



EVIDENCE OF ANOPHELES RESISTANCE TO PYRETHROID PESTICIDES: REPORT FROM GADAU KATAGUM ENDEMIC REGION BAUCHI STATE, NIGERIA

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ABSTRACT

Malaria continues to pose a significant health issue negatively. The high number of cases requires prevention, including controlling the *Anopheles gambiae s.l.*, mosquito. One of the control methods is the use of insecticides containing pyrethrin. WHO tube Bioassays in *Anopheles gambiae s.l.*, to determine resistance from pyrethroids expose in F1 adult mosquitoes to insecticides and a sensitivity test to temephos, measuring the activity of non-specific alpha and beta esterase enzymes. This study determined *Anopheles gambiae s.l.*, resistance from larvae to adult. The bioassay was used for the temephos sensitivity of *Anopheles* larvae. The LC99 value was analyzed using probit and compared with the diagnostic. WHO susceptibility test was conducted to determine pyrethroid resistance from adult mosquitoes. A mortality of less than 90% was declared as resistant. Measurement of alpha and beta esterase levels used Lee's microplate assay technique based on visual identification and absorbance value. *Anopheles gambiae s.l.*, were resistant to both class of pyrethroids. Adult mosquitoes have recorded resistant to both class of type i and ii pyrethroid with increase in time reaching about 60% with cypermethrin. At 24 hours Mortality Rates showed Cypermethrin with the highest at 90% followed by Permethrin (80%), Deltamethrin (75%), and Tetramethrin (70%). Based on the alpha esterase activity test, it was found that most of the mosquitoes showed very sensitive meanwhile, most of the mosquitoes were moderate resistance. This study suggests that *Anopheles gambiae s.l.*, the population from the endemic region in Bauchi, Nigeria are indicated to develop resistance to the pyrethroids insecticides.

Keywords: *Anopheles gambiae s.l.*, Malaria fever, Pyrethroid, Resistance

INTRODUCTION

The parasite species of the genus *Plasmodium* are the cause of malaria, a potentially fatal illness spread by the bite of an infected female *Anopheles* mosquito. Malaria, which is transmitted by the bite of female *Anopheles* mosquitoes carrying the parasite *Plasmodium*, is fatal. The WHO African Region continues to be the region most affected by malaria, accounting for 95% of all cases (234 million cases) and 96% of all deaths (593,000 deaths) as of 2021. More than 80% of malaria deaths in the African Region were in children under the age of five (WHO, 2022). Malaria can be prevented and treated using insecticides, mosquito nets, indoor residual spraying, and antimalarial drugs. Because vector management is so effective at avoiding infection and reducing the spread of the disease, it is a crucial component of strategies for managing and eliminating malaria. The two primary treatments are indoor residual spraying (IRS) and insecticide-treated nets (ITNs). (Kleinschmidt 2009; Ochomo *et al.*, 2013). *Anopheles* mosquitoes' increasing pesticide resistance poses a threat to the advancements gained in the worldwide fight against malaria. (Ngufor *et al.*, 2016). Inadequate access, net loss from daily stress exceeding replacement, and changes in mosquito behavior (such as biting before bed and sleeping outside, avoiding insecticide exposure) are further hazards to ITNs (PMI, 2018; WHO, 2022). According to a 2022 World Health Organization assessment, around 50% of the world's population is susceptible to contracting malaria. Globally, malaria caused over 247 million infections and 619,000 deaths in 2021. WHO 95% of malaria cases worldwide occurred in African countries (Kouamé *et al.*, 2022; Kendie *et al.*, 2023) with Nigeria accounting for 27% of cases and 31% of malaria-related deaths (WHO, 2022). In the Northeast, the frequency is roughly 32% in Bauchi State (NMIS, 2021).

However, in order to reduce the global malaria burden by 90% by 2030, WHO recommends that all countries implement Insecticide Treated Nets (ITNs), particularly Long Lasting Insecticidal Treated Nets (LLINs) and Indoor Residual Spray (IRS) as core interventions (Federal Ministry of Health, 2015). However, the sustainability and efficacy of this management strategy are being jeopardized by the rise of pesticide resistance (Ranson and Lissenden, 2016). The development of pesticide resistance in malaria vectors in Nigeria in recent years has put the effectiveness of these control measures in jeopardy (Ibrahim *et al.*, 2013; Habibu *et al.*, 2017; Ibrahim *et al.*, 2019; WHO, 2022). With the exception of carbamate in South East Nigeria, *Anopheles* mosquitoes were also found to be resistant to pyrethroids, organochlorines, and carbamate in Southern Nigeria (Busari *et al.*, 2023; Burton *et al.*, 2011). Furthermore, it was observed that *An. Coluzzii* in Northwest Nigeria exhibited high levels of resistance to DDT and permethrin, as well as the establishment of bendiocarb resistance (Hakizimana *et al.*, 2016). Research in Northern Nigeria has revealed that *Anopheles gambiae* is highly resistant to pyrethroids and DDT, while *Anopheles coluzzii* is less resistant to bendiocarb (Ibrahim *et al.*, 2019; Umar *et al.*, 2015; Charles *et al.*, 2014). In addition to resistance of these insecticides to *aedes aegypti* in central region of the country. (Muhammad *et al.*, 2024). The lack of knowledge regarding the precise level of pesticide resistance in the Sudano-Sahelian region of the Bauchi North thus served as the impetus for the current investigation. According to (Ohiri, *et al.* 2016), the results of this study could help the Nigerian National Control Programme choose which resistance management techniques to use in the region and inform the National Malaria Strategic Plan (NMSP) 2014–2020.

MATERIALS AND METHODS

Study Site and Mosquito Sampling

Blood-fed female *Anopheles* mosquitoes resting indoors were collected in the rainy season of July/August using battery-operated aspirators (John. W. Hock, Gainesville, FL, USA). The collection was done in the morning hours (5:30:00–6:00 a.m.) At (Gadau, Katagum Latitude 11° 58' longitude 10° 33') as shown in figure 1. Sudan Sahelian region of the north in Bauchi state.

The inhabitants of Gadau are mainly subsistent farmers and cultivated crops include sorghum, millet, maize, groundnuts and cowpea. They also practice irrigation farming due to the presence of the Gadan River which extends toward the Hadeja/Jama'are River. The farmers also keep and rear animals such as cattle, sheep and goats. The housing structures are made of sand screed blocks, mud and thatched.

Farmers do apply pesticides mainly Pyrethroids. Informed consent for the indoor collection was obtained from the two teachers, teaching the Almajiris student in their settlement. The blood-fed females obtained were maintained on 10 % sugar between 25 °C and 27 °C and 70–80 % relative humidity for 6–7 days. Gravid females were transferred into 1.5 mL tubes individually and forced to lay eggs, using established protocols (Ibrahim *et al.*, 2014). The F₀ parents were identified as belonging to the *An. gambiae s.l.* group using morphological keys (Gillies and Coetzee 1987). Egg batches were transferred into paper cups for hatching in the insectary. Eggs that hatched were pooled into bowls and supplemented with Tetramin TM baby fish food. The 3 to 4 days old F₁ females that emerged were mixed in cages and used for bioassays.



Figure 1: Sampling site access from Google and modified August 2024

Mosquito rearing

The mosquitoes were maintained following standard insectary conditions (reared at 29°C–32°C, 70–80% relative humidity and fed with 10% sucrose solution soaked in cotton wool). The larvae were maintained in deionized water and fed daily with fish feed under the same conditions as the adults. The emerging F₁ progenies (first filial generation) were used for insect susceptibility bioassays.

Morphological Species Identification

Mosquitoes were identified morphologically as *Anopheles gambiae s.l.* complex species using the procedure adopted for morphological identification keys (Gillies and Coetzee, 1987)

Biochemical Test

The biochemical test of nonspecific esterase is based on Lee's procedure (Lee H.L. 1990). Two hundred larval sample were homogenized individually in 0.5 ml cold PBS (0.02M; 7.5 pH) using micro pestle. With a micropipette, 65 µl of the clear homogenate was transferred to a microplate, where two replicates for each α esterase and β esterase. Sixty five µl each α and β naphthyl acetate solution (6 gram/L in ethanol dissolved in PBS with 1.5% concentration) as substrate was added and left for 60 seconds. Sixty five µl fast B blue salt solution (150 mg fast B blue salt in 15 ml aquadest and 35 ml sodium dodecyl sulphate 5% (W/V)) was used as a coupling reagent, and the colour changed immediately after 15 minutes. It turned to green until blue in α esterase activity and turned to dark pink in β esterase activity. The reaction was stopped

by adding 65 μ l 12% acetic acid solution into each well. The intensity of the final colour is an esterase activity indicator. A microplate reader also scanned the intensity of the final colour at 445 nm wavelength. The susceptibility status of mosquitoes based on α esterase activity was determined based on the absorbance value (AV), where the AV value of 0 - 0.65 is expressed as very sensitive, 0.65 - 0.90 as moderate resistance, and > 0.90 as highly resistant. While on β esterase activity with AV 0 - 0.45 as very sensitive; 0.45 - 0.65 as moderate resistance and > 0.65 as highly resistant.

WHO Insecticide Susceptibility Bioassays

WHO Bioassays were carried out on 2-4 days F1 (First filial generation) non-blood fed adult female progenies detect and characterize resistance to type i and a type ii pyrethroid (Permethrin 36.8%, Deltamethrin 2.5%, Cypermethrin 1.0% and Tetramethrin 0.5%) for one hour knock down and 24 hours' mortality. (WHO, 2016). Four replicates of 20 mosquitoes per tube were exposed to these insecticides impregnated papers for 1 hour, and knock down rate was recorded for 5 minute, 15 minute, 30 minute, 45 minute and 1 hour and then transferred to the holding tubes and fed with 10% sucrose solution soaked in cotton wool and kept overnight under standard insectary conditions. One tube each was used as a control for each insecticide tested, with 20

females exposed to control papers impregnated only with the carrier oil (QC controls for Pyrethroids. After 24 hours, Mortality rate was recorded for each insecticides and for each tubes. All bioassays were carried out at 27–30°C and 75–80% relative humidity. Resistance status of the population was established according to WHO criteria (WHO 2016) for which populations with mortality >98% are considered susceptible, populations with mortality of 90–98% are considered moderately resistant, and those with mortality >90% as resistant. Susceptible individuals are defined as individuals that did not survive a discriminating dose of the particular insecticide used (WHO 2016).

RESULTS AND DISCUSSION

Composition of the Mosquito Species Identified Morphologically

One thousand nine hundred and eighty five 1985 adult mosquitoes with a total of 341 (17.13%) found to be predominantly *Anopheles gambiae s.l* and the remaining were found to be *Culex quinquefasciatus* (82.87%) found as shown in the Figure 2; below. Mosquitoes were identified morphologically as *Anopheles gambiae s.l* complex and *Culex quinquefasciatus* species using the procedure adopted for morphological identification keys (Gillies and Coetzee, 1987).

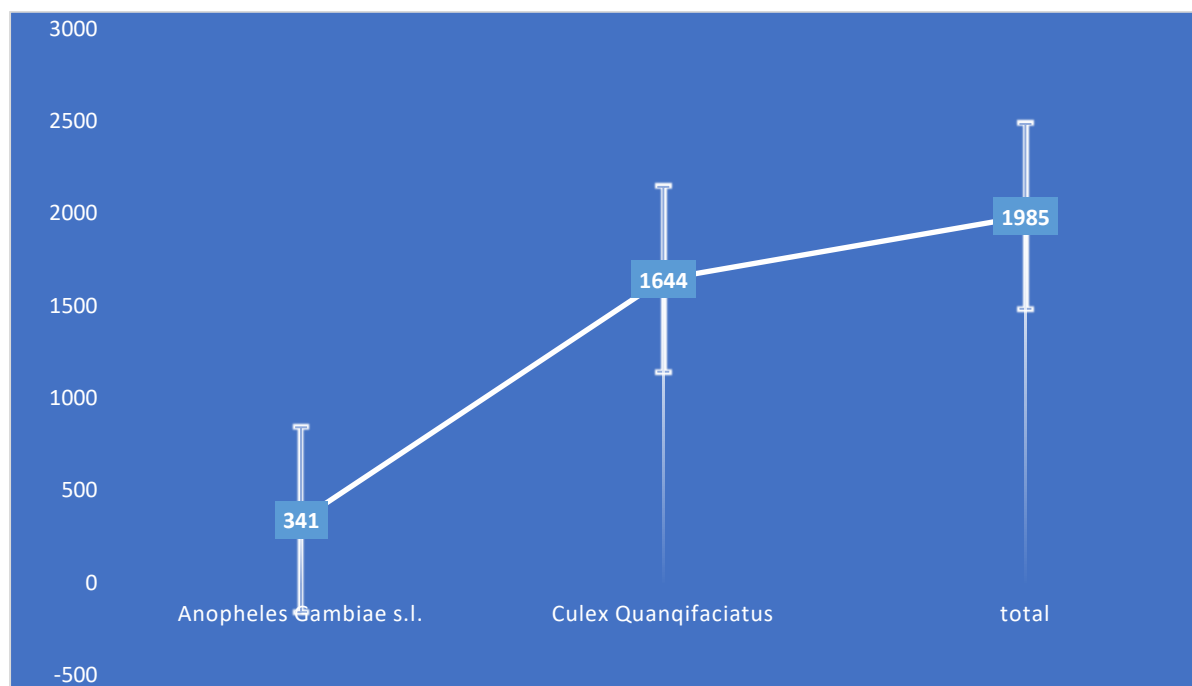


Figure 2: Summary of all vectors (mosquitoes species) Identified morphologically

Insecticide Susceptibility Bioassays

WHO Bioassays were carried out on 2-4 days F1 (First filial generation) non-blood fed adult female progenies detect and characterize resistance to type i and type ii pyrethroid for one hour knock down and 24 hours' mortality. (WHO, 2016). Four replicates of 20 mosquitoes per tube were exposed to these insecticides impregnated papers for 1 hour, and knock down rate was recorded for 5 minute, 15 minute, 30 minute, 45 minute and 60 minute. The average knock down in the four replicate indicated that an increasing rate or resistance with time. At Early Stages of 0–30 minutes Deltamethrin demonstrated rapid action with 10% knockdown at 0 minutes

and 25% by 30 minutes unusual for insecticides that typically require time to take effect. Cypermethrin showed delayed but accelerating knockdown with 10% at 30 minutes and rising to 60% at 60 minutes. At 45–60 minutes, permethrin and tetramethrin had minimal knockdown ($\leq 10\%$). Cypermethrin achieved 60% knockdown at 60 minutes correlating with its high final mortality rate. The control group shows minimal effect. After 24 hours Mortality Rates showed Cypermethrin with the highest at 90% followed by Permethrin (80%), Deltamethrin (75%), and Tetramethrin (70%). As shown in Figure 3

Average knock down rate

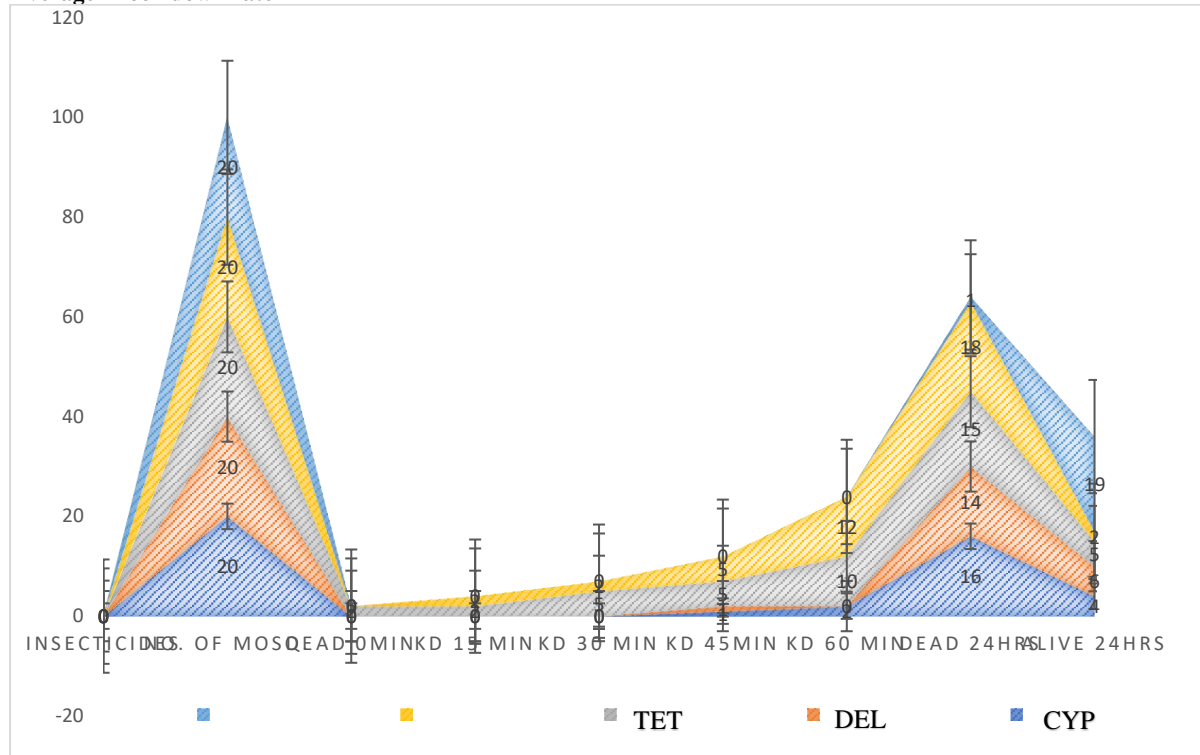


Figure 3: Average knock down rate with error bar representing data variability

Percentage Mortality

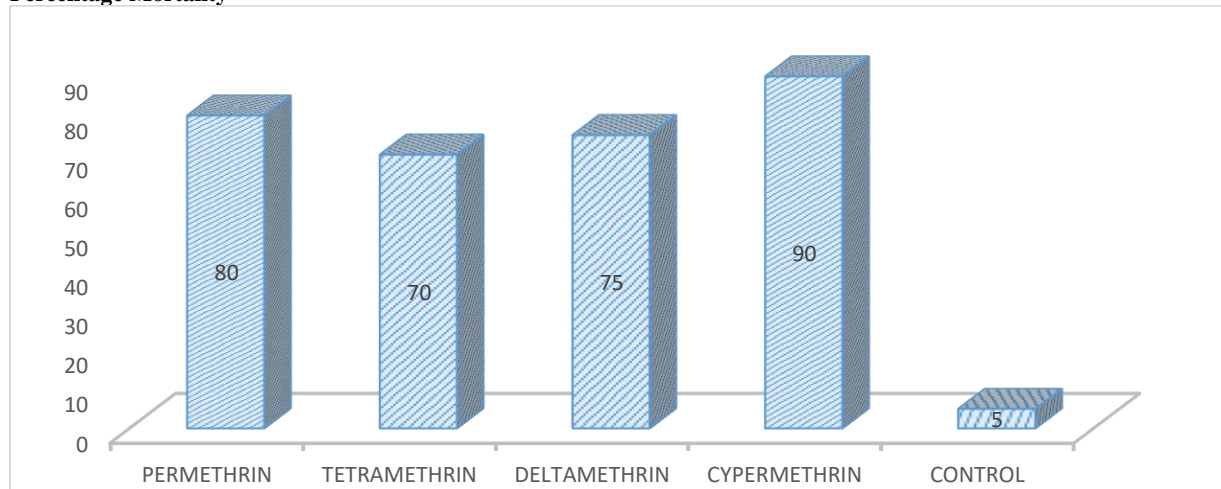


Figure 4: Percentage Mortality with error bar representing Data variability

Biochemical Test

Based on the results of biochemical tests can be seen as an increase in esterase activity. There was a green discoloration of α esterase and pink colour of β esterase to varying degrees.

Identifying resistance by visual has a high subjectivity level, so further examination is needed to determine the degree of absorbance value (AV). The α and β esterase test results based on the AV value can be seen in Table 1.

Results of α and β esterase activity

Table 1: The results of α and β esterase activity tests in Anopheles larvae

Esterase	Sensitivity	Qualitative	Quantitative %
Esterase α	Very sensitive	Yellow	<0,75
	Moderate resistant	Greenish blue	0,70-0,90
	Highly resistant	strong blue	>0,90
Esterase β	Very sensitive	Yellow	0-0,4,5
	Moderate resistant	Pink	0,4-0,6,5
	Highly resistant	Strong Pink	>0,6,5

Esterase α Very sensitive Yellow 0, 90 0 Esterase β Very sensitive Yellow 0-0,4 43,3 Moderate resistant Pink 0,4-0,6 48 Highly resistant Dark pink >0,6 8,7. Statistical analysis

using Anova as shown in Table 2 below SPSS /POSTHOC/DUNCAN C ALPHA Values of mortality rate among and in between groups with mean \pm S.D

Homogeneous Subsets

Table 2: Means for groups in homogeneous subsets

Insecticides	N	Subset for alpha = 0.05	
		1	2
Duncan ^a			
Control	4	0.00	
Cypermethrin	4	1.25	
Deltamethrin	4		4.00
Permethrin	4		5.25
Tetramethrin	4	0.78	
Sig.		0.121	0.121

Uses Harmonic Mean Sample Size = 4.000.

Discussion

Control and Reversal of Malaria was designed to reduce mosquitoes, particularly *Anopheles gambiae s. l.* but it has also increased the rate of resistance. Insecticide resistance has been reported worldwide in a number of locations, especially those that rely significantly on insecticide-based vector control methods (WHO 2020). Additionally, resistance to pyrethroids, the most commonly used kind of insecticide, has been reported on multiple occasions. In 2018 (Hassan *et al.*, 2018), additionally, reports of rising resistance to several pesticide classes are becoming more frequent (Ibrahim *et al.*, 2019). In addition, the findings are consistent with research by Ibrahim *et al.* (2019) and Mukhtar and Ibrahim (2022), which demonstrates that there are signs of cross-resistance, in which mosquitoes that are resistant to one class of pesticide also exhibit resistance to others, including carbamates and organophosphates. For example, populations of *Aedes aegypti* in the north central region of the country have been found to be resistant to pyrethroids, specifically deltamethrin and permethrin. According to the assessment of (Mahe *et al.*, 2022), the situation in Northern Nigeria is very worrisome. *Anopheles gambiae sensu lato* predominates in the area, with *Culex quinquefasciatus* accounting for the remainder. No other species were found, and (N'Dri *et al.*, 2023) recently reported on species cohabitation. The experimental results compare the efficacy of four insecticides type i (Permethrin, Tetramethrin) and type ii (Deltamethrin, Cypermethrin) against mosquitoes, with a control group. Knockdown rate; At Early Stages of 0–30 minutes deltamethrin demonstrated rapid action with 10% knockdown at 0 minutes and 25% by 30 minutes unusual for insecticides that typically require time to take effect. Cypermethrin showed delayed but accelerating knockdown with 10% at 30 minutes and rising to 60% at 60 minutes. At 45–60 minutes, permethrin and tetramethrin had minimal knockdown ($\leq 10\%$). Cypermethrin achieved 60% knockdown at 60 minutes correlating with its high final mortality rate. The control group shows minimal effect. This confirms the experiment's baseline - mortality in the absence of insecticide is low. (Mukhtar and Ibrahim 2022). The concentration of the insecticides used is crucial. Different concentrations might yield different results. Temperature, humidity, and other environmental factors can influence the effectiveness of insecticides. After 24 hours Mortality Rates showed Cypermethrin with the highest at 90% followed by Permethrin (80%), Deltamethrin (75%), and Tetramethrin (70%). Type i being more effective than type ii pyrethroids. The control group had a 5% mortality rate confirming experimental validity. The greater susceptibility to pyrethroid was recently reported by (Ibrahim *et al.*, 2019; Kendie *et al.*, 2023). The efficiency of pesticides can be impacted by

temperature, humidity, and other environmental conditions. Cypermethrin had the highest mortality rate after 24 hours at 90%, followed by Permethrin at 80%, Deltamethrin at 75%, and Tetramethrin at 70%. Pyrethroids of type I are more effective than those of type II. The control group's 5% death rate confirmed the experimental validity. Recent reports of increased pyrethroid susceptibility were made by (Ibrahim *et al.*, 2019; Kendie *et al.*, 2023) and to *aedes aegypti* species have been documented (Muhammad *et al.*, 2024). The most encouraging outcomes were seen with deltamethrin. To get firm conclusions, comprehend the long-term effects of these pesticides, and investigate environmental elements, more investigation should be adopted using more experimental approach. Esterase α and β biochemical assay findings show different sensitivity profiles and matching colorimetric indications. The sensitivity and resistance thresholds of Esterase α are higher than those of Esterase β . In particular, Esterase breakdown β is extremely sensitive at even lower concentrations (0-0.45%), and Esterase α exhibits very sensitive action at lower values (<0.75%). Since esterases are involved in the production of pesticides and other contaminants, environmental samples can be used to track their activity. According to a study by Machani *et al.* (2020), the qualitative color changes offer a simple visual indication for evaluating enzyme activity, with yellow denoting high sensitivity and stronger colors (pink or greenish blue) denoting increasing resistance. In contrast to Esterase β , which shows resistance at lower concentrations, the quantitative ranges imply that Esterase α becomes resistant at higher concentrations. In 2020 (Machani *et al.*, 2020) studies enable customized methods in enzymatic investigations or therapeutic applications by assessing the efficacy of substrates or inhibitors on these esterases in diverse biological or experimental settings. Resistance may be caused by a number of circumstances, including long-term and frequent pesticide application.

CONCLUSION

Multiple replicates, or repeating the experiment several times for each pesticide, would increase the experiment's robustness. This increases the statistical significance of the findings and helps to account for variability. It's critical to take pesticide resistance into account. A pesticide may not work as well if the mosquito population has become resistant to it. Additionally, these findings offer important new information for evaluating resistance levels and enzymatic activity in experimental contexts. Compared to β -esterase, the evidence indicates that Esterase α needs more quantitative measurements to show resistance. During simultaneous testing, it is possible to distinguish between different enzyme

types thanks to the noticeable color shifts (blue vs. pink spectra).

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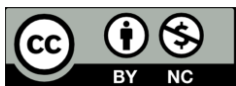
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