ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF ZOOLOGICAL SCIENCE

REPELLENCE AND LARVICIDAL EFFICACY OF SOME SELECTED PLANT OILS AND EXTRACTS AGAINST ADULT *Anopheles arabiensis* (Patton) AND LARVAE OF *Aedes aegypti* (Linnaeous) (Culicidae: Diptera) UNDER LABORATORY CONDITIONS

BY
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DECLARATION

I, the undersigned, declare that the thesis is my original work, has not been presented for degrees in any other university and all sources of material used for the thesis have been duly acknowledged.

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Place: College of Natural and Computational Sciences, Addis Ababa University

Date: _____

This MSc thesis has been submitted for evaluation and presentation with my approval as a university advisor.

Advisors

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LIST OF ABBREVIATIONS AND ACRONYMS

AMCA	American Mosquito Control Association
CDC	Center for Disease Prevention and Control
CEAG	Center for Environmental Advocacy and Governance
DDT	1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
FMoH	Federal Ministry of Health
GC-MS	Gas chromatography–mass spectroscopy
UNICEF	United Nations Children's Fund
WMR	World Malaria Report
YF	Yellow fever

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ABSTRACT

Insecticides and repellent chemicals are among the major tools of controlling disease vectors. In this study, the knock-down, adultcidal and repellence activities of crude ethyl alcohol extracts of the leaves of spearmint (Mentha spicata) and rosemary (Rosemarinus officinalis) were tested against the larval and adult stages of malaria vector Anopheles arabiensis. The larvacidal effect of seed powder of neem (Azadrichiata indica) was evaluated under laboratory condition against the fourth instar larvae of Aedes aegypti. Extracts of M. spicata and R. officinalis caused moderate knock-down and mortality activity after 1 h and 24 h of exposure, respectively, but higher repellency effects at 1 h. M. spicata caused average percent knock-down, mortality and repellency effects ranging from 8 to 32%, 0 to 28% and 95 to 97%, respectively at six different concentrations used. Whilst R. officinalis induced between 0 and 36%, 0 and 32% and 63 and 74% mean percent knock-down, mortality and repellency effects, in that order. Adult mosquito mortality increased with increasing concentration of the extracts but there was no significant variation among all concentrations of extracts of both plants used ($P>0.05$), except 0.1 and 1 percent concentrations which were significant ($P<0.05$). M. spicata showed much superior average percent repellency. No significant variation was shown on repellency effect among the different concentrations ($P>0.05$). A significantly higher repellency effect was observed in the different concentrations of extracts of R. officinalis ($P<0.05$) as compared to the control. The mean percent larval mortality against A. agypti caused by seed powder of A. indica at concentration of 5g/250ml was 58% at 24 h and 81% at 48 h. There was no significant difference in larval mortality between 24 and 48 h of exposure ($P>0.05$). In conclusion, M. spicata and R. officinalis can't be considered as sources of potential adulticides based on WHO standards as their extracts failed to produce >60% mortality but can be regarded as potential sources of

repellents against An. arabiensis. Besides, A. indica could be a potential source of larvicides against the larvae of Ae. aegypti.

Keywords: Adulticides, *Anopheles arabiesnis, Aedes agepti*, Larvicides, Plant extracts

1. INTRODUCTION

1.1 Background

Vector-borne diseases are amongst the most complex of all infectious diseases to prevent and control (WMR, 2014). Insect-borne diseases account over 17% of all infectious diseases (Karunamoorthi, 2015). Mosquitoes transmit over ten important human and/or animal diseases which include malaria, lymphatic filariasis, yellow fever, dengue fever, West Nile virus, rift valley fever, chikungunya, Japanese encephalitis, Venezuelan equine encephalitis, and Murray Valley Encephalitis, amongst others (Mukandiwa *et al*., 2016). Mosquito-borne diseases are common in more than 100 countries and about 700 million people are infected by mosquito-borne diseases globally, every year (Ghosh *et al*., 2012) while over one million people die from these diseases (AMCA, 2014). Of these, human malaria largely caused by *Plasmodium falciparum* and *P. vivax* is the most important particularly in the tropics and subtropics. Apart from malaria, *Aedes* transmitted viral diseases including dengue, yellow fever, chikungunya and Zika have been of considerable concern in recent years across much of the tropics and subtropics worldwide (Campbell *et al*., 2015).

Malaria, though a preventable protozoan disease of humans, is highly prevalent and widespread in many tropical countries and the malaria situation is serious in thes parts of the world. An estimated 3.3 billion people are at risk of malaria infection, of which 1.2 billion live in very high malaria risk areas. Malaria threatens the lives of 40% of the world's population representing over 2,200 million people. Each year, there are an estimated 300-500 million clinical cases. Malaria is estimated to kill more than one million people annually, the majority of whom are young children (UNICEF, 2000). About 90% of the world's malaria deaths occur in sub-Saharan Africa, (Hemingway, 2014). Despite control efforts, malaria remains a leading cause of morbidity and mortality worldwide (WMR, 2014).

Yellow fever (YF) is a viral infection caused by the yellow fever virus which is transmitted to humans and other vertebrate hosts by the bite of infected female *Aedes* mosquitoes (Mutebi and Barrett, 2002). The virus remains endemic in many parts of Africa and South America. It poses a serious public health threat in 45 endemic countries, 32 in Africa and 13 in Central and South

America, where altogether almost 900 million people are at risk (WHO, 2010). Declining vaccination rates and discontinuation of vector control measures in many parts of Africa have led to resurgence of yellow fever disease and periodic epidemics in East and West African countries (Beasley *et al*., 2015).

Epidemics of dengue, chikungunya, and Zika are reported through the Americas and an estimated 3.9 billion people living in 120 different countries are at risk (Shragai *et al*., 2017). Dengue is the most common mosquito-borne viral disease worldwide, with an estimated 390 million infections per year and 40% of the world's population at risk (Bhatt *et al*., 2013). Dengue fever is responsible estimated 50–100 million annual cases and tens-of thousands of more severe and sometimes fatal dengue hemorrhagic fever/shock syndrome (DHF/DSS syndromes (Weaver and Reisen, 2010).

Control of mosquito vectors has long been a critical part of the global strategy to manage mosquito-associated diseases, and insecticides are the most important component in this effort (Liu, 2015). However, development of resistance to insecticides is becoming a major issue and development of new insecticides is needed to maintain the current gains and reduce global burdens of transmission (Hemingway, 2014; Baskar *et al*., 2016). Mosquito control using synthetic chemical insecticides and their indiscriminate use has become a major concern.. The heavy reliance on chemical insecticides, recurrent and inappropriate insecticide applications are key sources for resistance (Karunamoorthi and Sabesan, 2012). Synthetic chemical insecticides also have an adverse effect on the environment, disturb ecological balance, majority of the chemical pesticides are harmful to man and animals, some of them are less degradable and spreading toxic effects (Govindarajan *et al*., 2011). Currently, serious issues concerning the ecotoxicology of chemical insecticides are raised (Popivanov *et al*., 2015). For these reasons, naturally occurring compounds and their derivatives are of increasing interest for the development of new insecticidal compounds against vectors of disease-causing pathogens (Anstrom *et al*., 2012)**.**

Plants are rich sources of bioactive compounds and constitute a number of secondary metabolites that can be utilized for the control of insects including mosquitoes as they are active against a limited number of species i.e. specific target insects and are biodegradable (Govindarajan *et al.,*

2011; Baskar *et al*., 2016). Essential oils fromAromatic plant and plants have demonstrated optimal potential for insecticidal activity against several species (Zoubiri and Baaliouamer, 2014). To date, the insecticidal activities of extracts from many plants have been assessed (Jide-Ojo *et al*., 2013). Globally, the mosquitocidal, larvicidal, ovicidal and repellent activity of extracts and essential oils of several plants against various species of mosquito vectors have been evaluated (Shaalan *et al*., 2005; Trongtokit *et al*., 2005; Kweka *et al*., 2008; Mullai *et al*., 2008; Remia and Logaswamy, 2010; Bagavan and Rahuman, 2011; Govindarajan *et al*., 2011; Chore *et al*., 2014; Krishnappa and Elumalai, 2014; Uniyal *et al*., 2014; Karunamoorthi, 2015; Yu *et al*., 2015; Ramkumar *et al*., 2016; Unachukwu *et al*., 2016). The two spicy plants spearmint (*Mentha spicata*) and rosemary (*Rosemarinus officinalis*) as well as neem tree (*Azadirachta indica*) have been assessed for their mosquitocidal effect against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*, (Mandal *et al*., 2011; Govindarajan *et al*., 2012; Nour *et al*., 2012). Likewise, the adulticidal and larvicidal activity of plant extracts against *An. arabiensis* have been evaluated (Karunamoorthi and Ilango, 2010; Maharaj *et al*., 2011; Taha *et al*., 2011; Edriss *et al*., 2012; Chalannavar *et al*., 2013; Mavundza *et al*., 2013).

The main advantage of using plant materials as alternative agents for the control of mosquitoes is that they are locally available, potentially less expensive, they can be propagated easily and sustainable, rich in bioactive chemicals, biodegradable and are active against a limited number of species eliciting no negative effects on the ecosystem (Govindarajan *et al*., 2011; Jouda, 2012; Guruprasad and Pasha, 2014). Therefore, plants could be considered as alternative agents for control of mosquitoes as they are rich in bioactive chemicals, active against a limited number of species and are biodegradable (Govindarajan *et al*., 2011).

In Ethiopia, malaria is the leading health problem. About 75% of the land of Ethiopia is malarious and more than 54 million people are at risk of infection. *Plasmodium falciparum* accounted for 60% and *P. vivax* to 40% of the infections (Alelign and Dejene, 2016).

Vector control has been one of the major mechanisms of malaria control program in the country. The program mainly focused on the use of indoor residual spray of 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDT) to control the principal vector. The good understanding of the partial endophilic behavior of the vector made the use of this chemical insecticide effective to control

the vector (Gish, 1992) and thus, the program was successful in its initial phase. However, with time the program couldn't stay last achieving the ultimate success due to the complexity of the process. Besides, the high dynamicity in disease symptom and way of transmission made the program very expensive. On the other hand recurrent climate changes and environmental modifications (Tulu, 1993) as well as outbreak of drug resistance of the parasites (Ghebreyesus and Alamrew, 1998; Mengesha *et al*., 1998) and insecticide resistance vectors (FMoH, 2002) is also becoming a threat to malaria control efforts. The emergence of resistance to various chemical insecticides by mosquitoes has been reported in different parts of the country (Balkew *et al*., 2010; Abate and Hadis, 2011; Yewhalaw *et al*., 2011).

Moreover, environmental concern and natural resource development programs have necessitated the utilization of appropriate technological and management techniques in an integrated approach to bring about an effective degree of vector suppression. Hence, the search for control tools that are cost effective, environmentally friendly and effective in reducing man vector contact is very much desired. Plant based mosquitocides and repellents are crucial to be investigated.

So far, in Ethiopia, the effects of essential oils and extracts of local plants against the adults of *An. arabiensis* (Betelehem Wondwosen, 2009; Massebo *et al*., 2013; Andemo *et al*., 2014), larva of *Ae. aegypti* (Massebo *et al*., 2009) and *An. arabiensis* (Massebo *et al*., 2009; Tigist Assefa, 2011; Tomass *et al*., 2011) have been evaluated. To our knowledge, published reports demonstrating the effect of *M. spicata*, *R. officinalis* and *A. indica* towards *An. arabiensis and Ae. Aegypti* are lacking. Therefore, this study was initiated and focused on evaluation of the mosquitocidal (adulticidal and larvicidal) and repellency effect these plants against the adult and larvae of *An. arabiensis* and *Ae. aegypti* under laboratory conditions.

1.2 Statement of the problem

In Ethiopia, mosquito-borne diseases such as malaria and yellow fever are prevalent and widespread infectious diseases among the human population. These diseases are transmitted principally by mosquito vectors such as *An. arabiensis* and *Ae. aegypti*. Mosquito vector control largely relied on the use of synthetic insecticides alongside ecological control. But, the development of resistance in mosquitoes to various synthetic insecticides utilized for the control of principal malaria vector, that is, *An. arabiensis* have been reported (Balkew *et al*., 2010; Abate and Hadis, 2011; Yewhalaw *et al*., 2011; Fetene *et al*., 2013; Asale *et al*., 2014). This emergence of insecticide resistance calls for the search of new insecticides. To date, there were several attempts to assess the adulticidal, larvicidal and repellency activity of essential oils (EOs) and extracts of indigenous plants against mosquito vectors. Nonetheless, the mosquitocidal activity and repellency effect of *M. spicata*, *R. officinalis* and *A. indica* against *An. arabiensis* and *Ae. aegypti* is not investigated.

1.3 Objectives

1.3.1. General objective

To evaluate the insecticidal efficacy of plant extracts against *An. arabiensis* and larvae of *Aedes aegypti* for integrated mosquito control.

1.3.2. Specific objectives

- To determine adulticidal effect of ethanol extracts of *Mentha spicata* and *Rosmarinus officinalis* against. *Anopheles arabiensis* in the laboratory.
- To evaluate the repellent effect of extracts of *Mentha spicata* and *Rosmarinus officinalis* towards laboratory reared *An. arabiensis* using a bioassay technique.
- To investigate the larvicidal effect of the extract of *Azadirachata indica* against the larval stages of *Aedes aegypti* under laboratory conditions.

2. LITERATURE REVIEW

2.1 Mosquito-borne Diseases

Mosquito-borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally (Ghosh *et al*., 2012). More than 40% of the world's populations live in malarious areas (Ghai and Gupta, 2000). Plate 1 presents countries with ongoing transmission of malaria. About 3.3 billion people −1/2 of the world's population –are at risk of contracting malaria. Malaria is a mosquito-borne disease affecting some 300 to 500 million people and causing 1.4 to 2.6 million deaths annually world-wide (Massebo *et al*., 2009). About 90% of mortality attributed to malaria is experienced by infants and young children (Pavela, 2015). Malaria is a major public health problem in Africa, parts of Asia, Latin America, the Middle East, Eastern Europe and the Pacific, 90% of malaria cases in the world occur in Africa south of the Sahara (UNICEF, 2000). In 2015, 214 million malaria cases and 438,000 deaths have been estimated to have occurred globally, 88% of which estimated to have occurred in WHO African countries (WHO, 2016). Malaria is the fourth leading cause of death of children under the age of five years (Alelign and Dejene, 2016).

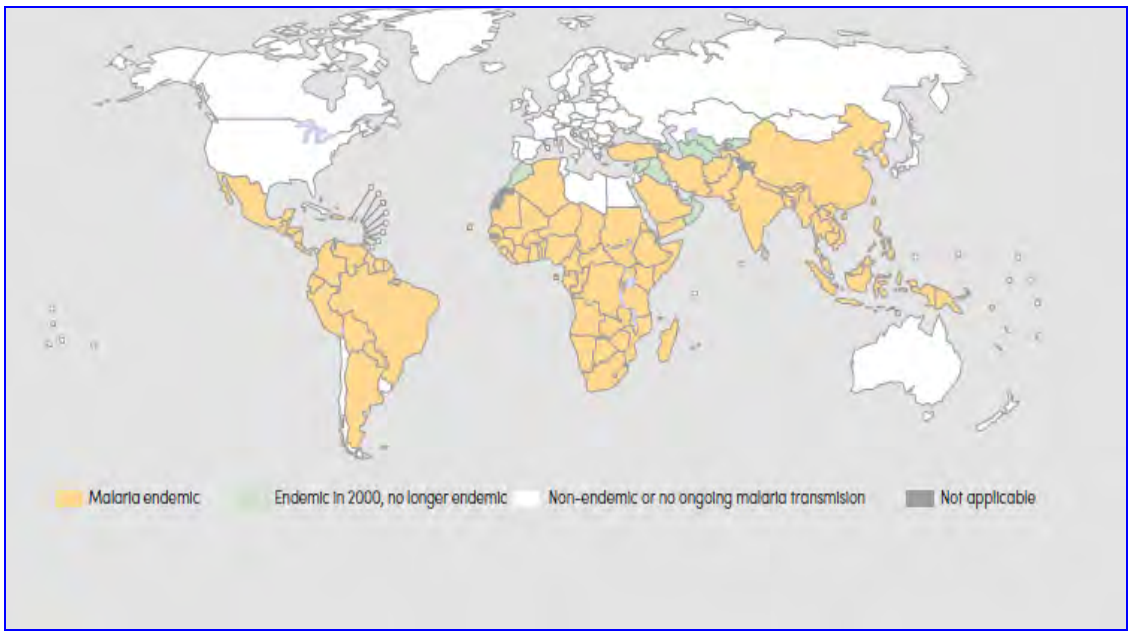

Plate 1: Countries with ongoing transmission of malaria, 2000 and 2015.

Source: WHO (2016).

In Africa, malaria is responsible for about 20-30% of hospital admissions and about 30-50% of outpatient consultations (UNICEF, 2000) and in south of Sahara, malaria is the leading health problem, with almost the entire population being at risk. More than 74% of the population lives in highly endemic areas while about 18% lives in epidemic prone areas (FMoH, 2002).

Yellow fever is one of the great burdens for public health in the endemic regions in Africa and South America. Worldwide there are forty-four endemic countries for yellow fever virus, all are located in Africa and Latin America; over 900 million people are at risk. In Africa, an estimated 508 million people live in 31 countries at risk. The remaining populations at risk are in 13 countries in Latin America (WHO, 2013). YF virus circulation and outbreaks of YF was observed in Brazil, Paraguay, Argentina, Colombia and Peru. Yellow is also fever is endemic in tropical and subtropical regions of Africa (Monath and Vasconcelos, 2015). The WHO estimates that approximately 200.000 yellow fever (YF) cases occur in South America and Africa each year (Tomori, 1999; Mutebi and Barrett, 2002).

Dengue fever (DF) is endemic in Africa, the Americas, eastern Mediterranean, South East Asia, and the Western Pacific, threatening more than 2.5 billion people (Guillena *et al*., 2010). An estimated 50 to 270 million dengue fever infections occur every year globally, of which two million cases evolve to severe dengue fever, and 21,000 would result in death (Ferreira, 2012).

Chikungunya virus emerged in the Americas in 2013 and has caused 1.8 million suspected cases from 44 countries and territories to date (Shragai *et al*., 2017).

Zika virus affects 32 countries and territories of the Americas (Vorou, 2016). In 2015–2016, outbreaks of Zika virus spread throughout the Americas, resulting in over 360,000 suspected cases, with likely many more going unreported (Shragai *et al*., 2017). In, 2016, local transmission of Zika infection had been reported from more than 20 countries and territories in the Americas and an outbreak numbering thousands of cases was under way in Cabo Verde in western Africa (Kindhauser *et al*., 2016).

In Ethiopia, among mosquito-borne illnesses transmitted by *An. arabiensis* and *Ae. aegypti* malaria, yellow fever and dengue fever have been reported. Areas of Ethiopia below 2,000 meters (m) are considered to be malarious (or potentially malarious). This comprises 75% of the

landmass where 68% of the human population lives (Ghebreyesus *et al*., 2005; FMoH, 2012; Ayele, *et al*., 2013). More than 54 million populations live in these malarious areas (Alelign and Dejene, 2016). More than 600,000 confirmed and more than 9 million clinical cases each year and cause about 70,000 deaths in all age stages each year in Ethiopia (Alemu *et al*., 2012). Malaria is mainly seasonal in the highland fringe areas, and of relatively longer transmission duration in lowland areas, river basins and valleys (FMoH, 2012). Ethiopia is one of the east African countries endemic to yellow fever. Though no cases/outbreaks were reported for 48 years, in January 15, 2014 outbreak of yellow fever was reported from Malle district of South Omo Zone of Southern Nation and Nationalities People Region with a total of three cases with two deaths (Etsehiwot Zemelak, 2014). In Ethiopia, dengue fever has been diagnosed among travelers that return to countries to which dengue was not endemic but never reported as occurring locally (Van *et al*., 2009; Amarasinghe *et al*., 2011; Were, 2012). In addition, Woyessa *et al*. (2014) reported that 56.8% (50/88) of samples were positive for dengue fever infection in Dire Dawa.

2.2 Aetiology of major mosquito-borne diseases

Human malaria is caused by infections of unicellular protozoan parasites of the genus *Plasmodium*. Four species including *Plasmodium falciparum* Welch, *P. vivax* Grassi & Feletti, *P. malariae* Feletti & Grassi and *P. ovale* Stephens infect human (Service, 2000; CDC, 2004; Pohlit *et al*., 2011). Globally, the most common malarial parasite is *P. vivax* but the most deadly is *P. falciparium* (CEAG, 2006; CDC, 2004).

Yellow fever (YF) is caused by the prototype member of the genus Flavivirus (family Flaviviridae), which contains approximately 70 positive-strand, single-strand RNA viruses, the majority of which are transmitted by arthropods (mosquitoes and ticks) (Monath and Vasconcelos, 2015). Almost half of these viruses are pathogens of humans and/or animals, and can be considered zoonotic viruses (Mutebi and Barrett, 2002). The YF virus genome is a single-stranded, positive-sense RNA molecule of approximately 10,760–11,008 nucleotides (Beasley *et al.,* 2015). Humans are infected sporadically when bitten by sylvatic mosquitoes that previously fed on a viremic monkey (so-called jungle yellow fever), mainly by *Ae. aegypti*, a species that breeds in water-containing vessels inside dwellings or in close proximity to them (so-called urban yellow fever (Monath and Vasconcelos, 2015).

Zika fever is an emerging zoonotic disease caused by zika virus, a Flavivirus related to yellow fever, dengue, West Nile, and Japanese encephalitis viruses, with a positive sense single-stranded RNA molecule 10,794 bases long (Hayes *et al*., 2009; Tilak *et al*., 2011).

In Ethiopia, malaria is caused by infection with the parasites *P. falciparum* and *P. vivax* with relative frequency of 60% and 40% respectively (FMoH, 2004; Deressa *et al*., 2006). Malaria caused by *P. malaria* (<1%) is found sporadically in some areas, while there has been no report about the occurrence of *P. ovale* induced malaria in the country (Deressa *et al*., 2006). However, these disease-agent proportions vary both spatially and temporally. Where malaria is epidemic, *P. falciparum* is the dominant parasite species that causes severe disease manifestations. Almost all deaths attributed to malaria happen due to infection by *P. falciparum* (FMoH, 2004).

2.3 Mosquito Vectors

There are about 3,400 different species of mosquitoes in the world belonging to 42 genera distributed among three subfamilies; Toxorhynchitinae, Anophelinae and Culicinae. They serve as vectors for a wide variety of human and veterinary pathogens and parasites (Pavela, 2015). They can transmit more diseases than any other group of arthropods. The most important man-biting mosquitoes belong to the genera *Aedes*, *Anopheles*, *Culex*, *Haemagogus*, *Mansonia*, *Sabethes* and *Psorophora* (Service, 2008). Bites of mosquitoes belonging to the genera *Anopheles* Meigen, *Aedes* Meigen, *Culex* L. and *Haemagogus* L. are a general nuisance and are responsible for the transmission of important tropical diseases such as malaria, hemorrhagic dengue and yellow fevers and filariasis (elephantiasis) (Pohlit *et al*., 2011).

Anopheles mosquitoes are the most efficient malaria vectors (Chalannavar *et al*., 2013). Approximately, 60 of the 460 described *Anopheles* species are able to transmit. In the main, malaria is transmitted by mosquitoes of the *An. gambiae* complex, *An. funestus* group*, An. nili* complex, and *An. moucheti* complex (Sokhna *et al*., 2013). *Anopheles arabiensis* is one of the dominant vectors of malaria (Sinka *et al*., 2010). In Africa, where the greatest burden of malaria mortality occurs, there are two major malaria vectors, *An. gambiae* and *An. funestus*, although several secondary vectors occur, which can take on a primary role (Hemingway, 2014). In Africa, the two main vectors are a complex of species referred to as *An. gambiae* sensu lato and *An. funestus* which is also probably a complex of species. *An. gambiae* sensu lato is made up of seven

species that are morphological indistinguishable but which are largely genetically isolated (Charles and Godfray, 2013).

Those most important malaria vectors in sub-Saharan Africa are members of *An. gambiae* species complex (Onyabe and Conn, 2001; Service, 2000). The complex consists of seven species *An. gambiae, An. arabiensis, An. melas, An. merus, An. quadriannulatus, An. bwambae* and *An. quadriannulatus* species B, a newly described species from Ethiopia, that vary in their ability to transmit malaria (Service, 2000; Toure *et al*., 2004). Two species of the *An. gambiae s*pecies complex, *An. gambiae* and *An. arabiensis* (Onyabe and Conn, 2001) in conjunction with *An. funestus* (Gary, 2005) are the most broadly distributed and the most efficient vectors of malaria in sub-Sahara Africa (Kameu *et al*., 2007; Takken and Knols, 1999).

Aedes aegypti (Linneaus) is the vector for urban YF epidemics in both Africa and South America (Jansen and Beebe, 2010; Wasserman *et al*., 2016). Dengue, chikungunya, and Zika are primarily transmitted by two mosquito species: *Ae. aegypti* (the yellow fever mosquito) and *Ae. albopictus* (the Asian tiger mosquito) (Shragai *et al*., 2017). Plate 2 presents predicted distribution of *Aedes aegypti.*

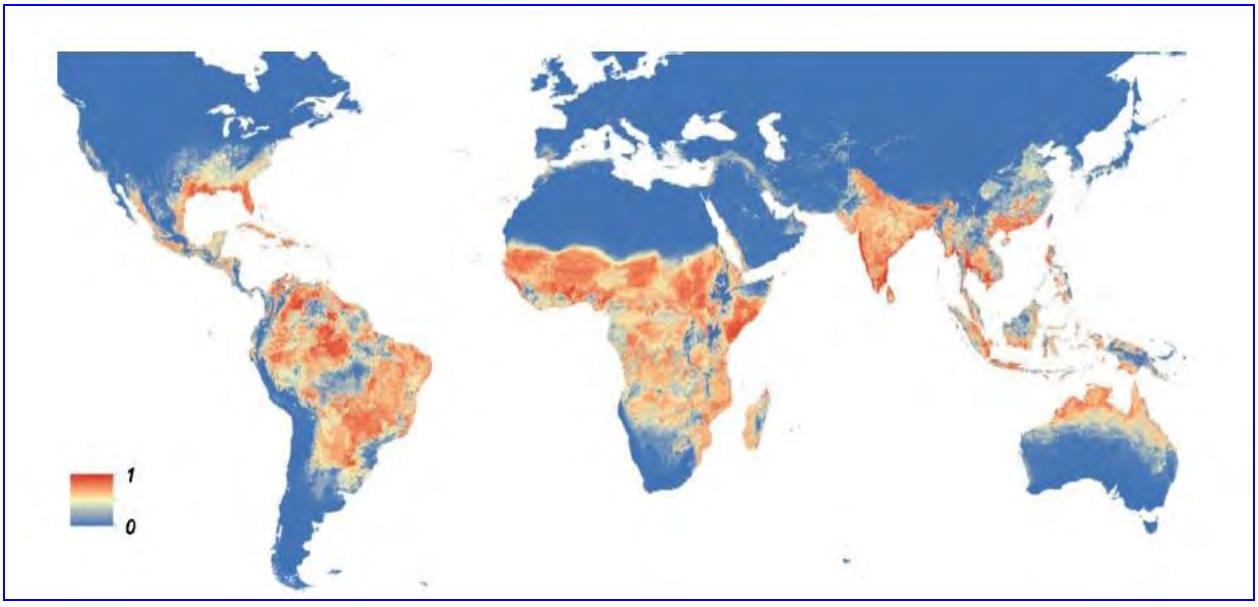

Plate 2: World map showing predicted distribution of *Aedes aegypti*.
Note: The map depicts the probability of occurrence (blue, none; red, highest occurrence).
Source: Tilak *et al*. (2016)

Malaria transmission in Ethiopia depends substantially on the main malaria vectors *An. arabiansis* and *An. pharoensis*, *An. nili* and *An. funesteus* are secondary vectors (TCC, 2000).

2.4 Biology and Life Cycle of Mosquitoes

Human malaria is transmitted exclusively through bites of infected female *Anopheles* mosquitoes (CEAG, 2006). *Anopheles* mosquitoes have a worldwide distribution occurring not only in tropical areas but also in temperature regions. Although there are approximately 430 different species of Anopheles, only approximately 70 species are malaria vectors and of these only 40 species are epidemiologically important (CDC, 2004; Service, 2000; Takken and Knols, 1999). Malaria vectors are divided in to primary and secondary vectors, but this can be misleading because a species may be classified as primary vector in some areas but be only a secondary vector in others (Service, 2000).

Malaria vectors go through four stages in their life cycle: egg, larva, pupa and adult (Plate 3). The first three stages are aquatic and last 5-14 days, depending on the species and ambient temperature (CDC, 2004). Larval stage mosquitoes feed on algae, yeast, bacteria, protozoa and numerous other plant and animal microorganisms found in the water (Service, 2000). Adult male Anopheles mosquitoes feed exclusively on sugar sources, for example nectar and honeydew, and therefore do not transmit the disease. Female mosquitoes also feed on sugar from different sources, but need blood for the development of their eggs. Depending on species, female mosquitoes may lay 100-300 eggs at a time and may lay an average of 1000-3000 at a single breeding site. The average life span of the female mosquito is 3 to 100 days; while the males survive 10 to 20 days (Yoseph, 2007). Most mosquitoes remain within 1 mile of the breeding site from which they emerged. A few species may range up to 20 miles or more (CDC, 2004; Yoseph, 2007).

Anopheles larvae thrive in many different types of large, more or less permanent, habitats ranging from fresh- and salt-water marshes, mangrove swamps, grassy ditches, rice fields, edges of streams and rivers to ponds and borrow pits (Service, 2000). The larval stages of the mosquitoes that comprise the *An. gambiaes pecies* complex frequently dwell in transient bodies of water in which suspended soil particles are abundant (Minakawa *et al*., 1999), particularly toward the end

of rainy season when these puddles begin to contract (Ye-ebiyo *et al*., 2003). These mosquitoes exploit turbid water as frequently as clear water. In contrast, many other kinds of mosquitoes appear to develop more readily in clear water than in turbid water (Savage *et al*., 1990).

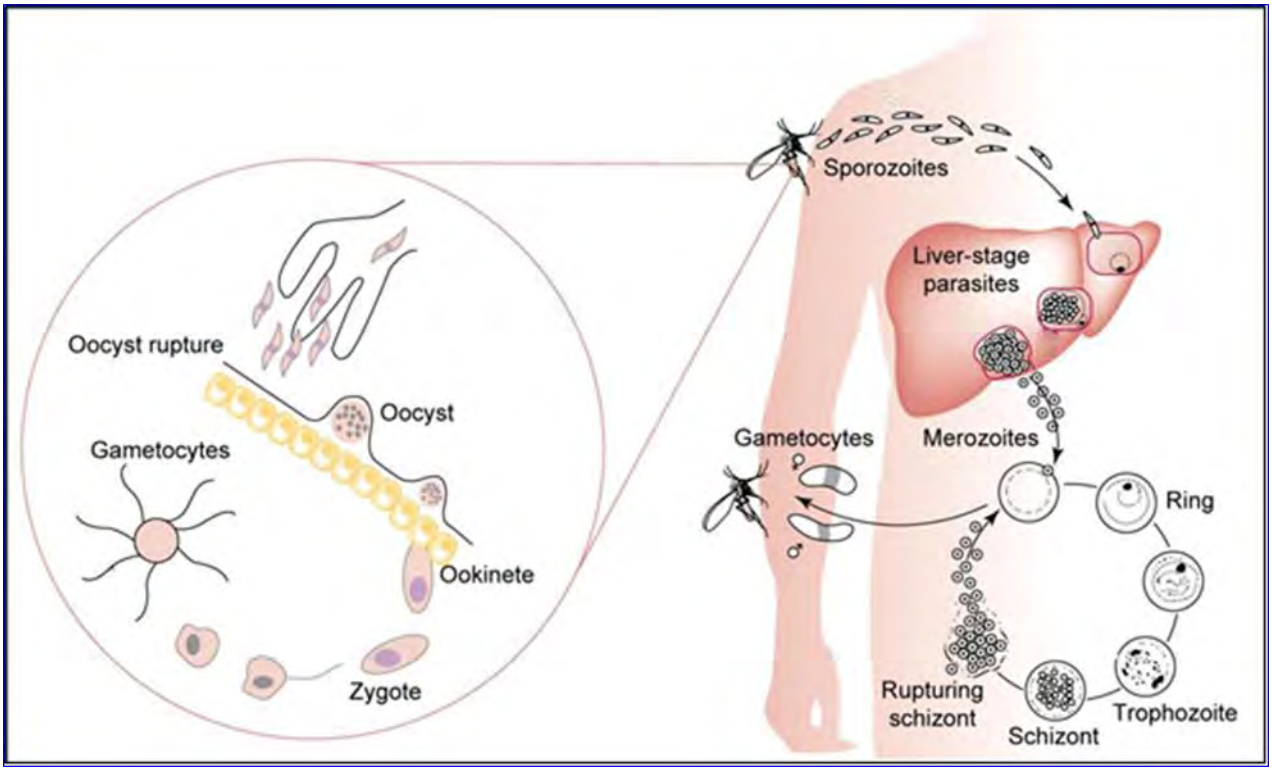

Plate 3: Life cycle of malaria parasite
Source: Targett (2005).

Unlike many other mosquito species, *Ae. aegypti* is a day-biting mosquito, and often feeds on multiple hosts during a single gonotrophic cycle (Jansen and Beebe, 2010).

2.5 Ecology of *Anopheles arabiensis* and *Aedes aegypti*

Transmission of mosquito-borne diseases depends on mosquito biology and population dynamics (Naish *et al*., 2013). Mosquitoes are found throughout the world excluding the places which are completely frozen. Three fourth (3/4) of the existing mosquito species were native to tropical and Subtropical region (Baskar *et al*., 2016). Mosquito biology and population dynamics in turn depends on climate for several purposes: mosquitoes require water to breed and warm temperature is important for larval development and adult feeding behavior (Naish *et al*., 2013).

Larvae and pupae of mosquitoes are always found in water (Forstinus *et al*., 2015). Larval habitats or breeding sites are always composed of water bodies, natural or man-made, permanent or temporary, large or small, freshwater or saline (Rejmánková *et al*., 2013). Typical habitats of *An. arabiensis* and *An. gambiae* are puddles, shallow ponds, burrow-pits, brick-pits, tyre tracks, ditches, human foot and animal hoof prints which are often created by the activities of humans or domestic animals (Forstinus *et al*., 2015). Permanent water sources in dry lands provide potential vectors with water for most of the year, ensuring year-round low level malaria transmission (Mala *et al*., 2011). Adult mosquito densities are seasonal and normally follow rainfall patterns, however this differs both across and within countries (Geissbühler, 2008).

Aedes aegypti is closely associated with human habitation and readily enters buildings to feed and to rest (Jansen and Beebe, 2010). It breeds in man-made containers of water and uniquely influenced by human water storage (Wasserman *et al*., 2016). Domestic vessels used for water storage are frequently the most abundant and productive habitats of immature stages of *Ae. aegypti* (Padmanabha *et al*., 2010). *It* feeds predominantly on human blood and bites multiple individuals in a single blood meal, lives in close association with human dwellings, and efficiently transmits YF virus in its saliva (Wasserman *et al*., 2016). *Ae. aegypti* is an early morning or late afternoon biter, but also bites at night if there is sufficient artificial light (Arya *et al*., 2012). In Dire Dawa artificial water containers including tire barrel, plastic drum, jerricans, mud pot flower pot, discarded sink, buckets, plastic bowl, dustbin, polythene sheet and discarded excavator were found breeding sites for *Ae. aegypti* (Getachew *et al*., 2015).

2.6 Mosquito Vector Control

The basis for protection against insect vectors particularly mosquitoes encompasses approaches such as protection against adults based on killing them, protection based on preventing insects from sucking blood using repellents and reduction of the population density of adults (Pavela, 2015). Insecticides remain as the mainstay in most of the vector control programmes and are commonly applied against adult insects through indoor residual sprays, fumigants, space sprays and treated bed nets (Karunamoorthi and Sabesan, 2012).

However, these days, long term application of chemical substances for the controlling, repelling and killing of hazardous insects are of serious concern for environment and human health

(Shooshtari *et al*., 2013). An effective prevention and control of malaria is threatened mainly by the emergence of insecticide resistance (Karunamoorthi and Sabesan, 2012). Plate 4 illustrates countries where insecticide resistance has been identified.

Thus, there is an urgent need to search new highly selective and biodegradable insecticides lacking long term toxicity to humans and mammals and to develop techniques that can be used to reduce insecticide use while maintaining human health at optimum.

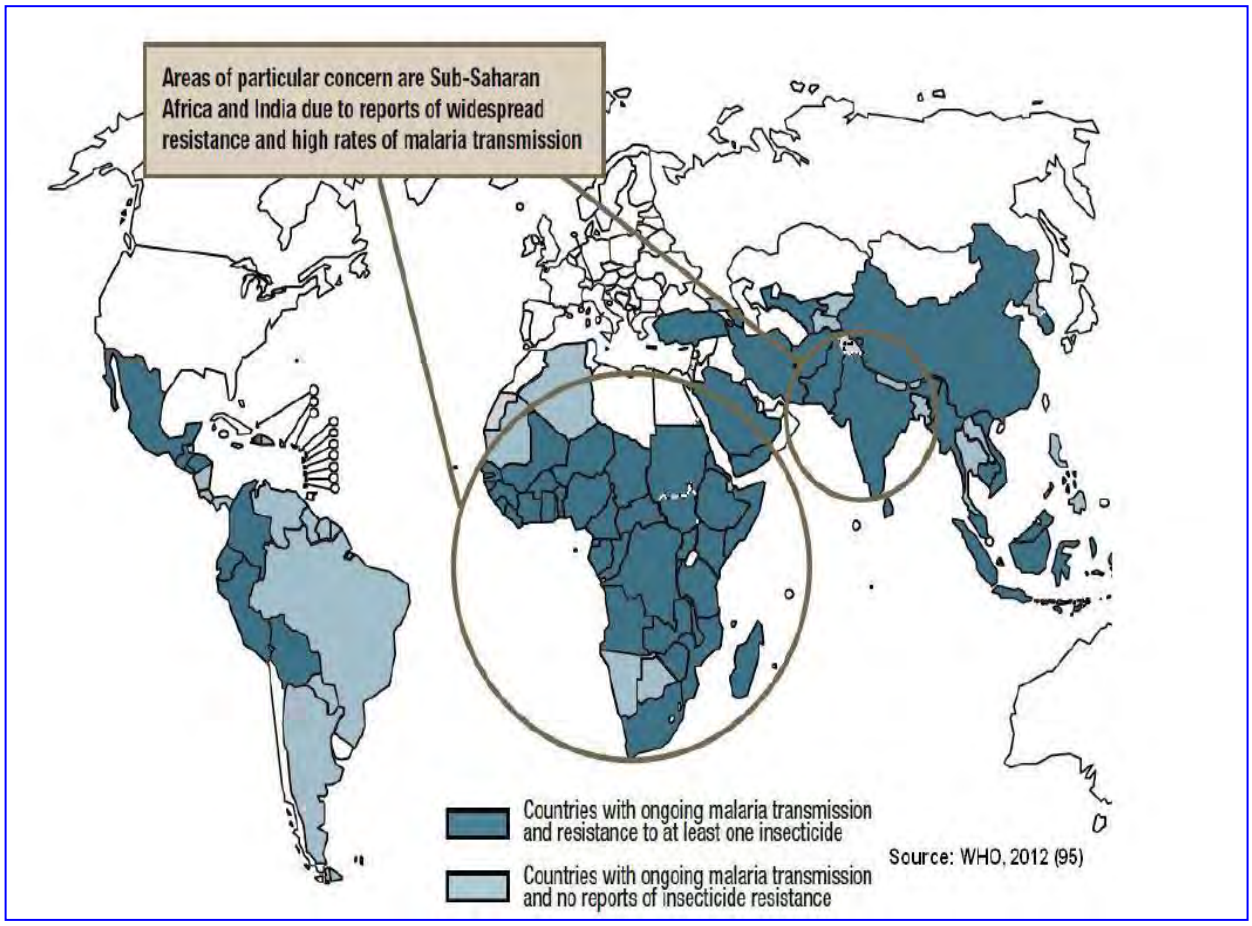

Plate 4: Countries with ongoing malaria transmission where insecticide resistance has been identified in at least one of their major vectors.
Source: Karunamoorthi and Sabesan (2012)

2.7 Botanical Mosquito Control Agents

Plants have historically been valuable sources of agents for the control of insects (Burfield and Reekie, 2005; Nerio *et al*., 2010). Botanicals can be used as alternative to synthetic insecticides or along with other insecticides (Govindarajan *et al*., 2011). Plant product phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal, and adulticidal properties (Elango *et al*., 2009). To date, more than 1,005 plants with insecticidal properties and 297 with repellents have been identified (Kuppusamy *et al*., 2016).

Steam distillation of aromatic plants produces essential oils (Koul *et al*., 2008). Essential oils have been widely used in traditional or herbal medicine, as fragrances in the perfume and cosmetic industries, as flavourings, additives or preservatives in the food industries as well as in aromatherapy (Koul *et al*., 2008; Mezzoug *et al*., 2016). Pesticides based on plant essential oils or their constituents have demonstrated efficacy against a range of stored product pests, domestic pests, blood feeding pests and certain soft-bodied agricultural pests, as well as against some plant pathogenic fungi responsible for pre- and post-harvest diseases (Koul *et al*., 2008).

Zoubiri and Baaliouamer (2014) have presented the review of 230 plants that have been reported to possess insecticidal activity. Aromatic plants are the sources of the natural insecticidal and larvicidal substances and include nicotine (*Nicotiana* L. spp.), quassin (*Quassia amara* L.), rotenone and rotenoids (*Derris* Lour. spp. and (Lonchocarpus Kunth spp. roots), pyrethrins like chrysanthamic acid and its derivatives present in pyrethrum [extracts of *Chrysanthemum cinerariifolium* (Trevir.) Vis. flowers] and azadirachtin (*Azadirachta indica* A. Juss. seed kernel) (Pohlit *et al*., 2011).

There are a number of works on bio-efficacy of plant extracts and essential oils against different mosquito species with the help of laboratory and field bioassay methods (Uniyal *et al*., 2014). The insecticidal and repellency activity of essential oils and plant extracts against mosquitoes has been evaluated (Manimaran *et al*., 2012; Prabhavathi *et al*., 2016). Extracts of *Azadirachta indica*, *Dysoxylum malabaricum*, *Khaya senegalensis*, *Lansium domesticum*, *Melia volkensii*, *Melia azedarach*, *Turraea abyssinica*, *Turraea wakefeldii*, *Turraea mombassana* and *Trichilia roka* showed potential larvicidal activity against vector mosquitoes (Baskar *et al*., 2016). The

solvent extracts of the leaves of *Ervatamia coronaria* and *Caesalpinia pulcherrimaplant* have been reported to be effective against *An. stephensi*, *Ae. aegypti*, and *Culex quinquefasciatus* (Govindarajan *et al*., 2011). The chloroform leaf extracts of *Ocimum canum* Sims have shown significant larvicidal, pupicidal and adulticidal activity against *Ae. aegypti* (Prabhavathi *et al*., 2016). A highest larval mortality was reported in acetone leaf extract of *Clausena dentata* (Willd) (Rutaceae) against *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* (Manjari *et al*., 2014). The leaf extract of *Citrullus vulgaris* plant extract was reported to be more effective against malarial vector, *An. stephensi*Liston (diptera culicidae), with maximum larvicidal, ovicidal, repellent and insect growth regulatory activity (Mullai *et al*., 2008). The chloroform extract of Seaweed (*Bryopsis pennata*) exhibited strong ovicidal activity and larvicidal activity against on *Ae*. *aegypti* and *Aedes albopictus* (Yu *et al*., 2015). Crude benzene and ethyl acetate extracts of the leaves of *Ervatamia coronaria* and *Caesalpinia pulcherrima* showed moderate larvicidal effects *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* (Diptera: Culicidae) (Govindarajan *et al*., 2011).

Plant-based repellents are extensively used in this traditional way throughout rural communities in the tropics (Maia and Moore, 2011). In recent years, plants extracts such as neem (*Azadirachta indica*, *A. b* Juss), citronella grass (*Cymbopogan nardus* Rendle), basil (*Ocimum basilicum* L., *Ocimum gratissimum* L., *Ocimum americanum* L.), clove (*Syzygium aromaticum* L.), prickly straggler (*Solanum trilobatum* L.), musk basil (*Moschosma polystachyum* L.) and thyme (*Thymus vulgaris* L.) have been studied as possible mosquito repellents (Gillij, 2007).

The mosquitocidal and repellency effects of extracts of several plants against *An. arabiensis have* been assessed. Methanol extracts of C*ymbopogon citratus* and *Croton macrostachyus* have been found toxic to the larvae of *An. arabiensis* and could serve as ideal potent larvicidal agent (Karunamoorthi and Ilango, 2010). The potential of the bark of *Olax dissitiflora* and leaves of *Aloe ferox* to be used as larvicides and adulticides against *An. arabiensis* mosquitoes have been reported (Mavundza, 2014).

So far, there is no evidence on the emergence of resistance to botanical mosquitocidal agents as they are not frequently used in vector control and there are mixtures of various related compounds with different modes of action (Rajkumar and Jebanesan, 2007).

In Ethiopia, there are limited reports on the evaluation of adulticicdal and repellent activities indigenous plants against mosquitoes vectors. Massebo *et al*. (2013) assessed the adulticidal activities of essential oils (EOs) of 11 plants against *A. arabiensis* and reported that all produced 100% mortality at a concentration ranging from 0.005 to 1 while *O. suave* acquired the highest level of toxicity at lowest concentration. Bekele *et al.* (2016) also noted that purified fractions of *Oreosyce africana* (leaf extract) possess a very high adulticidal effect against *A. arabiensis*. Out of 11 local plants tested, EOs of *Chenopodium ambrosioides* (aerial parts), *Ocimum lamiifolium* (leaves), *Schinus molle* L. (leaves and seeds), *Nigella sativa* L. (leaves), *Piper nigrum* L. (seeds), *Thymus vulgaris* L. (leaves) exhibited higher larvicidal activity against fourth instar laboratory reared larvae of *An. arabiensis* after 24 h of exposure (Massebo *et al*., 2013). Methanol seed extract of *Mellitia ferruginea* (Birbira) showed high mosquitocidal activity against larvae, pupae and adult stages of both the laboratory strain and field population of *An. arabiensis* (Andemo *et al*., 2014). EO of essential oil of *Tedh* (*Juniperus procera*) Cupressaceae exhibited significant mosquito repellent activity against *An. arabiensis* (Karunamoorthi *et al*., 2014).

Debella *et al.* (2007) assessed the effects of aqueous extracts of 33 plants and found that *Albizia gummifera* (seeds), *Balanites aegyptica* (fruits), *Hedera helix* (leaves and fruits), *Millettia ferruginea* (seeds) and *Warburgia ugandensis* (leaves) exhibited larvicidal activities ranging from 50-100% against laboratory reared *Aedes africanus Culex quinquefasciatus* and *Aedes agypti* at concentrations ranging from 23.6 to 263.5 ppm (Debella *et al*., 2007).

2.8 Description of test plants

2.8.1 Mentha spicata

Mint, belongs to the genus *Mentha* in the family *Labiatae* (*Lamiaceae*) that includes other commonly grown oil yielding plants such as basil, sage, rosemary, marjoram, lavender and thyme (Choudhury *et al*., 2006). *Mentha* is one of the most common herb which has been known for its medicinal and aromo-therapeutic properties since ancient times. The genus *Mentha* contains 25 to 30 species (Choudhury *et al*., 2006). Mint contains, among several others, the following properties: antifungal, antiviral, antimicrobial, insecticide, antioxidant, allergenic, diuretic, and stimulant (Almeida *et al*., 2012).

Mentha spicata L. is a perennial, rhizomatous herb with stems ascending to 30 to 50 cm high, strong scented. Stems are purplish and glabrous. Leaves are green above, pale green below and glabrous (Plate 5). Leaf blade ovate, ovate-oblong to lanceolate-oblong, 20-70×10-20 mm., rounded to acute at the apex, cuneate to truncate at the base, serrate at the margin (Asfaw and Demissew, 2009).

Mentha spicata is a long and widely-used flavouring and medicinal herb (Fletcher *et al*., 2005; Brahmi *et al*., 2016). It is an aromatic plant that can be used fresh or as dried leaves or powder, as a seasoning and flavouring herb, or traditionally as an herbal tea (Cirlini *et al*., 2016). It is commonly produced as a crop for its essential oil for food products, cosmetics and pharmaceuticals (Fletcher *et al*., 2010; Saleemand Al-Attar, 2016). It is a source of rosmarinic which is used in nutraceutical and cosmetic industries and owns activities against inflammatory lung diseases, autoimmune arthritis, heart disease and suppression of autoimmune rejectionin human skin transplant patients (Fletcher *et al*., 2005). The antibacterial activity of *M. spicata* and its effect on a fermented dairy product was demonstrated by (Golestan *et al*., 2016). The ability spearmint in reducing pain severity and its potential to be used as analgestic agent was confirmed in osteoarthritis patients (Mahboubi *et al*., 2017). The EO of *M. spicata* revealed insecticidal activity (Franzios *et al*., 1997) and has significant larvicidal properties against mosquito larvae. Govindarajan *et al.* (2012) reported that EO of *M. spicata* had a significant toxic effect against early third-stage larvae of *C. quinquefasciatus*, *Anopheles aegypti*, and *An. stephensi*.

In Ethiopia, *M. spicata* is a cultivated plant mostly grown in the backyards of homesteads and occasionally found growing wild between altitudes of 1500 and 2500 m. The leaves are used as flavouring in salads (Asfaw and Demissew, 2009). Water distillate of spearmint relieves hiccup and flatulence as well as the giddiness of indigestion (Choudhury *et al*., 2006). The ancients used spearmint to scent their bath and as a restorative (Sivarajan and Balachandran, 1994). In the fourteenth century, mint was used for whitening the teeth. Its distilled oil is still used to flavor toothpaste, confectionery, and chewing gum and also to perfume soaps. Spearmint has antifungal, antiviral, antimicrobial, insecticide, antioxidant, antiamoebic, antihemolytic, allergenic, CNS depressant, antihelmintic and antiancyclostomiasis activity (Sharma, 1993; Rastogi and Mehrotra, 1998).

Plate 5: Photograph of spearmint (*Mentha spicata*)

Photograph taken by Melete Berhe

Essential oil of *M. spicata* have acaricidal, insecticidal and repellency effect against spider mite, *Tetranychus urticaei* (Omar *et al*., 2009), stored food insect pest, *Callosobruchus chinensis* (Kedia *et al*., 2014) and larvae and adult of *Amblyomma hebraeum* (Acari: Ixodidae) (Mkolo *et al*., 2011), respectively. They were also reported to possess larvicidal properties against mosquitoes belonging *C. quinquefasciatus, Ae. aegypti, and An. stephensi*. Their toxic effect was attributed to three compounds namely carvone, cis-carveol, and limonene from 18 chemical compounds contained in essential oil obtained by gas chromatography–mass spectroscopy (GC-MS) (Govindarajan *et al*., 2012).

2.8.2 Rosmarinus officinalis

The plant, *Rosemarinus officinalis* (L.) (Rosemary: English; Azmarino, Yetibs ketel: Amhaic; Azmarino: Tigrigna) is a cultivated shrub 0.5 to 2 m high. Leaves fragrant, punctate, tomentose beneath, leaf-blade thick, linear, up to 3 cm long, obtuse at the apex, with strongly revolute margins (Plate 6). It is cultivated in Shewa, Illubabor, Sidamo floristic regions etc. between altitudes of 1,800 and 2,400 m (Asfaw and Demissew, 2009).

Rosmarinus officinalis has been regarded to possess medicinal uses, including use as a tonic, a digestive aid, to treat depression, headache, and muscle spam, and as an expectorant, promoter of

menstrual flow, and stimulant for production of bile (Banupriya and Maheshwari, 2013). However, in Ethiopia, the leaves of and stems of *R. officinalis* are used for flavouring roasted meat and pasta sauces. Medicinally, an infusion of the plant is used as hair rinse against dandruff (Asfaw and Demissew, 2009).

Essential oils of *R. officinalis* exhibited pest repellent activity against two stored product insects i.e. *Tribolium castaneum* and *Trogoderma granarium* (Khalil *et al*., 2015).

Plate 6: Photograph of *Rosmarinus officinalis*
Photograph taken by Melete Berhe

2.8.3 Azadirachta indica

Neem (*Azadirachta indica*) Juss is a tree in the mahogany family *Meliaceae*, one of the six species in the genus *Azadirachta*, and a native to India and Burma, growing in tropical and semi-tropical regions in Africa (Okigbo *et al*., 2010). Trees will reach up to 30 m tall with limbs reaching half as wide (Plate 7). The shiny dark green pinnately compound leaves are up to 30 cm long. Each leaf has 10–12 serrated leaflets that are 7 cm long by 2.5 cm wide. It will grow where rainfall is as little, and thrives in areas that experience extreme heat of up to 48°C (Muñoz-Valenzuela *et al.*, 2007).

Most of the plant parts of neem tree such as fruits, seeds, leaves, bark and roots contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer and antifungal uses (Girish and Shankara Bhat, 2008). Neem (*Azadirachta indica*) products showed antifeedant, oviposition deterrence, repellency, growth disruption, sterility and larvicidal action against insects (Elimam *et al*., 2009). The solvent extracts of *A. indica* have been reported to be effective against different species of insects. The hexane extract the leaves showed moderate toxicity against a red flour beetle *Tribolium castaneum* Herbst (Mostafa *et al*., 2012) whereas water extract was toxic to khapra beetle, *Trogodarma granariun* (Satti *et al*., 2010). Besides, extracts of *A. indica* showed significant mortality against all the vector mosquitoes (Baskar *et al*., 2016). The leaf extract of *Calotropis procera* Ait. (Asclepiadaceae) possess a remarkable larvicidal, adult emergence inhibitor, repellent and oviposition deterrent effect against both *An. arabiensis* and *Cx. quinquefasciatus* (Elimam *et al*., 2009).

Plate 7: Neem Tree

Source: Girish and Shankara Bhat (2008)

3. MATERIALS AND METHODS

3.1 Collection of plant materials

For the mosquitocidal bioassay three traditional medicinal species of plants that are *M. spicata* Crantz (Lamiaceae), *R. officinalis* (Lamiaceae) and *A. indica* (Meliaceae) were selected and used. *M. spicata* was collected from Merkato market while *R. officinalis* was obtained from the garden of the compound of College of Natural and Computational Sciences of Addis Ababa University. The seed powder of *A. indica* was obtained from dried neem seeds collected from Dire Dawa. The dried seeds were ground manually using mortar and pestle and the neem seed powder was stored in paper bags at room temperature until need for the experiment.

Following collection of *M. spicata* and *R. officinalis*, the plants were washed with distilled water and their leaves were dried under a shade in the insectary laboratory of Addis Ababa University, College of Natural and Computational Sciences, Department of Zoological Sciences. Then, the dried leaves were grounded with mortar and pestle, sieved and then the powder was stored in a brown bottle till used.

Taxonomic confirmation of *M. spicata* and *R. officinalis* was carried out at the National Herbarium (Department of Biology), College of Natural and Computational Sciences of Addis Ababa University. The samples were identified and voucher specimens were deposited at the herbarium maintained by College of Natural and Computational Sciences of Addis Ababa University, Ethiopia.

3.2 Soxhlet extraction

Fifty gram (50 g) of the fine powder of each plant was weighed and mixed with 250 ml of 97% ethanol and placed in a soxhlet apparatus (Plate 8). The extraction was performed for 3 hr. After extraction, the resulting extract was stored in refrigerator at 4^{o}C until use.

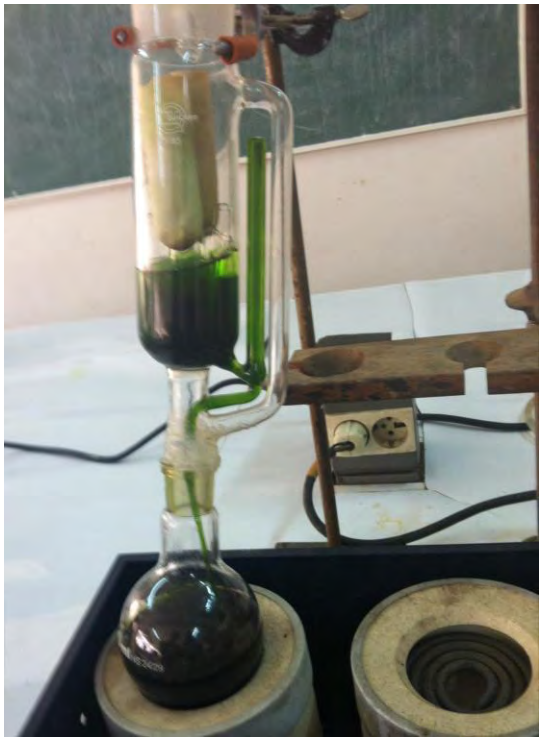

Plate 8: Soxhlet apparatus

Photograph taken by Melete Berhe

3.3 Test mosquitoes rearing

Laboratory reared female *An. arabiensis* mosquitoes were used in these tests. The colony were maintained under standard insectary condition at 27 ± 2°C, 80 ± 5% relative humidity and 12:12 (L:D). The egg, larva and pupa of *An. arabiensis* were reared in distilled water placed in larva trays. The larvae were fed on *fafa* (balanced food formulation from food manufacturers). On pupation, the pupae were transferred to standard 30 × 30 × 30 cm netting cages. After emergence, adult mosquitoes were held in cages and provided with sugar and distilled water socked cotton placed on the top of the cages. The female *An. arabiensis* were fed on rabbit for 3 hours in replicates. The cages were kept in insectary at ambient tropical temperature and under artificial light provided by fluorescence tube. The relative humidity was maintained by placing water basic place in the insectary (Gerberg, 1979).

3.4 Cone Bioassay

In cone bioassay, different concentrations of a 100 ml volume of *M. spicata* and *R. officinalis* extracts solution (0.1, 0.2, 0.4, 0.6, 0.8 and 1) were prepared with distilled water. A clean, dry, mosquitoe nets were dipped in to each concentration of prepared 100 ml volume of extract solution and they were air dried. Assays were initiated within 24 h of spraying. The mortality and knock down effect was carried using cone bioassay as per the recommendations of WHO (2013). Briefly, five susceptible non blood fed 2-5 day old female *An. arabiensis* mosquitoes were exposed to each piece of netting (25 cm×25 cm) for 3 min under standard WHO cone (Plate 9) after which they were held for 24 h with access to sugar solution. Knock-down was recorded 60 min after exposure and mortality after 24 h. One piece each from different nets was tested. Mosquitoes exposed to untreated net pieces were used as controls. These bioassays were carried out at 27^{o}C and 75% relative humidity.

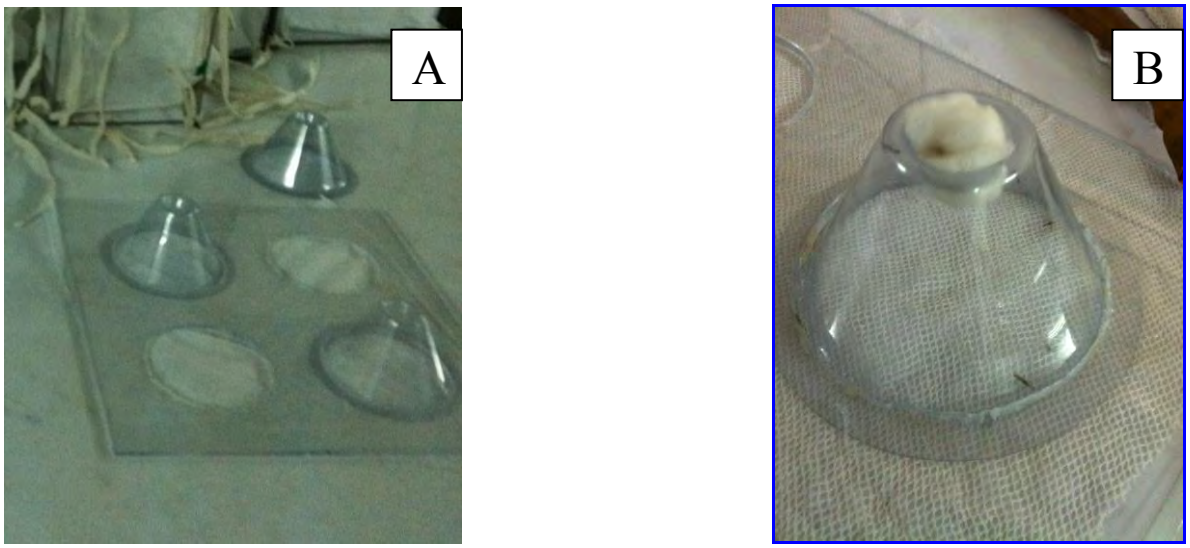

Plate 9. Cone test Bioassay.

A) Cones used for mortality assessment B) A cone with visible larvae during mortality trial Photograph taken by Melete Berhe

3.5 Tunnel Test

For the tunnel test, various concentrations of a 100 ml volume of *M. spicata* and *R. officinalis* extracts solution (0.1, 0.2, 0.4, 0.6, 0.8 and 1) were prepared with distilled water. Repellence evaluation was conducted according to WHO (2013) protocol. Briefly, non-blood-fed female *An.*

arabiensis aged 5-8 days were released into a 60-cm tunnel (25 cm×25 cm^2 section) made of glass (Figure 10). At each end of the tunnel, a 25-cm square cage covered with polyester netting was fitted (extension). The LN netting sample, held in a disposable cardboard frame, was placed at one third the length of the glass tunnel. The mosquito nets were dipped in to each concentration of prepared 100 ml volume of extract solution and they were air dried. The surface of netting available to the mosquitoes was 400 cm^2 (20 cm × 20 cm), with nine holes 1 cm in diameter; one hole was located at the centre of the square, and the other eight were equidistant and located 5 cm from the border. In the shorter section of the tunnel, a suitable bait (e.g. guinea-pig or rabbit) was placed, which is unable to move and was available for mosquito biting. One hundred female mosquitoes were introduced into the cage at the end of the longer section of the tunnel. They were free to fly in the tunnel but have to make contact with the piece of netting and locate the holes in it before passing through to reach the bait. After taking a blood meal, the mosquitoes would fly back to the cage at the end of this compartment and rest. A tunnel with untreated netting was used as a negative control. The percent of repellency of plant crude extract is calculated using formula provided by Ayrani and Auamcharoen (2016).

$$PR(\%) = Nc/(Nc+Nt)\times 100.$$

Where,

Nc = the number of insect present in the control (choice)

Nt = the number of insect present in the treatment (choice)

Plate 10: Tunnel test experimental setup

Photograph taken by Melete Berhe

3.6 Larvicidal Bioassay

For the larvicidal bioassay, a laboratory strain of *Ae. aegypti* larvae was used from Addis Ababa University, ALIPB. The larval bioassay tests were carried out at AAU, CNCS, DZS insectary. The bioassay was carried out on transparent larval rearing trays as follows**.** Plate 11 presents the dried seed and seed powder of *A. indica*. About 250 ml of distilled water was placed on each tray. Then, 5 g of seed powder of *A. indica* was placed into it and homogenized by stirring with a sterile Pasteur pipette. Then, 20 active early fourth instar larvae of *Ae. aegypti* were transferred into each transparent tray which contained 250 ml distilled water. Three replicates were carried out for the treatment trial and similar replicates of control were carried out simultaneously with only 250 ml of distilled water (Plate 12). Larval mortality was checked after 24 and 48 h. When the larvae failed to move when probed with a needle they are considered dead (WHO, 2005). The corrected percent of mortality was calculated by applying the following Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality (\%)}} \times 100$$

Plate 11: Dried neem seed and powder

Photograph taken by Melete Berhe

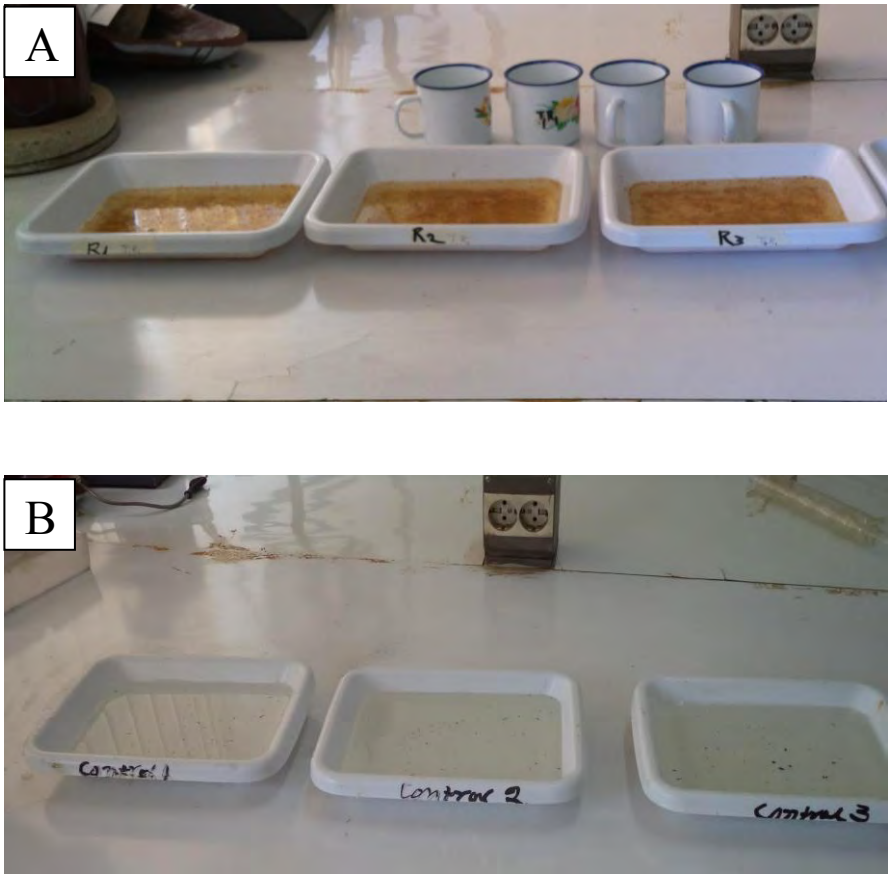

Plate 12: Larvacidal bioassay

A) Trays with seed powder of *Azadrichata indica* (treatment group) B) Trays with distilled water (control group)

3.7 Ethical Clearance

The ethical clearance was for conducting research was obtained from the College of Natural and computational Science Institutional Review Board (CNS-IRB) Committee, in its meeting held on November 11, 2016 with minutes CNSDO 119/09/2016.

3.8 Data Analysis

The design of the entire experiment was complete randomized design (CRD) and therefore the data generated in these experiments were recorded in a Microsoft excel spread-sheet, edited, and stored for future analysis. The statistical analysis was conducted using SPSS V 20 statistical package. The mean percent mortality and knock down effect along with their standard errors

were determined from the five replicates while that of percent repellency was calculated from the three replicates. The percentage of mortality, knock-down effect and repellency were expressed as mean percent ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to assess the significance difference in percent repellency exhibited by the various concentrations of each plant extract. When a significant difference was observed the Duncan multiple range comparison Post Hoc test assuming equal variances was used to identify those concentrations that showed difference in the percent repellency. For the comparison of larvicidal effects observed at 24 and 48 h of exposure paired student *t*-test was employed. A p-value <0.05 was considered significant.

4. RESULTS

4.1 Adulticidal effect of *Mentha spicata* and *Rosmarinus officinalis* leaf extracts

The adulticidal activities of ethyl alcohol leaf extracts of *M. spicata* and *R. officinalis* against laboratory populations of *An. arabiensis* were determined at 1 h of exposure. The results showed that extracts of *M. spicata* and *R. officinalis* exhibited moderate knock-down activity and mortality effects. In these assessments, the former caused average percent knock-down effect ranging from 8 to 32% whilst the latter induced between 0 and 28%. From the two, *M. spicata* had the greatest effect whilst that of *R. officinalis* had the lowest. In the contrary, the control (distilled water) showed no effect. In both plant extracts the knock-down effect increased or emerged with increasing plant extract concentration. The leaf extracts of *M. spicata* and *R. officinalis* exhibited a significantly higher knockdown effect at concentrations of 0.8 and 1 when compared with the control group ($F = 2.52$; $df = 34$; $P = 0.045$) and ($F = 6.93$; $df = 34$; $P < 0.001$), respectively (Table 1 and Appendix B).

With regard to mortality effect, the mean percent mortality of *M. spicata* varied between 8 and 36% whilst that of *R. officinalis* ranged from 0 to 32% at six different concentrations used (Table 1). The results of the present study revealed that adult mosquito mortality increased with increasing concentration of the extracts. Nonetheless, there is no significant variation in the mortality effect of the various concentrations of extracts of both plants used ($F = 2.32$; $df = 34$; $P = 0.60$), except the variation between concentrations of 0.1 and 1 which is significant ($F = 3.44$; $df = 34$; $P = 0.011$) (Table 1 and Appendix B).

Table 1: The mean percent knockdown effect of ethanol extracts of the leaves of *Mentha spicata* and *Rosemarinus officinalis* against adult *An. arabiensis* after 1-hour of exposure.

Treatment	Concentration	%Knockdown (mean±SE)	%Mortality (mean±SE)
Mentha spicata	0.1	8 ± 5^{ab}	8 ± 5^{ab}
	0.2	12 ± 8^{ab}	12 ± 5^{abc}
	0.4	20 ± 6^{ab}	20 ± 6^{abc}
	0.6	28 ± 10^{ab}	24 ± 12^{abc}
	0.8	32 ± 10^{b}	28 ± 10^{bc}
	1.0	32 ± 10^{b}	36 ± 12^{c}
Control	Distilled water	0 ± 0^{a}	0 ± 0^{a}
Rosemarinus officinalis	0.1	0 ± 0^{a}	0 ± 0^{a}
	0.2	0 ± 0^{a}	0 ± 0^{a}
	0.4	0 ± 0^{a}	20 ± 11^{ab}
	0.6	0 ± 0^{a}	20 ± 6^{ab}
	0.8	20 ± 6^{b}	20 ± 11^{ab}
	1.0	28 ± 10^{b}	32 ± 8^{b}
Control	Distilled water	0 ± 0^{a}	0 ± 0^{a}

Means with different superscript letters within the columns of concentrations of each botanical are different at $P<0.05$

4.2 Repellency Effect of *Mentha spicata* and *Rosmarinus officinalis* leaf extracts

The repellency effect of ethanol leaf extracts of *M. spicata* and *R. officinalis* against *An. arabiensis* was assessed at six different concentrations. Results in Table 2 showed that adult *An. arabiensis* preferred the untreated section (control). At all concentrations of leaf extract of *M. spicata* small percent of *An. arabiensis* were recorded on the treated section after one hour of exposure in contrast to the untreated section. *M. spicata* showed a higher average percent repellency ranging from 95 to 97%. Nonetheless, one-way analysis of variance (ANOVA) didn't show a significant variation on repellency effect among the six different concentrations of *M. spicata* used ($F = 0.85$; $df = 17$; $P = 0542$) (Table 2 and Appendix B).

Likewise, in the repellency effect evaluation of ethyl alcohol leaf extract of *R. officinalis* against *An. arabiensis* at six different concentrations, adult *An*. *arabiensis* preferred the untreated section (control) more commonly than the treated section. However, a large number of *An*. *arabiensis* entered to the treated section as compared to that of *M. spicata*. The mean percent repellency of *R. officinalis* was calculated to be between 63 and 74% at the six concentrations employed in this experiment (Table 2). Furthermore, a significant variation was observed among the six different concentrations used using Duncan multiple range test Post Hock test (F = 3.60; df = 17; *P* = 0.032) (Table 2 and Appendix B).

Table 2: The mean percent repellency and one-way analysis of variance of ethanol leaf extract of *Mentha spicata* and *Rosemarinus officinalis* against adult *Anopheles arabiensis* after 1 h of exposure.

	%Repellency Mean±SEM	
Concentration	*Mentha spicata*	*Rosemarinus officinalis*
0.1	95±0.7^{a}	63±2.6^{a}
0.2	96±0.9^{a}	66±3.5^{ab}
0.4	96±0.7^{a}	66±2.4^{ab}
0.6	96±0.7^{a}	70±0.3^{abc}
0.8	97±0.3^{a}	73±0.6^{bc}
1.0	97±0.3^{a}	74±2.3^{c}
F-value	0.848	3.609
P-value	0.542	0.032*

Means with different superscript letters within the columns of concentrations of each botanical are different at P<0.05.

*shows significance

4.3 Larvicidal Bioassay of seed powder of *Azadrichata indica*

The mosquito larvicidal activity seed of A. indica was also evaluated in this study. Testing of seed powder of A. indica for larvicidal activity was done at 5g/250ml (2%) concentration. In this

larvicidal bioassay, it was observed that the mean percent larval mortality against *Ae. aegypti* was 58% and 81% at 24 h and 48 h, respectively (Table 3). The remaining larvae of *Ae. aegypti* in the treatment group failed to transform to pupae while those in the control group were developed to pupae and then to the adult stage. Statistical analysis using paired student t-test didn't show a significant difference in larval mortality between 24 and 48 h exposure (P>0.05).

Table 3: Larvacidal activity seed powder of *Azadirachta indica* against fourth instar larvae of *Aedes aegypti* at 24 h and 48 h exposure.

		%Mortality					
		24 h			48 h		
Treatment Type	**Total (n)**	**# dead**	**%**	**Mean%±SE**	**# dead**	**%**	**Mean%±SE**
Azadrichiata indica	20	15	74	58±11	18	89	81±4
	20	8	37		16	79	
	20	13	63		15	74	
Control	20	1	5	5±0	1	5	5±0
	20	1	5		1	5	
	20	1	5		1	5	

$t = 2.328$; df = 2; P = 0.145

5. DISCUSSION

The present study demonstrated that ethyl alcohol leaf extracts of *M. spicata* and *R. officinalis* caused moderate mortality against adult *An. arabiensis*, however, it didn't exceed 50%. In line with the present finding, Maharaj *et al*. (2011) reported that only a single extract of *Ptaeroxylon obliquum* (Ptaeroxylaceae) and *Pittosporum viridiflorum* (Pittosporaceae), exhibited higher than 50% mortality among 381 crude extracts of South African plants screened for adulticidal bioactivity against *An. arabiensis*. In contrast to these and our study results, some botanical extracts with superior adulticidal activities against laboratory and field population of *An. arabiensis* have been reported in Ethiopia (Andemo *et al.,* 2014; Bekele *et al*., 2014). Thus, even though ethyl alcohol leaf extracts of both plants exhibited some degree of insecticidal activity against *An. arabiensis* particularly at higher dose rates, they cannot be considered as effective adulticide based on WHO standards as they failed to produce >60% mortality against the adult stages of the malaria vector *An. arabiensis* (Maharaj *et al*., 2011).

In the present study, ethyl alcohol leaf extract of *M. spicata* at six different concentrations were observed to be highly repellent and could be used to repel *An*. *arabiensis*. This repellent activity is comparable to previously reported by Chalannavar *et al*. (2013) that methanol and aqueous leaf extracts of *P. guajava* (pink fruit), *P. durbanensis, P. cattleianum*var. *cattleianum*, *P. guineense*, *G. densa*, *L. leonurus* flower and *D. cinerea*, provided a repellency ranging from 85 to 100% against *An. arabiensis* excluding aqueous extract of *G. densa*. Repellent chemicals will not become outdated as quickly as lethal ones because they provide less chance of the insects developing resistance (Kedia *et al*., 2014). Thus, extract of *M. spicata* have potential for the development of new naturally occurring repellents against *An. arabiensis* that can be used as an alternative for synthetic repellents. Formulation of mosquito repellent by pharmaceutical companies using *M. spicata* would be effective, economical and safe approach for malaria control programs in areas like Ethiopia where *An. arabiensis* has been incriminated as malaria vector. Bessides, *R. officinalis* could be considered to be the second-best repellent. In view of this repellency efficacy, it could also be regarded as beneficial malaria prevention and control.

Larvicides play a significant role in controlling mosquitoes in their breeding sites (Bagavan and Abdul Rahuman, 2011). Thus, this study demonstrated that the seed powder of A. indica has a potential as larvicides for the control of *Ae. aegypti*. Nonetheless, the observed larvicidal activity

is lower than that of leaf extract of Argentine Melia azedarach L. (Meliaceae) which showed a strong larvicide activity, with all larvae *Ae. aegypti* dying before pupation (Coria *et al*., 2008). The bioactivity of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant part, age of plant part, solvent used in extraction and mosquito species (Shaalan *et al*., 2005). Insecticidal activity of neem seed extract has also been reported on cabbage damaging Diamondback Moth (*Plutella xylostella* L.) (Lepidoptera: Plutellidae) in the Central Rift Valley of Ethiopia (Stotaw *et al*., 2009).

6. CONCLUSIONS

The results of this study revealed that ethyl alcohol leaf extracts of *M. spicata* and *R. officinalis* had moderate mosquitocidal effect against adults of *An. arabiensis*. Nonetheless, these plants might not be considered as excellent potential sources of insecticides as their mortality is <60%. In contrast, the leaf extracts of both plants produced a considerable amount of repellency against adult *An. arabiensis*. Especially, extract of *M. spicata* produced a highest repellency. Besides, seed powder of *A. indica* produced a significant amount of mortality against the fourth instar larvae of *Ae. aegypti*. Thus, this study showed the importance of using *M. spicata* and *A. indica* as an alternative for control by repellence of adult mosquitoes.

7. RECOMMENDATIONS

Based on the above conclusion, the following recommendations have been forwarded.

- Studies must be conducted to identify chemical ingredient/s for the mosquito repellent and larvicidal activities *M. spicata* and *A. indica*, respectively.
- Formulation of mosquito repellent and larvicides by pharmaceutical companies using the leaves *M. spicata* and seed of *A. indica* is economical and effective.
- Wide-scale studies need to focus on the adulticidal effects of other plants claimed for their insecticidal properties.

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9. APPENDICES

Appendix A. Raw data of botanical mosquitocidal assay

Appendix Table 1: The result of knock-down and mortality bioassay of ethanol extracts of the leaves of *Mentha spicata* and *Rosemarinus officinalis* against adult *Anopheles arabiensis*.

Treatment	Concentration	Replication	Total	#Knock-down (1 hr)	Total	#Died (24 hr)
Mentha spicata	0.1	1	5	1	5	1
		2	5	0	5	1
		3	5	0	5	0
		4	5	0	5	0
		5	5	1	5	0
	0.2	1	5	0	5	0
		2	5	2	5	1
		3	5	0	5	1
		4	5	1	5	1
		5	5	0	5	0
	0.4	1	5	1	5	1
		2	5	2	5	2
		3	5	1	5	1
		4	5	1	5	1
		5	5	0	5	0
	0.6	1	5	1	5	2
		2	5	3	5	3
		3	5	0	5	0
		4	5	2	5	0
		5	5	1	5	1
	0.8	1	5	2	5	1
		2	5	2	5	3
		3	5	1	5	2
		4	5	0	5	1

Table (*continued*)

		5	5	3	5	0
	1.0	1	5	2	5	1
		2	5	2	5	3
		3	5	3	5	0
		4	5	1	5	3
		5	5	0	5	2
Control	Distilled water		150	0	150	0
Rosemarinus officinalis	0.1	1	5	0	5	0
		2	5	0	5	0
		3	5	0	5	0
		4	5	0	5	0
		5	5	0	5	0
	0.2	1	5	0	5	0
		2	5	0	5	0
		3	5	0	5	0
		4	5	0	5	0
		5	5	0	5	0
	0.4	1	5	0	5	1
		2	5	0	5	1
		3	5	0	5	0
		4	5	0	5	3
		5	5	0	5	0
	0.6	1	5	0	5	1
		2	5	0	5	2
		3	5	0	5	0
		4	5	0	5	1
		5	5	0	5	1
	0.8	1	5	1	5	1
		2	5	2	5	0
		3	5	0	5	3
		4	5	1	5	1

Table (*continued*)

		5	5	1	5	0
	1.0	1	5	2	5	1
		2	5	1	5	1
		3	5	3	5	2
		4	5	0	5	3
		5	5	1	5	1
Control	Distilled water	-	150	0	150	0

Appendix Table 2: The results of repellency evaluation of ethanol extracts of the leaves of *Mentha spicata* and *Rosemarinus officinalis* against adult *Anopheles arabiensis* after 1 h of exposure.

Treatment	**Concentration**	**Replication**	**Total**	**# Choice**	**# No choice**
Mentha spicata	0.1	1	100	3	97
		2	100	3	97
		3	100	4	96
	Control	1	100	65	35
		2	100	65	35
		3	100	65	35
	0.2	1	100	3	97
		2	100	4	96
		3	100	2	98
	Control	1	100	72	28
		2	100	69	31
		3	100	56	44
	0.4	1	100	3	97
		2	100	3	97
		3	100	2	98
	Control	1	100	61	39
		2	100	60	40
		3	100	65	35
	0.6	1	100	3	97
		2	100	2	98

Table (*continued*)

		3	100	3	97
	Control	1	100	62	38
		2	100	61	39
		3	100	61	39
	0.8	1	100	2	98
		2	100	2	98
		3	100	3	97
	Control	1	100	78	22
		2	100	72	28
		3	100	74	26
	1	1	100	2	98
		2	100	3	97
		3	100	2	98
	Control	1	100	77	23
		2	100	56	44
		3	100	59	41
Rosemarinus officinalis	0.1	1	100	49	51
		2	100	33	67
		3	100	42	58
	Control	1	100	71	29
		2	100	69	31
		3	100	73	27
	0.2	1	100	30	70
		2	100	48	52
		3	100	32	68
	Control	1	100	72	28
		2	100	68	32
		3	100	70	30
	0.4	1	100	42	58
		2	100	37	63
		3	100	29	71
	Control	1	100	72	28
		2	100	68	32

Table (*continued*)

	3	100	70	30
0.6	1	100	32	68
	2	100	32	68
	3	100	33	67
Control	1	100	75	25
	2	100	79	21
	3	100	72	28
0.8	1	100	30	70
	2	100	28	72
	3	100	31	69
Control	1	100	78	22
	2	100	79	21
	3	100	82	18
1	1	100	28	72
	2	100	23	77
	3	100	19	81
Control	1	100	69	31
	2	100	71	29
	3	100	57	43

Appendix B Statistical Analysis Results

1. Analysis on knockdown effect and mortality of adult *Anopheles arabiensis*

1.1. Ethanol extract of *Mentha spicata*

a) Descriptives

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
percentknockdown	.00	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.10	5	8.0000	10.95445	4.89898	-5.6017	21.6017	.00	20.00
	.20	5	12.0000	17.88854	8.00000	-10.2116	34.2116	.00	40.00
	.40	5	20.0000	14.14214	6.32456	2.4402	37.5598	.00	40.00
	.60	5	28.0000	22.80351	10.19804	-.3143	56.3143	.00	60.00
	.80	5	32.0000	22.80351	10.19804	3.6857	60.3143	.00	60.00
	1.00	5	32.0000	22.80351	10.19804	3.6857	60.3143	.00	60.00
	Total	35	18.8571	19.96636	3.37493	11.9985	25.7158	.00	60.00
percentmortality	.00	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.10	5	8.0000	10.95445	4.89898	-5.6017	21.6017	.00	20.00
	.20	5	12.0000	10.95445	4.89898	-1.6017	25.6017	.00	20.00
	.40	5	20.0000	14.14214	6.32456	2.4402	37.5598	.00	40.00
	.60	5	24.0000	26.07681	11.66190	-8.3786	56.3786	.00	60.00
	.80	5	28.0000	22.80351	10.19804	-.3143	56.3143	.00	60.00
	1.00	5	36.0000	26.07681	11.66190	3.6214	68.3786	.00	60.00
	Total	35	18.2857	20.21731	3.41735	11.3408	25.2306	.00	60.00

B) Oneway ANOVA

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
percentknockdown	Between Groups	4754.286	6	792.381	2.521	.045
	Within Groups	8800.000	28	314.286		
	Total	13554.286	34			
percentmortality	Between Groups	4617.143	6	769.524	2.322	.060
	Within Groups	9280.000	28	331.429		
	Total	13897.143	34			

c) Post Hoc Tests

Homogeneous Subsets

percentknockdown

Duncan

concentration	N	Subset for alpha = 0.05	
		1	2
.00	5	.0000	
.10	5	8.0000	8.0000
.20	5	12.0000	12.0000
.40	5	20.0000	20.0000
.60	5		28.0000
.80	5		32.0000
1.00	5		32.0000
Sig.		.113	.068

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

percentmortality

Duncan

concentration	N	Subset for alpha = 0.05		
		1	2	3
.00	5	.0000		
.10	5	8.0000	8.0000	
.20	5	12.0000	12.0000	12.0000
.40	5	20.0000	20.0000	20.0000
.60	5	24.0000	24.0000	24.0000
.80	5		28.0000	28.0000
1.00	5			36.0000
Sig.		.071	.131	.071

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

2.1 Ethanol extract of *Rosemarinus officinalis*

a) Descriptives

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
percentknockdown	.00	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.10	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.20	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.40	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.60	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.80	5	20.0000	14.14214	6.32456	2.4402	37.5598	.00	40.00
	1.00	5	28.0000	22.80351	10.19804	-.3143	56.3143	.00	60.00
	Total	35	6.8571	14.50587	2.45194	1.8742	11.8401	.00	60.00
percentmortality	.00	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.10	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.20	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	.40	5	20.0000	24.49490	10.95445	-10.4144	50.4144	.00	60.00
	.60	5	20.0000	14.14214	6.32456	2.4402	37.5598	.00	40.00
	.80	5	20.0000	24.49490	10.95445	-10.4144	50.4144	.00	60.00
	1.00	5	32.0000	17.88854	8.00000	9.7884	54.2116	20.00	60.00
	Total	35	13.1429	18.75091	3.16948	6.7017	19.5840	.00	60.00

b) Oneway ANOVA

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
percentknockdown	Between Groups	4274.286	6	712.381	6.926	.000
	Within Groups	2880.000	28	102.857		
	Total	7154.286	34			
percentmortality	Between Groups	5074.286	6	845.714	3.442	.011
	Within Groups	6880.000	28	245.714		
	Total	11954.286	34			

c) Post Hoc Tests

Homogeneous Subsets

percentknockdown

Duncan

concentration	N	Subset for alpha = 0.05	
		1	2
.00	5	.0000	
.10	5	.0000	
.20	5	.0000	
.40	5	.0000	
.60	5	.0000	
.80	5		20.0000
1.00	5		28.0000
Sig.		1.000	.223

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

percentmortality

Duncan

concentration	N	Subset for alpha = 0.05	
		1	2
.00	5	.0000	
.10	5	.0000	
.20	5	.0000	
.40	5	20.0000	20.0000
.60	5	20.0000	20.0000
.80	5	20.0000	20.0000
1.00	5		32.0000
Sig.		.085	.279

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

2. Analysis on repellence of adult *Anopheles arabiensis*

2.1 Ethanol extract of *Mentha spicata*

a) Descriptives

Descriptives

percentrepellency

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
.10	3	95.3333	1.15470	.66667	92.4649	98.2018	94.00	96.00
.20	3	95.6667	1.52753	.88192	91.8721	99.4612	94.00	97.00
.40	3	95.6667	1.15470	.66667	92.7982	98.5351	95.00	97.00
.60	3	95.6667	1.15470	.66667	92.7982	98.5351	95.00	97.00
.80	3	96.6667	.57735	.33333	95.2324	98.1009	96.00	97.00
1.00	3	96.6667	.57735	.33333	95.2324	98.1009	96.00	97.00
Total	18	95.9444	1.05564	.24882	95.4195	96.4694	94.00	97.00

b) Oneway ANOVA

ANOVA

percentrepellency

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.944	5	.989	.848	.542
Within Groups	14.000	12	1.167		
Total	18.944	17			

c) Post Hoc Tests

Homogeneous Subsets

percentrepellency

Duncan

concentration	N	Subset for alpha = 0.05
		1
.10	3	95.3333
.20	3	95.6667
.40	3	95.6667
.60	3	95.6667
.80	3	96.6667
1.00	3	96.6667
Sig.		.196

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

2.2 *Rosemarinus officinalis*

a) Descriptives

Descriptives

percentrepellency

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
.10	3	63.0000	4.58258	2.64575	51.6163	74.3837	59.00	68.00
.20	3	66.0000	6.08276	3.51188	50.8896	81.1104	59.00	70.00
.40	3	66.3333	4.16333	2.40370	55.9910	76.6756	63.00	71.00
.60	3	69.6667	.57735	.33333	68.2324	71.1009	69.00	70.00
.80	3	73.0000	1.00000	.57735	70.5159	75.4841	72.00	74.00
1.00	3	74.0000	4.00000	2.30940	64.0634	83.9366	70.00	78.00
Total	18	68.6667	5.22438	1.23140	66.0686	71.2647	59.00	78.00

b) Oneway ANOVA

ANOVA

percentrepellency

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	278.667	5	55.733	3.609	.032
Within Groups	185.333	12	15.444		
Total	464.000	17			

c) Post Hoc Tests

Homogeneous Subsets

percentrepellency

Duncan

concentration	N	Subset for alpha = 0.05		
		1	2	3
.10	3	63.0000		
.20	3	66.0000	66.0000	
.40	3	66.3333	66.3333	
.60	3	69.6667	69.6667	69.6667
.80	3		73.0000	73.0000
1.00	3			74.0000
Sig.		.078	.065	.223

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

3. Analysis on larvicidal effect of *Azadrichiata indica* on larvae of *Aedes aegypti*

a) Descritpives

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	percentdeath48hr	80.6667	3	7.63763	4.40959
	percentdeath24hr	58.0000	3	19.00000	10.96966

b) Correlations

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	percentdeath48hr & percentdeath24hr	3	.465	.692

c) Paired-samples *t* test

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	percentdeath48hr - percentdeath24hr	22.66667	16.86219	9.73539	-19.22133	64.55466	2.328	2	.145

Appendix C. Photos of laboratory activities

Photo 1. Leaf of *Mentha spicata* and *Rosemarinus officinalis* spread under shade for drying.

Photo 2. Mosquito net immersed into leaf extract of *Mentha spicata*.

Photo 3. Cone bioassay

Photo 4. Tunnel test

Photo 5. Larvacidal bioassay.

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This is to certify that the thesis prepared by **Melete Berhe** entitled: **"THE REPELLENCE AND LARVICIDAL EFFICACY OF SOME SELECTED PLANT OILS AND EXTRACTS AGAINST ADULT *Anopheles arabiensis* AND LARVAE OF *Aedes aegypti* (Culicidae: Diptera) UNDER LABORATORY CONDITIONS"** submitted in partial fulfilment of the requirements for the Degree of Master of Science in Zoological Sciences (Insect Sciences) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

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Examiner	Signature	Date
3._____	_____	_____
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