JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

SCHOOL OF GRADUATE STUDIES

DEPARTEMENT OF BIOLOGY

Evaluation of the Efficacy and Residual Activity of three Candidate Insecticide Formulations against *Anopheles gambiae s.l.* in Jimma Zone, Southwestern Ethiopia

By

Abera Hailu

A Thesis Submitted to the Department of Biology, College of Natural Science, Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Ecological and Systematic Zoology Stream)

November, 2014

Jimma, Ethiopia

Jimma University
College of Natural Science
School of Graduate Studies
Department of Biology

Evaluation of the Efficacy and Residual Activity of Three Candidate Insecticide Formulations for *Anopheles gambiae s.l.* in Jimma Zone, Southwestern Ethiopia

By

Abera Hailu

A Thesis Submitted to the Department of Biology, College of Natural Science, Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Ecological and Systematic Zoology Stream)

Advisors

Delenasaw Yewhalaw (PhD)

Argaw Ambelu (PhD)

November, 2014

Jimma, Ethiopia

Acknowledgements

I would like to express my deepest gratitude and appreciation to my advisors' Dr. Delenasaw Yewhalaw and Dr**.** Argaw Ambelu of Jimma University for their initiation, advising and professional guidance, and for their financial and material support to do this study.

I would like to say thank you for Mr. Tafere Demis and Mr Abebe Asale of Jimma University staffs for their valuable comment and suggestion during defending the proposal at the Department of Biology, College of Natural Science Jimma University, Jimma Ethiopia.

I would like to say thank you for people who had direct and indirect contribution while I was under taking this program. My parents' especially my mother W/ro Fantu Beyane for her support, encouraging and appreciation from early stag till know. And my sisters, brothers and father thank you for their encouraging and appreciation.

I would like to say thank you to: Mr. Workineh Jaleta, M/Geta Tsege Ayalew and Mr. H/Geyorgies W/Rufael for their unreserved help and involvement during field larva collection and laboratory work.

I would also like to say thank you to Mr. Eba Alemayehu Head of Department of Biology for his encouraging and cooperating in need of his support. Dr. Getenet Assefa Livestock Directorate of Ethiopian Institute of Agricultural Research (EIAR) and Dr. Taye Kufa Director of Jimma Agricultural Research Center (JARC-EIAR) for their cooperation and supporting to pursue my MSc study. Mr. Kassa Biratu and Mr. Tarekegn Aregaw from JARC staffs for their help on writing this paper and in need of SPSS work.

I thank JU for the opportunity and Ethiopian Institute of Agricultural Research (EIAR) for sponsoring to follow this MSc study program. My respectful thanks to Gelo and Becho-Bore Kebel communities for their voluntariness and participation to do this work.

I also thanks to those individuals who supporting and encouraging me to pursue my MSc education next to the Almighty God Alpha- Omega.

Table of Contents

List of Tables

Tables Page

List of Figures

Figures Page

List of Plates

List of Abbreviations and Acronyms

a.i	Active Ingredient
An.	*Anopheles*
An. arabiensis	*Anopheles arabiensis*
An. funestus	*Anopheles funestus*
An. gambiae s.l.	*Anopheles gambiae sensu latu*
An. nili	*Anopheles nili*
An. pharoensis	*Anopheles pharoensis*
CDC	Centers for Disease Control and Prevention
CS	Capsule Suspension
DDT	Dichlorodiphenyltrichloroethane
GMAP	Global Malaria Action Plan
HH	Household
IRS	Indoor Residual spraying
JU	Jimma University
Kdr	Knockdown resistance
LLINs	Long- lasting Insecticide-treated Mosquito Nets
Pf	*Plasmodium falciparum*
Pv	*Plasmodium vivax*
RH	Relative Humidity
SW Ethiopia	Southwestern Ethiopia
WG	Water Dispersible Granules

WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme
WP	Wettable Powder

Abstract

One way of evaluating insecticide formulations against malaria vector is to undertake insecticide decay rate study for insecticide deposits on different Wall Surfaces using WHO cone assay. To assess the decay rate of an insecticide deposits against adult female Anopheles mosquitoes, cone bioassay test was conducted at different time intervals after the application of each candidate insecticide on different wall surfaces. Therefore, the residual life of three candidate pyrethroid insecticide formulations (deltamethrine 25%WG, lambdacyhalothrin 10%WP and lambdacyhalothrin 10%CS) was evaluated on three different wall surfaces under field conditions at two selected sites in Jimma zone. Deltamethrine 25%WG, lambdacyhalothrin 10%WP and lambdacyhalothrin 10%CS were sprayed at the rate of 0.02-0.025, 0.02- 0.03 and 0.02- 0.025 gm a.i /m^2, *respectively on different wall surfaces (painted, plastered and non plastered) of randomly selected houses. Mean knockdown and mortality rates of An. gambiae s.l. exposed to different wall surfaces sprayed with the different candidate insecticide formulations were determined from April to August 2014. During the assay, WHO cones were fixed at three pointes (0.5m, 1.0m & 1.5m) to the sprayed and control wall surfaces.* The results of the study showed that at week one, t*he highest mortality rates of mosquitoes exposed to painted surface sprayed with labdacyaholtherin 10% WP and deltametherin 25% WG were 89.3% and 88%, respectively while the lowest mortality rates of mosquitoes exposed to non-plastered and plastered surfaces sprayed with labdacyaholtherin 10% CS were 72% and 68.6%, respectively. The mean mortality rates of mosquitoes exposed to painted wall surfaces sprayed with the three insecticide formulations was 48%. There was significant difference in m*ean *knockdown and mortality rates of populations An. gambiae s.l. between time of test and insecticide formulations (p < 0.05.).* Moreover, *there was significant difference in mean knockdown* rates of *An. gambiae s.l.* among w*all surface types. There was no significant difference in mean mortality rates of An. gambiae s.l. among the three different wall surfaces (P > 0.05). In conclusion, populations of An. gambiae s.l. showed resistance against the three candidate insecticide formulations.*

1. INTRODUCTION

Malaria spread from one person to another by female mosquitoes of the genus Anopheles. There are about 400 different species of Anopheles mosquitoes, but only 30 – 40 of these are major importance (CDC, 2006). An estimated 3.2 billion people are at risk of malaria, of whom 1.2 billion are at high risk. In high-risk areas, more than one malaria case occurs per 1000 population. In 2013, 123 million people were protected from malaria by IRS around the world. In Africa, 55 million people, or 7% of the population at risk, lived in households that were regularly sprayed (WHO, 2014). WHO estimated that 207 million cases of malaria occurred globally in 2012 and 627 000 death. Most cases (80%) and deaths (90%) occurred in Africa and most deaths (77%) were in children under 5 years of age. In 2010, there were an estimated 216 million cases (WHO, 2011a) and 1.24 million deaths from malaria (Murray *et al*., 2012).

Malaria is one of the leading causes of morbidity and mortality in Ethiopia. An estimated 55.7 million people (68% of the population) were at risk of malaria infection and approximately 80% of the 736 districts in Ethiopia are considered "malarious". Malaria transmission is generally seasonal and unstable though patterns and intensity of transmission vary throughout the country due to differences in altitude, rainfall and population movement. *P. falciparum* accounts for 65-75% of infections, while *P. vivax* accounts for 25-35%. *P. ovale* and *P. malariae* are rare (MoH, 2013). *Anopheles arabiensis* is the main vector; *Anopheles pharoensis* is also widely distributed in the country and is considered to play a secondary role in malaria transmission and other secondary vectors include *An. funestus* and *An. nili* (MoH, 2007 & 2012). Their sensitivity to insecticides is also highly variable (CDC, 2006).

According to WHO report, 2007; IRS in Ethiopia was initiated in 1959 with the global malaria eradication campaign. Blanket spraying with DDT continued until the late 1970s in almost all affected areas. In the early 1980s, the eradication program was transformed into a control program with IRS as the major intervention. Blanket spraying was replaced by selective application. The use of only DDT continued until the early 1990s when time-limited replacement with malathion was considered in selected areas where vector populations resistant to DDT were encountered. During the 1990s, shortage of funding and supplies resulted in very scanty targeted spraying. Up to 2005, IRS was fully funded by government but is now partially supported by

Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM). In 2006 IRS was implemented in 2862 villages in all 10 administrative regions except in Addis Ababa. However, district specific data on IRS operations is not readily available. Since the early 1990s, IRS operations were decentralized and are now entirely managed by regional and district health teams. Owing to low technical capacity for IRS at district offices and the lack of a robust IRS reporting system to the federal MoH, monitoring of the IRS program is very difficult. Hence, there is no reliable list at national level of districts that implement IRS. To realize the full potential of IRS as a control tool, there is need to evaluate the effect of different surfaces on the availability of newer pyrethroid insecticides on sprayable surfaces in malaria vector control (Hemingway and Ranson, 2000).

The active ingredients of all WHO-recommended products for IRS come from only four classes of insecticide: pyrethroids, organochlorines, organophosphates and carbamates. According to WHO (2011b) report, all recommended LLINs were 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. The reliance of modern malaria control on pyrethroids and the increasing resistance of malaria vectors to these products put resent global efforts at risk. Determination of residual activity of insecticides is the essential information for the use of indoor spraying operation. The residual duration of pyrethroids recommended by WHO including alphacypermethrin, bifenthrin, cyfluthrin, deltamethrin, etofenprox, and lambdacyhalothrin WP, have estimated between two and six months (Najera & Zaim, 2001). Among the Synthetic pyrethroids the two; deltamethrin which is commonly available as formulations 2.5% and 5.0% of water dispersible powder (WP), 2.5% and 5.0% emulsifiable concentrate (EC) and 25% water dispersible granules (WG) forms. A dosage of 0.05 g/m^2 and residual efficacy (remains effective) for 2-3 months on mud and thatch surfaces, but 9 months has been reported for other surfaces. The second one is lambdacyhalothrin which is commonly available as formulations of 2.5% emulsifiable concentrate (EC) and as 10% wettable powder (WP) in preweighed sachets. A dosage of 0.025–0.05 g/m^2 and with residual efficacy (remains effective) for 2-3 months (MoH, 2012).

Two contemporary bioassays, the WHO cone bio-assay (WHO, 1998) and the bottle assay

developed at the Centers for Disease Control and Prevention (CDC) (Brogdon & McAllister, 1998), are used to determine the insecticide susceptibility of mosquito populations. Both methods test field-collected mosquitoes by contact exposure with either an insecticide-impregnated paper (WHO assay) or a glass surface coated with insecticide (bottle assay).

Identifying an appropriate and sustainable vector control strategy is therefore a major step toward achieving universal coverage of interventions, as emphasized in the Global Malaria Action Plan (GMAP) and contributing to Millennium Development Goals targets 4, 5, and 6 such as to reduce child mortality; improve maternal mortality and combat HIV/AIDS, malaria and other diseases, respectively (WHO, 2010). This requires understanding the relationship between the available tools and environmental or socio-economic factors that can affect the effectiveness of interventions. Such factors are manifold, but a major distinction can be made between intrinsic and extrinsic factors.

Intrinsic factors may be defined as characteristics belonging to the intervention itself, while extrinsic factors are mostly part of the environment or linked to human behavior and living conditions (socio-economic factors). As for vector control insecticide-based interventions, intrinsic factors include insecticide formulation, mode of action, dosage, properties (including knockdown, killing, exito-repellent effects) and type of treatment (IRS, LLINs, Sheets) (Hougard *et al*., 2002; Yates *et al*., 2005). Extrinsic factors which include physical and biological factors mostly affect the development and survival of the mosquito (behavior, resistance to insecticides, temperature, humidity, etc) (Sampath *et al*., 1998; Chandre *et al*., 2000), while human activities, behavior and living conditions may provide an additional risk of intervention failure or success (Sampath *et al*., 1998). Understanding and considering environmental, socio-economic and other factors that can jeopardize the effectiveness of malaria interventions should be given due considerations in the African context and especially when dealing with communities at different levels of incomes and living conditions.

Insecticide resistance is emerging as a major challenge to global malaria control efforts, especially in Africa. WHO and its partners have developed a Global Plan for Insecticide Resistance Management (GPIRM) that should form the basis of any national vector control strategy, including the use of IRS (WHO, 2012a). Fundamental to this plan is the building of capacity and systems for basic epidemiological and entomological monitoring, including

bioassays for insecticide susceptibility of the local vector populations. This information, together with information on local transmission ecology and epidemiology, e.g. length of transmission season and levels of transmission, will determine the appropriate selection of insecticides in order to mitigate or delay the further development of resistance (WHO, 2013b). Resistance management must become a primary consideration in the choice between alternative vector control methods. Presumably, some forms of vector control select more strongly for resistance than others, depending on the situation. Thus, the choice of alternative interventions for a vector control programme should consider not only maximizing the expected epidemiological benefits, but also minimizing the expected resistance costs (WHO, 2010).

According to WHO (2013a) report, effective IRS operations require: adequate political commitment and social acceptance of IRS; adequate programme and health system capacity to deliver good-quality, well timed and high-coverage IRS; adequate information on local vectors, especially insecticide susceptibility status and indoor versus outdoor feeding and resting behaviours; and adequate and sustainable financial, logistical and human resources. An outcome of IRS is that, when applied properly, it is a powerful malaria vector control intervention, rapidly reducing vector transmission capacity and malaria incidence. IRS provides maximum mass effect on the vector populations at high coverage levels.

IRS is applicable in many epidemiological settings, provided that it's operational and resource feasibility is considered in policy and programming decisions (WHO, 2013a).As insecticides are applied inside homes, there is significant chemical exposure for both the spray teams and the inhabitants. The various insecticides suitable for house spraying vary in the acute and chronic toxicity hazards they pose, particularly to sprayers. To protect themselves against acute intoxication, sprayers and people mixing or repackaging the concentrated pesticide formulations should wear protective clothing and receive proper training and supervision in safe pesticide handling (Chavasse & Yap, 1997).

IRS carried out correctly, is a powerful intervention to rapidly reduce adult mosquito vector density and longevity and, therefore, to reduce malaria transmission. The effectiveness of IRS as a malaria control intervention arises from the fact that many important malaria vectors are endophilic (GMAP, 2008). That is, when searching for blood meals they enter human habitations

or animal shelters where they rest on the walls, ceilings and other interior surfaces before and/or after feeding on the inhabitants. When a vector comes into contact with a sprayed surface, it absorbs lethal doses of insecticide, thereby reducing its lifespan. This results in a progressive decline in vector density and longevity, especially among older female mosquitoes, reduces overall vectorial capacity, and contributes to a reduction in malaria transmission. IRS is most effective against indoor feeding (endophagic) and indoor resting (endophilic) vectors (WHO, 2013c). However, there are overwhelming growing evidences of insecticide resistance of vectors against pyrethroid insecticides across Africa. Given their application in LLINs and IRS (Ranson *et al*., 2011; Coleman *et al*., 2006), the resistance to pyrethroid may compromise malaria control as LLINs may lose efficacy, although at present there are no studies linking insecticide resistance to LLIN control failure. Thus, this study was undertaken to evaluate the efficacy and residual effect of three candidate insecticide formulations (Pali 250 WG (deltamethrin 25% WG), Revival 100 WP, (lambdacyhalothrin 10% WP) and Revival 100 CS, (lambdacyhalothrin 10% CS)) against field populations' of *An. gambiae* s.l. mosquitoes on different indoor wall surfaces in order to guide future interventions.

1.1. Statement of the problem

There are increasing reports of malaria vectors that have developed resistance to the pyrethroids commonly used in LLINs and pyrethroid resistance is now firmly established throughout Africa (Ranson *et al*., 2011; Coleman *et al*., 2006). This resistance to pyrethroids may compromise malaria control as LLINs may lose efficacy, although at present there are no studies linking insecticide resistance to LLIN control failure. *An. arabiensis* is the primary malaria vector species in the southwest of Ethiopia, and is the only vector species of the *An. gambiae* complex found in Jimma, Tiro-Afeta, Omo-Nada and Kerssa districts. Studies done within these areas indicate that populations of *An. Arabiensis* were resistant to DDT, permethrin, deltamethrin, malathion (Yewhalaw *et al*., 2011 and 2010). Bottle bioassays revealed that populations of *An. arabiensis* from these study areas had low to moderate susceptibility to both permethrin and deltamethrin for the diagnostic dose and time used (Yewhalaw *et al*., 2012).

Therefore, this study was undertaken to evaluate the efficacy and residual effect of three candidate insecticide formulations (Pali 250 WG (deltamethrin 25% WG), Revival 100 WP,

(lambdacyhalothrin 10% WP) and Revival 100 CS, (lambdacyhalothrin 10% CS)) against field populations' of *An. gambiae* s.l. mosquitoes on different indoor wall surfaces in order to guide future interventions, in the context of southwest Ethiopia.

1.2. Objectives

1.2.1. General Objective

The main objective of this study was to evaluate the bio-efficacy and residual activities of three candidate insecticide formulations against *An. gambiae s.l.* populations in Jimma zone, Southwestern Ethiopia.

1.2.2. Specific Objectives

Specific objectives of this study were to:

- Determine the bio-efficacy of a candidate insecticide formulations, Pali 250 WG (deltamethrin 25% WG), Revival 100 WP (lambdacyhalothrin 10% WP) and Revival 100 CS (lambdacyhalothrin 10% CS) against field populations of *An. gambiae s.l.*,
- Asses the residual activity of a candidate insecticide formulations, Pali 250 WG (deltamethrin 25% WG), Revival 100 WP (lambdacyhalothrin 10% WP) and Revival 100 CS (lambdacyhalothrin 10% CS) against field populations of *An. gambiae s.l.* and

1.3. Significance of the Study

To determine the bio-efficacy and residual activity of deltamethrin 25% WG, lambdacyhalothrin 10% WP and lambdacyhalothrin 10% CS insecticides which provides information for insecticide choice for IRS and selection of appropriate indoor spraying operation. Identifying an effective insecticide is important to reduce the vector density and longevity to reduce their vectorial capacity. Which can to control important malaria vectors using IRS. To reduce vector life span in order to deny sporozoites of malaria the necessary time to develop inside mosquitoes. As a consequence the vectors die before they transmit the parasite even if they are contaminated with it. To Cause malaria vectors to avoid seeking the resting places where people live and therefore to reduce human vector contact which prevents vectors biting humans and transmitting the disease.

2. LITERATURE REVIEW

2.1. Biology, Ecology and Behavior of Anopheline Mosquitoes

Like all mosquitoes, anophelines go through four stages in their life cycle: egg, four larva stages, pupa and adult. The first three stages are aquatic and the last one is terrestrial. The whole life cycle lasts 5-14 days, depending on the species and the ambient temperature (Clements, 2000). Many species prefer habitats with vegetation, some breed in open sunlit pools while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants. The adult is the stage in which the female Anopheles mosquito acts as malaria vector. The adult females can live up to a month (or more in captivity) but most probably do not live more than 1-2 weeks in nature, while males live for about a week (Clements, 2000). Males feed on nectar and other sources of sugar; females also feed on sugar sources for energy but usually require blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature but usually takes 2-3 days in tropical conditions. Once the eggs are fully developed, the female oviposts and resumes host seeking (Foster, 1995).

Kersa District is malarious; the ecological condition in the district favors the existence of *Anopheles* mosquitoes associated with malaria transmission. Malaria is the most prevalent seasonal disease in the area accounting for 77.1% of all the reported disease in the health center in the 2006 and 2007. October to December is the peak transmission season (Assefa *et al*., 2010; Ketema *et al*., 2009). The district has 32 Kebels one urban and 31 rural where the rural Kebels are with more or less homogenous characteristics of house style as most are mud plastered grass roofed, similar socio-economic activity (Assefa *et al*., 2010; Yewalaw *et al*., 2010). The district catagories 20 Kebles as highly malarious, 10 Kebles as malaria case medium and 2 Kebles as malaria free. Gelo, Bulbul and Ankeso Kebles are among the highly malarious Kebles (Lelisa, 2013).

Gelo Keble shows semi-arid weather condition, where the flat nature of landscape dominated by scarcely distributed acacia trees. *An. gambiae s.l.* (71.8%) is the most abundant species, followed by *An. coustani s.l.* (22%) and *An. pharoensis* (6.2%) (Lelisa, 2013).

2.2. Anopheline Mosquitoes and Indoor Residual Spraying (IRS)

The first consideration to choose the insecticide to be used for IRS is its proven effectiveness on the target vector species and its safety for inhabitants, workers, animals, and environment. In addition to the susceptibility of target species to insecticides, the duration of residual effect of insecticides is essential information. The importance of a more precise definition of the duration of the residual effect is in the need for programming cycles so that the human population remains protected until a new spraying is conducted (Josiane *et al*., 2011).

Adult females of many mosquito species will bite humans, using the blood meals for egg production. Anophelines generally bite at night and usually rest on a surface (such as the wall of a house) before or after feeding (Kathleen, 2002).Vector control is the key intervention for global malaria control and elimination efforts. It is critical for the reduction and, ultimately, for the interruption of malaria transmission. The two most common vector control interventions are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Together these account for almost 60% of global investment in malaria control (GMAP, 2008). The number of people protected by IRS in the WHO African Region increased from 10 million in 2005 to 78 million in 2010. In total, 185 million people were protected by IRS in 2010; representing 6% of the global population at risk (WHO, 2011a). IRS can contribute to the elimination of malaria if rigorously applied.

Historically, IRS was largely responsible for the tremendous accomplishments of malaria programmes in Europe, Asia and the Americas that resulted in hundreds of millions of lives being saved between the 1940s and the 1980s. More recently, the scale-up of IRS in Africa has contributed, together with LLINs and improved diagnostic testing and treatment, to remarkable declines in malaria burden and all cause childhood mortality. IRS is highly effective when properly applied, but it requires national programme capacity, structures, and systems (WHO, 2013c). The rationale for IRS is based on the behavior of those *Anopheles* species that rest on walls before or after biting humans.

One of the principal methods that the World Health Organization (WHO) recommends for combating malaria is indoor residual spraying (IRS). This kind of spraying is the cornerstone of fighting malaria throughout the world, and as a result of IRS malaria incidents have been reduced

or even eliminated in some regions (WHO, 2013b). IRS is based on the application of appropriate long-lasting, residual insecticide to surfaces that the vectors come into contact with. Such surfaces include the internal walls, eaves and roofs of all houses and constructions (including those that are used for domestic animal housing) in malaria-infected areas. The effectiveness of IRS is, nonetheless, well established (WHO, 1995). Many different insecticides could be suitable for IRS. An appropriate insecticide should be highly toxic to the insect, safe for humans and non target organisms, persistent on the wall or ceiling surface, acceptable to the inhabitants of the house, easy to apply, and fairly inexpensive (Rozendaal, 1997).

The choice of class of insecticide for IRS has become a critical issue with the emergence of insecticide resistance. In 2009, pyrethroids were estimated to account for 77% of spray area covered, DDT accounted for 20% of sprayed areas, and carbamates and organophosphates represented a very small proportion of global usage for vector control (WHO, 2011b). According to WHO (2006), IRS can be effective in most epidemiologic settings, as follows. In areas with unstable malaria transmission, IRS will prevent seasonal increases in transmission, will prevent and control epidemics, and can eliminate local transmission of malaria. In areas with stable endemic malaria with moderately intense but seasonal transmission, IRS will prevent seasonal increases in transmission and reduce malaria prevalence and seasonal increases in morbidity and mortality. In areas with stable-hyper endemic malaria where transmission is intensely seasonal or perennial and without much seasonal flux, IRS will reduce malaria prevalence, incidence, morbidity, and mortality when applied more frequently than in the above instances.

According to Greek Ministry of Rural Development and Food (2013) report, the efficacy of IRS is mainly affected by the following factors. The biology and ethology of the *Anopheline* vectors of malaria in a region, such as endophily (resting inside houses) and endophagy (blood feeding inside houses) of mosquitoes as well as partial endophagy (resting in the habitat for a short period of time after blood feeding).Total and uniform coverage of spray able surfaces within habitats, such as walls, roofs and other possible resting places of disease vectors. The co-operation of the local population in full coverage of their houses, including avoiding work, such as plastering, washing and painting, that can negatively affect the residual activity of insecticides.

Indoor residual spraying (IRS) is one of the effective strategies against anopheline, such as *An. gambiae* s.l. and *An. funestus*, the main malaria vectors in Africa (Matola & Mgayuka, 1981; Najera et al., 2011). In 2007-2009, some countries (Botswana, Namibia, South Africa, and Swaziland) achieved ≥ 50% reduction in malaria cases by reaching > 70% coverage of IRS (WHO, 2010). Coverage of IRS is indeed increasing, but there is need to assess how far it is reaching the targeted populations and where else it would have added effect. In addition, a question mark hangs over their long-term effectiveness. In parts of Africa where infrastructure is especially weak, universal vector control coverage may not be achieved with IRS alone and LLINs will continue to be needed to achieve and sustain this goal.

2.3. Malaria Vector Control in Ethiopia

The national malaria vector control guideline shows that the National Strategic Plan for Malaria Control and Prevention in Ethiopia (NSP) 2006-2010 aimed to rapidly scale-up malaria control interventions to achieve a 50% reduction of the malaria burden, in line with global Roll Back Malaria (RBM) partnership objectives. The status of coverage of the major interventions was measured in the Malaria Indicator Survey (MIS) 2007. The MIS 2007 results show tremendous achievements by Ethiopia‘s malaria control program. Thus, between 2005 and 2007, insecticide-treated net (ITN) coverage increased 15-fold, with ITN use by children under five years of age and pregnant women increasing to nearly 45% in malaria-endemic areas and to over 60% in households that owned at least one ITN. Overall, 68% of households in malaria-endemic areas were protected by at least one ITN and/or indoor residual spraying of households with insecticide (IRS). The insecticides commonly used in the country include Dichlorodiphenyltrichloroethane (DDT), malathion and deltamethrin. Due to resistance of malaria vectors to DDT, the use of this insecticide for IRS has been discontinued in 2009. Deltamethrin is currently being used as an interim substitute insecticide for DDT in IRS operations. However, the selection of insecticides for IRS use in Ethiopia will be determined annually based on the insecticide resistance pattern of the vectors and other factors. Environmental management, supported by active participation of the community and use of larvicides are other preventive measures. This guideline incorporates

the three major vector control measures, namely environmental management, IRS, and LLINs (MoH, 2012).

2.3.1. Indoor Residual Spraying in Ethiopia

In Ethiopia, IRS was first implemented in the late 1950s. Though the malaria prevention and control program in the country has employed several organizational approaches, from the highly centralized vertical malaria eradication setting to an integrated and decentralized approach, IRS remains a key component of the national malaria prevention and control strategy. Though Ethiopia has a long history of conducting IRS, community knowledge, attitude and practices with regards to IRS are limited. Community acceptance of IRS is variable, with some areas having high levels of re-plastering of household walls following the application of insecticides. An integrated and intensive effort in SBCC regarding IRS is necessary, using the HEP, schools, community-based organizations (CBOs) and various media outlets. In most cases, malaria transmission follows the bimodal rainfall pattern in Ethiopia, with rainy seasons usually occurring in March-April and June/July-September. IRS campaigns are time-consuming and require sufficient lead time as well as access to the entire community in targeted areas. Sufficient time is required to treat all target communities before the onset of transmission to avert possible epidemics. The timing should also allow the spray team access to all targeted communities (i.e. avoid cut-off due to rain interference, denied road access, full streams and gorges).

The timing of IRS operations is usually determined by the residual efficacy period of the insecticide used and the length of the malaria transmission period. As a result, IRS operations in most parts of the country have taken place around the month of June. This timing was based on the six-month residual efficacy period of DDT and pyrethroids and the September-November main malaria transmission period. Because of mosquito resistance to DDT and pyrethroids, and the necessary switch to alternative insecticides with different residual efficacy (e.g. three months), an adjustment in the timing of spray operations is likely to take place in the future.

Bioassay test for insecticide deposits on different wall surfaces is used to assess the potency of an insecticide deposits to adult mosquitoes with proven susceptibility at various time intervals

after application on different surfaces and to detect the decline of toxic effect of the deposit. The mortality and knockdown results from the WHO cone bioassay test revealed that those insecticide formulations have a reduced efficacy, although it caused some mortality and knockdown rates compared to the unsprayed wall surfaces.Nearly all members of *An. gambiae* complex, that are potent vectors of malaria in tropical Africa, have shown various degrees of resistance to commonly applied insecticides like DDT (dichlorodiphenyltrichloroethane) and pyrethroids. *An. arabiensis*, and *An. gambiae s.s.* are the most important vectors of human malaria in sub-Saharan Africa particularly Ethiopia (Coetzee *et al*., 2000).

2.4. Insecticides for IRS use

Insecticide(s) for IRS operations must be selected based on evidence. Several insecticides have been recommended for use in IRS for malaria control by WHO (Table 1).

Table 1 : WHO recommended insecticides for indoor residual spraying against malaria vectors (WHO, 2009).

Insecticide compounds and formulations (1)	Class group (2)	Dosage (g a.i./m2)	Mode of action	Effective action
DDT WP	OC	1-2	Contact	>6
Malathion WP	OP	2	Contact	2-3
Fenitrothion WP	OP	2	contact & airborne	3-6
Pirimiphos-methyl WP & EC	OP	1-2	contact & airborne	2-3

Insecticide compounds and formulations (1)	Class group (2)	Dosage (g a.i./m2)	Mode of action	Effective action
Bendiocarb WP	C	0.1-0.4	contact & airborne	2-6
Propoxur WP	C	1-2	contact & airborne	3-6
Alpha-cypermethrin WP & SC	PY	0.02-0.03	Contact	4-6
Bifenthrin WP	PY	0.025-0.05	Contact	3-6
Cyfluthrin WP	PY	0.02-0.05	Contact	3-6
Deltamethrin WP, WG	PY	0.02-0.025	Contact	3-6
Etofenprox WP	PY	0.1-0.3	Contact	3-6
Lambdacyhalothrin WP, CS	PY	0.02-0.03	Contact	3-6

(1) CS: capsule suspension; EC = Emulsifiable concentrate; SC = suspension concentrate; WG = water dispersible granule; WP = wettable powder;

(2) OC= Organochlorines; OP= Organophosphates; C= Carbamates; PY= Pyrethroids.

For a given insecticide to be used for IRS, female *Anopheline* mosquito vectors must be susceptible to the insecticide selected. Insecticides may lose their efficacy if the target insects develop resistance. Susceptibility studies should be conducted on samples of the target insect population collected from the area. If resistance is observed, another insecticide, to which cross-resistance is unlikely, must be selected (WHO, 2012b). The most important quality of a residual insecticide is its long-acting effect on a given surface and high toxicity to vector mosquitoes. The toxicity should remain effective for a period long enough to cover the malaria transmission season (WHO, 2013b).

2.5. Insecticide Formulations

Insecticides are rarely applied in their pure form. They are available as special formulations adapted to the requirements of the various application methods. Residual insecticides for IRS operations are generally formulated as water-dispersible granules (WG), wettable powder (WP), emulsifiable concentrates, or suspension concentrates. Wettable powders (WP) are dry and powdery. They appear similar to a dust but contain additional wetting and dispersing agents so that water may be added for maximum effectiveness. Wettable powders are also more highly concentrated than dusts to contain more active ingredient. Wettable powder formulations do not form a true solution when water is added, so frequent agitation of the spray tank is required to keep the formulation in suspension.

Insecticide formulation types affect the residual life of insecticides. Efficacy of active ingredients on mosquitoes is modulated by type of substrate onto which the compound is applied (Etang *et al.*, 2011). Wettable powders (WP) and water dispersible granules (WG) insecticide formulations are best suited to very porous surfaces such as mud walls, while suspension concentrates (SC) or emulsifiable concentrates (EC) are more effective on finished cement, finished wood or lumber, or painted surfaces, especially those where oil-based paints have been applied (WHO, 2013b). Among the wall surfaces there were significant differences between painted and other surfaces as indicated by differences of mean knockdown in experimental mosquitoes. The suggestion is that the differences could be attributed to the nature of spray-able surface. This is dependent on the absorptive and adsorptive properties of the surface. The persistence of an insecticide sprayed on a surface varies with the type of insecticide, its formulation and the type of surface. Most insecticides last longer on wood and thatch than on mud. Mud surfaces, cement blocks, concrete and brick absorb the insecticide, and certain types of mud may also break it down chemically (WHO, 2013b).

Table 2: Major characteristics of the different formulations and their impact on IRS (WHO, 2013b).

FORMULATION	DESCRIPTION	ADVANTAGES	DISADVANTAGES
Wettable powder (WP) and water dispersible Granule (WG)	The a.i. is added to an inert powder containing a wetting and dispersing agent. Forms a suspension in water	Effective on porous surfaces (mud bricks/ concrete walls); Easy to transport, store and use; Relatively inexpensive	Ineffective on plastic sheeting, canvas tents, oil based paint; Spray tank needs occasional agitation/ shaking; Risk of exposure to dusts and spills during mixture.
Emulsifiable concentrate (EC)	The a.i. is dissolved in an oil based solvent and emulsifiers. When mixed with water it forms a milky, white oil-in water emulsion composed of finely suspended droplets carrying the insecticide.	Easy to mix with water; Few visible deposits; More effective on cement, wood or lumber; Effective on oil based painted surfaces; High concentration of a.i. in each container	Strong smell; Absorbed by porous surface; High dermal absorption increases risk for operators; Flammable.
Suspension concentrate/flow able concentrate (SC).	Contains tiny particles of a.i. suspended in a liquid (usually water). Forms crystalline particles, but smaller than those formed with	Safer for operators; Less visible residues than WP; Effective on cement, wood or lumber and on oil	Less effective on porous surface than WP.

FORMULATION	DESCRIPTION	ADVANTAGES	DISADVANTAGES
	WP and WG.	based paints.	
Capsule suspension (CS)	The a.i. is encapsulated in microscopic polymer capsules. Suspended in water for spraying.	Capsules release the insecticide slowly after spraying, extending compound's residual life.	Constant agitation is needed

2.6. Vector Resistance to Insecticides

Insecticide resistance can be defined as the ability of a population of insects to tolerate doses of an insecticide that would prove lethal to the majority of individuals in a normal population of the same species, developed as a result of selection pressure by insecticide. A population is termed resistant only when marked divergence from the norm has been confirmed by a standard test of a sample of the insects. The operational criteria of resistance has usually been taken as the survival of 20% or more individuals tested at the known diagnostic concentrations of commonly available pesticides using WHO test kits in the field. As DDT has been in use for IRS since 1955 in Ethiopia, the main malaria vector in Ethiopia has become resistant to DDT. Additionally, preliminary results showed resistance to deltamethrin, lambdacyhalothrin and malathion (MoH, 2012). This indicates the need to use alternative insecticides and vector control measures, prepare a comprehensive insecticide management strategy, and highlight the need for maximum precaution and continuous monitoring of the status of vectors' susceptibility/ resistance to insecticides at field level (MoH, 2012).

2.7. Insecticide resistance mechanisms

Typically two major mechanisms are assumed to be responsible for insecticide resistance: changes in the target site that reduce the binding of insecticides, and increases in the rate of insecticide metabolism that lower the amount of insecticide reaching the target site. Both of these resistance mechanisms are known to contribute to pyrethroid resistance in malaria vectors and are subjects of extensive research to determine their distribution and impact, and to develop improved methods of detection. Of these, target site resistance is best understood and molecular diagnostics to detect this resistance mechanism are now integrated into insecticide resistance monitoring strategies in some malaria control programmes (Ridl, *et al.,* 2008; Sharp, *et al*. 2007).

Metabolic resistance is more complex but recently advances have been made in identifying the key enzymes responsible for insecticide detoxification, paving the way for the development of molecular markers for this resistance mechanism. Although these two mechanisms clearly play a major role in conferring pyrethroid resistance, it is also important to consider other physiological or behavioural changes in the mosquito population that may impact on the efficacy of pyrethroid insecticides.

2.7.1. Target site resistance

The pyrethroid insecticides and the organochlorine insecticide (DDT) target the voltage-gated sodium channel on the insects' neurones (Naraheshi, 1996; Soderlund & Bloomquist, 1989). Their binding delays the closing of the sodium channel prolonging the action potential and causing repetitive firing, paralysis and eventual death of the insect. Alterations in the target site that cause resistance to insecticides are often referred to as knockdown resistance or *kdr* alleles in reference to the ability of insects with these alleles to withstand prolonged exposure to insecticides without being 'knockdown'. Several mutations in the sodium channel have been associated with resistance to pyrethroids in a variety of insects (Davies *et al*., 2007). One of the most common amino acid replacements, and so far the only residue associated with pyrethroid resistance in malaria vectors, is a substitution of the leucine residue found at codon 1014 with either phenylalanine (1014F) or serine (1014S). It is very clear that *kdr* gene is associated with resistance to pyrethroids and DDT but it is not evident that the presence of this resistance allele

alone is sufficient to result in control failure.

2.7.2. Metabolic resistance

Metabolic resistance occurs when elevated activities of one or more enzymes results in a sufficient proportion of the insecticide being sequestered or detoxified before it reaches the target site to impair the toxicity of the insecticide. The cytochrome P450s are the primary enzyme family responsible for pyrethroid metabolism (Feyereisen, 2005). There are 111 P450 enzymes in *An. gambiae (*Ranson & Hemingway, 2008*)* and, as in other insects, only a small number of these enzymes are capable of detoxifying insecticides.

Identifying the enzymes responsible has been facilitated by microarray based approaches to detect detoxification genes that are over expressed in resistant mosquitoes compared to susceptible populations from the same region (David *et al.,* 2005*)*. This approach has identified three 'candidate' P450 enzymes that were found to be repeatedly over expressed in pyrethroid resistant populations: CYP6M2, CYP6P3 and CYP6Z2 (Muller *et al.*, 2007). Functional characterization of these enzymes has shown that they are all able to bind to pyrethroid insecticides but only two of these, CYP6P3 and CYP6M2 can metabolise the insecticide (McLaughlin *et al.*, 2008). It is possible that elevated expression of an enzyme that can bind but not detoxify the insecticide may result in resistance by reducing the total bioavailability of insecticide (as is seen with over expression of carboxylesterases in organophosphate resistant populations of Culex mosquitoes (Raymond *et al*., 1998) provided that the enzymes are expressed in an appropriate tissue.

2.7.3. Cuticular resistance

Reduced uptake of insecticide, often referred to as cuticular resistance, is frequently described as a minor resistance mechanism (Plapp, & Hoyer, 1968). Certainly for pests where the major route of insecticide delivery is via ingestion, this is likely to be the case. However for malaria control, where insecticides are typically delivered on bed nets or on wall surfaces, uptake of insecticides is primarily through the appendages. Earlier experiments with DDT have shown that the hind legs make the greatest contact with insecticide treated surfaces and removal of this pair of legs reduces mortality in DDT susceptibility tests (Ungureanu & Burghele, 1959). A better

understanding of the processes involved in insecticide uptake could be translated into improvements in insecticide formulations to help overcome pyrethroid resistance.

2.7.4. Interactions between resistance mechanisms

Understanding patterns of cross resistance caused by alternative mechanisms is vital to the implementation of effective resistance management strategies. It is generally assumed that resistance renders the selecting insecticide, and all others with a similar mode of action, ineffective. For example, the high frequency of *kdr* mutations in malaria vectors is often attributed to extensive past use of DDT to control agricultural pests in Africa (Akogbeto & Noukpo, 2005). This assumption may hold true for target site resistance; it is clear that selection with DDT can cause cross resistance to pyrethroids and vice versa. However this may not necessarily be the case for metabolic resistance mechanisms. CYP6P3 and CYP6M2 are efficient at detoxifying pyrethroids but do not metabolize DDT. On other hand metabolic resistance may confer resistance to more than one class of insecticides. Bioassays using synergists should be utilized to elucidate the full spectrum of cross resistance prior to implementation of any resistance management strategies.

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

Wall bioassay test of field population of *An. gambae s.l.* was assessed using WHO cone bioassay tests to deltamethrin 25%WG, lambdacyhalotherin 10%WP and lambdacyhalotherin 10%CS, in Jimma, Ethiopia from April to August 2014. The two study sites were Kersa district (Gelo Keble) and Jimma town (Becho-Bore Keble) with an altitude of ranging from 1714-1748 and1710-1748 meters above sea level respectively Jimma zone, Oromia Regional State, Southwestern Ethiopia. They sites were selected purposively, while Gelo keble was selected randomly among Bulbul, Kitinbele and Ankeso Kebles and Bacho-Bore by preliminary survey results of *An. gambiae s.l.* larva among Bosa Kito, Ginjo Guduru and Seto Semero Kebles of Jimma town. Other factors considered were accessibility, severe and more frequent malaria epidemics, the density and availability of the principal vector *An. gambae s.l.* and outbreaks had been reported previously. Also, the local malaria vector, *An. gambiae s.l.* is known to feed and rest indoors and therefore more susceptible to IRS control strategy that was planned to take place.

Becho-Bore Keble of Jimma town, it is located 350Km southwest of Addis Ababa. The town's geographical coordinates are approximately 7°41' N latitude and 36° 50'E longitude. The town is found in an area of average altitude of about 1780 m above sea level. It lies in the climatic zone locally known as Woyna Dega which is considered ideal for agriculture as well as human settlement. The town is generally characterized by warm climate with a mean annual maximum temperature of 30°c and a mean annual minimum temperature of 14°c. The annual rainfall ranges from 1138 mm to 1690 mm. Maximum precipitation occurs during the three months period, June to August, with minimum rainfall in December and January.

Kersa district is about 318Km southwest from the capital, Addis Ababa, while Gelo Keble is on the main route to Addis Ababa 35Km Northeast of Jimma town. The district is bordered on the south by Dedo district, on the southwest by Seka Chekorsa district, on the west by Mana district, on the north by Limmu Kosa district, on the northeast by Tiro Afeta, and on the southeast by Omo Nada district(Fig 1). Serbo is the administrative town of Kersa district.

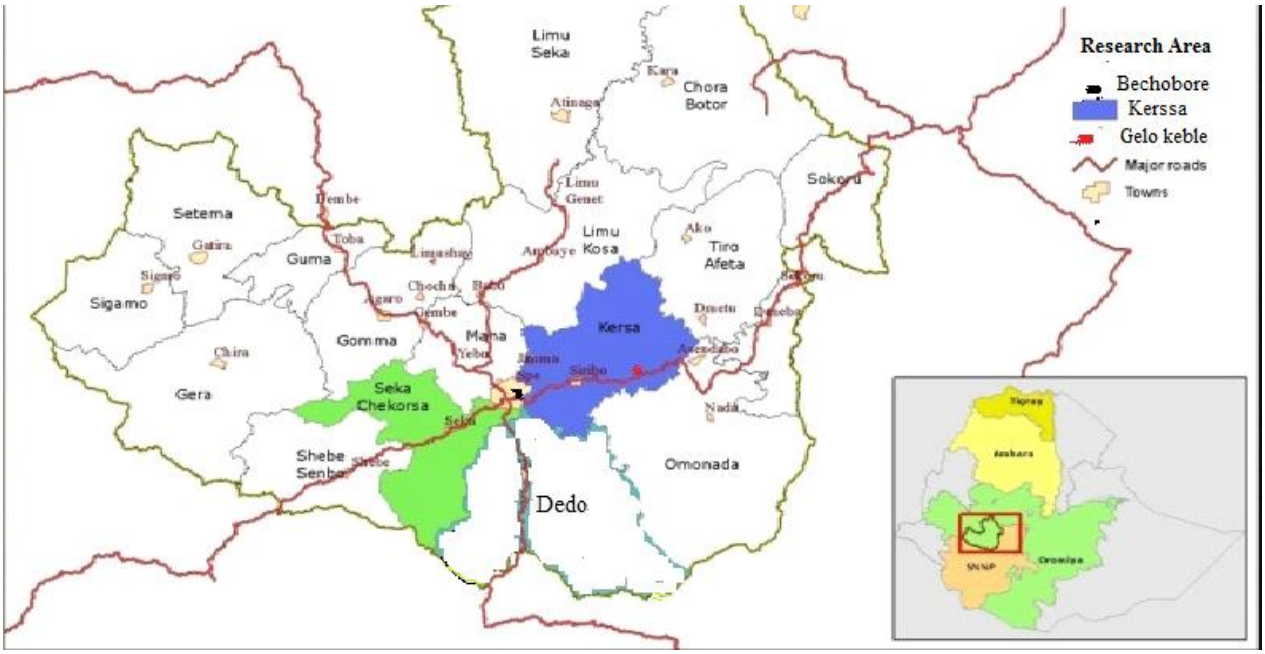


Figure 1. Map showing study sites

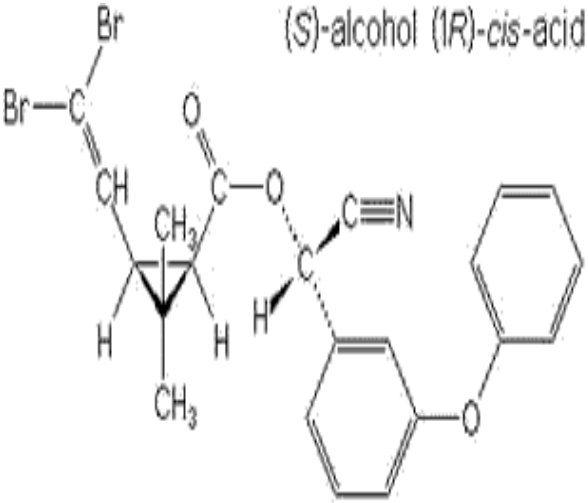
3.2. Candidate Insecticide Formulations

PALI 250 WG contains deltametrin 25% WG, REVIVAL 100 WP contains lambdacyhalothrin 10%WP and REVIVAL 100 CS contains lambdacyhalothrin 10% CS, all are synthetic pyrethroieds recommended by WHO for IRS application and they applied alone (www.tagros.com, manual).

Deltamethrin 25% WG, lambdacyhalothrin 10% WP and lambdacyhalothrin 10%CS are suitable for IRS application on the walls and animal shelter, in order to control the adult vector mosquitoes that land and rest on these surfaces. They reduce the life span of vector mosquitoes so that they can no longer transmit malaria parasites from one person to another, and reduces the density of the vector mosquitoes. They also repel mosquitoes and by doing so reduce the number of mosquitoes entering the sprayed room, and thus reduces human-vector contact.

The common, trade and IUPAC name of PALI 250 WG is deltamethrin, PALI 250 WG and(*S*)-a-cyano-3-phenoxybenzyl(1*R*,3R)-3-(2,2-dibromovinyl)-2,2dimethylcyclopropanecarbo-xylate respectively. The empirical formula for this candidate insecticide is $C_{22}H_{19}Br_2NO_3$, while its

molecular weight is 505.2gm. It is water dispersible granule (WG). PALI 250 WG prevents the Sodium ion channels of the insect nervous system from functioning, so that no transmission of nerve impulses can take place. The recommended dosage is 0.02- 0.025 gm/m^2 for malaria vector control. PALI 250 WG have ecotoxicity on birds, (*Japanese quails)* LD_{50} > 5000ppm, Fish, (*Poecilia reticulate)* LC_{50} (96 hr): 1.74 µg/l, (*Daphinia magna)* EC (24hr): 4.15µg/l, Bees, (*Apis indica)* LD_{50}: 0.52ppt and Earth worm, (*Lampito mauritii)* LD*50* > 1000 mg/kg dry soil. (www.tagros.com).

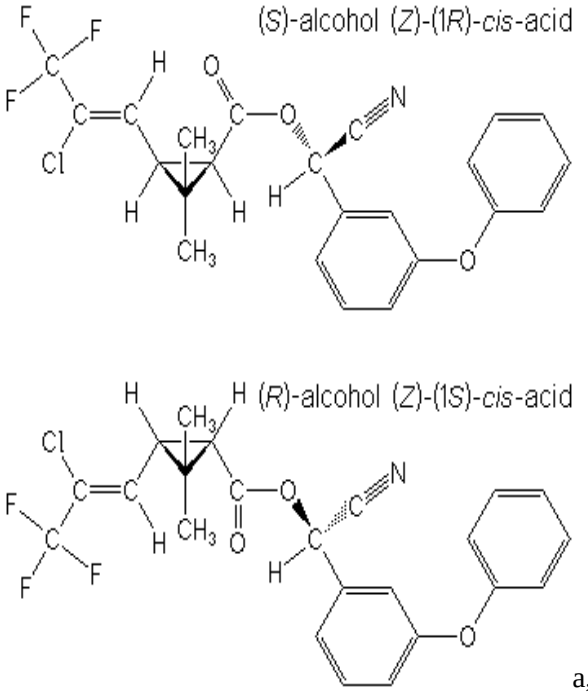


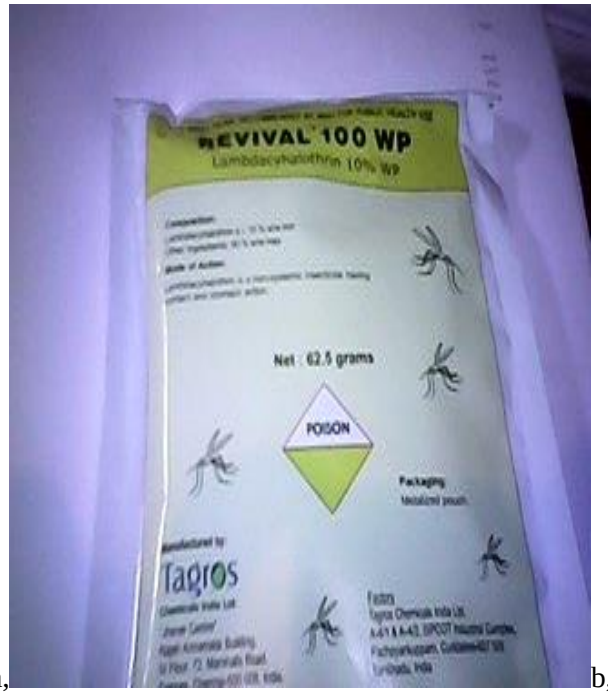
a,

b,

Plate 1. (a) Structural Formula of PALI 250 WG and (b) one sachet of PALI 250 WG

The common, trade and IUPAC name of REVIVAL 100 WP is lambdacyhalothrin, REVIVAL 100 WP and (*S*)- α-cyano-3-phenoxybenzyl (*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (*R*)-α-cyano-3-phenoxybenzyl (*Z*)-(1*S*,3*S*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate respectively. It is wettable powder form.

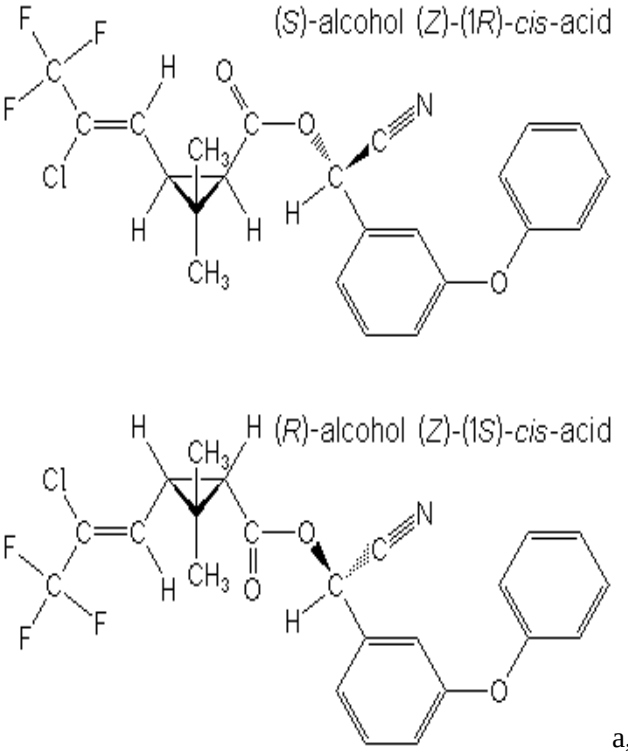


a,


b,

Plate 2. (a) Structural formula of REVIVAL 100WP and (b) one sachet of REVIVAL 100WP

The common , trade and IUPAC name of REVIVAL 100 CS is lambdacyhalothrin, REVIVAL 100 CS and (*S*)- α-cyano-3-phenoxybenzyl (*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (*R*)-α-cyano-3-phenoxybenzyl (*Z*)-(1*S*,3*S*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2 dimethylcyclopropanecarboxylate respectively. It is capsule suspension (CS). Their empirical formula for these candidate insecticides is $C_{23}H_{19}ClF_3NO_3$, while their molecular weight is 449.9gm. They prevent the Sodium ion channels of the insect nervous system from functioning, so that no transmission of nerve impulses can take place. The recommended dosage of REVIVAL 100 CS is 0.02- 0.025 gm/m^2 and REVIVAL 100 WP is 0.02- 0.03 gm/m^2 for malaria vector control. Both have ecotoxicity on birds, (*Japanese quails)* LD_{50} > 5000ppm, Fish, (*Cyripinus carpio)* LC_{50}: 0.49 g/l, (*Daphinia magna)* EC: 0.27 µg/l, Bees, (*Apis indica)* LD50: 0.068 µg and Earth worm, (*Eisenia fetida)* LD_{50} > 1000 mg/kg dry soil weight. (www.tagros.com and hand manual).



a,


b

Plate 3. (a) Structural formula of REVIVAL 100 CS and (b) one sachet of REVIVAL 100 CS

3.2.1. Mode of Action

PALI 250 WG, REVIVAL 100 WP and REVIVAL 100 CS are all non-systemic insecticides with contact and stomach action. When mosquitoes come in contact with sprayed wall and roof surfaces, they get in contact with the insecticide and become restless and either fly out of the house or get knockdown. Those insecticide formulations when mixed with water, they form a homogenous suspension (Technical Operation Manual).

3.3. Study Design

The design of the study for efficacy evaluation was completely randomized while, longitudinal for residual evaluation.

3.4. Collection and rearing of field populations of *An. gambiae s.l.*

Anopheles mosquito larvae and pupae were collected by dipping from a range of breeding sites (road paddies, brick pits, pools, marshes, surface water harvests mainly from Becho-Bore Kebele

of Jimma town, Jimma zone, Oromia Region. They were kept on larval tray for rearing and the pupae were collected using pipette and put in a beaker inside the cage to develop to adult. The collection sites were determined based on: a) anticipated high vector densities to allow collection of sufficient numbers for assays, b) based on previous susceptibility assays and/or historical use of insecticides, and c) ease of access to facilitate collections. Accordingly, Gelo Keble of Kersa district and Becho-Bore Keble of Jimma town were selected for the study.

Preliminary survey of *An. gambiae s.l.* larva and pupa collection from four Kebeles in Jimma town including Bosa Kito, Ginjo Guduru, Seto Semero and Bacho-Bore has been done among this Bacho-Bore Keble; there are abundant numbers (density). In this Kebele; there are brick makers such site is an ideal breeding site for them, even during the dry season brick makers bring water there and it makes a good breeding site.


a


b

Plate 4. (a) *An. gambiae s.l.* larvae & pupae collection by dipping (b) Larvae of *An. gambiae s.l.*

Larvae and pupae were collected from different breeding sites using dippers and then transported to the field insectary. Larvae were provided with bakery yeast to be reared to adults under standard conditions of temperature and relative humidity. Non-blood fed adult females of 2 – 5 days old were used for bioassays. The bioassays were carried out within marked areas on the wall of selected houses to assess the persistence of the residual activity on various sprayed wall surfaces. The inhabitants were informed not to alter the marked areas by re-plastering, or painting.


a


b,

Plate 5. (a) Collecting Pupae in beaker for adult emergence for the assay (b) Transferring 2-5 days old adult female *An. gambiae s.l.* from cage to cup by aspiration

3.5 Household and wall surface selection

Two "Kebeles" (the smallest administrative unit in Ethiopia) namely "Becho-Bore" from Jimma town and "Gelo" from Kersa district were selected from southwestern Ethiopia. Five houses with their wall made of mud but not plastered (up), five houses with their wall made of mud and plastered (p) and five houses with their wall made of mud, plastered and painted were randomly selected and coded from Becho-Bore Kebele. Similarly five houses with their wall made of mud

but not plastered and five houses with their wall made of mud and plastered were selected and coded randomly from Gelo Kebele. Four separate plots of wall surfaces (2sq.meter size each) were measured, marked and labeled with the name of insecticides (WG for PALI 250WG, WP for REVIVAL 100 WP, CS for REVIVAL 100CS and UNSPRAYED to be used as control) in each house. Three WHO Insecticide wall bioassay cones were fixed in each plots of wall surfaces at height of 0.5m, 1m, and 1.5 respectively from the ground. 3cm shoe nails were used to fix the perimeter of the cones in to the wall surfaces. Thus a total of 12 WHO Insecticide wall bioassay cones (9 for Insecticide formulations and three for control) were fixed in each house.

3.6Application of insecticide formulations on wall surfaces

Peoples living in the houses were informed to remain outside for three hours before re entering the treated houses. The application methods of the insecticides were based on the manufacturer recommendation; one sachet of each 20gm of PALI 250 WG, 62.5 gm of RIVIVAL 100WP and RIVIVAL 100 CS was used to cover an area of 200-250 square meters. The inner water soluble sachet was put directly into spray tank containing 10 liter of clean tape water for absorbent surfaces and 5 liter of clean tape water for non-absorbent surfaces. Before use the spray tank was closed and shaked well. To ensure that the soluble sachet is completely dissolved to prevent possible filter and nozzle clogging the tank was periodically shaked for 3 to 5 minutes.

Those formulations were applied using hand held compressor sprayer fitted with nozzle suitable for indoor residual application. 8002 nozzle for absorbent surface (mud, unpainted cement) and 8001 nozzle for non-absorbent surface (painted) have been used during spraying. Before spraying start the equipment was checked to ensure that all parts were in good working condition and operational.

For bio-efficacy and persistence evaluation of the candidate insecticide formulations, walls of the living room in each of the selected houses were sprayed with candidate insecticide formulations to make a homogenous residual deposit of the desired concentration; and a code (WP for lambdacyhaothrin 10% WP, WG for deltamethrin 25% WG and CS for lambdacyhaothrin 10% CS) were labeled for each respective insecticides at the visible and different parts of the wall. Internal walls of the selected houses were sprayed at a dosage of for the three insecticides; 25mg. a.i /m^2 deltamethrin 25% WG; 30mg a.i /m^2 lambdacyhaothrin 10% WP and lambdacyhaothrin

10% CS. Sprays were applied using Hudson® spryer. Pre-dosed sachets of insecticides were mixed based on the manufacturer's recommendations in ten litters and five litters of clean water in the sprayer for absorbent and non absorbent wall surfaces respectively. Bio-efficacy of IRS was assessed one week after treatment and then every month for the three months of the trial period.

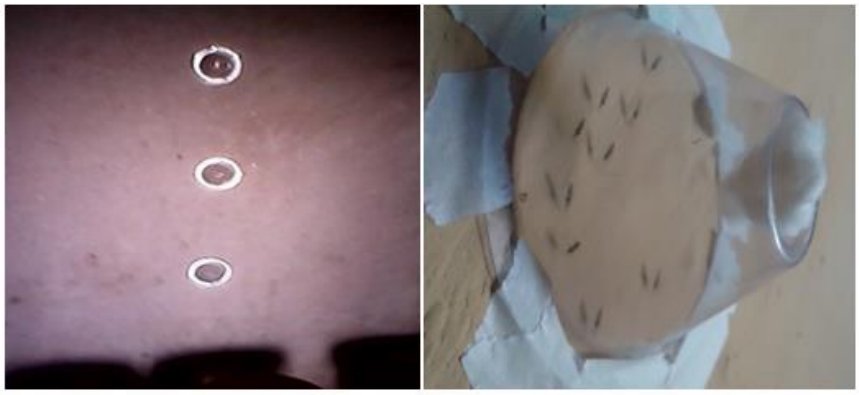
3.7. Wall Bioassay

Ten (2-5 day) aged non-blood fed female mosquitoes were introduced into Conical chambers of transparent plastic, 8.5cm in diameter at the base, 3.2cm diameter at the top and 5.5cm high for an exposure period of 30 minutes (plate 6a). Knockdown was counted and recorded for each respective cone after 30 minutes. After exposure mosquitoes were transferred in to 150-300ml size paper cups covered with nylon net fastened with rubber band; provided 10% sugar solution soaked in cotton wool placed on the nylon net provided and transported to the insectary room. The insectary room was maintained under standard conditions of temperature and relative humidity at (27 °C ± 2 °C and 80% ± 10% RH) (Plate 6b). Mosquitos' mortality was recorded 24hrs post exposure for each type of wall surface and insecticides (WHO, 2006).

Concurrently similar number of An. *gambiae s.l.* was used for all three types of insecticide formulations and control cones. The efficacy and residual activity of the three candidate insecticide formulations were monitored for three months. A total of 120 mosquitos were used per house per unit time. Total of 25 houses (10 house from Gelo Kebele and 15 houses from Becho-Bore Kebele) were selected for the trial. Thus, a total of 3000 (3-5 day old) female mosquitoes (25*120) were used in each round of the trial and grand total of 12,000 female anopheles mosquitoes were used for four round (first week, month one , month two and month three) experiment.

Mean percentage knockdown and mortality were computed for each treatment. Knockdown was calculated from the percentage of mosquitoes lying on their back or side. Mortality was calculated from the percentage of mosquitoes die out. WHO recommendation for assessing the significance of detected resistance is, 98% – 100% mortality at the recommended application of IRS insecticides indicates susceptibility; 80% –97% mortality at the recommended application of IRS insecticides suggests the possibility of resistance that needs to be confirmed and < 80%

mortality at the recommended IRS insecticides suggests resistance (WHO, 2006).



a

b

Plate 6. (a) Wall bio-assay test (b) 24hr holding period after exposure

4. DATA ANALYSIS

Data were analyzed using SPSS software package for windows version 20.0 and Excel MS 2007. To determine whether IRS was effective knockdown and mortality rates of populations of *An. gambiae s.l.* were calculated (WHO, 1998). Treatment was considered effective when knockdown and mortality rates of mosquitoes on exposed wall surfaces were greater than 95% and 85%, respectively. Mean knockdown and mortality rates of *An. gambiae s.l.* were compared among different wall surfaces, height of walls, time of test and treatments using Analysis of variance (ANOVA), for significant ANOVA post hoc was checked for mean separation. $P < 0.05$ and 95% Confidence interval (CI) was considered significant during the analysis. T-test was used to compare mean knockdown and mortality rates of *An. gambiae s.l.* between the two sites. Abbott's formula was used to correct mortality rates of *An. gambiae s.l.* when mean mortality on control wall surface was between 5-20% (WHO, 2006).

5. ETHICAL CONSIDERATIONS

This study was reviewed and approved by the Research and Ethics Committee of College of Natural Science, Jimma University. To conduct this study, the purpose was also explained and communicated to District and Kebele officials through official letters from Jimma University and oral and written consent were obtained from head of selected households (HHs) before the study. Study households were selected based on: permission from head of a household to execute this study after confirmation that the wall not swept or plastered until the end of the study; the house not sprayed with any residual insecticide for the last three months (to avoid any residual effect) and the house were not sprayed with any kind of insecticide or re-plastered until the test was finished.

6. RESULTS

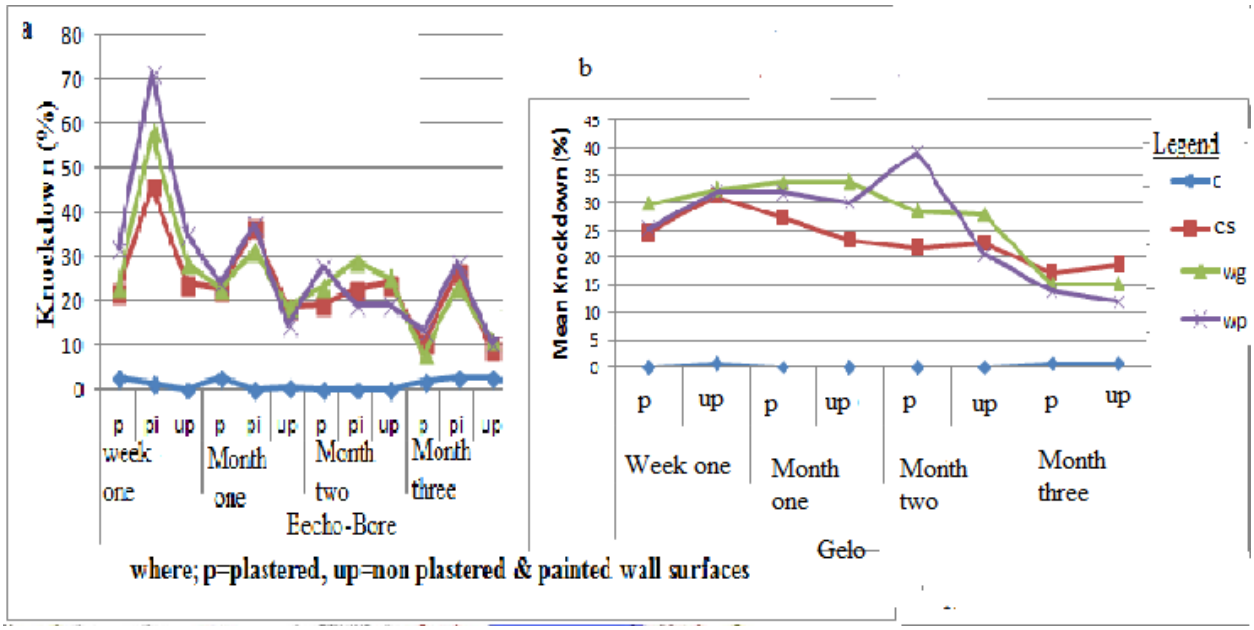
6.1. Knockdown rates of mosquitoes

Knockdown of populations *An. gambiae s.l.* after 30 minutes of exposure time on non-sprayed surfaces of painted, non-plastered and plastered wall surfaces were recorded. When comparing the two wall surfaces (non-plastered and plastered), the mean knockdown rates of populations of *An. gambiae s.l.* were always below 3%. The maximum mean knockdown of *An. gambiae s.l.* recorded was 2.6% for week one and month one on plastered, and also on painted–and non-plastered wall surfaces at month three (Fig. 2). For plastered wall surfaces, the maximum mean knockdown rates of populations of *An. gambiae s.l.* was 1.33% for week one, month one and month three in which there was no knockdown for month two. On the non-plastered wall surfaces, the maximum observed mean knockdown rate of *An. gambiae s.l.* was 1.67% for month three. There was no knockdown of populations of *An. gambiae s.l.* at month one and month two.

Mean knockdown rates of field populations of *An. gambiae s.l.* after exposure to sprayed wall surfaces of painted, non-plastered and plastered was presented in (Fig. 2). Different patterns of mean knockdown rates of *An. gambiae s.l.* were recorded among the three wall surfaces, by insecticide formulations, site and duration of the spray deposit (Fig. 2a).

The highest mean knockdown rate of An. gambiae s.l. was observed on painted wall surfaces (Fig. 2b). Mean knockdown rates of *An. gambiae s.l.* exposed to painted wall surfaces sprayed with lambdacyaholtherin 100WP, deltametherin 250 WG and lambdacyaholtherin 100 CS after one week was 71.33%, 58% and 46.0%, respectively. The lowest mean knockdown rate of *An. gambiae s.l.* recorded for non-plastered wall surfaces sprayed with lambdacyaholtherin 100WP and deltametherin 250 WG after three months was 11.3%. There was significant difference of mean knockdown rate of *An. gambiae s.l.* when the three candidate insecticide formulations (lambdacyaholtherin 10% WP, lambdacyaholtherin 10% CS and deltametherin 25% WG) sprayed on three types of wall surfaces (Table 3). Post spray mean knockdown rates of mosquitoes for lambdacyaholtherin 10% WP after a week on painted, plastered and non plastered wall surfaces was 71.33%, 28.68% and 33.67%, respectively. Mean knockdown rates of *An.*

gambiae s.l. on painted, plastered and non plastered wall surfaces after a week sprayed with deltametherin 25% WG was 58%, 26.67% and 30.67%, respectively. While mean knockdown rates of *An. gambiae s.l.* for week one on painted, plastered and non plastered wall surfaces sprayed with lambdacyaholtherin 10% CS was 46%, 23.33% and 27.67%, respectively.



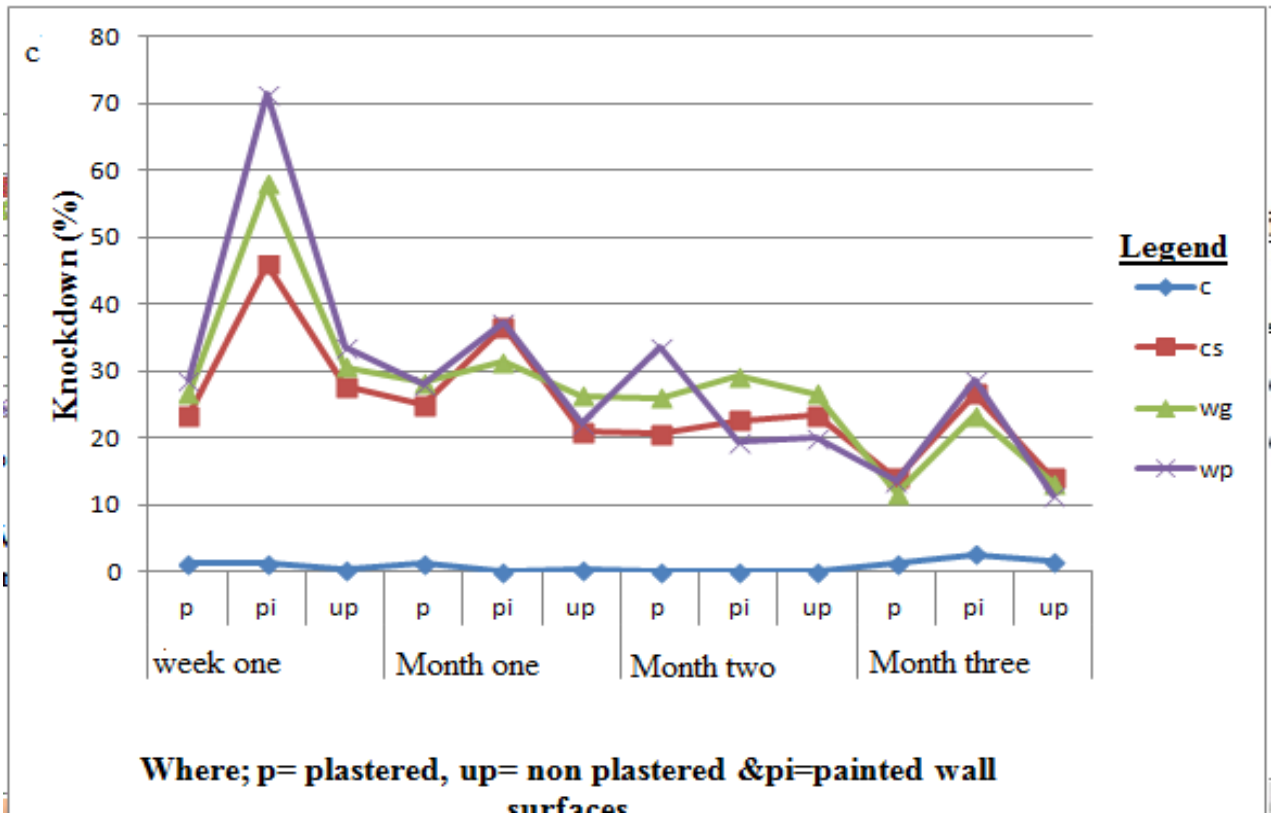


Figure 2. Mean knockdown rates (%) of field populations of *An. gambiae s.l.* exposed to different wall surfaces sprayed with candidate insecticide formulations and on control wall surfaces (a) by site and time of test (b) by time of test

6.2.Mortality rates of mosquitoes

As showed in Figure 3, mean mortality rates of the field populations of *An. gambiae s.l.* exposed to three control types of wall surfaces (painted, non-plastered and plastered). The mean mortality rates of *An. gambiae s.l.* on non sprayed wall surfaces was recorded on the three wall surfaces, the maximum value was 9.3% on non plastered and 4.0% on plastered wall surfaces during month one and month three respectively at Becho-Bore site. The mean mortality rates of *An. gambiae s.l.* during the study time were below 5.0%. No mortality of *An. gambiae s.l.* effect was observed after exposure of mosquitoes to control wall surfaces except month one at Becho-Bore site on non plastered wall surface which was 9.3%.

The mean mortality rate of *An. gambiae s.l.* for month one, on non plastered wall surface sprayed with labdacyaholtherin 10% CS, deltametherin 25% WG & labdacyaholtherin 10% WP insecticide formulations and on control wall surface was 60%, 58%, 52% & 9.3%, respectively.The corrected mean mortality rate of *An. gambiae s.l.* for lambdacyaholtherin 10% CS, deltametherin 25% WG & lambdacyaholtherin 10% WP insecticide formulations was 55.9%, 53.75 & 47.1%, respectively.

The effects of the three candidate insecticide formulations on mean knockdown and mortality rates of *An. gamiae s.l.* exposed to sprayed wall surfaces were assessed over three months. The mean mortality rates of *An. gambiae s.l.* exposed to the sprayed wall surfaces remained low throughout the trial period (week one, month one, month two and month three) (Fig. 3b). The highest mortality rates of *An. gambiae s.l.* sprayed with lambdacyaholtherin 10% WP on painted, plastered and non plastered wall surfaces during week one was 85.33%, 83.33% and 81.0%, respectively. Mean mortality rates of *An. gambiae s.l.* on painted, non plastered and plastered wall surfaces sprayed with deltametherin 25% WG during week one was 88%, 77.67% and 76.33%, respectively during week one. The mean mortality rates of *An. gambiae s.l.* sprayed with lambdacyaholtherin 10% CS was below 85.0% irrespective of time of test and wall surface types. The highest mean mortality rates of *An. gambiae s.l.* on painted, non-plastered and plastered wall surfaces during week one was 82.0%, 74.0% &72.67%, respectively and declined from week one to month three. Mean mortality rate of *An. gambiae s.l.* on painted wall surfaces at month three for all sprayed the three candidate insecticide formulations was 48%.The lowest mean mortality rate of *An. gambiae s.l.* mosquitoes recorded was 42.66% on plastered wall surface sprayed with deltametherin 25% WG at month three. There was no significant difference observed on mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted sprayed wall surfaces ($P > 0.05$).

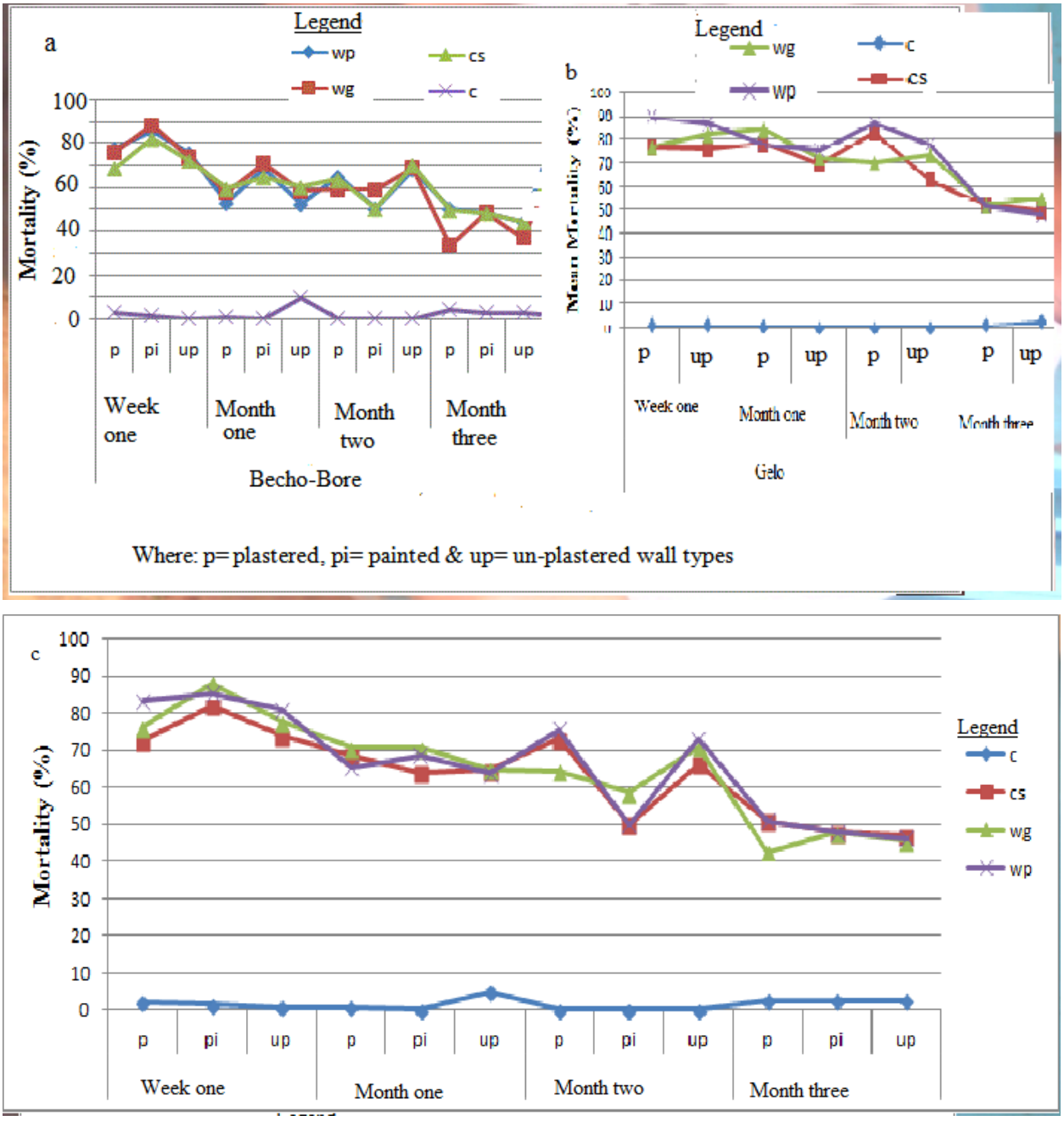


Figure 3. Mean mortality rates (%) of field population of *An. gambiae s.l.* exposed to wall surfaces sprayed with candidate insecticide formulations and on control wall surfaces (a) by site and time of test (b) by time of tests

Analysis of variance (ANOVA) revealed that there was a significant difference in mean knockdown and mortality rates of populations of *An.gambiae s.l.* among time of test and

treatments ($p < 0.05$). There was also significant difference in mean mortality rates of *An.gambiae s.l.* exposed to wall surface sprayed with lambdacyaholtherin 100 CS for both lambdacyaholtherin 100WP and deltametherin 250 WG. However, mean mortality rates of *An. gambiae s.l* exposed to wall surfaces sprayed with lambdacyaholtherin 100WP and deltametherin 250 WG were similar ($p > 0.05$).

The residual efficacy of the candidate insecticide formulations were varied between porous (plastered and non-plastered) and non porous (painted) sprayed wall surfaces. At week one, mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with deltamethrin 25%WG was 76.33%, 77.67% and 88.0%, respectively. Observed mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with lambdacyaholtherin 10% WP during week one was 83.33%, 81.0% and 85.33%, respectively. While observed mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with lambdacyaholtherin 10% CS during week one was 83.33%, 81.0% and 85.33%, respectively.

At month one observed mean mortality rates of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with deltamethrin 25%WG was 68.67%, 64.67% and 64.0%, respectively. Mean mortality rates of *An. gambiae s.l.* on plastered, non plastered and painted wall surfaces sprayed with lambdacyaholtherin 10% WP during month one was 70.67%, 65.0% and 70.67%, respectively. While during this time observed mean mortality rate of populations of *An. gambiae s.l.* exposed to plastered, non plastered and painted wall surfaces sprayed with lambdacyaholtherin 10% CS was 65.33%, 63.67% and 68.67%, respectively. Some of observed mean mortality rates of *An. gambiae s.l.* above 85% was on painted wall surfaces during week one. Mean mortality rates of *An. gambiae s.l.* exposed to painted wall surface sprayed with lambdacyaholtherin 10% WP and deltametherin 25% WG insecticide formulations was 85.33% and 88.0%, respectively at Becho-Bore and 89.3% on plastered and 86.6% on non-plastered wall surfaces sprayed with lambdacyaholtherin 10% WP insecticide formulation at Gelo site.

Mean knockdown and mean mortality rates of *An. gambiae s.l.* were compared among wall surface types, height of walls, time of test and treatments using analysis of variance (ANOVA).

Those having significant difference test of $p < 0.05$, post hoc tests was done to assess the efficacy of the given candidate insecticide formulations (Table 3). ANOVA reveals that the mean knockdown and mean mortality of *An. gambiae s.l.* among those factors such as time of test and treatments there was significant difference ($P < 0.05$). For wall surface types, mean knockdown of *An. gambiae s.l.* was significantly difference ($p < 0.05$) while mean mortality rates of *An. gambiae s.l.* there was no significant difference ($p > 0.05$). For height of test there was no significant difference ($p > 0.05$). Post hoc tests of multiple comparisons among treatments based on observed means of knockdown and mortality rates of *An. gambiae s.l.* the control was significantly difference than the three candidate insecticide formulations. Lambdacyaholtherin 100 CS insecticide formulation was also significantly different from both lambdacyaholtherin 100WP and deltametherin 250 WG. While there was no significant different between lambdacyaholtherin 100WP and deltametherin 250 WG insecticide formulations ($p > 0.05$).

Table 3 shows significant test of mean knockdown and mortality percentage rates of populations of *An. gambiae s.l.* the interaction among the independent variables. For mean knockdown rates of *An. gambiae s.l.* there was no significant difference between the two sites ($p = 0.758$). However, there was significant difference in mean mortality rates of *An. gambiae s.l.* between the two sites ($p = 0.000$).

Table 3: Mean knockdown and mortality (%) rates of populations of *An. gambiae s.l* by site, height of wall, time, treatments and wall surface types in Becho-Bore and Gelo Kebles, Jimma zone, Southwestern Ethiopia (April to August 2014)

Source	Dependent Variable	df	Mean Square	F test	p-value
Site	Knockdown	1		0.095	0.758
	Mortality	1		15.360	0.000*
Time	Mortality	3	26375.139	160.272	0.000*
	Knockdown	3	11458.108	82.160	0.000*
Treatments	Mortality	3	271020.556	1646.897	0.000*
	Knockdown	3	47683.941	341.918	0.000*

Source	Dependent Variable	df	Mean Square	F test	p-value
Wall type	Mortality	2	377.250	2.292	0.102
	Knockdown	2	9241.312	66.265	0.000*
Height	Mortality	2	250.833	1.524	0.218
	Knockdown	2	222.656	1.597	0.203
time * Treatments	Mortality	9	3501.065	21.275	0.000*
	Knockdown	9	1834.913	13.157	0.000*
time * Wall type	Mortality	6	1603.250	9.742	0.000*
	Knockdown	6	2538.368	18.201	0.000*
time * height	Mortality	6	89.306	0.543	0.776
	Knockdown	6	75.712	0.543	0.776
Treatments * Wall type	Mortality	6	383.028	2.328	0.031*
	Knockdown	6	1213.590	8.702	0.000*
Treatments * height	Mortality	6	433.056	2.632	0.015*
	Knockdown	6	79.462	0.570	0.755
Wall type * height	Mortality	4	195.250	1.186	0.315
	Knockdown	4	89.875	0.644	0.631
time * Treatments * Wall type	Mortality	18	358.806	2.180	0.003*
	Knockdown	18	554.350	3.975	0.000*
time * Treatments * height	Mortality	18	281.898	1.713	0.032*
	Knockdown	18	61.684	0.442	0.979
time * Wall type * height	Mortality	12	2890.000	1.756	0.051
	Knockdown	12	88.264	0.633	0.815
Treatments * Wall type * height	Mortality	12	86.944	0.528	0.897
	Knockdown	12	60.153	0.431	0.951
time * Treatments * Wall type * height	Mortality	36	94.361	0.573	0.980
	Knockdown	36	91.579	0.657	0.942

* Significant at $p < 0.05$

Table 4 indicates mean separation of knockdown and mortality of populations of *An. gambiae s.l.* (post hoc tests) among treatments. Based on mean separation of mortality rates of *An. gambiae s.l.*, there was no significant difference between deltamethrin 250 WG and lambdacyhalotherin 100 WP and deltamethrin 250 WG and lambdacyhalotherin 100 CS. While there was significant difference between lambdacyhalotherin 100 WP and lambdacyhalotherin 100 CS. The mean mortality of *An. gambiae s.l.* exposed to wall surfaces sprayed with deltamethrin 250 WG, lambdacyhalotherin 100 WP and with lambdacyhalotherin 100 CS insecticide formulations out of the exposed ten mosquitoes was 6.45, 6.65 and 6.36, respectively. Based on mean separation of knockdown of *An. gambiae s.l.*, there was no significant difference between deltamethrin 250 WG and lambdacyhalotherin 100 WP. While there was significant difference between lambdacyhalotherin 100 CS and deltamethrin 250 WG and lambdacyhalotherin 100 CS and lambdacyhalotherin 100 WP insecticide formulations. The mean knockdown of *An. gambiae s.l.* exposed to wall surfaces sprayed with deltamethrin 250 WG, lambdacyhalotherin 100 WP and lambdacyhalotherin 100 CS out of the exposed ten mosquitoes was 2.6, 2.7 and 2.4, respectively.

Table 4: Mean knockdown and mortality rates of populations of *An. gambiae s.l.* by treatments (April to August 2014).

Dependent Variable	Treatments	Mean	Mean ± SE	95% CI
Knockdown	Lambdacyhalotherin 100 WP	27.0[a]	27.0 2.0	(25.0, 29.0)
	Deltamethrin 250 WG	26.1[a]	26.1 1.9	(24.1, 28.0)
	Lambdacyhalotherin 100 CS	23.5[b]	23.5 1.6	(21.9, 25.1)
	Control (C)	0.8[c]	0.8 2.3	(-1.4, 3.1)
Mortality	Lambdacyhalotherin 100 WP	64.7[a]	64.7 2.7	(62.0, 67.4)
	Deltamethrin 250 WG	64.6[ab]	64.6 2.2	(62.4, 66.8)
	Lambdacyhalotherin 100 CS	62.0[b]	62.0 2.3	(59.7.0, 64.3)
	Control (C)	1.5[c]	1.5 2.5	(-0.1, 4.0)

Means with the same letter(s) in the same column are not significantly different from each other at $P < 0.05$

Table 5 indicates mean separation of knockdown and mortality of populations of *An.gambiae s.l.* (post hoc tests) among residual time. Except between month one and month two, there is significant difference among the residual times ($p < 0.05$).

Table 5: Mean knockdown (%) and mortality (%) rates of populations of *An. gambiae s.l.* by time in Becho-Bore and Gelo Kebles, Jimma zone southwestern Ethiopia (April to August 2014)

Dependent Variable	Time of tests	Mean	Mean ± SE	95% CI
Knockdown	Week one	26.07[a]	26.07 2.25	(23.82, 28.32)
	Month one	20.53[b]	20.53 2.25	(18.28, 22.78)
	Month two	18.60[b]	18.60 2.25	(16.35, 20.85)
	Month three	12.17[c]	12.17 2.25	(9.92, 14.42)
Mortality	Week one	59.60[a]	59.60 2.46	(57.12, 62.08)
	Month one	50.50[b]	50.50 2.48	(48.02, 52.98)
	Month two	50.27[b]	50.27 2.48	(47.79, 52.75)
	Month three	36.23[c]	36.23 2.48	(33.75, 38.71)

Means with the same letter(s) in the same column are not significantly different from each other at $P < 0.05$.

Mean separation of knockdown of population of *An. gambiae s.l.* (post hoc tests) among wall surface types, there was no significant difference between plastered and non plastered wall surfaces ($p > 0.05$). However, there was significant difference between absorbent and non absorbent wall surfaces ($p < 0.05$).

The mean knockdown rates of population of *An. gambiae s.l.* exposed to wall surfaces sprayed with all the three candidate insecticide formulations starting from week one to third month were below 95% and the mean mortality rates of population of *An. gambiae s.l.* was below 85% except week one on pained wall surface sprayed with deltametherin 25% WG and lambdacyhalothrin 10% WP insecticide formulations. Both mean knockdown and mean mortality rates of population of *An. gambiae s.l.* were decaling from the first test time to third month.

7. DISCUSSION

A total of 12,000 adult female 2-5 day old mosquitoes belonging to *An. gambiae s.l.* were used throughout the study period. For each test 3,000 adult female populations of *An. gambiae s.l.* were used to evaluate the bio efficacy and residual activity of the three candidate insecticide formulations.

The World Health Organization (WHO) recommends 12 insecticides in four classes (organochlorines, organophosphates, carbamates and pyrethroids) for indoor residual spraying (IRS) at specific doses (Najera & Zaim, 2002). These however differ in their residual life when sprayed on different wall surfaces. The effectiveness of insecticide depends on a complex set of factors. These include intrinsic toxicity, mode of action and stability and its effect on the vector (Najera *et al*., 1998).

The findings of this study revealed that there was possibility of resistant of populations of *An. gambiae s.l.* during week one exposed to all the three wall surfaces sprayed with labdacyaholtherin 10% WP insecticide formulation and on painted wall surface sprayed with deltametherin 25% WG and labdacyaholtherin 10% CS insecticide formulations having mean mortality rates of *An. gambiae s.l.* between 80% – 97% % (WHO, 2006). And for the rest residual time the mean mortality rates of *An. gambiae s.l.* exposed to wall surfaces sprayed with the three candidate insecticide formulations (deltametherin 25% WG, labdacyaholtherin 10% WP and labdacyaholtherin 10% CS) was below 80%. This shows the resistance occurrence of field population of *An. gambiae s.l*. to the corresponding insecticide formulations in the study sites; mean mortality rates of *An. gambiae s.l.* 25.7% for lambdacyhalothrin and 8% for deltametherin have been reported at Omo Nada (Asendabo) district of Jimma zone (PMI-AIRS, 2013).

The residual lifespan of IRS insecticide formulations is of key importance. Based on the mean separation of both knockdown and mortality rates of *An. gambiae s.l.* among residual time there was significance difference except between month one and month two. The mean mortality rates of *An. gambiae s.l.* for week one was 5.96 (95% CI, 5.712, 6.208); month one was 5.05 (95% CI, 4.802, 5.298); month two was 5.03 (95% CI, 4.779, 5.275) and month three was 3.62 (95% CI,

3.375, 3.871). Similar study by Okumu *et al*. (2012) showed that activity of the IRS declined significantly within two months.

Based on observed mean mortality rates of *An. gambiae s.l.* there was no significant when the insecticide formulations were sprayed on plastered, non-plastered and painted wall surfaces this clearly showed that the residual efficacy of the three candidate insecticide formulations was similar. This could be attributed to the strong resistance of the local *An. gambiae s.l.* population against pyrethroids. The mortality rates on the different sprayed wall surfaces remain ineffective in killing field populations of *An. gambiae s.l.* in week one, month one, month two and month three (Table 5). The mortality rates of *An. gambiae s.l.* on the different types of sprayed wall surfaces were low on painted, non-plastered and plastered (Fig. 3). This finding is consistent with the findings of a study by Yewhalaw *et al*. (2011) who reported the existence of multiple insecticide resistance in populations of *An. gambiae s.l.* in the study sites. The resistance levels of population of *An. gambiae s.l.* to the pyrethroids varied greatly across candidate insecticide formulations and time of test. The resistance levels to the pyrethroids varied greatly from susceptibility to resistance across treatments and time of test. Pyrethroid used in Africa for IRS and LLINs has increased greatly between 2002 - 2009 (Berg *et al*., 2012) and has probably accelerated the development and spread of pyrethroid resistance (Ranson *et al*., 2011; Czeher *et al*., 2008). Concurrent use of pyrethroids for indoor residual spraying and LLINs could increase the pressure for resistance development in vector populations (WHO, 2011c). There are concerns that increasing pyrethroid (deltamethrin) resistance will reduce effectiveness of both IRS and LLINs (PMI, 2014a). In 2009, 19 countries in the African region reported using pyrethroids for indoor residual spraying against malaria. These countries included Ethiopia, Kenya, Liberia, Madagascar, Mali, Mozambique, Nigeria, Rwanda, Senegal, Tanzania, and Uganda, all of which have high coverage rates of LLINs for malaria control (WHO, 2010).

Insecticide resistance is a major impediment in malaria vector control. There was rapidly spread of pyrethroid resistance in the past decade throughout Sub-Saharan Africa (PMI, 2014b). Anopheles mosquito resistance to insecticides has been detected in 64 countries with on-going malaria transmission, affecting all major vector species and all classes of insecticides (GPIRM, 2012). Current vector control tools remain effective; however, if left unchecked, insecticide

resistance could lead to a substantial increase in malaria incidence and mortality. The global malaria community needs to take coordinated action to prevent insecticide resistance from emerging at new sites, and to urgently address it at the sites where it has been identified (WHO, 2013a). *An. gambiae s.l.* was resistant to deltamethrin in Jimma and other project sites of African indoor residual spraying project (AIRS) in Ethiopia (PMI-AIRS, 2013). Moreover, populations of *An. arabiensis* developed resistance to permethrin, deltamethrin and lambdacyhalothrin (Yewhalaw *et al*., 2014). Another study conducted by Massebo *et al*. (2013) around southern Ethiopia also showed that populations of *An. arabiensis* were resistant to lambdacyhalothrin, cyfluthrin, alphacypermethrin and deltamethrin. A similar study conducted by Abate & Hadis (2011) in northern, northwestern, central and southern Ethiopia confirmed the development of high level pyrethroid resistance in populations of *An. gambiae s.l.*

Generally the findings of this study revealed that there was resistance of *An. gambiae s.l.* populations to the three candidate insecticide formulations (deltametherin 25% WG, labdacyaholtherin 10% WP and labdacyaholtherin 10% CS) with percentage mean mortality rate of below 80%. Recently, global malaria-control efforts rely heavily on a single class of insecticide the pyrethroids both for IRS and to treat bed nets. This class of insecticide is used in most IRS programmes, and it is the only insecticide used in WHO-recommended LLINs. However, increasing resistance of malaria vectors to pyrethroids and to other insecticides may jeopardize global malaria control efforts (WHO, 2013a). Recognizing the threat posed by insecticide resistance, WHO released the Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM, 2012). The residual life span and efficacy of most insecticides are affected by the chemical nature of the sprayed surface (Ansari *et al*., 1997). Therefore, the residual efficacy and the persistence of insecticide may vary on different types of surface. Currently insecticide resistance is the most critical challenge facing global malaria vector control efforts, and is central to the planning and implementation of an effective IRS programme. As outlined in the GPIRM, the insecticide resistance status of the local vectors must be determined before selecting the insecticides to be used in the IRS programme (WHO, 2013b). Pates and Curtis (2005), suggest that IRS is effective if the mosquito species concerned is endophilic and rests on the insecticide-treated surfaces for a sufficient time to pick up a lethal dose.

CONCLUSION

In view of the results, the evaluation of residual effects of the three candidate insecticide formulations (lambdacyhalothrin 10% WP, deltametherin 25% WG and lambdacyhalothrin 10% CS) on different wall surfaces (painted, plastered and non-plastered wall surfaces) had established a baseline set of data that can be used to show the occurrence of resistance of populations of *An. gambae s.l.* against those insecticide formulations before using by the national malaria control program for IRS in the study area. And to establish the efficacy of insecticide formulations at the selected application rates against the target vector species, before applying to all or most households in the community. Knowing how long a residual insecticide will last is important information for vector control, since it indicates the minimum interval between spraying to maintain the resistance of the insecticide**.**

RECOMMENDATION

Based on the research finding, the following recommendations are forwarded.

- Any insecticide formulations to be used for IRS should be tested in real use conditions at community level so that the results would guide the decision makers on the spray cycles.
- In the presence of the resistant *An. gambiae s.l.* populations in the study areas alternative new vector control tools should be used and an insecticide resistance management strategy plan should be developed and implemented.
- The IRS program to be effective against malaria control it is better to determine the dosage by studying at the application areas rather than somewhere else studied.

REFERRENCES

(WHO), (1998). Tests procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Report of the WHO Informal Consultation, Geneva, Switzerland. P.43.

Abate A, Hadis M., (2011). Susceptibility of *Anopheles gambiae s.l.* to DDT, malathion, permethrin and deltamethrin in Ethiopia. *Trop Med Int Health* 16(4)**:**486- 491.

Addis Ababa, Ethiopia.

Akogbeto MD, R Noukpo H., (2005). Use of agricultural insecticides in Benin. *Bull Soc Pathol Exot* 98:400-405.

Ansari MA, Mittal PK, Razdan RK, Batra CP., (1997). Residual Efficacy of Deltamethrin 2.5 WP (K-OTHRIN) Sprayed on Different Types of Surfaces against Malaria Vector *Anopheles culicifacies*. *Southeast Asian J Trop Med and Public Health* 28: 606-609.

Assefa A, Kassa M, Tadese G, Mohamed H, Animut A, Mengesha T., (2010). Therapeutic efficacy of Artemether/Lumefantrine (Coartem) against *Plasmodium falcifarum* in Kersa, SouthWest Ethiopia. *Parasit Vectors* 3 (1).

Berg Hvd., Zaim M, Singh R, Yadav A, Soares A, Ameneshewa B, Mnzava A, Hii J, Prasad Dash A, and Ejov M., (2012). Global Trends in the use of Insecticides for Vector-born disease control. *Enviro Health Perspect* http://dx.doi.org/10.1289/ehp.1104340 accessed on October 03, 2014

Brogdon WG, McAllister JC, (1998). SimpliÞcation of adult mosquito bioassays through use of time-mortality determinations in glass bottles. *J Am Mosq Control Assoc* 14: 159 - 164.

Centers for Disease Control and Prevention, (2006). Life cycle of Plasmodium species:

Source CDC http://www.dpd.cdc.gov/dpdx/HTML/Malaria.htm; accessed on November 15, 2013.

Chandre F, Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, Guillet P., (2000). Modifications of pyrethroid effects associated with *kdr* mutation in *Anopheles gambiae*. *Med Vet Entomol* 14:81–88.

Chavasse DC, Yap HH., (1997). Chemical methods for the control of vectors and pests of public health importance. WHO/CTD/WHOPES/97.2.WHO, Geneva.

Clements AN., (2000). The biology of Mosquitoes: development, nutrition and reproduction. Volume 1. 2nd edition. CABI publishing, UK.

Coetzee M, Craig M, Le sueur D., (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitol Today* 16: 74–77

Coleman M, Sharp B, Seocharan I, Hemingway J., (2006). Developing an evidence-based decision support system for rational insecticide choice in the control of African malaria vectors. *J Med Entomol* 43(4):663-668.

Czeher C, Labbo R, Arzika I, Moiroux N, Nkuni Z, Corbel V., (2008). Evidence of increasing Leu-pheknockdown resistance mutation in *Anopheles gambiae* from Niger following a nationwide long-lasting insecticide-treated nets implementation. *Malar J* 7:189

David JP, Strode C, Vontas J, Nikou D, Vaughan A, Pignatelli PM, Louis C, Hemingway J, Ranson H., (2005). The *Anopheles gambiae* detoxification chip: a highly specific microarray to study metabolic-based insecticide resistance in malaria vectors. *PNAS* 102(11):4080-4084.

Davies TG, Field LM, Usherwood PN, Williamson MS., (2007). DDT, pyrethroids and insect sodium channels. *IUBMB Life* 59(3):151-162.

Etang J, Nwane P, Mbida JA, Piameu M, Manga B, Souop D, Awono-Ambene P., (2011). Variations of insecticide residual bio-efficacy on different types of walls: results from a community-based trial in south Cameroon. *Malar J* 10:333.

Feyereisen R., (2005). Insect cytochrome P450. In: *Comprehensive Molecular Insect Science*. 4. Elsevier. Oxford, UK, 1-77.

Foster W., (1995). Mosquitoes sugar feeding and reproductive energetic. *Ann Rev Entomol* 40:

443-474.

Global Malaria Action Plan (GMAP), (2008). Roll Back Malaria Partnership, Geneva. http://www.rbm.who.int/gmap/index.html, accessed on December 17, 2013.

Global plan for insecticide resistance management in malaria vectors (GPIRM), (2012). Geneva, World Health Organization, (http://www.who.int/malaria/publications/atoz/gpirm/en/index.html, accessed June 25, 2014.

Greek Ministry of Rural Development and Food (2013). Lists of approved pesticides and biocides, *e_bulletin* http:// www.minagric.gr/syspest/; accessed on December 29, 2013.

Hemingway J, Ranson H., (2000). Insecticide resistance in insect vectors of human disease. *Ann Rev Entomol* 45: 371-391

Hougard JM, Duchon S, Zaim M, Guillet P, Bifenthrin., (2002). A useful pyrethroid insecticide for treatment of mosquito nets. *J Med Entomol* 39:526–533.

Josiane E, Philippe N, Jean AM, Michael P, Blaise M, Daniel S, Parfait AA., (2011). Variations of insecticide residual bio-efficacy on different types of walls: results from a community-based trial in south Cameroon. *Malar J* 10:333

Kathleen W., (2002). A Review of Control Methods for African Malaria Vectors, Environmental Health Project, Infectious Diseases and Nutrition, Bureau for Global Health, U.S. Agency for International Development, Washington, DC 20523.

Ketema S, Bacha K, Birhanu T, Petros B., (2009). Chloroquine-resistant *Plasmodium vivax* malaria in Serbo town, Jimma zone, South-West Ethiopia. *Malar J* 8: 177.

Lelisa K., (2013). Entomological Assessement of Anopheline mosquitoes in Kersa District Jimma Zone South-Western Ethiopia; MSc Thesis, Jimma University, Jimma Ethiopia, p 11.

Massebo F, Balkew M, Gebre-Michael T, Lindtjørn B., (2013). Blood meal origins and insecticide susceptibility of *Anopheles arabiensis* from Chano in SouthWest Ethiopia. *Parasit Vectors* 6:44.

Matola YG, Mgayuka SA., (1981). Malaria in the Pare area of Tanzania. Malaria 20 years after the end of residual insecticide spraying. *Trans R Soc Trop Med Hyg* 75:811–813.

McLaughlin LA, Niazi U, Bibby J, David JP, Vontas J, Hemingway J, Ranson H, Sutcliffe MJ, Paine MJ., (2008). Characterization of inhibitors and substrates of *Anopheles gambiae* CYP6Z2. *Insect Mol Biol* 17(2):125-135.

Ministry of Health (MOH), (2007). Malaria diagnosis and treatment guidelines for health workers. Addis Ababa, Ethiopia.

Ministry of Health (MOH), (2012). National Malaria Guidelines in Ethiopia. Third Edition;

Ministry of Health (MOH), (2013)**.** Summary Proceedings, 4th Annual Malaria Control Program Review Ethiopia and Nigeria. *In* The Carter Center, Atlanta, Georgia.

Muller P, Donnelly MJ, Ranson H., (2007). Transcription profiling of a recently colonised pyrethroid resistant *Anopheles gambiae* strain from Ghana. *BMC Genomics* 8:36.

Murray CJL, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD., (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet* 379:413- 431

Nájera JA, González-Silva M, Alonso PL., (2011). Some lessons for the future from the global malaria eradication programme (1955-1969) *PLoS Med* 8:1000412.

Najera JA, Kouzntsov RL, Delacollete C., (1998). Malaria Epidemics Detection and Control forecasting and Prevention. WHO Document WHO/MAL/98.1084, http://www.rbm.who.int/docs/najera_epidemics/naj11.htm accessed on January 09, 2014).

Najera JA, Zaim M., (2001). Malaria Vector control: Insecticides for indoor residual spraying.WHO/CDS/WHOPES/2001.3. http://209.61.208.233/LinkFiles/ *Dengue Bulletin* 25: pp 13

Najera JA, Zaim M., (2002). Malaria vector control: Decision making criteria and procedures for judicious use of insecticides. WHO/CDS/WHOPES/2002.5, WHO, Geneva, www.who.int/ctd/whopes/docs/judiciousUseRev.pdf , accessed on July 12, 2014.

Narahashi T., (1996). Neuronal ion channels as the target sites of insecticides. *Pharmacol Toxicol* 79(1):1-14.

Okumu FO, Chipwaza B, Madumla EP, Mbeyela E, Lingamba G, Moore J, Ntamatungro AJ, Kavishe DR, Moore S J., (2012). Implications of bio-efficacy and persistence of insecticides when indoor residual spraying and long-lasting insecticide nets are combined for malaria prevention. *Malar J* 11:378

Pates H, Curtis C., (2005). Mosquito behavior and vector control. *Annu Rev Entomol* 50: 53-70

Plapp FW, Hoyer RF., (1968). Insecticide Resistance in House Fly - Decreased Rate of Absorption as Mechanism of Action of a Gene That Acts as an Intensifier of Resistance. *J Eco Entomol* 61(5):1298-1301.

President's Malaria Initiative (PMI) Africa IRS (AIRS). (2013).Ethiopia End of Spray Report. Bethesda, MD. PMI|Africa IRS (AIRS) Project Indoor Residual Spraying (IRS 2) Task Order Four, *Abt Associates Inc*.

President's Malaria Initiative, (2014a). Ethiopia Malaria Operational Plan FY.

President's Malaria Initiative, (2014b). Indoor Residual Spraying (IRS) insecticide use and forecast for 2014. http://www.fightingmalariagov/technical/irs/PMI_IRS-Insecticide_Trends_080112xlsx accessed on November 01, 2014.

Ranson H, Hemingway J., (2008). Field-caught permethrin-resistant *Anopheles gambiae* over express CYP6P3, a P450 that metabolises pyrethroids. *PLoS Genet*, 4(11):e1000286.

Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V., (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol* 27:91-98.

Raymond M, Chevillon C, Guillemaud T, Lenormand T, Pasteur N., (1998). An overview of the evolution of overproduced esterases in the mosquito *Culex pipiens*. *Philos Trans R Soc Lond B Biol Sci* 353(1376):1707-1711.

Ridl F, Bass C, Torrez M, Govender D, Ramdeen V, Yellot L, Edu A, Schwabe C, Mohloai P, Maharaj R., (2008). A pre-intervention study of malaria vector abundance in Rio Muni, Equatorial Guinea: Their role in malaria transmission and the incidence of insecticide resistance alleles. *Malar J* **7**(1):194.

Rozendaal JA., (1997). Vector control: Methods for use by individuals and communities. WHO, Geneva

Sampath TRR, Yadav RS, Sharma VP, Adak T., (1998). Evaluation of lambdacyhalothrin impregnated bednets in a malaria endemic area of India. Part 1. Implementation and acceptability of the trial. *J Am Mosq Cont Ass* 14:431-436.

Sharp BL, Ridl FC, Govender D, Kuklinski J, Kleinschmidt I., (2007). Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial

Soderlund DM, Bloomquist JR., (1989). Neurotoxic actions of pyrethroid insecticides. *Annu Rev Entomol* 34:77-96.

Tagros chemical india Ltd.Technical Information Operation manual http://www.tagros.com/technical%20products/insecticides/insecticides_Deltamethrin_tagros.html; accessed February 01, 2014

Ungureanu C V, Burghele E., (1959). Investigations on the contact of the legs of mosquitos with resting surfaces and its relationship with DDT and dieldrin intoxication. *WHO/Mal/243*. *Malar J* 6:52.

WHO, (1995). Vector control for malaria and other mosquito- borne diseases. WHO Technical Report Series 857, World Health Organization, Geneva, Switzerland.

WHO, (2006). Guidelines for Testing Mosquito Adulticides for Indoor Residual Spraying and Treatment of Mosquito Nets. WHO/CDS/NTD/WHOPES/GCDPP/2006.3

WHO, (2007). Implementation of Indoor Residual Spraying of Insecticides for Malaria Control in the WHO African Region Report. World Health Organization, Geneva, Switzerland.

WHO, (2009). WHO recommended insecticides for indoor residual spraying against malaria vectors. World Health Organization, Geneva, Switzerland.

WHO, (2010). World Malaria Report. World Health Organization, Geneva, Switzerland. http://www.who.int/malaria/world_malaria_report/en/index.html

WHO, (2011a). The World Malaria Report. World Health Organization, Geneva, Switzerland. (http://www.who.int/malaria/ world_malaria_report/en/index.html; accessed on November 19, 2013).

WHO, (2011b).Global Insecticide Use for vector-borne disease control. World Health Organization, Geneva, Switzerland.

WHO, (2011c). The Technical Basis for Coordinated Action against Insecticide Resistance: Preserving the Effectiveness of Modern Malaria Vector Control. http://whqlibdoc.who.int/publications/2011/9789241501095_eng.pdf, accessed `on August 29, 2014

WHO, (2012a). Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM). World Health Organization, Geneva. (http://www.who.int/malaria/vector_control/ivm/gpirm/en/index.html, accessed on December 20, 2013).

WHO, (2012b). Guidelines for testing the efficacy of insecticide products used in aircraft, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland

WHO, (2012c). WHO interim position statement the role of larviciding for malaria control in sub-Saharan Africa. Geneva, World Health Organization, (http://www.who.int/malaria/ publications/atoz/larviciding_ position_statement/en/index.html, accessed July 25, 2014).

WHO, (2013a). World Malaria Report. World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland, http://www.who.int/malaria/publications/

WHO, (2013b). Indoor Residual Spraying. An operational manual for indoor residual spraying (IRS) for malaria transmission control and elimination. World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland

WHO, (2013c). Test procedures for insecticide resistance monitoring in malaria vector mosquito. World Health Organization, Geneva, (http://www.who.int/malaria/publications/ atoz/ en/index.html, accessed July 15, 2014).

WHO, (2014). World Malaria Report. World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland, http://www.who.int/malaria/publications/

Yates A, N'Guessan R, Kaur H, Akogbéto M, Rowland M., (2005). Evaluation of KO-Tab 1-

2-[3®]: a wash-resistant dip-it-yourself' insecticide formulation for long-lasting treatment of mosquito nets. *Malar J* 4:52

Yewhalaw D, Wassie F, Steurbaut W, Spanoghe P, Van Bortel W, Denis L, Tessema DA, Getachew Y, Coosemans M, Duchateau L, Speybroeck N., (2011). Multiple Insecticide Resistance: an Impediment to malaria Vector Control Program. *PLoS ONE* 6:1 e16066: doi: 10.1371/journal.pone.0016066

Yewhalaw D, Van Bortel W, Denis L, Coosemans M, Duchateau L, Speybroeck N., (2010). First evidence of high knockdown resistance frequency in *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. *Am J Trop Med Hyg* 83(1):122- 125.

Yewhalaw D, Asale A, Tushune K, Getachew Y, Duchateau L, Speybroeck N, (2012). Bio-efficacy of selected long-lasting insecticidal nets against pyrethroid resistant *An. Arabiensis* from SouthWestern Ethiopia. *Parasit Vectors* 5:159 doi:10.1186/1756-3305-5-159

Yewhalaw D, Asale A, Getachew Y, Hailesilassie W, Speybroeck N, Duchateau L., (2014). Evaluation of the efficacy of DDT indoor residual spraying and long-lasting insecticidal nets against insecticide resistant populations of *Anopheles arabiensis* Patton (Diptera: Culicidae) from Ethiopia using experimental huts, *Parasit Vectors* 7:131doi: 10.1186/1756- 3305-7-13

Annex 1: Mean knockdown (%) of field *An. gambiae s.l.* populations exposed to wall surface sprayed with insecticide formulations by time of tests & wall surface types

Residual time	Treatments	% Knockdown on wall surface types		
		plastered	Non plastered	painted
Week one	Deltamethrin 25%WG	26.67	30.67	58.00
	Labdacyaholtherin 10% WP	28.68	33.67	71.33
	Labdacyaholtherin 10% CS	23.33	27.67	46.00
	Control	1.33	0.33	1.33
Month one	Deltamethrin 25%WG	28.33	26.33	31.33
	Labdacyaholtherin 10% WP	28.00	22.33	37.33
	Labdacyaholtherin 10% CS	25.00	21.00	36.67
	Control	1.33	0.33	0.00
Month two	Deltamethrin 25%WG	26.00	26.67	29.33
	Labdacyaholtherin 10% WP	33.67	20.00	19.33
	Labdacyaholtherin 10% CS	20.67	23.33	22.67
	Control	0.00	0.00	0.00
Month three	Deltamethrin 25%WG	11.67	13.33	23.33
	Labdacyaholtherin 10% WP	13.67	11.33	28.67
	Labdacyaholtherin 10% CS	14.00	14.00	26.67
	Control	1.33	1.67	2.67

Annex 2 : Mean mortality (%) of field *An. gambiae s.l.* populations exposed to wall surface sprayed with insecticide formulations by time of tests & wall surface types

Residual time	Treatments	% Mortality on wall surface types		
		plastered	Non plastered	painted
Week one	Deltamethrin 25%WG	76.33	77.67	88.00
	Labdacyaholtherin 10% WP	83.33	81.00	85.33
	Labdacyaholtherin 10% CS	72.67	74.00	82.00
	Control	2.00	0.67	1.33
Month one	Deltamethrin 25%WG	70.67	65.00	70.67
	Labdacyaholtherin 10% WP	65.33	63.67	68.67
	Labdacyaholtherin 10% CS	68.67	64.67	64.00
	Control	0.67	4.67	0.00
Month two	Deltamethrin 25%WG	64.33	71.00	58.67
	Labdacyaholtherin 10% WP	75.67	73.00	50.00
	Labdacyaholtherin 10% CS	73.00	71.00	58.67
	Control	0.00	0.00	0.00
Month three	Deltamethrin 25%WG	42.67	45.67	48.00
	Labdacyaholtherin 10% WP	51.00	48.00	48.00
	Labdacyaholtherin 10% CS	51.00	47.00	48.00
	Control	2.67	2.67	2.67

N.B. WHO recommendation for assessing the significance of detected resistance is, 98% – 100% mortality at the recommended application of IRS insecticides indicates susceptibility; 80% – 97% mortality at the recommended application of IRS insecticides suggests the possibility of resistance that needs to be confirmed and < 80% mortality at the recommended IRS insecticides suggests resistance (WHO, 2006).

Jimma University

College of Natural Sciences

School of Graduate Studies

Department of Biology

Approved by:-

Name	Signature
Eba Alemayehu (Mr.)	_____
Department Head	

Research Advisors

Name	Signature
Delenasaw Yewhalaw (PhD)	_____
Argaw Ambelu (PhD)	_____

JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

SCHOOL OF GRADUATE STUDIES

DEPARTEMENT OF BIOLOGY

Evaluation of the Efficacy and Residual Activity of Three Candidate Insecticide Formulations Against Malaria Vector Mosquitoes In Jimma Zone, Southwestern Ethiopi

By

Abera Hailu

A Thesis Submitted to the Department of Biology, College of Natural Sciences, Jimma University in Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology (Ecological and Systematic Zoology Stream)

Approved by the examining board

Name Signature

aa

Chairman,

Research advisors

Delenasaw Yewhalaw (PhD)

ArgawAmbelu (PhD)

External Examiner

Xx

Internal Examiner

yy

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented in this or other university and all sources or materials used for this study have been acknowledged.

Name: Abera Hailu

Signature _____

Name of institution: Jimma University

Date of submission: _____

This thesis has been submitted for examination with my approval as university advisor.

Name of advisors	Signature	Date
1, Delnasaw Yewhalaw (PhD)	_____	_____
2, Argaw Ambelu (PhD)	_____	_____

In mammalian neuronal membrane pyrethroids selectively reduce the rate of closing of sodium channels both during depolarization and after repolarization of the nerve membrane.