**College of Public Health and Medical sciences Department of Environmental Health Science and Technology**


# Laboratory and field evaluation of mosquitocidal effect of *birbira* (*Mellitia ferruginea)* seed extract against *Anopheles arabiensis* in Ethiopia

BY

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A thesis submitted to the Department of Environmental Health Science and Technology, College of Public Health and Medical sciences, Jimma University; in partial fulfillment for the Degree of Master of Science in Environmental Science and Technology

**Laboratory and field evaluation of mosquitocidal effect of *birbira* (*Mellitia ferruginea)* seed extract against *Anopheles arabiensis* in Ethiopia**

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Jimma University

# APPROVAL SHEET

As thesis research advisor, I herby certify that I have read and evaluated this thesis prepared under my guidance by Abiy Andemo Kotacho entitled "evaluation of the mosquitocidal effect of "birbira" (*Mellitia ferruginea)* seed extracts against the primary malarial vector, *Anopheles arabiensis* (Diptera: Culicidae) under laboratory and simulated field condition in Ethiopia". I recommend it would be submitted as fulfilling the thesis requirement.

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| Name of co-advisor | Signature | Date |

As members of the examining board of the final M.Sc. thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Abiy Andemo Kotacho and examined the candidate. We recommend that it would be accepted as fulfilling the thesis requirement for the degree of Master of Science in Environmental Science and Technology.

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## DECLARATION

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## List of Abbreviations and Acronyms

| | |
|---|---|
| Bs | *Bacillus sphaericus* |
| Bti | *Bacillus thuringiensis var.israelensis* |
| CDC: | Center for Disease Prevention and Control |
| DDT | Dichlorodiphenyltrichloroethane |
| FMOH | Ethiopian Federal Ministry of Health |
| IVM | Integrated Vector Management |
| IRS | Indoor Residual Spraying |
| ITN | Insecticide Treated Nets |
| $LC_{50}$ | Lethal concentration that kills 50 % of the test organisms |
| $LC_{90}$ | Lethal concentration that kills 90 % of the test organisms |
| $LC_{99}$ | Lethal concentration that kills 99 % of the test organisms |
| LLINs | Long Lasting Insecticidal Nets |
| WHO | World Health Organization |
| SPSS | Statistical Package for Social Sciences |

## Abstract

Development of effective botanical mosquitocidal compounds is essential to combat increasing resistance rates and concern for the environment. Thus, the present study aims to evaluate the larvicidal, pupicidal and adulticidal potential of the methanolic extracts from “Birbira” (*M. ferruginea*) plant seeds against the primary malaria vector: Anopheles arabiensis mosquitoes under laboratory and simulated field condition. The ripen “Birbira” seeds were collected from the area around Jimma University and shade dried at room temperature. The dried plant seed materials were powdered using pestle and mortar. From each sample 100 gram of the sieved powder of the plant materials were macerated in 600ml of methanol for 72h in the ratio of 1:6. The extracts were evaporated to dryness in rotary evaporator to obtain 52.5 gram of residue. The residue was dissolved separately in acetone for stock solution from which different test concentrations were prepared through serial dilution for the bioassay test. The $LC_{50}$ values of the extracts for the larvicidal and pupicidal test of the laboratory Strain of An. arabiensis were 14.97 and 29.07 mg/L whereas the $LC_{50}$ values for the field population of An. arabiensis under simulated field condition were 31.04 and 54.58mg/L respectively. The $LC_{50}$ values for laboratory Strain of the adulticidal test were 46.03, 34.86 and 29.77mg/cm$^2$ at 1hour, 2hours and 3 hours exposure time respectively and the corresponding $LC_{50}$ values for the wild adults were 68.80, 56.57 and 44.55 mg/cm$^2$at 1hour, 2hours and 3 hours exposure. The $LC_{90}$ values for laboratory Strain of larvae and pupae were 36.65 and 79.99mg/L and 82.78 and 148.25 mg/L for the field population under simulated field test respectively. From the findings it can concluded that the extracts from the seeds of “Birbira” (*M. ferruginea*) showed potential mosquitocidal effect against An. arabiensis mosquito larvae, pupae and adult. This suggested that the plant seed extract may be further used as a component in integrated vector management program.

# Chapter one: Introduction

## 1.1 Background

Vector-borne diseases constitute the major cause of morbidity in most of the tropical and subtropical countries. Mosquitoes are the most deadly vector for several of these diseases like malaria, filariasis, Japanese encephalitis, dengue fever and yellow fever (Renugadevi, Ramanathan, Shanmuga, & Thirunavukkarasu, 2012). Malaria remains one of the highest priority insect transmitted diseases around the world, with Africa carrying the greatest burden (Maharaje *et al*., 2012).

Different strategies have been devised to reduce the prevalence of malaria globally. The malaria control strategy has a two-pronged approach, targeting the malaria parasite with anti-malarial drugs and controlling the vector through the use of insecticides, targeting both larval and adult life stages. However, over time success has been hampered by the development of insecticide resistance in mosquitoes (Maharaje *et al.*, 2012) and growing concerns about the potential health and environmental risks surrounding traditional chemical pesticides have led environmental protection agencies to ban or place severe restrictions on the use of many pesticides that were formerly used in mosquito control programmes (Soltani *et al.*, 2012). In Ethiopia, *An. arabiensis*, the most important malaria vector in the country, is strongly resistant to DDT and pyrethroids (Yewhalaw et al., 2011).

Vector control is by far the most successful method for reducing the incidences of diseases, but the emergence of widespread insecticide resistance and the potential environmental issues associated with conventional synthetic insecticides has indicated that additional approaches to control the proliferation of mosquito population would be an urgent priority research. In concern to quality & safety of life on controlling mosquito vectors has shifted steadily from the use of conventional chemicals toward alternative insecticides that are target-specific, biodegradable, environmentally safe and botanicals in origin (Kishore *et al.*, 2011). Control measures directed against the larval and other immature stages of mosquito vectors are useful components of malaria control programs in areas where mosquito breeding sites are accessible and relatively limited in number

and size (Tomass, Hadis, Taye, Mekonnen, & Petros, 2011). Larval anopheline mosquitoes can be controlled by using synthetic larvicidal chemicals such as temephos, fenthion, Malathion, chloropyrifos, and methoprene (ICMR, 2002). However, these life stages of anopheline mosquitoes are reported to develop also different level of physiological resistance against the aforementioned synthetic larvicides. In Ethiopia, indoor residual spraying (IRS) and insecticide-treated bed nets form the main malaria vector control and implicating wide distribution of resistance to this insecticide (Yewhalaw *et al., 2013).*

Botanical pesticides are promising in that they are effective, environment – friendly, easily biodegradable and also inexpensive. Besides to these, the development of resistant by vectors against plant derived bioactive molecules has not been reported so far (Senthilkumar, & Venkatesalu, 2012). Ethnobotanical and laboratory based studies have revealed the existence of insecticidal plants belonging to different families in different parts of the world. Crude solvent extracts of plant parts belonging to different families are shown to have various levels of bio activity against different developmental stages of malaria vector mosquitoes (Tomass *et al.,* 2011). Indeed extracts or even pure compounds from plant families have been established to be promising in the control of mosquitoes and other insect pests, being effective at various stages of the insect developmental growth (Jembere, Getahun, Negash, & Seyoum, 2011).

Birbira (*M. ferruginea)* is one of the plant families which is commonly known as a fish toxicant. In addition, extensive studies have been carried out for their medicinal properties and toxicity properties against aquatic organisms (Karunamoorthi, Bishaw, & Mulat, 2009). Thus, this experiment is initiated with the objectives of investigating the toxicity potential of the plant seed extract under laboratory and simulated field condition against one of the most important malaria vector; *An. arabiensis* in Ethiopia.

## 1.2 Statement of the problem

Mosquitoes are the most important single group of insects in terms of public health concern that can transmit a number of diseases including malaria and also cause millions of deaths each year (Senthilkumar and Venkatesalu, 2012). Malaria is one of the leading causes of illness and death in the world transmitted by the mosquito vector. According

WHO report of 2011, about 3.3 billion people were at risk of malaria in 2010 in which the disease killed estimated 655 000 persons and 86% of the victims were children under 5 years of age, and 91% of the deaths occurred were in the WHO African Region (WHO, 2011).

Following an increase in entomological surveillance in malaria-affected regions in recent years, sufficient data have now been collected to confirm already strong suspicions that the wide-scale use of insecticide-based malaria control strategies over the past decade has been associated with the development of resistance in several important vector species, including *Anopheles gambiae*, *An. funestus* and *An. Arabiensis.* Resistance to at least one class of insecticide has been identified in 64 countries with ongoing malaria transmission in which 27 of these are in sub-Saharan Africa. Thus the global malaria community is responding to the potential threat posed by emerging insecticide resistance; (WHO, 2013)

Extensive use of chemical insecticides for control of vector borne diseases has created problems related to physiological resistance to vectors adverse environmental effects, high operational cost and community acceptance (Renugadevi et al., 2012). Historically, the use of synthetic insecticides has been very effective in reducing malaria transmission. However, over time success has been hampered by the development of insecticide resistance in mosquitoes. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted undesirable effects on non-target organisms, and fostered environmental and human health concern that initiates a search for alternative control measures (El-Sheikh, Hanan, Bosly, Naglaa, & Shalaby, 2012).

In Africa, malaria threatens the lives and the livelihoods of more than 500 million Africans and exerts such a huge public health burden that it has been considered as one of the factors involved in the continued under development of the continent as the whole (Dadji, Jamesse, & Boyom, 2011). It continues to claim lives in African villages, despite repeated control programs that have reduced, but not eliminated, morbidity and mortality from the disease. Malaria has been identified as a key contributor to slow economic growth and investment in Africa because it experiences the most intense malaria transmission in the world. This can be due to vector resistant to chemical insecticides.

Countries in sub Saharan Africa are of greatest concern. These countries are characterized by high levels of malaria transmission and widespread reports of resistance. In some areas, resistance to all four classes of insecticides used for public health vector control has been detected (WHO, 2012).

In Ethiopia, malaria is one of the most important health problems with nearly 52 million (68%) of the populations being at risk to malaria infection. It is the leading cause of morbidity and mortality (Yewhalaw et al., 2010). Ethiopia has reported resistance to all four classes of insecticide, including widespread resistance to Dichlorodiphenyltrichloroethane (DDT) and an increasing frequency of resistance to pyrethroids (WHO, 2012). Near Jimma, Ethiopia, lower susceptibility of populations of An. *arabiensis* mosquitoes to DDT, permethrin, deltamethrin and malathion was documented (Yewhalaw *et al.*, 2010; 2011). This emergent resistance renders local IRS programs using DDT virtually ineffective, and the efficacy of permethrin and deltamethrin diminished (Yewhalaw *et al.*, 2011).

Consequently, substances which are alternatives to chemical pesticides should be developed. Even though many plant species from different families have been reported for mosquitocidal activity, only very few botanicals have moved from laboratory to the field use (Remia and Logaswamy, 2009). Therefore, the present study focuses to evaluate the mosquitocidal potential of the methanolic extract of birbira seed under the laboratory and simulated field condition.

## 1.3 Significance of the study

- It will provide baseline information on mosquito larvicidal, pupicidal and adulticidal effect of Birbira.
- It could be used as an alternative for malaria vector control in the presence of resistant mosquito populations and
- It could also be used as a component in integrated vector management

# Chapter two: Literature review

## 2.1 Biology and Ecology of Anopheline Mosquitoes

Mosquito serves as crucial vector for a number of arboviruses (arthropod-borne viruses) and parasites that are maintained in nature through biological transmission between susceptible vertebrate hosts by blood feeding arthropods responsible for encephalitis, dengue, malaria, rift valley fever, yellow fever and other infections (Kishore, Mishra, Tiwari, & Tripathi, 2011).

Malaria is caused by blood-borne protozoan parasites of the genus *Plasmodium*. *Plasmodium falciparum* is by far the most costly species in terms of its effects on both human life and economic progress. Mosquitoes of the genus *Anopheles* function as vectors for the *Plasmodium* parasite and are thus essential for disease transmission. Thus, *Anopheles* is the major genus responsible for malaria transmission .Vector-based malaria control is promising because the way of malaria infection is inextricably dependent on the lifecycle of these mosquitoes. There are over 400 species of mosquito in this genus; only 10% of these are suitable malaria vectors (Jose *et al.*, 2009). In Ethiopia, Plasmodium falciparum and Plasmodium vivax are predominant parasite species responsible for 60% and 40% of the infections, respectively. Plasmodium malaria and Plasmodium vale account for less than 1% of the cases (Yewhalaw et al., 2010).

Despite the many differences that exist within the genus, the lifecycle of all *Anopheles* mosquitoes is generally the same: Eggs hatch in water, where they undergo the transition to larvae. A larva of Anopheles mosquito hatches from the egg after about one or two days and generally floats parallel under the water surface, since it needs to breathe air. It feeds by taking up food from the water. When disturbed, the larva quickly swims towards the bottom but soon needs to return to the surface to breathe (WHO, 2002) There are four aquatic larval stages (first, second, third and fourth instar), followed by an aquatic pupal stage, before the adult emerges. Adults feed on nectar and other sugar sources and, within days of emergence, adult males form mating swarms into which females fly to mate. The female must then take a blood meal before she is able to lay 50-200 eggs. Most adults can live up to 2 weeks in the field, and longer under laboratory conditions, but within this

time period a female can take multiple blood meals and transmit malarial parasites. The knowledge on mosquito biology provides the basis for a variety of vector control approaches (Jose *et al.*, 2009).

## 2.2 Malaria vector control

Vector control is a central and critical component of all malaria control strategies. It relies primarily on two interventions: long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). The active ingredients of all WHO-recommended products for IRS come from only four classes of insecticide: pyrethroids, organochlorines (DDT), organophosphates and carbamates. All currently recommended LLINs are treated with pyrethroids. From the points of view of both safety and effectiveness, pyrethroids are the best insecticides ever developed for public health use. They accounted for the majority of IRS coverage worldwide in 2009 and were used in all LLINs (José, Ramirez, Lindsey, Garver, & Dimopoulos, 2009). The reliance of modern malaria control on pyrethroids and the increasing resistance of malaria vectors to these products put current global efforts at risk. Insecticide resistance is widespread and growing: it is now reported in nearly two thirds of countries with ongoing malaria transmission and the resistance occurred in all major vector species and to all classes of insecticides (WHO, 2012). The prevention of malaria in Ethiopia has relied mainly on early diagnosis and treatment of infection and reduction of human-vector contact by indoor residual spraying (IRS) and large-scale distribution of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (Yewhalaw et al., 2010).

Plant products have been used by traditional human communities in many parts of the world against the vectors and species of insects. The phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositional attractants and have deterrent activities (Prabhu, Murugan, Nareshkumar, Ramasubramanian, & Bragadeeswaran, 2011).

### 2.2.1 Environmental Management

Environmental management is defined by the WHO as “the planning, organization, carrying out and monitoring of activities for the modification and/or manipulation of

environmental factors or their interaction with man with a view to preventing or minimizing vector propagation and reducing man-vector pathogen contact" (Garg, 2010). There are three categories: environmental modification; manipulation to target the larval stages of the mosquito life-cycle; and non pesticide personal protection. Environmental management is typically applied to reduce the burden of malaria over the long term (Garg, 2010). Environmental management to control malaria consists of environmental modification, manipulation, and changes in human habitations and behavior ((Jose, 2009).

#### *2.2.1.1 Environmental manipulation*

It refers to activities that reduce larval breeding sites through temporary changes. The regular clearing of vegetation from water bodies or depending on the vector species elimination of shade or planting of shade trees may prevent egg deposition (vegetation management). Flushing streams, periodically changing the water level of reservoirs or changing water salinity can eliminate breeding sites, but the impact on non-target organisms must be critically evaluated (Garg, 2010).

#### *2.2.1.2 Environmental modification*

It aims to create a permanent or long-lasting effect on land, water or vegetation to reduce vector habitat and include draining wetlands by the creation of ditches or drains, land leveling, filling depressions or covering water tanks and stagnant water were among the approaches applied to prevent, eliminate or reduce the vector habitat. Initially, these interventions required significant costs but they contributed to the reduction or elimination of mosquito breeding habitats (Garg, 2010).

### 2.2.2 Biological Control

Biological methods consist of the utilization of natural enemies of targeted mosquitoes and of biological toxins to achieve effective vector management. They are typically most feasible with easily identifiable breeding places. Alternatives under this category include larvivorous fish, invertebrate predators, nematodes, Protozoa, fungi and bacteria (Walker, 2002). Larvivorous fish is most commonly used in this method, *Tilapia nilotica* and *Gambusa affinis* species, (IPEP, 2006). Biological control agents are more likely to cause

significant mortality in permanent habitats than in ephemeral habitats (William, 2005). Agents that effectively suppress larval populations under laboratory conditions often fail under less favorable field conditions. Furthermore, biological control agents tend to be more specific in terms of which mosquitoes they can control and which habitats they will work in (Walker, 2002).

Predatory fish that eat mosquito larvae, particularly in the family Cyprinodontidae, have been used for mosquito control. More recently, researchers have evaluated native fish species to identify appropriate local biological control agents. In spite of widespread recommendations for the use of fish and extensive laboratory data, reports of controlled field experiments evaluating the effectiveness of larvivorous fish in reducing malaria transmission are fairly limited. In rural areas, fish may be appropriate components of malaria control if breeding sites are well known and limited in number, but use of fish may be less feasible where natural breeding sites are extremely numerous. Fish may be particularly useful in controlling malaria vectors associated with rice fields (Bukhari, Takken, Constantianus, & Koenraadt, 2011).

Two different species of bacteria of the genus Bacillus, *thuringiensis israelensis* (Bti) and *B. sphaericus* (Bs), have been widely demonstrated to be effective larvicides against both anopheline and other mosquito species. Both Bti and Bs function as stomach poisons in the mosquito larva midgut. Bti is an important part of mosquito control in the United States Different formulations of Bti have been found effective against larvae of many mosquito species, including the malaria vectors *An. albimanus, An. sinensis, An. culcifacies, An. sundaicus, An. stephensi, An. gambiae, An. arabiensis,* and *An. maculatus.* The lethal effect of Bti on mosquito larvae is actually caused by toxins on the bacterial spore coat rather than an infection. Most formulations use dead spores and therefore do not persist or reproduce in the field. Bti generally requires fairly clean water to be effective, whereas Bs can be used successfully in water with some organic pollution (Walker, 2002).

### 2.2.3 Chemical Control

Early mosquito management relied on the use of Paris green (copper acetoarsenite) and petroleum byproducts, but the use of these chemicals has been discontinued because of their high toxicity and pollution of water sources. With the discovery of DDT the focus of malaria control strategies shifted to managing the adult mosquito population and resulted in the abandonment of early vector control approaches. Soon after, the appearance of insecticide-resistant mosquitoes, an increased public rejection of the application of DDT because of its ecological impact, and changes in the feeding behavior of certain vectors, among other factors, eroded this optimism. Currently, insecticide use still plays a significant role in malaria control programs involving the use of insecticide- treated nets and indoor residual spraying. However, Insecticide resistance in mosquitoes is another roadblock to proper malaria control. Such resistance has hampered eradication efforts and has been considered a serious threat to current malaria control strategies (Jose, 2009).

#### *2.2.3.1 Control of immature stages of mosquitoes*

Although control of the adult mosquitoes by using insecticides, either in indoor residual spraying or by insecticide-treated materials, are currently the most widely used strategy , the control of larvae at their breeding sites is another suitable option .The strategy may reduce population of adult mosquitoes by proper and selective larviciding in the breeding habitats of mosquitoes (Msangi *et.al.,* 2011). Control measures directed against the larval and other immature stages of mosquito vectors are useful components of malaria control programs in areas where mosquito breeding sites are accessible and relatively limited in number and size. Immature stages of mosquitoes including larvae are confined within relatively small aquatic habitats and cannot readily escape control measures. Larval anopheline mosquitoes can be controlled by using synthetic larvicidal chemicals such as temephos, fenthion, malathion, chloropyrifos, and methoprene. However, these life stages of anopheline mosquitoes are reported to develop different level of physiological resistance against the aforementioned synthetic larvicides and environmental hazards (Zewdneh *et al.,* 2011). Larviciding is killing of mosquito larvae and is always a supplementary measure in the integrated vector control programs. The organophosphate temephos is always used. Environmental management involves physical changes to the

mosquito larval breeding habitat, but mosquito suppression can also be achieved through treating the breeding sites directly with chemical or biological agents that kill the larvae. Chemical or biological larviciding for the control of malaria vectors is feasible and effective when breeding sites are relatively few or are easily identified and treated (Walker, 2002).

#### *2.2.3.2 Control of adult mosquitoes*

Indoor residual spraying or insecticide-treated materials are currently the most widely used strategy for the control of the adult mosquitoes *(*Msangi *et.al*., 2011*)*. Although four classes of insecticide are recommended by WHO for use against adult mosquitoes in public health programmes, in practice, modern-day malaria vector control has become highly dependent on just one class of insecticide – the pyrethroids but their use as larvicides is limited because of their toxicity to non-target aquatic organisms including fish. Currently, pyrethroids are used on all approved LLINs and are the basis of the vast majority of IRS programmes worldwide (WHO, 2013).

### 2.2.4 Botanicals (Phytochemicals)

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920s (Ghosh, Chowdhury, & Chandra, 2012). The use of powdered chrysanthemum as an insecticide comes from Chinese record. The other natural products like pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, *d*-limonene, camphor and turpentine were among some important phytochemical insecticides widely used in developed countries. The discovery of DDT's and the subsequent development of other organochlorines, organophosphates and pyrethroids suppressed natural product research as the problem for insect control were thought be solved. However, high cost of synthetic pyrethroids, environment and food safety concerns, the unacceptability and toxicity of many organophosphates and organochlorines, and increasing insecticide resistance on a global scale argued for stimulated research towards potential botanicals (Kishore *et al*., 2011).

At present, phytochemicals make up to one per cent of world's pesticide market. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Ghosh *et al.,* 2012).

### 2.2.5 Integrated vector management

Integrated vector management (IVM) is a decision-making process for the management of mosquito populations, involving a combination of methods and strategies for long-term maintenance of low levels of vectors. The purpose of IVM is to protect public health from diseases transmitted by mosquitoes, maintain healthy environment through proper use and disposal of pesticides and improve the overall quality of life through practical and effective pest control strategies (Ghosh *et al.,* 2012).

The main approaches of IVM include: *(i)* Source reduction and habitat management by proper sanitation, water management in temporary and permanent water bodies and channel irrigation. Vegetation management is also necessary to eliminate protection and food for mosquito larvae; *(ii)* Larviciding by application of dipteran specific bacteria, insect growth regulators, surface films and oils, expanded polystyrene beads, phytochemicals, organophosphates and organochlorines, *(iii)* Adulticiding by application of synthetic pyrethroids, organophosphates and synthetic or plant derived repellents, insecticide impregnated bed nets, genetic manipulations of vector species, *etc.*, *(iv)* Use of mosquito density assessment in adult and larval condition and disease surveillance; and *(v)* Application of biological control methods by using entomophagous bacteria, fungi, microsporidians, predators and parasites (Ghosh *et al*., 2012). Application of larvicide from botanical origin was extensively studied as an essential component of IVM and various mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin were developed (Ghosh *et al.*, 2012).

## 2.3 Insecticidal property of birbira (*M. ferruginea*)

The genus *Milletia* constitutes about 200 species in tropical and subtropical Africa, Asia and Australia. It occurs generally between 1000-2500 m above sea level and in the region where water is easily accessible such as streams or in rain forests. The seeds and roots of these plants are used as insecticides and pesticides in many parts of the world, and rotenone is responsible for their toxicity. One of the sources for rotenone from Ethiopian plants is *M. ferruginea* (Karunamoorthi *et al*., 2009).

Rotenone works by inhibiting a biochemical process in the fish cells, resulting in an inability of fish to use oxygen in the release of energy during normal body processes. It is highly toxic for aquatic life, especially to fish. Toxicity effects of rotenone to higher animals could be acute oral, dermal, inhalation, or chronic toxicity. It is more toxic when inhaled than when ingested and it is hundreds of times more toxic intravenously than orally. Fats and oils increase absorption of rotenone and it is highly irritating to the skin of rats (Ameha, 2004)

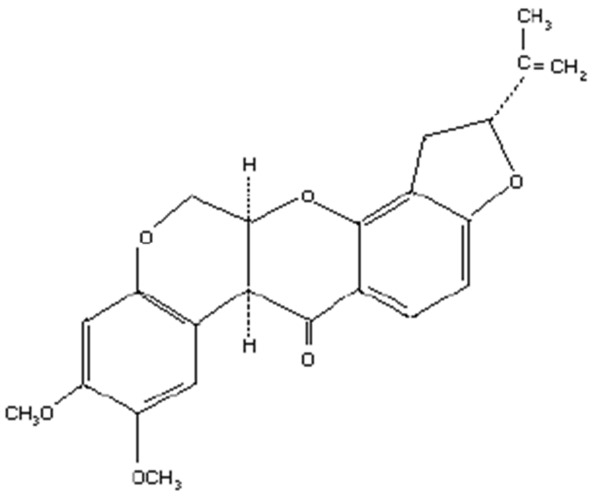


**Figure 1 Chemical structure of rotenone (chemical formula: $C_{23}H_{2206}$) (Ameha, 2004)**

# Chapter three: Objectives

## 3.1 General objectives

The main objective of this study is to investigate the toxicity potential of 'Birbira' seed extract against the primary malaria vector: *An. arabiensis*.

## 3.2 Specific objectives

- To evaluate the larvicidal effect of Birbira seed extract (*M. ferruginea)* against *An. arabiensis* mosquito larvae under laboratory and simulated field condition.

- o determine the ($LC_{50}$, $LC_{90}$ and $LC_{99}$) of Birbira (*M. ferruginea* )

## 3.3 Hypothesis

Methanol extract of "Birbira" seed could have potential mosquito larvicidal, pupicidal and adulticidal effect against An. *arabiensis* under laboratory and simulated field condition

# Chapter four: Materials and Methods

## 4.1 Study area and period

The laboratory test was done at the insectory of Adama WHO Malaria Control Training and research center. This study center is known in rearing of *An. arabiensis* mosquito vectors. It has well organized insectary laboratory facilities. Due to this, the center was selected to carry out the laboratory test using methanolic extract of birbira seed against laboratory reared larvae, pupae and adults of *An.arabinsis* mosquito vector.

The simulated field study was conducted in the village of Asendabo, Ethiopia (7° 45’ N, 37° 13’ E, elevation 1750 meters above sea level). Asendabo is located approximately 50 km far from Jimma, and is known to receive about 700mm of rainfall per year. In the wet season, the average temperature is approximately 20 °C. Malaria is endemic in the area where *An. arabiensis* is the dominant malaria vector and breeds prolifically in the borrow pits commonly found near houses (Trudel, & Bomblies, 2011). Furthermore, Asendabo is known to have mosquito field study site which is commonly called as Gilgel Gibe malaria vector field research center. For this reason, the site was chosen to execute the simulated field test against the mosquito vector. The overall laboratory and field study was conducted from February to April 2013.

## 4.2 Birbira plant specimen collection and preparation of plant powder

The plant materials of *M. ferruginea* ripen berries were collected from the trees growing in Jimma town, near Jimma University. The covers of the berries were removed by hands and the ripen seeds were separately collected to get de-pulped seeds. The de-pulped seeds were then shade dried before being crushed and powdered. The dried seed materials were crushed, powdered and meshed at 0.5mm mesh size using mortar and pestle. The meshed seed powder was then stored at 4ºC.

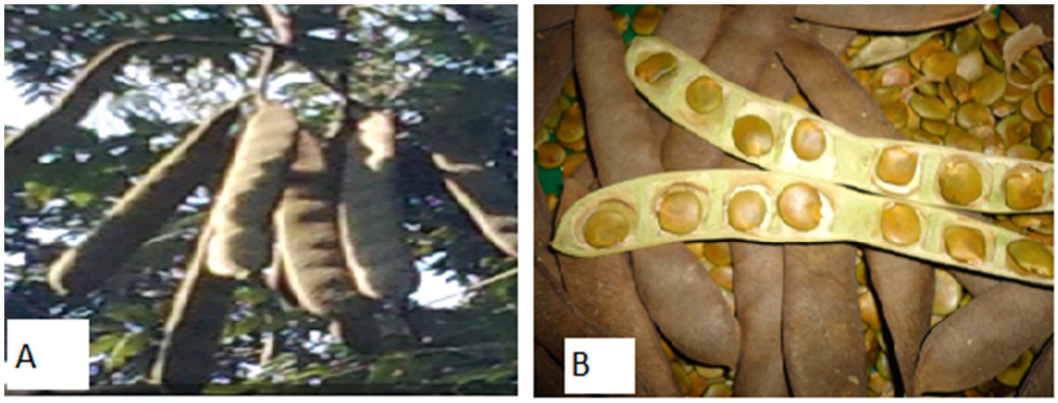


**Figure 2** Birbira*" (M. ferruginea)* tree with pods (A) and its seed (B)

**Figure 3** Preparation of Birbira (*M. ferruginea)* powder using crushed with mortar and pestle

### 4.3 Extraction of the plant seed and preparation of stock solutions

The powdered seed of Birbira (800gm) was macerated with 2.4 L methanol in 1:6 (W/V) using Erlenmeyer flasks and placed on bath shaker at room temperature for 72 hours. After 72 hrs, the mixture was filtered with Whatman no-1 filter paper. Finally, the extract was concentrated in the rotary evaporator in order to remove the solvent and the residue obtained was stored at 4ºC till used for experiments.

Crude methanol seed extract of Birbira (*M. ferruginea) was* not readily soluble in water. Therefore, stock solution was always prepared by adding the extract residue to acetone and different test concentration ranging from 5mg/L to 140mg/L were prepared through serial dilution and used for larvicidal, pupicidal and adulticidal activities under laboratory

and simulated field conditions (Zewdneh *et al., 2011;* Vievek and Sumangala*, 2008)*. The volume of stock solution was 20 ml of 1%, obtained by weighing 200mg of the powder and adding 20 ml of solvent to it. The control was set up with acetone solvent (WHO, 2005; Kamaraj *et al.,* 2010 and *Zewdneh et al., 2011)*.

**Figure 4** Birbira seed extract residue

**4.4 Mosquito sampling and rearing**

For laboratory bioassay; the larvae, pupae and adults of *An. arabiensis* were obtained from the insectary of Adama WHO Malaria Control Training and Research Center. The adults were given a blood meal from a rabbit placed in resting cages for blood feeding by females. Glass Petri dishes having water lined with filter paper was kept inside the cage for oviposition The larvae were made to feed with powdered yeast and the colony was kept at 27±2 °C with 80%-90% relative humidity until the formation of pupae. The pupae were then collected from the culture trays and transferred to glass cups containing water and the glass cup was kept in a mosquito cage for adult emergence.

For the simulated field trial, Wild population of *An. Arabiensis* mosquito larvae ($2^{nd}$ instar) were collected from the village of Asendabo around Gilgel Gibe River where *An.*

*Arabiensis* is the most dominant vector (Trudel and Bomblies, 2011) through dipping method from their natural breeding sites. The Larvae were then carefully taken to the Gilgel Gibe Vector Biology Laboratory and kept in plastic trays containing raw water for rearing and feed with yeast until they become early 3$^{rd}$ instar larvae for the simulated field application of larvicidal test. They were reared at room temperature (26$^{o}$C-29$^{o}$C). The field collected larvae population was also made to emerge into pupae and adults for the pupicidal and adulticidal activities of the plant extract under d simulate field condition. Pupae were transferred from the trays to a cup containing raw water and were maintained in cages (30cm×30cm×30 cm) where adults emerged. The adults were then continuously provided with sugar solution to feed on it.

## 4.5 Experimental design

Experimental study design was set under the laboratory and simulated field condition. The experiment consisted of three factors namely: larvicidal, pupicidal and adulticidal activities at the laboratory and field condition. Each treatment level was designed with four replicates, positive and negative controls. For laboratory and field larvicidal test, 25 mosquito vectors were used in each of the tests (WHO, 2005). For pupicidal and adulticidal activities 20 and 15 mosquitoes were used respectively (Muhammad and Umair, 2012; Nawaz *et al.*, 2011). .

## 4.6 Bioassays

A series of bioassays were implemented following the protocol recommended by the World Health Organization; guidelines for laboratory and field testing of mosquito larvicides (WHO, 2005) and guide lines for testing mosquito adulticides (WHO, 2006).

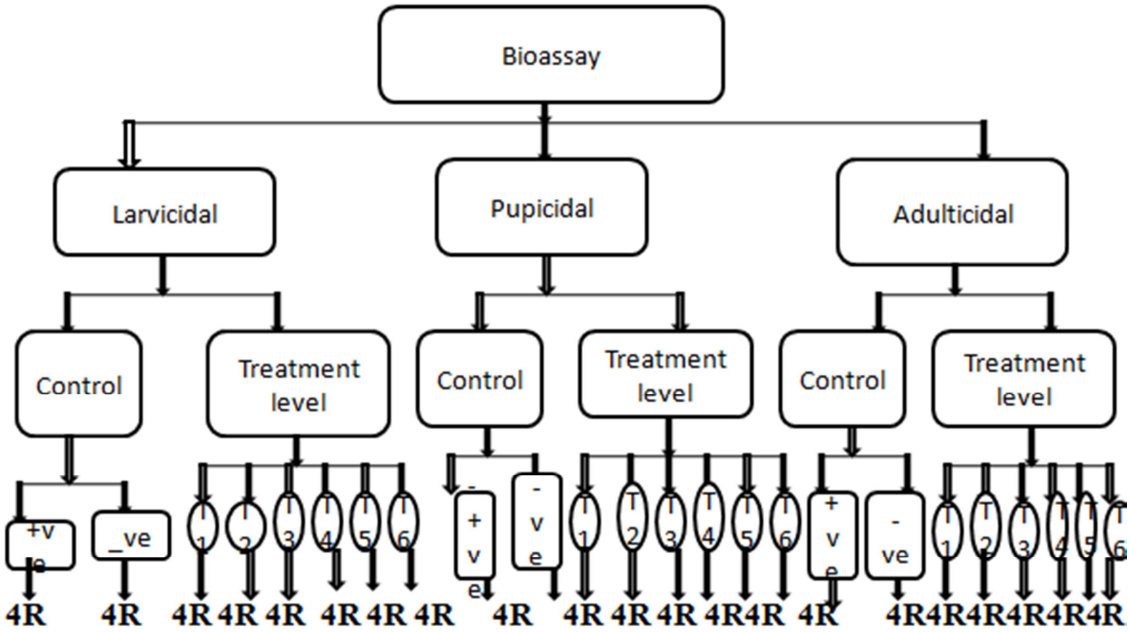


**Figure 5:** Schematic diagram of study design (R = replicates, T = treatments)

### 4.6.1 Test for larvicidal activity

The larvicidal bioassay was tested under laboratory and simulated field condition. An. arabiensis were used to test the larvicidal activity of birbira seed extract. For the laboratory trial of larvicidal activity, a laboratory colony of mosquito larvae was used from the insectary of Adama WHO malaria control training and research center. Batches of 25 late third instar larvae of *An. arabiensis* were transferred into each test concentration of methanol seed extract by means of droppers. Larval mortalities were recorded after 24 hours of exposure in each concentration of the test solutions.

For the simulated field larvicidal activity, methanolic extract of birbira seed was tested against field-collected mosquito larvae of *Anopheles arabiensis* around Gilgel Gibe River. For the larvicidal test, trays were filled with raw water and placed outdoor (in the natural environment). The trays were given 24 h for conditioning. Batches of 25 field-collected late third instar larvae of the *Anopheles arabiensis* mosquito species were transferred into each test trays and larval food was added (yeast). After 3 h of larval acclimation, the trays were treated with selected dosages of birbira seed extract (10, 20, 40, 60, 80 and 100 mg/L*)*. Equal volume of raw water was used as a negative control and

abates chemical (0.5 ml/L) was used as positive control. The trays were covered with nylon mesh screen to prevent other mosquitoes or other insects from laying eggs and to protect the water from falling debris. Four replicates were set up for each concentration and an equal number of controls were simultaneously used. Values of pH and water temperature were recorded throughout the evaluation. Larval mortalities were recorded after 24 hours of exposure in each concentration of the test solutions. Larvae were confirmed dead when they failed to move after probing them with a needle at their cervical region (WHO, 2005).

**Figure 6** Picture showing larvicidal test under laboratory condition

**Figure 7** Picture showing larvicidal test at simulated field condition

### 4.6.2 Test for pupicidal activity

A laboratory colony of mosquito pupae was used for the laboratory pupicidal activity while the wild pupae that were emerged from the field collected larvae were used for field pupicidal test. Experiments were carried out with a series of six to eight treatment levels for laboratory and field pupicidal test respectively, each with 4 replicates.

For laboratory trial, twenty freshly emerged pupae were shifted into a beaker of 250ml capacity that contained 200ml of distilled water. The extract of Birbira (*Milletia ferruginea*) seeds was added at different concentrations in to the test beaker and then placed in the mosquito cage at the laboratory where emerged adults were recorded after 48 hours. Each treatment was replicated four times and repeated at three different days. The same volume of negative control and 0.5mg/l of Temephos was used as positive control (El-Sheikh1 *et al.*, 2012).

For the field bioassay, twenty freshly emerged wild pupae were transferred in to a beaker of 250ml capacity holding 200ml of water and then treated with different concentration

of methanol extract of birbira seed. Finally, the treated beakers were placed in to the mosquito cages and then placed outdoor where emerged adults were counted after 48 hours.

**Figure 8** Picture showing pupicidal test at laboratory condition

### 4.6.3 Test for adulticidal

Laboratory colonies of *An. arabiensis* mosquitoes were reared and maintained at 27 ± $2^{0C}$ and 70-90 % per cent relative humidity in the insectory of Adama WHO Malaria Control Training and Research Center. Larvae were feed on yeast powder as nutrient. Adult mosquitoes were reared in cages and feed with glucose solution. Female mosquitoes were periodically blood-fed on rabbits for egg production and *An. arabiensis* mosquitoes were used for the adulticidal activities. For the adulticidal activity of wild adult, mosquito larvae were collected from their natural breeding site around Gilgel Gibe malaria field research center and brought in the center for emergence in to adults.

The bioassay procedure of WHO Guidelines for testing mosquito adulticides (WHO, 2006) was employed against the adult mosquito's species (*An.arabinsis*). The birbira seed extract was diluted with acetone and distilled water to make different test concentrations.

The diluted seed extract was impregnated on what man filter paper. What man filter paper consisting of only methanol was used as a negative control and propoxur was used as a positive control (Marimuthu *et al.*, 2011).

The bioassay was conducted in an experimental kit consisting of two plastic tubes. One tube was used to expose the mosquitoes to the plant seed extract and another tube was used to hold the mosquitoes before and after the exposure periods. Batches of 15 female adult mosquitoes (3 days old glucose feed, blood starved) were collected and gently transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1 h, 2h and 3h. At the end of 1h, 2h, 3h exposure periods, the mosquitoes were transferred back to the holding tube and kept 24 h for recovery period. A pad of cotton soaked with glucose solution was placed on the mesh screen in the tube during the holding period of 24 h. The test was undertaken at 27°C ± 2°C and 70% -90% relative humidity. Mortality of the mosquitoes was recorded after 24 hrs.

**Figure 9:** Adult mosquitoes in the holding and exposing tube

## 4.7 Study variables

### 4.7.1 Dependent variables

The dependent variable for this study is the number of dead mosquito larvae, pupae, and adults of An. arabiensis.

### 4.7.2 Independent variables

The independent variables include

- Concentration of the seed extracts,
- Exposure time,

## 4.8 Conceptual frame work

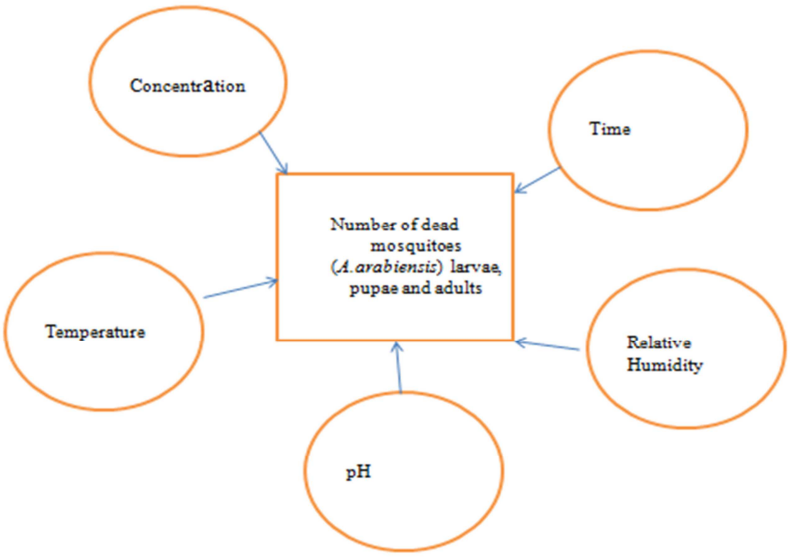


**Figure 10:** Conceptual frame work of the study

### 4.9 Data analysis

Data were coded, entered, and analyzed using SPSS window version 16.0. The average larval mortality data were subjected to probit analysis for calculating $LC_{50}$, $LC_{90}$, and $LC_{99}$. Statistical significance of the data and $R^2$ values were calculated using SPSS soft ware.package. $p < 0.05$ were considered significant during the analysis.

## 4.10 Data quality control

The bioassay test for each treatment was repeated three times with new batch of concentration and different batches of mosquito vectors. The test was carried out in the insectory laboratory of Adama Malaria Training and research center reference strain mosquito vector for about 31 years and each test was conducted in the presence of replicate controls. There was also progress report and close supervision by the advisors both at laboratory and field. During the bioassay test, there were malaria experts or entomological technicians who had been working in the NMCP for over 30 years at Asendabo field vector biology laboratory who provided me strong technical support.

### 4.11 Ethical considerations

The study protocol was read and approved by Jimma University College of public health and medical science research and ethical Committee.

# Chapter five: Results

## 5.1 Laboratory bioassay

### 5.1.1. Larvicidal effect

The larvicidal effect of birbira seed extract against laboratory strain of *An. arabiensis* showed 100 % mortality at 60mg/L. Similarly, 100 % mortality was observed in 0.5 mg/L temephos (positive control) and no mortality was observed in the negative control containing 200ml distilled water with 1ml of acetone. The p-value is less than 0.05 which shows that variation in the concentration of methanolic extract Birbira seed determines differences in mortality. The mortality response at different concentrations and the corresponding lethal concentrations at different points were given in the table 1 below.

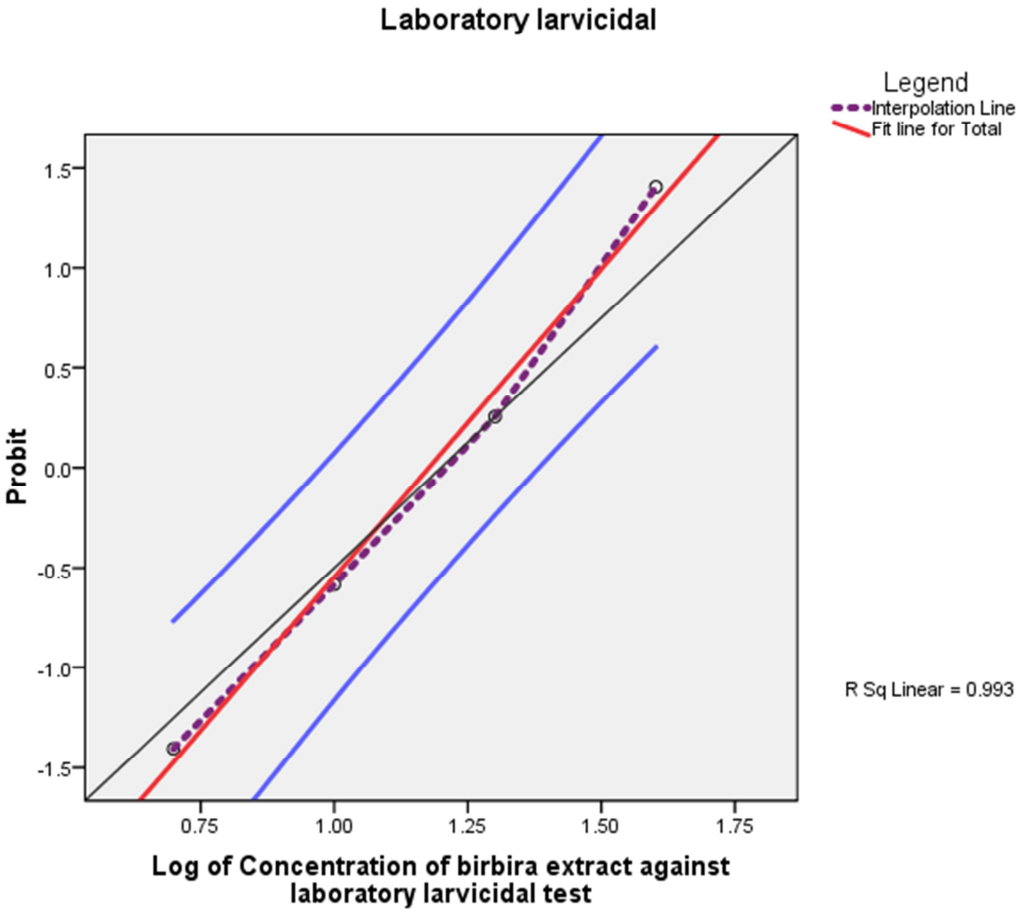

**Figure 11** Larvicidal effect of “Birbira” (*M. ferruginea*) seed extract against malaria vector mosquitoes; *Anopheles arabiensis* under laboratory condition

As indicated in the figure above (Figure 12) there is a linear relationship between concentrations of the Birbira seed extract and mortality of laboratory strain larvae. The R square value (0.993) above indicates that 99.3 % variation in mortality of laboratory strain larvae is explained by concentration difference of the seed extract.

### 5.1.2 Laboratory strain pupicidal

The pupicidal percent mortality for the laboratory strain pupae was increased by increasing extract concentration with p-value less than 0.05 that showed the finding is statistically significant. Thus, there is difference in mortality of laboratory strain pupae with variation in the concentration of the Birbira seed extract. The highest mortality was observed at 80ppm of the plant extract and 0.5ppm of temephos (positive control). No mortality was detected in the negative control containing 200ml of distilled water with 1ml of acetone.

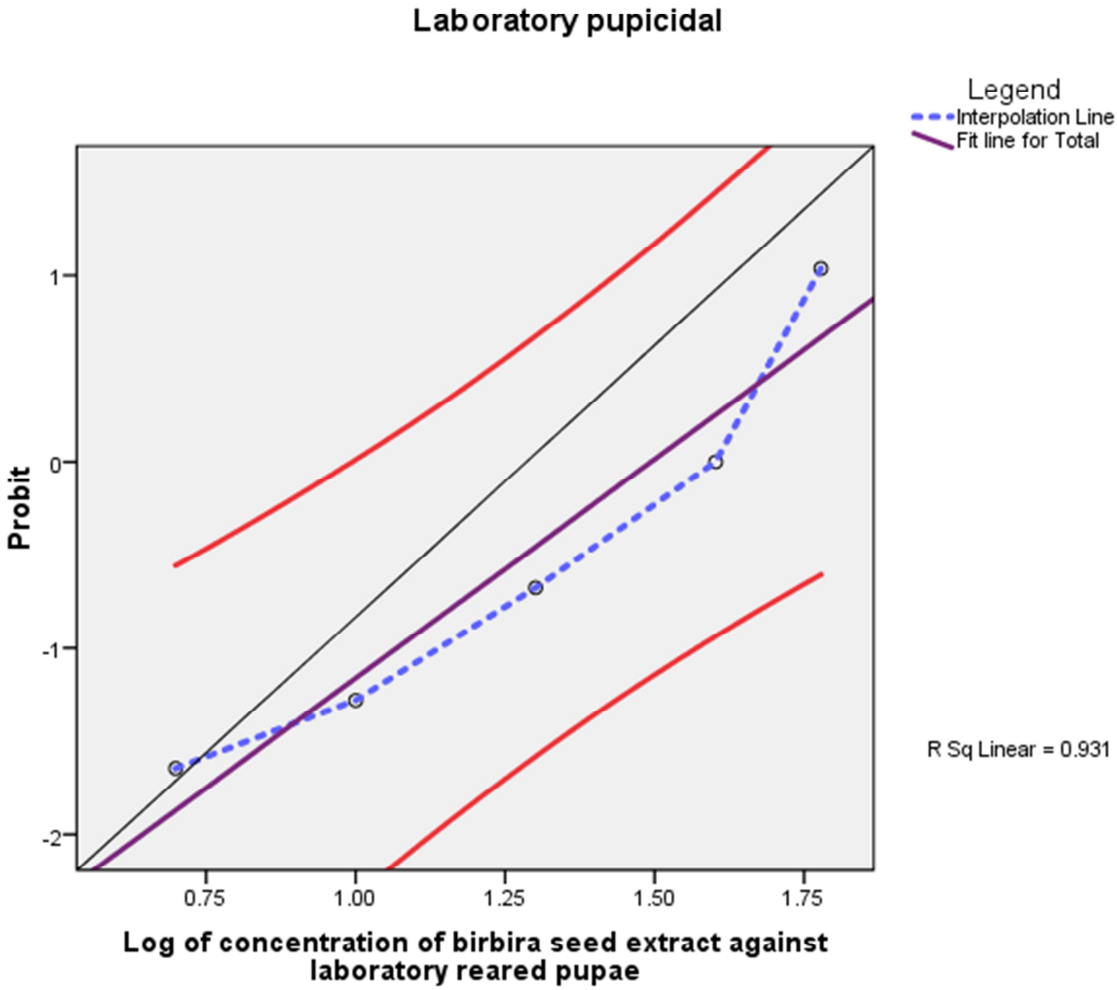


**Figure 13** Pupicidal effect of "Birbira" (*M. ferruginea)* seed extract against malaria vector mosquitoes; Anopheles arabiensis under laboratory condition

The graph above (Fig.13) indicates that the mortality of laboratory strain mosquito pupae is proportional with the concentration of the seed extract. It can also be seen from the R square value that about 93 % mortality of the laboratory strain is explained by difference in concentration of the seed extract.

### 5.1.3 Laboratory strain adult bioassay

The finding of Birbira seed extract against laboratory strain of An. arabiensis adult revealed that mortality of the adult mosquito increases with the extract concentration and exposure time .The highest mortality value was observed at 80ppm of two hours and 100ppm of three hours. The p-value at one hour, two hours and three hours is less than 0.01 which is statistically significant. From this statistical value it can be explained that the difference in mortality of the laboratory strain adult is different at different concentration and exposure time.

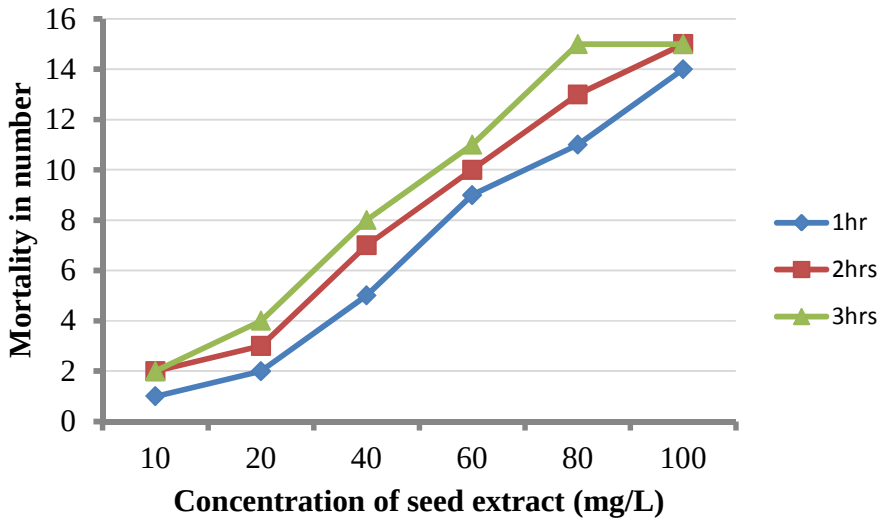


**Figure 14** Adulticidal effect of "*Birbira*" (*M. ferruginea)* seed extract against laboratory strain malaria vector mosquitoes; *Anopheles arabiensis*

The figure above shows that mortality of laboratory reared strain of *An. arabiensis* mosquito vector increases with concentration and exposure time. No mortality was observed in the control and the highest mortality was observed at 80mg/L of 3 hrs and 100mg/L of 2 hrs exposures and 100 % mortality was observed using propoxur as a positive control. It can also be seen from the graph that the mortality of increases with exposure time.

## 5.2 Simulated field bioassay

### 5.2.1 Larvicidal bioassay

The finding of the bioassay showed that larval mortality of wild populations of *An. arabiensis* was different according to concentration of the extract used used. The p-value for the bioassay is less than 0.001 that shows statistically significance of the result. Thus the variation in mortality of wild adult mosquito population is due to the difference of the seed extract.

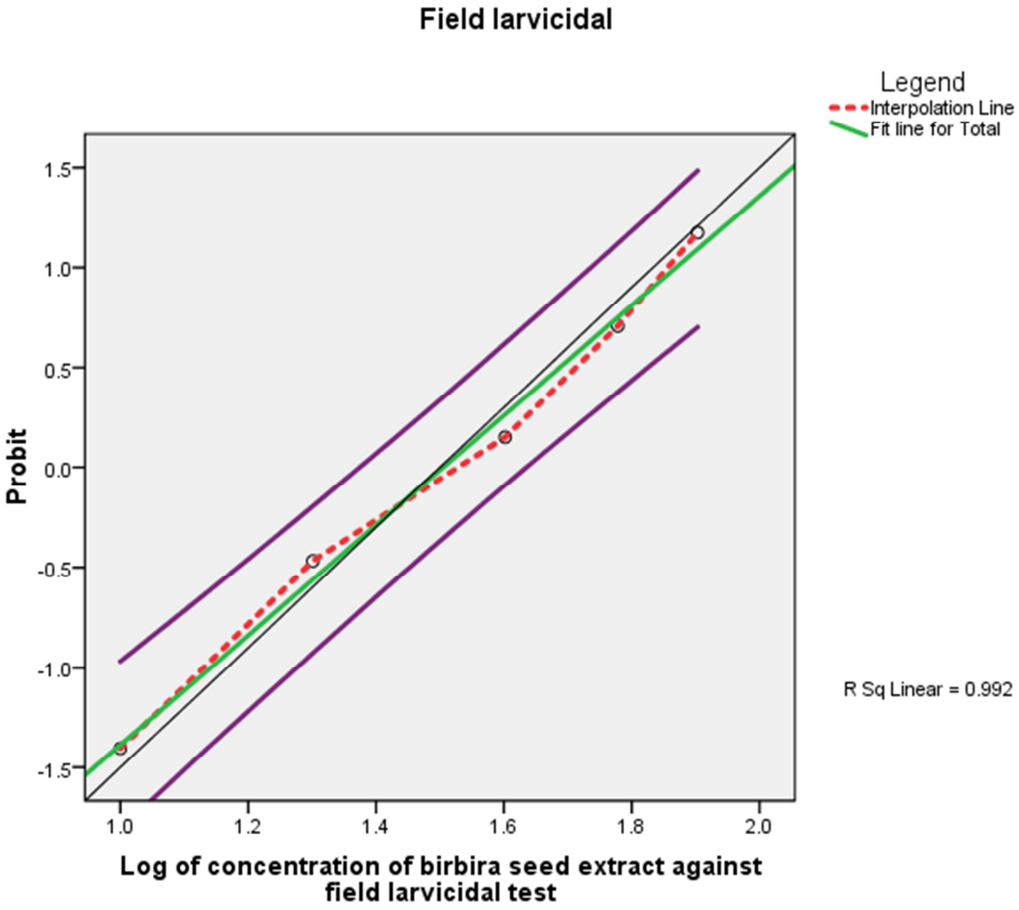

**Figure 15** Larvicidal effect of "*Birbira*" (*M. ferruginea)* seed extract against malaria vector mosquitoes; Anopheles arabiensis under simulated field condition

### 5.2.2 Wild Pupicidal bioassay

The bioassay result in wild pupicidal revealed that mortality of the pupae is different at different concentration of Birbira seed extract against an. arabiensis under simulated field condition. The highest mortality in the bioassay was observed at 140ppm. No mortality was observed in the negative control while 100 % mortality was detected at 0.5 ppm temephos.

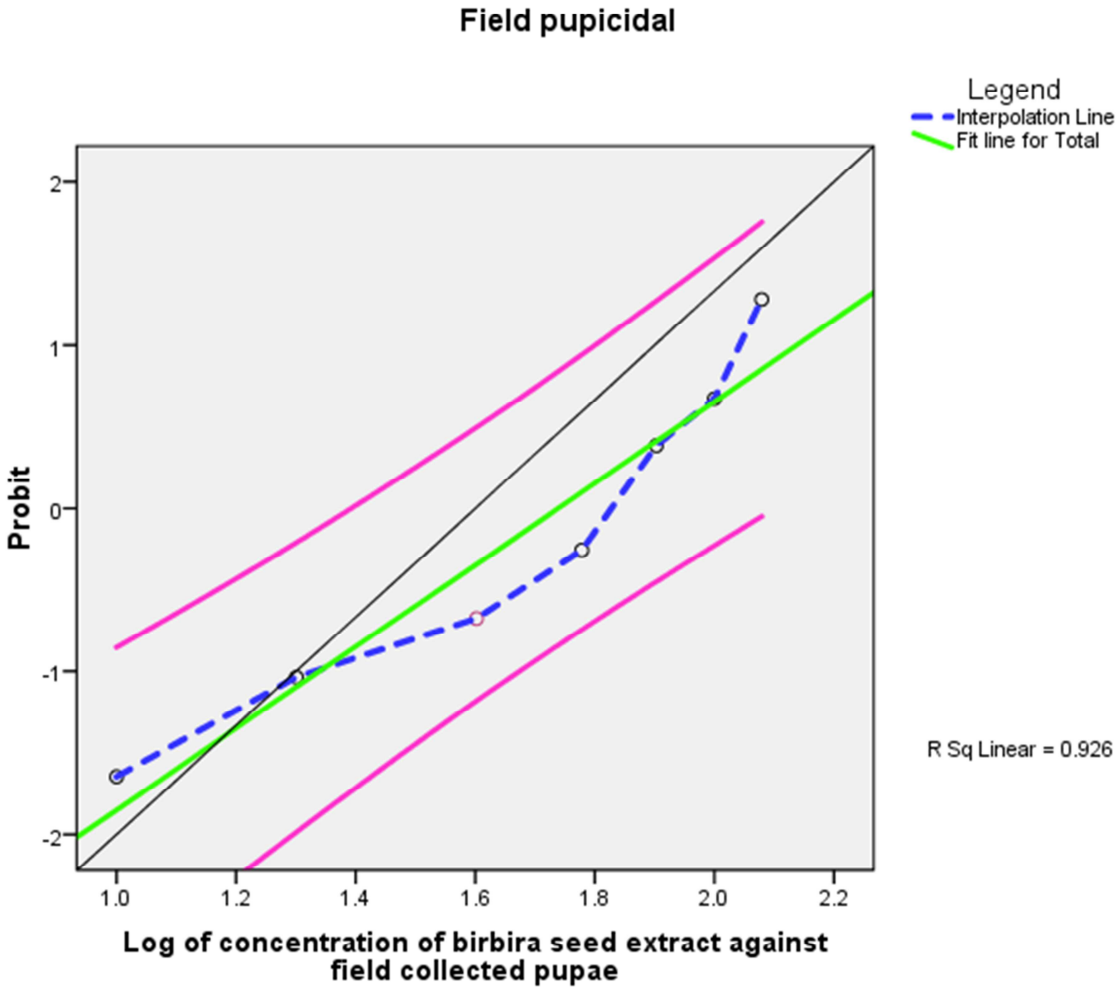


Figure 16. Pupicidal result of methanol extract of "*Birbira*" (*M. ferruginea) seed against* malaria vector mosquitoes; *Anopheles arabiensis under simulated field condition*

### 5. 2.3 Wild adulticidal bioassay

The finding of bioassay against wild population of An. arabiensis indicated that mortality of the wild population increases with concentration of the extract and exposure time. The p-value is ($p<0.001$) which is statistically significant that shows There was significant ($p < 0.05$)variation in mortality among different concentration of the extract and exposure time .

Table 6: Adulticidal result of methanol extract of "*Birbira*" (*M. ferruginea)* seed extract against wild malaria vector mosquitoes; *Anopheles arabiensis* in one hour, two hours and three hours exposur

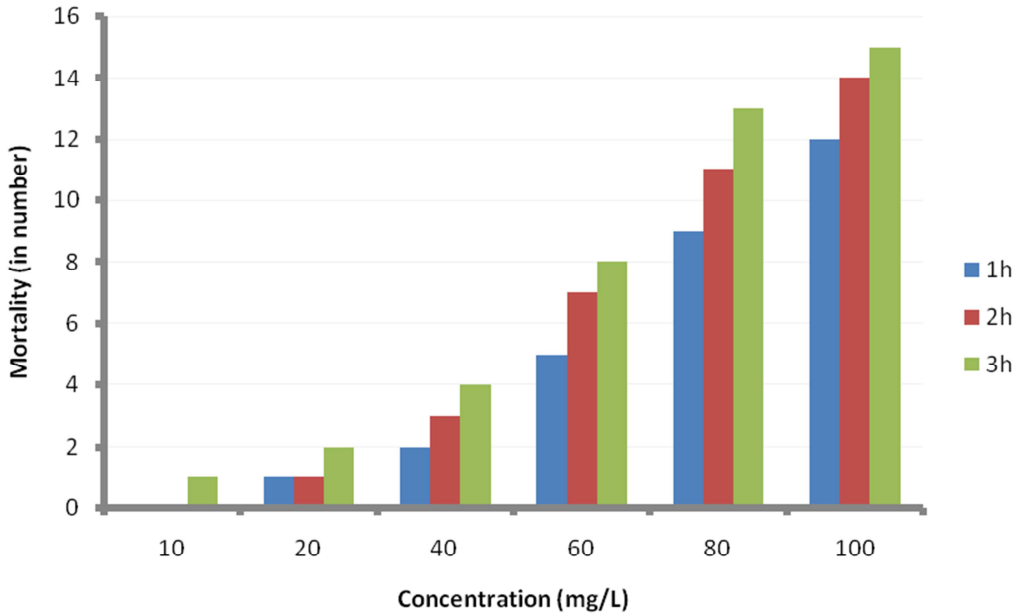


**Figure 17** Adulticidal effect of "*Birbira*" (*M. ferruginea) seed extract against wild population* malaria vector mosquitoes; *An. arabiensis in one hour, two hours and three hours exposure*

The figure 18 above revealed that mortality of wild adult mosquitoes of *An. arabiensis* increases with plant extract concentration and exposure time. It can be seen from the figure that highest mortality was observed at concentration of 100mg/L and 3 hrs exposure time.

In general the lethal concentration of wild pupae is greater than the lethal concentration of laboratory strain pupae and the lethal concentration of wild larvae population is greater than that of the laboratory strain larvae in all the lethal points.

Table 7: Summary on LC values for larvicidal and pupicidal result under laboratory and field condition

| LC point | Lab larvae | Lab pupae | Field larvae | Field pupae |
|---|---|---|---|---|
| $LC_{50}$ | 15 | 29 | 31 | 44 |
| $LC_{90}$ | 37 | 80 | 83 | 116 |
| $LC_{99}$ | 76 | 182 | 184 | 225 |

Lab=Laboratory, LC = Lethal concentration

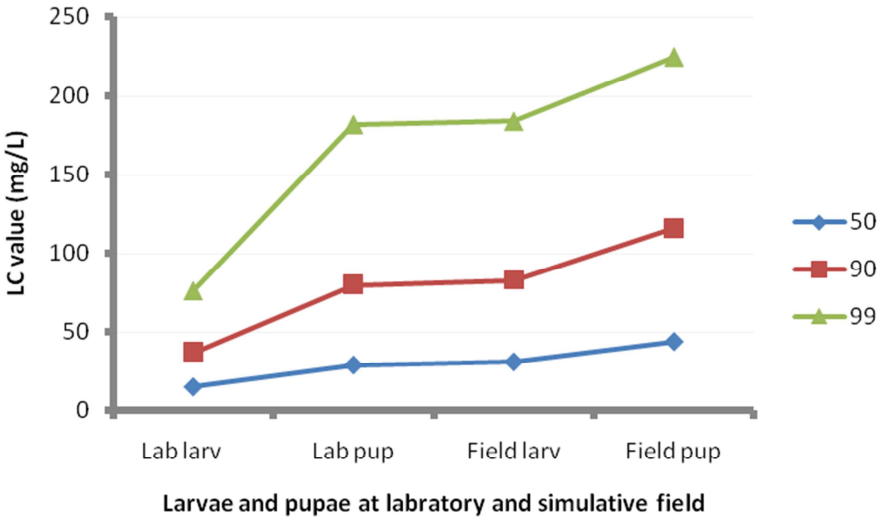


**Figure 18:** LC values for larvicidal and pupicidal result under laboratory and field condition

The figure 19 above shows that wild mosquito vectors of *An.arabinsis* have higher lethal concentration than the laboratory strains. The figure also indicates that pupae have got higher lethal concentration as compared to the larvae.

## Chapter six: Discussion

The present study revealed that, the mosquitocidal effect of ‘’*Birbira*’’ seed extract against *An.arabinsis* females varied according to the concentration of the extracts used. The concentrations tested have indicated mortalities proportional to the level of concentration at three different stages (larvae, pupae and adult) of *An. arabiensis* mosquitoes under laboratory and simulated field condition.

The larvicidal effect of the plant extract against 3$^{rd}$instar larvae of laboratory strain was different according to concentration of the extract under laboratory conditions. The larval mortality percent was increased by increasing extract concentration. The highest mortality for the crude extract of the birbira seed under laboratory trial was recorded at

60mg/L (100%). Temephos which was used as positive control, a standard insecticide, achieved 100% mortality at, 0.5mg /L. The $LC_{50}$, $LC_{90}$ and $LC_{99}$ values for the laboratory larvicidal tests were 14.97, 36.65 and 73.03 mg/L respectively ($p < 0.005$). Similar results were obtained by (Zewdneh *et al.,* 2011) using methanol extracts of *Jatropha curcasl* against *Anopheles arabiensis* larvae (Diptera:Culicidae); (El-Sheikh *et al.,* 2012) using methanolic extracts of leaves and seeds from, *Tribulus terrestris* (Zygophyllaceae) tested against 3rd instar larvae and adults of mosquito, *Anopheles arabiensis;* The results from the field trial of methanolic birbira see extract against 3rd instar wild mosquito vector showed that as the concentration of the extract increased, percentage mortality was also increased. The highest mortality for the simulated field trial was observed at 100mg/L (100%). Temephos was used as a positive control and caused 100% mortality at 0.5mg/L after 24 hours for the wild population. The $LC_{50}$, $LC_{90}$ and $LC_{99}$ values for the simulated field trial of the larvicidal tests were 31.04, 82.78, and184.15 respectively ($p < 0.05$). Similar finding was obtained by (El-Sheikh *et al.,* 2012) using methanolic extracts of leaves and seeds from, *Tribulus terrestris* (Zygophyllaceae) against 3rd instar larvae and adults mosquito, *Anopheles arabiensis*; (Shrankhla *et al.,* 2012) using methanol extract of *Pseudocalymma alliaceum* and *Allium sativum* against larvae of malaria vector. The LC values ($LC_{50}$, $LC_{90}$ and $LC_{99}$) for the field collected wild larvae were greater than the laboratory reared larvae at all points. This might be due to the fact that the wild larvae population is more resistant than the laboratory strain as the wild larvae have already adapted to the different environmental variation in contrast to the susceptible laboratory reared larvae.

Results obtained in the present study showed that the toxicity of methanol birbira seed extracts against 3rd instar larvae of *An. arabiensis* was also extended to the pupae causing 100% mortality at the concentrations of 80mg/L and 140mg/L for the laboratory and field trial of pupicidal test respectively. The highest pupicidal effect for the laboratory trial was observed at 80mg/L (100 %) while 100 % mortality under the field condition was observed at concentration of 140mg/L which is equivalent to 100 % mortality upon exposure to 0.5 mg/L of temephos chemical. Similar finding was obtained on the study conducted by (Muhammad and Umair, 2012) using plant extract evaluation of mosquitocidal activity of *Moringa oleifera* seeds against mosquito vector.

This showed that wild pupae are more resistant upon exposure to the plant extract than the laboratory reared ones. From the above larvicidal result it can also be seen that pupae are more resistant than the corresponding larvae of *An. Arabiensis* both at the laboratory and field condition. The $LC_{50}$, $LC_{90\ and\ LC99}$ values for the laboratory pupicidal activities were 29, 79, and 182 and 54, 148 and 334 mg/L for the simulated field trial of the pupicidal bioassay respectively ($p < 0.001$) both under laboratory and field condition of pupicidal result.0.625mg/$cm^2$(100 %) respectively. From this result of wild adulticidal test, it can be seen that with the same concentration of the extract having different exposure time results to variation in mortality ($p<0.01$). The $LC_{50}$, $LC_{90\ and\ LC99}$ values for the laboratory strain adulticidal activities were: at one hour; 0.29, 0.80 and 1.80mg/$cm^2$, at two hours; 0.22, 0.62 and 1.55mg/$cm^2$ and at three hours; 0.19, 0.48 and 1.03 mg/$cm^2$ respectively. For the simulated field trial of the adulticidal test, the $LC_{50}$, $LC_{90\ and\ LC99}$ values were 0.43,0.96 and 1.84mg/$cm^2$ for one hour exposure; 0.35, 0.69 and 1.22mg/$cm^2$ for two hours exposure and 0.28, 0.67 and 1.38mg/$cm^2$ for three hours exposure time respectively and it is statistically significant through all the test concentrations and exposure time ($p<0.001$). No mortality was observed in the control. This finding agree with results obtained by (Marimuthu and Rajamoha, 2011) using methanol extract of mosquito adulticidal and repellent activities of botanical extracts against malarial vector, Anopheles stephensi Liston (Diptera:Culicidae).

Results of log-probit analysis at 95% confidence level revealed that $LC_{50}$, $LC_{90\ and\ LC99}$ values gradually decreased with the exposure time and extract concentration. However the value increases for the wild mosquitoes as compared with laboratory reared .This might due to the fact that the field collected mosquitoes are more resistant than the laboratory reared which are more susceptible upon exposure to the plant extract in all the three stages of *An. arabiensis* mosquito vectors.

In general, the finding of the present study revealed that the methanol extract of birbira seed has potential mosquitocidal effect against the primary malaria vector; *An. arabiensis* larvae, pupae and adults of laboratory strain and wild population in Ethiopia. This could be due to the high dermal irritating or inhalation, or chronic toxicity nature of rotenone which is the dominant compound in the birbira seed.

# Chapter seven: Conclusions and recommendations

## 7.1 CONCLUSIONS

The present study revealed that the larvicidal, pupicidal and adulticidal effects of crude methanol seed extract of birbira (*Mellitia ferruginea)* against the late third instar larvae, pupae and adults of *An. Arabiensis*; important vector of malaria in Ethiopia both at the laboratory and field condition. The methanol extracts obtained from the seeds of *"Birbira"* (*Mellitia ferruginea)* possesses *the larvicidal, pupicidal and* adulticidal activity against Anopheles arabiensis mosquito species that could be utilized for development botanical insecticide as supplementary to synthetic insecticides. Hence, it could be concluded that methanol extracts birbira seeds *used* in the present study would act as larvicidal, pupicidal, adulticidal against *An. arabiensis* mosquito vector.

## 7.2 Recommendations

Future large scale field studies should point up on the larvicidal and pupicidal activities of methanol seed extract of birbira (*M. ferruginea)* against *An. arabiensis* mosquitoes in Ethiopia. Future field studies are also recommended to determine the residual activities of the methanol seed extract of birbira (*M. ferruginea)* under natural field condition. It is also recommended to conduct further study on larvicidal, pupicidal and adulticidal potential of the seed extract using different solvents to compare the lethal concentrations at the laboratory and field condition

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