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PERMETHRIN AND DELTAMETHRIN RESISTANCE STATUS OF FIELD POPULATION OF *ANOPHELES* MOSQUITOES IN ZUBA, FEDERAL CAPITAL TERRITORY OF NIGERIA

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ABSTRACT

There is limited data on malaria mosquito resistance in Zuba, Nigeria. This study assessed the resistance of Anopheles mosquitoes to WHO-recommended concentrations of permethrin and deltamethrin using CDC bottle bioassays. Twenty-five wild-caught mosquitoes were exposed in quadruplicate at various insecticide concentrations. Mortality data were analyzed using ANOVA at P=0.05. The recommended concentrations induced 84.4% and 72% mortality for permethrin and deltamethrin, respectively. Lower insecticide concentrations did not result in statistically significant mosquito mortality. The findings suggest developing resistance, particularly to deltamethrin and highlight the need for regular resistance monitoring in Zuba.

Keywords: *Anopheles* mosquito, Zuba, Insecticide, Resistance

INTRODUCTION

Malaria remains a significant public health challenge globally, with Sub-Saharan Africa—particularly Nigeria—bearing a disproportionate burden. The disease is primarily transmitted by *Anopheles gambiae (s.l.*) mosquitoes (WHO, 2023; Adeogun *et al.,* 2023). According to recent reports, Africa accounts for 92% of global malaria cases and over 93% of related deaths (WHO, 2023). Notably, Nigeria alone contributes approximately 25% of malaria cases across the continent (WHO, 2019; Adeniyi *et al.*, 2023).

Vector control remains a cornerstone of malaria prevention strategies in endemic regions (WHO, 2018; Verra *et al.,* 2020). The World Health Organisation (WHO) has long recommended the use of chemical insecticides—especially pyrethroids such as deltamethrin and permethrin—for mosquito control (WHO, 2015; WHO, 2018). However, the effectiveness of these insecticides is increasingly undermined by the emergence of resistance among mosquito populations in Nigeria, particularly through metabolic resistance mechanisms (Riveron *et al.,* 2013; Oduola *et al.,* 2017; Olagundoye and Adesoye, 2023; Adeniyi *et al.,* 2024; Adesoye *et al.,* 2024A).

In response to this challenge, the Centres for Disease Control and Prevention (CDC) and WHO suggested regular resistance monitoring as an essential component of vector control programs in malaria-endemic regions (Adeniyi *et al.,* 2023; CDC, 2024). Such surveillance enables early detection of resistance trends and facilitates timely adjustments to control strategies, helping to sustain the effectiveness of interventions (Liu, 2015; Brogdon and Chan, 2010). Routine resistance assessments can also inform the development of novel vector management approaches (Brogdon and Chan, 2010).

Despite these efforts, currently, to the best of our knowledge, there is a lack of documented data on the insecticide resistance status of malaria vectors in Zuba, Gwagwalada Area Council, Federal Capital Territory (FCT), Abuja. Therefore, this study aims to evaluate the resistance status of *Anopheles* mosquitoes in Zuba to two commonly used pyrethroids: deltamethrin and permethrin. The central hypothesis is that *Anopheles* mosquito populations in this area have developed measurable resistance to these insecticides. The findings will provide critical baseline data to inform local vector control programs, support evidence-based policy decisions, and contribute to broader efforts to mitigate the impact of malaria in Nigeria.

METHODOLOGY

Collection and Rearing of Immature Mosquito

Anopheles larval collections were conducted in June 2024, from temporary rain puddles in Zuba, FCT, (latitude of 9.1023 and longitude of 7.1952), Nigeria, following standard procedures. Larval sampling was conducted. For mapping the identified breeding locations, Garmin eTrex® GPS 10 personal navigators were used, following standard protocols (Service 1971; Adesoye *et al.,* 2023A). Larvae were collected by lightly submerging white dippers at a 45° angle into the breeding sites, skimming the water's surface, and then transferring the samples into labelled bottles for collection. Subsequently, these bottles were transported to the laboratory of the Centre for Malaria Control and Neglected Tropical Diseases, Suleja, Nigeria. The larval samples were maintained under regulated insectary conditions (temperature: 25–28°C, humidity: approximately 70–80%, and a 12-hour light/dark cycle) and fed yeast daily. Upon emergence, the adult mosquitoes were supplied with a 10% glucose solution soaked in cotton wool (Adesoye *et al.,* 2023B).

Preparation of Recommended and Lower Concentrations of Insecticides

A 1 ml sample of the original stock solution of technical-grade permethrin and deltamethrin, provided by the Centers for Disease Control and Prevention (CDC), were each diluted with 49 ml and 28.5 ml of 100% acetone, respectively, to prepare standard concentrations of 21.50 µg/ml and 12.50 µg/ml for the two insecticides.

These concentrations were used to separately coat 25 ml capacity CDC bottles, resulting in the recommended concentrations of 21.50 µg/bottle for permethrin and 12.50 µg/bottle (µg/b) for deltamethrin (Brogdom and Chan, 2010; Adesoye *et al.,* 2024B)

Lower concentrations of permethrin and deltamethrin were prepared following standard method outlined (Adesoye *et al.*, 2024C), yielding 1 ml of each concentration: 15 µg/bottle, 5 µg/bottle, 1 µg/bottle, 0.9 µg/bottle, 0.8 µg/bottle, 0.6 µg/bottle, 0.4 µg/bottle, 0.2 µg/bottle, and 0 µg/bottle (control, acetone only). This process is crucial for evaluating both lethal and sub-lethal effects of the insecticides on exposed mosquitoes. A summary of the dilutions is provided in Table 1 and Table 2.

Table 1. Serial Dilutions of Permethrin and Corresponding Volumes of Stock Solution and Acetone Used in CDC Bottle Bioassay

Serial No.	Concentration (µg/bottle)	Volume of Stock Taken (mL)	Volume of Acetone (mL)
1	15.0	0.9800	0.0200
2	10.0	0.8000	0.2000
3	5.0	0.4000	0.6000
4	1.0	0.0800	0.9200
5	0.9	0.0720	0.9280
6	0.8	0.0640	0.9360
7	0.6	0.0480	0.9520
8	0.4	0.0320	0.9680
9	0.2	0.0160	0.9840
Control	0.0	—	1.0000

Note: Stock solution and acetone were mixed to achieve each desired permethrin concentration per CDC bottle. The control group received acetone only.

Table 2. Serial Dilutions of Deltamethrin and Corresponding Volumes of Stock Solution and Acetone Used in CDC Bottle Bioassay

Serial No.	Concentration (µg/ bottle)	Volume of Stock Taken (mL)	Volume of Acetone (mL)
1	10.0	0.8000	0.2000
2	5.0	0.4000	0.6000
3	1.0	0.0800	0.9200
4	0.9	0.0720	0.9280
5	0.8	0.0640	0.9360
6	0.6	0.0480	0.9520
7	0.4	0.0320	0.9680
8	0.2	0.0160	0.9840
Control	0.0	—	1.0000

Note: Stock solution and acetone were mixed to achieve each desired permethrin concentration per CDC bottle. The control group received acetone only.

Mosquito Exposure to Permethrin and Deltamethrin Insecticides

Adult mosquitoes were maintained at 26 ± 2 °C, 75 ± 5 % relative humidity, and a 12:12 h light: dark photoperiod. They were then exposed to both the recommended and lower concentrations of the two insecticides to assess their lethal and sub-lethal effects. This was achieved by coating clean, dried, and well-labelled 25 ml capacity CDC bottles with each concentration. Subsequently, 25 adult mosquito samples were introduced into each bottle in four replicates, including a control, following a standard procedure (Adesoye *et al.*, 2024C; CDC, 2024). Mosquitoes were exposed to insecticides in CDC bottles for 30 minutes, after which they were transferred to holding cages for observation. Mortality was recorded continuously from the first minute of exposure up to 24 hours post-exposure.

Statistical Analysis

Information obtained from mosquito mortality was analysed using IBM-SPSS version 25.0 and expressed in tables as mean mortality (± SD) values. These were compared statistically among insecticide concentrations using Analysis of Variance at $P<0.05$ with the aid of Graph-Pad Prism 8.

RESULTS

The recommended concentration of permethrin (21.5 µg/b) by the Centres for Disease Control and Prevention (CDC) failed to achieve 100% mortality of adult *Anopheles* mosquitoes within 30 minutes of exposure, as shown in Table 3. The recommended concentration achieved 21.10±0.50 (84.4%) mosquito mortality, significantly higher ($P = 0.031$) than 18.00±0.00 (72%) mortality value elicited by 15.0µg/b permethrin concentration at the same time. Complete mortality was only attained after 35 minutes under the influence of 21.5 µg/b recommended permethrin concentration. The mosquito mortality rate, therefore declines with decreasing concentrations of permethrin insecticide.

Notably, the *Anopheles* mosquito population in Zuba appears to exhibit resistance to the recommended concentration of permethrin.

Table 3. Mortality Response of *Anopheles* Mosquitoes to Varying Lethal Concentrations of Permethrin Over Time in Zuba, FCT, Nigeria

Time (min)	21.5 µg/b	15.0 µg/b	10.0 µg/b	5.0 µg/b	1.0 µg/b	0.9 µg/b
0	–	–	–	–	–	–
15	15.00 ± 1.89^{b} (60)	10.00 ± 0.58^{a} (40)	7.00 ± 1.51^{a} (28)	–	–	–
30	21.10 ± 0.50^{c} (84.4)	18.00 ± 0.00^{b} (72)	7.00 ± 1.51^{a} (28)	–	–	–
35	25.00 ± 0.00^{c} (100)	21.00 ± 0.50^{c} (84)	10.00 ± 0.58^{a} (40)	1.00 ± 0.50^{a} (4)	–	–
40	25.00 ± 0.00^{c} (100)	24.80 ± 2.22^{c} (99.2)	21.00 ± 0.50^{c} (84)	1.00 ± 0.50^{a} (4)	–	–
45	25.00 ± 0.00^{c} (100)	25.00 ± 0.00^{c} (100)	21.00 ± 0.50^{c} (84)	4.00 ± 0.83^{a} (16)	–	–
60	25.00 ± 0.00^{c} (100)	25.00 ± 0.00^{c} (100)	25.00 ± 0.00^{c} (100)	23.00 ± 1.71^{c} (92)	15.00 ± 1.89^{b} (60)	–
24 hours	25.00 ± 0.00^{c} (100)	25.00 ± 0.00^{c} (100)	25.00 ± 0.00^{c} (100)	25.00 ± 0.00^{c} (100)	15.00 ± 1.89^{b} (60)	1.00 ± 0.50^{a} (4)

Note: Percent mortality is shown in parentheses. Superscripts marked with different letters within the same row indicate statistically significant differences (P < 0.05; n = 25). Here, µg/b denotes micrograms per 25 ml CDC bottle; - denote 0.00±0.00a (0) Mean mortality (± SD) and 0 percentage mortality

The Centre for Disease Control and Prevention (CDC)'s recommended concentration of deltamethrin (12.5 µg/b) failed to achieve 100% mortality of adult *Anopheles* mosquitoes within 30 minutes of exposure, as shown in Table 4. Complete mortality was only attained after 40 minutes of exposure to this concentration. Therefore, mosquito mortality rates increased with longer exposure times to various tested concentrations of deltamethrin. For instance, at a concentration of 12.5 µg/b, a mean mortality rate of 19.00±0.30 (76.0%) was observed after 30 minutes of exposure.

In contrast, a significantly higher (P = 0.04) mean mortality rate of 25.00±0.00 (100%) was recorded after 40 minutes of exposure to the same concentration. Additionally, the tested concentration of 0.9 µg/b demonstrated lethality (3% mortality) to the mosquito population only after 24 hours of exposure.

Therefore, the *Anopheles* mosquito population in Zuba is confirmed to be resistant to the recommended concentration of deltamethrin.

Table 4. Mortality Response of *Anopheles* Mosquitoes to Varying Lethal Concentrations of Deltamethrin Over Time in Zuba, FCT, Nigeria

Time (min)	12.5 µg/b	10.0 µg/b	5.0 µg/b	1.0 µg/b	0.9 µg/b
0	–	–	–	–	–
15	15.00 ± 1.89^{b} (60)	7.00 ± 1.51^{a} (28)	–	–	–
30	19.00 ± 0.30^{b} (76.0)	7.00 ± 1.51^{a} (28)	–	–	–
35	21.10 ± 0.50^{c} (84.4)	10.00 ± 0.58^{a} (40)	–	–	–
40	25.00 ± 0.00^{c} (100)	21.00 ± 0.50^{b} (84)	1.00 ± 0.50^{a} (4)	–	–
45	25.00 ± 0.00^{c} (100)	21.00 ± 0.50^{b} (84)	1.00 ± 0.50^{a} (4)	–	–
60	25.00 ± 0.00^{c} (100)	25.00 ± 0.00^{c} (100)	4.00 ± 0.83^{a} (16)	7.00 ± 1.89^{a} (60)	–
24 hours	25.00 ± 0.00^{c} (100)	25.00 ± 0.00^{c} (100)	20.00 ± 0.00^{c} (80)	10.00 ± 0.58^{a} (40)	0.75 ± 0.20^{a} (3)

Note: Per cent mortality is shown in parentheses. Superscripts marked with different letters within the same row indicate statistically significant differences (P < 0.05; n = 25). Here, µg/b denotes micrograms per 25 ml CDC bottle; - denote 0.00±0.00a (0) Mean mortality (± SD) and 0 percentage mortality

Mean mortality of 0.00±0.00 and percentage mortality of 0% were recorded for adult *Anopheles* mosquitoes exposed to 0.8 µg/b, 0.6 µg/b, 0.4 µg/b, 0.2 µg/b, and 0.0 µg/b sub-lethal concentrations of permethrin and deltamethrin, even 24 hours after exposure separately. These results were not statistically significant at the 0.05 threshold (P = 0.061) compared to the control experiments, as shown in Table 5. These concentrations of the two insecticides were thus sub-lethal to the malaria mosquito population in Zuba, as they resulted in 0% mortality even after 24 hours.

Table 5. Mean Mortality of Anopheles Mosquitoes in Zuba, FCT, Nigeria Under the Influence of Sub-lethal Concentrations of Permethrin and Deltamethrin

Time (min)	0.8 µg/b	0.6 µg/b	0.4 µg/b	0.2 µg/b	0.0 µg/b (Control)
0	–	–	–	–	–
15	–	–	–	–	–
30	–	–	–	–	–
35	–	–	–	–	–
40	–	–	–	–	–
45	–	–	–	–	–
60	–	–	–	–	–
24 hours	–	–	–	–	–

- denote 0.00±0.00a (0%) Mean mortality (± SD) and 0% mortality

DISCUSSION

Criteria for assessing pesticide resistance have been set by the World Health Organisation (WHO). When less than 80% of a population is killed after exposure to the WHO-approved concentration of an insecticide over 30 minutes of recommended exposure time in the case of the CDC bottle bioassay, it is classified as resistant. Mortality rates between 98% and 100% indicate susceptibility, while rates from 80% to 97% suggest possible or suspected resistance (WHO, 2018; WHO, 2023; Adesoye *et al.,* 2023A; Adesoye *et al.,* 2024B).

The current study revealed an 84.4% mortality rate of mosquito vectors at the recommended concentration of permethrin, suggesting that malaria mosquitoes in Zuba are suspected to be resistant to this insecticide (permethrin). In contrast, a 76.0% mortality rate was observed under the recommended concentration of deltamethrin insecticide, indicating that the same mosquito population in Zuba is confirmed to be resistant to deltamethrin.

The resistance of *Anopheles* mosquitoes in Zuba to deltamethrin and the suspected resistance to permethrin could be explained by a combination of environmental and biological factors. One likely cause is the prolonged exposure of mosquito populations to pyrethroid insecticides, commonly used in public health interventions like insecticide-treated nets and indoor residual spraying, as well as in agricultural settings. This continuous exposure may have created strong selective pressure, allowing resistant individuals to survive and reproduce (Hemingway *et al.,* 2016). Additionally, biochemical mechanisms such as the overexpression of detoxifying enzymes—particularly cytochrome P450s, glutathione S-transferases, and esterases—may contribute to metabolic resistance by breaking down the insecticides before they reach their target sites (Ranson & Lissenden, 2016; Adesoye *et al.,* 2023A). Furthermore, the presence of target-site mutations like knockdown resistance (*kdr*) in the voltage-gated sodium channel gene has been widely associated with reduced sensitivity to pyrethroids in *Anopheles* populations (Davies *et al.,* 2007).

Lethal concentration refers to the amount of an insecticide required to cause mortality in a specific proportion of an exposed population (Yuxian *et al.,* 2013; Awolola *et al.,* 2018; WHO, 2019; Islam *et al.,* 2021). This is typically expressed as LC_{50} and LC_{90}, representing the concentrations that result in the death of 50% and 90% of the exposed individuals, respectively. In contrast, sub-lethal concentrations do not cause immediate mortality but induce physiological or behavioural changes that can affect the insect's long-term survival, reproduction, or development. Such effects may include impaired movement, feeding inhibition, hormonal disruption, or reduced fertility, as reported by an article (Adesoye *et al.,* 2024C). Research has further indicated that exposure of mosquitoes to sub-lethal concentrations of insecticides is the main driver of resistance development in them (Nkya *et al.,* 2013; Adesoye *et al.,* 2023A; Adesoye *et al.,* 2024A).

The findings of this study indicate that concentrations of deltamethrin insecticide at 12.5 µg/bottle, 15 µg/bottle, 5 µg/bottle, 1 µg/bottle, and 0.9 µg/bottle are lethal to the mosquito population, causing varying percentages of mortality in them after exposure. This aligns with the other report (Adesoye *et al.*, 2024B), which demonstrated that the same concentrations induced mortality in *Anopheles gambiae* (Kisumu) when exposed to permethrin insecticide.

In contrast, the findings of another study (Adesoye *et al.*, 2024B) differ from the present study, as only the 0.2 and 0.4 µg/

bottle permethrin concentrations failed to induce any mortality at the 24-hour mark in their research. However, concentrations of 0.8, 0.6, 0.4, and 0.2 µg/bottle produced mortality rates that were not significantly different from the control experiment (0%) after 24 hours in the present study, rendering all these doses sub-lethal for the mosquito population. This discrepancy may be attributed to the use of wild mosquitoes in the current study, compared to the well-established susceptible *Anopheles gambiae* (Kisumu) strain used in the former research

CONCLUSION

The findings of this study provide clear evidence that the *Anopheles* mosquito population in Zuba, FCT, Nigeria, has developed resistance to deltamethrin and shows suspected resistance to permethrin, despite both being recommended concentrations by the CDC. These results highlight a trend indicating reduced efficacy of recommended insecticide concentrations, which poses a direct threat to the success of current malaria vector control strategies, such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS).

This resistance may contribute to sustained malaria transmission in the region, even in the presence of ongoing vector control efforts. Therefore, it is imperative for malaria control programs in Nigeria to incorporate routine insecticide resistance monitoring and adjust intervention strategies accordingly. This may include the integration of alternative insecticides, deployment of synergist-based nets, or rotation of active compounds to delay further resistance development.

LIMITATION OF THE STUDY

The present study is subject to important limitations. Firstly, the data were collected from a single location—Zuba, within the Gwagwalada Area Council—limiting the generalizability of the findings across broader ecological zones in Nigeria.

Secondly, while the study confirms phenotypic resistance through mortality assays, it does not identify the underlying resistance mechanisms (e.g., metabolic, target site mutations, or behavioural avoidance) contributing to reduced susceptibility. Future work may benefit from integrating molecular or biochemical assays to elucidate these mechanisms.

Conflict of Interest

The authors affirm that they have no conflicts of interest.

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