations

1. Using local plants for mosquito control by traditional method is accessible, affordable and easy to handle by local communities. In addition, it is more or less risk free though the smokes of burning may happen to accumulate soot on clothes. Therefore, it is important to integrate with the other available vector control methods such as ITNs which is at infancy stage in Ethiopia at the moment. Strong/ highly repellent plants may have potential for integration with other mosquito control methods or work complimentarily with other classical anti-mosquito methods.

2. Use of plants in the genus *Ocimum* is recommended because they can easily be cultivated within short period of time by the communities in malaria endemic areas and used particularly when the conventional control methods (indoor residual sprays and ITNs) somehow fail to reach them on time.

3. Since more studies can explore the effects of additional plant species against mosquitoes, conducting further study is necessary to evaluate the repellency of additional plant species for local use in different parts of the country.
4. The chemicals responsible for repellency should be extracted and commercialized.

8. REFERENCES

Abose, T., Yeebiyo, Y., Olana, D., Alamirew, D., Beyene, Y., Regassa, L. and Mengesha, A. (1998). *Re-orientation and definition of the role malaria vector control in Ethiopia*. Unpublished WHO Document WHO (MAL) 98.1085-31pp.

Adhanom, T., Deressa, W., Witten, K.H., Getachew, A. and Seboxa, T. (2006). Malaria. In: *Epidemiology and ecology of health and disease in Ethiopia,* pp. 556-577, (Berhane, Y., Haile Mariam, D. and Kloos, H., eds). Addis Ababa.

Ansari, M.A., Mittal, P.K, Razdana, R.K. and Sreeharia, U. (2005). Larvicidal and mosquito repellent activities of pine (*Pinus longifolia*, Family: Pinaceae) oil. *Journal of Vector Borne Diseases,* **42**: 95–99.

Atsbeha, A. (2005). *Evaluation of some plants for their repellency against some anopheline and culicine mosquitoes in Koka-Negewo, central Ethiopia*, MSc thesis Addis Ababa University.

Balázs, E. (2002). *Towards the safe use of modern biotechnology*: Report on the 7th International Symposium on the Biosafety of Genetically Modified Organisms held in Beijing (China). Environtal Biosafety Resource, **2**: 35–36.

Bang, Y. H. (1985). Integrated management of urban mosquito vectors of human diseases. *Journal of Communicable Diseases*, **17**: 1-10.

Barnard, D. R. (2004). Repellents and Toxicants for Personal Protection. WHO Pesticide Evaluation Scheme (WHOPES), Geneva, 49 Pp.

Baruah, I. and Das, S.C. (1996). Evaluation of methoprene (Altosid) and diflubenzuron (Dimilin) for control of mosquito breeding in Tezpur (Assam). *Indian Journal of Malariology,* **33**: 61-66.

Beidler, E. J. (1995). Biocontrol from a mosquito control director's point of view. *Journal of the American Mosquito Control Association*, **11**: 258-259.

Binka, F. N. and Adongo, P. (1997). Acceptability and use of insecticide impregnated bednets in northern Ghana. *Tropical Medicine and International Health,* **2**: 499–507.

Binka, F.N., Kubaje, A. and Adjuik, M. (1996). Impact of permethrin-impregnated bednets on child mortality in Kassena-Nankana District, Ghana: A randomized

trial. *Tropical Medicine and International Health*, **1**: 147–154.

Bowers, W. S. and Nishida, R. (1980). Juvocimenes: Potent juvenile hormone mimics from Sweet Basil. *Science,* **209**:1031-1032.

Breman, J. G. (2001). The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *American Journal of Tropical Medicine and Hygiene,* **64**: 1–11.

Bruce-Chwatt, L. J. (1985). *Essential Malariology*. 2nd ed., Alden press, London. 450Pp.

Carroll, S. P.and Loye, J. (2006). PMD, a registered botanical mosquito repellent with DEET-like efficacy. *Journal of the American Mosquito Control Association*, **22**: 507-514.

Carvalho, A.F.U., Melo, U. M. M., Craveiro, A. A., Machado, M. I. L., Bantim, M. B. and Rabelo, E. F. (2003). Larvicidal activity of the essential oil from *Lippia sidoides* Cham. against *Aedes aegypti* L. *Memorias do Instituto Oswaldo Cruz*, **98**: 569-571.

Chogo, J. B. and Crank, G. (1981). Chemical composition and biological activity of the Tanzanian plant, *Ocimum suave*. *Journal of Natural Products,* **44**: 308-311.

Choi, H.W., Breman, J.G., Teutsch, S.M., Liu, S., Hightower, A.W. and Sexton, J.D. (1995).The effectiveness of insecticide-impregnated bed nets in reducing cases of malaria infection: A meta-analysis of published results. *American Journal of Tropical Medicine and Hygiene*, **52**: 377–382.

Coetzee, M., Craig, M. and Sueur, D. (2000). Distribution of Africa malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today*, **16**: 74-77.

Coleman, P. G. and Alphey, L. (2004). Genetic control of vector populations: an imminent Prospect. *Tropical Medicine and International Health*, **9**: 433-437.

Curtis, C. F., Line, J. D., Baolin, L. and Renz, A. (1990). Natural and synthetic repellents. In: Curtis, C. .F. (ed.), *Appropriate Technology in Vector Contro*l. Wolfe, London, 75-92 pp.

D'Alessandro, U., Olaleye, B.O.P. and McGuire, W. (1995). Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. *Lancet*, **345**: 479–483.

Dame, D.A. (1984). Control of insects of veterinary importance by genetic techniques.

Preventive Veterinary Medicine, **2** :515-522.

Das, N.G., Baruah I., Talukdar, P.K and Das, S.C. (2003). Evaluation of botanicals as repellents against mosquitoes. *Journal of Vector Borne Diseases,* **40**: 49–53.

Depiny, J. M. O., Mbogo, C. M., Killeen, G., Knols, B., Beier, J., Carlson, J., Dushoff, J., Billingsley, P., Mwambi, H., Githure, J., Toure, A. M. and Mckenzie, F. E. (2004). A simulation model of African *Anopheles* ecology and population dynamics for the analysis of malaria transmission. *Malaria Journal*, **3**: 29-50.

Fontaine, R. E., Najjar, A. E. and Prince, J. S. (1961). The 1958 malaria epidemic in Ethiopia.*The American Journal of Tropical Medicine and Hygiene*, **10**: 795-803

Fontenille, D. and Lochouarn, L. (1999). The complexity of the malaria vectorial system in Africa. *Parassitologia,* **41**: 267-271.

Friis, I.B. (1995). Myrtaceae. In: *Flora of Ethiopia and Eritrea, Canellaceae* to *Euphorbiaceae,* vol. 2 Part 2., Pp71-106, (Edwards, S., Tadesse, M. and Hedberg, I., eds.). Addis Ababa, Ethiopia and Uppsala, Sweden..

Ghai, O. P. and Gupta, P. (2000). *Essential preventive medicine: a clinical and applied orientation.* 5, Ansari Road, New Delhi, 969 pp.

Gilles, H. M. and Warrell, D. A. (1993). *Bruce-Chwatt's essential malariology*, 3rd ed. Arnold, London, 340Pp.

Gillies, M.T. and Coetzee, M. (1987). *A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region).* South African Institute of Medical Research, Johannesburg.

Glogoza, P. A., McBride, D. K. and Anderson, A. W. (2000). *Mosquito management.* North Dakota State University Fargo, North Dakota 58105, 16pp.

(http://www.fao.org/docrep/x2230e/x2230e09.htm

http://www.ansci.cornell.edu/plants/medicinal/basil.html.

Holetz, F.B., Ueda-nakamura, T., Filho, B. P. D., Diógenes Cortez, A.G., Morgado-díaz, J. A. and Nakamura, C.V. (2003). Effect of essential oil of *ocimum gratissimum* on the rypanosomatid *Herpetomonas samuelpessoai*. *Acta Protozoologica*, **42**: 269 – 276.

Hunt, R. H., Coetzee, M. and Fetenne, M. (1998). *The Anopheles gambiae* complex*:* a new species from Ethiopia. *Transactions of Royal Society of Tropical Medicine and Hygiene*, **92**: 231-235.

Ibrahim, M. A., Kainulainen, P., Aflatuni, A., Tiilikkala, K. and Holopainen, J. K. (2001). Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference tolimonene and it suitability for control of insect pests. *Agricultural and food Science in Finland,* **10**: 343-259.

Jaenson, T. G. T., Garboui, S. and Lsson, K. P. (2006). Repellency of oils of lemon eucalyptus, Geranium, and Lavender and the mosquito repellent MyggA natural to *Ixodes ricinus*(Acari: Ixodidae) in the laboratory and field. *Journal of Medical Entomology*, **43**: 731-736.

Kokwaro, J. O. (1976). *Medicinal plants of East Africa.* East African Literature Bureau, Nairobi, Kenya, 284pp.

Krieger, R. I., Dinoff, T. M. and Zhang, X. (2003). Octachlorodipropyl Ether (S-2) Mosquito Coils Are Inadequately Studied for Residential Use in Asia and Illegal in the United States. *Environmental Health Perspectives*, **111**: 1439-1442.

Legner, E. F. and Sjogren, R. D. (1984). Biological mosquito control furthered by advances in technology and research. *Mosquito News*, **44**: 449-456.

Lindsay, S. W., Parson, L. and Thomas, C. J. (1998). Mapping the ranges of and relative abundance of the two principal African malaria vectors, *Anopheles gambiae sensu stricto* and *Anopheles arabiensis*, using climate data. *Proceedings of the Royal Society of London Series B*, **265**: 847-854.

Lindsay, S.W. and Gibson, M.E. (1988). Bednets revisited—old idea, new angle. *Parasitology Today,* **4**: 270–272.

Liu, N., Xu, Q., Zhu, F. and Zhang, L. (2006). Pyrethroid resistance in mosquitoes. *Insect Science*, **13**: 159-166).

Mboera, L.E.G., Kihonda, J., Braks, M.A.H. and Knols, B.G.J. (1998). Short report: Influence of Centers for Disease Control light trap position, relative to human-baited bednet, on catches of *Anopheles gambiae* and *Culex quinquefasciatus* in Tanzania. *American Journal of Tropical Medicine and Hygiene,* **59***:* 595-596.

Melman, S. A. and Hutton, M. P. (1985). Flea control on dogs and cats indoors and in

the environment. *The Compendium on Continuing Education*, **7:** 869-887.

Monath, T.P. (1994). Dengue: The risk to developed and developing countries. *Proceedings of the National Academy of Sciences of the United States of America*, **91:** 2395-2400.

MOH (2000). *Malaria Control Profile*: Federal Democratic Republic of Ethiopia Ministry of Health. Disease Prevention and Control Department, Addis Ababa Ethiopia.

MOH (2002). *Guideline for Malaria Vector Control in Ethiopia*. Addis Ababa Ethiopia. 14Pp.

MOH (2001). *National five years strategic plan for malaria control in Ethiopia:* 2001-2005. Ministry of Health, Addis Ababa.

Molyneux, D. H. (1998). Vector-borne parasitic diseases- an overview of recent changes. *International Journal of Parasitology*, **28**: 927-934.

Mosha, F. W., Njau, R. J. A. and Myamba, J. (1992). Biological efficacy of new formulations of mosquito coils and a critical review of test methods *pyrethrum post*, **17**: 47-51.

Mulla, M. S. and Su, T. (1999). Activity and biological effects of neem products against arthropods of medical and veterinary importance. *Journal of American Mosquito Control Association*, **15**: 133-152.

National Meteorological Service Agency (2006).

Nevill, C.G., Some, E.S. and Mungala, V.O. (1996). Insecticide-treated bednets beduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Tropical Medicine and International Health*, **1**:139–146.

Nigatu, W., Abebe, M. and Dejene, A. (1992). *Plasmodium vivax* and *P. falciparum* epidemiology in Gambella, south west Ethiopia. *Tropical Medicine and Paracitology*, **43**: 181-185.

Ntonifor, N.N., Ngufor C.A., Kimbi, H.K. and Oben, B.O. (2006). Traditional use of indigenous mosquito-repellents to protect humans against osquitoes and other insect

bites in a rural community of Cameroon. *East African Medical Journal,* **83**: 553-558.

Palsson, K. and Jaenson, T.G.T. (1999). Plant products used as mosquito repellents in Guinea Bissau, West Africa. *Acta Tropica,* **72**: 39-59.

Pathak, N., Mital, P.K., Singh, O.P., Sagar, D. V. and Vasudevan, O. (2000). Larvicidal action of essential oils from plants against the vector mosquitoes *Anopheles stephensi* (Liston) *Culex quiquefasciatus* Say and *Aedes aegypti* L. *International Pest Control,* **42**: 53-53.

Pitts, C. W., Holbrook, G. L., Gripp, S. I. and Hock , W.K. (2000). *Mosquito biology and control.* College of Agricultural Sciences, pathology, Pennsylvania State University, 6Pp.

Rajan, T.V, Hein, M., Porte, P. and Wikel, S. (2005). A double-blinded, placebo-controlled trial of garlic as a mosquito repellent: a preliminary study. *Medical and Veterinary Entomology,* **19**: 84-89.

Rowland, M., Downey, D., Rab, A., Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C., Lines, J. and Fayaz, M. (2004). DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. *Tropical Medicine and International Health,* **9**: 335–342.

Samba, E. M. (2000). *Statement on the American Summit on Roll Back Malaria*. African Health Monitor. A magazine of the WHO Regional office for Africa, **1:** 29-2.

Selene, M. M., Cavalcanti, E. S., Bertini, L. M., Oliveira, C. L.,. Rodrigues, J. R. and Cardoso,J. H. (2006). Larvicidal activity of essential oils from Brazilian croton species against *Aedes aegypti* L. *Journal of the American Mosquito Control Association*, **22**: 161-164.

Service, M. (1996). *Medical entomology for students,* 3rd ed. Cambridge University Press, Cambridge, 285 pp.

Service, M. W. (1993). Mosquitoes (Culucidae). In: *Medical Insects and Arachnids,* Pp. 120-240, (Lane, R. P. and Crosskey, R. W., eds). Chapman and Hall, London..

Seyoum, A., Kabiru, E. W., Lwande, W., Killeen, G. F., Hassanali, A. and Knols,

B.G. J. (2002b). Repellency of live potted plants against *Anopheles gambiae* from human baits in semi-field experimental huts. *American Journal of Tropical Medicine and Hygiene,* **8**: 191-195.

Seyoum, A., Killeen, G.F., Kabiru ,E.W., Knols, B.G.J. and Hassanali, A. (2003). Field efficacy of thermally expelled or live potted repellent plants against African malaria vectors in western Kenya. *Tropical Medicine and International Health,* **8**: 1005-1011.

Seyoum, A., Palsson, K., Kunga, S., Kabiru, E. W., Lwande, W. Killeen, G. F., Hassanali, A. and Knols, B. G. J. (2002a). Traditional use of mosquito-repellent plants in Western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: ethnobotanical studies and application by thermal expulsion and direct burning. *Transaction of the Royal Society of Tropical Medicine and Hygiene,* **96**: 225-231.

Shaaya, E., Kostjukovski, M., Eilberg, J. and Sukprakarn, C. (1997). Plant oils as fumigants and contact insecticides for the control of stored-product insects. *Stored Product Resources,* **33**: 7-15.

Sharma, V. P. (2001). Health hazards of mosquito repellents and safe alternatives. *Current Science*, **80**: 1-3.

Sharma, V. P. and Ansari, M. A. (1994). Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene. *Journal of Medical Entomology*, **31**: 505-507.

Siddall, J. B. (1976). Insect Growth Regulators and Insect Control. *Environmental Health Perspectives,* **14**: 119-126.

Silva, F., Santos, R.H.S, Andrade, N.J., Barbosa, L.C.A., Casali, V.W.D., Lima, R.R. and Passarinho, R.M. (2005). Basil conservation affected by cropping season, harvest time and storage period. *Pesq. agropec. bras. Brasília*, **40**: 323-328.

Sukumar, K, Perich, M.J. and Boobar, L.R. (1991). Botanical derivatives in mosquito control:a review. *Journal of American Mosquito Control Association,* **7**: 210–238.

Tawatsin, A., Wratten, S.D., Scott, R.R., Thavara, U. and Techadamrongsin, Y. (2001). Repellency of volatile oils from plants against three mosquito vectors. *Journal of Vector Ecology,* **26**: 76-82.

Trigg, J. K. (1996). Evaluation of eucalyptus-based repellent against *Anopheles* spp. in Tanzania. *Journal of American Mosquito Control Association*, **12**: 76-82.

Tulu, N.A. (1993). Malaria. **In**: *The Ecology of Health and Disease in Ethiopia*, PP. 341-352, (Kloos, H. and Zein, A. Z., eds.). West View Press.

Verrone, G.A. (1962). Outline for the determination of malarial mosquitoes in Ethiopia. Part I – Adult Female Anophelines. *Journal of the American Mosquito Control Association,* **22**: 37-49.

Waka, M., Hopkins, J.R. and Curtis, C. (2004). Ethnobotanical survey and testing of plants traditionally used against hematophagous insects in Eritrea. *Journal of Entomology*, **95**: 95-101.

Watanabe, K., Shono, Y., Kakiinizu, A., Okada, A., Matsuo, N., Satoh, A. and Nishimura, H. (1993). New mosquito repellent from *Eucalyptus camaldulensis. Journal of Agriculture and Food Chemistry*, **41**: 2164-2166.

White, G. D., Tesfaye, F., Boreham, P. F. L. and Lemma, G. (1980). Malaria vector capacity of *An. arabiensis* and *An. quadriannulatus* in Ethiopia. Chromosomal interpretation after 6 years storage of field preparations. *Transacctions of the Royal Society of Tropical Medicine and Hygiene*, **74**: 683-684.

WHO (1973). *Manual on larval control operations in malaria programmes*. Offset Publication no. 1. World Health Organization, Geneva.

WHO (1979). *Integrated control and its application to anti-alaria programs*. pp 14-20, WHO, Geneva).

WHO (1982). *Manual on Environmental Management for Mosquito Control with special emphasis on malaria vectors*. Geneva, Switzerland.

WHO (1983a). *Integrated disease vector control and community participation. Report of a WHO Regional Workshop, Pondicherry, India 3-8 October 1983*. WHO Mimeographed document SEAfVBC/20.

WHO (1983b). *Integrated vector control*. Seventh report of the WHO Expert Committee on Vector Biology and Control, WHO, Geneva.

WHO (1995). Vector control for malaria and other mosquito-borne diseases. *WHO Technical Report Series* No.857.1-73.

WHO (1996). *Report of the WHO informal consultation on the evaluation and testing of insecticides.* CTD/WHORES/1C/96.1, WHO, Geneva, 69 pp.

WHO (2000). WHO expert committee on malaria. *WHO Technical Report series* No.892. 11-62.

WHO (2004). *Bridging the Gap: linking research, training, and service delivery to reduce the malaria burden in Africa*. Brazzaville, Congo.

Yang, Y.C., Lee, E. H., Lee ,H. S., Lee, D. K. and Ahn, Y. (2004). Repellency of aromatic medicinal plant extracts and a steam distillate to *Aedes aegypti. Journal of the American Mosquito Control Association,* **20**:146-149.

Wongsiri, S. and A'ndre, R.G. (1984). Biological control of mosquitoes in Thailand. *Journal of Scientific Society of Thailand*, **10:** 73-88.

9. APPENDICES

Appendix 1 Pictures of the test plants taken during the study

Fig.5. A picture of *E. camaldulensis* taken from Shulki village

Fig.6. A picture of *C. citriodora* taken from Wendo Genet.

Fig.7. A picture of *O. basilicum* taken from Shulki village

Fig.8. A picture of *O. suave* taken from Wendo Genet.

Fig.9. Collection of mosquitoes from the cages and identification to species level

Appendix 2. Repellency status of the test plants in the first block (January) of the experiment by direct burning

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	12	2.4	2.30	0	6	66.67
		O. suave	13	2.6	2.19	0	6	63.89
		O. basilicum	8	1.6	1.52	0	4	77.78
		E. camaldulensis	7	1.4	0.89	0	2	80.56
		Control (charcoal)	36	7.2	3.11	4	12	-
2	*An. pharoensis*	*C. citriodora*	14	2.8	2.95	0	7	82.72
		O. suave	22	4.4	5.26	0	13	73
		O. basilicum	21	4.2	4.09	0	11	74.07
		E. camaldulensis	32	6.4	10.01	0	24	60.49
		Control (charcoal)	81	16.2	5.02	12	23	-
3	Culicines	*C. citriodora*	1	0.2	0.45	0	1	91.67
		O. suave	3	0.6	0.55	0	1	75.00
		O. basilicum	3	0.6	1.34	0	3	75.00
		E. camaldulensis	3	0.6	0.89	0	2	75.00
		Control (charcoal)	12	2.4	1.52	0	4	-

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

Appendix 3. Repellency status of the test plants in the second block (March) of the experiment by direct burning

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	14	2.8	2.95	1	8	77.78
		O. suave	18	3.6	3.36	0	9	71.43
		O. basilicum	12	2.4	2.88	0	6	80.95
		E. camaldulensis	18	3.6	2.70	1	8	71.43
		Control (charcoal)	63	12.6	4.51	9	20	-
2	*An. pharoensis*	*C. citriodora*	13	2.6	1.52	1	4	67.50
		O. suave	14	2.8	2.05	1	5	65
		O. basilicum	14	2.8	2.17	1	6	65.00
		E. camaldulensis	9	1.8	0.84	1	3	77.50
		Control (charcoal)	40	8.0	4.53	0	11	-
3	Culicines	*C. citriodora*	5	1.0	1.41	0	3	73.68
		O. suave	3	0.6	1.34	0	3	84.21
		O. basilicum	6	1.2	1.30	0	3	68.42
		E. camaldulensis	3	0.6	1.34	0	3	84.21
		Control (charcoal)	19	3.8	2.28	0	6	-

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

Appendix 4. Repellency status of the test plants in the third block (April) of the experiment by direct burning

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	32	6.4	6.02	0	15	75.38
		O. suave	32	6.4	10.45	0	25	75.38
		O. basilicum	31	6.2	3.96	1	12	76.15
		E. camaldulensis	52	10.4	9.56	1	23	60.00
		Control (charcoal)	130	26.0	15.38	13	51	-
2	*An. pharoensis*	*C. citriodora*	26	5.2	12.00	1	12	66.23
		O. suave	23	4.6	4.62	1	11	70
		O. basilicum	17	3.4	3.51	0	8	77.92
		E. camaldulensis	34	6.8	6.57	1	17	55.84
		Control (charcoal)	77	15.4	5.59	11	24	-
3	Culicines	*C. citriodora*	36	7.2	4.15	1	11	57.65
		O. suave	30	6.0	6.20	2	17	64.71
		O. basilicum	17	3.4	6.50	0	15	80.00
		E. camaldulensis	37	7.4	7.89	0	19	56.47
		Control (charcoal)	85	17.0	7.52	4	23	-

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

Appendix 5. Repellency status of the test plants in the fifth block (June) of the experiment by direct burning

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	427	85.4	33.80	47	131	61.84
		O. suave	435	87.0	56.46	33	179	61.13
		O. basilicum	463	92.6	50.96	17	151	58.62
		E. camaldulensis	528	105.6	40.56	55	150	52.82
		Control (charcoal)	1119	223.8	123.58	101	426	-
2	*An. pharoensis*	*C. citriodora*	38	7.6	9.84	1	25	78.65
		O. suave	53	10.6	9.66	1	21	70
		O. basilicum	53	10.6	12.01	2	31	70.22
		E. camaldulensis	56	11.2	8.61	3	25	68.54
		Control (charcoal)	178	35.6	21.86	4	61	-
3	Culicines	*C. citriodora*	57	11.4	13.28	0	33	35.96
		O. suave	50	10.0	15.26	5	41	43.82
		O. basilicum	79	15.8	13.18	2	33	11.24
		E. camaldulensis	43	8.6	10.43	2	29	51.69
		Control (charcoal)	89	17.8	17.04	2	44	-

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

Appendix 6. Repellency status of the test plants in the first block (January) of the experiment by thermal expulsion

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	10	2.0	2.83	0	7	83.61
		O. suave	13	2.6	3.63	0	9	78.69
		O. basilicum	9	1.8	1.30	1	4	85.25
		E. camaldulensis	13	2.6	2.35	1	7	78.69
		Control (charcoal)	61	12.2	9.28	4	28	-
2	*An. pharoensis*	*C. citriodora*	15	3.0	3.77	0	9	71.15
		O. suave	17	3.4	3.64	0	9	67
		O. basilicum	8	1.6	1.14	0	3	84.62
		E. camaldulensis	17	3.4	2.61	1	7	67.31
		Control (charcoal)	52	10.4	4.83	6	16	-
3	Culicines	*C. citriodora*	6	1.2	2.17	0	5	73.91
		O. suave	4	0.8	0.84	0	30	82.61
		O. basilicum	4	0.8	1.30	0	3	82.61
		E. camaldulensis	4	0.8	0.84	0	2	82.61
		Control (charcoal)	23	4.6	6.77	0	15	-

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

Appendix 7. Repellency status of the test plants in the second block (March) of the experiment by thermal expulsion

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	9	1.8	1.48	1	4	85.94
		O. suave	10	2.0	0.71	0	3	84.38
		O. basilicum	10	2.0	1.58	0	4	84.38
		E. camaldulensis	8	1.6	1.14	0	3	87.50
		Control (charcoal)	64	12.8	11.99	2	33	-
2	*An. pharoensis*	*C. citriodora*	7	1.4	1.34	0	3	88.89
		O. suave	9	1.8	2.00	0	5	86
		O. basilicum	11	2.2	1.00	0	2	82.54
		E. camaldulensis	11	2.2	3.27	0	8	82.54
		Control (charcoal)	63	12.6	9.91	7	32	-
3	Culicines	*C. citriodora*	3	0.6	1.34	0	3	86.96
		O. suave	5	1.0	1.22	0	3	78.26
		O. basilicum	7	1.4	2.61	0	6	69.57
		E. camaldulensis	2	0.4	0.89	0	2	91.30
		Control (charcoal)	23	4.6	2.79	0	7	-

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

Appendix 8. Repellency status of the test plants in the fourth block (May) of the experiment by thermal expulsion

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	225	45.0	29.27	9.0	81.0	79.45
		O. suave	278	55.6	31.76	21.0	92.0	74.61
		O. basilicum	200	40.0	24.53	4.0	72.0	81.74
		E. camaldulensis	414	82.8	76.69	10.0	209.0	62.19
		Control (charcoal)	1095	219.0	130.15	80.0	401.0	-
2	*An. pharoensis*	*C. citriodora*	16	3.2	3.11	0.0	7.0	80.95
		O. suave	17	3.4	4.28	1.0	11.0	80
		O. basilicum	16	3.2	2.68	0.0	6.0	80.95
		E. camaldulensis	17	3.4	2.61	1.0	7.0	79.76
		Control (charcoal)	84	16.8	13.70	0.0	33.0	-
3	Culicines	*C. citriodora*	32	6.4	7.83	1.0	20.0	49.21
		O. suave	33	6.6	2.88	4.0	11.0	47.62
		O. basilicum	32	6.4	3.91	1.0	12.0	49.21
		E. camaldulensis	31	6.2	5.54	1.0	15.0	50.79
		Control (charcoal)	63	12.6	9.76	4.0	27.0	-

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

Appendix 9. Repellency status of the test plants in the third (April) block of the experiment by thermal expulsion

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	40	6.7	6.71	1	18	69.47
		O. suave	43	7.2	6.77	1	19	67.18
		O. basilicum	41	6.8	6.24	2	19	68.70
		E. camaldulensis	65	10.8	8.80	1	25	50.38
		Control (charcoal)	131	21.8	13.04	11	47	-
		Coil	29	4.8	5.49	2	16	77.86
2	*An. pharoensis*	*C. citriodora*	20	3.3	2.50	0	7	65.52
		O. suave	27	4.5	6.72	0	18	53
		O. basilicum	20	3.3	3.56	0	10	65.52
		E. camaldulensis	28	4.7	4.26	0	10	51.72
		Control (charcoal)	58	9.7	4.62	8	20	-
		Coil	11	1.8	2.40	0	6	81.03
3	Culicines	*C. citriodora*	14	2.3	1.75	1	5	68.89
		O. suave	21	3.5	4.32	0	11	53.33
		O. basilicum	23	3.8	2.86	0	8	48.89
		E. camaldulensis	11	1.8	1.33	0	4	75.56
		Control (charcoal)	45	7.5	2.66	3	10	-
		Coil	10	1.7	1.51	0	4	78

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

Appendix 10. Repellency status of the test plants in the fifth (June) block of the experiment by thermal expulsion

	Test mosquito species	Test plant species	Number collected*	Mean	Sd	Minimum	Maximum	% repellency
1	*An. gambiae* s. l.	*C. citriodora*	391	65.2	42.88	11	130	59.52
		O. suave	425	70.8	32.52	29	126	56.00
		O. basilicum	467	77.8	52.66	19	152	51.66
		E. camaldulensis	530	88.3	43.60	10	126	45.13
		Control (charcoal)	966	161.0	67.81	76	240	-
		Coil	245	40.8	33.46	0	81	74.64
2	*An. pharoensis*	*C. citriodora*	45	7.5	3.83	2	13	60.53
		O. suave	42	7.0	6.42	2	19	63
		O. basilicum	52	8.7	4.18	3	14	54.39
		E. camaldulensis	55	9.2	9.24	2	25	51.75
		Control (charcoal)	114	19.0	8.67	10	33	-
		Coil	19	3.2	3.54	0	9	81.67
3	Culicines	*C. citriodora*	150	25.0	30.00	0	79	40.71
		O. suave	181	30.2	36.36	2	100	28.46
		O. basilicum	187	31.2	28.94	0	80	26.09
		E. camaldulensis	176	29.3	24.91	0	62	30.43
		Control (charcoal)	253	42.2	24.55	8	64	-
		Coil	54	9.0	9.61	0	23	79

*= Total number of mosquitoes collected during the five test nights
Sd= Standard deviation

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university, and that all sources of materials used for the thesis have been properly acknowledged.

Name of candidate Sisay Dugassa

Signature_____

Place Addis Ababa University

Date 20 / 07 / 07

This thesis has been submitted for examination with my approval as a university advisor(s),

Name of advisors:	Signature
Dr. Teshome Gebre-Michael	_____
Dr. Aklilu Seyoum	_____
Ato Meshesha Balkew	_____