AKENTEN APPIAH-MENKA UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT, MAMPONG.

ASSESSING CHEMICAL INSECTICIDE SUSCEPTIBILITY/RESISTANCE PROFILES OF MALARIA VECTORS IN THREE DISTRICTS OF THE UPPER WEST REGION OF GHANA, AND THEIR PUBLIC HEALTH IMPLICATIONS.

BY

ISAAC ARTHUR

2025

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BY

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A thesis submitted to the School of Graduate Studies, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, in partial fulfilment of the requirements for the award of a Master of Philosophy degree in Public Health at the Department of Public Health.

DECEMBER, 2025

DECLARATION

Candidate's Declaration

I hereby declare that this thesis, except for quotations and references contained in published works which have been duly acknowledged, is the result of my original work and that no part of it has been presented for another degree at this university or elsewhere.

Candidate's Name: Isaac Arthur

Signature…………………….………　　　Date…………………………………

Supervisor's Declaration

We certify that the preparation and presentation of the thesis were supervised in accordance with the guidelines on thesis supervision laid down by the Akenten Appiah Menka University of Skills Training and Entrepreneurial Development.

Principal Supervisor's Name: Dr. Daniel Hayford

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Co-Supervisor's Name: Dr. Kofi Sekyere Boateng

Signature ………………………………….　　　Date ……………………………

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DEDICATION

This project is dedicated to the Almighty God for his guidance and protection throughout my study. It is through his grace that I am alive to present this dissertation. It is also dedicated to my lovely family for their spiritual and physical support during my stay on the university campus, as well as for the success of this project and the programme. To all the people who have worked so hard to help me complete this project. May God bless you!

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CHAPTER SIX

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LIST OF ABBREVIATIONS

WHO	World Health Organisation
MoH	Ministry of Health
HBM	Health Belief Model
SD	Standard deviation
SPSS	Statistical Package for Social Sciences
PMI	President's Malaria Initiative
LLINs	Long-lasting insecticidal nets
IRS	Indoor Residual Spraying
AGAMAL	AngloGold Ashanti Malaria Control Programme
SMC	Seasonal Malaria Chemoprevention
MTTT	Mass testing, Treatment, and Tracking
ITNs	Insecticide-Treated Nets
CHAT	Community Health Advocacy Teams
CDC	Centres for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
IRAC	Insecticide Resistance Action Committee
IRM	Insecticide Resistance Management
IRS	Indoor Residual Spraying
KAP	Knowledge, Attitudes and Perception
Kdr	Knocked-down resistance gene
PCR	Polymerase Chain Reaction
PMI	President’s Malaria Initiative
RBM	Roll Back Malaria

RNA	Ribonucleic Acid
TDR	Tropical Diseases Research
uRDT	ultra-sensitive rapid diagnostic tests
KDT	Knockdown Time
RR	Resistance Ratio

ETHICAL CLEARANCE

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REGIONAL HEALTH DIRECTORATE - UPPER WEST

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INTRODUCTORY LETTER: MR. ISAAC ARTHUR

The bearer of this letter is a student of Philosophy in Public Health at the department of Public Health Education, Faculty of Environment and Health Education at the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong Campus. As a key part in partial fulfilment of the requirements for obtaining the award for Master of Philosophy degree, the student is undertaking a research work which will involve data collection in your institution on the topic: **"SUSCEPTIBILITY OF MALARIA VECTOR POPULATIONS OF THE UPPER WEST REGION TO THE NEW GENERATION OF INSECTICIDES APPROVED FOR VECTOR CONTROL OPERATIONS".**

Please provide him with the necessary support and cooperation as I wish to emphasize that this research is solely for academic purposes and as such ensure that the privacy and confidentiality of your staff and students are maintained.

Any form of misconduct related to this research should be reported to the Research Unit for appropriate action.

Thank you.

DR. (MED.) DAMIEN PUNGUYIRE

REGIONAL DIRECTOR OF HEALTH SERVICES – UWR

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ABSTRACT

Malaria remains a major public health concern in Ghana, including the Upper West Region, where the intensity of its transmission persists at high levels. The increasing emergence of insecticide resistance in *Anopheles* gambiae sensu lato vector populations is a major threat to the effectiveness of malaria vector control strategies. The current study assessed the susceptibility of malaria vector populations in Wa, Nadowli, and Lawra districts to older classes of key synthetic chemical insecticides, including pyrethroid (deltamethrin and permethrin), carbamate (bendiocarb), and organophosphate (pirimiphos-methyl); as well as a newer class: neonicotinoid (clothianidin). The evaluation of insecticide resistance in the malaria vector population was conducted using the WHO susceptibility bioassay guidelines and kits. Mosquito larvae were collected from selected breeding sites and reared to adults. Overall, 300 female *Anopheles gambiae* s.l. were exposed to varying concentrations of insecticides. Knockdowns and mortalities were recorded. Results revealed considerable variability in susceptibility across sites and insecticide classes. Knockdown rates (KDRs) ranged from 4.0% to 16.0% at lower deltamethrin concentrations but reached 100% at higher doses. Higher KDT_{50} and KDT_{95} values were estimated for wild mosquito populations compared with the standard Kisumu susceptible strain. The highest KDTs were calculated for the pyrethroid-based bioassays, with resistance ratios for $KDT_{50} >5$. This suggests significant physiological resistance in the wild mosquito population. For pyrethroid-based bioassays, mortality rates ranged from 20.0% for 0.05% deltamethrin to 100% for 0.50% deltamethrin. In addition, mortality rates for the permethrin ranged from 38.0% to 99.0%. For the carbamate and organophosphate bioassays, respectively, 0.10% bendiocarb induced mortalities ranging from 59.0% to 84.0% while pirimiphos-methyl achieved higher rates (87-99%). However, 2% clothianidin-based bioassays

yielded 100% mortality within 24 hours. These findings suggest considerable resistance in the wild *An. gambiae* s.l. populations to the older classes of insecticides but high susceptibility to the newer insecticide class, justifying the choice of indoor residual spraying insecticide formulation that includes clothianidin for the Upper West Region.

CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria continues to be one of the leading public health challenges worldwide, with Sub-Saharan Africa bearing the greatest burden. Despite ongoing global efforts to eradicate the disease, it persists as a major health concern. According to the World Health Organization (WHO,2024), interventions since the year 2000 have prevented approximately 2.2 billion malaria cases and 12.7 million deaths. Nonetheless, malaria remains a severe threat, particularly within the WHO African Region (Reference). The World Malaria Report (2024) estimated that in 2023, about 263 million cases and 597,000 deaths occurred globally, representing a nearly 11 million case increase compared to 2022, though the number of deaths remained nearly unchanged. Roughly 95% of malaria-related fatalities were recorded in the African Region, where many populations still lack access to vital preventive, diagnostic, and treatment services (World Malaria Report 2024, n.d.). In 2021 alone, about 229 million cases were documented worldwide, with more than 94% of these occurring in Africa (WHO, 2020). Since its introduction, indoor residual spraying (IRS) has played a central role in malaria vector control (Sovi et al., 2020). During the 1960s, WHO recommended widespread IRS deployment as a primary method for reducing malaria transmission (Sovi et al., 2020). Complementary strategies such as the use of long-lasting insecticidal nets (ITNs), larval source management, and structural modifications to reduce mosquito entry have been adopted to strengthen control efforts (WHO, 2024). However, several challenges, including insecticide resistance, logistical constraints, and financial limitations, have led some countries to scale down or discontinue IRS activities (Agossa et al., 2018).

In 2006, WHO reaffirmed IRS as a key intervention for controlling malaria transmission across various endemic settings (Oxborough et al., 2016). Since then, many African nations have expanded their IRS programs with support from partners such as the U.S. President's Malaria Initiative (PMI) and the Global Fund (Oxborough et al., 2016). Reports indicate that the IRS helped reduce malaria incidence and prevalence by approximately 14% and 16%, respectively, between 2000 and 2015 (Oxborough et al., 2016). In Ghana, the National Malaria Control Programme (NMCP), in collaboration with PMI and AngloGold Ashanti Malaria Limited (AGAMAL), has implemented IRS operations since 2008 in several northern districts to lower malaria transmission. The IRS primarily targets endophilic female *Anopheles* mosquitoes that rest on indoor walls after feeding (Dengela et al., 2018).

The success of IRS depends on multiple factors, such as the species composition and resting behaviour of mosquito populations (WHO, 2016); the quality of spray application and residual effectiveness of the insecticide used (Dengela et al., 2018); the formulation type, spray coverage, and the nature of wall surfaces (Agossa et al., 2018; Desalegn et al., 2018); as well as the timing of spray campaigns (Dengela et al., 2018). Moreover, community acceptance, perceptions, and participation also play a crucial role in determining the overall success of IRS interventions (Opiyo & Paaijmans, 2020).

Insecticide resistance among malaria vectors has been widely reported across Sub-Saharan Africa, particularly to the four WHO-approved chemical insecticide classes used in vector control (WHO, 2020). This rising resistance underscores the need for new insecticides with different modes of action that avoid cross-resistance with existing compounds (Ngufor et al., 2017). Clothianidin, a neonicotinoid, represents the first new insecticide class with a novel mechanism of action endorsed by WHO for IRS use over the years (IVCC, 2020). Given the increasing resistance to traditional insecticides, clothianidin is emerging as a preferred choice for IRS programs due to its odourless nature, low mammalian toxicity, and efficacy against resistant mosquito strains (Dagg et al., 2019). However, unlike the faster-acting pyrethroids, organophosphates, carbamates, and organochlorines, clothianidin acts slowly and typically requires 72 to 168 hours for its effects to become fully evident (Agossa et al., 2018; Oxborough et al., 2019).

1.2 Problem Statement

Malaria control efforts in Ghana continue to be threatened by the growing spread of insecticide resistance among *Anopheles* mosquito populations, particularly in regions where vector control interventions have been implemented for many years. The traditional classes of insecticides pyrethroids, carbamates, and organophosphates, have all shown varying degrees of reduced efficacy due to physiological and metabolic resistance mechanisms that are becoming increasingly widespread (WHO, 2024). Although periodic monitoring has been helpful in tracking whether resistance trends are strengthening, weakening, or stabilizing, recent reports, including the World Malaria Report (2024), emphasize that resistance patterns are rapidly evolving and require continuous, site-specific assessment. This situation poses a significant concern for IRS-dependent districts in northern Ghana, where malaria transmission remains persistently high.

In response to these challenges, AGA Mal introduced **Fludora® Fusion**, a dual-active IRS formulation combining clothianidin and deltamethrin, into its operational IRS use in 2023. While this formulation was designed to enhance residual efficacy and delay resistance selection, its long-term effectiveness cannot be assumed without empirical evidence from the local vector populations. Current data on the susceptibility of *Anopheles* mosquitoes in the Upper West Region to the neonicotinoid class (clothianidin) are limited, creating a critical knowledge gap that has implications for strategic decision-making, product rotation, and overall IRS sustainability. Regular assessment of susceptibility to both the new and traditional classes of insecticides is therefore essential to detect emerging tolerance early, safeguard operational effectiveness, and support evidence-based planning of vector control interventions. This study seeks to address this gap by evaluating the susceptibility status of malaria vector populations to the new generation of insecticides (Clothianidin) and the traditional WHO-approved insecticides across selected districts in the Upper West Region of Ghana.

1.3 Justification.

A study on the susceptibility/resistance of malaria vectors to both older and newer synthetic chemical insecticides is pivotal to malaria control. Contemporary insecticide-based malaria vector control programmes operate to effectively manage malaria vector populations and sustain the gains achieved against this infectious disease. Such a pragmatic strategy is based on a sound synthetic chemical insecticide resistance management (IRM) approach that informs the required programmatic decisions, ensuring the sustained effectiveness of vector control tools (Rivero et al., 2010; WHO, 2012; Mahande et al., 2012; Corbel & N'Guessan, 2013; Shah et al., 2020; Kamaraju et al., 2025). One of the justifications for the current research work on the older insecticide classes was the need to monitor and document the extent to which the loss of efficacy of these insecticides, as reported by WHO (2023,2024) has occurred in the Upper West Region, where large-scale use of such insecticides has occurred over the years. Older categories of insecticides, especially pyrethroids, organophosphates and carbamates, have been the cornerstone of vector control in the Upper West Region and several other parts of Ghana through IRS and ITNs (Gogue et al., 2020). A study to assess the performance of these insecticides remains pivotal in tracking the distribution and intensity of existing resistance, which can compromise their efficacy in IRS, ITNs, and larval control operations in the area. Furthermore, the planners and implementers of the malaria vector control interventions can choose to use older insecticide classes in rotation with newer ones as part of their insecticide resistance management strategy. Continued monitoring of these insecticides is crucial for determining when insecticide rotation may be necessary and for predicting its potential effectiveness (WHO, 2006; Maharaj et al., 2024).

Another considerable justification for assessing the performance of older insecticide classes is to detect potential reversal of vector resistance to susceptibility. An insecticide-resistant malaria vector population can regain susceptibility to a synthetic chemical insecticide when the active ingredient is withdrawn from use for a period. Continued monitoring of older insecticide active ingredients could help identify these cases, possibly enabling their reintroduction into the disease vector control operations. Some mosquito populations in Côte d'Ivoire, for example, reportedly regained susceptibility to malathion after it was temporarily substituted by another insecticide (The malERA Refresh Consultative Panel on Insecticide and Drug Resistance, 2017) (Wipf et al., 2022). Likewise, Zambia employed DDT in IRS operations from 2002 to 2010 and discontinued its use due to resistance in the *Anopheles* vector. Later, the vector's susceptibility to DDT was restored, and DDT was reintroduced into the control operations (National Assembly of Zambia, n.d.).

The introduction of newer insecticide classes for malaria vector control operations is an important strategy to overcome vector resistance to older classes. Nevertheless, mosquito vectors can develop resistance to the newer compounds with time. Therefore, continued monitoring of novel insecticides from the outset of their introduction into control operations is a proactive strategy to detect and manage insecticide resistance mechanisms before they become widespread.

The current research generated considerable data to support the comprehensive insecticide resistance management plan of the ongoing control programme by monitoring active ingredients of both older and newer insecticide classes. Information on both older and newer insecticides can help programme planners and implementers sustain or develop a more holistic strategy, including rotational or mosaic spraying, to maximise the lifespan of all approved insecticidal tools.

1.4 Aim of Study.

The current study determined the insecticide susceptibility and resistance profiles of the predominant malaria vector populations in the Wa Municipality, Nadoli District, and Lawra District of the Upper West Region of Ghana.

1.5.1 Specific Objectives.

The specific objectives of the current study were to determine:

i. The physiological susceptibility levels of *An. gambiae* s.l. populations to the older insecticide classes approved by WHO for vector control.
ii. The physiological susceptibility levels of *An. Gambiae*s.l. populations to the new generation of insecticide clothianidin, approved by WHO for vector control.
iii. To explore the implications of physiological susceptibility/resistance in the malaria vector to the newer and the older insecticide classes for malaria vector control programme operation in the three districts studied.

1.5.2 Research Questions

The research questions the current study sought to answer were:

i. To what extent are *Anopheles gambiae* s.l. populations in the three selected districts physiologically susceptible or resistant to the older WHO-approved insecticide classes?
ii. What is the physiological susceptibility status of *Anopheles gambiae s.l.* populations in the study districts to the new-generation active ingredient clothianidin?
iii. How do the detected susceptibility or resistance patterns to both older and newer insecticide classes influence malaria vector control decision-making and operational planning within the three study districts?

1.6 Scope

The current study explored physiological susceptibility and resistance in the main malaria vector populations in this region, namely, *An. gambiae* s.l. populations from the Nadoli District, Lawra District, and the Wa Municipality of the Upper West Region of Ghana, where there is no known published work on insecticide susceptibility or resistance. The study did not explore the physiological susceptibility and/or resistance status of other *Anopheles* vector populations in the studied districts and the municipality. Furthermore, it did not explore molecular and metabolic resistance mechanisms in the resistant mosquitoes. The study focused on deltamethrin of the pyrethroid class used in both insecticidal nets and IRS operations; permethrin of the pyrethroid class used in insecticidal nets; bendiocarb of the carbamate class, which serves as an active ingredient in IRS insecticide formulations; and pirimiphos methyl of the organophosphate class, which is also used in IRS products.

1.7 Limitation

Studies have shown not only spatial but also temporal or seasonal variation in insecticide resistance among malaria vector populations. The current study generated data on the physiological susceptibility and resistance profiles of *An. gambiae* s.l. populations during the rainy season.

The study did not include molecular assays to identify the specific mechanisms driving resistance.

1.8 Organisation of the Study

This thesis is structured into six main chapters. Chapter One presents the study's background, outlines the research problem, and details the study's objectives and the research questions addressed. Chapter Two provides a comprehensive review of the relevant literature, aligning with both the general and specific objectives of the study. Chapter Three describes the research design, study area, sampling methods, data collection instruments, and analytical procedures employed. Chapter Four presents the study's results, while Chapter Five discusses and interprets these findings in relation to existing research. The final chapter, Chapter Six, provides a summary of the key findings, conclusions drawn, and practical recommendations derived from the study.

CHAPTER TWO

LITERATURE REVIEW.

2.1 Burden of Malaria

The World Health Organization (WHO,2024) estimates that global malaria interventions have prevented approximately 2.2 billion infections and 12.7 million deaths since the year 2000. Despite these gains, malaria continues to pose a major global health challenge, with the most significant impact observed in the WHO African Region. The most recent World Malaria Report (2024) indicates that in 2023, there were about 263 million malaria cases and 597,000 related deaths worldwide, representing an increase of nearly 11 million cases compared to 2022, while mortality figures remained relatively unchanged. Roughly 95% of all malaria-related deaths occurred in the African Region, where large segments of the population still lack adequate access to preventive, diagnostic, and treatment services (World Malaria Report 2024, n.d.).

Globally, malaria cases rose from 227 million in 2019 to around 241 million in 2020 across 85 endemic countries, with the African continent continuing to experience the heaviest burden, accounting for nearly 96% of all cases and deaths reported worldwide (Magna et al., 2019). Within the same region, an estimated 11.6 million pregnant women, representing about 34% of the total, were at risk of infection, with the highest vulnerability recorded in West Africa (38%). Encouragingly, malaria mortality among children under five years of age declined from approximately 87% in 2000 to about 77% in 2020 (WHO, 2021). In Ghana, the disease remains a significant concern, contributing to roughly 2% of global malaria cases and about 3% of malaria deaths (WHO, 2020).

In 2021, there were approximately 247 million malaria cases worldwide, with significant morbidity and mortality concentrated in African nations, particularly in Western and Central Sub-Saharan Africa, which reported the highest incidence rates and a concerning increase in deaths from 1990 to 2019 (Darko et al., 2023). The disease's health impact is profound, contributing to an estimated 6.43 million deaths and 464 million disability-adjusted life years (DALYs) in 2019 alone (Shi et al., 2023).

2.2 Malaria Vectors and Transmission in Ghana.

There are over 400 species of Anopheles mosquitoes, but only about 30 are important in public health (WHO, 2020). The transmission of diseases, such as malaria, by the important ones is significantly influenced by various ecological, behavioural, and environmental factors. Urban areas, particularly Accra, exhibit diverse larval habitats for malaria vectors, with a notable prevalence of *Anopheles gambiae* sensu lato and the invasive *An. stephensi*, which thrives in polluted environments such as drainage ditches and irrigated farms (Sabtiu et al., 2024). *An. gambiae* complex is the predominant malaria vector in northern Ghana. The biting behaviour of these vectors varies, with indoor and outdoor biting rates being comparable, indicating a need for integrated control strategies that address both sites (Akuoko et al., 2024; Coleman et al., 2023). Mathematical models further emphasise the benefit of understanding vector dynamics and human interactions to develop effective control interventions in Ghana (Nana-Kyere et al., 2024*). An. gambiae s.l.* predominates as the malaria vector in Ghana, with significant indoor biting behaviour. The study found a sporozoite rate of 0.1%, indicating transmission potential, particularly in outdoor-biting mosquitoes in specific ecological zones (Akuoko et al., 2024).

Anopheles gambiae and *Anopheles funestus* are the predominant malaria vectors in Ghana, with *An. gambiae* exhibiting a higher *Plasmodium* infection rate (14.28) compared to *An. funestus*, indicating both species' significant roles in malaria transmission in the region. *Anopheles gambiae s.l.* predominates as the malaria vector in Ghana, with higher indoor biting rates (51.09%) than outdoor biting rates (48.91%). Sporozoite rates were higher in outdoor mosquitoes in specific locations, indicating varied transmission dynamics across ecological zones (Akuoko et al., 2024).

The region has been identified as having high malaria incidence, with studies indicating that Wa municipality reports the highest rates, reaching approximately 21.7% (Magna et al., 2019). Seasonal variations in malaria risk are pronounced, and interventions such as Seasonal Malaria Chemoprevention (SMC) have demonstrated a 67% reduction in under-five mortality during peak transmission months (Adjei et al., 2022). Furthermore, spatial analyses reveal substantial differences in malaria risk across districts, with specific areas experiencing relative risks reaching about 4.8% (Aheto et al., 2023). Despite the implementation of strategies such as indoor residual spraying (IRS) for some time now, community acceptance remains low due to various barriers (Suuron et al., 2020). Targeted interventions and active community participation remain vital in achieving effective malaria control across Ghana and the wider African region (Aheto et al., 2023; Magna et al., 2019). In Ghana, the primary mosquito vectors responsible for malaria transmission belong to the *Anopheles gambiae* and *Anopheles funestus* complexes. Globally, eight *Plasmodium* species are known to infect humans: *P.*

falciparum, *P. malariae*, *P. vivax*, *P. ovale wallikeri*, *P. ovale curtisi*, *P. knowlesi*, *P. cynomolgi*, *P. simium*, and *P. brasilianum* (Mourier et al., 2021). However, only five of these species, *P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale*, and *P. knowlesi*, are primarily responsible for the majority of malaria infections worldwide. In Ghana, *P. falciparum* accounts for more than 97% of reported malaria cases, while *P. malariae* and *P. ovale* often co-infect (WHO, 2020; Dao et al., 2023).

2.3 Malaria Interventions in Ghana.

Malaria intervention strategies in Ghana have been multifaceted, focusing on improving data quality, diagnostic accuracy, community engagement, and targeted case detection. A study on data coaching visits in Ghana demonstrated significant improvements in the quality and completeness of malaria service data, which is crucial for effective decision-making and planning at the district level. The intervention increased health workers' understanding of data standards and improved the reliability and timeliness of health management information system (HMIS) data (Asiedu et al., 2024). In terms of diagnostics, the use of ultra-sensitive rapid diagnostic tests (uRDT) in mass testing, treatment, and tracking (MTTT) interventions showed a significant reduction in asymptomatic parasite prevalence in intervention sites, highlighting the importance of sensitive diagnostic tools in identifying asymptomatic carriers who sustain transmission (Amoah et al., 2024). Additionally, a mathematical model emphasised the role of vaccines in reducing malaria cases, suggesting that integrating vaccination into intervention strategies could be cost-effective (Nana-Kyere et al., 2024). In low-endemic areas like Asutsuare, reactive case detection (RACD) has been recommended to identify asymptomatic infections, which are often missed but crucial for interrupting transmission (Aidoo et al., 2024). Furthermore, the implementation of Community

Health Advocacy Teams (CHAT) has proven effective in increasing awareness, access, and use of long-lasting insecticide nets (LLINs), demonstrating the potential of community-based interventions to support national malaria programs (Glozah et al., 2023). These studies underscore the importance of a comprehensive approach that includes data quality improvement, advanced diagnostics, community engagement, and targeted detection to combat malaria in Ghana effectively.

Vector control has been a key component of malaria prevention efforts in Ghana since the pre-independence era. Among the major strategies employed are indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs), both of which remain central to malaria control initiatives. In the Upper West Region, AngloGold Ashanti's malaria control programme focuses mainly on IRS and the distribution of insecticide-treated nets (ITNs). The IRS intervention, initiated in 2006, employs micro-encapsulated insecticide formulations designed to mitigate resistance development. This approach has led to notable declines in malaria incidence, with reported reductions of 39%-58% in IRS-targeted districts between 2015 and 2017 (Gogue et al., 2020b). Despite these achievements, sustaining community acceptance remains a concern, as cultural perceptions, limited awareness, and doubts about effectiveness continue to hinder full participation (Suuron et al., 2020). Studies indicate that combining IRS with ITNs enhances malaria transmission risk reduction, achieving a 58% decrease in transmission in communities utilising both interventions (Coleman et al., 2023). Overall, while the intervention has demonstrated positive impacts on malaria control, addressing community concerns and improving engagement are crucial for sustained effectiveness (Aabeyir, 2010). What are the multifaceted aspects of Ghana? Each should include a paragraph outlining whether the intervention was successful.

2.3.1 Multifaceted Aspects of Malaria Intervention in Ghana

Ghana's fight against malaria has employed a combination of interventions from vector control to drug-based prevention, reflecting a multipronged strategy that acknowledges the complexity of malaria transmission. The key aspects of malaria interventions in Ghana include the distribution of insecticide-treated nets (ITNs / LLINs), indoor residual spraying (IRS), preventive treatment during pregnancy, case management with effective antimalarial drugs, and, more recently, novel approaches such as chemoprevention campaigns and vaccine rollout. Each of these has contributed to varying extents to reductions in malaria burden, though challenges remain.

2.3.2 Insecticide-Treated / Long-Lasting Nets (ITNs / LLINs)

One of the foundational interventions in Ghana's malaria control strategy is the widespread distribution of insecticide-treated nets. Over the past two decades, mass net distribution campaigns, along with routine distribution through antenatal care (ANC) clinics and child welfare clinics, have expanded population access to nets. According to the most recent nationwide survey, about 74 % of households in Ghana own at least one ITN, and 52% of households have at least one net for every two people, representing major strides towards universal coverage (Tetteh et al., 2023). However, there remains a gap between ownership and consistent use: only around 43 % of the overall household population, 54 % of children under five, and 49 % of pregnant women reported sleeping under a net the night before the survey (Reed, 2019). Despite this shortfall, the widespread distribution and increased access to nets have significantly reduced malaria exposure and transmission risk, especially among the most vulnerable groups. The intervention can be considered *partially successful*, as coverage is high, but full impact is limited by suboptimal utilization.

2.3.3 Indoor Residual Spraying (IRS)

Indoor Residual Spraying has been another major component of Ghana's vector control efforts. Early implementation by a private sector actor, a large mining company in a Ghanaian township, demonstrated the potential of IRS: over two years, the area experienced a reduction of up to 74 % in hospitalized malaria cases (George, 2014). Because of this success, the IRS was scaled up to multiple districts under national malaria control strategies (Aregawi et al., 2017a). More recent national efforts under the high-burden, high-impact (HBHI) approach continue to deploy IRS where appropriate. Nevertheless, the IRS's reach remains limited compared to ITNs, in part because of the logistical demands, cost, and requirement for high coverage to maximize community-wide protection. As such, while the IRS has demonstrated ranked success in targeted communities, its national-scale impact remains constrained by operational challenges (WHO, 2024).

2.3.4 Intermittent Preventive Treatment in Pregnancy (IPTp)

Preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (SP) under directly observed therapy (DOT) has been institutionalized in Ghana's maternal health services. This intervention aims to reduce malaria-related morbidity and mortality among pregnant women and their unborn children (Aberese-Ako et al., 2020). While IPTp has achieved considerable uptake in antenatal care settings, the intervention has faced barriers, including inconsistent attendance at antenatal care, stock-outs of drugs, confusion over dosing timing, and socio-cultural practices that hinder full compliance (Aberese-Ako et al., 2020). Consequently, although IPTp has contributed to lowering malaria risk among pregnant women, its effectiveness has been uneven across regions and populations. In that context, IPTp can be considered partially successful, but with substantial implementation gaps.

2.3.5 Prompt Case Management with Effective Antimalarial Drugs

The adoption of artemisinin-based combination therapies (ACTs) for first-line treatment of uncomplicated malaria has been a cornerstone of Ghana's malaria control programme since the mid-2000s. This shift has ensured that malaria cases are treated with effective drugs, reducing parasite densities, preventing severe disease, and limiting onward transmission (Aregawi et al., 2017b). Health facility data and hospital records over a decade show significant declines in malaria admissions and deaths following the scale-up of ACT and net distribution. (WHO Africa, 2024). Nevertheless, challenges such as drug supply chain issues, delays in diagnosis, and access disparities in remote areas persist, limiting the uniform success of case management across the country. Overall, this strategy remains largely successful, albeit to varying degrees across different geographic and socioeconomic contexts.

2.3.5 Targeted Chemoprevention (Seasonal/High-Risk Campaigns) and Vaccine Introduction

In addition to long-standing interventions, Ghana has recently expanded its malaria control toolbox to include targeted chemoprevention campaigns in high-risk zones and the rollout of the first malaria vaccine. Under the high-burden, high-impact (HBHI) framework, the country has optimized existing tools to reduce malaria-related deaths(Li et al., 2024). As of 2023, national data show a marked reduction in parasite prevalence among children under five from 20.6% in 2016 to 8.6% and a significant drop in inpatient audited malaria deaths between 2018 and 2022 (WHO Africa, 2024). The combination of nets, IRS, chemoprevention, and vaccine deployment has strengthened Ghana's malaria control strategy, highlighting that the integrated, multifaceted approach is showing promising success. However, continued vigilance is needed to maintain coverage, address insecticide resistance, and ensure equitable access nationwide.

2.4 Insecticide Resistance

Malaria vector populations are increasingly exhibiting resistance to multiple classes of insecticides, including organochlorines, organophosphates, carbamates, pyrethroids, neonicotinoids, and pyrroles. This growing resistance undermines global efforts to eliminate malaria and underscores the urgent need for effective management strategies. Most malaria control interventions depend heavily on insecticide-based approaches, which have contributed to the problem.

Continuous and widespread use of these chemicals has led to mosquito resistance across all four major traditional insecticide classes: pyrethroids, carbamates, organophosphates, and organochlorines. This challenge has been particularly evident across Africa, including Ghana, where it threatens the success of vector control programmes (WHO, 2020). To address this concern, the World Health Organization has recommended the adoption of newer insecticide classes in indoor residual spraying (IRS) and long-lasting insecticidal net (LLIN) interventions (References). The aim is to reduce resistance development and preserve the effectiveness of ongoing malaria vector control strategies.

Recent studies highlight various resistance mechanisms across different Anopheles species. For instance, *Anopheles gambiae* in Tanzania exhibited widespread pyrethroid resistance, although pre-exposure to piperonyl-butoxide (PBO) restored susceptibility in many cases, indicating the presence of metabolic resistance mechanisms (Kabula et al., 2024). Conversely, *Anopheles funestus*, traditionally resistant through detoxification enzymes, has recently shown knock-down resistance (*kdr*) mutations, particularly linked to DDT exposure (Odero et al., 2024). In Ethiopia, *Anopheles stephensi* demonstrated resistance to multiple insecticides, necessitating the use of pirimiphos-methyl and PBO-pyrethroids for effective control (Samake et al., 2024). Additionally, *Anopheles coluzzii* in São Tomé and Príncipe displayed high resistance to pyrethroids and DDT, with partial susceptibility restoration via PBO, suggesting cytochrome P450-mediated resistance (Correa et al., 2024). These findings underscore the urgent need for adaptive vector control strategies to combat evolving resistance patterns (Kumar et al., 2024).

In Ghana, between 2008 and 2012, the President's Malaria Initiative (PMI) Indoor Residual Spraying (IRS) programme in northern Ghana utilized pyrethroid insecticides such as alpha-cypermethrin and deltamethrin (Oxborough et al., 2019). However, as resistance to pyrethroids began to rise and the programme's effectiveness was threatened, the initiative transitioned to pirimiphos-methyl, an organophosphate. This insecticide was applied for over six years because mosquito populations remained largely susceptible, and no suitable alternative was available at the time.

In 2018, the World Health Organization (WHO) prequalified a new IRS formulation, SumiShield 50WG, which contains clothianidin, a neonicotinoid active ingredient, and it was subsequently approved by the Ghana Environmental Protection Agency (EPA) for IRS operations (WHO, 2020). Following its introduction, the PMI IRS programme began applying clothianidin in March 2021, while AngloGold Ashanti Malaria Control (AGAMal) adopted the same product in April 2023. These changes were part of broader insecticide resistance management strategies in districts that had relied on pirimiphos-methyl for more than six years. Reports of emerging resistance to pirimiphos-methyl in specific communities prompted the National Malaria Control Programme (NMCP) to transition to the newer class of insecticides to sustain operational effectiveness.

In 2020, the programme also introduced Fludora® Fusion, a second clothianidin-based IRS formulation developed by Bayer Crop Science (IVCC, 2020). This product combines clothianidin and deltamethrin, making it the first dual-action IRS insecticide to receive WHO prequalification (WHO, 2020). Because neonicotinoids exhibit a lower affinity for vertebrate nicotinic acetylcholine receptors than for those of insects, they are less toxic to mammals and thus suitable for public health applications (Ngufor et al., 2017; Oxborough et al., 2019). Additionally, these compounds act more slowly than most conventional insecticides, prompting researchers to adjust their evaluation protocols to accommodate this delayed mode of action (Oxborough et al., 2019).

2.4.1 Mechanisms of Resistance.

Insecticide resistance occurs when mosquito populations are no longer effectively controlled by a standard insecticide dose, rendering them partially or fully resistant. This phenomenon represents an evolutionary adaptation within vector populations

(WHO, 2021). Several mechanisms have been implicated in resistance development, including target-site mutations, enhanced metabolic detoxification, reduced cuticular penetration, and behavioural avoidance. Each of these mechanisms contributes to the overall reduction in insecticide efficacy observed in malaria vectors.

2.4.2 Target-site Mutation.

Target-site mutations in mosquitoes, particularly in the context of insecticide resistance, are critical for understanding the efficacy of vector control strategies. The *kdr* (knockdown resistance) mutations, notably *L1014F* and *L1014S*, in the voltage-gated sodium channel (VGSC) have been extensively studied across various mosquito species, including *Anopheles gambiae* and *Aedes aegypti*. These mutations confer resistance to pyrethroids, the primary insecticides used in malaria control, with studies indicating that multiple mutations can significantly enhance resistance levels (Gueye et al., 2024; Uemura et al., 2024). In Anopheles populations from Senegal, the *L1014F* mutation was prevalent, while the N1575Y mutation was associated with resistance phenotypes (Gueye et al., 2024). Additionally, metabolic detoxification mechanisms, along with target-site mutations, have been implicated in neonicotinoid resistance in *Aedes aegypti*, highlighting the complexity of resistance mechanisms (Samal et al., 2022). Geographic and temporal trends in these mutations reveal significant variations across species and regions, emphasising the need for molecular surveillance to inform vector control strategies effectively (Hancock et al., 2022) ("Analysis of the Knockdown Resistance Locus (*kdr*) in *Anopheles stephensi*, *An. arabiensis*, and *Culex pipiens s.l.* for Insight Into the Evolution of Target-site Pyrethroid Resistance in Eastern Ethiopia", 2022).

2.4.3 Metabolic Resistance.

Metabolic resistance in malaria vectors, particularly Anopheles species, poses significant challenges to malaria control efforts. Studies have identified key genetic mutations, such as the *G454A* in *CYP9K1* and *E205D* in *CYP6P3*, which enhance the metabolic breakdown of pyrethroids, thereby reducing the efficacy of insecticide-treated bed nets (LLINs) (Kengne-Ouafo et al., 2024). In regions like East and Central Africa, the rapid spread of these mutations has been documented, with the *G454A* mutation becoming predominant in Anopheles funestus populations (Tagne et al., 2024). Additionally, research in Côte d'Ivoire confirmed strong resistance to pyrethroids, with metabolic resistance mechanisms being linked to the presence of *kdr*-West and *Ace-1* genes (BÉKÉ et al., 2024). The use of synergists such as Piperonyl butoxide (PBO) has shown potential to restore susceptibility, suggesting that metabolic resistance can be mitigated through strategic interventions (BÉKÉ et al., 2024). Overall, understanding these molecular mechanisms is crucial for developing effective resistance management strategies (Ingham & Nagi, 2024).

2.4.4 Reduced Penetration of Insecticide.

The reduced penetration of insecticides among malaria vectors, particularly in Anopheles species, poses a significant challenge to malaria control efforts. Insecticide resistance has escalated, with vectors developing resistance to various classes of insecticides, including pyrethroids, organophosphates, and newer compounds like chlorfenapyr (Zhu, 2024; Tchouakui et al., 2023). Studies indicate that resistance not only diminishes the efficacy of long-lasting insecticide-treated nets (LLINs) but also affects mosquito behaviour, such as host-seeking and feeding success (Barreaux, 2020; Tchouakui et al., 2021). For instance, in Uganda, high levels of resistance have been linked to a marked reduction in LLIN effectiveness, necessitating urgent intervention to prevent further escalation of resistance (Tchouakui et al., 2021). Moreover, the mechanisms of resistance are complex, involving genetic mutations and metabolic changes, which complicate management strategies (Zouré et al., 2021). Thus, addressing insecticide resistance is critical for sustaining malaria vector control and achieving global elimination goals.

2.4.5 Behavioural Resistance.

Behavioural resistance in malaria vectors, *Anopheles* mosquitoes, is increasingly recognised as a significant challenge to vector control efforts. Studies indicate that these vectors exhibit spatial sensory detection of pyrethroids, leading to behavioural adaptations that allow them to avoid insecticide exposure, such as reduced take-off responses in the presence of these chemicals (Kambou et al., 2024). Additionally, *Anopheles funestus* has shown novel knock-down resistance mutations, which may further complicate control strategies, as these mutations are linked to survival against banned insecticides like DDT (Odero et al., 2024). The overall increase in insecticide resistance, including behavioural changes, poses a substantial threat to malaria elimination efforts, necessitating ongoing monitoring and innovative management strategies to address both physiological and behavioural resistance (Meeûs, 2024).

2.5 New generation of Insecticides Recommended for vector control

The new generation of insecticides approved for malaria vector control primarily focuses on overcoming resistance to traditional pyrethroid insecticides. These next-generation insecticides are integrated into bed nets and other vector control tools to enhance efficacy against resistant mosquito populations. The World Health

Organisation (WHO,2020?) has recommended several classes of these new insecticides, including dual-active-ingredient bed nets combining pyrethroids with other chemistries such as chlorfenapyr, clothianidin, and pyriproxyfen. These combinations aim to counteract the widespread resistance observed in malaria vectors such as *Anopheles gambiae* (Böhmert et al., 2024).

2.5.1 Clothianidin

Clothianidin, a neonicotinoid insecticide, affects the development and survival of malaria vectors primarily through its interaction with nicotinic acetylcholine receptors, leading to delayed mortality in exposed mosquitoes, particularly *Anopheles gambiae* (Ngufor et al., 2017). The biochemical mechanism involves the metabolism of clothianidin by cytochrome *P450* enzymes, specifically *CYP6P9a*/b, which confer resistance by degrading the insecticide, thereby reducing its efficacy (Tchouakui et al., 2024; Zoh et al., 2021). Studies indicate that resistance mechanisms, including target-site mutations like *kdr* and over-transcription of *P450* genes, can significantly impact susceptibility to clothianidin, with resistant genotypes exhibiting higher survival rates (Zong et al., 2024; Fouet et al., 2023). Furthermore, combining clothianidin with pyrethroids like deltamethrin has shown improved control over resistant populations, suggesting a strategic approach to managing resistance (Ngufor et al., 2017). Overall, the effectiveness of clothianidin is influenced by both its novel mode of action and the evolving resistance mechanisms in malaria vectors.

CHAPTER THREE

METHODOLOGY

3.1 Study Design

This study adopted an experimental research design to investigate the susceptibility and resistance profiles of key malaria vector populations from one municipality and two districts in the Upper West Region of Ghana. These areas have ongoing insecticide-based vector control interventions. The design enabled the collection of field and laboratory data to evaluate and compare the responses of malaria vectors to both older and newer classes of insecticides used in Indoor Residual Spraying (IRS) formulations. The research process incorporated field collections, insectary rearing, laboratory bioassays, and computer-based data analysis to ensure comprehensive evaluation and accurate interpretation of results.

3.2 Study Area

The research was carried out in the Upper West Region of Ghana, focusing on three selected locations Lawra District (10°22'53.4" N, 2°49'50.9" W), Nadowli District (10°22'53.4" N, 2°40'32.6" W), and Wa Municipality (9°58'22.2" N, 2°35'45.6" W). The Upper West Region is located in the northwestern part of the country, spanning longitudes 1°25′ W to 2°45′ W and latitudes 9°30′ N to 11° N. It is Ghana's seventh-largest administrative region, covering approximately 18,476 square kilometres, which represents about 12.7% of the nation's total land area. The region shares a northern boundary with Burkina Faso and comprises eleven districts. Its estimated population is roughly 800,000, accounting for 3.5% of Ghana's total population, while the regional capital, Wa, has an estimated population of 108,715.

These districts were purposively chosen for their high malaria endemicity, consistent mosquito breeding sites, and distinct climatic conditions that favour vector survival and transmission. The region experiences a tropical climate characterized by a single rainy season from May to October, with annual rainfall averaging 100–115 cm. Relative humidity ranges between 70% and 90% during the wet season but may drop to around 20% in the dry months. Temperatures typically range from 14°C to 40°C, with an annual mean of 28°C, conditions highly conducive to the larval development of Anopheles mosquitoes. During the dry season (November to March), the area experiences the Harmattan, a cold and dusty wind blowing from the northeast. To sustain farming activities during this period, rainwater is harvested and stored in dugout dams and reservoirs. These water bodies often overflow during the rainy season, forming temporary marshes and pools that serve as ideal breeding sites for Anopheles mosquitoes. As the dry season progresses, many of these reservoirs shrink, creating smaller, shallow water pools that further promote mosquito proliferation.

3.2.1 Reasons for the Selection of the Study Sites

The Upper West Region was selected as the study area because it is a key operational zone for the Indoor Residual Spraying (IRS) programme implemented by AngloGold Ashanti Malaria Control (AGAMal) under Ghana’s National Malaria Elimination Programme (NMEP). The prolonged and repeated use of insecticides in IRS campaigns subjects local mosquito populations to constant chemical exposure, which may accelerate the development of insecticide resistance. Therefore, monitoring the susceptibility status of local *Anopheles* populations is essential for the early detection of resistance trends and for providing evidence-based recommendations to guide insecticide rotation and selection.

Furthermore, malaria vectors in Ghana have already demonstrated varying levels of resistance to pyrethroids, organophosphates, carbamates, and organochlorines, mainly due to their prolonged exposure to both IRS and long-lasting insecticidal nets (LLINs). This situation underscores the need for continuous resistance surveillance and management strategies to sustain the effectiveness of current vector control operations.

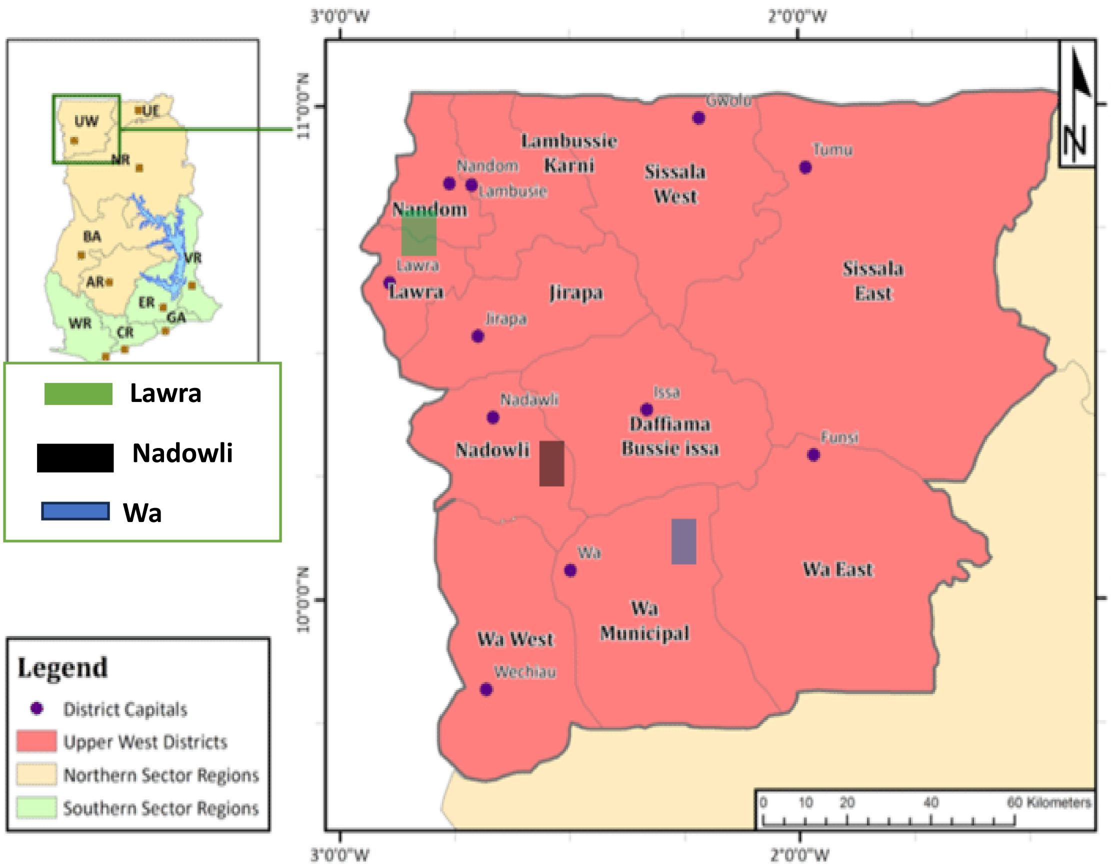

Figure 3. 2: Map showing the position of the study site in the Upper West Region.

3.3 Sampling Techniques

3.3.1 Larval Collection

Mosquito larvae were sampled from identified breeding habitats across the three selected study districts. Collections were carried out using standard 350-ml dippers and hand pipettes to gather *Anopheles* larvae and pupae from their natural aquatic environments. The specimens were placed in clean plastic containers as shown in Figure 3.1. To minimize the risk of repeatedly sampling the same sibling species, immature mosquitoes were collected at random from multiple breeding sites within each district. Larval sampling followed the standard dipping technique, in which Anopheles larvae were identified by their distinct horizontal resting posture, lying just beneath the water's surface film. Each breeding site was sampled systematically to obtain a representative distribution of the mosquito population. Collected larvae and pupae were placed in labelled plastic containers, with water sourced from the same breeding site to reduce stress and mortality during transport.

All samples were subsequently transferred to the AngloGold Ashanti Malaria Control Programme (AGAMal) insectary in Wa, where they were reared under controlled conditions to produce adult mosquitoes. The larvae were fed with finely ground fish meal (Tropical Fish Food Flakes) and maintained at an average temperature of 26 ± 2 °C, relative humidity of 80 ± 10 %, and a 12 h:12 h light/dark photoperiod. As pupae developed, they were gently collected and transferred into rearing cages to allow adult emergence. The newly emerged adult mosquitoes were maintained in holding cages and provided with a10% sucrose solution soaked in clean cotton wool as a food source.

Plate 3. 1: Picture of Larvae Collection

3.4 Laboratory Procedures

3.4.1 Mosquito Identification

Anopheles larvae were identified in the field by their characteristic parallel resting position just beneath the water surface. The collected larvae were placed into loosely capped 1,000 ml plastic containers and transported to the insectary for rearing to adulthood. Upon emergence, the adult mosquitoes were morphologically identified to differentiate *Anopheles* species using standard taxonomic identification keys outlined by the World Health Organization (2013).

3.4.2 Mosquito Breeding

In the insectary, larvae were sorted to retain only the *Anopheles* species for rearing. They were transferred into plastic bowls containing approximately 2 cm of water, and each bowl was clearly labelled to indicate the collection site and date (Figure 3.2). The larvae were maintained under controlled environmental conditions, with temperatures ranging from 27°C to 30°C and relative humidity of 80 ± 10%.

The developing larvae were fed approximately 100 mg of finely ground fish meal daily. Their growth and development were monitored regularly, and once pupation occurred, pupae were gently transferred into plastic cups using Pasteur pipettes. These cups were then placed into labelled emergence cages to allow adult mosquitoes to eclose. Emergent adults were sustained on a 10% sucrose solution absorbed on clean cotton wool. Female mosquitoes aged three to five days, which had not yet taken a blood meal, were subsequently selected and used for the insecticide susceptibility bioassays.

Plate 3. 2: Picture of Larvae processing in the insectary

3.4.2 WHO Insecticide Susceptibility Tests

The susceptibility of adult *Anopheles* mosquitoes to insecticides was evaluated following the World Health Organization (WHO, year?) standard bioassay procedures.

The WHO tube assay was employed to determine the resistance status of the mosquito populations. Non-blood-fed female adults were selected for testing because they exhibit lower control mortality and higher survival rates than males. All assays were performed indoors, under controlled environmental conditions, in a structure free of insecticide contamination, with strong airflow, minimal exposure to light, and within a range of temperature and humidity (WHO, 2022).

3.4.3 Insecticide-Impregnated Papers

The test utilized WHO-prepared insecticide-impregnated papers, representing both conventional and newer classes of insecticides approved for vector control. The active ingredients included Deltamethrin (0.05%, 0.25%, and 0.50%) and Permethrin (0.75% and 1.25%) from the *pyrethroid* class; Pirimiphos-methyl (0.25% and 1.25%) from the *organophosphate* class; and Bendiocarb (0.10%) from the *carbamate* group. For each insecticide, 3–5-day-old, unfed female mosquitoes were used. A total of 80–100 mosquitoes per insecticide type were exposed, divided into four replicates of 20–25 mosquitoes each. Control groups were maintained simultaneously using oil-treated (non-insecticidal) papers to account for natural mortality during testing.

3.4.4 Insecticide Susceptibility Test Procedure for Traditional Insecticides

Adult female mosquitoes were carefully aspirated from rearing cages and used for susceptibility testing. During each collection, no more than five mosquitoes were aspirated at a time to minimize stress or injury. The mosquitoes were first placed in holding tubes through a side filling opening. Each assay included four to five replicates, with 20-25 mosquitoes per replicate, representing approximately 100 females per site.

Before exposure, the mosquitoes were briefly held in the holding tubes for acclimatization. The test tubes were lined with a cylindrical sheet of insecticide-impregnated paper, held in position by a copper spring clip. The holding and exposure tubes were connected, and mosquitoes were gently introduced by sliding open the joining screw until all individuals had entered the exposure tube.

Mosquitoes were then exposed to the treated papers for intervals of 5, 10, 15, 20, 30, 40, 50, and 60 minutes. Following exposure, they were returned to the holding tubes by reversing the transfer process. A cotton pad soaked in a 10% sucrose solution was placed on the mesh covering of the holding tube to provide nourishment.

The mosquitoes were then kept for a 24-hour recovery period in a calm, shaded environment maintained at temperatures below 30°C. The platforms holding the tubes were placed in water-filled trays to prevent ant predation. After 24 hours, mortality counts were recorded, and dead mosquitoes were carefully removed for disposal. The data obtained were documented for subsequent analysis (WHO, 2022).

3.4.4.1 WHO Susceptibility Bioassays for Clothianidin

The susceptibility of *Anopheles* mosquitoes to clothianidin was assessed using the World Health Organization (WHO) bioassay protocol, with slight adjustments to the post-exposure holding period (WHO, 2020). Before testing, adult mosquitoes were held for approximately one hour to evaluate their physical condition. Individuals that appeared weak, injured, or unable to fly were removed to ensure only healthy specimens were used.\

For each test, 3- to 5-day-old female mosquitoes were exposed to filter papers impregnated with 13.2 mg (2%) clothianidin for 60 minutes. Each assay comprised four replicates, with 25 mosquitoes per replicate (totalling 100 exposed individuals). In addition, two replicates of 25 mosquitoes each served as the negative control, using untreated papers to assess natural mortality. During exposure, knockdown rates were observed and recorded at 30-minute intervals throughout the one-hour exposure period. After exposure, mosquitoes were gently transferred into clean holding tubes and provided with a 10% sucrose solution soaked in cotton wool. Mortality was first assessed 24 hours after exposure, followed by extended monitoring for delayed mortality over a further 120 hours (5 days) to capture any late effects of clothianidin. All assays were conducted under controlled insectary conditions, maintaining a temperature of 27 ± 2 °C and relative humidity of 75 ± 20%, in line with WHO recommendations (WHO, 2022). To validate test performance, a positive control was performed using the susceptible *Anopheles gambiae* (Kisumu strain) under the same experimental conditions and procedures.

Plate 3. 3: Picture of preparing the tube for the test

3.5 Data Collection Tools and Procedure

This study employed WHO-recommended tools and procedures to collect, rear, and test *Anopheles* mosquito populations across the three study districts. The methods were designed to ensure accuracy, consistency, and reproducibility, and to adhere to established entomological standards for vector surveillance and insecticide resistance monitoring (WHO, 2022).

Field sampling targeted aquatic stages of malaria vectors using a 350 mL mosquito dipper, with white trays for sorting larvae and removing debris or non-target organisms. Fine-tipped pipettes facilitated careful transfer of larvae into labelled containers, which were transported to the insectary in plastic buckets marked with site, date, and GPS coordinates. These procedures ensured standardized and systematic sampling across all study locations.

In the insectary, larvae were maintained in wide trays (30 × 40 cm) and fed powdered fish food daily. Pupae were transferred into emergence cups and then adult cages (30 × 30 × 30 cm) under controlled temperature and humidity. Adults were sustained with cotton balls soaked in a 10% sucrose solution until reaching the required age for susceptibility testing.

In 2022, susceptibility test kits were used for bioassays, including exposure and holding tubes, tube clips, mesh screens, and slide units (WHO, 2022). Insecticide-impregnated papers representing pyrethroids, carbamates, organophosphates, neonicotinoids, and newer formulations were applied, with control papers included in all tests. Manual aspirators ensured the gentle transfer of mosquitoes into tubes. Environmental conditions were monitored with a digital thermometer-hygrometer, and knockdown was recorded every 10 minutes for 60 minutes.

Data management combined manual and electronic systems. Observations, including knockdown counts, 24-hour mortality, and environmental conditions, were first recorded in laboratory notebooks and WHO data sheets, then entered into an Excel-based template for organization, cleaning, and preparation for analysis. Photographic documentation supplemented records when necessary to capture field or laboratory conditions.

3.5.1 Statistical Analyses

Data analysis was carried out using Microsoft Excel (version 2020). The software was also employed to generate all summary tables and graphical outputs. Interpretation of mosquito mortality results followed the World Health Organization's criteria for insecticide susceptibility. According to these guidelines, a mosquito population is considered *susceptible* when mortality is ≥98%, *possibly resistant* when mortality is 90%-97%, and resistant when mortality is <90% (WHO, 2022).

When mortality in control assays exceeded 5%, data were adjusted using Abbott's formula to account for natural mortality in the control group (Abbott, 1925). Tests were discarded if the control mortality was ≥20%. For control mortalities below this threshold, corrected mortality was computed as:

$$\text{Abbott's Formula: } = \frac{(\%\text{Test Mortality} - \%\text{Control Mortality})}{(100 - \%\text{Control Mortality})} \times 100$$

In this study, no mortality occurred in the control group, and therefore, mortality corrections were unnecessary. Mortality rates for test populations were calculated as:

$$\text{Observed Mortality} = \frac{\text{Total Number of Dead Mosquitoes}}{\text{Total Number of Exposed Mosquitoes}} \times 100$$

Statistical comparisons of susceptibility across the three study sites were performed using the Chi-square test to determine whether significant differences in mortality rates existed among the test populations. In addition, dose-response relationships were analyzed through probit regression analysis following the method described by Finney (1971). From this analysis, Knockdown Times (KDTs) were estimated - specifically, KDT_{50} and KDT_{95}, representing the time (in minutes) required to knock down 50% and 95% of the mosquito population, respectively.

The resistance ratio (RR) was calculated by comparing the KDT values of wild mosquito populations to those of the susceptible Kisumu reference strain, using the following relationship:

$$\text{RR}_{\text{KDT}} = \frac{\text{KDT (Wild Population)}}{\text{KDT (Kisumu Colony)}}$$

Thus, the $RRKDT_{50}$ and $RRKDT_{95}$ values were used to assess the magnitude of resistance. Knockdown percentages recorded during exposure to clothianidin-treated papers were expressed as cumulative proportions over time and presented graphically. According to WHO interpretation criteria, populations with mortality rates ≥98% were classified as susceptible, those with 90-97% as possibly resistant, and those with <90% as resistant (World Health Organization, 2016a).

3.6 Ethical Considerations

This study was approved by the Department of Public Health Education, Akenten Appiah Menka, of the University of Skills Training and Entrepreneurship Development, under protocol identification number M/DPHE/ADM/G/03/24/64. The study was also approved by the Research Unit of the Ghana Health Service and the Wa Municipal Health Directorate.

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents the results of the standard insecticide susceptibility tests conducted on *Anopheles gambiae* s.l. populations collected from three districts in the Upper West Region of Ghana. The mosquitoes were tested against three older classes of insecticides and one newer insecticide class used in malaria vector control: pyrethroids (deltamethrin and permethrin), organophosphates (pirimiphos-methyl), carbamates (bendiocarb), and neonicotinoids (clothianidin). The findings of the current research work are presented in three major categories: Knockdown rates (KDRs), knockdown times (KDT_{50} and KDT_{95}), resistance ratios (RRs), and susceptibility and resistance profiles established using mortality rates recorded after the 24-hour recovery period.

4.1.1 Knockdown Effects of Different Concentrations of the traditional class of insecticides on An. gambiae s.l. Populations of each site

4.1.1.1 Deltamethrin

The deltamethrin-induced knockdowns of *Anopheles gambiae* s.l. increased progressively with both diagnostic concentration and exposure time across all study sites. For the mosquitoes from the Lawra District, the lowest concentration (0.05% deltamethrin) was associated with the lowest knockdown rates, increasing from 0% at the 5th minute to 12% at the 60th minute. The 0.25% deltamethrin bioassay yielded significantly higher knockdown, reaching 86% after 60 minutes, while the highest concentration (0.50%) induced complete (100%) knockdown within the same period. For mosquitoes from Wa Municipal, the knockdown rate estimated at 0.05%

deltamethrin at the 60th minute was about 4%. The 0.25% deltamethrin induced a relatively higher knockdown of 34%, but the highest knockdown of 92% was recorded for 0.50% deltamethrin. Knockdown rates estimated for the Nadowli mosquitoes in the 0.05%, 0.25%, and 0.50% deltamethrin bioassays were 16%, 80%, and 100%, respectively.

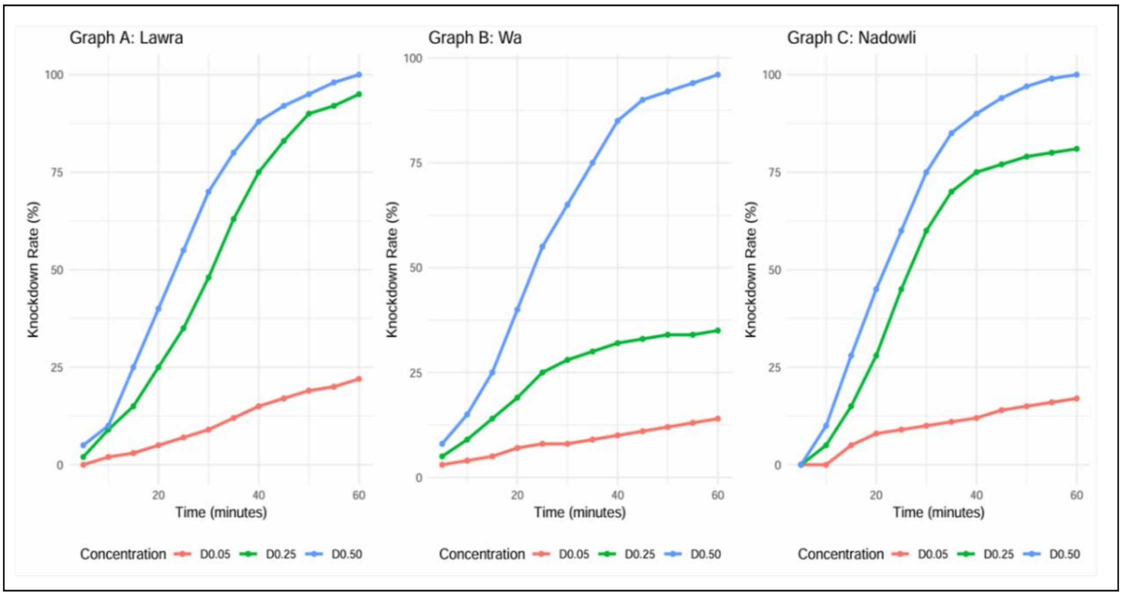

Figure 4.1: Time-dependent knockdown response of Anopheles gambiae s.l. to varying concentrations of Deltamethrin (0.05%, 0.25%, and 0.50%)

4.1.1.3 Permethrin

The knockdown rates of *An. gambiae* s.l. populations to Permethrin (1.25% and 0.75%) increased progressively with exposure time across all study sites. In the Lawra District, knockdown was generally higher with 1.25% permethrin than with 0.75% permethrin. Knockdowns caused by 0.75% deltamethrin ranged from 0% at the 5th minute to 22% at the 60th minute, whereas those by 1.25% deltamethrin ranged from 0% at the 5th minute to 47% after the 60th minute. For the Wa mosquitoes, the knockdown rates induced by 1.25% permethrin increased from 0% at the first 5 minutes of exposure to

92% at the 60th minute. In contrast, the 0.75% permethrin bioassay at Wa resulted in only 8% knockdown at the end of the exposure time. At Nadowli, a similar pattern occurred. The 1.25% permethrin caused more rapid knockdown, reaching 94% at the 60th minute, compared with 18% for the 0.75% permethrin. Across all the study sites, the higher permethrin concentration (1.25%) consistently induced greater and faster knockdowns. The Nadowli mosquitoes were knocked down to a higher extent than the Wa mosquitoes, which were also knocked down to a higher extent than the Lawra mosquitoes.

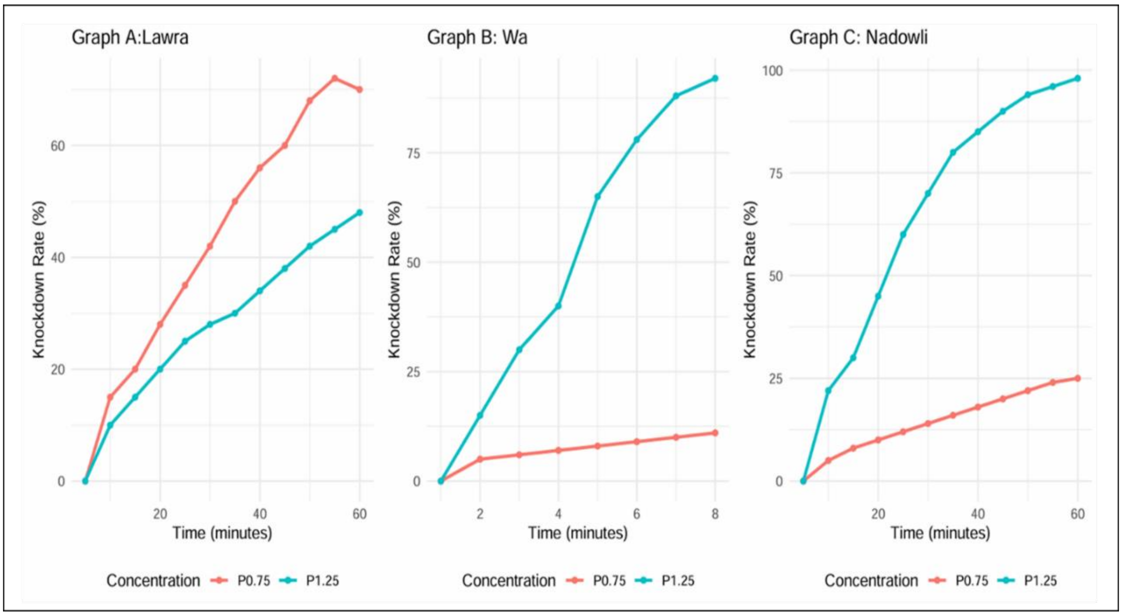

Figure 4.2: Time-dependent knockdown response of Anopheles gambiae s.l. to Permethrin at 0.75% and 1.25% concentrations

4.1.1.4 Pirimiphos-methyl

The knockdown rates recorded for *Anopheles gambiae* s.l. exposed to Pirimiphos-methyl at diagnostic concentrations of 0.25% and 1.25% increased progressively with both exposure time and insecticide concentration across all study sites. For the mosquitoes from the Lawra District, the knockdown rate was comparatively lower at 0.25%, with only 24% knockdown at the 60th minute. However, at the higher concentration (1.25%), knockdowns rose progressively from 0% at the 5th minute to 55% at the 60th minute. A similar trend was observed for the Wa Municipal. Knockdown rate at 0.25% remained relatively lower, reaching only 10% at the 60th minute. For 1.25% permethrin, knockdown increased relatively more rapidly, reaching 64% by the end of the exposure period. A comparable pattern was observed for the mosquitoes from the Nadowli District. For the 0.25% pirimiphos methyl bioassay, knockdown reached 12% at the 60th minute. Approximately 59% knockdown was observed with 1.25% pirimiphos methyl at the same time.

In all, mosquito populations in Lawra and Nadowli exhibited slightly higher knockdown rates than those from Wa. Across all sites, knockdown rates increased with time and concentration, confirming that Pirimiphos-methyl exerted a more substantial effect at higher doses, consistent with a concentration-dependent knockdown pattern.

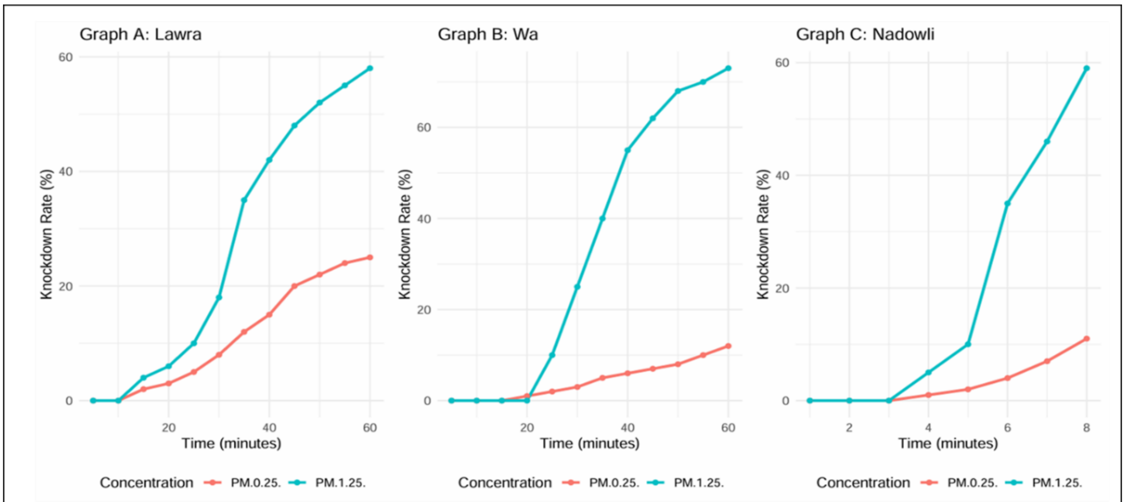

Figure 4.3: Time-dependent knockdown response of Anopheles gambiae s.l. to Pirimiphos-methyl (PM) at 0.25% and 1.25% concentrations

4.1.1.5 Bendiocarb

For the Lawra District, the knockdown rates of *Anopheles gambiae s.l.* exposed to 0.10% bendiocarb increased from 22% at the 30th minute to 58% at the 60th minute. The rates were higher at Wa, increasing from 0% at the 5th minute to 83% at the 60th minute. The highest rate of knockdown occurred between 30 and 40 minutes, where knockdown rose sharply from 40% to 71%. At Nadowli, no knockdown was observed until the 30th minute, when the rate was approximately 20%. AT the 60th minute, the rate was approximately 34%.

An. gambiae s.l. from Wa were more susceptible to Bendiocarb than those from Lawra and Nadowli. The comparatively lower knockdowns in the Nadowli district suggest a possible lower susceptibility or higher resistance to the insecticide. Overall, knockdown increased with exposure duration across all sites, demonstrating the time-dependent insecticidal activity of Bendiocarb on the vector populations.

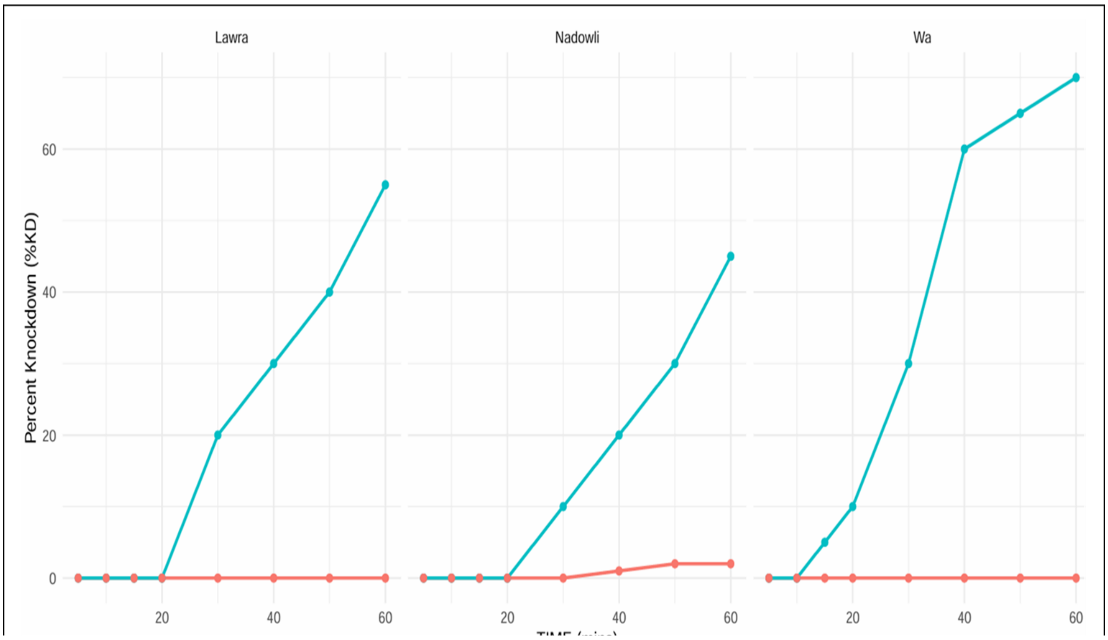

Figure 4.4: Time-dependent knockdown response of Anopheles gambiae s.l. to Bendiocarb (0.10%)

4.1.2. Pyrethroid Insecticides

4.1.2.1 Knockdown period of Deltamethrin 0.05%

Figure 4.5 illustrates the knockdown rates (KDRs) of *Anopheles gambiae* complex populations from Wa, Nadowli, and Lawra caused by 0.05% deltamethrin during a 60-minute exposure. Initially, no knockdown (KD) was recorded for any of the *An. gambiae* populations during the fifth minute of exposure at all study sites. No KDs were observed for the *An. gambiae* mosquitoes from Wa Municipality until the 60th minute, when a KDR of approximately 12% was noted. For the mosquito population from Lawra District, KDS began during the 20th minute of exposure, with a KD rate of about 1%. After this, KDS increased at ten-minute intervals until the 60th minute, when the KDR was around 12%. In Nandoli, KDs started earlier, during the 15th minute, with a KDR of roughly 8%. Overall, the KDR induced by 0.05% deltamethrin was significantly lower in the mosquito population from Wa municipality than in those from Nadowli and Lawra (χ^2 = 7.84, d.f. = 2, p = 0.020). However, there was no significant difference in KDR between Lawra and Nadowli (χ^2 = 0.664, d.f. = 1, p = 0.415).

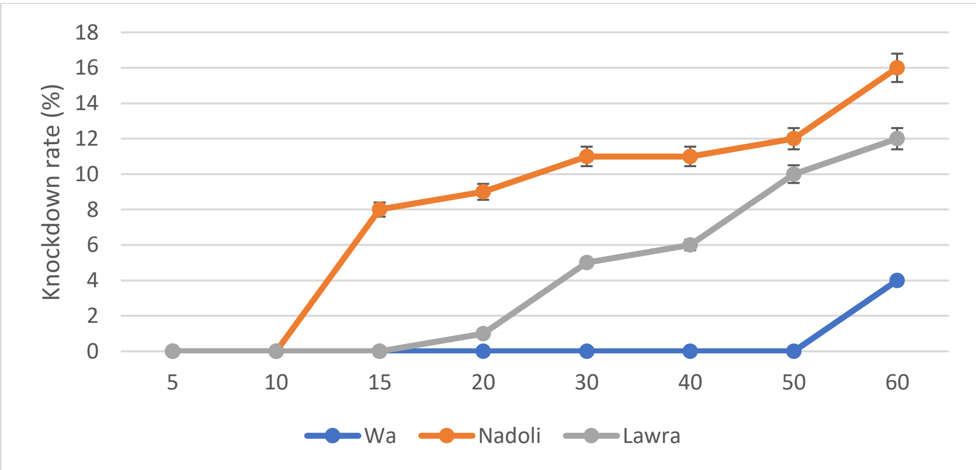

Figure 4. 5: Knockdown rates of Anopheles gambiae induced by Deltamethrin 0.05%

4.1.2.2 Knockdown periods of Deltamethrin 0.25%

Figure 4.6 shows the KDRs of *An. gambiae* populations from Wa, Nadowli, and Lawra during the 60-minute exposure to 0.25% deltamethrin. There was no record of KD at the 5th minute in Nadowli District, but the KDR gradually increased from the 10th minute (26%) to the 60th minute (80%). The 0.25% deltamethrin-induced KDR of the mosquitoes from the Wa municipality was approximately 2% at the 5th and 10th minutes. The rates then increased from about 14% at the 15th minute to approximately 34% by the 60th minute. For the mosquito population from the Lawra district, the KDR recorded at the 5th minute was approximately 1%. The KDR then gradually increased from about 9% during the 10th minute to approximately 86% at the 60th minute. Significant differences in 0.25% deltamethrin-induced KDRs were observed between the mosquito populations from the districts/municipalities explored, with the lowest rates observed for the population from Wa and the highest for the population from Nadowli ($\chi2$ = 72.83, d.f. = 2, p value = < 0.0001).

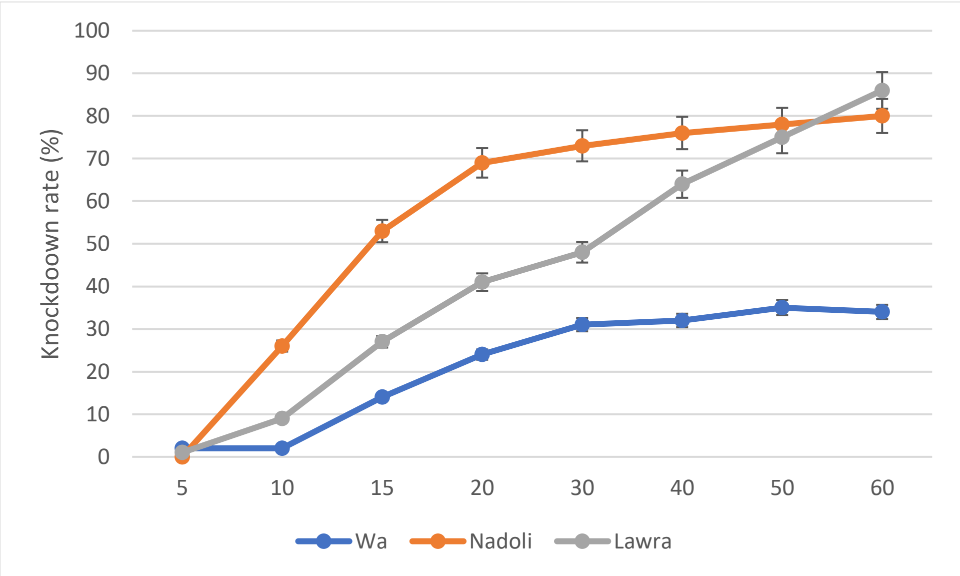

Figure 4.6: Knockdown Rates of *Anopheles gambiae* induced by Deltamethrin 0.25%

4.1.2.3 Knockdown periods of Deltamethrin 0.50%

Figure 4.7 displays the KDRs of the *An. gambiae* population from Wa, Nadowli, and Lawra during the 60-minute exposure to Deltamethrin 0.50%. At the 5th minute, KDRs of *3%* and 5% were recorded for Wa and Lawra, respectively, whereas no KDs were observed for the Nadowli mosquitoes. However, at the 10th minute, the highest KDR of about 28% was recorded for the Nadowli mosquitoes. By the 60th minute, 100% KDRS of *An. gambiae* were observed at Nadowli. There was a gradual increase in KDRS from 5% at the 5th minute to 100% at the 60th minute in the Lawra mosquitoes. Instead, KDRs for the Wa mosquitoes increased from approximately 3% at the 5th minute to 92% at the 60th minute. Overall, no significant differences in 0.5% deltamethrin-induced KDRs were observed for the mosquito from the three-study districts (χ^2 = 16.45, d.f. = 2, p < 0.001), albeit the KDR among the Nadowli mosquitoes was significantly higher compared to that of the Wa mosquitoes (χ^2 =8.33, df = 1, p = 0.004).

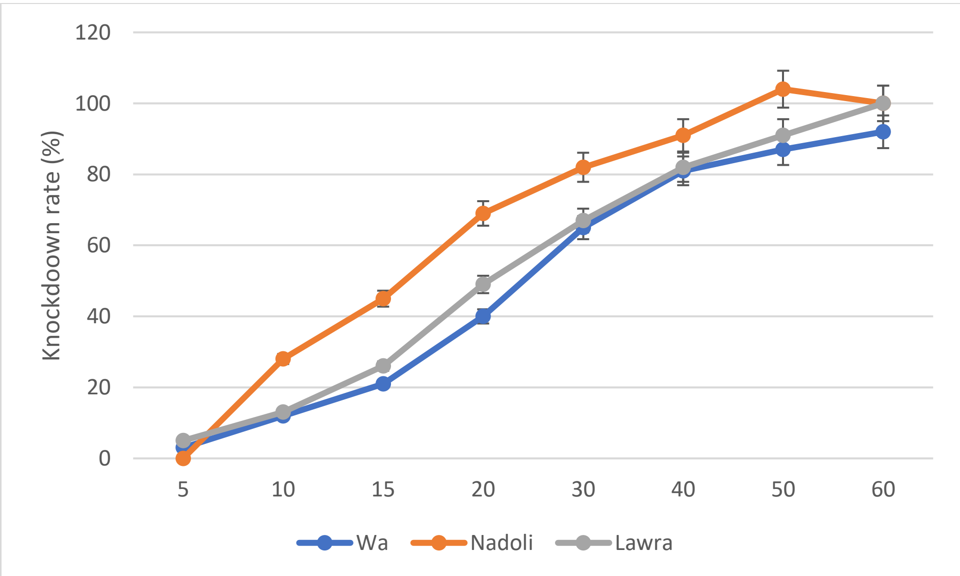

Figure 4.7: Knockdown Rates of *Anopheles gambiae* Induced by Deltamethrin 0.50%

4.1.2.4 Knockdown Rates of Permethrin 0.75%

KDRs of *Anopheles gambiae* from WA, Nadowli, and Lawra districts estimated through 0.75% permethrin-bioassays are shown in Figure 4.8. No KD was observed until the 15th minute, when a 1% KDR was recorded. KDR then increased gradually to 18% by the 60th minute. For the Lawra mosquitoes, KDR increased from 2% at the 10th minute to 22% in the 60th minute. This was the highest KDR caused by 0.75% Permethrin. For mosquitoes from the Wa municipality, a KDR of up to 3% was observed at the 30th minute, increasing to 8% by the 60th minute. There were significant differences in 0.75% permethrin-induced KDR between mosquitoes from the three districts ($\chi^2 = 7.74$, df = 2, $p = 0.021$).

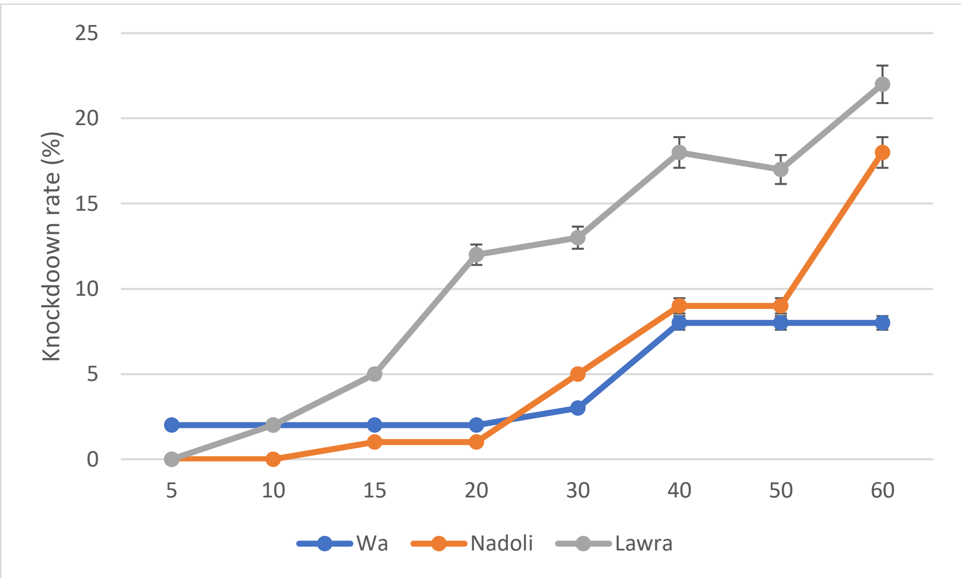

Figure 4.8: Knockdown rates of *Anopheles* mosquitoes exposed to Permethrin 0.75%

4.1.2.5 Knockdown periods of Permethrin 1.25%

Figure 4.9 shows the 1.25% Permethrin-induced KDRs estimated for *Anopheles gambiae* mosquitoes from Wa, Nadowli, and Lawra districts. For Wa municipal mosquitoes, KDRs increased from approximately 14% at the 10th minute to about 92% at the 60th minute. For the Lawra populace, KDs of *An. gambiae* reached 42% at the 60th minute. For the Nadowli mosquitoes, KDRs increased from 1% in the 5th minute to 94% in the 60th minute. Overall, however, there were significant differences in Permethrin-induced KDRs between mosquitoes from the study sites ($\chi^2 = 95.2$, d.f. = 2, $p < 0.0001$).

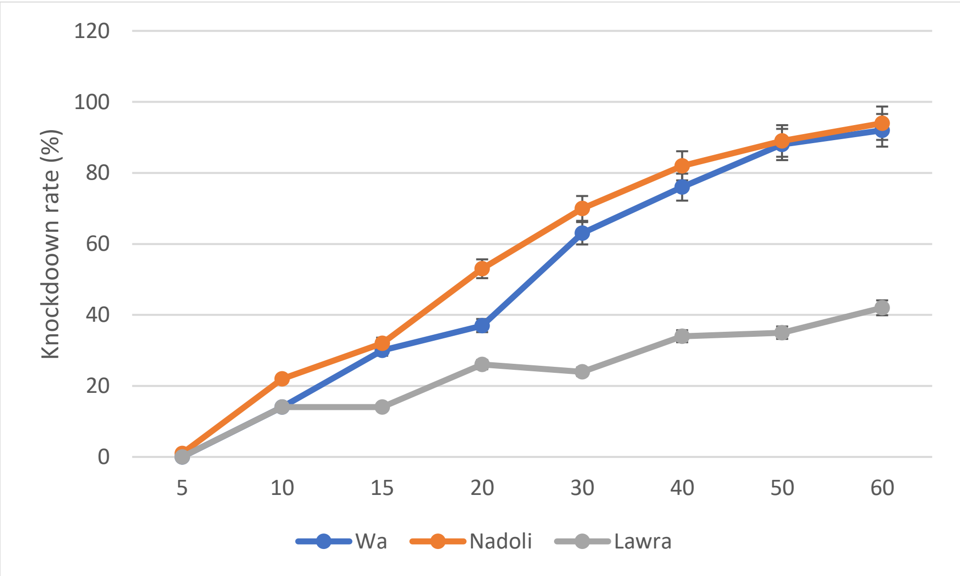

Figure 4.9: Knockdown rates of *Anopheles gambiae* exposed to Permethrin 1.25%

4.1.2.6 Organophosphates

4.1.2.6.1 Knockdown periods of Pirimiphos methyl 0.25%

KDRs estimated for the 0.25% Pirimiphos Methyl-*Anopheles gambiae* bioassays for Wa, Nadowli, and Lawra districts are shown in Figure 4.10. In the Lawra District, the KD was 0% until after the 30th minute, when a 2.0% KDR was observed. There was a gradual increase in the percentage KD, reaching 24% at the 60th minute. At the 60th minute, KDRs were 12% and 10% for the mosquitoes from Nadowli and Wa, respectively. There were significant differences in KDRs between the three categories of mosquitoes studied ($\chi^2 = 8.84$, df = 2, p = 0.012).

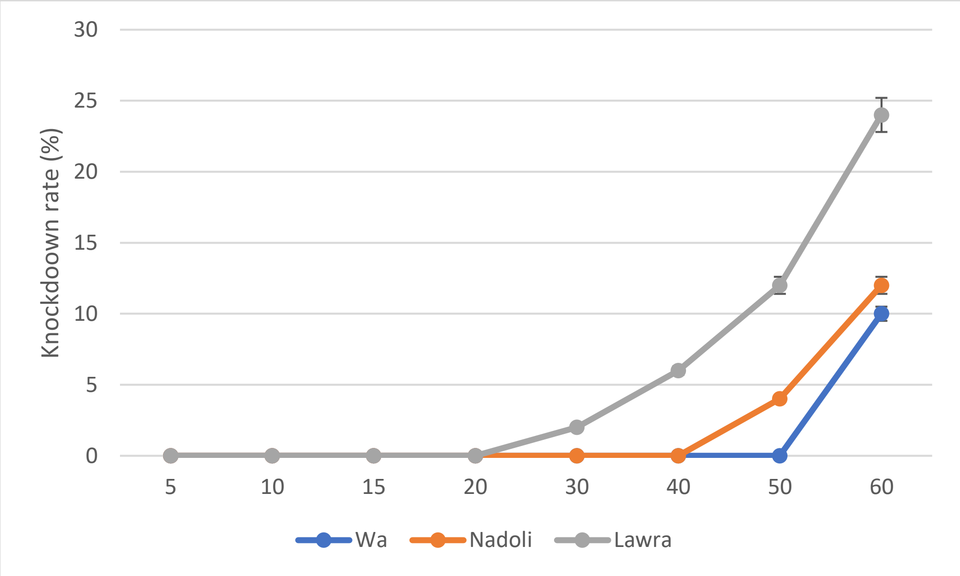

Figure 4.10: Knockdown Rates of *An. gambiae* exposed to Pirimiphos methyl 0.25%

4.1.2.7 Knockdown Rates of Pirimiphos methyl 1.25%

Figure 4.11 shows the KDRs estimated for 1.25% Pirimiphos Methyl-*An. gambiae* bioassays for the three districts explored. It took up to 15 minutes for the Lawra mosquito KD to reach 4.0%. By the 30th and the 60th minutes, KDRs were 17% and

55%, respectively. KDRs in Nadowli rose from 4% in 20 minutes to 59% in 60 minutes. Again, KDRs increased from 1% at the 20th minute to 64% at the 60th minute in the Wa mosquitoes. However, there were no statistically significant differences in KDR among the three mosquito populations examined ($\chi2 = 1.54$, d.f. = 2, $p = 0.46$).

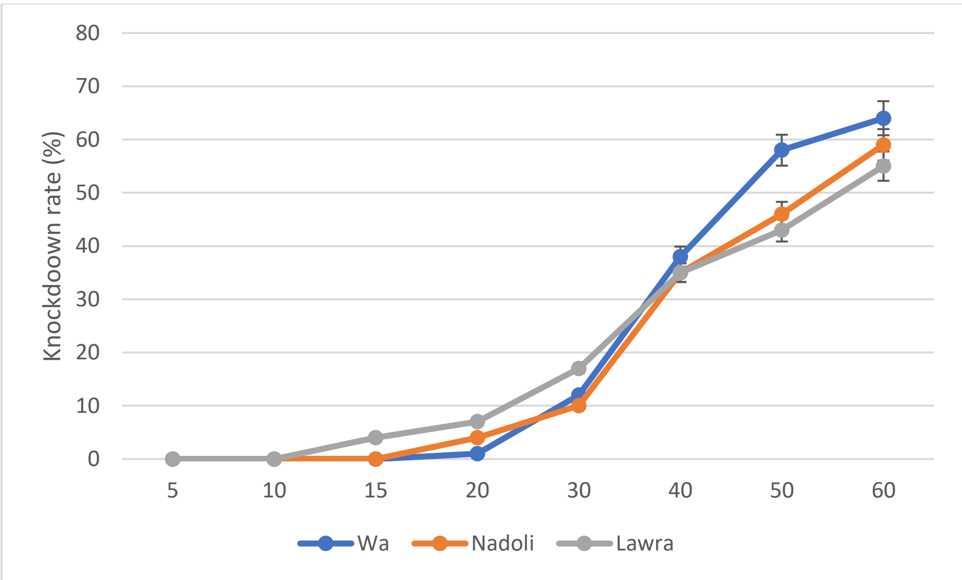

Figure 4.11: Knockdown Rates of *An. gambiae* exposed to Pirimiphos methyl 1.25%

4.1.2.8 Carbamates

4.1.2.8.1 Knockdown periods of Bendiocarb 0.10%

KDRs of *An. gambiae* mosquitoes from WA, Nadowli, and Lawra districts, as exposed to 0.10% Bendiocarb, are shown in Figure 4.12. No KDs were recorded for any of the mosquitoes from the three districts in the first 10 minutes. For the Wa mosquitoes, KDRs increased from 4% at the 15th minute to 83% at the 60th minute. For Lawra, KDRs began at 22% at the 30th minute and rose to 58% by the 60th minute. For

Nadowli, KDs started during the 40th minute with a KDR of 11% and increased to 34% at the 60th minute. There were significant variations in KDRs for the 0.10% Bendiocarb-*An. gambiae* bioassay for the three mosquito populations (χ^2 = 49.42, df = 2, p = < 0.0001).

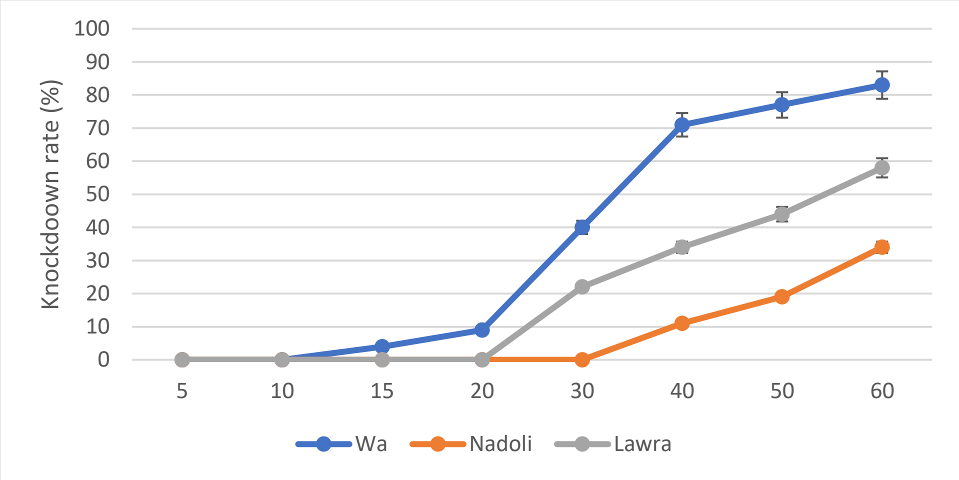

Figure 4.12: Knockdown rates of *Anopheles gambiae* exposed to carbamate 0.10%

Table 4.1: Knockdown Rate of *An. gambiae* exposure to insecticides at 60 minutes

Insecticide	District	N	%KDR	95% CI
Deltamethrin 0.05%	Wa	100	4.0	1.1-9.9
	Nadowli	100	16.0	9.4-25.1
	Lawra	100	12.0	6.3-20
Deltamethrin 0.25%	Wa	100	34.0	24.4-44.7
	Nadowli	100	80.0	70.9-87.6
	Lawra	100	86.0	77.6-92.6
Deltamethrin 0.50%	Wa	100	92.0	84.6-96.5
	Nadowli	100	100.0	96.4-100
	Lawra	100	100.0	96.4-100
Permethrin 0.75%	Wa	100	8.0	3.5-15.2
	Nadowli	100	18.0	14.3-31.4
	Lawra	100	22.0	11.0-26.9
Permethrin 1.25%	Wa	100	92.0	84.8-96.5
	Nadowli	100	94.0	86.7-98.7
	Lawra	100	42.0	38.6-55.3
Bendiocarb 0.10%	Wa	100	83.0	74.2-89.8
	Nadowli	100	34.0	24.8-44.2
	Lawra	100	58.0	47.7-67.8
	Wa	100	10.0	4.9-17.6

	Nadowli	100	12.0	6.4-20.0
Pirimiphos methyl 0.75%	Lawra	100	24.0	16.0-33.6
	Wa	100	64.0	53.8-73.4
Pirimiphos methyl 1.25%	Nadowli	100	59.0	48.7-68.7
	Lawra	100	55.0	44.7-65.0

4.1.3 24-hour Mortality Rate of Anopheles gambiae s.l. to Deltamethrin, Permethrin, Bendiocarb and Pirimiphos-methyl Across the Study Sites

The mortality rates of *Anopheles gambiae* exposed to the various diagnostic concentrations of insecticides after the 24-hour recovery period are summarised in Table 4.2. Mortality responses varied across insecticides and locations, indicating differences in vector population susceptibility.

4.1.3.1 *Deltamethrin (0.05%, 0.25%, and 0.50%)*

At the diagnostic concentration of 0.05% deltamethrin, low mortality rates were observed in all three study districts, with Wa at 20% (95% CI: 12.7-29.2) and Lawra at 22% (95% CI: 14.2-31.4) recording comparatively low mortality rates, suggesting high deltamethrin resistance. Mosquitoes from the Nadowli District, however, exhibited a relatively higher mortality rate of 71% (95% CI: 61.0-79.7), suggesting comparatively lower resistance or higher susceptibility. The differences among mosquitoes from the three districts were statistically significant ($\chi^2 = 71.071$, $p < 0.001$), indicating spatial variation.

When mosquitoes from the same batches were exposed to a higher concentration of the deltamethrin active ingredient [0.25% (5×)], mortality increased across all districts, with Wa (85.0%), Nadowli (82.0%), and Lawra (73.0%). However, susceptibility remained low, and statistical analysis showed no significant difference between districts ($\chi^2 = 4.875$, $p = 0.087$). At the highest concentration tested (0.50%, 10×), mortality rates ranged from 98.0% (95% CI: 93.0-99.8) for the Lawra mosquitoes to 100% for the mosquitoes from both Wa and Nadowli. No significant differences were observed between mosquitoes from the three districts ($\chi^2 = 4.03$, $p = 0.133$). This demonstrates that while the mosquitoes showed physiological resistance to the lower diagnostic concentration of deltamethrin, higher doses can overcome this resistance.

4.1.3.2 Permethrin (0.75% and 1.25%)

For permethrin at the standard diagnostic dose (0.75%), mortality rates ranged from 38.0% (95% CI: 28.5-48.3) for mosquitoes from both the Wa municipality and Lawra district to 57.0% (95% CI: 46.7-66.9) for those from the Nadowli District. The variations between mosquitoes from the three districts were statistically significant ($\chi^2 = 9.7519$, $p = 0.008$). When exposed to the 5× concentration (1.25%), mortality rates increased markedly to 98.0%, 99.0%, and 85.0% in Wa, Nadowli, and Lawra, respectively. The differences among sites remained statistically significant ($\chi^2 = 21.631$, $p < 0.001$), with mosquitoes from the Lawra District showing lower mortality than those from the other districts. These results suggest that *An. gambiae s.l.* populations from all sites are resistant to 0.75% permethrin but more susceptible to 1.25% permethrin.

4.1.3.3 Bendiocarb (0.10%)

Exposure to bendiocarb (0.10%) resulted in variable mortality among the sites. The highest mortality was recorded in Wa (84.0%, 95% CI: 75.3-90.6), followed by Lawra (78.0%, 95% CI: 68.6-85.7) and Nadowli (59.0%, 95% CI: 48.8-68.7). Statistical analysis showed a significant difference between sites ($\chi^2 = 17.561$, $p < 0.001$), suggesting heterogeneous resistance patterns to carbamates among the *An. gambiae s.l.* populations.

4.1.3.4 Pirimiphos-methyl (0.25% and 1.25%)

At the standard diagnostic concentration (0.25%), mortality rates of 87.0%, 94.0%, and 92.0% were recorded for the Wa, Nadowli, and Lawra mosquitoes, respectively. The differences in mortality rates between mosquitoes from the two districts and the municipal area were not statistically significant ($\chi^2 = 3.1746$, $p = 0.204$). At the 5× concentration (1.25%), mortality rates were 98% at Lawra and 99% at both Wa and Nadowli, with no significant difference observed ($\chi^2 = 0.506$, $p = 0.776$).

Table 4.2 **Mortality rates of *An. gambiae* after a 24-hour recovery period**

Insecticide & Concentration	District	N	%Mortality (24h)	95% (CI)	χ^2	P-value
Deltamethrin 0.05%						
	Wa	100	20.0	12.7 – 29.2		
	Nadowli	100	71.0	61.0 – 79.7		
	Lawra	100	22.0	14.2 – 31.4	71.071	3.691×10^{-16}
Deltamethrin 0.25% (5×)						
	Wa	100	85.0	76.5 – 91.4		
	Nadowli	100	82.0	72.9 – 89.1		
	Lawra	100	73.0	63.4 – 81.4	4.875	0.087
Deltamethrin 0.50% (10×)						
	Wa	100	100.0	96.4– 100.0		
	Nadowli	100	100.0	96.4– 100.0		
	Lawra	100	98.0	93.0 – 99.8	4.030	0.133
Permethrin 0.75%						
	Wa	100	38.0	28.5 – 48.3		
	Nadowli	100	57.0	46.7 – 66.9		
	Lawra	100	38.0	28.5 – 48.3	9.752	0.008
Permethrin 1.25% (5×)						
	Wa	100	98.0	93.0 – 99.8		
	Nadowli	100	99.0	94.6 – 100.0		
	Lawra	100	85.0	76.5 – 91.4	21.631	0.00002
Bendiocarb 0.10%						
	Wa	100	84.0	75.3 – 90.6		
	Nadowli	100	59.0	48.8 – 68.7		
	Lawra	100	78.0	68.6 – 85.7	17.561	0.0002
Pirimiphos-methyl 0.25%						
	Wa	100	94.0	87.4 – 97.8		
	Nadowli	100	92.0	84.8 – 96.5		
	Lawra	100	87.0	78.7 – 92.9	3.175	0.204
Pirimiphos-methyl 1.25% (5×)						
	Wa	100	99.0	94.6 – 100.0		

Nadowli	100	99.0	94.6 – 100.0		
Lawra	100	98.0	93.0 – 99.8	0.506	0.776

4.2 Knockdown Time (KDT_{50} and KDT_{95}) of Anopheles gambiae s.l. Populations after 60 minutes of Exposure to Different Classes of Insecticides.

The knockdown time (KDT_{50} and KDT_{95}) values for *Anopheles gambiae* s.l. from Wa, Nadowli, and Lawra districts showed greater variability in response to different insecticide classes and concentrations compared with the Kisumu susceptible strain. Overall, wild populations exhibited prolonged knockdown times relative to the Kisumu, reflected by resistance ratios ($RRKDT_{50}$ and $RRKDT_{95}$), which were greater than one (>1) across all insecticides and sites.

4.2.1 Pyrethroids

4.2.1.1 Deltamethrin

For the 0.05% Deltamethrin assay, while KDT_{50} for the standard *An. gambiae* s.l from Kisumu was 22.59 (95% CI: 19.20-25.94), those of the wild mosquitoes from the Upper West Region ranged from 78.23 for the Wa municipal to 113.22 for the Nadowli District. Accordingly, the estimated times in minutes for which 50% of wild mosquitoes were knocked down were about 3 to 5 times longer than those in Kisumu.

Likewise, the KDT_{95} for the Kisumu mosquitoes was 50.41, whereas those of the wild mosquitoes ranged from 95.16 for Wa to 208.98 for Nadowli. That is, the estimated times in minutes, for which 95% of the wild mosquitoes from the Upper West were approximately 2 to 4 times that of the Kisumu mosquitoes.

For the 0.25% deltamethrin assay, the KDT_{50} for Kisumu was approximately 13 minutes. On the other hand, the KDT_{50} values for the wild mosquitoes ranged from approximately 23 minutes to approximately 67minutes. Thus, the time that mosquitoes exposed to the diagnostic concentration of deltamethrin may be knocked down may range from approximately twice to 5 times that of Kisumu. Likewise, the KDT_{95} for Kisumu was about 28 minutes, and those of the wild ones ranged from approximately 69 minutes for Lawra to 141 minutes for Wa. In other words, the time required for 95% of the wild mosquito population to be knocked down ranged from approximately twice to five times that of Kisumu, for Lawra and Wa, respectively.

For the 0.50% Deltamethrin assay, the KDT50 for Kisumu was 12 minutes. However, the KDT50 for the wild mosquito ranged from approximately 19 minutes for Nadowli to about 28 minutes for Wa. Thus, the KDT50 values for the wild mosquitoes were approximately twice those of the Kisumu.

Similarly, the KDT95 for the Kisumu was 28 minutes, and those of the wild mosquitoes ranged from about 42 minutes for the Nadowli mosquitoes to 56 minutes for the Wa mosquito population. Compared with Kisumu, the KDT95 values for the wild mosquitoes were approximately twice those of Kisumu.

4.2.1.2 Permethrin

For the 0.75% Permethrin assay, the KDT_{50} for Kisumu was approximately 12 minutes, and that of the KDT_{95} was approximately 30 minutes. For the wild mosquitoes, *An. gmabiae* s.l, the KDT_{50} values ranged from about 87 minutes at Nadowli to 147 minutes at Wa. Likewise, the KDT_{95} ranged from about 138 minutes in Nadowli to 256 minutes in Wa. The resistance ratio for the KDT_{50} was relatively higher than that of the KDT_{95}. The resistance ratio for the KDT_{50} ranged from approximately 7 times that of the Kisumu in Nadowli and Laawra to about 12 times that of the Kisumu in Wa. On the other hand, the KDT_{95} values for both Nadowli and Lawra were 5 times that of Kisumu, whereas that of Wa was 8 times that of Kisumu. For the 1.25% Permethrin assay, the KDT_{50} for the Kisumu strain was 10.75 minutes (95% CI: 9.60-11.80), whereas those of the wild *An. gambiae* s.l. populations from the Upper West Region ranged from 24.37 minutes in Nadowli to 59.80 minutes in Lawra. Accordingly, the estimated time in minutes required for 50% of the wild mosquitoes to be knocked down was approximately 2 to 6 times higher than that for Kisumu. The resistance ratio for KDT_{50} ranged from 2.27 for Nadowli to 5.56 for Lawra.

Similarly, the KDT_{95} for the Kisumu population was 23.64 minutes (95% CI: 21.70-26.31), while those for the wild populations *An. gmabiae* s.l ranged from 54.25 minutes at Nadowli to 129.90 minutes at Lawra. The resistance ratio for KDT_{95} values was between 2.29 for Nadowli and 5.50 for Lawra, suggesting that the time required for 95% of the mosquito population to be knocked down in the wild populations was about two to five times longer than in Kisumu. Among the field populations, Lawra recorded the highest knockdown time for both KDT_{50} and KDT_{95}.

Table 4.3: Knockdown Time (KDT_{50}, KDT_{95}) and Resistance Ratios (RRKDT) of Anopheles gambiae s.l. Across Insecticides and Districts

Class of Insecticides	Insecticide (Conc.)	Locality	N	KDT_{50} (min)	95% CI	$RRKDT_{50}$	KDT_{95}	95% CI	$RRKDT_{95}$
Pyrethroid	Deltamethrin 0.05%	Kisum u	100	22.59	(19.20-25.94)	-	50.41	(44.48-59.40)	-
		Wa	100	78.23	()-	3.46	95.16	()-	1.89
		Nadowli	100	113.22	(77.30-394.30)	5.01	208.98	(131.99-832.46)	4.15
		Lawra	100	94.12	(80.25-121.74)	4.17	148.02	(120.73-203.76)	2.94
	Deltamethrin 0.25 %	Kisumu	100	13.03	(11.84-14.17)	-	27.86	(25.65-30.83)	
		Wa	100	66.65	(49.48-146.72)	5.12	140.68	(95.10-385.97)	5.05
		Nadowli	100	22.68	(-3.67-38.94)	1.74	70.60	(48. 91-217.57)	2.53
		Lawra	100	32.89	(27.33-39.32)	2.52	68.95	(58.06-89.25)	2.47
	Deltamethrin 0.50%	Kisumu	100	12.47	(11.19-13.67)	-	28.19	(25.88-31.30)	
		Wa	100	27.91	(23.50-32.61)	2.24	56.48	(48.80-69.35)	2.00
		Nadowli	100	18.97	(12.51-25.07)	1.52	42.20	(33.63-63.33)	1.50
		Law ra	100	24.84	(21.61-28.20)	1.99	49.70	(44.0.4-58.24)	1.76
	Permethrin 0.75%	Kisumu	100	12.34	(1.27-18.88)	-	29.61	(21.79-71.54)	
		Wa	100	146.51	(105.98-288.35)	11.87	255.55	(176.27-536.22)	8.63
		Nadowli	100	87.40	(76.23-107.60)	7.08	138.30	(115.69-180.41)	4.67
		Lawra	100	90.61	(64.19-162.57)	7.34	167.45	(119.72-338.11)	5.65
	Permethrin 1.25%	Kisumu	100	10.75	(9.60-11.80)	-	23.64	(21.70-26.31)	
		Wa	100	28.02	(23.44-32.89)	2.61	57.51	(49.49-71.14)	2.43
		Nadoli	100	24.37	18.80 – 29.92	2.27	54.25	45.34 – 71.24	2.29
		Lawra	100	59.80	47.99 – 88.93	5.56	129.90	96.99 – 224.74	5.50

4.2.1.3 Carbamates (Bendiocarb 0.10%)

For the 0.10% Bendiocarb assay, the KDT_{50} for the Kisumu susceptible strain was 13.44 minutes (95% CI: 11.69-15.00). However, the wild *An. gambiae* s.l. populations exhibited higher KDT_{50} values ranging from 38.12 minutes for Wa to 65.80 minutes for Nadowli. The resistance ratio for KDT_{50} ranged between 2.84 and 4.90, indicating that mosquitoes from these localities required approximately three to five times longer to achieve 50% knockdown compared with Kisumu.

The KDT_{95} values followed a similar trend, with 36.60 minutes (95% CI: 33.03-39.93) for Kisumu and 63.11-93.22 minutes for the field populations. The corresponding resistance ratios for KDT_{95} ranged from 1.72 at Wa to 2.55 at Nadowli. Thus, the KDT95 values for the wild mosquitoes were approximately twice those for the Kisumu mosquitoes.

4.2.1.4 Organophosphates

For the 0.25% Pirimiphos-methyl assay, the KDT_{50} for the susceptible An. gambiae s.l. Kisumu strain was 22.06 minutes (95% CI: 18.30-25.67). In contrast, the KDT_{50} for the wild *An. gambiae* s.l. populations from the Upper West Region ranged from 62.48 minutes in Wa to 74.92 minutes in Nadowli, with Lawra recording a value closely similar to 73.50 minutes. The resistance ratio for KDT_{50} ranged from 2.83 for Wa to 3.40 for Nadowli, indicating that the estimated times in minutes during which 50% of the wild mosquitoes from the Upper West were knocked down were approximately 2 to 4 times those of the Kisumu mosquitoes.

Similarly, the KDT_{95} for Kisumu was 52.61 minutes (95% CI: 46.09-62.72), while those of the wild populations ranged between 65.68 minutes for Wa and 106.51 minutes for Lawra. The resistance ratios for KDT_{95} ranged from 1.25 to 2.03; thus, the estimated times in minutes for which 95% of wild mosquitoes were knocked down were about 2 to 3 times those of Kisumu. The knockdown times were higher in the wild populations, with moderate differences in the ratios between KDT_{50} and KDT_{95}.

For the higher concentration of 1.25% Pirimiphos-methyl, the KDT_{50} for the Kisumu strain was 22.50 minutes (95% CI: 15.80-29.20), while those for the wild *An. gambiae* s.l. populations ranged from 49.42 minutes at Wa to 53.61 minutes at Lawra. The calculated resistance ratios for KDT_{50} ranged from 2.20 to 2.38, indicating that the wild populations required approximately twice the knockdown time as Kisumu.

The KDT_{95} for Kisumu was 54.20 minutes (95% CI: 44.70-63.70), whereas those for the wild populations *An. gmabiae* s.l ranged between 75.49 minutes for Wa and 89.82 minutes for Lawra. Correspondingly, the resistance ratios for KDT_{95} ranged from 1.39 to 1.66. Thus, the estimated times in minutes, for which 95% of the wild mosquitoes from the Upper West were approximately 1.39 and 1.66 times that of the Kisumu mosquitoes.

Table 4.4: Knockdown Time (KDT_{50}, KDT_{95}) and Resistance Ratios (RRKDT) of Anopheles gambiae s.l. Across Insecticides and Districts

Class of Insecticides	Insecticide (Conc.)	Locality	N	KDT_{50} (min)	95% CI	$RRKDT_{50}$	KDT_{95}	95% CI	$RRKDT_{95}$
Carbamate	Bendiocarb 0.10%	Kisumu	100	13.44	(11.69-15.0)	-	36.60	(33.03-39.93)	-
		Wa	100	38.12	(32.82-44.48	2.84	63.11	(54.41-79.41)	1.72
		Nadowli	100	65.80	(61.58-71.32)	4.90	93.22	(84.50-107.19)	2.55
		Lawra	100	52.22	(46.17-62.15)	3.89	83.04	(70.53-109.45)	2.27
Organopho sphate	Pirimiphos-methyl 0.25%	Kisumu	100	22.06	(18.30-25.67)	-	52.61	(46.09-62.72)	-
		Wa	100	62.48	(-244.98-369.95)	2.83	65.68	(-636.41-767.78)	1.25
		Nadowli	100	74.92	(67.83-96.54)	3.40	96.49	(82.25-141.76)	1.83
		Lawra	100	73.50	(673.31-83.99)	3.33	106.51	(93.45-129.71)	2.03
	Pirimiphos-methyl 1.25%	Kisumu	100	22.5	(15.8–29.2)	-	54.2	(44.7–63.7)	-
		Wa	100	49.42	(45.11-55.07)	2.20	75.49	(67.08-90.14)	1.39
		Nadowli	100	52.42	(49.92-55.38)	2.33	81.90	(76.39-89.17)	1.51
		Lawra	100	53.61	(50.53-57.38)	2.38	89.82	(82.72-99.33)	1.66

4.3 Knockdown Rate and 24-hour Mortality of Anopheles gambiae s.l. and Kisumu Strains Exposed to Clothianidin (2%).

For the Lawra District, the knockdown rate (KDR) of *An. gambiae s.l.* increased from 37% (95% CI: 27.56-47.24) at the 30th minute to 72% at the 60th minute. On the other hand, KDRs for the standard Kusumu mosquitoes increased from 65% to 100%. Thus, the KRDs for the wild mosquitoes from Lawra were significantly lower (p < 0.05), suggesting reduced susceptibility. However, 100% mortality was observed in both mosquito groups after the 24-hour recovery period. For the Wa municipal, KDRs ranged from 40% at the 30th minute to 65% at the 60th minute, whilst KDR for the Kisumu increased from 65% at the 30th minute to 100% at the 60th minute (p < 0.05). Mortality rates remained at 100% for both mosquito strains. At Nadowli, the lowest initial knockdown occurred at the 30th minute and increased to 66% after 60 minutes (p < 0.05). In contrast, Kisumu showed significantly higher values (78–100%). Nevertheless, mortality rates were 100% for both the wild and the Kisumu.

The mortality rates of *Anopheles gambiae* s.l. due to exposure to clothianidin, it varied between the three study sites. At 30 minutes, knockdown rates ranged from 28% for Nadoli to 60% for Wa. At the 60th minute, the lowest mortality rate of 65% was recorded for Wa, whereas the highest, 72%, was recorded for Lawra. Despite these variations, 100% mortality occurred after the 24-hour recovery period in all clothianidin-An treatments. *gambiae* s.l. assays.

Table 4.5: Knockdown Rate and 24-hour Mortality of *Anopheles gambiae* s.l. and Kisumu Strains Exposed to Clothianidin (2%)

Vector Species	N	30 min			60 min			24 h Mortality (%)
		KDR	95% CI	P-value	KDR	95% CI	P-value	
Lawra								
An. gambiae s.l.	100	37	27.56 – 47.24	0.012	72	62.13 – 80.52	0.048	100
Kisumu	100	65	54.82 – 74.27		100	96.38 – 100.00		100
Wa								
An. gambiae s.l.	100	40	30.33 – 50.28	0.021	65	54.82 – 74.27	0.033	100
Kisumu	100	65	54.82 – 74.27		100	96.38 – 100.00		100
Nadowli								
An. gambiae s.l.	100	28	19.48 – 37.87	0.008	66	55.85 – 75.18	0.041	100
Kisumu	100	78	68.61 – 85.67		100	96.38 – 100.00		100

CHAPTER FIVE

DISCUSSION

5.1 Overview

The current study examined how *Anopheles gambiae s.l.* from two districts, Lawra and Nadowli, and one municipal, Wa, in the Upper West Region of Ghana, responded to three older classes of synthetic chemical insecticides: pyrethroids (deltamethrin and permethrin), organophosphates (pirimiphos-methyl), carbamates (bendiocarb), and one newer class, neonicotinoids (clothianidin). The results of the current study revealed varying levels of insecticide susceptibility and resistance in the *An. gambiae* s.l. from the three locations to the various diagnostic concentrations of these insecticides.

5.2 Physiological susceptibility levels of An. gambiae to Four Insecticide Classes

5.2.1 Pyrethroids (Deltamethrin and Permethrin)

The results of the current research showed that deltamethrin assays yielded high knockdown and complete mortality at higher concentrations across all sites. However, the permethrin assays yielded lower knockdown rates and delayed mortality. The resistance ratios (RR) of *An. gambiae s.l* population of Wa was greater than 5, which indicates a confirmed resistance, while Nadowli and Lawra were less than five, indicating suspected resistance in field populations compared to the susceptible Kisumu strain. The strong knockdown caused by deltamethrin, particularly in Lawra, suggests partial susceptibility, indicating that resistance is developing but not yet fully established. Conversely, reduced permethrin efficacy across all districts indicates greater permethrin resistance than deltamethrin resistance. This pattern agrees with Pwalia et al. (2019) and Mugenzi et al. (2022), who reported widespread pyrethroid resistance in *An. gambiae* in southern Ghana and Nigeria due to long-term exposure to treated nets and agricultural insecticides.

The slower knock-down and elevated KDT values observed in Wa and Nadowli correspond with studies in Benin and Burkina Faso, where *An. gambiae* populations displayed similar pyrethroid resistance profiles (Hancock et al., 2020). The higher efficacy of deltamethrin compared to permethrin may be explained by structural differences; deltamethrin, a type II pyrethroid, possesses an alpha-cyano group that prolongs sodium channel opening, giving it higher potency and slower degradation by detoxifying enzymes (Ranson & Lissenden, 2016). However, this finding contradicts reports from southwestern Ghana (Mireji et al., 2021), where deltamethrin resistance was more intense than permethrin resistance. Such variations may arise from differences in insecticide usage history, genetic makeup of local mosquito populations, and environmental selection pressures. The relatively low susceptibility in Lawra could indicate reduced exposure to agricultural insecticides or a lower frequency of the kdr (L1014F) mutation. At the same time, the slower knockdown in Wa and Nadowli could result from higher kdr gene prevalence and higher expression of metabolic enzymes.

5.2.2 Organophosphate (Pirimiphos-Methyl)

Pirimiphos-methyl caused a rapid and complete knockdown at 1.25% across all sites, with the fastest knockdown observed in Nadowli. Even at lower concentrations (0.25%), mosquitoes showed high susceptibility, indicating that resistance to organophosphates remains low. The RRKDT of Wa and Lawra at 3.40 and 3.33, respectively, showed suspected resistance; only Wa had an RR of 2.88, indicating susceptibility at a 0.25% concentration of Pirimiphos-Methyl.

The observed susceptibility to pirimiphos-methyl is consistent with findings by Akpobolokemi et al. (2022) and Mugenzi et al. (2022), who reported strong efficacy of organophosphates against *Anopheles* in Ghana and Côte d'Ivoire. These studies attributed the preserved susceptibility to the limited household and agricultural use of organophosphates compared to pyrethroids. The results also align with the Ghana National Malaria Control Programme's resistance monitoring data (NMCP, 2023), which confirmed that organophosphate resistance remains uncommon in the Upper West Region. The high mortality in Nadowli suggests that *ace-1* mutations responsible for resistance to both organophosphates and carbamates are likely rare or absent in this population. Similar findings were observed in Mali and Cameroon, where populations without *ace-1* mutations remained fully susceptible to pirimiphos-methyl (Kwiatkowska et al., 2021). In contrast, studies in Kenya and Nigeria reported emerging resistance to organophosphates linked to *ace-1R* mutations and increased esterase activity (Adeleke et al., 2023).

5.2.3 Carbamate (Bendiocarb)

Bendiocarb induced low knockdown and partial mortality across all sites. The KDT values and resistance ratios (2.84, 4.99, and 3.89) indicated a suspected resistance, with Lawra showing the slowest knockdown. The reduced efficacy of bendiocarb indicates that resistance is developing gradually among *An. gambiae* populations. Similar findings were reported by Pwalia et al. (2019), who observed moderate carbamate resistance in *An. gambiae* from Accra and Kasoa. The leading cause is believed to be *ace-1R* mutations and the overproduction of detoxifying enzymes such as esterases, which make acetylcholinesterase less sensitive to carbamates (Weill et al., 2004). These mechanisms are often associated with cross-resistance to organophosphates because both insecticide classes target the same enzyme. The resistance found in this study was moderate compared to the more severe cases reported by N'Guessan et al. (2020) in Benin, where mortality fell below 60%. This difference may result from varying levels of pesticide pressure and mosquito genetic composition. Areas with intense agricultural pesticide use often show higher resistance, while the Upper West Region may have limited exposure to carbamates, which explains its partial susceptibility. The relatively consistent response across all three sites suggests that local mosquitoes experience similar selection forces, possibly from everyday use of crop protection chemicals or domestic insecticides.

Compared with pyrethroids, bendiocarb showed slightly better performance but was still weaker than pirimiphos-methyl and clothianidin. This pattern supports reports from Ghana and Burkina Faso, where carbamates remain intermediate in effectiveness, stronger than pyrethroids but less reliable than organophosphates (Mugenzi et al., 2022; Akpobolokemi et al., 2022). The slower knock-down observed may also relate to metabolic detoxification or cuticular thickening, which delays the penetration of carbamate molecules, as noted by Tchouakui et al. (2024). Additionally, the absence of significant differences among the sites could reflect low gene flow restriction between *An. gambiae* populations across the Upper West Region, promoting similar resistance profiles. When compared to newer insecticides like clothianidin, bendiocarb's reduced performance highlights the advantage of neonicotinoids, which remain highly effective even against populations showing *ace-1* or *kdr* mutations. However, if carbamates continue to be used in agriculture, they could still contribute to cross-resistance, reducing the effectiveness of related compounds. Therefore, monitoring both carbamate and organophosphate resistance together is important to prevent a wider spread of resistance.

5.3 Spatial knockdown at 60 minutes of *An. gambiae* to Insecticide for the Q three selected sites

5.3.1 Pyrethroid

The knockdown responses of *Anopheles gambiae s.l.* populations from Wa, Nadowli, and Lawra to the pyrethroid insecticides, deltamethrin and permethrin, demonstrated varying levels of susceptibility, indicating heterogeneous resistance patterns across the study sites. The results show that pyrethroid efficacy was generally low at standard WHO diagnostic concentrations but improved slightly with higher doses, suggesting the presence of strong resistance mechanisms in malaria vector populations.

At the lowest deltamethrin concentration (0.05%), knockdown rates were particularly low, especially in the Wa population, which recorded only about 4% knockdown at 60 minutes. This result implies a high level of pyrethroid resistance. The slightly higher KDRs observed in Nadowli (8%) and Lawra (12%) also remained far below the expected levels for susceptible mosquito populations. Similar patterns have been reported in northern Ghana, where *An. gambiae s.l.* populations showed reduced knockdown and mortality to deltamethrin due to widespread knockdown resistance (*kdr*) mutations and metabolic enzyme activity (Hamid-Adiamoh et al., 2020).

When the deltamethrin concentration was increased to 0.25%, knockdown improved across all sites, particularly in Lawra and Nadowli, where KDR reached approximately 86% and 80%, respectively, compared to 34% in Wa. The significant inter-site variation ($\chi^2 = 72.83$, $p < 0.0001$) suggests possible differences in resistance gene frequencies or insecticide exposure histories. This pattern aligns with the findings of Kouassi et al. (2020) in Côte d'Ivoire and Yadouleton et al. (2010) in Benin, who observed that increasing pyrethroid concentration temporarily enhances knockdown but does not always overcome resistance.

At the higher deltamethrin concentration (0.50%), knockdown rates improved drastically, with near-total knockdown (92-100%) in Nadowli and Lawra and a slightly lower response (92%) in Wa. These results indicate that although resistance is present, higher doses can still induce knockdown, a phenomenon previously noted by Kiuru et al. (2018) in Kenya, who observed dose-dependent knockdown responses among resistant *An. gambiae* populations.

The response to permethrin followed a similar pattern. At 0.75%, KDRs remained low across all districts, ranging from 8% in Wa to 22% in Lawra. However, at the higher concentration of 1.25%, knockdowns improved significantly, reaching 92% in Wa, 94% in Nadowli, and 42% in Lawra. Although these results show increased knockdown at higher doses, they still highlight varying levels of resistance across sites. The findings agree with those of Hamid-Adiamoh et al. (2020) and Barreaux et al. (2023), who reported widespread permethrin resistance in northern Ghana, often linked to agricultural pesticide use and long-term exposure to pyrethroid-treated bed nets.

5.3.2 Organophosphate (Pirimiphos-methyl)

The knockdown responses of *Anopheles gambiae s.l.* populations to Pirimiphos-methyl revealed moderate susceptibility across Wa, Nadowli, and Lawra. At the diagnostic concentration of 0.25%, knockdown rates were generally low, with Lawra recording 24%, Nadowli 12%, and Wa 10% at 60 minutes. Increasing the concentration to 1.25% substantially enhanced knockdown, with Wa (64%), Nadowli (59%), and Lawra (55%)

showing similar responses, though no significant differences were observed among sites ($\chi^2 = 1.54$, d.f. = 2, p = 0.46). These results indicate partial susceptibility and suggest that higher concentrations of Pirimiphos-methyl improve vector response.

The low but rising knockdown rates align with studies in Ghana that reported moderate susceptibility of *An. gambiae s.l.* to organophosphates (Debrah et al., 2025). These authors also observed similar delayed knockdown responses, attributing them to reduced target-site sensitivity or detoxification enzyme activity. Compared with lower concentrations, higher knockdowns at higher concentrations in this study reflect the continued operational effectiveness of organophosphates against *An. gambiae* populations in Kenya and observed pirimiphos-methyl resistance in *Anopheles arabiensis* and *Anopheles gambiae* s.s. study populations. Their results showed a G119S mutation in the resistance population and stated that resistance monitoring and management are urgently required. (Kitungulu et al., 2022). These findings suggest that *An. gambiae* populations in the Upper West Region remain moderately susceptible to Pirimiphos-methyl. The insecticide thus remains a viable alternative for indoor residual spraying (IRS) programs, offering a strategic advantage in managing resistance and maintaining vector control efficacy.

5.3.3 Carbamate (Bendiocarb 0.10%)

The knockdown responses of *Anopheles gambiae* s.l. populations exposed to Bendiocarb 0.10% revealed distinct variations in susceptibility across the three study districts. No knockdown was observed during the first 10 minutes of exposure, indicating a slow initial response to the carbamate. However, knockdowns increased steadily over time, with the Wa population recording the highest KDR (83% at 60 minutes), followed by

Lawra (58%) and Nadowli (34%). The observed differences were statistically significant ($\chi^2 = 49.42$, df = 2, $p < 0.0001$), signifying spatial heterogeneity in carbamate susceptibility among the mosquito populations.

The relatively higher knockdown rate in Wa suggests a greater susceptibility to Bendiocarb, while the lower responses in Nadowli and Lawra indicate emerging resistance. Similar findings have been reported in South-Western Nigeria, where there was proof of carbamate resistance in *A. gambiae* populations already harbouring resistance to DDT and permethrin, which is a clear indication that calls for the initiation of insecticide resistance management strategies to combat the multiple resistance identified (Oduola et al., 2012). Studies from Cameron draw attention to the probable implication of metabolic mechanisms in bendiocarb resistance in *An. gambiae* populations from Yaoundé and stresses the need for further studies leading to functional validation of detoxification genes involved in this resistance (Antonio-Nkondjio et al., 2016). The findings suggest that Bendiocarb 0.10 is less effective against some *An. gambiae* populations and shows resistance.

5.3.4 Mortality Rate of *Anopheles gambiae s.l.* Populations after 24-Hour Recovery

The strategic framework of the Ghana National Malaria Control Programme (NMCP) focuses primarily on prompt case management and vector control, especially through the widespread use of insecticide-treated nets (ITNs) among high-risk groups such as pregnant women and children under five years (Bawuah & Ampaw, 2021).

In this study, the susceptibility status of Anopheles gambiae sensu lato (s.l.) populations collected from Wa, Nadowli, and Lawra Districts was evaluated against three traditional WHO-approved insecticide classes following a 24-hour recovery period. Pyrethroids remain the most commonly used insecticides in malaria control programmes due to their proven success in long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and other household protection measures.

At the diagnostic concentration of deltamethrin (0.05%), however, mosquito mortality was generally low across all study sites, indicating widespread pyrethroid resistance. Mortality rates were lowest in Wa (20%) and Lawra (22%), while Nadowli recorded a relatively higher mortality rate of 71%, suggesting partial susceptibility among the mosquito population in that district.

Comparable findings have been reported in both northern Ghana and Mexico, where reduced susceptibility to deltamethrin among *An. gambiae s.l.* populations were attributed to knockdown resistance (kdr) gene mutations and enhanced metabolic enzyme activity. Elevated resistance levels also correlated strongly with the presence of kdr mutations, notably V410L, V1016I, and F1534C, which have been shown to play a major role in conferring deltamethrin resistance (Contreras-Perera et al., 2020; Hamid-Adiamoh et al., 2020).

When the concentration of deltamethrin was increased to 0.25% and 0.50%, mosquito mortality improved substantially, with near-complete mortality at the 10× diagnostic dose. This finding indicates that resistance in the tested populations was moderate and could be partially overcome by higher insecticide concentrations. This trend supports Carson et al.'s (2023) observation that metabolic resistance mechanisms may be suppressed at elevated doses, leading to improved knockdown and mortality outcomes. These findings underscore the importance of routine monitoring of kdr mutation frequencies as part of insecticide resistance surveillance and management strategies in Ghana's vector control programmes..

For permethrin (0.75%), mortality was again low in Wa and Lawra (38%) but relatively higher in Nadowli (57%), indicating heterogeneous resistance. These results are in line with studies conducted in Mozambique, which show resistance to pyrethroids in *An. funestus s.s.*, which was extremely high, much higher than reported in 2002 and 2009. No exposure killed more than 25.8% of the mosquitoes tested (average mortality, deltamethrin: 6.4%; lambda-cyhalothrin: 5.1%; permethrin: 19.1%) (Glunt et al., 2015) . When tested at a higher concentration (1.25%), mortality increased markedly to 85%-99%, suggesting that while resistance persists, the level may not yet compromise operational control effectiveness. The significant differences among districts further suggest localised variations in resistance drivers, possibly linked to differing agricultural insecticide exposure or vector control histories.

Bendiocarb (0.10%), a carbamate insecticide, elicited moderate to high mortality across the districts, with the Wa (84%) and Lawra (78%) populations showing relatively higher susceptibility than Nadowli (59%). The significant differences in mortality suggest that carbamate resistance is emerging, especially in Nadowli. This observation agrees with findings from Mali, where the study highlighted the implication of the G119S mutation in bendiocarb resistance in *An. gambiae* (s.s.), *An. arabiensis* and *An. coluzzii* populations from the three surveyed localities (Keïta et al., 2020). The results suggest that while bendiocarb remains effective in some areas, continued monitoring is crucial to detect early resistance trends before they compromise vector control outcomes.

In contrast, pirimiphos-methyl (0.25%) recorded consistently high mortality across all sites, indicating full susceptibility. Even at the diagnostic dose, mortality exceeded 87%, with no significant inter-district variation. At higher concentration (1.25%), complete mortality was achieved, confirming the continued efficacy of organophosphates against *An. gambiae s.l.* populations. These results align with findings that observed that pirimiphos-methyl remains one of the most potent insecticides for IRS due to its different mode of action, targeting acetylcholinesterase, which is less affected by *kdr*-mediated resistance (Keïta et al., 2021).

The findings reveal that *An. gambiae s.l.* populations in the selected district of the Upper West Region have developed varying degrees of resistance to deltamethrin, permethrin, carbamate, and pirimiphos-methyl, showing high resistance at low concentrations. In contrast, at higher concentrations (x10), they showed low mortality rates, and others even showed susceptibility after a 24-hour recovery period. The persistence of pyrethroid resistance poses a challenge to the continued use of LLINs, emphasising the need for integrated vector management strategies.

5.4 Neonicotinoid (Clothianidin)

Field populations exhibited significantly lower knock-down rates at 30 and 60 minutes compared to the Kisumu strain, but all achieved 100% mortality after 24 hours. The Lawra population had the slowest early knockdown, while Nadowli showed slightly faster response at later time points. The delayed knockdown but full mortality aligns with clothianidin's mode of action, which targets nicotinic acetylcholine receptors and causes gradual paralysis rather than rapid knockdown. These findings are comparable to those from northern Ghana (Kemevor et al., 2023) and Benin (Azizi et al., 2024), where mosquitoes remained fully susceptible, albeit with delayed mortality. Similar trends were reported in Tanzania (Fouet et al., 2024) and Nigeria (Adeleke et al., 2023), where clothianidin performed better than older pyrethroids and carbamates, proving its potential as a key alternative in resistance management.

The slower initial response in Lawra could indicate early tolerance to agricultural pesticides, as farmers often use neonicotinoids on crops such as maize and vegetables. Repeated environmental exposure can increase detoxifying enzymes, especially cytochrome P450s, which reduce the insecticide's effectiveness. This explanation is supported by Fouet et al. (2024), who found reduced clothianidin potency in farming areas due to metabolic enzyme activity. However, the complete mortality after 24 hours suggests that the resistance mechanism remains weak and has not yet affected control outcomes. Compared with other insecticides in this study, clothianidin clearly outperformed deltamethrin, permethrin, and bendiocarb, demonstrating that it remains a reliable option even where pyrethroids and carbamates fail. It is delayed but complete, unlike the faster knockdown seen with pyrethroids, and it has a longer residual effect, making it ideal for indoor residual spraying (IRS). This agrees with research by Ngufor et al. (2023) and Kemevor et al. (2023), who highlighted that clothianidin provides sustained control for weeks after spraying.

This finding contrasts with early resistance signs reported in Cameroon (Ngufor et al., 2023) and in some East African settings, where repeated IRS rounds led to lower mortality. The difference may be due to fewer IRS campaigns and less neonicotinoid exposure in Ghana. Additionally, Ghanaian *An. gambiae* populations may have different genetic backgrounds, such as lower expression of P450 genes compared to those in

Cameroon. The high overall susceptibility here also mirrors observations for other neonicotinoids, such as imidacloprid and acetamiprid, in Kenya (Owuor et al., 2024), which continue to show full activity against resistant *Anopheles* strains. Clothianidin's superior performance and long-lasting mortality demonstrate its strength compared to older insecticides. However, constant monitoring is needed to ensure that early tolerance signs, especially from agricultural exposure, do not develop into complete resistance. The inclusion of clothianidin in IRS rotations alongside pirimiphos-methyl could help manage cross-resistance and sustain vector control efficacy in the Upper West Region.

5.4.1 Cross-Resistance and Resistance Mechanisms of An. gambiae and Kisumu strain

The prolonged knockdown times and high resistance ratios across all insecticide types, compared with the susceptible Kisumu strain, suggest that several mechanisms are acting together to make mosquitoes more resistant. This pattern of multiple resistance mechanisms has been widely reported in West Africa and can explain why no single insecticide class works perfectly in all areas. Target-site mutations such as *kdr* (L1014F/S) and *ace-1R* are key factors conferring resistance to pyrethroids, carbamates, and organophosphates in mosquitoes. When these mutations occur together, they can make mosquitoes tolerant to multiple chemical groups. In Ghana and Benin, such combined mutations have been found in *An. gambiae*, leading to overlapping resistance between deltamethrin, bendiocarb, and pirimiphos-methyl.

Another important factor is metabolic detoxification, in which enzymes such as cytochrome P450S (CYP6M2, CYP6P3), esterases, and glutathione S-transferases break down or deactivate the insecticide before it reaches its target. Studies by Mitchell et al.

(2023) and Ranson et al. (2022) showed that high enzyme activity can reduce the impact of pyrethroids and even newer compounds like neonicotinoids. Compared with other insecticides, clothianidin appears less affected by this mechanism, which explains its better performance in this study.

Cuticular resistance also plays a part. Mosquitoes may develop thicker or chemically altered outer layers that slow the absorption of insecticides. This has been seen in *An. gambiae* from Ghana and Burkina Faso (Tchouakui et al., 2024). As a result, even strong insecticides like deltamethrin take longer to knock mosquitoes down. In contrast, organophosphates like pirimiphos-methyl are less affected by this mechanism because they act more rapidly once absorbed.

Environmental factors also contribute. The use of agricultural insecticides such as pyrethroids and neonicotinoids on crops increases exposure and selection pressure. Studies in Ghana and Cameroon have shown that pesticide runoff into mosquito breeding sites raises the chances of resistance development (Acheampong et al., 2025). These pressures may explain why mosquitoes in farming districts like Wa and Nadowli were less susceptible. In contrast, Lawra, which may have fewer agricultural activities, showed slightly higher sensitivity.

Across insecticide classes, pyrethroids are most affected by these combined mechanisms, followed by carbamates. Organophosphates and neonicotinoids remain more effective because resistance genes and metabolic pathways for these compounds are not yet widespread. The results of this study are consistent with findings in Nigeria, Mali, and Burkina Faso, where similar multi-mechanism resistance has been reported, though with varying intensities. This comparison emphasises that managing resistance requires a multi-insecticide approach and strict regulation of pesticide use in health and agriculture.

The findings show that pyrethroid resistance is entrenched in *An. gambiae* populations in the Upper West Region, whereas organophosphates and neonicotinoids remain highly effective. The emerging resistance to carbamates and partial tolerance to deltamethrin call for a shift toward integrated resistance management strategies, including rotation with non-pyrethroids. Continuous molecular monitoring for *kdr* and *ace-1R* mutations, along with metabolic assays, should be incorporated into regional surveillance. Further, local agricultural pesticide use should be regulated to minimise cross-resistance pressures.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary of Key Findings

The results demonstrated significant variability in the susceptibility of *An. gambiae s.l.* populations from Lawra, Wa, and Nadowli to the traditional insecticide classes. Pyrethroid resistance was widespread across all three districts, with diagnostic-dose knockdown remaining low for both deltamethrin and permethrin. Lawra recorded the weakest knockdown response (12% for deltamethrin and 22% for permethrin at 60 minutes), while Wa and Nadowli exhibited slightly higher but still sub-optimal values. Even at 10× concentrations, complete restoration of susceptibility was inconsistent. For carbamates, bendiocarb produced moderate resistance patterns, with reduced KDT_{50} and KDT_{95} values and corresponding resistance ratios ($RRKDT_{50}$ = 2.84-3.89), suggesting a gradual decline in susceptibility. In contrast, the organophosphate pirimiphos-methyl showed comparatively strong performance, with mortality values of 87-99% across sites, and relatively low resistance ratios ($RRKDT_{50}$ = 2.20-3.40). These observations confirm a marked erosion of efficacy for pyrethroids and carbamates, while organophosphate susceptibility remains largely preserved in the study area.

Clothianidin performed strongly across all districts, producing consistently high mortality within 24 hours and demonstrating minimal evidence of physiological resistance. The uniform response of the vector populations to clothianidin indicates that neonicotinoids remain a viable and practical option for IRS and complementary vector control strategies. The marked contrast in response patterns between clothianidin and the older insecticide classes emphasizes the operational value of new-generation insecticides in areas where traditional chemicals have experienced prolonged selective pressure.

The combined knockdown and mortality data indicate that resistance patterns differ across insecticide classes and across geographical sites, with operational implications for ongoing IRS programmes. Pyrethroid resistance, evident in extended KDT values, elevated resistance ratios, and reduced 24-hour mortality, points to the diminishing usefulness of pyrethroid-based interventions in the Upper West Region. Similarly, moderate bendiocarb resistance highlights the need for cautious deployment of carbamates. The strong susceptibility to pirimiphos-methyl and clothianidin suggests that organophosphates and neonicotinoids currently offer the most reliable control options. Resistance drivers such as *kdr* and *ace-1R* mutations, increased detoxification enzyme activity, cuticular thickening, and local agricultural pesticide use further underscore the need for integrated resistance management, including rotation of insecticide classes and routine resistance monitoring. Collectively, these findings enhance the evidence base for programme decisions by AGAMal and the NMCP regarding IRS product selection, rotation schedules, and long-term resistance management strategies.

6.2 Conclusion

The study concluded that *An. gambiae* mosquitoes in the Upper West Region have developed strong resistance to pyrethroids and moderate resistance to carbamates, but remain highly susceptible to organophosphates and neonicotinoids. This pattern suggests that continuous use of pyrethroid-based insecticides for indoor spraying and treated nets could worsen resistance and reduce their effectiveness. This finding implies that malaria control programs relying only on pyrethroid-treated nets may lose effectiveness over time. It calls for the urgent adoption of alternative insecticides and combination products to sustain protection. However, the strong performance of pirimiphos-methyl and clothianidin offers hope for adequate control. Their inclusion in rotation or combination programs can help slow the spread of resistance. The high mortality rates observed indicate that organophosphates and neonicotinoids could serve as backup insecticides in resistance management plans. This suggests that these compounds should be prioritised in Integrated Vector Management (IVM) to reduce malaria transmission. The results also stress the importance of improving surveillance systems to detect resistance early and promoting cooperation between agriculture and public health to regulate insecticide use. Strengthened surveillance will help identify emerging resistance quickly, enabling authorities to respond in a timely manner. Closer collaboration between the health and agriculture sectors will reduce cross-resistance to pesticides caused by unregulated use on farms, ensuring that both agricultural productivity and malaria control goals are sustained.

6.3 Recommendations

Based on the findings of the study, the following were recommended;

1. The **AGAMAl** should strengthen and optimise clothianidin-based IRS cycles**:** Full susceptibility across all districts indicates that clothianidin formulations (e.g., SumiShield, Fludora Fusion) should remain a core component of AGAMal's IRS strategy.
2. The **AGAMAl** should enhance regular monitoring of pyrethroid, carbamate, organophosphate, and neonicotinoid susceptibility within IRS operational areas to support timely decisions on IRS product choice, prevent premature resistance development, and improve programme efficiency

3. The Ghana National Malaria Control Programme (NMCP) should alternate insecticides with different modes of action during indoor residual spraying and net

distribution campaigns. This will help prevent mosquitoes from developing strong resistance to a single chemical group. The NMCP, Anglogold Ashanti Malaria Control Programme (AGAMal), in collaboration with the Ministry of Health (MoH), should design a national insecticide rotation plan that includes pyrethroids, organophosphates, and neonicotinoids.

4. **Further Study:** Conduct longitudinal bioassays and molecular resistance profiling to track the evolution of pyrethroid, carbamate, organophosphate, and neonicotinoid resistance markers over time in AGAMal IRS areas

6.4 Statement of Novelty

This study provides one of the most recent and district-specific characterizations of physiological susceptibility and resistance patterns of *An. gambiae s.l.* populations in the Upper West Region of Ghana, using both older and new-generation insecticides. Its novelty lies in simultaneously evaluating clothianidin, a newly introduced IRS active ingredient, alongside traditional classes, and linking phenotypic resistance outcomes with operational programmatic implications. By presenting district-level differences in susceptibility, resistance ratios, and knockdown dynamics, the study generates actionable evidence that directly supports data-driven IRS decision-making, strengthens local resistance-management strategies, and contributes new insights to Ghana's evolving malaria vector control landscape.

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APPENDICES

Appendix 1. Data collection form for testing insecticide susceptibility of adult mosquitoes in the WHO tube tests

Form 1: To be completed in black or blue ink only. Do not use a pencil or correction fluid.

Date (dd/mm/yyyy):	**Technician's name:**	
Location of mosquito collection:	**Coordinates** Latitude: Longitude:	
Period of mosquito collection: Start date (dd/mm/yyyy): End date (dd/mm/yyyy):	**Collection method:**	
Insecticide tested and concentration:	**Date of paper impregnation (dd/mm/yyyy):**	**No. of times the same papers have been used before:**
Mosquito species:	**Mosquito stage and origin:** F0 adults (from wild larvae), F0 adults (wild collected), F1 adults (from wild larvae), F1 adults (progeny of wild adults)	
Age of females (days):	**Feeding status:** unfed; sugar-fed and starved; other, specify	
Start time of exposure (hh:mm):	**End time of exposure (hh:mm):**	
Temperature during exposure + holding period (°C): Max: Min:	**Relative humidity during exposure + holding period (%):** Max: Min:	

	Tube	**Number of mosquitoes introduced**	**Number of knocked down mosquitoes 1 h after exposure**	**No. of dead and alive mosquitoes at 24 h after 1 h exposure**		**Mortality at 24 h after 1 h exposure**
				No. dead	**No. alive**	**Mortality (%)**
Wild mosquitoes exposed to DC[a] of the insecticide	**Tube 1**					
	Tube 2					
	Tube 3					
	Tube 4					
Wild control mosquitoes	**Control tube 1**					
	Control tube 2					

Final results (all tubes)

	Knocked down after 1 h exposure (%) (at the end of 1 h exposure)	**Mortality % (at 24 h)**	**Abbott's corrected mortality % (24 h)**
Wild mosquitoes exposed to the DC[a] of a test insecticide			

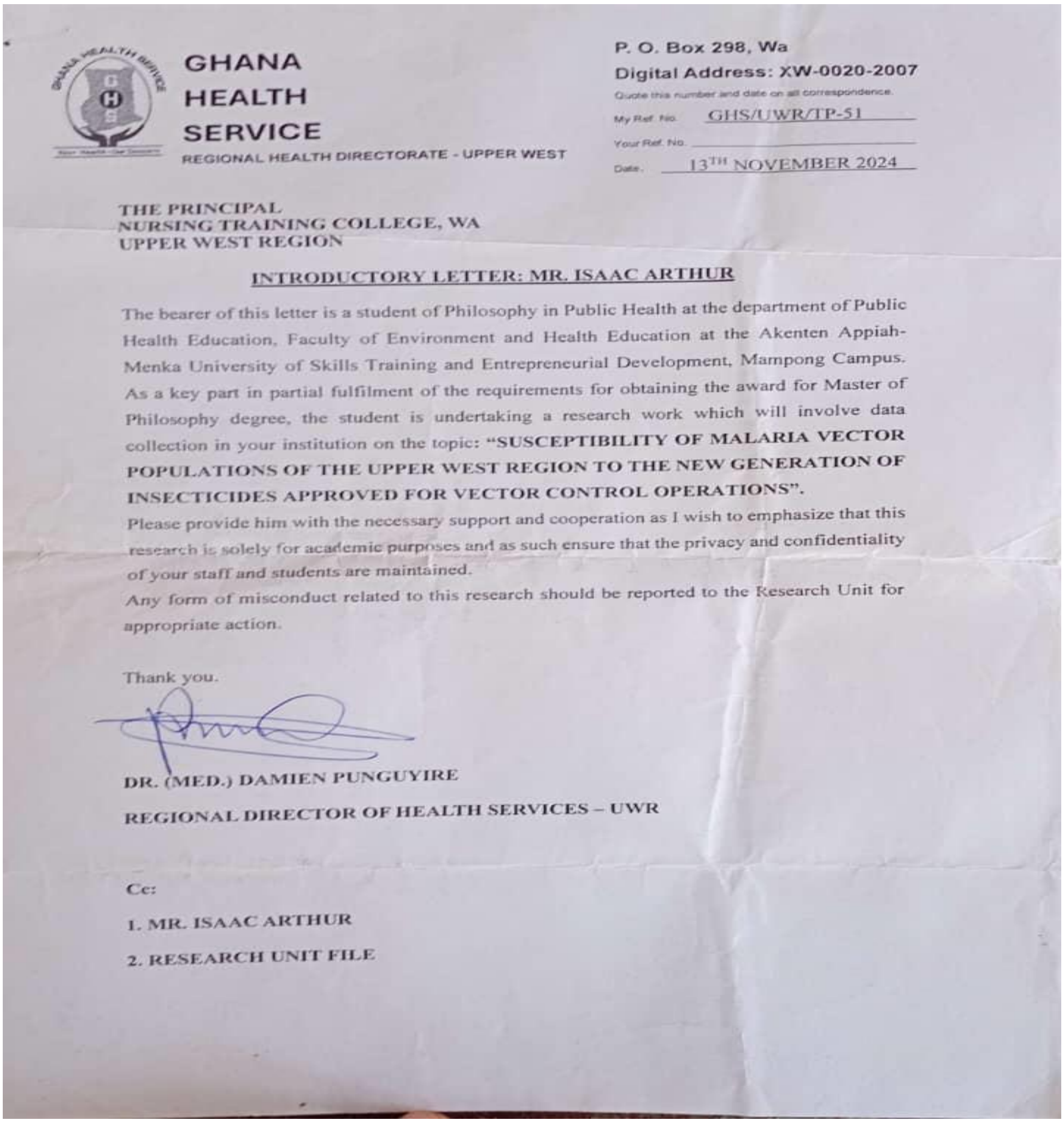
Test result: The vector population is ________________(susceptible/resistant/possibly resistant) to the insecticide. Comments, if any:

Verified by Supervisor: ________ Date: ________

Form 2: Knockdown Sheet

Time/minutes	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Control 1	Control 2
0						
10						
20						
30						
40						
50						
60						

Appendix IV Ethical approval form



GHANA HEALTH SERVICE
REGIONAL HEALTH DIRECTORATE - UPPER WEST

P. O. Box 298, Wa
Digital Address: XW-0020-2007
Quote this number and date on all correspondence.
My Ref. No. GHS/UWR/TP-51
Your Ref. No.
Date. 13TH NOVEMBER 2024

THE PRINCIPAL
NURSING TRAINING COLLEGE, WA
UPPER WEST REGION

INTRODUCTORY LETTER: MR. ISAAC ARTHUR

The bearer of this letter is a student of Philosophy in Public Health at the department of Public Health Education, Faculty of Environment and Health Education at the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong Campus. As a key part in partial fulfilment of the requirements for obtaining the award for Master of Philosophy degree, the student is undertaking a research work which will involve data collection in your institution on the topic: **"SUSCEPTIBILITY OF MALARIA VECTOR POPULATIONS OF THE UPPER WEST REGION TO THE NEW GENERATION OF INSECTICIDES APPROVED FOR VECTOR CONTROL OPERATIONS".**
Please provide him with the necessary support and cooperation as I wish to emphasize that this research is solely for academic purposes and as such ensure that the privacy and confidentiality of your staff and students are maintained.
Any form of misconduct related to this research should be reported to the Research Unit for appropriate action.

Thank you.

DR. (MED.) DAMIEN PUNGUYIRE
REGIONAL DIRECTOR OF HEALTH SERVICES – UWR

Cc:
1. MR. ISAAC ARTHUR
2. RESEARCH UNIT FILE