

**Potency and implications of use of insecticide mixtures in
management of resistant *Anopheles gambiae* s.l. (Diptera:
Culicidae) mosquitoes.**

BY

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14/68FG003

ZOOLOGY (ENTOMOLOGY OPTION)

**A Ph.D. THESIS PRESENTED TO DEPARTMENT OF ZOOLOGY, FACULTY OF
LIFE SCIENCES, UNIVERSITY OF ILORIN, ILORIN, NIGERIA, IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF
PHILOSOPHY (Ph.D) DEGREE IN ZOOLOGY (ENTOMOLOGY)**

DECLARATION

I hereby declare that this thesis entitled “Potency and implications of use of insecticide mixtures in management of resistant *Anopheles gambiae* s.l. (Diptera: Culicidae) mosquitoes” was written by me and it is a record of my Ph.D research work. I also proclaim that neither the whole work nor any part of it has been, is been, or is to be submitted for another degree at this or any other University or examining body.

Signature:.....

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April, 2021

CERTIFICATION

This is to certify that this study, written by OMOTAYO, Ahmed Idowu (14/68FG003) has been read and approved as meeting part of the requirements of Department of Zoology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria for the award of Doctor of Philosophy in Zoology.

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DEDICATION

This thesis is dedicated to all moralists and agents of social justice in the world.

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ABSTRACT

The preponderance of insecticide resistance development in malaria vectors constitutes a major challenge in malaria management. The dire need to stem this challenge imposed by resistant mosquito populations informed this study aimed at exploring the possibility of deploying sequentially programmed insecticide mixtures. The specific objectives were to: (i) determine susceptibility status of *Anopheles gambiae* in Lagos state; (ii) determine the prevalence of resistant genes; (iii) determine the susceptibility status of resistant populations of *Anopheles gambiae* to insecticide mixtures; (iv) compare susceptibility statuses of mixtures and single insecticides; and (v) determine and compare metabolic enzymes activities in *Anopheles gambiae* exposed to individual insecticides and insecticide mixtures.

Susceptibility status was determined using WHO susceptibility test protocol. Twenty-five (25) female adult mosquitoes of 3 – 5 days old were exposed in four (4) replicates to insecticide treated papers impregnated with WHO diagnostic doses of DDT, Permethrin, Pirimiphos-methyl and Propoxur. Exposure to mixtures was done by slight modification of WHO method. WHO treated papers were cut into half and combined in the following format; DDT/Pirimiphos-methyl, DDT/Propoxur, Permethrin/Pirimiphos-methyl and Permethrin/Propoxur into WHO susceptibility bottles for exposure of mosquitoes to mixtures. Resistant genes were determined using Allele-Specific Polymerase Chain Reaction assay. Activities of metabolic enzymes; esterase, monooxygenase and glutathione-S-transferase were determined using naphthyl acetate end point assay, monooxygenase titration assay and glutathione-S-transferase assay respectively. Susceptibility status was analysed by calculating percentage mortality. Knockdown times (KDT₅₀ and KDT₉₅) were determined by probit analysis and used in calculating resistance reduction index. Prevalence of resistant genes was calculated using percentages. Paired student t-Test was employed to determine the significant difference at $P < 0.05$. Results from metabolic assays were interpreted spectrophotometrically and analysed using standard graphs.

The findings of the study were that:

- (i) *Anopheles gambiae* from Kosofe, Lagos mainland and Ojo were absolutely susceptible (100%) to Propoxur but resistant to DDT (44%, 46%, 29%), Permethrin (50%, 48%, 96%) and Pirimiphos-methyl (25%, 38%, 32%) respectively.
- (ii) The allelic frequency of resistant gene; *kdr* were low in Kosofe (11.6%), Lagos Mainland (16.4%) and Ojo (6.7%). No *ace-1^R* gene was found.
- (iii) *Anopheles gambiae* from Kosofe, Lagos mainland and Ojo were susceptible to all propoxur mixtures and Permethrin/Pirimiphos-methyl (99%, 97%, 99%) but resistant to DDT/Pirimiphos-methyl (43%, 29%, 35%) respectively.
- (iv) Resistance reduction indices in mixtures involving DDT and permethrin mixtures had higher knockdown impacts. Whereas mixtures involving pirimiphos-methyl

mixtures had lower knockdown impact, mixtures involving propoxur mixtures had comparable knockdown impact when compared with individual insecticides.

- (v) There was significant elevation ($P=0.039$) of esterase activities in populations exposed to DDT/Pirimiphos-methyl when compared with DDT-exposed populations. GST activities was significantly elevated ($P=0.016$) in Permethrin/Propoxur-exposed populations when compared with Permethrin-exposed populations. For monooxygenase, there was significant decrease in activities of DDT/Pirimiphos-methyl-exposed ($P=0.031$) and Permethrin/Propoxur-exposed ($P=0.023$) populations when compared to Pirimiphos-methyl-exposed and Permethrin-exposed populations respectively.

The study concluded that insecticide mixtures are potent in the management of resistant *Anopheles gambiae* populations. The study recommended that Permethrin/Propoxur, DDT/Propoxur and Permethrin/Pirimiphos-methyl can be employed in the management of multiple resistance in *Anopheles gambiae*.