ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Evaluation of the Mosquitocidal Activities of Essential Oils of some Local Aromatic Plants under Laboratory and Simulated Field Conditions

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirement of the Degree of Masters of Science in Biology (Insect Science)

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July 2006

Acknowledgement

I am especially grateful to Dr. Teshome Gebre-Michael, principal advisor, for his continuous and useful guidance and strong support throughout the study period. His consistent encouragement, friendly approach and kind hospitality is highly appreciated. I am also very grateful to him for reading and commenting on this paper and clarifying some points.

I would like to extend my gratitude to Dr. Makuria Tadesse, co advisor, for his support and encouragement throughout the investigations. I am also very grateful for his collaboration and constructive comment on this thesis.

My special thanks goes to Ato Meshesha Balkew, co-advisor, for his valuable advice, providing some materials and information during the study. His friendly approach is specially appreciated.

Dr. Tesfaye Bekele, director of Essential Oil Research Center, is gratefully acknowledged for his collaboration and encouragement. The organization as whole is acknowledged for providing the laboratory and library facilities.

I am also thankful to Dr. Bekele Jembere for his important information on the extraction of essential oils. Prof. Sebsibe Demissew and Dr. Ensermu Kelbesa are gratefully acknowledged for their identification or confirmation of test plants. My thanks goes to Dr. Teketel Yohannis who introduced me to the preparation of different concentrations of essential oils.

I would like to thank W/t Azeb Mulugeta, Ato Wosen Sisay and Misganew Kassie, the laboratory assistant in Aklilu Lemma Institute of Pathobiology, for their helpful guidance in the identification of different instars of mosquito larvae and maintaining the colony throughout the study period.

Laboratory assistant in the Essential Oil Research Center, W/o Adench Tesfaye and Tedelech Atew are highly acknowledged for their collaboration in distillation and quantification of essential oils.

I am indebted to Ato Marid Negash, Research Assistant of Insect Science Stream, for his assistance in analyzing the data.

Finally, my appreciation goes to my mother and friends who shared my struggle during the study periods.

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Abstract

The essential oils of 11 plants were evaluated for larvicidal activities against laboratory reared *Anopheles arabiensis* and *Aedes aegypti* by exposing third-fourth instar larvae in white enamel cups of 350 ml size. Five plants essential oil were assayed against anopheline mosquito larvae in the simulated field conditions. Tests were also conducted on essential oils of 11 plants against laboratory reared adult *An. arabiensis* by bottle bioassay. The residual activities of essential oils of 7 plants were tested against adult *An. arabiensis* in the laboratory.

Of the essential oils of 11 plants tested, *Chenopodium ambrosioides* (LC_{50} = 17.5 ppm; LC_{90} = 33.2 ppm), *Ocimum lamiifolium* (LC_{50} = 20.9 ppm; LC_{90} = 39.9 ppm) and *Schinus molle* (leaves) (LC_{50} = 21.0 ppm; LC_{90} = 37.3 ppm) essential oils showed highest activity against *An. arabiensis* larvae in the laboratory. However, *Mentha spicata* (LC_{50} = 85.9 ppm; LC_{90} = 128.4 ppm) showed the lowest efficacy. Similarly, *O. lamiifolium* (LC_{50} = 8.6 ppm; LC_{90} = 13.4 ppm), *C. ambrosioides* (LC_{50} = 9.1 ppm; LC_{90} = 14.3 ppm), *Piper nigrum* (LC_{50} = 9.1 ppm; LC_{90} = 13.5 ppm) and *S. molle* (leaves) (LC_{50} = 9.6 ppm; LC_{90} = 15.0 ppm) essential oils had the highest larvicidal activities against *Ae. aegypti* larvae in the laboratory. Whereas, *M. spicata* (LC_{50} = 67.8 ppm; LC_{90} = 96.4 ppm) had the lowest larvicidal activity.

In mortality-time bioassays, *Lippia adoensis* provided the highest activity against *An. arabiensis* and *Ae. aegypti* larvae, whereas essential oils of *O. suave* (fresh) and *O. suave* (dry) showed the lowest larvicidal activity. *Ae. aegypti* larvae died at shorter time than *An. arabiensis* larvae.

In simulated field condition, *O. lamiifolium* (LC_{50} = 34 ppm; LC_{90} = 63.7 ppm) showed the highest activity against field collected larvae of anopheline mosquito followed by *C. ambrosioides* (LC_{50} = 47.3 ppm; LC_{90} = 97.9 ppm) essential oil. The essential oil of *P. nigrum* (LC_{50} = 110ppm; LC_{90} = 162 ppm) showed the lowest efficacy against anopheline mosquito larvae in the field conditions.

Of the 11 plants essential oils tested, *O. suave* (fresh) (LC_{50} = of 0.0014 ml% v/v) and (LC_{90} = 0.0027 ml% v/v) had the highest adulticidal activity against *An. arabiensis* in the laboratory followed by *Thymus vulgaris* with LC_{50} and LC_{90} values of 0.0028 and 0.005 ml% (v/v), respectively. The lowest adulticidal activity was obtained from essential oils of *S. molle* (leaves), *Eucalyptus globulus* and *P. nigrum*. *O. suave* (fresh) at lower concentrations (0.05 ml% v/v) caused 100% mortality instantaneously with in 5 minutes. However, *P. nigrum* caused 100% mortality with in 5 minutes at higher concentration (2.5 ml% v/v). In residual activity test, *O. suave* (fresh) essential oil showed the highest potency against *An. arabiensis* (persists for 15 days). However, the lowest residual activity against *An. arabiensis* adult was obtained from the essential oil of *E. citriodora* (persist only for 2 days).

1. INTRODUCTION

1.1. Malaria

Mosquito borne diseases contribute significantly to disease burden, death, poverty, and social debility in tropical countries. Among these diseases, malaria affects some 300-500 million people and kills three million people annually throughout the world (WHO, 1995; Ghai and Gupta, 2000). More than 40% of the world's populations live in malarious areas (Ghai and Gupta, 2000). It is also a highly complex disease vectored by many different mosquito species.

1.1.1. Malaria in Africa

In Africa, malaria is responsible for 90% of the continents human disease burden and for more than 10% of disability to adjust life years (Breman *et al.*, 2004). It also constitutes nearly 25% of all childhood mortality. In Sub-Saharan alone, malaria causes high levels of human suffering and mortality. More than 74% of the populations live in highly endemic areas while about 18% lives in epidemic prone areas and only 7% lives in low or malaria free areas. The annual clinical cases are in magnitude of 350 to 500 million with more than one million deaths, most of them among children under five years of age (Brown, 2002; Renshaw and Silver 2001).

The economic burden of malaria to the country, the family and the individual is immense. It has been estimated that it causes a reduction of 1.3% in the annual per capita economic growth rate of malaria endemic countries (WHO, 1993b). The economic effects of malaria are especially noticeable in rural areas where malaria strikes at the time of the year when there is greatest need for agricultural work. The situation is worsening with the evolution of resistance to cheap and easily available drugs and insecticides (WHO, 1992), changes in environmental conditions leading to increasing epidemics, civil unrest coupled with population movements and economic development programmes in risk areas such as wetlands, desert fringes, and highlands. Indeed malaria has spread into areas, which previously had low or no transmission (WHO, 1993a; Brown, 2002).

1.1.2. Malaria in Ethiopia

Malaria is a leading public health problem in Ethiopia where, an estimated 48 million people (68% of the population) live in areas at risk of malaria. Each year more than 5 million malaria cases are estimated to occur in the country (Tulu, 1993; MOH, 2002; WHO, 2004). The country had experienced the worst malaria epidemics in 1958 with an estimated three million malaria cases and 150,000 deaths (MOH, 2003). In 2002-2003, the disease was the primary cause of reported morbidity and mortality, accounting for 16% of outpatient visits, 20% of hospital admissions and 27% of hospital deaths. In 2003, large-scale malaria epidemics occurred from April to December resulting in 2 million clinical and confirmed cases and 3000 deaths, affecting 3368 localities in 211 districts (MOH, 2005).

The main cause of seasonal malaria epidemics in Ethiopia is the rainfall associated breeding behavior of the major vector *An. arabiensis*. Malaria transmission in Ethiopia is unstable and characterized by frequent and often large-scale epidemics. Most of the epidemic affected areas are high lands or fringe areas where the population has no immunity to malaria and thus all age groups are equally affected (Abose *et al*., 1998). In Ethiopia, highland malaria epidemics are due to degraded environment and resettlement schemes bringing non-immunes to lower altitude where transmission is already established (Tulu, 1993; Molyneux, 1998). In warm zones, malaria endemicity range from moderate to high. Tulu (1993) reported that malaria in the low lands has contributed to over population, over cultivation, deforestation, soil erosion, and drought and famine in temperate and cold zones of the Ethiopian highlands.

MOH (2002) reported the socioeconomic burden resulting from malaria:

i. The high morbidity and mortality rate in the adult population significantly reduces production activities.
ii. The prevalence of malaria in many productive parts of the country prevents the movement and settlement of people in resource-rich low lying river valleys; on the flip side, the concentration of population in non-malaria risk high land areas has resulted in a massive environmental and ecological degradation of the country to repeated droughts, famine and over all object poverty.

iii. The increased school absenteeism during malaria epidemics significantly reduces learning capacity of students.

iv. Coping up with malaria epidemics overwhelms the capacity of the health services in Ethiopia and thus substantially increases public health expenditures.

Thus, malaria in Ethiopia, is not only a health issue, it is also a food security and environmental issue.

1.2. Malaria vectors

The malaria vectorial system is very complex. Approximately 70 species of *Anopheles* have been implicated in malaria transmission worldwide (Service, 1996). They are a widespread species displaying a capacity to adapt rapidly to increasing ecological and environmental change, with an increasing number of species complexes, thus indicating a high level of genetic diversity and plasticity (Molyneux, 1998).

1.2.1. Malaria vectors in Africa

Members of the *An. gambiae* complex are the most important vectors of malaria in sub-Sahara Africa. The complex consists of about seven species: *An. gambiae* s.s, *An. arabiensis, An. quadriannulatus* spp.A, *An. bwamnbe, An. merus, An. melas* and *An. quadriannulatus* spp. B (Hunt *et al*., 1998). Two species of the complex: *An. gambiae* s.s. and *An. arabiensis*, are both most broadly distributed and the most efficient vectors of malaria (Tulu, 1993; Fontenille and Lochouarn, 1999; Coetzee *et al*., 2000).

The range and relative abundance of *An. arabiensis* and *An. gambiae* s.s appear to be strongly influenced by climatological factors, especially total annual rain falls (Lindsay *et al.*, 1998). Generally, *An. arabiensis* tends to predominate in arid savannas, where as *An. gambiae* s.s. is the dominant species in humid forest zones. However, in most Africa, the two species occur in sypmpatry (Coetzee *et al*., 2000). *An. funestus, An. bwamnbe, An. merus,* and *An. melas* are also an important species in malaria transmission in localized areas (Coetzee *et al*., 2000). *An. funestus* is particularly important at the end of rainy season (Depiny *et al*., 2004).

1.2.2. Malaria vectors in Ethiopia

In Ethiopia, 43 *Anopheles* species have been documented. But, *An. arabiensis* in the *An. gambiae* complex is the most widespread and principal vector (Tulu, 1993; Abose *et al.*, 1998). *An. nili*, *An. pharoensis* and *An. funestus are* the secondary important vectors of malaria (Gebre-Mariam, 1988; Tulu, 1993; Abose *et al*., 1998). *An. quadriannulatus* spp.B in the *An. gambiae* complex is zoophilic and has no role in malaria transmission (Coetzee, 2004).

1.3. Malaria vectors control

Worldwide, mosquito control depends primarily on indoor residual house spraying, insect growth regulators, biological control methods, insecticide treated mosquito nets (ITNs), larvicides and environmental management (Butler, 1997). However, the service delivery, species diversity (Tulu, 1993), development of insecticide resistance in most African countries by most of the important vector species (Brown and Pal, 1971; WHO, 1992; Coetzee *et al*., 2000), choice of control strategy and cost for some of these materials (Paul *et al*., 2006), makes them unsuitable for mosquito control, especially in areas of the world that are subjected to the greatest human health threats from mosquitoes.

Fontenille and Lochouarn (1999) indicated that any vector control measure should consider (i) the vector species implicated and the population dynamics, (ii) the biology and behaviour, the vectorial capacity and the vectorial competence of each vector species, (iii) the genetic structure and the gene flow among and between the populations.

1.3.1. Adult mosquito control

The adult control method consists of indoor residual insecticides, insecticide treated bed nets, and repellents such as synthetic (Tawatsin *et al.,* 2001) and traditional (Curtis *et al.,* 1989) repellents.

1.3.1.1. Indoor chemical spraying

Chemical insecticides are one of the major tools for controlling vector populations and for reducing the transmissions of human pathogens (Paul *et al*., 2006). Due to the introduction of synthetic organic insecticides for vector control in the 1940s and 1960s, the prevalence of vector-borne diseases decrease in many parts of the world. The commonly used insecticide during the 50's and 60's was dichlorodiphenyltrichloroethane (DDT) (Mabaso *et al*., 2004). DDT is still used in some Africa countries for the control of epidemics (e.g. South Africa and Ethiopia), although its use is know very much restricted. Other groups of insecticides such as organophosphate, carbamates and pyrethroids are available for indoor spraying.

Indoor spraying is one of the most valuable tools in malaria vector control. It was the strategy used in the most successful eradication programmes of the 50's and 60's (Mabaso *et al*., 2004). Indoor house spraying involves the spraying of inside walls with a long lasting insecticide. A variety of insecticides are available for indoor spraying, and selection of which insecticide to use will depend on the local situations (e.g. susceptibility of local vectors, vector behavior, areas to be sprayed) and resources available (cost of the insecticide and human power) (Service, 1996).

Service (1996) suggested that indoor house spraying is considered as appropriate and cost effective where:

i. A high percentage of structures in an operational area have adequate sprayable surfaces and can be well sprayed.
ii. The majority of vector population rest indoors and
iii. The vector is susceptible to the insecticide in use.

In Ethiopia, vector control is carried out mainly by means of chemical measures, particularly indoor residual house spray (MOH, 2002). Highly malarious localities are sprayed twice a year while moderately malarious localities are sprayed once a year (Abose *et al*., 1998). Currently, insecticide treated bed nets (ITNs) are also introduced.

However, the incidence of many vector-borne pathogens is increasing and vector control is becoming increasingly difficult because of several species becoming physiologically

resistant to many of the conventional insecticides (WHO, 1992) and due to the limited number of insecticides is available for use in public health (Paul *et al*., 2006).

In Ethiopia, high and epidemiologically significant resistance to DDT was reported from Gambella and Arbaminch areas (Abose *et al*., 1998) though the level of *An. arabiensis* resistance to DDT was moderately low in some other localities (Nigatu *et al*., 1994; Balkew *et al.,* 2003).

1.3.1.2. Insecticide treated mosquito nets (ITNs)

Bed nets are traditionally used to ward off mosquitoes and have been advocated as a means of personal protection against malaria vector in Africa. However, torn or incorrectly tucked nets provide little additional protection and mosquitoes are adept at feeding through nets on exposed limbs (Port and Boreham, 1982). For these reasons the application of a residual insecticide to bed nets was suggested as a means of reinstating the effectiveness of torn or incorrectly used nets as a man vector barrier. Insecticide treated nets provide good protection from mosquitoes after people go to bed and when used widely in the community. However, there remains the problem of biting and disease transmission when people are sitting out side houses before going to bed (Port and Boreham, 1982).

Nowadays, pyrethroid-treated bed nets are an effective means for controlling malaria vectors and preventing diseases for large scale deployment in highly endemic areas (Curtis *et al.,* 2003; Lengeler and Snow, 1996). However, over reliance on a single class of insecticides for bed net treatment probably may lead to the evolution of resistance. Thus, Zaim and Guillet (2002) pointed out that there is an urgent need for the alternative insecticides to control the vectors of human diseases.

The use of insecticide treated bed nets can be considered as an option for reducing contact with *An. arabiensis*, but would be ineffective against *An. pharoensis* because most man-vector contact with this species occurs out doors during the early hours of the evening (Abose *et al.,* 1998).

1.3.1.3. Synthetic repellents

Majorities of commercial repellents are prepared by using chemicals like N, N- diethyl-m-toluamide (DEET) and diethyl phthalate (DMP). DEET is the most commonly used synthetic repellent which is effective against a broad spectrum of biting insects (Novak, 1985: Osimitz and Grothaus, 1995). They are used in protecting people from mosquito bites and therefore also reduce transmission of mosquito-borne diseases (Tawatsin *et al.,* 2001). DEET is generally safer for topical use if applied as recommended. However, unpleasant smell, oily feeling to some users and potential toxicity has been reported (Robbins and Cherniack, 1986; Sudakin and Trevathan, 2003). Many people consider that DEET and related compounds are a health and environmental hazard (Aquino *et al*., 2004). DEET does not readily degrade by hydrolysis at environmental PHs and has been identified as a ubiquitous pollutant in aquatic ecosystem, the effects of which are unknown (Aquino *et al*., 2004). Due to the deleterious effects associated with some synthetic repellents, some prefers to use natural insect repellent products. Natural products are safe for human when compared to synthetic compounds and rapidly degrade in the environment (Sharma and Nagpal, 1993). Moreover, they may be economically more optimal alternatives to synthetic chemicals for use in low-income areas of world where problems with vector-borne infections tend to predominate.

1.3.1.4. Botanical repellents

The repellent properties of plants to mosquitoes and other pest insect were well known before the advent of synthetic chemicals. Use of traditional repellents is widespread among different cultures and very common in many communities (Curtis *et al.,* 1989). Herbs of the basil family (Labiatae) are used as mosquito repellents in East Africa (Kokwaro, 1976). People in Northern Ghana used fresh strong scented leaves around their beds to drive away mosquitoes. They also control mosquito by burning green or a dried leaf of special plants (Binka and Adongo, 1997). In Tanzania, branches of *Ocimum suave* are often placed around the windows and doors to keep the mosquito's away (Chogo and Crank, 1981). Palsson and Jaenson (1999) experimentally tested different plants against mosquito in Guinea-Bissau and found smouldering of *Hyptis suaveolens* (Lamiaceae) and smoking of

the bark of *Daniellia oliveri* (Caesalpiniaceae) are effective mosquito repellents. Seyoum *et al., (*2002) tested different plants against *An. gambiae* .s.s. in Kenya and found thermal expulsion of leaves and seeds of *Ocimum suave* and *O. kilimandsharicum* and leaves of *Eucalyptus citriodora* were effective repellents. Leaves of *Eucalyptus citrodora* also showed repellency by direct burning. The intact potted plants including *O. americanum, Lantana camara* and *Lippia ukambensis* showed significant repellency against *An. gambiae* s.s. (Seyoum *et al.,* 2003). The leaf extracts of *O. suave* showed a strong repellent effect to mosquito in Tanzania (Chago and Crank, 1981). In Ethiopia, Astbaha (2005) evaluated repellency of some selected local plants against *An. arabiensis, An. pharoensis* and some culicine mosquitoes. He reported that the thermal expulsion of seeds of *Shinus molle, Melia azedarach*, and *Ruta challepensis* were good repellents of mosquitoes. Direct burning of the seeds of *R. challepensis* (78%), *S. molle* (58.3%) and *M. azedarach* (54.3%) repel mosquitoes significantly.

Rajkumar and Jebanesan (2005) tested the leaf extracts of *Solanum trilobatum* against the malaria vector *An. stephensis* and observed 70 to 120 minutes' protection. Tawatsin *et al*., (2001) noted that the volatile oils of *Curcuma longa, Cymbopogon winterianus* and *Ocimum americanum* were topical repellent against both day and night biting mosquitoes. Das *et al*. (2003) reported the repellent effects of essential oils of *Zanthoxylum limonella* and *Citrus aurantifolia*. Oshaghi *et al.* (2003) tested the repellent effect of extracts and essential oils of *Citrus limon* and *Melissa officinalis* against *An. stephensi* and they found that lemon oil was equally repellent with DEET against *An. stephensi* hence the oil could serve as alternative to DEET.

Jaenson *et al*. (2006) reported that ethyl acetate extracts of *Hyptis suaveolens, Rhododendon tomentosum* and *Myrica gale* and extracts from leaves of *R. tomentosum M. gale* and *Achillea millefolium* significantly reduced probing and biting activity of *Ae. aegypti*, respectively. In Zimbabwe, Lukwa *et al*. (1999) reported that 50% of the study subjects use *Lippia jaunica* as mosquito repellent by smoking and placing fresh plant parts. In Western Kenya, *L. ukambensis* was tested in live and intact potted form, and showed significant repellency (45.2%) against *An.gambiae* s. l. (Seyoum *et al.,* 2003).

1.3.2. Mosquito larval control

Larval control involves the killing of immature stages of mosquitoes by application of various forms of chemicals to the breeding sites. Larvicides target larvae in the breeding habitat before they can mature into adult mosquitoes and disperse. The most common chemicals are insecticides of various groups, insect growth regulators, bacterial larvicides and botanicals (Service, 1996).

Killeen *et al.* (2002) explained that larval control has advantages over the adult control in that adults are highly mobile flying insects that can readily detect and avoid many control measures while mosquito eggs, larvae and pupae are confined within relatively small aquatic habitats and can not readily escape control measures. Amer and Mehlhorn (2006a) also noted that larviciding can reduce overall pesticide use in a control programme by reducing or eliminating the need for the ground or aerial application of chemicals to kill adult mosquitoes.

1.3.2.1. Chemical control

Chemical larvicides are chemicals of various kinds, which can be applied to the surface of water to breeding place to destroy mosquito larvae, as well as their eggs and pupae. However, continued and heavy usage of some larvicides has created serious problems such as toxicity to non-target organisms and accumulations in food chains and non-target organisms (Norris *et al*., 2003; Hemingway and Craig, 2004). However, natural pesticides, especially derived from plants, provide a more environmentally safe vector control methods (Minjas and Sarda, 1986), and non-toxic to non-target organisms (Sukumar *et al.*, 1991) than the use of synthetic chemicals.

1.3.2.2. Biological control

Biological control of mosquito vectors involves the introduction of natural enemies into mosquito breeding sites. These could be in the form of parasites or predatory animals. Use of biological control agents require a good understanding of the agents and the mosquitoes to be controlled as well as their local environment. The most widely employed biological

control agent is the larvivorous fish, or bacteria and fungi as pathogens or toxins to the insects (Lacey and Orr, 1994; Scholte *et al*., 2004).

1.3.2.2.1. Pathogens

Pathogens are microbial larvicides that are naturally occurring bacterium, fungi and virus. Mosquito larvae eat the product that is made up of the dormant spore form of the bacterium and an associated pure toxin (Lacey and Orr, 1994).

Bacillus thuringiensis israelensis (Bti) infects and kills mosquito larvae when consumed by the larvae in the aquatic medium. The proteins released from the bacteria act as a stomach poison, damaging mid-gut cells of mosquito larvae. It is highly selective, killing only mosquitoes and larvae of a few other related flies (Lacey and Orr, 1994). *Bti* is used in irrigation ditches, floodwater and fresh water or salt-water marshes. However, bacterial products are expensive and their larvicidal activity is short duration (Lacey and Undeen, 1986). *Bacillus sphericus* is fairly persistent in a wide variety of mosquito habitats (Lacey *et al*., 1988). It causes high mortality initially and the survivors of treatments experience longer pupal periods, decreased survival as adults, and lower energy reserves (Mulla *et al*., 1991). *Anopheles* larvae that survived exposure to the bacterium were also less competent vectors of plasmodium species. Like *B. thuringiensis*, it has shortcomings such as cost of production and relatively short residual activity (Lacey and Orr, 1994).

The fungal pathogen of mosquito larvae are recognized as significant potential biological control agents. These include *Lagenidium giganteum*, *Culicinomyces clavisporus* and *Coelomomyces* spp. The infectious zoospores actively swim towards the mosquito larva, and if a larva contacted, they settle on its surface, encyst, and eventually penetrate the cuticle when the proliferate is in the larval body or ingested by the larvae, resulting in the death of larvae (Scholte *et al*., 2004). A few trials have been done in rice fields in the California, USA (Lacey and Undeen, 1986) by using *L. giganteum.*

1.3.2.2.2. Larvivarous fishes

Many fish species are potential predators of mosquito larvae. Fish with potential for mosquito control are those which show preference for mosquito larvae over other types of food, are small in size, have high reproductive rate in small water bodies and are locally available. The most promising species are the *Gambusia affinis* and *Poecilia reticulata* (Service, 1996). However, it takes time for the fish to establish themselves, so not suitable in out break situations.

1.3.2.3. Botanical larvicides

One potential alternative or additional control method/ material is application of selected botanical derivatives against the target mosquito species (Perich *et al*., 1995). Photo-chemicals derived from plant resources can act as larvicides and insect growth regulators (Venkatachalam and Jebanesan, 2001). Many plant extracts of terrestrial origin have been reported to suppress mosquito larvae populations (Chavan and Nikam, 1982). For example, in Pakistan, Islam *et al.* (2003) evaluated a pure compound phenyl ethyl-D glucopyranoside, which is isolated from *Sida rhombitolia*, against *Culex quinquefasciatus*. They observed mortality of larvae and many larvae failed to ecdyse to prefect pupae due to disturbance in molting and metamorphosis. Thomas *et al.* (2004) evaluated the essential oil extracted from *Ipomoea cairica* against the larvae of vector species of mosquitoes and observed a remarkable larvicidal property. In the Northeast of Brazil, Cavalcanti *et al.* (2004) noted that essential oil of *Cymbopogon citratus* was active larvicides against third instars larvae of *Aedes aegypti*. In Nigeria volatile oils of *Ocimum gratissium, C. citratus* and *Ageratum conyzoides* were also showed larvicidal activity against *Aedes aegypti (*Sosan *et al.,* 2001). Bassole *et al.* (2003) reported the larvicidal activity of *C. proximus* essential oils against *Ae. aegypti* and *An. gambiae* third and fourth instar larvae.

Ameen *et al*. (1985) reported the larvicidal effects of *Derris elliptica* (Leguminosae) root against fourth instar larvae of *Ae. aegypti.* Chapagain and Wiesman (2005) tested the larvicidal effect of aqueous extracts of the fruit pulp, seed kernel, roots, bark, and leave of *Balanitie aegyptiaca* (Zygophyllaceae) against the larvae of the *Cx. pipens* mosquitoes. Singh *et al.* (2001) reported the larvicidal activity of *Solanum nigrum* extract against *An.*

culicifacies, Ae. aegypti and *Cx. quinquefasciatus* in the laboratory with LC_{50} values of 0.027, 0.032 and 0.027ml% in water, respectively. Neem oils obtained from crushed seeds of *Azadirachita indica* was effective larvicide against *Ae. aegypti* and *Cx. quinquefasciatus* larvae (Sinniah *et al.,* 1994). Pushpalatha and Muthukrishnan (1999) reported the larvicidal activity of seed and leaf extracts of *Calophyllum inophyllum* and leaf extract of *Rhinacanthus nasutus* against *Cx. quinquefasciatus, Ae. aegypti* and An. *stephensi* larvae. The leaf extract of *Agave americana* showed strong larvicidal activity against *Anopheles, Aedes* and *Culex* larvae (Dharmshaktu *et al*., 1987). Singh and Bansal (2003) tested the larvicidal properties of *Solanum xanthocarpum* against vectors of malaria and dengue larvae. Monzon *et al*. (1994) reported the larvicidal activity of five Philippine plants against *Ae. aegypti* and *Cx. quinquefasciatus* larvae and found that *Anona squamosa* showed the highest larvicidal activity with LC_{50} values of 2.4196 and 4.7693 g% (w/v), respectively.

Ethanol extracts of 83 plant species belong to the Asteraceae were tested for larvicidal activity against *Ae. fluviatilis* larvae (Macedo *et al.,* 1997). Mwaiko and Savaeli (1994) reported the larvicidal activity of lemon peel against the immature stages of *Cx. quinquefasciatus.* Chansang *et al.* (2005) reported the larvicidal activity of aqueous extract of *P. retrofractum* against *Cx. quinquefasciatus* and *Ae. aegypti* larvae. *M. spicata* variety *varidis* has been evaluated for larvicidal, oviposition deterrent and repellent properties against various stages of *An. stephensi* (Tripathi *et al.,* 2004). Ethanolic extracts from *Ginkgo biloba* were evaluated against different strains of *Cx. pipiens pallens* larvae (Sun *et al.,* 2006).

Pitasawat *et al*. (1998) screened the larvicidal effects of ten plants species and found three plants essential oils (*Kaempferia galangal, Illicium vernum* and *Spilanthes acmella*) to have larvicidal properties against *Culex quinquefasciatus*. Similarly, Jang *et al*. (2002) reported the larvicidal activity of methanol extracts of 26 leguminous seeds and 20 grains against *Ae. aegypti* and *Cx. pipiens pallens* larvae. Recently, Jantan *et al.* (2005) evaluated the leaf essential oils of 8 *Cinnamomum* species of plants for larvicidal activity against *Ae. aegypti* and *Ae. albopictus* and found 5 species (*C. impressicostatum, C. microphyllum, C. pubescens, C. mollissimum* and *C. rhyncophyllum*) to have significant larvicidal effects. More recently, Amer and Mehhorn (2006a) evaluated the larvicidal effects of essential oils

of 41 plants against *Aedes*, *Anopheles* and *Culex* mosquitoes and reported 13 plants oils (*Cinnamomum camphora*, *Thymus serpyllum*, *Amyris balsamifera*, *Citrus limon*, *Juniperus virginiana*, *Boswellia carteri*, *Anethum graveolens*, *Myrtus communis*, *J. communis*, *Piper nigrum*, *Lippia citriodora*, *Helichrysum italicum* and *Santalum album)* to have significant effects.

1.4. Test plants bearing essential oils

1.4.1. Essential oils

Essential oils are the volatile and aromatic oils obtained by hydro-distillation of botanicals. Most essential oils are primarily composed of terpenes and their oxygenated derivatives. Different parts of the plants can be used to obtain essential oils, including the flowers, leaves, seeds, roots, stems, bark and wood. They are composed of volatile liquid and solid compounds which vary widely in regard to their composition and boiling points (Guenther, 1949).

1.4.2. Description of test plants

C. ambrosioides L. (Amedmado or gimy -Amh) (Chenopodiaceae Family) or Mexican tea originated in Central America but is now distributed throughout of the world. It is a herb that grows to a height of 40cm. The leaves are oval and toothed. The flowers are small and green, and the seeds are very small and green when fresh and black when dry. The plant has a very strong odor (Friis and Gilbert, 2000). *C. ambrosioides* has been used as an anthelmintic for many years. In the early 1900s it was one of the major anthelmintics used to treat ascarids, and hook worms in humans, cats, horses, and pigs. It is still used to treat worm infections in humans in many countries. In many Latin American countries, the whole plant or leaves are ground and added to water. This mixture is then consumed to treat worm infections (Kliks, 1985). It has been also used as a traditional protectant for stored beans and groundnuts in the Congo (Peteroson *et al.*, 1989). The oil is used externally to treat athlete's foot and insect bites. The plant is used as a fumigant against mosquitoes and is also added to fertilizers to inhibit insect larval development. It is also used as a fragrance component in creams, perfumes, and soaps.

Chiasson *et al*. (2004) reported that the essential oil extraction of *C. ambrosioides* was effective against the adult and egg stages of *Tetranychus urticae* (spider mites) and *Panonychus ulmi* (European red mite). They also reported that the extract from *C. ambrosioides* was effective against *Mytus persicae* (peach aphid).There are many compounds in *Chenopodium.* The major components of oil of *Chenopodium* are ascaridole; a monoterpene (60-80%), isoascaridole, p-cymene, limonene, and x-terpinene (Chiasson *et al*., 2004). The level of the different compounds varied depending on the part of the plant, and whether it is dried or fresh plant material.

Eucalyptus citriodora Hook and *E.* globules Labill. (Bahirzaf -Amh) (Myrtaceae Family) are well known for their essential oils. They are used in Brazilian folk medicine for the treatment of various medical conditions such as cold, flue, fever, and bronchial infections (Silva *et al.*, 2003). Hot water extracts of dried leaves of *E. citriodora* are traditionally used as analgesic, anti-inflammatory, and anti-pyretic remedies for the symptoms of respiratory infections, such as cold, flue, and sinus congestion (Kokwaro, 1976). The crushed leaves emit delightful lemon scent. The leaves and a lemon scented oil rich in citronella are used in perfumery and as flavoring agents besides possessing pesticidal properties (Isman, 2000). Singh *et al*. (2005) reported that the volatile oils of *E. citriodora* possess weed suppressing ability and used as bioherbicide for weed control. *E. citriodora* is insect repellent (Kokwaro, 1976). The wood is good for saw-timber, used for general construction, poles, railroad ties, and tool handles.

Cubans placed the leaves under the sheets of patients with high fever, and inhaled the steam from boiled leaves for cold and various pulmonary problems. Cubans also poultice the leaves on to ulcers, wounds, and other skin ailments (Watt and Breyer-Brandwijk, 1962). In Western Africa, Palsson and Jaenson (1999) tested smoke of the leaves of *Eucalyptus* and it showed 72.2% repellency against mosquitoes. Seyoum *et al.* (2002) noted that the fresh leaves of *E. citriodora* were an effective repellent against *An. gambiae*. In Western Kenya, thermal expulsion of the leaves of *E. citriodora* resulted in highest repellency against *An. gambiae.* s.s. (Seyoum *et al.*, 2003). Trigg (1996) reported the comparable repellent activity of *E. citriodora* extract with DEET against *Anopheles* mosquitoes.

Components (cymol and limonene) of the essential oils of *E. camalulensis* and *E. cameroni* have significant insecticidal action, being lethal to stored product pests (Ibrahim, 2001). Limonene has shown insecticidal properties against human blood-sucking insects when tested against early fourth instar larvae of mosquito *Culex quinquefasciatus.* Limonene treated water was less favorable than untreated water for oviposition by females of the mosquito (Kassir *et al.,* 1989). The essential oils of *E. citriodora* and *E. globulus* were active against female *Pediculus humanus capitis* (Yang *et al*., 2004).

Lippia adoensis Hochst.ex Schau.(Koseret -Amh) (Family Verbenaceae) are herbs, shrubs and small trees (Terblanche and Kornelius, 1996). The leaves and flowers are first rubbed between the palms to get the maximum scent emitted, and when sniffed the subject is set sneezing which then clears the nose (Kokwaro, 1976). *L. adoensis* is endemic to the afro-montane region of Ethiopia. Wild *Lippia* species has lemon like odor, which can be used to differentiate it from the cultivated species, which have rather sweet fragrance. For the treatment of malaria, a decoction of boiled leaves is taken and the whole body bathed in the same fluid. Pounded leaves can also be applied on cut wounds, or soaked in water and the juice drunk for the treatment of tapeworm and for indigestion (Kokwaro, 1976). In Ethiopia, the cultivated *L. adoensis* is traditionally used to flavor butter for a special dish known as" kitfo" (Abegaz *et al.,* 1993). It is also a source of essential oils and food constituents (Wiley, 2005).

The essential oil and its main constituent, thymol, of *L. sidoides* were shown to have potent larvicidal activity against *Ae. aegypti* (Carvalho *et al*., 2003; Cavalcanti *et al.,* 2004). In the Northeast of Brazil, Cavalcanti *et al*. (2004) reported the larvicidal activity of essential oil *L. sidoides* against third instars larvae of *Ae. aegypti.* Bassole *et al.* (2003) also reported the larvicidal activity of *L. multiflora* essential oils against *Ae. aegypti* and *An. gambiae* larvae. The intact potted *L. ukambensis* showed significant repellency against *An. gambiae* s.l. (Seyoum *et al.,* 2003).

M. spicata L. (Anana -Amh) (Family Labiatae) is cultivated in home gardens at altitude up to 2500m a. s. l. in Ethiopia (Fichtl and Adi, 1994). The essential oil and Piperitenone oxide, major component of essential oil, of *M. spicata viridis* showed larvicidal, ovicidal, oviposition deterrent, developmental toxicity, and repellent properties against various

stages of *An. stephensi* (Tripathi *et al*., 2004). The higher efficacy was observed in Piperitenone oxide than the crude essential oil of *M. spicata* variety, *viridis* in all bioassay. Ansari *et al.* (2000) reported that the essential oils of mint are widely used in flavoring, pharmaceuticals, medicines, and pesticides. Tripathi *et al*. (2000) reported the insecticidal properties of *M. spicata* essential oils against stored product insects. Pathak *et al*. (2000) reported the larvicidal activity of *M. piperita* against *An. stephensi*. Traboulsi *et al*. (2002) noted the larvicidal activity of *M. microcorphylla* extract against larvae of *Cx. pipiens molestus.* They identified the main components of its extract; piperitenone, pulegone, piperitenone oxide and menthone that caused larval mortality.

Nigella sativa L. (Tqur azmud -Amh) (Family Ranunculaceae) is known to contains 20 species. It has been used as a herbal medicine for many years. It is also used as a food additive and flavor in many countries (Fichtl and Adi, 1994). *N. sativa* volatile oil has recently be shown to possess 67 constituents, many of which are capable of inducing beneficial pharmacological effects in humans. The seeds of *N. sativa* have been used traditionally for treatment of asthma, cough, bronchitis, headache, fever and influenza (Ghannadi, 2005). Furthermore, black cumin seeds are of importance as a carminative and spice or remedy used as condiment in bread and other dishes (Merfort *et al.,* 1997).

In Ethiopia some species are widely cultivated and their seeds are used as spice in preparing hot pepper sauce and other dishes. They are also used to flavor some types of breed. The seeds are also mixed with melted butter, wrapped in a piece of cloth and sniffed to relieve some types of headaches (Teketay, 2000). The essential oil of black cumin seeds and the major constituents of essential oil, thymoquinone, carvacrol, t-anethole and 4-terpineol, were shown to have antioxidant activity (Burits and Bucar, 2000). The extract of *N. sativa* has antimicrobial, antiviral, antihelmintic, anti-inflammatory and immunomodulatory activities. However, no literature was available on larvicidal and adulticidal activity of its essential oil against mosquitoes.

Ocimum suave Willd. and *O. lamiifolium* Hochst.ex Benth. (Damakasse -Amh) (Lamiaceae Family) species are strongly aromatic plants. *O. suave* is common in the up land forest areas of East Africa. The plant is branched, erect shrub to 2m in height. *O. suave* is used as

a traditional medicine for the treatment of human aliments such as stomachache, cough, and influenza (Makonnen *et al*., 2003). The strongly scented leaves of the plant is rubbed between the palms and snuffed as a treatment for blocked nostrils. The leaves are also used for the abdominal pains, sore eyes, ear troubles, and for coughs. An infusion of leaves acts as a disinfectant and as an insecticide (Kokwaro, 1976). It is also used as a perfume, an insect repellent (particularly mosquitoes) and a grain protection (Hassanali *et al*., 1990). It is also used as repellents, toxicants and protectants against stored product pest insects (Bekele *et al.,* 1996; 1997). The oil of *O. suave* was active repellent and kills all active stages of the tick *Rhipicephalus appendiculatus* (Mwangi *et al.,* 1995). Periodic thermal expulsion of leaves and seeds of *O. suave* and *O. kilimandscharicum* showed repellency against *An. gambiae* (Seyoum *et al.* 2002; 2003). The intact potted plants of *O. americanum* provided significant repellency (37.9%) against *An. gambiae. s.l* (Seyoum *et al*., 2003).

Ocimum lamiifolium is widely distributed in different parts of Ethiopia at an altitude range of 1600-2000 m. a. s. l. (Makonnen *et al*., 2003). Its leaves are medically used at home to give remedy to different ailments like the common cold (Fitchl and Adi, 1994). However, the larvicidal and adulticidal activity of the essential oil of this plant has not been evaluated.

In the Northeast of Brazil, Cavalcanti *et al*. (2004) found that essential oils of *O. gratissium* and *O. americanum* were active larvicides against third instars larvae of *Aedes aegypti*. The extract of *O. canum* in Tanzania was effective against *An. gambiae* larvae (Lukwa, 1994). The essential oils of *O. gratissium* and *O. americanum* were effective against third instar larvae of *Aedes aegypti* (Cavalcanti *et al*., 2004). Chavan and Nikam (1982) also reported the larvicidal activity of essential oil of *O. basilicum* against the larvae of *Anopheles stephensi* mosquito. Essential oil of *O. gratissium* was also shown potent larvicidal activity against *A. aegypti* (Sosan *et al*., 2001). Some studies have shown that the essential oils of the leaves of *O. canum* possess antibacterial (Bassole *et al.,* 2005) and insecticidal (Bassole *et al.,* 2003) properties.

Piper nigrum L. (Qundo berberie -Amh) (Family Piperiacae) has been used in traditional medicine for treatment of various diseases. Black pepper is called king of spices because it is one of the oldest spices with an aroma that is universally pleasant and appetizing. It is a stimulant of digestive juices as well as nerves. *P. amapaense* is used for treatment of dizziness. *P. aduncum* showed aromatic, styptic, antimicrobial and antimycotic activity.

Park *et al.* (2002) evaluated the insecticidal activity of essential oil from fruits of *P. nigrum* against third instar larvae of *Culex pipiens pallens, Aedes aegypti and Ae. togoi* and found that the different components of its essential oils had different larvicidal activity. Pipernonaline, a piperidine alkaloid derived from *P. longum* showed the larvicidal activity against *Ae. aegypti* larvae (Lee, 2000). Yang *et al*. (2002) reported that Pipernonaline, components of essential oils of *P. longum* from fruits had strong larvicidal activities against the fourth stage larvae of *Ae. aegypti*. Chansang *et al*. (2005) noted the larvicidal activity of *P. retrofractum* against *Ae. aegypti* and *Cx. quinquefasciatus* larvae. Some studies have shown the strong larvicidal activity of *P. aduncum* against *Ae aegypti* larvae (Pohlit *et al.*, 2004). Piperine is the major bioactive component of essential oil (Rathnawathic and Buckle, 1983).

Schinus molle L. (Tqur-berberie -Amh) (Family Anacardiaceae) is graceful evergreen tree. It is heat and drought resistant tree that is widely planted in all but the coldest and wettest areas and some times regenerating naturally (Gilbert, 1989). It is widely grown as ornamental and shade tree especially in the towns and cities of the rift valley in Ethiopia. Its branches are commonly placed on dining tables in hotels, restaurants and resident places to repel flies in some parts of the country. Thermal expulsion of seeds of *S. molle* showed repellent activity against anopheline and culicine mosquitoes (Astbaha, 2005).

The essential oil of *S. molle* comprise 50 constituents. The oil exhibited the maximum fungitoxic activity against some common storage and animal pathogenic fungi (Dikshit *et al*., 1986). The toxicity of the oil persisted up to 80^0c and 90 days of storage. The essential oil from fresh leaves also showed the antibacterial and antifungal activities (Gundidza, 1993). However, no information is available on the larvicidal and adulticidal activity of its essential oil.

*Thymus vulgari*s L. (Tosgn -Amh) (Family Lamiaceae) is very rich in essential oils and these are active ingredients responsible for most of the medicinal properties. In particular, Thyme is valued for its antiseptic and antioxidant properties. It also an excellent tonic and is used in treating respiratory diseases and a variety of other ailments. El-Gengaihi *et al*. (1996) evaluated the biological activity of Thyme oil and Thymol, its major constituents against mite. Mansur *et al.* (2000) reported the larvicidal and adulticidal activity of *T. capitatus* extracts against *Cx. pipiens*. They also reported that the high larvicidal activity was due to the high level of carvacrol, main component of *T. capitatus* essential oils.

Current mosquito control strategies depend primarily on synthetic insecticides. However, widespread use of synthetic insecticides in public health and agriculture programs for the control of vector and pest species has created different problems, such as the development of physiological resistance in major vector species (WHO, 1992; Saelim *et al*., 2005), environmental pollution, and toxic hazards to human and other non-target organisms due to their broad spectrum of activity (Minjas and Sarda, 1986; Hemingway and Craig, 2004). This has increase the interest in developing potential alternative or additional control methods and materials. The identification and eventual use of local plants in the control of mosquito may be very valuable for developing countries. Besides being more readily available, they are more economical to use and the methods employed are usually simpler (Redwane *et al*., 2002; Monzon *et al.,* 1994). In the present study, essential oils of the some local aromatic plants were tested against larvae of *An. arabiensis* and *Ae. aegypti* in the laboratory and anophelines in the simulated field conditions, and against *An. arabiensis* adults in the laboratory.

2. OBJECTIVES OF THE STUDY

❖ General objectives:

- To determine the insecticidal effects of essential oils of some local aromatic and medicinal plants against mosquitoes both in the laboratory and simulated field conditions.
- To create alternative or additional options using local aromatic and medicinal plant resources for feature integrated management approaches of malaria vector control.

❖ Specific objectives:

- To determine the larvicidal properties of essential oils of some selected aromatic plants found in Ethiopia against *An. arabiensis* and *Ae. aegypti* larvae in the laboratory.
- To determine the larvicidal properties of essential oils of some selected aromatic plants found in Ethiopia against anopheline mosquito larvae in the simulated field conditions.
- To determine the adulticidal effects of the essential oils of some aromatic plants found in Ethiopia against *An. arabiensis* adults.
- To determine mortality-time rates of some essential oils against *An. arabiensis and Ae. aegypti* larvae and *An. arabiensis* adults.
- To determine the residual activities of different essential oils against *An. arabiensis* adults.

3. MATERIALS AND METHODS

3.1. Collection of plant materials and distillation of essential oils

Essential oils bearing plant species which are with high levels of traditional medicinal value and some reported in the literature to have medicinal and /or insecticidal properties were used in this study. These include *Chenopodium ambrosioides* L., *Eucalyptus citriodora* Hook, *E. globules* Labill., *Lippia adoensis* Hochst.ex Schau., *Mentha spicata* L., *Nigella sativa* L., *Ocimum lamiifolium* Hochst.ex Benth. , *O. suave* Willd. , *Piper nigrum* L., *Schinus molle* L., and *Thymus vulgaris* L. (Appendix 1). However, no work has been done on the mosquito larvicidal and adulticidal activities of these plants in Ethiopia.

The samples of test plants were collected from different localities of the country (Table 1). The accessible parts of the plants were collected for extraction. Taxonomic identification or confirmation of plants was preformed by botanists of the National Herbarium (ETH), Department of Biology, Addis Ababa University. Prior to extraction of essential oils, moisture content of each sample was determined by using oven drying method at 100^0c. Essential oils were extracted from leaves and/or seeds of the different test plants by hydro-distillation method using a Clevenger-type apparatus for 3 hours (Fig. 1) (Guenther, 1949). *C. ambrosioides* was also extracted by ethanol in Soxhlet apparatus. The production and quantification of essential oils of test plants were preformed at the laboratory of Essential Oil Research Center (EORC) of Ethiopian Institute of Agricultural Research (EIAR).

Table 1. Plant species, plant locality, collection times and plant part(s) processed for essential oil distillation.

Plant species	Locality	Collection dates	Plant parts
C. ambrosiodes	Addis-Ababa	November 2005	Aerial parts (dry)
L. adoensis	Entoto (Addis Ababa)	December 2005	Leaves (dry)
O. lamiifolium	Hosanna and its vicinities	December 2005	Leaves (dry)
O. suave	Gibe valley	February 2006	Leaves (dry)
O. suave	Gibe valley	February 2006	Leaves (fresh)
S. molle	Debera-zait	January 2006	Seeds (fresh)
S. molle	Debera-zait	January 2006	Leaves (fresh)
P. nigrum	Tepi	April 2006	Seeds (dry)

200g dried or freshly collected leaves or seeds were placed in a distillation flask and 1 liter of aqua-distilled water was added. Distillation was repeated to obtain sufficient oils for the experiments. The oils thus obtained were separated from water and stored in airtight containers at 4^{0c} until later use for bioassays.

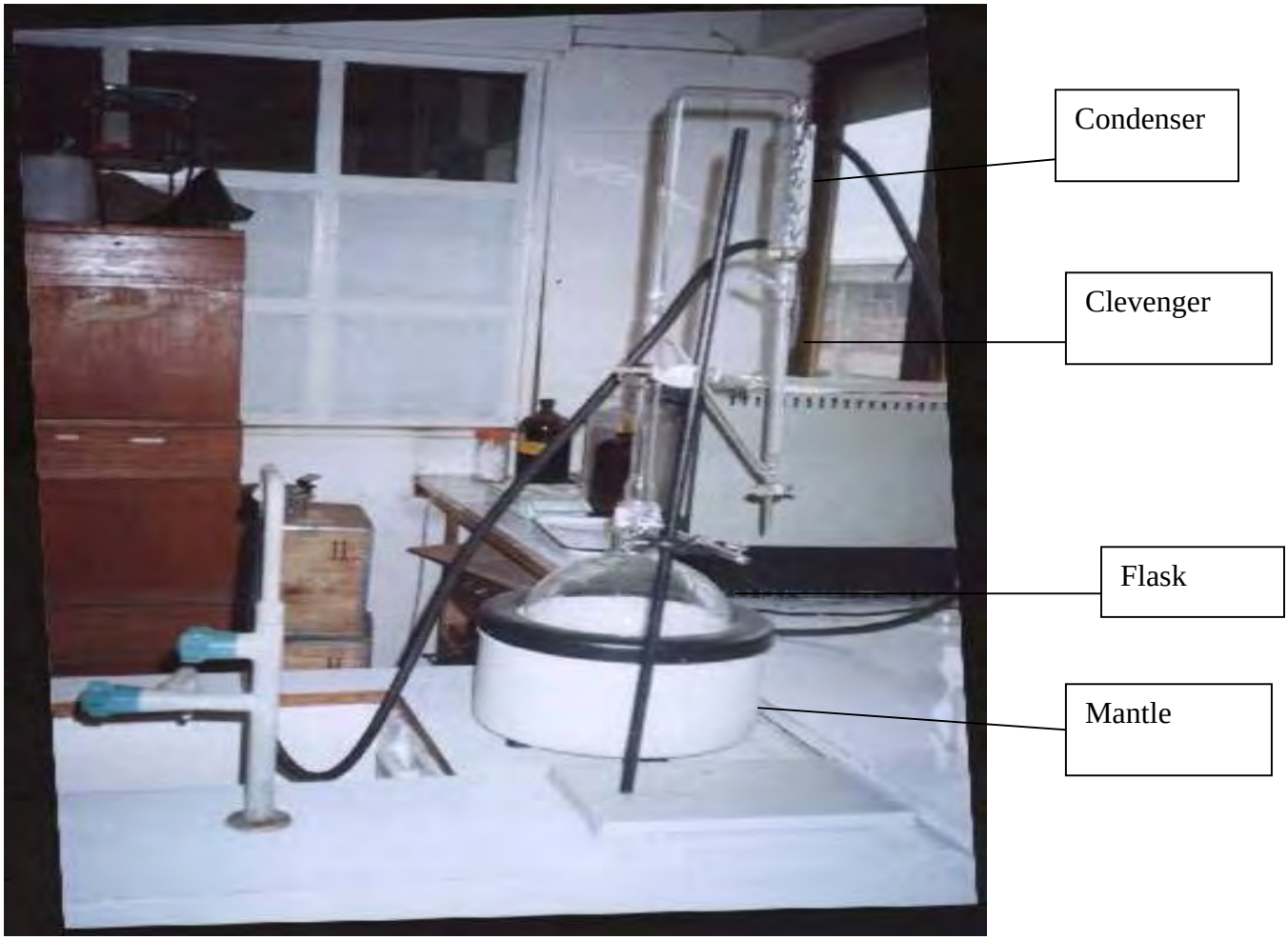

Fig. 1. A Clevenger-type apparatus utilized for essential oil hydro-distillation.

3.2. Maintenance of test mosquitoes

Tests were conducted on *Anopheles arabiensis* $F_{51\text{-}61}$ (Bishoftu population) and *Aedes aegypti* $F_{49\text{-}60}$ (Matahara population) already maintained in the Insectary of Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University at 25-27^{0}c and 60-70% relative humidity. According to ALIPB, larvae were fed with a mixture of powdered dried animal food pellets, dry milk and yeast. Adults maintained in wire mesh nylon cages (30×30×30 cm) and fed with sterile 10% sugar solution soaked in cotton pads placed in petridishes. In addition to sugar feeding, females were allowed to take blood meals from the restrained rabbit three times a week for oviposition. Two developmental stages, larvae and adult females were continuously available for the experiments.

3.3. Larvicidal bioassay in the laboratory

3.3.1. Dose-response bioassay

The larval toxicity tests were carried out following the standard World Health Organization larval bioassay test method (WHO, 2005). The white enamel cups with capacity of 350ml were used for the larvicidal bioassays in the laboratory (Fig. 2). Appropriate amount of each essential oil was dissolved in acetone to prepare 5ml of stock solution for each concentration. For example, 0.05ml of particular essential oil dissolved in 4.95ml acetone to produce 5ml of 1% stock solution. When 1ml of this stock solution was introduced into 149ml of distilled water, the final concentration became 66.67 ppm (Appendix, 2). Fresh stock solution was prepared for each concentration.

Twenty-five healthy and active third to fourth instar larvae from colonies of *An. arabiensis* and *Ae. aegypti* in 19ml distilled water were transferred in to 350ml of white enamel cups which contained 130ml distilled water. 1ml of stock solution was added to each cup contained 149ml distilled water. Then, the final solution became 150ml**.**

The experiments were carried out at 25-27^{0}c and 60-70% relative humidity. Four replicates for treatment and two replicates for control (with 149ml of distilled water and 1ml of acetone) were carried out for each concentration and species of mosquito larvae.

Symptoms of treated larvae were observed and recorded immediately and at timed intervals at least for 5 hours. Final mortality and survival were recorded after 24 hours of exposure period. The moribund and dead larvae in four replicates were combined and expressed as a percentage of larval mortality of each concentration. Dead larvae were those that failed to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showed the characteristic diving reaction when the water was disturbed.

The mortalities of treated larvae were corrected using WHO (1996) formula, viz.

$$\frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100,$$

when control mortality was between 5% and 20%. However, in this case no dead larvae were observed in the controls after 24 hours exposure.

Fig. 2. White enamel cups used for larvicidal bioassays in the laboratory.

3.3.2. Mortality-time tests

In time response experiments, the mortality data were collected at a fixed concentrations such as 666.67 and 333.3 ppm until all test larvae were died. The concentrations used by Carvalho *et al*. (2003) were used as a baseline for the selection of these concentrations. Preparation of test solutions and bioassays were conducted similar to that of dose response experiments.

3.4. Larvicidal bioassay under simulated field condition

3.4.1. Study area

Larvicidal efficacies of essential oils of different plants under simulated field situations were evaluated in May 2006 in Sille village (1100 m. a. s. l.) in southern Ethiopia approximately 525 km from Addis Ababa and 20km south of Arba-Minch.

3.4.2. Test protocol

The simulated field trials were conducted according to the methods recommended by World Health Organization (WHO, 2005) and Mwaiko and Savaeli (1994). Artificial containers (plastic bowels) with shallow depth were used for the simulated field larvicidal bioassay. The containers were burrowed into the ground but not covered with soil. Water from the natural breeding habitats of larvae was added into the containers. Other bigger living organisms were filtered out by nylon mesh screen.

C. ambrosioides, O. lamiifolium, O. suave (fresh), *P. nigrum* and *Schinus molle* were evaluated here against the field population of anopheline larvae in the simulated field conditions. Batch of 40 field collected third and fourth instar larvae of anopheline mosquito in 29ml of water were released into each bowel containing 270ml of water. 5ml of stock solution was prepared by dissolving the calculated amount of the target essential oil in acetone (Appendix 3). After 1 hour of larval acclimatization, each container was treated with 1m of stock solution. The containers were covered with broken pieces of clay pots (‘Gal’) to prevent other mosquitoes from laying eggs, falling debris and rain. Four replicates were carried out for treatment and two replicates as control of each concentration. All containers were examined for 5 hours and final mortality was recorded after 24 hours.

3.5. Adulticidal bioassay under laboratory condition

3.5.1. Dose -response bioassay

Adulticidal mosquito bioassays were conducted in Pyrex bottles (250ml) (Fig. 3) recommended by Brogdon and McAllister (1998b) and Petersen *et al.* (2004). 5ml of the stock solution of each essential oil was prepared for each concentration. Acetone was used as diluent to prepare stock solution of different concentrations (0.00075-1ml%;v/v) of the essential oils. The inner walls of the bottles were evenly coated with 1ml stock solution of each concentration of each essential oil and acetone (for controls) by gentle shaking and rolling the bottles on their side. Then, the bottles were allowed to dry at least for two hours in the laboratory room in a vertical position. Fresh test solutions were prepared for each concentration. Four replicates for treatment and two replicates for controls were prepared.

Ten adult blood deprived females (3 to 5 days old) of *An. arabiensis* were aspirated into each bottle. The opening of the bottles was covered with nylon mosquito nets and held with rubber bands. Adult mosquitoes in the Pyrex bottles were fed with 10% sucrose solution placed on top of nylon mosquito nets. Bioassays were conducted in the laboratory at room temperature. Adults were observed for any behavioral changes and mortality from the treatment time to the end of the experiment. Final mortality of mosquito was recorded at 1 hour as recommended by Petersen *et al*. (2004). Adults were identified as dead if they were lying on their backs or sides and were unable to maintain any flight after gentle tap of the bottle (Petersen *et al.*, 2004) and collected at the bottom of bottle.

In this study, because of time limitation, adulticidal activity of essential oils was only tested against the laboratory colony of *An. arabiensis.*

Fig. 3. Picture of Pyrex bottles used for adulticidal bioassay in the laboratory.

3.5.2. Mortality-time tests

In time response experiments**,** the stock solutions were prepared as described for the dose response experiments. The concentration that caused 100% mortality in the dose response tests during 1 hour was used as a baseline to select different concentrations of each essential oils. The tests were carried out similar to the dose response. Data were collected at a fixed concentration until all test mosquitoes were died.

3.6. Bioassay for residual toxicities of essential oils

Residual effects of essential oils against adult mosquitoes were conducted in cages (15x15x15 cm) made of nylon netting (Fig. 4). The cages were soaked in 1ml% (v/v); the concentration that caused 100% mortality instantaneously in bottle bioassay, of selected plants essential oil solution for 15 minutes. Acetone was used as diluent. The cages were allowed to dry in open air in the laboratory at room temperature at least for 2 hours. The dried cages were then placed inside the polyethylene bag to provide moisture. Finally, 10 female *An. arabiensis* (3-5 days old) were aspirated into the cages. Sugar soaked cotton pads on a filter paper was placed on the upper surface of each cage for the mosquitoes. Four replicates of each treatment and two replicates for control (soaked in acetone of equal

volume) were prepared for the experiment. Mortality was recorded after 24 hours of exposure. The experiments were continued until less than 25% mortality was recorded for each essential oil. The mean percent mortality rates were the means of four replicates.

Due to time shortage and unavailability of some essential oils, the oils that were found in excess and provided some promising activity in adulticidal bioassay were only selected for residual efficacy tests.

Fig. 4. Cages used for residual activity test in the laboratory.

3.7. Data analysis

LC_{50} (the concentration at which 50% of test larvae were died) and LC_{90} (the concentration at which 90% of test larvae were died) and the 95% confidence intervals were calculated by probit analysis using SPSS computer soft ware programs version 11.0. The means and standard errors were calculated by descriptive statistics. LC_{50} and LC_{90} values were judged as significantly different between the essential oils ($p < 0.05$) if the confidence intervals did not overlap for larvae and adults bioassay (Bassole *et al.*, 2003; Petersen *et al*., 2004).

The LC_{50} and LC_{90} values were used to evaluate and compare the larvicidal potency of each plant against *An. arabiensis* and *Ae. aegypti*, and adulticidal potency against *An. arabiensis.*

The lower the values obtained indicate the more potent or effective activity of the plant's larvicidal and adulticidal potential. The relative toxicity, defined as the toxicity of each essential oil relative to the least toxic essential oil against the mosquito species, was calculated as the LC_{50} value of least toxic essential oil divided by LC_{50} value of each essential oil and LC_{90} value of least toxic essential oil divided by LC_{90} value of each essential oil (Ameen *et al*., 1985).

4. RESULTS

4.1. Essential oils content of tested plants

The oil contents of the test plants ranged from 0.44 to 2.8g% (w/w) based on dry weight ratios (Table 2). The highest oil yield was harvested from the seeds of *S. molle* (2.8%), which was followed by *P. nigrum* (1.8%), *C. ambrosioides* (1.6%) and *S. molle* (leaves) (1.6%). The lowest oil yield was recorded from distillation of *O. lamiifolium* (0.44%). The percentage of essential oils contents was calculated by the formula (EORC, Unpublished) viz. $\frac{\text{Oil yield}}{\text{sample weight -\% moisture content}}$

Table 2. Percentage of essential oil and moisture contents of samples of tested plants.

Plant species	Moisture content (g%)	Oil yield (g/ 200g)	Essential oils content (g% ;w/w)
C. ambrosiodes	11	2.9	1.6
L. adoensis	8.9	2.3	1.3
O. lamiifolium	8.2	0.8	0.44
O. suave (dry)	8	1	0.54
O. suave (fresh)	44.9	1	0.9
S. molle (leaf)	45	1.8	1.6
S. molle (seed)	46	3.0	2.8
P. nigrum	8	3.3	1.8

4.2. Larvicidal activities of essential oils under laboratory condition

4.2.1. Dose-response relationships

Table 3 shows the LC_{50} and LC_{90} values of essential oils of different plants against third and fourth instar *An. arabiensis* larvae. The volatile oils of *C. ambrosioides* , *O. lamiifolium, S. molle* (leaves) and *N. sativa* showed the highest larvicidal activities against third and fourth instar *An. arabiensis* larvae after 24 hours exposure. The bioassay results also showed that the effects of essential oils from *O. lamiifolium, C. ambrosioides,* P. *nigrum* and *S. molle* (leaves) were with the highest larvicidal activity against third and fourth instar *Ae. aegypti* larvae after 24 hours exposure(Table 4). However, the essential oils of *M. spicata* and *E. globulus* showed the least lethal effects against *An. arabiensis* larvae. Similarly, *M. spicata, E. globulus* and *O. suave* (dry) exhibited the least larvicidal activities against *Ae. aegypti* larvae .

Essential oils of *C. ambrosioides, N. sativa, O. lamiifolium, P. nigrum, S. molle* (seeds), *S. molle* (leaves) and *T. vulgaris* caused up to 100% mortality in *An. arabiensis* larvae at 66.67 ppm (Table 5). Similarly, *C. ambrosioides, O. lamiifolium, P. nigrum* and *S. molle* (leaves) caused up to 100% mortality in *Ae. aegypti* larvae at 33.3 ppm (Table 6). The least toxic essential oils were from *E. globulus* and *M. spicata,* which resulted 100% mortality of *An. arabiensis* and *Ae. aegypti* at about 166.67 and 133.3 ppm, respectively (Tables 5 and 6).

The essential oils from *C. ambrosioides, O. lamiifolium, S. molle* (leaves) and *N. sativa* showed relatively 4.9, 4.1, 4.1 and 3.7 times more toxicity to *An. arabiensis* larvae than *M. spicata*, respectively at LC_{50} level (Table 3). Similarly, the essential oils from *O. lamiifolium, P. nigrum, C. ambrosioides* and *S. molle* (leaves) showed relatively 7.9, 7.5, 7.5 and 7.1 times more toxicity than *M. spicata* against *Ae. aegypti* larvae, respectively at LC_{50} level (Table 4).

Observations were carried out on the exposure times of test larvae to essential oils. All larvae became restless and frequently sank down and floated up quickly, particularly after treatments of higher concentrations (10% and 5%) of most essential oils. Then, the

restlessness persisted, and tremor and convulsion at the bottom of the enamel cup were observed. When the larvae were exposed to essential oils of lower concentrations, some immediately floated to surface and showed restlessness. Finally, the larvae paralyzed and sank to the bottom of the enamel cups.

Table 3. LC_{50} and LC_{90} (ppm) values of the essential oils of different plants against *An. arabiensis* larvae after 24 hours exposure.

Plants oil	LC_{50} (95% CI)	LC_{90}(95% CI)	Relative toxicity at LC_{50} (LC_{90})	Chi-square (df)	p
C. ambrosioides	17.5 (13.26-22.2)	33.2 (27.3-45.4)	4.9 (3.9)	2.1 (2)	0.35
O. lamiifolium	20.9 (16.2-26.7)	39.9 (32.6-55.5)	4.1 (3.2)	2.1 (2)	0.35
S. molle (leaves)	21.0 (16.8-26.3)	37.3 (30.9-50.3)	4.1 (3.4)	1.87 (2)	0.32
N. sativa	23.4 (18.2-28.5)	45.4 (38.8-55.9)	3.7 (2.8)	1.67 (3)	0.64
S. molle (seeds)	26.5 (18.2-32.6)	52.8 (45.2-66.4)	3.2 (2.4)	2.3 (2)	0.34
P. nigrum	33.5 (28.5-37.9)	48.2 (43.0-57.0)	2.6 (2.7)	1.99 (2)	0.36
T. vulgaris	33.7 (27.4-39.4)	57.5 (50.2-70.1)	2.5 (2.2)	2.04 (2)	0.36
E. citriodora	40.3 (33.2-47.6)	65.4 (56.3-81.7)	2.1 (1.9)	1.8 (2)	0.4
C. ambrosioides (extract)	49.4 (44.0-54.5)	68.2 (61.7-81.2)	1.7 (1.9)	0.35 (2)	0.83
O. suave (dry)	52.8 (47.9-58.9)	75.2 (67.2-91.2)	1.6 (1.7)	0.61 (2)	0.74
O. suave (fresh)	53.5 (47.9-59.6)	75.3 (67.4-91.1)	1.6 (1.7)	0.65 (2)	0.64
L. adoensis	56.4 (47.7-65.6)	90.3 (79.5-109.7)	1.5 (1.4)	1.2 (2)	0.5
E. globulus	68.3 (57.1-78.3)	109.7 (97.3-130)	1.3 (1.2)	0.62 (2)	0.89
M. spicata	85.9 (76.6-96.1)	128.4 (115-148)	1 (1)	0.28 (3)	0.96

95% CI = 95% confidence intervals in parts per million (ppm), chi-square values (x^2) are significant at $p < 0.05$ level.

Table 4. LC_{50} and LC_{90} (ppm) values of the essential oils of different plants against *Ae. aegypti* larvae after 24h exposure.

Plants oil	LC_{50} (95% CI)	LC_{90} (95% CI)	Relative toxicity at LC_{50} (LC_{90})	Chi-square (df)	p
O. lamiifolium	8.6 (7.3-10.0)	13.4 (11.5-17.5)	7.9 (7.6)	6.4 (2)	0.04
P. nigrum	9.1 (7.9-10.5)	13.5 (11.7-16.9)	7.5 (7.5)	3.8 (2)	0.15
C. ambrosioides	9.1 (7.8-10.7)	14.3 (12.2-18.6)	7.5 (7.1)	4.22 (2)	0.12
S. molle (leaves)	9.6 (8.2-11.4)	15.0 (12.8-19.9)	7.1 (6.8)	3.3 (2)	0.2
S. molle (seeds)	14.5 (11.4-18.4)	28.5 (23.3-38.6)	4.7 (3.6)	7.0 (3)	0.07
T. vulgaris	17.3 (12.2-22.0)	36.6 (30.3-48.2)	3.9 (2.8)	2.7 (2)	0.26
O. suave (fresh)	29.8 (23.5-35.0)	50.9 (44.6-61.8)	2.3 (2.0)	2.6 (2)	0.26
N. sativa	32.1 (27.1-36.7)	48.4 (42.9-57.6)	2.1 (2.1)	0.15 (2)	0.93
C. *ambrosioides*(extract)	37.4 (30.8-43.2)	62.8 (55.2-75.7)	1.8 (1.6)	0.97 (3)	0.81
E. citriodora	38.7 (31.3-46.5)	65.5 (55.9-82.6)	1.8 (1.9)	1.46 (2)	0.48
L. adoensis	47.1 (40.5-54.6)	68.7 (60.2-83.1)	1.4 (1.5)	0.13 (2)	0.94
E. globulus	52.9 (41.8-63.6)	102.0 (87.7-125.9)	1.3 (1)	2.4 (3)	0.48
O. suave (dry)	55.0 (50.4-60.4)	72.2 (65.6-84.8)	1.2 (1.4)	1.2 (2)	0.56
M. spicata	67.8 (59.4 -76.3)	96.4 (86.3-113.8)	1.0 (1.1)	0.52 (2)	0.77

95% CI = 95% confidence intervals in parts per million (ppm), chi-square values (x^2) are significant at $p < 0.05$ level.

Table 5. Larvicidal activities of essential oils of different plants against *An. arabiensis* larvae after 24 hours exposure.

	Mean (M ±SE) percent mortality of larvae at different concentrations (ppm)									
Plants oil	6	6.67	13.3	33.3	50	66.67	100	133.3	166.67	Control
C. ambrosioides	ND	12 ±0.4	47 ±1.2	88±0.41	NR	100	ND	ND	ND	0
O. lamiifolium	ND	10 ±0.3	40 ±0.42	77 ±1.03	NR	100	ND	ND	ND	0
S. molle (leaves)	ND	8 ±0.75	36 ±0.4	81 ±0.96	NR	100	ND	ND	ND	0
N. sativa	12±0.4	31 ±0.9	77 ±0.85	90 ±0.28	NR	100	ND	ND	ND	0
S. molle (seeds)	ND	7 ±0.47	25 ±0.62	67 ±0.7	80 ±0.4	100	ND	ND	ND	0
P. nigrum	ND	ND	7 ±0.47	40 ±0.7	96 ±0.0	100	ND	ND	ND	0
T. vulgaris	ND	ND	18 ±0.28	43 ±0.47	77 ±1.1	100	ND	ND	ND	0
E. citriodora	ND	ND	4 ±0.0	45 ±0.25	NR	88 ±0.4	100	ND	ND	0
C. ambrosioides (ex)	ND	ND	ND	12 ±0.4	56 ±0.4	85 ±0.8	100	ND	ND	0
O. suave (dry)	ND	ND	ND	16 ±0.4	38 ±0.3	80 ±0.4	100	ND	ND	0
O. suave (fresh)	ND	ND	ND	15 ±0.45	35±0.5	80 ±0.8	100	ND	ND	0
L. adoensis	ND	ND	ND	16 ±0.4	NR	72 ±0.4	92± 0.4	100	ND	0
E. globulus	ND	ND	ND	12 ±0.4	NR	50 ±0.3	86 ±1.3	96 ±0.4	100	0
M. spicata	ND	ND	ND	6 ±0.5	NR	29 ±1.1	64 ±0.4	92 ±0.4	100	0

ND= not done, NR = not recorded, (ex) = extract, SE = standard error, M = means of four replicates.

Table 6**.** Larvicidal activities of essential oils of different plants against *Ae. aegypti* larvae after 24 hours exposure.

	Mean percent mortality of larvae at different concentrations (ppm)								
Plants oil	6	6.67	13.3	33.3	50	66.67	100	133.3	Control
O. lamiifolium	10 ±0.3	48 ±0.4	88±0.4	100	ND	ND	ND	ND	0
C. ambrosioides	10 ±0.3	40 ±0.41	84±0.4	100	ND	ND	ND	ND	0
P. nigrum	8 ±0.4	36 ±0.4	88 ±0.4	100	ND	ND	ND	ND	0
S. molle (leaves)	10 ±0.3	36 ±0.4	80 ±07	100	ND	ND	ND	ND	0
S. molle (seeds)	5 ±0.2	36 ±0.71	53 ±2	94 ±0.9	100	ND	ND	ND	0
T. vulgaris	ND	18 ±0.28	50 ±1	80 ±0.9	100	ND	ND	ND	0
O. suave (fresh)	ND	ND	13 ±0.2	68 ±0.6	82 ±0.3	100	ND	ND	0
N. sativa	ND	8 ±0.57	52 ±0.7	92±0.6	NR	100	ND	ND	0
C. ambrosioides (ex)	ND	ND	8 ±0.4	47 ±1.4	76 ±0.4	90 ±0.3	100	ND	0
E. citriodora	ND	ND	7 ±0.5	48±0.4	NR	88 ±0.4	100	ND	0
L. adoensis	ND	ND	3 ±0.5	19±0.7	NR	88 ±0.4	100	ND	0
E. globulus	ND	ND	12 ±0.3	40 ±0.4	NR	56 ±0.4	88 ±0.4	100	0
O. suave (dry)	ND	ND	ND	8 ±0.4	28 ±1.6	84 ±0.4	100	ND	0
M. spicata	ND	ND	ND	8±0.4	NR	43 ±1.1	94 ±0.3	100	0

ND= not done, NR = not recorded, (ex) = extract, SE= standard error, M = means of four replicates.

4.2.2. Mortality-time tests

The results of mortality-time experiments showed that the essential oils of different plants at similar concentration required varying lengths of time to induce lethality of the two different mosquito larvae (Table 7). All the essential oils at 666.67 ppm caused 100% mortality against *Ae. aegypti* larvae within shorter times (20-120min), while they required a bit longer times (50-180min) to cause the same 100% mortality against *An. arabiensis* larvae. The shortest possible exposure time against *An. arabiensis* was recorded by using essential oils from *L. adoensis, O. lamiifolium, C. ambrosioides* and *P. nigrum*. The longest time against the same species was recorded for *O. suave* (fresh) and *O. suave* (dry) essential oils. The shortest time against *Ae. aegypti* was also recorded for the essential oil of *L. adoensis* The longest exposure time against the same species was recorded for *M. spicata, O. suave* (fresh) and *O. suave* (dry) essential oils.

At concentration 333.3 ppm, all the essential oils also caused 100% mortality of larvae of *An. arabiensis* and *Ae. aegypti*, but they required longer time (50 - 290 min) and (50-250 min), respectively (Table 7). Against *An. arabiensis*, the shortest treatment time was recorded for *L. adoensis* and *P. nigrum* essential oils, while the longest time was recorded for *T. vulgaris, M. spicata, O. suave* (fresh) and *O. suave* (dry*)* essential oils. Against *Ae. aegypti*, the shortest time was recorded for *L. adoensis* essential oil.

Apparently, *Ae. aegypti* larvae died in shorter time than *An. arabiensis* at concentration 666.67 and 333.3 ppm (Table 7)

Table 7. Time (minutes) (M±SE) required to induce 100% mortality of *An. arabiensis* and *Ae. aegypti* larvae at 666.67 and 333.3 ppm concentrations of different plants essential oils.

Plant oil	666.67 ppm	666.67 ppm	333.3 ppm	333.3 ppm
	An	Ae	An	Ae
L. adoensis	50 ±4.56	20 ±2.04	80 ±4.56	50 ±4.56
P. nigrum	50 ±2.04	30 ±2.04	80 ±4.1	60 ±6.45
O. lamiifolium	50 ±3.53	30 ±2.88	90 ±3.53	60 ±2.88
C. ambrosioides	50 ±3.53	30 ±5.1	90 ±2.88	60 ±2.88
S. molle (leaves)	60 ±4.56	50 ±2.04	90 ±3.53	70 ±2.04
S. molle (seeds)	60 ±4.6	45 ±2.04	90 ±4.56	60 ±4.56
*C. ambrosioides (*extract)	90 ±2.88	50 ±3.53	120 ±3.53	90 ±3.53
E. citriodora	100 ±4.1	30 ±2.04	120 ±4.56	60 ±4.1
E. globulus	115 ±2.04	60 ±3.53	150 ±4.56	100 ±4.5
N. sativa	120 ±4.56	90 ±4.56	250 ±4.1	120 ±4.5
T. vulgaris	140 ±4.56	90 ±4.56	270 ±8.89	180 ±7.3
M. spicata	150 ±5.4	120 ±5.4	270 ±5.77	240 ±6.3
O. suave (fresh)	180 ±9.1	120 ±5.4	270 ±4.1	240 ±9.1
O. suave (dry*)*	180 ±5.4	120 ±2.04	290 ±4.56	250 ±4.1

An = *Anopheles arabiensis* , Ae = *Aedes aegypti* , SE= standard error, M = means times of four replicates, No mortality was observed in control treatments.

4.3. Larvicidal effects of essential oils under simulated field condition

Results on the toxicity of essential oils against anopheline mosquitoes larvae in the simulated field conditions are shown in Table 8. Essential oil of *O. lamiifolium* had shown the highest larvicidal activity against field population of anopheline larvae. However, *P. nigrum* showed the lowest activity against the field population of anopheline mosquito larvae. Against field population of anopheline mosquito larvae, essential oil from *O. lamiifolium* showed relatively 3.3 times more toxicity than *P. nigrum* essential oil, at LC_{50} level.

Essential oils of *O. lamiifolium, C. ambrosioides, S. molle* (leaves), *O. suave* (fresh) and *P. nigrum* caused 100% mortality of anopheline larvae in the simulated field conditions at 133.3, 166.6, 166.6, 166.6 and 200 ppm, respectively (Table 10).

Table 9 shows the LC_{50} and LC_{90} values of essential oils against laboratory reared *An arabiensis* and field population of anopheline mosquito larvae. The result showed the highest toxicities of all tested essential oils to laboratory reared *An. arabiensis* than field anopheline mosquitoes population.

Table 8. LC_{50} and LC_{90} (ppm) values of the essential oils of different plants against anopheline mosquito larvae in the simulated field condition.

Plants oil	LC_{50} (95% CI)	LC_{90} (95% CI)	Relative toxicity at LC_{50} (LC_{90})	Chi-square (df)	p
O. lamiifolium	34 (27.6-40.2)	63.7 (54.9-79.4)	3.3 (2.6)	4.4 (2)	0.11
C. ambrosioides	47.3 (42.0-56.9)	97.9 (89.6-114.4)	2.3 (1.7)	15 (3)	0.001
S. molle (leaves)	63.5 (57.0 -71.4)	100.7 (89.8-119)	1.7 (1.6)	4.1 (2)	0.13
O. suave (fresh)	86.4 (76.4-94.6)	127.6 (117.2-144)	1.3 (1.3)	0.45 (2)	0.8
P. nigrum	110.6 (99.7-121.4)	162.9 (148.9-183)	1 (1)	2.3 (2)	0.32

95% CI = 95% confidence intervals in parts per million (ppm), chi-square values (x^2) are significant at $p < 0.05$ level.

Table 9. Comparison of LC_{50} and LC_{90} (ppm) values of the essential oils of different plants against laboratory reared *An. arabiensis* and field population of anopheline mosquito larvae.

Plant oil		Laboratory reared *An. arabiensis*	Field anophelines spp.	Relative toxicity
O. lamiifolium	LC 50 (95% CI)	20.9 (16.2-26.7)	34 (27.6-40.2)	1.6
	LC 90 (95% CI)	39.9 (32.6-55.5)	63.7 (55.9-79.4)	1.6
C. ambrosioides	LC 50 (95% CI)	17.5 (13.3-22.2)	47.3 (42.0-56.9)	2.7
	LC 90 (95% CI)	33.2 (27.3-45.4)	97.9 (89.6-114.4)	2.9
S. molle (leaves)	LC 50 (95% CI)	21.0 (16.8-26.3)	63.5 (55.6-71.4)	3.0
	LC 90 (95% CI)	37.3 (30.9-50.3)	100.7 (89.8-119.0)	2.7
O. suave (fresh)	LC 50 (95% CI)	53.5 (47.9-59.6)	86.4 (76.4-94.6)	1.6
	LC 90 (95% CI)	75.3 (67.4-91.1)	127.6 (117.2-144.2)	1.7
P. nigrum	LC 50 (95% CI)	33.5 (28.9-37.9)	110.6 (99.7-121.4)	3.3
	LC 90 (95% CI)	48.2 (43.0-57.0)	162.9 (148.9-183.8)	3.4

95% CI = 95% confidence intervals in parts per million (ppm).

Table 10. Mortality response of field anopheline mosquitoes population to different concentrations of different essential oils after 24 hours exposure.

	Mean (M±SE) percent larval mortality at different concentrations (ppm)							
Plants oil	200	166.67	133.3	100	66.67	33.3	16.67	Control
O. lamiifolium	ND	ND	100	NR	88.8 ±1.32	61 ±1.7	15 ±0.8	0
C. ambrosioides	ND	100	93.8±0.3	NR	82.5 ±0.4	47 ±0.8	7.5 ±0.4	0
S. molle (leaves)	ND	100	NR	94.4 ±0.25	42.5 ±0.4	20 ±0.8	ND	0
O. suave (fresh)	ND	100	92.5 ±0.6	63.8 ±0.96	28.8 ±0.9	ND	ND	0
P. nigrum	100	87.5 ±0.4	NR	45.6 ±0.8	11.3 ±0.6	ND	ND	0

ND= not done, NR = not recorded, SE = standard error, M = means of four replicates.

4.4. Adulticidal activities of essential oils under laboratory condition

4.4.1. Dose-response relationships

LC_{50} and LC_{90} values obtained with essential oils against adults of *An. arbiensis* are presented in Table 11. The results revealed that the *O. suave* (fresh) essential oil showed the highest activity against *An. arbiensis* adults. *P. nigrum, E. globulus* and *S. molle* (leaves) showed the lowest activity against *An. arbiensis* adults.

Essential oil from *O. suave* (fresh) caused 100% mortality of *An. arabiensis* adults at lowest concentration (0.005 ml%, v/v) after 1hour exposure. *P. nigrum* the least toxic in this case caused 100% mortality of *An. arabiensis* at higher concentration (1ml%, v/v) (Table 12).

Table 11. LC_{50} and LC_{90} (ml%, v/v) values of the essential oils of different plants against *An. arabiensis* adults after 1h exposure.

Plants oil	LC_{50} (95% CI)	LC_{90} (95% CI)	Relative toxicity at LC_{50} (LC_{90})	Chi-square (df)	p
O. suave (fresh)	0.0014 (0.0008-0.002)	0.0027 (0.002-0.006)	278.6(270.4)	1.13 (2)	0.56
T. vulgaris	0.0028 (0.002-0.004)	0.005 (0.004-0.008)	139.3 (146)	0.06 (2)	0.97
O. suave (dry)	0.005 (0.004-0.006)	0.008 (0.007-0.01)	78 (91.3)	0.14 (2)	0.93
M. spicata	0.027 (0.017-0.038)	0.047 (0.037-0.077)	14.4 (15.5)	0.35 (2)	0.84
L. adoensis	0.03 (0.021-0.041)	0.05 (0.042-0.082)	13 (14.6)	0.15 (2)	0.93
C. ambrosioides	0.06 (0.048-0.073)	0.09 (0.075-0.12)	6.5 (8.1)	1.1 (2)	0.58
E. citriodora	0.069 (0.055-0.081)	0.093 (0.081-0.127)	5.6 (7.8)	1.06 (2	0.87
O. lamiifolium	0.12 (0.03-0.18)	0.24 (0.18-0.56)	3.3 (3.0)	1.98 (2)	0.34
N. sativa	0.13 (0.073-0.18)	0.24 (0.19-0.496)	3 (3)	0.8 (2)	0.67
S. molle (leaves)	0.22 (0.081-0.34)	0.55 (0.4-0.96)	1.8 (1.3)	1.3 (3)	0.73
E. globulus	0.24 (0.11-0.34)	0.46 (0.35-0.79)	1.6 (1.6)	1.05 (2)	0.59
P. nigrum	0.39 (0.16-0.52)	0.73 (0.59-1.15)	1 (1)	1.05 (2)	0.59

95% CI = 95% confidence intervals in percentage (ml%, v/v), chi-square values (x^2) are significant at $p < 0.05$ level.

Table 12. Toxicity of essential oils of different plants against *An. arabiensis* adults after 1hour exposure.

Plants oil	Concentrations (ml% v/v)	Mean (M± SE) %mortality	Plant oil	Concentrations (ml % v/v)	Mean (M±SE) % mortality
O. suave (fresh)	0.005 0.0025 0.001 0.00075	100 85 ±0.28 45 ±0.28 15 ±0.28	*E. citriodora*	0.25 0.1 0.075 0.05	100 90 ±0.0 75 ±0.28 10 ±0.0
T. vulgaris	0.0075 0.005 0.0025 0.001	100 90 ±0.0 40 ±0.41 15 (0.28	*N. sativa*	0.5 0.1 0.25 0.075	100 90 ±0.0 55 ±0.57 15 ±0.57
O. suave (dry)	0.01 0.0075 0.005 0.0025	100 85 ±0.28 45 ±0.28 10 ±0.0	*O. lamiifolium*	0.5 0.25 0.1 0.075	100 90 ±0.0 60 ±0.41 20 ±0.41
L. adoensis	0.075 0.05 0.025 0.01	100 85 ±0.28 40 ±0.0 10 ±0.0	*E. globules*	0.75 0.5 0.25 0.1	100 90 ±0.0 65 ±0.28 15 ±0.25
M. spicata	0.075 0.05 0.025 0.01	100 95 ±0.28 50 ±0.28 10 ±0.0	*S. molle* (leaves)	0.75 0.5 0.25 0.1 0.075	100 80 ±0.41 60 ±0.41 40 ±0.41 2 0 ±0.41
C. ambrosioides	0.1 0.075 0.05 0.025	100 75 ±0.28 25 ±0.28 10 ±0.0	*P. nigrum*	1 0.75 0.5 0.25	100 85 ±0.28 70 ±0.41 25 ±0.28

M = means of four replicates, SE = standard error, no mortality was observed in controls.

4.4.2. Mortality-time tests

Each concentration of essential oils required various lengths of time to kill 100% of the exposed *An. arabiensis* (Table 13). Accordingly, *O. suave* (fresh) killed 100% of the mosquitoes in 5 minutes with the smallest concentration of 0.05 ml/100ml (v/v), while *P. nigrum* had the highest concentration (2.5 ml/100ml, v/v) to cause 100% mortality of the mosquitoes in the same period of time. Similarly, at longer times of exposure (15-60 min), the same plant had the smallest concentration than the rest of the plants. *P. nigrum* was the weakest of all tested essential oils.

Table 13. The mean times (minutes) at which 100% mortality of *An. arabiensis* was obtained at different concentrations of essential oils.

Plants oil	Concentrations (ml/100ml, v/v)	Mean times (M±SE)	Plant oil	Concentrations (ml/100ml,v/v)	Mean times (M±SE)
O. suave (fresh)	0.05 0.025 0.01 0.005	5 ±0.0 15 ±1.4 30 ±2.04 60 ±4.56	*E. citriodora*	1 0.75 0.5 0.25	5 ±0.0 15 ±2.04 30 ±2.04 60 ±4.10
O. suave (dry)	0.075 0.05 0.025 0.01	5±0.0 15 ±2.04 35 ±2.04 60 ±4.56	*N. sativa*	1 0.75 0.5	5 ±0.0 25 ±2.88 60 ±3.53
T. vulgaris	0.25 0.1 0.075 0.0075	5 ±0.0 15 ±2.04 30 ±2.88 60 ±3.53	*O. lamiifolium*	1.5 1 0.75 0.5	5 ±0.0 15 ±2.04 30 ±3.50 60 ±3.50
L adoensis	0.5 0.25 0.1 0.075	5±0.0 15 ±2.88 25 ±1.4 60 ±2.04	*E. globulus*	2 1.5 1 0.75	5 ±0.0 10 ±2.04 20 ±2.04 60 ±2.04
M. spicata	0.5 0.25 0.1 0.075	5 ±0.0 15 ±3.5 30 ±3.5 60 ±2.04	*S. molle* (leaves)	2 1.5 1 0.75	5 ±0.0 15 ±2.04 30 ±2.88 60 ±4.56
C. ambrosioides	0.5 0.25 0.1	5 ±0.0 20 ±2.04 60 ±2.88	*P. nigrum*	2.5 2 1.5 1	5 ±0.0 15 ±2.04 30 ±2.04 60 ±2.88

M = means of four replicates, SE = standard error, no mortality was observed in control treatments.

4.4.3. Residua toxicities of essential oils

The result showed that oil of *O. suave* (fresh) had the longest residual activity (15 days) against adult *An. arabiensis* followed by *S. molle* (leaves), *O. suave* (dry) and *O. lamiifolium*, respectively (Fig. 5). *O. suave* (fresh) oil persisted to cause 100% mortality against adult *An. arabiensis* for up to 5 days, and still caused higher mortality (80%) by day 10. Its residual toxicity declined sharply and caused only 5% mortality at day 15. The lowest residual toxicity was observed for *E. citriodora* essential oil. The oil caused 100% mortality only at day 1.

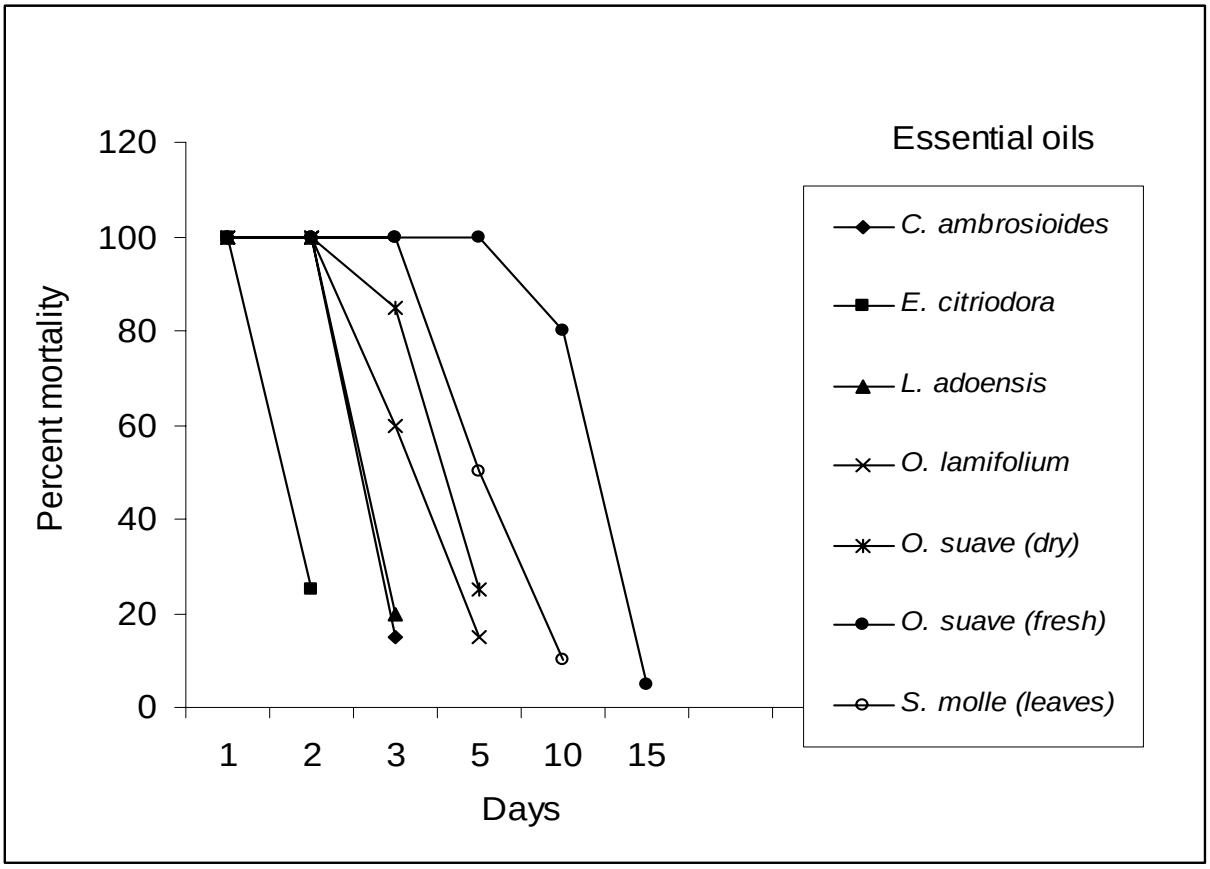

Fig. 5. The residual toxicity of essential oils of different plants *against An. arabiensis* adults.

5. DISCUSSION

In present study, larvicidal responses varied according to the mosquito species and essential oils from different plant species in the laboratory. However, larvicidal effects in the simulated field conditions and adulticidal responses in the laboratory varied according to the origin of essential oils from tested plant species.

From the LC_{50} and LC_{90} values, the essential oils from *C. ambrosioides*, *O. lamiifolium*, *S. molle* (leaves) and *N. sativa* had the highest larvicidal activity against third and fourth instar *An. arabiensis* larvae after 24 hours of exposure. The bioassay results of the essential oils from *O. lamiifolium*, *C. ambrosioides, Piper nigrum* and *S. molle* (leaves) also showed the highest levels of larvicidal activity against third and fourth instar *Ae. aegypti* larvae after 24 hours of exposure. Earlier studies involving the essential oils obtained from various plants viz. *Lippia sidoides, Ocimum gratissimum* and *O. americanum* recorded LC_{50} values of 63, 60, and 67 ppm, respectively (Cavalcanti *et al*., 2004). The essential oils from *L. adoensis* with LC_{50} value of 47.1 ppm, *O. lamiifolium* with LC_{50} value of 8.6 ppm, *O. suave* (fresh) with LC_{50} value of 29.8 ppm and *O. suave* (dry) with LC_{50} value of 55.0 ppm in present study showed more toxicity to *Ae. aegypti* larvae than the previously reported essential oils. *O. lamiifolium* essential oil with LC_{50} value of 8.6 ppm is 6.9 and 7.8 times more toxic than *O. gratissimum* and *O. americanum* against *Ae. aegypti* larvae, respectively. Essential oil of *O. suave* (fresh) showed 2.0 and 2.24 times more toxicity in *Ae. aegypti* larvae than *O. gratissimum* and *O. americanum*, respectively. When compared with *L. sidoides*, *L. adoensis* essential oil is 1.3 times more toxic to *Ae. aegypti* larvae. Recently, *L. citriodora* had also been identified as effective larvicides against *Ae. aegypti* and *An. stephensi* larvae with LC_{50} values of 100 ppm and 10 ppm, respectively (Amer and Mehlhorn, 2006a).

Interestingly, all essential oils evaluated in present study were more potent as compared to the effects of *Croton sonderianus* (LC_{50} = 104 ppm) and *C. argyrophyloides* (LC_{50} = 102 ppm) extracts against *Ae. aegypti* larvae (Morais *et al.*, 2006). Even the strong larvicidal essential oil, *C. zenhtneri* (LC_{50} = 28 ppm), against *Ae. aegypti* had the lower larvicidal activity when compared with essential oils from *O. lamiifolium*, *C. ambrosioides, S. molle* (leaves), *P. nigrum, S. molle* (seeds) *and T. vulgaris.* In an evaluation of 41 plants essential

oils for larvicidal effects against *An. stephensi* and *Ae. aegypti*, 13 essential oils exhibited high larvicidal activities after 24 hours exposure with LC_{50} values ranged from 9.7 to 101.4 ppm for *An. stephensi* and 1 to 104.4 ppm for *Ae. aegypti* larvae (Amer and Mehlhorn, 2006a). The LC_{50} values in present study ranged from 17.5 to 85.9 ppm for *An. arabiensis* and 8.6 to 67.8 ppm for *Ae. aegypti* larvae, which seems encouraging for future development of selected candidate plant materials for mosquito control.

M. spicata in present study showed larvicidal activity with LC_{50} value of 85.9 ppm against *An. arabiensis* larvae. Similarly, *Mentha spicata viridis* with LC_{50} value of 82.95 ppm was reported as larvicides against *An. stephensi* larvae (Tripathi *et al*., 2004). Larvicidal activity of essential oil of *Pinus longifolia* against *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* were noted with LC_{50} values of 112.6 ppm, 85.7 ppm and 82.1 ppm, respectively (Ansari *et al*., 2005). The result obtained in the present investigation showed LC_{50} values between 8.6-67.8 ppm against *Ae. aegypti* showing more larvicidal activity than *P. longifolia* essential oil.

Extract from *Piper retrofractum* has also shown larvicidal activity against *Cx. quinquefasciatus* and *Ae. aegypti* larvae with LC_{50} values of 135 and 79 ppm, respectively (Chansag *et al.*, 2005). When compared with essential oil of *P. nigrum* with LC_{50} value of 9.1 ppm against *Ae. aegypti* larvae, *P. retrofractum* is 8.7 times less toxic to *Ae. aegypti* larvae. But in another works, essential oil of *P. nigrum* revealed larvicidal activity with LC_{50} value of 50 and 100 ppm against *Ae. aegypti* and *An. stephensi* ,respectively (Amer and Mehlhorn, 2006a).

The essential oils with the larvicidal activities in this study such as from *O. lamiifolim* (LC_{50} = 8.6 ppm), *C. ambrosioides* (LC_{50} = 9.1 ppm), *P. nigrum* (LC_{50} = 9.1 ppm), *S. molle* (leaves) (LC_{50} = 9.6 ppm), *S. molle* (seeds) (LC_{50} = 14.5 ppm) and *T. vulgaris* (LC_{50} = 17.3 ppm) were highly toxic to *Ae. aegypti* larvae than that of *Daucus carota* (LC_{50} = 37.95 ppm), *Angelica glauca* (LC_{50} = 56.31 ppm) and *Saussarea lappa* (LC_{50} = 65.25 ppm) oils (Tare *et al*., 2004). The activity of *O. sanctum* (LC_{50} = 29.75 ppm) and *M. piperita* (LC_{50} = 26.192 ppm) (Pathak *et al*., 2000) as compared with *O. lamiifolium* (LC_{50} = 8.6 ppm) and *M. spicata* (LC_{50} = 67.8 ppm), *O. lamifolium* showed 3.46 times more toxicity in *Ae.*

aegypti, while that of *M. spicata* was 2.59 times less effective than *M. piperita* against *Ae. aegypti* larvae. Essential oil of *O. suave* (dry) (LC_{50} = 55 ppm) had shown less larvicidal activity than *O. sanctum* against *Ae. aegypti* larvae, while *O. suave* (fresh) (LC_{50} = 29.8 ppm) had similar level of larvicidal efficacy with that of *O. sanctum* essential oil against *Ae. aegypti* larvae.

Monzon *et al*. (1994) tested five Philippine plants against *Ae. aegypti* larvae and found that the essential oil of *E. globulus* showed the least larvicidal activity. Though the essential oil of *E. globulus* in present study showed poor larvicidal activity against *An. arabiensis* and *Ae. aegypti* larvae, it had more potency than *E. globulus* from Philippine (LC_{50} 92.0123 and LC_{90} 810.6377 g%;w/v) against *Ae. aegypti* larvae. Petroleum ether extract of thyme plant, *T. capitatus* was found to be toxic (LC_{50} = 49.0 ppm) against the larvae and adults of *Cx. pipiens* (Mansur *et al.,* 2000). Similarly, Amer and Mehlhorn (2006a) reported the larvicidal activity of *T. serpyllum* with LC_{50} values of 1 and 10 ppm against *Ae. aegypti* and *An. stephensi* larvae, respectively. However, the effects of essential oil of *T. vulgaris* in this study showed larvicidal activity with LC_{50} values of 33.7 and 17.3 ppm against *An . arabiensis* and *Ae. aegypti* larvae, respectively.

The activity of each essential oil is different from one species of mosquito to another species. *An. arabiensis* larvae showed similar level of susceptibility to both dry *O. suave* and fresh *O. suave*, and *S. molle* (leaves) and *S. molle* (seeds) essential oils. However, essential oils of *O. suave* (fresh) and dry *O. suave*, and *S. molle* (leaves) and *S. molle* (seeds) essential oils had shown different larvicidal efficacy against *Ae. aegypti* larvae. Similarly, Amer and Mehlhorn (2006a) noted similar level of susceptibility of *An. stephensi* larvae when exposed to the essential oils of *Cinnamomum camphora, C. limon, Boswellia carteri, Anethum graveolens, Myrtus communis, Juniperus communis, Piper nigrum* and *Helichrysum italicum,* while they have shown different level of activities against *Ae. aegypti* larvae.

Mosquitocidal activity of essential oil from *S. molle* (leaves) (LC_{50}= 9.6 ppm) is 1.5 times more toxic than *S. molle* (seeds) (LC_{50}= 15.4 ppm) against *Ae. aegypti* larvae. Likewise, Sukumar *et al.* (1991) reported that different parts of the same plant might induced different activity in the same mosquito species. *O. suave* (fresh) (LC_{50} = 29.8 ppm) was also shown 1.8 times more toxicity than dry *O. suave* (LC_{50}= 55.0 ppm) essential oil in *Ae. aegypti* larvae. Guenther (1949) noted the possibility of variations of chemical composition of essential oils when the plant parts were dried before extraction. This variation in chemical composition may result the differences in activity of essential oil from dry and fresh parts of the same plant. The activity of essential oil of *E. citriodora* with LC_{50} value of 40.3 ppm against *An. arabiensis* and with LC_{50} value of 38.7 ppm against *Ae. aegypti* is 1.7 and 1.4 times more toxic than *E. globulus* with LC_{50} value of 68.3 ppm against *An. arabiensis* and with LC_{50} value of 52.9 ppm against *Ae. aegypti* larvae, respectively. The essential oil of *O. lamiifolium* (LC_{50}= 20.9 ppm) is 2.53 and 2.56 times more toxic than *O. suave* (dry) (LC_{50}= 52.8 ppm) and *O. suave* (fresh) (LC_{50}= 53.5 ppm) against *An. arabiensis* larvae, respectively. Against *Ae. aegypti* larvae, *O. lamiifolium* (LC_{50}= 8.6 ppm) essential oil is 6.4 and 3.46 times more toxic than *O. suave* (dry) (LC_{50}= 55.0 ppm) and *O. suave* (fresh) (LC_{50}= 29.8 ppm), respectively.

C. ambrosioides essential oil (LC_{50}= 17.5 ppm) appeared to be about 2.8 times more effective than *C. ambrosioides* oleoresin (ethanol extract) (LC_{50}= 49.9 ppm) against *An. arabiensis* larvae. However, *C. ambrosioides* essential oil (LC_{50}= 9.1 ppm) depicted to be 4.1 times more toxic than *C. ambrosioides* extract (ethanol extract) (LC_{50}= 37.4 ppm) against *Ae. aegypti* larvae. Thomas *et al*. (2000) similarly reported that the essential oil of *Cannabis sativa* obtained by hydro-distillation was more toxic than ethanolic extraction against mosquitoes.

The effects of the same plant essential oil also varied against the two mosquito species. When the LC_{50} and LC_{90} values were considered, the essential oils were found to be relatively more toxic to the larvae of *Ae. aegypti* than *An. arabiensis,* except the essential oils from *N. sativa* and *O. suave* (dry). The tested oils from *O. lamiifolium, P. nigrum, C. ambrosioides* and *S. molle* (leaves) exhibited LC_{50} values < 10 ppm, but none of the

essential oils exhibited the same level of activity against *An. arabiensis* larvae. However, none of the essential oils exhibited LC_{50} values > than 100 ppm against *An. arabiensis* and *Ae. aegypti* larvae. Tested oils, for instance, from *C. ambrosioides, O. lamiifolium S. molle* (leaves*), S. molle* (seeds), *P. nigrum, T. vulgaris, O. suave (*dry), *C. ambrosioides* extract and *M. spicata* revealed to be 1.9, 2.4, 2.2, 1.8, 3.7, 1.95, 1.8, 1.3 and 1.26 times more toxic to *Ae. aegypti* than *An. arabiensis* larvae, respectively. These variations are suggested to be not abnormal. Thomas *et al.* (2000), Sivagnaname and Kalyanasundaram (2004), Vatandoost and Vaziri (2004), Ansari *et al.* (2005) and Jang *et al*. (2005) reported similar results in that the same essential oil from single plant species exhibit various degrees of toxicity against different mosquito species. Furthermore, Amer and Mehlhorn (2006a) also noted the difference in sensitivities of the mosquito species in reaction to essential oil toxicity. Novak (1985) previously reported that *Ae. aegypti* larvae were generally more susceptible to essential oils than *An. gambiae* larvae. Pathak *et al*. (2000) also noted the susceptibility of *Ae. aegypti* larvae to essential oils of *O. canum* and *Murraya koenigil* than *An stephensi* larvae. *Cannabis sativa* essential oil was more toxic against *Ae. aegypti* larvae than *An. stephensi* larvae (Thomas *et al*., 2000). Bassole *et al.* (2003), Ansari *et al*. (2000) and Amer and Mehlhorn (2006a) reported similar observations. However, *Tagetes erecta* and *M. piperita* essential oils showed more toxicity against *An. stephensi* larvae than *Ae. aegypti* larvae (Pathak *et al*., 2000). Thomas *et al.* (2004) similarly reported the higher toxicity of *Ipomoea cairica* essential oil against *An. stephensi* than *Ae. aegypti* larvae. Minjas and Sarda (1986) also showed that the crude extracts containing saponin fruit pods of *Swartzia madagascariensis* produced higher mortality in the larvae of *An. gambiae* than in the larvae of *Ae. aegypti*. Amer and Mehlhorn (2006a) also reported high susceptibility of *An. stephensi* (LC_{50} = 10 ppm) than *Ae. aegypti* larvae (LC_{50} = 100 ppm) when they exposed to *L. citriodora* essential oil. The difference in susceptibility is due to the difference in physiological response among mosquito species and accumulated genetic differences (Monzon *et al*., 1994; Shaalan *et al*., 2005) and levels of toxicity among the active insecticidal ingredients from each plant (Monzon *et al*., 1994).

The symptoms observed in treated larvae might be due to nerve poisoning, i.e. excitation, convulsions, paralysis, and death. Choochote *et al.* (2004) also reported similar observations in the larvae treated with essential oils. They also showed discoloration, unnatural positions and uncoordination (Choochote *et al*., 2004). Corbet *et al*. (2000) reported that the volatile components affected the mosquito larvae by poisoning. However, methanol extract of *Derris urucu* resulted in the mortality in *Ae. aegypti* larvae by disrupting the peritrophic matrix and rupturing of the mid-gut cells (Gusmao *et al*., 2002).

During exposure time and mortality response assays**,** *L. adoensis* essential oil at 666.67 and 333.3 ppm gave 100% mortality of *Ae. aegypti* larvae with in 20 min and 50 min, respectively. The pure (100%) essential oil of *L. sidoides* caused 100% larval mortality in *Ae. aegypti* almost instantaneously (1 to 5min) (Carvalho *et al*., 2003). This indicated that the mortality time decreased in larval bioassay when the concentration of essential oil increased as similar to the current investigations. More recently, Amer and Mehlhorn (2006a) sowed that the dosage effect had an inverse relation with time, at higher dose mortality induced immediately. The decrease in mortality time with increasing in concentration could be due to the increase of uptake of active ingredients by the mosquitoes (Kabir *et al*., 2003). At higher concentration 100% mortality was achieved instantaneously, showing that the dilution of active components delayed death time or required longer time (Carvalho *et al*. 2003).

Essential oil from *O. lamiifolium* showed the highest larvicidal activity under the simulated field condition. When compared with the effects of *O. suave* (fresh), it showed 2.65 times more toxicity against the field population of anopheline mosquito larvae. Previous studies involving the essential oils obtained from *C. proximus*, *L. multiflora* and *O. canum* indicated LC_{50} and LC_{90} values of 69.7 ppm and 121.6 ppm, 61.9 ppm and 136.7 ppm, and 301.6 ppm and 582.9 ppm against field collected *An. gambiae* s.l., respectively (Bassole *et al.,* 2003). LC_{50} values obtained from *C. ambrosioides*, *O. lamiifolium and S. molle* (leaves) oils against anopheline larvae in the simulated field conditions were lower than *C. proximus* and *O. canum* but *L. multiflora* showed similar level of activity with *S. molle* (leaves) essential oil. *O. lamiifolium* with LC_{50} values of 34 ppm and LC_{90} values of 63.7

ppm had shown 8.9 and 9.15 times more larvicidal activity than *O. canum* essential oil, respectively at LC_{50} and LC_{90} level. Mwaiko and Savaesi (1994) also noted the mortality of *Cx. quenquefasciatus* larvae in small scale natural breeding sites and simulated field conditions when treated with lemon peel oil.

The laboratory reared *An. arabiensis* larvae seemed to be more susceptible to essential oils than field population of anopheline larvae. No essential oil exhibited similar activity against laboratory reared *An. arabiensis* and field population of anopheline mosquitoes. Essential oils from *O. lamiifolium, C. ambrosioides, O. suave* (fresh), *P. nigrum* and *S. molle* (leaves) were relatively 1.6, 2.7 , 1.6 , 3.3 , and 3.0 times more toxic to laboratory reared *An. arabiensis* than field population of anopheline mosquito larvae *(An. arabiensis* are predominant species in the area (Adugna *et al.,* 1998; Taye *et al*., 2006)), respectively at LC_{50} level. Recently, George and Vincent (2005) evaluated the larvicidal activity petroleum ether seed extract of *Annona squamosa* and *apaongamia glabra* against field collected and laboratory reared *Cx. quiquefasciatus* larvae and noted that the field collected larvae were apparently better adapted to adjust to stress variations in the environment and hence required a higher concentration of extract to bring about the required mortality. More recently, Sun *et al.* (2006) evaluated the larvicidal effects of ethanol extract of *Ginkgo biloba* against laboratory and field strain of *Cx. pipiens pallens* and reported that the field strain were more resistant than laboratory reared strain. The possible reasons are that the field strains were genetically more heterogeneous (Kabir *et al*., 2003) and are routinely exposed to diverse insecticides.

Thought several plants from different families have been reported for mosquitocidal activity, only very few botanicals have moved from the laboratory to field use, like neem based insecticides (Awad and Shimaila, 2003;Vatandoost and Vaziri, 2004), which might be due to the light and heat instability of phytochemicals compared to synthetic insecticides (Green *et al*., 1991). However, Mwaiko and Savaesi (1994) reported that light did not affect the larvicidal activity of essential oil from lemon peel. Similarly, Amer and Mehlhorn (2006b) tested the essential oils solution stored in light and dark against different mosquito species and found the identical toxicity of essential oils against the tested mosquito species. It is clear from the data obtained that the essential oils in this study were also shown some

promising results in the simulated field conditions regardless of light effects. However, further investigation on the persistency of essential oils may be needed.

The essential oils tested by bottle bioassay against adults of *An. arabiensis* showed some promising results. The mosquitocidal effects of *O. suave* (fresh) (LC_{50} = 0.0014 ml%; v/v), *T. vulgaris* (LC_{50} = 0.0028 ml%; v/v) and *O. suave* (dry) (LC_{50} = 0.005 ml %; v/v) were 278.6, 139.3 and 78 times more toxic than that of *P. nigrum* (LC_{50} = 0.39 ml%; v/v) oil against adult *An. arabiensis*, respectively. *Tagetes minuta* flora extract showed adulticidal activity against *Ae. aegypti* and *An. stephensi* with LC_{50} values of 0.15 and 0.16 ml%, respectively (Perich *et al.*, 1995). Choochote *et al*.(2004) also reported adulticid activity of *Apium graveolens* with LC_{50} value of 6.6gm/cm^2 against *Ae. aegypti*. Anti-oviposition activity of *Imperata cylindria* against *Cx. quinquefasciatus* was previously reported (Mohsen *et al*.,1995). In contrast, neem products did not showed adulticidal activity against different mosquito species (Singh *et al*., 2003).

The adulticidal bioassay of the essential oils against *An. arabiensis* with respect to time response showed that *O. suave* (fresh) was the most potent essential oil followed by *O. suave* (dry) and *T. vulgaris*. *O. suave* (fresh) caused 100% mortality in *An arabiensis* in 5 minutes at 0.05 ml%(v/v) concentration. However, *O. lamiifolium* caused 100% mortality of *An. arabiensis* in 5 minutes at 30 fold more concentration (1.5 ml% (v/v). In comparison with *E. citriodora, E. globulus* resulted 100% mortality in *An. arabiensis* at 2 times more concentration. The synthetic adulticides such as permethrin, lambda-cyhalothrin, malathion, chlorphoxin and fentirothion resulted 100% mortality in *An. albimanus* exposed to insecticide treated papers at 0.25, 0.025, 5, 5 and 1% in 30, 75, 75, 105 and 150 minutes, respectively (Brogdon and McAllister 1998b). The tested essential oils in present study caused 100% mortality of *An. arabiensis* at lower concentrations and shorter times though the methods were different. This indicated the promising activity of essential oils as alternative adulticides in mosquito control programs. With an increase in concentration, the mortality time decreased in adulticidal bioassay. The decrease in mortality time with increasing in concentration could be due to the increase of uptake of active ingredients by the mosquitoes (Kabir *et al*., 2003).

At higher concentrations of each plant essential oil following exposure for 5-30min, almost all adult mosquitoes showed signs of paralysis, i.e. unable to fly and lay at the bottom of the exposure bottle immediately. In a similar experiment, Quraishi (1977) reported that different mosquito species exhibited typical symptoms of nerve poisoning at initial excitation, followed by paralysis and finally death that caused by pyrethrins.

Regarding the residual effects of the essential oils, the highest effect was revealed by *O. suave* (fresh). However, *E. citriodora* essential oil showed the lowest residual activity against *An arabiensis* adults. The purpose of impregnation of the cages with the essential oils was to find out if any of the essential oils could be potential candidates in impregnation of the bed nets with the view to searching alternative or additional insecticides to the current pyrethroids used in treating mosquito bed nets to which mosquitoes might develop resistance (Zaim and Guillet 2002). To date, no literature is available on the residual activity of the essential oils against adult mosquitoes for comparison. However, the result revealed that the essential oils had residual activity though they required further studies to identify the active component and to produce more effective formulations to elongate their residual activity.

As whole, essential oils individually evaluated in this study had higher LC_{50} values than that of the synthetic larvicides such as permethrin and chlorfenapyr (Paul *et al*., 2006). However, they showed higher adulticidal activities than some synthetic adulticides reported by Brogdon and McAllister (1998b). Higher LC_{50} values of plant products are expected and acceptable, considering that they are generally more biodegradable, have lower non-target toxicity and environmentally friendly. Furthermore, unlike conventional insecticides, which are based on a single active ingredient, plant derived insecticides comprise variety of components with different mechanisms of action. Thus the chances of insects developing resistance to plant products seem likely to be low (Dhar *et al.,* 1996; Saxena, 1987). Insecticide resistance has been a problem in all insect groups that serve as vectors of emerging diseases (Brogdon and McAllister, 1998a).

6. CONCLUSION

Larvicidal effects of essential oils of 11 plants tested (*Chenopodium ambrosioides, Eucalyptus citriodora, E. globulus, Ocimum lamiifolium, O. suave, Schinus molle, Lippia adoensis, Piper nigrum, Mentha spicata, Thymus vulgaris* and *Nigella sativa*) against laboratory reared *An. arabiensis* and *Ae. aegypti* were carried out in this study. The larvicidal activity against *An. arabiensis* was much pronounced in the essential oils of *O. lamiifolium, C. ambrosioides, S. molle* (leaves) and *N. sativa.* Against *Ae. aegypti*, the highest larvicidal activity was exhibited due to application of essential oils of *O. lamiifolium, C. ambrosioides, P. nigrum* and *S. molle* (leaves). The lowest larvicidal efficacy was obtained when the essential oils of *E. globulus* and *M. spicata* tested against both laboratory reared *An. arabiensis* and *Ae. aegypti*. The results indicated the presence of potential diversity of plants used as an alternative or additional method of malaria vector control in Ethiopia. No work has been done on the larvicidal activities of essential oils of *O. lamiifolium, C. ambrosioides, S. molle* (leaves) and *N. sativa* against mosquitoes.

Time-mortality bioassays with essential oils of above 11 plants against laboratory reared *An. arabiensis* and *Ae. aegypti* were also conducted in present study. The activity of essential oil of *L. adoensis* had the highest kill with in a shorter time than other essential oils. However, essential oils of *O. suave* (fresh) and *O. suave* (dry) caused persistently longer time with 100% mortality against both tested mosquito species.

In simulated field conditions, essential oils of *C. ambrosioides, O. lamiifolium, S. molle* (leaves), *O. suave* (fresh) and *P. nigrum* were evaluated against field population of anopheline larvae in Sille, South of Arba-Minch. Essential oil of *O. lamiifolium* showed the highest larvicidal activity against the field population of anopheline mosquitoes. However, the lowest larvicidal activity against the field population of anopheline mosquitoes was recorded due to application of *P. nigrum* essential oil. The field population of anopheline larvae were shown more resistant to essential oils than the laboratory reared *An. arabiensis* larvae.

Adulticidal effects of essential of oils the above 11 plants were tested by the bottle bioassay method against *An. arabiensis* reared in the laboratory. The essential oil from *O. suave* (fresh)

showed the most adulticidal activity against *An. arabiensis* followed by *T. vulgaris* and *O. suave* (dry) essential oils. *P. nigrum* essential oil resulted the least adulticidal activity against *An. arabiensis* than other essential oils tested. This is the first work on adulticidal activities of essential oils of all plants tested against *An. arabiensis*.

In time mortality response of essential oils against laboratory reared *An. arabiensis* adults, essential oil of *O. suave* (fresh) caused up to 100% mortality even at lower concentration (0.05 ml% v/v) in a shorter time (5 minutes). Similarly, oils of *P. nigrum* induced 100% mortality in *An. arabiensis* adults in 5 minutes but it was only at higher concentration (50 times more concentration).

The residual activity of essential oils of *C. ambrosioides*, *O. lamiifolium*, *S. molle* (leaves), *O. suave* (fresh), *O. suave* (dry), *E. citriodora* and *L. adoensis* were conducted by soaking cages with 1ml% (v/v) of essential oil solution for 15 minutes. From tested plants essential oils, *O. suave* (fresh) treatment showed the highest residual activity against laboratory reared *An. arabiensis* adults (persisted for 15 days), whereas *E. citriodora* had the lowest residual effects in *An. arabiensis* adults (effective only for 1 day).

7. RECOMMENDATIONS

1. From the laboratory bioassays, the essential oils from *Ocimum lamiifolium*, *Chenopodium ambrosioides* and *Schinus. molle* (leaves) had shown the highest promising results in both tested mosquito species. Further studies on the isolation and identification of the active components, and formulation preparations for enhancing potency and stability is suggested to be pertinent.

2. Though the essential oils were effective at higher doses in the simulated field bioassays, they might be used as larvicides selectively in small breeding places such as hoof prints, irrigation channels, small ponds etc., where water is stagnant and are the main breeding places for the major malaria vector, *An. arabiebsis*, of Ethiopia. However, it is very important to evaluate the larvicidal activity of the essential oils under natural breeding habitats, their stability to light and heat and their residual activities.

3. From the laboratory bioassays, the essential oils from *Ocimum suave* (fresh), *Thymus Vulgaris and Ocimum suave* (dry) were shown the highest promising results against *An. arabiensis* adults. Further studies on the isolation and identification of the active components, and formulation preparations for enhancing potency and stability might be requited.

4. Further studies on the adulticidal and larvicidal mode of action of essential oils and their effects on non-target organisms and environment might be needed.

5. It is also important to test the larvicidal and adulticidal activities of the above plants essential oils on other medically important vectors since the response of insects to a particular essential oil varied from one species to another.

6. Conservation and propagation of local medicinal plants are also recommended.

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Appendix 1. Plants tested for mosquitocidal properties

C. ambrosioides

E. citriodora

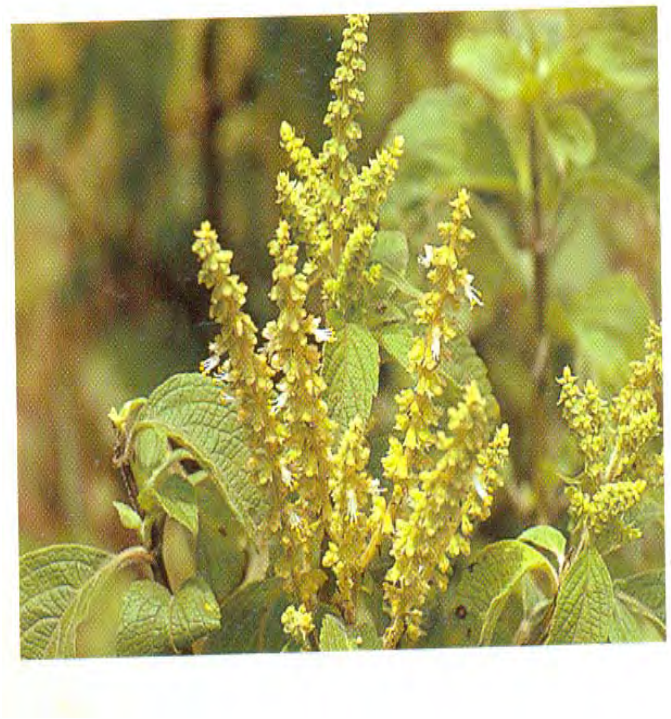
E. globulus

L. adoensis

M. spicat

N. sativa

O. lamiifolium



O. suave

S. molle

Source: Fichtl and Adi (1994)

Appendix 2. Preparation of different concentrations of essential oils for larvicidal test in the laboratory.

Initial solution (ml%; v/v)	Initial solution in PPM	Final concentration in each cup when 1ml of stock solution added to 150ml of distilled water
10	100000	666.67
5	50000	333.3
2.5	25000	166.67
2	20000	133.3
1.5	15000	100
1	10000	66.67
0.75	7500	50
0.5	5000	33.3
0.2	2000	13.3
0.1	1000	6.67
0.09	900	6

PPM = parts per million

Appendix 3. . Preparation of different concentrations of essential oils for larvicidal test in the simulated field condition.

Initial solution (ml%; v/v)	Initial solution in PPM	Final concentration in each cup when 1ml of stock solution added to 30ml of distilled water
6	60000	200
5	50000	166.67
4	40000	133.3
3	30000	100
2	20000	66.67
1	10000	33.3
0.5	5000	16.67

PPM = parts per million