



## Arid Zone Journal of Basic and Applied Research

Faculty of Science, Kashim Ibrahim University  
Maiduguri, Nigeria

Journal homepage: <https://www.azjournalbar.com>



### Research Article

## Assessment of Pyrethroid Insecticide Resistance on *Anopheles gambiae* in Potiskum, Yobe State, Nigeria

Mohammed Ahmed<sup>1</sup>, and Usman N Gadzama<sup>2</sup>.

<sup>1</sup>Department of Fisheries, University of Maiduguri

<sup>2</sup>Department of Biological Sciences, University of Maiduguri.

\*Corresponding author's Email: [Muhammada889@gmail.com](mailto:Muhammada889@gmail.com), [doi.org/10.55639/607.special.012](https://doi.org/10.55639/607.special.012)

#### ARTICLE INFO:

#### ABSTRACT

#### Keywords:

Malaria,  
Vector,  
Insecticide,  
Breeding.

Malaria is a tropical disease associated with over 400,000 annual mortality globally. The burden of malaria remains high in Nigeria. Insecticide resistance in malaria vectors is a growing concern in many countries which requires immediate attention. The emergence of Pyrethroid resistance on *Anopheles gambiae* has become a serious concern to the future success of malaria control. It is, therefore, crucial to monitor the level and type of insecticide resistance in *An. gambiae*, where reduced efficacy of insecticide-treated nets (ITNs) and IRS has previously been reported. *Anopheles* larvae were collected from breeding sites and they were fed with Cabin biscuits until they emerge as adults. The adults were further fed with 10% sugar solution. Bioassay were performed on female *Anopheles* Mosquitoes and their susceptibility to insecticide-impregnated papers (0.75%Permethrin, 0.05%Delthamethrin, and 0.05%Alphacypermethrin) following WHO guidelines were determine. Morphological identification was conducted by adopting Coetztes' method of identification. Bioassay mortality decreased for all the insecticides that were tested in Sites A, B and C. The mortality rate for Permethrin ranged from 75% to 90%, Deltamethrin and Alphacypermethrin showing similarly lower rates, all below the WHO grading scale of  $\geq 98\%$  for susceptibility to base line insecticides. Synergist bioassay enhanced the efficacy of Permethrin and Alphacypermethrin, restoring susceptibility, but Deltamethrin resistance persisted with mortality of 97% below WHO grading scale in site A. This study showed that, resistance to Permethrin and Alpha-cypermethrin in *An. gambiae* populations were evident, which is aligned with metabolic enzymes resistance. Notwithstanding, this study suggest the use of Permethrin and Alphacypermethrin in combination with Piperonyl butoxide. Molecular approach should be employed to determine exact resistance on *Anopheles gambiae*.

**Corresponding author:** Mohammed Ahmed., **Email:** [Muhammada889@gmail.com](mailto:Muhammada889@gmail.com)  
Department of Fisheries, University of Maiduguri

#### INTRODUCTION

Malaria is a life-threatening disease and the parasites are transmitted via the bites of infected female *Anopheles* mosquitoes [World Health

Organization (WHO), 2022]. Mosquitoes belong to the phylum Arthropoda, class *Insecta*, and order *Diptera* (Gaugler, 2016). Over 3,530

species of mosquitoes exist worldwide which are grouped under 43 genera; all these genera are included in the family *Culicidae* (Goutam *et al.*, 2013). *Aedes*, *Culex* and *Anopheles* mosquitoes are medically important because of their capacity to transmit human diseases (Gubler, 2010). Mosquito species frequently bite humans and serve as carriers for several infectious diseases that affect millions of people annually in Sub-Saharan Africa including Nigeria (Molavi, 2003). Diseases like malaria, zika virus, filariasis, and yellow fever are transmitted by mosquitoes (Dawn *et al.*, 2001).

However, the variety of aquatic habitats available often leads to significant mosquito populations, causing both nuisance through biting and the transmission of disease-causing organisms (Adefolabi *et al.*, 2013). All mosquito species require aquatic habitats for laying eggs and for the development of larvae and pupae as well as the type of water in these habitats influences where mosquitoes lay their eggs, thereby affecting the productivity of these sites and ultimately impacting the mosquitoes' ability to transmit diseases (Afolabi *et al.*, 2013). Many species breed in both natural and artificial settings such as concrete gutters, stagnant pools, discarded plastics, abandoned tires, and even open unused drums (Afolabi *et al.*, 2013).

The vectors of malaria are primarily female *Anopheles* mosquitoes. These mosquitoes usually bite between dusk and dawn, making bed nets treated with insecticides a crucial intervention in preventing malaria transmission, indoor residual spraying of insecticides and preventive antimalarial medication for at-risk populations are also important strategies in malaria control (WHO, 2023).

Malaria control in many endemic countries relies on vector control with long-lasting insecticidal nets and indoor residual spraying (National Health Policy, 2020); globally, these two interventions have averted about 78% of malaria-related deaths and morbidity (Bhatt *et al.*, 2015). Nevertheless, as they rely on efficacious insecticides, the emergence and spread of insecticide resistance may represent a major threat (Ranson and Lissenden, 2016; Opondo *et al.*, 2016 and Alout *et al.*, 2016) although LLINs would still offer personal protection against mosquito bites (Ochomo *et*

*al.*, 2017). Recently, IRS has been implemented in many African countries including, Gambia, Ghana, Ethiopia and Nigeria. The choice of the insecticide for use in IRS should be based on the susceptibility of the local vectors to the WHO approved insecticides (WHO, 2018).

Vector control is one of the most effective methods to control vector-borne diseases, with insecticide-based interventions predicted to have averted the majority of malaria cases since 2000 (Bhatt *et al.*, 2015). However, because they reduce vector survival and reproduction, the continued and widespread use of chemical-based vector control tools (VCTs) has contributed to the evolution of insecticide resistance (IR) and evasion, particularly in *Anopheles* mosquito populations (Huijben and Paaijmans, 2018; and Killeen *et al.*, 2017), and threatens progress towards malaria elimination targets (WHO, 2021).

Preventing malaria transmission by targeting the main vectors remains a challenge for National Malaria Elimination Programmes (WHO, 2021). Only four classes of insecticides, pyrethroids, organochlorines

(Dichlorodiphenyltrichloroethane), carbamates and organophosphates are currently approved for indoor residual spraying (IRS) and only pyrethroids for insecticide treated bed nets (ITNs) and long-lasting insecticidal nets (LLINs) (Edi *et al.*, 2012). High coverage with IRS and especially ITNs has contributed to the decrease in the number of malaria cases throughout Sub-Saharan Africa over the past decade (WHO, 2012).

However, resistance to pyrethroids and DDT is now widespread in many African countries including Nigeria (Ranson *et al.*, 2011). Though currently less common, resistance to carbamates and organophosphates, the available alternatives for IRS, is also being reported (Aizoun *et al.*, 2013), and resistance to all four classes has now been found in mosquitoes (Edi *et al.*, 2012). The choice of the insecticide for use in IRS should be based on the susceptibility of the local vectors to the WHO approved insecticides (WHO, 2018).

## **MATERIALS AND METHODS**

### **Study Area**

This study was conducted in Potiskum, Yobe State, Nigeria. Potiskum is situated at 11°43'N and 11°04'E, it has an area of 559 Km<sup>2</sup> and a

population of 565,000 (Nigerian Metro Area Population, 2024).

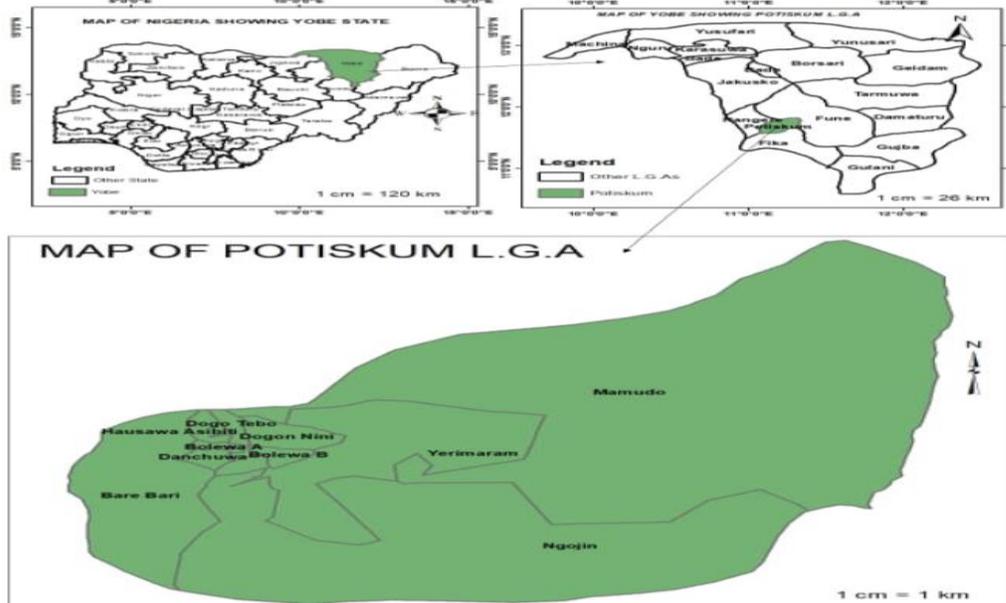


Figure 1: Map of Potiskum Local Government, Yobe State, Nigeria.

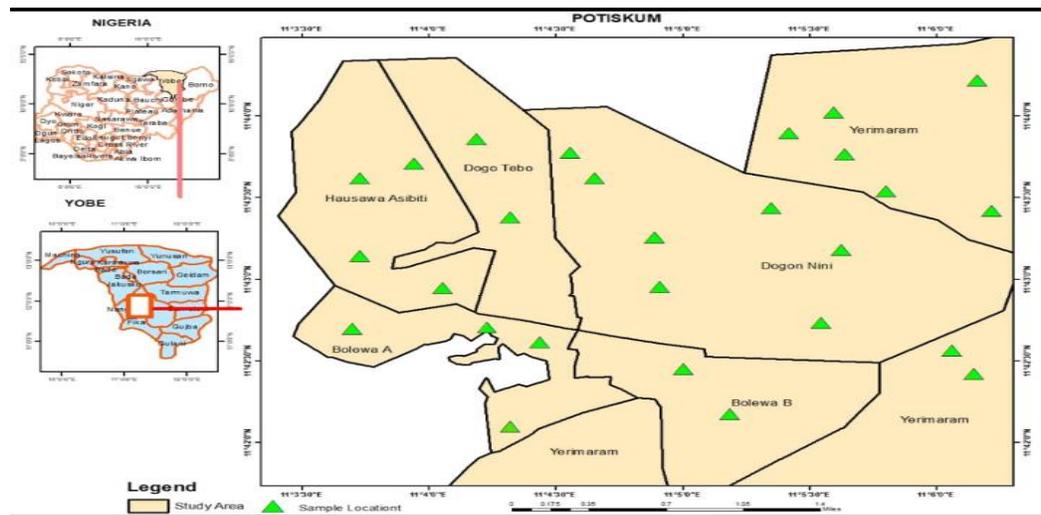


Figure 2: Map of the study area indicating sampling sites

**Field Collection of Larvae from breeding sites and Laboratory Rearing to Adults**

Anopheles mosquitoes (Larvae) were collected as earlier after identifying breeding sites, using ladle. The larvae were transported to the laboratory, sorted and transferred to small transparent containers, following which, they were fed with grinded cabin biscuits and yeast tablets placed in the breeding cages for further development to pupae. The pupal stages are non-feeding, hence were only monitored until emergence of adult forms. Newly emerged adult

forms were maintained in the insectary (temperature 26°C to 30°C and humidity of 75% ± 10% in 12 hours dark and 12 hours light cycles) (Leite et al., 2024).

Adult mosquitoes in the breeding cages were fed with 10% sugar solution soaked in a cotton wool and allowed approximately three (3) days in the insectary condition before selecting the females and subjecting them to bioassay tests.

**Pyrethroid Insecticide Susceptibility Studies of Anopheles Mosquitoes Test Conditions**

Susceptibility testing was thus conducted using female mosquitoes, only. Initial susceptibility test using varying concentrations was performed on adult female aged 3 to 5 days that are non-blood fed.

#### Sample Size

One hundred and fifty (150) adult female *Anopheles* mosquitoes were used to perform single set of WHO insecticide susceptibility test. Of these, ~100 were exposed to various technical grade insecticides provided and tested in four (4) replicates each 25 mosquitoes. The remaining 50 mosquitoes served as controls (i.e. two replicates each 25 mosquitoes).

#### Set up and Procedures for WHO Susceptibility/Resistance Bioassay

Four (4) replicates of 25 non-blood fed adult female *Anopheles* mosquitoes each were used per insecticide. For clarity during assay testing, WHO bioassay tubes were color labelled as shown and described in Fig. 5 below;

Green Dot: Serves as the holding tube, in which Adult female *Anopheles* mosquitoes (F0) exposed to plain whitepapers were held prior to exposure to insecticide impregnated test papers

#### Mortality calculations and adjustments

$$i. \quad \text{Control Mortality (\%)} = \frac{\text{Number of control mosquitoes that died}}{\text{Total number of control mosquitoes}} \times 100$$

Mortality adjustments when control mortality is high

- ii. If the control mortality is <5%, no correction of mortality is necessary.
  - ❖ When control mortality is  $\geq 5\%$  and  $\leq 20\%$ , the observed mortality in insecticide-exposed mosquitoes must be corrected using Abbott's formula:

$$iii. \quad \text{Corrected Treatment Mortality (\%)} = \frac{(\% \text{ treatment mortality} - \% \text{ control mortality})}{(100 - \% \text{ control mortality})} \times 100$$

$$iv. \quad \text{Treatment Mortality (\%)} = \frac{\text{Number of treatment mosquitoes that died}}{\text{Total number of control mosquitoes}} \times 100$$

(WHO, 2022)

#### Synergist Bioassay

For the synergist assay, only Deltamethrin(0.05%) in sites A, B and C resistant populations were used. The experiment involves three bioassay exposures:

- i. Piperonylbutoxide(PBO) only
- ii. Insecticide only and
- iii. Control.

Synergist bioassay was performed on resistant population of female *Anopheles* mosquitoes to technical grade insecticides. One hundred and fifty mosquitoes were transferred to six holding tubes (25 in each) as earlier described.

Red Dot: Serves as exposure tube. In this tube, WHO (Insecticide impregnated) test papers are rolled and inserted prior to exposure. Adult female *Anopheles* mosquitoes (F0) were transferred from the holding tubes into these (Exposure) tubes and knock down monitored for 60 minutes at intervals of 10, 15, 20, 30, 40, 50 and 60 minutes respectively.

Insecticide control: Following exposure, all mosquitoes were transferred back to the holding tubes and mortality was read 24 hours post exposure.

Yellow Dot: Serves as control tubes, in this tube, WHO papers are rolled and inserted prior to exposure that served as controls. These are kept site by site with the exposure tubes and readings are taken concurrently with the mosquitoes exposed to insecticide impregnated test papers. Coordinates of the breeding sites were taken and the *Anopheles* mosquito larvae were then transported in well labelled transparent plastic containers ((125cm×55cm)) to the Research laboratory of Yobe State University, Damaturu Yobe State.

The female *Anopheles* mosquitoes were transferred to exposure tubes containing 4% PBO for one hour.

The mosquitoes were later on transferred to holding tubes and allowed to stay for one hour before being transferred to exposure tube containing impregnated test papers. These were monitored to stand for 1 hour and knock down were recorded at an interval of 10,15,20,30,40,50 and 60 minutes respectively, before being transferred to holding tubes.

Mosquitoes in holding tubes were allowed for 24hours after which mortality was recorded.

#### Morphological Identification

Exposed *Anopheles* mosquitoes were identified according to Coetzee's method of morphology identification was adopted, (Coetzee, 2020). The method provides morphological keys to differentiate between species of *Anopheles* mosquitoes.

**Data Analysis**

WHO standard criteria for determining resistance and susceptibility status of the baseline diagnostic dose of both insecticide and the synergist tests (Susceptibility (S) = Mortality  $\geq$  98%, Possible Resistance (PR) = Mortality of 90-97% and Resistance (R) = Mortality  $\leq$  89%) was adopted. Data were analyzed using a statistical package for social science (SPSS) software. One-way analysis of variance (ANOVA) was used to analyze the data in this study. P values  $P < 0.05$  was regarded as statistically significant. Multiple comparison test (Duncans MRT) was used to determine which of the site is significant among.

**RESULTS**

Table 1 shows the mortality of female *Anopheles* mosquitoes collected from sites (A, B and C). After post exposure to  $\times 1$  discriminating concentrations of Pyrethroids (0.75% Permethrin, Deltamethrin and alphacypermethrin). Results obtained showed that, Site A (Bolewa A, Bolewa B, Hausawa Asbiti) had the highest mortality (90%) when compared to site B and C (83.7% and 75%) which showed low mortality. Similarly, alphacypermethrin produced high mortality in Site A (Bolewa A, Bolewa B, Hausawa Asbiti) than in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) and C. On the other hand, Deltamthrin showed low mortality in both Site A (Bolewa A, Bolewa B, Hausawa Asbiti) and B while percentage mortality in Site C (Stadium, Yindiski and Dogonini Area) (Stadium, Yindiski and Dogonini Area) was high.

**Table 1:** Single bioassay post exposure of female *Anopheles* mosquitoes to pyrethroids

Sites	Pyrethroids						
	Permethrin		Deltamethrin		alphacypermethrin		Discriminating Concentration
A	%Mortality $\pm$ SD	Status	%Mortality $\pm$ SD	Status	%Mortality $\pm$ SD	Status	
B	90.0(100) $\pm$ 2.00 <sup>a</sup>	PR	66.3 (100) $\pm$ 1.53 <sup>a</sup>	R	66.3(100) $\pm$ 2.52 <sup>b</sup>	R	$\times 1$
C	83.7 (100) $\pm$ 2.52 <sup>b</sup>	R	65.0(100) $\pm$ 1.00 <sup>a</sup>	R	84.0(100) $\pm$ 1.00 <sup>a</sup>	R	$\times 1$
	75.0 (100) $\pm$ 1.00 <sup>c</sup>	R	70.7 (100) $\pm$ 1.53 <sup>a</sup>	R	73.0(100) $\pm$ 3.21 <sup>b</sup>	R	$\times 1$

Mortality  $\geq 90\%$  and  $< 98\%$ =Possible Resistant (PR); Mortality  $< 90\%$ =Resistant (R), SD=Standard Deviation Mean $\pm$  SD in the same column with the same letters were not significantly different ( $p < 0.05$ ) using Duncan's multiple range test and Vice versa. A = Bolewa A, Bolewa B and Hausawa Asbiti, B = College of Administration and Management Technology, Federal College of Education and Low-cost Area and C = Stadium, Yindiski and Dogonini Area.

Table 2 shows mortality of female *Anopheles* mosquitoes collected from the sites (A, B and C) for a synergist bioassay after 24 hours post exposure to Pyrethroids (Permethrin, Deltamethrin and alphacypermethrin). It was observed that Permethrin has high mortality in Site A (Bolewa A, Bolewa B, Hausawa Asbiti), while in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) and C shows low mortality. Deltamethrin shows low mortality in

all the sites with low mortality in sites (A, B and C) when compared to other two sister insecticides (Permethrin and alphacypermethrin). However, alphacypermethrin shows a significant mortality in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) with low mortality in Site A (Bolewa A, Bolewa B, Hausawa Asbiti) and C (Stadium, Yindiski and Dogonini Area).

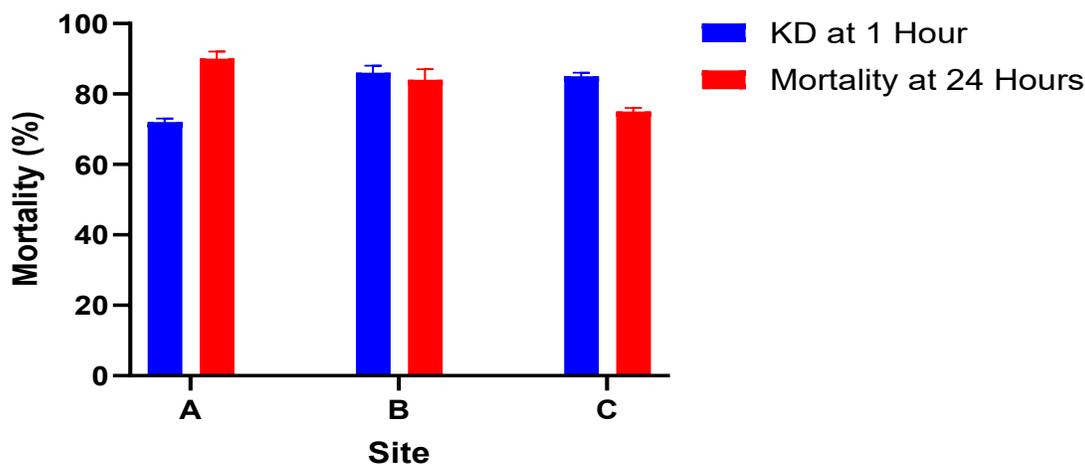
**Table 2:** Synergist bioassay post exposure of female *Anopheles* mosquitoes to pyrethroids

Pyrethroids							
	Permethrin		Deltamethrin		alphacypermethrin		
Sites	%Mortality±SD	Status	%Mortality±SD	Status	%Mortality±SD	Status	Discriminating Concentration
A	99.7(100)±0.58a	S	88.0(100)±2.00 <sup>a</sup>	R	98.0(100)±2.52 <sup>b</sup>	S	×1
B	99.3.(100)±1.15a	S	84.0(100)±1.00 <sup>b</sup>	R	99.3(100)±1.00 <sup>a</sup>	S	×1
C	99.3(100)±0.58a	S	89.0(100)±2.00 <sup>a</sup>	R	99.0(100)±3.21 <sup>b</sup>	S	×1

Mortality 98%≥ indicates Susceptible; ≥90 and <98%=Possible Resistant; <90=Resistant, SD=Standard Deviation. Mean ± SD in the same column with the same letters were not significantly different (p<0.05) using Duncan’s multiple range test and Vice versa. A = Bolewa A, Bolewa B and Hausawa Asbiti, B = College of Administration and Management Technology, Federal College of Education and Low-cost Area and C = Stadium, Yindiski and Dogonini Area.

Figure 1 illustrates the knockdown time (KDT) at 60 minutes and mortality at 24 hours post-exposure of Permethrin in female *Anopheles* mosquitoes from Sites A, B and C. It was observed that the female *Anopheles* mosquitoes show high mortality in Site A (Bolewa A, Bolewa B, Hausawa Asbiti) to Permethrin after 24hours in Site A (Bolewa A, Bolewa B, Hausawa Asbiti). However, there was decrease in the mortality to Permethrin in Site B (College of Administration and Management Technology, Federal College of Education and

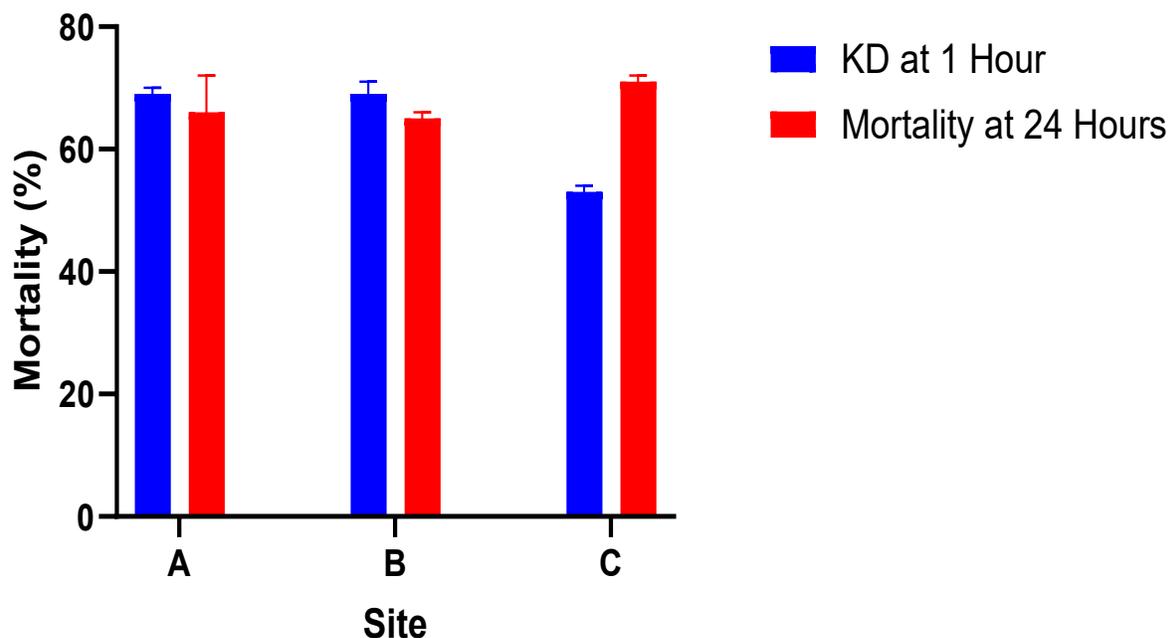
Low-cost Area) and C after 24 hours of holding. The knockdown at 60 minutes tends to be very high by female *Anopheles* mosquitoes in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) and C. Nevertheless, there was a significant decrease in the knockdown effect for Site A (Bolewa A, Bolewa B, Hausawa Asbiti) after one hour of exposure to Permethrin.



**Figure 4.1:** Single Bioassay knockdown and % mortality at one and 24hours post exposure to Permethrin. A = Bolewa A, Bolewa B and Hausawa Asbiti, B = College of Administration and Management Technology, Federal College of Education and Low-cost Area and C = Stadium, Yindiski and Dogonini Area.

Figure 2 illustrates the knockdown time (KDT) at 60 minutes and mortality at 24 hours post-exposure of Deltamethrin in female *Anopheles* mosquitoes from Sites A, B and C. It was observed that there was a high knockdown at 60 minutes by *Anopheles* mosquitoes in Site A (Bolewa A, Bolewa B, Hausawa Asbiti) and B after exposure (hpe). In Site C (Stadium, Yindiski and Dogonini Area) (Stadium, Yindiski and Dogonini Area) at 60 minutes of exposure

(hpe), the numbers of knockdown mosquitoes were found to be very low as compared to Site A (Bolewa A, Bolewa B, Hausawa Asbiti) and B. The mortality of female *Anopheles* mosquitoes was observed to be high in Site C (Stadium, Yindiski and Dogonini Area) (Stadium, Yindiski and Dogonini Area) after 24 hours of holding (hpe). In similar vein, there was a low mortality in Site A (Bolewa A, Bolewa B, Hausawa Asbiti) and B after 24 hours of post exposure.



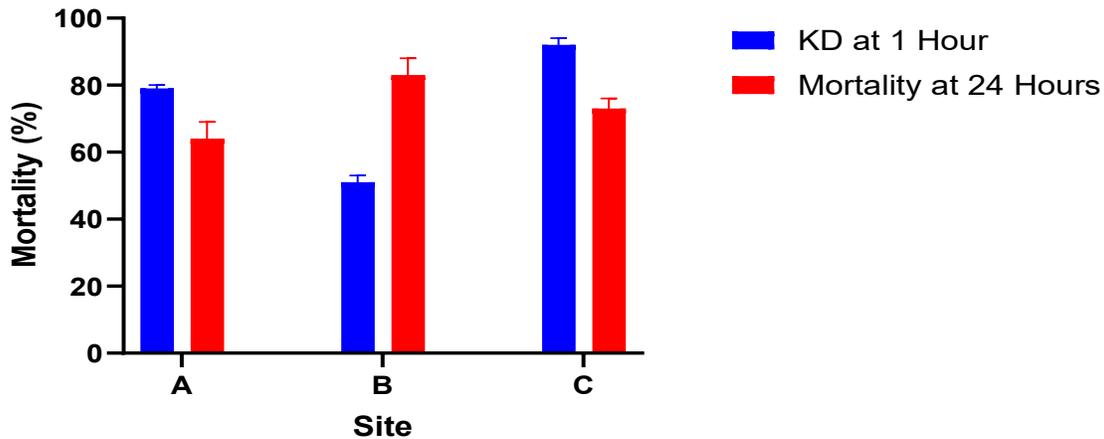
**Figure 4.2:** Single Bioassay knockdown and % mortality at one and 24 hours post exposure to Deltamethrin. A = Bolewa A, Bolewa B and Hausawa Asbiti, B = College of Administration and Management Technology, Federal College of Education and Low-cost Area and C = Stadium, Yindiski and Dogonini Area.

Figure 3 illustrates the knockdown time (KDT) at 60 minutes and mortality at 24 hours post-exposure of alphacypermethrin in female *Anopheles* mosquitoes from Sites A, B and C. It was observed to that the knockdown effect of *Anopheles* mosquitoes to alphacypermethrin was observed be high in Site C (Stadium, Yindiski and Dogonini Area) (Stadium, Yindiski and Dogonini Area) at 60 minutes. In Site A (Bolewa A, Bolewa B, Hausawa Asbiti) and B, the knock down effect after one hour of exposure (hpe) to alphacypermethrin, was a significant decrease.

Although, the decrease in mortality was low in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) than in Site A (Bolewa A, Bolewa B, Hausawa Asbiti). The Mortality was high in Site C (Stadium, Yindiski and Dogonini Area) (Stadium, Yindiski and Dogonini Area) while a significant decrease in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) after 24 hours of post exposure was observed. The mortality in

Site A (Bolewa A, Bolewa B, Hausawa Asbiti) was observed to be the lowest as compared to all

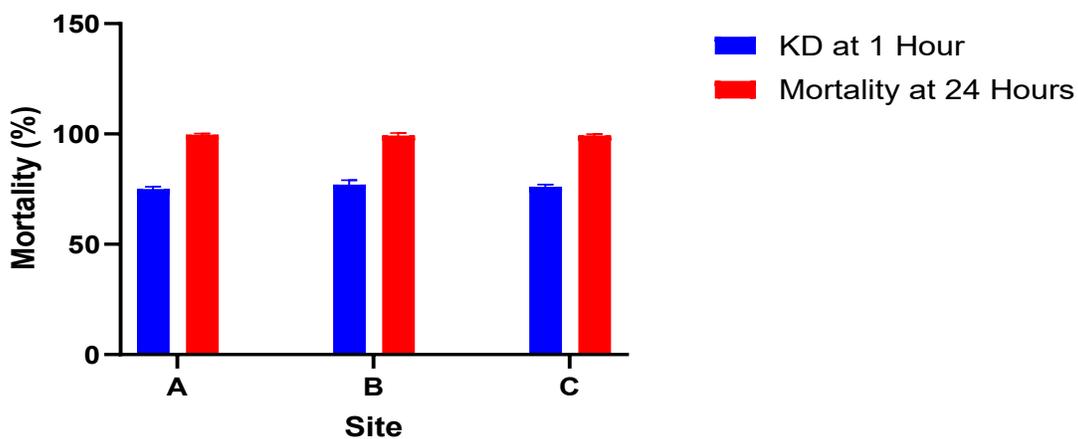
the sites after 24 hours of post exposure.



**Figure 4.3:** Single Bioassay knockdown and % mortality at one and 24hours post exposure to Alphacypermethrin. A = Bolewa A, Bolewa B and Hausawa Asbiti, B = College of Administration and Management Technology, Federal College of Education and Low-cost Area and C = Stadium, Yindiski and Dogonini Area.

Figure 4 illustrates the knockdown time (KDT) at 60 minutes and mortality at 24 hours post-exposure to a combination with Piperonyl Butoxide (PBO) and Permethrin in female *Anopheles* mosquitoes from Sites A, B and C. It was observed that there was high mortality in sites (A and B) after 24 hours, and Site C (Stadium, Yindiski and Dogonini Area) showed

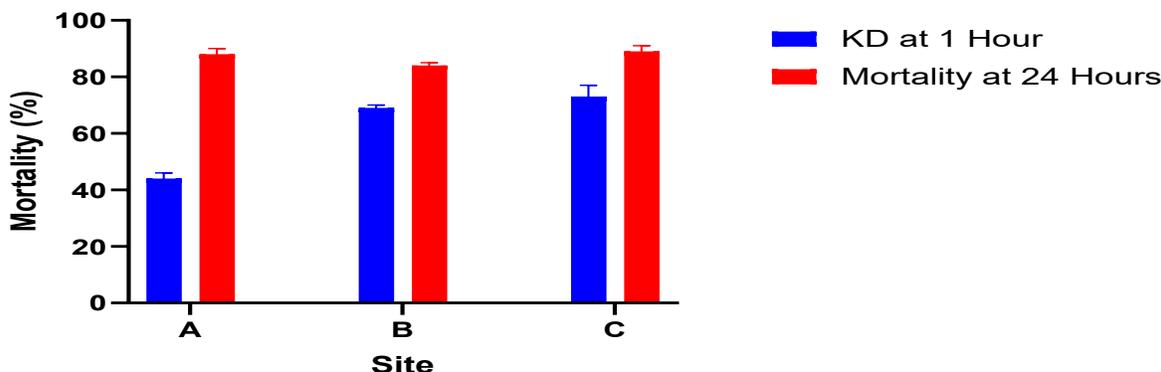
low mortality. The knockdowns of female *Anopheles* mosquitoes at 60 minutes were observed to be high in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area). In Site A (Bolewa A, Bolewa B, Hausawa Asbiti) and C, there was low knockdown of mosquitoes to the aforementioned insecticide when compared to side B.



**Figure 4.4:** Synergist bioassay knockdown and % mortality at one and 24hours post exposure to PBO+ Permethrin. A = Bolewa A, Bolewa B and Hausawa Asbiti, B = College of Administration and Management Technology, Federal College of Education and Low-cost Area and C = Stadium, Yindiski and Dogonini Area.

Figure 5 illustrates the knockdown time (KDT) at 60 minutes and mortality at 24 hours post-exposure to Deltamethrin in combination with Piperonyl Butoxide (PBO) and Deltamethrin in female *Anopheles* mosquitoes from Sites A, B and C. Knockdown of female *Anopheles* mosquitoes were high in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost

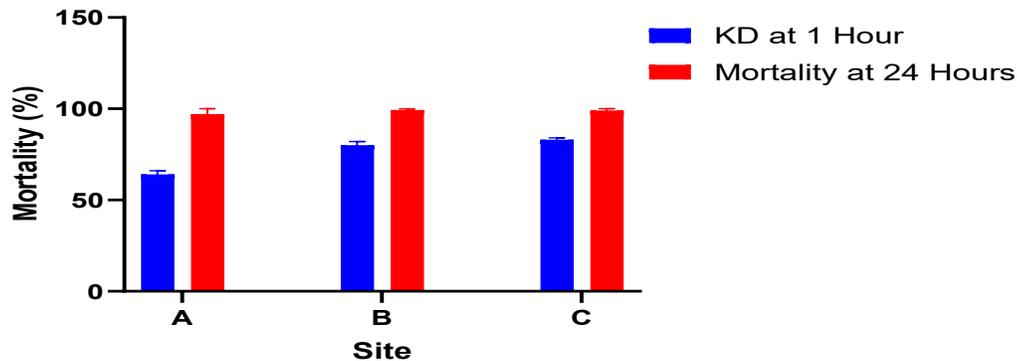
Area) and C while KD in Site A (Bolewa A, Bolewa B, Hausawa Asbiti). However, % mortality was found to be high in Site A (Bolewa A, Bolewa B, Hausawa Asbiti) and C after the synergist bioassay and low mortality was observed in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) after the synergist bioassay.



**Figure 4.5:** Synergist bioassay knockdown and % mortality at 1 and 24hours post exposure to PBO + Deltamethrin. A = Bolewa A, Bolewa B and Hausawa Asbiti, B = College of Administration and Management Technology, Federal College of Education and Low-cost Area and C = Stadium, Yindiski and Dogonini Area.

Figure 5 illustrates the knockdown time (KDT) at 60 minutes and mortality at 24 hours post-exposure to Deltamethrin in combination with Piperonyl Butoxide (PBO) and alphacypermethrin in female *Anopheles* mosquitoes from Sites A, B and C. It was observed that the knockdown of *Anopheles* mosquitoes was observed to be high in Site A (Bolewa A, Bolewa B, Hausawa Asbiti) after 60minutes of exposure (hpe) to PBO+ alphacypermethrin. Although, in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) the knockdown by the insecticide to

*Anopheles* mosquitoes have decreased in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) after 60 minutes. The lowest number of knockdowns by female *Anopheles* mosquitoes was found to be the lowest in Site A (Bolewa A, Bolewa B, Hausawa Asbiti). The observed mortality was high in Site B (College of Administration and Management Technology, Federal College of Education and Low-cost Area) and C after synergist bioassay at 24hours (hpe). Conversely, there is decrease in the mortality in Site A (Bolewa A, Bolewa B, Hausawa Asbiti) after the synergist bioassay.



**Figure 4.6:** Synergist bioassay knockdown and % mortality at one and 24hours post exposure to PBO + Alphacypermethrin. A = Bolewa A, Bolewa B and Hausawa Asbiti, B = College of Administration and Management Technology, Federal College of Education and Low-cost Area and C = Stadium, Yindiski and Dogonini Area.

## DISCUSSION

This study was conducted to assess the status of field population of *Anopheles* mosquitoes in Potiskum town of Yobe State, Nigeria. Elsewhere in West Africa, evidence of insecticide resistance in malaria vectors has been reported by several authors (Hien *et al.*, 2017; Chabi *et al.*, 2018). This study has revealed that *Anopheles gambiae* are resistant to base line pyrethroids insecticides (Permethrin, Deltamethrin and alphacypermethrin) across all the sites.

The results of this study reveal resistance to Permethrin across Sites A, B, and C in *Anopheles* mosquitoes' populations. The percentage mortalities observed 90% in Site A, 83% in Site B, and 75% in Site C. This reflect a concerning resistance pattern, with two of the sites (B and C) falling below the WHO defined resistance threshold of 90% mortality and therefore resistance across all the sites. In Site A, where the mortality was 90%, the population reveal "possible resistance" as seen in Table 1 and Figure 1 according to WHO criteria. This indicates that while a proportion of the mosquito population is still susceptible to Permethrin, there is a growing presence of individuals with resistance. Site B, with a mortality of 83%, shows a more resistance when compared to site A as seen in Table 1 and Figure 1 The low mortality suggests that a significant proportion of the mosquito population has developed

mechanisms to survive exposure to Permethrin. This level of resistance is likely the result of prolonged and possibly indiscriminate use of pyrethroid-based interventions, both in public health and agriculture. The widespread use of Permethrin-treated bed nets (ITNs) and indoor residual spraying (IRS) may contribute to the selection pressure, particularly if these control measures are not used correctly. Site C presents the most alarming case, with a mortality of only 75%. This reflects a high level of resistance, indicating that the majority of the mosquito population can survive exposure to Permethrin as also seen in Table 1 and Figure 4 Such compromises the effectiveness of pyrethroid-based vector control measures, potentially leading to increased mosquito longevity and a greater risk of malaria transmission. After one-hour post-exposure to Permethrin, the number of mosquitoes knocked down at sites A, B, and C were 75, 77, and 76, respectively as seen in Figure 1. These values suggest a similar level of susceptibility to Permethrin across all three sites. The slight variations in knockdown numbers could be attributed to differences in environmental factors, mosquito population densities, or levels of previous exposure to Permethrin or other pyrethroids at each site. These findings are similar to that of Sani *et al.*, 2023; Ndiaye *et al.*, 2021; Balde *et al.*, 2020 and Diop *et al.*, 2018 and as well against the findings

of Hassane *et al.*, 2022; Tegbaru *et al.*, 2019 and Diouf *et al.*, 2020.

The mean observed mortality of *Anopheles* populations exposed to Deltamethrin ( $\times 1$  discriminating concentrations) baseline insecticide after 24 hours were 66%, 65%, and 70.5% for sites A, B, and C, respectively. These results indicate that the insecticide was less effective in inducing mortality among the *Anopheles* mosquito populations across all sites, as the values are well below the WHO benchmark of  $\geq 98\%$  mortality, which confirms full susceptibility. The mortality suggests that *Anopheles gambiae* populations in these sites are not adequately controlled by this insecticide. Site A and Site B, with mortality of 66% and 65%, respectively as seen in Table 1 and Figure 2, show similar levels of reduced effectiveness. Site C, with a slightly higher mortality of 70.5%, indicates better results but still does not meet the standard for susceptibility. This finding supports the findings of Riagaud *et al.*, 2017; Aikpon *et al.*, 2013 and Hassane *et al.*, 2022. And as well against the findings of Senghor *et al.*, 2017; Tegbaru *et al.*, 2019 and Diouf *et al.*, 2020.

The knockdowns for *Anopheles gambiae* exposed to Deltamethrin after 1 hour were 44%, 68%, and 73% sites (A, B and C). These varying rates suggest differences in mosquito susceptibility to Deltamethrin across the sites. The lower knockdown rate of 44% indicates a relatively weaker response to the insecticide, possibly reflecting some degree of resistance as seen in Figure 2. The rates of 66% and 73% show increasing effectiveness to Deltamethrin. The results of these findings are similar to that of Boye *et al.*, 2021; Rigaud *et al.*, 2017; Aikpon *et al.*, 2013 and Hassane *et al.*, 2022 and go against the findings of Senghor *et al.*, 2017; Tegbaru *et al.*, 2019 and Diouf *et al.*, 2020.

The observed mortality of *Anopheles* mosquitoes to alphacypermethrin after 24 hours were 66%, 84%, and 73% for sites A, B, and C, respectively, all indicating resistance below the WHO susceptibility threshold of  $\geq 98\%$  and therefore resistant. Site A, with a mortality of 66%, reflects high resistance, demonstrating the least susceptibility to the insecticide as seen in Table 4.1 and Figure 3. Site C, with a mortality of 73%, exhibits moderate resistance, suggesting slightly better control than site A and Site B,

with the highest mortality of 84%, indicates low resistance, as mosquitoes in this area show a stronger response to alphacypermethrin as also seen in Table 4.1 and figure 4.3. Similarly, the knockdowns after 1 hour were 65%, 80%, and 82% for sites A, B, and C, respectively. Site A (65%) shows low knockdown efficiency, aligning with its higher resistance as seen in Figure 4.3. Site B (80%) shows moderate knockdown, and Site C (82%) exhibits relatively higher knockdown as seen in Figure 4.3. The result of these findings is similar to that of Augustin *et al.*, 2019; Amadou *et al.*, 2020 and Belaynesh *et al.*, 2018 and subsequently deviating from the work of Hassane *et al.*, 2022; Moussa *et al.*, 2021; and Lamine *et al.*, 2020.

The susceptibility of *Anopheles* population to Permethrin, Deltamethrin, and alphacypermethrin was assessed following pre-exposure to 4% piperonyl butoxide (PBO) for 1 hour, followed by exposure to the insecticides for another hour. The mortality observed after 24 hours provide insights into the effectiveness of PBO in enhancing the efficacy of these insecticides. The results are discussed as follows:

Mortality for Permethrin were 99.7%, 99.3%, and 99.3% in sites A, B, and C, respectively as against Chouaibou *et al.*, Cameroon; Edi *et al.*, 2014 and Yawson *et al.*, 2020.. All values fall within the "susceptible" category (greater than 98%), indicating that Permethrin remains highly effective against *Anopheles gambiae* in all three sites. The pre-exposure to PBO likely played a role in mitigating any potential metabolic resistance by inhibiting cytochrome P450 enzymes, which are commonly associated with pyrethroid resistance this was similar to the findings of Olusegun *et al.*, 2020; Okia *et al.*, 2018 and Norris *et al.*, 2015.

The mortality for Deltamethrin were 88%, 84%, and 89% in sites A, B, and C, respectively. These values fall into the "resistance" category (less than 90%), even after pre-exposure to PBO. The persistence of resistance indicates that metabolic mechanisms may not be the sole factor driving Deltamethrin resistance. Other resistance mechanisms, such as target site mutations (e.g., kdr mutations), could be contributing to the reduced efficacy. This finding was similar to that of Reddy, 2019 and

Boussougou *et al.*, 2021 and at the same time deviated with the findings of Boudhraa *et al.*, 2019 and Wondji, *et al.*, 2018).

Alphacypermethrin, mortality were 97%, 99.3%, and 99.0% in sites A, B, and C, respectively as against the findings of Hemingway *et al.*, 2016 and Corbel *et al.*, 2007. Sites B and C fall into the "susceptible" category (greater than 98%), suggesting that alphacypermethrin remains effective in these locations after PBO pre-exposure. However, the 97% mortality observed in site A falls within the "possible resistance" category (90–97%), indicating a potential early-stage resistance. Pre-exposure to PBO appears to have partially restored susceptibility in site A, implying that metabolic resistance mechanisms are involved. This is similar with work of Djouaka *et al.*, 2016; Ngufor *et al.*, 2020 and Mavridis *et al.*, 2019.

The species of *Anopheles gambiae* S.l. () were identified morphologically. They were not different with those identified in Lareunion, Djibouti, Mauritius, Eswatini, South Africa, Somalia, Cameroon, Democratic Republic of Congo, Zimbabwe, Botswana, Togo, Ethiopia, Ghana, South Sudan as reported by Coetzee, 2020.

### Conclusions

Concussively, with all tested insecticides showing mortality below the WHO susceptibility threshold  $\geq 98\%$ . Synergist improved the effectiveness of Permethrin and alphacypermethrin, while resistance to Deltamethrin persisted, indicating the involvement of both metabolic and non-metabolic mechanisms, such as kdr mutations. Regular resistance monitoring and public awareness campaigns are important in maintaining the effectiveness of malaria control interventions (the use of LLINs and ITNs) and reducing the public health impact of insecticide resistance.

### References

Adeleke, M. A., Akinsanya, B., & Durojaiye, A. (2013). The role of oxygen levels and predation in shaping mosquito larvae survival. *Journal of Environmental Biology*, *34*(4), 543-550.

Abd Farag, E. A., Bansal, D., Mardini, K., Sultan, A. A., Al-Thani, M. H., Al-

Marri, S. A., . . . Schaffner, F. (2021). Identification and characterisation of mosquitoes from different locations in Qatar in 2017–2019. *Parasite*, *28*(84).

- Adeparusi, E. O., Oduola, A. M., Kolawole, O. M., Gilles, J. R., and Boyle, D. R. (2014). Dominant malaria vector species in Nigeria: Modelling potential distribution of *Anopheles gambiae sensu lato* and its siblings with MaxEnt. *PLoS One*, *9* (1), e84337.
- Afolabi, O. J., Simon-Oke, I. A., and Osomo, B. O. (2013). Distribution, abundance and diversity of mosquitoes in Akure, Ondo State, Nigeria. *Journal of Parasitology and Vector Biology*, *5* (10), 132–136.
- Ahmad, A., Gao, Y., Fang, J., and Guo, D. (2020). Alphacypermethrin resistance and its associated mechanisms in *Culex pipiens pallens* from Jiangsu Province, China. *Pesticide Biochemistry and Physiology*, *168*, 114-121.
- Awolola, T. S., Ibrahim, K., Okorie, T., Koekemoer, L. L., and Coetzee, M. (2019). Species composition and biting activities of *Anopheles* mosquitoes in selected rural and urban communities in Southwestern Nigeria. *Journal*, *56*, 190-197.
- Aïkpon, R., Djouaka, R., Akogbeto, M., and Dabiré, K. R. (2013). Bendiocarb resistance in *Anopheles gambiae* s.l. populations from Benin. *Parasites and Vectors*, *6*, 192.
- Alout H, Dabiré RK, Djogbénou LS, Abate L, Corbel V, Chandre F, (2016). Interactive cost of *Plasmodium* infection and insecticide resistance in the malaria vector *Anopheles gambiae*. *Sci Rep.*; *6*:29755.
- Augustin, J., Trinh, Q. T., Pham, T. K., Tran, N. D., Nguyen, T. A., & Nguyen, H. L. (2019). Resistance to Alphacypermethrin in malaria vectors. *Malaria Journal*, *18*(1), Article 42.
- Amadou Diouf, Ousmane Diedhiou, and Birame B. Sarr. (2020). "Insecticide resistance in *Anopheles gambiae* from Mali. *Journal of Medical Entomology*, *57*(2), 457-463.

- Balde, A. M., Camara, M., and Diallo, A. (2020). Resistance of *Anopheles gambiae* to pyrethroids in Guinea: Implications for malaria vector control. *Journal of Vector Borne Diseases*, *57* (3), 345–352. DOI: 10.4103/jvbd.JVBD\_37\_20
- Bhatt S, Weiss D, Cameron E, Bisanzio D, Mappin B, Dalrymple U. (2015) The effect of malaria control on *Plasmodium falciparum* in Africa. *Nature*. 2015; 526:207.
- Beier, J. C., Barrera, R., and Bosio, C. (2009). Turbidity effects on the survival of *Anopheles* larvae. *Vector-Borne and Zoonotic Diseases*, *9*(5), 639-646.
- Benelli, G., & Mehlhorn, H. (2016). Declining malaria, rising of dengue and Zika virus: Insights for mosquito vector control. *Parasitology Research*, *115* (5), 1747–1754.
- Brook, B. W., Sodhi, N. S., & Bradshaw, C. J. A. (2007). Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, *23*(8), 453–460.
- Blaustein, L., and Kiflawi, M. (2001). Effect of dissolved oxygen concentration on the survival of *Anopheles* larvae. *Journal of Vector Ecology*, *26* (1), 87-95.
- Belaynesh Tegbaru, Mulugeta Tsegaye, and Abiy T. Y. (2018). "Monitoring resistance to insecticides in *Anopheles gambiae* in Ethiopia." *Parasites and Vectors*, *11* (1), 85.
- Boye, A., Ba, M., Ndiaye, M., Ndir, O., Faye, O., and Diagne, M. (2021). Insecticide resistance in *Anopheles gambiae* populations from Senegal. *Pest Management Science*, *78* (7), 1552-1561.
- Boudhraa, K., Elhaj, S., and Trabelsi, M. (2019). "Insecticide Susceptibility in *Anopheles gambiae* from Tunisia. " *Malaria Journal*, *18* (2): 107–115. DOI: 10.1186/s12936-019-2662-7
- Boussougou-Sambé, K., Boussougou, S. M., Mmbando, A. S., Nguertom, A., and Kenga, S. (2021). "Assessment of Resistance Intensity to Pyrethroids in *Anopheles gambiae* Using WHO Standard Diagnostic Concentrations and PBO Synergist. " *Malaria Journal*, *20*(1): 12–25. DOI: 10.1186/s12936-021-03624-7
- Beck, M. W., Onstad, D. D., Clark, G. M., and Myer, F. D. (2000). Larvicidal effects of common botanical control agents on the mosquito *Aedes aegypti*. *Journal of the American Mosquito Control Association*, *16* (2), 165-169.
- Bentley, M. D., and Mendes, A. M. (2005). The physiology of mosquito mouthparts. *Cellular and Molecular Life Sciences*, *62* (1), 200-212.
- Bock, R. R., and Rowland, M. (2001). The morphology and physiology of the antennae of the mosquito, *Anopheles gambiae*. *Journal of Insect Physiology*, *47*(10), 1043-1054.
- Bøe, M. E., and McLaughlin, A. (2016). Mode of action of pyrethroids and DDT. *Insecticides Advances in Integrated Pest Management*, *1*. Vol. 23 (4), 155-156
- Brogdon, W. G., and Chan, A. (2010). *Guideline for evaluating insecticide resistance in mosquitoes*. World Health Organization. 4, 125-127
- Campbell, M. M., and Lainhart, W. (2008). Development and validation of a new spatial risk model for malaria transmission in the highlands of western Kenya. *American Journal of Tropical Medicine and Hygiene*, *78* (2), 229-239.
- Centers for Disease Control and Prevention (CDC). (2024). About Permethrin-treated clothing and gear. In CDC.
- Centers for Disease Control and Prevention (CDC). (2021). *Anopheles mosquitoes*. Retrieved from <https://www.cdc.gov>
- Center for Disease Control and Prevention (CDC). (2013) National center for Emerging and Zoonotic Infectious Diseases (NCEZID). (2013). *Strategic Plan 2013-2018*.
- Charlwood, D., Billingsley, P. F., and Bundy, D. A. P. (2017). Evolutionary responses of mosquitoes to malaria parasites. *Parasitology*, *144* (1), 16-30.
- Coetzee, M. (2020). Key to the females of Afrotropical *Anopheles* Mosquitoes

- (Diptera: Culicidae). *Malaria Journal*, *19*, 70.
- Chabi J, Eziefule MC, Pwalia R, Joannides J, Obuobi D, Amlalo G, *et al.*, (2018) Impact of urban agriculture on the species distribution and insecticide resistance profile of *Anopheles gambiae*s. and *Anopheles coluzzii* in Accra metropolis, Ghana. *Adv Entomol.* 2018; 6:198–211.
- Chouaibou, Moussa, Fodjo, Blaise K., and Yapou, Patrice (2021). Persistent resistance in *An. gambiae* despite PBO exposure in Cameroon. *Parasites & Vectors*, *Volume 14*, Article 78, 78–85.
- Corbel, Vincent, N'Guessan, Raphael, and Martin, Thomas (2020). Impact of metabolic resistance on PBO-Alphacypermethrin synergist in Burkina Faso. *Insect Science and its Application*, *Volume 40*, 1, 105–117.
- Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, Akogbeto M, Hougard JM, (2007) Rowland M: Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Trop*, 101:207-216.
- David, J. P., Ismail, H. M., Chandor-Proust, A., and Paine, M. J. (2013). Role of cytochrome P450s in insecticide resistance: Impact on the control of mosquito-borne diseases and use of insecticides on Earth. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1612), 20120429.
- Dawn, H. G., Kirk, A. S., Carl, O., and Paul, B. (2001). Cooperative Extension. College of Agriculture and Life Sciences, *the University of Arizona*, *26* (2), 307–367.
- De Jong, R., and Knols, B. G. J. (1995). The proboscis of the malaria mosquito *Anopheles gambiae*: a review of its structure, function, and behavior. *Journal of Insect Physiology*, *41* (10), 937-948.
- Dennett, C. (2021). Mosquito behavior and the impact on disease transmission. *Trends in Parasitology*, *32* (9), 669-679.
- Djouaka, Rodrigue, Aholoukpè, Henri, and Agbéké, Onanina (2016). Restoring Alphacypermethrin efficacy with PBO in *Anopheles gambiae* populations in Benin. *Journal of Vector Borne Diseases*, *Volume 53*, 4, 272–278.
- Diop, A., Ouédraogo, S., and Sawadogo, N. (2018). High resistance to Permethrin in *Anopheles gambiae* populations in Burkina Faso. *Acta Tropica*, *184*, 13-19. DOI: 10.1016/j.actatropica.2018.04.011.
- Diouf, A., Kone, A., Traore, S., Keita, A., and Coulibaly, Y. (2020). Monitoring of Permethrin resistance in *Anopheles gambiae* from Mali. *Journal of Vector Borne Diseases*, *57*(1), 42-50. <https://doi.org/10.4103/0972-9062.295027>
- Edi, C. V., Koudou, G. B., Jones, C. M., Weetman, D., and Ranson, H. (2012). Multiple-insecticide resistance in *Anopheles gambiae* mosquitoes, Southern Cote d'Ivoire. *Emerging Infectious Diseases*, *18*, 1508–1511.
- Edi, Constant V., N'Guessan, Raphael, and Asidi, Anicet (2014). Resistance mechanisms in malaria vectors in Côte d'Ivoire. *Malaria Journal*, *Volume 13*, 438, 438–445.
- Ernest A.A. (2013): Effect of pit latrines on dug-well water quality - a case study of the asankrangwa community in the wassaamenfi west district of Ghana. Department of Environmental science; College of Science. Kwame Nkrumah University of Science and Technology. Vol. 23 (4), 15-17
- Eaton, A. D., Clesceri, L. S., Rice, E. W., & Greenberg, A. E. (2005). *Standard Methods for the Examination of Water and Wastewater* (21st ed.). Washington, DC: *American Public Health Association*. ISBN: 978-0875530475
- Edi, C. V., N'Guessan, R., Beugré, R. K., Chouaibou, M., Jenkins, A. M., Pigeault, R., ... & Koudou, B. G. (2014). Restoration of pyrethroid susceptibility in resistant *Anopheles gambiae* populations by synergists. *Parasites & Vectors*, *7*(1), Article 556.

- Federal Ministry of Health, Nigeria. (2021). *National Malaria Strategic Plan 2021-2025*.
- Foley, A. H., Danahar, A. J., Bhatt, S., Amato, T. S., and Shaman, J. (2021). Potential distribution of the primary malaria vector *Anopheles gambiae* Giles [Diptera: Culicidae] in Southwest Nigeria under current and future climatic conditions. *Basic and Applied Zoology*, **10**(1), 43-52.
- Foster, W. A., and Walker, E. D. (2002). Mosquitoes (Culicidae). In R. W. Merritt and K. W. Cummins (Eds.), *An introduction to the aquatic insects of North America* (pp. 351-373).
- Gad, S. C. (2007). *Animal Models in Toxicology* (2nd ed., pp. 123-456). CRC Press.
- Gaugler, R. (2016). *Medical and Veterinary Entomology*. 3rd Edition. Academic Press. ISBN: 9780128102345.
- Gopalakrishnan, R., and Kamala, S. (2006). Effects of pH and salinity on mosquito larvae survival. *Journal of Vector Biology*, **21**(3), 132-138.
- Goutam Chandra, A. G., Anupam Ghosh, I. B., Indrani Bhattacharjee, and S. K. Ghosh. (2013). Use of larvivorous fish in biological and environmental control of disease vectors. In *Biological and Environmental Control of Disease Vectors* (pp. 25-41).
- Gubler, D. J., W. Takken and B.G.J. Knol. (2010). "The Global Threat of Emergent/Re-emergent Vector-borne Diseases." In *Vector Biology, Ecology and Control*, s, Springer, Dordrecht, pp. 39-62. ISBN: 9789048190884.
- Harbach, R. E. (2004). The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bulletin of the British Museum (Natural History) Entomology*, **52**(1), 1-173.
- Hay, S. I., Sinka, M. E., Okara, R. M., Kabaria, C. W., Mbithi, P. M., Tago, C. C., and Snow, R. W. (2010). Developing global maps of the dominant *Anopheles* vectors of human malaria. *PLoS Medicine*, **7**(2), e1000209.
- Hemingway, J., and Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology*, **45** (1), 371-391.
- Hassane Mohamed, A. M. M., A. C. Cissé, and B. A. B. Soro. (2022). "Evaluation of alphacypermethrin + PBO mixture long-lasting insecticidal net VEERALIN® LN against pyrethroid resistant *Anopheles gambiae*." *Parasites and Vectors*", **15**(1), 109.
- Hassane, M., Diallo, A., Sanogo, S., Traore, D., and Diop, A. (2022). Permethrin resistance in *Anopheles gambiae* populations. *Pest Management Science*, **78**(4), 1162-1168.
- Hemingway, Janet, Corbel, Vincent, and Ranson, Hilary (2016). Challenges in overcoming resistance in *Anopheles gambiae* in Mozambique. *Annual Review of Entomology, Volume 61*, 239-257.
- Harte, J., Holdren, J. P., Schneider, S. H., & Shirley, C. (1991). *Toxics A to Z: A guide to everyday pollution hazards*. University of California Press **4**(6), 97-06.. ISBN: 978-0520072172
- Harbach, R. E., & Kitching, R. L. (2012). Effects of water turbidity on the growth of mosquito larvae. *Parasites and Vectors*, **5**, 33. <https://doi.org/10.1186/1756-3305-5-33>
- Horsfall, W. R. (1955). Ecology of the *Anopheles gambiae* complex in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **49**(6), 497-506.
- Hien SA, Soma DD, Hema O, Bayili B, Namountougou M, Gnankine O, Evidence that agricultural use of pesticides selects pyrethroids resistance within *Anopheles gambiae* populations from cotton growing areas in Burkina Faso, West Africa. *PLoS ONE* 23-27
- Hoffmann, F., and Dulbecco, R. (2012). *Bioassays: A comprehensive guide to testing methods*. 35-41
- Huijben, S., and Paaijmans, K. P. (2018). Putting evolution in elimination: Winning our ongoing battle with evolving malaria

- mosquitoes and parasites. *Evolutionary Applications*, *11*, 415–430.
- Huang, J., Walker, E. D., and Otienoburu, P. E. (2010). The role of aquatic vegetation in structuring mosquito habitats. *Journal of Medical Entomology*, *47* (3), 329-338.
- Jing, Q., & Tan, J. (2011). The effects of environmental temperature on the development of *Anopheles* larvae. *Parasites & Vectors*, *4*, 23-30.
- Killeen, G. F., and Knols, B. G. J. (2003). Sexual selection in malaria mosquitoes. *Evolutionary Biology*, *3*, 1-22.
- Killeen, G. F., Seyoum, A., Holloway, M., Okumu, F. O., and Bradley, J. (2017). Developing an expanded vector control toolbox for malaria elimination. *Global Health*, *2*, e000211.
- Kunz, P. Y., Focke, M., and Tresch, S. (2016). Toxicity of Permethrin and Deltamethrin against larvae of *Culiseta longiareolata* (Diptera: Culicidae): Effects of larval age and temperature. *Journal of Medical Entomology*, *53*(2), 368-77.
- Lefebvre, V., Maire, O., Pinet, P., and Ogor, P. (2015). Contrasting patterns of variability and stability in the community ecology of pelagic larvae of temperate reef fishes. *Journal of Marine Systems*, *146* (1), 1-10.
- Leite, L. N., Bascuñán, P., Dotson, E. M., & Benedict, M. Q. (2024). Considerations for Rearing and Maintaining *Anopheles* in the Laboratory. *Cold Spring Harbor Protoc.*
- Lamine Traore, Amadou Bougouma, and Seydou Ba (2020). "Effectiveness of pyrethroid-treated nets against *Anopheles gambiae* in Mali." *Journal of Vector Borne Diseases*, *57*(3), 195-201.
- Malaria Consortium. (2018). Insecticide resistance status of *Anopheles gambiae* complex in Ondo and Anambra States, Nigeria. Retrieved from Malaria Consortium.
- Mavridis, Konstantinos, Wondji, Charles S., and Vontas, John (2019). Alphacypermethrin restoration post-PBO in Tanzanian vectors. *PLOS ONE*, *Volume 14*, *5*, e0217803.
- Manguin, S., Carnevale, P., Robert, V., & Delorme, S. (2010). *Anopheles* mosquitoes and malaria transmission in the tropical world. Vol. 43 (4), 133-135
- Minakawa, M., Sonye, G., Munga, S., Barrera, R., & Beier, J. C. (2008). The effects of environmental factors on the distribution of *Anopheles* mosquitoes and their larvae in East Africa. *Journal of Vector Ecology*, *33* (1), 65-73.
- Moussa Ndiaye, Aboulaye Diop, and Oumar Faye. (2021). "Monitoring insecticide resistance in *Anopheles gambiae* in Senegal." *Malaria Journal*, *20* (1), 278.
- Molavi, A. (2003). Africa's malaria death toll still outrageously high. *National Geographic News*, *12*. 23-32.
- Martinez-Torres, D., Chandre, F., Williamson, M. S., Darriet, F., Bergé, J. B., Devonshire, A. L., and Pauron, D. (1998). Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae*s. *Insect Molecular Biology*, *7* (2), 179-184.
- Mamuya, N. S., Unamba, C. P. C., and Ndung'u, M. W. (2018). Efficacy of Alphacypermethrin and Deltamethrin for impregnating mosquito nets against *Anopheles gambiae* and *Anopheles arabiensis* in western Kenya. *Parasites & Vectors*, *11* (1), 425.
- Merritt, R. W., Cummins, K. W., and Berg, M. B. (2008). *An introduction to the aquatic insects of North America* (Vol. 4). Kendall/Hunt Publishing Company. 77-79
- Muturi, E. J., Lampman, R., & Muriu, S. (2012). Effects of water turbidity and temperature on the survival and development of *Anopheles* larvae. *Journal of Vector Ecology*, *37*(1), 12-21.
- Müller, P., Warr, E., Stevenson, B. J., Pignatelli, P. M., Morgan, J. C., Steven, A., and Hemingway, J. (2008). Field-caught Permethrin-resistant *Anopheles gambiae* overexpress CYP6P3, a P450 that metabolizes pyrethroids. *PLoS Genetics*, *4* (11), e1000286.

- Mwangungulu, S. P., Sumaye, R. D., Limwagu, A. J., Siria, D. J., Kaindoa, E. W., Okumu, F. O., and Govella, N. J. (2017). Mosquito larval habitats in a rural Tanzanian village: Characteristics, dynamics and implications for control. *Malaria Journal*, *16*(1), 1-10.
- Ngufor, C., Tchicaya, E., Koudou, B., N'Guessan, R., Rowland, M., and Ranson, H. (2020). Efficacy of Fludora® Fusion (a mixture of Deltamethrin and clothianidin) for indoor residual spraying against pyrethroid-resistant malaria vectors: Laboratory and experimental hut evaluation. *Parasites & Vectors*, *13* (1), 1-11.
- Njiru, B. N., Mukabana, W. R., and Takken, W. (2006). Effects of larval competition on the reproductive potential of *Anopheles gambiae* mosquitoes. *Emerging Infectious Diseases*, *12* (8), 1230-1236.
- Nkengurutse, R., and Gassie, G. (2015). Efficacy of biopesticides and pyrethroids against housefly (*Musca domestica* L.) populations in poultry farms of Rwanda. *Journal of Entomology and Zoology Studies*, *3*(4), 1-7.
- Norris, L. C., Main, B. J., Lee, Y., Collier, T. C., Fofana, A., Cornel, A. J. ... and Lanzaro, G. C. (2017). Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. *Proceedings of the National Academy of Sciences*, *114*(41), 10773-10778.
- National Population Commission. (2006). Population and housing census of Nigeria. Abuja: NPC.
- Norris, Laura C., and Norris, Donald E. (2015). Synergistic impact of PBO with Permethrin on resistant *An. gambiae* in Southern Malawi. *Journal of Medical Entomology*, *Volume 52*, 3, 531-539.
- Ndiaye, K., Sow, D., and Ndir, O. (2021). Insecticide resistance in *Anopheles gambiae* in Senegal: A persistent threat to malaria control. *Malaria Journal*, *20*(1), 118. DOI: 10.1186/s12936-021-03843-0.
- Olusegun-Joseph, Tola S., Aigbodion, Felix I., and Fawole, Anthony M. (2020). Efficacy of piperonylbutoxide synergist on resistant mosquitoes in Lagos, Nigeria. *Animal Research International*, *Volume 17*, 3, 3821-3828.
- Okiya, Maureen, Simonsen, Peter E., and Opiyo, Elizabeth (2018). Improved efficacy of Permethrin on resistant *Anopheles gambiae* after PBO pre-exposure. *Journal of Vector Ecology*, *Volume 43*, 2, Pages 297-304.
- Ochomo, D., Bayoh, N. M., Walker, E. D., Abongo, B. O., Ombok, M., Ouma, C., ... & Vulule, J. M. (2017). Pyrethroid resistance in *Anopheles gambiae*. *Malaria Journal*, *16*(1), Article 1854.
- Oduola, A.O., Oduola, O.A., and Olayemi, I.K. (2020). High pyrethroids /DDT resistance in major malaria vector *Anopheles gambiae* populations in Nigeria. PLOS ONE, *15*(3), e0247944. <https://doi.org/10.1371/journal.pone.0247944>.
- Okechukwu, G. N., Dia, I., and Gimnig, J. E. (2023). Spatial distribution and ecological niche modeling of geographical spread of *Anopheles gambiae* complex in Nigeria using real-time data. *Scientific Reports*, *13* (1), 40929.
- Olenec, J. P., and DeJong, R. J. (2017). The mosquito proboscis: An anthropological perspective on the curve. *Parasites & Vectors*, *10* (1), 478.
- Ponlaw, L., Linton, Y. N., Mori, A., et al., (2008). Blood feeding patterns of *Anopheles gambiae* and *Anopheles arabiensis* in two villages in rural Tanzania. *Malaria Journal*, *7*(1), 12.
- Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., and Corbel, V. (2016). Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control? *Trends in Parasitology*, *27*, 91-98.
- Ranson, H., Jensen, B., Vulule, J. M., Wang, X., Hemingway, J., & Collins, F. H. (2000). Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated

- with knockdown resistance to DDT and pyrethroids. *Insect Molecular Biology*, **9**(5), 491–497.
- Romero, A., Potter, M. F., and Haynes, K. F. (2010). Evaluation of piperonylbutoxide as a resistance-breaking agent for pyrethroid-resistant bed bugs. *Journal of Economic Entomology*, **103**(1), 208–213.
- Reddy, M. R., Odhiambo, G., and Mutungi, S. (2019). "Pyrethroid Resistance in *Anopheles gambiae* Across Nigeria: Mechanisms and Effects." PLOS ONE, **14**(10).
- Rigaud, T., Elanga, M., Djeukam, R., Ait-Lahou, T., and Doudou, B. (2017). Insecticide resistance status of *Anopheles gambiae* in Chad. *Malaria Journal*, **16**(1), 131. <https://doi.org/10.1186/s12936-017-1941-7>
- Roberts, D. R., and Andre, R. G. (1994). Insecticide resistance issues in vector-borne disease control. *American Journal of Tropical Medicine and Hygiene*, **50**, 21–34.
- Sahu, S. S., Thankachy, S., Dash, S., Nallan, K., Swaminathan, S., Kasinathan, G., and Purushothaman, J. (2020). Evaluation of long-lasting indoor residual spraying of Deltamethrin 62.5 SC-PE against malaria vectors in India. *Malaria Journal*, **19** (1), 1–15.
- Salmaso, M., Schiavon, M., Perinelli, R., Bovo, S., and Barbato, G. (2016). Pyrethroids in indoor dust: Occurrence, indoor fate, human exposure assessment. *Chemosphere*, **146**, 471–86.
- Scott, T. W., and Takken, W. (2012). Feeding strategies of anthropophilic mosquitoes result in increased risk of pathogen transmission. *Trends in Parasitology*, **28**(3), 114–121.
- Singh, O. P., and Adelaja, O. J. (2019). Current status of major malaria vector mosquitoes in Nigeria: A comprehensive review. *Journal of Vector Borne Diseases*, **56**(1), 1–10.
- Soderlund, D. M. (2015). Mode of action of pyrethroids. *Annual Review of Entomology*, **60**(1), 487–505.
- Sani, M., Diabaté, A., Sanogo, D., and Faye, O. (2023). Permethrin resistance in *Anopheles gambiae* populations from southern Mali. *Parasites and Vectors*, **16**(1), 52. DOI: 10.1186/s13071-023-05547-77.
- Senghor, M., Diallo, A., Sene, F., and Faye, A. (2017). Insecticide resistance in *Anopheles gambiae* in Senegal. *Malaria Journal*, **16**(1), 271. <https://doi.org/10.1186/s12936-017-1941-7>
- Sokolove, P. G., and Tantawy, A. E. (2009). Mosquito metamorphosis: Assays for analyzing adult emergence and wing expansion. *Journal of Visualized Experiments* (28), 1222.
- Tang, L., and Wang, J. J. (2021). Synergistic effects of piperonylbutoxide on pyrethroids in mosquito control: implications for cost efficiency. *Journal of Vector Ecology*, **46**(1), 45–52.
- Takken, W., & Verhulst, N. O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology*, **58**(1), 433–453.
- Trung, N. D., Nam, V. S., Quang, N. T., Hai, N. H., Tuan, N. A., and Yen, N. T. (2009). Survival and development of *Aedes aegypti* larvae under different water qualities in southern Vietnam. *Tropical Biomedicine*, **26** (2), 221–229.
- Tegbaru, B., Alemayehu, H., Taye, M., and Desta, B. (2019). Insecticide resistance in *Anopheles gambiae* populations in Ethiopia. *Parasites and Vectors*, **12** (1), 230.
- U.S. Environmental Protection Agency (EPA). (2024). Permethrin, d-phenothrin (Sumithrin®): *Synthetic pyrethroids for mosquito control*.
- Wondji, C. S., McCall, P. J., and Tchouakui, M. (2018). "Pyrethroid Susceptibility in *Anopheles gambiae* Populations in Kenya." *BMC Infectious Diseases*, **18**(1): 92–103. DOI: 10.1186/s12879-018-3033-4
- World Health Organisation (2012) Global Plan for Insecticide Resistance Management in *Malaria Vectors*. Geneva: World Health Organization. 132.

- World Health Organization. (2023). World Malaria Report 2023. Geneva, France: World Health Organization. 1-200.
- World Health Organization. (2022). Global report on malaria. Geneva, France: World Health Organization. 1-100.
- World Health Organization. (2021). *Anopheles* mosquitoes. Retrieved from <https://www.who.int/news-room/q-a-detail/malaria-and-the-Anopheles-mosquito>
- World Health Organization. (2021). Global Technical Strategy for Malaria 2016–2030, 2021 Update. Geneva, France: World Health Organization. 1-50.
- World Health Organization. (2019). World Malaria Report 2019. Geneva, France: World Health Organization. 1-186.
- World Health Organization. (2018). Vector Control: Methods for Use by Individuals and Communities. Geneva, France: WHO. pp. 95-102.
- World Health Organization. (2018). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva, France: World Health Organization. 1-52.
- World Health Organization. (2016). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. Geneva: WHO.
- World Health Organization. (2012). *Global Plan for Insecticide Resistance Management in Malaria Vectors*. Geneva, France: World Health Organization. 1-70.
- Yawson, A. E., Essandoh, J., Oteng, E. K., & Weetman, D. (2020). Cytochrome P450-mediated resistance to permethrin in *Anopheles gambiae* populations. *Pest Management Science*, *76*(1), 172–180.