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Studies on the Impact of Bendiocarb Indoor Residual Spraying on Insecticide Resistance of *Anopheles arabiensis* Patton and other Entomological Determinants of Malaria Transmission in the Upper Blue Nile River Basin, Bahirdar Zuria District, Northwestern Ethiopia

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This is to certify that the thesis prepared by Alemayehu Abate Abeje, entitled: Studies on the Impact of Bendiocarb Indoor Residual Spraying on Insecticide Resistance of *Anopheles arabiensis* Patton and other Entomological Determinants of Malaria Transmission in The Upper Blue Nile River Basin, Bahirdar Zuria District, Northwestern Ethiopia and submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in Zoological Sciences (Insect Science) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Studies on the Impact of Bendiocarb Indoor Residual Spraying on Insecticide Resistance of *Anopheles arabiensis* Patton and other Entomological Determinants of Malaria Transmission in the Upper Blue Nile River Basin, Bahirdar Zuria District, Northwestern Ethiopia

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In Ethiopia, malaria vector control using indoor residual spraying has been used against *Anopheles arabiensis* Patton for decades. However, data for the effective residual life of the insecticides used for indoor residual spraying on sprayed surfaces and the impact of these interventions on different entomological risk factors for malaria transmission is insufficient or absent. Therefore, a comparative study was carried out in Andassa and nearby villages, Bahir Dar Zuria District, Upper Blue Nile River Basin, Northwest Ethiopia to estimate the effective residual life of bendiocarb on treated surfaces and the impact of the application of indoor residual spraying on insecticide resistance status, host preferences, sporozoite and entomological inoculation rate, abundance and resting habits of *An. arabeinsis* Patton. The effects of indoor residual spraying on the composition and abundance of other Anopheles mosquitoes were also assessed.

Susceptibility of *Anophels arabiensis* to the insecticide used for indoor residual spraying and its residual life on sprayed surfaces was evaluated based on World Health Organization test procedures. Adult mosquitoes were collected from houses located

nearby mosquito breeding sites using Center for Disease Control and preventions light trap, pyrethroid spray sheet collection and artificial pit shelters made nearby pyrethroid spray sheet sampling houses. Collected mosquitos were sorted into species using their external morphology and sibling species of the *Anopheles gambiae* complex were identified by Polymerase Chain Reaction based molecular methods. Enzyme-linked immunosorbent assay was run to determine blood meal sources and Plasmodium infection rates in *Anopheles arabiensis.*

The effective residual life of bendiocarb on treated surfaces was higher on plastered wall surfaces than on non-plastered wall surfaces. Resistance to bedndiocarb was suspected in *Anophels arabiensis* (95%) after three successive spraying cycles within three-year's time in Andassa, while the vector remained susceptible (99%) in Tikurit, which received only one round spray. Susceptibility status of the vector to fenithrothion, primiphosmethyl and propoxur was unaffected (100%) due to the application of bendiocarb indoor residual spraying. Susceptibility of the vector to DDT, delthamethrin and malathion was improved progressively at both study sites. East African kdr mutation *(kdr-e*) was detected in *Anopheles arabiensis* Patton sampled from both study groups.

In total, 8,859 Anopheles mosquitoes were collected among which 70.15% (n = 6, 215), 21.99% (n = 1,948) and (7.86%, n = 696) of them were *Anopheles arabienesis*, *Anopheles pharoensis* and *Anopheles coustani*, respectively. The abundance of *Anopheles arabienesis* drastically declined after spray, while indoor residual spraying did not influence its resting habits. General estimating equation model showed that indoor residual and time (month and years) played significant role to affect mosquito abundance. The model also showed that sampling methods had significant effect on the number of

mosquitoes caught. In contrast, the role of the number of human hosts who slept the previous night in mosquito sampling houses was not significant. The relative adjusted reduction in *Plasmodium falciparum* infection and entomological inoculation rate in *Anopheles arabiensis* was 100% after indoor residual spraying, while the reduction in human blood meal index was between 3% and 10%.

In conclusion, bendiocarb was effective to reduce vector density, sporozooite rate and entomological inoculation rate in *Anopheles arabiensis* without affecting the resting habits of the vector. However, effective residual life of bendiocarb is too short on non-plastered wall surfaces to cover the main malaria transmission season; wall plastering is, therefore, recommended to increase insecticide persistence on treated surfaces. East African kdr mution is widely distributed in populations of the main malaria vector signifying the need to implement insecticide resistance management to restore susceptibility of the vector to DDT/pyrethroid and maintain the efficacy of long-lasting insecticide treated mosquito nets that have been used as frontline defense in the control of malaria vectors in Ethiopia in general and the present study site in particular. Resistance to bendiocarb was suspected after three spraying cycles within three-year times, while efficacy to DDT and pyrethroid was not restored within three years' time implying that prevention is better than restoration of insecticide resistance in disease vectors. Therefore, insecticide restitance management should be in place in time.

Key words: Ethiopia, *Anopheles arabiensis*, Bendiocarb, Sprorozoite rate, EIR

Dedication

To victims of malaria and to those who are working hard to make the world free of malaria.

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Acronyms

BBI Bovine blood meal Index

CDC Center for Disease Control and Prevention

DDT Dichloro-diphenyl-Trichloroethne

EIR Entomological inoculation rate

ELISA Enzyme-Linked Immunosorbent Assay

EPHI Ethiopian Public Health Institute

FF Fresh fed

GEE General estimating equation

GR Gravid

HBI Human blood meal index

IRS Indoor residual spraying

KDR Knock down resistance

LLINs Long-lasting insecticide treated mosquito nets

Masl meter above sea le

PCR Polymerase chain reaction

PSC Pyrethrum spray sheet collection

s.l sensu lato

SG Semi gravid

SR Plasmodium sporozoite infection rate

UBI Unknown blood meal sources index

UF Unfed

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CHAPTER 1: General introduction

Studies confirmed that the existing malaria control measures such as indoor residual spraying (IRS) and long-lasting insecticide treated mosquito nets (LLINs) have had a demonstrable impact on malaria transmission in different malarious countries in sub-Saharan Africa [Okumu & Moore, 2011; Zhou *et al*., 2010] including Ethiopia [Deribew *et al*., 2017]. Chemical based indoor malaria vector intervention tools break malaria transmissions either by deterring, repelling or killing mosquitoes that enter and rest on treated surfaces [WHO, 2006a]. Therefore, the efficacy of these intervention tools is a function of the behaviour [Pates and Curtis, 2005] and susceptibility of the vector under consideration to the insecticide used for IRS or LLINs treatment [Hemingway & Ranson, 2000].

Change in the abundance of vector population due to IRS interventions was observed in the archipelago of Sa˜o Tome´ and Principe in 1980's [Sousa *et al.,* 2001]. The impact of IRS campaign on vector composition, abundance and density was also observed in the Pare region of Tanzania in 1950's where *An. funestus* was eliminated and the abundance of *An. gambiae* s.l was reduced to one-fifth of its pre-intervention density [Pringle, 1967]. Published reports also indicated that *An. funestus* and the S form of *An. gambiae* eliminated in Equatorial Guinea on Bioko Island in 2004 soon after the IRS campaign [Sharp *et al*., 2007].

Successes in reducing malaria morbidity and mortality by scaling up LLINs distribution and IRS have been reported from different malarious countries in Africa [Bayoh *et al.*,

2010; Greenwood, 2008]. Giardina *et al*. [2014] demonstrated the association of ITNs and IRS interventions with parasitaemia risk reduction in Rwanda, Tanzania, Senegal, Angola, Liberia and Mozambique. Similarly, success stories in reducing malaria morbidity and mortality have been reported in Ethiopia due to LLINs and IRS interventions [Deribew *et al*., 2017]. However, the effectiveness of IRS and LLINs is threatened by the widespread distribution of insecticide resistance and behavioral adaptations in malaria vector populations. Therefore, appropriate monitoring system of insecticide resistance and vectors' behavioral ecology should be set as an integral part of planning and evaluation in any disease vector control programs [WHO, 1998].

In Ethiopia, chemical-based vector control had been started in the 1960s and currently it has been intensified by scaling up of IRS coverage and LLINs distribution. DDT had been the choice of insecticide for IRS operations until 2007 in many malarious areas of the country except at a few places where malathion was used for DDT resistance vector populations. Following the detection of DDT resistance in the major malaria vector populations, DDT was replaced by delthamethrin in 2007. Due to the scaling-up of the distributions of pyrethroid insecticides treated LLINs, the building up of delthamethrin resistance in different vector populations and by considering the possibilities of cross resistance-between DDT and pyrethroid insecticides, IRS control program again shifted from delthamethrin to carbamate in 2010 [FDREMOH, 2012]. However, the buildup of carbamate resistance populations has been reported, so that control programmers need to look for alternative insecticides for IRS application [Alemayehu *et al.*, 2013, unpublished report].

1.1 Statement of the problem

Currently, studies have shown that *An. arabiensis,* which is the major malaria vector in Ethiopia, has developed different levels of physiological resistance against a range of insecticides recommended by WHO for malaria vector control [Abate & Hadis, 2011; Yehualaw *et al.*, 2010, Balkew *et al.*, 2003; Abose *et al.*; 1998]. The widespread of insecticide-resistant genes in populations of the major malaria vector across the country could put the efficacy and effectiveness of the country's vector control program using IRS and LLINs at risk as it had been demonstrated in Benin [N'Guessan *et al*., 2007] and in South Africa [Hargreave *et al*., 2000]. However, data for the impact of insecticide resistance on malaria control interventions and the contribution of these interventions to the development of insecticide resistance in disease vectors are either absent, inaccessible or not updated. Therefore, measuring the role of these insecticide based indoor vector control interventions to the development of insecticide resistance and the impact of insecticide resistance on the effectiveness of these interventions on entomological malaria transmission risk factors is valuable to develop effective insecticide resistance management system in the country.

The vector could also change its feeding and resting behaviour if they are to survive under the influence of the insecticides used for IRS application and LLINs treatment [Russel *et al*., 2011; Bugoro *et al*., 2011]. Therefore, quantifying vector's reaction to insecticides (used for vector control in the form of IRS and LLINs) with respect to its behavioural ecology is equivalently important to toxicity studies [WHO, 2006a]. In Ethiopia, data for behavioural resistance of the major malaria vector against the insecticides used for vector control and the impact of resistance on the existing chemical

based indoor interventions in the control of malaria transmission risk factors is either not available or not updated.

Thus, the general objective of PhD work was to assess the impact of bendiocarb IRS on insecticide resistance status and other malaria transmission risk factors associated with *An. arabiensis.*

CHAPTER 2: Literature review

2.1 Overview of malaria transmission cycle

Human malaria is a vector borne disease caused by different single-celled Plasmodium species that are transmitted among human hosts by Anopheles mosquitoes, while taking their blood meals. Malaria infection is initiated when the infective stage of the parasite (sporozoite) is injected with the saliva of blood feeding female mosquito [Cox, 2010; Simonetti, 1996; www.cdc.gov/malaria/about/biology] (Figure1C-1). Once entering the circulatory system of the human host, the parasites reproduce asexually in the liver (exoerythrocytic) (Figure 1 A 2-4) and in red blood cells (erythrocytic) (Figure 1B 5-6). Schizonts ruptured from the liver cells (Figure 1A-4); invade the red blood cell (Figure 1B-5) and pass through different developmental stages. After maturation, the schizonts released out from bursting red blood cells and segregate into the asexual and sexual forms (Figure 1B-6B). The proportion of matured schizonts transformed into sexual forms are fewer than the asexual forms [Smalley *et al.,* 1981]. The infective stage of the asexual form of the parasite re-invade other red blood cells (Figure1 B-5) and the sexual forms (the male and female gametocytes) produce the zygote within 60 minutes of blood ingestion. The macrogamete differentiates into ookinate and forms an oocyst that can produce many thousands of invasive sporozoites between 7-12 days. Then the sporozites escape and invade the salivary glands of the vector where they stay until they are injected into a new host when the vector takes its blood meal [Sinden 1984; Carter & Graves 1988] (Figure1 C 9-12) and the cycle continues if transmission is not intercepted or controlled.

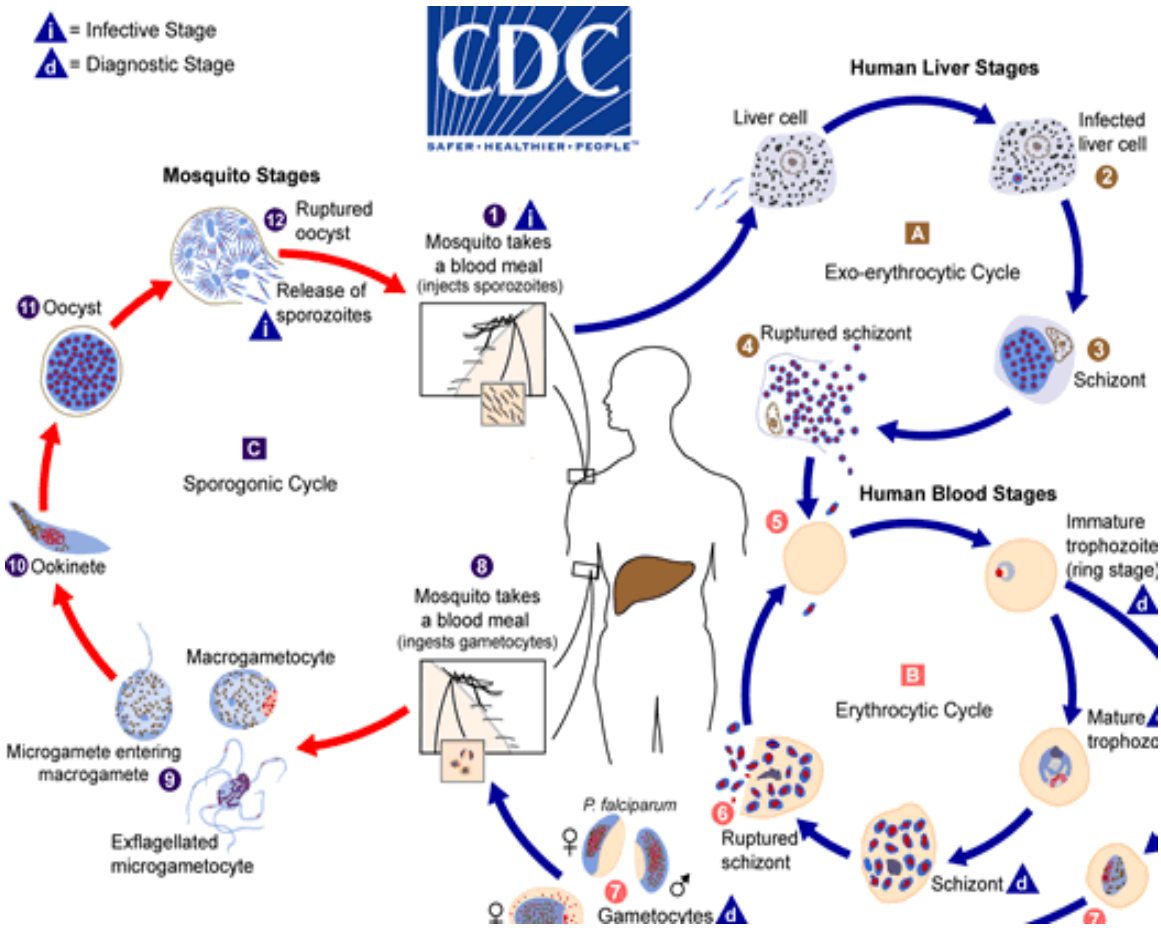


Figure 1: Overview of malaria transmission (Adopted from CDC)

2.2 Malaria parasites and vectors

The parasites that cause malaria in human include *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale*. A very rare zoonotic transmission of P. knowlesi and P. cynomology occurs when the Anopheles mosquito bites a human host after biting infected non-human primates. *Plasmodium falciparum* and *P. vivax* are the most prevalent human malaria parasite [WHO, 2011]. There are over 400 species of Anopheles mosquitoes among which about 30- 40 of them can transmit these malaria parasites among individuals of the human host in nature [WHO, 2015, Service, 2000].

2.3 The Global distribution and burden of malaria

According to WHO [2015], the distribution of malaria went down from 106 countries in 2000 to 95 countries in 2015. Correspondingly, malaria cases declined form 260 million to 214 million cases, while the number of deaths reduced from 839, 000 to 430,000 globally. The burden of the disease was/is still high in Africa where 88% percent of cases and deaths occurred. This would be attributed to three most competent Anopheles mosquito species that have high selective host preferences and adaptive feeding behavior. The presence of these highly specialized vectors, i.e. *An.gambiae* and *An. funestus* that feed almost exclusively on human host and the anthrophilic and zoophilic *An. arabiensis* [Kiswewski *et a*l., 2004] combined with the existence and prevalence of the most life threatening malaria parasite (*P. falciparum*) [WHO, 2015] under favorable climatic and ecological conditions makes malaria burden higher in Africa [Gallup & Sachs, 2001]. Barat *et al*. [2004] also argued that since most of the nations are poor they cannot afford the cost of living and vector control intervention tools (such as purchasing insecticide treated mosquito nets, insecticides for indoor residual spraying and cost of indoor residual operations etc.) so that the probability of contracting malaria by these people could be higher.

2.4 Strategies to prevent and control malaria transmissions

Varieties of malaria prevention and control tools have been developed against the parasite and the vector. Preventive therapies, early diagnosis, case treatment, and management and different entomological intervention tools for vector control combined with education and communication are the main malaria controlling tools that have been used in the Glob [WHO, 2015]. The primary objective of chemotherapy is to reduce the prevalence

of the parasite, while vector control intercepts the contact between the host and the vector so that the transmission of the disease would be controlled [Coseman *et al*., 1992].

Entomological interventions for malaria control have been suggested after the incrimination of mosquitoes as malaria vectors by Ronald Ross in 1897 and, Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava between 1898 and 1900 [Cox, 2010]. Since then a range of vector control tools including larval control by environmental management and larviciding, and the control of mosquito bites using adulticides, house improvement, repellent and biological control have been developed and used. Among those tools developed and used so far indoor residual spraying (IRS) and long-lasting insecticide treated mosquito nets (LLINs) are the primary vector control tools that are being used currently worldwide [Pluses *et al*., 2010; WHO, 2006b; Curtis, 1996]. Indoor residual spraying and LLINs are efficient against vectors of malaria that prefer to bite and rest indoors [Bockarie *et al*., 2009; Gillies & De Meillon, 1968], but less effective for vectors that are biting and resting outdoors [Okumu *et al*., 2013]. The efficacy of these intervention tools also depends up on the susceptibility of the vectors to the insecticides used for IRS operation and LLINs impregnation.

2.5 Potential risk factors contributing for malaria transmission

Malaria transmission risk factors are attributes or circumstances that might boost the probability of being infected with malaria parasite at a given time and place [Castillo-Salgado, 1992]. These risk factors are pertinent to the human host, the parasite, vector, geographical and environmental factors. Temperature, humidity, availability of vectors'

breeding sites and altitude are among geo-environmental factors that contribute most for both vector and parasite development and distribution. Vector density, vectorial capacity and competence, vectors' biting and resting habits and host preferences are important entomological factors that could influence the intensity of malaria transmission [Lindsay *et al*., 1990; Hoek *et al*, 1998; Drakeley *et al*., 2003]. The development of insecticide resistance by the vector and drug resistance by the parasite are also among the most important malaria transmission risk factors threatening the efficacy of the current malaria control strategies [Guyant *et al*., 2015]. Sonko *et al*. [2014] reported that malaria transmission pattern is also positively correlated with socioeconomic status of a given country i.e. the poorest countries have high level of transmission intensity, while the richest countries have low level of malaria transmission intensity. This has been seen in Africa where malaria burden is high compared with the burden of the same disease in other malarious countries in the world.

2.5.1 Parasite prevalence

2.5.1.1 Prevalence of infectious human malaria parasite reservoir

The transmission of malaria parasite from one human host to a new host occurs through the bites of blood feeding female Anopheles mosquito. Once entering its new host the parasite invades and replicates in the red blood cells of its host to produce the merozoites. Merozoites, which are emerged from mature schizonts, are capable of invading other red blood cells and produce shcizonts; or developing into transmission forms called the gametocytes. The gametocytes (male and female gametocytes) are the only blood stages of the parasite that are cable of infecting mosquitoes and keep onward infection and

transmission [Churcher *et al*., 2013]. Therefore, as the number of human malaria parasite reservoirs having these gametocytes increases in a given population the probability of contracting malaria will increase or the reverse [Mendis *et al*., 1987]. Compared to entomological factors, the contribution of infectious human parasite reservoirs to malaria transmission is smaller [Cosemans *et al*., 1992]

2.5.1.2 Prevalence of infective stages of malaria parasites

Human malaria parasites have asexual and sexual reproductive stage in the human host and malaria vectors, respectively. The mature asexual stage of the parasite in the human host is responsible for all clinical symptoms of malaria, while the non-relapsing blood stage of sexual reproduction in the vector is responsible for the transmission of malaria from human-to-malaria vectors and sustain the life cycle of the parasite [Bousema & Drakeley, 2011]. The efficacy of the transmission of gametocytes from human-to-mosquito is confirmed by the detection of oocytes and sporozites in the vector [Sattabongkot *et al.*, 1991]. Studies indicated that the probability of a vector getting infected with gametocytes might increase as the density increases in the blood, but the association between blood gametocyte density and mosquito infectivity is not linear [Churcher *et al*., 2013] because the process of the transmission is influenced by (i) immunities of both the human host and the vector to the parasite (ii) genetic and environmental factors and (iii) parasite and microbiota competition in the vector [Buckling *et al*, 1997; Buckling *et al*, 1999; Boissie`re *et al*, 1989].

2.5.2 Entomological malaria transmission risk factors

Several entomological determinants are involved for the transmission of malaria. Nevertheless, this review limits its scope to vectorial capacity and competence, longevity, biting and resting habits and blood meal source preferences. Insecticide resistance also affects disease transmission.

2.5.2.1 Vectorial capacity and vector competence

Vectorial capacity and competence are considered as fundamentals of malaria transmission risk factors. Vector competence is described as the measure of the natural susceptibility of the vector for parasite development that is governed by intrinsic factors (genetics) of the parasite and its vector [Osta *et al,* 2004; Oyewole *et al.*, 2010]. Vectorial capacity is defined as the ability of a vector to transmit the disease, which is expressed as the rate of new infections received daily by a single host. It is influenced by vector density (proportion of density of vectors in relation to density of hosts), longevity (daily survival of vectors), frequency of bites on hosts per vector per day and extrinsic incubation period of the parasite (sporogonic cycle) and vector competence [Rradrianasolo & Colluzzi, 1989, Garret-Johns, 1964]. It varies from species to species and from locality to locality depending on different factors. The anthrophilic *An. gambiae s.s,* and zoophilic and anthrophilic *An. arabiensis,* for example, are important human malaria vector species in West and East African countries, respectively, i.e. *An. gambiae s.s* is the primary vector in West Africa, while *An. arabiensis* is in East Africa. Similarly, the role of *An. arabiensis* in malaria transmission in East and West Africa is different, i.e. it is the primary vector in East African countries including Ethiopia (Abose *et al*., 1998),

while the contribution of this vector in malaria transmission comes after *An. gambiae s.s* in West African countries [Sinka *et al*, 2012]. Therefore, any malaria vector control strategy should take vectors' bionomics and behavior into consideration to intercept disease transmission effectively.

2.5.2.2 Vector density

The proportion of malaria vectors (vector density) in relation to host density is an important factor in malaria transmission. As vector density increases the intensity of disease transmission might increase if (i) the life span of the vector is long enough for the parasite to develop into its infective stage (sporozoite) and (ii) these sporozoite infected vector mosquitoes can contact and take blood meals from its human host [Protopopoff *et al*., 2009; Bradya *et al*., 2015].

2.5.2.3 Longevity of the vector

Survival of an adult female Anopheles mosquito is a determinant factor for malaria transmission because the parasite requires a portion of the lifespan of its vector (about 10-14 days in tropical countries) to complete its sporogonic cycle. Therefore, if the lifespan of the vector is less than the parasites incubation period, the probability of getting infective bites will be low so that the intensity of disease transmission will also be low or the reverse [Bradya *et al*., 2015]. Thus, any vector control measure affecting vector longevity will reduce disease transmission.

2.5.2.4 Vector host preference, feeding frequency and resting habits

Female Anopheles mosquitoes require blood meals for egg production [Clements, 1992]. Mosquitoes use different vertebrate animals as sources of their blood meals and usually synchronize their living environment with the environment of their blood meal sources to increase the chance of landing on the right host by minimizing the costs associated with time and energy required to searching for their hosts. Indoor resting habits help them to avoid harsh environmental factors and to be protected from its natural enemies (predators) so that increases the rate of its survival. For example, the anthrophilic Anopheles mosquitoes have adopted indoor biting and resting habits and adjusted their feeding time with the times when the host is either becoming inactive at time such as during sleep or when it shows the least defensive reactions [Haddow, 1946; Lindsay *et al*., 1992]. Malaria vectors' host preference is determined genetically and modified in nature by relative abundance and accessibility of their hosts [Clements, 1999]. Different Anopheles species have differences in their host preferences, i.e. some species like *An. gambiae s.s* are anthrophilic, while others such as A*n. quadrianulatus* are zoophilic. On the other hand, *An. arabiensis* is both anthrophilic and zoophilic where the degree of association between this vector and its host varies depending on the abundance and accessibility of the respective hosts mentioned [Gillies 1967, Bryan *et al*., 1987). Diversity in the feeding patterns of malaria vectors also occurs between different populations of the same species and even within the same population [Lyimo & Ferguson, 2009] due to differences in host-associated cues released in a heterogeneous environment [Takken & Knowls, 1999; Zwiebel & Takken, 2004].

In the case of human malaria, the frequency of Anopheles bites on its host is also an important factor [Tirados *et al.,* 2006] because biting frequency increases the likelihood that a mosquito could come in contact with a carrier of gametocytes (infected host) and able to transmit the infective stage of the parasite. This probability is more important for vectors that exclusively bite only humans and reflects the degree of human-vector contact so that it can be used as an indicator in the implementation and evaluation of the impact of control measures [Garrett-Jones *et al.,* 1980].

2.5.3 Insecticide and drug resistance

Insecticide resistance is the term used to describe the situation in which vectors are no longer killed by the standard dose of insecticide (they are no longer susceptible to the insecticide) or manage to avoid coming into contact with the insecticide [WHO, 2012; IRA, 2011]. Drug resistance is defined as the ability of a parasite strain to survive and /or to multiply despite the administration and absorption of a drug given in doses equal or higher than those are usually recommended, but within the limits of tolerance of the patients [WHO, 1967]. WHO [1986] modified the definition of drug resistance by including a sentence "The form of the drug active against the parasite must be able to gain access to the parasite or the infected erythrocyte for the duration of the time necessary for its normal action".

Currently, insecticide resistance is widespread in countries having ongoing malaria transmission and affecting all species of malaria vectors and classes of insecticides [WHO, 2012]. Nevertheless, it is not uniformly distributed among vector species and can greatly differ from one locality, province, country, region and continent to another. The

highest levels of insecticide resistance were reported in Africa where malaria burden is still the highest in the World [WHO, 2010a]. Drug resistance has been documented in *P. falciparum, P. vivax* and *P. malariae* among the five Plasmodium species known to infect human in nature. Drug resistance also evolved to nearly every antimalarial drug in use [WHO, 2010b]. It is widely distributed in *P. falciparum* compared to *P. vivax* and *P. malariae.*

2.5.3.1 Insecticide resistance mechanisms in malaria vectors

Insecticide resistance mechanisms that the insects use to resist the impact of the insecticides on them could be grouped into four distinct categories including behavioral resistance, cuticular resistance, target-site insensitivity, and metabolic resistance.

2.5.3.1.1 Behavioral resistance

It is the ability of the insect to avoid insecticide treated surfaces without having the lethal effect of the insecticide [Grieco *et al*., 2007]. The insect could move away either after making tarsal contact with insecticide treated area (contact irritancy) or before making a direct contact with insecticide treated surfaces (spatial repellency) [Roberts *et al*., 1997]. Inhibition is also another form of behavioral resistance where some chemicals, such as DEET, mask or jam the presence of a host through the inhibition of odor-mediated receptors [Ditzen *et al.*, 2008]. Insecticide avoidance is not genetically based shift in behavior but it is developed due to repeated exposure to an insecticide [Chandre *et al*., 1999; Roberts *et al*., 1997; Muirhead-Thomson, 1960]. Reports indicated that a shift in feeding behavior from indoor to outdoor; and in biting time from night to early morning occurred because of behavioral resistance development in *An. funestus* populations

[Moiroux *et al.*, 2012; Russel *et al*., 2011) following the distribution and use of pyrethroid treated long-lasting insecticides. Such kind of behavioral resistance would compromise the impact of the existing vector control tools such as LLINs and IRS because these insecticides based vector control tools are developed to be used indoors.

2.5.3.1.2 Cuticluar resistance

Malaria vectors take the lethal dose of the insecticide while resting on insecticide treated surfaces such as walls, ceilings or mosquito nets through its tarsal contact. However, if the vector develops resistance, its tarsal cuticle will be thickened or its permeability will be decreased so that the absorption or penetration of insecticides would be prevented or reduced [http://dx.doi.org/10.5772/56117]. Cuticular resistance is mentioned in mosquitoes but characterized rarely. Few recent studies demonstrated cuticular resistance in pyrethroid resistance *An. funestus* and *An. gambiae* populations [Wood *et al*., 2010; Awolola *et al*., 2009]. Thus, much more work is required to characterize its role and level of significance in the control of malaria transmission.

2.5.3.1.3 Target site resistance

Target site resistance occurs due to point mutation that modifies the structure of the action site of the insecticide so that the insecticide cannot bind and impact effectively. Acetylcholinesterase (whose role is the hydrolysis of the neurotransmitter acetylcholine), the c-aminobutyric acid (GABA) receptors (chloride-ion neurotransmission channels in the insect's nervous system), and the sodium channels (responsible for raising the action potential in the neurons during the nerve impulses) are the target sites of organophosphorus and carbamate, cyclodiene (organochlorine) and pyrethroid and

organochlorine insecticides, respectively [Fournier, 2005; Soderlund & Knipple., 2003]. Alterations in the target site that cause resistance to insecticides are often referred as knockdown resistance (kdr). Knockdown resistance is the ability of the insect to tolerate prolonged exposure to insecticides without being 'knocked-down" [Donnelly *et al*., 2009]. Target site resistance to pyrethroids and DDT in *An. gambiae* is due to a substitution of an amino acid at a single codon in the sodium channel membrane gene. Two kdr alleles occur in *An. gambiae*, a leucine to phenylalanine substitution, known as West African knock down (*kdr-w*) [Martinez-Torres *et al*., 1998] and a leucine to serine substitution known as East African Knockdown (*kdr-e*) [Santolamazza *et al*., 2008]. G119S mutation (i.e. glycine to serine substitution at position 119) responsible for carbamate and organophosphate resistance has been reported in *An. gambiae and An. albimanus*, principally at the heterozygous state [Weill *et al*., 2004]. Recent sequence analysis of some resistant mosquitoes collected in Benin revealed the presence of a duplication of the ace-1 gene in *An. gambiae* M and S forms [Djogbenou *et al*., 2008].

2.5.3.1.4 Metabolic resistance

Metabolic resistance also known as detoxification mechanism occurs due to sequestration, metabolism and/or detoxification of the insecticide, largely through the overproduction of specific enzymes [Ranson *et al.*, 2011; Hemingway & Karunaratne, 1998; Hemingway *et al*., 1998]. Carboxylesterases (efficient against organophosphate and carbamate insecticides), glutathione S-transferases or GSTs (efficient against organophosphates, organochlorine, and pyrethroid insecticides) and cytochrome P450-dependent monoxygenases (efficient against most insecticide types, frequently in conjunction with other enzymes) are the three main categories of enzymes involved in

metabolic resistance [Montella *et al*, 2012; Russell *et al*., 2011; Hemingway & Ranson, 2000; Hemingway *et al*., 2004). The overproduction of these enzymes may be achieved via two non-exclusive mechanisms: gene amplification increasing the gene's copy number and gene expression via modifications in the promoter region or mutations in trans-acting regulatory genes [Hemingway *et al*., 1998; Rooker *et al*., 1996]. In addition, in some mosquito species, carboxylesterase resistance to the insecticide malathion has been associated with a qualitative change in the enzyme (a few amino acid substitutions can increase the rate of hydrolysis of the enzyme [Hemingway *et al*., 2004].

2.5.4 Geographical and climatic factors

Malaria transmission is influenced by different geographical and climatic factors because of the effect of these factors on the biology and ecology of malaria parasites and its vectors. Topography is among geographical factors that affect malaria distribution and transmission in such a way that as altitude increases temperature gets cooler [Hay *et al*., 2002] and impacting the development of malaria vectors and the parasite they transmit [Minakawa, 2006]. Flat land surfaces collect, contain and maintain water where the immature stages of the vectors could be reared [Minakawa *et al*., 2005].

Temperature, rainfall and humidity are also important climatic factors that influence the dynamics of malaria vector population. Rainfall provides water in the breeding sites for mosquitoes to lay their eggs, and ensures the availability of a suitable relative humidity (at least 50% to 60%) to prolong mosquito survival. Relative humidity below 60% shortens the life span of the mosquitoes. But, heavy rains, which results in flooding can have a flushing effect, cleansing immature stages (larvae and pupae) from their breeding

sites [Paaijmans *et al*., 2007; Reiter, 2001]. The daily survival of the vector is also dependent on temperature. At temperatures between 16°C and 36°C, the daily survival is about 90%. This survival drops rapidly at temperatures above 36°C. The highest proportion of vectors surviving the incubation period (the time being infected and infective) is observed at temperatures between 28° C and 32°C [Craig, 1999; Craig, 2004]. The gonotrophic cycle (the time between two blood meals of the vector) is short at higher temperatures because the digestion speed increases. Therefore, higher temperatures result in more frequent vector-host contact that would increase the probability of contracting the disease. Similarly, the development of the parasite within the mosquito (sporogonic cycle) is dependent on temperature. The sporogonic cycle takes about 9 to 10 days at temperatures of 28°C, but stops at temperatures below 16°C [Lindsay *et al.*, 1996].

2.5.5 Socio-economic risk factors

House types and proximity to mosquito breeding sites, knowledge and awareness, occupation, household revenue, access to health facilities, [Yadav *et al*., 2014), population size [Kreuels *et al*., 2008], occupation and human movement [WHO, 2003] and presence of animals in or nearby residential houses [Mayagaya *et al*., 2015] are some of the factors related to socioeconomic issues that could influence the level of risk of malaria transmission in a community or population. The risk of exposure to mosquito biting could be high in poorly constructed houses characterized by having eaves, unscreened windows and doors, with poorly fitted windows and doors, gaps between walls and floors etc. because all these allow mosquitoes to have access into the house and

increase the probability of its contact with its host [Hiscox *et al*., 2013; Yadav *et al.*, 2014]. Vectors have limited flight range so that they would prefer to houses located nearby their breeding sites to find their hosts for blood meals than houses located at distant. The misconception and beliefs about malaria could be setbacks to control malaria so that policy makers and control programmers need to consider communities' knowledge, attitude and perceptions towards malaria while developing control strategies. Household revenue, accessibility of antimalarial drugs and vector control tools and health facilities have inverse relationship with transmission risk; as the household revenue increases the capacity to cover the costs associated with malaria prevention and treatment increases so that the risk decreases [Yadav *et al.*, 2014]. The probability of receiving mosquito bites is high for those who are having nocturnal activities outdoors. The risk of contracting malaria for migrants coming from non-malariaous areas to malarious area is high because these people do not have immunity against malaria [WHO, 2003]. Zooprophlaxis (the use of animals as alternative host) to divert malaria vectors away from the human host is also considered as a strategy to minimizes the risk of malaria transmission [WHO, 1982]. But, the availability of alternative hosts in huge number near or inside residential houses could increase the number of mosquitoes attracted due to the odors (cues that help vectors to locate their hosts) emitted by them so that the chance of human hosts to receive more mosquito bites and infection would be high [Iwashita *et al*., 2014]. Furthermore, the presence of alternative hosts such as livestock in a greater number nearby human settlements could also make mosquito breeding sites (larval habitats) by their hoof prints and watering sites [Minakawa *et al*., 2002]. Therefore, the effectiveness of zooprophlaxis depends up on the type of vector species involved; i.e. the

effect could be high on *An. arabiensis* [Kaburi *et al*., 2009] than on the anthrophilic and endophilic *An. gambiae* s.s. [Bogh *et al*., 2002]. The number of available hosts also affects the level of transmission risk because as the number of hosts increases the number of mosquito biting per individual host decreases [WHO, 2003].

2.6 Status of malaria in Ethiopia

2.6.1 Country profile

The Federal Democratic Republic of Ethiopia (FDRE) is in the horn of Africa bordered by Eritrea, Sudan, Kenya and by Somalia and Djibouti in the north, west, south and east, respectively. It lies within the tropics between 3 and 15 degrees North altitude and 33 degrees and 48 degrees East altitudes. The total landmasses of the country cover 1.12 million square kilometers. The country has nine regional states and two city administrations [FDREMOH, 2006; Figure 2 A & B]. The total population of the country projected for 2017 was estimated to be 94, 351,001 [FDRECSA, 2013]. Ethiopia has extremely varied topography with altitudes ranging from 136 meters below sea level in the Dalol Depression (Afar) on the northern border to 4,490 meters in the highest mountain, Ras Dejen in the Semen Mountains north of Lake Tana [Figure 2C]. There are vast areas of plateaux and marginal slopes between the two extremes.

The country has heterogeneous climates and climatic elements such as rainfall (Figure 2E) and temperature (Figure 2D) etc. that are affected by altitude, i.e. as altitude increases temperature decreases and vise versa. Altitude and rainfall have positive relationship; i.e. highlands receive high rainfalls annually while the mean annual rainfall is low in the lands.

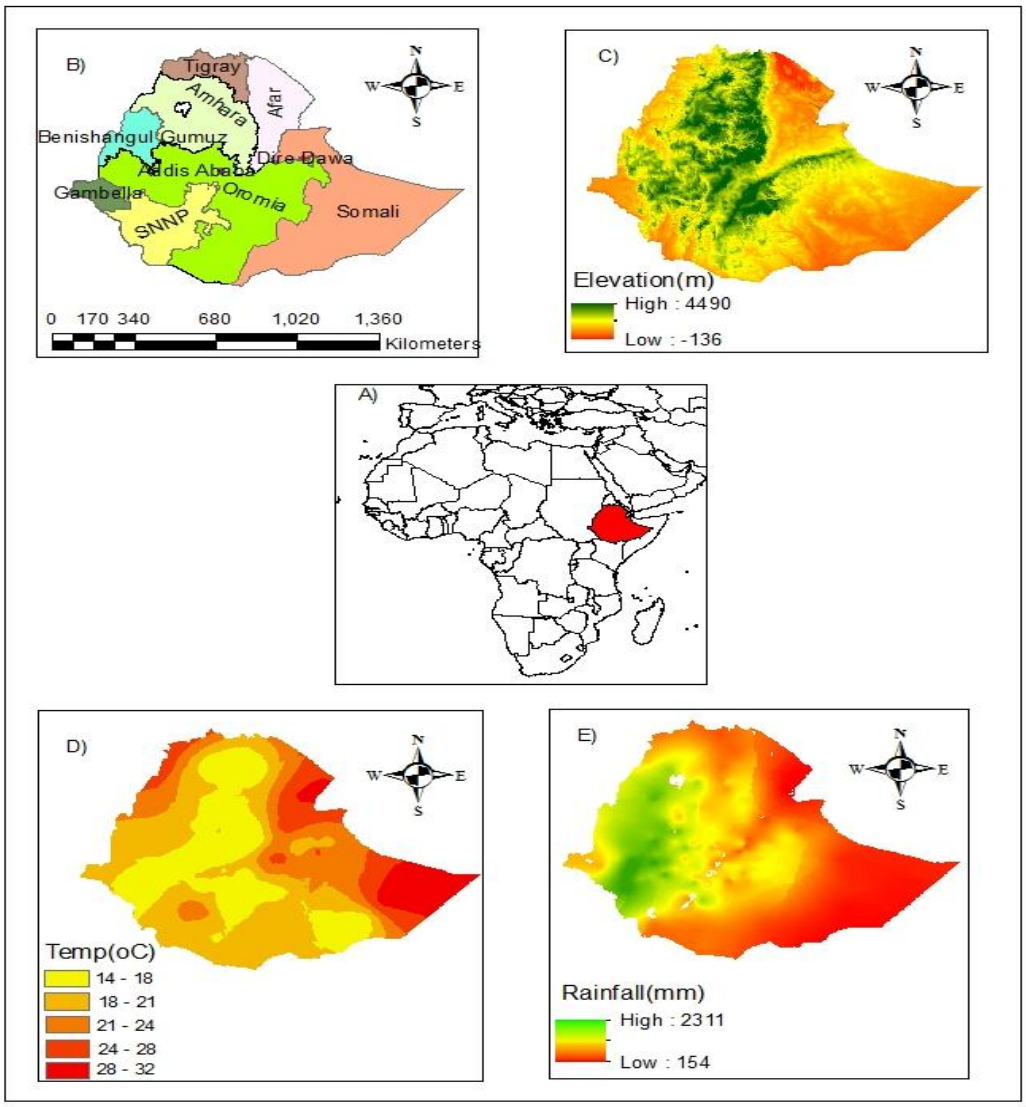


Figure 2: Resource map of Ethiopia based on EthioGIS (1999) (A) Location map of Ethiopia in Africa (B) Regional States of Ethiopia (C) Altitude above sea level (D) Annual daily average temperature (E) Mean Annual rainfall

2.6.2 Distribution and transmission of malaria

According to the Federal Democratic Republic of Ethiopia Ministry of Health [FDRMOH, 2012] about 75% of the land masses of the country is malarious and malaria continues to be one of the major health problems to 68% of the population inhabiting in these areas. Temperature and rainfall in combination with topography are considered as the most important factors to determine malaria distribution in the country and areas below 2,000 meters are known to be malarious. Malaria has also been reported up to 2,500 meters recently [FDREMOH, 2006]. The intensity of malaria transmission is the highest in the low land areas below 1,500 masl that receives ≥ 500 mm rainfalls and lowest in Semi-arid low land areas below 1,500 masl that receives < 500 mm rain falls annually. Malaria transmission intensity is higher in areas having altitudes ≥ 1,500 and < 1,750 masl; and lower in areas ≥ 1,750 and < 2,000 masl. Occasional malaria transmissions occur in an epidemic form in the highlands that have elevations ≥ 2,000 and ≤ 2,500 masl. Areas having altitudes > 2500 masl are free of malaria (Figure 3).

Transmission is either seasonal or perennial depending on the area under consideration. The major malaria transmission season occurs between September and December following the June -September rains, while the minor transmission season occurs between April and May following the February - March rains. Areas with bimodal transmission pattern are limited and restricted to a few areas that receive the small/Belg rains. The major transmission season occurs in almost every part of the country. Perennial or year round transmission is confined to Western and Northwestern parts of the country that are located nearby river basins [FDRMOH, 2006]. Malaria transmission period varied from

location to location in the county. The highest transmission period is over six months in areas having permanent mosquito breeding sites with suitable climatic and environmental factors for both vector and parasite development. The largest parts of the country have a transmission period between 3 and 6 months following the heavy rainy season. Certain parts of the country also have < 3 months malaria transmission period (Figure 4).

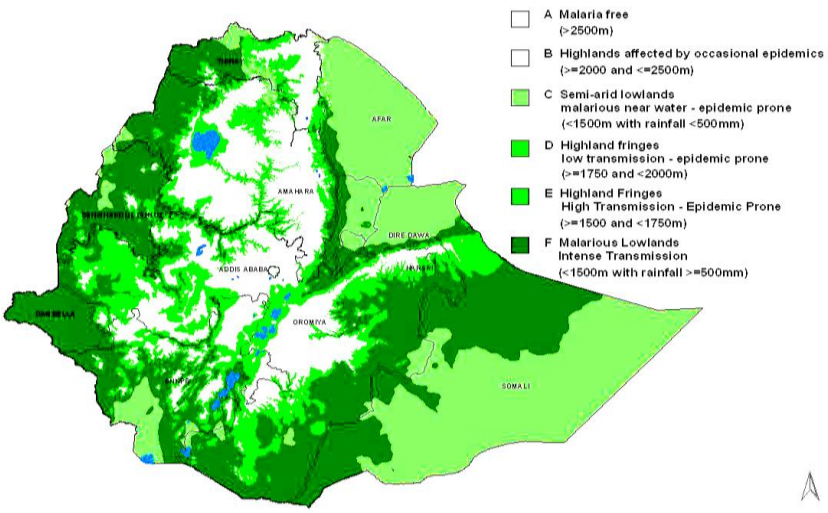


Figure 3: Map of malaria distribution in Ethiopia (Adopted from FDREMOH Malaria National Strategic Plan 2014-2020)

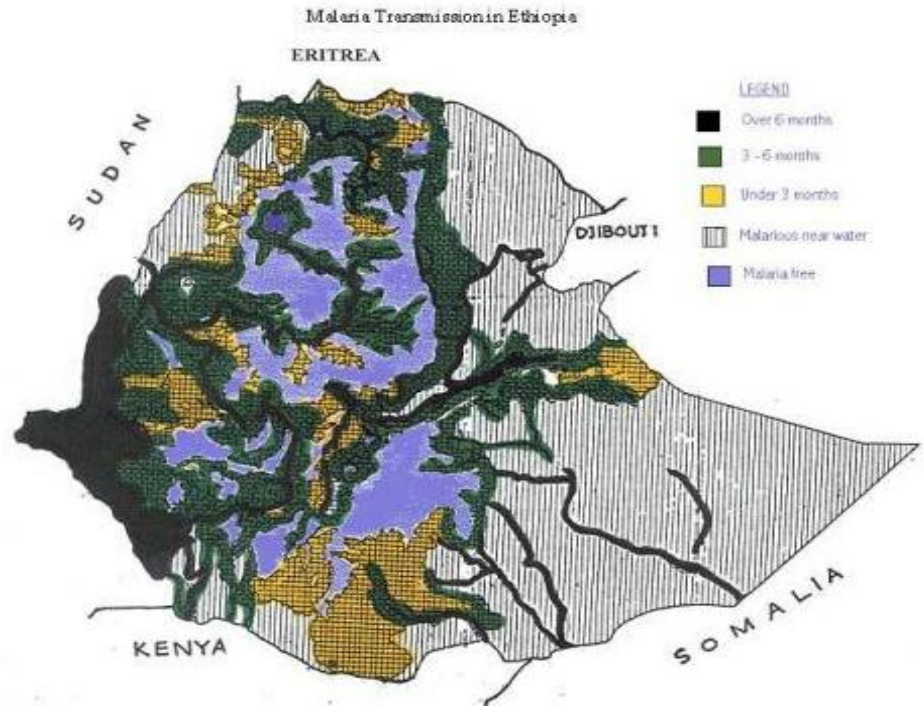


Figure 4: Map showing malaria transmission period in Ethiopia (Adopted from FDREMOH, 2006)

2.6.3 Dominant malaria vectors and parasites

About 42 Anopheline species were identified in Ethiopia [Mekuria, 1983; Geberemariam, 1988] among which four of them namely *An. arabiensis, An. funestus, An. pharoensis* and *An. nili* are incriminated as important vectors of malaria in the country. *An. arabiensis* is the only sibling species of the *An. gambiae c*omplex involved in malaria transmission. *Plasmodium falciparum* and *P. vivax* are the dominant malaria parasites constituting 59% and 41% prevalence, respectively [WHO, 2015].

2.6.4 Malaria intervention strategies

In Ethiopia, malaria prevention and control strategies target both the vector and the parasite. Vector control intervention tools include IRS, LLINs, larval control via environmental management and larviciding. Indoor residual spraying and LLINs are the most widely used chemical based indoor vector control intervention tools in the country. Long-lasting mosquito nets are distributed free of charge and the cost of insecticides and IRS operation is covered by the government. The country scaled up the distribution of LLINs and more than 60% of the population had access to one LLIN in 2014. Indoor residual coverage was more than 20% in the same year. Diagnosis and case treatment with artemisinin-based combination therapy (ACT's) and after being diagnosed either by rapid diagnosis test (RDT) or microscope are also used routinely and implemented broadly. Both diagnosis and treatment services are given free of charge at government's health facilities [WHO, 2015].

2.6.5 Impact of malaria control interventions on malaria and entomological malaria transmission risk factors

Reports indicated that malaria has been declined heavily in Ethiopia following the scale up of LLIN distribution and IRS coverage. About 50-70% of reduction reported in cases and incidence between 2000 and 2015 [WHO, 2015, Deribew *et al*., 2017]. However, the correlation between these chemicals based indoor malaria vector control intervention tools and the contribution of these intervention tools either separately or in combination on different entomological factors is not clear. Altitude, rainfall and temperature are considered as key determinants of malaria transmission in the country [FDREMOH, 2006]. However, the association between these geographic and climatic factors on entomological factors is also not clear and becomes an important gap that needs to be addressed at different ecological settings in the country. All malaria vector control interventions primarily targets *An. arabiensis. An. arabiensis* is both anthrophilic and zoophilic and bites its host either indoor (endophilic) or outdoor (exophagic). The vector also has both indoors and outdoors resting habits. *An. arabiensis* has developed different levels of resistance at least for an insecticide from all classes of insecticides and the distribution of insecticide resistance is high in the country (Abate & Hadis, 2011; Yehualaw *et al*., 2010; Balkew *et al*., 2003

CHAPTER 3: General Materials and Methods

3.1 Study site

The present study was conducted in Andassa and nearby villages, Bahir Dar Zuria District, Amhara Regional State, Upper Blue Nile River Basin, and Northwestern Ethiopia (Figure 3). The district has proximity to Lake Tana and crossed by the mighty Blue Nile River and its tributaries, i.e., Andassa River, Tikurit and Yudoket streams. The area was selected purposively by considering malaria endemicity and associated risk factors for malaria transmission such as the existence of permanent, semi-permanent and temporary vector breeding sites, presence of important malaria vectors, and socio-economic characteristics. The total study area covered about 47.13 km^2. The area has bimodal rainfall patterns, with a long rainy season between June and September, and a short rainy season between March and April. According to unpublished report from the district health office, the study area receives between 820-1250 mm rainfalls annually, while the minimum and maximum temperatures range between 10^oC and 32^oC. Traditional small-scale irrigation and rain fed farming, and animal husbandry are the main livelihoods of the inhabitants. Vegetables, maize, sugarcane, teff, millet and fruits are important crops of the study area (Figure 6). Indoor residual spraying and LLINs are in the first line of defense against *An. arabiensis* (important vector of the study area). The vector has developed different levels of insecticide resistance to insecticides of different classes recommended for both LLIN treatment and IRS operation [Abate & Hadis, 2011].

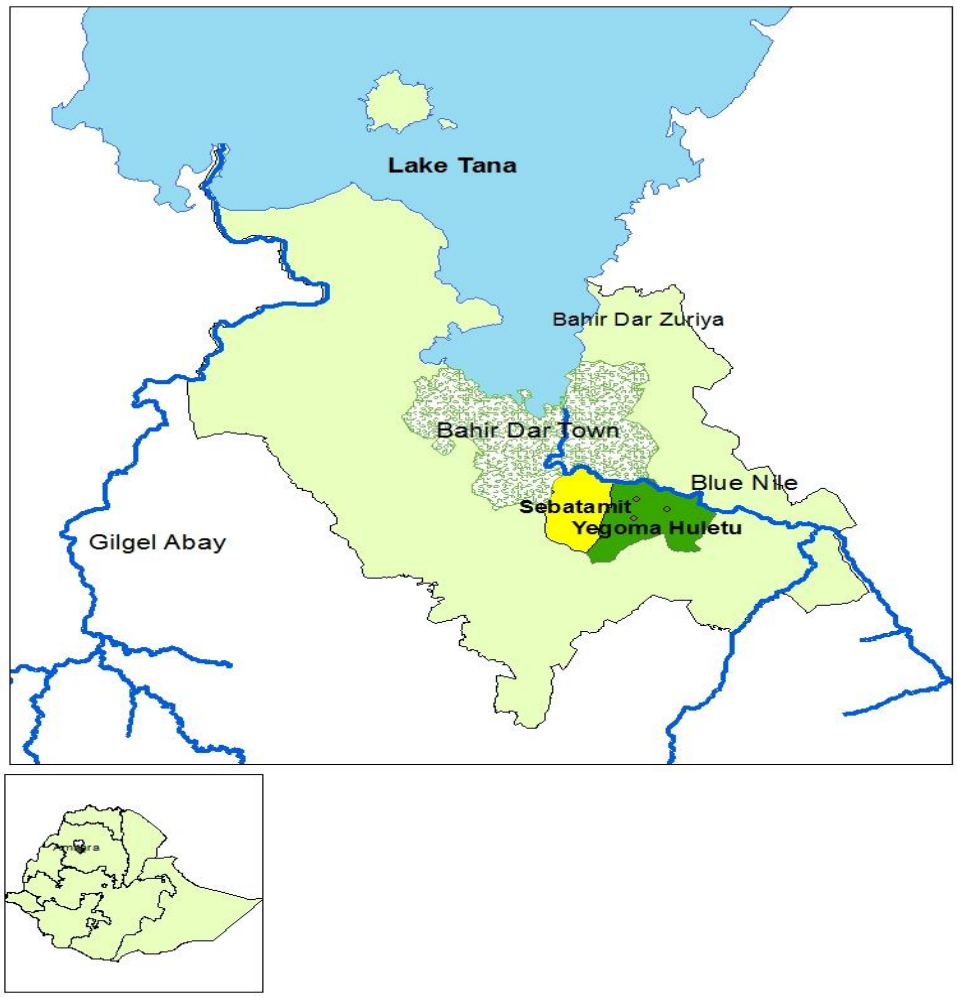


Figure 5: Relative location map of the study site

Figure 6: Some environmental features of the study site (A) topography (B) Sugercane and Maize (C) Tef (*Eragrostis tef*) and (D) Papaye & Chat (*Catha edulis*)

3.2 Study design

A comparative study was carried out in Yegoma Huletu kebele at three villages namely Andassa (N11°30'.146", E 037°29'.278'', Alt., 1,716), Tigeza (N11°29'.4", E 037°27'.522", Alt., 1,682), Tikurit (N 11°30' .498", E 037°28'.028 ", Alt., 1, 740), Tikurit-Dewel (N 11°31'.142", E 037°23'.523", Alt., 1,775) (Figure 5). The study group (Annex I) categorized the study villages after a field visist into two study groups, Andassa and Tigeza in one group, named as Andassa; and Tikurit and Tikurit-Dewel in the second group, and named as Tikurit. Grouping was made based on their proximity to each other, while other factors were considered similar.

Twenty-four houses (12-houses for PSC and 12-houses for CDC light trap collection) located nearby mosquito breeding sites (Figure 7) were selected and used for mosquito sampling. Twelve pit shelters were also constructed with a depth of one meter and width of 1.2 meter under shed trees and nearby PSC mosquito sampling houses. Mosquito sampling was done in the same houses and pit shelters throughout the study period. Entomological data were collected from the two study groups before intervention (September 2013 and 2014) and after the intervention (October to December 2013 and 2014; and September 2015). Data were compared between the study sites and within the same study site (group) before and after the intervention.

Figure 7: Mosquito sampling houses situated nearby mosquito breeding sites

3.3 Indoor residual application

Bendiocarb was sprayed at a rate of 400 mg/m^2 in Andassa in October 5-11/2013 and 2014, and in both study groups in September 4-10/2015. The formulation was prepared based on the guidelines given on the pre-dosed sachets by the manufacturer. Spray men, who carried out the spray, were recruited from local community and they were given hands on training using (Figure 8) WHO IRS modus operandi [WHO, 2013b]. The spray

men were provided with protective gears (hand gloves, goggles and masks) and sprayed the inside parts of the walls and roofs using manual pressure sprayer of the HUNDSON XPERT type. Over 90% of the structures (both human and animal shelters) were sprayed. The researcher with the support of field technical assistants supervised the quality of spraying.

Figure 8: Spray man exercising IRS application (left) and implementing IRS operation (right)

3.4 Bioassays

3.4.1 WHO insecticide susceptibility testing

Before the application of IRS, susceptibility of wild *An.arbiensis* to bendicarb (0.1%) was determined following WHO susceptibility test procedures (WHO, 2013a, Figure 9). Pupae and larvae of *An. arabiensis* were collected from their breeding sites using larval sampling procedures (WHO, 1975) and reared into adult in field insectarium (Figure 9). Susceptibility status of the vector was also examined against DDT, delthamethrin and malathion to assess whether susceptibility status of the vector was getting reversed and to examine cross-resistance between different insecticide groups. Bendiocarb was the choice

of insecticide used for IRS application during the study period, while propoxur and premiphosmethyl are alternatives for IRS application. Mosquitoes recorded as either dead (susceptible) or alive (resistant) were stored individually over silica gel (Figure 9) or 100% ethanol for PCR based molecular analyses. Abbots formula [Abbott, 1925] was used to correct control mortality when percent of control mortality was between 5% and 20%.

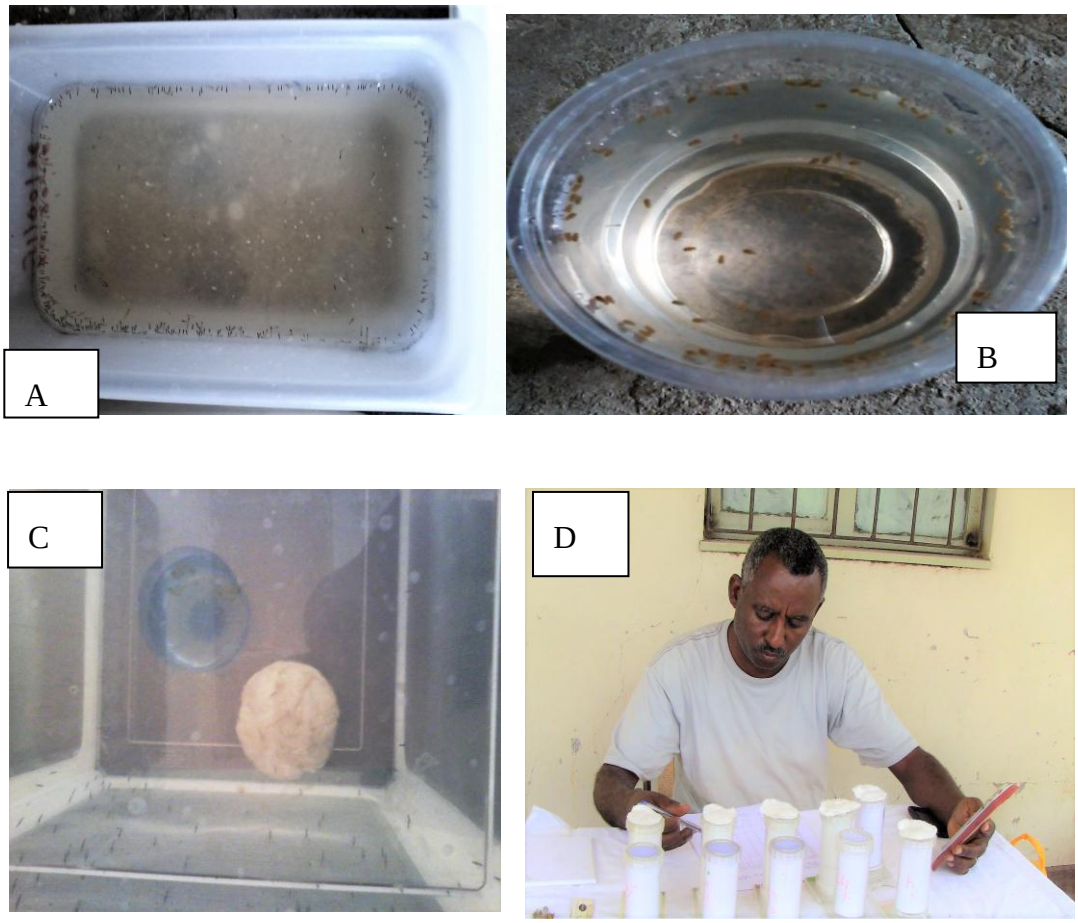
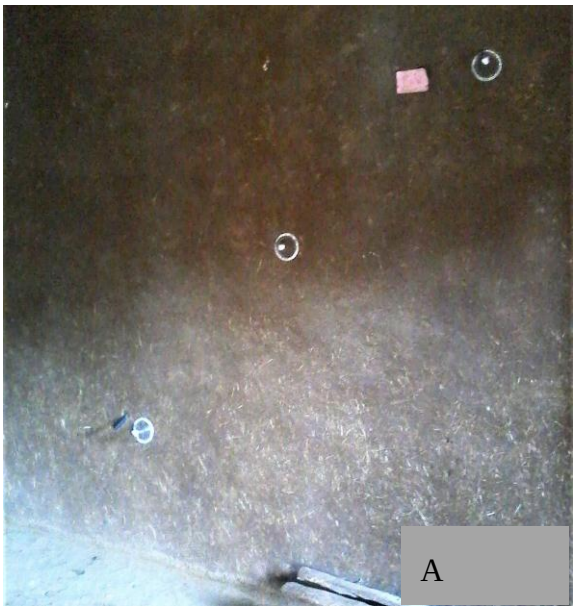


Figure 9: Rearing of *An. arabiensis* (A) larvae (B) Pupae (C) Adult under field insectarium and (D) Susceptibility testing

3.4.2 WHO wall bioassay

Wall bioassays (WHO, 2**0**06a) were carried out on two wall surfaces types. These surfaces types were mud-walls plastered with cow dung and dried before sprayed and mud walls without being plastered with cow dung. Cow dung plastering by mixing cow dung with water is a common practice by dwellers in these and other parts of the country to make the inside of mud wall surfaces smooth and fill out cracks. Sprayed houses were not re-plastered until the end of the study period. The efficacy and the residual life of bendiocarb were estimated using eight sprayed houses (four houses with mud walls plastered with cow dung and four houses without plastering). Non-sprayed wall surfaces of the same type with the sprayed ones (one house with mud walls plastered with cow dung and the other without plastering) were used for the control group. Ten sugar fed two- to three - days old mosquitoes were introduced into cones firmly fixed near the bottom, in the middle and on top parts of sprayed wall surfaces (Figure 10).



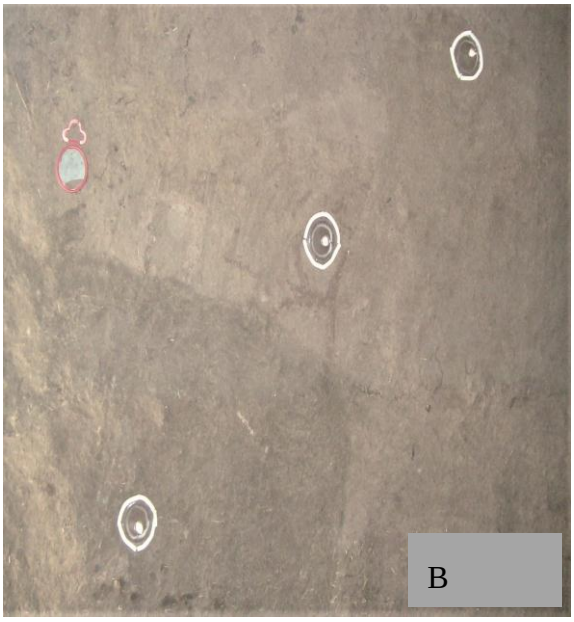


Figure 10: WHO wall cone bioassay to estimate the effective residual life of bendiocarb sprayed on (A) Non-plastered (B) cow dung plastered mud wall surfaces using susceptible female *An.arabiensis*

3.5 Adult Mosquito sampling

Adult mosquitoes were collected using three sampling methods specifically CDC light trap (Figure 11 A) PSC (Figure 11 B) and pit shelter (Figure 11 C) sampling methods. Mosquito sampling was done from residential houses for two consecutive days/month. Data collection was done during the main malaria transmission season

Figure 11: Adult mosquito sampling using (A) CDC light trap (B) PSC (C) Pit shelter sampling methods

3.6 Species identification and preservation

3.6.1 Morphological methods of species identification

Mosquitoes that were collected by the three sampling methods were sorted into species and sexes, counted and recorded (Figure 12). Each species was identified using external morphology (Verone, 1962; Gillies & Coetzee, 1987). Species of the *An. gambiae* complex were then preserved individually in a tube containing either silica gel (Figure 12 B) or 100% alcohol for further PCR based molecular analyses because they are morphologically identical [Wilkins *et al.*, 2006].

Figure 12: Mosquito (A) Sorting, morphometric identification & counting (B) Mosquito preserved individually in Eppendorf tube containing silica gel

3.6.2 Molecular species identification

The primary strategy for *An. gambiae* complex species identification is the use of universal forward primer (IMP-UN) for all species in a cocktail with four species-specific

reverse primers [Wilkins *et al.*, 2006]. These cocktails of primers produce a 636 bp fragment for *An. quadriannulatus*, 528 bp *An.merus*, 463 bp *An. gamb*ae and 387 bp *An. arabiensis*.

3.6.2.1 DNA extraction

Each mosquito preserved in alcohol was grinded individually in a tube containing 50 µl of grinding buffer with a sterile blue knots pestle until no identifiable mosquito parts remained. The pestle was then rinsed with additional 50 µl of grind buffer into the same tube. The tubes were then put in dry bath for 30 minutes to kill nucleases released after grinding the mosquitoes so that they do not degrade the DNA. Thirteen microliter of 8M potassium acetate was added to each tube while still warm and mixed up by tapping. The tubes were then left on ice for an hour to precipitate out the mosquito parts, other insoluble and proteins denatured by SDS. The solution was micro centrifuged at a maximum speed (13, 2000 rpm) at 4^{O} C for 20 minutes and the supernatants were transferred into the new tubes immediately after the spin without disturbing the precipitate. Two hundred micro litter of 100% ethanol was added to each tube containing the supernatant and left at -20 ^{O} C for incubation overnight and to precipitate out the DNA. The next day the tubes were micro centrifuged at a maximum speed for 20 minutes to pellet the DNA and two hundred micro litter of 70% ice-cold ethanol added to each tube, micro centrifuged for 5 minutes again. The 70% ethanol was then pipetted out and the DNA pellets dried in air. The DNA pellets were dissolved in 20-50 µl of sterile H_2O depending on the size of DNA pellet and stored at -20 ^{O} C for short term and -80 ^{O} C for long term storage.

3.6.3 Detection of knock down resistance

Knockdown resistance to pyrethroid insecticides and DDT is commonly associated with a single base-pair point mutation caused by a substitution of leucine to phenylalanine substitution (TTA/TTT) (West African type) or a substitution of leucine to serine (TTA/TCA) (East African type). The intentional mismatch PCR primer method described by Wilkins *et al*., [2006] with a minor modification by CDC was used to detect kdr in *An. arabiensis*.

3.6.4 Blood meal sources and sporozoite rate determination

Enzyme-linked immunosorbent assay (ELISA) originally described by Beier *et al.,* [1988)] and CS-ELISA [Wirtz *et al*., 1987] protocols were adopted and used for blood meal host source and sporozoite rate analyses, respectively. Blood fed mosquitoes were preserved individually in tubes containing silica gel (Figure 12B) were used to determine their blood meal host sources and sporozoite infection rate. Heads-thoraxes of mosquitoes were separated from their abdomens and each body part (abdomen/ head-thorax) was given a corresponding ID number and kept individually in tubes for blood meal host source and sporozoite infection rate.

3.6.5 Determination of entomological inoculation rate

Entomological inoculation rate is a measure of transmission intensity expressed as the number of infective bites per person per unit time (e.g., per day, month, year) [Lines *et al.,* 1991]. It is calculated by multiplying the human-biting rate (HBR) by the proportion of sporozoite positive mosquitoes.

3.6 6 Data entry and analyses

The data collection tools were developed by the research team and completed by the principal investigator. Data were also entered to the computer using a template designed in EPIINFO software by the investigator. The data were then exported first into excel sheet and then into SAS version 9.2. Frequency distribution of all variables was run to assess missing values and anomalies (outliers) in the data set. Normality test was also performed.

3.6.7 Ethical Clearance

Ethical clearances were obtained from the Scientific and Research Ethics Committee of Ethiopian Public Health Institute and the Amhara Regional Health Bureau (ARHB). Community members were informed about the study prior to initiation and verbal consent was obtained from heads of mosquito collection sentinel houses.

CHAPTER 4: Effective residual life of bendiocarb 80% WP on sprayed surfaces

4.1 Introduction

The history of indoor residual spraying (IRS) begun in 1960s and remains a powerful malaria vector control tool in Ethiopia. It has been implemented for decades in the country. One round of IRS application has been implemented in most malarious areas of the country usually in the end of the main rainy season, while two rounds of IRS have been implemented in some areas especially at times of epidemic [FDREMOH, 2012].

In IRS operations, indoor spray able surfaces and eaves of dwellings are sprayed with the recommended concentration of the insecticide chosen for IRS application before the onset of malaria transmission to avert possible epidemics [FDREMOH, 2012]. The purpose of IRS is to shorten the lifespan of indoor resting malaria vectors, reduce vector density and intercept human-vector contact [FDREMOH, 2012; Akogbéto *et al*., 2011], which would be influenced by the residual efficacy of the insecticide, vector's behavior and ecology. Residual efficacy of the insecticide sprayed indoors depends up on the effective life of the insecticide on sprayed surfaces and susceptibility of the vector to the insecticide sprayed [WHO, 2006a]. The effective residual life of the insecticide on sprayed surfaces is an important factor to determine the number of spraying cycles required to cover malaria transmission season under consideration [Ferro *et al*., 1995]. Physical and chemical properties of spray able surfaces of dwellings are among the attributes that impact the active residual life of the insecticide sprayed for vector control [Arias *et al*., 2004; de Arias *et al*., 2003; Hadaway & Ballow, 1963]. Climatic factors are

also among the factors that could influence the bio-efficacy of the insecticide sprayed indoors for vector control [Bordas *et al*., 1953; Bami, 1961; Smith & Hocking, 1962; http://www.who.int/whopes/Insecticides_IRS_Malaria_09].

DDT was the choice of insecticide for IRS operation that had been used for decades in many malarious areas of Ethiopia except at a few places where malathion was used for DDT resistance vector populations. This was continued until 2007 when DDT was replaced by delthamethrin due to the development of DDT resistance in the major malaria vector populations [FDREMOH, 2012]. Payable to the occurrences of delthamethrin resistance in different vector populations, in view of the possibility of cross-resistance between DDT and pyrethroid insecticides and the scaling up of the distributions of pyrethroid treated LLINs, IRS control program again replaced delthamethrin by bendiocarb (carbamate group) in 2010 and still in use for IRS operations in different parts of the country.

The residual efficacy of bendiocarb with the recommended concentration could last between 2 and 6 months depending on the nature of spray able surfaces [Cullen & De Zulueta, 1964]. Studies indicated that the residual effective life of bendiocarb 80% was not consistent across published reports [Maharaj, 2004; Mpofu *et al*., 1991; Etang *et al*., 2011; Djènontin*et al*., 2013]. This necessitates the need to determine the effective residual life of any insecticide chosen for IRS intervention at local ecological setups. Besides, susceptibility of the same or different vector populations to insecticides could vary from region to region and/or from locality to locality. Thus, any malaria vector control program involving IRS operations should primarily confirm the susceptibility of

the vector to the insecticide chosen for IRS and establish the effective residual life of the insecticide on common sprayed surface types of the structures under natural conditions to determine spraying cycles.

Studies have shown that different populations of *An. arabiensis* (the main malaria vector in Ethiopia) have developed different levels of resistance at least to one insecticide per insecticide class including bendiocarb. Both published and unpublished reports indicated that the level of insecticide susceptibility status of the malaria vector varies from locality to locality [Tangena *et al*., 2013; Sibanda *et al*., 2011]. Therefore, insecticide resistance data should be considered, while choosing insecticides for IRS operation. Substrate types could influence insecticide persistence on sprayed surfaces, so that the effective life of the insecticide should be estimated to determine spraying cycles. Studies on the impact of different wall substrates on the residual efficacy of insecticides commonly used for IRS in Ethiopia are limited [Yemane *et al*., 2016].

Therefore, the specific objective of the present work was to determine the effective residual activities of bendiocarb 80% WP sprayed on common surfaces of human dwellings against *An. arabiensis* in Andassa, Bahirdar Zuria District, North west Ethiopia.

4.2 Materials and methods

4.2.1 Study site

The study was carried out in Andassa and its surrounding villages described in detail in section 3.1 of this thesis. The study site is found at about 20 km southeast of Bahirdar

town, the capital of Amhara Regional State. Most of the residential houses were/are constructed of wood framework mortared by mud and roofed by corrugated iron. Mud walls were/are again either plastered by cow dung diluted in water or without plastering.

4.2.2 Wall cone bioassays

Cone bioassays of four replicates/surface type/ round (120 mosquitoes/surface type) were carried out using WHO [2006a] supplied bioassay kit after 5, 35 and 65 days of the spray. Description of IRS application is given in section 3.3 of this thesis. For the control group, 30 mosquitoes/surface type/round were exposed on non-sprayed wall surfaces of the same type with the sprayed ones. Mosquitoes were exposed for 30 minutes and transferred into labeled paper cups covered with untreated white netting. Knock down rate (kdr) was recorded 60 minutes after being transferred into paper cups, while mortality was recorded after holding them for 24 hours at a temperature of 26 ± 2^{o} C and 70-80% relative humidity (RH). Mosquitoes were provided with 15% sugar solution during the holding period. Abbots formula (1925) was used to correct control mortality when percent of control mortality was between 5% and 20%.

4.2.3 Data analyses

Mortalities of the bioassay tests were calculated according to WHO cone test protocol [WHO, 2006a] to determine the effective residual activities of the insecticide. Poisson regression model was fitted to see the association of days after spray, wall plastering, insecticide spray and position of wall surfaces with mosquito mortality. The Poisson

regression coefficients along with their 95% confidence interval and p-value were used to determine the strength, direction and significance of association.

4.2.4 Ethical clearance

Verbal consent was obtained from heads of the households where wall bioassay tests were conducted. The study did not involve human subjects or animals.

4.3 Results

4.3.1 Impact of wall surfaces on the effective residual life of bendiocarb

The effective residual life of bendiocarb 80% WP on mud walls plastered with cow dung and non-plastered mud walls was estimated based on its killing effect on susceptible female *An. arabiensis* exposed to these surface types. Efficacy was considered effective when percent mortalities in exposed mosquitoes were ≥ 80 % [WHO, 2006a]. Bendiocarb was 100% effective in killing of *An. arabiensis* for about a month on both types of wall surfaces. After a month, efficacy decreased on both surface types. However, the efficacy of the insecticide deteriorated faster on non-plastered walls than plastered walls.

Table 1 presents the results of Poisson regression model. According to this model wall plastering, spray and time period had highly significant effect ($P \leq 0.001$, 95% CI) on mosquito mortality, while the impact of position was not significant ($P = 0.20$, 95% CI). Wall spraying [Coef = 3.637542: 95% CI (2.654363, 4.620721)] and wall plastering [Coef = 0.282841: 95% CI (0.1232043, 0.4424778)] had positive relationship with mosquito mortality, while the association between mosquito mortality and time lags was negative [Coef = -0.600159: 95% CI = -0.8157261, -0.3845919], i.e. as the time lags

increased after spray mosquito mortality decreased. However, there was no significant difference in mosquito mortality between day 5 and 35 (P = 0.917, 95% CI = -0.3845919, 0.16757) after spray. The effect of wall spray was the strongest of all factors considered in this model followed by time lags and plastering.

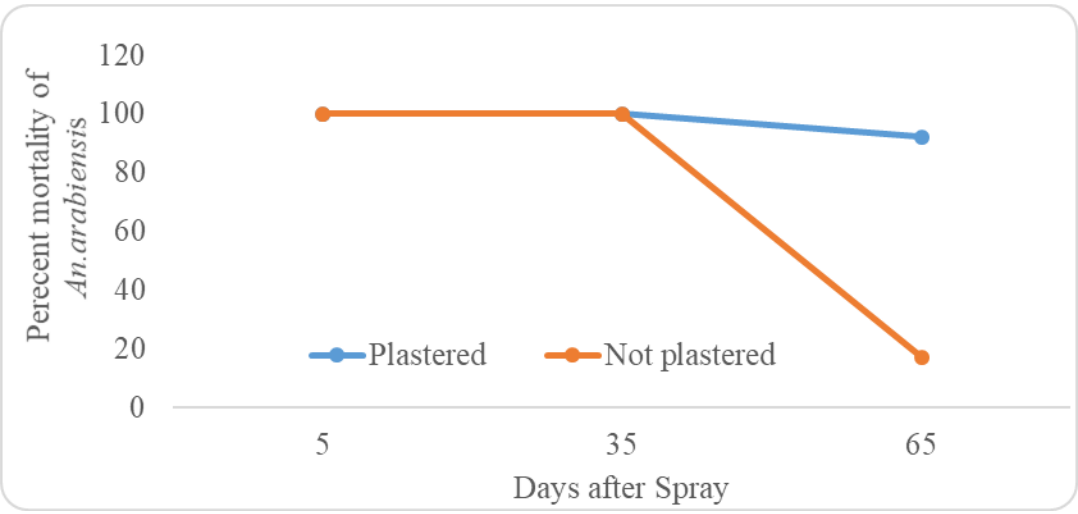


Figure 13: Estimated effective residual life of bendiocarb 80% WP sprayed (Blue) cow dung plastered surface & (Red) non-plastered mud wall surface based on percent mortality of bendiocarb susceptible female *An. arabiensis* against time

Table 1: Effective residual activities of bendiocarb 80% WP sprayed on plastered and non-plastered indoor wall surface types estimated based on mortality of susceptible *An.arabiensis* in Andassa & nearby villages,

Dead	**Coef**	**Std.Err.**	**Z**	**P > |z|**	**[95% Conf. interval]**	
Plastering	0.282841	0.0814488	3.47	0.001	0.1232043	0.4424778
Spray	3.637542	0.501631	7.25	0.000	2.654363	4.620721
Days after spray						
35	-0.0094612	0.0903237	-0.0100	0.917	-0.01864924	0.16757
65	-0.600159	0.1099852	-5.46	0.000	-0.8157261	-0.3845919
Position						
Middle	0.0195496	0.0980357	0.20	0.842	-0.1725969	0.2116961
High	-0.0196712	0.0990117	-0.20	0.843	-0.2137305	0.1743882

4.4 Discussions

The residual efficacy of an insecticide chosen for IRS intervention for the control of malaria vectors is evaluated based on the criteria set by World Health Organization Pesticide Evaluation Scheme (WHOPES) [WHO, 2006a]. Based on this recommendation, the residual efficacy of an insecticide is considered effective if mosquito mortality is ≥ 80% at 24 hours post exposure to sprayed surfaces for 30 minutes. In reference to WHOPES recommendation, the residual effective life of bendiocarb 80% WP was longer on cow dung plastered walls than on mud walls without plastering. Similar residual performance of this insecticide was reported from experimental hut trials in Ethiopia [Yemane *et al*., 2016]. However, the results from experimental hut trails indicated that the residual effective life of bendiocarb on mud walls without plastering was >2 months, while the present study reported <2 months. The potential reason for the differences observed between the present and experimental trial might be attributed to differences in their experimental set ups, i.e. the present study used residential houses where real life activities of dwellers might influence persistence of the insecticide while these were avoided in experimental houses. Furthermore, experimental hut trials were conducted in semi-arid area where humidity was reported to be low, while the present study was carried out in an area where humidity was expected to be high due to the available water sources and high temperature. Humidity is considered as an important factor to impact on the residual efficacy of an insecticide on sprayed surfaces [Maharaj *et al*., 2004].This efficacy study also demonstrated that the residual effective life of bendiocarb was >2 months on plastered mud walls. This is in line with the results of other studies reported from different countries including Ethiopia [Yemane *et al*., 2016; Djènontin*et al*., 2013;

Tangena *et al*, 2013; Etang *et al*., 2011; Sibanda *et al*., 2011]. Although the residual life of the insecticide was not identical across these studies, the residual efficacy of bendiocarb was between 2 and 6 months, which is in line with WHOPES recommendations [WHO, 2009]. The differences observed in these reports might be due to different attributes associated with the natural conditions of each specific study sites.

Because the purpose of this study was to estimate the effective residual life of bendiocarb 80% WP on different surface types under natural conditions, it was conducted on two wall surface types only for the reason that the residential houses of the study site were having either of these surfaces types during the study period. Therefore, this study did not involve other wall surface types such as walls made of cemented blocks and painted walls that are common in Semi-urban and urban areas in the country. The complete period of the residual efficacy of the insecticide was not established on plastered walls because it was not possible to monitor the residual efficacy of the insecticide beyond 65 days. Therefore, similar studies are recommended to better understand the impact of different wall surface types and other factors on the residual efficacy of this and alternative insecticides and establish the complete period of effective residual life of the insecticide (s) under natural conditions.

4.5 Conclusion

This study demonstrated mixed results. The effective residual efficacy of bendiocarb 80% WP on non-plastered mud wall surfaces is too short to cover malaria transmission season in areas having >1 month transmission. Therefore, alternative insecticides should be considered for IRS in areas having >1 month malaria transmission season and >20%

houses with non-plastered mud wall surfaces. On the other hand, the residual effective life of the insecticide was >2 months on plastered mud walls suggesting that bendiocarb would be the recommended insecticide for IRS in areas having plastered mud walls to cover at least 2-3 months of malaria transmission season.

4.6 Recommendation

Cow dung is easily available to rural communities in Ethiopia, so that community education to plaster their houses with cow dung would improve the effective residual life of bendiocarb.

CHAPTER 5: The effect of bendiocarb 80% WP indoor residual spraying on insecticide resistance status of *An. arabiensis*

5.1 Introduction

Insecticide resistance is a natural and heritable characteristic in an insect population that could be manifested by the failure of an insecticide product to provide the intended level of control when used as recommended for a particular species at a particular time and place. The magnitude of resistance development in an insect population is dependent up on the volume and frequency of the applications of the insecticides used against them and the inherent characteristics of the insect species involved [Pates & Curtis, 2005; Brooke & Koekemoer, 2010]. Studies indicated that a long term wide application of IRS and use LLINs augmented the selection of insecticide resistance gene frequencies [Stump *et al*., 2004; Reimer *et al*., 2005; Padonu *et al*., 2012].

Ethiopia has a long history of using IRS for its malaria vector control program and IRS remains to be one of the core interventions to control malaria vector. In most malarious parts of the country, a one-blanket round of IRS has been implemented for decades. There are also some areas where two rounds of IRS have been implemented mainly when epidemics occur. DDT has been the choice of insecticide for the country's IRS operations for decades and was replaced by delthamethrin in 2007. Malathion was also used in a limited extent over 10 years in some areas where high DDT resistance was detected [FDREMOH, 2012]. Delthamethrin was also replaced by bendiocarb in 2010 due to the development of delthamethrin and other pyrethroid insecticide resistance in populations

of the main malaria vector and by considering the impact of LLINs on resistance development.

Insecticide resistance is reversible and manageable. It could be the result of repeated application of the same insecticide, use of similar insecticides for agricultural pests or use of insecticides of different groups having similar mode of action. Therefore, the use of two or more insecticides having different modes of action alternatively within a given period could delay the development of insecticide resistance at a significant level. The removal of its selection pressure would help to restore susceptibility of vector population that was resistant to the insecticide under consideration [IRAC, 2011]. Therefore, knowing the time required to delay or restore resistance to susceptibility is vital to use the available limited insecticides recommended for vector control.

Studies reported different levels of insecticide resistance in *An. arabiensis* against a range of insecticides of different classes including DDT, malathion, permethrin, delthamethrin, lambdacyhalothrin and bendiocarb [Abose *et al*.; 1998; Balkew *et al.*, 2003; Abate & Hadis, 2011; Yehualaw *et al.*, 2011,]. The vector developed resistance not only to insecticides that had been used either for IRS or LLINs, but also to insecticides that had never been used before, which would be the outcome of cross-resistance. Therefore, if insecticide resistance is not managed, the success achieved in the country so far by means of IRS and LLIN to control malaria would be at risk.

Thus, the specific objective of this study was to estimate effect of the application of bendiocarb 80% WP indoor residual spraying on insecticide resistance status of *An.arabiensis.*

5.2 Materials and methods

5.2.1 Study site and study design

A comparative study was made in Andassa and Tikurit villages. Descriptions of the study villages are given in section 3.1 of this monograph. Susceptibility status of *An. arabiensis* to different insecticides was assessed for three consecutive years. Andassa received three round sprays, i.e., one spray in each of the years 2013, 2014 and 2015, while Tikurit was sprayed only in 2015.

5.2.2 WHO insecticide susceptibility testing

Groups of 2-3 -days old females reared under field insectarium were selected using WHO tubes and exposed to papers treated with different insecticides including DDT (4%), delthamethrin (0.05%), malathion (5%), bendiocarb (0.1%), propoxour (1%) and premiphosmethyl (1%) at a temperature of 26^{o} C ± 2^{o} C and 80% ± 10% relative humidity. Insecticide treated papers were purchased from the WHO reference Centre at University Sains, Penang, Malaysia. A minimum of 100 mosquitoes/insecticide type were exposed for a standard one-hour susceptibility test and mortality was recorded after 24 hours holding period. The teses were condicted in 2013, 2014 and 2015.

5.2.3 Detection of Knockdown resistance

Two separate PCR reactions were run, one to detect *kdr-e* and the other *kdr-w* at a time (Figure 14 & 15). Extracted DNA at step 3.6.3 was stored at -80^{0}C and used for kdr PCR amplification. The master mix formulation for detecting the DNA changes responsible for kdr in *An. arabiensis* was consisted of Acustar II (consisted of Taq polymerase 5 units/

µl, dATP, dCTP, dGTP, dTTP, $Mgcl_2$, 5xbuffer) molecular grade water, primers and DNA templates. East and west resistance alleles were distinguished using east or west primers along with the WT (Table 2). The final volume of the master mix for kdr amplification was 25 µl (Table 2). Thermal cycling for kdr PCR reaction was programed at Bio-Rad PCR machine for 30 cycles with an initial denaturing step at 95 ^{0} C for 30 seconds, annealing at 57 ^{0} C for 30 seconds (for kdr east) or at 59 ^{0} C for 30 seconds (for kdr west), extension at 72 ^{0} C for 30 seconds and at 72 ^{0} C for 5 minutes.

Table 2: Primers and their sequences used to identify DNA changes in an.arabiensis

Primer name	**Primer sequence**
IPCF-F	GATAATGTG GATAGATTCCCC GACCAT G
AltRev- R	TGC CGT TGG TGC AGA CAA GGA TG
EAST:WT-R	GGTCCATGTTAATTTGCATTACTTACGAaTA
East: F	CTTGGCCACTGTAGTGATAGGAAAaTC
IPCF-F	GATAATGTG GATAGATTCCCC GACCAT G
AltRev- R	TGCCGTTGGTGCAGACAAGGATG
WEST: WT-R	GGTCCATGTTAATTTGCATTACTTACGAaTA
West –F	CTT GGC CAC TGT AGT GAT AGG AAA TgTT

5.2.3.1 Visualization of amplified DNA on a 2% agarose gel

The PCR products were separated by electrophoresis on 2% agarose TBE gels. A 1.4 gms of agarose gel was dissolved in 70 ml of buffer and stained by 7 µl of gel red. The solution was then microwaved for 2 minutes and cooled by running cold tap water on the outside of heating flask poured into an electrophoresis tray where 14 or 20 well comb was inserted. The comb was removed after the agarose gel was completely cooled. Three micro litters of the PCR product was loaded into each well and run at 70-75 volts for 2 and ½ hours. A negative control was also loaded in a well per assay. The amplicons were visualized with an ultraviolet trans illumination gel documentation system (Alpha Imager 2200, San Leandro, California, USA). The predicted DNA bands on the gel were compared to a one kb reference DNA ladder (Figure 15 & 16).

5.2.4 Data analyses

Insecticide susceptibility tests results were calculated according to WHO [2013] bioassay test protocol. Chi-square test was used to determine level of significance of differences in knockdown rates and mortalities within and between the study sites and years. Line graphs used to show susceptibility status of *An.arabiensis* to different insecticides against time. Gel photographs show the bands of resistance alleles in DDT and delthamethrin resistant *An. arabiensis* after separating them on 2% agarose gel.

5.2.5 Ethical clearance

The study did not involve any human subject or animals so that no ethical clearance was required.

Table 3: PCR components for kdr detection in *An.Arabiensis*

Components	**Concentration**	**Volume/25 µl rxn**
Accustar II		12.5 µl
IPCF-F	2.5 µM	2 µl
EAST AltRev-R	2.5 µM	2 µl
EAST:WT-R	5 µM	2 µl
EAST:WT-F	2.5 µM	2 µl
H_2O	Biological Grade	2.5 µl
DNA templates	Extracted from mosquitoes	2 µl
Total volume/well		25 µl
Accustar II		12.5 µM
IPCF-F	2.5 µM	1 µl
West AltRev-R	2.5 µM	1 µl
West:WT-R	25 µM	1 µl
West:WT-F	8.8 µM	3 µl
H_2O	Biological Grade	4.5 µl

DNA templates	Extracted from mosquitoes	2 µl
Total volume/well		25 µl

5.3 Results

5.3.1 Effect of IRS on insecticide resistance status of *An.arabiensis*

Susceptibility of *An. arabiensis* to fenithrothion, primiphosmethyl and propoxur was 100% in both study villages for three successive years. The same was true for Bendiocarb in Tikurit. However, in Andassa, resistance to bendiocarb was suspected after three spraying cycle in three years' time because susceptibility of the vector to this insecticide was < 98 %. However, there was no significant difference in susceptibility of the vector to bendiocarb within the same study site between years, i.e. the level of significance for susceptibility (χ^2 = 0.25, P= 0.8825 in Andassa; χ^2 = 0.02, P= 0.99 in Tikurit) (Figure 14, Tables 4).

Susceptibility status of *An.arabiensis* to malathion also steadily increased in both villages with time [Figure 14]. While susceptibility of *An.arabiensis* was below 98% in Andassa, the vector was 100% susceptible to malathion in Tikurit in 2015. Reversion of resistance to susceptibility was significant within and between study villages [Tables 4]. Mortality due to malathion significantly increased from 2013 to 2015 (Andassa: χ^2 = 8.04, p = 0.02; Tikurit: $\chi^{2\,=}$ 8.04, p = 0.0018).

An. arabiensis was resistant to DDT and delthamethrin in both study villages throughout the study period (Figure14 & Table 4). However, the level of susceptibility of the vector to these insecticides improved progressively and significantly with time. Mortality and

knock down effect due to DDT significantly increased from 2013 to 2015 (knock down: $\chi^2 = 117.87$, $P < 0.0001$ and mortality: $\chi^2 = 66.32$, $p < 0.0001$) in Tikurit. The same was true for deltamethrin (kd: $\chi^2 = 7.29$, $p = 0.004$ and mortality: $\chi^2 = 37.75$, $P<0.0001$). Similarly, mortality and knock down effect due to DDT significantly increased from 2013 to 2015 (kd: $\chi^2 = 198.66$, $p < 0.0001$ and mortality: $\chi^2 = 82.94$, $p < 0.0001$) in Andassa. The trend was also similar for deltamethrin (kd: $\chi^2 = 26.09$, $p < 0.0001$ and mortality: $\chi^2 = 48.22$, $p < 0.0001$). The differences in the reversion of resistance to susceptibility of the vector to DDT and delthamerin between the study villages were not significant ($p > 0.05$) (Table 4).

5.3.2 Detection of DDT and Delthamethrin kdr mutation in *An.arbiensis*

Figure 15 &16 show the presence or absence of kdr alleles in *An.arbiensis* and the changes in kdr mutations with time. The East African kdr (*kdr-e*) was detected in subsample of *An.arabiensis* screened for the presence of kdr mutations, while the West African kdr (*kdr-w*) was absent. Gel photographs showed bands of 314 bp signifying that PCR reactions were successful. Bands of 156 indicated that the vector had homozygous resistant allele. Other bands showed heterozygous resistance alleles of the vector. The change from homozygous to heterozygous kdr was higher for DDT than delthmamethrin within three years' time.

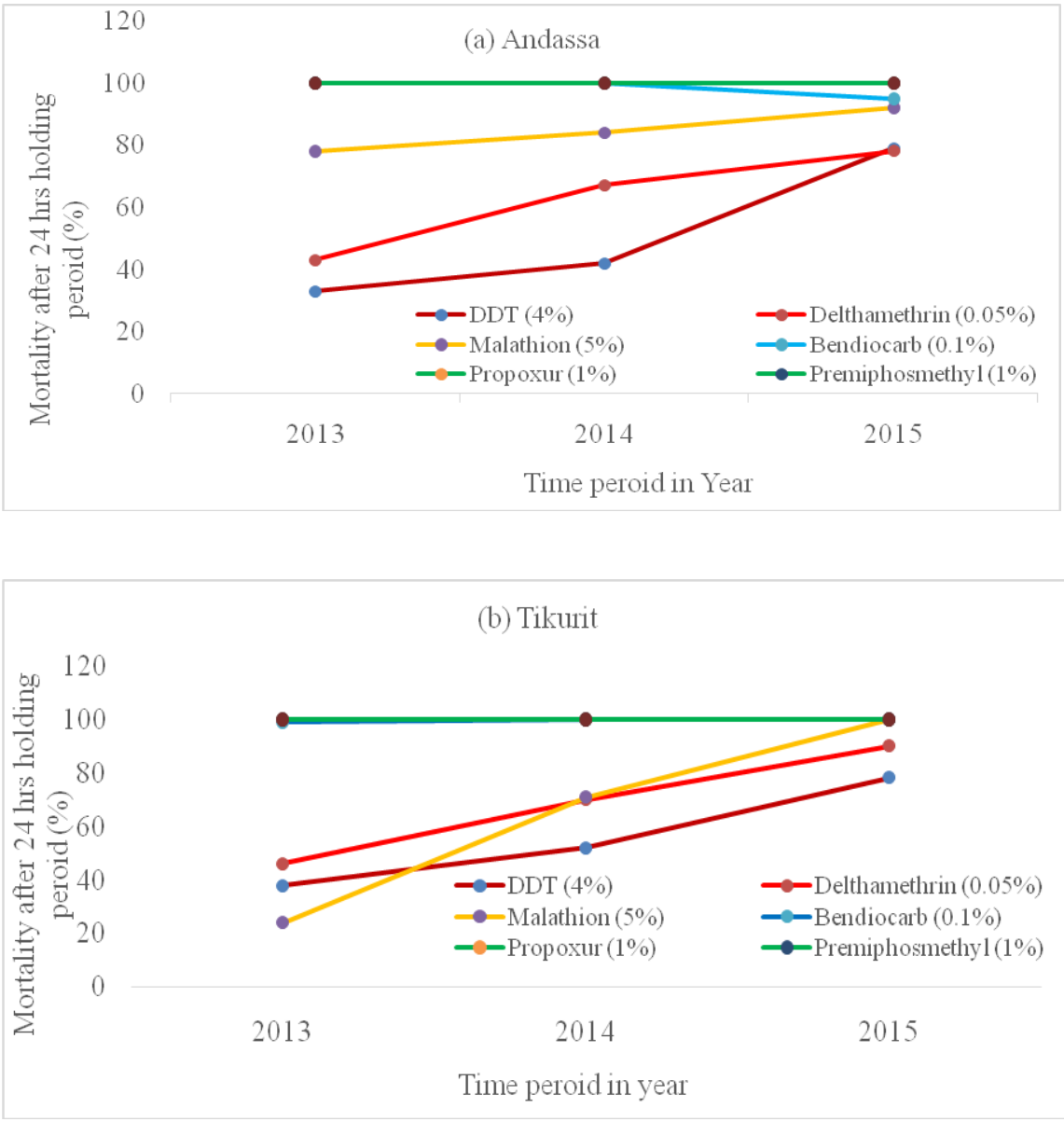


Figure 14: Susceptibility status of *An.arabiensis* to DDT (brown) delthamethrin (red) malathion (yellow) bendiocarb (blue) propoxur & premiphosmethyl (green) in (a) Andassa & (b) Tikurit at different times

Table 4: Change in susceptibility status of *An.arabiensis* exposed to different insecticides in Andassa & Tikurit (2013-2015)

		Year				
Insecticide	**Effect**	**2013**	**2014**	**2015**	χ^2	**P value**
			DDT			
Andassa	Knock down	15	21	20	4.54	1.04
Tikurit	Knockdown	23	33	63		
Andassa	Mortality	33	42	79	1.89	0.39
Tikurit	Mortality	38	52	78		
			Delthamethrin			
Andassa	Knock down	55	78	90	1.45	0.48
Tikurit	Knockdown	73	81	100		
Andassa	Mortality	43	67	78	0.2	0.9
Tikurit	Mortality	46	70	90		
			Malathion			
Andassa	Mortality	78	84	92	15201111	0
Tikurit	Mortality	24	71	100		
			Bendiocarb			
Andassa	Mortality	100	100	95	0.7493	0.96
Tikurit	Mortality	99	100	99		

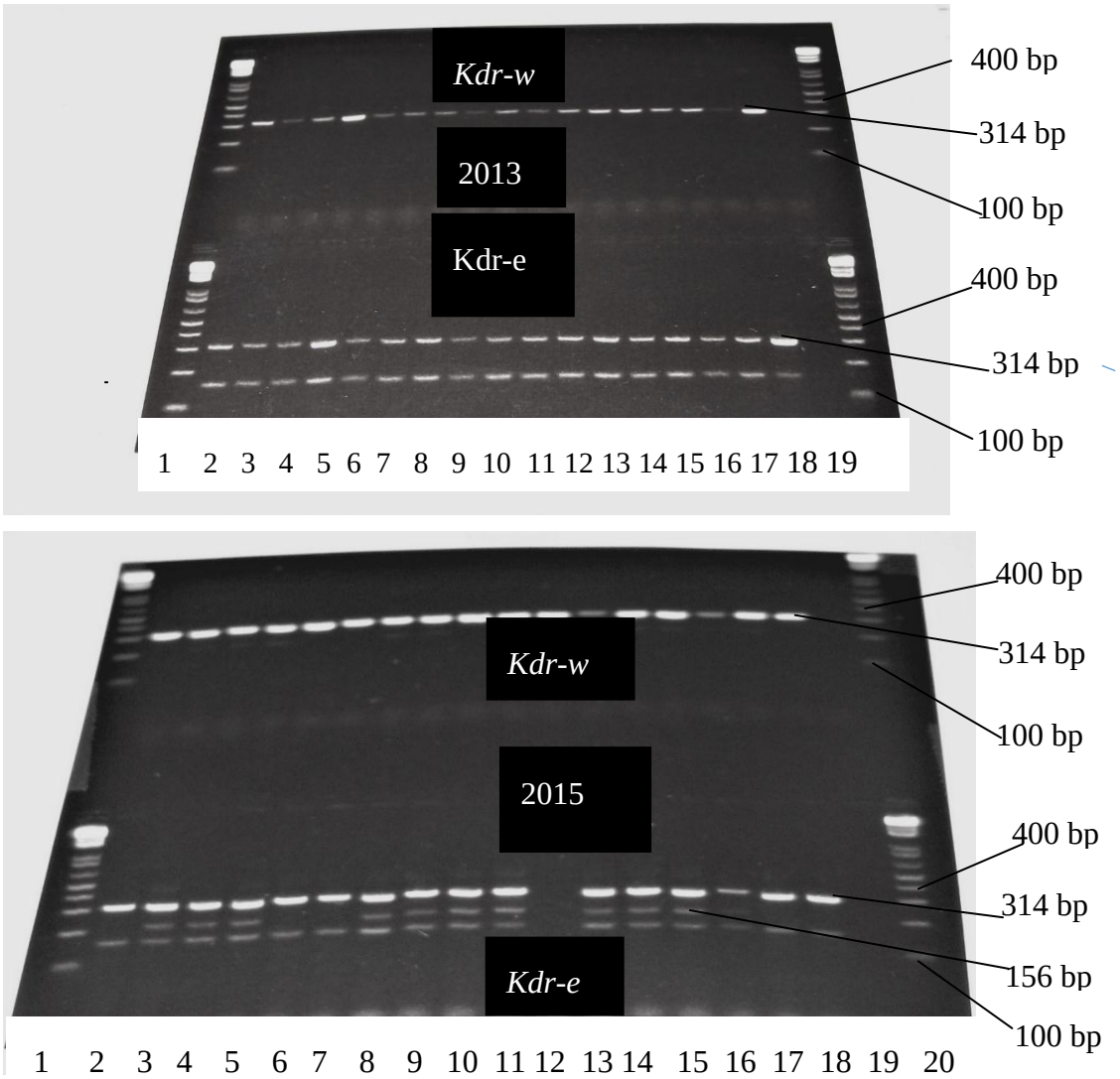


Figure 15: Gel photographs showing bands of DDT resistant kdr alleles in *An.arabiensis* after separation on 2% agarose gel: lane 1 & 20 1kb ladder, 19 negative control (2013) 2-18 homozygous resistant (2015) 2, 6, 7, 16-18 homozygous resistant & 3-5, 8-11, 13-15 heterozygous resistant, 12 not amplified, no *kdr-w* detected both in 2013 & 2015

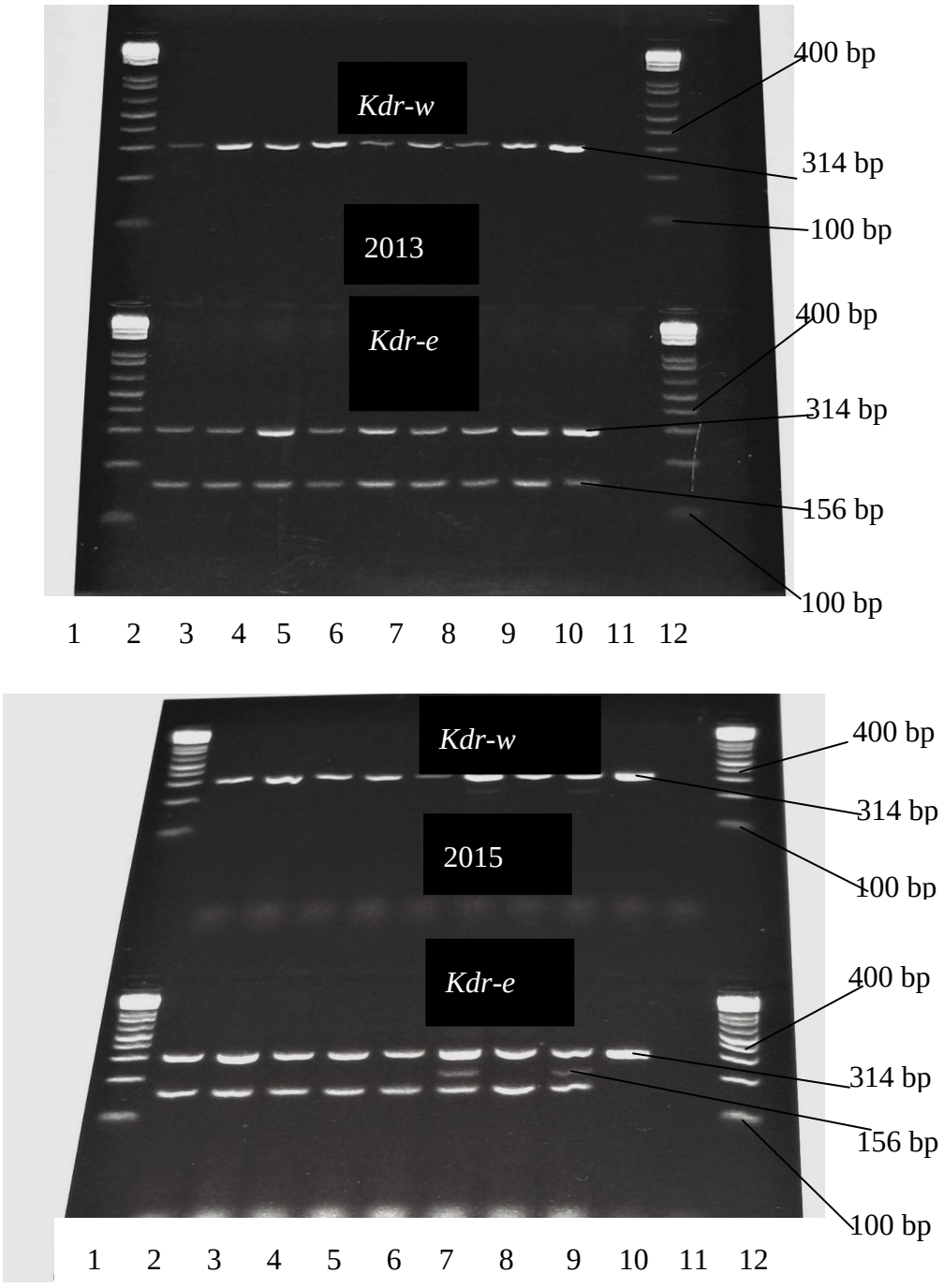


Figure 16: Gel photographs showing bands of delthamethrin resistant kdr alleles in *An.arabiensis* after separation on 2% agarose gel: lane 1 & 12 1kb ladder, 11 negative control (2013) 2-10 homozygous resistant (2015) 2-6 & 8 homozygous resistant, 7 & 9 heterozygous resistant, 10 not amplified, no kdr –w detected both in 2013 & 2015

5.4 Discussion

Genes conferring resistance to insecticides are natural and heritable in an insect population. Insecticide resistance in a given vector population is the selection of these heritable characteristics. The selection process would be slow if the resistance genes are rare or present at low rate in vector population and the effect of selection pressure for resistance lasts shorter [IRAC, 2011]. The operational life of an insecticide could vary with the amount and the frequency of its application [Koella *et al*., 2012]. The significant increase in insecticide resistance in malaria vector populations globally especially in Africa is due to the extensive application of insecticides in the form of IRS and LLINs at large scale for vector control [WHO, 2012].

Resistance to DDT (dichlorodiphenyltrichloroethane) was first reported in Aedes mosquito a year after the introduction of this insecticide for mosquito control in 1946 [Brown, 1986]. DDT resistance was also detected in malaria vectors developed by the end of 1960s following its widespread application during the era of malaria eradication started in 1955 [Hemingway *et al*., 2000; Kelly-Hope *et al*., 2008]. DDT had been used for about four decades in Ethiopia and populations of the main malaria vector in the country are resistant to DDT throughout the country. In the present study, resistance to bedniocarb was suspected after three spraying cycles in three years' time implemented one round spray/year. Compared with DDT, the effective residual life of bendiocarb is shorter. This could be attributed to the differences in their mode of action and the inherit characteristics of the vector species involved. The differences observed in the residual life of bendiocarb between Tikurit and Andassa could be justified by the difference in the frequency of bendiocarb IRS spraying cycles, i.e. Tikurit received one spraying cycle

while three spraying cycles were made in Andassa signifying the need to alternate bendiocarb by other insecticide having different mode of action every 2-3 years.

Insecticide resistance is reversible when its selection pressure is removed [IRAC, 2011]. Published data on the time required to revert resistance to susceptibility are limited and difficult to discuss the present data in relation to other studies. The present study demonstrated that resistance reverted to susceptibility every year progressively. Susceptibility of *An.arabiensis* to malathion was reverted from 24% to 100% in Tikurit in three years while the same vector was found to be resistant in Andassa. The possible reason for the differences observed in the rate of reversion in malathion resistance in the two study villages might be due to the selection pressure exerted by bendiocarb IRS in Andassa since both insecticides have similar mode of action. The trend in the reversion of DDT and delthamethrin resistance to susceptibility was similar in both study villages justifying that the impact of bendiocarb IRS application or other selection pressure for these insecticides is low or absent.

Two mutations, which are known as *kdr-e* and *kdr-w*, occurring at the same locus in the voltage-gated sodium channel gene are known to confer knockdown resistance (kdr) to pyrethroids and DDT. *Kdr-e* involves a leucine-serine substitution while *kdr-w* involves a leucine-phenylalanine substitution. Several studies indicated that *kdr-w* was predominated in the region of West African [Fanello *et al*., 2000; Diabate *et al*., 2002; Awolola *et al.*, 2005; Dabire *et al*., 2009a] while *kdr-e* was in East Africa [Ranson *et al*., 2000; Verhaeghen *et al*., 2006] in earlier times. However, *kdr-e* alleles have been spread out in populations of *An.arabiensis* in West African countries including Burkina Faso and Benin [Djegbe *et al.*, 2011; Badolo *et al*., 2012] recently. Similarly, *kdr-w* has been

distributed in vector populations in Uganda [Verhaeghen *et al*., 2006] and Ethiopia [Balkew *et al*, 2010; Yehualaw *et al*., 2010]. In this study, only *kdr-e* was detected and may be attributable to DDT and delthamethrin resistance in *An.arabiensis.* The results of the present study are in agreement with previous studies reported from different African countries [Djegbe *et al.*, 2011; Badolo *et al*., 2012] where both *kdr-w* and *kdr-e* were found within the same country. However, in Ethiopia Balkew *et al*. [2010] and Yehualaw *et al*. [2010] reported *kdr-w*, which is inconsistent with the present results. The differences observed between the previous and present studies in Ethiopia might be due to the recent inflow of *kdr-e* genes in *An. arabiensis* populations. The differences observed between the previous and the present study in Ethiopia needs to be verified.

5.5 Conclusion

The present study confirmed that bendiocarb would be effective against *An. arabiensis* for three years under bendiocarb IRS operation. Therefore, alternative insecticides with different mode of action should be used to replace every two to three years to delay resistance development to bendiocarb.

5.6 Recommendation

The present study was conducted in two study villages only for three consecutive years. Furthermore, population genetics varies from region to region or locality to locality. Therefore, a longitudinal study should be carried out in representative study sites in the country to determine the time to either rotate insecticides in IRS operations to delay IR development or restore susceptibility of the vector to the insecticides that the vector already developed resistance.

CHAPTER 6: Impact of bendiocarb indoor residual spraying on the composition, abundance and resting habits of malaria vectors

6.1 Introduction

Entomological interventions for malaria control have been suggested after the incrimination of mosquitoes as malaria vectors by Ronald Ross in 1897 and, Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava between 1898 and 1900 [Cox, 2010]. Since then a range of vector control tools including larval control by environmental management and larviciding, and the control of mosquito bites using chemical adulticides, house improvement, repellent and biological control have been developed and used. Among these tools developed so far, IRS and LLINs are the primary vector control tools that are being used currently [Curtis, 1996; WHO, 2006a; Pluses *et al*., 2010]. Indoor residual spraying and LLINs are efficient against vectors of malaria that prefer to bite and rest indoors [Gillies & De Meillon, 1968; Bockarie *et al*., 2009] but less effective for vectors that are biting and resting outdoors [Okumu *et al.*, 2013]. The efficacy of these intervention tools also depend up on the susceptibility of the vectors to the insecticides used for IRS operation and LLINs impregnation.

In the last decade, reports indicated that malaria deaths have been reduced by 33% subsequent to the massive scale up of LLINs distribution and IRS coverage [Hemingway, 2014]. However, the growth of resistance against the insecticides used for IRS and mosquito net treatment in malaria vector populations have become a growing threat to these insecticides based vector control operations and to sustain the success achieved so

far. Vectors' physiological insecticide resistance to at least one insecticide used for malaria control has been reported by 64 countries [WHO, 2013a]. Malaria vectors may also challenge the role of IRS and LLINs in malaria control by changing their indoor biting and resting habit to outside of the human homes [Charlwood & Graves, 1987] and by altering their active biting time from late to earlier hours of the night [Yohannes & Boelee, 2012; Gatton *et al*., 2013]. These intervention tools could also diminish the abundance of predominant vector species and favor secondary and other less important vectors to boost and maintain disease transmission [Wilikes *et al*., 1996]. Thus, assessing the impact of these entomological intervention tools on entomologic malaria transmission risk factors should be an integral part of any vector control program.

Similarly, IRS and LLINs are the frontline vector control tools in Ethiopia where 70.5% of the households living in areas < 2,000 meters or ≥ 2,500 meters above sea level are protected by IRS and LLINs [EMIS, 2015]. These insecticide-based indoor intervention tools have been used primarily against *An. arabiensis*, the main malaria vector in the country. *An. pharoensis, An.funestus* and *An. nili* are also important vectors [Abose *et al*., 1998]. Success stories in the reduction of malaria related mortality and morbidity are attributed to the use of IRS and LLINs vector control operations and improved health system. Thus, the country is trying to sustain the achievements made so far and to eliminate malaria from hypo endemic areas by 2015 and from the country by 2020 by increasing IRS coverage and LLINs distribution; and improving case detection and treatment in the health system in general [Woyessa *et al*., 2013]. However, data for the measurable impacts of these frontline vector control intervention tools on entomological malaria transmission factors are either insufficient, unavailable or missing.

Therefore, the specific objective of this study was to assess the impact of indoor residual spraying on the composition, abundance and resting habits of malaria vectors

6.2 Materials and methods

6.2.1 Study area and study design

A comparative study was conducted in Andassa and nearby villages. Details of the study area and study design are given under sections 3.1 & 3.2 of this thesis.

6.2.2 Adult mosquito sampling and identification

Mosquito sampling was carried out in the months of September (before the IRS application in 2013 and 2014) and October (one month after intervention), November (two months after intervention) and December (three months after intervention) in the same year. Similar data were collected in September 2015 after IRS application in both Andassa and Tikurit. Adult mosquitoes were collected using PSC, CDC light traps and pit shelter sampling methods. Pyrethroid spray catches and pit shelter collections were carried out from six houses and six pit shelters per study arm, respectively, between 6:30 and 11:00 AM. For indoor mosquito collection, pyrethroid aerosol (Roach killer, M/S Kafr EI Zayat, Egypt with registration No.ET/HHP/130) was applied by one trained spray man on the outside and the investigator from inside of the house simultaneously after room surfaces were covered by white spray sheets. After 12 minutes the spray sheets were taken cautiously out of the house and knocked down mosquitoes were collected using forceps and labeled paper cups covered with white netting. Prior to spraying the aerosols, all food items and animals were taken outside of the house. Mosquitoes resting

inside the pit shelters were searched out using torch light, mouth aspirators and paper cups. CDC light trap collections were also made in six houses per study group between 6:00 to 6:30 PM. Traps were hung down from wooden sticks, which were placed across the top of the walls parallel to the foot end of the bed. Mosquitoes that were collected by each sampling method were sorted into species and sexes before being counted. Each species was identified using morphemically keys [Verone, 1962; Gillies & Coetzee, 1987]. A PCR molecular technique was employed for sibling species of *An. gambiae* s.l.

6.2.2.1 PCR based identification of *An.gambiae* complex

Extracted DNA (extraction was made as described at 3.10.3) was used for PCR amplifications. PCR reactions were consisted of Accustar^{TM} II (a mixture of Taq Polymerase, $MgCl_2$, dNTPs and buffer), primers, biological grade water and DNA template, which made 25 µl in total volume (Table 5). Positive and negative DNA and no template controls using sterile water instead of DNA controls were also used in parallel. Thermal cycling was programed at Bio-Rad PCR machine for 30 cycles with an initial denaturing step at 95 ^{0}C for 30 seconds, annealing at 60 ^{0}C for 30 seconds, extension at 72 ^{0}C for 30 seconds, at 72 ^{0}C for 5 minutes and held at -12 ^{0}C for further analyses.

6.2.2.2 Visualization of Amplified DNA

The PCR products were separated by electrophoresis on 2.5% agarose TBE gels, and stained with ethidium bromide. A 1.8 gms of agarose gel was dissolved in 90 ml of buffer and stained by 9 µl of gel red. The solution was microwaved for 2 minutes and cooled by running cold tap water on the outside of heating flask. The solution was then poured into an electrophoresis try where 14 or 20 well comb was inserted. The comb was removed

after the agarose gel was completely cooled. Two micro litters of the PCR product was loaded into each well and run at 70-75 volts for 2½ hours. The amplicons were visualized with an ultraviolet Trans illumination gel documentation system (Alpha Imager 2200, San Leandro, California, USA). The predicted DNA bands on the gel were compared to a 100bp reference ladder.

Table 5: PCR components used for the identification of *An.gambiae* complex

Anopheles species ID	**Primer sequence**	**Vol./25µl rxn**
IMP-UN-F	GCTGCGAGTTGTAGAGATGCG	1 µl
AR-3T-R	GTG TTA AGT GTC CTT CTC CGT C	1 µl
AG-3T-R	GCT TAC TGG TTT GGT CGG CAT GT	1 µl
ME-3T-R	CAA CCC ACT CCC TTG ACG ATG	1 µl
QD-3T-R	GCA TGT CCA CCA ACG TAA ATCC	1 µl
H_2O	Biological grade	5.5 µl
DNA templates	Extracted from mosquitoes	2 µl
Accustar II		12.5 µl
Total volume/well		25 µl

6.2.3 Data analysis

After entering data in excel spread sheets, they were analyzed using descriptive and inferential statistics. Descriptive statistics was used to show the abundance of indoor and outdoor resting habits of Anopheles mosquitoes across the season and based on

categorical variables such as study villages, insecticide spray status, sampling methods, sampling months and years. Bar graphs were used to show total number of mosquitoes recorded against categorical variables.

Before performing formal inferential statistics, normality and homogeneity of variance tests were done using Shapiro-Wilk test, and Levene's test. Both tests turned out to be significant, suggesting that the groups to be compared were not normally distributed and they did not have the same variance. Therefore, parametric tests such as ANOVA could not be used. Furthermore, the data generally lacked the third basic assumption of independence because data were temporally correlated. Therefore, Generalized Estimating Equations (GEEs) were employed with reasonable statistical efficiency to analyze the data. The GEE approach is based on the concept of estimating equations and provides a very general approach for analyzing correlated responses.

The GEE with negative binomial distribution was preferred for analysis. The negative binomial distribution is defined by two parameters, the mean and a dispersion parameter, which measures the degree of clumping or aggregation in the distribution (White & Bennetts, 1996). The mean response (mosquito abundance) was modeled as a negative binomial regression model using the explanatory variables such as housing category (two levels, i.e., sprayed and non-sprayed with insecticide), sampling houses (sprayed and non-sprayed), four sampling periods or months (i.e., September, October, November, December), and three types of sampling methods (CDC light trap, PSC and Pit). The response variable, i.e., number of mosquitoes, for individual houses was assumed to be equally correlated, implying an exchangeable correlation structure. The concept of

correlation refers to data collected repeatedly (from September to December) from each house and across years. Data recorded from each house are technically correlated enough because they come from the same house.

6.3 Results

6.3.1 Mosquito composition and their abundance

In this study, 20,243 mosquitoes were collected in total during the main malaria transmission season (September to December) in the years 2013, 2014 and 2015. Overall, 43.8% (n = 8,859) belonged to the different Anopheles species, while 56.2% (n = 11,384) of them were culicines. *Anopheles arabiensis, An. pharoensis* and *An.coustani* were the only Anopheles species identified during the study period. The results of molecular identification using polymerase chain reaction revealed that all Anopheles mosquitoes that were identified as *An. gambiae* s.l morphologically were *An.arabiensis* (Figure 17). *An. arabienesis* was the most abundant species (70.2%, N = 6,215) while *An. coustani* was the least abundant (7.9%, N= 696). *Anopheles pharoensis* was the second most abundant malaria vector species next to *An. arabiensis* contributing 22% (n = 1,948) of the Anopheles mosquitoes recorded.

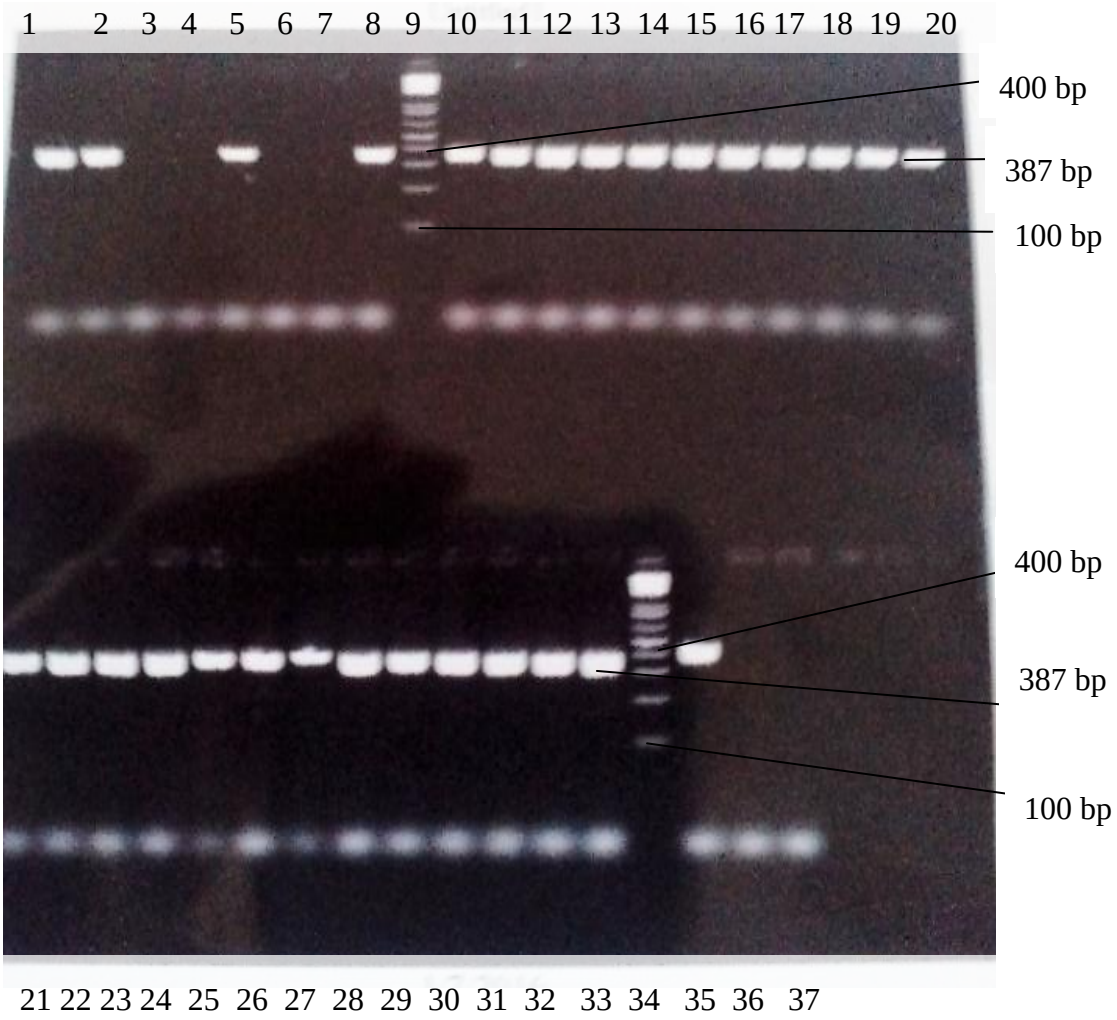


Figure 17: Gel photographs of *An.gambiae* complex species identified using IMP method. Lane 9 & 34 contain 100 bp marker, lane 1, 2 , 5, 8, 10-20, 21-33 & 35 are bands of *An.arabiensis*, lane 3, 4, 6, 7, 36 & 37 not amplified

6.3.2 Impact of IRS on the abundance of Anopheles mosquitoes

Anopheles arabiensis: the abundance of *An.arabiensis* was high before the application of IRS in 2013 and 2014. Numbers diminished in 2015 after IRS application compared to the two-years baseline data that had been recorded before IRS application in both study villages (Figure 18 A). In contrast to non-sprayed village, the number of *An. arabiensis* also declined dramatically after IRS application in 2013 and 2014 in the sprayed village

(Andassa) (Figure 18 B). Similar results were also demonstrated in sprayed village following IRS application in October compared with the previous month before IRS application both in 2013 and 2014 (Figure 18 B).

Anopheles pharoensis: the impact of IRS on the abundance of *An. pharoensis* was not consistent and strong (Figure 19 A & B) compared to the impact on *An. arabiensis*. Reduction in the abundance of *An. pharoensis* was more pronounced and consistent with time i.e. highest in September (before IRS application) and lowest in December (three months after IRS application) (Figure 19 A & B).

Anopheles coustani

The abundance of *An. coustani* recorded in sprayed village both before IRS and after IRS was negligible to validate the impact of IRS on its abundance. However, in non-sprayed village the numbers decreased after spray in September 2015 compared to the numbers recorded in September 2013 and 2014 before spray. The abundance of *An. coustani* was also declined in October 2013 but increased in October in 2014 in non-sprayed village (Tikurit) when IRS was not applied (Figure 20 B).

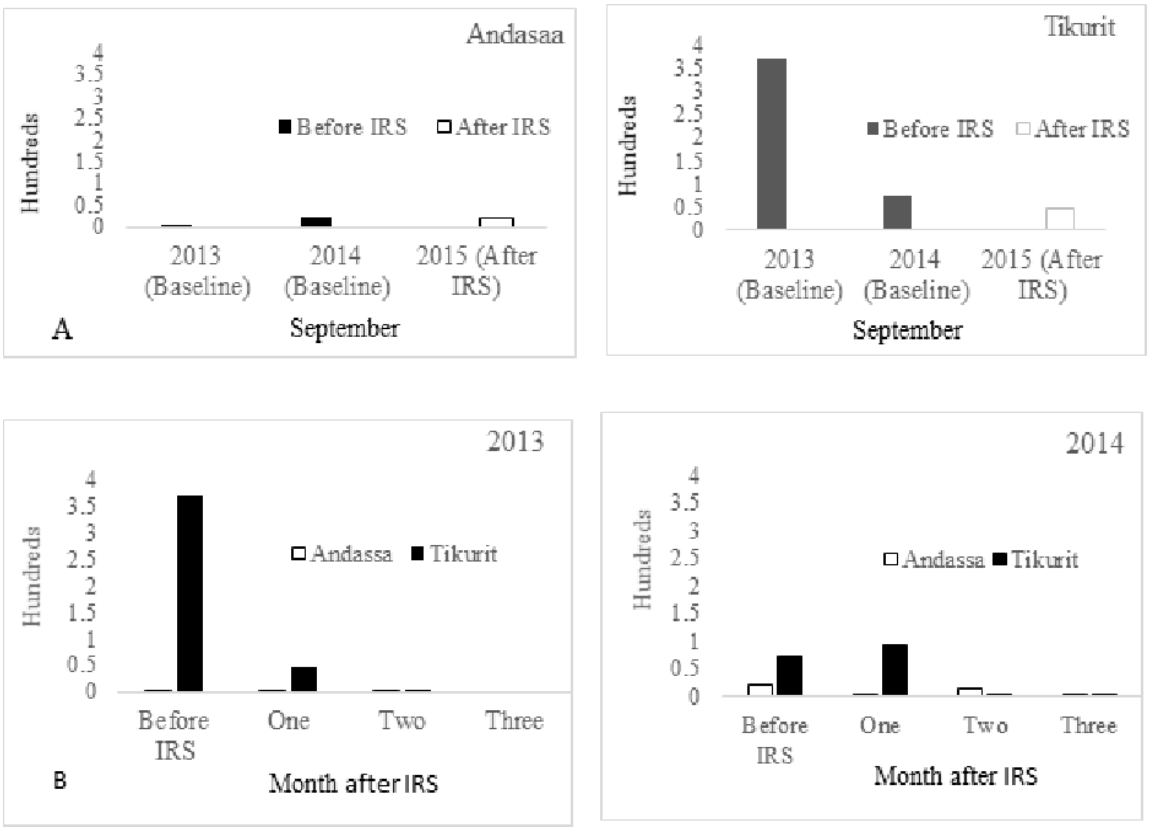


Figure 18: Bar graphs showing the effect of bendiocarb IRS on the abundance of adult *An.arabiensis* estimated based on (A) two years baseline data collected in 2013 & 2014 before spray & in 2015 after spray (B) data collected before IRS and one, two & three month after IRS in 2013 & 2014 in Andassa & Tikurit

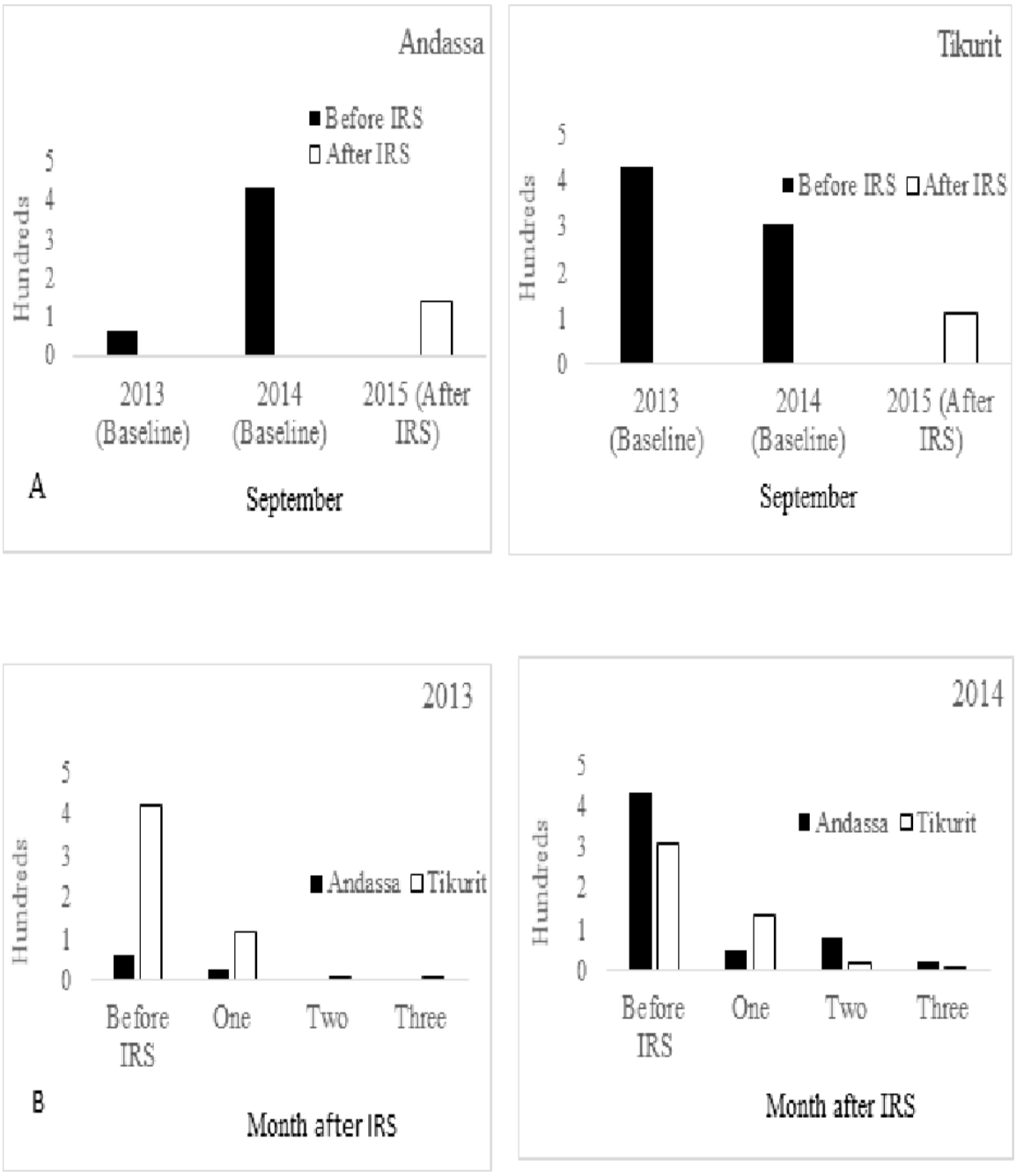


Figure 19: Bar graphs showing the effect of bendiocarb IRS on the abundance of adult *An.pharoensis* estimated based on (A) two years baseline data collected in 2013 & 2014 before IRS & 2015 after IRS (B) data collected before IRS & one, two & three month after IRS in 2013 & 2014 in Andassa & Tikurit

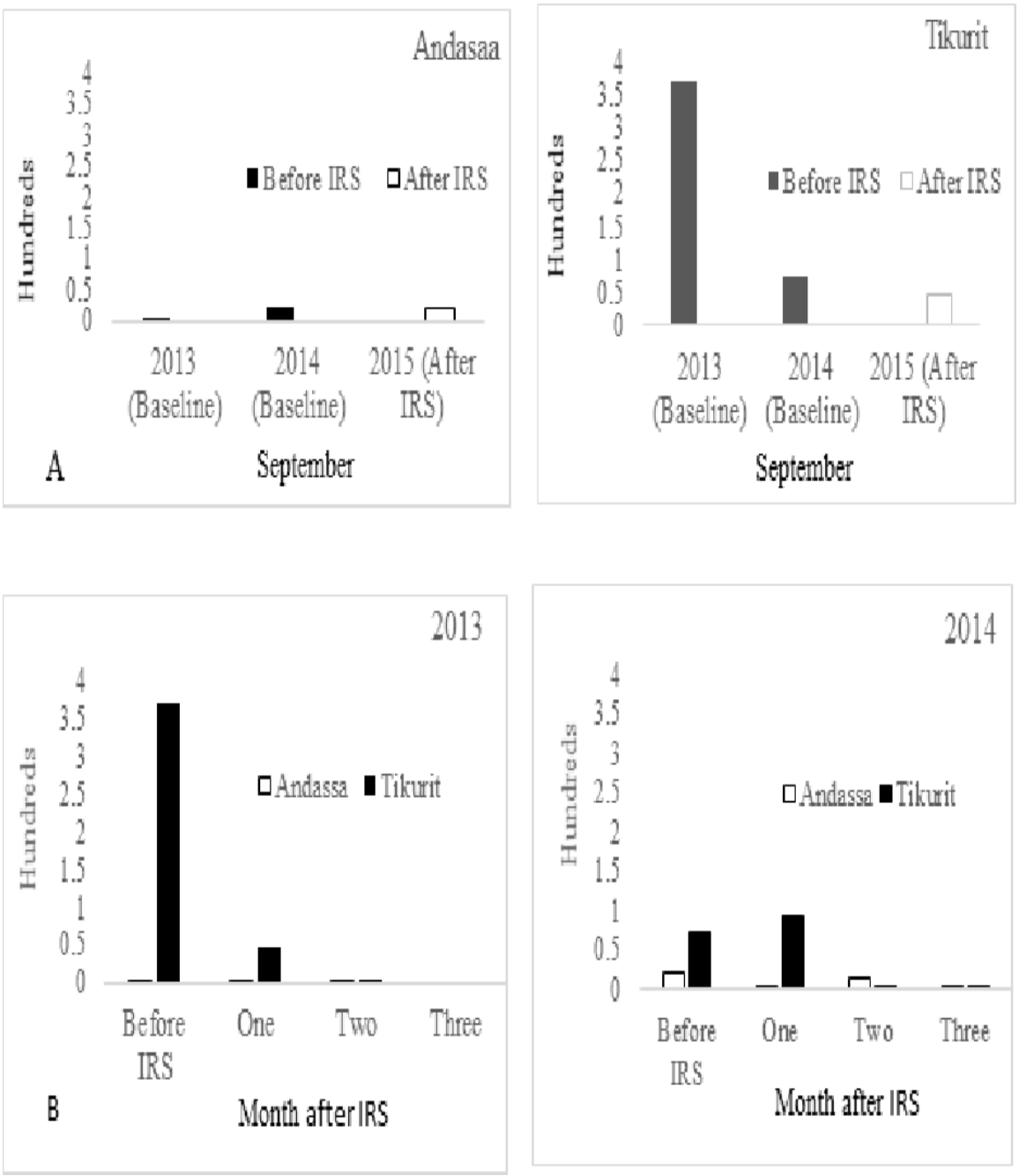


Figure 20: Bar graphs showing the effect of bendiocarb IRS on the abundance of adult *An.coustani* estimated based on (A) two years baseline data collected in 2013 & 2014 before IRS and in 2015 after IRS (B) data collected before IRS and one, two & three month after IRS in 2013 & 2014 in Andassa & Tikurit villages

6.3.3 Impact on indoor and outdoor resting density

The impact of IRS application on indoor and outdoor resting density of Anopheles mosquitoes were estimated by comparing the number of mosquitoes captured by PSC and Pit shelter.

Anophels arabiensis

The number of outdoor resting *An. arabiensis* captured were too few to compare with the number captured indoors. However, the result indicates that no change was observed in the resting habits of the vector due to spraying (Figure 21 A, B & C).

Anopheles pharoensis

Several indoor resting *An. pharoensis* were recorded only in September 2014 in sprayed village (Figure 22 A & C). However, the numbers of *An. pharoensis* captured in other years either indoor or outdoor were negligible (Figure 19 A, B & C) to demonstrate the impact of IRS application on the resting habits of this species.

Anopheles coustani

Few *An. couustani* captured indoors and outdoors by PSC (n=7) and Pit (n=3) during the whole study period so that the numbers were too small to validate the impact of IRS application on the resting habits of this vector species.

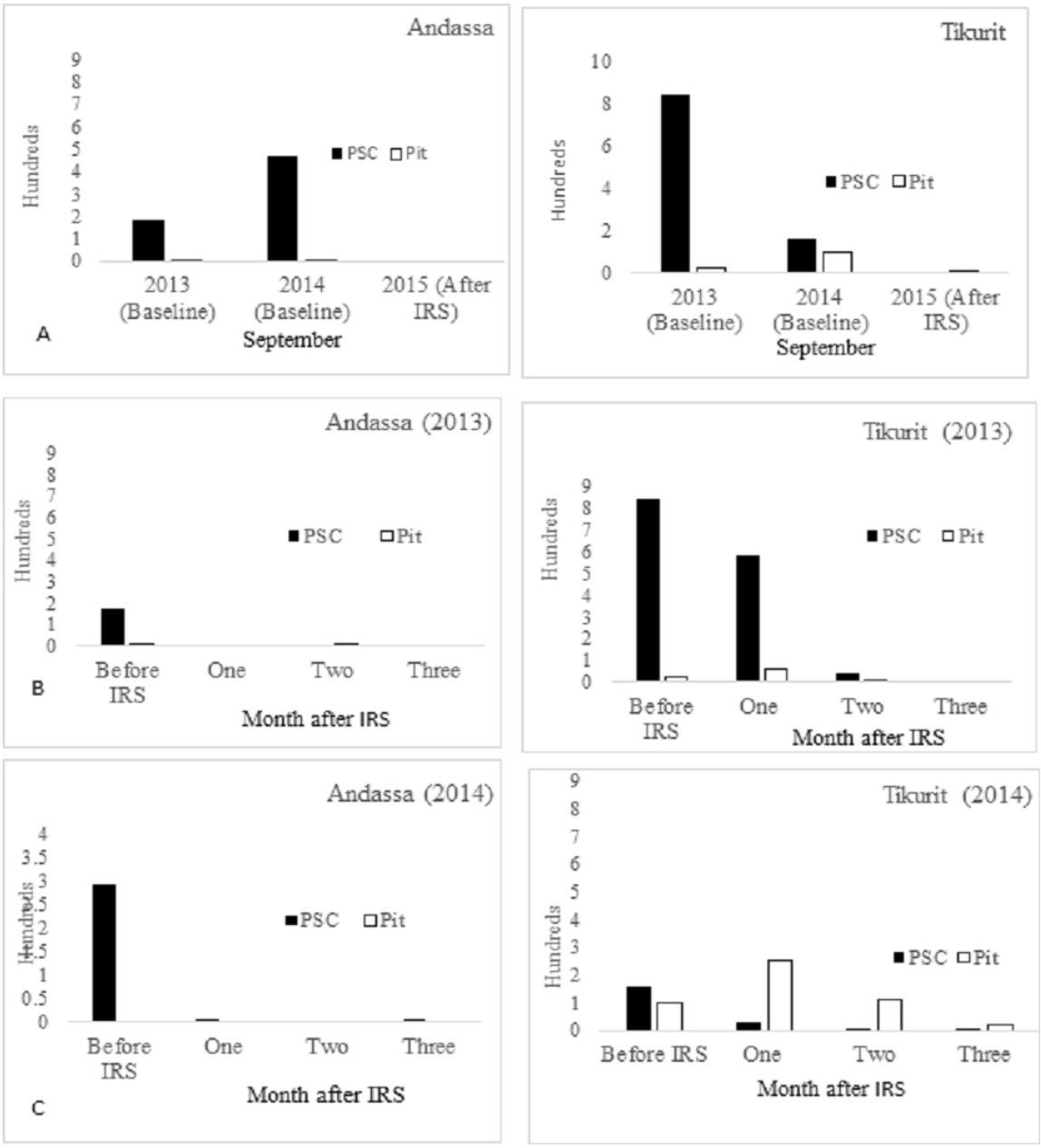


Figure 21: Bar graphs showing the effect of bendiocarb IRS on indoor & outdoor density of *An.arabiensis* estimated based on the number of mosquitoes collected using PSC & Pit shelter sampling methods (A) two years baseline data collected in 2013 & 2014 before IRS & in 2015 after IRS; before IRS & one, two & three month after IRS in (B) 2013 and (C) 2014 in Andassa and Tikurit villages

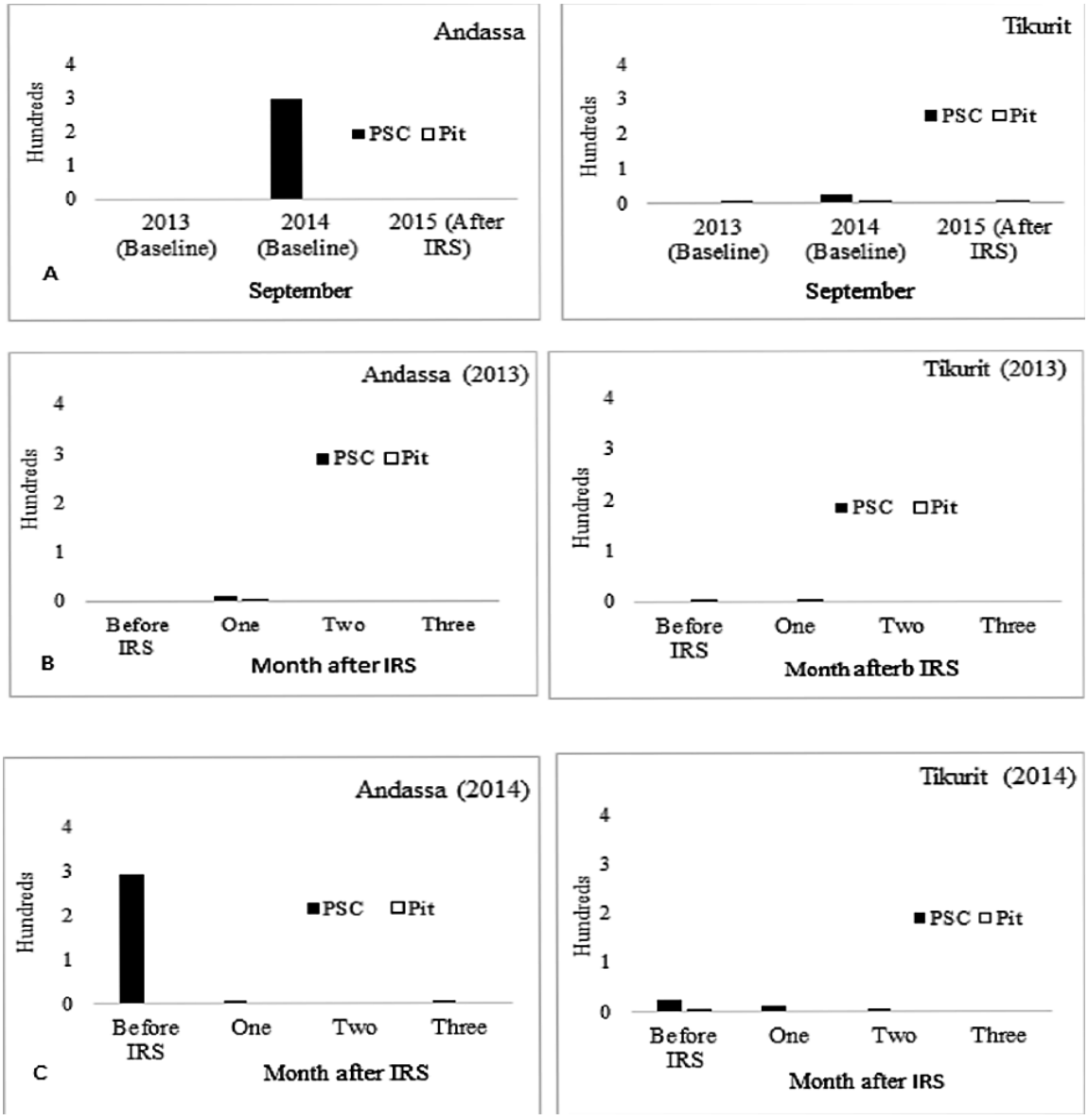


Figure 22: Bar graphs showing the effect of bendiocarb on indoor & out door density of *An.pharoensis* estimated based on the number of mosquitoes collected using PSC & Pit shelter sampling methods (A) two years baseline data collected in 2013 & 2014 before IRS and in 2015 after IRS; before IRS and one, two & three months after IRS in (B) 2013 and (C) 2014 in Andassa & Tikurit villages

6.3.4 Generalized estimating equations

Analyses of maximum likelihood parameter estimate was made using GEE to estimate the effect of explanatory variables including sampling villages, months, methods and hosts (human and cattle) on the number of mosquitoes collected. The parameter estimates table for each species contains parameter estimates, standard errors, confidence intervals, Z scores, and values for the parameter estimates.

Anopheles arabiensis

The parameter estimates table for *An. Arabiensis* is displayed in Table 6. All parameter estimates except human hosts significantly contributed for the model ($P<0.05$). That meant all effects or explanatory variables (spraying categories, months, and sampling methods) and their sublevels played significant role in the buildup of mosquito abundance except the number of persons. The role of the number of persons is not strong enough. Because the column in the model matrix corresponding to the parameter for spraying category at non-sprayed villages was found to be linearly dependent, or aliased, with columns corresponding to parameters preceding it in the model, i.e., spraying category at sprayed villages, and therefore PROC GENMOD of SAS assigned it zero for both the parameter estimate and its standard error.

Anopheles pharoensis

Except the sprayed villages category, all explanatory variables played significant ($P<0.01$) role on the abundance of *Anopheles pharoensis* (Table 7).

Table 6: Analyses of maximum likelihood parameter estimate for *An.arabiensis* abundance against explanary variables

Parameter	Levels*	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-square	Pr > Chi-square
Intercept		1	0.7265	0.2236	0.2882	1.1648	10.55	0.0012
Villages	SP	1	-2.5958	0.2379	-3.0620	-2.1296	119.10	<.0001
	NSP	0	0.0000	0.0000	0.0000	0.0000	.	.
Methods	CDC	1	2.0287	0.2332	1.5717	2.4857	75.70	<.0001
	PSC	1	1.2611	0.2724	0.7273	1.7949	21.44	<.0001
	Pit	0	0.0000	0.0000	0.0000	0.0000	.	.
Months	BIRS	1	1.8974	0.2833	1.3422	2.4527	44.86	<.0001
	OML	1	1.0440	0.2576	0.5392	1.5488	16.43	<.0001
	TML	1	-1.1040	0.2861	-1.6648	-0.5433	14.89	0.0001
	THML	0	0.0000	0.0000	0.0000	0.0000	.	.
Persons		1	-0.0602	0.0355	-0.1298	0.0093	2.88	0.0897
Cattle		1	0.0783	0.0344	0.0108	0.1458	5.17	0.0230
Dispersion		1	4.1231	0.3165	3.5027	4.7435		

*Sp=sprayed, NSP=non-sprayed, CDC=CDC light trap, PSC=pyrethroid spray sheet collection, Pit=pit shelter collection, BIRS=before indoor residual spraying, OML=one month later, TML=two month later, THML=three month later

Table 7: Analyses of maximum likelihood parameter estimates for *An. pharoensis* abundance against explanary variables

Parameter	Levels	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > Chi-square
Intercept		1	-5.3332	0.5111	-6.3351	-4.3314	108.87	<.0001
Villages	SP	1	-0.2919	0.2447	-0.7715	0.1878	1.42	0.2330
	NSP	0	0.0000	0.0000	0.0000	0.0000	.	.
Months	B IRS	1	2.2427	0.3012	1.6523	2.8331	55.43	<.0001
	OML	1	1.3870	0.3323	0.7357	2.0383	17.42	<.0001
	TML	1	-1.2177	0.3759	-1.9545	-0.4808	10.49	0.0012
	THML	0	0.0000	0.0000	0.0000	0.0000	.	.
Methods	CDC	1	5.8581	0.4372	5.0012	6.7151	179.52	<.0001
	PSC	1	2.3545	0.4360	1.5000	3.2090	29.17	<.0001
	Pit	0	0.0000	0.0000	0.0000	0.0000	.	.
Persons		1	-0.1177	0.0444	-0.2047	-0.0308	7.04	0.0080
Cattle		1	0.3145	0.0443	0.2278	0.4013	50.49	<.0001
Dispersion		1	3.0761	0.3392	2.4113	3.7408		

*Sp=sprayed, NSP=non-sprayed, CDC=CDC light trap, PSC=pyrethroid spray sheet collection, Pit=pit shelter collection, BIRS=before indoor residual spraying, OML=one month later, TML=two month later, THML=three month later

Table 8: Analyses of maximum likelihood parameter estimates for *An. coustani* abundance against explanary variables

Parameter	Levels	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-square	Pr > Chi-square
Intercept		1	-5.9966	0.9394	-7.8379	-4.1553	40.75	<.0001
Villages	SP	1	-2.1093	0.4031	-2.8994	-1.3193	27.38	<.0001
	NSP	0	0.0000	0.0000	0.0000	0.0000	.	.
Months	B IRS	1	2.2912	0.4889	1.3330	3.2495	21.96	<.0001
	OML	1	1.1321	0.5401	0.0736	2.1906	4.39	0.0361
	TML	1	-2.6452	0.9169	-4.4423	-0.8481	8.32	0.0039
	THML	0	0.0000	0.0000	0.0000	0.0000	.	.
Methods	CDC	1	5.8708	0.7924	4.3177	7.4239	54.89	<.0001
	PSC	1	0.9462	0.8936	-0.8051	2.6975	1.12	0.2896
	Pit	0	0.0000	0.0000	0.0000	0.0000	.	.
Persons		1	0.0223	0.0593	-0.0940	0.1385	0.14	0.7073
Cattle		1	0.1627	0.0782	0.0094	0.3159	4.33	0.0375
Dispersion		1	4.5936	0.8171	2.9920	6.1951		

*SP stands for sprayed villages, NSP for non-sprayed villages, CDC for CDC light trap, PSC for pyrethroid spray sheet collection, Pit for pit shelter collection, BIRS before indoor residual spraying, OML for one month later, TML for two month later, THML three month later

Anopheles coustani

Except the PSC sampling method and number of persons, all explanatory variables played significant (P<0.01) role on the number of *Anopheles coustani* collected (Table 8).

6.4 Discussion

The main aim of indoor residual spraying is to kill and reduce the density of indoor feeding and resting disease vectors [Pates &*Curtis*, 2005]. In the present study, *An. arabiesnsis* is the only sibling species of the *An.gambiae* complex found in this study, which similar with other studies reported previously [Fettene *et al*., 2013; Balkew *et al*., 2010; Yehualaw *et al*., 2010]. The abundance of *An.arabiensis* dramatically declined following IRS application. However, the proportion of indoor and outdoor resting density was not changed throughout the study periods indicating that the insecticide did not influence the vector to alter its resting habit. This would be potentially attributed to the less irritant effect of bendiocarb [Padonou *et al*, 2012]. Thus, the insecticide was effective to bring an impact on the abundance of *An. arabiensis.* Ansari and Rasdan [2004] reported similar observations on *An. culcifacies* and other mosquitoes. On the other hand, Ossè *et al.* [2012] reported that bendiocarb indoor residual spraying decreased the number of *An. gambiae* s.l resting indoors while the rate of exophily was high after intervention. The differences observed between the present and previous studies in exhophily rates might be associated either with the differences in sampling methods used for mosquito collections or the background variations of vector populations or both. In the present study, indoor and outdoor densities of the vector were estimated using PSC and Pit shelter collection methods, respectively, while PSCs and exit window traps were used to estimate endophily rates in the previous study.

The effect of IRS application on the abundance and resting habits of *An. pharoensis* and *An. coustani* was different from the effect on *An. arabiensis*, i.e. the impact on *An. pharoensis* was not as strong as it was on *An. arabiensis* while the abundance of *An. coustani* appeared to be not influenced by IRS application. The difference could be due to the difference in indoor resting habits of these vectors, *An. arabiensis* is more endophagic and endophilic than *An. pharoensis* and *An. coustani* so that the level of exposure to the insecticide sprayed could vary accordingly. The difference in indoor resting habits of each species could be demonstrated by the number of each species captured indoors by PSC in the present study, i.e. among 2,690 of them collected indoors by PSC, 87% (n = 2,341) and 12.7 (n = 342) were *An. arabiensis, An. pharoensis,* respectively. The number of *An. coustani* captured indoors by PSC were less than 1% (n = 7).

A substantial reduction in the composition of Anopheles mosquitoes was seen at IRS villages one month after the intervention while the proportion was unchanged at non-IRS villages signifying that IRS-induced significant change in the abundance and proportion of vectors. This is consistent with the results of earlier studies documented from east Africa where large scale IRS operations replaced *An. funestus* Gillies by *An.rivulorum* Leeson (Gillies & Smith, 1960). Similar reports from Kenya and Tanzania also reported a significant shift in vector dominance from *An.gambiae* s.s to *An. arabiensis* [Mutuku *et al*., 2011; Russell *et al*., 2011; Zhou *et al*., 2013; Bayoh *et al*, 2010] following the massive scale up of LLIN distribution. The shifts in vector species composition from highly endophilic and endophagic to less endophilic and endophagic species due to successful indoor entomological interventions inevitably alter disease transmission patterns from indoor to outdoor transmission via secondary sources of transmission.

Therefore, the need to develop efficient outdoor vector control measures appears to be important [Ferguson *et al*., 2010] against secondary sources of malaria transmission.

6.5 Conclusion

The present findings demonstrated that the strategy of indoor residual spraying (IRS) was effective in the control of *An. Arabiensis* without affecting its resting behavior while the impact was not noteworthy on *An. pharoensis* and *An. coustani*.

6.6 Recommendation

Changes in species dominance after the application of the IRS would maintain disease transmission by these secondary sources of transmission so that the need to study the ecology and behavior of *An. pharoensis* and *An. coustani* is recommended to develop effective intervention tools other than IRS, which would be suitable for these malaria vector species.

CHAPTER 7: Effect of bediocarb indoor residual spraying on entomological inoculation rate of *Anopheles arabiensis*

7.1 Introduction

Current malaria vector control strategies rely heavily on IRS and LLINs. The impact of these interventions tools on entomological malaria transmission risk factors needs to be evaluated. The level of exposure to infective mosquito bites could be measured using EIR in the vector [Macdonald, 1957]. The EIR is defined as the number of infective bites received by an individual per unit time (night, month or year). It is the product of human biting rate (HBR) and sporozoite rate (SR) [WHO, 1975; Lines *et al*., 1991].

The human landing catch (HLC) is the most commonly used method to determine the human biting rate because it is the direct measure of human-vector contact [WHO, 1975]. However, due to ethical and logistic constraints associated with HLC, light trap catches (LTC), pyrethrum spray sheet catches (PSC) and exit trap catches could be used as alternatives to human landing catches [Lines *et al.*, 1991; Mbogo *et a*l., 1993] to estimate the HBR. In this study, Center for Disease Control and Prevention (CDC) light trap and PSC mosquito sampling methods were used to estimate the HBR.

Malaria is a public health problem in Ethiopia. Indoor residual spraying and LLNs are the frontline pillars of malaria vector intervention tools that have been used in all malarious parts of the country. However, studies on the impact of these interventions on EIR are either limited or unavailable [Abose *et al*., 1998; Taye *et al*., 2006]. Besides, EIR varies

from region to region, even from locality to locality. Therefore, narrowing this knowledge gap would be valuable for vector control programers.

Thus the present study was carried out to assess the impact of the current vector control strategy specifically bendiocarb IRS on BMI, SR and EIR.

7.2 Materials and methods

7.2.1 Study site and study design

A comparative study was carried out in Andassa and Tikurit villages. The study villages are described under section 3.1 of the monograph. The study was conducted for two consecutive years. Andassa received two round sprays in 2013 and 2014 while no spray was implemented in Tikurit. Susceptibility status of *An.arabiensis* to bendiocarb was confirmed before the application of IRS.

7.2.2 Mosquito sampling

Adult female *An.arabiensis* were collected from 24 residential houses (12 houses/ village; and 6 houses/sampling method) using Pyrethroid spray (PSC) and CDC light traps sampling methods (Figure 11A-C). Mosquitoes that were collected by each sampling method before and after IRS were then stored individually in tubes containing silica gel to process and determine their BMI and SR in the lab (Figure 12-B).

7.2.3 Blood meal host source and sporozoite rate determination

Enzyme-linked immunosorbent assay (ELISA) originally described by Beier *et al.* [1988] and CS-ELISA [Wirtz *et al*., 1987] protocols were adapted and used for BMI and SR

analyses, respectively. Blood fed mosquitoes preserved individual in tubes containing silica gel were used to determine their BMI and SR. Heads-thoraxes of mosquitoes were separated from their abdomens, and each body part (abdomen / head-thorax) was given a corresponding ID number and kept individually in tubes for the analyses.

7.2.3.1 Blood meal sources determination

Mosquito abdomen, which was kept individually in tubes containing silica gel, was ground in a tube containing 100 µl of phosphate buffer saline (PBS) with a plastic pestle fitted with foot-operated grinder. The pestle was rinsed twice with 200 µl of PBS to achieve the final volume of 500 µl. The samples were either incubated at room temperature for 3 hours and then tested, or stored at 4^0C and tested the next day. Mosquitoes were tested to assess the blood meal origin of human and bovine only because these hosts were the predominant hosts of the vector during the study period. A 96- well ELISA plate was used and 50 µl of the positive control for the blood meal host being tested was loaded. Wells A2-A5 had 50 µl of the negative controls and wells A6-A8 were blanks containing 50 µl of blocking buffer. The plate was then covered and incubated for 3 hours. The mosquito triturate was then aspirated by multichannel pipet and the plate was washed 3 times with 200 µl PBS-Tween20 (5%). For a full 96-well plate, the peroxidase conjugate anti-host IgG antibody was prepared by adding 4800 µl of blocking buffer and 19.2 µl of anti-host and 1µl of 1: 100,000 of each of the negative control [Beier *et al*., 1988]. Fifty microliter of peroxidase conjugate was added to each well and the plate was covered and incubated for 1 hour at room temperature. The plate was then washed 3 times with 200 µl PBS-Tween20 (5%) and the 1 component ABTS peroxidase substrate was added to each well. PBS-tween20 was aspirated by

multichannel pipet and plates were bang between washes. After 30 minutes of covered incubation at room temperature, the plate was read with the Spectramax 340 plate reader (Molecular Devises) at 414 nm.

7.2.3.2 Sporozoite rate determination

Head-thorax of a mosquito, which was kept individually in step tubes containing silica gel, was ground in 1.5 µl micro centrifuge grinding tube containing 50 µl PBS with a plastic pestle fitted with foot-operated grinder. The pestle was rinsed twice with 100 µl of PBS and dried with tissue paper to prevent contamination between mosquito samples.

A 96-well ELISA PVC plate was coated with 50 µl of capture monoclonal antibodies (mAb) of each Plasmodium sporozoite species (Pf, Pv-2010 and Pv-247) in each well of the ELISA plates (a separate plate used for each species), covered and incubated for half an hour. After the well contents were aspirated, plates were banged upside-down on paper towel 5 times. The wells were then filled with 200 µl blocking buffer (BB), covered with lid and incubated for 1 hour at room temperature. Well contents aspirated and plate banged on paper towel 5 times. Samples and controls were loaded into the plate (well 1A positive control, 1B-1H, negative control and the rest of the wells with mosquito triturate) and covered and incubate for 2 hours. Well contents aspirated and the plates were banged upside-down on paper towel 5 times and washed 2 times with 200 µl of PBS-Tween. The wells were aspirated and plates were banged upside down five times with each wash. Then 50 µl of peroxidase conjugate solution of each plasmodium sporozoite species (Pf, Pv-2010 and Pv-247) was added to each well covered and incubated for 1 hour. After aspirating the well contents and banging the plates, wells were

washed 3 times with 200 µl of PBS-Tween, aspirated and plates were banged 5 times with each wash. Finally, 100 µl of the substrate solution was added per well, covered with cover plate and incubated for 30 minutes. The results were then read visually at the Spectramax 340 plate reader (Molecular Devises) at 405-414 nm. All positive samples were retested for confirmation.

7.2.4 Determination of entomological inoculation rate

Plasmodium EIR of *An.arabiensis* was determined based on CDC and PSC catches. The EIR was estimated from PSC samples as described by the World Health Organization [WHO, 1975] using the formula: number of FF mosquitoes caught by PSC/no. human occupants who spent the previous night in sprayed house) × (number of human fed mosquitoes / number of mosquitoes tested for human blood meal) × (number of sporozoite positive ELISAs / number of mosquitoes tested i.e. HBR × CSP rate. The human-biting rate was calculated by dividing the total number of freshly fed *An. arabiensis* caught in PSC by the total number of occupants who slept in the houses in the previous night of mosquito collection and multiplied by the HBI. The HBI was calculated as the proportion of Anopheles mosquitoes that fed on humans to the total Anopheles analyzed for blood meal origin [Garrett-Jones, 1964]. EIR from CDC light traps catches ware estimated using the standard method, 1.605 (number of circumsporozoite-positive ELISA results from CDC light trap / no. of mosquitoes tested) × (number of mosquitoes collected by CDC LT /no. of CDC LT catches), and the alternative method, 1.605 (no. positive ELISA/no. catches) [Drakeley *et al*., 2003].

7.2.5 Data analyses

The relative adjusted reduction in human blood feeding index (HBI), sporozoite rate (SR) and the entomologic inoculation rate (EIR) of the vector after intervention was calculated using Henderson & Tilton [1955] formula: PR=100 -$\frac{C1T2}{C2T1} \times 100$, where C1 and C2; and T1 and T2 describe either the number of *An.arabienis* or percentages of BMI, SP or EIR in sprayed (T) and non-sprayed villages (C) before IRS (subscript 1) and after IRS (subscript 2). This formula takes into account that changes in the mosquito population and parasite prevalence are taking place at the same level and rate in both sprayed and non-sprayed villages, i.e. the reductions were adjusted for the background differences. This formula was used only when the denominators were non-zero.

7.3 Results

7.3.1 Effect of IRS on the abundance of *An.arabiensis*

Table 9 shows the abundance and abdominal status of *An. arabiensis* collected before and after spray. The relative adjusted reduction in the abundance of *An.arabiensis* was between 91.4% and 96.1%. The abundance and abdominal status of *An.arabiensis* varied by sampling method, spray status, study village and year. Among 5,425 *An.arabiensis*, 3,111 of them were collected by CDC light trap while 2,314 of the vector were by PSC. The numbers of semi-gravid and gravid *An.arabiensis* were smaller in CDC light trap catches than in PSC collections. The proportions of unfed *An.arabiensis* were higher in CDC light trap catches than PSC collections. Fresh fed *An.arabiensis* was dominant in PSC collections (>75%) while < 54 % of them were FF in CDC light trap catches. The

abundance of these FF mosquitoes was declined after IRS in sprayed villages (n=62) while the number of FF remained high in non-sprayed villages (n =1,690). The abundance of unfed, gravid and semi-gravid mosquitoes also decreased after spray.

7.3.2 Effect of IRS on HBI

Among 3,451 FF *An.arabiensis* collected, 1,574 (45.61%) of them were tested to determine their blood meal sources and sporozoite infection rate. The relative adjusted reduction in *An.arabiensis* human blood feeding index (HBI) due to IRS implementation varied from 3% to 10% except in 2014 when no human blood was detected in any of the three mosquitoes that were collected and tested. Despite IRS implementation reduced HBI, a non-negligible proportions of *An.arabiensis* still fed on humans (Table 10).

7.3.3 Effect of IRS on SR

The estimated sporozoite rate in *An.arabiensis* was low in both sprayed and non-sprayed villages especially after IRS implementation. As indicated by ELISA test, *P.falciparum* was more prevalent than *P.vivax* in both sprayed and non-sprayed villages. *Pv*-247 was the only subspecies detected during the study period. There was no any mixed infection detected in the vector in both study villages during the study period. Neither *P.falciparum* nor *P.vivax* was not detected in *An.arabiensis* collected from sprayed villages after the implementation of IRS. Similar results were observed for *Pv*-247 in non-sprayed villages except in 2013 when SR was 0.57% in *An.arabiensis* caught by CDC light trap. The relative adjusted reduction in *P.falciparum* infection in *An.arabiensis* in sprayed villages

was 100% after IRS. A similar result was observed for *Pv*-247 EIR in 2013 in *An.arabiensis* collected by CDC light traps (Table 11).

7.3.4 Effect of IRS on EIR

The reduction in EIR after the implementation of IRS had similar trends with the reduction in SR because EIR is the product of SR and HBI. Compared with CDC light trap catches, EIR was high in PSC catches, i.e. *Pf*-EIR in *An.arabiensis* was 452 infective bites/month/house in PSC catches while this was 32.2 infective bites in CDC light trap catches. *Pv*-247 EIR was 226 and 16 infective bites/month/house in *An.arabiensis* collected by PSC and CDC light traps, respectively. The relative adjusted reduction in *Pf*-EIR in *An arabiensis* was 100% after the implementation of IRS. A similar result was observed for *Pv*-247 EIR in 2013 in *An.arabiensis* caught by CDC (Figure 12).

7.4 Discussion

The aim of vector control using IRS and LLINs interventions is to reduce vectors' abundance, survival, contact with human and feeding frequency [WHO, 2011]. Vector abundance is an important determinant of malaria transmission [Garrett-Jones 1964] and thus factors that increase or decrease vector abundance could have an impact on the intensity of disease transmission. The present study demonstrated that IRS implementation brought about 91.4% to 96.1% reduction in the abundance of *An.arabiensis* signifying that the abundance of this vector could not be reduced to non-detectable level by the implementation of IRS. Previous similar studies in Ethiopia are either missing or unavailable to compare and contrast with the present study. However, studies from Zambia [Chanda *et al*., 2012] validated that the effect of IRS on the density

of *An.arabiensis* was not as strong as on *An.gambiae s.s* and *An.funestus* due to its exophilic and wide-ranging feeding behavior. Alegana *et al.* [2016] also confirmed that IRS intervention reduced the density of *An. funestus* and *An. gambiae* s.l disproportionally, twice high on *An.funestus* compared with *An. gambiae* s.l. Thus, malaria transmission through the bites of *An.arabiensis* could not be intercepted entirely by the application of IRS so that the impact of IRS should be complemented by and integrated with other vector control interventions.

Blood meal source analyses indicated that *An.arbiensis* was found to have strong preferences to bovine and other hosts over human hosts. Similar results from other parts of the country were published in previous studies [Hadis *et al*, 1997; Habtewold *et al*., 2001; Masebo *et al*., 2015] where *An.arbiensis* demonstrated strong blood meal preferences of bovine over human hosts. Similar results were also reported from neighboring Eritrea [Waka *et al*., 2005] and Kenya [Collins *et al*., 1987]. Contrary to zoophilic, strong athrophyilic tendency was observed in *An arabiensis* in The Zambia [Fornadel *et al.*, 2007; Fornadel & Norris, 2008], Malawi [Mzilahowa *et al.*, 2012] and Mozambique [Thompson *et al*., 1997]. The potential reason for the differences observed in the anthrophyilic tendency of *An.aranbiensis* between Eastern and southern African countries would be justified by the differences in their ecological setups and the impact of these ecological differences on the ecology and behavior of *An.arabiensis* populations in these two sub-African regions. The application of IRS in the present study showed the reduction in the rate of anthrophyily of *An. arabiensis* signifying that zooprophylaxis could be considered as a potential vector control strategy for zoophilic vector species in areas having similar ecological setups with the present study site. On the contrary, a

considerable proportion of *An.arabiensis* still fed on human hosts suggesting that zooprophylaxis alone cannot intercept malaria transmission. Thus, zooprophylaxis would advance the effectiveness of malaria interventions if it is used in an integrated way with other vector control intervention measures.

Either data are unavailable or no previous attempts were made about the impact of IRS on SR in Ethiopia. However, studies from other African countries [Coleman *et al*., 2017; Ossè *et al*., 2013] demonstrated that the implementation of IRS reduced SR to non-detectable level, which is consistent with the results of the present study. And these would substantiate the contribution of IRS implementation in reducing malaria transmission risks in general and SR in particular in the present study area and others having similar ecological set ups.

In the present study, *P.falciparum* was more prevalent than *P. viva* in *An.arabiensis.* No *An.arabiensis* was found positive for either *P.faciparum* or *P. vivax* in sprayed villages after IRS. Although too few *An.arabiensis* were recorded in sprayed villages after IRS, it would have been necessary to process thousands of mosquitoes to find any of them were infected by malaria parasites. There was no any mixed infection detected. The proportion of plasmodium infected *An.arabiensis* was also low in non-sprayed villages indicating that SR might be low in naturally occurring vector population. Contradictory results about the prevalence of *P. falciparum* and *P.vivax* in *An.arabiensis* have been reported from different parts of Ethiopia at different times.

Table 9: Abundance and abdominal status of *An.arbiensis* collected by PSC & CDC light traps catches

Before spray							After Spray					Adjusted reduction (%)
CDC light trap collection												
Year	Village	Row Total	UF	FF	SG	G	Row Total	UF	FF	SG	G	
2013	Sprayed	103	46	57	0	0	12	6	6	0	0	91.4
	Non-sprayed	599	356	240	0	3	811	341	468	0	2	
	Column Total	702	402	297	0	3	823	347	474	0	2	
2014	Sprayed	139	56	83	0	0	67	18	48	0	1	94.3
	Non sprayed	146	69	71	0	6	1234	583	650	0	1	
	Column Total	285	125	154	0	6	1301	601	698	0	2	
Pyrethrum spray collection												
2013	Sprayed	176	13	151	10	2	6	1	5	0	0	95.8
	Non-sprayed	769	33	666	49	21	624	19	543	48	14	
	Column Total	945	46	817	59	23	630	20	548	48	14	
2014	Sprayed	471	16	302	86	67	3	0	3	0	0	96.1
	Non-sprayed	228	25	129	33	41	37	6	29	0	2	
	Column Total	699	41	431	119	108	40	6	32	0	2	

Table 10: Effect of bendiocarb IRS on blood meal sources (BMS) of *An.arabiensis*

		Before spray		After spray		
Year	**Host**	**Sprayed (n)**	**Non-sprayed (n)**	**Sprayed (n)**	**Non-sprayed (n)**	**Adjusted reduction (%)**
			CDC light trap collection			
2013	HBI	19.30 (57)	18.18 (176)	16.67 (6)	17.61 (176)	-10.83
	BBI	31.58 (57)	42.05 (176)	33.33 (6)	40.91 (176)	+8.48
	Mix	19.30 (57)	1.7 (176)	16.67(6)	0 (176)	
	Un	29.82 (57)	38.07 (176)	33.33 (6)	41.48 (176)	+2.58
2014	HBI	18.75 (80)	18.57 (70)	16.67 (48)	17.05 (176)	-3.17
	BBI	33.75 (80)	44.29 (70)	37.50 (48)	46.02 (176)	+6.93
	Mix	7.5 (80)	0 (70)	0 (48)	0 (176)	
	UN	40 (80)	31.14 (70)	45.83 (48)	36.93 (176)	-3.39
			Pyrethrum spray sheet collection			
2013	HBI	20.71(140)	25 (176)	20 (5)	25 (176)	-3.43
	BBI	36.43 (140)	51.70 (176)	40 (5)	55.11 (176)	+5.16
	Mix	20 (140)	0 (176)	20 (5)	0 (176)	
	UN	22.8 (140)	23.30 (176)	20 (5)	21.02 (176)	-2.76
2014	HBI	19.89 (176)	18.75(80)	0 (3)	17.24 (29)	100
	BBI	32.95 (176)	48.75 (80)	33.33 (3)	48.28 (29)	+2.14
	Mix	24.43 (176)	21.25 (80)	66.67 (3)	0 (29)	
	UN	22.73 (176)	30.00 (80)	0 (3)	34.48 (29)	0

HBI= Human blood index, BBI= Bovine blood index, UN= unknown hosts, n= number of mosquitoes tested for their blood meal origin

Table 11: Effect of IRS on SR in *An.arabiensis* estimated based on CDC light trap and PSC collections

		Before spray		After spray		
Year	**Parasite**	**Sprayed (n)**	**Non-sprayed (n)**	**Sprayed (n)**	**Non-sprayed (n)**	**Adjusted reduction (%)**
			CDC light trap collection			
2013	Pf	1.75 (57)	1.14 (176)	0 (6)	0.57 (176)	100
	Pv-247	1.75 (57)	0.57 (176)	0 (6)	0. 57 (176)	100
	Pv-210	0 (57)	0 (176)	0 (6)	0 (176)	
	Mixed	0 (57)	0 (176)	0 (6)	0 (176)	
2014	Pf	2.5 (80)	1.43 (70)	0 (48)	1.70 (176)	100
	Pv-247	0 (80)	0 (70)	0 (48)	0 (176)	
	Pv-210	0 (80)	0 (70)	0 (48)	0 (176)	
	Mixed	0 (80)	0 (70)	0 (48)	0 (176)	
			Pyrethrum spray sheet collection			
2013	Pf	1.43 (140)	1.14 (176)	0 (5)	1.14 (176)	100
	Pv-247	0.71 (140)	0.57 (176)	0 (5)	0 (176)	
	Pv-210	0 (140)	0 (176)	0 (5)	0 (176)	
	Mixed	0 (140)	0 (176)	0 (5)	0 (176)	
2014	Pf	1.70 (176)	1.25 (80)	0 (3)	0 (29)	
	Pv-247	0 (176)	0 (80)	0 (3)	0 (29)	
	Pv-210	0 (176)	0 (80)	0 (3)	0 (29)	
	Mixed	0 (176)	0 (80)	0 (3)	0 (29)	

P f=*Plasmodium falciparum*, Pv-247= *Plasmodium vivax* 247, Pv-2010=*Plasmodium vivax* 2010, n=number of mosquitoes tested for CSP ELISA

Table 12: Effect of IRS on EIR in *An.arabiensis* based on CDC light trap & PSC collections

		Before spray		After spray		
Year	**EIR**	**Sprayed**	**Non-sprayed**	**Sprayed**	**Non-sprayed**	**Adjusted reduction (%)**
			CDC light trap collection			
2013	Pf	16	32.2	0	4.47	100
	Pv-247	16	16.1	0	4.47	100
	Pv-210	0	0	0	0	
	Mixed	0	0	0	0	
2014	Pf	26.76	13.38	0	13.34	100
	Pv-247	0	0	0	0	
	Pv-210	0	0	0	0	
	Mixed	0	0	0	0	
			Pyrethrum spray sheet collection			
2013	Pf	101.58	452.01	0	151.62	100
	Pv-247	50.44	226	0	0	
	Pv-210	0	0	0	0	
	Mixed	0	0	0	0	
2014	Pf	249.2	88.83	0	0	
	Pv-247	0	0	0	0	
	Pv-210	0	0	0	0	
	Mixed	0	0	0	0	

EIR= entomological inoculation rate, Pf=*Plasmodium falciparum*, Pv-247= *Plasmodium vivax* 247, Pv-2010=*Plasmodium vivax* 2010

Masebo *et al*. [2013] reported the dominance of *P.falciparum* over *P.vivax* while Taye *et al*. [2006] reported the dominance of *P.vivax* over *P. falciparum* in South west Ethiopia. Animut *et al*. [2013] reported the dominance of *P.vivax* over *P.falciparum* in South central Ethiopia. Differing from all these Taye *et al*. [2016] reported that no *An.arabiensis* was found positive either for *P.falciparum* or *P.vivax* in South west Ethiopia. Except Taye and his colleagues [2006] other investigators used either CDC and/or PSC mosquito sampling method so that the differences observed in the prevalence of malaria parasites in *An.arabiensis* could be potentially justified by the differences in ecological set ups of the study sites and time period in which the study was conducted. Otherwise, this would be a question of validation.

Malaria transmission intensity, which normally expressed by EIR, is highly variable with annual EIRs ranging from < 1 to >1,000 infective bites per person per year in Africa [Beier *et al,* 1999]. Variations in EIR in malaria vectors could be due to different factors such as ecological heterogeneity at continental, regional and country level [Hay *et al*., 2000; Ndenga *et al,* 2006; Animut *et al*, 2013] and season (dry or wet) [Shililu *et al*, 2003; Mabaso *et al*., 2013; Animut *et al.*, 2013]. For example, the burden of malaria is high in tropical countries having warm temperature, heavy rainfall, high humidity, and efficient Anopheles vectors than non-tropical countries [Breman, 2001]. Previous studies indicated that the impact of wet or dry season on EIR is inconsistent, i.e. Published reports indicated that EIR is higher during wet season [Shililu *et al*, 2003; Drakeley *et al*, 2003; Kent *et al*., 2007]or vice versa [Shililu *et al* ., 1998; Himeidan *et al*., 2011].

In the present study, a very high *Pf*-EIR was observed in the vector in both years and study villages although SR and HBI were low. The trend was also similar for *Pv*-247

EIR in both study villages before IRS in 2013. These findings would be justified by the occurrences of high mosquito density before IRS during the study periods. The level of EIR of both parasites went to zero in sprayed villages after the implementation of IRS suggesting that IRS application is 100% effective to control disease transmission. In contrast, previous studies reported that EIR was 90% lower in the ITN community and 93% lower in the IRS community, relative to the community without intervention [Shaukat *et al*., 2010]. The differences observed between the present and previous studies would be attributed to heterogeneity in the ecology and behavior of the vector.

Variation in EIR could also differed by mosquito collection methods [Shaukat *et al*., 2010]. Krafsur [1977] indicated that PSC might underestimate the HBR, which again under-rate EIR. Previous studies also reported CDC light traps were more efficient than PSC to estimate EIR [Masebo *et al*., 2013; Ndiath *et al*., 2011, Fornadel *et al*., 2010]. Contrary to these, a study from Bioko Island, Equatorial Guinea, demonstrated that CDC light traps failed to determine the human-biting rate of the anthropogenic *An. gambiae s.s* [Overgaard *et al*., 2012]. Different from all previous reports, the present study indicated that higher EIRs were recorded from PSC catches than CDC light trap catches. Both CDC and PSC are reported to have shortcomings in mosquito sampling. While CDC light traps attract fed indoor resting mosquitoes [Lines *et al*., 1991; Petrarca *et al*., 1991], PSC tends to miss mosquitoes that leave the house after feeding and includes those entering the house after feeding outdoor [Mboera, 2005]. Therefore, estimating the HBR using either CDC light trap or PSC has limitations and the need to develop standard HBR remains high. Thus, the differences observed

between the present and previous studies might be associated with limitation stated for each sampling method.

7.5 Conclusion

This study was linked with IRS application to assess its effect on EIR and other entomological risk factors for malaria transmission. The results illustrated that IRS was strong enough to reduce mosquito abundance, sporozoite rate and EIR, which are the most important determinants for malaria transmission. Therefore, IRS operations would be effective to control disease transmission in the present and other areas having similar ecological set up with the present study villages.

7.6 Recommendation

The present study presented that the application of bendiocarb IRS was effective to intercept the transmission of malaria.Similar studies require in sentinel study sites in the country because the country have varied ecological and other risk factors contributing to the transmission of malaria. The role of other determinants, which influence the effectiveness of IRS operations, needs to be assessed. Impact assessement should be an integral part of IRS programs to discover any change contributing for control failure.

CHAPTER 8: General conclusions and Recommendations

8.1 General conclusions

This study was linked with bendiocarb 80% WP IRS applications (a part and parcel of IRS operation in the country) to assess its effectiveness in the control of entomological risk factors for the transmission of malaria. The findings of the study demonistrated that bendiopcarb IRS was strong enough to reduce the abundance, sporozoite rate and EIR of malaria vectors, which are the most important determinants for malaria transmission. However, the effective residual life of the insecticide was too short on non-plastered mud wall surfaces to cover malaria transmission season in areas having >1 month transmission. Therefore, alternative insecticides should be considered for IRS in areas having >1 month malaria transmission season and >20% houses with non-plastered mud wall surfaces. On the other hand, the residual effective life of the insecticide was >2 months on plastered mud walls suggesting that bendiocarb would be the recommended insecticide for IRS in areas having plastered mud walls to cover at least 2-3 months of malaria transmission season.

Resistance to bendiocarb was suspected after three round sprays in three years time. On the other hand, susceptibility of the vector to other insecticdes to which the vector already had developed resistance was improved at significant level but not restored whin three years time. This signifies that the restoration of susceptible vector populations requires more time than the establishment of resistant vector populations due to IRS operations. Therefore, insecticde resistance monitoring and evaluation every 2-3 years and the choice of an insecticide for IRS opearation should be made accordingly to delay the occurances of residtance in populations of disease vectors.

The present findings also demonstrated that the impact of bendiocarb IRS on *An. pharoensis* and *An. coustani* was not remarkable. However, a huge number of *An. pharoensis* and a non-negligible number of *An. coustani* was captured both indoors and out doors.The role of *An. pharoensis* as malria vector in Ethiopia comes next to *An.arabiensis*. Studies indicated that *An. coustani* is a potential malaria vector. Therefore, both *An. pharoensis* and *An. coustani*, particularly, *An.pharoensis* could mainatain malaria transmission. Thus, any IRS program targeting *An.arabiensis* should take the impact of the same operation on *An.pharoensis* and other potential vectors into consideration.

8.2 Recommendations

♦ Cow dung plastering on mud walls before the application of IRS increased the effective residual life of bendiocarb sprayed on it. Cow dung is easily available in rural Ethiopia so that community education is recommended to improve the persistence of the insecticide by cow dung plastering and cover malaria transmission season.

♦ The need to study the ecology and behavior of *An. pharoensis* and *An. coustani* is recommended to develop effective intervention tools, which would be effective for the control of these malaria vector species.

♦ The present study presented that the application of bendiocarb IRS was effective to intercept the transmission of malaria. Similar studies should also be carried out in sentinel study sites because the country has varied ecological setups and other risk factors contributing to the transmission of malaria.

♦ Impact assessment on entomological disease transmission risk factors needs to be an integral parts of vector control program to maintain the effectiveness of vector control interventions

♦ The country requires to build its capacity in terms of expertise, resources allocation and laboratories with a minimum requirement to support field research activities

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Apennedix

Appendix I. Project appraisal after visiting the study villages in Bahirdar Town, Dr. Wubegzier Mekonen, Professor Emana Getu, Dr. Mamuye Hadis and Alemayehu Abate from right to left