Jimma University

School of Graduate Studies

Department of Biology

DETERMINATION OF THE RESIDUAL EFFICACY OF BROFLANILIDE (VECTRON ™ T500) INSECTICIDE FOR INDOOR RESIDUAL SPRAYING ON DIFRRENT WALL SURFACES: AN EXPERIMENTAL HUT STUDY

By:HabtamuZegeye

Advisors: Delenasaw Yewhalaw (PhD, Professor)

Co-advisor: Eba Alemayehu (PhD)

"Research thesis submitted to the department of Biology**,** *School of Graduate Studies, Jimma University in partial fulfillment of the requirement, for the degree of Master of Science in Biology (Applied Entomology)*

Approved by the boards of examiners:

_____	_____	_____
Advisor	Signature	Date
_____	_____	_____
Co-advisor	Signature	Date
_____	_____	_____
Examiner	Signature	Date

ACKNOWLEDGMENTS

First and foremost, I would like to glorify and bring the greatest of all thanks to GOD for the strength and patience he has given for me to withstand all burdens and rigorous to complete my thesis work. Next, I would like to express my deepest gratitude to my advisor Dr. Eba for his helpful comments, guidance, his timely support and encouragement throughout my thesis work from the start till the end.

I am delighted to extend my heartfelt thanks to may devisor Delenesaw Yewhalaw for his help starting from proposal development to the final part of thesis writing, Special acknowledgement to Jimma University Tropical Infectious Research center(JUTIRC) for providing financial support for the completion of my thesis work,I extend my sincere appreciation to my family, Yewalashet Gizaw, for their unwavering support and motivation. I would also like to express my gratitude to my friends and colleagues, Worku Meles Mosisa Ayansa, for their valuable advice, insightful comments, and dedicated assistance throughout the completion of this thesis work

Contents

ACKNOWLEDGMENTS i
LIST OF TABLES iv
LIST OFFIGURES v
Acronyms/Abbreviations vi
ABSTRACT vii
1. INTRODUCTION 1
1.1. Statement of the problem 3
1.2. OBJECTIVES 4
1.2.1. General objective 4
1.2.2. Specific objectives 4
2. LITERATURE REVIEW 5
2.1 *Anopheles gambiae* 5
2.2 Life-cycle of the Anopheles mosquito 5
2.2.1 Egg stage; 5
2.2.2 Larval stage 5
2.2.3 Pupa stage 6
2.2.4 Adult stage 6
2.3 Habitats Anopheles species 6
2.4 Patterns of feeding and resting 7
2.5 Vector-borne diseases 7
2.6 Vector control 7
2.7 The need for chemical control 8
2.8 The threat of insecticide resistance 9
2.9 A limited number of effective insecticides 9
2. 10Insecticideresistance status 10
2.11 Defining IRS 10
3. MATERIALS AND METHODS 12
3.1. STUDY AREA AND PEROD 12
3.2STUDY DESIGN 13
3.2.1 Treatment Combination 13

3.2.2. Preparing the spray mixture 15
3.3 ASSESSMENT OF INSECTCIDAL SPRAY QUALITY 15
3.3.1 ASSESSEMET OF RESIDUAL ACTIVITY 16
3.4. DATA MANAGEMENT 17
3.4.1. Data analysis 17
CHAPTER FOUR 18
4.1. Results 18
4.1.1. Insecticide spray quality/ Filter paper data 18
4.1.2Mosquito mortality 24hrs post exposure 19
4.1.3Mosquito mortality 48hrs post exposure 21
4.1.4Mosquito mortality 72hrs post exposure 24
5. Discussion 30
6. Conclusion and recommendation 32
6.1Conclusion 32
6.2 Recommendation 33

LIST OF TABLES

Table 1. Trial design ……..……..……..…13

Table 2. Mean percent mortality rates of *An. arabiensis* 24hrs post exposure …….20

Table 3. Mean percent mortality rates of *An. arabiensis* 48hrs post exposure…….....22

Table 4. Mean percent mortality rates of *An. arabiensis* 72hrs post exposure…….....…25

LIST OFFIGURES

Figure 1.Active ingredient contents on filter papers sprayed (a) ……..18

Figure 2.Active ingredient contents on filter papers sprayed (b) ……..19

Figure 3.percentage mortality rates of *An. arabiensis* exposed 24hrs…….. …21

Figure 4.percentage mortality rates of *An. arabiensis* exposed 48hrs……...23

Figure 5.percentage mortality rates of *An. arabiensis* exposed 72hrs……...…24

Figure 6.percentage mortality72h post exposure from cemented wall surface type ……....26

Figure 7.percentage mortality 72h post exposure from dung wall surface type……..27

Figure 8.percentage mortality 72h post exposure from mud wall surface type…….....28

Figure 9.percentage mortality 72h post exposure from painted wall surface type……...….29

Acronyms/Abbreviations

CFV	Constant Flow Valve
DDT	Dichloro-diphenyl-trichloethane
HPLC	high performance liquid chromatography
IRAC	Insecticide Resistance Action Committee
IRS	Indoor residual spraying
ITNs	Insecticide-treated benders
IVM	Integrated vector management
LLINs	long-lasting insecticidal nets
MDA	mass drug administration
NCA	non-competitive antagonist
TIDRC	tropical & Infectious Diseases Research Center
WHO	World Health Organization
WP	wet table powder

ABSTRACT

The rotational use of insecticides with different modes of action for indoor residual spraying (IRS) is recommended for improving malaria vector control and managing insecticide resistance Therefore, a more diversified portfolio of IRS insecticides is required. Previous tests of propoxur, bendiocarb & pirimiphos-methyl have indicated that residual activity is significantly influencedby the wall surface. Inparticular, Bendiocarb showed poor residual lifespan on dung & mud walls. The most commonly used surface in areas where IRS is conducted. An essential component of IRS programs is insecticide resistance monitoring. The detection of resistance to most existing insecticides underscores the urgent need for new insecticides with novel chemistries, capable of delivering enhanced and prolonged control over insecticide-resistant vector populations. Broflanilide is a newly discovered insecticide being considered for malaria vector control.Thus objective of this study was to assess the efficacy of VECTRON^{TM}T500by sapling the insecticide formulation on different wall surfaces Throughout the nine-month hut trial, monthly wall cone bioassay mortality on VECTRON T500 treated hut walls remained > 80%. IRS with broflanilide shows potential to significantly improve the control of malaria transmitted by parathyroid-resistant mosquito vectors and could thus be a crucial addition to determine its decay rates in Ethiopia

Key word*:* Indoor residual spraying, *VECTRON^{TM}T50*0, insecticides, resistance

.

1. INTRODUCTION

Malaria continues to be a universal public health problem. For example, in 2023, 608,000 deaths and 249 million cases were informed. Of these deaths, about 67% (272,000) were under-five years children (WHO, 2023).In Africa the World Health Organization reported, around 233,000 million malaria cases and 580,000 deaths (WHO, 2023). Beyond morbidity and mortality, malaria causes huge economic loss (Sicuri *et al*., 2013). Malarias is caused by a hematophagous protozoan of the genu*s Plasmodium.* This pathogen is transmitted to humans through the bite of an infected *Anopheles*female mosquito.

Ethiopia is one of the African countries where malaria is endemic and 68% of their populations are at risk of contracting malaria infection. According to a report by EARHI (2023), there were over 1.2 million malaria cases reported on June 25, 2018, and this number increased to 2.4 million by October 2023.This is mainly connected with the geography and climate condition, which are more suitable for the reproduction of malaria vector (Petros andGeshea,2014). A retrospective study done in Ethiopia over 16 years period (2000–2016) also showed that the load of malaria remains high and accounts over five million cases and thousands of deaths annually (Girum*et al.,* 2019). Due to this, various intervention approaches, such as the use of insecticide-treated mosquito nets, indoor residual spraying (considered the most effective due to its impact on mosquitoes' indoor biting and resting habits (Huho et al., 2013), early diagnosis and prompt treatment are being implemented to combat malaria. Additionally, prevention and control strategies encompass operational research, surveillance, environmental management, and monitoring and evaluation systems, providing crucial information (Taffese et al., 2018; Gari and Lindtjørn, 2018).

Indoor residual spraying is one of the main vector control methods used for preventing malaria in many malaria-endemic countries (WHO, 2019).Indoor residual spraying can reduction malaria transmission by dropping female mosquito density and durability when the indoor residual spraying product is applied inside residential houses. The residual insecticide on the potential resting surfaces such as internal walls, eaves and ceilings is effective against female mosquitoes that contact these surfaces and are killed (WHO, 2006) . Reduction in malaria morbidity and mortality was observed in endemic countries in Africa and Asia that increased significantly the coverage of Indoor residual spraying during the last 20years.

Only two non-parathyroid insecticides are currently listed by WHO Prequalification Unit Vector Control Product Assessment Team (WHOPQT/VCP) as IRS formulation products. They are clothianidin (a neonicotinoidinsecticide; available as SumiShield 50WG and its co formulated with deltamethrin as Fludora Fusion) andpirimiphos-methyl (an oregano phosphorus insecticide formulated as Actellic 300CS) (WHO, 2020).However, to properly implement an insecticide resistance management strategy based on the rotation of insecticides with different modes of action, IRS products containing at least 3 different insecticideswill be required. Therefore, finding additional alternative insecticides with novel modes of action to vector control has become a priority (WHO, 2018). VECTRON™T500, containing the active ingredientbroflanilide a novel insecticide formulation developed by Mitsui ChemicalsAgro, Inc., (MCAG; Tokyo, Japan) for Indoor residual spraying (IRS) use to control malaria vectors or other pests (Katsuta*et al.,*2019). It has thepotential to control mosquitoes that have become resistant to pyrethroids and other knownclasses of conventional insecticides (Lee *et al.,* 2020). Broflanilide has been categorized as a member of a newgroup, Group 30: GABA-gated chloride channel allosteric modulators, by the Insecticide Resistance Action Committee (IRAC). It targets the GABA-receptor of chloride channels in the nervous system of insects (IRAC, 2020).

Broflanilide is a meta-diamide insecticide that has a distinct mode of action compared to conventional insecticides currently used in public health (Nakao, and Banba, 2016). It has a lower action onthe mosquitoes than pyrethroids insecticide. There is currently no known cross-resistance to broflanilide via mechanisms of resistance to other public health insecticides. It has also shown low acute toxicity to non-target aquatic organisms (Jia *et al.,* 2020), which demonstrates its high potential for use in public health and agriculture. In addition, the residual efficacy of this dose must be evaluated against the target mosquitoes. In this study, our aim was to establish the optimal dosage and effectiveness of VECTRON™ T500, a new product. We determined the formulation of the active ingredient and evaluate its residual activity. Furthermore, we also assessed the residual effectiveness of this dosage against the target mosquitoes.

1.1. Statement of the problem

Resistance has been observed in world wide. Including malaria in malaria transmitted mosquitoes. Mosquitoes are typical R-strategists (animals that reproduce fast and produce a large number of offspring), and can adapt fast to environmental changes. Due to this and the widespread use of insecticides inboth agriculture and public health, resistance has emerged quite rapidly in malaria vectors. Insecticide-resistant phenotypes are favored where mosquitoes are exposed to sub-lethal doses of the insecticide. Under these conditions, resistant individuals have a better chance to survive and reproduce; this means selection pressure towards resistant populations.

Insecticides are essential for controlling the mosquito vector that transmits malaria. The pressure on mosquitoes to become resistant to these insecticides has increased along with the disease control efforts. The success of control efforts is currently in question due to the sharp growth in the strength and distribution of this resistance in recent years.

1.2. OBJECTIVES

1.2.1. General objective

- To Assess the residual efficacy of VECTRON ™ T500 against susceptible *Anopheles arabiensis strains*

1.2.2. Specific objectives

- To determine the effect of different wall surface types (Mud, dung, painted and cemented wall types) against the residual efficacy of VECTRON ™ T500
- To determine effect of different wall substrate types on the persistence of VECTRON ™ T500Compare residual efficacy of VECTRON ™ T500with comparator (Actelic 300CS)
- To document Decay rate of VECTRON ™ T500 across study month
- To determine the post-exposure mortalities of (24, 48 and 72 hrs) against VECTRON ™ T500

1.3. Significant of the study

If work is done on a range of wall surfaces, such as mud, dung, painted, and cemented, the results will last for a long time. Works on a range of wall surface types, such as mud, dung, painted, and cemented wall types, demonstrate long-term residual efficacy. The results of this study show that VECTRON TM T500 should be used on wild populations of *An. arabiensis* and other Anopheles species in order to determine the drug's effectiveness for use in IRS in Ethiopia and other areas. Additionally, the results of this study can be used by other academics and stakeholders for a variety of decision-making processes and future research.

2. LITERATURE REVIEW

2.1 *Anopheles gambiae*

The *An.gambiae*complex consists of at least seven morphologically indistinguishable species of mosquitoes in the genus *Anopheles*. The complex was recognized in the 1960s and includes the most important vectors of malaria in sub-Saharan Africa, particularly of the most dangerous malaria parasite, *P. falciparum* (De Silva, & Marshall, 2012). It is one of the most efficient malaria vectors complex known. The *An. gambiae* mosquito additionally transmits *WuchereriaBancroft* which causes *Lymphatic filariasis*, a symptom of which is *elephantiasis* (Sodahlon *et al* ,. 2016).

2.2 Life-cycle of the Anopheles mosquito

There are four stages in the life cycle of a mosquito: egg, larva, pupa and adult . During its life-cycle the mosquito undergoes two changes (metamorphoses), from larva to pupa and frompupato adult ((Ezeakacha, 2015).

2.2.1 Egg stage;

The adult An. female mates once and continues to lay eggs throughout its lifespan.Females must take a blood meal every 2-3 days. Blood is needed to develop eggs. Females will lay a batch of eggs before taking the next blood mealEggs are laid on water (rain pools, ponds, riversides, lakes, etc.) in batches of 50–200 eggs. The length of time the eggs take to hatch into larvae largely depends on temperature: At about 30ºC, eggs hatch into larvae in about 2-3 days. In temperate zones (16ºC), about 7-14 days (Collins *et al.,* 2006).

2.2.2 Larval stage

The larva has a well-developed head with “mouth brushes” used for feeding (filter feeders). The larva feeds on micro-organisms (e.g. algae, bacteria) and organic matter in the water where they breed. The An. larva has no respiratory siphon. It lies parallel to surface of water in order to breath. There are four developmental stages of larva known as instars (denoted as L1 to L4,).The development from larva to pupa last about 5-10 days in normal tropical temperatures, depending on the species. Water temperature affects the time required for development, which is shorter in warmer waters (Ezeakacha, 2015)

2.2.3 Pupa stage

The pupa is shaped like a comma and stays at the surface of the water. It has a pair of respiratory trumpets through which it breathes when at the surface. No feeding goes on during this stage but the pupa is motile and responds to stimuli. This is the resting or inactive stage during which there is a major transformation from living in water to emerging and living out of water. The pupa stage takes about 2-5 days (Ezeakacha, 2015)

2.2.4 Adult stage

The adult usually emerges from the pupa at dusk. After emerging from the pupa, the adult mosquito rests for a short time in order to harden its body. Shortly after emergence the mosquitoes mate .The males form large swarms, usually around dusk, and females fly into the swarms to mate. Both male and femalemosquitoes feed on nectar for energy. After mating, the female mosquito searches for a blood meal for the development of her eggs. For some species one feed is enough to develop the eggs. In other species two feeds are required, at least for the development of the first batch of eggs. Duration from egg to adult Anopheles may vary between 7 days at 31ºC and 20 days at 20ºC (Ezeakacha, 2015)

2.3 Habitats *Anopheles* species

Although malaria is nowadays limited to tropicalareas, most notoriously the regions of sub-Saharan Africa, many *Anopheles* species live in colder latitudes. Indeed, malaria outbreaks have, in the past, occurred in colder climates, for example during the construction of the Rideau Canal in Canada during the 1820s (McLaren, 2022). Since then, the *Plasmodium* parasite (not the*Anopheles*mosquito) has been eliminated from first world countries. The CDC warns, however, that "*Anopheles* that can transmit malaria is found not only in malaria endemic areas, but also in areas where malaria has been eliminated. The latter areas are thus constantly at risk of reintroduction of the disease (Carnevale & Manguin, 2021).

2.4 Patterns of feeding and resting

Most Anopheles mosquitoes are crepuscular (active at dusk or dawn) or nocturnal (active at night).Some feed indoors (endophagic), while others feed outdoors (exophagic). After feeding, some blood mosquitoes prefer to rest indoors (endophilic), while others prefer to rest outdoors (exophilic) (Tirados *et al.*, 2006). Though this can differ regionally based on local vector ecotype, and vector chromosomal makeup, as well as housing type and local microclimatic conditions. Biting by nocturnal, endophagic*An.* mosquitoes can be markedly reduced through the use of insecticide-treated bed nets or through improved housing construction to prevent mosquito entry (e.g. window screens). Endophilic mosquitoes are readily controlled by indoor spraying of residual insecticides. In contrast, exophagic/exophilic vectors are best controlled through source reduction (destruction of the breeding sites) (Degefa, *et al*., 2017).

2.5 Vector-borne diseases

The socioeconomic burden associated with tropical diseases such as malaria, dengue, filariasis and trypanosomiasis is a serious impediment to development in many tropical countries, and most of these diseases are a major cause of poverty (Beaumier*et al.,* 2013). It is estimated that malaria alone has reduced the gross national product of the African continent by more than 20% over the past 15 years. Vector-borne diseases account for a very significant part of total morbidity due to infectious diseases, and occur not only in the tropics but also in many temperate countries. For example, the recent progression of West Nile virus in North America, of Lyme disease in Europe, Chikungunya in the Indian Ocean and the worldwide spread of the vector Aedesalbopictus (the “tiger mosquito”) are serious and largely uncontrolled developments (IRAC, 2006).

2.6 Vector control

There is no effective drug or vaccine for important diseases such as dengue, dengue hemorrhagic fevers, and Chagas disease (Beaumier *et al*., 2013). The only way to control these diseases is to prevent transmission by insect vectors. Vector control, personal protection and community participation are the pillars of the WHO strategies for insect-transmitted disease control(Chanda*et al.,* 2016). Unfortunately, mass malaria chemo-prophylaxis cannot be implemented for technical and economic reasons, especially in Africa. The effective treatment of

malaria cases is increasingly complex and expensive because of drug resistance. In high-transmission areas (which include most parts of Africa) malaria incidence cannot be reduced if, in parallel with early diagnosis and treatment, transmission is not controlled through very effective vector-control and/or personal-protection interventions. Vector control may also be important for diseases that are controlled primarily by preventive mass drug administration (MDA) (Webster, *et al.,* 2014). The current strategy of the Global Alliance to Eliminate Lymphatic Filariasis is unlikely to achieve complete elimination of infection if MDA is not supplemented by transmission-control interventions in some areas(WHO,2016). Many other examples that emphasize the need for vector control can be given for most tropical areas as well as developed (Raghavendra *et al*., 2011).

2.7 The need for chemical control

Insecticides remain the most important element of integrated approaches to vector control. The recent restriction on the use of DDT by the Convention on Persistent Organic Pollutants (POPs) has dramatically underlined the high degree of reliance of malaria or *leishmaniosis* control programmers on residual insecticides such as DDT (WHO, 2010) . To reduce this reliance, WHO is promoting integrated vector and pest management, including alternative measures such as biological control or environmental management when and where they are effective and applicable. WHO also promotes the safe and targeted use of insecticides when there is no alternative? For example, a very successful Chagas disease control program in the Americas has been entirely based on indoor spraying of pyrethroid insecticides. Onchocerciasis (river blindness) has been successfully controlled for thirty years in eight countries of West Africa by weekly applications of safe larvicides (Brattig *et al*., 2021).

New technologies such as insecticide-treated bed nets (ITNs) and insecticide-treated materials (ITMs) are now highly promoted and used to prevent diseases transmitted at night by mosquitoes and sand flies (Kitchen, *et al.,* 2009). Although applying insecticides on nets instead of walls is dramatically reducing the total amount of insecticide used for malaria prevention, ITNs remain highly dependent on a single class of insecticides; the synthetic pyrethroids (Okumu, & Moore, 2011) . Most insecticides belonging to other chemical groups do not have all the required attributes in terms of efficacy and safety to be used on mosquito nets. The massive

effortscurrently developed to control malaria, especially in Africa, may be jeopardized by the widespread development of parathyroid resistance (Yewhalaw and Eliningaya, 2016)

2.8 The threat of insecticide resistance

Although public health accounts for only a very small fraction of overall insecticide quantities applied, many vector species of public health importance have already developed resistance to one or more insecticides van den (van den Berg,*et al*., 2021). Development of resistance is a complex and dynamic process and depends upon many factors. Most commonly, when the frequency of resistant insects in a vector population increases, Efficacy of the treatment decreases up to the point where the insecticide has to be replaced by another one (Dusfour*et al.,* 2019).. Increasing the dosages in an attempt to maintain efficacy is not a recommended option because of environmental and safety concerns, increased cost of the insecticide and the resistance genes can be driven to even higher frequencies (Muniz-Junior*et al.,* 2023). Replacing an insecticide with a new one has important cost, logistic and sociological implications that will be discussed later in this document.

In addition, a significant reduction of morbidity and mortality can be achieved only if the efficacy of vector-control interventions is continuously maintained at a very high level. Almost all public health insecticides are also used in agriculture. When vectors breed within or close to agricultural crops, they can be exposed to the same or similarinsecticidal compounds and develop resistance. This phenomenon is of particular relevance for malaria vectors. Moreover, many insecticides are also massively used to control domestic pests, and therefore, impact the vector species which are resting indoors. These so-called “endophilic” vectors are among the most dangerous ones because of their close contact with humans. It is common for a single vector-mosquito population to be exposed to a given insecticide (e.g. a pyrethroid) at the larval stage through agricultural spraying and then again at the adult stage through household pest control, as well as vector-control programmers (IRAC, 2006).

2.9 A limited number of effective insecticides

Although there is a relatively long list of public health insecticide products that can be used to control adult vectors, these products are all members of a small number of chemical groups with discrete modes of action (Matthews *et al.,* 2014). The list is further shortened by similarities in

the mode of action across some of these chemical groups and the phenomenon of crossresistance. Cross-resistance explains why, in some situations, vector populations can develop resistance very rapidly to newly introduced insecticides. Furthermore, in some circumstances, resistance can persist in populations for very long periods after regular use of an insecticide has ceased. In these cases, resistance to new insecticides is inherited from the past as a result of the previous use of other insecticides. Such situations reinforce the importance of understanding which target(s) insecticides are acting upon, and precisely identifying the mechanisms involved once resistance has appeared in a vector population (IRAC, 2006).

2. 10. Insecticide resistance status

Malaria is a devastating parasitic disease transmitted by *Anopheles* mosquitoes. Africa bears the heaviest burden of malaria due to occurrence there of the most strong malaria parasite, *Plasmodium falciparum*, and the most efficient vectors, *An. gambiae* s.s and *An.funestus* (Danlel*et al*., 2021). Although malaria continues to be a leading cause of mortality in Africa, sustained vector control interventions based on indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) have contributed to a significantturndown in malaria related mortality over the last two decades (Danlel *et al*., 2021).Until recently, pyrethroid insecticides were the only class of insecticides approved for use in LLINs,and these pesticides are also commonly used in IRS along with carbamates,organophosphates and organ chlorines. Unfortunately, the development of resistance of malaria vectors to these insecticide classes has become a major global threat to their long-term use in the fight against malaria (Danlel *et al.,* 2021).

As VECTRON TM T500 is prospectiveapplicant insecticide &likely to be careful for widespread use in the near future for IRS programmers'.it is essential to have an estimate of the persistence of this insecticide formulation in phase IIstudy conducted using experimental huts in Ethiopia

2.11 Defining IRS

IRS is the application of a long-lasting, residual insecticide to potential malaria vector resting surfaces such as internal walls, eaves and ceilings of all houses or structures (including domestic

animal shelters) where such vectors might come into contact with the insecticide (WHO , 2015).

When carried out correctly, IRS is a powerful intervention to rapidly reduce adult mosquito vector density and longevity and, therefore, to reduce malaria transmission. The effectiveness of IRS as a malaria control intervention arises from the fact that many important malaria vectors are endophilic (Killeen*et al.,* 2013).That is, when searching for blood meals they enter human habitations or animal shelters where they rest on the walls, ceilings and other interior surfaces before or after feeding on the residents. When a vector comes into contact with a sprayed surface, it absorbs a lethal dose of insecticide, thereby reducing its lifespan(Ritchie,*et al.,*2021). This results in a progressive decline in vector density and longevity, especially among older female mosquitoes, and a reduction in overall Victoria capacity, thereby contributing to a reduction in malaria transmission. IRS is most effective against indoor feeding (endophagic) and indoor-resting (endophilic) vectors. IRS was the primary malaria control method used during the Global Malaria Eradication Campaign (1955–1969). The campaign did not achieve its stated objective, but 37 of the 143 countries that were endemic in 1950 were free of malaria by 1978 and there was a sharp reduction in the burden of disease in other countries (WHO, 2015).

One significant difference between the use of IRS and the use of treated mosquito nets is the point at which each intervention works to greatest effect. IRS may provide some small amount of protection to an individual house by repelling and reducing the number of vectors that come into the house(Killeen, 2014). However, the greatest impact of an IRS intervention takes place after feeding, when the *anopheline* mosquito is more likely to rest on a sprayed surface and pick up a lethal dose of insecticide, thus preventing it from going on to transmit the malaria parasite to others in the vicinity(WHO, 2015). This means that for IRS to be effective, there must be high coverage1 (usually > 85%) of all structures that are potential resting places in order to obtain the "mass effect" on the vector population: in other words, being the only sprayed house in the neighborhood will do little to protect the residents. LLINs, however, inhibit feeding before the mosquito can inoculate the person with sporozoites, and insecticide component of net provide a degree of lethal effect on the vector. This provides both personal protection and, at high coverage rates, a "mass effect" on the vector population. Therefore, being the only house in the neighborhood with residents sleeping under a treated net will provide some degree of protection, even if the neighbors are not covered (WHO, 2015).

3. MATERIALS AND METHODS

3.1. STUDY AREA AND PEROD

Jimma, the largest city in the southwest, approximately 350 kilometers from Addis Ababa, Ethiopia. Along with The town's coordinates are 36° 50' E longitude and 7°41' N latitude. There are 207,573 residents there. 1780 meters above sea level It is situated in the "Woyna Daga" climate zone. One of the largest universities in Ethiopia, Jimma University, is situated there, and its economy is diverse. The study was conducted at the Tropical & Infectious Diseases Research Center (TIDRC) in the southwest Ethiopian region of Sekoru from March 2021 to August 2022.

Rearing and Origin of Susceptible strain

Insectary reared *An. arabiensis Sekoru* strain. However, the susceptibility of the VECTRON TMT500 is not known. Two to three-day-old female mosquitoes fed labium with a sugar solution (10%) were used for the bioassays.

HUT DESIGN

Seven test huts constructed at TIDRC. Jimma University Sekoru for the evaluation of different insecticide formulations were renovated and used for the study. The huts are of the ‘’tukul’’ type, which is a circular hut. Constructed using the wattle and daub technique. Consisting of a frame of eucalyptus wood plastered with mud. The roof is made of framed eucalyptus wood beams covered with grass. The interior diameter of the huts is approximately 2.5–3.5 m, and the height of the walls between 2 and 3m. Each type of wall in a single hut is demarcated and labeled as rough mud. Dung panted and cemented. Each hut was sprayed uniformly by an experienced spray operator, non-stop, starting from the door and moving clockwise to cover the wall surface of the hut. The treatment was assigned to huts randomly (WHO, 2013).

3.2STUDY DESIGN

This is a mixture longitudinal experimental conducted Once per month. and lased for 11 months. During the experiment all bioassays were conducted on the same day. But as this may not be logistically possible, all bioassays were conducted within 7 days. The study ran for about nine months until the average mortality in the VECTRON ™ T 500 across all huts declined<80% for two consecutive months as measured by cone bioassay. The total number of mosquitoes needed per site per month is 10 mosquitoes x 3 cones x 4 surfaces x 7 huts = 840 mosquitoes (Bayili *et al*., 2022)

3.2.1 Treatment Combination

The treatment included: VECTRON ™ T500, Actellic300CS Positive control and water (Negative). The new IRS product used in this study is VECTRON ™ T500 from MITSUI CEMICAL AGRO. The surfaces at least, one month older. Since preparation were used to allow P^H to stabilize and be representative of typical building material of Ethiopia. The surface tested include Mud, Dung, plastered,painted(a layer of paint over smooth mud and lime)&cement.

Table 1.Trial design

HUT ID	Treatment	Commercial Name	Concentration	Wall position	Substrate			
					Mud (1wallper hut)	Dung(1wallper hut)	Painted (Mud) (1wall per Hut)	Cement (4 walls per hut)
	Positive control	-		high	X	X	X	X
	Positive control			Medium				-
	Positive control			Low				-
1	Negative control			High	X	X	X	X
	Negative control			Medium				
	Negative control			Low				-
2	Pirimiphos-Methyl	Actellic 300CS	1000mg/m^2	High	X	X	X	X
	Pirimiphos-Methyl	Actellic 300CS	1000mg/m^2	medium				
	Pirimiphos-Methyl	Actellic 300CS	1000mg/m 2	Low				
3	Broflanilide	VectronT500	100mg/m^2	High	X	X	X	X
	Broflanilide	VectronT500	100mg/m^2	Medium				
	Broflanilide	VectronT500	100mg/m^2	Low				

There are four treatments three wall position and 4 wall surfaces. Thus, this is a 4 by 3 by 4 factorial experiment with a total of 48 treatments , wall position andsurface The pH level is measured using a pH meter or litmus paper before spraying, and spraying is conducted if the pH falls within the range of 7-8. Three huts are used for each dose of 100mg/m2 of the VECTRON™ T500 insecticide, with additional huts used as positive and negative controls. The internal wall and ceiling areas (m2) of these huts are calculated to determine the volume of insecticide solution required

3.2.2. Preparing the spray mixture

The active component of **VECTRON**™500 is wet table powder (WP) formulation broflanilide. It is a meta-diamide insecticide that functions as a GABA-gated chloride channel allosteric modulator. The insecticide is applied by a trained operator using a hand-held compression sprayer that complies with WHO specifications, fitted with flat fan nozzles and a red CFV (Constant Flow Valve) (Laimböck, 1991)

The target dose of the VECTRON™ T500 insecticide was 100mg/m^2. To achieve this target, one sachet of VECTRON™ T500 was mixed in 7.5L of water and applied to 2.5m^2area using an 8002E nozzle fitted with a red CFV

3.3 ASSESSMENT OF INSECTCIDAL SPRAY QUALITY

In addition to recording the volume of spray, to evaluate the quality of the spray, 3 filter paper (Whitman No. 4) placed on the walls (low, 50 cm from the floor. center and high.50 cm from the junction with the ceiling on the walls) were sprayed for each hut. These should be supported on pins to keep papers clear of the wall surface to prevent them soaking up excess run off spray. The location of the paper marked on hut walls to ensure subsequent bioassays are not conducted in locations papers prevented the spray from touching the walls. The paper collected after spraying. Storedinaluminumfoil.Carefullylabeled with: the date of spraying. Treatment ID, hut number & operator initials. All filter paper sample were stored at 4^oC until transported to the laboratory for analysis. The insecticide concentration analyzed using high performance liquid chromatography (HPLC). as previously described (Tangna*et al.,* 2005). at the quality control Research lab of LSTM (Markpaine'sgroup).

3.3.1 ASSESSEMET OF RESIDUAL ACTIVITY

WHO cone bioassays were used to observe the durability of insecticides on sprayed walls. Relative humidity and temperature were recorded daily.until experiments were fully completed Susceptible *An. arabiensis strains* were used from TIDRC insectaries for this study. The strain is fully susceptible to various classes of insecticides. A batch of ten female mosquitoes was transferred into paper cups covered with netting. Sugar solution-soaked cotton wool was placed on each cup. and mosquito weretransfer to the experimental huts in a box covered with wetted towel maintain humidity, Temperature and humidity were monitored by putting thermo hygrometer inside a wooden box. In each hut. A minimum of three cones were fixed to the walls at different heights (high, middle, and low) of the indoor walls to evaluate the bio-efficacy of insecticides at different heights (WHO 2006). Then, mosquitoes from each paper cup were transferred into the cones by using a mouth aspirator (a separate aspirator used for insecticide/dose).

Cones were fixed to the walls using small nails. After 30 minutes of exposure, the mosquito returned to the paper cups with a sugar solution on cotton wool. which was kept in a wooden box covered with a moist tower to maintain temperature and relative humidity within a recommended range (27^{o}C, 2^{o}C, and 80% RH), and mortality was counted after 24, 48, and 72 hours. A mosquito is considered alive if it can stand the right way up. and a mosquito is considered knocked down if it is immobile and unable to stand. or unable to takeoff or fly in a coordinated manner either during or within 60 minutes of exposure, mosquitoes are considered dead if there is no sign of life; immobile; and cannot stand for at least 24 hours after exposure. When control mortality is between 5% and 20%, experiment mortality is corrected using Abbott's formula (1925), and if mortality exceeds 20% in the control, the test is rejected and repeated. When assessing mortality, the position of the cone on the wall was recorded alongside data for each batch of mosquitoes. This allowed the correlation of mortality data with each specific part of the hut wall, which can be particularly useful if one cone is repeatedly showing low activity.

3.4. DATA MANAGEMENT

3.4.1. Data analysis

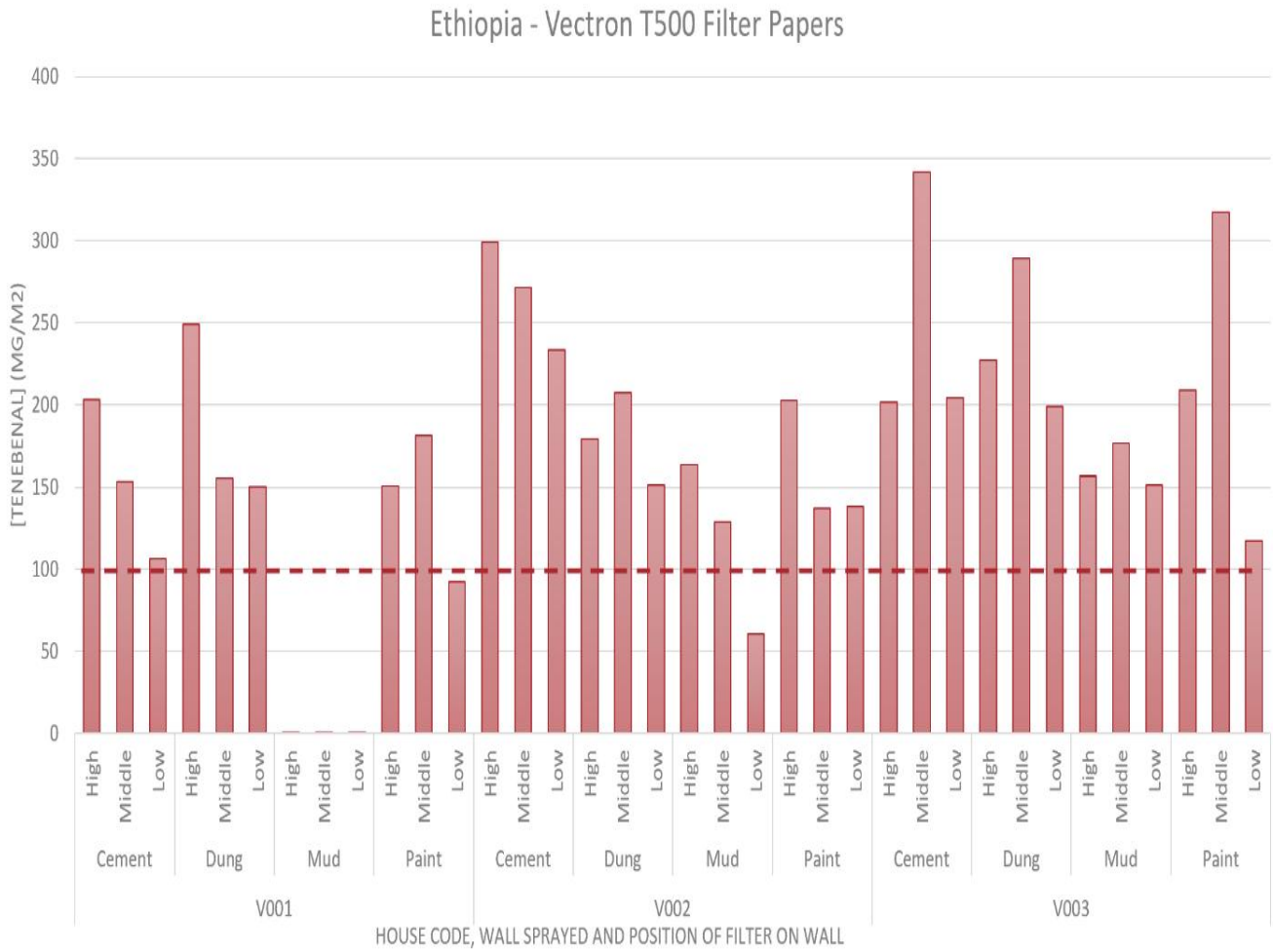
The *An. arabiensis* female mortality rate after exposure to a treated wall is estimated using a binomial regression model based on the number of weeks following treatment. Keeping in mind the experiment's location and the substrate that was treated. The number of months was predicted with a 95% confidence interval, after which this likelihood would fall below 80% (Djenontin *et al*., 2013). Analysis was performed with R (version 3.1).

CHAPTER FOUR

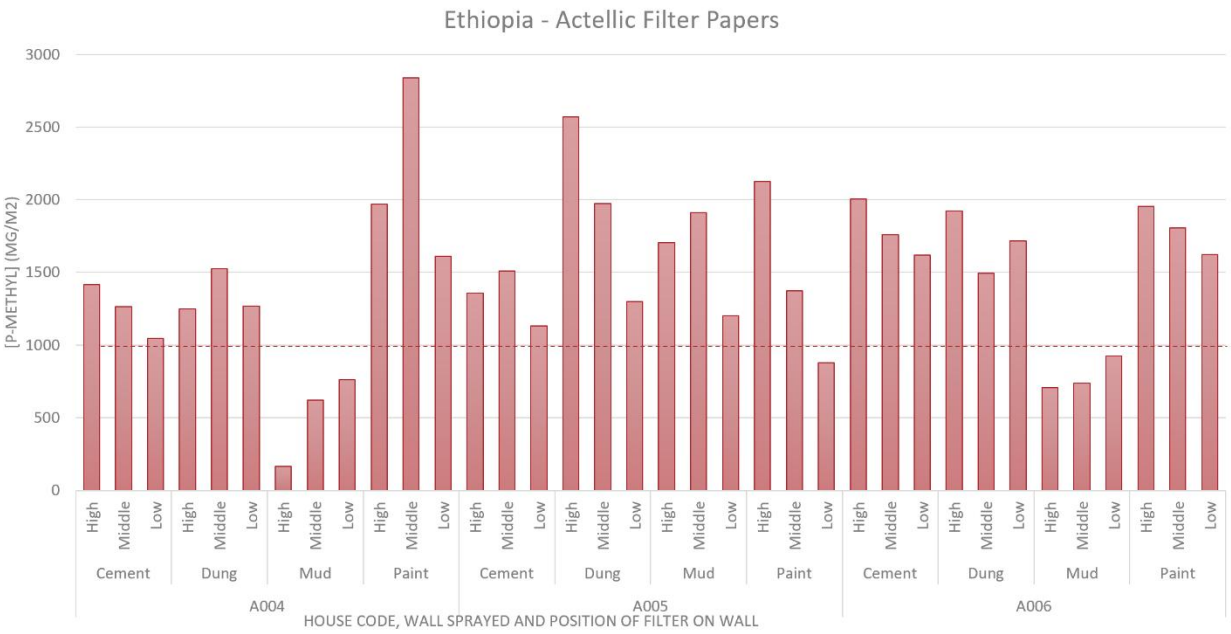
4.1. Results

4.1.1. Insecticide spray quality/ Filter paper data

The results from the filter paper chemical analysis showed a difference in insecticide concentration levels across experimental huts; however, the vast majority of experimental huts were treated with an actual dose of active ingredients (Figures 2a, b). Distinctions in insecticide application rates during IRS are due to the volume of variables involved.



a



b

Figure *2.Active ingredient contents on filter papers sprayed with(a) VECTRON ™ T500 and (b) Actellic 300CS on different wall surfaces of experimental huts, Ethiopia*

4.1.2Mosquito mortality 24hrs post exposure

The 24-hour mean percent mortality of *An. arabiensis* that was exposed to four different wall surfaces (cement, mud, and dung-painted) treated with either VECTRON™ T500 or Actellic 300CS is shown in Figure 2. Both VECTRON™ T500 and Actellic 300CS had advanced mortality rates compared to the controls for each of the wall surface types. Overall (nine months) 24-hour mortality rates across all wall surface types were 86% and 83% for VECTRON™ T500 and Actellic 300CS, respectively (Table 2).

Table 1.Mean percent mortality rates of *An. arabiensis* 24hrs post exposure to different wall surface types treated with VECRTRON™ T500 (100mg/m^2) and Actellic 300CS (1000mg/m^2) (January- October 2022)

Treatment	Wall type	Monthly percent mortality by month (24hrs post exposure)									
		1st month	2st	3st	4tt	5th	6th	7th	8th	9th	10th
VECTRON™T 500	Cement	96	100	100	100	99	88	94	100	100	99
	Dung	88	77	77	94	91	31	82	93	94	74
	Mud	83	74	94	92	89	66	87	93	99	89
	Painted	91	72	81	87	88	46	82	91	96	80
Actellic 300CS	Cement	100	100	99	99	98	94	74	78	75	60
	Dung	100	100	99	86	92	71	63	31	38	58
	Mud	100	97	100	77	97	93	78	66	54	57
	Painted	100	100	100	99	98	90	89	71	79	63
Control	Cement	13	0	0	0	7	7	3	0	3	3
	Dung	7	7	0	0	0	0	3	0	0	7
	Mud	13	0	0	3	0	3	0	0	3	3
	Painted	13	10	3	0	0	0	0	0	3	0

The 24-hour mortality rate of *An. arabiensis* exposed to the Actellic 300CS insecticide formulation was higher for all surface types during the first four months after the application of the insecticide on three wall surfaces (cement, dung, and mud), then started to significantly decline after the fifth month of the insecticide application on almost all wall surfaces. However, the 24-hour mortality rates of *An. arabiens treated with* VECTRON™ T500 were relatively lower for the first two months and consistently increased (except for the month of June 2022) across all wall surfaces. The VECTRON™ T500 cement wall surface type yielded a relative higher mortality rate (98%) compared to the other surface types, whereas the painted wall surface type yielded a relatively higher mortality rate (89%) for Actellic 300CS 24 hours post-exposure (Figure 3).

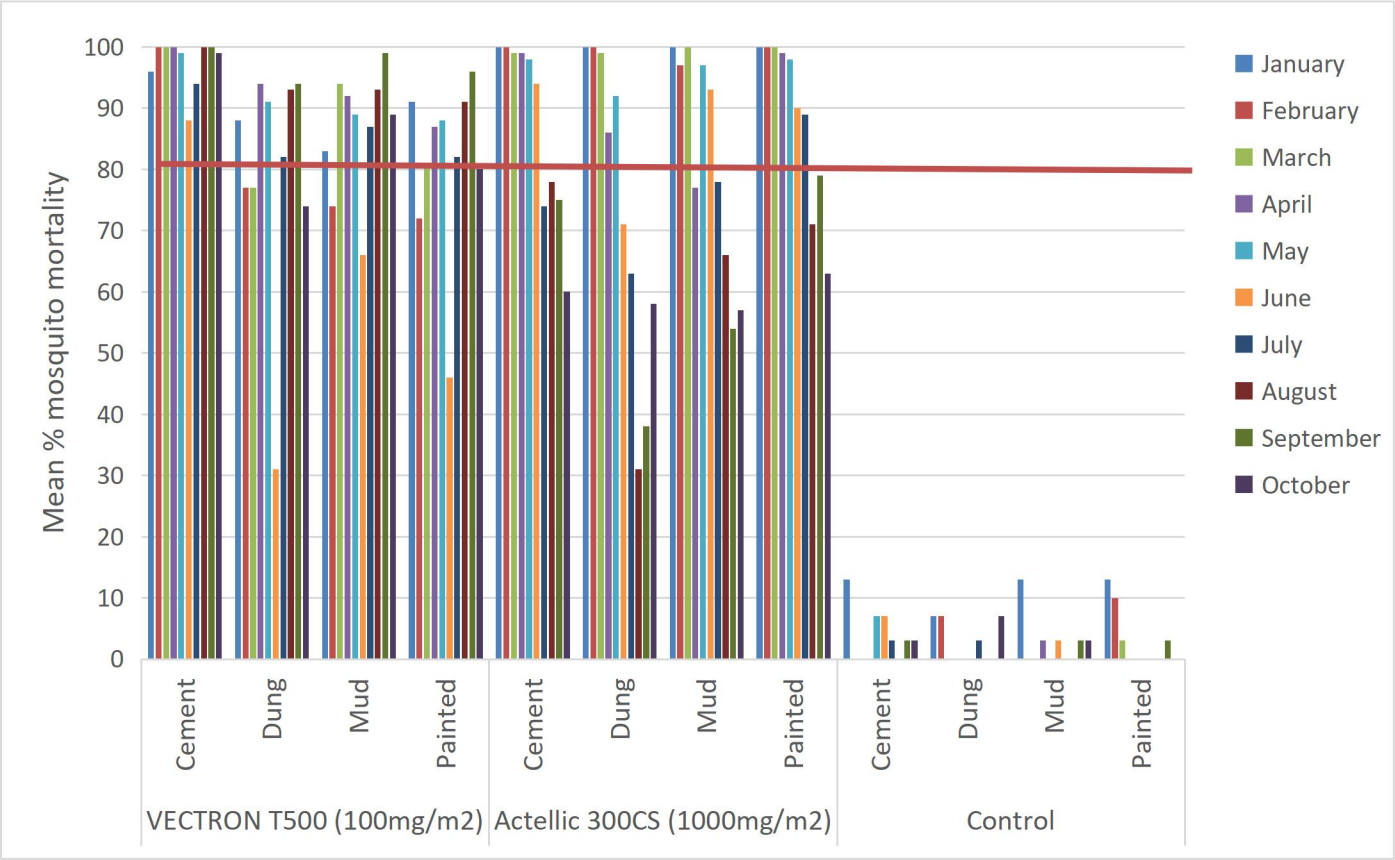


Figure 3. Mean percentage mortality rates of *An. arabiensis* exposed to different wall surface types*(cemented mud dung &painted)* treated with VECTRON™ T500 (100mg/m^2) and Actellic 300CS (1000mg/m^2) over time (24hrs post exposure)

4.1.3 Mosquito mortality 48hrs post exposure

The mean percent mortality of *An. arabiensis* 48 hours post-exposure to the four different wall surfaces (cemented mud, dung, and painted) treated with VECTRON™ T500 and Actellic 300CS is shown in Figure 4. Both VECTRON™ T500 and Actellic 300CS yielded higher mortality rates compared to controls in all wall surface types. Overall (nine months) mortality rate across all wall surface types was 98% and 87% for VectorTM T500 and Actellic 300CS, respectively (Table 3).

Table 3. Mean percent mortality rates of *An. arabiensis* 48hrs post exposure to different wall surface types (cemented dung mud &painted) treated with VECTRONTM T500 (100mg/m2) and Actellic 300CSs

Treatment	Walltype	Monthly percent mortality by month (48hrs post exposure)									
		1st month	**2st**	**3st**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**	**10th**
VECTRON™ T500	Cement	97	100	100	100	100	100	100	100	100	99
	Dung	99	100	99	100	99	83	97	98	100	94
	Mud	88	89	100	99	97	88	100	100	100	100
	Painted	100	87	99	100	97	81	98	100	100	100
Actellic300CS	Cement	100	100	100	100	98	98	78	81	80	64
	Dung	100	100	100	90	97	82	72	68	43	69
	Mud	100	99	100	87	98	97	84	71	61	61
	Painted	100	100	100	100	98	96	93	74	87	68
Control	Cement	13	0	7	7	13	13	7	0	10	3
	Dung	7	7	0	0	0	3	3	7	3	7
	Mud	13	0	0	7	3	3	0	0	3	3
	Painted	13	10	3	3	0	0	0	0	3	0

The 48-hour mortality rate of *An. arabiensis* exposed to the Actellic 300CS insecticide formulation was higher for all surface types in the first four months after the application of the insecticide on three of the wall surfaces (cement, dung mud, and painted), but started to considerably decline after the fourth month of the insecticide application on all wall surfaces. However, 48-hour mortality rates of *An. arabiensis* treated with VECTRON™ T500 were relatively lower for the first two months and then steadily increased, which was remarkably higher than Actellic 300CS across all wall surfaces. The VECTRON™ T500 cement wall surface type yielded a relative higher mortality rate (100%) compared to the other surface types, whereas the painted wall surface type yielded a relative higher mortality rate (92%) for Actellic 300CS in 48 hours post-exposure (Figure 4).

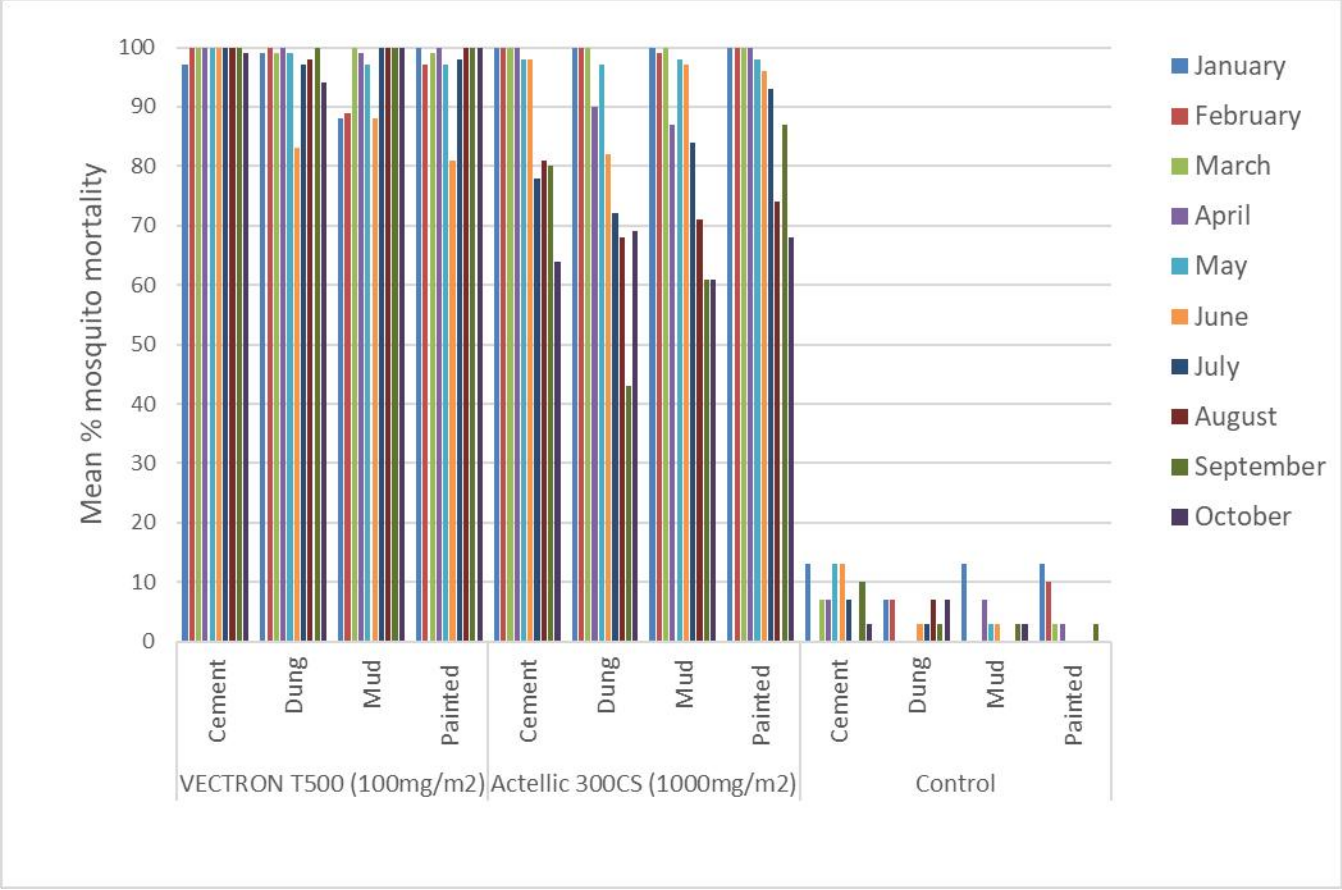


Figure 4. Mean percentage mortality rates of *An. arabiensis* exposed to different wall surface types treated with VECTRON™ T500 (100mg/m^2) and Actellic 300CS (1000mg/m^2)over time (48hrs post exposure)

4.1.4Mosquito mortality 72hrs post exposure

The mean percent mortality of *An. arabiensis* 72 hours post-exposure to the four different wall surface types treated with VECTRON™ T500 and Actellic 300CS is presented in Figure 5. Both VECTRON™ T500 and Actellic 300CS yielded higher mosquito mortality rates compared to control across all wall surface types. Overall (nine months) 72-hour post-exposure mortality rate in all wall surface types for VECTRON™ T500 and Actellic 300CS was 99% and 89%, respectively.

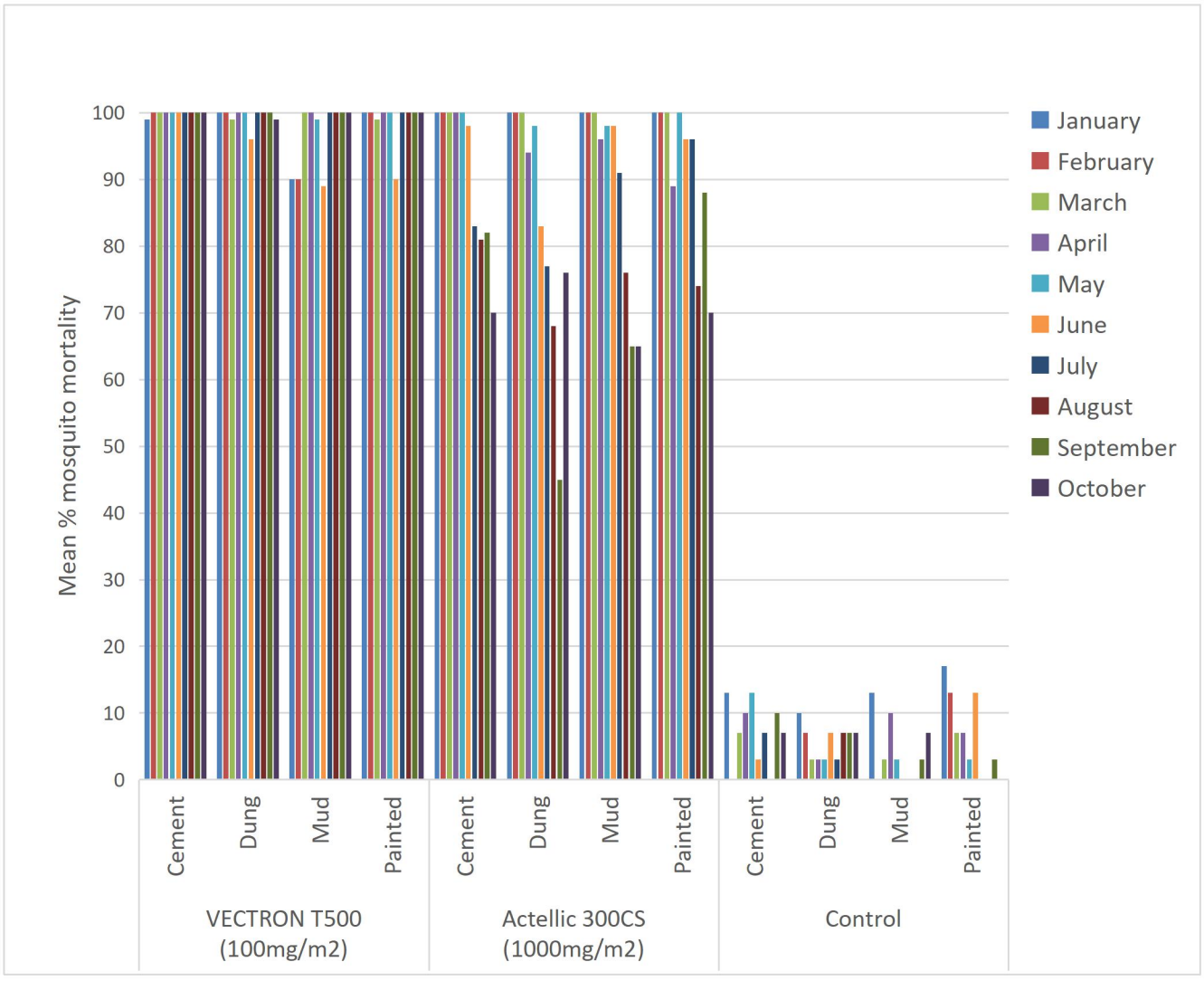


Figure 5. Mean percentage mortality rates of *An. arabiensis* exposed to different wall surface types treated with VECTRON™ T500 (100mg/m^2) and Actellic 300CS (1000mg/m^2)(72hrs post exposure

Table4.Mean percent mortality rates of *An. arabiensis* 72hrs post exposure to different wall surface types treated with VECTRON^{TM}T500 (100mg/m^2) and Actellic 300CS (1000mg/m^2) (January-October 2022).

Treatment	Wall type	Monthly percent mortality by month (72hrs post exposure)									
		1thmonth	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**	**10th**
VECTRON T500 (100mg/m^2)	Cement	99	100	100	100	100	100	100	100	100	100
	Dung	100	100	99	100	100	96	100	100	100	99
	Mud	90	90	100	100	99	89	100	100	100	100
	Painted	100	100	99	100	100	90	100	100	100	100
Actellic 300CS (1000mg/m^2)	Cement	100	100	100	100	100	98	83	81	82	70
	Dung	100	100	100	94	98	83	77	68	45	76
	Mud	100	100	100	96	98	98	91	76	65	65
	Painted	100	100	100	89	100	96	96	74	88	70
Control	Cement	13	0	7	10	13	3	7	0	10	7
	Dung	10	7	3	3	3	7	3	7	7	7
	Mud	13	0	3	10	3	0	0	0	3	7
	Painted	17	13	7	7	3	13	0	0	3	0

The results of 72-hour post-exposure mosquito mean mortality from the wall cone bioassays presented in Figures 5 to 8 showed that VECTRON™ T500 performed better on all wall substrates, with mortality remaining over 80% across the nine months but Actellic 300 CS below 80% after five months. The cemented wall surface type yielded a relatively higher mortality rate compared to the other surface types (Figure 6-9).

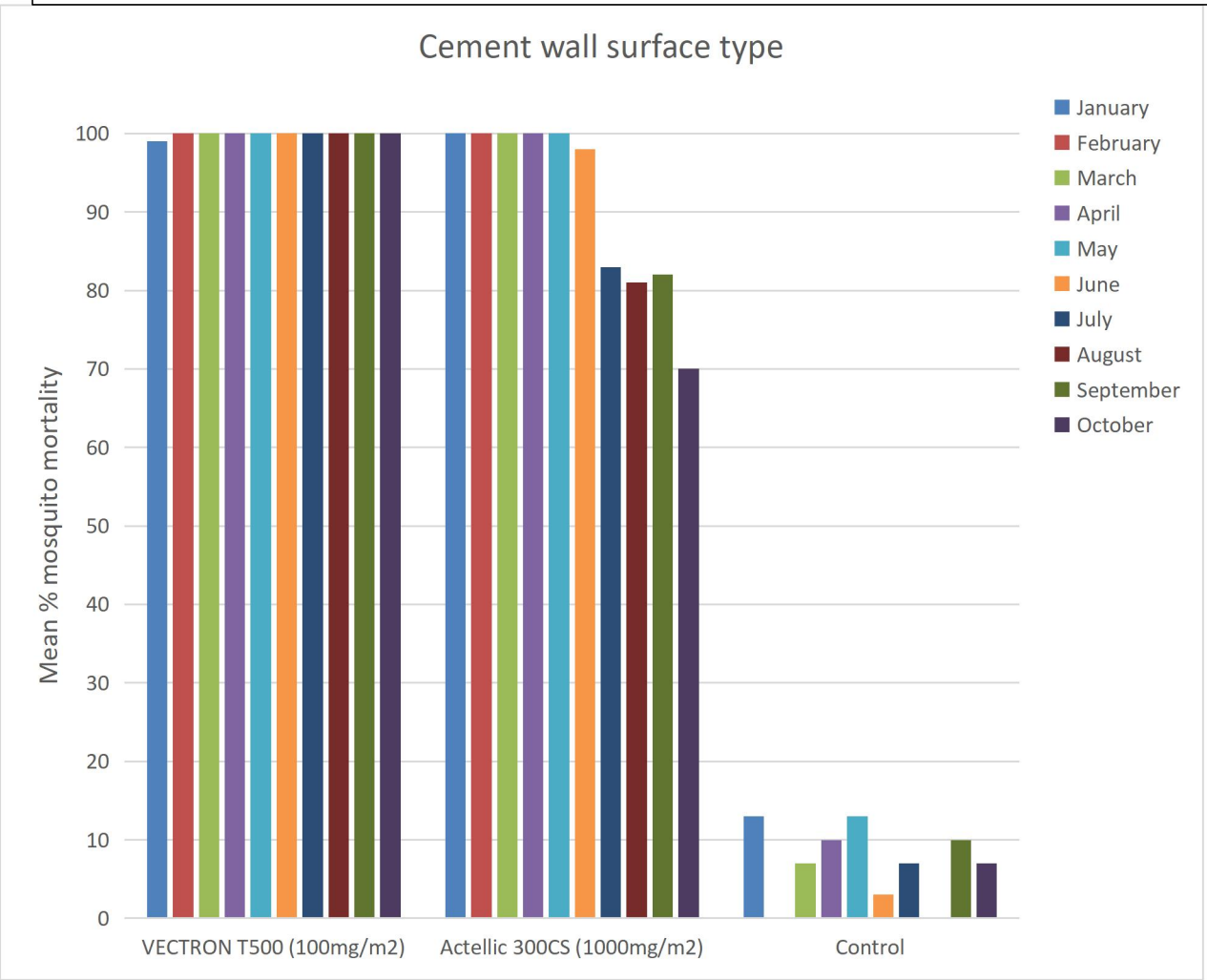


Figure 6.*An.arabiensis* mean percentage mortality72h post exposure from cemented wall surface type

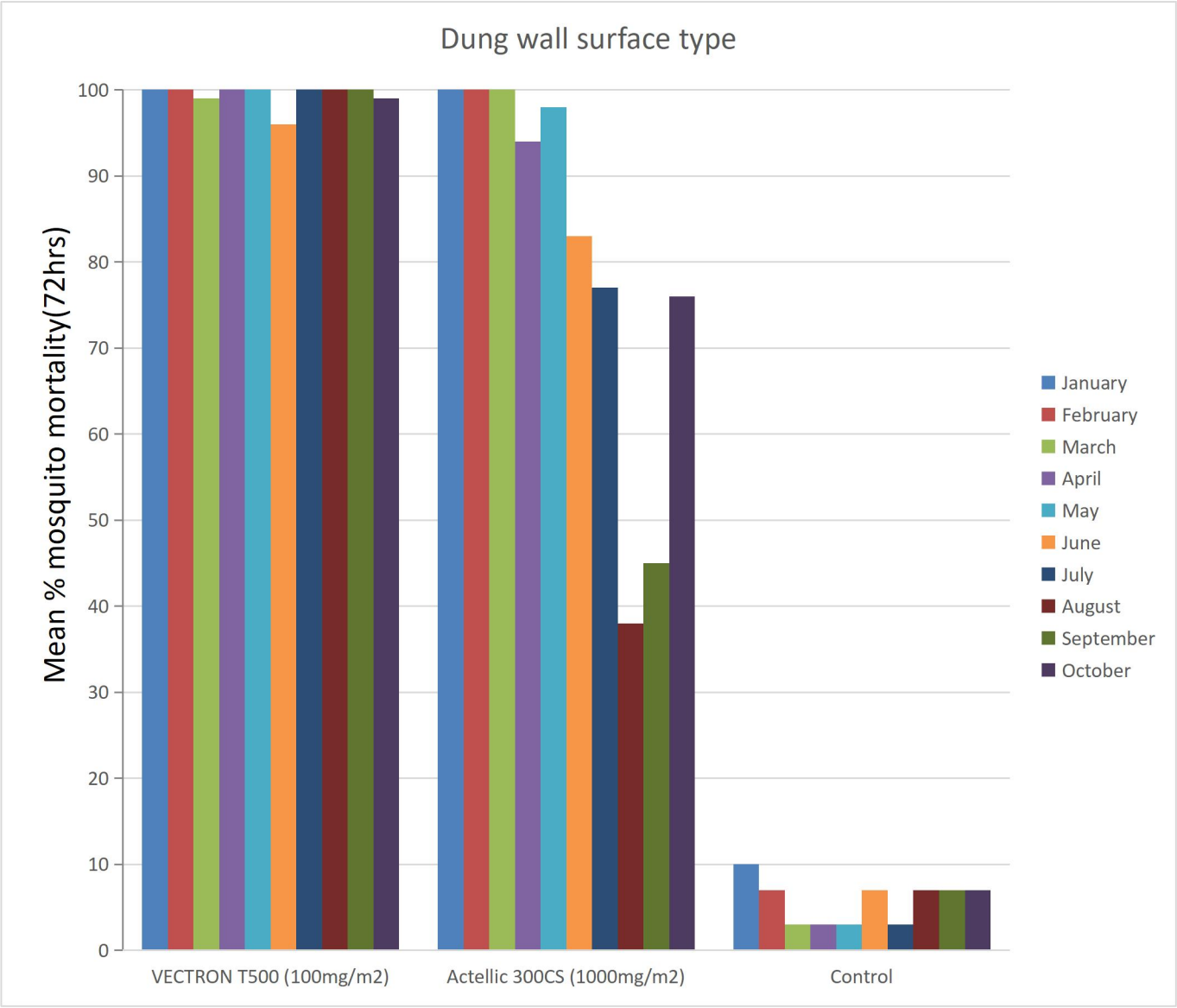


Figure 7.*An. arabiensis* mean percentage mortality 72h post exposure from dung wall surface type

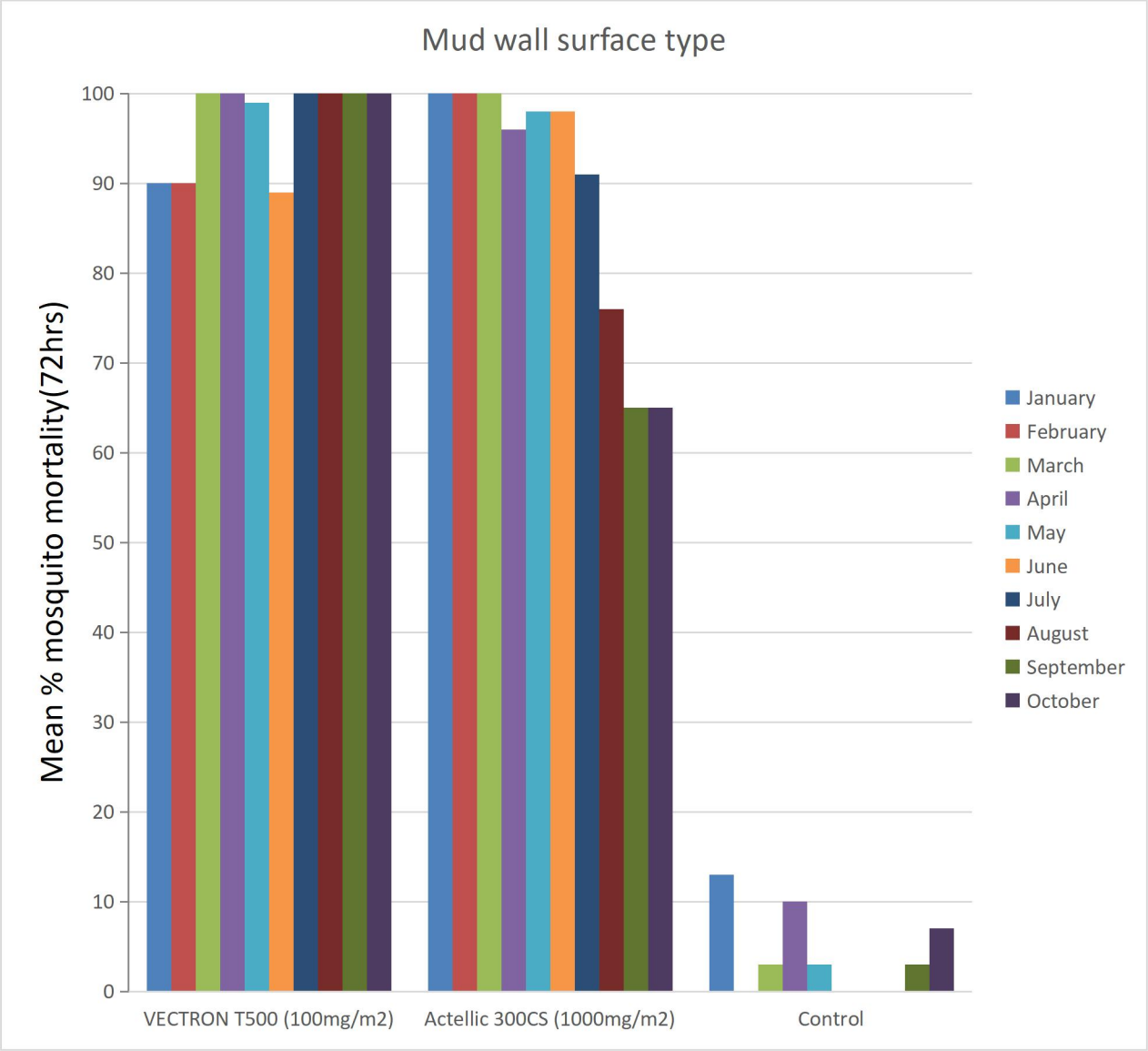


Figure 8.*An. arabiensis* mean percentage mortality 72h post exposure from mud wall surface type

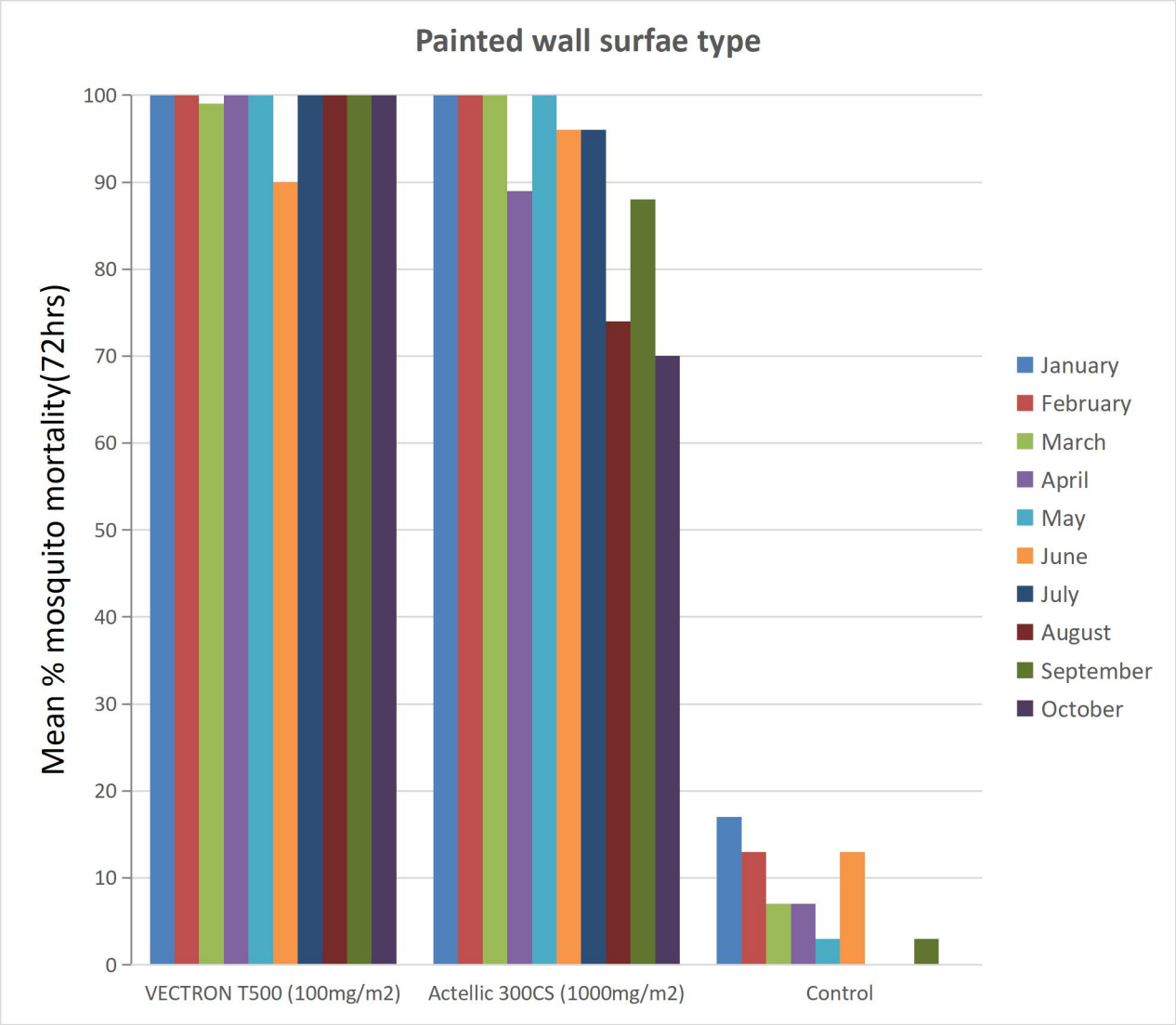


Figure 9.*An. arabiensis* mean percentage mortality 72h post exposure from painted wall surface type

5. Discussion

The two main methods used to fight malaria vectors are indoor residual spraying (IRS) and the use of insecticides applied through long-lasting insecticidal nets (LLINs). Regretfully, the World Health Organization (WHO) has approved a number of insecticide classes for use in public health, but the primary malaria vector species are resistant to them all (WHO, 2016). Effectively addressing insecticide resistance stands as a significant challenge in the realms of malaria control and elimination. The implementation of insecticide resistance management strategies is pivotal for sustained malaria control efforts. These strategies necessitate the development of novel insecticides with modes of action that effectively target mosquito strains resistant to conventional treatments (Fongnikin *et al.,* 2020).

According to WHO policy recommendations, a new IRS pesticide must demonstrate its effectiveness against vector mosquitoes through experimental hut studies, ideally demonstrating non-inferiority to current IRS treatments. (WHO, 2017; WHO, 2019). The overall mortality of *An. arabiensis* raised in the lab was the subject of our investigation, and the results showed that VECTRONTM T500 and Actellic 300CS are not inferior. Actellic 300CS has a history of effectively suppressing malaria and mosquito-borne illnesses. As a result, VECTRONTM T500 satisfied WHO requirements to be included to the IRS pesticide recommendation list. In laboratory cone bioassays, on substrates consisting of mud dung, painted, and cement block, the mortality of susceptible and pyrethroid-resistant vector mosquito strains with VECTRON T500 remained > 80% for nine months. (WHO, 2021) Mud walls, often known as dung walls, are frequently seen as a more difficult substrate for

The diminishing use of IRS for malaria control is attributed to the high costs and logistical challenges associated with sustaining annual campaigns in highly endemic areas (Oxborough, 2016). IRS formulations with extended efficacy, requiring less frequent application, are more desirable (WHO, 2021). In our study, VECTRON™ T500 exhibited remarkable residual efficacy, inducing 98% mortality in situ wall cone bioassays for nine months post-IRS application.

The emergence of vector resistance to public health insecticides poses a significant threat to malaria elimination targets (WHO, 2022). Preserving the efficacy of new malaria control

insecticides is paramount, and the rotational deployment of IRS insecticides with diverse modes of action is recommended for pre-emptive management of insecticide resistance (WHO, 2012). However, this strategy has been underexplored due to the limited availability of insecticide modes of action for IRS (Mnzava *et al.* 2015). The inclusion of broflanilide (VECTRON™ T500) in the WHO prequalified IRS products, presenting a new mode of action with no observed cross-resistance, enhances the capacity to manage insecticide resistance and maintain the effectiveness of newly developed IRS insecticides.

Despite the pursuit of universal Insecticide-Treated Net (ITN) coverage, most IRS campaigns are deployed against a background of medium to high ITN coverage. While current WHO guidance discourages a combined ITN and IRS approach (WHO, 2023), persistent malaria in hyper endemic countries with high ITN coverage suggests the limitations of relying solely on ITNs (WHO, 2022). Introducing a long-lasting IRS like VECTRON™ T500 later in the operational life of ITNs in high transmission areas could effectively reduce malaria transmission while minimizing costs associated with annual IRS campaigns. The impact of such combinations depends on interactions between the IRS insecticide and the ITN insecticide, necessitating further studies to guide the deployment of VECTRON™ T500. Co-deployment with new dual active ingredient ITNs may also present an opportunity to manage insecticide resistance by introducing different modes of action simultaneously (WHO, 2012).

Laboratory and experimental hut trials confirm broflanilide (VECTRON™ T500) as effective against DDT, pyrethroid, organophosphates, and carbamates resistant mosquitoes. In experimental hut studies, a 100 mg/m2 dose yielded over 80% mortality for 9 months on various surfaces. Results align with previous studies in Benin and Tanzania (Snetselaa.*et al*,2021: Govoetchan *et al* 2022). Cone bioassays on treated walls indicated VECTRON™ T500's performance matching Actellic 300CS for 9 months post-spraying, suggesting its potential for prolonged vector control in malaria-endemic African regions. Notably, the co-deployment of VECTRON™ T500 with other IRS insecticide formulations in a rotation strategy may help manage insecticide resistance and extend their effective lives. The observed differences in Ethiopia between previous and present studies warrant further verification.

6. Conclusion and recommendation

6.1Conclusion

VECTRON T500 showed high activity against susceptible strains of *An. arabiensis,* which lasted 9 months or more on local mud, dug, and painted and cement substrates in both laboratory bioassays and experimental hut studies. Indoor residual spraying with broflanilide WP VECTRON™ T500 shows potential to provide substantial and prolonged control of malaria transmitted by pyrethroid-resistant mosquito vectors when applied for IRS. Its addition to the current list of WHO-approved IRS insecticides will provide a suitable option to facilitate the rotation of IRS products with different modes of action.

6.2 Recommendation

Based on the result of this study, the following recommendation was forwarded: determination of residual efficacy and unique mode of action. Broflanilide VECTRON T500 showed high activity against both parathyroid-susceptible and resistant strains of *An. arabiensi.* It is recommended that it could be an idea product to be considered as a potential insecticide suitable for indoor residual spraying in malaria-wide-spread countries *An. arabiensis* is the outcome of this insecticide formulation in the Sekoru district. highlights the need for more research in Sub-Saharan Africa and other regions of Africa

REFERENCE

Abbot WS. (1925).Methodof computing the effectiveness of insecticide. *Journal of economic entomology*. 18:2652

Bayili, K., Ki, H. D., Bayili, B., Sow, B., Ouattara, A., Small, G., ...& Diabate, A. (2022). Laboratory and experimental hut trial evaluation of VECTRON™ T500 for indoor residual spraying (IRS) against insecticide resistant malaria vectors in Burkina Faso. *Gates Open Research*, *6*.

Beaumier, C. M., Gillespie, P. M., Hotez, P. J., & Bottazzi, M. E. (2013). New vaccines for neglected parasitic diseases and dengue. *Translational Research*, *162*(3), 144-155.

Brattig, N. W., Cheke, R. A., & Garms, R. (2021). Onchocerciasis (river blindness)–more than a century of research and control. *ActaTropica*, *218*, 105677.

Carnevale, P., &Manguin, S. (2021). Review of issues on residual malaria transmission. *The Journal of Infectious Diseases*, *223*(Supplement_2), S61-S80.

Cibulskis. R. E. Alonso. P Aponte. J. Aregawi. M. Barrette. A. Begeron. L. Fergus. C. A. knox.T.Lynch. M. Patouillard E. Schwerte S. Stewart S.& Williams R. (2016). Malaria: Globalprogram 200-2015 and future challenges. *Infectious diseases of poverty. 5(1). 61.*https://doi.org/10.1186/s40249-016-051-8

Chanda, *et al,* (2016). Optimizing Strategic Insecticide Resistance Management Planning in Malaria Vectors. *Insecticides Resistance*, 155-187.

Collins, D. A., Conyers, S. T., & Cardwell, S. K. (2006). The effect of sub-zero temperature on the mortality of Ephestiaelutella (Hübner).

De Silva, P. M., & Marshall, J. M. (2012). Factors contributing to urban malaria transmission in sub-Saharan Africa: a systematic review. *Journal of tropical medicine*, *2012*.

Degefa, T., Yewhalaw, D., Zhou, G., Lee, M. C., Atieli, H., Githeko, A. K., & Yan, G. (2017). Indoor and outdoor malaria vector surveillance in western Kenya: implications for better understanding of residual transmission. *Malaria journal*, *16*, 1-13.

Diabate. A. Brengues.C. Baldet. T. Dabire. K . R. Hougard. J. M. Akogbeto.M. &Chandre. F(2004). The spread of the leu-phekdr mutation through Anopheles gambiae complex in Burkina Faso : genetic introgression and de novo phenomena Tropical Medicine & international Health. 9(12). 127-1273

Djenontin.A. Aimihous. O. Sezonlin. M.Damilen G. B. Osse.R. Soukou. B. & Akogbeto.M.(2013). The residual life of bendiocarb on different sunstrates under Laboratory and fild condition in Benin Western. *BMC Research Notes. 6.1-6*

Dusfour, *et al,*(2019). Management of insecticide resistance in the major Aedes vectors of arboviruses: Advances and challenges. *PLoS neglected tropical diseases*, *13*(10), e0007615.

Ezeakacha, N. F. (2015). *Environmental impacts and carry-over effects in complex life cycles: the role of different life history stages*. The University of Southern Mississippi.

Fongnikin. A. Houet. N. Agbevo. A. Odjo. A. Syme. T.N. Guessan.R. &Ngufor. C. (2020). Efficacy of Fludora@ Fusion (a mixture of deltamethrin and clothianidin) for indoor residual spraying against pyrethroid –resistant malaria vectors: Laboratory and experimental hut evaluation parasites & Vectors. 13. 1-11.

Ethiopia_ News. (2023). ¬ - Amidst Alarming Surge in Malaria Cases, Amhara Regional Health Institute to Launch Antimalarial Chemical Spraying ProgramallAfrica.com 9 OCTOBER

Gari T, & lindtiorn.B (2018). Reshaping the vector control strategy for malaria elimination in Ethiopia in the context of current evidence and new tools: opportunities and challenges.*Malaria Jornal.17(1).454*

Girum T, shumbel.T.& shewangizaw.M. (2019). l Burden of malaria in Ethiopia, 2000–2016: findings from the global health estimates. Trop Dis Travel Med Vaccines.5.1-7

Govoetchan, R. Fongnikin.A.Smaii.G.Gbegbo.M.To.djinou.D.& Hgufor.C (2022). VECTRON™ T500, a new brofanilide insecticide for indoor residual spraying, provides prolonged control of pyrethroid-resistance vector.*Malaria Journal.21(1).324*

Killeen, G. F., Seyoum, A., Sikaala, C., Zomboko, A. S., Gimnig, J. E., Govella, N. J., & White, M. T. (2013). Eliminating malaria vectors. *Parasites & vectors*, *6*, 1-10.

Killeen, G. F. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malaria journal*, *13*, 1-22.

Laimböck, F. J. (1991). The potential of small loop-scavenged spark-ignition single cylinder two-stroke engines. *SAE transactions*, 1030-1103.

Matthews, G., Bateman, R., & Miller, P. (2014). *Pesticide application methods*. John Wiley & Sons.

McLaren, G. (2022). *'Not so very fine and healthy as has been reported': Settlers, Malaria, and the Construction of the Rideau Canal (1826-1832)* (Doctoral dissertation, Concordia University).

Muniz-Junior,et al (2023). Are lower pesticide doses better? An evolutionary perspective on integrated pest management. *Ecological Modelling*, *482*, 110408.

Mnzava, A. P. (2015). Implementation of the global plan for insecticide resistance management in malaria vectors: Progress, challenges and the way forward. Malar

Nakao T, Banba. S: (2016). Broflanilide: A meta-diamide insecticide with a novel mode of action Bioorganic Med Chem.Med *chem.:24(3):372-377.10.1015/j.bmc.2015.08.00*

Ngufor, C. *et al-*. (2017). Which intervention is better for malaria vector control: Insecticide mixture long-lasting insecticidal nets orstandard pyrethroid nets combined with indoor residual spraying.

Namountougou, M., Soma, D. D., Kientega, M., Balboné, M., Kaboré, D. P. A., Drabo, S. F., ...&Gnankine, O. (2019). Insecticide resistance mechanisms in Anopheles gambiae complex populations from Burkina Faso, West Africa. *Actatropica*, *197*, 105054..

Okumu, F. O., & Moore, S. J. (2011). Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future. *Malaria journal*, *10*, 1-13.

Oxborough, R. M. (2015). *Laboratory and experimental hut evaluation of mosquito net and indoor residual spray (IRS) insecticides for improved malaria control* (Doctoral dissertation, London School of Hygiene & Tropical Medicine).

Oxborough, R. M. (2016). Trends in US President's Malaria Initiative-funded indoor residual spray coverage and insecticide choice in sub-saharan Africa (2008-2015). urgent need for afordable long-lasting insecticide Malaria .

Petros B, Geshere G. (2014). Trend of malaria prevalence in Ilu Galan, BakoTibe, and Danno districts of West Shoa Zone, OromiyaRegion, Ethiopia. *Int J Biolchm Sci.31:678-9*..

Raghavendra, Kamaraju, *et al*. (2011)"Malaria vector control: from past to future." *Parasitology research* 108: 757-779.

Sicuri, E., Vieta, A., Lindner, L., Constenla, D., &Sauboin, C. (2013). The economic costs of malaria in children in three sub-Saharan countries: Ghana, Tanzania and Kenya. *Malaria journal*, *12*, 1-14.

Snetselaar, J., Rowland, M. W., Azizi, S., Mawa, B., Malone, D. J., & Kirby, M. J. (2023). Laboratory evaluation of broflanilide (TENEBENAL™) against Anopheles gambiae in Moshi, Tanzania–delayed mortality, cross-resistance, and residual efficacy. *Frontiers in Tropical Diseases*, *4*, 1097189

Sodahlon, Y., Malecela, M., &Gyapong, J. O. (2016). Lymphatic filariasis (elephantiasis). *Neglected Tropical Diseases-Sub-Saharan Africa*, 159-186.

Taffese HS, Hemming-Schroeder E, Koepfli C, Tesfaye G, Lee MC, Kazura J, *et al.* (2018). Malaria epidemiology and interventions in Ethiopia from 2001 to 2016. Infect Dis Poverty.7:1–9.doi: 10.1186/s40249-018-0487-.

Tangena, J.-A.*et al*. (2020). Indoor residual spraying for malaria control in sub-Saharan Africa 1997 to 2017: An adjusted retrospective analysis.

Tirados, I., Costantini, C., Gibson, G., &Torr, S. J. (2006). Blood-feeding behaviour of the malarial mosquito Anopheles arabiensis: implications for vector control. *Medical and veterinary entomology*, *20*(4), 425-437.

van den Berg, H., da Silva Bezerra, H. S., Al-Eryani, S., Chanda, E., Nagpal, B. N., Knox, T. B., ... &Yadav, R. S. (2021). Recent trends in global insecticide use for disease vector control and potential implications for resistance management. *Scientific reports*, *11*(1), 23867

Webster, *et al,* (2014). The contribution of mass drug administration to global health: past, present and future. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1645), 20130434

WHO, (2013). Guidelines for Laboratory and Field-Testing of Long-Lasting Insecticidal Nets.

WHO (2010). *Report on the Second meeting of the regional Scientific and Technical Advisory Committee of the WHO*. No. WHO-EM/VBC/110/E..

WHO.(2016) "Strengthening the assessment of lymphatic filariasis transmission and documenting the achievement of elimination.".

WHO, (2019). Data requirements and protocol for determining non-inferiority of insecticide-treated net and indoor residualspraying products within an established WHO policy . Geneva.

WHO, (2016). Global Report on Insecticide Resistance in Malaria Vectors: 2010 - 2016.

WHO, (2006). Guidelines for testing mosquito adulticide for indoor residual spraying and treatment of mosquito nets. .Geneva.WHO/CDS/NTD/WHOPES/ GCDPP/2006.3

WHO, (2006). Indoor residual spraying: use of indoor residual spraying for scaling up global malaria contro and elimination.WHO position statement. https://apps.who.int/iris/handle/10665/69386 (World Health Organisation,)

WHO,, (2012). . Global Plan for Insecticide Resistance Management in Malaria Vectors .

WHO, (2021). Preferred product characteristics: indoor residual spraying products for malaria transmission control in areas with insecticide-resistant mosquito populations; Draft for public consultation. Geneva, World Health Organization,

WHO, (2023). World Malaria Report.2019.

WHO, (2015).*Indoor residual spraying.an operational manual for indoor residual spray(irs) for malaria transmission control and elimination,.*

WHO, (2018). Global Report on Insecticide Resistance in Malaria Vectors: 2010–2016 (World Health Organization, .

WHO, (2020). *List of WHO Prequalified Vector Control Products.*(002):1-5

WHO,(2023). . Guidelines for Malaria Vector Control (World Health Organization,.

WHO, (2019). *Guidelines for Malaria Vector Control.*

WHO, (2020). *World Malaria Report.*

Yewhalaw,Delenasaw, and Eliningaya J. Kweka, (2016). "Insecticide resistance in East Africa—history, distribution and drawbacks on malaria vectors and disease control." *Insecticides resistance* 39 : 189-215