FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 3, June, (Special Issue) 2024, pp 539 - 545
DOI: https://doi.org/10.33003/fjs-2024-0803-2602

BIOLOGICAL FITNESS COSTS OF GLUTATHIONE-S-TRANSFERASE (GST)-MEDIATED PERMETHRIN RESISTANCE IN *ANOPHELES GAMBIAE* GILES (DIPTERA: CULICIDAE)

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

[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 authors' email: oludesoye@gmail.com Phone: +2347038118580
ORCID: https://orcid.org/0009-0003-0362-947X

ABSTRACT

Glutathione-S-transferase (GST)-mediated resistance development has been well documented in *Anopheles gambiae* Giles (Diptera: Culicidae). However, its biological consequences in this malaria vector are merely addressed. The present study aims to determine the implications of such a response in *An. gambiae* Kisumu population following exposure to a concentration of permethrin selection across multiple generations. Generations of adult mosquitoes exposed to 0.2 µg/bottle of permethrin were compared with unexposed controls to analyze resistance development, life events, and GST levels. Data obtained were analyzed using SPSS. Analysis of Variance was used to determine statistical differences at 95%. Resistance development and inference on filial generation where the population becomes resistant to recommended concentration (full-blown resistance) were determined using the *R*-Program. The fecundity of the selected population declined progressively over generations. With an increase in the activity of GST enzyme as stated in the previous study, the resistance of the Kisumu population progressed significantly (P = 0.041) against 5.0 µg/bottle and 10.0 µg/bottle from f_1 to f_4 generations in response to generational selection by 0.2 µg/bottle. This population would infer full-blown resistance at the 154th generation as a result of generational exposure to 0.2 µg/bottle. Mosquito resistance development is detrimental to malaria vectors as it reduces oviposition capability, increases the longevity of immature stages with filial generations, and delays full-blown resistance of susceptible vectors.

Keywords: Selection pressure, Vector control, Cost fitness, Mortality, Sub-lethal concentration, CDC bottle

INTRODUCTION

Anopheles gambiae Giles sensu stricto (s.s.) is the primary vector causing high malaria incidence and mortality in Nigeria and sub-Saharan Africa (Adeogun *et al.,* 2023; Koffi *et al.,* 2023). 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; Verra *et al.,* 2020; Dufera *et al.,* 2018). 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 (Nakatani *et al.,* 2014).

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, 2014, 2018). However, this approach appears to have encountered a hurdle, with reports on the emerging resistance development, particularly metabolic resistance, in mosquito vectors (Awolola *et al.,* 2018; Chukwuekezie *et al.,* 2020; Kpanou *et al.,* 2021).

Insecticide resistance refers to alterations in the physiology of an insect, 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, 2015). Following the proposition of Vatandoost *et al.* (2004), the 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 (Yadouleton, 2010).

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 (Gassmann *et al.,* 2009; 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 empowers entomologists to gauge the biological robustness of resistant mosquitoes accurately, facilitating more effective and judicious vector management strategies.

Previously, we documented the evidence of metabolic resistance development (with enzyme activity levels) in *An. gambiae* (Kisumu), a susceptible strain of *An. gambiae* s.s., when exposed to permethrin over generations (Adesoye *et al.*, 2023). The current study aims to assess the consequences of such resistance development and the biological costs of fitness according to the same previous study within Kisumu population following exposure to permethrin across multiple generations.

MATERIALS AND METHODS

Source of *An. gambiae* (Kisumu) Population

A large population of *An. gambiae* (Kisumu) was obtained from the insectary of the Molecular Entomology and Vector Control Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos Nigeria (NIMR). The insectary has housed a large colony of the Kisumu population since 2012. The resistance profile of the insectary mosquito population is usually confirmed every four generations in the laboratory, and the results have always indicated over the years that mosquitoes are fully susceptible to pyrethroids, including permethrin.

Insecticide Source and Dilutions

The 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) according to Brogdon & Chan (2010). Various wall coating concentrations of permethrin were obtained by a slightly modified CDC (2015) procedure to obtain 1 ml each of 0.2 µg/bottle, 5 µg/bottle, 10 µg/bottle, and 0 µg/bottle (control) in four replicates. Modifications to the CDC protocol allows to prepare lethal and sub-lethal (lower than the standard 21.50/bottle) concentrations stated above. All steps of the procedure were meticulously carried out to minimized errors.

Mosquito Rearing and Exposure to Permethrin

Furthermore, modification to the CDC procedure became necessary so that the population could produce subsequent generations after 24 hours of exposure to 0.2 µg/bottle which has been previously established to serve as metabolic resistance selection pressure in Kisumu by Adesoye *et al.* (2023). It was stated in their study that exposing a Kisumu population to 0.2 µg/bottle of permethrin will not result in their mortality but trigger (acts selection pressure) metabolic resistance development in them over generations. Therefore, 25 samples of Kisumu mosquitoes were raised and exposed to 0.2 µg/bottle of permethrin over generations as described by Adesoye *et al.* (2023).

All eggs laid by the mosquito population (fecundity) per generation, the number of days it took eggs to develop into larvae, the number of days it took larvae to develop into pupae, number of days it took pupae to develop into adult stages were all noted respectively and recorded accordingly for the 0.2 µg/bottle concentrations exposure in four replicates as well as in the control experiments. 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:

$$\text{Fitness Cost} = \frac{\text{Control population} - \text{Population of vector under investigation}}{\text{Control(as in unexposed)}} \times 100$$

Resistance/Susceptibility Determination

The resistance/susceptibility of the various filial generation adult mosquitoes was determined. A slightly modified CDC protocol (Brogdon & Chan, 2010) was adopted for use. In this case, a permethrin reference slightly above 0.2 µg/bottle, i.e., 0.6 µg/bottle, as well as 5.0 µg/bottle and 10 µg/bottle were selected through a 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, and mortality was recorded over 1 hour as described by Adesoye *et al.* (2023).

Determination of Resistance Development and Inferring the Full-blown Filial Generation

Predictive analysis was used to determine the generation at which the mosquito population exposed to 0.2 µg/bottle would acquire the same activity of the enzyme about an established reference point for wild *An.* population. Glutathione-S-Transferase (GST) enzyme that showed progressive average activity as *An. gambiae* (Kisumu) progressed under 0.2 µg/bottle exposure in the Adesoye *et al.* (2023) study and was used to predict the full-blown resistance development.

Data Analysis

Information obtained on mosquito fecundity, life-cycle parameters, and resistance/susceptibility assay were analyzed and calculated as means and percentages and expressed in tables using Statistical Package for Social Sciences (SPSS version 20.0, Inc., Chicago, IL, United States 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. Resistance development and inference on filial generation with full-blown resistance were determined using the *R*-Program

RESULTS AND DISCUSSION

The mosquito population exposed to 0.2 µg/bottle permethrin at sub-lethal concentration survived four filial generations 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. Mean mortality of *An. gambiae* (Kisumu) populations exposed generationally to 0.2 µg/bottle showed no significant (P = 0.062) mortality of not more than 1.00 (4%). Correlation with Abbott's formula was not necessary, as control mortalities were less than 5% throughout the generational test (WHO, 2015; Adesoye *et. al.*, 2023).

Biological Fitness Cost for Permethrin Resistance Development in *Anopheles gambiae* (Kisumu)

The biological fitness costs associated with resistance development (increase in activities of GST metabolic enzyme) in *An. gambiae* (Kisumu) exposed population over generations in terms of fecundity and durations of egg, larval and pupal development, and mortality are shown in Table 1. The average fecundity in unexposed (control) Kisumu population was 876.67 ± 68.07 eggs per generation of 25 females. On exposure to 0.2 µg/bottle permethrin, a lower and insignificantly (P= 0.521) different mean number of eggs (638.67 ± 1.53) were observed by the same number of female mosquitoes. Subsequent generations laid distinctly and significantly (P = 0.021) lower number of eggs, 150.67 ± 11.02 at f_1, 39.33 ± 0. 58 at f_2, 103.67 ± 1.17 at f_3 and 100.00 ± 2.00 at f_4, as activity of GST progresses.

Table 1: Life table for *Anopheles gambiae* (Kisumu) population exposed to a sub-lethal concentration of 0.2 µg/bottle of permethrin over generations

	f_0		f_1		f_2		f_3		f_4		Control	
Attributes		**% mortality**		**% mortality**		**% mortality**		**% mortality**		**% mortality**		**% mortality**
Mean fecundity/Plate n=25	638.67±1.53a	-	150.67±11.02b	-	39.33±0.58b	-	103.67±1.17b	-	100.00±2.00b	-	876.67±68.07a	-
Egg-Larvae (days)	2.00±0.00a	0	2.00±0.00a	0	2.00±0.00a	0	2.00±0.00a		2.00±0.00a	-	2.00±0.00a	0
Larvae-pupae (days)	10.00±0.00a	0	10.00±0.00a	0	10.67±0.58a	0	13.33±0.58b	4	13.00±0.00b	4	10.00±0.00a	0
Pupa– Adult (days)	2.00±0.00a	0	2.00±0.00a	0	2.33±0.58a	1	4.33±1.15b	1	15.00±0.00b	4	2.00±0.00a	0
GST activity (mmol/min/mg/protein); n=30 (Adesoye *et al.*, 2023)	1.990±0.82a		2.60±1.46b		3.06±1.45c		3.52±1.25bc		-		1.130±0.77d	

Subscripts with same alphabets along row are not significantly different at P> 0.05

There was a 90% fecundity cost due to resistance development in the f_4 generation of the Kisumu population when compared to the susceptible population in the control experiment. Also, it cost a bit resistant f_3 population 88.1% fecundity when compared to the control. There were 31.00% and 66.0 % cost due to resistance development in the f_4 generations of the Kisumu mosquito population larvae and pupa developmental cycle, respectively as GST activity increases (Table 2).

Table 2: Biological fitness cost Associated with resistance development in *An. gambiae* (Kisumu) exposed to of 0.2 µg/bottle over generations

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

Resistance progression among generations of permethrin exposed *An. gambiae* population

Table 3 shows mean mortality and mortality rates (%) of *An. gambiae* (Kisumu) adult population exposed persistently to 0.2 µg/bottle with 0.6 µg/bottle of the insecticide to determine their susceptible status. Mortalities were not recorded throughout the one-hour duration of the experiment in unexposed *A. gambiae* (Kisumu) adults (Control) and all the 4 filial generations of the 0.6 µg/bottle exposed mosquitoes. Hence, the exposed mosquitoes were 100% resistant to 0.6 µg/bottle permethrin test case over the entire generations.

Table 3: Mean Mortality and Mortality Rate of *Anopheles gambiae* (Kisumu) Population Exposed to 0.6 µg/bottle Sub-lethal Concentrations of Permethrin Over generations

Concentrations	0.6 (µg/bottle)				
Time (Minutes)	f_1	f_2	f_3	f_4	**Control**
0	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)
15	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)
30	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)
35	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)
60	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)

n=25; Subscripts with different alphabets along row are significantly different at P<0.05

Table 4 shows the mean mortality and mortality rates (%) of *Anopheles gambiae* (Kisumu) adult population exposed persistently to 0.2 µg/bottle of permethrin while tested with a CDC bottle coated with 5.0 µg/bottle for susceptibility status per generation. The population which had been resistant to the 5.0 µg/bottle in previous generations, became resistant with 18.25±0.50 (73.0%) at f_4, after 1 full hour of exposure to CDC bottle coated at permethrin concentration of 5.0 µg/bottle. Hence, the activity of GST enzyme after third generation was used to predict using *R*-program the future generation in which the population would development resistance fully to the recommended concentration used in human household since the enzyme activity after the f3 result in resistance in the population after the f_3.

Table 4: Mean Mortality and Mortality Rate of *Anopheles gambiae* (Kisumu) Population Exposed to 5.0 µg/bottle Sub-lethal Concentrations of Permethrin Over generations

Concentrations	5.0 (µg/bottle)				
Time (Minutes)	f_1	f_2	f_3	f_4	**Control**
0	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a	0.00±0.00a(0)	0.00±0.00a(0)
15	3.00±0.82c(12)	0.50±1.00b(2)	0.00±0.00a	0.00±0.00a(0)	0.00±0.00a(0)
30	25.00±0.00c(100)	1.00±1.15b(4)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)
35	25.00±0.00c(100)	24.75±0.50a(99)	22.25±2.63c(90)	18.25±0.50b(73)	0.00±0.00a(0)
60	25.00±0.00c(100)	24.75±0.50a(99)	22.25±2.63c(90)	18.25±0.50b(73)	0.00±0.00a(0)

n=25; Subscripts with different alphabets along row are significantly different at P<0.05

Table 5 shows the mean mortality and mortality rates (%) of *Anopheles gambiae* (Kisumu) adult population exposed persistently to 0.2µg/bottle of Permethrin when tested with a CDC bottle quoted with 10.0 µg/bottle. There were significantly higher (*P*=0.041) recorded mortality after 1 hour in 10 µg/bottle (25.00±0.00: 100) test case in Kisumu population persistently exposed to the permethrin concentration in the f_1 generation. Also, similar record was obtained in the f_2 generation in which significantly higher (*P*=0.041) mortality after 1 hour in 10 µg/bottle (25.00±0.00: 100) test case in Kisumu population persistently exposed. The population however became suspected resistant (20.75±2.22; 83) at f4 generation after 1 hour test with 10 µg/bottle. Generally, it took up to 15 minutes (delayed mortality) at f_4 generation to record a single mortality in 5 µg/bottle and 10 µg/bottle of permethrin.

Table 5: Mean Mortality and Mortality Rates of *Anopheles gambiae* (Kisumu) Population Exposed to 10.0 µg/bottle Sub-lethal Concentrations of Permethrin Over Generations

Concentrations	**10.0 (µg/bottle)**				
Time (Minutes)	**f_1**	**f_2**	**f_3**	**f_4**	**Control**
0	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)	0.00±0.00a(0)
15	2.50±0.58a(9)	1.25±0.50a(5)	4.00±1.63b(16)	1.25±0.96a(5)	0.00±0.00a(0)
30	3.00±0.82a(12)	2.25±1.89a(9)	13.50±9.95b(52)	2.75±0.96a(11)	0.00±0.00a(0)
35	25.00±0.00a(100)	25.00±0.00a(100)	22.50±1.91ab(88)	20.75±2.22a(83)	0.00±0.00a(0)
60	25.00±0.00a(100)	25.00±0.00a(100)	22.50±1.91ab(88)	20.75±2.22ab(83)	0.00±0.00a(0)

n=25; Subscripts with different alphabets along row are significantly different at P<0.05

There was consistent and progressive increase in GST level with advancing generations under 0.2µg/bottle exposure. Thus, the activity of the enzyme was used to predict the full-blown resistance development of the population. According to Gunasekaran *et al*. (2015), 195.3398 nmol/mg protein was the reference quantity or activity level of GST enzyme in wild *Anopheles* mosquitoes resistant to permethrin, just as 3.52±1.25 nmol/mg protein is the GST activity resistant to 5.0 µg/bottle of the insecticide at f3 under the present study. The enzyme activity marched the reference point at 154th forecasted generation (Table 6).

Table 6: Predictive Generation and Rate of Full-blown Resistance Development of *An. gambiae* (Kisumu) Population Exposed 5.0 µg/bottle after persistently to 0.2 µg/bottle of Permethrin over generations

Predictive Enzyme Activity (mmol/min/mg/protein)	**Predictive Filial Generation**
.3.52	.3
.	.
.195.3398*	.154

*Forecast of Enzyme Activity for Resistance Development

Discussion

We discovered that an increase in the activity of the resistance enzyme, GST, in major malaria vectors imposes a fitness cost on the population. Adi & Murad (2012) noted 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. Addressing the toxicity of insecticides can be resource-intensive, demanding energy and resources for adaptation and survival. Insect vectors employ various behavioral (Carrasco *et al.,* 2019), physiological (Liu, 2015), and genetic (Riveron *et al.,* 2013) mechanisms to cope with toxic insecticides, often resulting in resistance through the constitutive overexpression of detoxification enzymes (Li *et al.,* 2014) or the induction of mutations in target sites (Awolola *et al.,* 2018). Such behaviors incur significant costs and may impact reproduction/fecundity, dispersal ability, and other aspects of insect fitness. Fitness costs associated with the development of pesticide or insecticide resistance have also been observed in various insects from other orders, including Hemiptera (Puinean *et al.,* 2010) and Lepidoptera (Gassmann *et al.,* 2009).

The duration of complete developmental stages (metamorphosis) in exposed mosquitoes across generations noticeably extended with a significant rise in the activity level of GST within the population compared to the control experiment. Puinean *et al.* (2010), and Feng *et al.* (2009) documented a parallel observation in the development of metabolic resistance in *Nilaparvata lugens* against neonicotinoid insecticide.

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 (Rivero *et al.,* 2011).

The *An. gambiae* (Kisumu) population initially exhibited absolute susceptibility but gradually reduced in its susceptibility status over time of exposure to Permethrin. This indicates a general increase in resistance within the mosquito population as it was continuously exposed to 0.2 µg/bottle of Permethrin across generations. Interestingly, the population showed higher susceptibility to 10 µg/bottle compared to 5 µg/bottle after generational exposure to 0.2 µg/bottle. This suggests that the susceptibility of any mosquito population to an insecticide tends to rise with an increase in the concentration of that particular insecticide.

However, escalating the concentration of insecticide and/or relying excessively on its application over time may have adverse effects on human health. 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 (Awolola *et al.,* 2018). In this study, the mosquito population became resistant to 5 µg/bottle by the 4th generation (with a mortality rate of 73%), whereas the same population was only suspected to be resistant (with an 85% mortality rate) at the same generation against 10 µg/bottle.

There was a significant difference in average mortality of the mosquito population between the f1 and f4 generations as they were exposed independently to 10 µg/bottle and 5 µg/bottle. Thus, resistance mechanisms (GST activity) build up over generation as the population of mosquitoes are being exposed to insecticide selection pressure concentration persistently over a generation (Adesoye *et al.,* 2023). This is evident as the mortality rate of mosquitoes reduces with 5 µg/bottle over generation's exposure to 0.2 µg/bottle. Increased enzyme activities over generation therefore accounts for the observed reduction in mortality over

generation. This is in line with the report of de França *et al.* (2017) that higher concentrations of insecticides could multiply physiological responses such as metabatic enzyme activities and the behaviour of insects.

It required up to 30 minutes of exposure, particularly by the fourth filial generation, before mortality was observed in the mosquito population exposed to 5 µg/bottle. This corresponds with the findings of Vulule *et al.* (1999), who proposed that the delay in mortality observed in *An. gambiae* mosquitoes previously exposed to long-lasting insecticide nets (LLINs) when exposed to standard concentrations are due to the development of physiological mechanisms to survive the use of nets in malaria vector control.

However, 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. Their 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, hence, the reason for the present study could not advance beyond f4 generation even without mortality. These delayed mortalities, along with the associated fitness costs generally observed in vectors developing resistance, are adequate to significantly reduce their potential for malaria transmission. This could partly explain why insecticide resistance is not inevitably linked to LLIN failure or other insecticide-based methods of malaria management.

Gunasekaran *et al.* (2015) reported a level of 195 mmol/min/mg/protein of GST enzyme responsible for the development of resistance in wild *An. gambiae* populations. However, the statistical analysis utilized in the current study has revealed that the Kisumu population, persistently exposed to 0.2 µg/bottle, will develop resistance to the same standard concentration of Permethrin used in homes or the wild by the 154^{th} generation. The prolonged duration for the population in the present study to achieve resistance could be attributed to their continuous exposure to 0.2 µg/bottle of a single insecticide (permethrin alone) across generations, as opposed to wild mosquito populations exposed to various concentrations of different insecticides in their natural environment over generations. The future research will be focus on the determination of response of wild and fully resistant *An.* mosquitoes to permethrin sub-lethal concentrations.

CONCLUSION

The minimal, sub-lethal permethrin insecticide concentration in the present study was 0.2 µg/bottle and thus, acted as resistance selection pressure. As the generation of Kisumu mosquitoes progressed, there was a significant reduction in mosquito susceptibility with a significant increase in the activity of metabolic enzymes (glutathione-s-transferase) upon consistent exposure to 0.2 µg/bottle of permethrin. The vector population however experienced resistance development detrimental to them as it reduced their ovipositional capability, increased durations of immature stages, and delayed full-blown resistance in susceptible vectors till the predicted 154^{th} generation. The study evaluated resistance development over limited generations, however, the information provided thereof could allay fear that comes with resistance development in malaria vectors. Thus, it helps creates quality time for the development of a more effective vector control management strategy.

ACKNOWLEDGMENT

Moral support from Mrs. Titilope Adegbola towards the success of this research work is well appreciated

AVAILABILITY OF DATA AND MATERIALS

The datasets analysed during the current study are available from the corresponding author (OAA) on reasonable request.

REFERENCES

Adeogun, A., Babalola, A.S., Okoko, O.O., Oyeniyi, T., Omotayo, A., Izekor, R.T., Adetunji, O., Olakiigbe, A., Olagundoye, O., Adeleke, M., Ojianwuna, C., Adamu, D., Daskum, A., Musa, J., Sambo, O., Oduola, A., Inyama, P., Samdi, L., Obembe, A., Dogara, M., Poloma, K., Mohammed, S., Samuel, R., Amajoh, C., Musa, A., Bala, M., Omo-Eboh, M.E., Sinka, M., Idowu OA, Ande A, Olayemi I, Yayo A, Uhomoibhi P, Awolola S. and Salako B. (2023). Spatial distribution and ecological niche modeling of geographical spread of *Anopheles gambiae* complex in Nigeria using real time data. *Sci Rep.*, 13, 13679. https://doi.org/10.1038/s41598-023-40929-5

Adesoye, A.O., Adeogun, A., Olagundoye, O.E., Oyeniyi, T.O., Izekor, R.T., Adetunji, O.O., Babalola, A.S., Adediran, D.A., Isaac, C., Adeleke, T., Awolola, T.S. and Ande, A.T. (2023). Metabolic resistance mechanisms evident in generations of *Anopheles gambiae* (kisumu) adults exposed to sub-lethal concentrations of permethrin insecticide. *pajols* 7(3), 750-758

Adeniyi, Kamoru A., Abubakar, Abubakar Sodiq, Adesoye, Oluwaseun Adegbola, Joshua Babalola Balogun, Akinsete, Israel, and Adeogun Adedapo O. (2024). Knowledge Evaluation of Mosquito Control Practices Within the Central Region of Jigawa State, North-West Nigeria. *FJS*., 8(3), 73

Adi, K. and Murad, G. (2012). Fitness costs associated with insecticide Resistance. *Pest Manag*. *Sci*., 68, 1431 –1437

Awolola, T.S., Adeogun, A., Olakiigbe, A.K., Oyeniyi, T., Olukosi, Y.A., Okoh, H., Arowolo, T., Akila, J., Oduola, A. and Amajoh, C.N. (2018). Pyrethroids resistance intensity and resistance mechanisms in *Anopheles gambiae* from malaria vector surveillance sites in Nigeria. *PLoS ONE*, 13(12), e0205230. doi: 10.1371/journal.pone.0205230.

Brogdom, W.G. and Chan, A. (2010). Guidelines for evaluating insecticide resistance in vectors using cdc bottle bioassay/methods in *Anopheles* research CDC Atlanta USA: 2010 CDC technical Report. P. 28.

Carrasco, D., Thierry, L., Nicolas, M., Pennetier,, C., Fabrice, K. and Cohue, A. (2019). Behavioural adaptations of mosquito vectors to insecticide control. *Science Direct*, 34, 48-53

Centre for Diseases Control and Prevention (CDC) (2015). CDC bottle bioassay: dissection of parasitic diseases and malaria. Global Health. http://www.cdc.gov/parasites/education_training/lab/bottlebioassay [Accessed 10/2/2024]

Chukwuekezie, O., Nwosu, E., Nwangwu, U., Dogunro, F., Onwude, C., Agashi, N., Ezihe, E., Anioke, C., Anokwu, S., Eloy, E., Attah, P., Orizu, F., Ewo, S., Okoronkwo, A., Anumba, J., Ikeakor, I., Haruna, S. and Gnanguenon, V. (2020). Resistance status of *Anopheles gambiae* (s.l.) to four

commonly used insecticides for malaria vector control in South-East Nigeria. *Parasites Vectors*, 13, 152. https://doi.org/10.1186/s13071-020-04027-z

de França, S., Breda, M., Douglas, R.S., Alice, M.N., Araujo, A. and Guedes, C.A. (2017). The sublethal effects of insecticides in insects. biological control of pest and vector insects. *InTechOpen* ISBN 978-953-51-3036-9, 360. DOI:10.57-72/66461

Dufera, M., Dabsu, R. and Tiruneh, G. (2020). Assessment of malaria as a public health problem in and around Arjo Didhessa sugar cane plantation area, Western Ethiopia. *BMC public health*, 20, 1-10.

Feng, Y.T., Wu, Q.J., Xu, B.Y., Wang, S.L., Chang, X and Xie, W. (2009). Fitness costs and morphological change of laboratory-selected thiamethoxam resistance in the B type *Bemisia tabaci* (Hemiptera: Aleyrodidae). *J Appl Entomol*., 133, 466–472

Gassmann, A.J., Carriere, Y. and Tabashnik, B.E. (2009). Fitness costs of insect resistance to *Bacillus thuringiensis*. *Annu Rev Entomol.*, 54, 147 –163

Gunasekaran, K.S., Muthukumaravel, S.S., Sahu, T. and Vijayakumar, P. (2015). Glutathione S.Transferase activity in indian vectors of malaria: a defense mechanism against DDT. *J of Medical Entomology*, 48(3), 561–569

Koffi, A.A., Camara, S., Ahoua, A., Alou, L.P., Oumbouke, W., Wolie, R.Z., Tia, I.Z., Sternberg, E.D., Yapo, F.H., Koffi, F.M., Assi, S.B., Cook, J., Thomas, M.D. and N'Guessan, R. (2023). *Anopheles* vector distribution and malaria transmission dynamics in Gbêkê region, central Côte d'Ivoire. *Malar J*., 22, 192. https://doi.org/10.1186/s12936-023-04623-1

Kpanou, C.D., Sagbohan, H.W., Dagnon, F., Padonou, G.G., Ossè, R., Salako, A.S., Sidick, A., Sewadé, W., Sominahouin, A., Condo, P., Ahmed, S.H., Impoinvi, D. and Akogbéto M. (2021). Characterization of resistance profile (intensity and mechanisms) of Anopheles gambiae in three communes of northern Benin, West Africa. *Malar J*., 20, 32. https://doi.org/10.1186/s12936-021-03856-2

Liu, N. (2015). Insecticides resistance in mosquitoes: impact, mechanisms, and research directions. *Annu. Rev. Entomol*., 60, 537-559.

Nakatani, K., Ishikawa, H., Aono, S. and Mizutani Y. (2014). Identification of essential histidine residues involved in heme binding and hemozoin formation in heme detoxification protein from *Plasmodium falciparum*. *National Academic Science*, 4, 6137.

Puinean, A.M., Denholm, I., Millar, N.S., Nauen, R. and Williamson, M.S. (2010). Characterisation of imidacloprid resistance mechanisms in the brown planthopper, NilaparvatalugensStål. (Hemiptera: Delphacidae). *PesticBiochem Physiol*., 97, 129-132

Riveron, J.M., Irving, H., Ndula, M., Barnes, K.G. and Ibrahim, S.S. (2013). Directionally selected cytochrome P450 alleles are driving the spread of pyrethroid resistance in the major malaria vector *Anopheles funestus*. *Proc. Natl. Acad. Sci., USA*, 110, 1252-1257

UNICEF (2024). Malaria: Monitoring the situation of children and women. https://data.unicef.org/topic/child-health/malaria/ [Accessed 10/2/2024]

Vatandoost, H., Ezeddinloo, L., Malivia, A.M. and Mobedi, E. (2004). Enhanced tolerance of house mosquito to different insecticides due to agriculture and household pesticides in sewage system of Tehran, Iran. *Iranian J*. Env. *Health Sci Eng*., 1,42-45

Verra, F., Angheben, A., Martello, E., Giorli, G., Perandin, F. and Bisoffi Z. (2018). A systematic review of transfusion-transmitted malaria in non-endemic areas. *Malar. J*., 17, 36. https://doi.org/10.1186/s12936-018-2181-0

Vulule, J.M., Beach, R.F., Atieli, F.K., McAllister, J. and Brogdon, W. (1999). Elevated oxidase and esterase levels associated with permethrin tolerance in *anopheles gambiae* from kenyan villages using permethrin- impregnated nets. *Med. Vet. Entomol.*, 1, 239–244.

World Health Organization (WHO) (2015). Question and Answer on the Global Plan for Insecticide Resistance Management in Malaria Vector. http://www.who.int/malaria/media/insecticide_resistance_management_qa/en/ [Accessed 11/2/2024]

World Health Organization (WHO) (2018). World Malaria Report. Geneva, Switzerland: WHO Press. https://www.who.int/publications/i/item/9789241565653 [Accessed 11/2/2024]

World Health Organization (WHO) (2022). World Malaria Report. 2022. World Health Organization, Geneva, Switzerland. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022 [Accessed 14/2/2024]

World Health Organization (WHO) (2014). Fact Sheet Number 94, WHO, Geneva, 2014. http://www.who.int/mediacentre/factsheets/fs094/en/ [Accessed 15/2/2024]

Yadouleton, A.W., Padonou, G., Asidi, A., Moiroux, N., Bio-Banganna, S., Corbel, V., N'guessan, R., Gbenou, D., Yacoubou, I., Gazard, K and Akogbeto, M.C. (2010). Insecticide resistance status in *Anopheles gambiae* in southern Benin. *Malar J.*, 9, 83. https://doi.org/10.1186/1475-2875-9-83