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Research Article

Evaluation of Generational Implications of Metabolic Resistance Development in Malaria Mosquitoes against Permethrin Insecticides

***Oluwaseun Adegbola Adesoye[1,2], Adedapo O. Adeogun[2], Tolulope A. Oyeniyi[2], Olalekan E. Olagundoye[2], Romoke T. Izekor[2], Oluwakemi O. Adetunji[2], Ayodele S. Babalola[2], Israel O. Akinsete[3], Adeniyi Kamoru A.[4], Callistus A. Akinleye[5], Adewale D. Adediran[2], Chidinma Isaac[2], Samson T. Awolola[2], and Adeolu T. Ande[6]**

[1]Department of Biological Sciences, University of Abuja, Abuja, Nigeria
[2]Molecular Entomology and Vector Control Research Laboratory, Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria
[3]Department of Animal Biology, Federal University of Technology, Minna, Nigeria
[4]Department of Biological Sciences, Federal University, Dutse, Nigeria
[5]Department of Community Medicine, Osun State University, Osogbo, Nigeria
[6]Department of Zoology, University of Ilorin, Ilorin, Nigeria
****Corresponding Author's email***: oluwaseun.adesoye@uniabuja.edu.ng

ABSTRACT

Enzymes mediated resistance development and their activities are well documented in *Anopheles gambiae* vectors. However, biological consequences of such resistance development in malaria vectors are obscure. The present study aims to determine the implications of such response in *An. gambiae* (Kisumu) population following exposure to a concentration of Permethrin selection pressure across multiple generations. Adult Kisumu mosquitoes exposed to 0.2 µg/ CDC bottle over generations were compared with unexposed control populations by way of resistance status, cost of fitness, and correlating metabolic enzymes. Data obtained were analysed using SPSS. Analysis of Variance was employed to determine statistical differences at 95%. Fecundity rates of the mosquito population dropped progressively over generations. With increase in activity of metabolic enzymes, resistance status of the mosquito population progressed significantly ($P < 0.05$) over generations with 5.0 µg (18.00±0.00; 72%) at f_4 generation. Mosquito resistance development is therefore a disadvantage to malaria vectors as it reduces ovipository capability, and increases longevity of immature stages (life cycle) with filial generations.

Keywords: Selection Pressure; Vector Control; Cost fitness; Sub-lethal Concentration; CDC Bottle

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INTRODUCTION

The major cause of high morbidity and mortality rates associated with malaria in Nigeria and across sub-Saharan Africa is attributed to the *Anopheles gambiae* sensu stricto (s.s.) as the major malaria vectors (WHO, 2018, Koffi *et al.,* 2023; Adesoye *et al.,* 2023a). On a global scale, the year 2022 witnessed no less than 249 million documented cases of malaria, leading to 608,000 fatalities spanning 85 countries (WHO, 2022). Malaria claims the lives of 1 to 3 million individuals annually, with a child under the age of 5 succumbing to the disease approximately every minute (UNICEF, 2024).

Despite concentrated efforts to combat malaria in endemic regions, it persists as a substantial public health concern in over 106 countries of the world (WHO, 2022; Adesoye *et al.*, 2023b; Verra *et al.,* 2020). The disease spreads through various routes, including blood transfusion from infected individuals and vertical transmission from mother to child during pregnancy, but primarily through the bites of female *Anopheles* mosquitoes (Adeniyi *et al.,* 2023).

To address the challenge of malaria in endemic regions, the World Health Organization (WHO) has advocated for the use of chemical insecticides, notably Permethrin, to control mosquitoes (WHO, 2018). However, this approach appears to have encountered a hurdle, with reports emerging of resistance development, particularly metabolic resistance, in mosquito vectors (Oduola *et al.,* 2017; Olagundoye and Adesoye, 2023; Adeniyi *et al.,* 2024b; Adesoye *et al.,* 2024).

Insecticide resistance, as defined by the WHO, refers to alterations in the physiology of an insect (WHO, 2015; Awodoyin *et al.,* 2024), such as *An. gambiae* s.s in this context, that enhance its ability to tolerate or counteract the effects of one or more insecticides. WHO has established criteria for assessing resistance: if less than 80% mortality is observed in a sample population after exposure to the WHO-recommended concentration of a particular insecticide, the population is deemed resistant. Mortality rates between 80% and 97% suggest suspected resistance, while mortality rates between 98% and 100% indicate susceptibility to the insecticide (WHO, 2015; Adesoye *et al.,* 2023a; Adesoye *et al.,* 2023b).

The acquisition of Permethrin resistance by mosquitoes entails a significant allocation of resources toward the synthesis of enzymes and other necessities to support the development and survival of insecticide resistance. Consequently, it is expected that this development will impose pressure on other physiological functions within the mosquito, potentially leading to reduced performance in certain areas. This diminished performance is commonly referred to as fitness costs associated with resistance development, a phenomenon observed in various insect species (Puinean *et al*., 2010). A comparison of the performance between susceptible and resistant individuals within a population provides insights into the extent of these fitness costs. Understanding the physiological status of resistant mosquitoes in comparison to their susceptible counterparts would allow entomologists to gauge the biological robustness of resistant mosquitoes accurately, facilitating more effective and judicious vector management strategies.

Evidence of metabolic resistance development in *An. gambiae* (Kisumu), a strain of *An. gambiae* s.s., when exposed to Permethrin over generations has been documented (Adesoye *et al.,* 2023). The current study aims to revalidate and assess the implication of such resistance development on resistance/susceptibility and costs of fitness within a population of the same strain of mosquitoes following exposure to Permethrin across multiple generations.

MATERIALS and METHODS

An. gambiae (Kisumu) Population

A large population of *An. gambiae* (Kisumu) was obtained from the insectary of the Molecular Entomology and Vector Control Insectary, Nigerian Institute of Medical Research, Yaba, Lagos Nigeria (NIMR). The insectary houses a large colony of Kisumu population since 2012 till date. The resistance profile of the insectary mosquito population is usually confirmed after generations to be susceptible to pyrethroids.

Range Finding Test of Permethrin Against Mosquitoes

Original stock solution of technical grade Permethrin was supplied by the Centre for Disease Control (CDC) for this study. One ml from the solution was diluted with 49 ml (100%) acetone to give a standard concentration of 21.50 µg to be used to coat a 250 ml capacity CDC bottle (to give 21.50 µg/250 ml bottle or 21.50/bottle) in accordance with manufacturer's instructions. Various randomly selected wall-coating concentrations of Permethrin were obtained by slightly modified CDC (2015) procedure to obtain in four replicates 1 ml each of 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). This modification to the CDC procedure became necessary so we can determine (i) concentration that could allow the population reproduce and give rise to subsequent generations even after exposure to it (Permethrin selection pressure), (ii) determine minimal Permethrin concentrations that could be used to evaluate resistance status of exposed mosquito. Therefore, 25 samples of mosquitoes were raised and exposed separately to every preparation of Permethrin over generations as described by Adesoye *et al.* (2023).

Exposure of Mosquito Population to Permethrin Selection Pressure

A separate mosquito population was exposed in four replicates to the predetermined Permethrin selection pressure from generation to generation (Adesoye *et al.,* 2023). All eggs laid by the mosquito population (fecundity) per generation, number of days it took eggs to develop to larvae, number of days it took larvae to develop into pupae, number of days it took pupae to develop to adult stages were all noted respectively and recorded as well as in the control experiments.

Activity Measurement of Metabolic Enzymes in Exposed Mosquitoes

Thirty non-blood female adult mosquitoes were selected from the reared population of exposed mosquitoes after each generation, and analysed for metabolic enzyme activities. Control of these experiments was made of 30 non-blood female adult mosquitoes not exposed to the insecticide. Assays of monooxygenase (P450) and Glutathione S-transferase (GST), the major detoxification enzymes, were carried out using procedures described by Adesoye *et al.* (2023).

Determination of Cost of Fitness

The average number of eggs values obtained for fecundity and average duration (in days) to develop from one developmental stage to another were compared with control which is 100% susceptible over generations and were expressed in terms of fitness cost. The following formula was used to evaluate this:

$$\textbf{Fitness Cost} = \frac{\textbf{Control population} - \textbf{Population under investigation}}{\textbf{Control (as in unexposed)}} \textbf{ x 100}$$

Resistance/Susceptibility Determination

The resistance/susceptibility of the various filial generation adult mosquitoes were determined. In this case, a permethrin reference slightly above Permethrin selection pressure were selected the range finding test. For each generation of both exposed and unexposed mosquitoes, 25 individuals were picked randomly and introduced into CDC bottles previously coated to reflect appropriate permethrin concentrations. Four replicates of each dose and control were made, the mortality was recorded over 1 hours.

Data Analysis

Information obtained on mosquito fecundity, life-cycle parameters, metabolic enzyme activities and resistance/susceptibility assay were analyzed and was calculated as means and percentages and expressed in Chart and Tables using Statistical Package for Social Sciences (SPSS version 20.0, Inc., Chicago, IL, United State of America, USA) with Graph Prism Statistical software (Prism, GraphPad Software, San Diego, CA, USA). These were compared statistically using Analysis of Variance (ANOVA) at $P = 0.05$.

RESULTS

Range Finding Test

Table 1 shows that 0.4µg/b and 0.2µg/b could act as a selection pressure for this experiment. This is because no mortality, 0.00±0.00 (0), not significantly different (P=0.061) from the control while the mosquito population was exposed to them over 24 hours. The mortality of mosquitoes population decreases with decreases in concentration.

Mosquitoes under 0.4 µg/bottle Permethrin exposure could laid no eggs. However, the mosquito population exposed to 0.2 µg/bottle. Permethrin sub-lethal concentration survived four filial generations and showed no significant (P = 0.062) mortality of 0.00 (0%) throughout but did not make the fifth under this study because of significant reductions in the number of eggs laid by the same population over generations. According to WHO (2015) and report from Adesoye *et al.* (2023), correlation with Abbott's formula was not necessary as mortalities in control experiments were less than 5% throughout the generational tests.

Resistance Status of Exposed Mosquito Population

The same mosquito species which produced 4.0% mortality under 0.6 µg/bottle exposure in range finding test has become absolutely resistant (0.0%) at f4 generation after persistent exposure to 0.2 µg/bottle over generations under the same conditions. However, Table 2 showed that the same population produced 18.00±0.00 (72%) against 5.0 µg/bottle at the f4 generation with generational exposure to 0.2 µg/bottle. It should be recalled that the mosquito species produced 100 % mortality (susceptibility) under similar condition.

Activities of Metabolic Enzymes Recovered from Mosquito Population Exposed to 0.2 µg/bottle

As shown in Table 3, the mean GST enzyme activity obtained from unexposed (Control) mosquitoes was 1.13±0.77 mmol/min/mg/protein. GST enzyme, unlike monooxygenase enzymes, showed significant (P< 0.25) upregulation as the generation of *An. gambiae* (Kisumu) mosquito progresses. Monooxygenase activity only increases significantly (P< 0.05) after the third generation of persistent exposure to the 0.2 µg/bottle of Permethrin.

Biological Cost of Fitness Associated with Resistance Developed in the Mosquito Population

In Table 4, there was a 90% fecundity cost (reduction) due to resistance development in the f_4 generation of the mosquito population when compared to the susceptible population of the control experiment. There were 31.00% (increased) and 66.0 % cost due to resistance development in the f_4 generations of the mosquito population larvae and pupa developmental cycle respectively as activities of detoxifying enzymes increases.

Table 1: Mortality Rates (%) of Mosquito Population Exposed to Range of Permethrin Concentrations

Time (min)	21.5µg/b	15.0µg/b	10.0µg/b	5.0µg/b	1.0µg/b	0.9µg/b	0.8µg/b	0.6µg/b	0.4µg/b	0.2µg/b	Control
0	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00a(0)	0.00±0.00^{a}(0)
30	100.00±00^{a}(100)	90.80±2.22^{a}(90.8)	26.00±0.50^{a}(26)	10.80±0.96^{b}(10.8)	1.00±0.51^{b}(1)	0.00±0.02^{b}(0)	0.00±0.02^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00$_{b}$(0)	0.00±0.00^{b}(0)
35	100.00±0.00^{a} (100)	100.00±0.00^{a}(100)	42.00±0.58^{a}(42)	72.75±12.50^{a}(72)	7.00±1.50^{b}(7)	1.00±0.50^{b}(1)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00b(0)	0.00±0.00^{b}(0)
45	100.00±0.00^{a}(100)	100.00±0.00^{a}(100)	84.75±12.51^{a}(84)	80.00±0.02^{a}(80)	15.00±1.89^{b}(15)	3.00±0.50^{b}(3)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00$_{b}$(0)	0.00±0.00^{b}(0)
60	100.00±0.00^{a} (100)	100.00±0.00^{a}(100)	84.75±12.51^{a}(84)	80.00±0.02^{a}(80)	37.00±1.41^{a}(37)	4.00±0.82^{b}(4)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00$_{b}$(0)	0.00±0.00^{b}(0)
24hrs	100.00±0.00^{a} (100)	100.00±0.00^{a} (100)	100.00±0.00^{a}(100)	100.00±0.00^{a}(100)	36.00±1.42^{a}(36)	4.00±0.83^{b}(4)	4.00±0.82^{b}(4)	4.00±0.82^{b}(4)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)	0.00±0.00^{b}(0)

Mean values with different alphabets along the row are significantly different at *P*<0.05; N= 25

Table 2 Resistance Status of Mosquito Population Against 5.0 µg/bottle After generational Exposure to a Sub-lethal concentration

Concentrations	5.0 (µg/bottle)				Control
Time (Minutes)	f1	f2	f3	f4	
0	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)
15	3.00±0.82^{c}(12)	0.50±1.00^{b}(2)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)
30	25.00±0.00^{c}(100)	1.00±1.15^{b}(4)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)
35	25.00±0.00^{c}(100)	24.75±0.50^{a}(99)	22.25±2.63^{c}(90)	18.25±0.50^{b}(73)	0.00±0.00^{a}(0)
60	25.00±0.00^{c}(100)	24.75±0.50^{a}(99)	22.25±2.63^{c}(90)	18.00±0.00^{b}(72)	0.00±0.00^{a}(0)

Mean values with different alphabets along the row are significantly different at *P*<0.05; N= 25

Table 3: Mean Activities of Metabolic Enzymes in Mosquito Population Exposed to 0.2 µg/bottle over Generations

Metobolic Enzymes	f_0 (0.4µg/b)	f_0(0.2µg/b)	f_1 (0.2µg/b)	f_2(0.2µg/b)	f_3 (0.2µg/b)	Control
Monooxygenase (P450/mg protein)	0.014±0.029^{a}	0.003±0.003^{b}	0.003±0.003^{b}	0.004±0.002^{b}	0.005±0.002^{c}	0.001±0.001^{bc}
GST (mmol/min/mg/protein)	1.950±0.93^{a}	1.990±0.82^{a}	2.60±1.46^{b}	3.06±1.45^{c}	3.52±1.25^{bc}	1.130±0.77^{d}

N = 30; Subscripts with different alphabets along a row are significantly different at *P*<0.05

Table 4: Fitness Cost Resulted from Increased Enzyme Activities in Exposed Mosquito Population to 0.2 µg/bottle

Biological Features	The actual value recorded in the susceptible population (Control)	Fitness costs (%)				
		f_0	f_1	f_2	f_3	f_4
Fecundity	876.67 ± 68.07	- 27	+83.8	+84	+88.1	-90
Egg-Larvae (days)	2.00 ± 0.00	0.0	0.0	0.0	0.0	0.0
Larvae-pupae (days)	10.00 ± 0.00	0.0	0.0	-6.7	-33	-31
Pupae–Adult (days)	2.00 ± 0.00	0.0	0.0	-16.5	-115	-66

DISCUSSION

Permethrin resistance, like other pyrethroids, arises when susceptible *An. gambiae* vectors are exposed to the insecticide or its derivatives in sub-lethal concentration as a selection pressure which will not necessarily lead to their death. Therefore, 0.2 µg/bottle sub-lethal Permethrin insecticide concentration in the present study acted as resistance selection pressure. As the generation of mosquitoes progressed, there was significant reduction in the mosquito susceptibility with significant increase in the activity of metabolic enzymes upon consistent exposure to 0.2 µg/bottle of permethrin. Thus, the present study revalidates the earlier findings by Adesoye *et al.* (2023).

Increase in the activity of the resistance enzymes in the malaria population in the present study imposes a fitness cost on the population. This is in line with the report of Adi and Murad (2012), which stated that insects face numerous stressors in their environment, many of which are harmful chemical insecticides/pesticides that lead to the development of resistance at the expense of fitness. As vectors struggles to overcome the toxicity of insecticides, they do so by losing substantial biological resource because the process demand energy and resources for adaptation and survival (Adi and Murad 2012; Liu, 2015; Awolola *et al.,* 2018; Carrasco *et al.,* 2019).

The duration of complete developmental stages (metamorphosis) in exposed mosquitoes across generations noticeably extended with a significant rise in the activity level of metabolic enzymes within the population compared to the control experiment. Moreover, there was a notable reduction in the number of eggs laid (fecundity) over generations in the exposed population compared to the control experiment. This indicates that the development of metabolic resistance in *Anopheles gambiae* vectors adversely affects their ability to reproduce. This outcome aligns with the findings of a study on *Culex pipiens*, which demonstrated the development of metabolic resistance against Organophosphate insecticides (Puinean *et al.,* 2010; Riveron *et al.,* 2013).

Human tends to increase the concentration of insecticides and/or relying excessively on their application when population of pest or vector develop resistance. Such practice, over time may have adverse effects on human health (Adesoye *et al.,* 2023C; Soladoye *et al.,* 2021; Adesoye *et al.,* 2024a; Adesoye *et al.,* 2024b). This can lead to both short-term negative health impacts, known as acute effects, and long-term negative health effects, which can manifest over months or years following human exposure. Acute health impacts may include symptoms like eye stinging, rashes, blisters, blindness, nausea, dizziness, diarrhea, and even death. Chronic consequences may include conditions such as cancers, birth abnormalities, reproductive harm, neurological and developmental toxicity, immunotoxicity, and disturbances in the endocrine system (Nkya *et al.,* 2013; Awolola *et al.,* 2018).

It has been observed that such mosquito populations may suffer long-term damage as a result of insecticide exposure, which diminishes their ability to transmit disease (Gunasekaran *et al.,* 2015). The report indicated that even highly resistant strains of the primary malaria vector *An. gambiae* experience a reduction in fertility of more than 50%, following exposure to long-lasting insecticidal nets (LLINs), consistent with the findings of the current study. Thus, the reason for the present study could not advance beyond f4 generation even without mortality against Permethrin selection pressure.

CONCLUSION

As generation of mosquito population progressed, there was significant increase in the mosquito resistance with increase in the activity of metabolic enzymes upon consistent exposure to Permethrin selection pressure. As population progress in resistance status, population experience a disadvantage with reduced ovipositional capability, increased life cycle duration. This information can be capitalized upon by experts for better vector management program.

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Conflict of Interest

Authors declare that there is no conflict of interests.

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