



**HABITAT CHARACTERIZATION AND INSECTICIDE RESISTANT STATUS OF
ANOPHELES GAMBIAE (S.L.) IN MALARIA ENDEMIC DISTRICTS OF SIDAMA
REGION, SOUTH ETHIOPIA**

M.Sc. THESIS

MEDAN TAYE HUSSE

HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

MAY, 2024

**HABITAT CHARACTERIZATION AND INSECTICIDE SUSCEPTIBILITY STATUS
OF *ANOPHELES GAMBIAE* (S.L.) IN MALARIA ENDEMIC DISTRICTS OF SIDAMA
REGION**

MEDAN TAYE HUSSE

**A THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGY, COLLEGE OF
NATURAL AND COMPUTATIONAL SCIENCES, HAWASSA UNIVERSITY
HAWASSA, ETHIOPIA**

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

**MASTER OF SCIENCE IN BIOLOGY
(SPECIALIZATION: BIOMEDICAL SCIENCE)**

MAY, 2024

ADVISORS' APPROVAL SHEET SCHOOL OF GRADUATE STUDIES

HAWASSA UNIVERSITY ADVISORS' APPROVAL SHEET

(Submission Sheet-1)

This is to certify that the thesis entitled “**HABITAT CHARACTERIZATION AND INSECTICIDE SUSCEPTIBILITY STATUS OF *ANOPHELES GAMBIAE* (S.L.) IN MALARIA ENDEMIC DISTRICTS OF SIDAMA REGION**” submitted in partial fulfillment of the requirements for the degree of Master's with specialization in **Biomedical Science**, the Graduate Program of the **Department of Biology**, and has been carried out by **Medan Tave Husse** ID. No. **GPBiSc/R/0007/14**, my supervision. Therefore I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

Name of advisor

Signature

Date

**EXAMINER'S APPROVAL SHEET-I SCHOOL OF GRADUATE STUDIES
HAWASSA UNIVERSITY EXAMINERS' APPROVAL SHEET-1**

(Submission Sheet-2)

We, the undersigned, members of the Board of Examiners of the final open defense by **Medan Taye Husse** have read and evaluated his thesis entitled *“Habitat Characterization and Insecticide Susceptibility status of Anopheles gambiae (s.l.) in malaria endemic districts of Sidama Region,* and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree.

_____	_____	_____
Name of Major Advisor	Signature	Date
_____	_____	_____
Name of Internal Examiner-I	Signature	Date
_____	_____	_____
Name of Internal Examiner-II	Signature	Date
_____	_____	_____
Name of External examiner	Signature	Date
_____	_____	_____
SGS Approval	Signature	Date

DECLARATION

I hereby declare that this M.Sc. thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: _____

Signature: _____

Place and Date of Submission: _____

ACKNOWLEDGEMENTS

First of all I would like to thank almighty God for his endless mercy and unconditional love throughout all my journey. I would like to thank my advisor Melese Birmeka (PhD) who sacrifices his time to make this work to sound better. Then I have special gratitude for my family and friends for their tremendous support. I would like to thank Wolaita Sodo University from bottom of my heart for granting me this opportunity of master's degree with financial support. Finally I have special gratitude to Hawassa University for providing me various facilities and financial support at the time of conducting my study.

ACRONYMS/ ABBREVIATIONS

AChE: Acetylcholinesterase

CDC: Center for Disease Control and Prevention

DDT: Dichlorodiphenyltrichloroethane

EMR: Eastern Mediterranean Region

FMOH: Federal Ministry of Health

GFFATM: Global Fund to Fight AIDs Tuberculosis and Malaria

GSTs: Glutathione-S-transferases

IRS: Indoor Residual Spraying

ITN: Insecticide Treated Net;

Kdr: Knockdown Resistance

LAMP: Loop-mediated Isothermal Amplification

LLIN: Long Lasting Insecticidal Net;

MFOs: mixed-function oxidases

NIRS: Near-infrared Spectroscopy

NSPE: National Strategic Plan for malaria Prevention control and elimination in Ethiopia

PMI: President Malaria Initiatives

WHO: World Health Organization

TABLE OF CONTENTS

Contents	Pages
DECLARATION.....	v
ACKNOWLEDGEMENTS	vi
ACRONYMS/ ABBREVIATIONS	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
ABSTRACT.....	xiv
1. INTRODUCTION.....	1
1.1 Background of the study	1
1.2. Statement of the problem	3
1.3. Significance of the study	4
1.4 Objectives.....	4
1.4.1 General objective	4
1.4.2 Specific Objectives	4
1.5 Scope of the study	5
1.6 Limitation of the study	5
2. LITERATURE REVIEW	6

2.1 The mosquito.....	6
2.2 Mosquito vector for malaria.....	6
2.3 Malaria vector species Available in Ethiopia.....	7
2.4 Life cycle of mosquito.....	7
2.6 Identification of male and female mosquito.....	8
2.7 Mosquito species Identification	8
2.7.1 Morphological identification	8
2.7.2 Molecular identification	9
2.8 Mosquito rearing in Laboratory	9
2.8.1 Equipment /supplies for mosquito rearing.....	10
2.8.2 Microclimate required for insectary	10
2.8.3 Anopheles Mosquito rearing procedure	12
2.9 Insecticide and resistance behavior of Anopheles mosquito.....	13
2.9.1 Insecticide against Mosquito	13
2.9.2 Mosquito killing mechanism of insecticide.....	14
2.9.3 Insecticide resistance mechanism of Mosquito	14
2.10 Insecticide susceptibility test for Anopheles mosquito.....	17
3. MATERIALS AND METHODS	19
3.1. Description of the study area.....	19

3.2. Study design	20
3.3. Study population	20
3.4. Sample size determination and sampling methods	21
3.5 Habitat characterization.....	21
3.6 Mosquito Collection and rearing.....	22
3.7 Insecticide susceptibility tests	23
3.8 Species Identification	24
3.9 Data Analysis	24
3.9.1 Habitat characterization.....	24
3.9.2 Insecticide susceptibility test.....	25
3.10 Quality Control.....	26
3.11 Ethical approval.....	26
4. RESULTS	27
4.1 Insecticide resistance status of <i>A. gambiae</i> in study sites to selected insecticides.....	27
4.2 Knockdown Time KDT_{50}	28
4.3 Anopheles larvae species composition.....	29
4.4 Anopheles larval productivity in different habitat types.....	29
4.5 Association between larval density and habitat variables.....	31
5. DISCUSSIONS.....	35

6. CONCLUSIONS AND RECOMMENDATIONS.....	40
6.1. Conclusions	40
6.2. Recommendations	40
REFERENCES.....	41
APPENDIX.....	77

LIST OF TABLES

Tables	Pages
Table 1: A summary of percentage mortality 24 h after a 1-h exposure to different classes of insecticides on field collected F1 progeny of <i>An. gambiae</i> (n=100 per insecticide) from Loka Abaya, Aleta chuko and Dale woredas	28
Table 2: Density of <i>Anopheles</i> larvae in different habitat types in Aleta Chuko, Dale and Loka Abaya Woredas study sites	30
Table 3: Multiple regression analysis showing the key predicting factors for <i>Anopheles</i> larvae density	31
Table 4: Correlation of some larval habitat characteristics with the average <i>Anopheles</i> larval density sampled	32
Table 5: Environmental variables and distribution of <i>Anopheles</i> larvae at Aleta Chuko district (2024)	33

LIST OF FIGURES

Figures	Pages
Figure 1: Life cycle of mosquito (Image courtesy of Louise Malmgren/NRI).....	8
Figure 2: Study area map (From ArcGIS)	20
Figure 3: Mean \pm 95% CL summary results of knockdown time (KDT ₅₀) (minutes) of field collected <i>Anopheles gambiae</i> F1 progeny (n=100 per insecticide) from malaria endemic districts tested against different classes of insecticides	29
Appendix Figure 1: Road puddle from study area.....	77
Appendix Figure 2: Ditches (A) and Water canal from study area (B)	77
Appendix Figure 3: Insecticide susceptibility test	78
Appendix Figure 4: <i>Anopheles</i> species identification	78

ABSTRACT

The prevalence of malaria in Ethiopia has decreased somewhat in recent decades, but it is currently increasing as a result of the introduction of insecticide-resistant *Anopheles* mosquitoes. Therefore, the purpose of this study was to assess the anopheles mosquito's insecticide susceptibility status and habitat characterization in a selected study area at November. Cross sectional study was used to conduct the study. The presence of *Anopheles* larvae was confirmed in all water-holding habitats, and information about the habitat's perimeter, water depth, light intensity, current flow, temperature, pH, turbidity, and proximity to the closest house was noted. Additionally, information about vegetation coverage, permanence, surface debris, emergent plant, habitats and substrate type of larval holding habitats were recorded for the purpose of characterizing the habitat. Sample of anopheles larvae were taken to Akililu Lemma Institute of Pathobiology from study area in November 2024. The larvae were then reared there, their insecticide susceptibility tested, and their species was identified using the Coetzee 2020 identification key. In order to test for insecticide susceptibility, 100 adult *Anopheles* mosquitoes were exposed to each insecticide that the World Health Organization (WHO) recommended (propoxur 0.1%, deltamethrin 0.05%, and alpha-cypermethrin 0.45%). The knockdown times (KDT), percentage knockdown, and percentage mortalities of the exposed mosquitoes were noted. *Anopheles gambiae* s.l., the species of mosquito that was identified, was resistant to propoxur (0.1%) and deltamethrin (0.05%), but it was completely susceptible to alpha-cypermethrin (0.45%) across all study sites. In all research sites, alpha-cypermethrin insecticide took less time (18.67-24.23min) to kill 50% of *Anopheles gambiae* s.l. In every study area, the KDT_{50} of *Anopheles gambiae* exposed to propoxur insecticide was high, ranging from 51.95 to 56.25 minutes. *Anopheles* mosquito species that dominate study area was *Anopheles gambiae* s.l.; it was susceptible to Alpha cypermethrin and resistant to deltamethrin and propoxur and it prefer to breed in habitat which exposed to sun light, had stone substrate and had no canopy cover. *Anopheles gambiae* (s.l) that inhabit Chuko, Dale and Loka Abaya was susceptible to Alphacypermethrin and resistant to Deltamethring & Propoxure. This species was highly productive in habitat like ditches, burrow pits and road puddle which exposed to sun light, without canopy cover and with stony substrate.

Keywords/phrases: Alpha-cypermethrin, *Anopheles gambiae*, Delthametrin, insecticide susceptibility, Propoxur

1. INTRODUCTION

1.1 Background of the study

Malaria is the most prevalent serious mosquito-borne disease worldwide (WHO, 2008). The female Anopheles mosquito, which varies depending on the region, is the vector of this infectious disease, which is caused by the parasite Plasmodium and spreads from infected to healthy individuals (Pimenta *et al.*, 2015). According to Saraiva *et al.* (2016), the main Anopheles species that spread malaria are *An. gambiae*, *An. stephensi*, *An. dirus*, *An. coluzzii*, *An. albimanus*, *An. funestus*, and *An. arabiensis*.

The WHO's African Region accounts for the majority of the increase in malaria cases, which is expected to reach 247 million worldwide in 2021 from 245 million in 2020 in 84 malaria-endemic countries (including the French Guiana territory) (WHO, 2022). Over 50% of the global population is susceptible to the illness, which is made worse by failing healthcare systems, rising drug and pesticide resistance, natural disasters, and climate change (Martens and Hall, 2000).

These days, the subtropics bear the brunt of the malaria epidemic. *Plasmodium falciparum* is the most dangerous parasite species that infects humans in the African Sahara; it is estimated to cause roughly 250 million cases and approximately 1 million deaths annually (WHO 2008). Different Anopheles mosquito species carry malaria throughout sub-Saharan Africa, and the risk of infection and illness varies greatly between locations (De Silva and Marshall, 2012; Doumbe-Belisse *et al.*, 2021).

Malaria has been a persistent public health issue in Ethiopia for a number of years. *Anopheles arabiensis* is the primary vector of malaria, with *Plasmodium falciparum* and *Plasmodium vivax* being the two most common parasites (Temesgen Ashine *et al.*, 2023). Temperature, precipitation, and altitude all affect the seasonality of transmission (Bødker *et al.*, 2003; Abebe Alemu *et al.*, 2011). An estimated 68% of the population is at risk of contracting malaria, with over half (60%) of the country's population living in malaria-prone areas (PMI, 2019). According to Gessesew Bugssa and Kiros Tedla (2020), the primary methods for preventing and controlling malaria in Ethiopia are the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Malaria continues to be the leading cause of morbidity and mortality in Ethiopia despite the achievements (WHO, 2014) and efforts made thus far. According to estimates, the nation will be responsible for 1.7% of cases and 1.5% of deaths from malaria in 2021, making it a major public health concern (Brhane Berheet *et al.*, 2019).

Pyrethroids are the primary class of insecticide approved for use in net impregnation, out of five main classes: carbamates, neonicotinoid, organochlorines, organophosphates, and pyrethroids (WHO, 2022). October 2017 saw the prequalification of the first neonicotinoid product for indoor residual spraying (WHO, 2021). The majority of pesticides were applied by residual spraying, which was followed by larviciding and space spraying. All of the insecticide classes Organochlorine, Organophosphate, Carbamate, Pyrethroid, and Neonicotinoid—are used for residual spraying; Organophosphate, Carbamate, and Pyrethroid are used for space spraying; and Organophosphate is used for larviciding. Pyrethroids are the most effective insecticide for treating nets (WHO, 2021).

Numerous studies have revealed that *Anopheles* species found in various parts of Ethiopia exhibit high levels of resistance to insecticides such as deltamethrin, permethrin, pirimiphos-methyl, bendiocarb, propoxur, DDT, and others (Solomon Yared *et al.*, 2020; Delelegn Woyessa and Delenasaw Yewhalaw, 2024; Fassil Adugna *et al.*, 2023; Silesh Barasa *et al.*, 2023; Esayas Kinfet *et al.*, 2021).

1.2. Statement of the problem

In Ethiopia one of malaria controlling measure is implementation of IRS (Indoor residual spraying) using different insecticides including organophosphate, organochlorine and carbamate (Eba Alemayehu *et al.*, 2017). But the emergence of insecticide resistance in malaria vectors has presented a significant threat to control the disease. Emergence of insecticide resistant variety of *Anopheles* mosquito was reported from different region of Ethiopia (Meshesha Balkew *et al.*, 2003; Eba Alemayewu *et al.*, 2017; Esayas Kinfet *et al.*, 2021).

All efforts to control vectors rendered useless if insecticide-resistant mosquito species emerge. If an organization fighting malaria sprays insecticides hundreds of times while an insecticide-resistant mosquito variety emerges in a particular area, significant change will not occur. As a result, this study was carried out to assess the presence of insecticide-resistant

Anopheles mosquitoes and the characteristics of their habitat in the Sidama region's malaria endemic districts, Aleta Chuko, Dale and Loka Abaya which are well known for their malariousity.

1.3. Significance of the study

Finding of this study is important to identify type of Anopheles mosquito which dominate study area. It is also essential to evaluate the presence of insecticide resistant anopheles mosquito species in study area which lay down scientific base for government to develop effective intervention of controlling malaria vector. Information of this finding gives clues to identify the types and characteristics of habitat in which mosquitoes prefer to breed. The research findings also serve as a starting point to enable other researcher to conduct further studies regarding malaria vector in study area. Additionally result of this study is input for the science realm.

1.4 Objectives

1.4.1 General objective

General objective of this study was to examine habitat characteristics and insecticide susceptibility status of Anopheles mosquito in malaria endemic districts of Sidama Region.

1.4.2 Specific Objectives

- To identify types of anopheles mosquito species that inhabit study area.
- To evaluate insecticide resistance status of anopheles mosquito that inhabit study area to selected insecticides
- To identify the environmental variables and habitat characteristics associated with the occurrence of Anopheles larvae.

1.5 Scope of the study

The study addressed three Woredas of Sidama Regional state which are well known for their malariosity; Loka Abaya, Chuko and Dale Woreda. This study excluded another Woredas in Sidam Regional State.

1.6 Limitation of the study

A number of factors made the investigation extremely difficult, including the constantly changing weather, which made it difficult to locate enough mosquitoes for a sample. It was quite difficult to gather and transport sampled mosquitoes from the field to the Addis Ababa-based insectary lab as there is no insectary lab facilities near study area.

It was also challenging to get insecticide impregnated paper, insecticide susceptibility bioassay kit as it is available only in research institute via WHO.

2. LITERATURE REVIEW

2.1 The mosquito

There are 43 genera and more than 3,530 species of mosquitoes in the world. The Culicidae family includes all of these genera (Chandira *et al.*, 2013). Because they can spread diseases that infect humans through mosquitoes, Anopheles, Aedes, and Culex are among these genera that are significant to medicine (Gubler, 2010). Anopheles is capable of spreading malaria and lymphatic filariasis, while Aedes is able to spread the Zika virus, Rift Valley fever, yellow fever, lymphatic filariasis, and chikungunya. Culex is a vector for Japanese encephalitis, lymphatic filariasis, and West Nile fever (WHO, 2020).

2.2 Mosquito vector for malaria

The malaria-causing Plasmodium species is carried by female Anopheles mosquitoes. Male mosquitoes are incapable of spreading malaria or other illnesses because they do not bite (Nicoletti, 2020). Anopheles mosquitoes have been described in over 400 species; of these, about 70 species—including *Anopheles gambiae*, *Anopheles stephensi*, *Anopheles arabiensis*, *Anopheles funestus*, and others have the potential to transmit malaria to humans (Sinka *et al.*, 2012). Anopheles mosquito species are found throughout the world and vary depending on the environment, with the exception of Antarctica, where they require a hot season to be active (Nicoletti, 2020).

2.3 Malaria vector species Available in Ethiopia

To date, Ethiopia has recorded over 40 different species of Anopheles (FMoH, 2016; Irish *et al.*, 2020). The four Anopheles species that are medically significant malaria vectors in Ethiopia are *An. arabiensis*, *An. funestus*, *An. pharoensis*, and *An. nili* (Kassahun Jaleta *et al.*, 2013; Delenesaw Yewhalaw *et al.*, 2010). *An. arabiensis* is a significant malaria vector that is common in Ethiopia, according to Yohannis Mekonnen *et al.* (2005). *An. funestus* and *An. pharoensis* are two other common malaria vectors that are predominant in malarial endemic areas (NSPE, 2010; Anmut Assemie, 2022). The least frequent, more confined, and little-studied species is Anopheles nili. It can be located in Ethiopia's northwest, western, and southwestern regions (Kassahun Jaleta *et al.*, 2013). *An. stephensi* was recently identified in eastern Ethiopia (Meshesha Balkew *et al.*, 2020).

2.4 Life cycle of mosquito

Mosquitoes undergo a complete metamorphosis, in which eggs hatch into larvae, then larva into a pupal phase, finally hatching as fully adult flies (Hardy *et al.*, 2022).

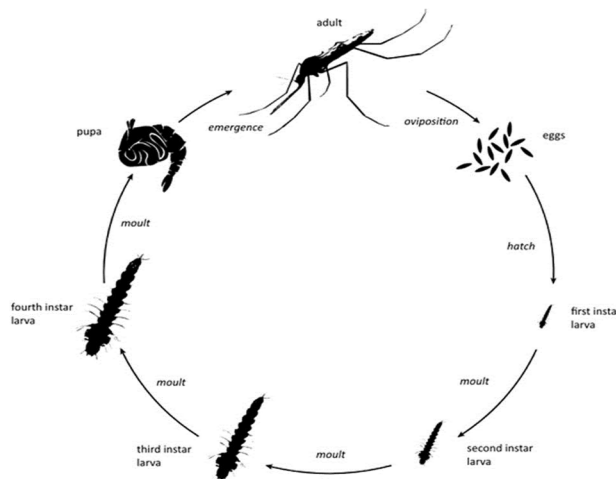


Figure 1: Life cycle of mosquito (Image courtesy of Louise Malmgren/NRI)

2.6 Identification of male and female mosquito

Using a microscope or magnifying glass, there are three methods to differentiate between female predators and harmless male mosquitoes. Males are larger than females, and females have a proboscis that resembles a needle that they use for biting. According to the National Environment Agency (2020), females have far fewer hairy antennae than bushy, hairy ones on males.

2.7 Mosquito species Identification

There are about 400 species of Anopheles mosquito and 70 of these species are potential vector to malaria (Sinka et al., 2012). These species are identified by using morphological and molecular identification keys (Mala *et al.*, 2011; Mishra *et al.*, 2021).

2.7.1 Morphological identification

In aquatic environments, mosquito species can be distinguished from one another both as adults and as larvae by their external morphology (Mala *et al.*, 2011; Erlank *et al.*, 2018; González *et al.*, 2021). A morphology identification key was created by Sallum *et al.* (2020); Becker *et al.* (2020), and Cotzee (2020). The gross morphology of the species, the external morphology of the head, mouthparts, antennae, proboscis, and patches of pale and black scales on the wings and legs, and the terminal abdominal segments can all be used to classify adult mosquitoes into different species (Cotzee, 2020).

2.7.2 Molecular identification

The "gold standard" diagnostic method used today to differentiate between specimens that are morphologically identical and closely related is PCR. Sequence variations in the rDNA's second internal transcribed spacer (ITS2) are common to *Anopheles* mosquitoes and serve as useful markers for diagnostic assays (Walton *et al.*, 2007; Mishra *et al.*, 2021). PCR procedures, like the ones created to identify individuals within the *An. gambiae* complex (Bass *et al.*, 2007; Bass *et al.*, 2008), are crucial instruments for both fundamental and practical investigations. But it has a number of disadvantages, including being expensive, labor-intensive, and time-consuming (Müller *et al.*, 2013).

In order to address the drawbacks of traditional PCR-based approaches, researchers have recently focused more on elective strategies, most notably the near-infrared spectroscopy (NIRS) technique (Mayagaya *et al.*, 2009) and the loop-mediated isothermal amplification (LAMP) technique (Fallahi *et al.*, 2015). A gene can be amplified and detected in a single step with the LAMP technique, which uses strand displacement reaction to amplify DNA at a constant temperature. NIRS, on the other hand, gathers a sample's density distribution of the near-infrared energy absorbed, which is subsequently examined for traits that identify biological samples (Müller *et al.*, 2013).

2.8 Mosquito rearing in Laboratory

In laboratories, mosquito colonies are needed in order to conduct studies on vector biology, vector-parasite interactions, insecticide susceptibility, vaccine studies etc., For this reason it is

important to maintain the original gene pool, physiological and behavioral characteristics of the insects under study as much as possible (Lazzari et al., 2004)

2.8.1 Equipment /supplies for mosquito rearing

Equipments used to rear mosquito in laboratory include cage, aspirator, tray, heater and humidifier (Araya Eukubay, *et al.*, 2017) Cages have a wooden or metal frame that is covered in aluminum, cotton, or plastic netting and function to hold adult anopheles mosquito (Araya Eukubay, *et al.*, 2017). Aspirator is used to collect adult mosquito from resting place easily (Birungi *et al.*, 2021; John W. Hock Company). Trays are water resistant containers used to hold immature mosquito stage (Araya Eukubay, *et al.*, 2017). Temperature and humidity in the rearing room is controlled by heater and humidifier respectively (Padmanabha *et al.*, 2011; Moura *et al.*, 2021).

2.8.2 Microclimate required for insectary

Temperature has a major impact on several biological aspects of mosquitoes, including growth rates. Higher temperatures hasten larval development and increase the need for higher concentrations of larvicides for control, according to research on the effects of temperature on *Aedes aegypti* larvae (Padmanabha *et al.*, 2011; Moura *et al.*, 2021). Temperature variations affect *Culex pipiens* populations' resistance to chemical insecticides, survival rates, and egg hatching percentages, according to Lahondère and Lazzari's (2012) research. Furthermore, research on *Culex quinquefasciatus* mosquitoes revealed that a number of factors, such as adult

longevity, emergence rates, larval growth rates, and immature survivorship, are negatively impacted by rising temperatures (Ukubuiwe *et al.*, 2018).

Since moisture has a significant impact on mosquito lifespan, moisture control is crucial. A humidifier is used to regulate humidity. Moisture will eventually cause the micro switches to fail because these must be installed in the room, so it's advised that hygrometers and humidifiers be changed on a regular basis. Additionally, small commercial mist/aerosol generators can be used to control humidity. These feature an electric motor spinning a flat disc at a high speed, forcing a fine mist of water droplets out of a nozzle on top. Frequent measurements of humidity should be made, either daily with a vortex hygrometer or continuously with a recording thermohygrometer. Relative humidity in the $80\pm 10\%$ range is frequently mentioned as the range needed to maintain an adult (Araya Eukubay *et al.*, 2017).

The length of the day and the intensity of the light affect how various life cycle stages—mating, feeding, oviposition, and pupation—develop. With a timer and switch integrated into the light source, controlling the length of the lighting is simple. The best kind of light source is a regular daylight fluorescent tube. You can have a separate red light for when you need to work in the dark. It's usually advised to follow a 12-hour light and 12-hour dark photoperiod. In order to allow them to carry out their daily activities in the morning and feed on adult female mosquitoes in the afternoon, species that feed on blood at night can be housed in well-lit rooms with lights turned off at noon and on at midnight (Araya Eukubay *et al.*, 2017).

2.8.3 Anopheles Mosquito rearing procedure

A pad of cotton wool soaked in 10% sucrose solution was placed on top of the covering screen of a plastic cup measuring 8.5 cm in diameter and 11 cm in depth, which was lined with filter paper. The cup was used to house wild-caught, fully engorged adult females that were collected from traps set up by humans or animals. In order to maintain humid conditions in the cup and slow down the quick drying of the soaked cotton wool, it was covered with a clear plastic bag. To maintain humidity and temperature, it was kept in a humid chamber with a picnic foam box (18 x 26 x 39 cm). After that, it was moved to the insectarium for biological research and colonization. The insectarium was used for all of the experiments, and it was kept at 27 ± 2 °C, 70–80% relative humidity, and lit for about 12 hours a day with a combination of fluorescent lighting and natural daylight coming through a glass window (Choochote and Saeung, 2013).

Since using live animals raises ethical concerns, adult female mosquitoes can be raised artificially through blood-feeding systems in order to produce eggs (Hila and Obara, 2023). The system's foundation can be a glass feeder that is heated to 37°C and contains a mixture of red blood cells and heat-inactivated serum to achieve a hemoglobin concentration of less than 40% (Rucci *et al.*, 2023). After about 30 minutes of feeding, females can lay eggs. A wet filter paper in a beaker should be left inside the cage for the duration of the night in order to collect the eggs (Costa-da-Silva, 2023). After that, the eggs, along with some food and distilled water, can be transferred to a plastic tray for the larvae's growth and hatching (Martin-Martin *et al.*, 2023). This method replicates natural blood-feeding processes and supports mosquito colony maintenance for research purposes (Leite *et al.*, 2024).

Every day on the fifth and eighth days of their upbringing, the developing larvae are fed two Purina cat food tablets and their density and population are tracked. After five days, on the eighth day, the population of larvae is diluted by adding two tablets of purina cat food and a pinch of ground fish food to each tray, which takes about thirty minutes (Das *et al.*, 2007). The larvae are fed cat food every day from day 9 to day 12. The water is replaced with fresh water on the twelfth day, when the larvae are nine days old, and food is added (two cag food tablets and a pinch of grounded cat food). At this point, the pupae begin to develop. Nets are placed over the trays to keep adults from escaping (approximately 8–10 minutes). Pupae are permitted to become adults for the following two to three days starting on the thirteenth day. To prevent adults from escaping, food is given to the larvae and pupae on a daily basis by carefully removing the net. On the sixteenth day, the adults are gathered into a cage using a vacuum-connected aspirator.

2.9 Insecticide and resistance behavior of Anopheles mosquito

There are five main classes of insecticides approved for use against mosquito vector; Pyrethroid, Organophosphate, Organochlorine, Carbamate and Neonicotenoid (WHO, 2022). As insecticide design strategy to kill mosquito, mosquito also develop strategy to resist insecticide against them (Yang *et al.*, 2020; Kampango *et al.*, 2022; Mack *et al.*, 2021; TF *et al.*, 2023).

2.9.1 Insecticide against Mosquito

Pyrethroids are the primary class of insecticide approved for use in net impregnation, out of five main classes: carbamates, neonicotinoid, organochlorines, organophosphates, and pyrethroids (WHO, 2022). October 2017 saw the prequalification of the first neonicotinoid product for indoor residual spraying (WHO, 2021). The majority of pesticides were applied by residual

spraying, which was followed by larviciding and space spraying. All of the insecticide classes—Organochlorine, Organophosphate, Carbamate, Pyrethroid, and Neonicotinoid—are used for residual spraying; Organophosphate, Carbamate, and Pyrethroid are used for space spraying; and Organophosphate is used for larviciding. Pyrethroids are the most effective insecticide for treating nets (WHO, 2021).

2.9.2 Mosquito killing mechanism of insecticide

Pyrethroids and dichlorodiphenyltrichloroethane (DDT) kill mosquito vectors by focusing on their sodium channels. Insecticides cause repetitive nervous system discharge and depolarization of the nerve membrane after binding to sodium channels, which ultimately leads to the death of the insect (Eldefrawi and Eldefrawi, 2020). The enzyme acetylcholinesterase (AChE), which is essential to the nervous system for hydrolyzing the neurotransmitter acetylcholine and playing a role in stopping nerve impulses, is the target of organophosphate and carbamate insecticides (Kaushal *et al.*, 2021; Ranjan and Jindal, 2022).

2.9.3 Insecticide resistance mechanism of Mosquito

The primary resistance mechanisms can be broadly categorized into two groups. Resistance to both metabolic processes and target sites. Changes in the mosquito's enzymatic system cause metabolic resistance, which speeds up the insecticide's usual detoxification process. The process of detoxification stops the insecticide from going to where the mosquito is supposed to work. Three enzymatic systems are thought to be significant insecticide metabolites for malarial vectors. Glutathione-S-transferase, monooxygenase, and esterase. Mutations that change the

protein receptors that pesticides are intended to target give rise to target site resistance. In this instance, the insecticide can no longer bind to the receptor's intended target site. Therefore, insects are either unaffected by pesticides or less susceptible to them (WHO, 2016).

Both DDT and pyrethroids mainly work by binding to the sodium channel, changing its gating characteristics, and keeping it open for an abnormally long period of time to produce their toxic, insecticidal effects (Silver *et al.*, 2014; Ravula and Yenugu, 2021). The nervous system's sodium channels become insensitive to DDT and pyrethroids due to structural modifications in the form of point mutations or substitutions brought about by single nucleotide polymorphisms. This occurs when the insecticides' affinity for binding proteins is reduced or completely eliminated (Rinkevich *et al.*, 2013).

There have been reports of *An. arabiensis* insecticide resistance to permethrin and DDT from different parts of Ethiopia (Meshesha Balkew *et al.*, 2003). Knockdown resistance is a crucial mechanism linked to both pyrethroid and DDT resistance. Two mutations in the voltage-gated sodium channel gene's domain II are linked to pyrethroid and DDT resistance in *Anopheles gambiae* sensu stricto (Ranson *et al.*, 2000; Tchigossou *et al.*, 2018; Kouadio *et al.*, 2023). The first mutation, called West African kdr (L1014F), is a nucleotide alteration where a phenylalanine (TTT) is substituted for a leucine residue (TTA). In West Africa, this mutation is common at varied frequencies. Yawson *et al.* (2004); Fanello *et al.* (2003). Leucine (TTA)-serine (TCA) substitution at the same codon characterizes the second mutation, the East African kdr (L1014S), which was first discovered in western Kenya (Ranson *et al.*, 2000; Stump *et al.*, 2004). Numerous African countries have reported *An. gambiae*s.s. populations containing both

East African and West African kdr mutations. Pinto and colleagues, 2006; Verhaeghen and colleagues, 2006).

Different species of mosquitoes have been found to carry the ace-1 and ace-2 genes, which encode AChE1 and AChE2, respectively. However, only AChE1 has been linked to mosquito resistance to OP and carbamate insecticides (Alout *et al.*, 2008). Two mutations from the AChE1 active site in mosquitoes cause insensitivity or decreased sensitivity to OP and carbamate insecticides, despite the fact that several mutations linked to the development of insecticide resistance in other insect species have been reported. Numerous mosquito species, including *Anopheles albimanus*, *Culex vishnui*, *Culex pipiens*, and *Culex quinquefasciatus*, have been reported to substitute G119S (Weill *et al.*, 2003). According to 3-D modeling, steric hindrance would result from the G-to-S substitution, decreasing the accessibility of inhibitors and substrate (Alout *et al.*, 2008). According to a functional analysis of ace-1 in the baculovirus–insect cell system, the F455W substitution in ace-1 is what causes OP resistance in *Culex tritaeniorhynchus* (Nabeshima *et al.*, 2004). Strong evidence for the significance of this mutation in resistant insects in general has been provided by the substitution of F455W in carbamate-resistant *Myzus persicae* (Nabeshima *et al.*, 2004), OP-resistant B-biotype whitefly *Bemisia tabaci* (Alon *et al.*, 2008), and OP-resistant two spotted spider mite *Tetranychus urticae* (Aazawa *et al.*, 2003).

Three main metabolic detoxification gene families cytochrome P450s (P450s), esterases, and glutathione S-transferases (GSTs) are involved in the detoxification of pesticides in mosquitoes. P450s perform a wide range of physiological and biochemical roles and make up one of the largest gene families in all living things. P450s are essential for the activation of endogenous

compounds and/or detoxification of xenobiotics (Feyereisen, 2005). According to Ranson and Hemingway (2005), GSTs are soluble dimeric proteins that play a significant role in the metabolism, detoxification, and excretion of numerous endogenous and exogenous substances. The transcriptional upregulation of insect P450s and GSTs, which raises levels of enzymatic activity and protein synthesis, is a noteworthy characteristic. Thus, insecticide resistance (Liu *et al.*, 2015) and tolerance to plant toxins (Li *et al.*, 2007) are developed, and the metabolic detoxification of pesticides and plant toxins is improved. It has also been suggested that the development of insecticide resistance is influenced by the amplification or duplication of genes encoding P450s (Riveron *et al.*, 2013). The majority of organisms contain esterases, a diverse group of enzymes. Since the amplification and/or sporadic overexpression of esterase genes increases the production of detoxification proteins, their overproduction has been thoroughly studied (Liu *et al.*, 2015; Samal *et al.*, 2022).

2.10 Insecticide susceptibility test for *Anopheles* mosquito

The World Health Organization uses a technique called the "WHO Susceptibility Bioassay" to track insecticide resistance in mosquito populations. Since its initial development in 1960, this procedure has undergone multiple modifications (Praulins *et al.*, 2022). In response to widespread resistance to organochlorine insecticide, the test kit was created in 1958 to check for the emergence of resistance to both organochlorine and organophosphate insecticide (Pinto *et al.*, 2019). A procedure for using the WHO Insecticide Susceptibility Bioassay, also known as the WHO Tube Bioassay, has been released by the World Health Organization (WHO) in order to track adult resistance to a variety of insecticides that are frequently used to control mosquito populations (WHO, 2022). This method of resistance monitoring involves gathering adult wild

female mosquitoes or their larvae, which are then raised to adulthood in a testing facility. The insecticide's discrimination concentration (DC) is subsequently applied to the treated filter paper, after which knockdown and mortality can be recorded (Praulins *et al.*, 2022). And a WHO guideline will be interpreted based on the assay result. According to WHO (2013), mosquitoes are classified as susceptible (S) if their mortality rate exceeds 98%, suspected resistant (R) if their mortality rate is between 90% and 97%, and resistant (R) if it is less than 90%.

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was carried out in Aleta Chuko, which is situated in the southwest of the Sidama region, as well as the Dale and Loka Abaya districts. The Districts are well-known for their flat terrain and close proximity to Ethiopia's Abaya Lake. The Districts are situated between 1650 and 2100 meters above sea level. The distance from Aleta chuko, Dale and Loka Abaya to Addis Ababa is 281, 261 and 283 Km respectively. Mean annual rainfall of Aleta chuko, Dale and Loka Abaya is 1961.9, 1264.2 and 999.4 respectively. Mean annual rain fall of 19.1 °c, 18.3°c and 20.5°c is belong to Aleta chuko, Dale and Loka Abaya respectively. There are 1 primary hospital and 24 health posts at Aleta chuko districts, 10 health centers and 32 health posts at Dale district and 1 primary hospital, 5 health center and 22 health posts at Loka Abaya district. About 190,081 population are living in Aleta chuko, 289,593 living in Dale and 116,434 population living in Loka Abaya.

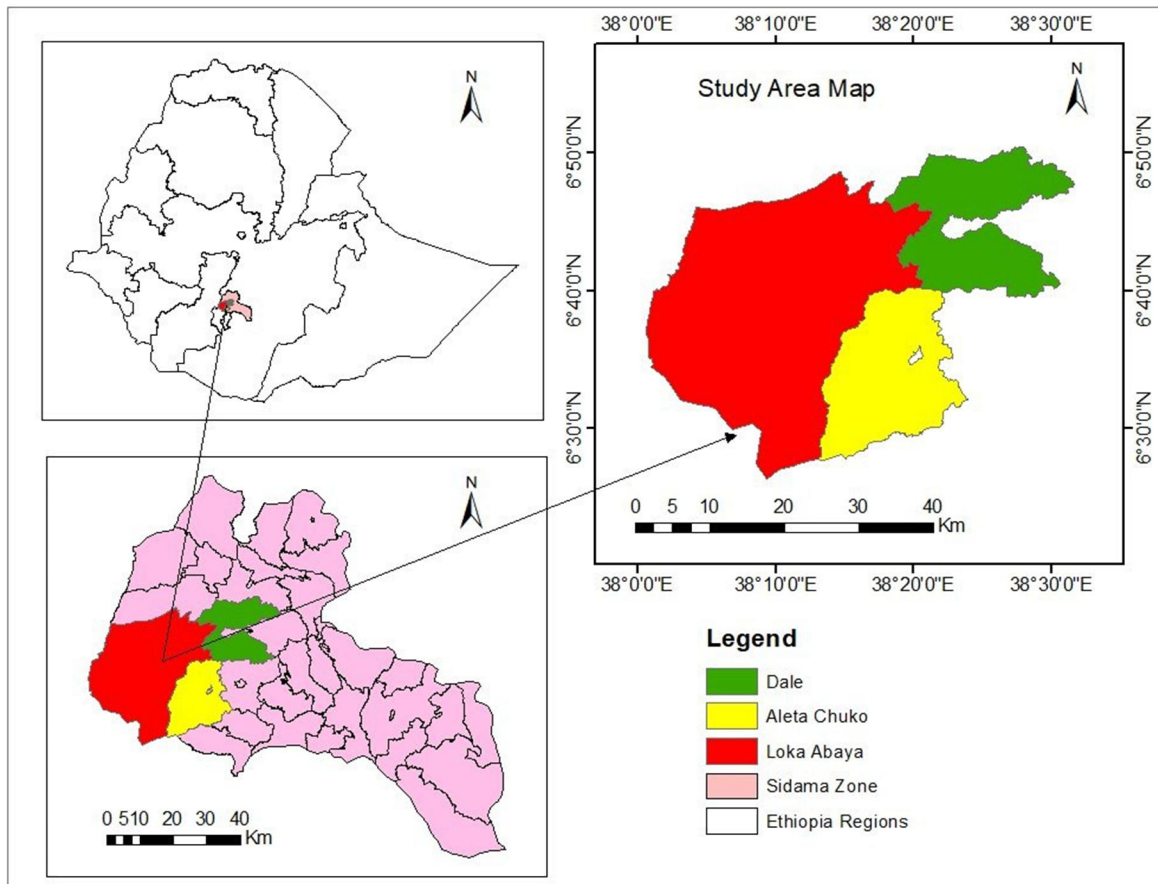


Figure 2: Study area map (From ArcGIS)

3.2. Study design

Cross sectional study was employed that includes entomological data collection of the study area.

3.3. Study population

Anopheles mosquito that inhabit study sites.

3.4. Sample size determination and sampling methods

150 female anopheles mosquitoes were used to test susceptibility to each selected insecticides and the larvae were collected from the field with standard dipping method.

3.5 Habitat characterization

The habitats of Anopheles larvae were identified. Environmental factors including the size of the habitat, the depth of the water, the presence of water flow, direct sunlight, water temperature at the time of sampling, water turbidity, pH, and the distance to the nearest house were recorded for every habitat that supported Anopheles larvae, with the exception of those that were empty. Additional environmental factors encompassed the extent of vegetation, the stability of the habitat, the existence of algae, the amount of surface debris, the amount of emergent plants, the type of habitat, and the type of substrate. Using a meter stick, the water depth of a habitat was measured from various locations based on its size, and the average depth was determined. If the distance was less than 100 meters, it was estimated; if it was greater, it was measured with a tape measure. After that, distance was divided into three classes: ≤ 100 m, 101–200 m, and 201–300 m, are the three distance ranges. Visual observation was used to determine the amount of surface debris, the presence of algae, and the coverage of emergent plants. The three categories of vegetation cover were determined by visual observation: open (no vegetation), tree (large trees within a range of 10-15 m where shade and foliage could reach), and shrub (woody plants smaller than a tree within a range of 10-15 m).

Using a tape measure, the habitat perimeter was measured and divided into three categories: less than 10 meters, between 10 and 100 meters, and greater than 100 meters. A measure of habitat

stability was the amount of time the habitat held water following a rainfall. According to Mwangangi *et al.* (2010), a habitat was classified as temporary if it retained water for two weeks or less and permanent if it did so for more than two weeks following a rainstorm. Water samples were put in glass test tubes and placed against a white background to measure turbidity. The samples were then categorized into three levels: low, medium, and highly turbid (Dejene Getachew *et al.*, 2020). If the habitat could receive direct sunlight at all times of the day, it was visually classified as sunlit; if not, it was described as shaded. The type of substrate was classified as gravel when the stones were smaller than sand, stone if the pool was lined with large stones (rocks larger than 10 cm in diameter), and mud otherwise. At the time of collection, a water thermometer was used to record the temperature of the water, and pH was determined using pH indicator paper (Oljira Kenea *et al.*, 2011).

3.6 Mosquito Collection and rearing.

After the extended rainy season (November) in each study site, anopheline mosquito larvae were collected by dipping from a variety of breeding sites, including ditches, water canals, road puddles, rain pools, and burrow pits. Every larvae was maintained in a 5-liter plastic jar. 1.5 liters of habitat water were added to a plastic jar with lots of holes on top so that there would be enough aeration and plant debris to feed the larvae. The specimens were brought to the Aklilu Lema Institute of Pathobiology at Addis Ababa University to be raised in a laboratory.

Choochote and Saeung (2013) employed detailed techniques in the rearing of larvae. Every field testing room in every study site had a temperature and relative humidity within 25 ± 2 °C and $80 \pm 10\%$, respectively. Each site's larvae and pupae were transferred to plastic trays labeled with

the site's measurements (25 cm × 35 cm × 6 cm) and filled with one liter of naturally flowing water close to the village. There were about eighty larvae in each rearing tray. Every day, the mosquito larvae were fed fish food containing Tetramin (TM). In order to avoid scum accumulation, the trays were replenished with water when the level dropped below the necessary level due to evaporation. The pupae were placed in adult emergence cages, measuring 30 cm by 30 cm by 30 cm. Adult female mosquitoes that were 2-3 days old were utilized for insecticide susceptibility testing and species identification. Adult mosquitoes were morphologically sexed upon eclosion using differences in antennas (Buxton *et al.*, 2020).

3.7 Insecticide susceptibility tests

Using WHO papers and bioassay tubes, 150 adult female anopheles mosquitoes, two to three days old, not fed blood, were exposed for one hour to varying dosages of 0.05% Deltamethrin, 0.45% Alpha-cypermethrin, and 0.1% Propoxur. The selection of insecticides was based on their operational significance in the national malaria control program at the time of the study. Propoxur is currently used in Ethiopia for IRS, and deltamethrin is included in LLINs. In cooperation with the WHO, insecticide-impregnated paper was acquired from the Aklilu Lema Institute of Pathobiology. Six holding tubes were filled with batches of twenty-five female mosquitoes each, and they were kept upright for an hour. After securing the holding and exposure tubes together, mosquitoes were moved from one to the other. One was used as a control in two green dotted tubes, and the other was blown through the opened slit to four red dotted exposure tubes lined with impregnated insecticide. Before the exposure period, damaged mosquitoes from the transfer were replaced. After an hour of being housed in the test tubes, the number of mosquitoes that had been knocked down was counted and recorded every ten minutes

for a total of sixty minutes. Following an exposure period of one hour, the mosquitoes were carefully placed into holding tubes. Adults were given food in the form of a 10% sugar solution on top of the net screen. For a full day, mosquito-containing tubes were maintained in a lab at 25 ± 2 °C and 70–80% relative humidity. Deaths were noted following a 24-hour observation period. Absolute ethanol was used to preserve every test specimen.

3.8 Species Identification

A 10x magnification trinocular dissecting microscope (Amscope SZMT2/MU100010APTINA COLOR CMOS) was used for morphological identification. Using standard identification keys, the mosquitoes were classified into species based on physical characteristics of their abdomen, legs, wings, and palps (Nour *et al.*, 2019; Akeju, 2022).

3.9 Data Analysis

SPSS statistical for Windows (IBM Corp., Armonk, NY), version 25, was used to analyze the Habitat characterization and insecticide susceptibility test data. For every test, values were deemed significantly different if $p < 0.05$.

3.9.1 Habitat characterization

Tables were used to describe the number of immature Anopheles mosquitoes sampled and the larval breeding habitats. The relationship between pH, temperature, and water depth and the density of Anopheles larvae was examined using correlation analysis. The number of Anopheles larvae (early or late) divided by the total number of dips taken from each larval habitat was used to calculate the Anopheles larval density. Log transformed $\log_{10}(x+1)$ larval density was used

to increase distribution normalcy. The environmental factors linked to the presence of *Anopheles* larvae were found using multiple regression analysis. The presence or absence of algae, the permanence of the habitat (temporary or permanent), the presence or absence of surface debris, the intensity of light (sunlit or shaded), and the movement of the water (still or flowing) were the two variables that were compared between the samples using the Mann-Whitney U test. The water turbidity, water perimeter, distance to the closest house, canopy cover, emergent plant coverage, habitat type, and substrate type were all compared between samples in more than two groups using the Kruskal-Wallis H test. Larval densities from sites with various habitat characteristics were compared using these non-parametric tests. IBM

3.9.2 Insecticide susceptibility test

Data were entered into Microsoft Excel spreadsheets after being recorded on the corresponding laboratory data forms in the insectary and laboratory. These steps included error checks and corrections. For all test runs with matching negative and positive controls, the WHO bioassay knockdown was recorded every 10 minutes for one hour, and the final mortality was recorded at twenty-four hours. When the negative control mortality was between 5% and 20%, experiments with negative control mortality greater than 20% were discarded, and if the control mortality was less than 5%, the formula was ignored. In these situations, Abbot's formula was used to adjust the percentage mortality. 98%–100% mortality in the sample population suggested that the tested insecticide was susceptible to the population. Less than 90% of deaths in the tested species indicated resistance, while deaths between 90 and 98% suggested potential resistance (WHO, 2022). The Statistical Package for the Social Sciences (SPSS) software (Version 25) was used to

perform a probit analysis on the knockdown time 50% (KDT₅₀) of all tested female mosquitoes for each insecticide.

3.10 Quality Control

Other predator insects were removed from collected water containing sample mosquito larvae, sufficient air was allowed to enter in to larva containing container during transportation to prevent death of larvae due to shortage of air and temperature & humidity was controlled at insectary room to create suitable environments for larvae taken from field.

3.11 Ethical approval

This study was conducted after reviewed and approved by Ethical review board of Hawassa University biology department.

4. RESULTS

4.1 Insecticide resistance status of *A. gambiae* in study sites to selected insecticides

In this study, 7908 *An. gambiae* mosquitoes were tested to ascertain their level of insecticide resistance using WHO guidelines. According to WHO (2013), mortality falling between 98 and 100% indicates susceptibility, 90 to 97% indicates potential resistance for which more research is necessary, and less than 90% indicates resistance, necessitating the management of resistance against insecticides used to control malaria vectors.

Insecticide resistance was observed in the Dale, Aleta Chuko, and Loka Abaya districts primarily due to deltamethrin and propoxur of the pyrethroid and carbamate insecticide classes, respectively (Table 1). Aleta Chuko mosquitoes demonstrated pyrethroid resistance (deltamethrin, 0.05%; carbamate, propoxure, 0.1%) (89% and 18% mortality, respectively), whereas Loka Abaya mosquitoes demonstrated notable carbamate resistance (propoxure, 0.1%) (22% mortality). In addition, Dale showed resistance to carbamate (propoxur 0.1%) and pyrethroid (deltomethrin 0.05%) with mortality rates of 88% and 38%, respectively. In the Loka Abaya district, potential resistance (90–97% mortality and 91% mortality) to deltamethrin 0.05% was confirmed (Table 1). Mosquitoes from every district were found to be susceptible to pyrethroids (alpha-cypermethrin 0.45%) with a mortality rate of approximately 98% (Table 1). The alpha-cypermethrin and pyrethroid susceptibilities of Loka Abaya and Aleta Chuko mosquitoes (0.45% and 98% mortality, respectively) were similar. Mosquitoes in the Dale district were found to be susceptible to pyrethroids, with alpha-cypermethrin having a 0.45% mortality rate (Table 1).

Table 1: A summary of percentage mortality 24 h after a 1-h exposure to different classes of insecticides on field collected F1 progeny of *An. gambiae* (n=100 per insecticide) from Loka Abaya, Aleta chuko and Dale woredas

Insecticides		Districts and Resistance status		
Class	Name	Loka Abaya	Aleta Chuko	Dale
Pyrethroid	Alpha-cypermethrin (0.45%)	98(S)	98(S)	99(S)
	Deltamethrin (0.05%)	91(PR)	89(R)	88(R)
Carbamate	Propoxur (0.1%)	22(R)	18(R)	38(R)

Letters in the parentheses indicate resistance status of tested mosquitoes (S: susceptible, PR: potential resistance and R: resistant)

4.2 Knockdown Time KDT_{50}

Propoxur (0.1%) had the highest mosquito KDT_{50} (Fig. 4.1) among the districts sampled. Aleta Chuko recorded the highest value (56.25 minutes), while Dale recorded the lowest value (51.95 minutes). Compared to other insecticide classes, pyrethroids (deltamethrin, 0.5%; alpha-cypermethrin, 0.45%; Fig. 4.1) showed the lowest KDT_{50} . Deltamethrin (0.5%) had the highest values (23.57 min, 24.4 min, and 26.23 min) in Loka Abaya, Aleta Chuko, and Dale districts, whereas alpha-cypermethrin (0.45%) had the lowest values (20.82 min, 18.67 min, and 24.23 min, respectively) in Aleta Chuko.

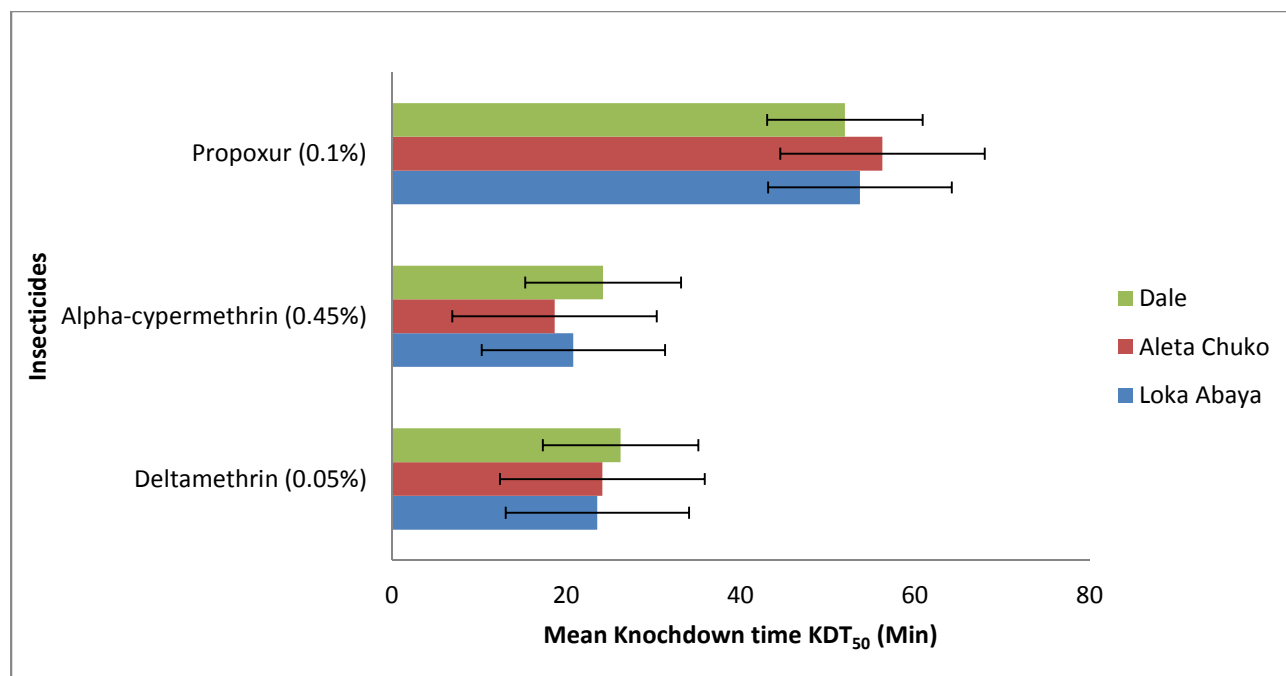


Figure 3: Mean \pm 95% CL summary results of knockdown time (KDT_{50}) (minutes) of field collected *Anopheles gambiae* F1 progeny ($n=100$ per insecticide) from malaria endemic districts tested against different classes of insecticides

4.3 *Anopheles* larvae species composition

A total of 4489 adult *Anopheles* mosquitoes from 1 species were identified based on their morphology. *An. gambiae sensu lato* (s.l.) was identified from Loka Abaya, Aleta Chuko, and Dale study sites out of the entire *Anopheles* species. *Anopheles gambiae* (s.l.) comprised 100% across all study sites.

4.4 *Anopheles* larval productivity in different habitat types

Larval sampling findings and types of productive larval habitat in the study area are presented in Table 2. In Aleta Chuko, seven different types of habitat have been identified, including ditches, swamps, burrow pits, water canals, rain pools, and road puddles. Except for the water canal, all of these types of habitats were also found in Loka Abaya and Dale. Larval habitats were ditches

(Loka Abaya, n = 10, Aleta Chuko, n = 17, and Dale, n = 12), road puddles (Loka Abaya, n = 9, Aleta Chuko, n = 13, and Dale, n = 11), and rain pools (Loka Abaya, n = 23, Aleta Chuko, n = 27, and Dale, n = 23). Anopheles mosquitoes were used to sample a total huge populations of immature Anopheles mosquitoes were gathered from ditches and burrow pits at Loka Abaya. Larvae were abundant in rain pools and ditches at Aleta Chuko, with 11.5 and 10.7 larvae per dip and 0.1 and 0.2 pupae per dip. Large populations of immature Anopheles mosquitoes were collected from ditches and burrow pits at Dal

Table 2: Density of Anopheles larvae in different habitat types in Aleta Chuko, Dale and Loka Abaya Woredas study sites

Study site	Habitat type (n)	Total no. of dips	Total Larval count	No.of larvae/dip (mean±se)	Total pupal count	No. of pupae/dip (mean±se)
Aleta Chuko	Rain pool(27)	136	1545	11.5±1.2	13	0.1±0.1
	Road puddle (13)	46	374	8.0±1.1	5	0.1±0.2
	Ditches(17)	55	582	10.7±1.3	12	0.2±0.3
	Swamp(4)	20	205	10.3±0.7	2	0.1±0.2
	Burrow pits(6)	61	321	5.3±0.4	20	0.3±0.2
	Marshes(4)	19	159	8.3±0.6	1	0.1±0.1
	Water canal(4)	28	32	1.2±0.3	0	0±0
	Total	365	3218	8.8	53	0.1
Dale	Rain pool(23)	138	706	5.2±0.4	5	0.04±0.1
Woreda	Road puddle(11)	52	360	7.1±1.5	2	0.04±0.1
	Ditches(12)	56	505	9.1±1.1	6	0.1±0.2
	Swamp(3)	27	84	3.1±0.2	0	0±0

	Burrow pits(7)	65	590	9.2±0.8	9	0.1±0.2
	Marshes(3)	15	125	8.3±0.4	0	0±0
	Total	353	2370	6.7	22	0.06
Loka Abaya	Rain pool(23)	133	685	5.2±0.9	5	0.1±0.2
	Road Puddle(9)	58	365	6.5±1.4	2	0.04±0.1
	Ditches(10)	59	510	8.9±1.5	6	0.1±0.2
	Swamp(3)	29	86	2.9±0.3	0	0±0
	Burrow pits(5)	47	510	11±1	9	0.2±0.2
	Marshes(4)	20	164	8.2±1.1	0	0±0
	Total	346	2320	6.7	22	0.06

4.5 Association between larval density and habitat variables

According to the multiple regression model, temperature at the time of collection ($p = 0.000$), water depth ($p = 0.000$), substrate type (0.001), canopy cover ($p = 0.011$), and habitat type ($p = 0.041$) were the best predictors of Anopheles larval densities (Table 3). The ANOVA table's F-ratio demonstrated a statistically significant correlation between the larval densities and the physicochemical parameters observed during the larval sampling, with $F_{14, 58} = 46.235$, $p < 0.0001$, and $R^2 = 0.918$.

Table 3: Multiple regression analysis showing the key predicting factors for Anopheles larvae density

Variable	B	95% C.I. for B		t	Sig
		Lower	Upper		
Temperature	0.861	0.749	0.972	15.319	0.000
PH	-2.124	-3.971	-0.277	-2.3	0.025

Turbidity	0.503	-0.239	1.244	1.356	0.180
Intensity of Light	0.76	-1.163	2.683	0.79	0.432
Canopy Cover	1.317	0.307	2.327	2.609	0.011
Surface debris	0.093	-1.381	1.568	0.127	0.9
Algae	0.708	-0.495	1.911	1.177	0.244
Emergent Plants	0.342	-0.334	1.019	1.013	0.315
Water depth	-0.033	-0.045	-0.022	-5.67	0.000
Substrate type	1.345	0.549	2.14	3.381	0.001
Water Perimeter	-0.017	-0.964	0.930	-0.036	0.972
Habitat stability	0.426	-0.637	1.489	0.802	0.426
Distance to nearest house	-0.017	-0.77	0.736	-0.044	0.965
Habitat type	-0.183	-0.358	-0.007	-2.076	0.041
Constant	-8.676	-15.735	-1.616	-2.46	0.017

CI confidence intervals, B the unstandardized coefficient value, Sig significant at $p < 0.05$

Larval densities and water temperatures were positively correlated ($r = 0.936$, $p = 0.045$) (Table 4). A strong negative correlation was observed between the depth and density of Anopheles larvae ($r = 0.485$, $p = 0.000$). However, pH and Anopheles larval densities were not correlated ($r = 0.116$, $P = 0.323$).

Table 4: Correlation of some larval habitat characteristics with the average Anopheles larval density sampled

Physicochemical characteristics	Mean±SD	Correlation coefficient (R^2)	p-value
Water Temperature	27.096 ±2.832	0.936**	0.045
Water depth	44.27±42.08	-0.495**	0.000

Water pH	7.173±0.302	-0.116	0.323
-----------------	-------------	--------	-------

SD standard deviation *Correlation is significant at the 0.05 level

For *An. Gambiae* there were sufficient numbers of late instar larvae available for the examination of environmental factors. *An. gambiae* larval density did not significantly differ between permanent and temporary habitats (U=486.5, p=0.060), habitats with or without algae (U=582, p=0.854), with or without surface debris (p>0.05), between habitats of small and large water perimeter (p>0.05), between habitats closer and farther from human habitation (p>0.05), or between clean, moderately turbid, or turbid water (p>0.05), according to the results of the Mann-Whitney U tests and the Kruskal-Wallis H test (Table 5). It was, however, significantly higher in habitats with sun lit (U=136.5, p=0.024), in habitats with stone substrate ($\chi^2=19.534$, df=2, p=0.000), and in habitats with no canopy cover close to the larval habitat ($\chi^2=11.216$, df=2, p=0.004).

Table 5: Environmental variables and distribution of Anopheles larvae at Aleta Chuko district (2024)

Environmental factors	<i>An. gambiae</i> (s. l.)			
	Mean rank	U	P	
Presence of algae				
Present	37.69	582	0.854	
Absent	38.7			
Intensity of light				
Sunlit	54.44	136.5	0.024	
Shaded	36.04			
Surface debris				
Present	43.03	407.5	0.279	
Absent	36.53			
Habitat Stability				
Permanent	34.35	486.5	0.060	
Temporary	44.13			
	<i>An.gambiae</i> (s.l.)			
	Mean rank	X ²	df	p
Turbidity				
High	44.46	1.795	2	0.408

Medium	38.46			
Low	34.37			
Canopy cover				
Open	33.29	11.216	2	0.004
Shrub	53.25			
Tree	55.7			
Emergent plant				
Absent	37.56	2.112	3	0.550
Grass	35.03			
Weeds	51.38			
Grass+weeds	44.5			
Substrate type				
Mud	31.6	19.534	2	0.000
Stone	60.39			
Gravel	53.7			
Water perimeter(m)				
<10m	38.72	1.321	2	0.517
10 to 100m	35.8			
>100m	48.63			
Distance to nearest house(m)				
≤100m	38.57			
101 to 200m	37.62			
201-300m	30.25	1.041	2	0.594

5. DISCUSSIONS

In this study, the most commonly observed larval habitats were rain pools, ditches, and road puddles. These small water bodies hold water and can serve as larval sites only during the rainy season (Dejene Getachewet *et al.*, 2020). A similar cross-sectional study conducted in the same country indicates that rain pools, ditches, and tire tracks/road puddles were the major contributors during the long rainy season (Arega Tsegayeet *et al.*, 2023; Dawit Hawaria *et al.*, 2020) ditches, burrow pits, and rain pools contribute a large number of anopheles larvae in the study area This is in line with other studies that showed that ditches burrow pits and rain pools supported the greatest number of mosquito larvae (Nicholas *et al.*, 2021; Mihretu Tarekegnet *et al.*, 2022). In the present study, larval breeding habitats such as road puddles and swamps have lower larval densities compared to other sites. This is in contrast with other findings that show road puddles and swamps had higher larval density (Arega Tsegayeet *et al.*, 2023; Babale *et al.*, 2023). This could be due to less number of larvae in the habitat at the time of sampling which could be attributed to habitat disturbance by human and animal activity. Temperature, pH, water depth, canopy cover, and habitat types were the most important variables for Anopheles larval density at the time of sampling. Densities of *An. gambiae* were found to be significantly associated with sunlit habitats, no canopy cover, and stone substrates. This is in line with studies conducted elsewhere (Oljira Keneaet *et al.*, 2011; Gimnig *et al.*, 2001). Generally, Anopheles larval density was not significantly associated with water pH, water temperature, water turbidity, algal content, and distance to the nearest house. The mean water temperature was 27.1 ± 2.8 which may have at a range of suitable water temperature for the Anopheles larvae to survive and develop into an adult as was stated by Bayoh and Lindsay (2003). The distance to the nearest house was

measured only from habitats containing *Anopheles* larvae; therefore, most habitats were close (300 m) to the nearest house, and a statistically significant association was not found between distance to the nearest house and larval density.

The results presented in the present study showed that *An. gambiae* s.l. from all study districts had a high resistance status for Deltamethrin (0.05%) with a possible resistance rate (91%) in Loka Abaya and the least resistance rate (88%) in Dale. This resistance development for Deltamethrin (0.5%) in Loka Abaya, Aleta Chuko and Dale district as had been showed in Ethiopia (Delelegn Woyessa and Delenasaw Yewhalaw, 2024; Fasil Adugna *et al.*, 2023; Silesh Barasa *et al.*, 2023) and Uganda (Oruni *et al.*, 2024). This resistance of Anopheline mosquitoes to deltamethrin may be due to its impregnation on LLIN. This is in agreement with earlier findings (Fasil Adugna *et al.*, 2023; Alemayehu Dagne *et al.*, 2020). These findings are consistent with those reported by Chukwuekezie *et al.* (2020) that *An. gambiae* (sensu stricto) is highly resistant to deltamethrin insecticidal agents. Oduola *et al.* (2012) suggested that resistance to deltamethrin was observed in *An. gambiae* populations in five different localities from Lagos, South-Western Nigeria.

Similar to Kouassiet *al.*, (2020), *An. gambiae* were resistant to deltamethrin and alpha-cypermethrin insecticides in most of the sites. In contrast, Fasil Adugna *et al.* (2023) reported that approximately 87.2% of *An. gambiae* in Lake Tana, northwest Ethiopia, died by using deltamethrin insecticide with the lowest percentage of resistance. Rakotoson *et al.* (2017) also suggested that no confirmed resistance was recorded when *An. gambiae* (s.l.) populations tested for deltamethrin but suspected resistance was observed. *An. gambiae* from Loka Abaya showed

possible resistance to Deltamethrin insecticide; this may be the result of climate change, particularly the effects of temperature and the previous use of pyrethroids in this community.

Anopheles gambiae s.l. with Propoxur insecticide had its highest resistance rate (38%) in Dale and its lowest resistance rate (22%) in Loka Abaya. Propoxur insecticide had the highest mortality rate (78%) in Loka Abaya and the lowest (62%) in Dale. This study showed that *Anopheles gambiae* s.l. develops resistance to propoxure in all study sites. This is in line with findings in Nigeria (Shehu *et al.*, 2023) and West Africa (Awoke Minwuyelet *et al.*, 2023). In contrast, mosquito population susceptible to propoxur was reported from different parts of Ethiopia from 2012 to 2024 (Meshesha Balkew *et al.*, 2012; Esayas Kinfeet *et al.*, 2021; Tebiban Chanyalew *et al.*, 2021; Misrak Abraham *et al.*, 2017 Silesh Barasa *et al.*, 2023; Delelegn Woyessa and Delenasaw Yewhalaw, 2024). This contradictory result may be the first evidence that *An. gambiae* has resistance to propoxur in Ethiopia. These findings are somewhat agreed with that of Oduola *et al.* (2012), who reported that mortality rates between 25 -77% from propoxur exposure in some localities.

The lowest mortality rate of *An. gambiae* was recorded in all three districts (Loka Abaya, Aleta Chuko, and Dale) through Alpa-cypermethrin and Deltamethrin insecticides. Alpha-cypermethrin (0.45%) treated *An. gambiae* s.l. are susceptible ($\geq 98\%$ mortality) in all study sites. Susceptibility to alpha-cypermethrin may be attributed to the fact that alpha-cypermethrin is new for the mosquito population in the study area. In contrast, populations of *An. gambiae* s.l. proved resistance to apha-cypermethrin at Gambela and Gondar respectively (Tebiban

Chanyalew *et al.*, 2022; Silesh Barasa *et al.*, 2023). Similar findings have also been reported from Côte d'Ivoire (Ekra *et al.*, 2023) that *An. gambiae* is resistant to alpha-cypermethrin.

The existence of resistance to two classes of insecticides in populations of *An. gambiae* in three districts of the Sidama region is worrisome and an indication of the threatened sustainability of malaria vector control programs utilizing any of these insecticides. The major challenge arising from these results is that the choice of Pyrethroid and Carbamate may not be a suitable alternative in the event of switching to another class of insecticide. According to Coetzee (2020), *An. gambiae* (in broad sense) was identified as the identification key. Further molecular investigations were not conducted. This is consistent with other studies (Assalif Demissew *et al.*, 2020; Alemnesh Hailemariam *et al.*, 2022; Dejene Getachew *et al.*, 2019; Werissaw Haileselassie *et al.*, 2021) which reported that *An. gambiae* s. l. is the most widely distributed species in Ethiopia.

Assessment and selection of pesticides based on their time of action for vector control is an essential component that has a bearing on the management of insecticide resistance. The KDT₅₀ determines the time that enables 50% of the mosquito population to be knocked down by an insecticide. As reflected by the trend in KDT₅₀ in the findings, knockdown was more rapid for pyrethroids i.e. deltamethrin and alpha-cypermethrin. Okafor *et al.*, 2023; Muhammet and Elcin, 2015) reported similar observations. Propoxur showed a delayed effect of knockdown to 50% of the mosquito population within 1 hour and it is less effective to kill mosquitoes in the study area. In contrast, Propoxur has a knockdown of 100% after 1 h of exposure (Alemayehu Dagne *et al.*,

2020; Silesh Barasa *et al.*, 2023) and is a candidate insecticide for vector control activities in Ethiopia.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Anopheles gambiae s.l is most predominating species found in malarious district of Sidama region .It is susceptible to Alpha-cypermethrin (0.45%) insecticide and resistant to deltamethrin (0.05%) and propoxure (0.1%). Ditches, burrow pits, and rain pools were habitat types in which Anopheles larvae found in large number. Larvae of *Anopheles gambiae* s.l in study area occur in habitat with stone substrate, exposed to sunlight and had no canopy cover.

6.2. Recommendations

Based on the findings of this study, using deltamethrin to treat bed net and propoxur for indoor spraying techniques should not provide important protection in the future, instead, it is better to use alpha-cypermethrin to treat bed net and other unusual insecticides for indoor spraying technique around the study area. Using Pyrethroid and Carbamate class may not be an effective insecticide to *An. gambiae* populations in the Sidama region,Ethiopia. Hence, there is a need to incorporate resistance and integrated vector management approaches to malaria vector control As this study focused primarily on malaria vector susceptibility, further studies, especially molecular studies, are needed to identify anopheles species in a strict sense.

REFERENCES

- Abebe Alemu, Gemedo Abebe, Wondewossen Tsegaye, Lemu Golassa. 2011. Climatic variables and malaria transmission dynamics in Jimma town, South West Ethiopia. *Parasites & vectors*, 4: 1-11.
- Akeju, A.V. 2022. Morphometric variations in morphological features of *Anopheles gambiae* complex in Akure North Local Government area of Ondo State Nigeria. *International Journal of Tropical Insect Science*, 42(5): 3551-3561.
- Alemayehu Dagne, Behailu Taye and Solomon Yeshanew. 2020. Susceptibility status of malaria vector *Anopheles gambiae* in settlement villages of southwestern Ethiopia. *Indian Journal of Entomology*, 82(2): 209-212.
- Alemnesh Hailemariam Bedasso, Abebe Asale Guto, Abate Waldetensai, Araya Eukubay, Getachew Eticha Bokore, Solomon Kinde, Fekadu Gemechu, Yared Debebe, Mesfin Aklilu, Geremew Tasew, Fekadu Massebo, Abebe Teshome, Tilahun Kebede, Bedri Abdulatif, Achamyesh Sisay, Hiwot Solomon and Eliningaya, J.K. 2022. Malaria vector feeding, peak biting time and resting place preference behaviors in line with Indoor based intervention tools and its implication: scenario from selected sentinel sites of Ethiopia. *Heliyon*, 8(12).
- Al-Eryani, S.M., Irish, S.R., Carter, T.E., Lenhart, A., Aljasari, A., Montoya, L.F., Awash, A.A., Mohammed, E., Ali, S., Esmail, M.A. and Hussain, A. 2023. Public health impact of the spread of *Anopheles stephensi* in the WHO Eastern Mediterranean Region countries in Horn of Africa and Yemen: need for integrated vector surveillance and control. *Malaria Journal*, 22(1): 187.
- Ali, R. S. M. and Parasitology, I. 2019. Genetic and Morphological Studies of *Anopheles Maculatus* (Diptera: Culicidae) In Indonesia.

- Alon, M., Alon, F., Nauen, R. and Morin, S. 2008. Organophosphates' resistance in the B-biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) is associated with a point mutation in an ace1-type acetylcholinesterase and overexpression of carboxylesterase. *Insect Biochemistry and Molecular Biology*, 38(10): 940-949.
- Alout, H., Djogbénu, L., Berticat, C., Chandre, F. and Weill, M. 2008. Comparison of *Anopheles gambiae* and *Culex pipiens* acetylcholinesterase 1 biochemical properties. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 150(3): 271-277.
- Amenya, D.A., Naguran, R., Lo, T.C., Ranson, H., Spellings, B.L., Wood, O.R., Brooke, B.D., Coetzee, M. and Koekemoer, L.L. 2008. Over expression of a cytochrome P450 (CYP6P9) in a major African malaria vector, *Anopheles funestus*, resistant to pyrethroids. *Insect molecular biology*, 17(1): 19-25.
- Anazawa, Y., Tomita, T., Aiki, Y., Kozaki, T. and Kono, Y. 2003. Sequence of a cDNA encoding acetylcholinesterase from susceptible and resistant two-spotted spider mite, *Tetranychus urticae*. *Insect biochemistry and molecular biology*, 33(5): 509-514.
- Anmut Assemie. 2022. Distribution and abundance of larval malaria vectors in Bibugn District, East Gojjam, Ethiopia. *International Journal of Tropical Insect Science*, 42(2): 1713-1719.
- Araya Eukubay, Getachew Eticha, Wendatir Nigatu, Adugna Wayessa, Wendimeneh Mekuriaw, Yonas Wuletaw, Alemnesh H/Mariam, Fitsum Tesfaye, Tefera Asfaw and Esayas Kinfe. 2017. *Anopheles* mosquito rearing and insectary handling guideline.
- Arega Tsegaye, Assalif Demissew, Dawit Hawaria, Ashenafi Abossie, Hallelujah Getachew, Kassahun Habtamu, Teshome Degefa, Wang, X., Lee, M., Zhou, G., Delnasaw Yewhalaw and Yan, G. 2023. *Anopheles* larval habitats seasonality and environmental

- factors affecting larval abundance and distribution in Arjo-Didessa sugar cane plantation, Ethiopia. *Malaria Journal*, 22(1): 350.
- Assalif Demissew, Dawit Hawaria, Solomon Kibret, Abebe Anmut, Arega Tsegaye, Lee M.C., Yan, G. and Delenasaw Yewhalaw. 2020. Impact of sugarcane irrigation on malaria vector *Anopheles* mosquito fauna, abundance and seasonality in Arjo-Didessa, Ethiopia. *Malaria journal*, 19: 1-8.
- Awoke Minwuyelet, Delenasaw Yewhalaw, Sciarretta, A. and Getnet Atenafu. 2023. Susceptibility Status of Principal Malaria Vectors to Insecticides Commonly Used for Malaria Control in Africa: Meta-analysis and Systemic review, 19 June 2023, PREPRINT (Version 1) available at Research Square [<https://doi.org/10.21203/rs.3.rs-3020410/v1>].
- Babale, S.K., Salim, H., Yakudima, I.I., Kabir, B.M., Mamman, R., Chiroma, U.M. and Ibrahim, S.K. 2023. Seasonal assessment of pupal habitat productivity of malaria vector: *Anopheles gambiae s.l.* as influence by physico-chemical conditions at selected breeding habitats in Niger, Nigeria. *EUREKA: Life Sciences*, (2): 38-55.
- Bass, C., Williamson, M.S. and Field, L.M. 2008. Development of a multiplex real-time PCR assay for identification of members of the *Anopheles gambiae* species complex. *Acta tropica*, 107(1): 50-53.
- Bass, C., Williamson, M.S., Wilding, C.S., Donnelly, M.J. and Field, L.M. 2007. Identification of the main malaria vectors in the *Anopheles gambiae* species complex using a TaqMan real-time PCR assay. *Malaria journal*, 6: 1-9.
- Bayoh, M.N. and Lindsay, S.W. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Bulletin of entomological research*, 93(5): 375-381.

- Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M. B., Dahl, C. and Kaiser, A. 2020. *Mosquitoes: identification, ecology and control*. Springer Nature.
- Birungi, K., Mabuka, D.P., Balyesima, V., Namukwaya, A., Chemoges, E.W., Kiwuwa-Muyingo, S., Collins, C.M., Tripet, F. and Kayondo, J.K. 2021. Eave and swarm collections prove effective for biased captures of male *Anopheles gambiae* mosquitoes in Uganda. *Parasites & Vectors*, 14(1): 281.
- Bødker, R., Akida, J., Shayo, D., Kisinza, W., Msangeni, H.A., Pedersen, E.M. and Lindsay, S. W. 2003. Relationship between altitude and intensity of malaria transmission in the Usambara Mountains, Tanzania. *Journal of medical entomology*, 40(5): 706-717.
- Brhane Berhe, Fitsum Mardu, Haftom Legese and Hadush Negash. 2019. Seasonal distribution and seven year trend of malaria in North West Tigray: 2012–2018, Ethiopia; 2019. *Tropical diseases, travel medicine and vaccines*, 5: 1-7.
- Buxton, M., Wasserman, R.J. and Nyamukondiwa, C. 2020. Spatial *Anopheles arabiensis* (Diptera: Culicidae) insecticide resistance patterns across malaria-endemic regions of Botswana. *Malaria journal*, 19: 1-9.
- Camara, S., Koffi, A.A., Ahoua Alou, L.P., Koffi, K., Kabran, J.P.K., Koné, A., Koffi, M.F., N'guessan, R. and Penetier, C. 2018. Mapping insecticide resistance in *Anopheles gambiae* (s.l) from Côte d'Ivoire. *Parasites & Vectors*, 11: 1-11.
- CDC. 2006. Center for Disease control and prevention Department of Health and Human Services, Center for Disease Control and Prevention /<http://www.cdc.gov/malaria/biology/> Retrieved on December 20, 2009.
- CDC. 2016. History of Malaria, an Ancient Disease, Division of Parasitic Diseases and Malaria, USA.

- Chandra, G., Ghosh, A., Bhattacharjee, I. and Ghosh, S.K. 2013. Use of larvivorous fish in biological and environmental control of disease vectors. *Cameron MM, Lorenz LM, eds*, 25-41.
- Choochote, W. and Saeung, A. 2013. Systematic Techniques for the Recognition of Anopheles Species Complexes. InTech. doi: 10.5772/54853.
- Chukwuekezie, O., Nwosu, E., Nwangwu, U., Dogunro, F., Onwude, C., Agashi, N., Ezihe, E., Anioke, C., Anokwu, S., Eloy, E. and Attah, P. 2020. Resistance status of *Anopheles gambiae* (sl) to four commonly used insecticides for malaria vector control in South-East Nigeria. *Parasites & vectors*, 13: 1-10.
- Cohee, L. M., Nankabirwa, J. I., Greenwood, B., Djimde, A., & Mathanga, D. P. (2021). Time for malaria control in school-age children. *The Lancet Child & Adolescent Health*, 5(8), 537-538.
- Clesse, L., Du Jonchay, T., Cetina, A.E., François, S.A.L.A., Canitrot, C. and Berro, F. 2022. *U.S. Patent Application No. 17/294,435*.
- Coetzee, M. 2020. Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae). *Malaria Journal*, 19(1): 1-20.
- Costa-da-Silva, A.L. 2023. Membrane Feeding Devices to Blood Feed Mosquitoes in the Laboratory. *Cold Spring Harbor Protocols*, 2023(3): pdb-top107655.
- Das, S., Garver, L. and Dimopoulos, G. 2007. Protocol for mosquito rearing (*An. gambiae*). *Journal of Visualized Experiments: JoVE*, (5).

- Dawit Hawaria, Assalif Demissew, Solomon Kibret, Lee, M.C., Delenasaw Yewhalaw and Yan, G. 2020. Effects of environmental modification on the diversity and positivity of anopheline mosquito aquatic habitats at Arjo-Dedessa irrigation development site, Southwest Ethiopia. *Infectious diseases of poverty*, 9: 1-11.
- De Silva, P. M. and Marshall, J. M. 2012. Factors contributing to urban malaria transmission in sub-Saharan Africa: a systematic review. *Journal of tropical medicine*, 2012. <https://doi.org/10.1155/2012/819563>.
- Dejene Getachew, Meshesha Balkew and Habte Tekie. 2020. Anopheles larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin, southwestern Ethiopia. *Malaria Journal*, 19: 1-13.
- Dejene Getachew, Meshesha Balkew and Habte Tekie. 2020. Anopheles larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin, southwestern Ethiopia. *Malaria Journal*, 19: 1-13.
- Dejene Getachew, Teshome Gebre-Michael, Meshesha Balkew and Habte Tekie. 2019. Species composition, blood meal hosts and Plasmodium infection rates of Anopheles mosquitoes in Ghibe River Basin, southwestern Ethiopia. *Parasites & vectors*, 12: 1-15.
- Dejenie Tadesse, Yohannes Mekonnen and Assmelash Tsehaye. 2011. Characterization of mosquito breeding sites in and in the vicinity of Tigray micro-dams. *Ethiopian journal of health sciences*, 21(1): 57-66.
- Delelegn Woyessa and Delenasaw Yewhalaw. 2024. Bionomics, seasonal abundance and insecticide susceptibility of Anopheles (Diptera: Culicidae) in low and high malaria transmission settings in Ethiopia. <https://doi.org/10.21203/rs.3.rs-3307406/v2>.

- Delenasaw Yewhalaw, Van Bortel, W., Denis, L., Coosemans, M., Duchateau, L. and Speybroeck, N. 2010. First evidence of high knockdown resistance frequency in *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. *The American journal of tropical medicine and hygiene*, 83(1): 122.
- Doumbe-Belisse, P., Kopya, E., Ngadjou, C.S., Sonhafouo-Chiana, N., Talipouo, A., Djamouko-Djonkam, L., Awono-Ambene, H.P., Wondji, C.S., Njiokou, F. and Antonio-Nkondjio, C. 2021. Urban malaria in sub-Saharan Africa: dynamic of the vectorial system and the entomological inoculation rate. *Malaria Journal*, 20: 1-18.
- Eba Alemayehu, Abebe Asale, Kasahun Eba, Kefelegn Getahun, Bryon, A., Morou, E., Vontas, J., Van Leeuwen, T., Duchateau, L. and Delenasaw Yewhalaw. 2017. Mapping insecticide resistance and characterization of resistance mechanisms in *Anopheles arabiensis* (Diptera: Culicidae) in Ethiopia. *Parasites & vectors*, 10(1): 1-11.
- Ekra, A.K., Edi, C.A., Gbalegba, G.C.N., Zahouli, J.Z., Danho, M. and Koudou, B.G. 2023. Can neonicotinoid and pyrrole insecticides manage malaria vector resistance in high pyrethroid resistance areas in Côte d'Ivoire?
- Eldefrawi, M.E. and Eldefrawi, A.T. 2020. Nervous-system-based insecticides. In *Safer Insecticides* (pp. 155-207). CRC Press.
- Enayati, A., Hanafi-Bojd, A. A., Sedaghat, M. M., Zaim, M. and Hemingway, J. 2020. Evolution of insecticide resistance and its mechanisms in *Anopheles stephensi* in the WHO Eastern Mediterranean Region. *Malaria Journal*, 19: 1-12.
- Erlank, E., Koekemoer, L.L. and Coetzee, M. 2018. The importance of morphological identification of African anopheline mosquitoes (Diptera: Culicidae) for malaria control programmes. *Malaria Journal*, 17: 1-7.

- Esayas Kinfu, Irish, S., Alemnesh Hailemariam, Yonas Wuletaw, Sintayehu Abate and Habte Tekie. 2021. Susceptibility of *Anopheles gambiae*sl, and *Anopheles funestus* sl to seven insecticides in southern Ethiopia. *Ethiopian Journal of Public Health and Nutrition (EJPHN)*, 4(2): 153-159.
- Fallahi, S., Mazar, Z.A., Ghasemian, M. and Haghghi, A. 2015. Challenging loop—mediated isothermal amplification (LAMP) technique for molecular detection of *Toxoplasma gondii*. *Asian Pacific journal of tropical medicine*, 8(5): 366-372.
- Fanello, C., Petrarca, V., Della Torre, A., Santolamazza, F., Dolo, G., Coulibaly, M., Allouche, A., Curtis, C.F., Toure, Y.T. and Coluzzi, M. 2003. The pyrethroid knock-down resistance gene in the *Anopheles gambiae* complex in Mali and further indication of incipient speciation within *An. gambiae* ss. *Insect molecular biology*, 12(3): 241-245.
- Fasil Adugna Kendie, Melaku Wale, Endalkachew Nibret and Zena Ameha. 2023. Insecticide susceptibility status of *Anopheles gambiae* (sl) in and surrounding areas of Lake Tana, northwest Ethiopia. *Tropical Medicine and Health*, 51(1): 3.
- Federal Ministry of Health (FMOH). 2013. Malaria. <http://www.moh.gov.et/malaria>.
- Federal Ministry of Health. 2018. Annual Health Sector Performance Report, 2018. Addis Ababa.
- Feyereisen, R. 2005. Insect cytochrome P450. *Comprehensive molecular insect science*, 1-77.
- Fikadu Massebo, Meshesha Balkew, Teshome Gebre-Michael and Lindtjørn, B. 2013. Entomologic inoculation rates of *Anopheles arabiensis* in Southwestern Ethiopia. *The American journal of tropical medicine and hygiene*, 89(3): 466.

- FMOH. 2011. Ethiopia national malaria indicator survey. Addis Ababa: Federal Ministry of Health.
- FMOH. 2014. Health and health-related indicators. Addis Ababa: Federal Ministry of Health; 2014: 32.
- FMOH. 2016. Five-year National Operational Plan for Malaria Prevention and Control in Ethiopia 2016 – 2020, Addis Ababa.
- Gessew Bugssa and Kiros Tedla. 2020. Feasibility of malaria elimination in Ethiopia. *Ethiopian journal of health sciences*, 30(4).
- Gimnig, J.E., Ombok, M., Kamau, L. and Hawley, W.A. 2001. Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. *Journal of medical entomology*, 38(2): 282-288.
- Global Fund to Fight AIDS Tuberculosis and Malaria. 2009. Early Evidence of Sustainable Impact on Malaria. <http://www.theglobalfund.org/documents-/publications/onepagers-/Malaria.pdf>.
- González, M.A., Cevidanes, A., Goiri, F., Barandika, J.F. and García-Pérez, A.L. 2021. Diversity and distribution of larval habitats of mosquitoes (Diptera: Culicidae) in northern Spain: from urban to natural areas. *Journal of Vector Ecology*, 46(2): 173-185.
- Gubler, D.J. 2010. The global threat of emergent/re-emergent vector-borne diseases. *Vector biology, ecology and control*, 39-62.
- Hardy, H., Hopkins, R., Mnyone, L. and Hawkes, F.M. 2022. Manure and mosquitoes: life history traits of two malaria vector species enhanced by larval exposure to cow dung, whilst chicken dung has a strong negative effect. *Parasites & Vectors*, 15(1): 472.

- Hila, A.M.J. and Obara, G.B. 2023. Evaluation of Blood-feeding Methods for Rearing Dengue Mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Philippine Journal of Science*, 152(3): 903-912.
- Irish, S.R., Kyalo, D., Snow, R.W. and Coetzee, M. 2020. Updated list of Anopheles species (Diptera: Culicidae) by country in the Afrotropical Region and associated islands. *Zootaxa*, 4747(3): zootaxa-4747.
- Kabula, B., Kisinza, W., Tungu, P., Ndege, C., Batengana, B., Kollo, D., Malima, R., Kafuko, J., Mohamed, M. and Magesa, S. 2014. Co-occurrence and distribution of East (L1014S) and West (L1014F) African knock-down resistance in *Anopheles gambiae* sensu lato population of Tanzania. *Tropical Medicine & International Health*, 19(3): 331-341.
- Kampango, A., Hocke, E.F., Hansson, H., Furu, P., Haji, K.A., David, J.P., Konradsen, F., Saleh, F., Weldon, C.W., Schiøler, K.L. and Alifrangis, M. 2022. High DDT resistance without apparent association to kdr and Glutathione-S-transferase (GST) gene mutations in *Aedes aegypti* population at hotel compounds in Zanzibar. *PLoS neglected tropical diseases*, 16(5): e0010355.
- Kassahun Jaleta, Hill, S.R., Emiru Seyoum, Meshesha Balkew, Teshome Gebre-Michael, Ignell, R. and Habte Tekie. 2013. Agro-ecosystems impact malaria prevalence: large-scale irrigation drives vector population in western Ethiopia. *Malaria journal*, 12: 1-11.
- Kaushal, J., Khatri, M. and Arya, S.K. 2021. A treatise on Organophosphate pesticide pollution: Current strategies and advancements in their environmental degradation and elimination. *Ecotoxicology and Environmental Safety*, 207: 111483.
- Kouadio, F.P.A., Wipf, N.C., Nygble, A.S., Fodjo, B.K., Sadia, C.G., Vontas, J., Mavridis, K., Müller, P. and Mouhamadou, C.S. 2023. Relationship between insecticide resistance

- profiles in *Anopheles gambiae* sensu lato and agricultural practices in Côte d'Ivoire. *Parasites & Vectors*, 16(1): 270.
- Kouassi, B.L., Edi, C., Tia, E., Konan, L.Y., Akéré, M.A., Koffi, A.A., Ouattara, A.F., Tanoh, A.M., Zinzindohoue, P., Kouadio, B. and Andre, M. 2020. Susceptibility of *Anopheles gambiae* from Côte d'Ivoire to insecticides used on insecticide-treated nets: evaluating the additional entomological impact of piperonyl butoxide and chlorfenapyr. *Malaria journal*, 19: 1-11.
- Krettli, A.U. and Miller, L.H. 2001. Malaria: a sporozoite runs through it. *Current Biology*, 11(10): 409-412.
- Lahondère, C. and Lazzari, C.R. 2012. Mosquitoes cool down during blood feeding to avoid overheating. *Current biology*, 22(1): 40-45.
- Lazzari, C. R., Minoli, S. A., & Barrozo, R. B. 2004. Chemical ecology of insect vectors: the neglected temporal dimension. *Trends in Parasitology*, 20(11), 506-507.
- Leite, L.N., Bascuñán, P., Dotson, E.M. and Benedict, M.Q. 2024. Anopheles egg collection, disinfection, and hatching. *Cold Spring Harbor Protocols*, 2024(3): pdb-prot108186.
- Li, X., Schuler, M.A. and Berenbaum, M.R. 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annu. Rev. Entomology*, 52, 231-253.
- Liu, N., Li, M., Gong, Y., Liu, F. and Li, T. 2015. Cytochrome P450s—Their expression, regulation, and role in insecticide resistance. *Pesticide biochemistry and physiology*, 120: 77-81.
- Mack, L.K., Kelly, E.T., Lee, Y., Brisco, K.K., Shen, K.V., Zahid, A., van Schoor, T., Cornel, A.J. and Attardo, G.M. 2021. Frequency of sodium channel genotypes and association

- with pyrethrum knockdown time in populations of Californian *Aedes aegypti*. *Parasites & vectors*, 14: 1-11.
- Mala, A.O., Irungu, L.W., Shililu, J.I., Muturi, E.J., Mbogo, C.C., Njagi, J.K. and Githure, J.I. 2011. Dry season ecology of *Anopheles gambiae* complex mosquitoes at larval habitats in two traditionally semi-arid villages in Baringo, Kenya. *Parasites & vectors*, 4: 1-11.
- Mangani, C., Frake, A. N., Chipula, G., Mkwaila, W., Kakota, T., Mambo, I., ...& Walker, E. (2022). Proximity of residence to irrigation determines malaria risk and *Anopheles* abundance at an irrigated agroecosystem in Malawi. *The American Journal of Tropical Medicine and Hygiene*, 106(1), 283.
- Marten, P. and Hall, L. 2000. Malaria on the move: Human population movement and malaria transmission. *Emerging Infectious Diseases Journal Home*, 6: 28-45
- Martin-Martin, I., Williams, A.E. and Calvo, E. 2023. Determination of Mosquito Probing and Feeding Time to Evaluate Mosquito Blood Feeding. *Cold Spring Harbor Protocols*, 2023(6): pdb-top107659.
- Maulidya, F., Zulkarnaim, Z. and Taufiq, A.U. 2023. Development of insectarium media combined with QR code on animalia subject for the ten-grade students. *JPBIO (Jurnal Pendidikan Biologi)*, 8(1): 12-19.
- Mayagaya, V.S., Michel, K., Benedict, M.Q., Killeen, G.F., Wirtz, R.A., Ferguson, H.M. and Dowell, F.E. 2009. Non-destructive determination of age and species of *Anopheles gambiae* sl using near-infrared spectroscopy. *American Journal of Tropical Medicine and Hygiene*, 81(4): 622.
- Mekonnen Yohannes, Mituku Haile, Tedros Adanoum Ghebreyesus, Witten, K.H., Asefaw Getachew, Byass, P. and Lindsay, S.W. 2005. Can source reduction of mosquito larval

- habitat reduce malaria transmission in Tigray, Ethiopia? *Tropical Medicine & International Health*, 10(12): 1274-1285.
- Meshesha Balkew, Alemayehu Getachew, Shelleme Chibsa, Dereje Olana, Reithinger, R. and Brogdon, W. 2012. Insecticide resistance: a challenge to malaria vector control in Ethiopia. *Malaria Journal*, 11(1): 139.
- Meshesha Balkew, Mumba, P., Dereje Dengela, Gedeon Yohannes, Dejene Getachew, Solomon Yared, Sheleme Chibsa, Murphy, M., George, K., Lopez, K. and Janies, D. 2020. Geographical distribution of *Anopheles stephensi* in Eastern Ethiopia. *Parasites & vectors*, 13: 1-8.
- Meshesha Balkew, Teshome Gebre-Michael and Habte Tekie. 2003. Insecticide susceptibility level of *Anopheles arabiensis* in two agro-development localities in eastern Ethiopia. *Parassitologia*, 45(1): 1-3.
- Mihretu Tarekegn, Habte Tekie, Yitbarek Wolde-Hawariat and Sisay Dugassa. 2022. Habitat characteristics and spatial distribution of *Anopheles* mosquito larvae in malaria elimination settings in Dembiya District, Northwestern Ethiopia. *International Journal of Tropical Insect Science*, 42(4): 2937-2947.
- Mishra, S., Sharma, G., Das, M.K., Pande, V. and Singh, O.P. 2021. Intragenomic sequence variations in the second internal transcribed spacer (ITS2) ribosomal DNA of the malaria vector *Anopheles stephensi*. *PloS one*, 16(6): e0253173.
- Misrak Abraham, Fekadu Massebo and Lindtjørn, B. 2017. High entomological inoculation rate of malaria vectors in area of high coverage of interventions in southwest Ethiopia: implication for residual malaria transmission. *Parasite epidemiology and control*, 2(2): 61-69.

- MOH. 2021. National malaria elimination strategic plan. Federal Democratic Republic of Ethiopia Ministry of Health. August, 2020, Addis Ababa, Ethiopia.
- Molavi, A. 2003. Africa's malaria death toll still outrageously high. *Nat Geo News*, 12.
- Moura, L., de Nadai, B.L., Bernegossi, A.C., Felipe, M.C., Castro, G.B. and Corbi, J.J. 2021. Be quick or be dead: high temperatures reduce *Aedes aegypti* (Diptera: Culicidae) larval development time and pyriproxyfen larvicide efficiency in laboratory conditions. *International Journal of Tropical Insect Science*, 41(2): 1667-1672.
- Muhammet, M. and Elcin, E. 2015. Evaluation of insecticide resistance and biochemical mechanisms of *Culex pipens* L. in four localities of east and middle Mediterranean basin in Turkey. *Int. J. Mosq. Res.*, 2(3): 39-44.
- Mullen, G.R. and Durden, L.A. (Eds.). 2009. *Medical and veterinary entomology*. Academic press.
- Müller, P., Pflüger, V., Wittwer, M., Ziegler, D., Chandre, F., Simard, F. and Lengeler, C. 2013. Identification of cryptic Anopheles mosquito species by molecular protein profiling. *PLoS One*, 8(2): e57486.
- Mwangangi, J.M., Shililu, J., Muturi, E.J., Muriu, S., Jacob, B., Kabiru, E.W., Mbogo, C.M., Githure, J. and Novak, R.J. 2010. Anopheles larval abundance and diversity in three rice agro-village complexes Mwea irrigation scheme, central Kenya. *Malaria journal*, 9, 1-10.
- Nabeshima, T., Mori, A., Kozaki, T., Iwata, Y., Hidoh, O., Harada, S., Kasai, S., Severson, D.W., Kono, Y. and Tomita, T. 2004. An amino acid substitution attributable to insecticide-insensitivity of acetylcholinesterase in a Japanese encephalitis vector mosquito, *Culex tritaeniorhynchus*. *Biochemical and biophysical research communications*, 313(3): 794-801.

- Namountougou, M., Soma, D.D., Kientega, M., Balboné, M., Kaboré, D.P.A., Drabo, S.F., Coulibaly, A.Y., Fournet, F., Baldet, T., Diabaté, A. and Dabiré, R.K. 2019. Insecticide resistance mechanisms in *Anopheles gambiae* complex populations from Burkina Faso, West Africa. *Acta tropica*, 197: 105054.
- National environmental agency. 2020. <https://www.nea.gov.sg/corporate-functions/resources/research/wolbachia-aedes-mosquito-suppression-strategy/male-mosquitoes-do-not-bite>
- NIAID.(2007). “Understanding malaria,” Fighting an Ancient Scourge, No. 07-7139, U.S. Dept. of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA.
- Nicholas, K., Bernard, G., Bryson, N., Mukabane, K., Kilongosi, M., Ayuya, S. and Mulama, D. H. 2021. Abundance and distribution of Malaria vectors in various aquatic habitats and land use types in Kakamega County, highlands of Western Kenya. *Ethiopian Journal of Health Sciences*, 31(2).
- Nicoletti, M. 2020. Three scenarios in insect-borne diseases. *Insect-Borne Diseases in the 21st Century*, 99.
- Nour, M.M., Aboud, M.A.E. and Ali, N.O.M. 2019. Morphological identification, species composition and distribution of mosquitoes in Kosti region, White Nile State, Central Sudan. *Malaysian Journal of Medical and Biological Research*, 6(1): 7-18.
- NSPE. 2010. National Strategic Plan for Malaria Prevention Control and Elimination in Ethiopia.
- Nwane, P., Etang, J., Chouaïbou, M., Toto, J.C., Mimpfoundi, R. and Simard, F. 2011. Kdr-based insecticide resistance in *Anopheles gambiae* ss populations in Cameroon: spread of the L1014F and L1014S mutations. *BMC research notes*, 4(1): 1-9.

- Oduola, A.O., Idowu, E.T., Oyebola, M.K., Adeogun, A.O., Olojede, J.B., Otubanjo, O.A. and Awolola, T.S. 2012. Evidence of carbamate resistance in urban populations of *Anopheles gambiae s.s.* mosquitoes resistant to DDT and deltamethrin insecticides in Lagos, South-Western Nigeria. *Parasites & vectors*, 5: 1-8.
- Okafor, M.A., Ekpo, N.D., Opara, K.N., Udoidung, N.I., Ataya, F.S., Yaro, C.A., Batiha, G.E.S., Alexiou, A. and Papadakis, M. 2023. Pyrethroid insecticides susceptibility profiles and evaluation of L1014F kdr mutant alleles in *Culex quinquefasciatus* from lymphatic filariasis endemic communities. *Scientific Reports*, 13(1), 18716.
- Okoye, P.N., Brooke, B.D., Koekemoer, L.L., Hunt, R.H. and Coetzee, M. 2008. Characterisation of DDT, pyrethroid and carbamate resistance in *Anopheles funestus* from Obuasi, Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102(6): 591-598.
- Oladepo, O., Tona, G.O., Oshiname, F.O. and Titiloye, M.A. 2010. Malaria knowledge and agricultural practices that promote mosquito breeding in two rural farming communities in Oyo State, Nigeria. *Malaria journal*, 9(1): 1-9.
- Oliveira, E.E., Du, Y., Nomura, Y. and Dong, K. 2013. A residue in the transmembrane segment 6 of domain I in insect and mammalian sodium channels regulate differential sensitivities to pyrethroid insecticides. *Neurotoxicology*, 38: 42-50.
- Oljira Kenea, Meshesha Balkew and Teshome Gebre-Michael. 2011. Environmental factors associated with larval habitats of anopheline mosquitoes (Diptera: Culicidae) in irrigation and major drainage areas in the middle course of the Rift Valley, central Ethiopia. *J Vector Borne Dis*, 48(2): 85-92.
- Olusi, T.A., I.A. Simon-Oke, and A.V. Akeju. 2021. "Composition, habitat preference and seasonal variation of malaria vector larval and pupa stage in Akure North Local

- Government Area of Ondo State, Nigeria." *Bulletin of the National Research Centre*, 45(2021): 1-8.
- Oruni, A., Lynd, A., Njoroge, H., Onyige, I., van't Hof, A.E., Matovu, E, and Donnelly, M.J. 2024. Pyrethroid resistance and gene expression profile of a new resistant *An. gambiae* colony from Uganda reveals multiple resistance mechanisms and overexpression of Glutathione-S-Transferases linked to survival of PBO-pyrethroid combination. *Wellcome Open Research*, 9: 13.
- Oyewole, I.O., Momoh, O.O., Anyasor, G.N., Ogunnowo, A.A., Ibidapo, C.A., Oduola, O.A., Obansa, J.B. and Awolola, T.S. (2009). Physico-chemical characteristics of Anopheles breeding sites: Impact on fecundity and progeny development. *African Journal of Environmental Science and Technology*, 3(12).
- Padmanabha, H., Lord, C.C. and Lounibos, L.P. 2011. Temperature induces trade-offs between development and starvation resistance in *Aedes aegypti* (L.) larvae. *Medical and veterinary entomology*, 25(4): 445-453.
- Pimenta, P.F., Orfano, A.S., Bahia, A.C., Duarte, A.P., Ríos-Velásquez, C.M., Melo, F.F., Pessoa, F.A., Oliveira, G.A., Campos, K.M., Villegas, L.M. and Rodrigues, N.B. 2015. An overview of malaria transmission from the perspective of Amazon Anopheles vectors. *Memórias do Instituto Oswaldo Cruz*, 110(1): 23-47.
- Pinto, J., Lynd, A., Elissa, N., Donnelly, M.J., Costa, C., Gentile, G., Caccone, A. and Rosário, V.D. 2006. Co-occurrence of East and West African kdr mutations suggests high levels of resistance to pyrethroid insecticides in *Anopheles gambiae* from Libreville, Gabon. *Medical and Veterinary Entomology*, 20(1), 27-32.

- Pinto, J., Palomino, M., Mendoza-Uribe, L., Sinti, C., Liebman, K. A. and Lenhart, A. 2019. Susceptibility to insecticides and resistance mechanisms in three populations of *Aedes aegypti* from Peru. *Parasites & vectors*, 12: 1-11.
- PMI. 2019. President Malaria Initiatives -Ethiopia. Malaria Operation plan financial year 2018.
- Rakotoson, J.D., Fornadel, C.M., Belemvire, A., Norris, L.C., George, K., Caranci, A., Lucas, B. and Dengela, D. 2017. Insecticide resistance status of three malaria vectors, *Anopheles gambiae* (sl), *An. funestus* and *An. mascarensis*, from the south, central and east coasts of Madagascar. *Parasites & vectors*, 10: 1-17.
- Ranjan, A. and Jindal, T. 2022. *Toxicology of organophosphate poisoning*. Springer International Publishing.
- Ranson, H. and Hemingway, J. 2005. Mosquito glutathione transferases. *Methods in enzymology*, 401: 226-241.
- Ranson, H., Jensen, B., Vulule, J. M., Wang, X., Hemingway, J. and Collins, F.H. 2000. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect molecular biology*, 9(5): 491-497.
- Ravula, A.R. and Yenugu, S. 2021. Pyrethroid based pesticides—chemical and biological aspects. *Critical Reviews in Toxicology*, 51(2): 117-140.
- Regnault-Roger, C., Vincent, C. and Arnason, J.T. 2012. Essential oils in insect control: low-risk products in a high-stakes world. *Annual review of entomology*, 57: 405-424.
- Ricardo T, Parisa K, Katherine, A. and Douglas, G. 2014. "Plasmodium life cycle and the pathogenesis of malaria. From innate sensing of malaria parasites." *Nature Reviews Immunology*, 14: 744–757.

- Rinkevich, F.D., Du, Y. and Dong, K. 2013. Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids. *Pesticide biochemistry and physiology*, 106(3): 93-100.
- Riveron, J.M., Tchouakui, M., Mugenzi, L., Menze, B.D., Chiang, M.C. and Wondji, C.S. 2018. Insecticide resistance in malaria vectors: an update at a global scale. In *Towards malaria elimination-a leap forward*. Intech Open.
- Rucci, K.A., Pueta, M. and Díaz, A. 2023. Effects of blood meal source and seasonality on reproductive traits of *Culex quinquefasciatus* (Diptera: Culicidae). *bioRxiv*, 2023-06. <https://doi.org/10.1101/2023.06.30.546984>.
- Sallum, M. A. M., Obando, R. G., Carrejo, N. and Wilkerson, R. C. 2020. Identification keys to the Anopheles mosquitoes of South America (Diptera: Culicidae). II. Fourth-instar larvae. *Parasites & vectors*, 13: 1-25.
- Samal, R. R., Panmei, K., Lanbiliu, P. and Kumar, S. 2022. Metabolic detoxification and ace-1 target site mutations associated with acetamiprid resistance in *Aedes aegypti* L. *Frontiers in Physiology*, 13: 988907.
- Santolamazza, F., Calzetta, M., Etang, J., Barrese, E., Dia, I., Caccone, A., Donnelly, M.J., Petrarca, V., Simard, F., Pinto, J. and della Torre, A. 2008. Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and west-central Africa. *Malaria journal*, 7(1): 1-8.
- Saraiva, R.G., Kang, S., Simões, M.L., Angleró-Rodríguez, Y.I. and Dimopoulos, G. 2016. Mosquito gut antiparasitic and antiviral immunity. *Developmental & Comparative Immunology*, 64: 53-64.

- Seid Tiku Mereta, Delenasaw Yewhalaw, Boets, P., Abdulhakim Ahmed, Duchateau, L., Speybroeck, N., Vanwambeke, S.O., Worku Legesse, De Meester, L. and Goethals, P.L. 2013. Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasites & vectors*, 6: 1-16.
- Shehu, I.K., Ahmad, H.B., Olayemi, I.K., Solomon, D., Ahmad, A.H. and Salim, H. 2023. Insecticide susceptibility status in two medically important mosquito vectors, *Anopheles gambiae*, and *Culex quinquefasciatus* to three insecticides commonly used in Niger State, Nigeria. *Saudi journal of biological sciences*, 30(2): 103524.
- Silesh Barasa, Ayalew Jejaw, Mulugeta Aemero, Dagimawi Woldesenbet and Wagaw Abebe. 2023. Assessing Insecticide Susceptibility Status of Anopheles Mosquitoes in Gondar Zuria District, Northwest Ethiopia.
- Silva, A.P.B., Santos, J.M.M. and Martins, A.J. 2014. Mutations in the voltage-gated sodium channel gene of anophelines and their association with resistance to pyrethroids—a review. *Parasites & vectors*, 7(1): 1-14.
- Silver, K.S., Du, Y., Nomura, Y., Oliveira, E.E., Salgado, V.L., Zhorov, B.S. and Dong, K. 2014. Voltage-gated sodium channels as insecticide targets. *Advances in insect physiology*, 46: 389-433.
- Sinka M.E., Bangs, M.J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P. and Temperley, W.H. 2012. A global map of dominant malaria vectors. *Parasite & Vectors*, 5: 69.
- Solomon Tesfaye, Vileikyte, L., Rayman, G., Sindrup, S.H., Perkins, B.A., Baconja, M., Vinik, A.I., Boulton, A.J.M. and Toronto Expert Panel on Diabetic Neuropathy. 2011. Painful

diabetic peripheral neuropathy: consensus recommendations on diagnosis, assessment and management. *Diabetes/metabolism research and reviews*, 27(7): 629-638.

Solomon Yared, Araya Gebressielasie, Damodaran, L., Bonnell, V., Lopez, K., Janies, D. and Carter, T.E. 2020. Insecticide resistance in *Anopheles stephensi* in Somali Region, eastern Ethiopia. *Malaria journal*, 19(1): 1-7.

Stump, A.D., Atieli, F.K., Vulule, J.M. and Besansky, N.J. 2004. Dynamics of the pyrethroid knockdown resistance allele in western Kenyan populations of *Anopheles gambiae* in response to insecticide-treated bed net trials. *The American journal of tropical medicine and hygiene*, 70(6): 591-596.

Szmitko, P.E., Kohn, M.L. and Simor, A.E. 2009. Plasmodium falciparum malaria occurring 8 years after leaving an endemic area. *Diagnostic microbiology and infectious disease*, 63(1): 105-107.

Tchigossou, G., Djouaka, R., Akoton, R., Riveron, J.M., Irving, H., Atoyebi, S., Moutairou, K., Yessoufou, A. and Wondji, C.S. 2018. Molecular basis of permethrin and DDT resistance in an *Anopheles funestus* population from Benin. *Parasites & vectors*, 11: 1-13.

Tebiban Chanyalew, Gadisa Natea, Desalegn Amenu, Delenasaw Yewhalaw and Eba Alemayehu Simma. 2021. Mosquito Species Composition and Insecticide Resistance Status of *Anopheles arabiensis* in Itang Special Woreda, Gambella, Southwestern Ethiopia. <https://doi.org/10.21203/rs.3.rs-1179058/v1>.

Tebiban Chanyalew, Gadisa Natea, Desalegn Amenu, Delenasaw Yewhalaw and Eba Alemayehu Simma. 2022. Composition of mosquito fauna and insecticide resistance status of *Anopheles gambiae sensu lato* in Itang special district, Gambella, Southwestern Ethiopia. *Malaria Journal*, 21(1): 125.

- Temesgen Ashine, Adane Eyasu, Yehenew Asmamaw, Eba Simma, Endalew Zemene, Epstein, A., Brown, R., Nigatu Negash, Kochora, A., Reynolds, A.M. and Bulto, M.G. 2023. Spatiotemporal distribution of *Anopheles stephensi* in different eco-epidemiological settings in Ethiopia, 27 December 2023, PREPRINT (Version 1) available at Research Square [<https://doi.org/10.21203/rs.3.rs-3793340/v1>].
- TF, T., Osse, R., Zoukifilou, S.D., Gbenouga, A., Houessinon, F., Gounou, I., Sidick, A., Messenger, L.A. and Akogbeto, M. 2023. Insecticide Resistance in *Aedes aegypti* Mosquitoes: First Evidence of Kdr F1534C, S989P and V1016G Triple Mutation in Benin, West Africa.
- Ukubuiwe, A.C., Olayemi, I.K., Arimoro, F.O., Omalu, I.C.J., Baba, B.M., Ukubuiwe, C.C., Odeyemi, M.O. and Adeniyi, K.A. 2018. Influence of rearing-water temperature on life stages' vector attributes, distribution and utilisation of metabolic reserves in *Culex quinquefasciatus* (Diptera: Culicidae): implications for disease transmission and vector control. *The Journal of Basic and Applied Zoology*, 79(1): 1-15.
- Vatandoost, H., Hanafi-Bojd, A. A., Nikpoor, F., Raeisi, A., Abai, M. R. and Zaim, M. 2022. Situation of insecticide resistance in malaria vectors in the World Health Organization of Eastern Mediterranean region 1990–2020. *Toxicology Research*, 11(1): 1-21.
- Verhaeghen, K., Van Bortel, W., Roelants, P., Backeljau, T. and Coosemans, M. 2006. Detection of the East and West African kdr mutation in *Anopheles gambiae* and *Anopheles arabiensis* from Uganda using a new assay based on FRET/Melt Curve analysis. *Malaria Journal*, 5(1): 1-9.
- Walton, C., Somboon, P., O'loughlin, S.M., Zhang, S., Harbach, R.E., Linton, Y.M., Chen, B., Nolan, K., Duong, S., Fong, M.Y. and Vythilingum, I. 2007. Genetic diversity and molecular identification of mosquito species in the *Anopheles maculatus* group using the ITS2 region of rDNA. *Infection, Genetics and Evolution*, 7(1): 93-102.

- Wang, Y., Wang, Y., Xu, J. and Huang, X. 2023. Risk factors of lower respiratory tract infection caused by *Stenotrophomonas maltophilia*: Systematic review and meta-analysis. *Frontiers in Public Health*, 10(2023): 1035812.
- Webb Jr, J. L. 2014. Malaria Control and Eradication Projects in Tropical Africa, 1945–1965. In *The Global Challenge of Malaria: Past Lessons and Future Prospects* (pp. 35-56).
- Weill, M., Chandre, F., Brengues, C., Manguin, S., Akogbeto, M., Pasteur, N., Guillet, P. and Raymond, M. 2000. The kdr mutation occurs in the Mopti form of *Anopheles gambiaes*. s. through introgression. *Insect molecular biology*, 9(5): 451-455.
- Weill, M., Lutfalla, G., Mogensen, K., Chandre, F., Berthomieu, A., Berticat, C., Pasteur, N., Philips, A., Fort, P. and Raymond, M. 2003. Insecticide resistance in mosquito vectors. *Nature*, 423(6936): 136-137.
- Werissaw Haileselassie, Endalew Zemene, Lee M.C., Zhong, D., Zhou, G., Behailu Taye, Alemayehu Dagne, Wakgari Deressa, James, W.K., Yan, G. and Delenasaw Yewhalaw. 2021. The effect of irrigation on malaria vector bionomics and transmission intensity in western Ethiopia. *Parasites & vectors*, 14: 1-11.
- White, N.J. 2011. Determinants of relapse periodicity in Plasmodium vivax malaria. *Malaria journal*, 10: 297. <https://doi.org/10.1186/1475-2875-10-297>
- WHO. 2022. *World malaria report 2022*. World Health Organization.
- WHO. 2020. World Malaria Report, World Health Organization (WHO, 2020), Geneva, Switzerland, <https://www.who.int/news-room/fact-sheets/detail/malaria>.
- WHO. 2018. Global report on insecticide resistance in malaria vectors: 2010–2016.

- WHO. 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.
- WHO. 2014. World malaria report. Geneva: World Health Organization.
- WHO. 2013. Larval source management: a supplementary malaria vector control measure: an operational manual.
- WHO. 2013. Training Module on Malaria Control Malaria Entomology and Vector Control. Guide for Participants, WHO, Geneva, Switzerland.
- WHO. 2009. Malaria rapid diagnostic test performance. WHO, Geneva, Switzerland
- WHO. 2008. World Malaria Report 2008. WHO Global Malaria Programme
[<http://www.who.int/malaria/wmr2008/>].
- Wondji, C.S., Dabire, R.K., Tukur, Z., Irving, H., Djouaka, R. and Morgan, J.C. 2011. Identification and distribution of a GABA receptor mutation conferring dieldrin resistance in the malaria vector *Anopheles funestus* in Africa. *Insect Biochemistry and Molecular Biology*, 41: 484–491
- Xu, Q., Zhang, L., Li, T., Zhang, L., He, L., Dong, K. and Liu, N. 2012. Evolutionary adaptation of the amino acid and codon usage of the mosquito sodium channel following insecticide selection in the field mosquitoes.
- Yang, F., Schildhauer, S., Billeter, S.A., Hardstone Yoshimizu, M., Payne, R., Pakingan, M.J., Metzger, M.E., Liebman, K.A., Hu, R., Kramer, V. and Padgett, K.A. 2020. Insecticide resistance status of *Aedes aegypti* (Diptera: Culicidae) in California by biochemical assays. *Journal of Medical Entomology*, 57(4): 1176-1183.

Yawson, A.E., McCall, P.J., Wilson, M.D. and Donnelly, M.J. 2004. Species abundance and insecticide resistance of *Anopheles gambiae* in selected areas of Ghana and Burkina Faso. *Medical and veterinary entomology*, 18(4): 372-377.

APPENDIX

Appendix Figures



Appendix Figure 1: Road puddle from study area



Appendix Figure 2: Ditches (A) and Water canal from study area (B)



Appendix Figure 3: Insecticide susceptibility test



Appendix Figure 4: Anopheles species identification

