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THE PMI VECTORLINK NIGERIA PROJECT

ANNUAL ENTOMOLOGY REPORT

OCTOBER 2021–SEPTEMBER 2022

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ACRONYMS

AIRS	Africa Indoor Residual Spraying Project
CDC	(U.S.) Centers for Disease Control and Prevention
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
FCT	Federal Capital Territory
HBR	Human Biting Rate
ITN	Insecticide-treated Net
Kdr	Knock down resistance
LGA	Local Government Area
LT	Light Trap
NIMR	Nigeria Institute for Medical Research
NMEP	National Malaria Elimination Program
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	(U.S.) President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SOP	Standard Operating Procedure
SPR	Sporozoite Rate
WHO	World Health Organization

EXECUTIVE SUMMARY

Malaria vector surveillance and insecticide resistance monitoring activities provide stakeholders with evidence-based data that can inform malaria vector control decisions. The U.S. President's Malaria Initiative (PMI) VectorLink Project is currently supporting vector surveillance and insecticide resistance monitoring activities across five ecological zones in Nigeria. From October 2021 to September 2022, pyrethrum spray catches (PSCs) and human-baited U.S. Centers for Disease Control and Prevention (CDC) Light Traps (LTs) were used to collect mosquitoes both indoors and outdoors to determine species composition, behavior, seasonality, biting rates, infectivity rates, blood meal sources, and entomological inoculation rates (EIRs) of malaria vectors across sentinel sites. Additionally, VectorLink Nigeria collected information on household-level human behavior through direct human observations during routine entomological surveys in Kebbi and Sokoto. The human behavior data together with biting rates from CDC light trap collections was used to estimate exposure to mosquito bites in Kebbi and Sokoto. CDC bottle bioassays were used to determine the insecticide resistance status, intensity, and underlying resistance mechanisms.

A total of 86,799 *Anopheles* mosquitoes were collected from six sentinel sites using human-baited CDC LTs (indoors/outdoors) and PSCs. *Anopheles gambiae* s.l. was the most abundant species across most sites, ranging from 13.8% in Akwa Ibom to 100% in Kebbi. Other *Anopheles* species identified in varying abundance were *An. marshallii* complex (Akwa Ibom only), *An. funestus,* and *An. rufipes.* Other localized species observed were *An. squamosus, An. coustani, An. pretoriensis*, and *An. moucheti.*

A total of 3,004 *An. gambiae* s.l. mosquitoes collected by PSCs and CDC LTs between October 2021 and September 2022 were subjected to species-specific PCR assays. Results showed that 2,366 (78.8%) were identified as *An. coluzzii,* 603 (20.1%) were *An. gambiae* s.s., and 35 (1.2%) were *An. arabiensis.* For the first time, *An. coluzzii* was the dominant vector species found both indoors and outdoors using CDC LTs across all sentinel sites. The highest proportion of *An. coluzzii* was collected indoors in Kebbi (96.0%) followed by Sokoto (83.8%). The highest proportion of *An. coluzzii* collected outdoors was also in Kebbi (94.0%) followed by Ebonyi (91.7%). The highest proportion of *An. gambiae* s.s. were recorded outdoors in Akwa Ibom (40.0%) with the next highest found indoors in Ebonyi (27.4%). The proportion of *An. arabiensis* found indoors and outdoors was generally quite low, with the highest percentages reported from Oyo (2.9% indoors and 2.6% outdoors). There were no hybrid species recorded in any of the sites. For mosquitoes caught using PSCs, *An. coluzzii* was the dominant vector species found in all sites with the highest proportion recorded in Kebbi (95.8%) followed by Sokoto (86.1%) and Ebonyi (82.8%).

Plasmodium falciparum sporozoite infection rates of *An. gambiae* were recorded indoors in Ebonyi and Plateau only (2.2% and 1.8% respectively) for mosquitoes collected using CDC LTs. Sporozoite infection rate of *An. coluzzii* were recorded indoors (0.3%) and outdoors (0.5%) in Kebbi. No sporozoite positive *An. arabiensis* samples were found indoors or outdoors across sites. For mosquitoes collected using PSCs, the highest sporozoite infection rate was recorded in *An. arabiensis* in Ebonyi (20.0%); this was the only site where *An. arabiensis* tested positive for sporozoites. For *An. coluzzii,* the sporozoite rate was highest in Akwa Ibom (5.4%), followed by Ebonyi (0.7%) and Kebbi (0.6%). The only infection rate recorded in *An. gambiae* was in Oyo (1.1%).

A total of 772 *An. funestus* s.l. mosquitoes collected by CDC LTs and PSCs in Ebonyi, Oyo, Plateau, and Sokoto were subjected to species-specific PCR assays. *Anopheles funestus* s.s. was found to predominate across the sites both indoors and/or outdoors, ranging from 75% to 100%. Unamplified samples were found in Plateau (25.0%) and Oyo (1.1%), and all were collected outdoors. *Anopheles leesoni* was recorded only in Oyo indoors (0.6%). For members of *An. funestus* group collected using PSCs, *An. funestus* s.s. predominated in all three sites (Ebonyi, Oyo, and Plateau). *Anopheles funestus* s.s. collected using CDC LTs were positive for *P. falciparum*

sporozoites in Oyo (outdoors, 3.4%) and Plateau (indoors, 1.6%). No other members of *An. funestus* group tested positive for *Plasmodium* sporozoites. For *An. funestus* s.s. collected by PSC methods, no sporozoite positivity rates were recorded across the various sites.

The indoor resting density of *An. marshallii* complex mosquitoes varied across the months, ranging from 0-0.5 mosquitoes/room/day between October 2021 to July 2022, before peaking in August 2022 (2.7 mosquitoes/room/day). The mean indoor biting rates of *An. marshallii* complex peaked in January (21.8 bites per person per night (b/p/n)) and again in August 2022 (18.3 b/p/n). Outdoors, biting rate peaked in December 2021 (5.3 b/p/n), February (3.8 b/p/n) as well as in August 2022 (3.3 b/p/n). One sample tested positive from the PSC collection resulting in a 0.7 positivity rate for *An. marshallii* from Akwa Ibom.

The indoor resting density of *An. gambiae* s.l. mosquitoes varied across the sites and months, ranging from 0.1 mosquitoes/room/day in Oyo during January 2022 to 29.4 mosquitoes/room/day in Sokoto in August 2022, with a second highest peak in Kebbi in September 2022 with 24.7 mosquitoes/room/day. The indoor resting density of *An. marshallii* complex mosquitoes remained under 0.5 mosquitoes/room/day except for a peak of 2.7 mosquitoes/room/day in August 2022 (Figure 8). The highest mean indoor biting rates of *An. gambiae* s.l. were found in September in Plateau (85.7 b/p/n). In Kebbi, indoor biting of *An. gambiae* s.l. peaked in April, July-September in Kebbi (31.3, 25.9 -29.5 b/p/n, respectively). Outdoor biting rates of *An. gambiae* s.l. remained under the peak of 11.8 b/p/n in Kebbi in April 2022. The mean indoor biting rates of *An. marshallii* complex peaked in January 2022 (21.8 b/p/n), and outdoor biting peaked in December 2021 (5.3 b/p/n). The mean indoor biting rates of *An. funestus* were recorded in Oyo, Plateau, and Sokoto. Rates in Plateau peaked in September 2022 (6.6 b/p/n), while in Oyo, an initial peak was observed in May 2022 (4.8 b/p/n). Mean outdoor biting rates of *An. funestus* peaked in June 2022 (2.9 b/p/n) in Oyo.

Estimates from the human behavior data and biting rates indicated variabilities on time and place of exposures in Kebbi and Sokoto. Most human exposure in Kebbi occurs indoors in the middle of night (11 p.m.-6 a.m.) primarily due to people being in bed but not using nets at the time when most biting activities occurring indoors. In Sokoto, most human exposure occurs indoors during the early evening hours before bedtime (before 10 p.m.) when most people are not protected under nets.

The highest indoor EIR was recorded with *An. gambiae* in Ebonyi (12.1 infective bites/person/year) and Plateau (10.9 infective bites/person/year), and for *An. coluzzii* in Kebbi (5.8 infective bites/person/year) Outdoor EIR was recorded for *An. coluzzii* in Kebbi at 6.6 infective bites/person/year. EIRs were recorded for *An. funestus* s.s. in Oyo and Plateau. The highest EIR was recorded outdoors in Oyo (9.5 infective bites/person/year), followed by indoors EIR in Plateau (5.2 infective bites/person/year). There were no infective bites recorded among *An. funestus* s.s. indoors in Oyo and outdoors in Plateau.

Across the sites, human blood index (HBI) analysis detected increased human blood meal preference in *An. gambiae* s.s.*, An. coluzzii*, *An. arabiensis*, and *An. marshallii* collected using PSCs compared with CDC LTs.

Insecticide susceptibility test results indicated that pyrethroid resistance was widespread in *An. gambiae* s.l. mosquitoes at all sentinel sites. Full susceptibility of *An. gambiae* s.l. populations exposed to alpha-cypermethrin was recorded in all LGAs in Bayelsa, Benue, Cross River, Federal Capital Territory (FCT), Oyo, Sokoto, and Zamfara. Susceptibility to alpha-cypermethrin was only observed in 1/6 LGAs in Bauchi and Enugu, 2/6 LGAs in Kaduna, and 7/9 LGAs in Kebbi. No susceptibility to alpha-cypermethrin was observed in Akwa Ibom, Ebonyi, Nasarawa, or Plateau. *Anopheles gambiae* s.l. were found to be susceptible to deltamethrin in all LGAs in Benue, Cross River, Kebbi, Oyo, and Zamfara, and a subset of LGAs in Bauchi, Bayelsa, Enugu, FCT, Nasarawa, and Sokoto. No susceptibility to deltamethrin was observed in *An. gambiae* s.l. populations from Akwa Ibom, Ebonyi, Kaduna, and Plateau. Resistance to permethrin was recorded in *An. gambiae* s.l. populations across all states, except in select LGAs in Bayelsa, Kaduna, and Sokoto. No *An. gambiae* s.l. tested in any LGAs in Akwa Ibom, Ebonyi, and Plateau were found to be susceptible to the three pyrethroids.

Insecticide resistance intensity in the vector populations were determined by subjecting *Anopheles* mosquitoes across the different ecozones to insecticide resistance test assays with different concentrations of the three pyrethroids. *Anopheles gambiae* s.l. was susceptible to 1X alpha-cypermethrin across all LGAs in Bayelsa, Benue,

Cross River, FCT, Oyo, Sokoto, and Zamfara. Pre-exposure of *An. gambiae* s.l. mosquitoes to piperonyl butoxide (PBO) synergist before exposure to pyrethroids increased mortality to varying degrees across sites. In cases where full susceptibility (mortality greater than or equal to 98%) was not restored with PBO exposure, it suggests the existence of mechanisms unrelated to the activity of mixed function oxidases. Where tested, susceptibility to alpha-cypermethrin was fully restored in *An. gambiae* s.l. mosquitoes pre-exposed to PBO in all LGAs of Ebonyi, Enugu, Kebbi, and Nasarawa. Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO restored susceptibility to alpha-cypermethrin in the three LGAs in Akwa Ibom, 1/6 LGAs in Bauchi and FCT, and 2/6 LGAs in Kaduna. Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO did not restore susceptibility to alpha-cypermethrin in any LGAs in Plateau. Where tested, susceptibility of *An. gambiae* s.l. mosquitoes to deltamethrin were restored across all LGAs in Akwa Ibom, Bauchi, Ebonyi, FCT, Nasarawa, and Sokoto.

The percentage knockdown of *An. gambiae* s.l. exposed to chlorfenapyr at 60 minutes varied across LGAs in the various sentinel sites. Mortality rates in *An. gambiae* s.l. were between 98-100% after the 48-hour holding period in all LGAs in Bauchi, Bayelsa, Benue, Cross River, Ebonyi, FCT, Kebbi, Nasarawa, Oyo, Plateau, and Zamfara. *Anopheles gambiae* s.l. populations from all LGAs across all ecozones were susceptible to chlorfenapyr with 100% mortality at 72 hours. The percentage knockdown of *An. gambiae* s.l. mosquitoes after 30 minutes of exposure to clothianidin also varied across the sites. At 30 minutes, the percentage knockdown of *An. gambiae* s.l. mosquitoes exposed to clothianidin varied across the sites: Akwa Ibom (44-63%), Bauchi (73-83%), Bayelsa (81-92%), Benue (76-94%), Cross River (28-87%), Ebonyi (33-75%), Enugu (0-71%), FCT (3-33%), Kebbi (41-56%), Nasarawa (12-40%), Oyo (11-62%), Plateau (10-12%), Sokoto (5-41%), and Zamfara (51-77%). Mortality rates of *An. gambiae* s.l. 24 hours post-exposure were 99–100% in 11 out of 15 sites, 94-99% in Akwa Ibom, 94-100% in FCT, and 82-96% in Nasarawa. An assessment of *kdr* mutations in deltamethrin-resistant *An. gambiae* s.l. indicated the presence of both *kdr-w* and *kdr-e* point mutations. The *kdr-w* gene frequencies varied by ecozone, types of pyrethroids, and vector composition. *Kdr-w* gene frequencies in *An. coluzzii* ranged from 0.38 in Ebonyi to 0.56 in Bayelsa. For *An. gambiae* s.s., gene frequencies ranged from 0.33 in Ebonyi to 1.00 in Cross River. For *An. arabiensis,* gene frequencies ranged from 0.17 in Ebonyi to 0.50 in Bayelsa and Sokoto. The *kdr-e* gene frequencies also varied by ecological zones and ranged from 0.00 in Benue and Oyo to 0.17 in Bayelsa for *An. coluzzii*. In *An. gambiae* s.s.*,* the *kdr-e* gene frequency ranged from 0.00 in Benue, FCT, Nasarawa, Oyo, and Plateau to 0.50 in Bayelsa and Cross River. These varied in *An. arabiensis* from 0.00 in Akwa Ibom, Bayelsa, Ebonyi, and Sokoto to 0.08 in Kaduna. In general, *kdr-w* frequencies were higher in *An. gambiae* s.s. versus *An, coluzzii.* For permethrin, *kdr-w* gene frequencies in *An. coluzzii* ranged from 0.37 in Enugu and Nasarawa to 0.53 in FCT while *kdr-w* gene frequencies in *An. gambiae* s.s. ranged from 0.25 in Bayelsa to 0.55 in Cross River. *Kdr-w* gene frequencies in *An. arabiensis* ranged from 0.25 in Bauchi, Bayelsa, and Enugu to 1.00 in Sokoto.

I. INTRODUCTION

Malaria remains a major public health problem in Nigeria. According to the 2022 World Malaria Report, Nigeria accounts for 27% of malaria cases worldwide and 31% of malaria deaths—the most of any country and is one of 11 high burden to high impact (HBHI) countries. The detection of *An. stephensi*—an invasive malaria vector species which thrives in urban environments and has been found to be resistant to many insecticides—in Nigeria has further heightened concerns and warranted additional entomological monitoring.

Nigeria has five diverse geo-ecological zones, each supporting a variety of *Anopheles* species involved in malaria transmission. The major malaria vectors in the country are the members of the *An. gambiae* complex (*An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis)* and *An. funestus* s.l. Secondary malaria vectors include *An. nili, An. moucheti, An. pharoensis, An. coustani,* and *An. longipalpis* (PMI VectorLink Annual Entomology Report, 2021).

In 2012, the U.S. President's Malaria Initiative (PMI), through the Africa Indoor Residual Spraying (AIRS) Project, started entomological surveillance in Nasarawa State. In 2014, the National Malaria Elimination Program (NMEP), in collaboration with the AIRS Project, expanded to six entomological monitoring sites to support evidence-based decision-making for malaria vector control activities.

With the transition from AIRS Project to the PMI VectorLink Project in 2017, the number of entomological monitoring sites was increased to seven plus two insecticide resistance monitoring sites, bringing the total to nine states. Currently, VectorLink is supporting longitudinal vector surveillance and insecticide resistance monitoring in six states and insecticide resistance monitoring only in an additional nine states.

VectorLink strengthens the capacity of local universities to implement vector surveillance and insecticide resistance monitoring. Each sentinel site is coordinated by a well-trained Principal Investigator chosen from universities located in the PMI-focus states. Through VectorLink, each sentinel site recruits field staff comprising of technicians and mosquito collectors trained on entomological methods. VectorLink also provides basic equipment for entomological monitoring. Each VectorLink-supported sentinel site and insecticide resistance monitoring team works in conjunction with the Malaria Control Program division of the State Ministry of Health. VectorLink also partners with the Nigeria Institute for Medical Research (NIMR) to conduct laboratory analyses which are processed at the PMI-supported laboratory in Nasarawa State University Keffi.

Longitudinal vector surveillance is conducted monthly, while insecticide resistance monitoring occurs once per year. The data generated from both activities provide valuable information on vector distribution, behavior, and susceptibility to insecticides. Data generated have been used to inform insecticide-treated net (ITN) procurement decisions and can guide the choice of other vector control interventions in the future. The plan is for these sites to continue to be monitored on a regular basis to track vector susceptibility and dynamics over time.

From October 2021 to September 2022, VectorLink Nigeria conducted longitudinal vector surveillance in six sites and insecticide resistance monitoring in 15 states (11 PMI-focus states and four non-PMI focus states), assessing species composition, density, feeding time, location (indoors or outdoors), seasonality, and insecticide susceptibility status of the major malaria vectors. The intensity and mechanism of insecticide resistance across the different ecozones of Nigeria were also determined. This report summarizes entomological monitoring activities completed from October 2021 to September 2022.

1.1 VECTOR SURVEILLANCE AND INSECTICIDE RESISTANCE MONITORING SITES AND AFFILIATED INSTITUTIONS

During the period covered by this report, VectorLink Nigeria implemented both longitudinal vector surveillance and insecticide resistance monitoring in six sentinel sites and insecticide resistance monitoring only in nine additional sites (Tables 1 and 2). Annex 1 contains GPS coordinates of sampling site locations.

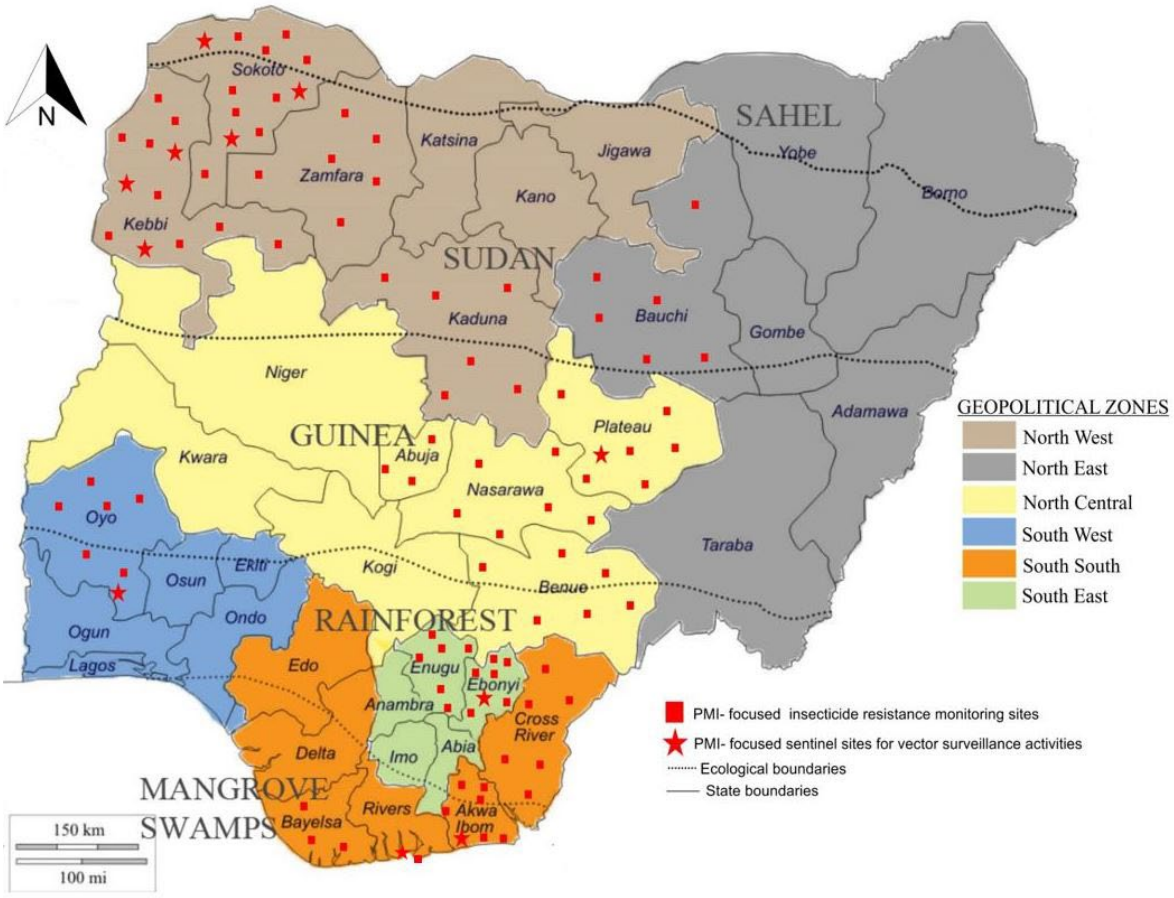
TABLE 1: LONGITUDINAL VECTOR SURVEILLANCE AND INSECTICIDE RESISTANCE MONITORING SITES AND AFFILIATED INSTITUTIONS

Geopolitical Zone	State/Institution	Local Government Areas (LGA)/Sentinel Site	Ecozone(s)
South West	Oyo/University of Ibadan	Akinyele/Elekuru	Rainforest/Guinea Savannah
South East	Ebonyi/State Univ Abakaliki	Ezza North/ Umuaghara	Rainforest
South South	Akwa Ibom/University of Uyo	Mpat Enin/Ibekwe Akpannya	Mangrove swamps/Rainforest
North Central	Plateau/University of Jos	Shendam/Tumbi	Guinea Savannah
North West	Sokoto/Usmanu Danfodiyo University Sokoto	Rabah/Angwan Sarki, Shiyyar Magali Sokoto North/Magajin Gari Ward, Waziri B Ward. Shagari/Chofal A, Chofal B	Sahel Savannah
North West	Kebbi/Federal University Birnin Kebbi	Bunza/Bunza, Maidahini Kalgo/Kalo, Hirishi Argungu/Gotomo, Alwasa	Sahel Savannah

TABLE 2: ADDITIONAL INSECTICIDE RESISTANCE MONITORING SITES AND AFFILIATED INSTITUTIONS

Geopolitical Zone	State/Institution	LGA/Insecticide Resistance Monitoring Site	Ecozone(s)
North East	Bauchi/Abubakar Tafawa Balewa University	Ningi, Misau, Shira, Dass, Bauchi, Toro	Sudan Savannah
North Central	Benue/Federal University of Agriculture Makurdi	Apa, Gwer, Obi, Tarkaa, Ukum, Vandeikya	Guinea Savannah
South South	Cross River/University of Calabar	Abi, Akampka, Calabar Municipal, Etung, Ogoja, Obudu	Rainforest/Mangrove swamps
North West	Zamfara/Federal University Gusau	Bakura, Birnin Magaji, Bungudu, Gummi, Maradun, Maru	Sahel Savannah
North West	Kaduna/Ahmadu Bello University Zaria	Giwa, Igabi, Jema'a, Lere, Markarfi, Sabon Gari	Guinea Savannah
North Central	Nasarawa/Nasarawa State University Keffi	Keana, Keffi, Kokona, Obi, Toto, Wamba	Guinea Savannah
South East	Enugu/National Arbovirus and Vectors Research Centre Enugu	Awgu, Enugu South, Ezeagu, Igbo-Eze, Nsukka, Isi-Uzo	Rainforest
North Central	FCT/University of Abuja	AMAC, Gwagwalada, Kwali	Guinea Savannah
South South	Bayelsa/Niger Delta University, Yenagoa	Ogbia, Sagbama, Yenagoa	Mangrove swamps

FIGURE 1: MAP OF NIGERIA SHOWING THE SENTINEL SITES AND INSECTICIDE RESISTANCE MONITORING SITES



From October 2021 to September 2022, *Anopheles* mosquitoes were collected monthly from six sentinel sites located in five ecozones of Nigeria (Figure 1). Mosquitoes were caught using human-baited U.S. Centers for Disease Control and Prevention (CDC) light traps (LTs) indoors and outdoors, and pyrethrum spray catches (PSCs). Details for each method are shown in Table 3. Data collected from longitudinal surveillance sites were collated and used to calculate the indicators in Table 4, which are also described in the sections on the respective mosquito collection methods below. *Anopheles* larvae were collected using ladles and reared to adults for insecticide susceptibility tests.

TABLE 3: LONGITUDINAL MONITORING ADULT MOSQUITO COLLECTION METHODS

Collection Method	Time	Frequency	Sample
Human-baited CDC LTs	6:00 p.m. to 6:00 a.m.	Three nights per site per month	Four houses per site using two CDC LTs per house per night (indoors/outdoors)
PSCs	6:00 a.m. to 8:00 a.m.	Three days per site per month	32 houses per site (10-12 houses per day)

TABLE 4: ENTOMOLOGICAL SURVEILLANCE INDICATORS

Indicator	Definition
Indoor resting density	Number of adult female vectors collected indoors per room per day. This was estimated from PSC collections.
Human biting rate	Number of female *Anopheles* vectors attempting to feed or freshly fed, per person per unit time. This was estimated from CDC LT collections.
Parity rate	Proportion of adult female vectors that laid eggs. This was estimated through ovary dissection.
Sporozoite rate	Proportion of adult female vectors harboring sporozoites in their salivary glands. This was estimated using ELISA method.
Human blood index	Proportion of blood-fed adult female vectors that fed on humans. This was determined with PCR method.
Entomological inoculation rate	Number of infectious bites by adult female vectors per person per unit time. Calculated as the product of human biting rate and sporozoite rate.
Resistance status	Classification of adult female vectors as confirmed resistant, possibly resistant, or susceptible following bioassay tests.
Resistance intensity	Classification of adult female vector populations as having high, moderate, or low intensity of resistance following bioassay tests at different concentrations.

1.2 CDC LIGHT TRAP COLLECTION

Field teams placed two human-baited CDC LTs—one indoors and one outdoors—at four houses per sentinel site for three nights each month to measure mosquito biting time and location. Collection cups were changed hourly throughout the night. The teams followed the methods outlined by Yohannes and Boelee (2012) and VectorLink Standard Operating Procedure (SOP) #1[1]. The teams sent all samples collected from the field to the centrally located insectary at Nasarawa State University Keffi for further processing and later sent to NIMR for analyses to identify sibling species and determine sporozoite rate and bloodmeal source. The mean indoor and outdoor human biting rate (HBR) was calculated as the number of mosquitoes collected per human-baited CDC LT per night. The entomological inoculation rate (EIR), defined as the number of infective bites per person per night, was calculated as the HBR multiplied by the sporozoite infection rate, on a monthly basis and over one year.

1.2.1 HUMAN BEHAVIOR OBSERVATIONS

Human and vector behaviors have the potential to threaten vector control interventions such as ITNs. The efficacy of this control measure partly relies on appropriate use by humans. Human-baited CDC LTs provide the opportunity to assess vector biting throughout the night. To understand how human and vector behaviors may be impacting the efficacy of vector control in Nigeria, VectorLink Nigeria collected information on household-level human behavior through direct human observations during routine entomological surveys in Kebbi and Sokoto. Human behavior was assessed during three nights per month in four households each in six communities in Kebbi and Sokoto in July through September 2022. During mosquito collections using CDC LTs, two collectors per house (1 indoors and 1 outdoors) collected data on the number of people indoors and outdoors. Data was collected hourly by the team from 6 p.m. to 8 a.m. A paper-based data collection form was used to record the location of household members at the start of each hour of collection throughout the night. Data collected included: number of person(s) indoors (out of bed, in bed without a net, and in bed with a net) and outdoors (out of bed, in bed without a net, and in bed with a net). Also, the number of animals both indoors and outdoors were recorded. The mean hourly HBR of each site, representing the number of bites per

[1] Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/

person per hour, was estimated and multiplied by the proportion of people at a given time and location to estimate exposure for each location and time.

1.3 Pyrethrum Spray Catches

The team randomly sampled 32 houses per sentinel site per month using the PSC method (World Health Organization (WHO), 1975) to collect indoor-resting mosquitoes (VectorLink SOP #3). The mean indoor resting density was determined by calculating the number of mosquitoes per house per day over the course of the month.

1.4 Identification of Malaria Vectors

Anopheles mosquito samples collected by the field teams using the two mosquito collection methods were morphologically identified to the species level according to methods described by Coetzee (2020). All *Anopheles* specimens collected were labelled and stored individually over silica gel in Eppendorf tubes for further processing. All samples collected were sent to the centrally located insectary at Nasarawa State University Keffi where samples were verified for accuracy of morphological identification and sorted for shipment to NIMR for molecular analysis and to determine sporozoite rate and blood meal source.

1.5 Determination of Parity Rate

To determine parity rate, the team dissected ovaries from about 20% of randomly selected, unfed, female *An. gambiae* s.l. specimens captured with human-baited CDC LTs. The teams used methods as described by Gillies and Wilkes (1963) and the WHO (2003). Mean parity rate was determined by dividing the number of parous females by the total number dissected and confirmed by observing the degree of coiling by the ovarian tracheoles (WHO, 2013).

1.6 PCR Identification of Members of *An. gambiae* Complex and *An. funestus* Group

Polymerase chain reaction (PCR) assays were carried out on mosquito samples collected to identify members of the *An. gambiae* complex and *An. funestus* group at NIMR, Yaba Lagos. PCR was conducted on approximately 10% of the total number of samples caught, including both those that had been caught indoors and outdoors by CDC LTs and by PSCs. The team amplified the extracted DNA using the *An. gambiae* species-specific multiplex PCR (Scott *et al.* 1993; Fanello *et al.* 2002). The multiplex PCR assay (Koekemoer, *et al.*, 2002) was used to determine members of the *An. funestus* group.

1.7 *Plasmodium* Sporozoite and Blood Meal Assays

The team also performed enzyme-linked immunosorbent assays (ELISAs) for sporozoite antigen on about 20% of randomly selected mosquitoes collected from the field using CDC LT and PSC methods to estimate the *Plasmodium* infection rate in the mosquito population. These were carried out according to methods described by Burkot *et al.* (1984). Positive samples were boiled and retested according to Durnez *et al* 2011. The blood meal index of the selected mosquitoes was also determined by ELISA testing of human, bovine, and goat blood sources of *Anopheles* mosquitoes (Beier *et al.,* 1988).

1.8 Insecticide Resistance Monitoring

Adult female *An. gambiae* s.l. mosquitoes (3-5 days old) reared from wild-caught larvae were exposed to pyrethroid (alpha-cypermethrin, deltamethrin, and permethrin) and organophosphate (pirimiphos-methyl) insecticides using CDC bottle bioassay methods (Brogdon and Chan, 2010). Resistance intensity assays were carried out with different doses (1X, 2X, 5X, and 10X) of pyrethroids to determine insecticide resistance intensity across all locations where pyrethroid resistance was detected. The test results were interpreted according to WHO guidelines (WHO, 2016). Susceptibility tests on chlorfenapyr (100 µg per bottle) and

clothianidin (4 µg per bottle) using the CDC bottle assay were carried out on *An. gambiae* Kisumu strain mosquitoes (control) and wild-caught *An. gambiae* s.l. from all insecticide resistance monitoring sites.

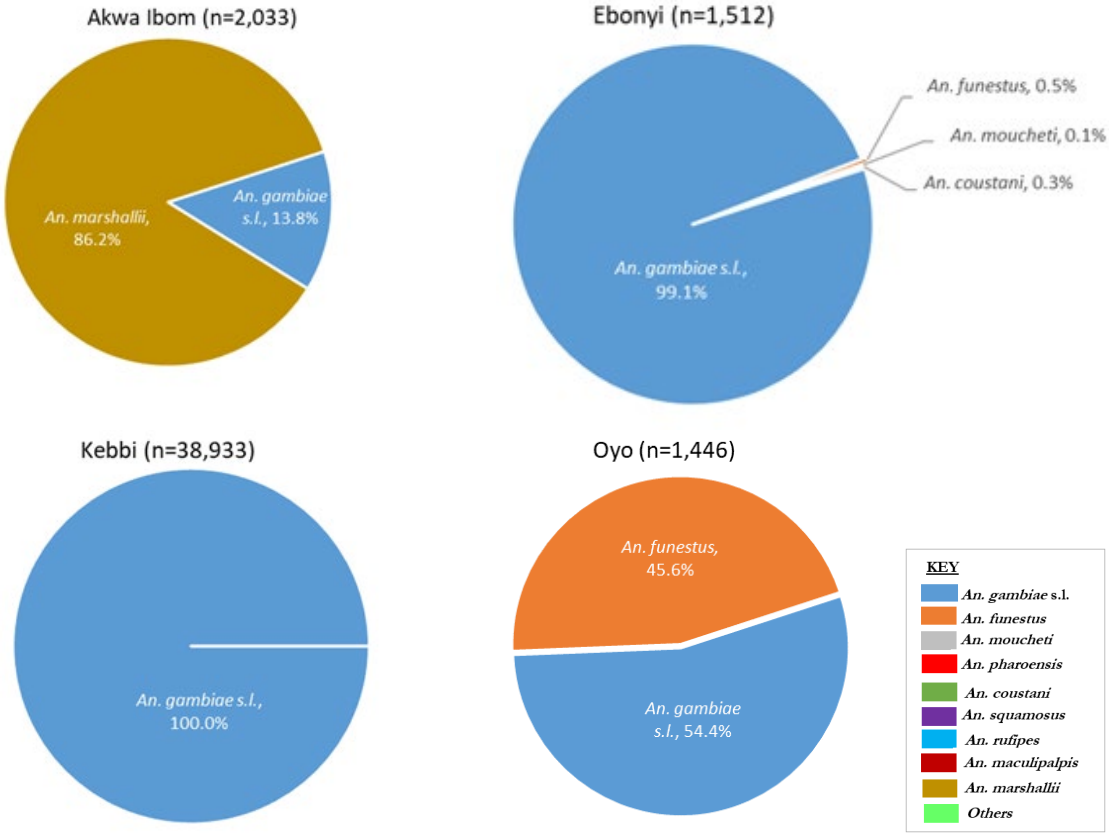
Synergist assays using piperonyl butoxide (PBO) were also carried out using standard methods to determine mechanisms of resistance in the *An. gambiae* s.l. mosquitoes. The knockdown resistance (*kdr)* genotype frequencies were determined among *An. gambiae* s.l. using allele-specific PCR assays. Surviving mosquitoes from intensity and synergist assays across all sites were analyzed for *kdr* alleles.

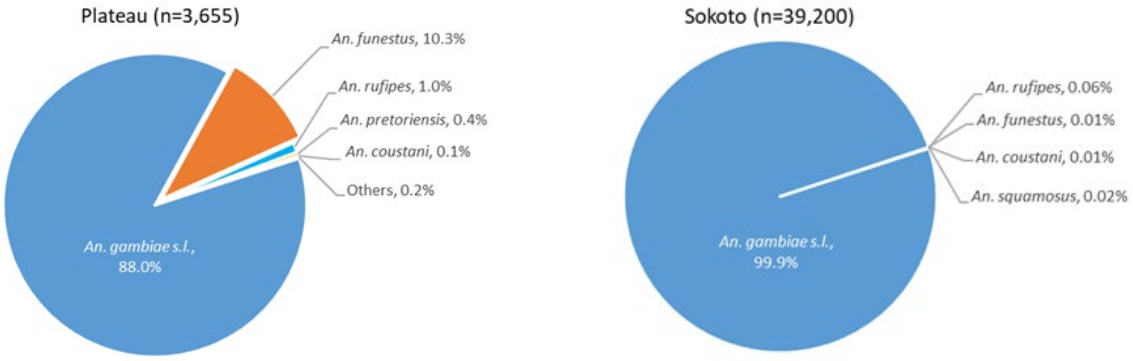
2. RESULTS

2.1 MOSQUITO ABUNDANCE AND SPECIES COMPOSITION

A total of 86,799 *Anopheles* mosquitoes were collected from six sentinel sites using human-baited CDC LTs (indoors/outdoors) and PSCs. *Anopheles gambiae* s.l. was the most abundant species across most sites, ranging from 13.8% in Akwa Ibom to 100% in Kebbi (Figure 2). Other *Anopheles* species identified in varying abundance were *An. marshallii* complex (Akwa Ibom only), *An. funestus,* and *An. rufipes.* Other localized species observed were *An. squamosus, An. coustani, An. pretoriensis*, and *An. moucheti.* In Plateau, a small number of other species—*An. maculipalpis* (n=1), *An. pharoensis* (n=2), and unidentified samples (n=9)—were collected. Annex 3 provides the number of each species collected by site and collection method.

FIGURE 2: *ANOPHELES* SPECIES COMPOSITION ACROSS SITES





2.2 MOLECULAR IDENTIFICATION OF MEMBERS OF THE *AN. GAMBIAE* COMPLEX AND DETERMINATION OF SPOROZOITE RATES

A total of 3,004 *An. gambiae* s.l. mosquitoes collected by PSCs and CDC LTs between October 2021 and September 2022 were subjected to species-specific PCR assays. All samples successfully amplified and the results indicated that 2,366 (78.8%) were *An. coluzzii,* 603 (20.1%) were identified as *An. gambiae* s.s.*,* and 35 (1.2%) were *An. arabiensis* (Annex 4).

For the first time, *An. coluzzii* was the dominant vector species found both indoors and outdoors using CDC LTs across all sentinel sites. The highest proportion of *An. coluzzii* was collected indoors in Kebbi (96.0%) followed by Sokoto (83.8%). The highest proportion of *An. coluzzii* collected outdoors was also in Kebbi (94.0%) followed by Ebonyi (91.7%). The highest proportion of *An. gambiae* s.s. identified relative to other sites was recorded outdoors in Akwa Ibom (40.0%) and followed by Ebonyi indoors (27.4%). The proportion of *An. arabiensis* found indoors and outdoors were generally quite low across all sites, with the highest proportions reported indoors (2.9%) and outdoors (2.6%) in Oyo. There were no hybrid species recorded in any of the sites (Figure 3).

For mosquitoes caught using PSCs, *An. coluzzii* was the dominant vector species found in all sites with the highest proportion recorded in Kebbi (95.8%) followed by Sokoto (86.1%) and Ebonyi (82.8%). The highest proportion of *An. gambiae* was recorded in Akwa Ibom (36.7%) followed by Plateau (27.6%). *Anopheles arabiensis* was found in all sites with the highest proportion relative to other species recorded in Akwa Ibom and Sokoto (1.7%) followed by Ebonyi (1.4%) and Plateau (1.2%). No hybrid forms (*An. gambiae* s.s./*An. coluzzii*) were collected using PSCs in any of the sites (Figure 4).

Plasmodium falciparum sporozoite infectivity rates of *An. gambiae* indicated that infection rates were recorded indoors in Ebonyi (2.2%) and Plateau (1.8%) only in mosquitoes collected using CDC LTs (Table 5). Sporozoite infectivity rate of *An. coluzzii* indicated that infection rates were recorded indoors (0.3%) and outdoors (0.5%) in Kebbi. No sporozoite positive *An. arabiensis* samples were found indoors or outdoors across sites.

For mosquitoes collected using PSCs, the highest sporozoite infection rate was recorded in *An. arabiensis* in Ebonyi (20.0%) which happens to be the only infection rate recorded among *An. arabiensis.* For *An. coluzzii,* the sporozoite rate was highest in Akwa Ibom (5.4%), followed by Ebonyi (0.7%), Kebbi (0.6%), Sokoto (0.5%), and Oyo (0.4%). The only infection rate recorded in *An. gambiae* was in Oyo (1.1%) (Figure 6).

FIGURE 3: PROPORTION OF *AN. GAMBIAE* S.S., *AN. COLUZZII*, *AN. ARABIENSIS*, AND HYBRID SPECIES COLLECTED BY INDOOR AND OUTDOOR CDC LIGHT TRAPS ACROSS SENTINEL SITES

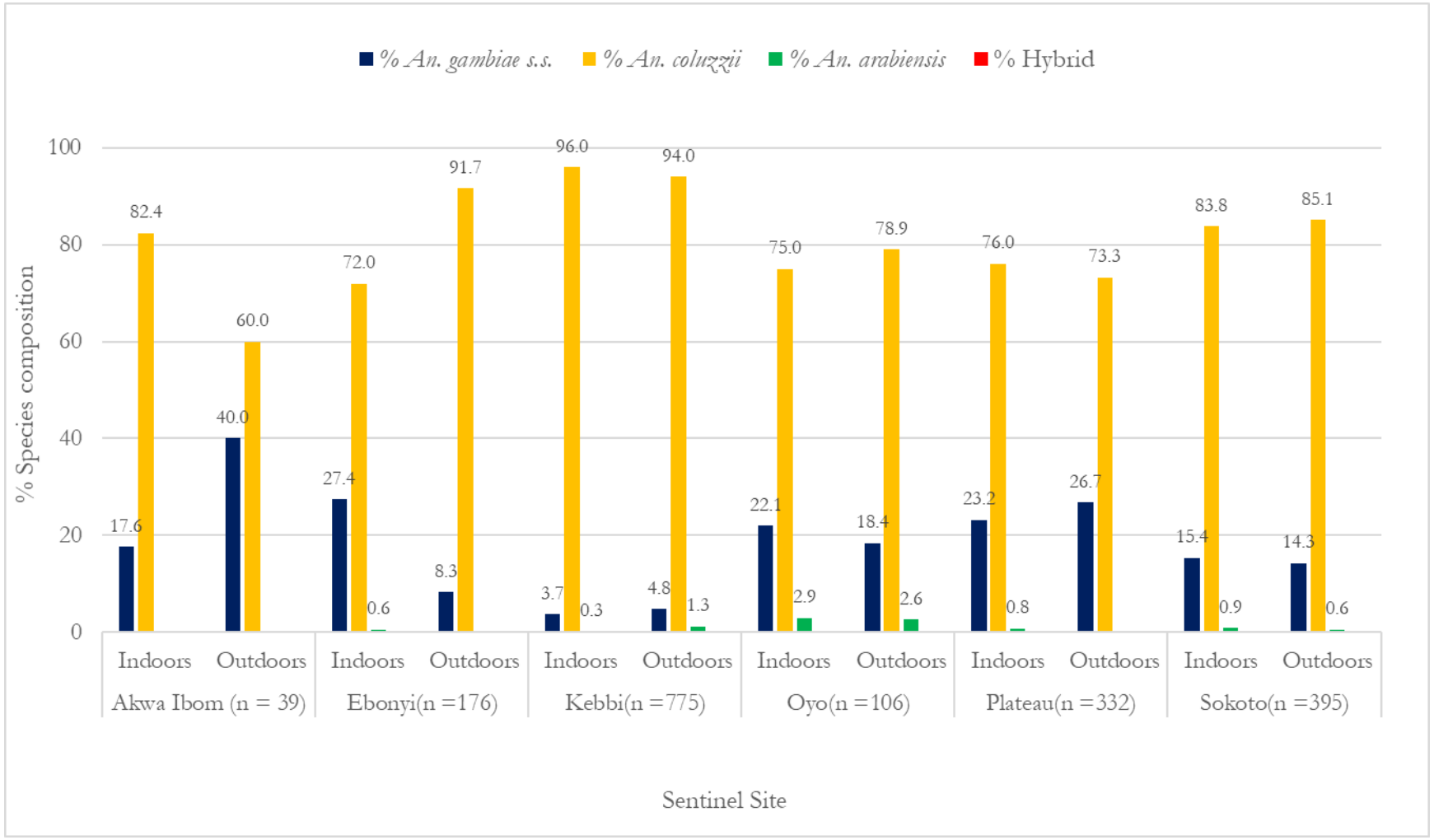


FIGURE 4: PROPORTION OF *AN. GAMBIAE* S.S., *AN. COLUZZII*, *AN. ARABIENSIS*, AND HYBRID SPECIES COLLECTED BY PSC ACROSS SENTINEL SITES

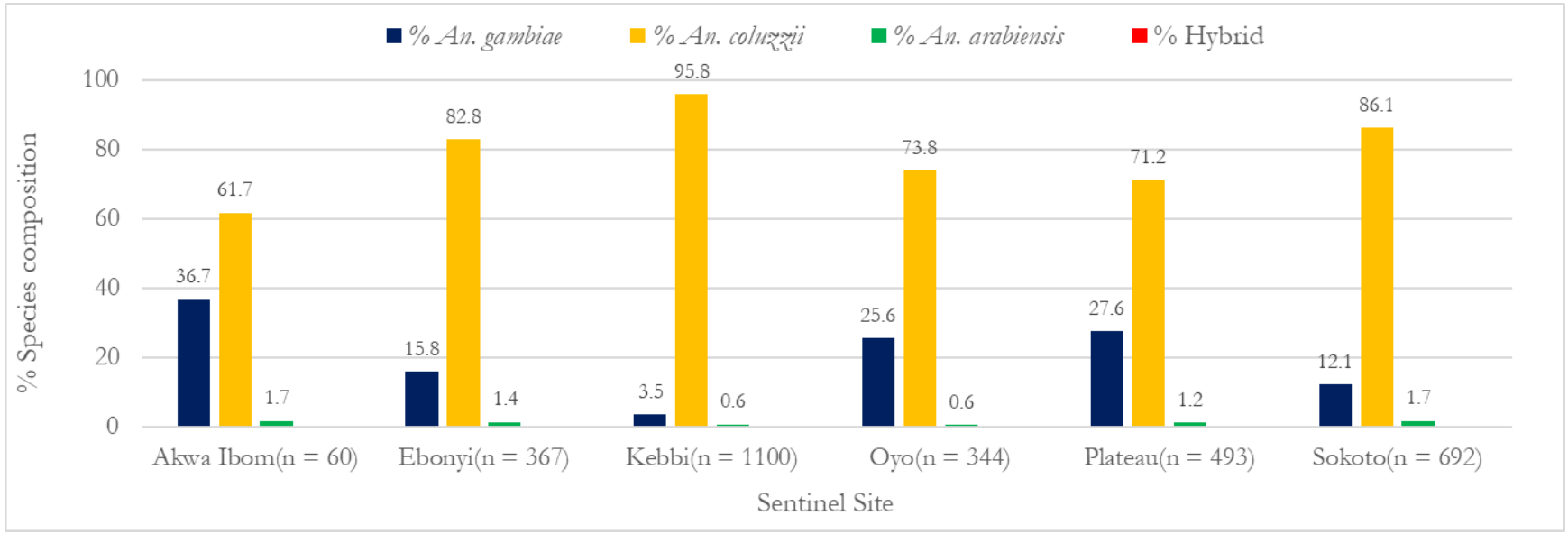


TABLE 5: SPOROZOITE POSITIVITY RATES OF *AN. GAMBIAE* S.S., *AN. COLUZZII*, AND *AN. ARABIENSIS* MOSQUITOES COLLECTED BY CDC LIGHT TRAPS ACROSS SITES

Site	Total Analyzed	*An. gambiae*						*An. coluzzii*						*An. arabiensis*					
		Number identified (%)		No. Positive for Sporozoites		SPR (%)		Number identified (%)		No. Positive for Sporozoites		SPR (%)		Number identified (%)		No. Positive for Sporozoites		SPR (%)	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Akwa Ibom	39	6 (15.4)	2 (5.1)	0	0	0.0	0.0	28 (71.8)	3 (7.7)	0	0	0.0	0.0	0 (0)	0 (0)	0	0	0.0	0.0
Ebonyi	176	45 (25.6)	1 (0.6)	1	0	2.2	0.0	118 (67)	11 (6.3)	0	0	0.0	0.0	1 (0.6)	0 (0)	0	0	0.0	0.0
Kebbi	775	14 (1.8)	19 (2.5)	0	0	0.0	0.0	362 (46.7)	374 (48.3)	1	2	0.3	0.5	1 (0.1)	5 (0.6)	0	0	0.0	0.0
Oyo	106	15 (14.2)	7 (6.6)	0	0	0.0	0.0	51 (48.1)	30 (28.3)	0	0	0.0	0.0	2 (1.9)	1 (0.9)	0	0	0.0	0.0
Plateau	332	57 (17.2)	23 (6.9)	1	0	1.8	0.0	187 (56.3)	63 (19)	0	0	0.0	0.0	2 (0.6)	0 (0)	0	0	0.0	0.0
Sokoto	395	36 (9.1)	23 (5.8)	0	0	0.0	0.0	196 (49.6)	137 (34.7)	0	0	0.0	0.0	2 (0.5)	1 (0.3)	0	0	0.0	0.0
Total	**1,823**	**173 (9.5)**	**75 (4.1)**	**2**	**0**	**1.2**	**0.0**	**942 (51.7)**	**618 (33.9)**	**1**	**2**	**0.1**	**0.3**	**8 (0.4)**	**7 (0.4)**	**0**	**0**	**0.0**	**0.0**

Note: In=Indoor CDC LT, Out=Outdoor CDC LT, SPR=Sporozoite Positivity Rate

TABLE 6: SPOROZOITE POSITIVITY RATES OF *AN. GAMBIAE* S.S., *AN. COLUZZII*, AND *AN. ARABIENSIS* MOSQUITOES COLLECTED BY PYRETHRUM SPRAY COLLECTIONS ACROSS SITES

Site	Total Analyzed	*An. gambiae*			*An. coluzzii*			*An. arabiensis*		
		Number identified (%)	No. Positive for Sporozoites	SPR (%)	Number identified (%)	No. Positive for Sporozoites	SPR (%)	Number identified (%)	No. Positive for Sporozoites	SPR (%)
Akwa Ibom	60	22 (36.7)	0	0.0	37 (61.7)	2	5.4	1 (1.7)	0	0.0
Ebonyi	367	58 (15.8)	0	0.0	304 (82.8)	2	0.7	5 (1.4)	1	20.0
Kebbi	1,100	39 (3.5)	0	0.0	1,054 (95.8)	6	0.6	7 (0.6)	0	0.0
Oyo	344	88 (25.6)	1	1.1	254 (73.8)	1	0.4	2 (0.6)	0	0.0
Plateau	493	136 (27.6)	0	0.0	351 (71.2)	0	0.0	6 (1.2)	0	0.0
Sokoto	692	84 (12.1)	0	0.0	596 (86.1)	3	0.5	12 (1.7)	0	0.0
Total	**3,056**	**427 (14)**	**1**	**0.2**	**2,596 (84.9)**	**14**	**0.5**	**33 (1.1)**	**1**	**3.0**

2.3 MOLECULAR IDENTIFICATION OF MEMBERS OF THE *AN. FUNESTUS* GROUP AND DETERMINATION OF SPOROZOITE RATES

A total of 772 *An. funestus* mosquitoes collected by CDC LTs and PSCs in Ebonyi, Oyo, Plateau, and Sokoto were subjected to species-specific PCR assays (Annex 5). Of these, 439 (56.9%) mosquitoes were collected using CDC LTs (Figure 5), while 333 (43.1%) were collected using PSCs (Figure 6). *Anopheles funestus* s.s. predominated across the sites both indoors and/or outdoors ranging from 75% to 100%. Unamplified samples were collected outdoors in Plateau (25.0%) and Oyo (1.1%) (Figure 5), while *An. leesoni* was recorded only in Oyo indoors (0.6%). For members of *An. funestus* group collected using PSCs, *An. funestus* s.s. was the predominant species in all three sites (Ebonyi, Oyo, and Plateau) (Figure 6).

FIGURE 5: PROPORTION OF MEMBERS OF *AN. FUNESTUS* GROUP COLLECTED BY CDC LIGHT TRAPS INDOORS AND OUTDOORS ACROSS SENTINEL SITES

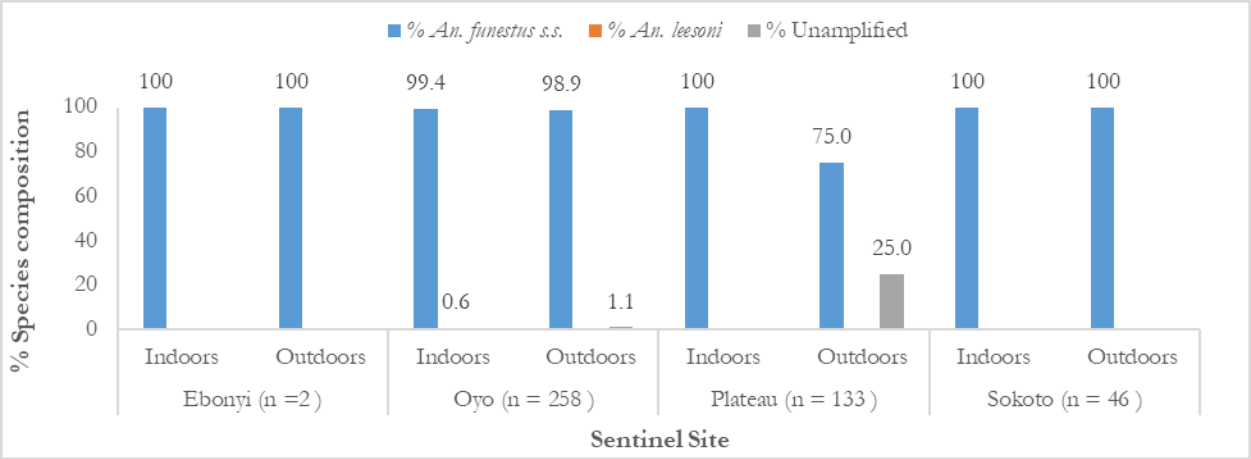
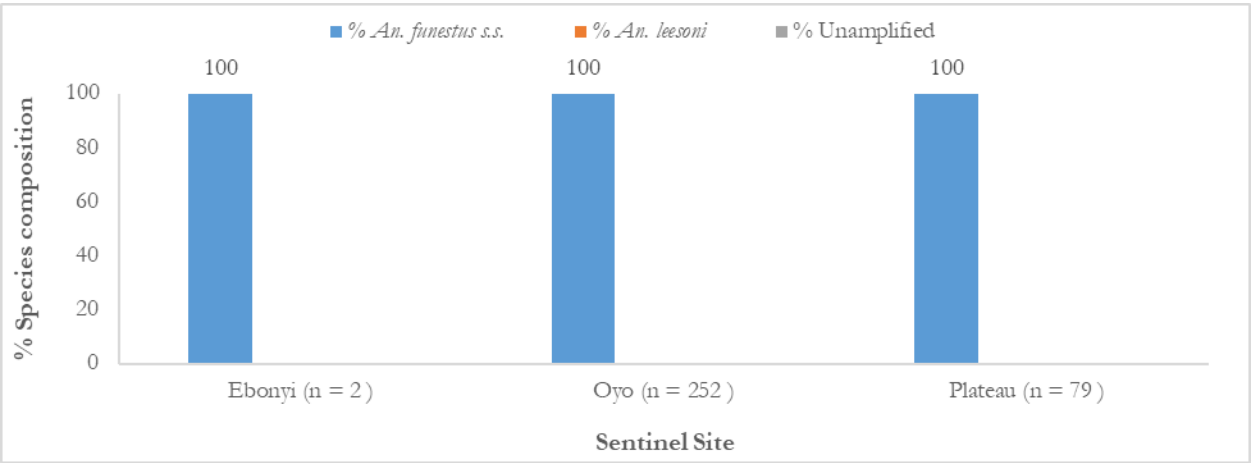


FIGURE 6: PROPORTION OF MEMBERS OF *AN. FUNESTUS* GROUP COLLECTED BY PSC ACROSS SENTINEL SITES



The highest *Plasmodium falciparum* sporozoite rates for *An. funestus* s.s. collected using CDC LTs was recorded outdoors in Oyo (3.4%) followed by Plateau indoors (1.6%). No other members of *An. funestus* group tested positive for *Plasmodium* sporozoite (Table 7). For *An. funestus* s.s. collected by PSC methods, no sporozoite positivity rates were recorded in any site (Table 8).

TABLE 7: SPOROZOITE POSITIVITY RATES OF *AN. FUNESTUS* MOSQUITOES COLLECTED BY CDC LIGHT TRAP INDOORS AND OUTDOORS ACROSS SITES

Site	Total Analyzed	*An. funestus* s.s.						*An. leesoni*						Unamplified					
		Number identified (%)		No. Positive for Sporozoites		SPR (%)		Number identified (%)		No. Positive for Sporozoites		SPR (%)		Number identified (%)		No. Positive for Sporozoites		SPR (%)	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Ebonyi	2	1 (50.0)	1 (50.0)	0	0	0.0	0.0	0 (0)	0 (0)	0	0	0.0	0.0	0 (0)	0 (0)	0	0	0.0	0.0
Oyo	258	168 (65.1)	88 (34.1)	0	3	0.0	3.4	1 (0.4)	0 (0)	0	0	0.0	0.0	0 (0)	1 (0.4)	0	0	0.0	0.0
Plateau	133	125 (94.0)	6 (4.5)	2	0	1.6	0.0	0 (0)	0 (0)	0	0	0.0	0.0	0 (0)	2 (1.5)	0	0	0.0	0.0
Sokoto	46	22 (47.8)	24 (52.2)	0	0	0.0	0.0	0 (0)	0 (0)	0	0	0.0	0.0	0 (0)	0 (0)	0	0	0.0	0.0
Total	**439**	**316 (72.0)**	**119 (27.1)**	**2**	**3**	**0.6**	**2.5**	**1 (0.2)**	**0 (0)**	**0**	**0**	**0.0**	**0.0**	**0 (0)**	**3 (0.7)**	**0**	**0**	**0.0**	**0.0**

TABLE 8: SPOROZOITE POSITIVITY RATES OF *AN. FUNESTUS* MOSQUITOES COLLECTED BY PSC ACROSS SITES

Site	Total Analyzed	*An. funestus* s.s.			*An. leesoni*			Unamplified		
		Number identified (%)	No. Positive for Sporozoites	SPR (%)	Number identified (%)	No. Positive for Sporozoites	SPR (%)	Number identified (%)	No. Positive for Sporozoites	SPR (%)
Ebonyi	2	2 (100)	0	0.0	0 (0)	0	0.0	0 (0)	0	0.0
Oyo	252	252 (100)	0	0.0	0 (0)	0	0.0	0 (0)	0	0.0
Plateau	79	79 (100)	0	0.0	0 (0)	0	0.0	0 (0)	0	0.0
Sokoto	0	0 (0)	0	0.0	0 (0)	0	0.0	0 (0)	0	0.0
Total	**333**	**333 (100)**	**0**	**0.0**	**0 (0)**	**0**	**0.0**	**0 (0)**	**0**	**0.0**

2.4 SPOROZOITE POSITIVITY RATES OF *AN. MARSHALLII* MOSQUITOES COLLECTED BY CDC LT AND PSC IN AKWA IBOM

An. marshallii complex mosquitoes caught using CDC LTs indoors and outdoors as well as PSC in Akwa Ibom were screened for *Plasmodium falciparum* circumsporozoite antigens. Whereas no sporozoite infection was recorded in any of the analyzed mosquitoes caught using CDC LT, a sporozoite infection rate of 0.7% was recorded among samples caught using PSC method (Table 9).

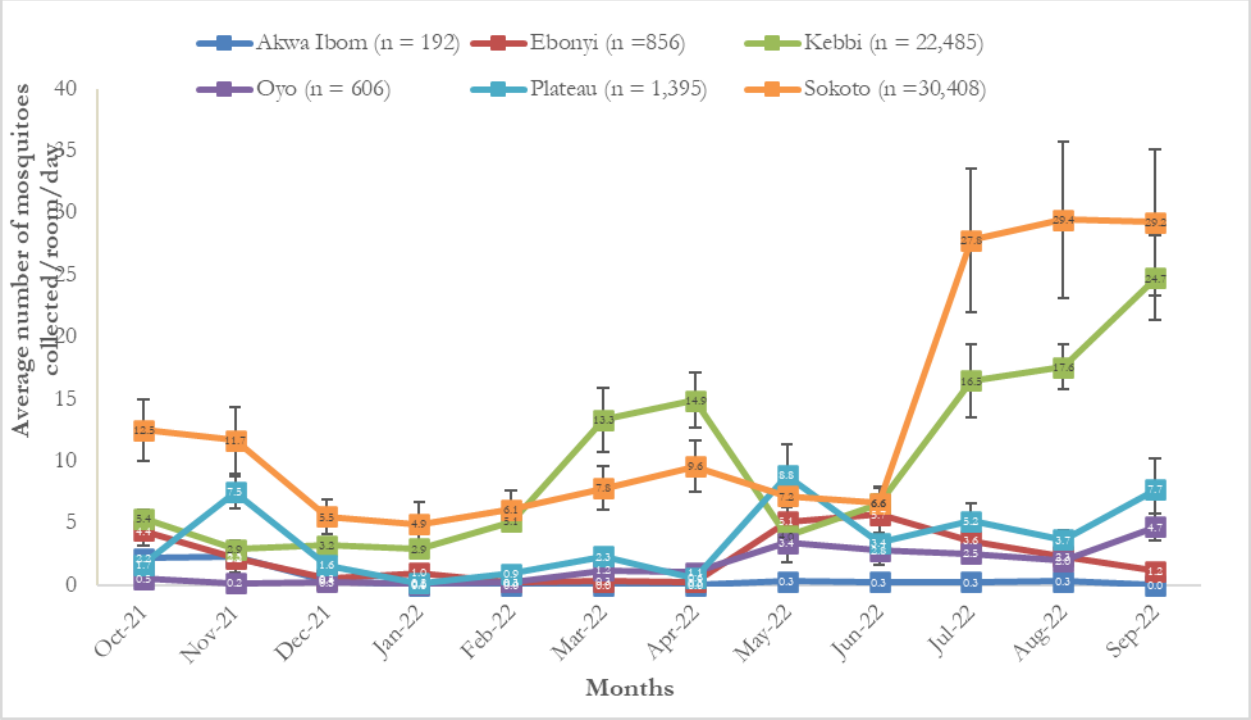
TABLE 9: SPOROZOITE POSITIVITY RATES OF *AN. MARSHALLII* MOSQUITOES COLLECTED BY CDC LT AND PSC AT AKWA IBOM SENTINEL SITE

Site	Method of Mosquito Collection	Total Analyzed	*An. marshallii.*		
			Number identified (%)	No. Positive for Sporozoites	SPR (%)
Akwa Ibom	CDC Indoors	507	507 (100)	0	0.0
	CDC Outdoors	128	128 (100)	0	0.0
	PSC	142	142 (100)	1	0.7

2.5 MONTHLY INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L.

The indoor resting density of *An. gambiae* s.l. mosquitoes varied across the sites and months, ranging from 0.1 mosquitoes/room/day in Oyo during January 2022 to 29.4 mosquitoes/room/day in Sokoto in August 2022 (Figure 7 and Annex 6). In Sokoto, higher indoor resting densities were also observed in July 2022 (27.8 mosquitoes/room/day) and September 2022 (29.2 mosquitoes/room/day), as well as a steady increase in Kebbi during that same time frame (July 2022: 16.5 mosquitoes/room/day – September 2022: 24.7 mosquitoes/room/day).

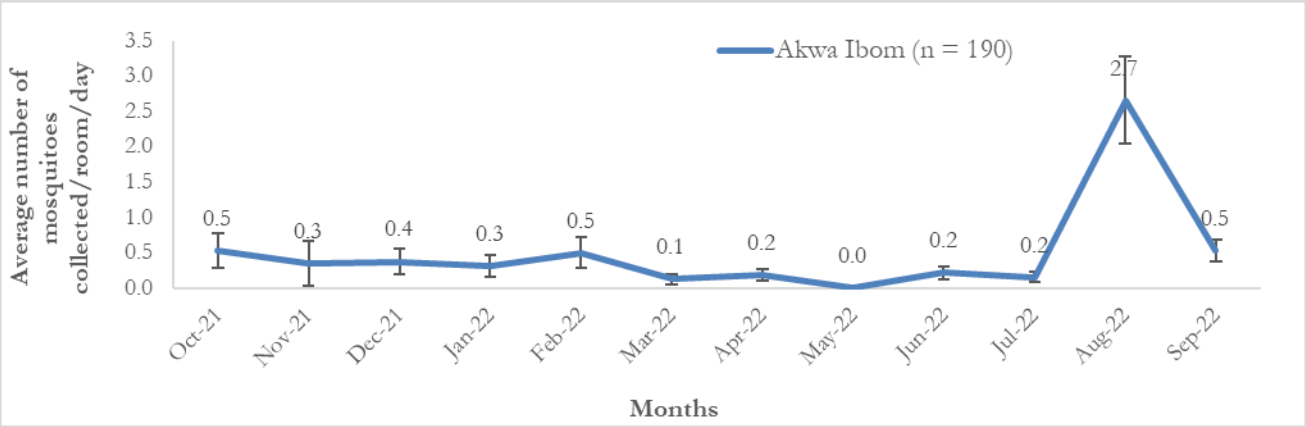
FIGURE 7: MONTHLY INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. ACROSS SITES



2.6 MONTHLY INDOOR RESTING DENSITY OF *AN. MARSHALLII* COMPLEX IN AKWA IBOM SITE

The indoor resting density of *An. marshallii* complex mosquitoes varied across the months, ranging from 0-0.5 mosquitoes/room/day between October 2021 to July 2022, before peaking in August 2022 (2.7 mosquitoes/room/day) (Figure 8).

FIGURE 8: MONTHLY INDOOR RESTING DENSITY OF *AN. MARSHALLII* COMPLEX IN AKWA IBOM



2.7 HUMAN BITING RATES

2.7.1 HUMAN BITING RATE OF *AN. GAMBIAE* S.L. ACROSS SITES

The mean indoor biting rates of *An. gambiae* s.l. were highest in April (31.3 b/p/n), July and August (25.9 b/p/n), and September (29.5 b/p/n) in Kebbi, and peaked in June in Ebonyi (22.3 b/p/n), and in September in Plateau (85.7 b/p/n) and Sokoto (24.4 b/p/n) (Figure 9). Smaller peaks were observed in *An. gambiae* biting rates outdoors in Kebbi (11.8 b/p/n) and September (7.7 b/p/n) in Sokoto (Figure 10).

FIGURE 9: MONTHLY INDOOR HUMAN BITING RATES OF *AN. GAMBIAE* S.L. ACROSS SITES

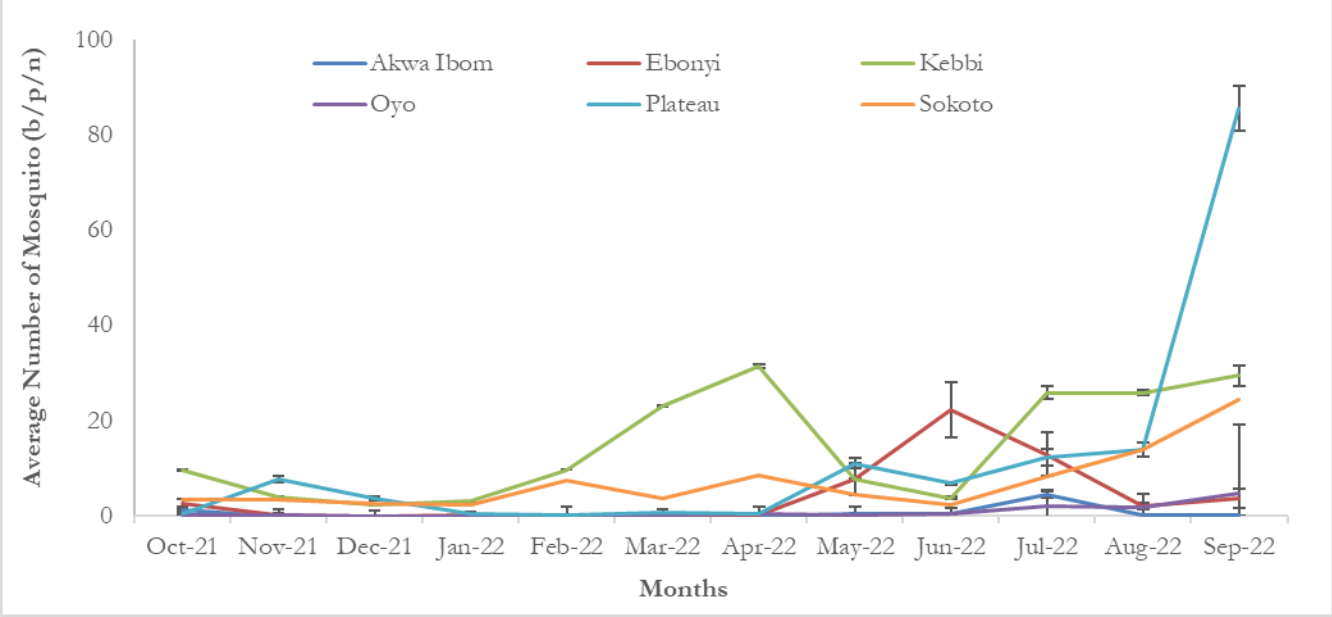
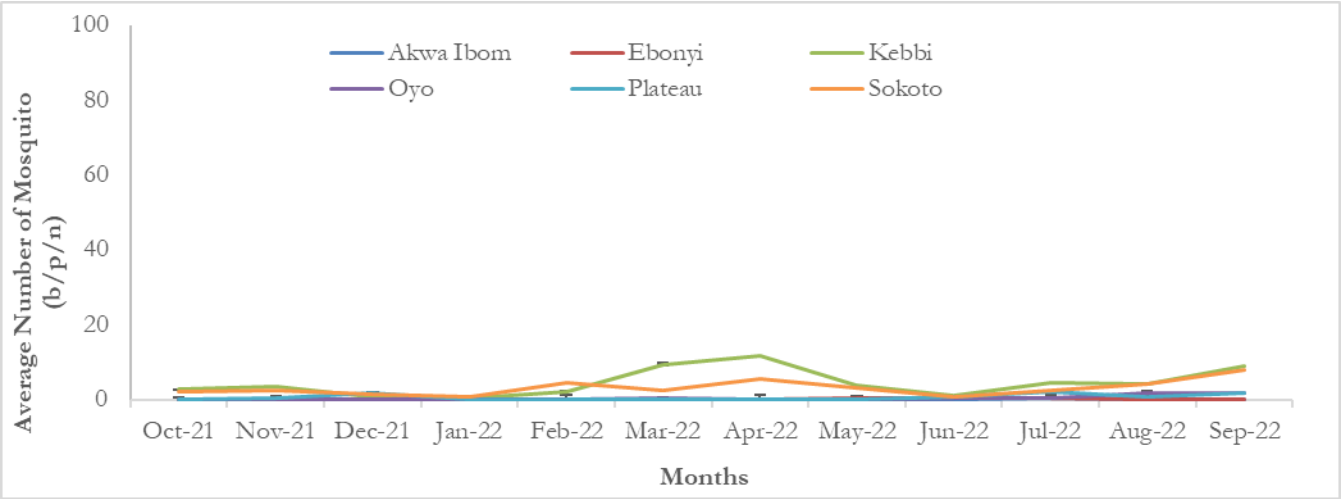


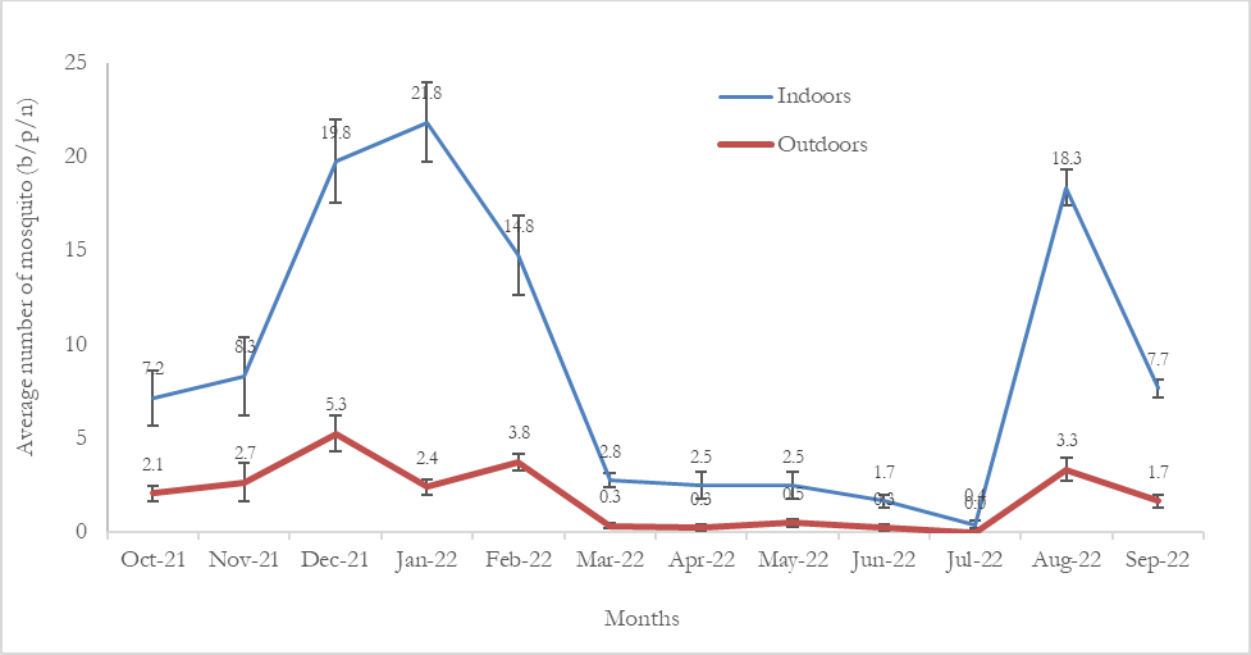
FIGURE 10: MONTHLY OUTDOOR HUMAN BITING RATES OF *AN. GAMBIAE* S.L. ACROSS SITES



2.7.2 HUMAN BITING RATES FOR *AN. MARSHALLII* COMPLEX IN AKWA IBOM

The mean indoor biting rates of *An. marshallii* complex peaked in January (21.8 b/p/n) and again in August 2022 (18.3 b/p/n). Outdoors, biting rate peaked in December 2021 (5.3 b/p/n), February (3.8 b/p/n) as well as in August 2022 (3.3 b/p/n) (Figure 11).

FIGURE 11: MONTHLY INDOOR AND OUTDOOR HUMAN BITING RATES OF *AN. MARSHALLII* COMPLEX IN AKWA IBOM



2.7.3 HUMAN BITING RATES FOR *AN. FUNESTUS* IN OYO, PLATEAU, AND SOKOTO

The mean indoor biting rates of *An. funestus* were recorded in Oyo, Plateau and Sokoto. This peaked in September (6.6 b/p/n) in Plateau, May (4.8 b/p/n) and in Oyo (Figure 12). The highest outdoor biting peak was recorded in June (2.9 b/p/n) in Oyo (Figure 13).

FIGURE 12: MONTHLY INDOOR HUMAN BITING RATES OF *AN. FUNESTUS* IN OYO, PLATEAU AND SOKOTO (OCTOBER 2021-SEPTEMBER 2022)

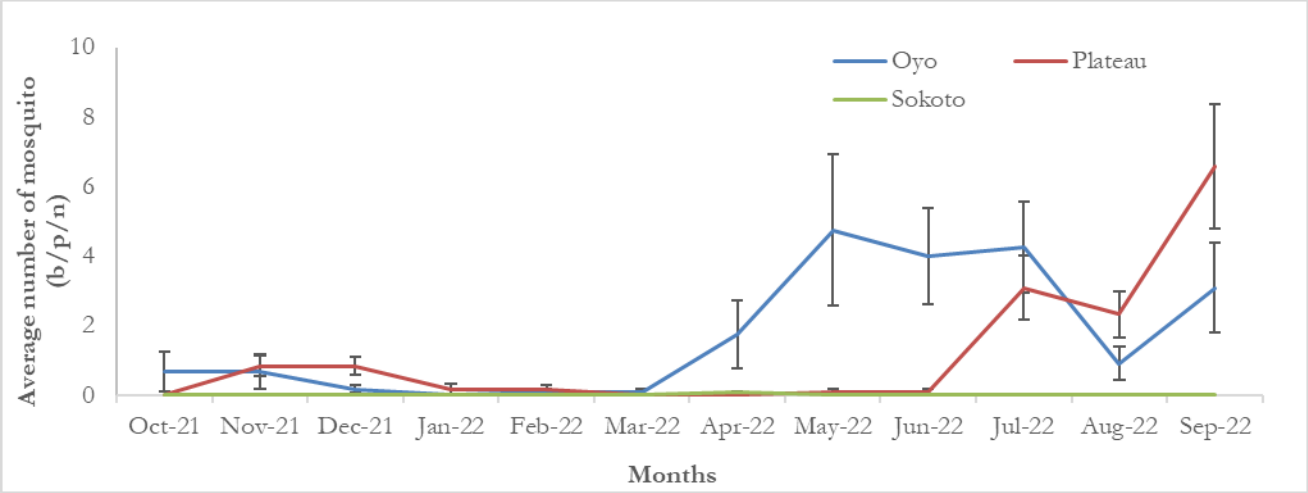
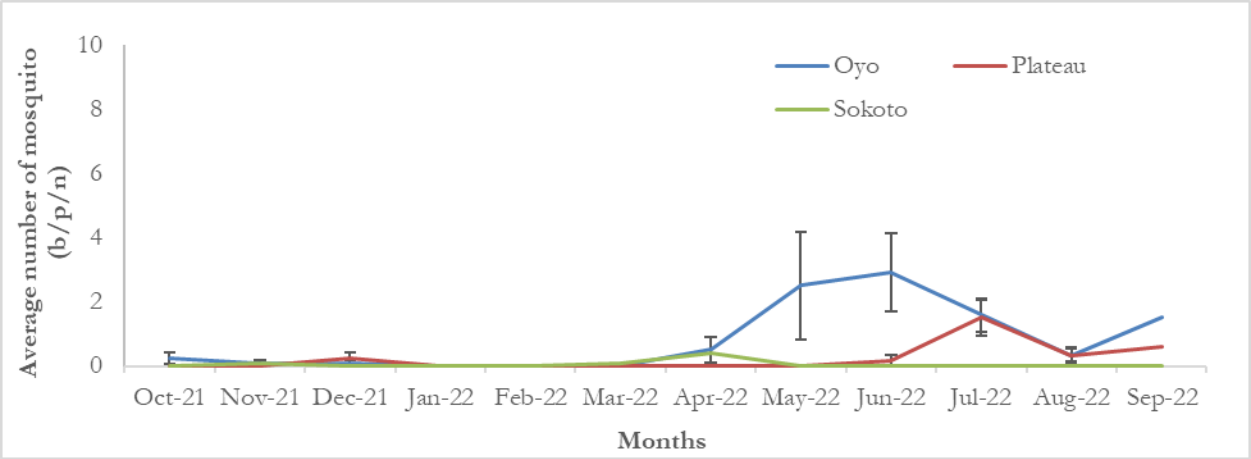


FIGURE 13: MONTHLY OUTDOOR HUMAN BITING RATES OF *AN. FUNESTUS* IN OYO, PLATEAU AND SOKOTO (OCTOBER 2021-SEPTEMBER 2022)

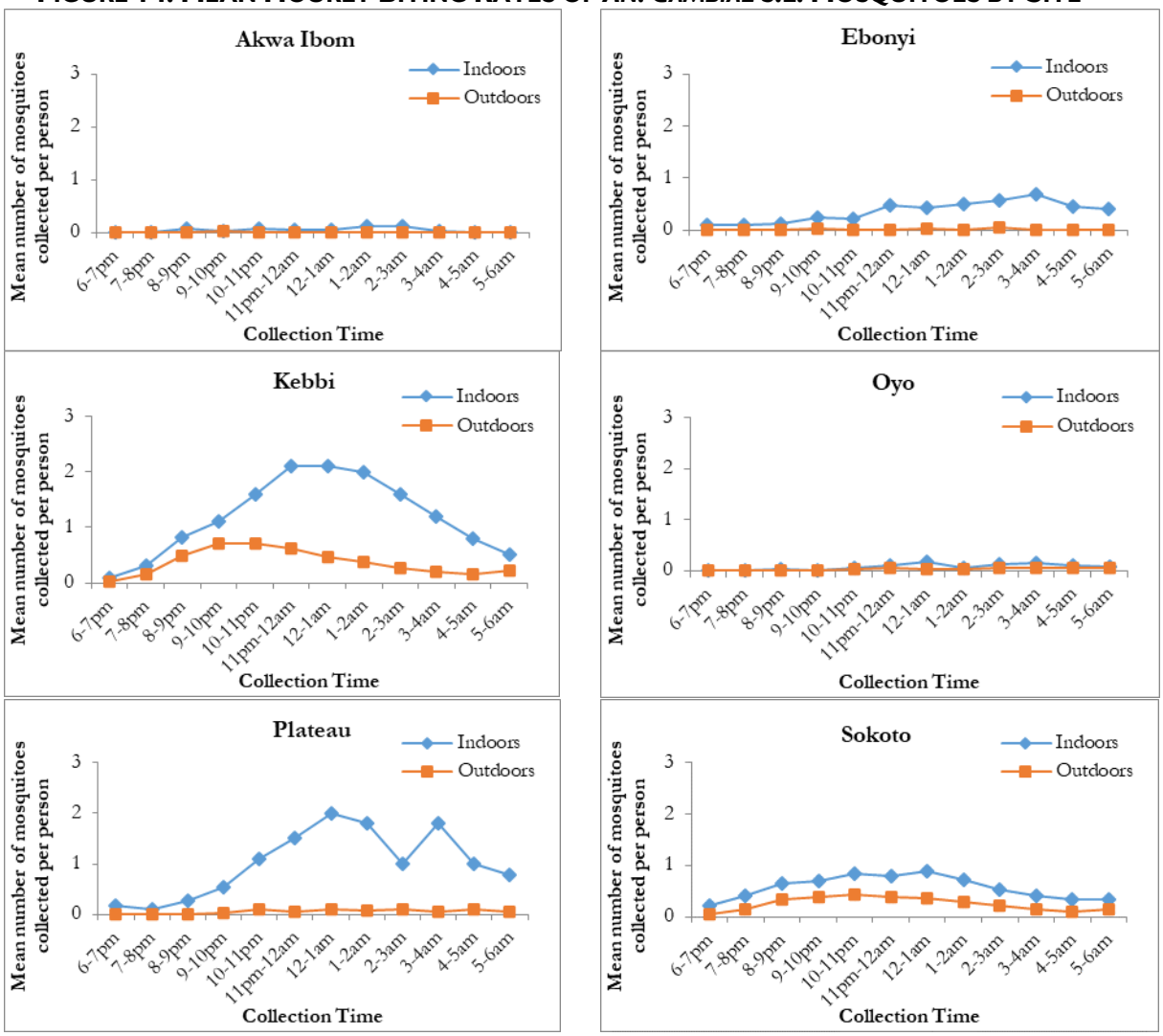


2.8 BITING TIME OF *ANOPHELES* MOSQUITOES

2.8.1 BITING TIME OF *AN. GAMBIAE* S.L. ACROSS SITES

Across sites, the average number of mosquitoes caught biting per hour was generally higher indoors than outdoors, with peaks varying by site and typically found in the late evening to early morning hours. In Kebbi, indoor biting peaked between 11 p.m.-2 a.m., while Plateau and Sokoto peaked between 12-1 a.m. Biting in Ebonyi peaked between 3-4 a.m.; there was also an uptick in biting in Plateau during this time. Outdoors, the hourly biting rates were lower in all sites, and peaked between 9-10 p.m. in Kebbi and between 10-11 p.m. in Sokoto (Figure 14).

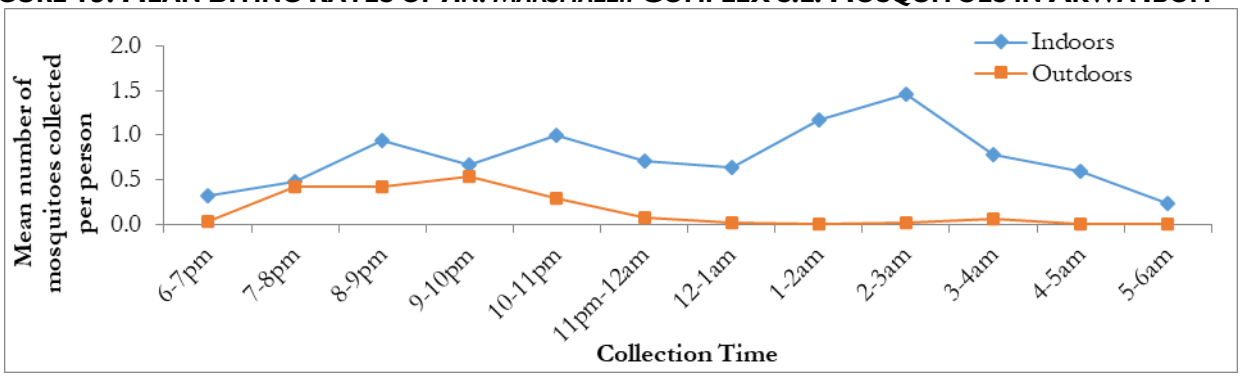
FIGURE 14: MEAN HOURLY BITING RATES OF *AN. GAMBIAE* S.L. MOSQUITOES BY SITE



2.8.2 BITING TIME OF *AN. MARSHALLII* COMPLEX S.L. IN AKWA IBOM

The average number of *An. marshallii* caught biting per hour in Akwa Ibom was generally higher indoors, with three peaks, one between 8-9 p.m., one between 10-11 p.m., and another between 2-3 a.m. (Figure 15). Outdoor biting began to increase first between 7-8 p.m. before peaking between 9-10 p.m.

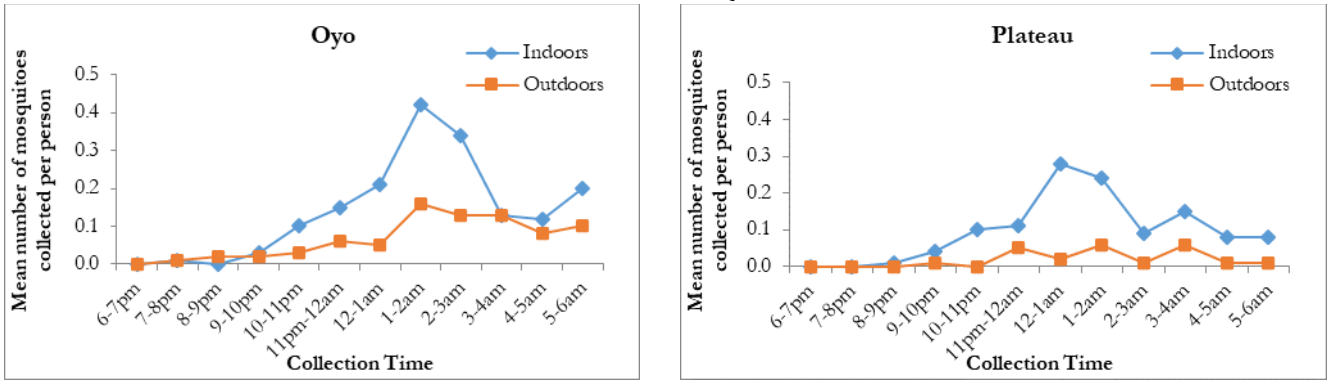
FIGURE 15: MEAN BITING RATES OF *AN. MARSHALLII* COMPLEX S.L. MOSQUITOES IN AKWA IBOM



2.8.3 BITING TIME OF *AN. FUNESTUS* S.L. IN OYO AND PLATEAU

The average number of *An. funestus* mosquitoes caught biting per hour was higher indoors compared to outdoors in Oyo and Plateau. The hourly number of mosquito bites in Oyo, peaked indoors at 1-2 a.m., while those of Plateau peaked between 12-1 a.m. (Figure 16).

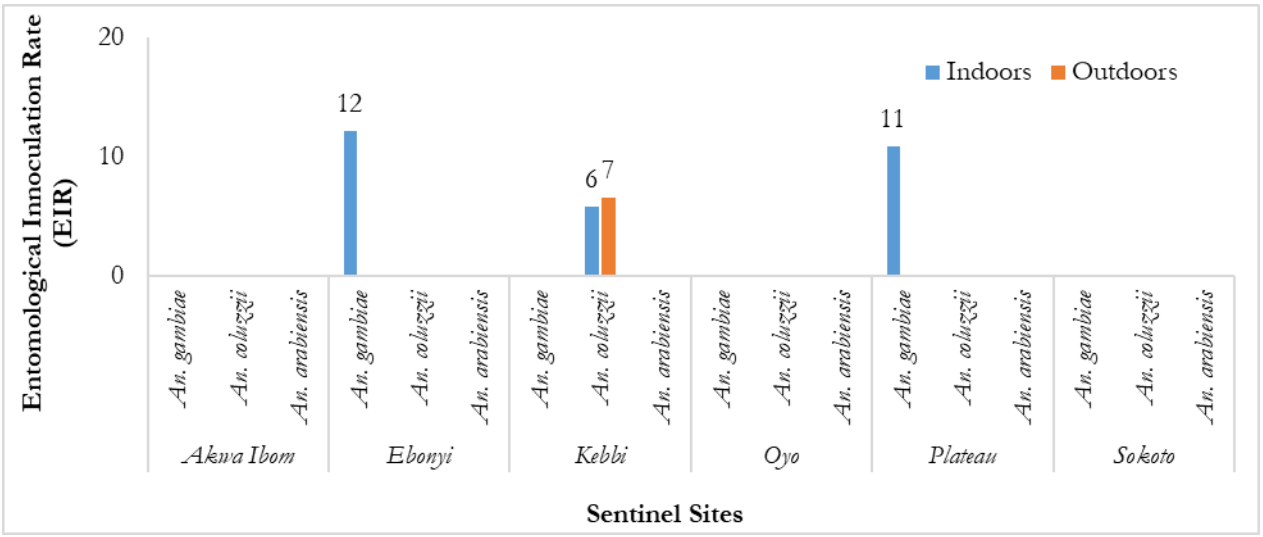
FIGURE 16: MEAN BITING RATES OF *AN. FUNESTUS* MOSQUITOES FOR OYO AND PLATEAU



2.9 ENTOMOLOGICAL INOCULATION RATES ACROSS SITES

EIRs were recorded indoors in two of the five sites among *An. gambiae*. There were no infective bites recorded both indoors and outdoors among *An. coluzzii* and *An. arabiensis* in any of the sentinel sites. The highest indoor EIR was recorded with *An. gambiae* in Ebonyi (12.1 infective bites/person/year) and then in Plateau (10.9 infective bites/person/year). No outdoor EIR was recorded among the three species across the various sites (Figure 17 and Annex 7).

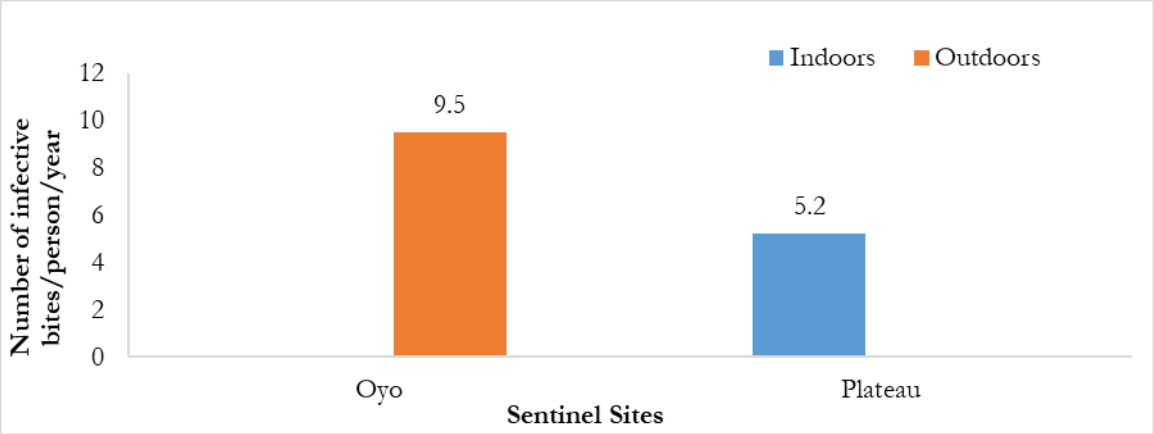
FIGURE 17: ANNUAL ENTOMOLOGICAL INOCULATION RATES ACROSS SITES



2.9.1 ENTOMOLOGICAL INOCULATION RATES IN *AN. FUNESTUS* S.S. ACROSS SITES

EIRs were recorded for *An. funestus* s.s. in Oyo and Plateau. The highest EIR was recorded outdoors in Oyo (9.5 infective bites/person/year), followed by indoors in Plateau (5.2 infective bites/person/year). There were no infective bites recorded among *An. funestus* s.s. indoors in Oyo and outdoors in Plateau (Figure 18 and Annex 9).

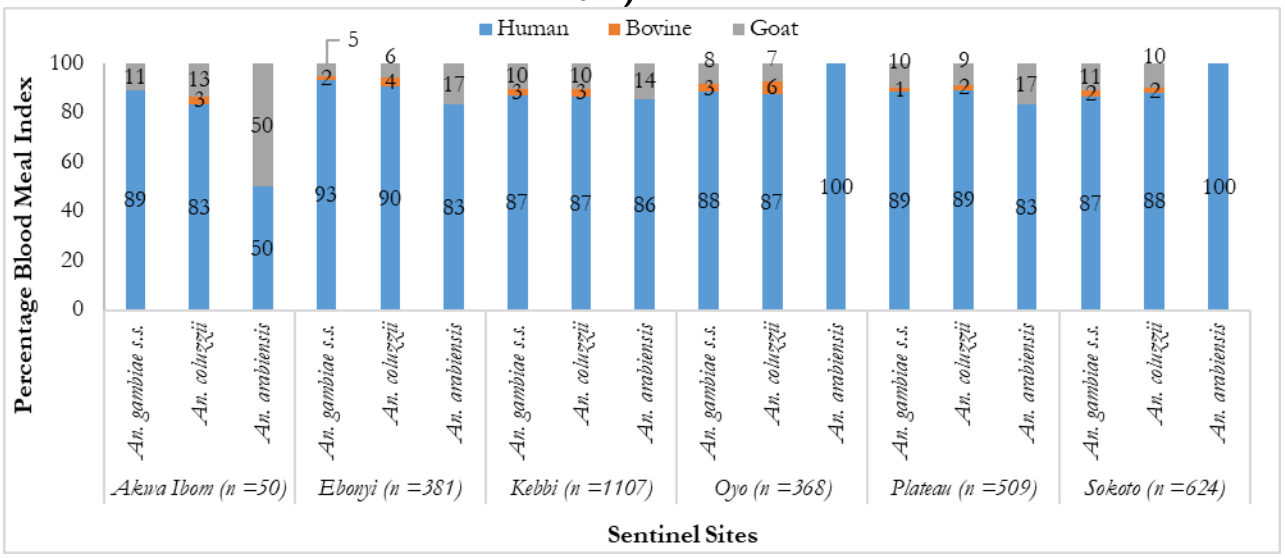
FIGURE 18: ANNUAL INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES OF *AN. FUNESTUS* S.S.



2.10 HUMAN BLOOD INDEX

Across the sites, human blood index (HBI) analysis detected human blood meals in varying proportions in *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* collected. Compared to CDC LTs, the PSC method of collection provided more blood fed mosquitoes required for the determination of blood meal sources (Figures 19-21). The percentage of mosquitoes collected by PSC that fed on humans (50-100%) was higher compared to those that fed on bovine (1-6%) and goat (5-50%) blood meals (Figure 21). The human blood meal patterns observed from the analysis of mosquitoes from all sites showed that all three blood meal sources were found in both *An. gambiae* s.s. and *An. coluzzii* in Ebonyi, Kebbi, Oyo, Plateau, and Sokoto. *Anopheles arabiensis* from Oyo and Sokoto fed only on human blood meal, while those analyzed in Akwa Ibom, Ebonyi, Kebbi and Plateau fed only on human and goat blood (Figure 19).

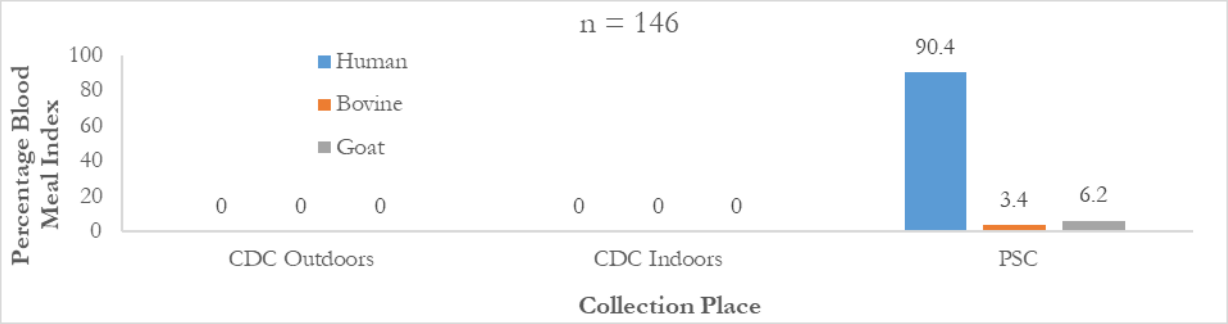
FIGURE 19: BLOOD MEAL SOURCES OF *AN. GAMBIAE* S.S., *AN. COLUZZII*, AND *AN. ARABIENSIS* FROM PYRETHRUM SPRAY CATCH COLLECTIONS ACROSS SITES (OCTOBER 2021-SEPTEMBER 2022)



2.11 BLOODMEAL SOURCES IN *AN. MARSHALLII* COMPLEX

Because of the preponderance of *An. marshallii* complex in Akwa Ibom, it became necessary to examine the blood meal preferences of the species and establish the level of human-vector interactions in the area. Of the 146 blood fed mosquitoes identified as *An. marshallii*, all that tested positive for blood meal were collected from PSC. No blood meal was detected among *An. marshallii* complex mosquitoes caught using CDC LTs. Blood meal analysis of *An. marshallii* samples collected from PSC indicated that 90.4% fed on humans, compared to 3.4% and 6.2% that fed on bovine and goat blood meal, respectively (Figure 20).

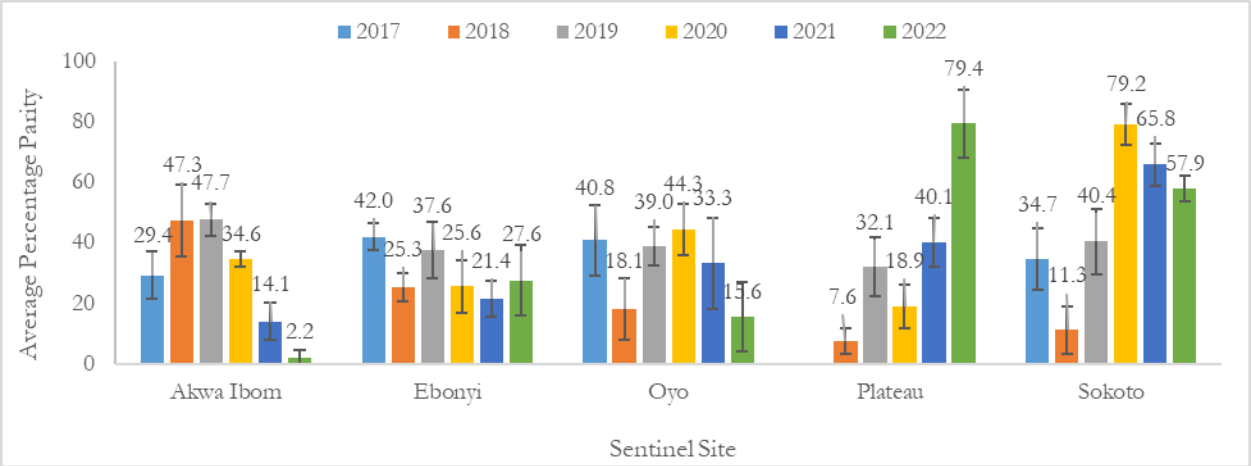
FIGURE 20: PERCENTAGE BLOOD MEAL INDEX FOR *AN. MARSHALLII*, OCTOBER 2021-SEPTEMBER 2022



2.12 PARITY RATE

Unfed, female *An. gambiae* s.l. specimens captured with human-baited CDC LTs were dissected across the sentinel sites to determine the parity rates of the mosquitoes. Average parity rates of *An. gambiae* s.l. mosquitoes from 2017-2022 were calculated and compared (Figure 21). Across the six years, the highest parity rates were recorded in Sokoto in 2020 (79.2%) and Plateau in 2022 (79.4%). There was a significant difference in the average percentage of parous mosquitoes in Sokoto ($F_{4,46}$=0.7.2795, p=0.0001) and in Akwa Ibom ($F_{4,45}$=2.787, p=0.0376) where there was marked reduction in 2022. The average percentage of parous mosquitoes recorded within five years in Oyo ($F_{4,46}$=0.776650, p=0.5461), Plateau ($F_{3,35}$=1.9167, p=0.1449), and Ebonyi ($F_{4,46}$=1.0604, p=0.3869) were not statistically significant (Figure 21).

FIGURE 21: PARITY RATES OF DISSECTED MOSQUITOES IN SENTINEL SITES (2017-2022)



2.13 HUMAN BEHAVIOR OBSERVATIONS

2.13.1 HUMAN EXPOSURE TO MALARIA VECTORS IN KEBBI STATE

In Kebbi, biting occurs predominantly indoors at bedtime with peak indoor biting rate of 3.11 bites per person per hour (b/p/h) occurring between 1-2 a.m. The outdoor biting rate in Kebbi peaked at 0.89 b/p/h between 9-10 p.m. (Figure 22). The human behavior data indicated that most people were outdoors (not in bed) before 10 p.m., and most people were indoors without bed nets between 11 p.m. and 6 a.m. in the morning. At the peak indoor biting hour of 1-2 a.m., 57% of the people were in bed, but not protected under nets. When considered along with high biting rates indoors, this might have contributed to a high proportion of exposure (0.57) occurring indoors while people are in bed in Kebbi (Figure 23 & Table 10). The proportion of exposure indoors for individuals out of bed was 0.13. Overall, the proportion of vector bites occurring indoors for unprotected individuals is estimated as 0.70 (70%). The proportion of exposure prevented by using nets indoors in Kebbi was estimated as 0.16 (16%). The proportion of exposure occurring outdoors for unprotected individuals was generally low and estimated as 0.14 (14%). The proportion of exposure prevented for individuals using nets outdoors was 0.01 (1%). Overall, the proportion of exposure prevented both indoors and outdoors is estimated as 0.17 and this leaves a gap in protection of about 0.83 (83%) in Kebbi (Table 10).

In summary, most human exposure in Kebbi occurs indoors in the middle of night (11 p.m.-6 a.m.) and primarily is due to people being in bed not under nets at the time when most biting activities occurring indoors.

FIGURE 22: HUMAN BEHAVIOR PROPORTION FOR KEBBI SENTINEL SITE

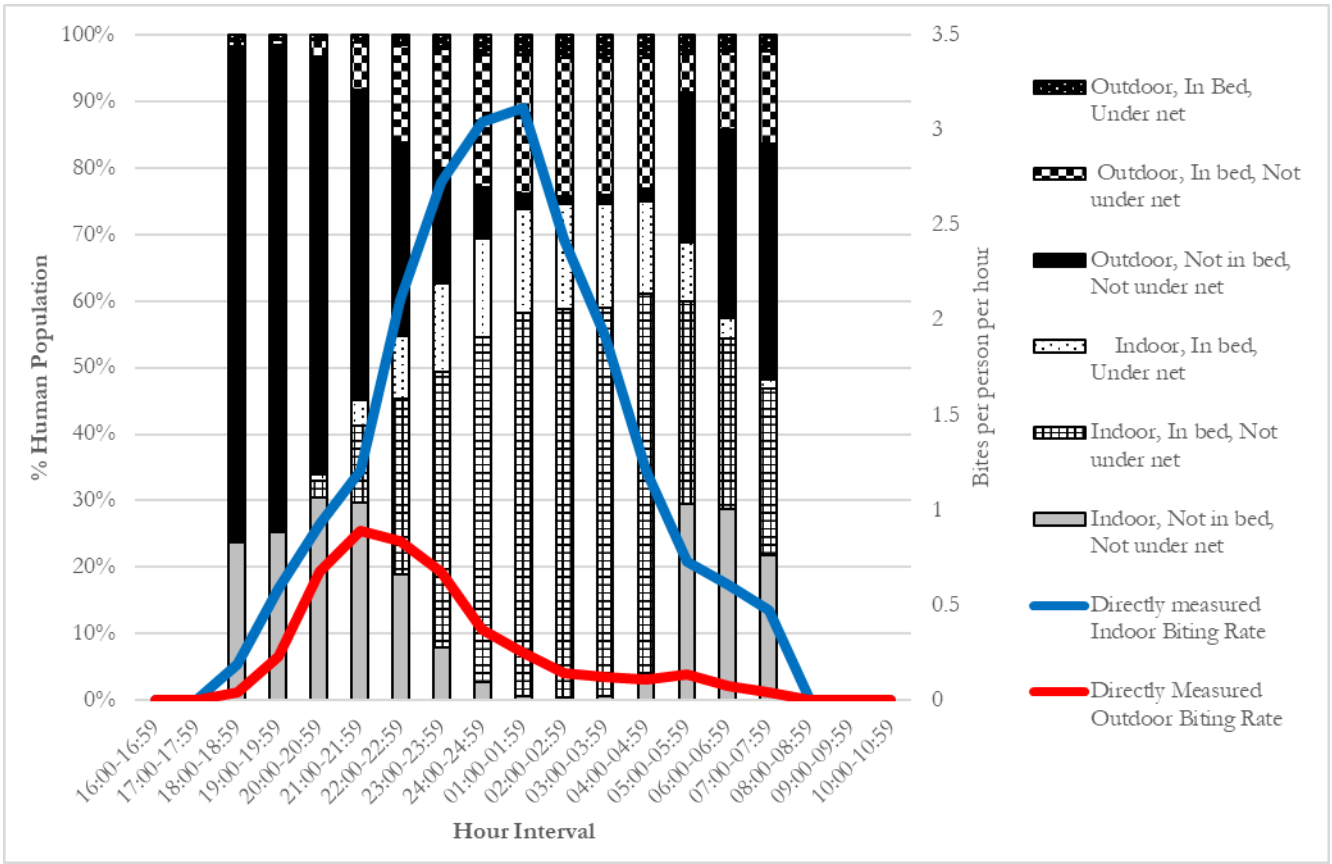
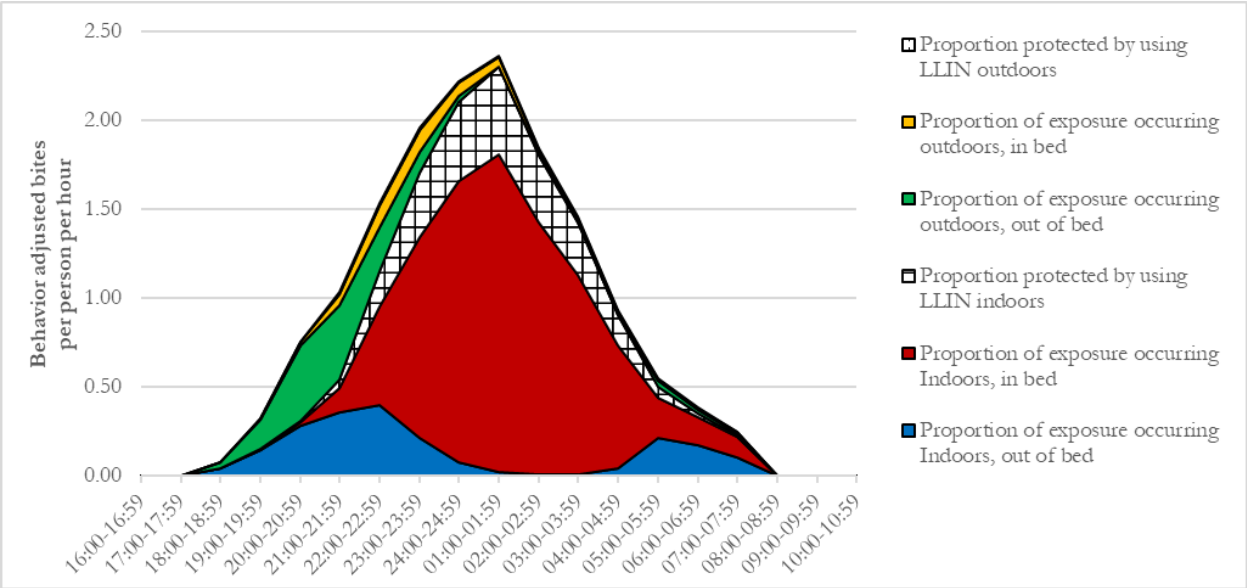


FIGURE 23: BEHAVIOR ADJUSTED BITING RATES FOR KEBBI SENTINEL SITE



2.13.2 HUMAN EXPOSURE TO MALARIA VECTORS IN SOKOTO

In Sokoto, the peak indoor biting of 1.43 b/p/h was recorded between midnight and 1 a.m. There was also significant biting occurring indoors before bedtime between 8 to 10 p.m. (1.18 – 1.20 b/p/h). The outdoor peak was between 10-11 p.m. with 0.50 b/p/h (Figure 24). Like Kebbi, most people were outdoors (not in bed) for the periods before 10 p.m. However, most people indoors were under net at the peak biting hours, though still large proportion of people indoors were sleeping without nets. At the peak indoor biting hour of midnight to 1 a.m., about 20% of the people slept under net, whereas about 13% were sleeping without nets indoor. This coupled with lower biting rate contributed to the reduced proportion of exposure indoors at bedtime as compared to Kebbi. The proportion of exposure indoors for individuals sleeping without net was 0.20, whereas the proportion of exposure occurring indoors for individuals out of bed was 0.26 (Figure 25 and Table 10). Overall, the proportion of vector bites occurring indoors for unprotected individuals is estimated as 0.46 (46%) in Sokoto. The proportion of exposure prevented by using nets indoors in Sokoto was estimated as 0.33 (33%). The proportion of exposure occurring outdoors for unprotected individuals was estimated as 0.16 (16%) and the proportion of exposure prevented for individuals using nets outdoors was about 0.05 (5%). Overall, the proportion of exposure prevented both indoors and outdoors is estimated as 0.38 and this leaves a gap in protection of about 0.62 (62%) in Sokoto (Table 10). In summary, most human exposure in Sokoto occurs indoors during early evening hours (before 10 p.m.) when most people are not protected under nets.

FIGURE 24: HUMAN BEHAVIOR PROPORTION FOR SOKOTO SENTINEL SITE

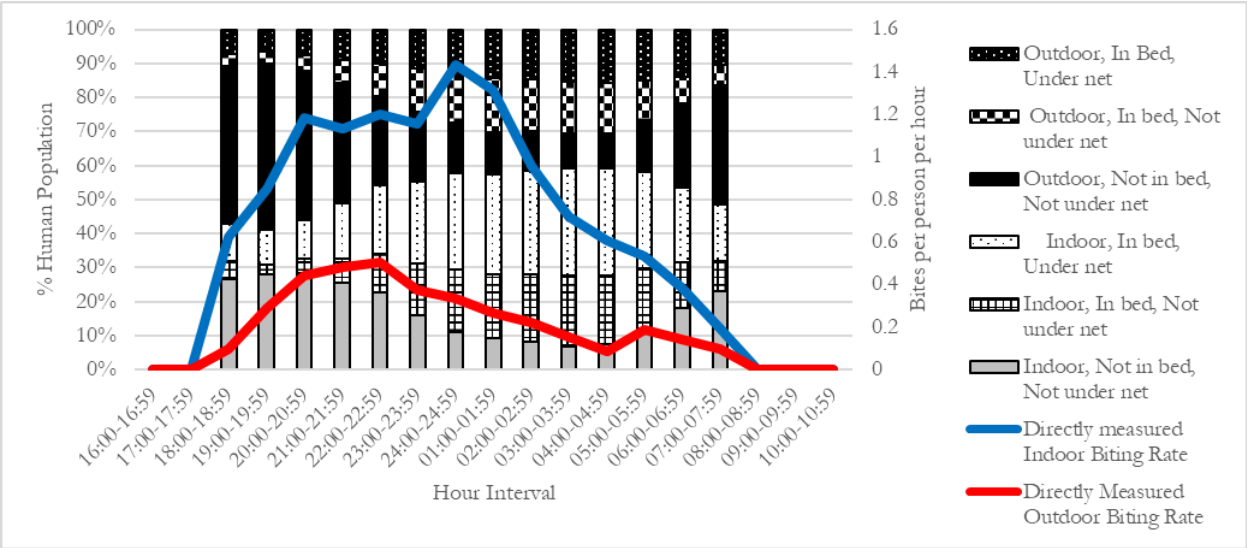


FIGURE 25: BEHAVIOR ADJUSTED BITING RATES FOR SOKOTO SENTINEL SITE

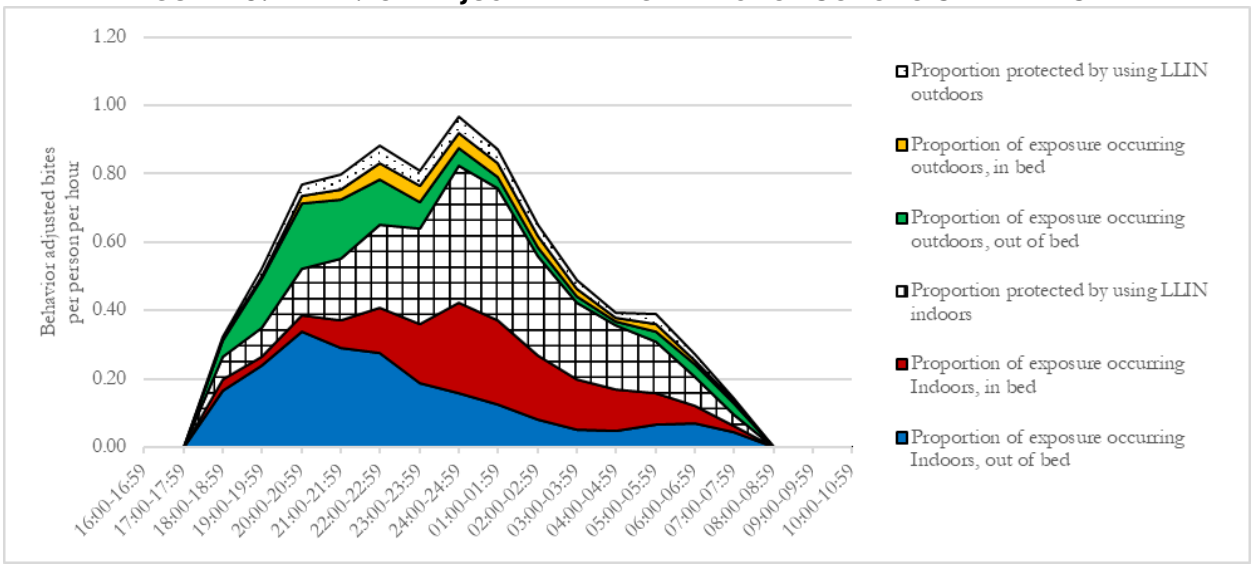


TABLE 10: SUMMARY OF THE PROPORTION OF EXPOSURE INDOORS AND OUTDOORS IN KEBBI AND SOKOTO, EXTRACTED FROM FIGURES 23 AND 25

Parameters Measured	Kebbi	Sokoto
Proportion of exposure occurring indoors, out of bed	0.13	0.26
Proportion of exposure occurring indoors, in bed	0.57	0.2
Proportion protected by using net indoors	0.16	0.33
Proportion of exposure occurring outdoors, out of bed	0.10	0.12
Proportion of exposure occurring outdoors, in bed	0.04	0.04
Proportion protected by using net outdoors	0.01	0.05
Proportion protected by using net indoors and outdoors	0.17	0.38
Gap in protection	**0.83**	**0.62**

2.13.3 Proportion of Domestic Animals Indoors and Outdoors in Kebbi and Sokoto

Keeping domestic animals such as goats and sheep both indoors and outdoors is a common practice in Kebbi and Sokoto. The mean number of domestic animals observed indoors and outdoors for the time of collections are summarized in Figures 26 and 27. A higher number of domestic animals were observed in Sokoto than Kebbi. The proportion of domestic animals was higher indoors than outdoors in Sokoto, while the opposite was observed in Kebbi. This has not resulted in a higher biting rate in Sokoto and may also be unlikely to have an impact on the proportions of human exposures reported in these locations.

Figure 26: Average Number of Domestic Animals Indoors and Outdoors in Kebbi

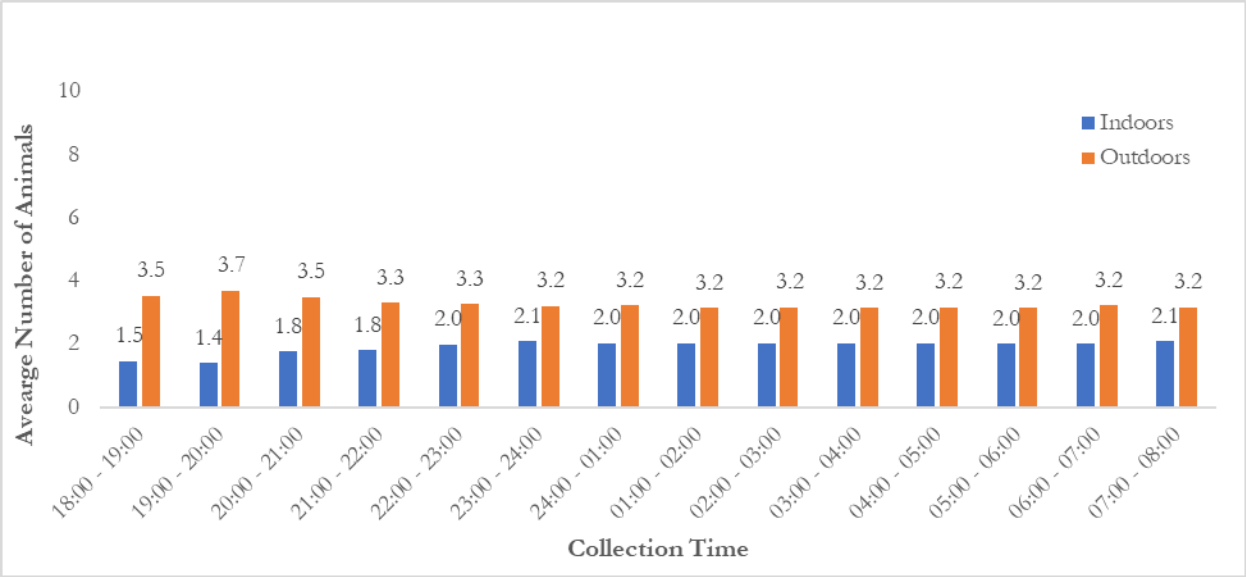
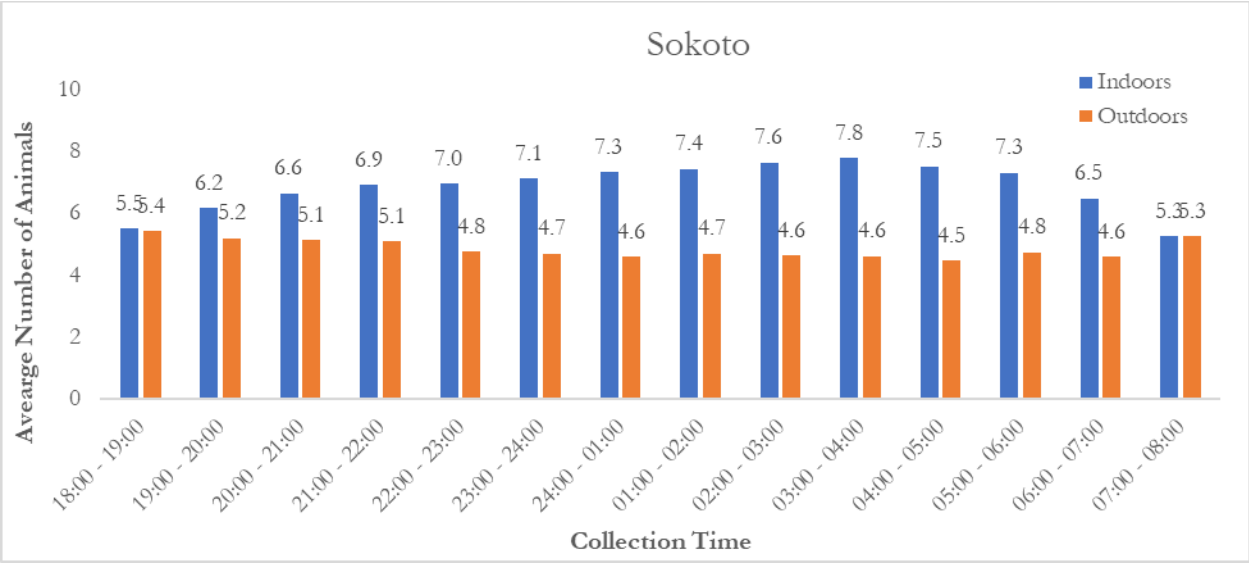


Figure 27: Average Number of Domestic Animals Indoors and Outdoors in Sokoto



2.14 INSECTICIDE SUSCEPTIBILITY AND MECHANISMS OF RESISTANCE

Insecticide susceptibility test results indicated that pyrethroid resistance was widespread in *An. gambiae* s.l. mosquitoes at all sentinel sites (Table 11). Pyrethroid resistance patterns varied within and among the states.

Full susceptibility of *An. gambiae* s.l. populations exposed to alpha-cypermethrin was recorded in all LGAs in Bayelsa, Benue, Cross River, FCT, Oyo, Sokoto, and Zamfara. Susceptibility to alpha-cypermethrin was only observed in 1/6 LGAs in Bauchi and Enugu, 2/6 LGAs in Kaduna, and 7/9 LGAs in Kebbi. No susceptibility to alpha-cypermethrin was observed in *An. gambiae* s.l. mosquitoes from Akwa Ibom, Ebonyi, Nasarawa, or Plateau (Table 11).

Anopheles gambiae s.l. were susceptible to deltamethrin in all LGAs in Benue, Cross River, Kebbi, Oyo, and Zamfara. Susceptibility to deltamethrin was observed in *An. gambiae* s.l. populations in 2/6 LGAs in Bauchi, 1/3 LGAs in Bayelsa, 1/6 LGAs in Enugu, 2/3 LGAs in FCT, 3/6 LGAs in Nasarawa, and 2/9 LGAs in Sokoto. No susceptibility to deltamethrin was observed in *An. gambiae* s.l. populations from Akwa Ibom, Ebonyi, Kaduna, and Plateau (Table 11).

Resistance to permethrin was recorded in *An. gambiae* s.l. populations across all states except in 2/3 LGAs in Bayelsa, 1/6 LGAs in Kaduna, and 4/9 LGAs in Sokoto. Among the states where resistance was recorded in all LGAs, possible resistance was recorded in 1/6 LGAs in Bauchi and 2/6 LGAs in Zamfara (Table 11).

The vector was not susceptible to any pyrethroids in any LGAs of Akwa Ibom, Ebonyi, and Plateau (Table 11).

Anopheles gambiae s.l. mosquitoes showed susceptibility to pirimiphos-methyl in all LGAs in Akwa Ibom, Bauchi, Bayelsa, FCT, Cross River, Ebonyi, Enugu, Kaduna, Nasarawa, Kebbi, Sokoto, and Zamfara. Resistance to pirimiphos-methyl was observed in *An. gambiae* s.l. mosquitoes from one LGA in Oyo (Ibarapa North) and possible resistance in one LGA in Benue (Tarka) (Table 11).

TABLE 11: CDC BOTTLE BIOASSAY TEST RESULTS FOR *AN. GAMBIAE* S.L. IN 2022

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Alpha-cypermethrin		Deltamethrin		Permethrin		Pirimiphos-methyl	
Sentinel Site	LGA	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status
Akwa Ibom	Abak	79%	R	90%	PR	31%	R	100%	S
	Itu	75%	R	88%	R	18%	R	98%	S
	Mkpat Enin	78%	R	86%	R	22%	R	100%	S
	Nsit Ubium	82%	R	91%	PR	32%	R	99%	S
	Onna	81%	R	89%	R	29%	R	99%	S
	Ukanafun	76%	R	89%	R	31%	R	100%	S
Bauchi	Darazo	87%	R	82%	R	89%	R	99%	S
	Dass	78%	R	80%	R	86%	R	100%	S
	Itas/Gadau	91%	PR	98%	S	91%	PR	98%	S
	Katagun	98%	S	98%	S	88%	R	98%	S
	Ningi	82%	R	85%	R	89%	R	100%	S
	Toro	79%	R	82%	R	86%	R	100%	S
Bayelsa	Ogbia	99%	S	97%	PR	98%	S	100%	S
	Sagbama	98%	S	98%	S	99%	S	100%	S
	Yenagoa	100%	S	92%	PR	93%	PR	100%	S
Benue	Apa	100%	S	99%	S	85%	R	100%	S
	Gwer	100%	S	98%	S	87%	R	99%	S
	Obi	100%	S	99%	S	88%	R	98%	S
	Tarka	99%	S	99%	S	86%	R	97%	PR
	Ukum	100%	S	99%	S	82%	R	99%	S
	Vandeikya	100%	S	99%	S	83%	R	99%	S

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Alpha-cypermethrin		Deltamethrin		Permethrin		Pirimiphos-methyl	
Sentinel Site	LGA	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status
Cross River	Biase	100%	S	100%	S	59%	R	100%	S
	Boki	100%	S	100%	S	48%	R	99%	S
	Calabar South	100%	S	100%	S	49%	R	100%	S
	Obanliku	100%	S	100%	S	73%	R	100%	S
	Obubra	100%	S	99%	S	53%	R	100%	S
	Yala	100%	S	100%	S	56%	R	99%	S
Ebonyi	Abakaliki	81%	R	84%	R	34%	R	100%	S
	Ebonyi	82%	R	85%	R	40%	R	100%	S
	Ezza North	95%	PR	89%	R	30%	R	100%	S
	Ezza South	79%	R	81%	R	35%	R	100%	S
	Izzi	80%	R	78%	R	37%	R	100%	S
	Ohaozara	86%	R	91%	PR	36%	R	100%	S
Enugu	Awgu	86%	R	100%	S	43%	R	100%	S
	Enugu-East	100%	S	89%	R	49%	R	100%	S
	Enugu-South	85%	R	71%	R	80%	R	100%	S
	Isi-Uzo	39%	R	40%	R	NA*	NA*	100%	S
	Nsukka	86%	R	76%	R	71%	R	100%	S
	Udi	89%	R	89%	PR	57%	R	100%	S
FCT	AMAC	99%	S	69%	R	0%	R	98%	S
	Gwagwalada	100%	S	99%	S	54%	R	100%	S
	Kwali	100%	S	100%	S	49%	R	100%	S
Kaduna	Giwa	98%	S	68%	R	52%	R	99%	S
	Igabi	47%	R	71%	R	64%	R	100%	S
	Jema'a	72%	R	40%	R	60%	R	98%	S
	Lere	99%	S	92%	PR	98%	S	100%	S
	Makarfi	70%	R	65%	R	55%	R	99%	S
	Sabon Gari	92%	PR	77%	R	72%	R	99%	S
Kebbi	Argungu	100%	S	100%	S	78%	R	100%	S
	Augie	100%	S	100%	S	72%	R	100%	S
	Bunza	99%	S	100%	S	80%	R	100%	S
	Fakai	94%	PR	100%	S	85%	R	100%	S
	Gwandu	99%	S	100%	S	83%	R	100%	S
	Jega	98%	S	100%	S	73%	R	100%	S
	Kalgo	99%	S	100%	S	78%	R	100%	S
	Maiyama	99%	S	100%	S	76%	R	100%	S
	Suru	97%	PR	100%	S	84%	R	100%	S
Nasarawa	Karu	96%	PR	98%	S	54%	R	100%	S
	Keffi	87%	R	96%	PR	46%	R	99%	S
	Kokona	92%	PR	98%	S	38%	R	99%	S
	Nasarawa	96%	PR	97%	PR	44%	R	100%	S
	Nasarawa Eggon	91%	PR	97%	PR	44%	R	100%	S
	Obi	83%	R	99%	S	36%	R	100%	S
Oyo	Akinyele	100%	S	100%	S	9%	R	100%	S
	Atiba	100%	S	100%	S	11%	R	100%	S
	Ibarapa North	100%	S	100%	S	77%	R	80%	R
	Itesiwaju	100%	S	100%	S	69%	R	100%	S
	Saki West	100%	S	100%	S	8%	R	100%	S
	Surulere	100%	S	99%	S	33%	R	100%	S

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Alpha-cypermethrin		Deltamethrin		Permethrin		Pirimiphos-methyl	
Sentinel Site	LGA	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status
Plateau	Bokkos	88%	R	88%	R	71%	R	99%	S
	Jos-east	92%	PR	87%	R	72%	R	100%	S
	Jos-south	88%	R	87%	R	54%	R	100%	S
	Kanam	67%	R	84%	R	66%	R	100%	S
	Mangu	89%	R	86%	R	71%	R	100%	S
	Pankshin	88%	R	84%	R	68%	R	100%	S
Sokoto	Bodinga	100%	S	100%	S	82%	R	100%	S
	Gudu	100%	S	86%	R	100%	S	100%	S
	Kware	100%	S	91%	PR	81%	R	100%	S
	Rabah	100%	S	87%	R	76%	R	100%	S
	Shagari	100%	S	99%	S	100%	S	100%	S
	Sokoto North	100%	S	94%	PR	100%	S	100%	S
	Sokoto South	100%	S	96%	PR	100%	S	100%	S
	Tambuwal	100%	S	79%	R	76%	R	100%	S
	Wamakko	100%	S	85%	R	94%	PR	100%	S
Zamfara	Bakura	100%	S	100%	S	94%	PR	100%	S
	Birnin Magaji	100%	S	100%	S	89%	R	100%	S
	Bungudu	100%	S	100%	S	67%	R	100%	S
	Gummi	100%	S	100%	S	69%	R	100%	S
	Maradun	100%	S	100%	S	89%	R	100%	S
	Maru	100%	S	100%	S	90%	PR	100%	S

S = Susceptible, R = Resistant, PR = Possibly Resistant. Note: Diagnostic time is 30 minutes. A minimum of 100 mosquitoes were exposed each insecticide.
*Not tested due to insecurity.

2.15 INSECTICIDE RESISTANCE INTENSITY

Insecticide resistance intensity in the vector populations were determined by subjecting *Anopheles* mosquitoes across the different ecozones to insecticide resistance test assays with different concentrations of three pyrethroids. *Anopheles gambiae* s.l. was susceptible to 1X alpha-cypermethrin across all LGAs in Bayelsa, Benue, Cross River, FCT, Oyo, Sokoto, and Zamfara (Figures 30, 31, 32, 35, 39, 41, and 42). Susceptibility was also recorded in 1/5 LGAs in Enugu, 2/6 LGAs in Kaduna, and 7/9 LGAs in Kebbi (Figures 34, 36, and 37). *Anopheles gambiae* s.l. mosquitoes that were resistant to alpha-cypermethrin at 1X concentration in Bauchi and Enugu (5/6 LGAs) and Ebonyi (all six LGAs) became susceptible at 2X concentration (Figures 29, 34, and 33) indicating resistance was only at the diagnostic dose. *Anopheles gambiae* s.l. showed low resistance intensity (mortality between 98-100% at 5X dose) to alpha-cypermethrin in all six LGAs in Akwa Ibom and Plateau (Figures 28 and 40).

Complete susceptibility of *An. gambiae* s.l. to 1X deltamethrin was recorded in all LGAs in Benue, Cross River, Kebbi, Oyo, and Zamfara (Figures 31, 32, 37, 39, and 42). Susceptibility was also recorded in 2/6 LGAs in Bauchi, 1/3 LGAs in Bayelsa, 2/3 LGAs in FCT, 3/6 LGAs in Nasarawa, and 1/9 LGAs in Sokoto (Figures 29, 31, 38, and 41). Susceptibility to deltamethrin at 2X concentration was observed in *An. gambiae* s.l. populations from all LGAs in Akwa Ibom, Bauchi, Ebonyi, Enugu, Kaduna, Nasarawa, and Sokoto (Figures 28, 29, 33, 34, 36, 38, and 41). Low intensity of resistance to deltamethrin (mortality between 98-100% at 5X dose) was recorded in 1/3 LGAs in Bayelsa and FCT, 2/6 LGAs in Kaduna, and all six LGAs of Plateau (Figures 30, 35, 36, and 40).

Complete susceptibility to permethrin at 1X was recorded in 2/3 LGAs of Bayelsa, 1/6 LGAs in Kaduna, and 4/6 LGAs of Sokoto (Figures 30, 36, and 41). Complete susceptibility of *An. gambiae* s.l. was observed at 2X permethrin concentration test across all six LGAs in Bauchi, Benue, and Zamfara, and in 1/6 LGAs in Enugu

and Kaduna where resistance to 1X concentration were recorded (Figures 29, 31, 34, 36, and 42).

Low permethrin resistance intensity (mortality between 98–100% at 5X dose) was recorded in *An. gambiae* s.l. from 1/3 LGAs in Bayelsa, 5/6 LGAs in Cross River, 2/6 LGAs in Enugu, 1/3 LGAs in FCT, 4/6 LGAs in Kaduna, 4/9 LGAs in Kebbi, all LGAs in Oyo, and 2/6 LGAs in Plateau (Figures 30, 32, 34-37, and 39-40).

Moderate permethrin resistance intensity (mortality greater or equal to 98% at 10X dose) was observed in *An. gambiae* s.l. populations from all six LGAs in Akwa Ibom and Nasarawa (Figures 28 and 38) and 1/6 LGAs in Cross River, 3/6 LGAs in Ebonyi, 2/3 LGAs in FCT, 5/9 LGAs in Kebbi, and 3/6 LGAs in Plateau (Figures 32, 33, 35, 37, and 40).

High permethrin resistance intensity (mortality less than 98% at 10X dose) was recorded in 3/6 LGAs in Ebonyi and 1/6 LGA in Plateau (Figures 33 and 40).

FIGURE 28: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT AKWA IBOM

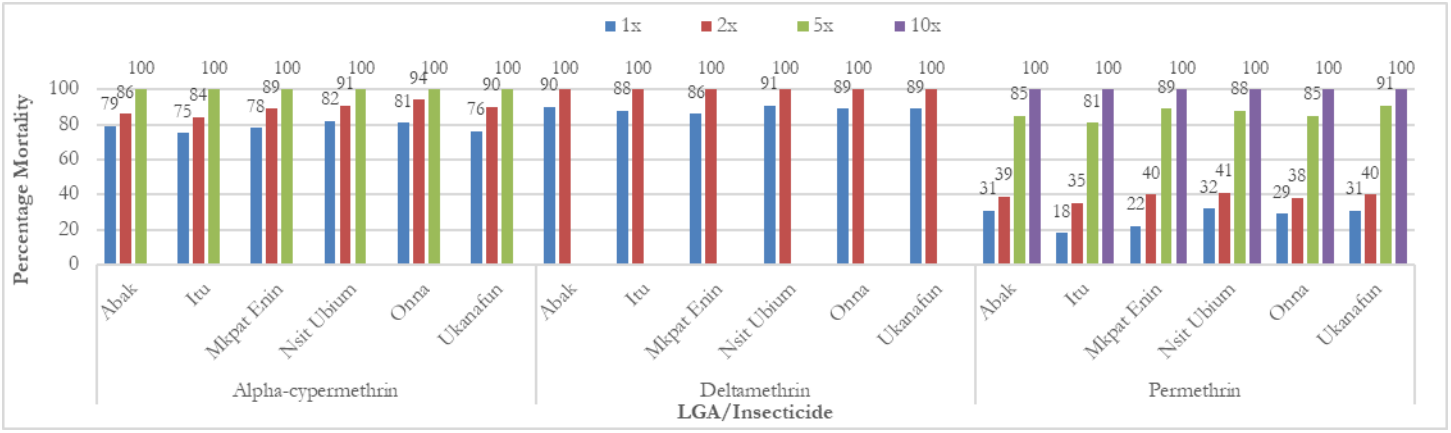


FIGURE 29: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT BAUCHI

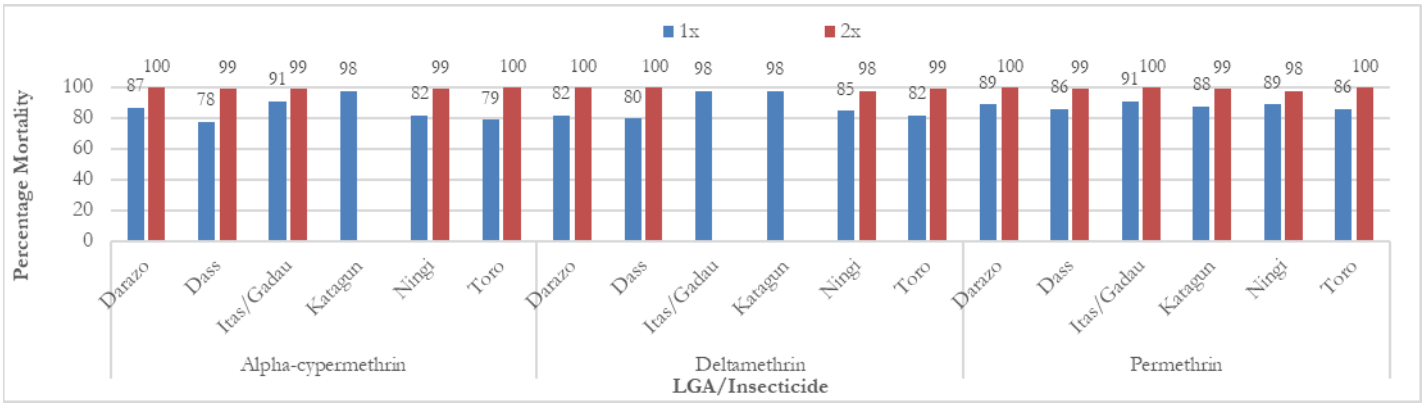


FIGURE 30: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT BAYELSA

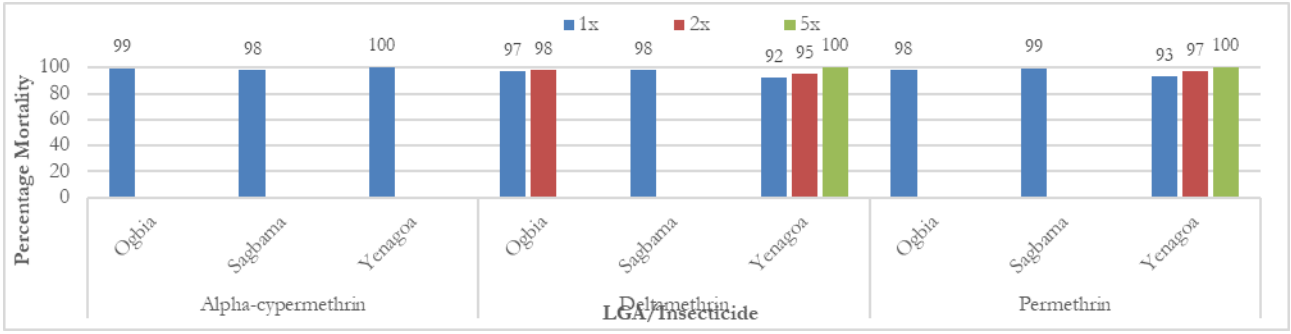


FIGURE 31: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT BENUE

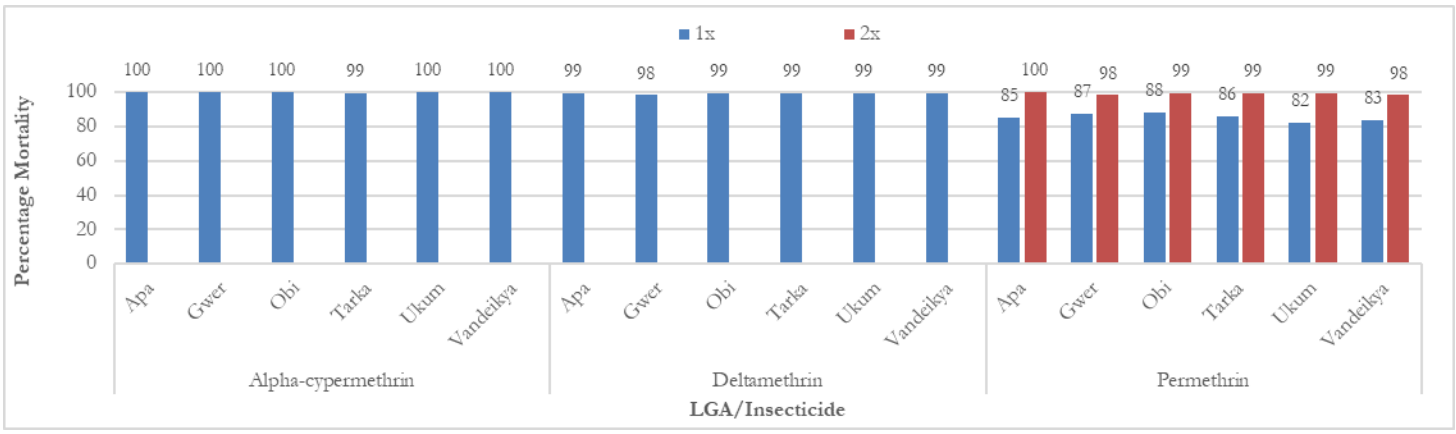


FIGURE 32: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT CROSS RIVER

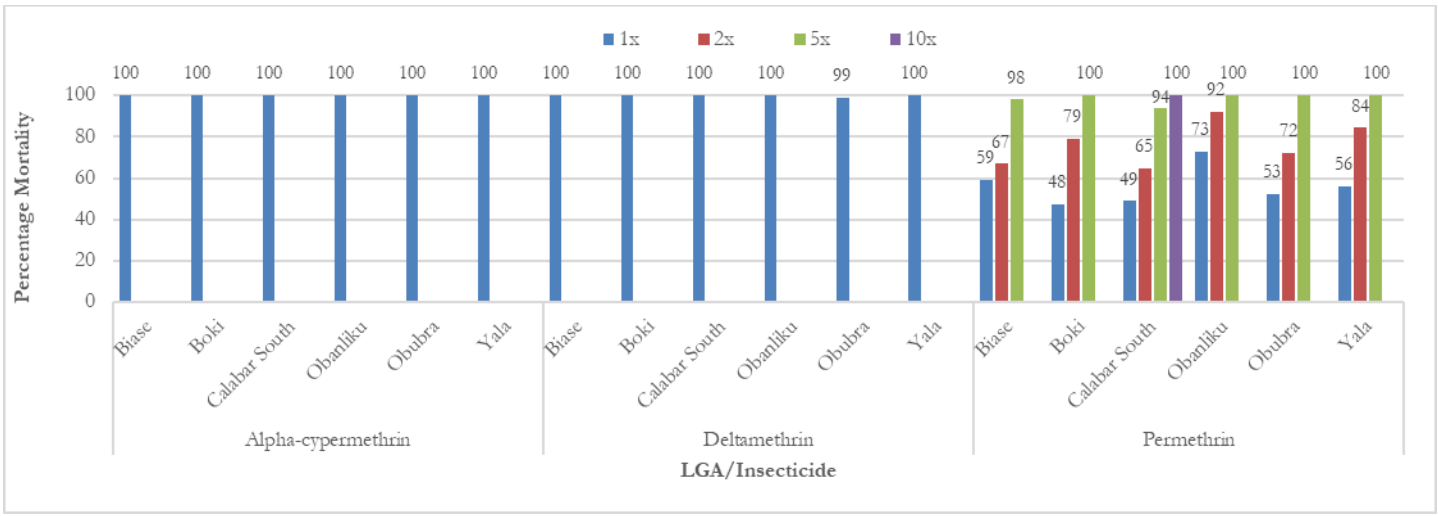


FIGURE 33: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT EBONYI

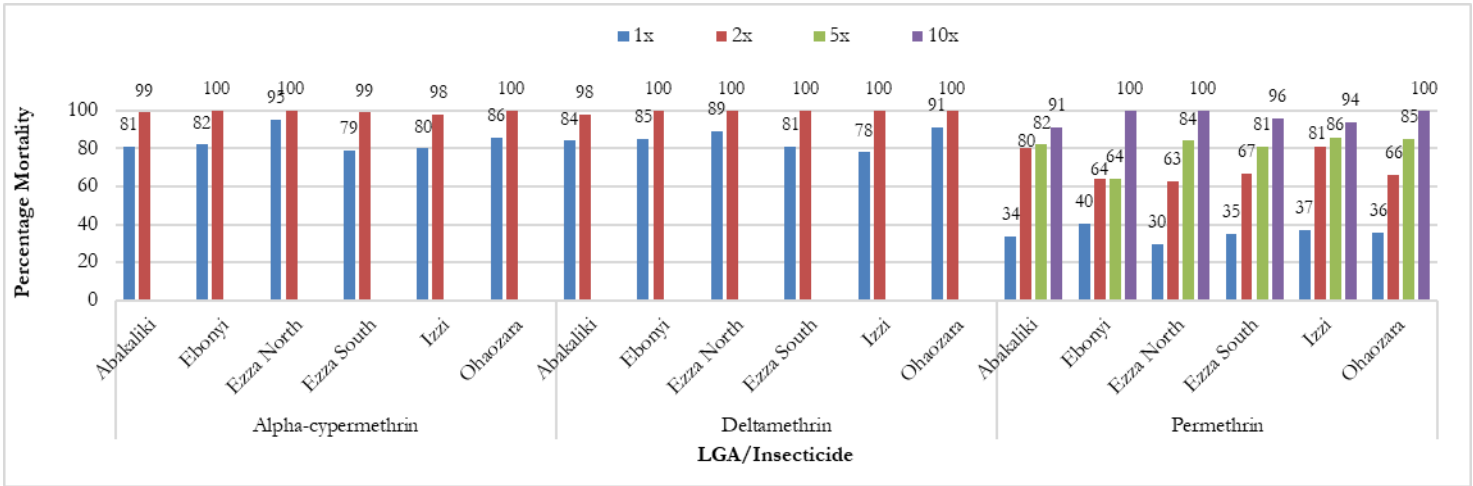


FIGURE 34: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT ENUGU

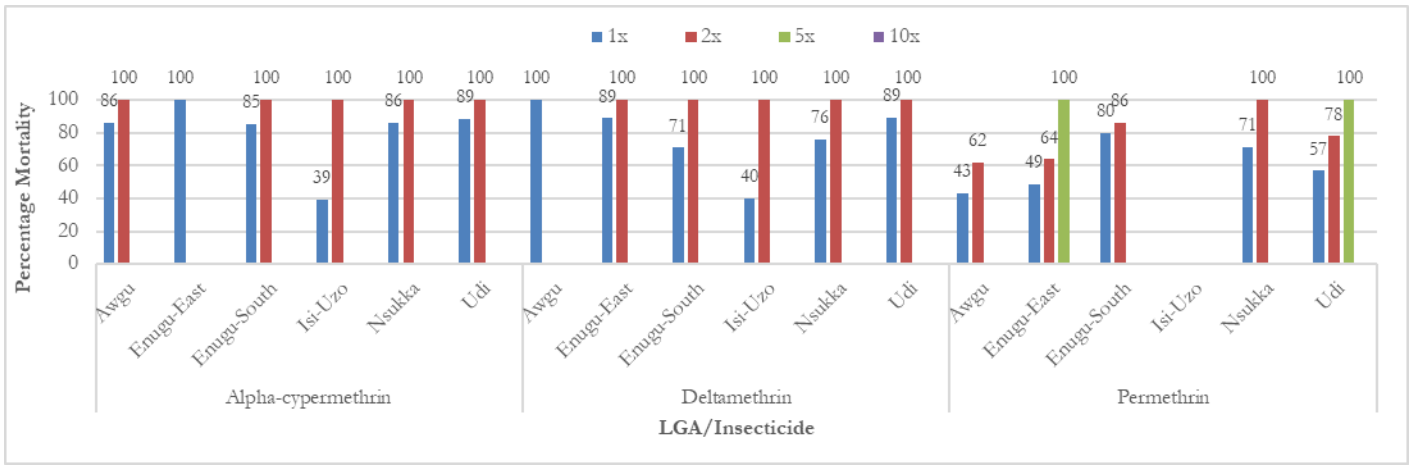


FIGURE 35: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT FCT

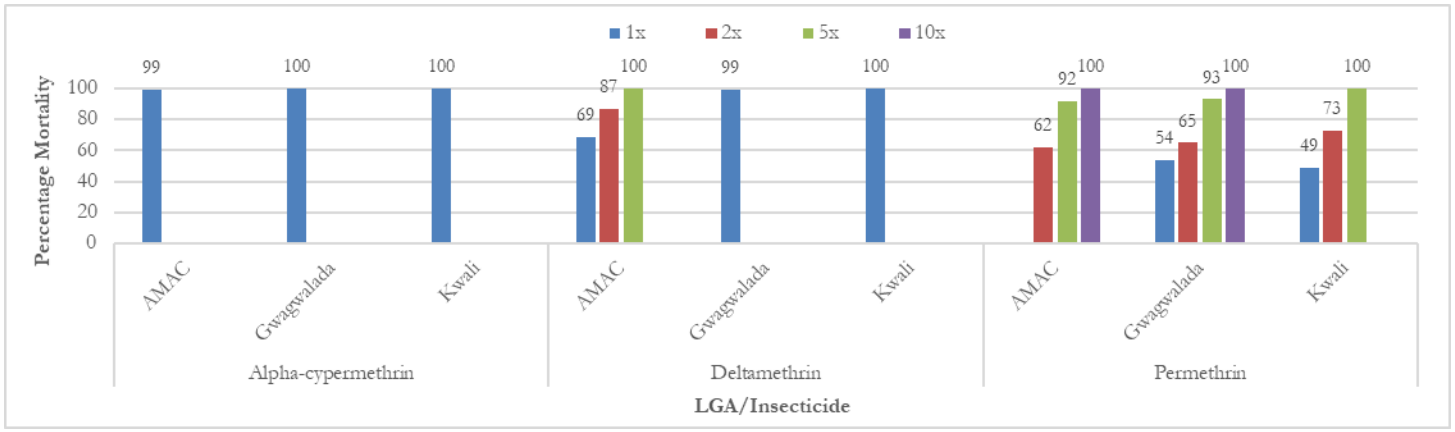


FIGURE 36: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT KADUNA

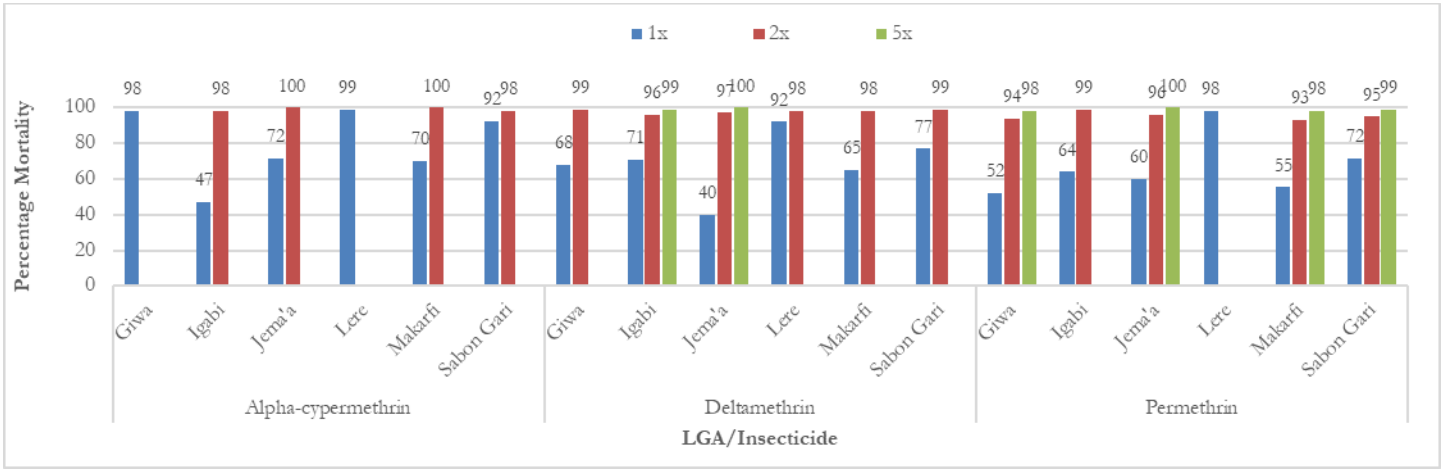


FIGURE 37: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT KEBBI

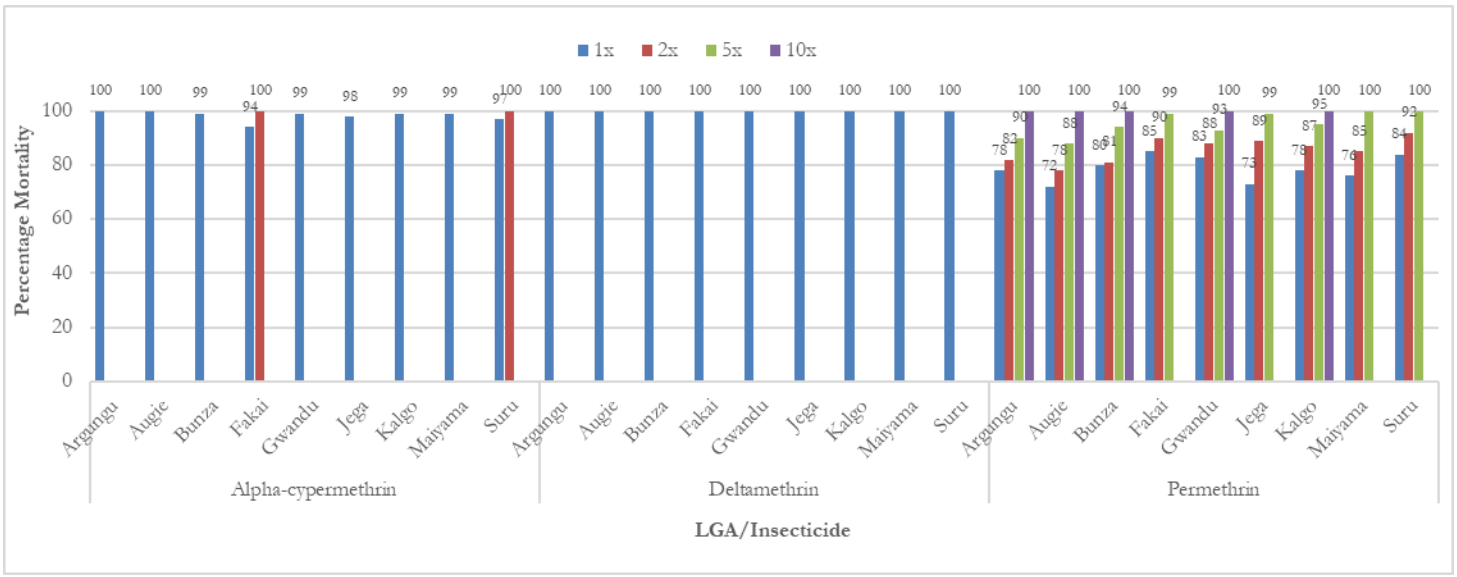


FIGURE 38: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT NASARAWA

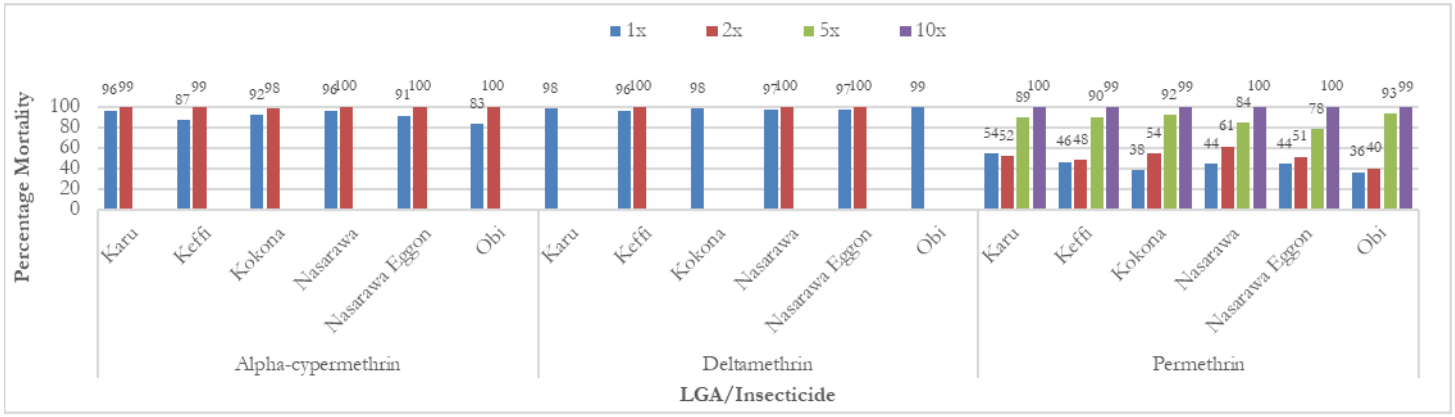


FIGURE 39: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT OYO

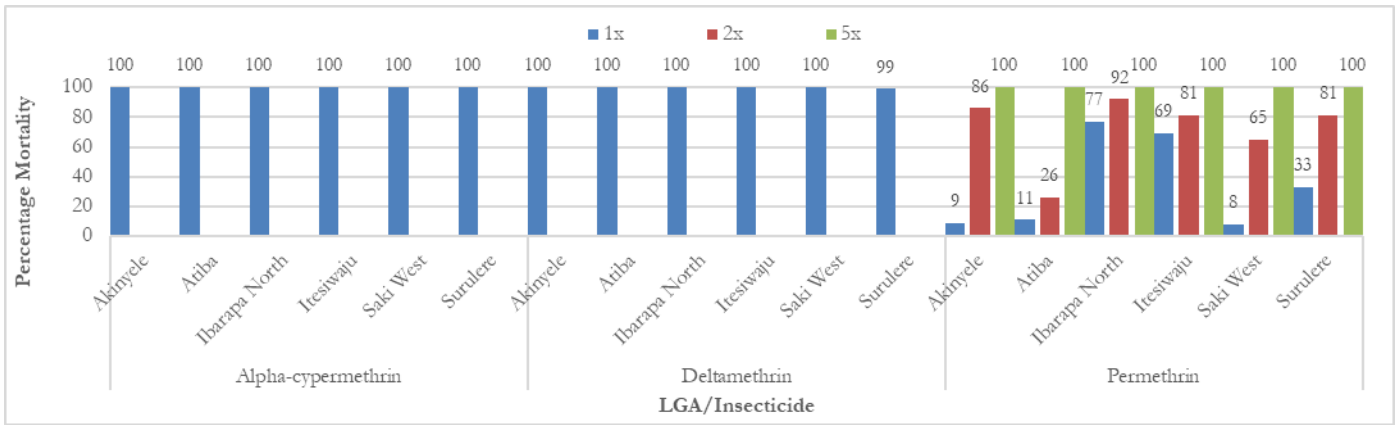


FIGURE 40: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT PLATEAU

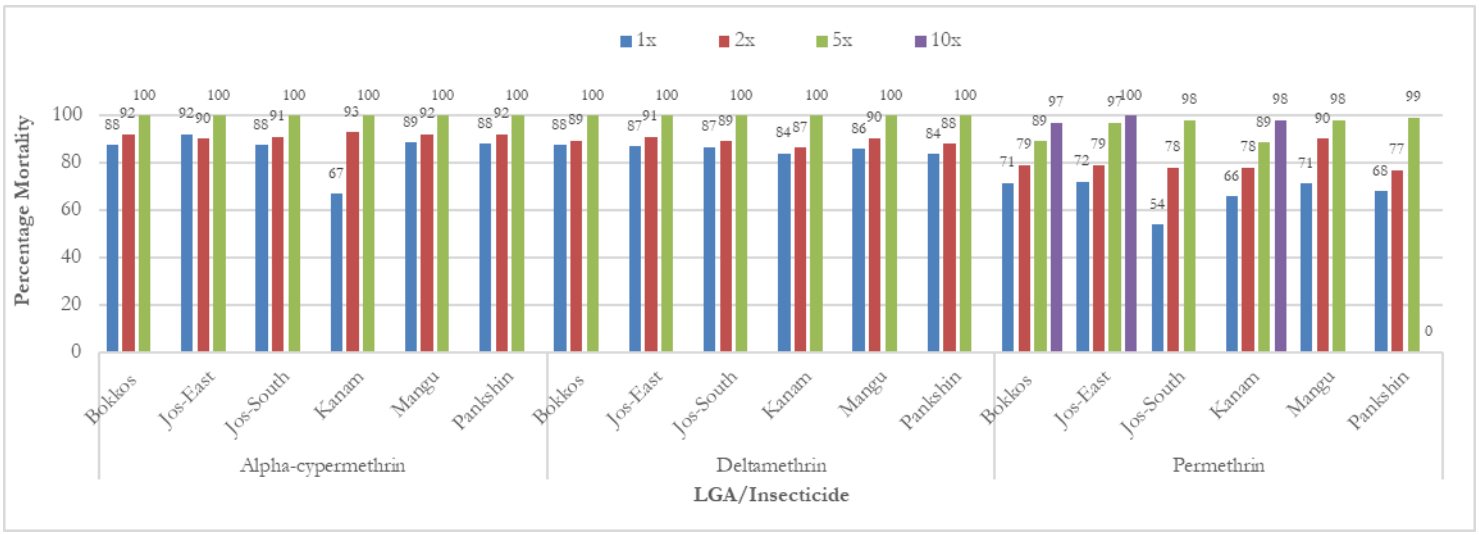


FIGURE 41: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT SOKOTO

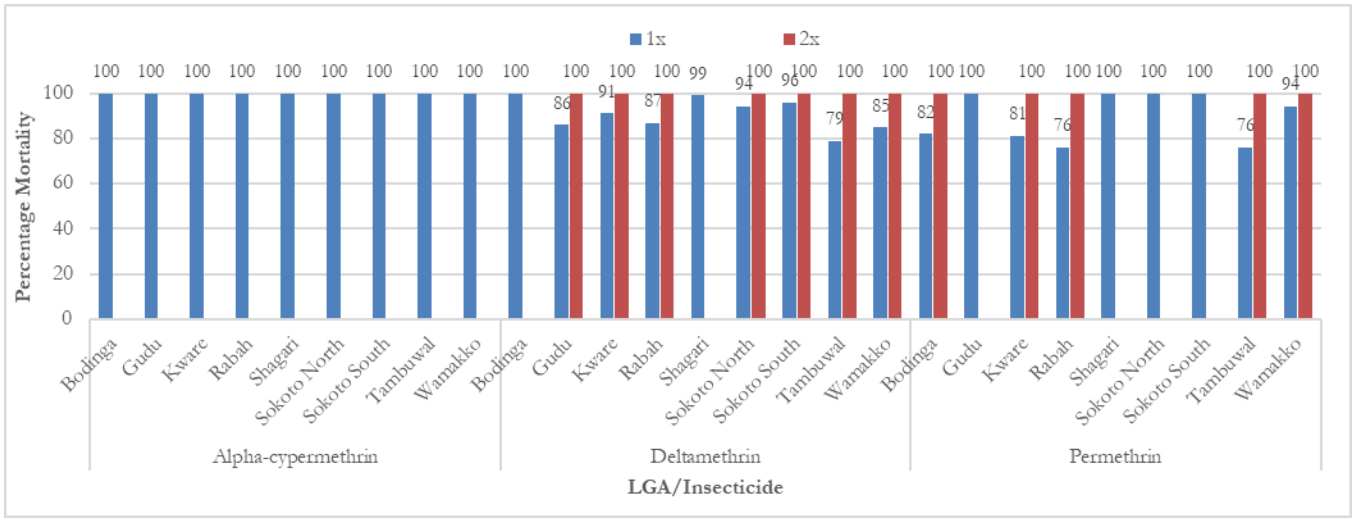
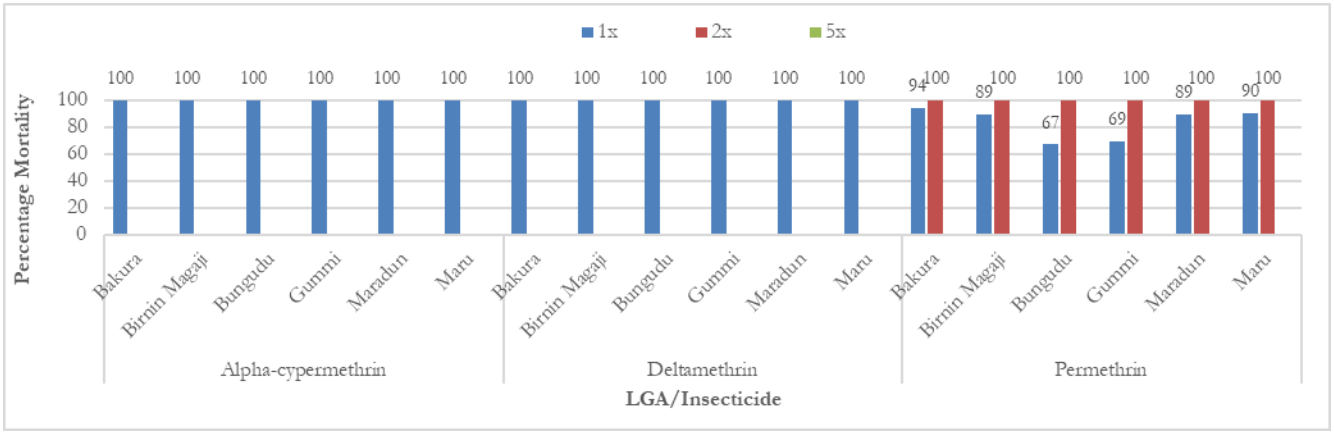


FIGURE 42: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT ZAMFARA



2.16 SYNERGIST ASSAYS

Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO synergist before exposure to pyrethroids (alpha-cypermethrin, deltamethrin, and permethrin) increased mortality at varying degrees across sites (Figures 41–55). In cases where full susceptibility (mortality greater than or equal to 98%) was not restored with PBO exposure, it suggests the existence of mechanisms unrelated to the activity of mixed function oxidases.

Where tested, susceptibility to alpha-cypermethrin was fully restored in *An. gambiae* s.l. mosquitoes pre-exposed to PBO in all LGAs of Ebonyi, Enugu, Kebbi, and Nasarawa (Figures 48, 49, 52, and 53). Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO did not affect susceptibility to alpha-cypermethrin in 3/6 LGAs in Akwa Ibom, 1/6 LGAs in Bauchi, and 2/4 LGAs Kaduna (Figures 43, 44, 51). Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO did not restore susceptibility to alpha-cypermethrin across any LGAs in Plateau (Figure 55).

Where tested, PBO restored susceptibility of *An. gambiae* s.l. mosquitoes to deltamethrin across all sites in Akwa Ibom, Bayelsa, Ebonyi, FCT, Nasarawa, and Sokoto (Figures 43, 45, 46, 50, 53, and 57). Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO restored susceptibility to deltamethrin in 1/5 LGAs of Bauchi, 4/5 LGAs in Enugu, and 2/6 LGAs in Kaduna (Figures 44, 49, and 52). Across all six LGAs of Plateau, pre-exposure of *An. gambiae* s.l. mosquitoes to PBO did not restore susceptibility to deltamethrin (Figure 55).

It was also observed that susceptibility to permethrin was fully restored in *An. gambiae* s.l. populations tested in one LGA from Bayelsa, and all LGAs in Ebonyi and Sokoto (Figures 44, 48, and 56). Susceptibility to permethrin after pre-exposure to PBO was observed in 3/6 LGAs in both Bauchi and Zamfara, 4/5 LGAs in Enugu, and 1/3 LGAs in FCT (Figures 44, 49-50, and 57). PBO did not fully restore susceptibility in *An. gambiae* s.l. populations in any LGAs in Akwa Ibom, Benue, Cross River, Kaduna, Kebbi, Nasarawa, Oyo, and Plateau (Figures 43, 46, 47, and 51-55).

FIGURE 43: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM AKWA IBOM

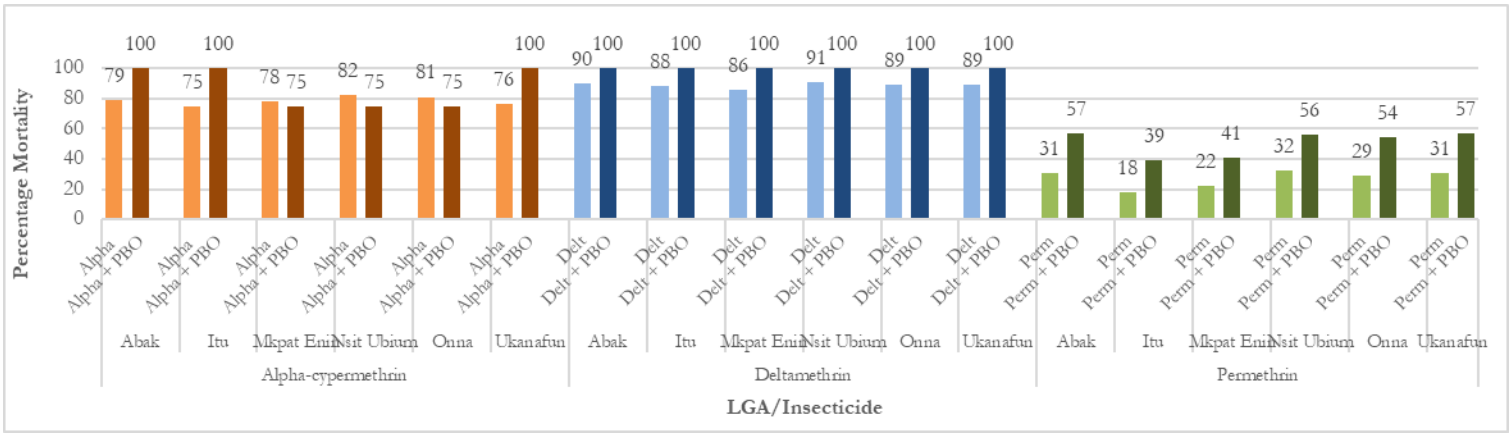


FIGURE 44: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM BAUCHI

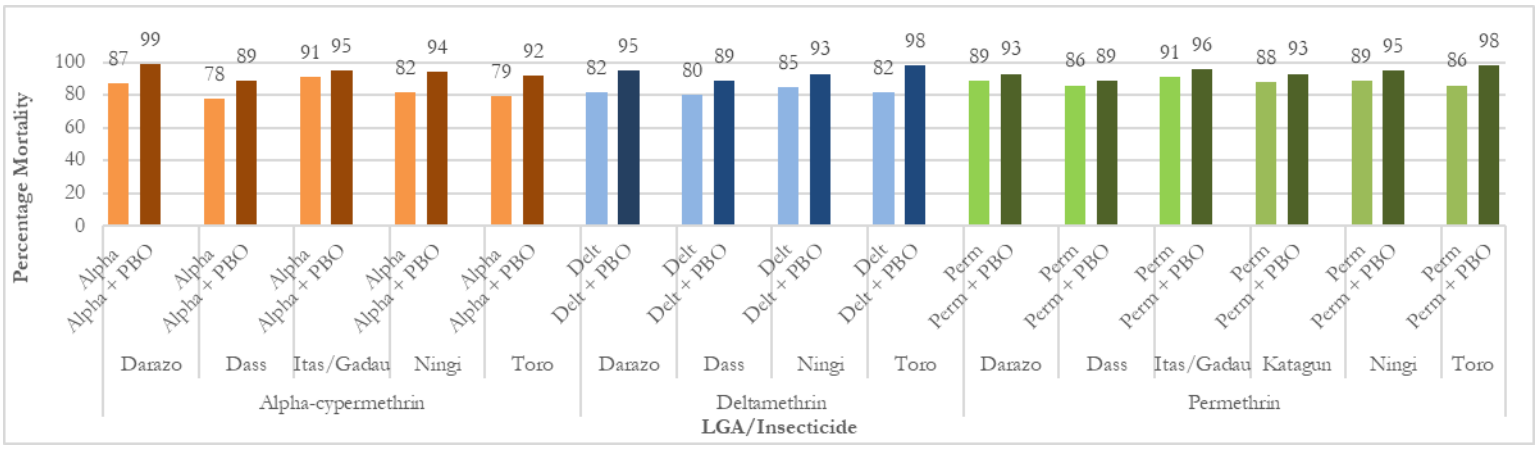


FIGURE 45: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM BAYELSA

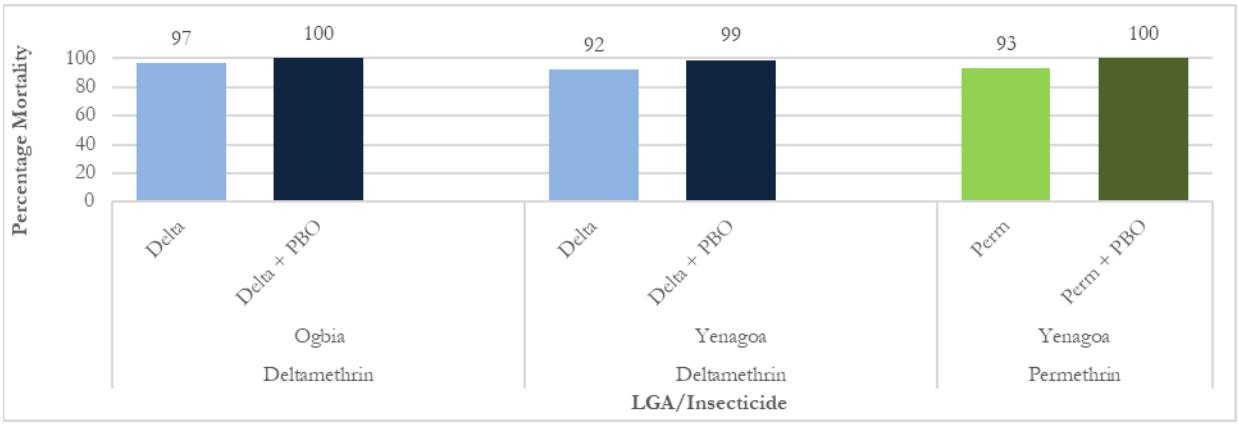


FIGURE 46: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM BENUE

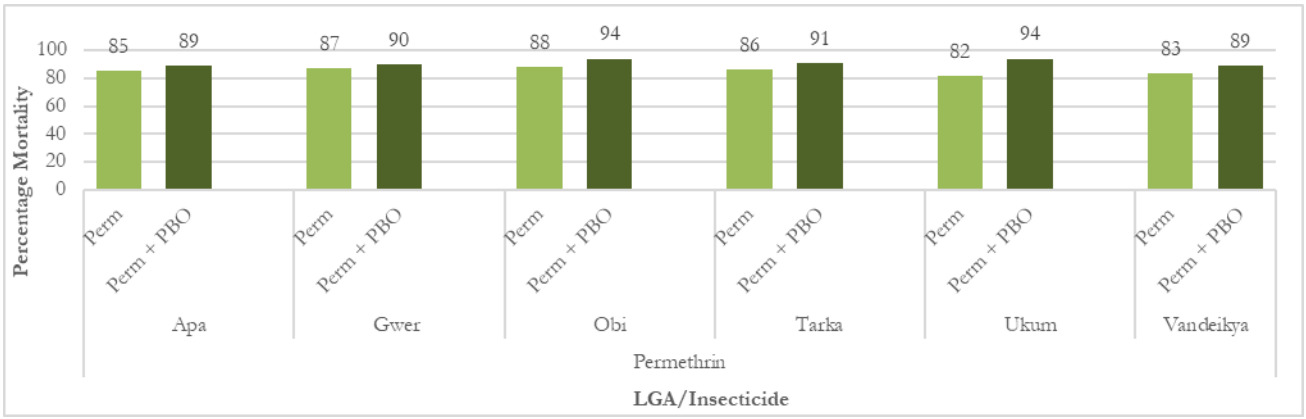


FIGURE 47: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM CROSS RIVER

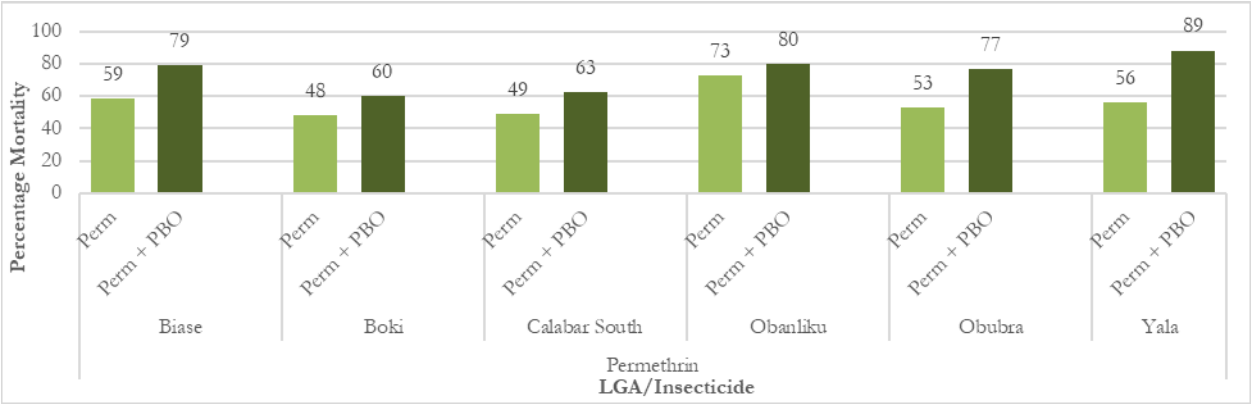


FIGURE 48: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM EBONYI

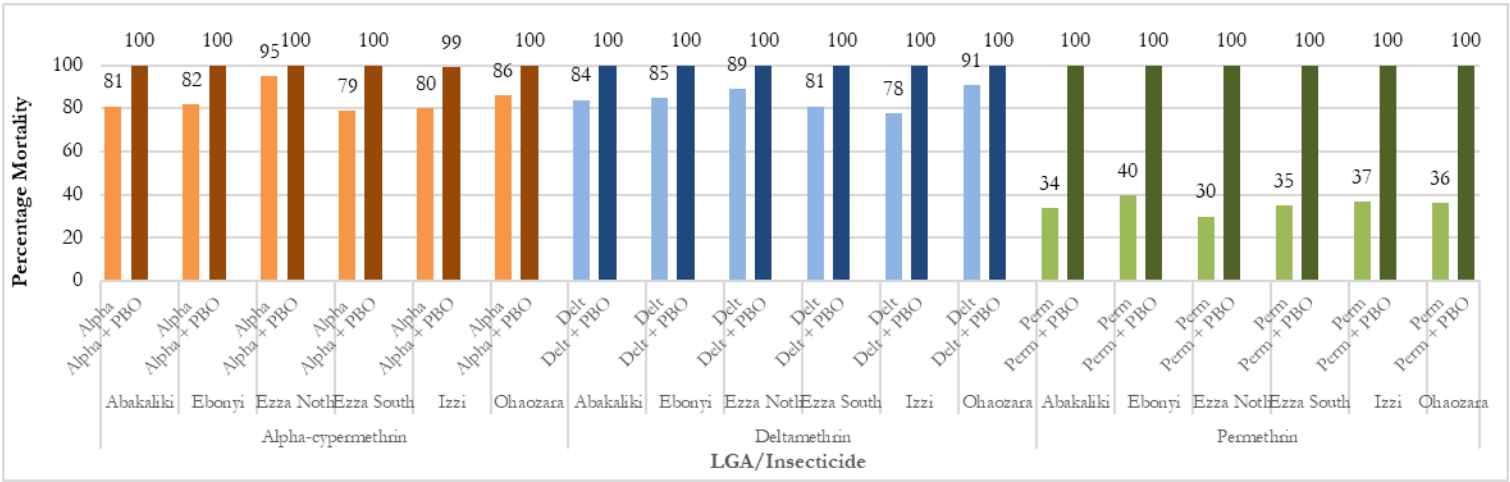


FIGURE 49: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM ENUGU

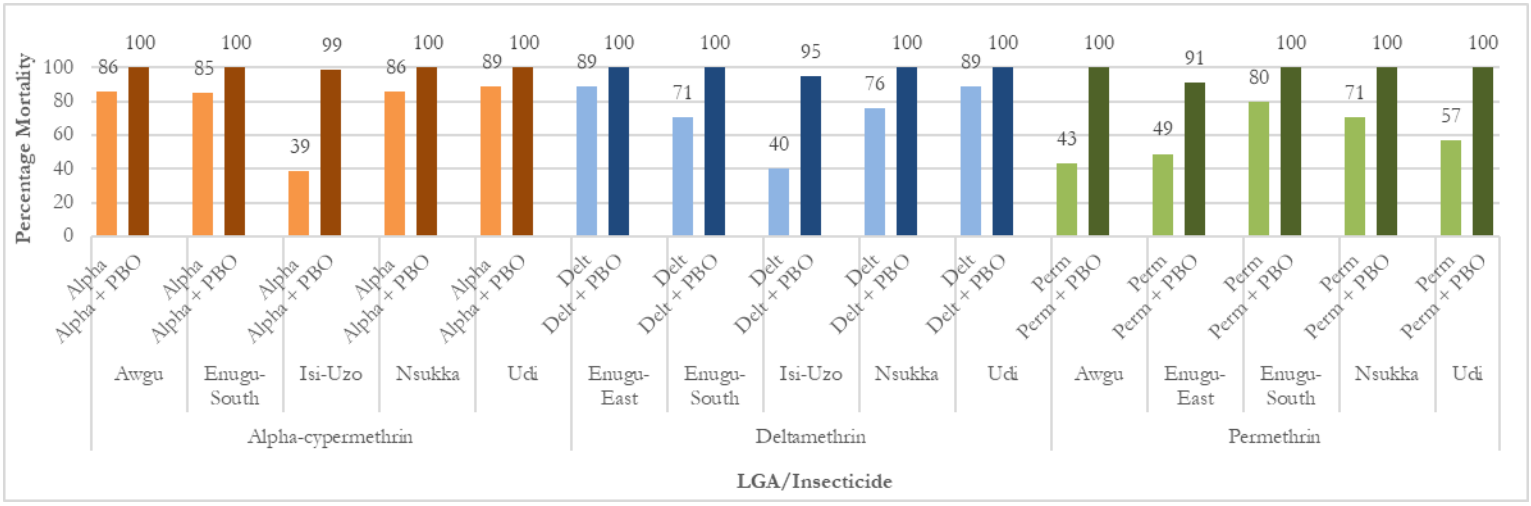


FIGURE 50: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM FCT

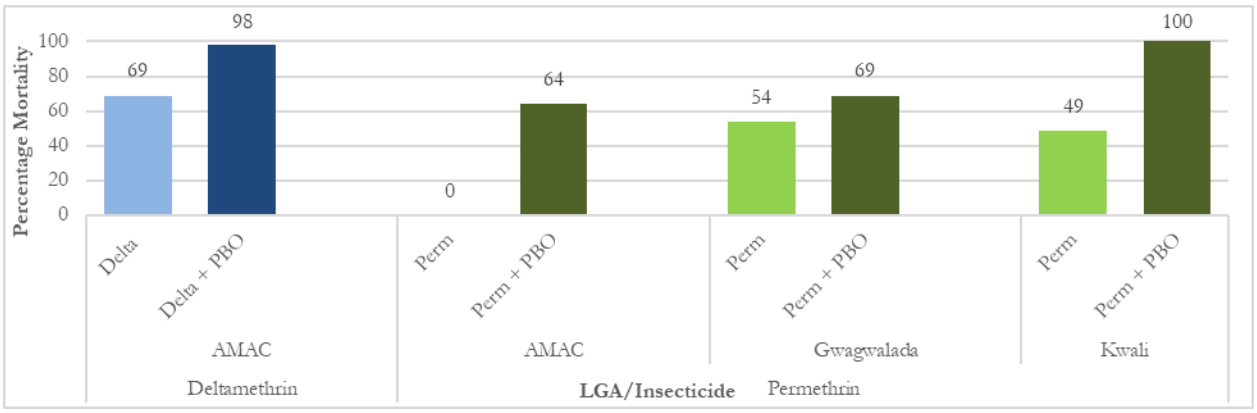


FIGURE 51: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM KADUNA

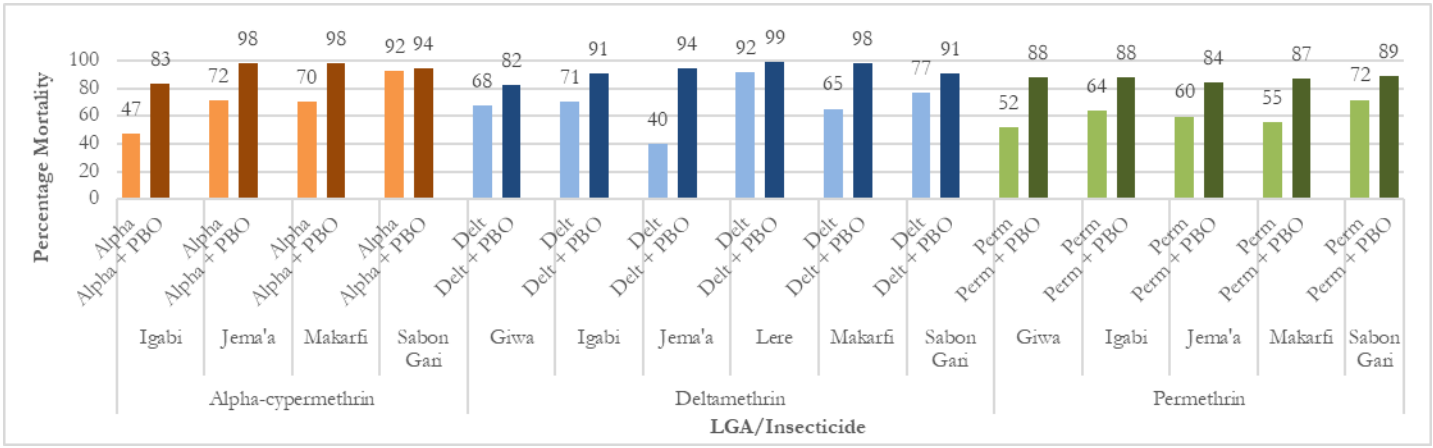


FIGURE 52: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM KEBBI

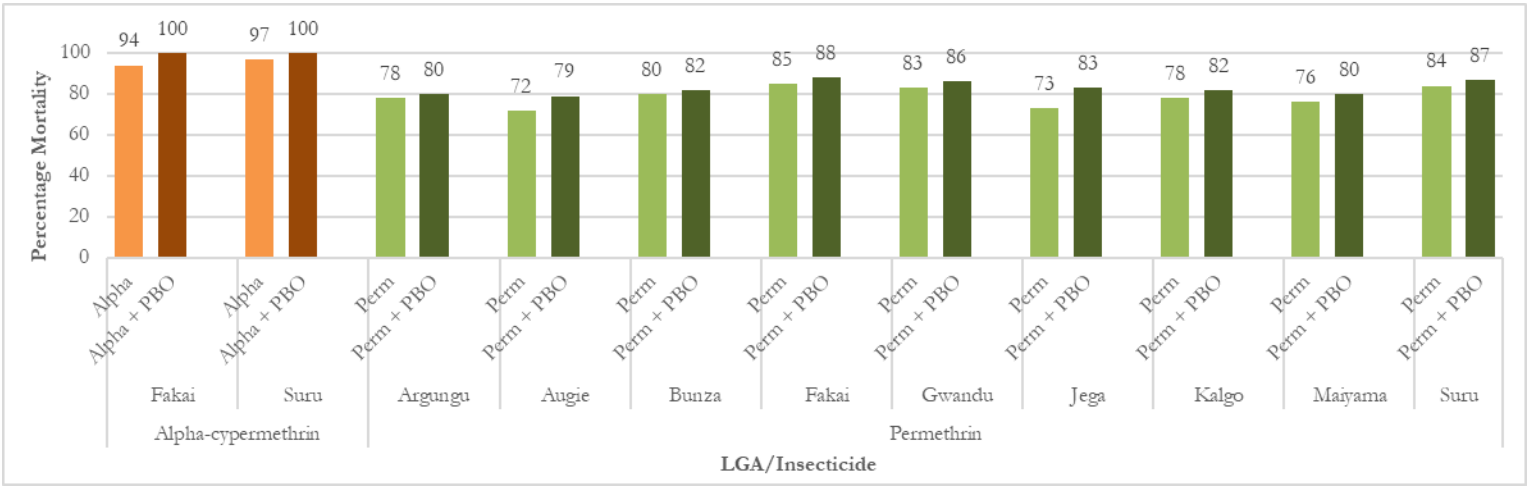


FIGURE 53: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM NASARAWA

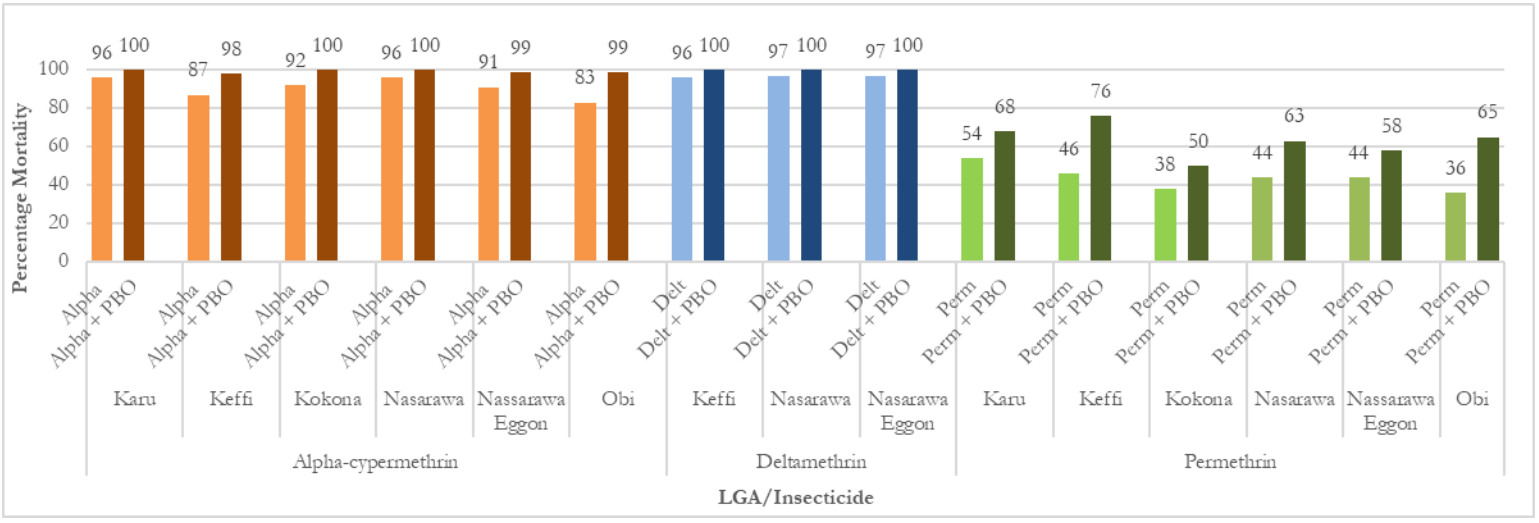


FIGURE 54: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM OYO

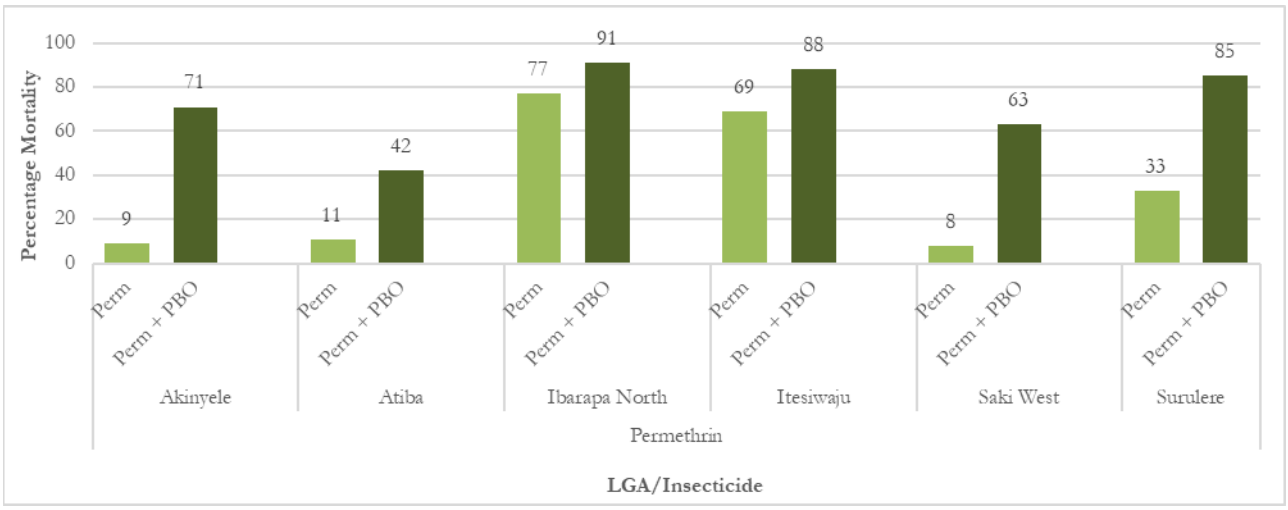


FIGURE 55: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM PLATEAU

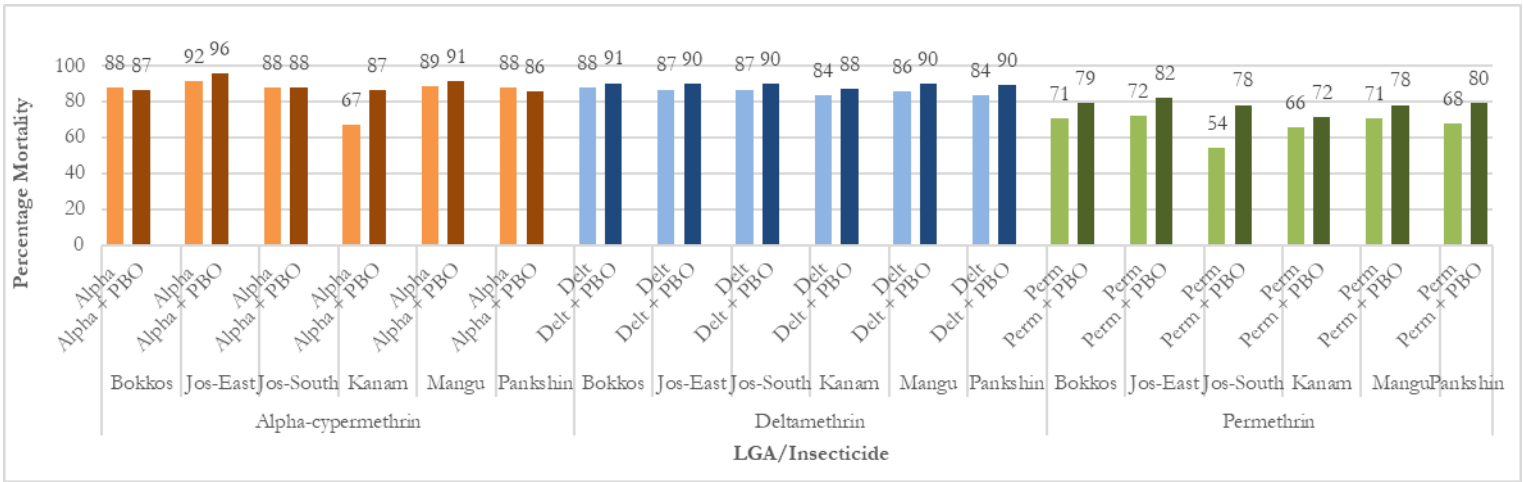


FIGURE 56: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM SOKOTO

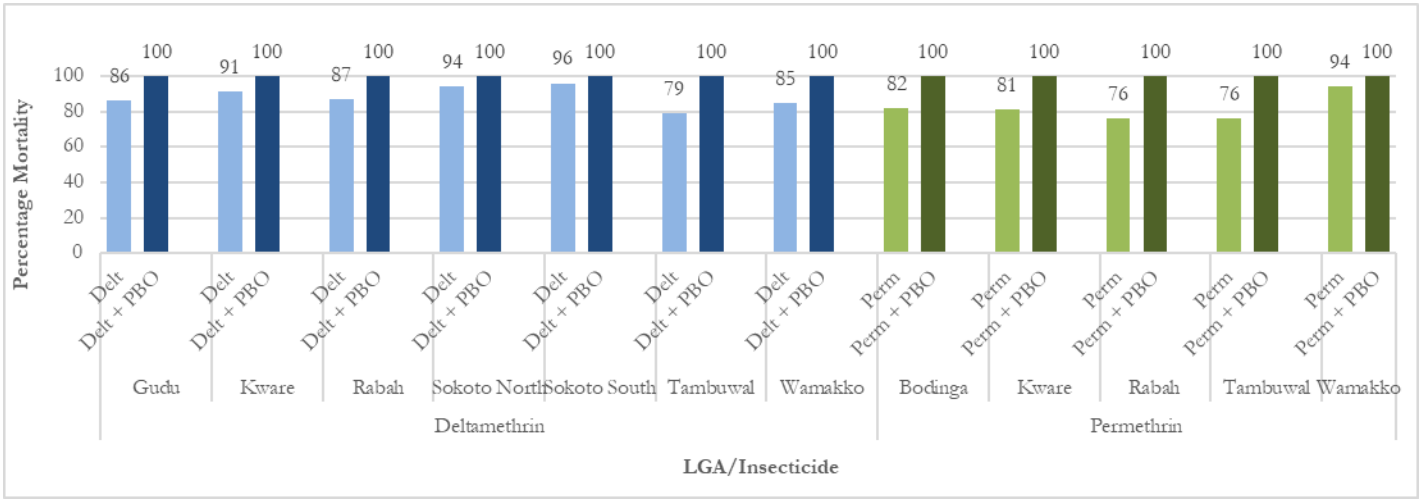
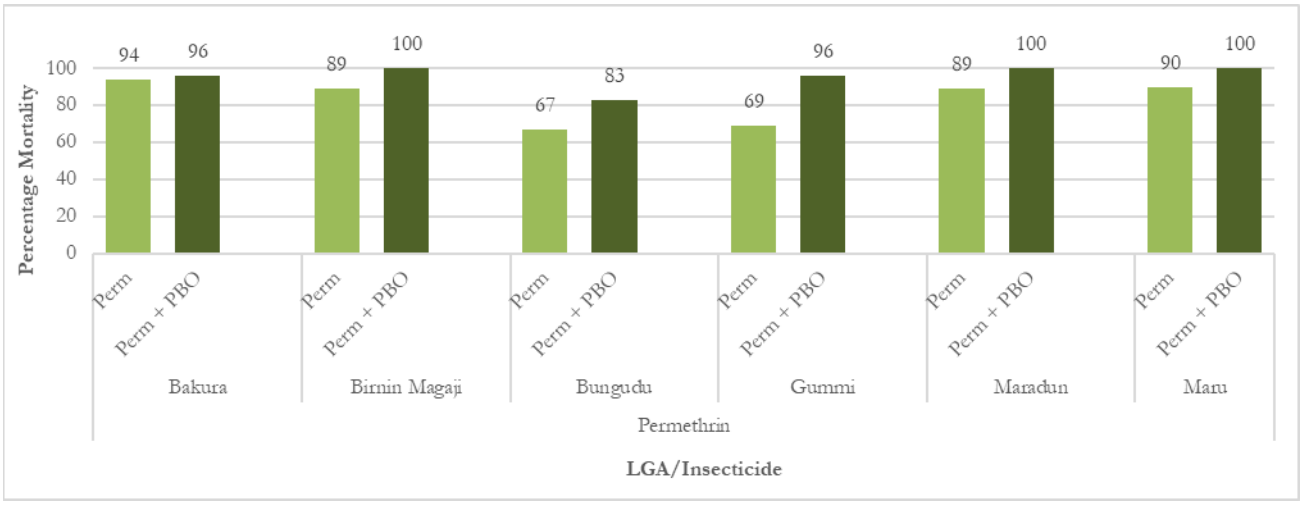


FIGURE 57: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM ZAMFARA



2.17 DETERMINATION OF SUSCEPTIBILITY STATUS OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR

The percentage knockdown of *An. gambiae* s.l. exposed to chlorfenapyr at 60 minutes varied across LGAs in Akwa Ibom (71–89%), Bauchi (83–98%), Bayelsa (82-96%), Benue (71–88%), Cross River (60–81%), Ebonyi (47-76%), Enugu (13-38%), FCT (8-22%), Kaduna (64-96%) Kebbi (76–84%), Nasarawa (50–66%), Oyo (45-64%), Plateau (18-74%), Sokoto (54–88%), and Zamfara (83–95%) (Figures 58-72).

The percentage mortality after the 24-hour holding period also varied in Akwa Ibom (93-99%), Bauchi (94–100%), Bayelsa (96-100%), Benue (100%), Cross River (95-100%), Ebonyi (97-100%), Enugu (62-97%), FCT (90-100%), Kaduna (53-99%) Kebbi (100%), Nasarawa (88-100%), Oyo (79-99%), Plateau (100%), Sokoto (77-100%), and Zamfara (99-100%) (Figures 58-72).

Full susceptibility (98-100% mortality) of *An. gambiae* s.l. to chlorfenapyr occurred at 24 hrs post exposure in all LGAs in Benue, Kebbi, Plateau, and Zamfara (Figure 61, 67, and 72). Mortality rates of *An. gambiae* s.l. were between 98-100% after the 48-hour holding period in all LGAs in Bauchi, Bayelsa, Benue, Cross River, Ebonyi, FCT, Kebbi, Nasarawa, Oyo, Plateau, and Zamfara (Figures 59-63, 65, 67, 68-70, and 72). *Anopheles gambiae* s.l. populations from all LGAs across all ecozones were susceptible to chlorfenapyr with >99% mortality at 72 hours (Figures 58-72).

FIGURE 58: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT AKWA IBOM

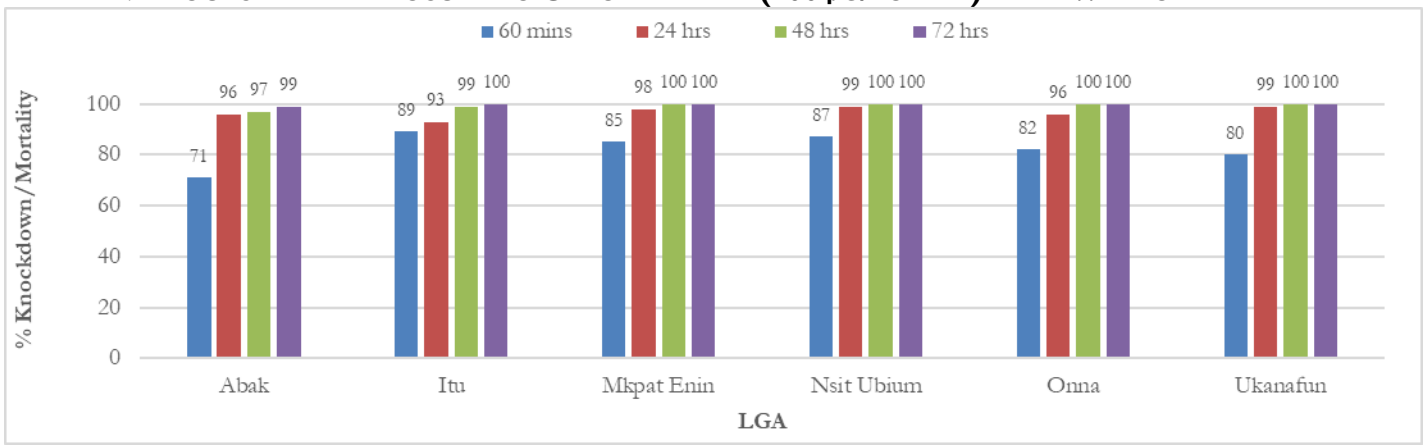


FIGURE 59: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT BAUCHI

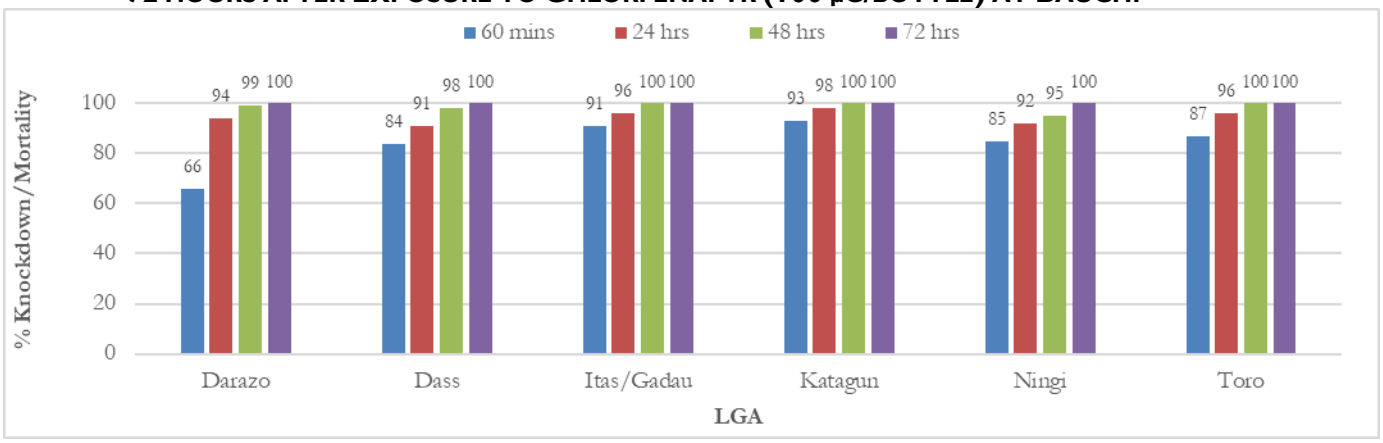


FIGURE 60: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT BAYELSA

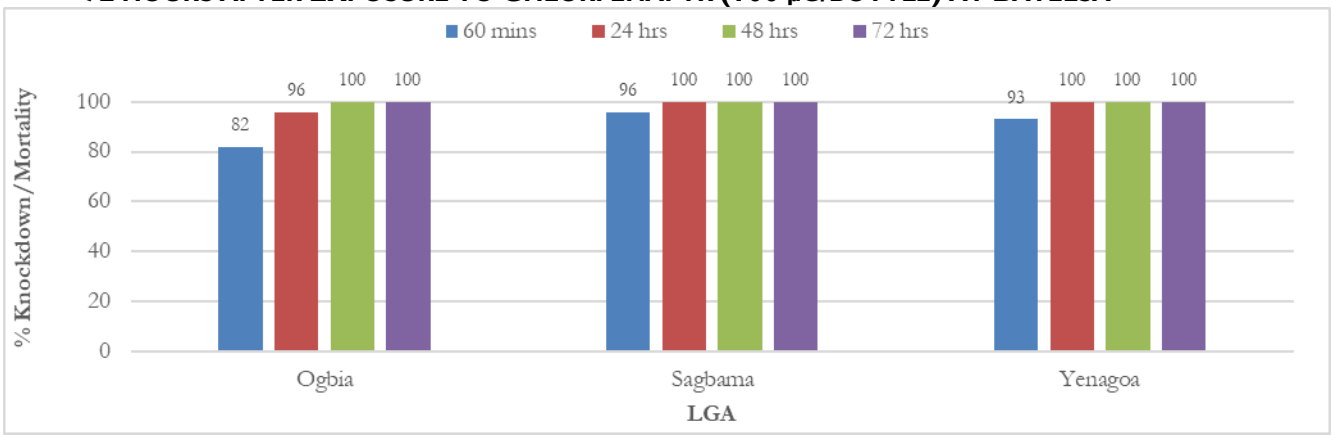


FIGURE 61: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT BENUE

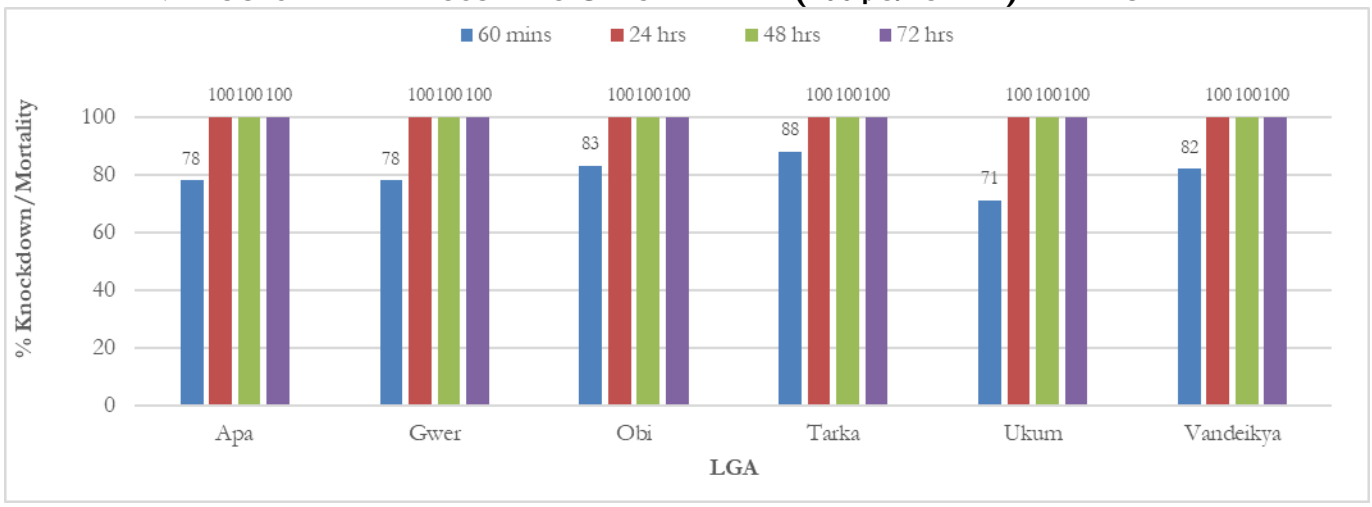


FIGURE 62: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT CROSS RIVER

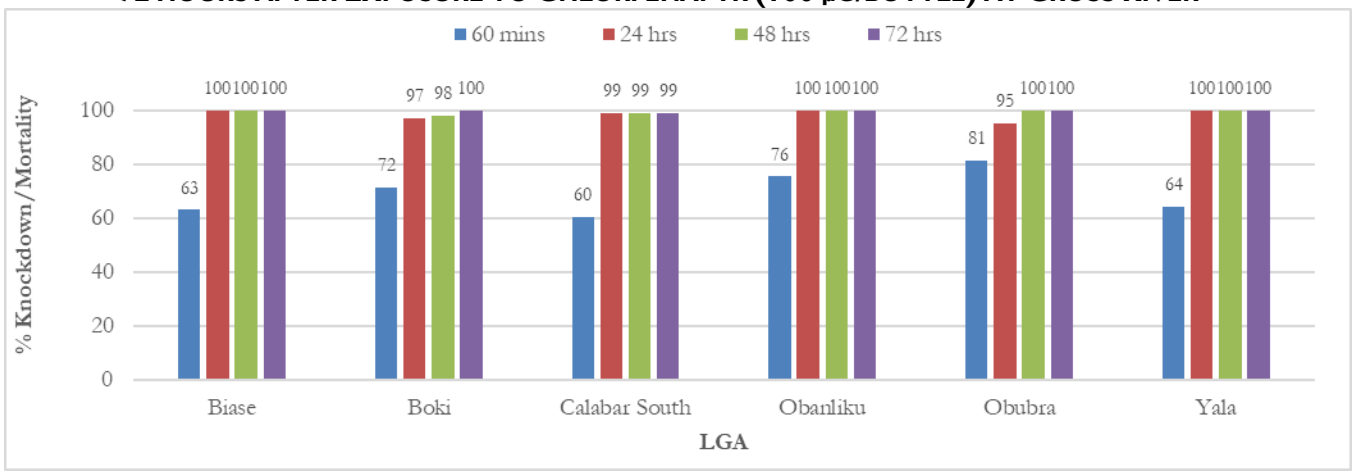


FIGURE 63: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT EBONYI

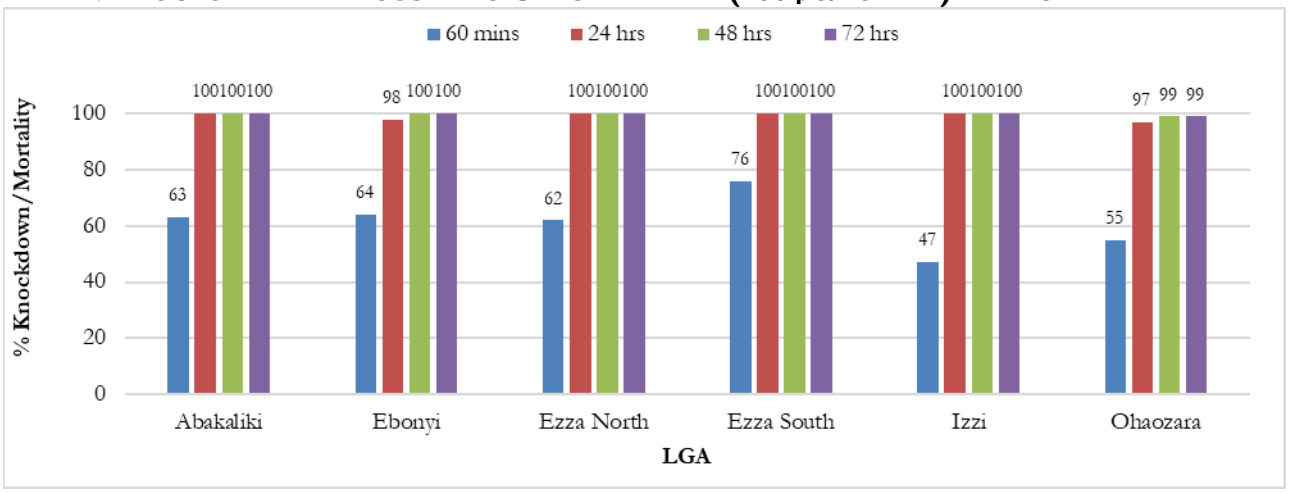


FIGURE 64: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT ENUGU

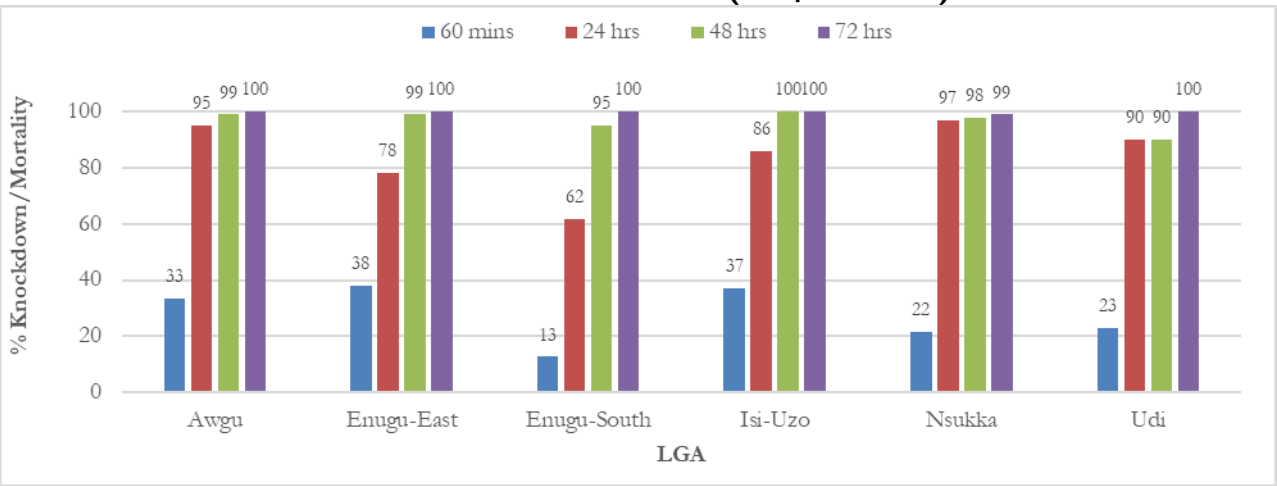


FIGURE 65: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT FCT

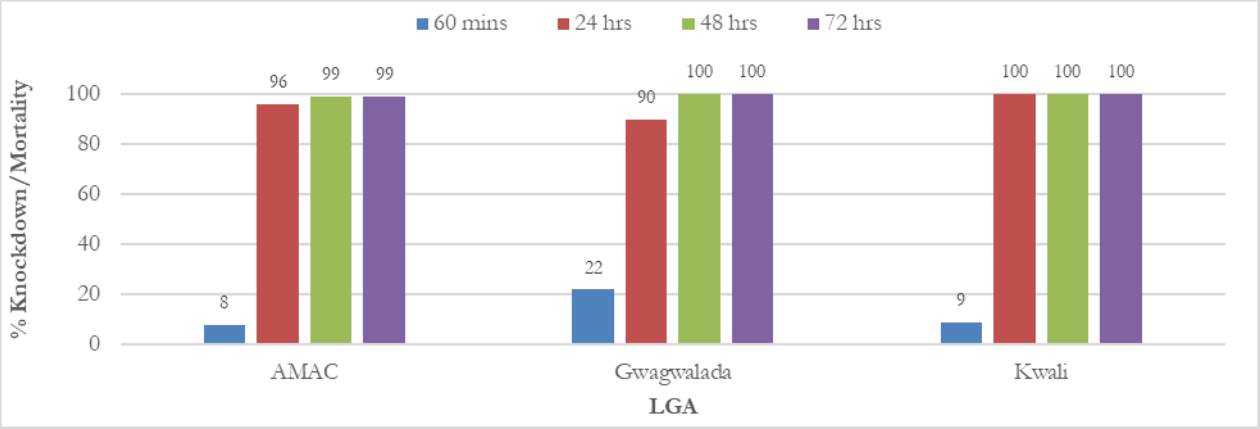


FIGURE 66: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT KADUNA

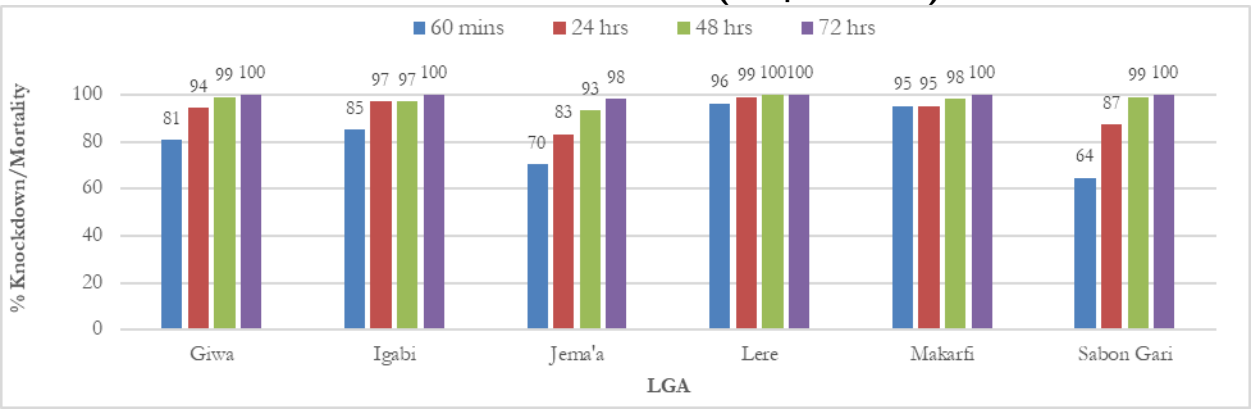


FIGURE 67: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT KEBBI

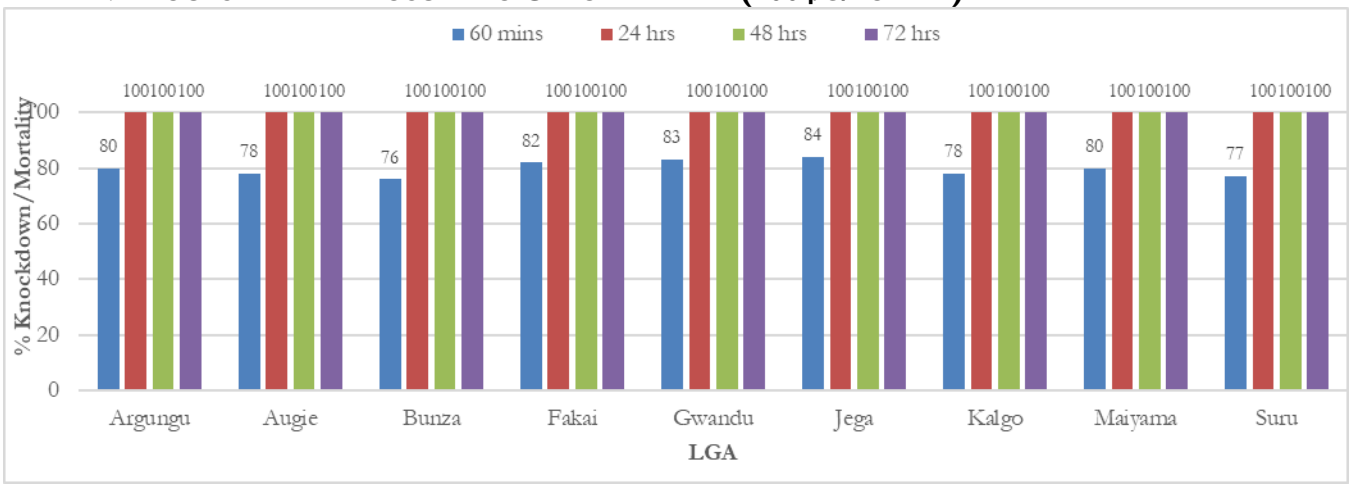


FIGURE 68: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT NASARAWA

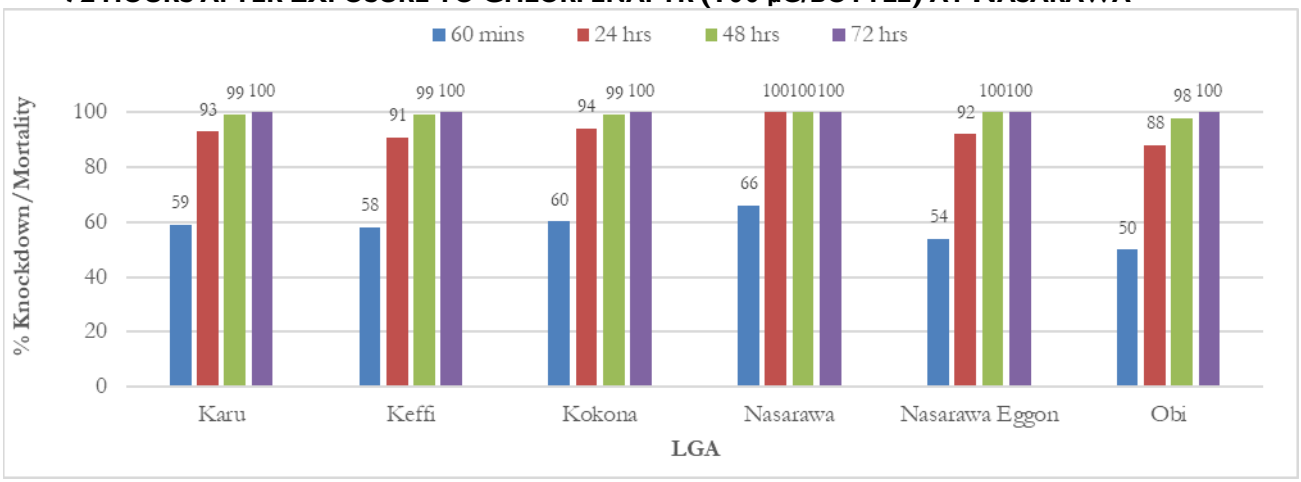


FIGURE 69: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT OYO

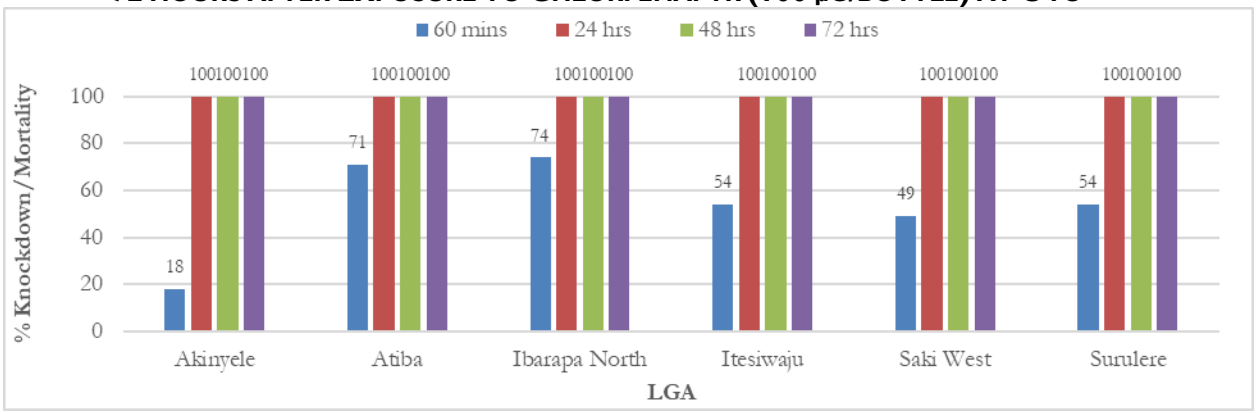


FIGURE 70: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) IN PLATEAU

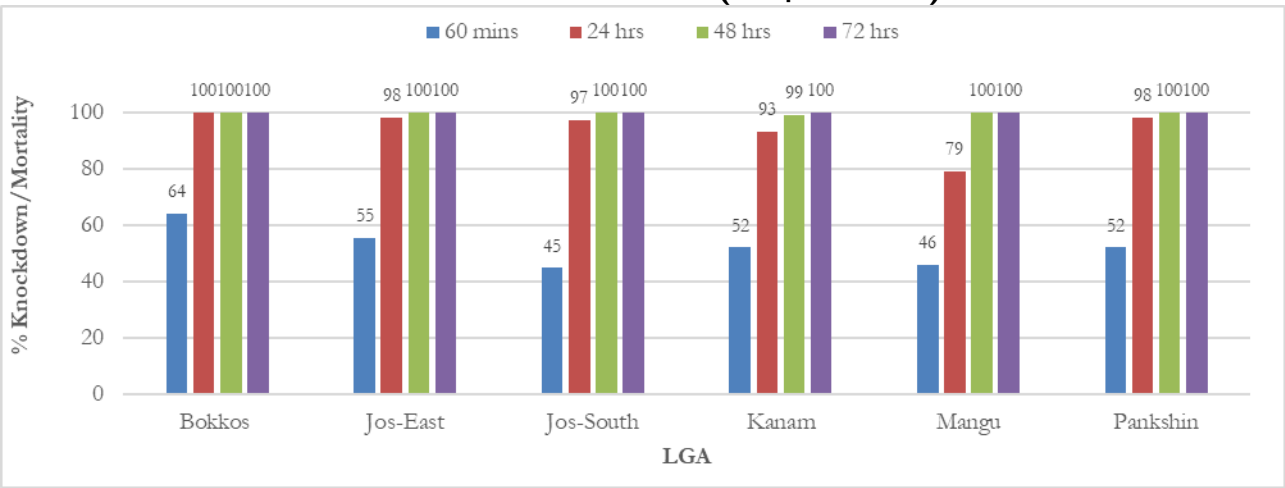


FIGURE 71: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT SOKOTO

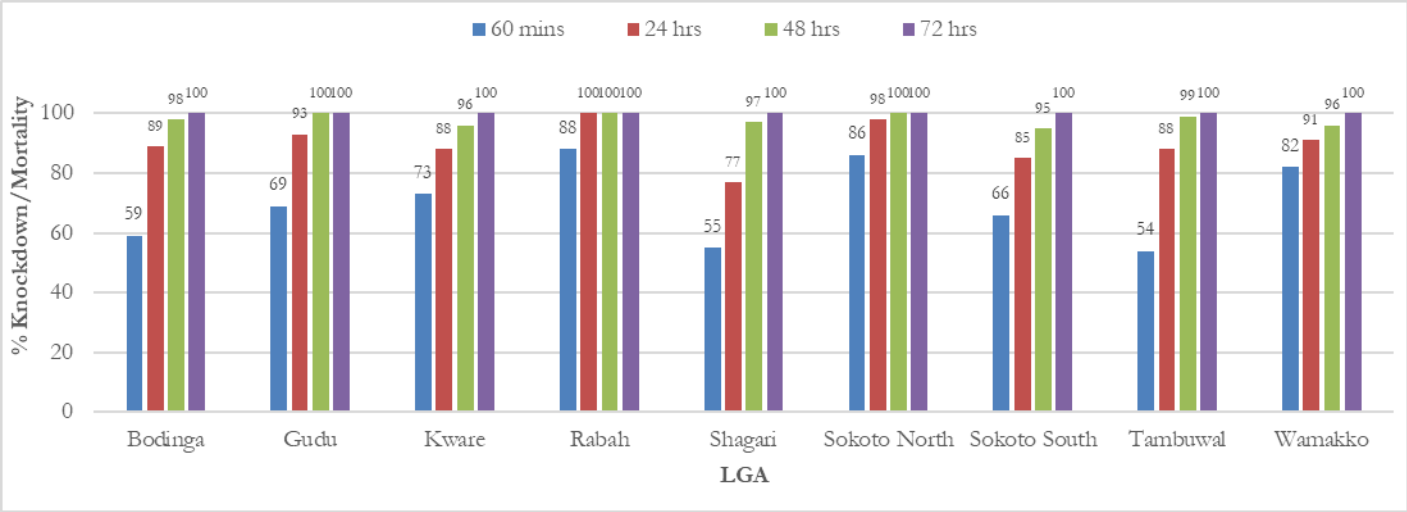
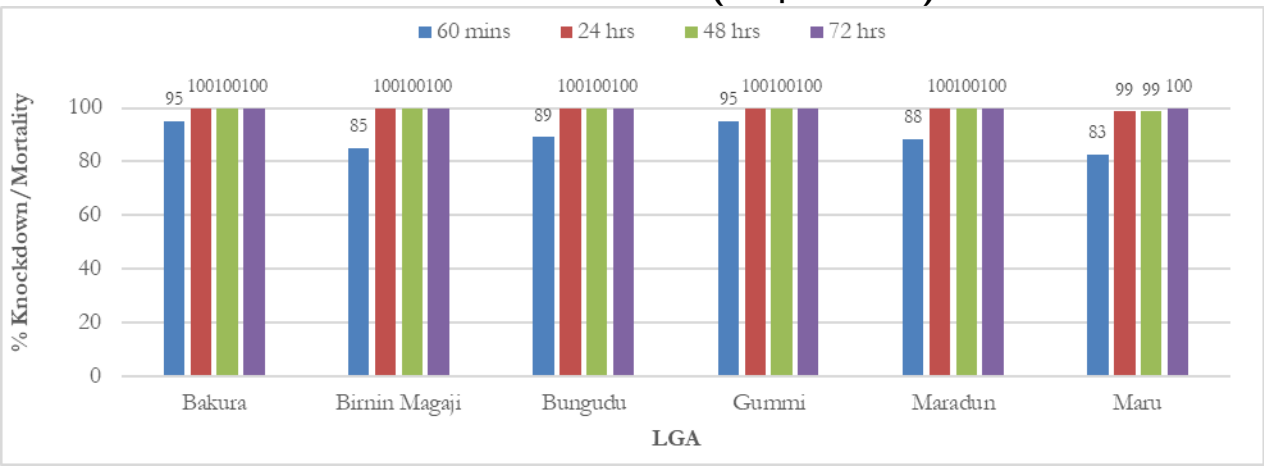


FIGURE 72: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT ZAMFARA



Note: Test conditions during bioassays: 25.8-28.7°C, 67-82% RH

2.18 DETERMINATION OF SUSCEPTIBILITY STATUS OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN USING CDC BOTTLE BIOASSAY

As shown in Table 12, the percentage knockdown of *An. gambiae* s.l. mosquitoes at 30-minutes exposure to clothianidin varied across the sites: Akwa Ibom (44-63%), Bauchi (73-83%), Bayelsa (81-92%), Benue (76-94%), Cross River (28-87%), Ebonyi (33-75%), Enugu (0-71%), FCT (3-33%), Kaduna (21-70%) Kebbi (41-56%), Nasarawa (12-40%), Oyo (11-62%), Plateau (10-12%), Sokoto (5-41%), and Zamfara (51-77%).

At 60 minutes, the percentage knockdown of *An. gambiae* s.l. mosquitoes exposed to clothianidin increased across all sites: Akwa Ibom (71-88%), Bauchi (87-97%), Bayelsa (92-98%), Benue (100%), Cross River (79-99%), Ebonyi (56-89%), Enugu (24-100%), FCT (36-82%), Kaduna (74-91%), Kebbi (62-93%), Nasarawa (37-64%), Oyo (52-97%), Plateau (50-61%), Sokoto (69-88%), and Zamfara (85-100%).

Mortality rates of *An. gambiae* s.l. at 24 hours post-exposure to clothianidin were between 98-100% in all LGAs in 12 out of 15 sites. Mortality rates were less than 98% after 24 hours in 4/6 LGAs in Akwa Ibom, 1/3 LGAs in FCT, and all six LGAs in Nasarawa (Table 12).

TABLE 12: CDC BOTTLE BIOASSAY RESULTS (PERCENT MORTALITY AFTER 24 HOURS) FOR *AN. GAMBIAE* S.L. TO CLOTHIANIDIN

Sentinel Site	LGA	Number Tested	Test Time		
			30 mins	60 mins	24 hrs
Akwa Ibom	Abak	100	61	87	98
	Itu	100	55	78	97
	Mkpat Enin	100	51	78	94
	Nsit Ubium	100	44	71	94
	Onna	100	63	88	99
	Ukanafun	100	53	77	97
Bauchi	Darazo	100	73	95	100
	Dass	100	77	93	100
	Itas/Gadau	100	78	87	100
	Katagun	100	73	93	100
	Ningi	100	83	96	100
	Toro	100	77	95	100
Bayelsa	Ogbia	100	92	98	100
	Sagbama	100	81	92	100
	Yenagoa	100	92	97	100
Benue	Apa	100	94	100	100
	Gwer	100	91	100	100
	Obi	100	87	100	100
	Tarka	101	76	100	100
	Ukum	102	81	100	100
	Vandeikya	103	76	100	100
Cross River	Biase	103	87	97	100
	Boki	101	87	99	100
	Calabar South	100	55	90	100
	Obanliku	104	39	85	100
	Obubra	100	28	79	99
	Yala	104	69	96	100
Ebonyi	Abakaliki	100	75	89	100
	Ebonyi	100	35	56	100
	Ezza North	100	35	60	99
	Ezza South	100	66	81	100
	Izzi	100	70	86	99
	Ohaozara	100	33	65	100
Enugu	Awgu	100	21	48	100
	Enugu-East	102	52	100	100
	Enugu-South	101	71	100	100
	Isi-Uzo	102	69	100	100

Sentinel Site	LGA	Number Tested	Test Time		
			30 mins	60 mins	24 hrs
Enugu	Nsukka	102	27	100	100
	Udi	101	0	24	100
FCT	AMAC	100	3	39	100
	Gwagwalada	100	33	82	94
	Kwali	100	19	36	99
Kaduna	Giwa	100	35	84	99
	Igabi	102	41	90	98
	Jema'a	99	21	90	99
	Lere	100	70	74	99
	Makarfi	100	61	91	100
	Sabon Gari	100	42	87	100
Kebbi	Argungu	100	48	93	100
	Augie	100	56	88	100
	Bunza	100	44	80	100
	Fakai	100	47	78	100
	Gwandu	100	45	79	100
	Jega	100	54	77	100
	Kalgo	100	51	81	100
	Maiyama	100	53	62	100
	Suru	100	41	84	100
Nasarawa	Karu	100	12	61	94
	Keffi	100	15	37	82
	Kokona	100	18	37	94
	Nasarawa	100	40	50	94
	Nasarawa Eggon	100	20	57	91
	Obi	103	35	64	96
Oyo	Akinyele	100	33	80	100
	Atiba	100	11	52	100
	Ibarapa North	100	52	89	100
	Itesiwaju	100	62	95	100
	Saki West	100	62	97	100
	Surulere	100	45	83	100
Plateau	Bokkos	103	10	50	100
	Jos-East	103	12	60	100
	Jos-South	103	11	55	100
	Kanam	103	10	61	100
	Mangu	101	10	57	100
	Pankshin	102	11	51	99
Sokoto	Bodinga	100	5	77	100
	Gudu	100	10	72	100
	Kware	100	41	83	100
	Rabah	100	41	88	100
	Shagari	100	35	74	100
	Sokoto North	100	16	80	100
	Sokoto South	100	11	69	100
	Tambuwal	100	35	71	100
	Wamakko	100	26	87	100
Zamfara	Bakura	100	72	95	100
	Birnin Magaji	100	57	85	100
	Bungudu	100	77	95	100
	Gummi	100	51	87	100
	Maradun	100	53	100	100
	Maru	100	75	100	100

2.19 *KDR* GENE FREQUENCY IN *AN. GAMBIAE* S.L. EXPOSED TO ALPHA-CYPERMETHRIN, DELTAMETHRIN, AND PERMETHRIN ACROSS SITES

Frequency of *kdr* genes in Alpha-cypermethrin Resistant *An. coluzzii* and *An. gambiae* s.l.

Assessment of *kdr* mutations in alpha-cypermethrin resistant *An. gambiae* s.l. indicated the presence of both *kdr-w* and *kdr-e* point mutations across the sentinel sites. *Kdr-w* gene frequencies in *An. coluzzii* ranged from 0.00 in FCT to 1.00 in Benue. *Kdr-w* gene frequencies in *An. gambiae* s.s. ranged from 0.29 in Ebonyi to 0.75 in Kebbi. Additionally, *kdr-w* gene frequencies in *An. arabiensis* ranged from 0.17 in Ebonyi and Nasarawa to 0.57 in Kaduna. On the other hand, *kdr-e* gene frequencies in *An. coluzzii* ranged from 0.00 in FCT, Bayelsa, and Benue to 0.14 in Kebbi. *Kdr-e* gene frequencies in *An. gambiae* s.s. ranged from 0.00 in Bayelsa and Kebbi to 0.1 in Bauchi while those in *An. arabiensis* ranged from 0.00 in Bauchi, Enugu, Kebbi and Nasarawa to 0.33 in Ebonyi. Hybrid was detected in Kaduna and though the *kdr-w* and *kdr-e* gene frequencies were both 0.00 (Table 13). Higher *kdr-w* gene frequencies were detected in *An. gambiae* s.s. than in *An. coluzzii* in Kebbi (0.75 vs. 0.50) (p=1.0000) and in Kaduna, higher *kdr-w* frequencies were recorded in *An. arabiensis* than in *An. coluzzii* (0.57 vs 0.45) (p=1.0000), but these did not vary significantly (Table 13).

TABLE 13: FREQUENCY OF *KDR* GENES IN ALPHA-CYPERMETHRIN-RESISTANT *AN. COLUZZII* AND *AN. GAMBIAE* S.S. ACROSS SITES

State	Species Identified	Alpha-cypermethrin Resistant								
		Number Tested for *kdr*	*Kdr-w*				*Kdr-e*			
			RR	Rr	rr	*kdr* frequency	RR	Rr	rr	*kdr* frequency
Akwa Ibom	*An. coluzzii*	48	14	20	14	0.50	1	3	44	0.05
	An. gambiae s.s.	83	27	29	27	0.50	0	14	69	0.08
	An. arabiensis	3	0	3	0	0.50	0	1	2	0.17
Bauchi	*An. coluzzii*	23	3	15	5	0.46	2	2	19	0.13
	An. gambiae s.s.	56	8	31	17	0.42	2	7	47	0.10
	An. arabiensis	6	1	2	3	0.33	0	0	6	0.00
Bayelsa	*An. coluzzii*	1	0	1	0	0.50	0	0	1	0.00
	An. gambiae s.s.	2	0	2	0	0.50	0	0	2	0.00
Benue	*An. coluzzii*	1	1	0	0	1.00	0	0	1	0.00
Ebonyi	*An. coluzzii*	64	6	40	18	0.41	2	4	58	0.06
	An. gambiae s.s.	33	1	17	15	0.29	0	2	31	0.03
	An. arabiensis	3	0	1	2	0.17	1	0	2	0.33
Enugu	*An. coluzzii*	58	4	36	18	0.38	0	7	51	0.06
	An. gambiae s.s.	19	2	15	2	0.50	0	2	17	0.05
	An. arabiensis	1	0	1	0	0.50	0	0	1	0.00
FCT	*An. coluzzii*	1	0	0	1	0.00	0	0	1	0.00
Kaduna	*An. coluzzii*	39	3	29	7	0.45	3	2	34	0.10
	An. gambiae s.s.	14	0	11	3	0.39	0	1	13	0.04
	Hybrid	1	0	0	1	0.00	0	0	1	0.00
	An. arabiensis	23	4	18	1	0.57	1	3	19	0.11
Kebbi	*An. coluzzii*	7	0	7	0	0.50	0	2	5	0.14
	An. gambiae s.s.	2	1	1	0	0.75	0	0	2	0.00
	An. arabiensis	2	0	2	0	0.50	0	0	2	0.00
Nasarawa	*An. coluzzii*	43	5	19	19	0.34	0	4	39	0.05
	An. gambiae s.s.	14	1	9	4	0.39	0	1	13	0.04
	An. arabiensis	3	0	1	2	0.17	0	0	3	0.00
Plateau	*An. coluzzii*	38	3	30	5	0.47	1	6	31	0.11
	An. gambiae s.s.	23	4	14	5	0.48	0	1	22	0.02
	An. arabiensis	7	1	5	1	0.50	0	2	5	0.14
Total		**618**	**89**	**359**	**170**	**0.43**	**13**	**64**	**541**	**0.07**

Frequency of *kdr* Genes in Deltamethrin-Resistant *An. coluzzii* and *An. gambiae* s.s.

An assessment of *kdr* mutations in deltamethrin-resistant *An. gambiae* s.l. indicated the presence of both *kdr-w* and *kdr-e* point mutations (Table 14). The *kdr-w* gene frequencies varied by ecozone, types of pyrethroids and vector composition. *Kdr-w* gene frequencies in *An. coluzzii* ranged from 0.38 in Ebonyi to 0.56 in Bayelsa. *Anopheles gambiae* s.s. gene frequencies ranged from 0.33 in Ebonyi to 1.00 in Cross River. For *An. arabiensis,* gene frequencies ranged from 0.17 in Ebonyi to 0.50 in Bayelsa and Sokoto. The *kdr-e* gene frequencies also varied by ecological zones and ranged from 0.00 in Benue and Oyo to 0.17 in Bayelsa in *An. coluzzii*. In *An. gambiae* s.s.*,* the *kdr-e* gene frequency ranged from 0.00 in Benue, FCT, Nasarawa, Oyo, and Plateau to 0.50 in Bayelsa and Cross River. These varied in *An. arabiensis* from 0.00 in Akwa Ibom, Bayelsa, Ebonyi and Sokoto to 0.08 in Kaduna. In general, *kdr-w* frequencies were higher in *An, gambiae* s.s. versus *An, coluzzii*. *Kdr-w* mutations did not vary significantly in *An. gambiae* s.s. and *An. coluzzii* in Bayelsa (0.67 vs 0.56) (p=1.0000) while *An. gambiae* was higher than *An. arabiensis* (0.54 vs 0.50) in Sokoto but did not also vary significantly (p=1.0000). *Kdr-e* gene frequencies was higher in *An. gambiae* than in *An. coluzzii* (0.50 vs 0.17) in Bayelsa while *kdr-e* gene frequencies were 0.08 in *An. arabiensis* and *An. gambiae* respectively in Kaduna though these did not vary significantly.

TABLE 14: FREQUENCY OF *KDR* GENES IN DELTAMETHRIN-RESISTANT *AN. COLUZZII* AND *AN. GAMBIAE* S.S. ACROSS SITES

State	Species Identified	Deltamethrin Resistant								
		Number Tested for Kdr	*Kdr-w*				*Kdr-e*			
			RR	Rr	rr	Kdr frequency	RR	Rr	rr	Kdr frequency
Akwa Ibom	*An. coluzzii*	19	0	16	3	0.42	1	3	15	0.13
	An. gambiae s.s.	62	9	36	17	0.44	4	9	49	0.14
	An. arabiensis	2	0	1	1	0.25	0	0	2	0.00
Bauchi	*An. coluzzii*	21	3	13	5	0.45	0	5	16	0.12
	An. gambiae s.s.	53	7	30	16	0.42	0	4	49	0.04
Bayelsa	*An. coluzzii*	9	3	4	2	0.56	0	3	6	0.17
	An. gambiae s.s.	3	1	2	0	0.67	0	3	0	0.50
	An. arabiensis	1	0	1	0	0.50	0	0	1	0.00
Benue	*An. coluzzii*	5	0	5	0	0.50	0	0	5	0.00
	An. gambiae s.s.	3	0	3	0	0.50	0	0	3	0.00
Cross River	*An. gambiae* s.s.	1	1	0	0	1.00	0	1	0	0.50
Ebonyi	*An. coluzzii*	69	7	38	24	0.38	1	0	68	0.01
	An. gambiae s.s.	18	1	10	7	0.33	1	1	16	0.08
	An. arabiensis	3	0	1	2	0.17	0	0	3	0.00
Enugu	*An. coluzzii*	58	7	33	18	0.41	1	10	47	0.10
	An. gambiae s.s.	28	3	17	8	0.41	0	2	26	0.04
	An. arabiensis	7	1	3	3	0.36	0	1	6	0.07
FCT	*An. coluzzii*	17	1	13	3	0.44	0	4	13	0.12
	An. gambiae s.s.	6	2	4	0	0.67	0	0	6	0.00
Kaduna	*An. coluzzii*	64	7	37	20	0.40	1	4	59	0.05
	An. gambiae s.s.	33	3	23	7	0.44	1	3	29	0.08
	An. arabiensis	19	2	14	3	0.47	0	3	16	0.08
Nasarawa	*An. coluzzii*	9	2	4	3	0.44	0	1	8	0.06
	An. gambiae s.s.	5	0	4	1	0.40	0	0	5	0.00
Oyo	*An. coluzzii*	1	0	1	0	0.50	0	0	1	0.00
	An. gambiae s.s.	1	0	1	0	0.50	0	0	1	0.00
Plateau	*An. coluzzii*	62	4	40	18	0.39	1	2	59	0.03
	An. gambiae s.s.	24	1	22	1	0.50	0	0	24	0.00
Sokoto	*An. coluzzii*	73	3	67	3	0.50	2	8	63	0.08
	An. gambiae s.s.	25	2	23	0	0.54	2	1	22	0.10
	An. arabiensis	3	0	3	0	0.50	0	0	3	0.00
Total		**704**	**70**	**469**	**165**	**0.43**	**15**	**68**	**621**	**0.07**

Frequency of *kdr* genes in Permethrin-Resistant *An. coluzzii* and *An. gambiae* s.s.

Kdr-w gene frequencies in *An. coluzzii* ranged from 0.37 in Enugu and Nasarawa to 0.53 in FCT while *kdr-w* gene frequencies in *An. gambiae* s.s. ranged from 0.25 in Bayelsa to 0.55 in Cross River. *Kdr-w* gene frequencies in *An. arabiensis* ranged from 0.25 in Bauchi, Bayelsa, and Enugu to 1.00 in Sokoto. Higher *kdr-w* gene frequencies were observed in *An. gambiae* than *An. coluzzii* in Cross River (0.55 vs. 0.48) (p=1.000) while in Sokoto, higher *kdr- w* gene frequencies were recorded in *An. arabiensis* than *An. coluzzii* (1.00 vs 0.49) (p=1.000), but did not vary significantly. For *kdr-e* mutations, gene frequencies in *An. coluzzii* ranged from 0.04 in Nasarawa and Zamfara to 0.25 in Bayelsa. In *An. gambiae* s.s., *kdr-e* gene frequencies ranged from 0.00 in Bayelsa to 0.31 in FCT. For *An. arabiensis,* they ranged from 0.00 in Bauchi, Kebbi and Sokoto to 0.50 in Bayelsa and Plateau. Higher *An. arabiensis* gene frequencies were recorded in Bayelsa than *An. coluzzii* (0.50 vs 0.25). This was the same for *An. arabiensis* and *An. gambiae* in Nasarawa (0.36 vs 0.09). These did not vary significantly (p=0.1573).

TABLE 15: FREQUENCY OF *KDR* GENES IN PERMETHRIN-RESISTANT *AN. COLUZZII* AND *AN. GAMBIAE* S.S. ACROSS SITES

State	Species Identified	Permethrin Resistant								
		Number Tested for Kdr	*Kdr-w*				*Kdr-e*			
			RR	Rr	rr	Kdr frequency	RR	Rr	rr	Kdr frequency
Akwa Ibom	*An. coluzzii*	54	12	27	15	0.47	0	6	48	0.06
	An. gambiae s.s.	84	15	58	11	0.52	1	3	80	0.03
Bauchi	*An. coluzzii*	10	1	6	3	0.40	0	1	9	0.05
	An. gambiae s.s.	57	6	33	18	0.39	0	4	53	0.04
	An. arabiensis	4	0	2	2	0.25	0	0	4	0.00
Bayelsa	*An. coluzzii*	4	1	2	1	0.50	1	0	3	0.25
	An. gambiae s.s.	4	0	2	2	0.25	0	0	4	0.00
	An. arabiensis	2	0	1	1	0.25	0	2	0	0.50
Benue	*An. coluzzii*	83	3	80	0	0.52	1	12	70	0.08
	An. gambiae s.s.	7	0	7	0	0.50	0	1	6	0.07
	An. arabiensis	10	0	8	2	0.40	0	1	9	0.05
Cross River	*An. coluzzii*	108	17	69	22	0.48	1	24	83	0.12
	An. gambiae s.s.	29	6	20	3	0.55	1	1	27	0.05
Ebonyi	*An. coluzzii*	87	8	53	26	0.40	3	7	77	0.07
	An. gambiae s.s.	47	4	29	14	0.39	1	3	43	0.05
	An. arabiensis	4	2	1	1	0.63	1	0	3	0.25
Enugu	*An. coluzzii*	67	6	37	24	0.37	1	6	60	0.06
	An. gambiae s.s.	39	3	24	12	0.38	0	7	32	0.09
	An. arabiensis	8	0	4	4	0.25	1	0	7	0.13
FCT	*An. coluzzii*	33	8	19	6	0.53	0	3	30	0.05
	An. gambiae s.s.	16	6	2	8	0.44	3	4	9	0.31
Kaduna	*An. coluzzii*	69	10	46	13	0.48	1	9	59	0.08
	An. gambiae s.s.	26	2	16	8	0.38	0	4	22	0.08
	An. arabiensis	22	4	11	7	0.43	0	5	17	0.11
Kebbi	*An. coluzzii*	148	16	121	11	0.52	2	22	124	0.09
	An. gambiae s.s.	29	3	24	2	0.52	3	4	22	0.17
	An. arabiensis	7	0	6	1	0.43	0	0	7	0.00
Nasarawa	*An. coluzzii*	97	9	53	35	0.37	0	7	90	0.04
	An. gambiae s.s.	34	3	19	12	0.37	2	2	30	0.09
	An. arabiensis	7	0	5	2	0.36	1	3	3	0.36
Oyo	*An. coluzzii*	61	1	55	5	0.47	1	13	47	0.12
	An. gambiae s.s.	23	1	19	3	0.46	0	7	16	0.15
	An. arabiensis	16	3	11	2	0.53	0	3	13	0.09
Plateau	*An. coluzzii*	87	9	60	18	0.45	1	6	80	0.05
	An. gambiae s.s.	49	3	29	17	0.36	0	4	45	0.04
	An. arabiensis	2	0	2	0	0.50	1	0	1	0.50
Sokoto	*An. coluzzii*	72	20	31	21	0.49	4	7	61	0.10
	An. gambiae s.s.	18	2	10	6	0.39	0	2	16	0.06
	An. arabiensis	1	1	0	0	1.00	0	0	1	0.00
Zamfara	*An. coluzzii*	66	11	35	20	0.43	0	5	61	0.04
	An. gambiae s.s.	15	0	10	5	0.33	0	3	12	0.10
	An. arabiensis	3	0	2	1	0.33	1	0	2	0.33
Total		**1,609**	**196**	**1,049**	**364**	**0.45**	**32**	**191**	**1,386**	**0.08**

3. DISCUSSION AND CONCLUSION

3.1 SPECIES COMPOSITION

Anopheles gambiae s.l., consisting of efficient vectors including *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis,* remained the primary and most abundant major malaria vector found, with varied composition and widespread distribution across surveyed sites in Nigeria. Its significant occurrence in all six states indicates its ability to adapt and utilize varying breeding sites. *Anopheles funestus* was another major malaria vector reported, but was more limited in its distribution when compared with *An. gambiae* s.l. Other secondary malaria vectors found but with limited distribution and abundance included *An. moucheti, An. pharoensis, An. coustani, An. squamosus, An. rufipes,* and *An. pretoriensis.* Other localized species found in select sites included *An. squamosus, An. rufipes*, *An. pretoriensis,* and *An. marshallii.*

The percentage composition of *An. funestus* mosquitoes in 2018 (0.1-4.0%, four sites) increased in 2019 (1.0-16.9%, five sites) and 2020 (0.2-48.1%, four sites). There was a significant increase in 2021 (0.9-51.2%) from three sites (Ebonyi, Plateau, and Oyo) while in 2022, there was a slight decrease in *An. funestus* preponderance from four sites (0.01-45.6%). Increased abundance of *An. funestus* indicates its potential to significantly contribute to malaria transmission, particularly in areas where suitable breeding conditions for this species are available. *Anopheles funestus* prefer to breed in water bodies that are stable, either permanent or semi-permanent, and contain aquatic vegetation. This pattern of occurrence has been consistent in the last five years (AIRS Nigeria Final Entomology Report 2017, PMI VectorLink Nigeria Final Entomology Report 2018, 2019, 2020, and 2021). The breeding sites of *An. funestus* in the three surveillance sites are similar, reinforcing habitat specificity.

There was a predominance of *An. marshallii* complex (86.2%) among mosquitoes collected from the vector surveillance site in Akwa Ibom compared those of *An. gambiae* complex (13.8%), which was perceived to be the predominant malaria vector in the area. In previous year's collections, further laboratory analysis conducted following failed amplifications of the suspected *An. gambiae* species confirmed the identity as *An. marshallii.* This year's identification agrees with our previous findings (PMI VectorLink Nigeria Final Entomology Report 2021). All 1,752 *An. marshallii* complex mosquitoes caught during this period were from Akwa Ibom, a mangrove swamp area biting mainly indoors. This could contribute to malaria transmission in the area, hence the need to continue to screen these species for sporozoites.

A total of 11 *Anopheles* mosquito species were morphologically identified across the six sentinel sites giving a total of 86,779 *Anopheles* mosquitoes (Annex 3). This is consistent with previously reported numbers in 2018, 2019, and 2020, though in 2021, 12 *Anopheles* species were identified (PMI VectorLink Nigeria Final Entomology Report 2018, 2019, 2020 and 2021). Similar numbers of *Anopheles* mosquitoes were identified from a study on *Anopheles* abundance and diversity from the Guinea and Savannah transitional forest zone of Nigeria (Oduola *et al.,* 2013). The ability of *An. gambiae* s.l. to utilize different breeding habitats, coupled with secondary and localized vectors that leverage specific habitats and seasonal conditions, accounts for variation in occurrence, predominance and abundance across the different ecological zones in Nigeria. The collective or individual roles of these vectors during both the rainy and dry seasons may be responsible for sustaining malaria transmission all year round. All three members of the *An. gambiae* complex (*An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis*) were found at varying proportions in each sentinel site. The only members of the *An. funestus* group molecularly identified were *An. funestus* s.s. and *An. leesoni* (Figures 5 and 6). However, the few unidentified from the PCR assay results suggest an improvement in molecular identification of *An. funestus* group.

For the first time, the composition of the vector species identified from *An. gambiae* species specific PCR analysis showed *An. coluzzii* as the dominant vector species found both indoors and outdoors across all sentinel sites using both CDC LT and PSC methods. This is in contrast with our previous findings in collections from 2018/2019 and 2019/2020, where *An. gambiae* was the dominant vector species found both indoors and outdoors across the five sentinel sites. This variation began in 2020/2021 collections where *An. gambiae* predominated indoors in 4/5 sites and outdoors in 2/5 sites. This further changed in the 2021/2022 collections indicating a trend in the manner in which *An. coluzzii* dominance has occurred (PMI VectorLink Annual Entomology Report, 2018, 2019, 2020, 2021). The interplay of factors responsible for this shift requires further analysis to understand the dynamics, particularly given the change occurred across all ecological zones in Nigeria. In Benin, *An. coluzzii* predominated during the dry season and accounted for most malaria transmissions (Akogbeto, 2018). This data requires monitoring over time to be able to determine the precursors and implications.

3.2 HUMAN BITING RATE AND VECTOR BITING TIME

The mean indoor biting rates of *An. gambiae* s.l. peaked in September in Plateau (85.7 bites/person/night) and in April, July-September in Kebbi (31.3, 25.9, and 29.5 b/p/n, respectively). Increased outdoor biting was recorded in Kebbi in March, April and September (9.4 b/p/n, 11.8 b/p/n, and 8.8 b/p/n, respectively). Biting rate has been found to be largely dependent on mosquito abundance which is influenced by rainfall patterns and strongly reflects the productivity period of mosquitoes when habitats are neither flooded nor dry but are stable enough to ensure rapid emergence of adult mosquitoes. The early rains are the best period to apply control measures to reduce mosquito abundance and limit the peak biting activities. In agreement with our previous observations (PMI VectorLink Annual Entomology Report 2021), biting rates did not increase much until July through September. Similarly, a small peak in outdoor biting was recorded in April in Kebbi with a smaller peak in September recorded in Kebbi and Sokoto, which stresses the need for those who sleep outdoors to protect themselves by sleeping under nets. Both indoor and outdoor biting rates in the other sites were generally low. Biting rate activities for most of the surveillance sites occurred both outdoors and indoors, increasing during the late rainy season (August-September) compared to previous findings where peak biting rates were observed during the early rainy season (April-June) (PMI VectorLink Annual Entomology Report 2020, 2021). Delayed rainfall may be responsible for the shift in the monthly mosquito peak biting.

The biting rate of *An. marshallii* peaks during the dry season and late rains, an indication of its non-dependence on the rainy season with permanent breeding sites that become stable during the dry season. If this species is found to be a malaria vector, the biting activity suggests it could perpetuate transmission during the dry season followed by transmission largely by *An. gambiae* at the onset of the rains.

The average number of mosquitoes caught biting per hour was generally higher indoors, with the highest collected between 11 p.m. and 2 a.m. in Kebbi and between 12-1 a.m. in Plateau. This agrees with previous findings which show the peak biting time as after midnight in most sentinel sites (PMI VectorLink Annual Entomology Report 2019; 2020; and 2021). Incidental shifts in peak biting time (10-11 p.m.) were reported in Plateau in 2020 as was recorded in Kebbi (11 p.m.-12 a.m.) in 2022 and may require further investigation of human activity at night and other environmental factors such as temperature and precipitation, which have been reported to influence the mosquito biting time (Dambach *et al.,* 2018). There was increased indoor biting for *An. gambiae* s.l. in September in Plateau (85.7 bites/person/night). This is in contrast with our findings in the previous year where *An. gambiae* s.l. indoor biting peaked in the month of July in Plateau (103.8 bites/person/night (b/p/n) (PMI VectorLink Annual Entomology Report 2021). The shift from July to September could be due to a delayed rainy season. This further agrees with the findings of Kabbale *et al.,* (2013) working in Budiope county of Uganda who found that although the abundance of *An. gambiae* s.l. was rainfall-dependent in some areas, both *An. gambiae* s.l. and *An. funestus* mosquitoes thrived all year round regardless of the amount of rainfall.

The high indoor biting rate and late night is also in consonance with the report of Moiroux *et al.* (2012) working in Benin who reported significant changes in the host-seeking behavior of *An. funestus* after scaling up universal coverage of ITNs in southern Benin. The fact that most of the bites from *An. gambiae* s.l. and *An. funestus* occur

during hours of the night when most people are in bed was the source of the enthusiasm for the use of insecticide-treated nets for malaria control in Africa (Maxwell *et al.,* 1998).

Due to the high rate of early morning biting by *An. funestus* in Oyo, extended morning collection by alternative non-LT method (such as HLC) may be considered.

The mean indoor biting rates of *An. marshallii* complex in Akwa Ibom peaked in January (21.8 b/p/n) with an earlier peak in December 2021 (19.8 b/p/n) and increased outdoor biting recorded in the same month of December 2021 (5.3 b/p/n), as well as in the month of August (3.3 b/p/n). This agrees with our previous findings in the same state where the mean indoor biting rates of *An. marshallii* complex peaked in September (26.6 b/p/n) with an earlier smaller peak in November 2020 (23.9 b/p/n) and December 2020 (10.1 b/p/n) indicating that *An. marshallii* complex preponderance occur more during the drier seasons (PMI VectorLink Annual Entomology Report 2021).

Early evening bites by *Anopheles* mosquitoes were on the rise particularly outdoors in Akwa Ibom, where samples have been identified as *An. marshallii.* Teshome *et al (*2021) working in Ethiopia established that *An. pharoensis* contributed exclusively to outdoor transmission whereas malaria transmission by *An. arabiensis* occurred both indoors and outdoors. This has highlighted the need for screening secondary vectors like *An. marshallii* for sporozoites which were mainly found outdoors. Residual malaria transmission due to early evening and outdoor biting vectors could pose a challenge to malaria control and/or elimination efforts. Additional control tools targeting early evening and outdoor biting malaria vectors are required to complement the current control interventions to control residual transmission.

3.2.1 Human Exposure to Mosquito Bites in Kebbi and Sokoto

Estimates from the human behavior data and biting rates indicated variabilities in time and place of exposure to mosquito bites in Kebbi and Sokoto. Most human exposure in Kebbi occurs indoors in the middle of night (11 p.m.-6 a.m.) and primarily due to people being in bed not using nets at the time when most biting activities occur indoors. In Sokoto, most human exposure occurred indoors during the early evening hours (before 10 p.m.) when most people are not protected under nets. This can inform the remedial measures needed in the two settings. In Kebbi, the high proportion of exposure occurring indoors during bedtime in the middle of the night can primarily be prevented with effective social behavior and communications to inform the community on the importance of using nets throughout the night. In Kebbi, where the highest proportion of exposure occurs before bedtime, supplemental preventive measures such as repellents may be needed to protect the communities from mosquito bites in the early evening. The estimated gap in protection, 83% in Kebbi and 62% in Sokoto, can be considered as high and needs attention by control programs.

3.3 Sporozoite Infection Rate

Plasmodium falciparum sporozoite rates of *An. gambiae* were recorded indoors in Ebonyi and Plateau only (2.2% and 1.8% respectively) for mosquitoes collected using CDC LTs. This is lower than what was reported in the previous year in Oyo (11.1%) and Plateau (2.2%) (PMI VectorLink Annual Entomology Report 2021).

Incidentally, there was no sporozoite positivity recorded in *An. coluzzii* and *An. arabiensis* both indoors and outdoors across the various ecozones. This does not reflect a low vectoral capacity but could be due to fewer numbers or no infected mosquitoes drawn during the sampling of mosquitoes for the test assays. Previous reports from the different sites have confirmed *An. gambiae*, *An. coluzzii,* and *An. arabiensis* as vectors of malaria in Nigeria (PMI VectorLink Nigeria Annual Entomology Report 2019, 2020, and 2021). *Anopheles gambiae* s.s. and *An. coluzzii* are both efficient transmitters of *P. falciparum*. Compared to 2021, where *An. gambiae* (from Oyo and Plateau) and *An. coluzzii* (from Ebonyi, Oyo, Plateau, and Sokoto) tested positive for sporozoites, only *An. gambiae* s.s. collected indoors in this reporting period in Ebonyi and Plateau were positive for *P. falciparum.* Similarly diverging from last year's results (where infection rates in *An. coluzzii* indoors ranged from 0.7% in Sokoto to 3.7% in Plateau and an infection rate of 6.7% was recorded outdoors only in Oyo), *An. coluzzii* analyzed this year were not found to be positive for sporozoites. Mosquitoes collected using PSCs had more positive samples compared to the other species and collection methods. In 2019, sporozoites in *An. arabiensis*

outdoors were recorded in Nasarawa (2.9%), Plateau (2.2%), and Sokoto (0.9%), while in 2021 and 2022, no sporozoite infection was recorded among *An. arabiensis* in any other sites (PMI VectorLink Nigeria Annul Entomology Report 2019; 2020 and 2021). The lack of sporozoite infection rate among *An. arabiensis* could be due to the small sample size analyzed.

The highest *P. falciparum* sporozoite rates in *An. funestus* s.s. collected indoors using CDC LTs was recorded outdoors in Oyo (3.4%) followed by Plateau indoors (1.6%). No other members of *An. funestus* group tested positive for *Plasmodium* sporozoites. This agrees with our previous finding where *An. funestus* was recorded indoors (2.6%) and outdoors (6.0%) in Oyo as well as indoors (2.8%) in Plateau (PMI VectorLink Annual Entomology Report 2021). Positivity for *P. falciparum* circumsporozoites among *An. funestus* s.s. collected indoors and outdoors further establishes its role as a major malaria vector. Though *An. funestus* is less studied in Nigeria compared to *An. gambiae* s.l., its role in malaria transmission has been reported (Awolola *et al*., 2003). *Anopheles marshallii* complex mosquitoes caught using CDC LT methods were screened for *P. falciparum* circumsporozoite antigens in Akwa Ibom and no sporozoite infection was recorded. This contrasts with our previous report from the same area which shows that 0.6% sporozoite infection was recorded in *An. marshallii* mosquitoes last year (PMI VectorLink Annual Entomology Report 2021). Given their high densities and endophily equivalent to primary vectors and the previous incrimination with *Plasmodium* sporozoites, *An. marshallii* may contribute to malaria transmission in Akwa Ibom. Further morphological and molecular identification studies towards further characterization of this species is recommended. Continued monitoring is essential for understanding their temporal contributions to malaria transmission in the area.

3.4 Entomological Inoculation Rate

EIRs were recorded indoors in two of the five sites among *An. gambiae* s.s., with the highest indoor EIR contribution recorded in *An. gambiae* from Ebonyi (12.1 infective bites/person/year) followed by Plateau (10.9 infective bites/person/year), and in *An. coluzzii* in Kebbi (5.8 infective bites/person/year). Outdoor EIR was recorded for *An. coluzzii* in Kebbi at 6.6 infective bites/person/year. There were no infective bites recorded among *An. arabiensis* (indoors or outdoors) across the sites, which is consistent with the previous year's report (PMI Vector Link Annual Entomology Report 2021).

EIRs were recorded for *An. funestus* s.s. in Oyo and Plateau with the highest EIR recorded outdoors in Oyo (9.5 infective bites/person/year), followed by indoors EIR in Plateau (5.2 infective bites/person/year). This agrees with our earlier findings that the highest EIR among *An. funestus* was recorded outdoors in Oyo (31.7 infective bites/person/year), followed by the indoor EIR also in Oyo (14.1 infective bites/person/year) (PMI VectorLink Annual Entomology Report 2021). The EIR in this current report and the previous year confirms the role of major vectors *An. gambiae* s.s., *An. coluzzii*, *An. arabiensis,* and *An. funestus* in malaria transmission in Nigeria. However, interpretation of the trends must be done with caution given the limited samples analyzed. The lack of EIR in a particular year or a particular species does not necessarily mean the vector species is inactive; the samples randomly selected for analysis simply may not have been infected.

3.5 Blood Meal Sources

The blood meal analysis indicates that *An. gambiae* s.s.*, An. coluzzii,* and *An. arabiensis* collected using CDC LTs and PSCs varied in their preference for human blood meal. However, samples collected from PSC provided a adequate sampling of blood fed mosquitoes to be analyzed when compared to mosquitoes from CDC LT. The outcome of this analysis clearly demonstrated that a significant proportion of the major vectors had ready access to human blood meal and is consistent with previous reports (PMI VectorLink Annual Entomology Report 2019; 2020; and 2021). The co-occurrence of *An. gambiae* s.s., *An. coluzzii,* and *An. arabiens*is with preference for human blood meal further attest to behavior which makes them important malaria transmitters. An understanding of the feeding behavior of these vectors will ensure proper planning and consideration of all factors that may potentiate malaria risks. Caution should be exercised in interpreting the blood meal preferences of mosquitoes collected from CDC LTs, as only few blood-fed mosquitoes collected in CDC LTs were analyzed. Overall, despite the zoophilic nature of *An. arabiensis,* a significantly higher number of human blood meals ($p<0.0001$) followed by goat blood meal sources were detected. This agrees with the findings of Ogola

et al., (2017) from Kenya, which found a higher proportion of human blood meals among both *An. arabiensis* and *An. funestus* collected indoors. Generally, the prevalence of animal blood meal in human dwellings indicates that livestock live near humans. This can support higher malaria transmission by attracting infected mosquitoes to human habitations (Iwashita *et al.,* 2014). Among the blood fed mosquitoes identified as *An. marshallii,* the majority (90.4% by PSC) were positive for human blood, indicating a possible preference for human blood over animal blood, though bovine (3.4%) and goat (6.2%) blood meals were also present amongst the samples. None of the *An. marshallii* complex mosquitoes caught using ;CDC LT tested positive for any blood meal.

3.6 INSECTICIDE SUSCEPTIBILITY

Resistance to the three pyrethroids tested varied according to type of pyrethroid. Pyrethroid resistance patterns also varied within and among the states and across different ecozones. This is consistent with a known trend across most African countries (Soumaila *et al.,* 2022). Recent global reports of countries that reported insecticide resistance monitoring data to the WHO further indicated that pyrethroid resistance was detected in at least one malaria vector in 87% of countries and to organophosphates in 60% (World Malaria Report 2022).

Compared with deltamethrin, full susceptibility to alpha-cypermethrin was recorded across *An. gambiae* s.l. populations from all LGAs in seven states (Bayelsa, Benue, Cross River, FCT, Oyo, Sokoto, and Zamfara). Four states (Benue, Cross River, Oyo, and Zamfara) recorded full susceptibility to deltamethrin across all LGAs tested.

Limited areas have recorded susceptibility to permethrin in Bayelsa (2/3 LGAs), Kaduna (1/6 LGAs), and Sokoto (4/6 LGAs). This is consistent with results previously reported in the last three years. Similar findings have recently been recorded in neighboring countries such as Niger (Soumaila *et al.,* 2022). The factors responsible for continued susceptibility to permethrin amidst widespread resistance require further investigation. Permethrin-only ITNs are not recommended due to the widespread and intense resistance in Nigeria.

The insecticide resistance data here show that *An. gambiae* mosquitoes react differently to different classes of insecticides and emphasize the importance of consistently monitoring the susceptibility status of these mosquitoes across ecozones. These results suggest possible use of alpha-cypermethrin and deltamethrin, especially for treating ITNs, under an insecticide resistance management plan in areas where the vector is susceptible to these insecticides. In areas where full susceptibility to deltamethrin or alpha-cypermethrin plus PBO is found, nets treated with the dual active ingredients should be considered.

Complete susceptibility to pirimiphos-methyl was recorded across all LGAs recorded in 13 states. This agrees with findings from WHO (2022) which reported low resistance of local *Anopheles* mosquitoes to pirimiphos-methyl compared to pyrethroids in most countries.

3.7 RESISTANCE INTENSITY

Although the WHO (2022) reported that high intensity to pyrethroids has been detected in western Africa more frequently than other regions of the world, during the period of this report, moderate resistance was not recorded in both alpha-cypermethrin and deltamethrin. The intensity assays should also be considered in determining the degree of pyrethroid resistance present in the *Anopheles* population. Resistance at operationally significant doses of 5X and 10X discriminating concentrations were recorded from the intensity bioassays with permethrin only. These results indicate that the increasing resistance intensity being selected in field populations of mosquitoes will reduce the efficacy of pyrethroid-based interventions. Insecticide resistance intensity recorded in *An. gambiae* s.l. mosquitoes across the different ecozones reveals that different resistance management options are needed.

3.8 SYNERGIST ASSAYS

Pre-exposure to PBO substantially increased susceptibility with increases in mortality for alpha-cypermethrin, deltamethrin, and permethrin tested. This indicated the impact of PBO in significantly increasing *An. gambiae*

s.l. mortality for the three pyrethroids tested across all sites. The exception was in Plateau, where PBO did not restore susceptibility to any of the pyrethroids in any LGA. This finding showed the presence of P450 enzymes as the predominant resistance mechanism across most of the sites. Similar results have been recorded across 35 countries and 364 sites according to the WHO (2022). Overall findings indicated significantly higher mosquito mortality against alpha-cypermethrin and deltamethrin after pre-exposure to PBO across most ecozones. Mortality to permethrin was however recorded in the rainforest area of Ebonyi and Enugu and Sokoto in the Sahel. This trend could support the choice of alpha-cypermethrin or deltamethrin-based PBO ITNs to be prioritized in many of the sites. The use of PBO ITNs with deltamethrin or alpha-cypermethrin-based combination have shown high performance particularly in western African countries where vector populations are highly resistant to pyrethroids (Kouassi *et al.*, 2020; Soumaila *et al.,* 2022).

3.9 Determination of Susceptibility Status of *An. gambiae* s.l. to Chlorfenapyr

Anopheles gambiae s.l. mosquitoes were susceptible (98-100% mortality) to chlorfenapyr at 72 hours across all LGAs at the dose of 100 µg/bottles. This confirms the suitability of chlorfenapyr as an option for controlling highly pyrethroid-resistant vectors (Oxborough *et al.,* 2021). Higher mortality of mosquitoes could be observed using chlorfenapyr, particularly in areas where insecticide detoxification was the main resistance mechanism, suggesting that ITNs with chlorfenapyr may be appropriate in Plateau, which is located in the Guinea savannah ecozone (Kouassi *et al.*, 2020).

3.10 Determination of Susceptibility Status of *An. gambiae* s.l. to Clothianidin

The percentage knockdown of *An. gambiae* s.l. mosquitoes after 30 and 60 minutes of exposure to clothianidin varied across the sites using the CDC bottle bioassay method. Complete susceptibility after the 24-hour holding period was recorded across all sites except three, where possible resistance of 94-96% was recorded. In Nasarawa, suspected resistance of 91-97% was recorded in 5/6 LGAs and 82% in one of the LGAs. Four of six LGAs in Akwa Ibom (Abak, Itu, Mkpat Enin, and Onna) recorded mortality rates ranging from 94-97% while one LGA in the FCT recorded a mortality rate of 94%. Further investigation is required in the LGAs of concern.

3.11 *Kdr* Gene Frequencies

Assessing the *kdr* mutations, an important mechanism associated with pyrethroid resistant *An. gambiae* s.l., indicated the presence of both *kdr-w* (1014F) and *kdr-e* (1014S) point mutations in alpha-cypermethrin, deltamethrin, and permethrin tested mosquitoes across the sites. This is in contrast with our findings in the ecological zones in previous years (PMI VectorLink Annual Entomology Report 2020; 2021). This, however, is in consonance with previous findings in the same locations three years earlier where both *kdr-w* and *kdr-e* point mutations were present (PMI VectorLink Annual Entomology Report 2019). Where metabolic resistance is ruled out, mutations in the target sites of insecticides are often involved. Though it is not evident that the presence of this resistance allele alone is sufficient to result in control failure, the *kdr-w* allelic frequencies in mosquitoes resistant to alpha-cypermethrin, deltamethrin, and permethrin increased this year compared to last year (PMI VectorLink Annual Entomology Report 2021). There is need for continued monitoring of the spread and gene frequencies of these mutations in *An. gambiae* s.l. populations. Analysis of the dynamics and trends over time may indicate the presence of selection pressure among the mosquito population.

In addition to the phenotypic resistance found in this study, more information is provided on the frequency and distribution of physiological resistance mechanisms such as the *kdr-w* mutation, which is one of the most important mechanisms for pyrethroid resistance. The significance of this finding this year is the identification of the *kdr-w* and *kdr-e* alleles in *An. arabiensis* in Nigeria, in addition to *An. coluzzii* and *An. gambiae* s.s., previously reported. This is in contrast with our earlier findings in the past three years across the various ecological zones where *kdr-w* and *kdr-e* were not detected in *An. arabiensis* (PMI VectorLink Annual Entomology Report, 2019;

2020; and 2021). This finding is in agreement with previous reports from several other African countries that indicated the widespread of *kdr-w* mutations in the three major vector species of the *An. gambiae* complex. Increased selection pressure due to the increased pyrethroid ITN coverage over time as well as the culture of using pyrethroid insecticides for crop protection in agriculture, in combination, or alone might have been sufficient to drive *kdr-w* mutations to the high frequencies in *An. gambiae* s.l. Previous findings elsewhere indicated that large-scale countrywide distribution of ITNs led to an increased frequency of *kdr-w* mutations in Niger (Czeher *et al.,* 2008). Use of pyrethroids at the household level and in small vegetable cultivation has also been reported to drive the *kdr* mutation to a higher frequency in Mali (Fanello *et al.,* 2003).

This study also demonstrated that multiple insecticide-resistance mechanisms have evolved in *An. gambiae* s.l. in Nigeria. The extent and variety of phenotypic resistance and the physiological mechanisms associated with it, serve as a 'wake-up call' for ongoing support of evidenced-based decision making in programming involving insecticide-based malaria control efforts. Findings in this study highlight the need for routine resistance monitoring to update the information base for rational deployment of the existing tools for effective vector control in Nigeria. It is noteworthy that the implications and operational impact of resistance to malaria control efforts needs to be evaluated urgently. There is need for appropriate and correct vector control strategies to be put in place in a context of insecticide resistance management. Additionally, innovative vector control tools that include new active ingredients for ITNs might be needed to complement or replace the existing strategies in areas of pyrethroid resistance.

ANNEXES

ANNEX 1: GPS COORDINATES OF LONGITUDINAL SAMPLING SITE LOCATIONS

State	Location of Sampling Sites	Latitude	Longitude	Name of Nearest Health Facility	GPS Coordinate of Health Facility
Akwa Ibom	Itu	5.055905	7.888948		
	Nsit Ubium	4.742735	7.948834		
	Abak	4.984058	7.790945		
	Ukanafun	4.90385	7.6055		
	Onna	4.63676	7.87237		
	Mkpat Enin	4.7708499,	7.735482	Primary Health Care Mkpat Enin	4.784669, 7.731115
Ebonyi	Izzi	6.307358	8.169770		
	Abakaliki	6.32306	8.11201		
	Ezza South	6.149010	7.955550		
	Ohaukwu	6.397660	7.940440		
	Ezza North	6.328900	8.069780	Comprehensive Health Centre, Okposi Umuoghara	6.338803, 8.060135
	Ebonyi	6.330530	8.089530		
	Onicha	6.11163	7.8232		
	Ishielu	6.39073	7.82864		
	Ohaozara	6.046900	7.755300		
Kebbi	Argungu	12.69677	4.44737	General Hospital Argungu	12.74382, 4.52205
	Augie	12.89322	4.59985		
	Bunza	12.20108	3.93373		
	Fakai	11.50855	5.11251		
	Gwandu	12.48938	4.62779		
	Jega	12.1012	4.46592		
	Kalgo	12.38984	4.04318		
	Maiyama	12.0826	4.3677		
	Suru	11.92397	4.18217		
Oyo	Ibarapa North	7.64457	3.1731		
	Itesiwaju	8.21071	3.5482		
	Akinyele	7.550300	3.947000	Elekuru Primary Health Center	3.82523, 7.59228
	Orelope	8.79413	3.80192		
	Saki West	8.64879	3.20508		
	Surulere	6.50246	3.35903		
Plateau	Bassa	9.93333	8.73333		
	Jos South	8.642080	9.813490		
	Bokkos	9.93333	8.73333		
	Kanam	9.49114	10.15453		
	Mangu	9.36223	9.18163		
	Shendam	8.825520	9.459720	Nyuun Primary Health Center	569248.5, 977431.8
	Pankshin	9.3286	9.44143		
Sokoto	Kware	13.21794	5.26564		
	Bodinga	12.825000	5.022100		
	Tambuwal	12.698000	4.859000		
	Wamakko	13.231260	5.117600		
	Sokoto South	13.06106	5.23732		
	Rabah	13.122540	5.505310	General Hospital Rabah	13.12375, 5.49889
	Gudu	13.411600	5.480000		

ANNEX 2: *ANOPHELES* MOSQUITOES COLLECTED BY DIFFERENT METHODS AND SUBJECTED TO PCR ACROSS SITES (OCTOBER 2021-SEPTEMBER 2022)

Sentinel Site	Total PCR +ve from CDC LT (Indoors)	Total PCR +ve from CDC LT (Outdoors)	Total PCR -ve from CDC LT (Indoors)	Total PCR -ve from CDC LT (Outdoors)	Total Analyzed from CDC LT	Total PCR +ve from PSC	Total PCR -ve from PSC	Total Analyzed from PSC	Overall Total Analyzed
Akwa Ibom	34	5	0	0	39	60	0	60	99
Ebonyi	164	12	0	0	176	367	0	367	543
Oyo	68	38	0	0	106	344	0	344	450
Kebbi	377	398	0	0	775	1,100	0	1,100	1,875
Plateau	246	86	0	0	332	493	0	493	825
Sokoto	234	161	0	0	395	692	0	692	1087
Total	**1,123**	**700**	**0**	**0**	**1,823**	**3,056**	**0**	**3,056**	**4,879**

ANNEX 3: *ANOPHELES* CAUGHT BY SPECIES, METHOD, AND SITE (OCTOBER 2021-SEPTEMBER 2022)

Mosquito Species	Akwa Ibom			Ebonyi			Kebbi			Oyo			Plateau			Sokoto			Overall			
	CDC LT			CDC LT			CDC LT			CDC LT			CDC LT			CDC LT			CDC LT			
	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	Total
An. gambiae s.l.	83	6	192	623	20	856	12,658	3,790	22,485	124	56	606	1,726	93	1,395	6,078	2,670	30,408	21,292	6,635	55,942	83,869
An. funestus s.l.	-	-	-	1	-	6	-	-	-	245	117	298	170	34	171	1	7	-	417	158	475	1,050
An. coustani	-	-	-	3	-	1	-	-	-	-	-	-	3	-	-	1	1	-	7	1	1	9
An. nili	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
An. pharoensis	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	2	-	-	2
An. pretoriensis	-	-	-	-	-	-	-	-	-	-	-	-	2	9	3	-	-	-	2	9	3	14
An. rufipes	-	-	-	-	-	-	-	-	-	-	-	-	9	7	21	2	21	-	11	28	21	60
An. squamosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	-	-	11	-	-	11
An. maculipalpis	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	1
An. moucheti	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	2
An. marshallii	1,292	270	190	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	1,292	270	193	1,755
Other	-	-	-	-	-	-	-	-	-	-	-	-	3	3	-	-	-	-	3	3	-	6
Total	**1,375**	**276**	**382**	**628**	**20**	**864**	**12,658**	**3,790**	**22,485**	**369**	**173**	**904**	**1,916**	**146**	**1,593**	**6,093**	**2,699**	**30,408**	**23,039**	**7,104**	**56,636**	**86,779**

In=Indoor CDC Light Trap, Out=Outdoor CDC Light Trap, PSC=Pyrethrum Spray Catch

ANNEX 4: PCR IDENTIFICATION OF MEMBERS OF THE *AN. GAMBIAE* COMPLEX

Sentinel Site	CDC Indoors					CDC Outdoors					PSC					Totals				
	No. PCR +ve	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*
Akwa Ibom	34	6	28	0	0	5	2	3	0	0	60	22	37	0	1	99	30	68	0	1
Ebonyi	164	45	118	0	1	12	1	11	0	0	367	58	304	0	5	543	104	433	0	6
Kebbi	377	14	362	0	1	398	19	374	0	5	1,100	39	1054	0	7	1,875	72	1,790	0	13
Oyo	68	15	51	0	2	38	7	30	0	1	344	88	254	0	2	450	110	335	0	5
Plateau	246	57	187	0	2	86	23	63	0	0	493	136	351	0	6	825	216	601	0	8
Sokoto	234	36	196	0	2	161	23	137	0	1	692	84	596	0	12	1,087	143	929	0	15
Total	**1,123**	**173**	**942**	**0**	**8**	**700**	**75**	**618**	**0**	**7**	**3,056**	**427**	**2,596**	**0**	**33**	**4,879**	**675**	**4,156**	**0**	**48**

ANNEX 5: PCR IDENTIFICATION OF MEMBERS OF THE *AN. FUNESTUS* COMPLEX

Sentinel Site	Total Analyzed	Total by Method			CDC LT Indoors			CDC Outdoors			PSC		
		CDC LT Indoors	CDC LT Outdoors	PSC	*An. funestus* s.s.	*An. funestus* (Other)	*An. leesoni*	*An. funestus* s.s.	*An. funestus* (Other)	*An. leesoni*	*An. funestus* s.s.	*An. funestus* (Other)	*An. leesoni*
Ebonyi	4	1	1	2	1	0	0	1	0	0	2	0	0
Oyo	510	169	89	252	168	0	1	88	1	0	252	0	0
Plateau	212	125	8	79	125	0	0	6	2	0	79	0	0
Sokoto	46	22	24	0	22	0	0	24	0	0	0	0	0
Total	**772**	**317**	**122**	**333**	**316**	**0**	**1**	**119**	**3**	**0**	**333**	**0**	**0**

ANNEX 6: INDOOR RESTING DENSITY OF *ANOPHELES* BY SITE

Sentinel Sites	Month	# of Rooms	Total # of *Anopheles* Caught	Indoor Resting Density
Akwa Ibom	Oct-21	32	70	2.2
	Nov-21	32	73	2.3
	Dec-21	32	12	0.4
	Jan-22	32	1	0.0
	Feb-22	32	0	0.0
	Mar-22	32	0	0.0
	Apr-22	32	0	0.0
	May-22	32	10	0.3
	Jun-22	32	8	0.3
	Jul-22	32	8	0.3
	Aug-22	32	10	0.3
	Sep-22	32	0	0.0
			Mean	**0.5**
Ebonyi	Oct-21	32	140	4.4
	Nov-21	32	69	2.2
	Dec-21	32	17	0.5
	Jan-22	32	32	1.0
	Feb-22	32	8	0.3
	Mar-22	32	10	0.3
	Apr-22	32	8	0.3
	May-22	32	164	5.1
	Jun-22	32	182	5.7
	Jul-22	32	115	3.6
	Aug-22	32	74	2.3
	Sep-22	32	37	1.2
			Mean	**2.2**
Kebbi	Oct-21	192	1,037	5.4
	Nov-21	192	557	2.9
	Dec-21	192	613	3.2
	Jan-22	192	563	2.9
	Feb-22	192	987	5.1
	Mar-22	192	2,559	13.3
	Apr-22	192	2,857	14.9
	May-22	192	763	4.0
	Jun-22	192	1,261	6.6
	Jul-22	192	3,162	16.5
	Aug-22	192	3,376	17.6
	Sep-22	192	4,750	24.7
			Mean	9.8

Sentinel Sites	Month	# of Rooms	Total # of *Anopheles* Caught	Indoor Resting Density
Oyo	Oct-21	32	16	0.5
	Nov-21	32	6	0.2
	Dec-21	32	8	0.3
	Jan-22	32	3	0.1
	Feb-22	32	8	0.3
	Mar-22	32	39	1.2
	Apr-22	32	34	1.1
	May-22	32	109	3.4
	Jun-22	32	89	2.8
	Jul-22	32	81	2.5
	Aug-22	32	63	2.0
	Sep-22	32	150	4.7
			Mean	1.6
Plateau	Oct-21	32	53	1.7
	Nov-21	32	240	7.5
	Dec-21	32	52	1.6
	Jan-22	32	5	0.2
	Feb-22	32	30	0.9
	Mar-22	32	74	2.3
	Apr-22	32	19	0.6
	May-22	32	283	8.8
	Jun-22	32	109	3.4
	Jul-22	32	166	5.2
	Aug-22	32	117	3.7
	Sep-22	32	247	7.7
			Mean	3.6
Sokoto	Oct-21	192	2,399	12.5
	Nov-21	192	2,242	11.7
	Dec-21	192	1,061	5.5
	Jan-22	192	943	4.9
	Feb-22	192	1,173	6.1
	Mar-22	192	1,504	7.8
	Apr-22	192	1,840	9.6
	May-22	192	1,377	7.2
	Jun-22	192	1,267	6.6
	Jul-22	192	5,337	27.8
	Aug-22	192	5,653	29.4
	Sep-22	192	5,612	29.2
			Mean	13.2

ANNEX 7: INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES BY SITE

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		An. gambiae s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Akwa Ibom	Oct-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Apr-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	May-22	0	0	5	0	0	0	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-22	11	2	42	4	0	0	0.88	0.20	3.53	0.30	0.00	0.00	0.000	0.000	0.00	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-22	0	0	2	0	0	0	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-22	0	0	1	0	0	0	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	TOTAL	15	2	68	4	0	0	1.22	0.20	5.70	0.30	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Ebonyi	Oct-21	1	0	29	0	0	0	0.12	0.00	2.38	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-22	1	0	1	0	0	0	0.04	0.00	0.04	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Apr-22	0	0	1	0	0	0	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	May-22	5	0	89	4	0	0	0.39	0.00	7.44	0.33	0.00	0.00	1.000	0.00	0.000	0.000	0.00	0.00	12.142	0.000	0.000	0.000	0.000	0.000
	Jun-22	126	0	142	0	0	0	10.51	0.00	11.82	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-22	43	1	113	5	0	0	3.58	0.08	9.43	0.42	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-22	10	0	15	2	0	0	0.83	0.00	1.25	0.17	0.00	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-22	13	0	30	0	1	0	1.06	0.00	2.48	0.00	0.12	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	TOTAL	171	2	448	18	4	0	14.25	0.14	37.35	1.53	0.32	0.00	0.022	0.000	0.000	0.000	0.000	0.00	12.142	0.000	0.000	0.000	0.000	0.000

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		An. gambiae s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Oyo	Oct-21	0	0	2	0	0	0	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-21	0	0	2	0	0	0	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-22	1	1	1	4	0	0	0.08	0.08	0.08	0.33	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Apr-22	0	0	6	0	0	0	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	May-22	0	0	2	2	0	0	0.00	0.00	0.17	0.17	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-22	0	0	5	3	0	0	0.00	0.00	0.42	0.25	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-22	3	2	23	2	0	0	0.27	0.13	1.90	0.13	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-22	6	2	15	18	0	2	0.50	0.18	1.25	1.47	0.00	0.18	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-22	18	6	36	15	3	0	1.52	0.47	3.04	1.28	0.28	0.00	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	TOTAL	27	10	93	44	4	1	2.28	0.86	7.75	3.68	0.30	0.12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Kebbi	Oct-21	0	0	689	194	0	0	0.00	0.00	9.57	2.69	0.00	0.00	0.00	0.00	0.000	0.038	0.00	0.00	0.000	0.000	0.000	3.213	0.000	0.000
	Nov-21	9	8	274	214	0	25	0.13	0.11	3.80	2.97	0.00	0.34	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-22	17	0	202	21	8	7	0.23	0.00	2.80	0.29	0.12	0.10	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-22	0	0	695	153	0	0	0.00	0.00	9.65	2.13	0.00	0.00	0.00	0.00	0.021	0.000	0.00	0.00	0.000	0.000	5.751	0.000	0.000	0.000
	Mar-22	0	0	1,662	675	0	0	0.00	0.00	23.08	9.38	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Apr-22	0	42	0	807	0	0	0.00	0.59	0.00	11.20	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	May-22	68	56	489	213	0	0	0.95	0.78	6.79	2.96	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-22	34	21	237	56	0	0	0.47	0.29	3.29	0.78	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-22	0	0	1,862	316	0	0	0.00	0.00	25.86	4.39	0.00	0.00	0.00	0.00	0.000	0.025	0.00	0.00	0.000	0.000	0.000	3.401	0.000	0.000
	Aug-22	0	0	1,867	303	0	0	0.00	0.00	25.93	4.21	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-22	0	0	2,122	631	0	0	0.00	0.00	29.47	8.76	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	TOTAL	470	181	12,154	3,561	34	48	6.53	2.51	168.81	49.46	0.47	0.66	0.000	0.000	0.003	0.005	0.000	0.000	0.000	0.000	5.751	6.614	0.000	0.000

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		An. gambiae s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Plateau	Oct-21	4	2	2	0	0	0	0.30	0.17	0.20	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-21	37	2	56	3	0	0	3.10	0.14	4.65	0.28	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-21	4	0	42	20	0	0	0.30	0.00	3.53	1.67	0.00	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-22	2	0	3	0	0	0	0.14	0.00	0.28	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-22	2	0	2	0	0	0	0.13	0.00	0.13	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-22	3	0	8	0	0	0	0.21	0.00	0.63	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Apr-22	0	0	6	1	0	0	0.00	0.00	0.50	0.08	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	May-22	22	0	106	2	4	0	1.85	0.00	8.87	0.17	0.37	0.00	0.000	0.00	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-22	3	4	79	6	0	0	0.23	0.37	6.61	0.46	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-22	55	5	92	18	0	0	4.55	0.38	7.70	1.53	0.00	0.00	0.077	0.000	0.000	0.000	0.00	0.00	10.850	0.000	0.000	0.000	0.000	0.000
	Aug-22	61	4	106	6	0	0	5.10	0.31	8.81	0.52	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-22	177	7	815	13	35	0	14.77	0.56	67.94	1.11	2.95	0.00	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	TOTAL	400	25	1,312	68	14	0	33.33	2.07	109.34	5.68	1.17	0.00	0.018	0.000	0.000	0.000	0.000	0.00	10.850	0.000	0.000	0.000	0.000	0.000
Sokoto	Oct-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-21	26	0	222	0	0	0	0.36	0.00	3.09	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-21	18	0	163	95	0	0	0.25	0.00	2.26	1.32	0.00	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-22	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-22	158	28	385	304	0	0	2.20	0.38	5.34	4.23	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-22	9	26	254	157	0	0	0.13	0.36	3.53	2.18	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Apr-22	0	0	596	398	21	0	0.00	0.00	8.28	5.53	0.29	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	May-22	0	0	316	235	0	0	0.00	0.00	4.39	3.26	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-22	0	0	164	41	0	0	0.00	0.00	2.28	0.57	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-22	225	58	365	115	0	0	3.12	0.80	5.07	1.60	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-22	332	102	664	174	0	15	4.61	1.41	9.22	2.42	0.00	0.20	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-22	1054	148	585	408	117	0	14.63	2.06	8.13	5.66	1.63	0.00	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	TOTAL	935	381	5,091	2,272	52	17	77.92	31.79	424.25	189.33	4.33	1.38	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

ANNEX 8: ANNUAL EIR FOR ALL SENTINEL SITES

Sentinel Sites	Mosquito Species	Estimated EIR over 12 months	
		Indoors	Outdoors
Akwa Ibom	*An. gambiae* s.s.	0.0	0.0
	An. coluzzii	0.0	0.0
	An. arabiensis	0.0	0.0
Ebonyi	*An. gambiae* s.s.	12.1	0.0
	An. coluzzii	0.0	0.0
	An. arabiensis	0.0	0.0
Kebbi	*An. gambiae* s.s.	0.0	0.0
	An. coluzzii	5.8	6.6
	An. arabiensis	0.0	0.0
Oyo	*An. gambiae* s.s.	0.0	0.0
	An. coluzzii	0.0	0.0
	An. arabiensis	0.0	0.0
Plateau	*An. gambiae* s.s.	10.9	0.0
	An. coluzzii	0.0	0.0
	An. arabiensis	0.0	0.0
Sokoto	*An. gambiae* s.s.	0.0	0.0
	An. coluzzii	0.0	0.0
	An. arabiensis	0.0	0.0

ANNEX 9: INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES BY SITE FOR *AN. FUNESTUS* S.S.

Sentinel Site	Month	Number Identified		HBR		SPR		Monthly EIR	
		In	Out	In	Out	In	Out	In	Out
Oyo	Oct-21	8	3	0.7	0.3	0.00	0.00	0.00000	0.00000
	Nov-21	8	1	0.7	0.1	0.00	0.00	0.00000	0.00000
	Dec-21	2	1	0.2	0.1	0.00	0.00	0.00000	0.00000
	Jan-22	0	0	0.0	0.0	0.00	0.00	0.00000	0.00000
	Feb-22	1	0	0.1	0.0	0.00	0.00	0.00000	0.00000
	Mar-22	1	0	0.1	0.0	0.00	0.00	0.00000	0.00000
	Apr-22	21	6	1.8	0.5	0.00	0.00	0.00000	0.00000
	May-22	57	30	4.8	2.5	0.00	0.05	0.00000	3.87500
	Jun-22	48	35	4.0	2.9	0.00	0.05	0.00000	4.60526
	Jul-22	51	19	4.3	1.6	0.00	0.00	0.00000	0.00000
	Aug-22	11	4	0.9	0.3	0.00	0.10	0.00000	1.03333
	Sep-22	37	18	3.1	1.5	0.00	0.00	0.00000	0.00000
	TOTAL	245	117	20.4	9.8	0.00	0.03	0.0	9.5
Plateau	Oct-21	0	0	0.0	0.0	0.00	0.00	0.00000	0.00000
	Nov-21	10	0	0.8	0.0	0.00	0.00	0.00000	0.00000
	Dec-21	10	3	0.8	0.3	0.10	0.00	2.58333	0.00000
	Jan-22	2	0	0.2	0.0	0.50	0.00	2.58333	0.00000
	Feb-22	2	0	0.2	0.0	0.00	0.00	0.00000	0.00000
	Mar-22	0	0	0.0	0.0	0.00	0.00	0.00000	0.00000
	Apr-22	0	0	0.0	0.0	0.00	0.00	0.00000	0.00000
	May-22	1	0	0.1	0.0	0.00	0.00	0.00000	0.00000
	Jun-22	1	2	0.1	0.2	0.00	0.00	0.00000	0.00000
	Jul-22	37	18	3.1	1.5	0.00	0.00	0.00000	0.00000
	Aug-22	28	4	2.3	0.3	0.00	0.00	0.00000	0.00000
	Sep-22	79	7	6.6	0.6	0.00	0.00	0.00000	0.00000
	TOTAL	170	34	14.2	2.8	0.02	0.00	5.2	0.0

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