



U.S. PRESIDENT'S MALARIA INITIATIVE



VECTORLINK NIGERIA FINAL ENTOMOLOGY REPORT

APRIL – SEPTEMBER 2018

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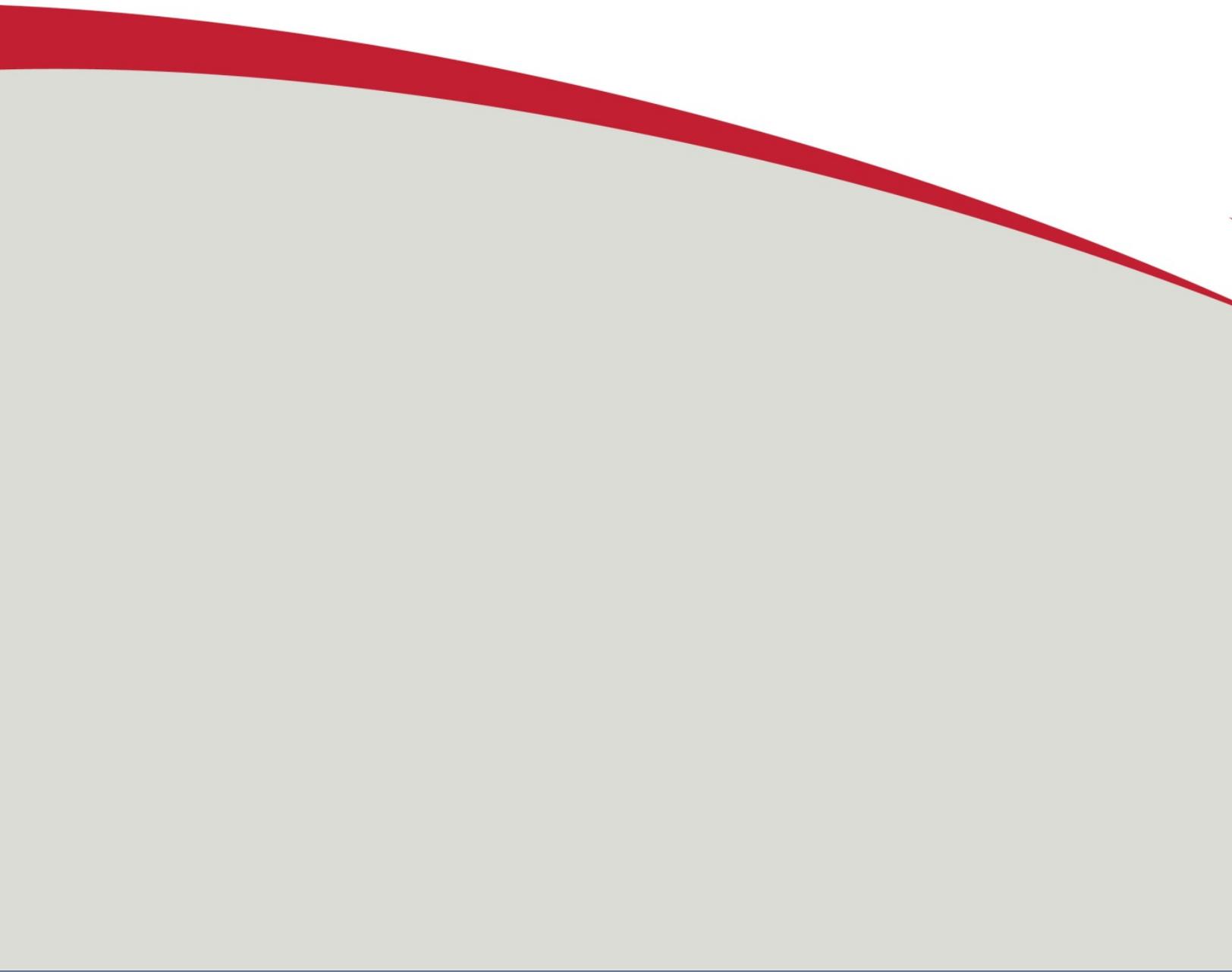
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ACRONYMS

AIRS	Africa Indoor Residual Spraying project
CDC	Centers for Disease Control and Prevention
CDC LT	CDC Light Trap
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
GPIRM	Global Plan for Insecticide Resistance Management
HBR	Human Biting Rate
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
Kdr	Knock down resistance
LGA	Local Government Area
LLIN	Long-Lasting Insecticidal Net
NMEP	National Malaria Elimination Program
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
WHO	World Health Organization

EXECUTIVE SUMMARY

From April to September 2018, the U.S. President's Malaria Initiative (PMI) VectorLink Project conducted both longitudinal vector surveillance and insecticide resistance monitoring at seven sentinel sites and insecticide resistance monitoring at two additional sites in Nigeria. Pyrethrum spray catches (PSCs) and human-baited CDC light traps (CDC LTs) situated both indoors and outdoors were used to sample mosquitoes and determine the species composition, indoor resting density, biting rates, longevity, infectivity rates, human blood indices, and entomological inoculation rates (EIRs) of malaria vectors at each site. WHO tube test kits and CDC bottle bioassays were used to determine the susceptibility of vector populations. Insecticide resistance intensity was also determined for *Anopheles gambiae* s.l. The underlying mechanisms of resistance were assessed using molecular methods and synergist assays.

A total of 23,097 *Anopheles* mosquitoes were collected from seven sentinel sites using human-baited CDC LTs and PSCs. *An. gambiae* s.l. was the most abundant species at each site, ranging from 62.7 to 99.8 percent of mosquitoes collected in Akwa Ibom and Sokoto, respectively. A total of 2,646 *An. gambiae* s.l. mosquitoes were identified by PCR from PSC and CDC LTs collections, including *An. gambiae* 1447 (54.7%), *An. coluzzii* 575 (21.7%), *An. arabiensis* 608 (23%), and a hybrid of *An. gambiae* and *An. coluzzii* 16 (0.6%).

An. gambiae was the dominant species both indoors and outdoors in Akwa Ibom, Nasarawa Doma, and Plateau. In Nassarawa Eggon and Sokoto, its predominance was restricted indoors. In Ebonyi, *An. coluzzii* was the predominant species indoors (43.9%). *Plasmodium falciparum* sporozoite rates in *An. gambiae* collected indoors ranged from 2.7% in Nassarawa (Doma) to 11.9% in Ebonyi. Outdoors, the sporozoite rates ranged from 2.2% in Nassarawa (Doma) to 7.4 percent in Ebonyi. The sporozoite infection rate of *An. coluzzii* collected indoors ranged from 0 percent in Oyo to 12.5 percent in Bauchi. EIRs varied according to the vector species and the ecozone. Overall comparative contributions of EIR between members of *An. gambiae* s.l. indicated that the highest numbers of infective bites indoors and outdoors were from *An. coluzzii* and *An. arabiensis*, respectively. The indoor EIR of *An. coluzzii* varied from 0 in Oyo to 137.39 and 147.71 infective bites per person per year in Akwa Ibom and Plateau, respectively. All *An. coluzzii* mosquitoes analyzed from Oyo and all *An. gambiae* mosquitoes caught in Sokoto fed on human blood.

Insecticide susceptibility test results indicated that pyrethroid resistance was widespread in *An. gambiae* s.l. at all sites. In Akwa Ibom and Bauchi, possible resistance was detected to lambda-cyhalothrin, deltamethrin, and alpha-cypermethrin. Pyrethroid resistance was widely recorded in mosquitoes from Ebonyi, Oyo, Sokoto, Nasarawa, and Plateau. *An. gambiae* mosquitoes in Ebonyi were susceptible to alpha-cypermethrin. Intensity assay tests indicated that deltamethrin resistance was recorded in *An. gambiae* s.l. from Akwa Ibom and Oyo at 2X but not at 5X or 10X the diagnostic concentration. *An. gambiae* s.l. mosquitoes in Bauchi, Plateau, Benue, and Sokoto were fully susceptible to deltamethrin at 2X. Deltamethrin intensity assays in Ebonyi indicated varying high resistance intensities at 2X, 5X and, in some cases, 10X the diagnostic concentration across the Local Government Areas (LGAs). Piperonyl butoxide (PBO) synergist assays reversed pyrethroid (deltamethrin, alpha-cypermethrin, and permethrin) resistance in *An. gambiae* s.l. to varying degrees. In Sokoto, Ebonyi, and Akwa Ibom, the mortality of permethrin-exposed *An. gambiae* s.l. was 17-81 percent compared to 94-100 percent mosquito mortality following pre-exposure to PBO. While PBO significantly restored susceptibility (98-100%) in permethrin-resistant *An. gambiae* s.l. populations from all locations in Ebonyi, this was not the case among *An. gambiae* s.l. mosquitoes tested from Nasarawa, Oyo, and Plateau.

Findings demonstrate the contributions of *An. gambiae*, *An. coluzzii*, and *An. arabiensis* to malaria transmission across all ecozones of Nigeria. Results provide data for Nigeria's National Malaria Program to deploy effective insecticides via LLINs or IRS based upon resistance profiles of major vectors in the states surveyed, consistent with the insecticide resistance management plan. As data indicate, transmission occurs both indoors and outdoors, in the longer term interventions to reduce outdoor transmission will likely be required.

I. INTRODUCTION

Nigeria contributes the highest proportion of malaria cases (25%) and death due to malaria (19%) of all countries in the world (WHO, 2018). The country has five ecozones that each support a variety of *Anopheles* species involved in malaria transmission. Since 2014, the U.S. President's Malaria Initiative (PMI), through the Africa Indoor Residual Spraying Project and now the VectorLink Project, supports entomological data collection from seven sentinel sites representing the five ecozones of Nigeria. The project captured PMI entomological indicators in all sentinel sites. Information collected from these sites support the National Malaria Elimination Program (NMEP) in making data-driven decisions for programming vector control activities.

The Nigeria Federal Ministry of Health's National Malaria Elimination Program (NMEP), in collaboration with VectorLink Nigeria, established six malaria vector surveillance and insecticide resistance monitoring sites in the country in 2014. Each of the sentinel sites has well-trained staff and the facilities and basic equipment needed for entomological monitoring. In 2018, the project expanded to seven sentinel sites and two insecticide resistance monitoring sites actively involved in monthly vector surveillance and insecticide resistance monitoring to inform decisions for the LLIN mass campaign and other vector control interventions in the future. The plan is for these sites to continue to be monitored on a regular basis. To build sustainable entomology capacity and institutionalize surveillance activities, VectorLink partners with local universities and research institutes to implement entomological monitoring at each sentinel site.

Between April and September 2018, VectorLink Nigeria coordinated vector surveillance and insecticide resistance monitoring activities to generate data on malaria vector species composition, density, feeding time, location (indoors or outdoors), seasonality, insecticide susceptibility status of the major malaria vectors, and the intensity and mechanism of resistance in different ecozones of Nigeria. These data support NMEP in evidence-based decision making on the optimal time and place to implement vector control. This report summarizes entomological monitoring activities completed between April and September 2018.

I.1 STUDY SITES AND COLLECTION AND ANALYTICAL METHODS

VectorLink Nigeria implements both vector surveillance and insecticide resistance monitoring in seven sentinel sites and insecticide resistance monitoring only in two additional sites (Tables 1 and 2).

Table 1: Longitudinal Vector Surveillance and Insecticide Resistance Monitoring Sites And Affiliated Institutions

Geopolitical Zone	State/Institution	LGAs/Sentinel Sites	Ecozone(s)
South West	Oyo/University of Ibadan	Akinyele/Olorisaoko	Rainforest/Guinea Savannah
South East	Ebonyi/State Univ Abakaliki	Ezaa North/ Umuaghara	Rainforest
South	Akwa Ibom/University of Uyo	Mpat Enin/Ibekwe Akpannya	Mangrove swamps/rainforest
North East	Bauchi/ATBU	Dass/Gwantar	Sudan Savannah
North Central	Nasarawa/State Univ Keffi	Doma, Nasarawa Eggon	Guinea Savannah
North Central	Jos/University of Jos	Shendam/Tumbi	Guinea Savannah
North West	Sokoto/Usmanu Danfodiyo University Sokoto	Rabah/Angwan Sarki	Sahel Savannah

Table 2: Additional Insecticide Resistance Monitoring Sites and Affiliated Institutions

Geo-political Zone	State/Institution	LGAs/Insecticide Resistance Monitoring Sites	Ecozone(s)
North Central	Benue/Federal University of Agriculture Makurdi	Makurdi, Gboko, Katsina-Ala, Kwande, Otukpo, and Oju	Guinea Savanah
North West	Zamfara/Usmanu Danfodiyo University Sokoto	Gusau, Chafe, Anka, Kaura Namoda Talata-Mafara and Maru,	Sahel Savanah

*LGAs in Kebbi and Cross River will be determined in collaboration with PIs once these individuals are selected.

From April to September 2018, *Anopheles* mosquitoes were collected monthly from seven sentinel sites located in five ecozones of Nigeria (Figure 1). Mosquitoes were caught using human-baited Centers for Disease Control and Prevention light traps (CDC LTs) indoors and outdoors, and Pyrethrum Spray Catches (PSCs). The design for adult mosquito collection is shown (Table 3).

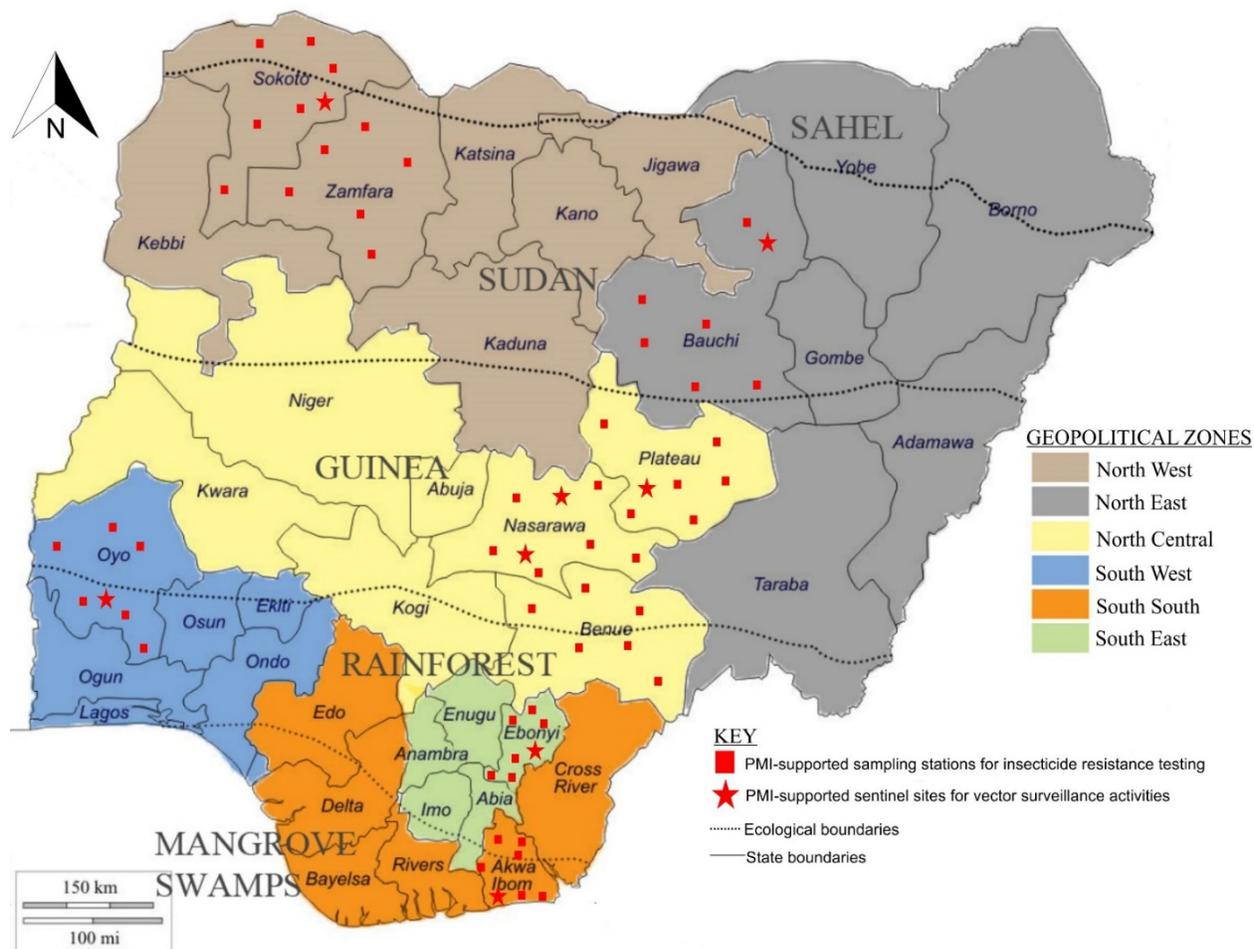


Figure 1: Map of Nigeria showing the Sentinel Sites and Insecticide Resistance Monitoring Sites

Table 3: Longitudinal Monitoring Adult Mosquito Collection Methods

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

The team collected adult mosquitoes monthly in all the sentinel sites using PSCs and CDC LTs. Anopheline larvae were collected using ladles and reared to adults for insecticide susceptibility tests. Data collected from longitudinal surveillance sites were collated used to measure the indicators in Table 4, which are also described in the sections on the respective mosquito collection methods described below. Annex A details the specific location of the sampling sites and the name of the nearest health facility to the sentinel site.

Table 4: Entomological Surveillance Indicators and Timelines

Indicator	Purpose	Timeline 2018	Frequency
Vector composition, density, distribution, and seasonality	To evaluate the indoor resting density of vectors at the sentinel sites	April through September 2018	Every month from April to September 2018
Vector feeding time and location	To evaluate the indoor/outdoor, biting behavior of the vector	April through September 2018	Every month from April through September 2018
Determination of Parity	To determine the physiological age and parity rate of female Anopheles mosquitoes	April through September 2018	Every month from April through September 2018
Vector Infectivity	To determine the proportion of Anopheles mosquitoes infected with Plasmodium sporozoites	April – September 2018	Every month from April through September 2018
Human Blood Index	Proportion of Anopheles mosquitoes that fed on human blood	April – September 2018	Every month from April through September 2018
Vector susceptibility studies, resistance mechanism analysis, and intensity assays	To evaluate the susceptibility of local mosquitoes to insecticides as well as the mechanisms and intensity of resistance.	April – September 2018	Every other year, unless resistance is detected

1.2 CDC LIGHT TRAP COLLECTION

Field teams placed 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). The teams sent all samples collected from the field to the centrally-located insectary at Nasarawa State University Keffi for further processing and analyses. The mean human biting rate (HBR) was calculated as the number of mosquitoes attracted to the human-baited CDC LT (both indoor and outdoor) per night. The entomological inoculation rate (EIR), defined as the number of infectious bites per person per night, was calculated as the HBR multiplied by the sporozoite infection rate, on a monthly basis and over a period of six months.

1.3 PYRETHRUM SPRAY CATCHES

The team randomly sampled 32 houses per sentinel site per month using the PSC method (WHO 1975) to sample indoor-resting mosquitoes. The teams sent all samples collected from the field to the centrally-located insectary at Nasarawa State University Keffi for further processing and analysis. The mean indoor resting density was determined by calculating the number of mosquitoes per house per night during 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 Gillies and De Meillon (1968), Gillet (1972), Gillies and Coetzee (1987), and Kent (2006). The teams labeled all non-dissected *Anopheles* specimens and stored them individually over silica gel in Eppendorf tubes for further processing. The team sent all samples collected to the centrally-located insectary at Nasarawa State University Keffi.

1.5 DETERMINATION OF PARITY RATE

To determine physiological age and parity rate, the team dissected ovaries from 20 percent 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). This was done each month for six months (Detinova 1962, Detinova and Gillies 1964).

1.6 PCR IDENTIFICATION OF MEMBERS OF AN. GAMBIAE COMPLEX

Polymerase chain reaction (PCR) assays were carried out on mosquito samples collected to identify members of the *An. gambiae* s.l. complex and *An. funestus* group at the Nigeria Institute for Medical Research (NIMR), Yaba Lagos. PCR amplification was carried out using the *An. gambiae* species-specific multiplex PCR (Scott *et al.* 1993). PCR products were separated in agarose gel stained with ethidium bromide and visualized under an ultraviolet (UV) trans-illuminator. The PCR diagnosis bands for this assay included: a 464 base pair band for *An. melas*, 390 base pair for *An. gambiae* s.s., and 315 base pair for *An. arabiensis*. All PCR negative tests were repeated for confirmation PCR was conducted on approximately 10% of the samples caught (those that had been caught indoors and outdoors by CDC LTs and by PSCs). To make up the required number, 10 percent of mosquitoes collected monthly, which is a subset of the overall number of mosquitoes collected were randomly selected from each method, both from indoor and outdoor collections. The team amplified extracted DNA using the *An. gambiae* species-specific multiplex PCR (Scott *et al.* 1993; Fanello *et al.* 2002).

1.6.1 PLASMODIUM SPOROZOITE ASSAYS

To estimate the *Plasmodium* infection rate in the mosquito population, the team also performed enzyme-linked immunosorbent assays (ELISAs) on a proportion of randomly-selected mosquitoes collected from the field. These were carried out according to methods described by Burkot *et al.* (1984). The blood meal index of the selected mosquitoes was determined by ELISA testing of animal blood sources of *Anopheles* mosquitoes.

1.7 INSECTICIDE RESISTANCE MONITORING AND DETERMINATION OF THE DIAGNOSTIC DOSE FOR CHLORFENAPYR

Adult *Anopheles* mosquitoes (3-5 days old) were reared from wild caught larvae. Insecticide susceptibility status of *An. gambiae* s.l. to pyrethroid (deltamethrin and permethrin, lambda-cyhalothrin and alpha-cypermethrin) organochlorine (DDT), organophosphate (pirimiphos-methyl), and carbamate (proprhexur and bendiocarb) insecticides were determined using WHO susceptibility test kit and CDC bottle bioassay methods (WHO, 2013; Brogdon and Chan, 2010). Insecticide resistance tests were carried out with different doses (x1, x2, x5, and x10) to determine the intensity of pyrethroid resistance in locations where resistance was detected. 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 *kdr* genotype frequencies were determined among *An. gambiae* s.l. using allele-specific PCR assays. Surviving mosquitoes from intensity and synergist assays were analyzed for *kdr* alleles. To determine the diagnostic dosage of chlorfenapyr, susceptibility tests were carried out on Kisumu strain mosquitoes (control) and *An. gambiae* s.l. from Ebonyi, Nasarawa, and Oyo using the CDC bottle assay with various concentrations of insecticide (12.5, 25, 50, 100, and 200 µg per bottle).

2. RESULTS

2.1 MOSQUITO ABUNDANCE AND SPECIES COMPOSITION

A total of 23,097 *Anopheles* mosquitoes were collected from seven sentinel sites using human-baited CDC LTs (indoors/outdoors) and PSCs (Annex B).

An. gambiae s.l. was the most abundant species across all the sites ranging from 62.7% in Akwa Ibom (mangrove swamp) to 99.8% in Sokoto (Sahel savannah) (Figure 2). Other *Anopheles* species identified in varying abundance were *An. moucheti*, *An. coustani*, *An. nili*, *An. funestus*, and *An. pharoensis*. Other localized species observed were *An. squamosus*, *An. maculipalpis*, *An. longipalpis*, and *An. rufipes*. Annex B provides the number of each species collected by site and collection method. The major malaria vectors in Nigeria are the members of the *An. gambiae* s.l. complex (*An. gambiae*, *An. coluzzii*, and *An. arabiensis*) and *An. funestus*. *An. nili*, *An. moucheti*, *An. pharoensis*, *An. coustani*, and *An. longipalpis* are considered secondary vectors of malaria in the country.

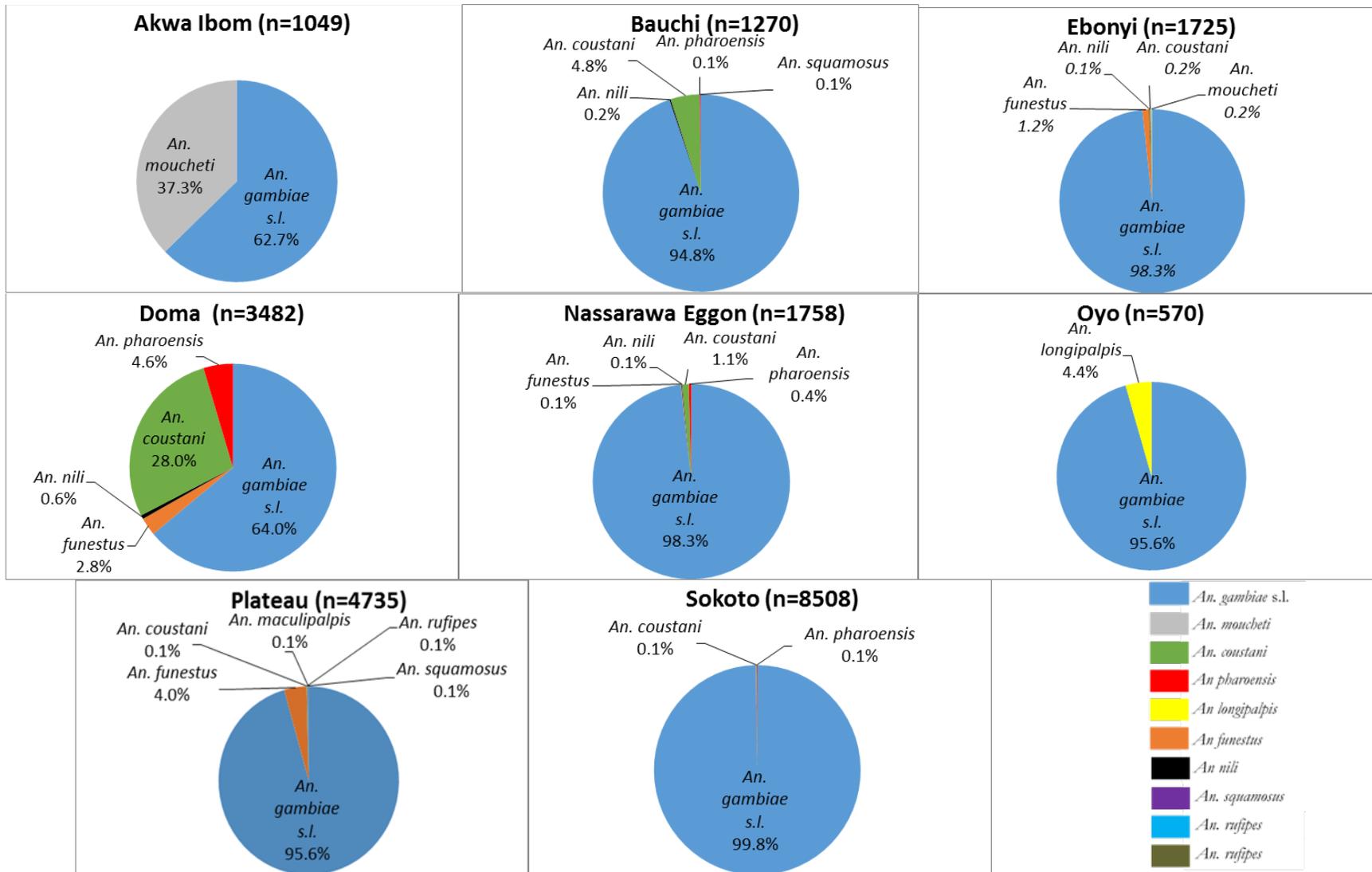


Figure 2: Species Composition across Sites (April-September 2018)

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

Between April and September 2018, 2,646 *An. gambiae* s.l. mosquitoes were collected by PSCs and CDC LTs. 1,447 were identified as *An. gambiae* (54.7%), 575 were *An. coluzzii* (21.7%), 608 were *An. arabiensis* (23%), and 16 were hybrid *An. gambiae/An. coluzzii* (0.6%) (Annex C). Of the total 2,646 mosquitoes collected, 1,457 were from CDC LT collections. Of these, *An. gambiae* (762, 52.3%) was the predominant species compared to *An. coluzzii* (307, 21.1%), *An. arabiensis* (377, 25.9%), and hybrids forms of *An. gambiae/An. coluzzii* (11, 0.8%) (Annex C).

An. gambiae was the dominant species both indoors and outdoors in Akwa Ibom, Nassarawa Doma, and Plateau, compared to Nassarawa Eggon and Sokoto, where its predominance was restricted to indoors. This contrasted with Ebonyi (rainforest) where *An. coluzzii* was the predominant species collected indoors (43.9%). Hybrid forms were also recorded outdoors in Ebonyi (1.4%), and both indoors (0.6%) and outdoors (1.7%) in Nassarawa Doma (Figure 3). A total of 1,189 *An. gambiae* s.l. mosquitoes collected by PSC were identified by PCR. This consists of 685 *An. gambiae* (57.6%), 268 *An. coluzzii* (27.5%), 231 *An. arabiensis* (19.4%), and 5 hybrid *An. gambiae/An. coluzzii* (0.4%) (Annex C). The trends and seasonality of the Anopheline species over time is shown in Annex D.

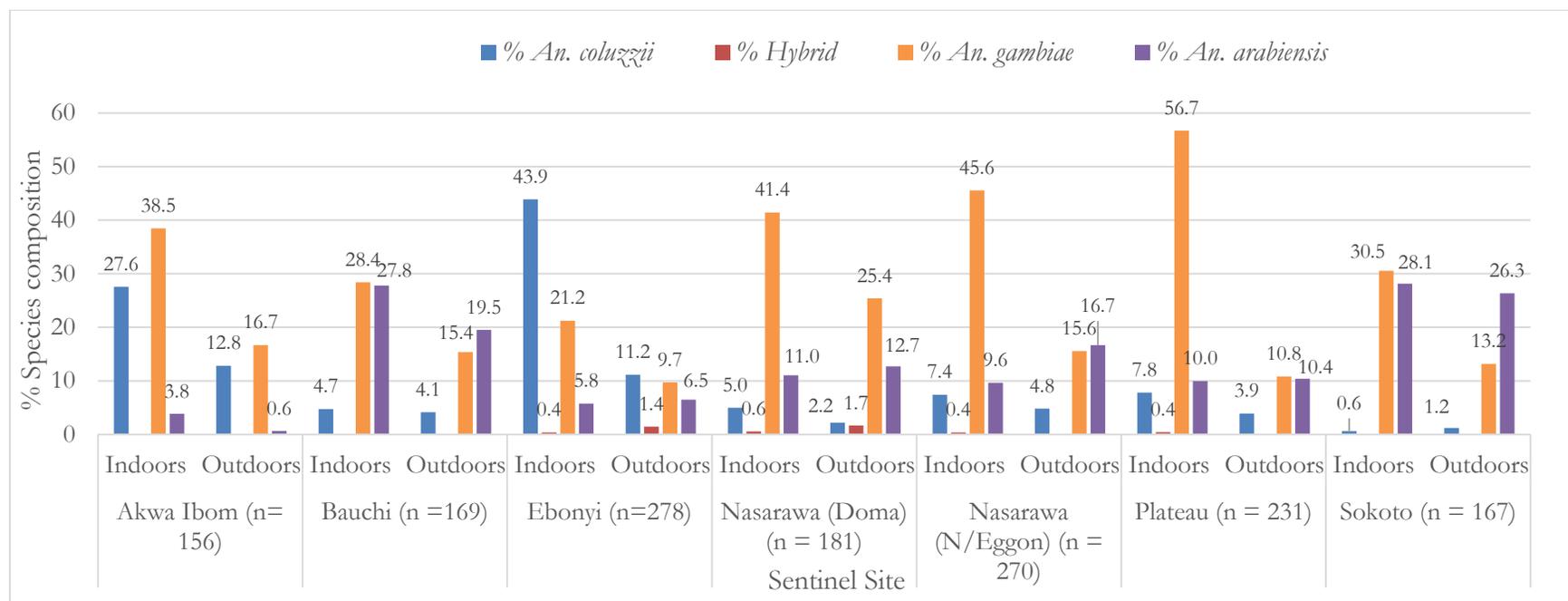


Figure 3: Proportion of *An. coluzzii*, *An. gambiae*, *An. arabiensis*, and Hybrid Species Collected Indoors and Outdoors across Sentinel Sites

As shown in Table 5, *Plasmodium falciparum* sporozoite rates of *An. gambiae* collected indoors ranged from 0.0% in Oyo to 11.9% in Ebonyi. Outdoors, the sporozoite rates in *An. gambiae* ranged from 0.0% in Oyo to 8.0% in Plateau. Sporozoite infection rates in *An. coluzzii* collected indoors varied from 0.0% in Oyo to the highest sporozoite infection rate of 12.5 % in Bauchi. Sokoto had the highest rate of *An. arabiensis* collected indoors that tested positive for *P. falciparum* (8.5%) while Plateau had the highest rate of those collected outdoors (8.3%). *An. coustani* were tested for sporozoite antigen and only one tested positive in 2018. None of the *An. moucheti* tested were positive.

Table 5: Sporozoite Positivity Rates of *An. coluzzii*, *An. gambiae*, and *An. arabiensis* Mosquitoes across Sites

Site	Total Analyzed	<i>An. coluzzii</i>						<i>An. gambiae</i>						<i>An. arabiensis</i>					
		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	259	43 (16.6)	20 (7.7)	3	0	7.0	0.0	60 (23.2)	26 (10.0)	3	1	5.0	3.8	6 (2.3)	1 (0.4)	0	0	0.0	0.0
Bauchi	284	8 (2.8)	7 (2.5)	1	0	12.5	0.0	48 (16.9)	26 (9.2)	2	1	4.2	3.8	47 (16.5)	33 (11.6)	2	0	4.3	0.0
Ebonyi	548	122 (22.3)	31 (5.7)	1	0	0.8	0.0	59 (10.8)	27 (4.9)	7	2	11.9	7.4	16 (2.9)	18 (3.3)	0	0	0.0	0.0
Nassarawa (Doma)	343	9 (2.6)	4 (1.2)	0	0	0.0	0.0	75 (21.9)	46 (13.4)	2	1	2.7	2.2	20 (5.8)	23 (6.7)	1	0	5.0	0.0
Nasarawa (Nassarawa Eggon)	530	20 (3.8)	13 (2.5)	1	0	5.0	0.0	123 (23.2)	42 (7.9)	6	2	4.9	4.8	26 (4.9)	45 (8.5)	1	0	3.8	0.0
Oyo	184	0 (0.0)	0 (0.0)	0	0	0.0	0.0	0 (0.0)	1 (0.5)	0	0	0.0	0.0	0 (0.0)	4 (2.2)	0	0	0.0	0.0
Plateau	464	18 (3.9)	9 (1.9)	1	0	5.6	0.0	131 (28.2)	25 (5.4)	4	2	3.1	8.0	23 (5.0)	24 (5.2)	0	2	0.0	8.3
Sokoto	539	1 (0.2)	2 (0.4)	0	0	0.0	0.0	51 (9.5)	22 (4.1)	4	0	7.8	0.0	47 (8.7)	44 (8.2)	4	3	8.5	6.8

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

2.3 HUMAN BITING RATES ACROSS SITES

The mean indoor biting rates of *An. gambiae* s.l. peaked in September at Akwa Ibom, Bauchi and Ebonyi while in Nasarawa Doma, Nassarawa Eggon, and Plateau, the peak occurred in July. In Sokoto, indoor and outdoor biting rates peaked in August. The biting pattern in Oyo was not clear due to low numbers of mosquitoes collected across all months (Figure 4 and 5).

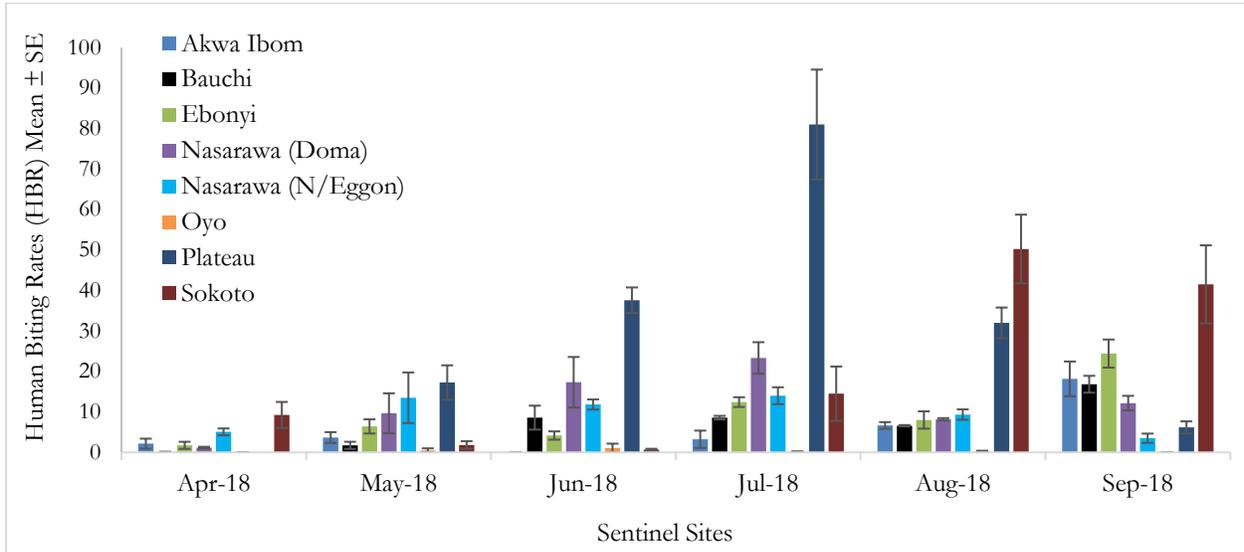


Figure 4: Monthly Indoor Human Biting Rates (HBR) of *An. gambiae* s.l. by Site

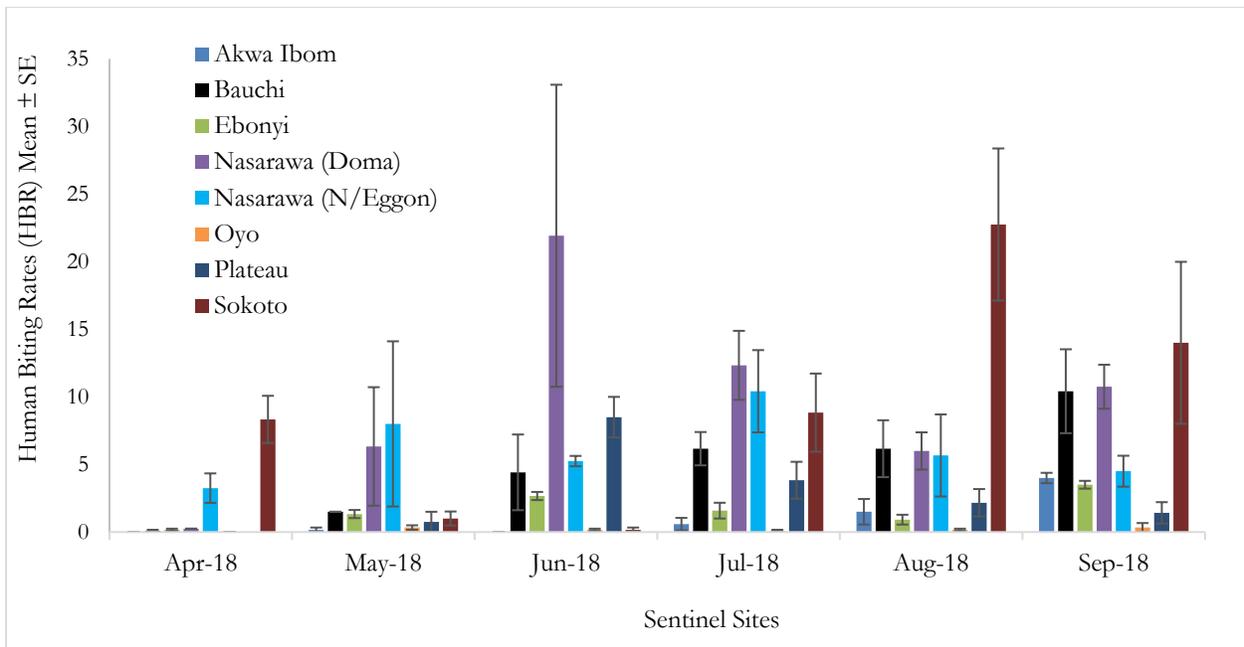


Figure 5: Monthly Outdoor Human Biting Rates (HBR) of *An. gambiae* s.l. by Site

2.4 MONTHLY INDOOR RESTING DENSITY (IRD) OF *AN. GAMBIAE* S.L.

The Indoor Resting Density (IRD) varied across the sites and across months, ranging from 0 mosquitoes per house per night in Plateau in April to 68.2 mosquitoes per house per night in Sokoto in August (Figure 6).

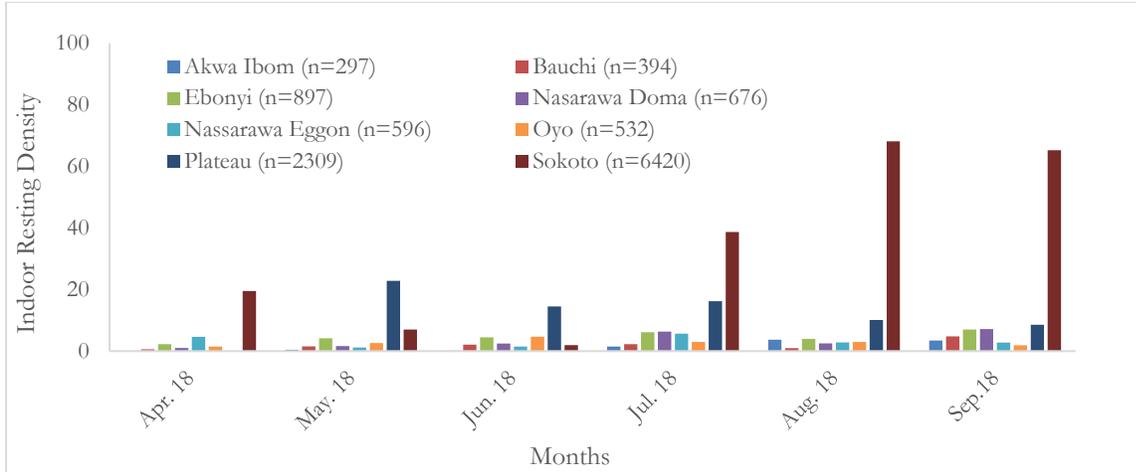


Figure 6: Indoor Resting Density (IRD) across Sites

2.5 ENTOMOLOGICAL INOCULATION RATES ACROSS SITES

EIRs varied across months according to the vector and ecozone. The highest numbers of infective bites indoors and outdoors were from *An. coluzzii* and *An. arabiensis* respectively (Figure 7). Indoor EIR of *An. coluzzii* varied from 0 in Oyo to 137.39 infective bites per person and 147.71 infective bites per person over six months in Akwa Ibom and Plateau, respectively. The highest outdoor EIR (33.8 infective bites per person over six months) by *An. arabiensis* was observed in Plateau. See Annex E for more details.

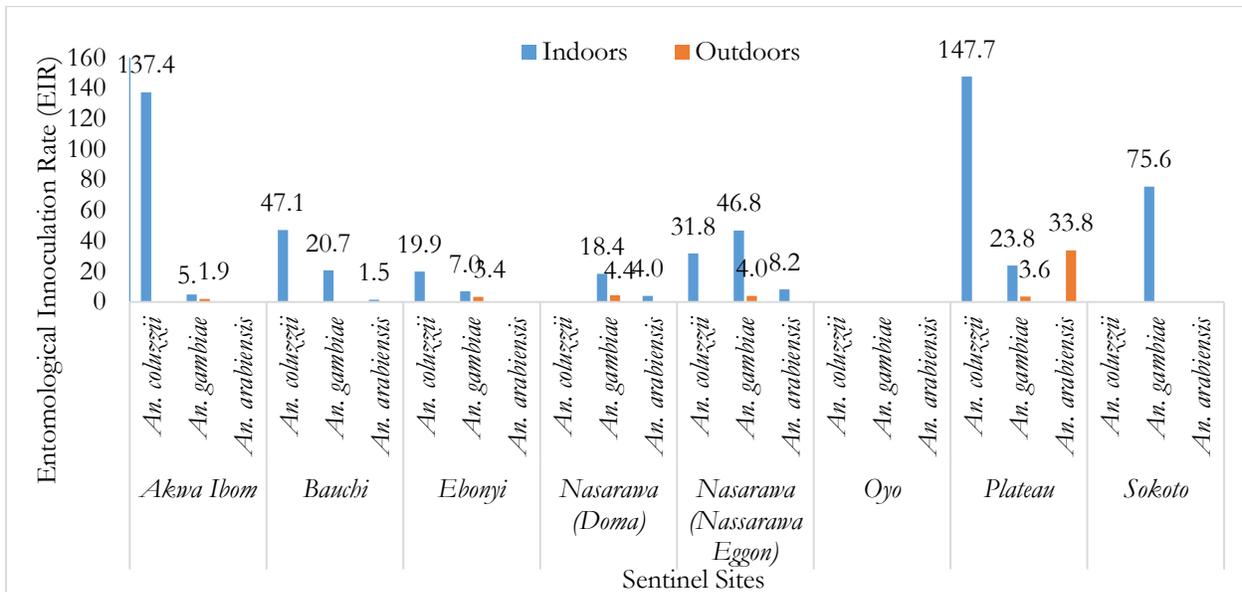


Figure 7: Entomological Inoculation Rates across Sites

2.6 HUMAN BLOOD INDEX

All *An. coluzzii* mosquitoes analyzed from Oyo and all *An. gambiae* mosquitoes from Sokoto had fed on human blood. *An. arabiensis* also recorded 100% human blood meal source in Akwa Ibom site. This was followed by *An. coluzzii* and *An. gambiae* mosquitoes from Akwa Ibom (96% and 93%, respectively). *An. coluzzii* mosquitoes from Bauchi had fed on goat blood (42%) while *An. gambiae* mosquitoes had fed on bovine blood (23%) (Figure 8). In Nasarawa Eggon, 27 percent of *An. coluzzii* and 25 percent of *An. gambiae* mosquitoes had fed on bovine blood while in Doma, 24 percent of *An. gambiae* had fed on bovine blood (Figure 8).

In Ebonyi and Oyo sites, 70 and 60 percent of *An. arabiensis* had human blood meals, respectively, while in Sokoto, 48 percent were found to be fed on bovine blood (Figure 8).

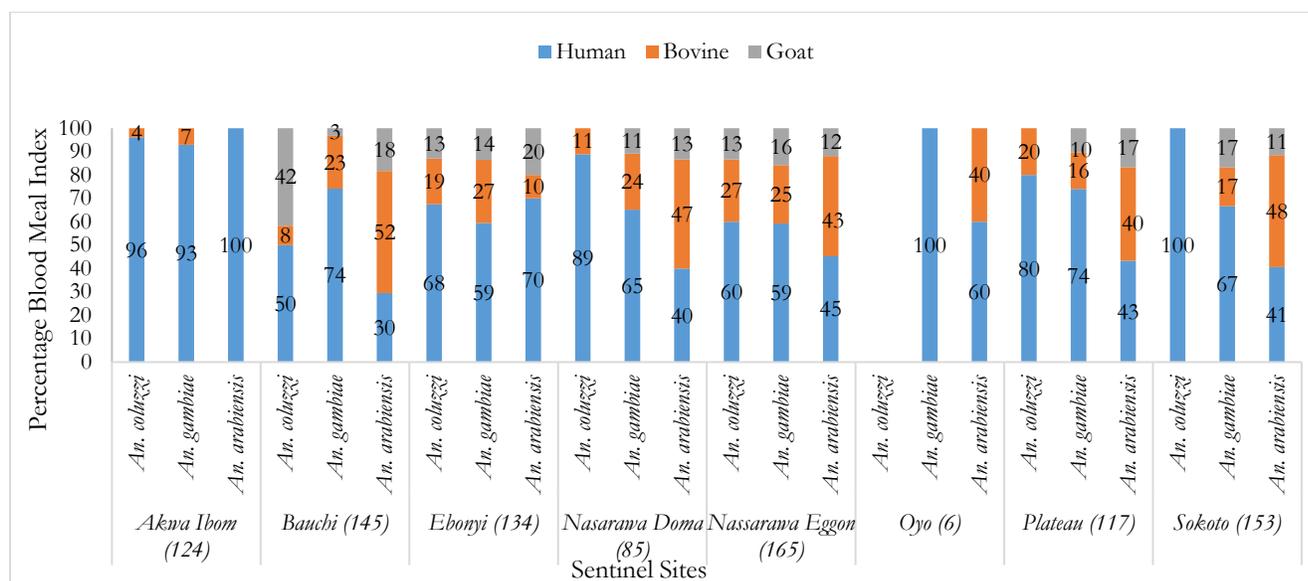


Figure 8: Blood Meal Sources of *An. gambiae*, *An. coluzzii*, and *An. arabiensis* across Sites, April-September, 2018

2.7 INSECTICIDE SUSCEPTIBILITY AND MECHANISMS OF RESISTANCE

The WHO tube test kits and CDC bottle bioassays were used to determine the susceptibility of vector populations at the different sites. Insecticide susceptibility test results indicated that pyrethroid resistance was widespread in *An. gambiae* s.l. mosquitoes at all the sentinel sites across all the ecozones. In Akwa Ibom and Bauchi, possible resistance was detected to lambda-cyhalothrin, deltamethrin, and alpha-cypermethrin. Pyrethroid resistance was widely recorded in mosquitoes from Ebonyi, Oyo, Sokoto, Nassarawa, and Plateau (Table 6). *An. gambiae* s.l. mosquitoes in Ebonyi, Ezza North, Izzi, and Ohaozara LGAs in Ebonyi were susceptible to alpha-cypermethrin.

The *An. gambiae* s.l. mosquitoes in all sites were susceptible to both carbamate (bendiocarb and propoxur) and organophosphate (pirimiphos-methyl) insecticides. However, organophosphate resistance was observed in *An. gambiae* s.l. mosquitoes in Bodingo, Ilela, and Wamakko LGAs in Sokoto (Tables 6 and 7). Further insecticide susceptibility results indicated strong vector resistance to DDT (organochlorine) in four of the seven sentinel sites where the *An. gambiae* s.l. populations were tested (Table 6 and 7).

Table 6: WHO Tube Test Method Results (Percent Mortality after 24 Hours) for *An. gambiae* s.l.

WHO Tube Bioassay (Percent Mortality after 24 hrs)																	
Class of Insecticides		Pyrethroid								Carbamate				Organophosphate		Organochlorine	
Insecticides		Lambda-cyhalothrin		Permethrin		Deltamethrin		Alpha-cypermethrin		Bendiocarb		Propoxur		Pirimiphos methyl		DDT	
Sentinel Site	LGA	Percentage Mortality	Status														
Akwa Ibom	Abak	95%	PR	68%	R	92%	PR	92%	PR	100%	S	100%	S	100%	S	50%	R
	Ikot Ekpene	96%	PR	56%	R	91%	PR	90%	PR	100%	S	100%	S	100%	S	57%	R
	Itu	94%	PR	43%	R	89%	R	90%	PR	100%	S	100%	S	100%	S	30%	R
	Mkpat Enin	94%	PR	56%	R	94%	PR	91%	PR	100%	S	100%	S	100%	S	71%	R
	Nsit Ubium	95%	PR	50%	R	91%	PR	90%	PR	100%	S	100%	S	100%	S	58%	R
	Oron	94%	PR	63%	R	93%	PR	91%	PR	100%	S	99%	S	100%	S	64%	R
Bauchi	Bauchi	92%	PR	88%	R	80%	R	95%	PR	98%	S	98%	S	99%	S	65%	R
	Dass	95%	PR	94%	PR	82%	R	87%	R	100%	S	99%	S	100%	S	70%	R
	Misau	75%	R	85%	R	93%	PR	71%	R	98%	S	96%	PR	100%	S	85%	R
	Ningi	100%	S	96%	PR	100%	S	100%	S	99%	S	99%	S	98%	S	77%	R
	Shira	90%	PR	83%	R	87%	R	91%	PR	98%	S	99%	S	98%	S	68%	R
	Toro	98%	S	96%	PR	94%	PR	100%	S	100%	S	100%	S	100%	S	60%	R
Ebonyi	Ebonyi	68%	R	13%	R	65%	R	100%	S	-	-	100%	S	100%	S	-	-
	Ezza North	22%	R	35%	R	59%	R	100%	S	-	-	100%	S	100%	S	-	-
	Ezza South	54%	R	24%	R	61%	R	85%	R	-	-	100%	S	100%	S	-	-
	Izzi	42%	R	35%	R	25%	R	100%	S	-	-	100%	S	100%	S	-	-
	Ohaozara	15%	R	42%	R	81%	R	100%	S	-	-	100%	S	100%	S	-	-
	Ohaukwu	1%	R	68%	R	72%	R	83%	R	-	-	100%	S	100%	S	-	-

WHO Tube Bioassay (Percent Mortality after 24 hrs)

Class of Insecticides		Pyrethroid								Carbamate				Organophosphate		Organochlorine	
Insecticides		Lambda-cyhalothrin		Permethrin		Deltamethrin		Alpha-cypermethrin		Bendiocarb		Propoxur		Pirimiphos methyl		DDT	
Sentinel Site	LGA	Percentage Mortality	Status														
Nasarawa	Akwanga	78%	R	70%	R	85%	R	89%	R	99%	S	100%	S	100%	S	-	-
	Doma	73%	R	74%	R	80%	R	85%	R	100%	S	100%	S	100%	S	-	-
	Karu	79%	R	72%	R	80%	R	90%	PR	100%	S	100%	S	100%	S	-	-
	Keana	77%	R	71%	R	80%	R	86%	R	99%	S	98%	S	100%	S	-	-
	Keffi	68%	R	76%	R	68%	R	83%	R	100%	S	100%	S	100%	S	-	-
	Kokona	73%	R	66%	R	83%	R	82%	R	100%	S	100%	S	100%	S	-	-
	Lafia	70%	R	69%	R	81%	R	78%	R	98%	S	99%	S	100%	S	-	-
	Nasarawa	73%	R	77%	R	83%	R	87%	R	100%	S	99%	S	99%	S	-	-
	Nassarawa Eggon	77%	R	73%	R	82%	R	88%	R	98%	S	100%	S	100%	S	-	-
	Obi	82%	R	77%	R	76%	R	83%	R	100%	S	100%	S	100%	S	-	-
	Toto	69%	R	75%	R	78%	R	84%	R	100%	S	100%	S	100%	S	-	-
Wamba	80%	R	68%	R	83%	R	83%	R	100%	S	100%	S	100%	S	-	-	
Oyo	Afijio	84%	R	87%	R	94%	PR	93%	PR	100%	S	-	-	100%	S	-	-
	Akinyele	52%	R	61%	R	61%	R	83%	R	98%	S	-	-	100%	S	-	-
	Atiba	31%	R	48%	R	38%	R	7%	R	100%	S	-	-	100%	S	-	-
	Egbeda	67%	R	37%	R	70%	R	88%	R	100%	S	-	-	100%	S	-	-
	Ibarapa East	17%	R	48%	R	38%	R	100%	S	100%	S	-	-	100%	S	-	-
	Oluyole	21%	R	42%	R	53%	R	100%	S	100%	S	-	-	100%	S	-	-

WHO Tube Bioassay (Percent Mortality after 24 hrs)

Class of Insecticides		Pyrethroid								Carbamate				Organophosphate		Organochlorine	
Insecticides		Lambda-cyhalothrin		Permethrin		Deltamethrin		Alpha-cypermethrin		Bendiocarb		Propoxur		Pirimiphos methyl		DDT	
Sentinel Site	LGA	Percentage Mortality	Status														
Plateau	Langtang North	70%	R	80%	R	75%	R	94%	PR	100%	S	100%	S	100%	S	-	-
	Langtang South	68%	R	76%	R	72%	R	97%	PR	100%	S	100%	S	100%	S	-	-
	Mikang	76%	R	83%	R	88%	R	95%	PR	100%	S	100%	S	100%	S	-	-
	Quanpan	70%	R	71%	R	85%	R	97%	PR	100%	S	100%	S	100%	S	-	-
	Shendam	62%	R	72%	R	82%	R	94%	PR	100%	S	100%	S	100%	S	-	-
	Wase	80%	R	80%	R	77%	R	100%	S	100%	S	100%	S	100%	S	-	-
Sokoto	Bodingo	80%	R	80%	R	70%	R	100%	S	100%	S	100%	S	70%	R	60%	R
	Gudu	96%	PR	100%	S	71%	R	100%	S	100%	S	100%	S	100%	S	65%	R
	Illela	83%	R	82%	R	80%	R	69%	R	100%	S	100%	S	73%	R	52%	R
	Rabah	70%	R	68%	R	73%	R	76%	R	98%	S	100%	S	95%	PR	56%	R
	Tambawal	75%	R	70%	R	95%	PR	100%	S	100%	S	100%	S	100%	S	51%	R
	Wamakko	80%	R	100%	S	73%	R	100%	S	100%	S	100%	S	100%	S	75%	R

S = Susceptible, R = Resistant, PR = Possibly Resistant

Table 7: CDC Bottle Bioassay Test Results for *An. gambiae* s.l.

CDC Bottle Bioassay (Percent Mortality After 30 Minutes/45 Minutes for DDT/60 Minutes Pirimiphos-methyl)																	
Class of Insecticides		Pyrethroid								Carbamate				Organophosphate		Organochlorine	
Insecticides		Lambda-cyhalothrin		Permethrin		Deltamethrin		Alpha-cypermethrin		Bendiocarb		Propoxur		Pirimiphos methyl		DDT	
Sentinel Site	LGA	Percentage Mortality	Status														
Akwa Ibom	Abak	59%	R	41%	R	53%	R	45%	R	100%	S	100%	S	100%	S	52%	R
	Ikot Ekpene	47%	R	30%	R	56%	R	53%	R	100%	S	100%	S	100%	S	59%	R
	Itu	54%	R	17%	R	48%	R	42%	R	100%	S	100%	S	100%	S	59%	R
	Mkpat Enin	57%	R	33%	R	54%	R	42%	R	100%	S	99%	S	100%	S	54%	R
	Nsit Ubium	58%	R	32%	R	63%	R	45%	R	100%	S	100%	S	100%	S	59%	R
	Oron	49%	R	35%	R	60%	R	56%	R	100%	S	100%	S	100%	S	40%	R
Bauchi	Bauchi	100%	S	86%	R	95%	PR	99%	S	99%	S	100%	S	100%	S	87%	R
	Dass	92%	PR	91%	PR	97%	PR	90%	PR	100%	S	98%	S	100%	S	85%	R
	Misau	98%	S	96%	PR	94%	PR	92%	PR	95%	PR	100%	S	100%	S	92%	PR
	Ningi	100%	S	81%	R	90%	PR	89%	R	100%	S	100%	S	100%	S	95%	PR
	Shira	97%	PR	87%	R	92%	PR	99%	S	93%	PR	98%	S	100%	S	94%	PR
	Toro	94%	PR	88%	R	93%	PR	91%	PR	98%	S	100%	S	100%	S	89%	R
Benue	Gboko	99%	S	84%	R	100%	S	99%	S	100%	S	-	-	99%	S	-	-
	Katsina-ala	100%	S	83%	R	98%	S	93%	PR	100%	S	-	-	100%	S	-	-
	Kwande	99%	S	82%	R	99%	S	93%	PR	100%	S	-	-	100%	S	-	-
	Makurdi	100%	S	92%	PR	72%	R	100%	S	100%	S	-	-	99%	S	-	-
	Oju	100%	S	71%	R	98%	S	95%	PR	100%	S	-	-	100%	S	-	-
	Otukpo	97%	PR	79%	R	93%	PR	98%	S	100%	S	-	-	100%	S	-	-

CDC Bottle Bioassay (Percent Mortality After 30 Minutes/45 Minutes for DDT/60 Minutes Pirmiphos-methyl)

Class of Insecticides		Pyrethroid								Carbamate				Organophosphate		Organochlorine	
Insecticides		Lambda-cyhalothrin		Permethrin		Deltamethrin		Alpha-cypermethrin		Bendiocarb		Propoxur		Pirimiphos methyl		DDT	
Sentinel Site	LGA	Percentage Mortality	Status														
Ebonyi	Ebonyi	85%	R	17%	R	78%	R	65%	R	-	-	100%	S	100%	S	-	-
	Ezza North	83%	R	32%	R	79%	R	24%	R	-	-	100%	S	100%	S	-	-
	Ezza South	90%	PR	34%	R	81%	R	86%	R	-	-	100%	S	100%	S	-	-
	Izzi	73%	R	31%	R	77%	R	35%	R	-	-	100%	S	100%	S	-	-
	Ohaozara	77%	R	25%	R	77%	R	16%	R	-	-	100%	S	100%	S	-	-
	Ohaukwu	80%	R	48%	R	81%	R	86%	R	-	-	100%	S	100%	S	-	-
Nasarawa	Akwanga	96%	PR	66%	R	94%	PR	90%	PR	100%	S	99%	S	100%	S	-	-
	Doma	96%	PR	78%	R	95%	PR	84%	R	100%	S	100%	S	100%	S	-	-
	Karu	99%	S	83%	R	94%	PR	90%	PR	100%	S	100%	S	100%	S	-	-
	Keana	90%	PR	76%	R	92%	PR	97%	PR	100%	S	100%	S	99%	S	-	-
	Keffi	98%	S	77%	R	95%	PR	90%	PR	99%	S	100%	S	100%	S	-	-
	Kokona	89%	R	80%	R	92%	PR	89%	R	100%	S	100%	S	100%	S	-	-
	Lafia	85%	R	80%	R	90%	PR	88%	R	100%	S	100%	S	100%	S	-	-
	Nasarawa	92%	PR	83%	R	92%	PR	80%	R	100%	S	100%	S	100%	S	-	-
	Nassarawa Eggon	98%	S	70%	R	98%	S	84%	R	99%	S	100%	S	100%	S	-	-
	Obi	95%	PR	72%	R	90%	PR	94%	PR	100%	S	99%	S	100%	S	-	-
	Toto	92%	PR	65%	R	91%	PR	96%	PR	98%	S	100%	S	100%	S	-	-
Wamba	94%	PR	67%	R	86%	R	90%	PR	100%	S	99%	S	100%	S	-	-	

CDC Bottle Bioassay (Percent Mortality After 30 Minutes/45 Minutes for DDT/60 Minutes Pirimiphos-methyl)

Class of Insecticides		Pyrethroid								Carbamate				Organophosphate		Organochlorine	
Insecticides		Lambda-cyhalothrin		Permethrin		Deltamethrin		Alpha-cypermethrin		Bendiocarb		Propoxur		Pirimiphos methyl		DDT	
Sentinel Site	LGA	Percentage Mortality	Status														
Oyo	Afijio	81%	R	41%	R	23%	R	82%	R	100%	S	-	-	96%	PR	-	-
	Akinyele	100%	S	65%	R	57%	R	97%	PR	100%	S	-	-	100%	S	-	-
	Atiba	84%	R	27%	R	59%	R	39%	R	100%	S	-	-	80%	R		
	Egbeda	74%	R	5%	R	78%	R	92%	PR	100%	S	-	-	100%	S	-	-
	Ibarapa East	76%	R	22%	R	27%	R	80%	R	100%	S	-	-	100%	S	-	-
	Oluyole	75%	R	22%	R	33%	R	80%	R	100%	S	100%	S	100%	S	-	-
Plateau	Langtang North	69%	R	79%	R	94%	PR	79%	R	100%	S	100%	S	100%	S	81%	R
	Langtang South	75%	R	73%	R	76%	R	82%	R	100%	S	100%	S	100%	S	82%	R
	Mikang	76%	R	74%	R	85%	R	84%	R	100%	S	100%	S	100%	S	57%	R
	Quanpan	78%	R	79%	R	76%	R	82%	R	100%	S	100%	S	100%	S	44%	R
	Shendam	78%	R	74%	R	75%	R	76%	R	100%	S	100%	S	100%	S	48%	R
	Wase	78%	R	77%	R	76%	R	83%	R	100%	S	100%	S	100%	S	73%	R
Sokoto	Bodingo	74%	R	72%	R	67%	R	100%	S	100%	S	100%	S	60%	R	55%	R
	Gudu	98%	S	100%	S	80%	R	100%	S	100%	S	100%	S	100%	S	60%	R
	Illela	81%	R	79%	R	72%	R	66%	R	100%	S	100%	S	80%	R	49%	R
	Rabah	69%	R	64%	R	73%	R	79%	R	99%	S	100%	S	94%	PR	51%	R
	Tambawal	77%	R	76%	R	75%	R	100%	S	100%	S	100%	S	100%	S	50%	R
	Wamakko	74%	R	100%	S	70%	R	100%	S	100%	S	100%	S	65%	R	69%	R

CDC Bottle Bioassay (Percent Mortality After 30 Minutes/45 Minutes for DDT/60 Minutes Pirimiphos-methyl)

Class of Insecticides		Pyrethroid								Carbamate				Organophosphate		Organochlorine	
Insecticides		Lambda-cyhalothrin		Permethrin		Deltamethrin		Alpha-cypermethrin		Bendiocarb		Propoxur		Pirimiphos methyl		DDT	
Sentinel Site	LGA	Percentage Mortality	Status														
Zamfara	Anka	100%	S	100%	S	-	-	99%	S	100%	S	-	-	100%	S	-	-
	Chafe	100%	S	-	-	-	-	100%	S	100%	S	-	-	100%	S	-	-
	Gusau	97%	PR	81%	R	-	-	100%	S	100%	S	-	-	100%	S	-	-
	Kaura Namoda	100%	S	81%	R	-	-	100%	S	100%	S	-	-	100%	S	-	-
	Maru	100%	S	-	-	-	-	100%	S	100%	S	-	-	100%	S	-	-
	Talata Mafara	-	-	76%	R	-	-	100%	S	100%	S	-	-	100%	S	-	-

S = Susceptible, R = Resistant, PR = Possible Resistant

Note: Diagnostic time is 30 minutes for all except DDT, which is 45 minutes.

2.8 INSECTICIDE RESISTANCE INTENSITY

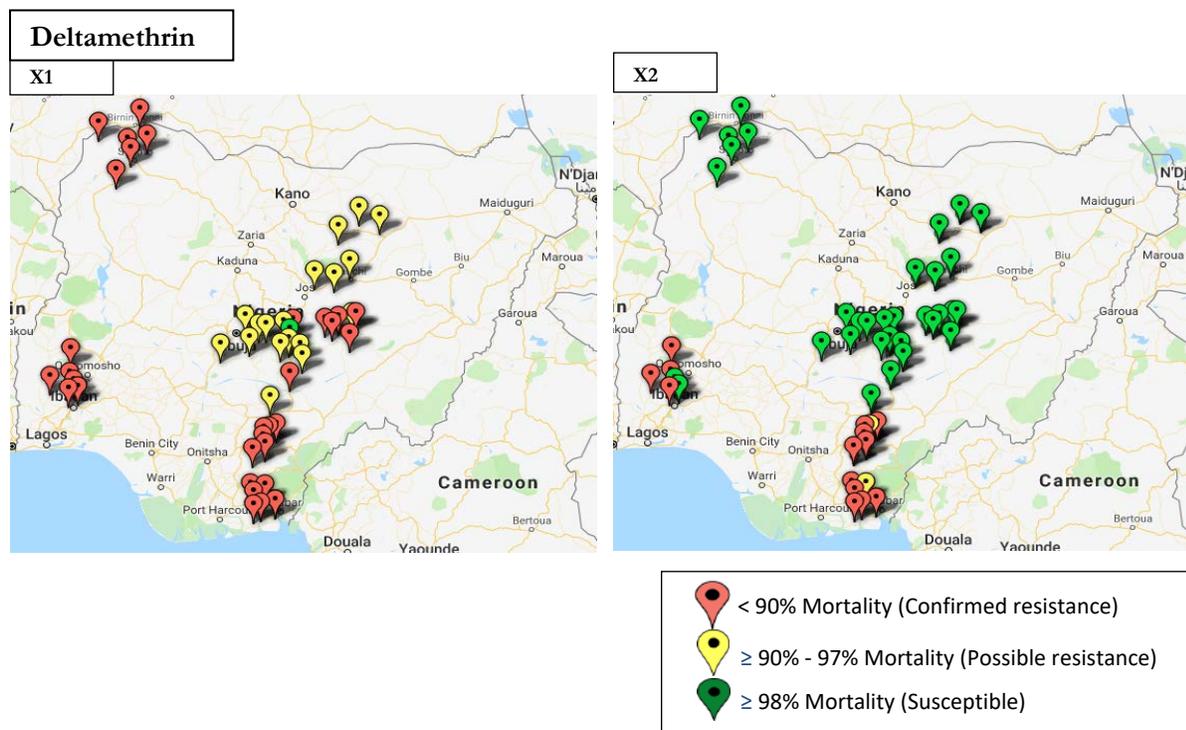
Pyrethroid insecticide resistance intensities in *An. gambiae* mosquitoes across the ecological zones in Nigeria is described in Figure 9. Deltamethrin resistance was recorded in *An. gambiae* s.l. mosquitoes from Akwa Ibom and Oyo at twice the diagnostic concentration. *An. gambiae* s.l. mosquitoes in Bauchi, Plateau, Benue, and Sokoto were fully susceptible to deltamethrin at two times the diagnostic concentration. Results of deltamethrin intensity assays in Ebonyi provided evidence of varying high resistance intensities at 2X, 5X and in some cases 10X the diagnostic dose across the LGAs. *An. gambiae* s.l. mosquitoes tested from all six LGAs in Ebonyi were susceptible to 2X diagnostic dosage of alpha-cypermethrin (Figures 10-17).

Permethrin resistance at 5X was observed in the *An. gambiae* s.l. mosquitoes at all six LGAs in both Akwa Ibom and Ebonyi. *An. gambiae* s.l. mosquitoes in more than one of these locations were resistant at 10x concentration (Figures 10 and 13). In Bauchi, resistance was observed at 2X concentrations in the *An. gambiae* s.l. mosquitoes in two of the four study sites. The same mosquitoes were found to be susceptible at 5-times the diagnostic concentration (Figure 11).

An. gambiae mosquitoes in Benue recorded resistance intensities at 2X and 5X across all four LGAs and at 10X concentration in two LGAs. Intensity of permethrin resistance was observed to be high in *An. gambiae* s.l. in Nasarawa and Oyo (Figures 14 and 15).

Full susceptibility to permethrin was observed in *An. gambiae* s.l. mosquitoes from Nasarawa exposed to 5X and 10X intensity assays (Figure 14). Intensity assays from Oyo showed 10X resistance of permethrin in *Anopheles* mosquitoes from five out of the six LGAs. Permethrin resistance intensity in the *Anopheles* mosquitoes from Plateau and Sokoto did not exceed the 5X the starting concentration level (Figure 15-17).

The intensity of alpha-cypermethrin susceptibility of *An. gambiae* s.l. mosquitoes from Oyo did not exceed five-times the original concentration (Figure 15). There were security challenges in Plateau and Zamfara that affected the completion of insecticide resistance tests in those locations.



Deltamethrin

X5



Key

-  < 98% Mortality (Moderate to High Intensity resistance)
-  ≥ 98% Mortality (Low Intensity Resistance)

Deltamethrin

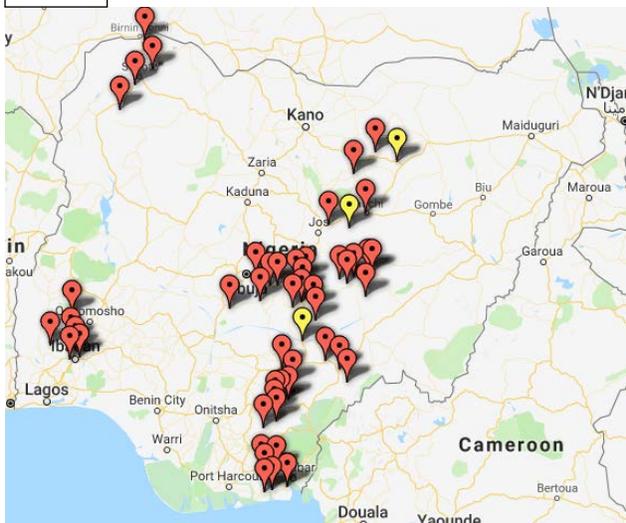
X10



-  < 98% Mortality (High Intensity Resistance)
-  ≥ 98% Mortality (Moderate Intensity Resistance)

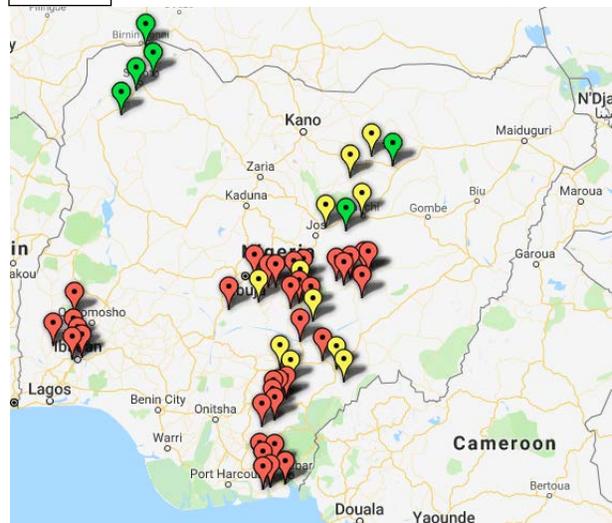
Permethrin

X1



Permethrin

X2



-  < 90% Mortality (Confirmed resistance)
-  ≥ 90 - 97% Mortality (Possible resistance)
-  ≥ 98% Mortality (Susceptible)

Permethrin

X5



Key

-  < 98% Mortality (Moderate to High Intensity)
-  ≥ 98% Mortality (Low Intensity Resistance)

Permethrin

X10



-  < 98% Mortality (High Intensity Resistance)
-  ≥ 98% Mortality (Moderate Intensity Resistance)

Alpha-cypermethrin

X1



Alpha-cypermethrin

X2



Key

-  < 90% Mortality (Confirmed resistance)
-  ≥ 90% - 97% Mortality (Possible resistance)
-  ≥ 98% Mortality (Susceptible)

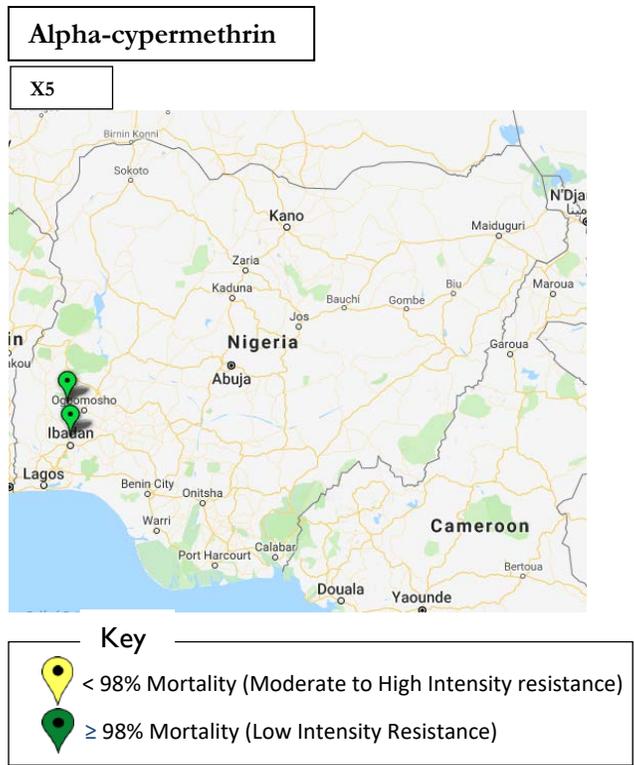


Figure 9: Distribution of Pyrethroid Resistance Intensities in *An. gambiae* s.l. Mosquitoes Exposed to Deltamethrin, Permethrin, and Alpha-Cypermethrin across the Sentinel Sites in Nigeria

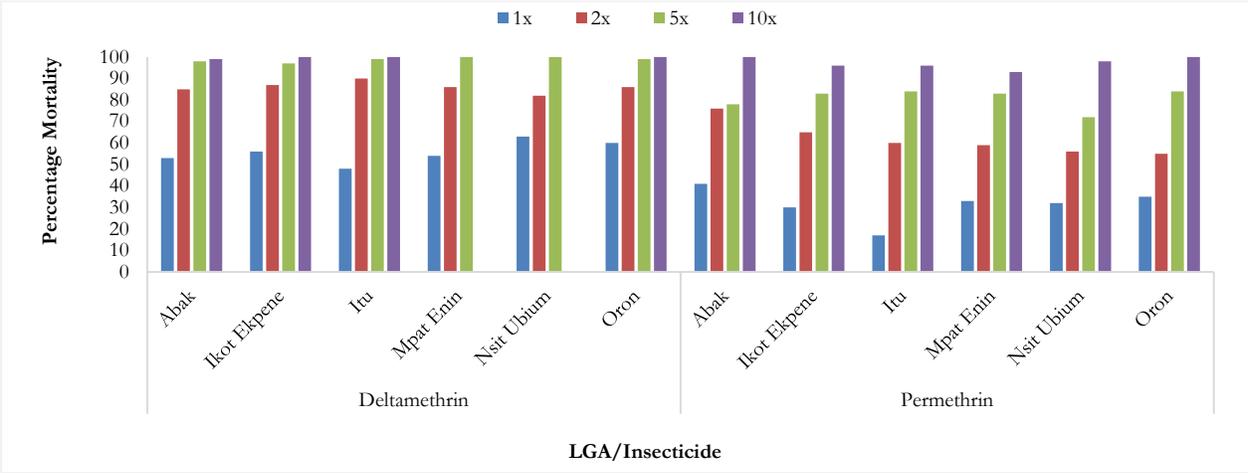


Figure 10: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Akwa Ibom

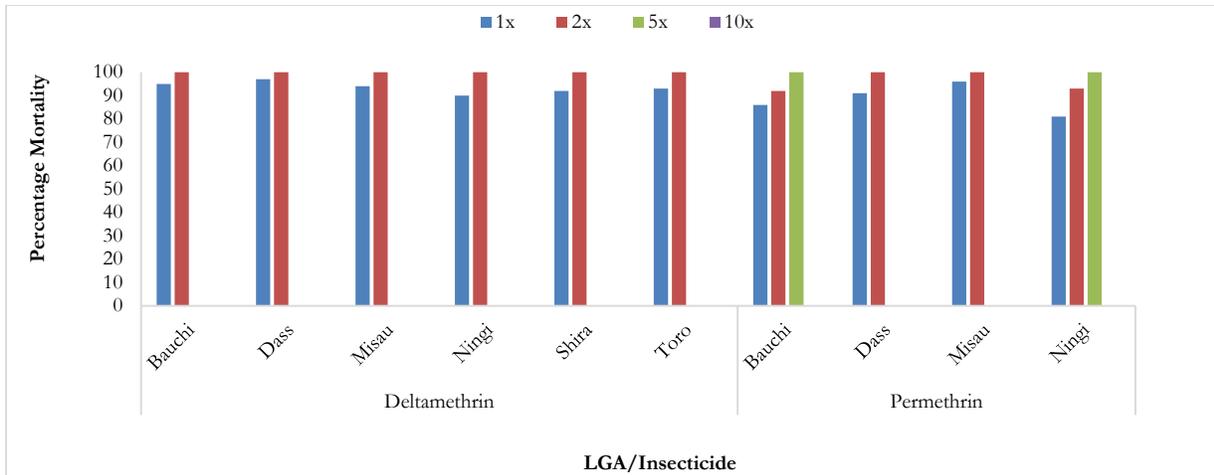


Figure 11: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Bauchi

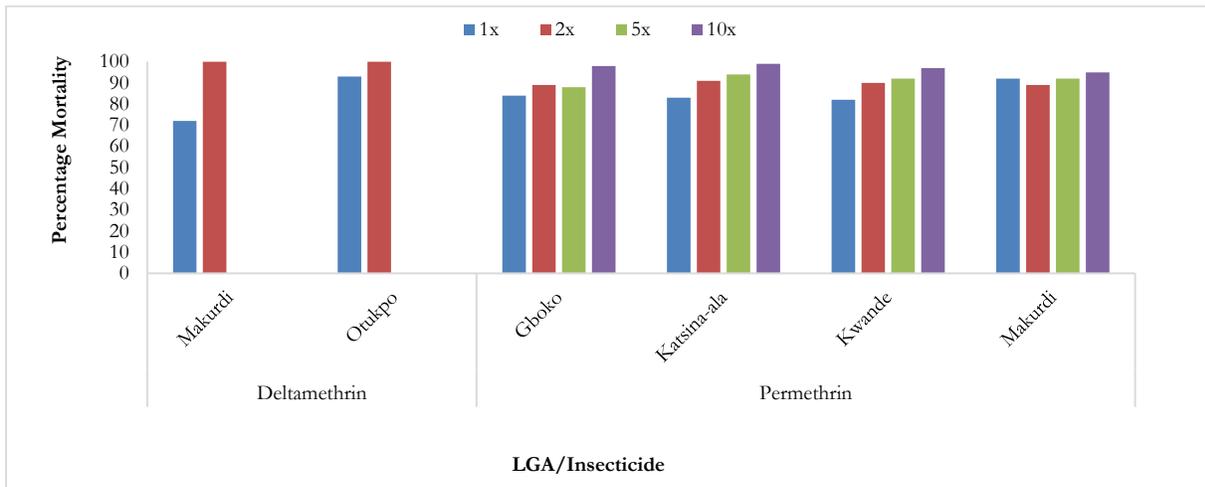


Figure 12: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Benue

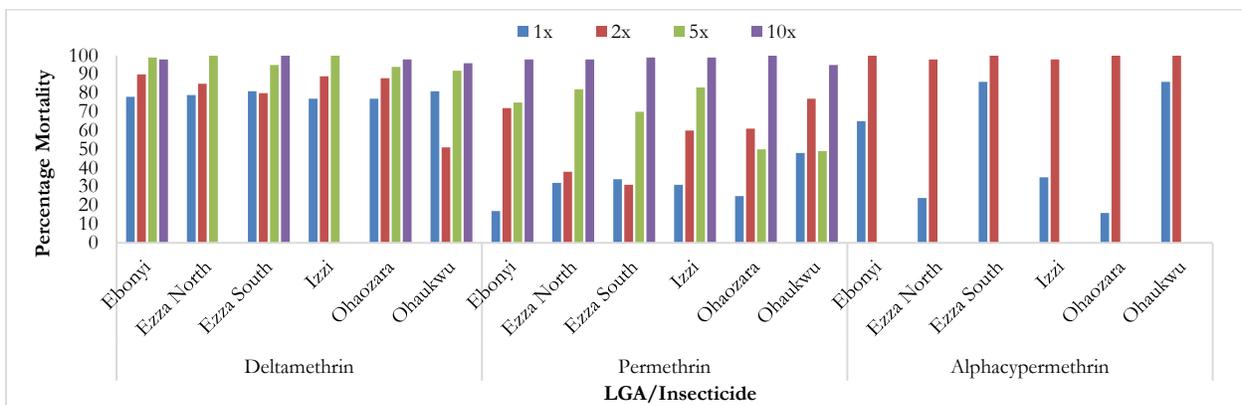


Figure 13: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Ebonyi

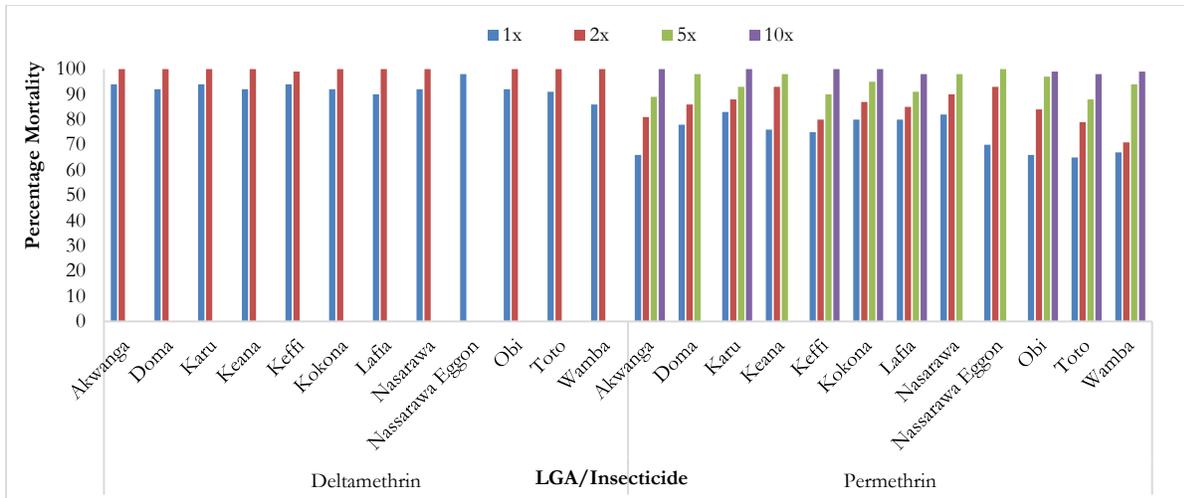


Figure 14: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Nasarawa

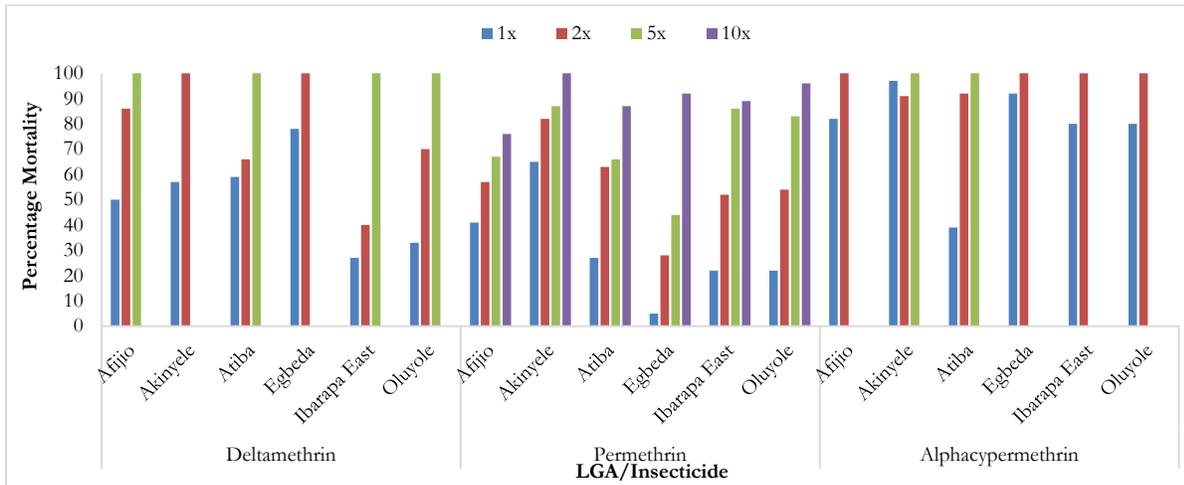


Figure 15: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Oyo

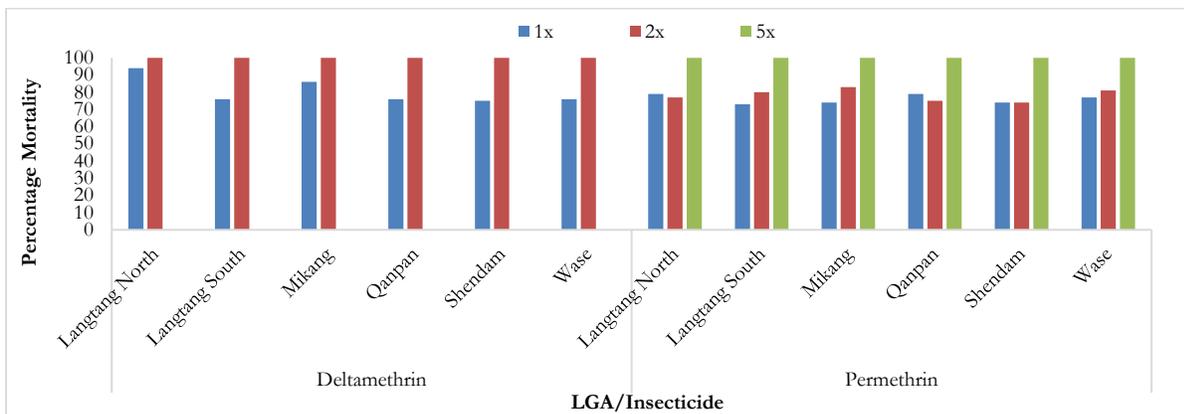


Figure 16: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Plateau

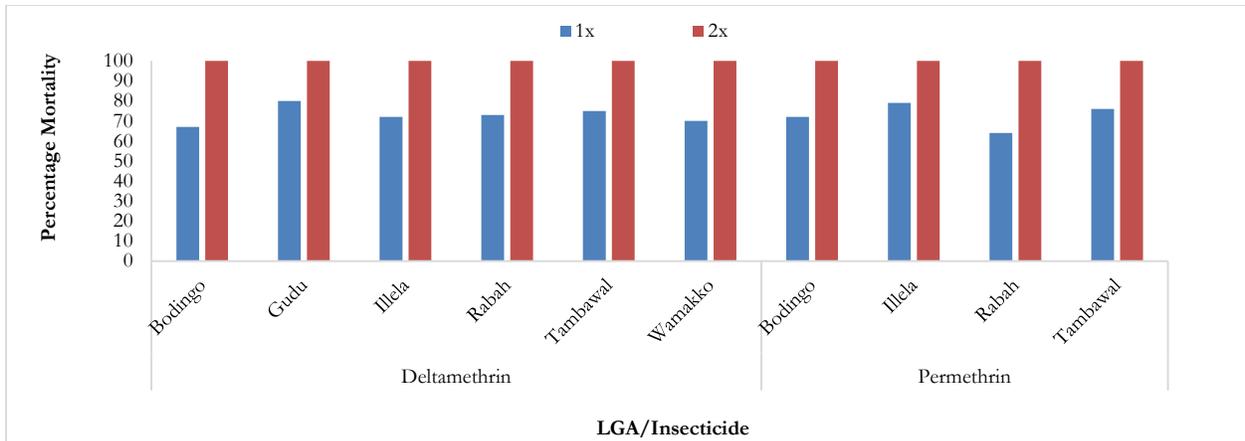


Figure 17: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Sokoto

2.9 SYNERGIST ASSAYS

PBO synergist assays reversed pyrethroid (deltamethrin, alpha-cypermethrin, and permethrin) resistance in *An. gambiae* s.l. mosquitoes at varying degrees across all sentinel sites (Figure 18-25). In some cases, complete restoration of susceptibility was not observed with PBO exposure, suggesting the existence of mechanisms unrelated to oxidase detoxification (Figure 18-19).

In Sokoto, Ebonyi, and Akwa Ibom, the mortality of *An. gambiae* s.l. exposed to permethrin alone was 17-81% compared to 94-100% mortality in *Anopheles* mosquitoes pre-exposed to PBO. Piperonyl butoxide significantly restored susceptibility (98-100%) in permethrin-resistant *An. gambiae* s.l. populations from five out of six locations in Ebonyi, this was not the case in *An. gambiae* s.l. mosquitoes from Nasarawa, Oyo, and Plateau (Figures 21-24). The lowest mortality (47%) was observed in permethrin-resistant *An. gambiae* s.l. mosquitoes (from Egbeda, Oyo state) pre exposed to PBO (Figure 22). PBO significantly restored susceptibility in deltamethrin-resistant *An. gambiae* s.l. mosquitoes from most LGAs in Plateau, Sokoto, Nasarawa, Ebonyi, Benue, Bauchi, and Akwa Ibom. Full susceptibility to alpha-cypermethrin was observed following pre-exposure to PBO in *An. gambiae* s.l. from Benue and Oyo that were resistant to alpha-cypermethrin (Figures 18-25).

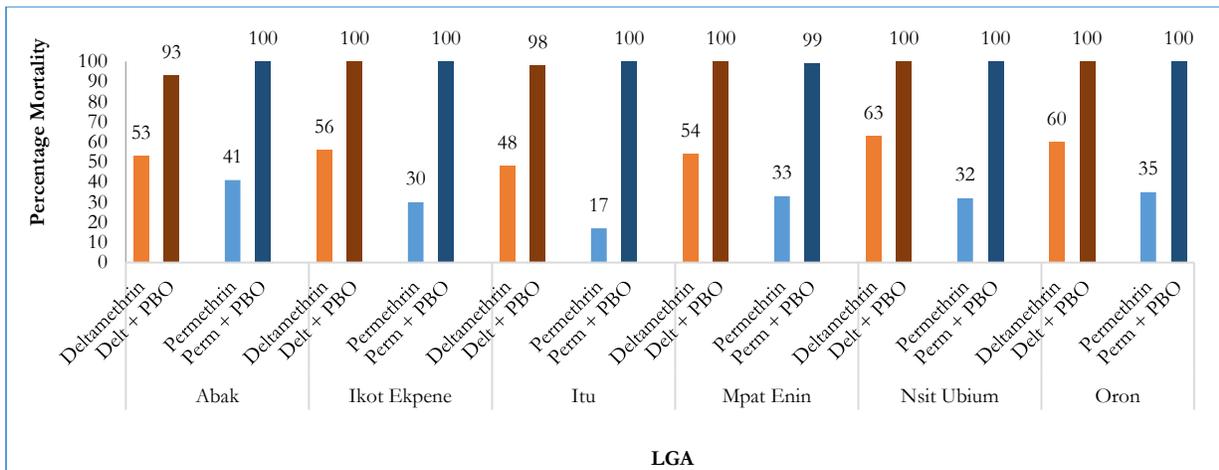


Figure 18: Synergist Bottle Assay Results for *An. gambiae* s.l. from Akwa Ibom

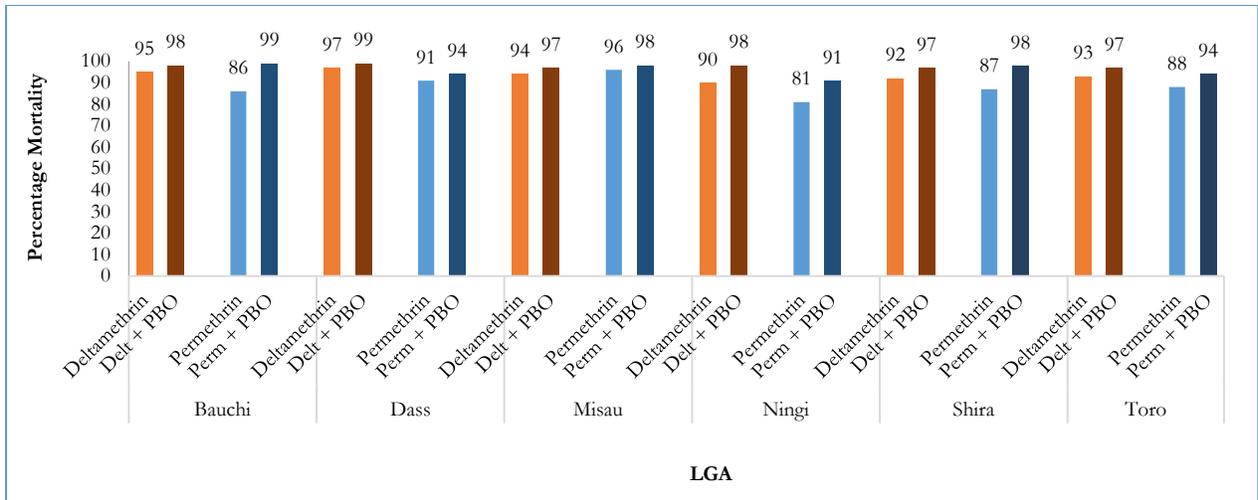


Figure 19: Synergist Bottle Assay Results for *An. gambiae* s.l. at Bauchi

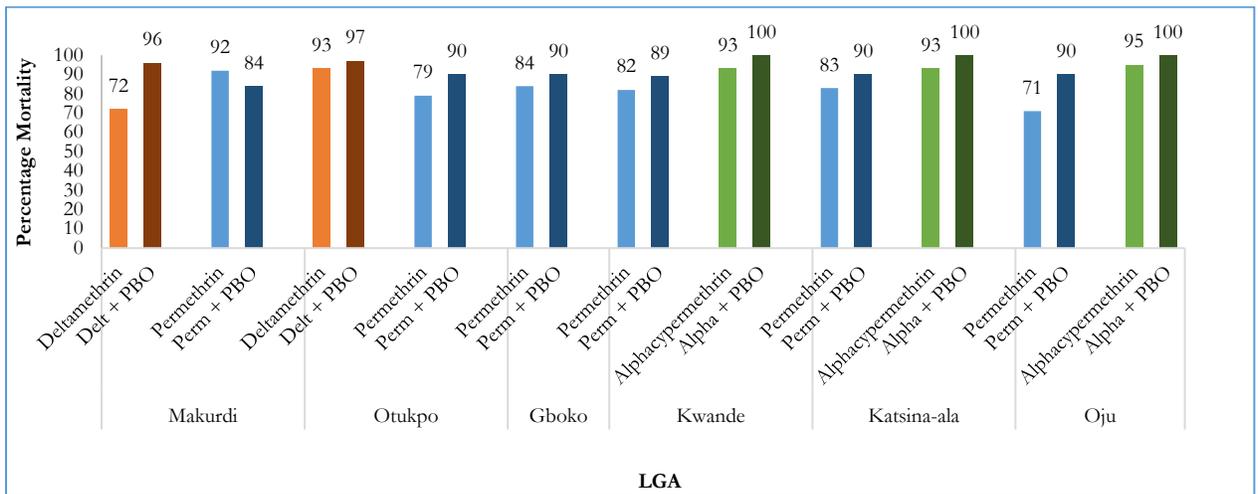


Figure 20: Synergist Bottle Assay Results for *An. gambiae* s.l. at Benue

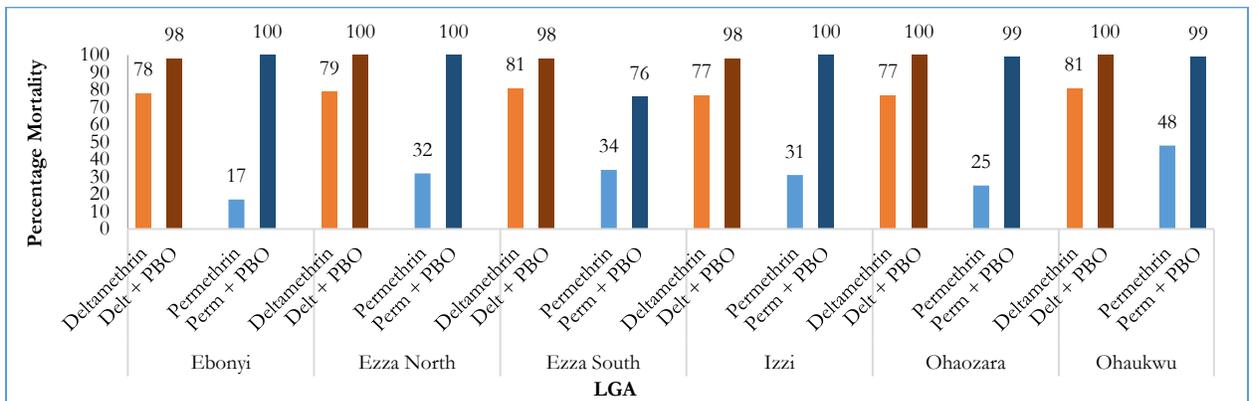


Figure 21: Synergist Bottle Assay Results for *An. gambiae* s.l. at Ebonyi

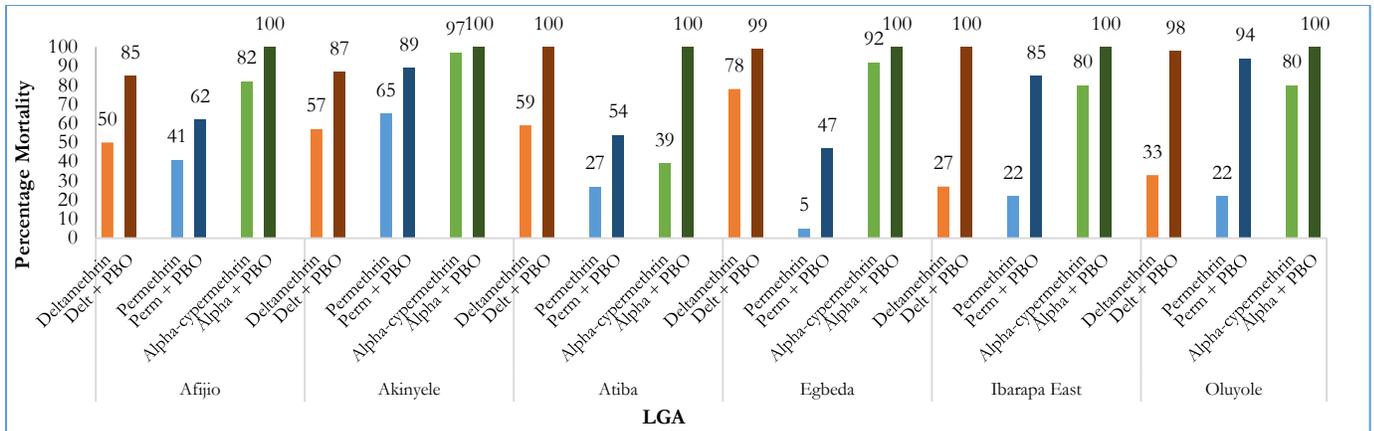


Figure 22: Synergist Bottle Assay Results for *An. gambiae* s.l. at Oyo

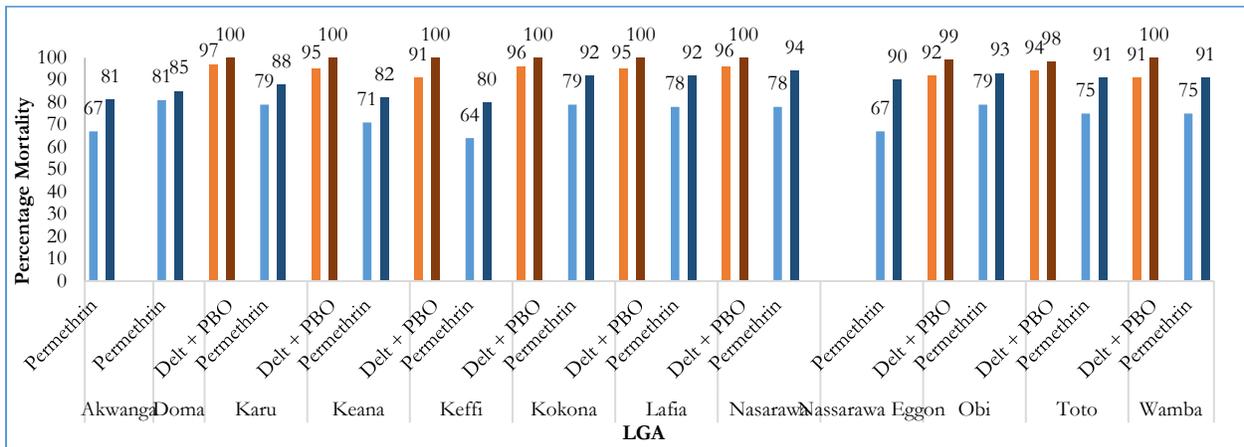


Figure 23: Synergist Bottle Assay Results for *An. gambiae* s.l. at Nasarawa

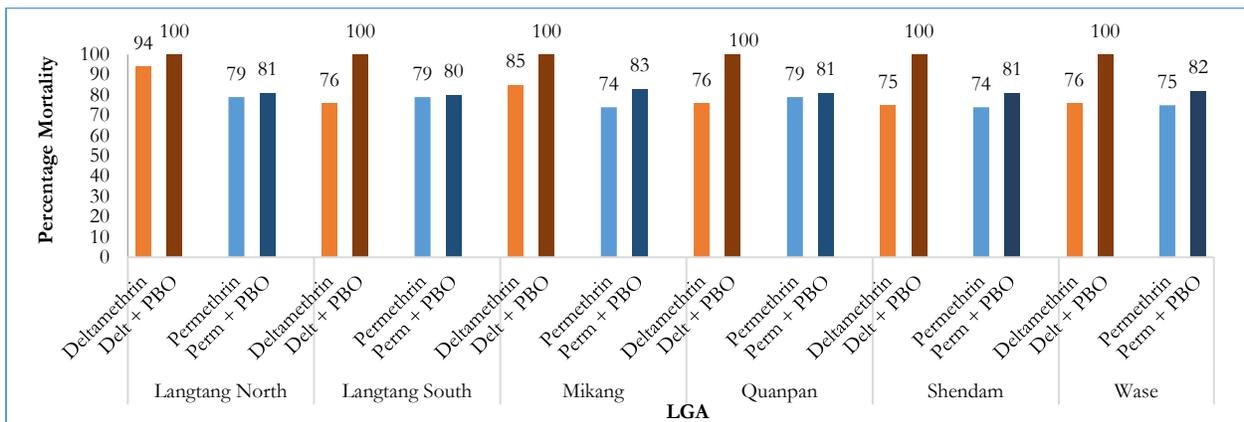


Figure 24: Synergist Bottle Assay Results for *An. gambiae* s.l. at Plateau

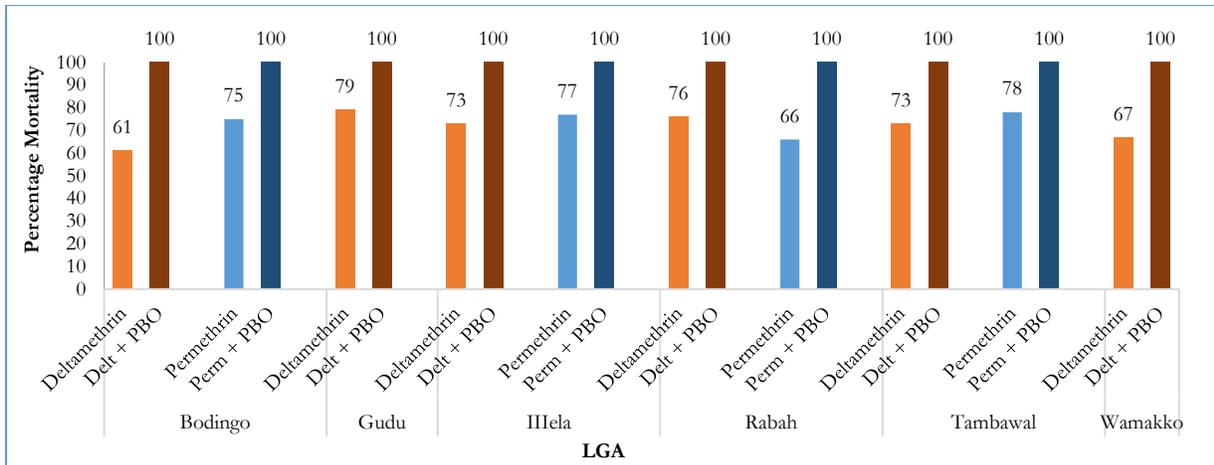


Figure 25: Synergist Bottle Assay Results for *An. gambiae* s.l. at Sokoto

2.10 DETERMINATION OF THE DIAGNOSTIC DOSE FOR CHLORFENAPYR

Percentage knockdown of *An. gambiae* s.l. from Nasarawa, Oyo, and Ebonyi after 60 minutes exposure to chlorfenapyr showed some variability among the sites (Figure 26). Despite high pyrethroid resistance in Ebonyi, Nasarawa, and Oyo, CDC bottle assay tests with chlorfenapyr at doses 50 µg per bottle and above yielded 100% mortality of *An. gambiae* s.l. after the 72-hour holding periods (Figure 27).

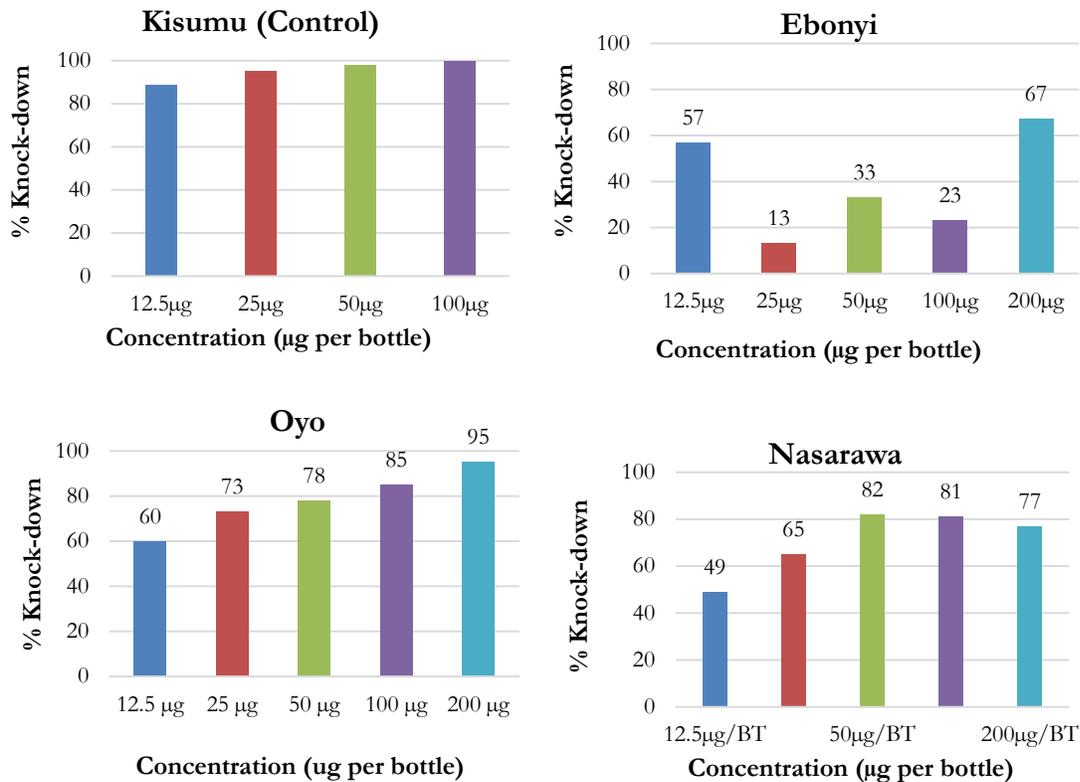


Figure 26: Percentage Knockdown of *An. gambiae* s.l. after 60 Minutes Exposure to Various Concentrations of Chlorfenapyr (µg/bottle) in Ebonyi, Nasarawa, and Oyo

Test conditions during bioassay: 25.7-27.1°C, 79.1-85.6 %RH

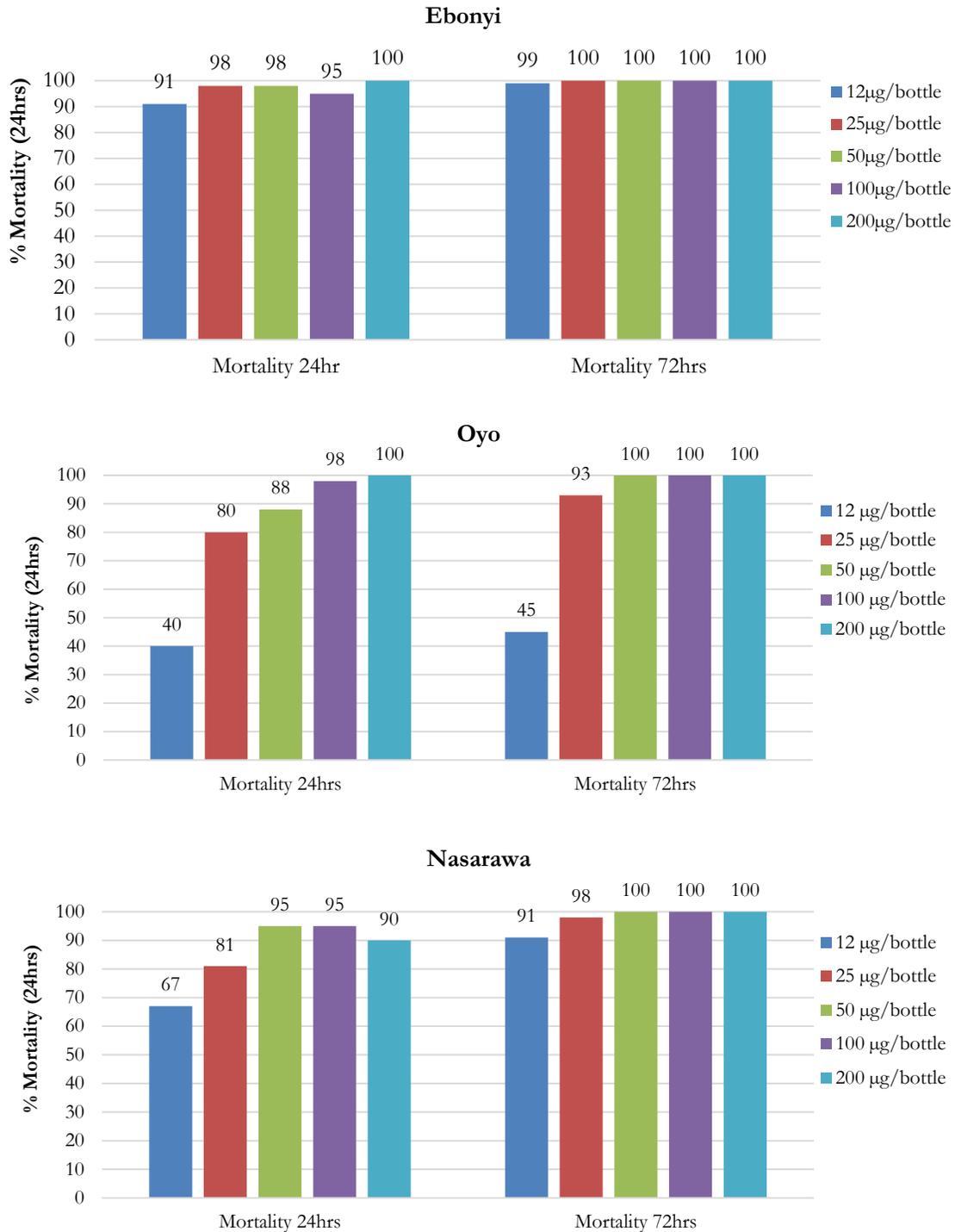


Figure 27: Percentage Mortality of *An. gambiae* s.l. after 24h and 72h Holding Periods at Ebonyi, Nasarawa, and Oyo when Exposed to Various Concentrations of Chlorfenapyr

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

2.11 KDR GENE FREQUENCY IN *AN. GAMBIAE* S.L. EXPOSED TO DELTAMETHRIN AND DDT ACROSS SITES

Assessment of *kdr* mutations in pyrethroid resistant *An. gambiae* s.l. indicated the presence of both *kdr-w* and *kdr-e* point mutations. This is the first time *kdr-e* is being reported in Akwa Ibom (deltamethrin-resistant *An. gambiae* s.l. mosquitoes) and Ebonyi (deltamethrin- and permethrin-resistant *An. gambiae* s.l. mosquitoes). The frequency of the *kdr-w* and *kdr-e* gene mutations in *An. gambiae* s.l. mosquitoes tested was generally low, ranging from 0.06 in Ebonyi to 0.36 in Sokoto for deltamethrin-exposed *An. gambiae* s.l. mosquitoes (Table 8). The *kdr* gene frequency was also low in permethrin-exposed *An. gambiae* s.l. from 0.08 in Ebonyi to 0.49 in Benue (Table 9).

The frequency of East *kdr* (*kdr-e*) gene mutation in *An. gambiae* s.l. mosquitoes tested was generally low, appearing in Ebonyi (0.02) and Akwa Ibom (0.07) for deltamethrin-exposed *An. gambiae* s.l. (Table 8) and in Ebonyi (0.01) for permethrin-exposed *An. gambiae* s.l. mosquitoes (Table 9). The *kdr-w* gene frequencies in deltamethrin- and permethrin-resistant *An. gambiae* s.l. were similar in Ebonyi, Plateau, and Sokoto.

Table 8: Frequency of *kdr* Genes in Deltamethrin-Resistant *An. gambiae* s.l.

Insecticide	State	Number Tested for <i>kdr</i>	<i>kdr-w</i>				<i>kdr-e</i>			
			RR	Rr	rr	<i>kdr</i> Frequency	RR	Rr	rr	<i>kdr</i> Frequency
Deltamethrin	Akwa Ibom	59	5	8	46	0.15	2	4	53	0.07
	Bauchi	38	8	4	26	0.26		0	38	0.00
	Ebonyi	169	7	6	156	0.06	3	2	164	0.02
	Nasarawa	167	27	31	109	0.25	0	0	167	0.00
	Plateau	56	10	9	37	0.26	0	0	56	0.00
	Sokoto	125	39	12	74	0.36	0	0	125	0.00

Table 9: Frequency of *kdr* Genes in Permethrin-Resistant *An. gambiae* s.l.

Insecticide	State	Number Tested for Kdr	<i>kdr-w</i>				<i>kdr-e</i>			
			RR	Rr	rr	<i>kdr</i> Frequency	RR	Rr	rr	<i>kdr</i> Frequency
Permethrin	Benue	43	18	6	19	0.49		0	43	0.00
	Ebonyi	418	23	22	373	0.08	6	0	412	0.01
	Oyo	193	28	38	127	0.24	0	0	193	0.00
	Plateau	84	20	7	57	0.28	0	0	84	0.00
	Sokoto	46	12	3	31	0.29	0	0	46	0.00
	Zamfara	34	0	0	34	0.00	0	0	34	0.00

3. DISCUSSION AND CONCLUSION

3.1 SPECIES COMPOSITION

From April to September 2018, VectorLink investigated the principal vectors of the malaria parasite in all the ecozones of Nigeria. Results show that members of *An. gambiae* s.l. (*An. coluzzii*, *An. gambiae*, and *An. arabiensis*) are the predominant vectors across all ecozones of the country. Consistent with previous AIRS reports, *An. gambiae* predominated across all sites except Ebonyi, where *An. coluzzii* was most abundant. *An. funestus* was much less abundant and only found in four sites. Continuous monitoring of these vectors in the context of LLIN interventions will provide further understanding on the impact of these interventions on the distribution of vectors and their role in transmission. *An. coustani*, a secondary vector in Nigeria, was collected in Nassarawa, where it is known to contribute to malaria transmission both indoors and outdoors (Inyama *et al.*, 2017). Effective malaria control programs should therefore include tools that target both indoor and outdoor transmission.

3.2 VECTOR BEHAVIOR AND BITING RATE

The mean indoor biting rates of *An. gambiae* s.l. peaked in September at Akwa Ibom, Bauchi, and Ebonyi compared to Nasarawa Doma, Nassarawa Eggon, and Plateau where the peak occurred in July. The peak biting rates recorded during the rainy season (June-September) occurred indoors, suggesting an important role for LLINs in malaria prevention. However, despite the presence of LLINs, *An. gambiae* s.l. maintained a high index of human-vector contact as indicated by high human biting rates and human blood indices, which might be due to resistance to the pyrethroid insecticides used to treat bed nets.

3.3 SPOROZOITE INFECTION RATE

Plasmodium falciparum sporozoite rates in *An. gambiae* indoors varied across the various ecological zones with the highest infection rate recorded in Ebonyi. *An. arabiensis* contributed the highest *P. falciparum* sporozoite rates indoors (8.5%) and outdoors (8.3%) in Sokoto and Plateau, respectively. These areas are known as having large farming communities where residents spend more time outdoors at night tending to their crops, exposing them to repeated *Anopheles* mosquito bites. Continuous exposure to malaria for a community close to vegetable farming was also reported by Yadouleton *et al.* (2010). It is known that most communities in Ebonyi engage in mass rice farming and other agricultural practices. Such observations suggest that rice cultivation and vegetable farming are key factors associated with high malaria transmission risk due to more breeding sites and increased human-vector contact and could explain transmission observed in Ebonyi and Bauchi. This suggests the need to promote the use of LLINs among the farming communities in Ebonyi. Because both species are similarly attracted to humans, *An. gambiae* and *An. coluzzii* had similar sporozoite rates at 11.9% (in Ebonyi) and 12.5% (in Bauchi). Given the similar affinity for humans, it is likely that *An. gambiae* and *An. coluzzii* have the same host-seeking behavior suitable for the development of the parasite during the sporogonic cycle. *An. arabiensis* is very flexible over its ecological range and behavior. It is both exophilic (resting outdoors) and endophilic (resting indoors), anthropophilic (feeding on human blood) and zoophilic (feeding on animals) (Coetzee *et al.*, 2000).

3.4 ENTOMOLOGICAL INOCULATION RATE

EIRs varied according to the vector and the ecozone. Overall comparative contributions of EIRs between members of *An. gambiae* s.l. indicated that the highest numbers of infective bites indoors and outdoors were from *An. coluzzii* and *An. arabiensis*, respectively. In Plateau, EIRs outdoors were higher than indoors for *An. arabiensis*. Similar findings were previously recorded in Sokoto (Inyama *et al.* 2018).

The project recorded high indoor EIRs for *An. coluzzii* in Plateau and Akwa Ibom, in contrast with previous results which suggested that *An. gambiae* contributed the highest EIR in Akwa Ibom and Nasarawa (169.6 and 102.9, respectively) (Inyama *et al.* 2018). The Nigeria Malaria Indicator Survey (2015) showed that net usage among pregnant women was less than 50% in Akwa Ibom, Nasarawa, and Plateau states which is far less than the 80% target. High EIRs recorded in these areas may be attributed to inconsistent use of LLINs. Increased community sensitization and education could be considered.

3.5 BLOOD MEAL SOURCES

The high proportion of *An. coluzzii*, *An. gambiae*, and *An. arabiensis*, that fed on human blood in Oyo, Sokoto, and Akwa Ibom (respectively) might relate to the low bed net use rate of the community during the collection period. LLIN usage is less than 50% in all the three states (NMIS, 2015). The higher rates of bovine blood meal in *An. arabiensis* from Bauchi (52%) and Sokoto (48%) showed the number of *Anopheles* mosquitoes that fed on cattle outdoors. Similarly, increased goat blood meal source (42%) was recorded among *An. coluzzii* in Bauchi, where animal husbandry is common and a key economic activity. In these regions, people and domesticated animals are in close vicinity to malaria vectors at night. Increased bed net coverage of humans and the presence of domesticated animals in the same houses at night likely plays a key role in the number of these *Anopheles* mosquitoes feeding on bovine and goat blood.

3.6 INSECTICIDE SUSCEPTIBILITY

Except for only few localities in Sokoto, Plateau, and Oyo where susceptibility to alpha-cypermethrin was observed, we saw widespread permethrin, lambda-cyhalothrin, deltamethrin, and alpha-cypermethrin resistance in *An. gambiae* s.l. across all ecozones in Nigeria. This is a major concern for program managers considering that pyrethroid-impregnated LLINs are the main vector control intervention in Nigeria. Data presented show some level of consistency with previous *An. gambiae* resistance data (AIRS Report, 2017). Decisions about LLIN deployment in these states must continue to be guided by data and the guidelines provided by the National Insecticide Resistance Monitoring Plan. Introduction of next-generation nets such as PBO nets and Interceptor G2 nets should be considered. Other types of pyrethroids that demonstrated to be effective control from the bioassays should be considered for insecticide resistance management.

VectorLink Nigeria observed susceptibility to carbamate and organophosphate across most sentinel sites, which aligns with previous findings (AIRS Report, 2017). The choice of these class of insecticides for vector control is not advised.

3.7 RESISTANCE INTENSITY AND MECHANISMS

The confirmed levels of resistance at 5- and especially at 10-times the diagnostic dosage highlights an urgent need to develop and implement appropriate resistance management strategies in line with WHO guidelines. Where resistance intensity assay results indicated that the *An. gambiae* s.l. populations are fully susceptible at higher diagnostic concentrations (as was observed in deltamethrin-exposed mosquitoes from Bauchi, Plateau, Benue, and Sokoto), the resistance should continue to be monitored in subsequent years. Further increased intensity of resistance beyond two-times observed in deltamethrin-exposed populations of *An. gambiae* s.l. from Oyo, Akwa Ibom, and Ebonyi, attest to the fact that intensity of resistance in *An. gambiae* s.l. clearly varies across locations and will require monitoring to detect and forestall increased intensity. However, there is an urgent need to consider management strategies in Ebonyi where cases of 5- and 10-times the intensity of deltamethrin resistance were recorded. This could include deployment of next-generation LLINs in these areas. Similar cases of resistance intensity was reported in Ebonyi in 2017 (AIRS Report, 2017). High permethrin resistance intensity in *An. gambiae* s.l. populations from Nasarawa, Benue, Akwa Ibom, Ebonyi, and Oyo qualifies these regions for insecticide resistance management strategies.

The restoration of insecticide susceptibility in pyrethroid-resistant *An. gambiae* s.l. populations pre-exposed to PBO across all sentinel sites indicates the potential of PBO to control pyrethroid-resistant mosquitoes. It is recommended that PBO+deltamethrin nets can be deployed to manage deltamethrin resistance in Plateau, Sokoto, Nasarawa, Ebonyi, Benue, and Akwa Ibom. Given that PBO did not restore susceptibility in one of

the six LGAs in Akwa Ibom, resistance results should be monitored at the LGA level. The choice of a non-pyrethroid for IRS could be considered in regions like Afijio and Akinyele (both in Oyo), where deltamethrin and permethrin resistance were detected despite pre-exposure to PBO. In Bauchi, full susceptibility was not restored in *An. gambiae* s.l. mosquitoes exposed to both permethrin and deltamethrin combinations with PBO in Bauchi. Alpha-cypermethrin + PBO and chlorfenapyr bioassays should be initiated in this area.

Alpha-cypermethrin treated nets are recommended for Zamfara. If possible, efforts should be made to generate susceptibility data for deltamethrin and synergist assays, which were not able to be completed due to security challenges. In general, there is need to fully characterize the involvement of other metabolic enzymes in pyrethroid-resistant mosquito populations where PBO has failed to restore full susceptibility.

3.8 KNOCKDOWN GENE FREQUENCIES

The knockdown (*kdr*) point mutation is an important mechanism of pyrethroid resistance. We found two types of these point mutations; the 1014F (*kdr-w*) and 1014S (*kdr-e*). The *kdr-w* allele in *An. gambiae* mosquitoes in Nigeria has been closely correlated with survival to pyrethroid–DDT exposure. The epidemiological implications of the occurrence of both mutations are yet to be fully understood, particularly because the east *kdr* allele is just being reported in Nigeria. Our concern remains the emergence of an additional point mutation resistance mechanism in *An. gambiae* populations from Akwa Ibom and Ebonyi where higher intensities of permethrin and deltamethrin resistance have been reported. Though it is not evident that the presence of this resistance allele alone is sufficient to result in control failure, there is need to continually monitor the spread and gene frequencies of these mutations in the *An. gambiae* s.l. populations.

ANNEXES

ANNEX A: GPS COORDINATES OF LARVAL COLLECTION SITES AND HEALTH FACILITIES

State	Location of Sampling Sites	Latitude	Longitude	Name of Nearest HF
Oyo	Afijio	7.792333	3.904167	
	Atiba	7.877833	3.944667	
	Akinyele	7.550300	3.947000	Olorisa-Oko Primary Health Center
	Egbeda	7.992000	4.208833	
	Ibarapa East	7.541167	3.419500	
	Oluyole	7.222500	3.857500	
Plateau	Langtang North	9.133333	9.783330	
	Lantang South	8.642080	9.813490	
	Mikang	9.016930	9.539600	
	Qua'an pan	8.977620	9.264100	
	Shendam	8.825520	9.459720	PHC Nyuum, Tumbi Private clinic
	Wase	9.094650	9.930680	
Sokoto	Illela	13.430000	5.150000	
	Bodinga	12.825000	5.022100	
	Tambuwal	12.698000	4.859000	
	Wamakko	13.231260	5.117600	
	Rabah	13.122540	5.505310	General Hospital Rabah
	Gudu	13.411600	5.480000	

State	Location of Sampling Sites	Latitude	Longitude	Name of Nearest HF
Bauchi	Ningi	11.044710	9.572530	
	Misau	11.305730	10.474310	
	Shira	11.428900	9.963100	
	Dass	10.083360	9.633120	Gwantar Health Facility
	Bauchi	8.825520	9.459720	
	Toro	10.067120	9.126200	
Ebonyi	Izzi	6.307358	8.169770	
	Ezza South	6.149010	7.955550	
	Ohaukwu	6.397660	7.940440	
	Ezza North	6.328900	8.069780	Comprehensive Health Centre, Okposi Umuoghara
	Ebonyi	6.330530	8.089530	
	Ohaozara	6.046900	7.755300	
Nasarawa	Akwanga	8.901000	8.412000	
	Doma	8.400000	8.350000	Primary Health Care Centre Alagye
	Karu	9.132000	7.895000	
	Keana	8.147000	8.798000	
	Keffi	8.952000	7.891000	
	Kokona	8.847000	7.893000	
	Lafia	8.594000	8.557000	
	Nasarawa	8.536000	7.674000	
	Nassarawa Eggon	8.775000	8.531000	
	Obi	8.502000	8.543000	
	Toto	8.436000	7.283000	
	Wamba	8.956000	8.608000	

State	Location of Sampling Sites	Latitude	Longitude	Name of Nearest HF
Akwa Ibom	Itu	5.055905	7.888948	
	Nsit Ubium	4.742735	7.948834	
	Abak	4.984058	7.790945	
	Ikot Ekpene	5.190752	7.72.633	
	Oron	4.797047	8.238453	
	Mkpat Enin	4.7708499 ,	7.735482	Primary Health Care Mkpat Enin

ANNEX B: ANOPHELES CAUGHT BY SPECIES, METHOD, AND SITE, APRIL-SEPTEMBER, 2018

Mosquito Species	Doma			Ezza North			Gwantar			Ibekwe Akpannya			Nassarawa Eggon			Olorisa –oko			Rabah			Tumbi			Total (In)	Total (Out)	Total (PSC)	Overall	%
	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC					
<i>An. gambiae</i> s.l.	862	691	674	686	122	888	509	345	350	405	75	178	687	445	596	25	13	507	1412	661	6420	2088	200	2242	6674	2552	11855	21081	91.3%
<i>An. funestus</i>	64	34	0	11	3	7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	110	14	67	186	51	74	311	1.3%
<i>An. nili</i>	14	7	0	0	1	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	14	9	2	25	0.1%
<i>An. constani</i>	369	608	0	0	3	0	12	8	41	0	0	0	10	10	0	0	0	0	7	0	0	2	1	0	400	630	41	1071	4.6%
<i>An. pharoensis</i>	89	68	2	0	0	0	1	0	1	0	0	0	3	5	0	0	0	0	8	0	0	0	0	0	101	73	3	177	0.8%
<i>An. malculipalpis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	2	1	0	3	0.0%
<i>An. moucheti</i>	0	0	0	0	2	2	0	0	0	250	22	119	0	0	0	0	0	0	0	0	0	0	0	0	250	24	121	395	1.7%
<i>An. pretoriensis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0.0%
<i>An. rufipes</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	2	1	0	3	0.0%
<i>An. squamosus</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	3	2	0	5	0.0%
<i>An. longipalpis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	25	25	0.1%
Grand Total	1398	1408	676	697	131	897	523	353	394	655	97	297	701	461	596	25	13	532	1427	661	6420	2206	220	2309	7632	3344	12121	23097	100.0%

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

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

Sentinel Site	CDC LT Indoors					CDC LTs Outdoors					PSCs					Totals				
	No. PCR +ve	<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>	No. PCR +ve	<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>	No. PCR +ve	<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>	PCR +ve	<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>
Akwa Ibom	109	43	0	60	6	47	20	0	26	1	40	28	0	11	1	196	91	0	97	8
Bauchi	103	8	0	48	47	66	7	0	26	33	62	3	0	44	15	231	18	0	118	95
Ebonyi	198	122	1	59	16	80	31	4	27	18	210	145	1	43	21	488	298	6	129	55
Nasarawa (Doma)	105	9	1	75	20	76	4	3	46	23	115	12	1	89	13	296	25	5	210	56
Nasarawa (N/Eggon)	170	20	1	123	26	100	13	0	42	45	151	15	1	111	24	421	48	2	276	95
Oyo	0	0	0	0	0	5	0	0	1	4	147	22	0	103	22	152	22	0	104	26
Plateau	173	18	1	131	23	58	9	0	25	24	170	28	2	120	20	401	55	3	276	67
Sokoto	99	1	0	51	47	68	2	0	22	44	294	15	0	164	115	461	18	0	237	206
Total	957	221	4	547	185	500	86	7	215	192	1189	268	5	685	231	2646	575	16	1447	608

ANNEX D: *AN. COLUZZII*, *AN. GAMBIAE*, *AN. ARABIENSIS*, AND HYBRID SPECIES COLLECTED INDOORS AND OUTDOORS ACROSS SITES

State	Month	# CDC Indoors				# CDC LTs Outdoors				Total	% CDC LTs Indoors				% CDC LT Outdoors			
		<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>	<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>		% <i>An. coluzzii</i>	% Hybrid	% <i>An. gambiae</i>	% <i>An. arabiensis</i>	% <i>An. coluzzii</i>	% Hybrid	% <i>An. gambiae</i>	% <i>An. arabiensis</i>
Akwa Ibom	April	3	0	6	0	0	0	0	0	9	33.3	0.0	66.7	0.0	0.0	0.0	0.0	0.0
	May	9	0	9	2	0	0	0	0	20	45.0	0.0	45.0	10.0	0.0	0.0	0.0	0.0
	June	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
	July	11	0	15	0	3	0	5	1	35	31.4	0.0	42.9	0.0	8.6	0.0	14.3	2.9
	August	11	0	15	2	2	0	1	0	31	35.5	0.0	48.4	6.5	6.5	0.0	3.2	0.0
	September	9	0	15	2	15	0	20	0	61	14.8	0.0	24.6	3.3	24.6	0.0	32.8	0.0
	Total		43	0	60	6	20	0	26	1	156	27.6	0.0	38.5	3.8	12.8	0.0	16.7
Bauchi	April	0	0	1	0	0	0	1	0	2	0.0	0.0	50.0	0.0	0.0	0.0	50.0	0.0
	May	1	0	7	9	0	0	2	5	24	4.2	0.0	29.2	37.5	0.0	0.0	8.3	20.8
	June	2	0	11	8	3	0	8	3	35	5.7	0.0	31.4	22.9	8.6	0.0	22.9	8.6
	July	2	0	14	21	4	0	9	11	61	3.3	0.0	23.0	34.4	6.6	0.0	14.8	18.0
	August	2	0	7	4	0	0	4	9	26	7.7	0.0	26.9	15.4	0.0	0.0	15.4	34.6
	September	1	0	8	5	0	0	2	5	21	4.8	0.0	38.1	23.8	0.0	0.0	9.5	23.8
	Total		8	0	48	47	7	0	26	33	169	4.7	0.0	28.4	27.8	4.1	0.0	15.4
Ebonyi	April	11	0	6	1	0	0	0	0	18	61.1	0.0	33.3	5.6	0.0	0.0	0.0	0.0
	May	24	0	12	2	4	2	2	4	50	48.0	0.0	24.0	4.0	8.0	4.0	4.0	8.0
	June	32	0	12	0	9	2	13	3	71	45.1	0.0	16.9	0.0	12.7	2.8	18.3	4.2
	July	37	1	15	6	8	0	7	1	75	49.3	1.3	20.0	8.0	10.7	0.0	9.3	1.3
	August	6	0	6	4	3	0	1	3	23	26.1	0.0	26.1	17.4	13.0	0.0	4.3	13.0
	September	12	0	8	3	7	0	4	7	41	29.3	0.0	19.5	7.3	17.1	0.0	9.8	17.1
	Total		122	1	59	16	31	4	27	18	278	43.9	0.4	21.2	5.8	11.2	1.4	9.7

State	Month	# CDC Indoors				# CDC LTs Outdoors				Total	% CDC LTs Indoors				% CDC LT Outdoors					
		<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>	<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>		% <i>An. coluzzii</i>	% Hybrid	% <i>An. gambiae</i>	% <i>An. arabiensis</i>	% <i>An. coluzzii</i>	% Hybrid	% <i>An. gambiae</i>	% <i>An. arabiensis</i>		
Doma	April	0	0	13	0	0	0	0	0	3	16	0.0	0.0	81.3	0.0	0.0	0.0	0.0	0.0	18.8
	May	3	1	13	6	0	2	7	6	6	38	7.9	2.6	34.2	15.8	0.0	5.3	18.4	15.8	15.8
	June	2	0	31	4	1	0	24	11	73	73	2.7	0.0	42.5	5.5	1.4	0.0	32.9	15.1	15.1
	July	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	0.0
	August	3	0	10	8	0	0	0	0	21	21	14.3	0.0	47.6	38.1	0.0	0.0	0.0	0.0	0.0
	September	1	0	8	2	3	1	15	3	33	33	3.0	0.0	24.2	6.1	9.1	3.0	45.5	9.1	9.1
	Total	9	1	75	20	4	3	46	23	181	181	5.0	0.6	41.4	11.0	2.2	1.7	25.4	12.7	12.7
N/Eggon	April	1	0	25	2	1	0	3	8	40	40	2.5	0.0	62.5	5.0	2.5	0.0	7.5	20.0	20.0
	May	2	0	32	7	0	0	6	6	53	53	3.8	0.0	60.4	13.2	0.0	0.0	11.3	11.3	11.3
	June	8	0	20	7	7	0	10	7	59	59	13.6	0.0	33.9	11.9	11.9	0.0	16.9	11.9	11.9
	July	6	1	34	1	5	0	22	4	73	73	8.2	1.4	46.6	1.4	6.8	0.0	30.1	5.5	5.5
	August	2	0	7	5	0	0	1	10	25	25	8.0	0.0	28.0	20.0	0.0	0.0	4.0	40.0	40.0
	September	1	0	5	4	0	0	0	10	20	20	5.0	0.0	25.0	20.0	0.0	0.0	0.0	50.0	50.0
	Total	20	1	123	26	13	0	42	45	270	270	7.4	0.4	45.6	9.6	4.8	0.0	15.6	16.7	16.7
Oyo	April	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	0.0
	May	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	0.0
	June	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	0.0
	July	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	0.0
	August	0	0	0	0	0	0	0	1	1	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
	September	0	0	0	0	0	0	1	3	4	4	0.0	0.0	0.0	0.0	0.0	0.0	25.0	75.0	75.0
	Total	0	0	0	0	0	0	1	4	5	5	0.0	0.0	0.0	0.0	0.0	0.0	20.0	80.0	80.0

State	Month	# CDC Indoors				# CDC LTs Outdoors				Total	% CDC LTs Indoors				% CDC LT Outdoors			
		<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>	<i>An. coluzzii</i>	Hybrid	<i>An. gambiae</i>	<i>An. arabiensis</i>		% <i>An. coluzzii</i>	% Hybrid	% <i>An. gambiae</i>	% <i>An. arabiensis</i>	% <i>An. coluzzii</i>	% Hybrid	% <i>An. gambiae</i>	% <i>An. arabiensis</i>
Plateau	May	5	0	30	6	2	0	0	5	48	10.4	0.0	62.5	12.5	4.2	0.0	0.0	10.4
	June	6	0	36	6	5	0	15	3	71	8.5	0.0	50.7	8.5	7.0	0.0	21.1	4.2
	July	4	1	38	9	0	0	6	5	63	6.3	1.6	60.3	14.3	0.0	0.0	9.5	7.9
	August	1	0	10	0	2	0	1	8	22	4.5	0.0	45.5	0.0	9.1	0.0	4.5	36.4
	September	2	0	17	2	0	0	3	3	27	7.4	0.0	63.0	7.4	0.0	0.0	11.1	11.1
	Total		18	1	131	23	9	0	25	24	231	7.8	0.4	56.7	10.0	3.9	0.0	10.8
Sokoto	April	0	0	5	5	0	0	6	7	23	0.0	0.0	21.7	21.7	0.0	0.0	26.1	30.4
	May	0	0	12	7	0	0	4	4	27	0.0	0.0	44.4	25.9	0.0	0.0	14.8	14.8
	June	0	0	0	3	0	0	1	0	4	0.0	0.0	0.0	75.0	0.0	0.0	25.0	0.0
	July	1	0	19	19	0	0	4	13	56	1.8	0.0	33.9	33.9	0.0	0.0	7.1	23.2
	August	0	0	8	6	1	0	6	6	27	0.0	0.0	29.6	22.2	3.7	0.0	22.2	22.2
	September	0	0	7	7	1	0	1	14	30	0.0	0.0	23.3	23.3	3.3	0.0	3.3	46.7
	Total		1	0	51	47	2	0	22	44	167	0.6	0	30.5	28	1.2	0	13.2

ANNEX E: INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR							
		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>			
		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	Apr-18	9	0	2	0	0	0	2.17	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.88	0.00	0.00	0.00
	May-18	20	0	2	0	11	0	4.95	0.00	0.42	0.00	2.70	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.44	0.00	0.00	0.00
	Jun-18	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jul-18	17	2	3	1	0	2	4.13	0.58	0.74	0.31	0.00	0.60	0.09	0.00	0.00	0.20	0.00	0.00	11.63	0.00	0.00	0.00	1.94	0.00	0.00	0.00
	Aug-18	31	12	3	0	7	0	7.86	3.00	0.80	0.03	1.73	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.64	0.00	0.00	0.00
	Sep-18	75	21	4	4	5	0	18.87	5.14	0.90	0.97	1.25	0.00	0.22	0.00	0.00	0.00	0.00	0.00	125.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		161	32	13	5	20	4	40.14	7.98	3.17	1.27	5.09	0.98	0.07	0.00	0.05	0.04	0.00	0.00	137.39	0.00	4.95	1.94	0.00	0.00	0.00	0.00
Bauchi	Apr-18	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-18	1	0	10	0	1	0	0.31	0.00	2.48	0.00	0.23	0.00	0.00	0.00	0.00	0.50	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.78	0.00
	Jun-18	10	11	10	3	2	3	2.45	2.84	2.41	0.78	0.42	0.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jul-18	6	12	22	5	2	10	1.39	3.08	5.40	1.13	0.49	2.44	0.00	0.00	0.07	0.00	0.05	0.00	0.00	0.00	11.95	0.00	0.72	0.00	0.00	0.00
	Aug-18	12	0	4	0	2	0	3.04	0.00	0.95	0.00	0.53	0.00	0.50	0.00	0.00	0.00	0.00	0.00	47.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Sep-18	14	0	9	0	1	0	3.61	0.00	2.33	0.00	0.13	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	8.75	0.00	0.00	0.00	0.00	0.00
		40	37	52	20	7	11	9.88	9.15	12.88	5.11	1.83	2.83	0.13	0.00	0.04	0.04	0.04	0.00	47.10	0.00	20.70	0.00	1.50	0.00	0.00	0.00

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR							
		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>			
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out		
Ebonyi	Apr-18	13	0	1	0	13	0	3.21	0.00	0.20	0.00	3.36	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.05	0.00	0.00	0.00
	May-18	49	6	2	0	30	38	12.16	1.60	0.40	0.10	7.58	9.60	0.00	0.00	0.17	0.50	0.00	0.00	0.00	0.00	0.00	0.00	2.05	1.61	0.00	0.00
	Jun-18	36	12	1	3	0	9	9.09	2.88	0.34	0.75	0.00	2.24	0.00	0.00	0.08	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.86	1.74	0.00	0.00
	Jul-18	95	10	2	1	113	7	23.76	2.38	0.49	0.29	28.32	1.71	0.03	0.00	0.13	0.00	0.00	0.00	19.91	0.00	2.02	0.00	0.00	0.00	0.00	0.00
	Aug-18	36	5	1	0	18	46	9.00	1.18	0.33	0.05	4.50	11.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Sep-18	153	16	1	1	28	57	38.22	4.08	0.32	0.21	7.04	14.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		425	50	8	6	246	101	106.21	12.44	1.98	1.38	61.47	25.29	0.01	0.00	0.12	0.07	0.00	0.00	19.91	0.00	6.97	3.35	0.00	0.00	0.00	0.00
Doma	Apr-18	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-18	16	0	8	0	2	0	3.95	0.00	1.99	0.00	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jun-18	11	7	48	72	0	0	2.81	1.83	11.95	18.00	0.04	0.04	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.56	0.00	0.00	0.00
	Jul-18	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aug-18	14	0	6	0	4	0	3.50	0.00	1.46	0.00	1.03	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.99	0.00
	Sep-18	13	18	7	9	0	1	3.32	4.61	1.83	2.19	0.07	0.26	0.00	0.00	0.13	0.07	0.00	0.00	0.00	0.00	0.00	0.00	6.88	4.38	0.00	0.00
		75	38	72	70	2	1	18.65	9.47	18.06	17.49	0.62	0.33	0.00	0.00	0.03	0.02	0.05	0.00	0.00	0.00	18.44	4.38	3.99	0.00	0.00	0.00

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
N/Eggon	Apr-18	2	3	58	3	0	3	0.54	0.81	14.58	0.75	0.01	0.67	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	17.50	0.00	0.00	0.00
	May-18	8	0	55	0	0	0	1.98	0.00	13.67	0.00	0.06	0.00	0.00	0.00	0.03	0.17	0.00	0.00	0.00	0.00	13.24	0.00	0.00	0.00
	Jun-18	32	18	7	3	8	17	8.11	4.59	1.82	0.71	1.92	4.29	0.00	0.00	0.05	0.00	0.14	0.00	0.00	0.00	2.73	0.00	8.23	0.00
	Jul-18	25	20	19	11	0	2	6.15	5.04	4.84	2.84	0.08	0.44	0.17	0.00	0.06	0.05	0.00	0.00	31.76	0.00	8.83	4.00	0.00	0.00
	Aug-18	16	0	4	0	2	0	4.00	0.00	1.02	0.00	0.61	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	4.52	0.00	0.00	0.00
	Sep-18	4	0	4	0	1	0	1.05	0.00	1.04	0.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		81	58	87	27	6	22	20.33	14.46	21.65	6.73	1.50	5.43	0.05	0.00	0.05	0.05	0.04	0.00	31.76	0.00	46.82	4.00	8.23	0.00
Oyo	Apr-18	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	0.00	0.00	0.00	0.00	0.00	0.00
	May-18	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	0.00	0.00	0.00	0.00	0.00	0.00
	Jun-18	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	0.00	0.00	0.00	0.00	0.00	0.00
	Jul-18	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	0.00	0.00	0.00	0.00	0.00	0.00
	Aug-18	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	0.00	0.00	0.00	0.00	0.00	0.00
	Sep-18	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	0.00	0.00	0.00	0.00	0.00	0.00
		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	0.00	0.00	0.00	0.00	0.00	0.00

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR							
		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		<i>An. arabiensis</i>			
		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	May-18	25	3	21	0	1	0	6.31	0.64	5.13	0.00	0.37	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.59	0.00	0.00	0.00
	Jun-18	56	22	24	6	2	3	14.06	5.54	6.00	1.44	0.38	0.65	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	0.00	0.00	0.00
	Jul-18	76	0	40	0	1	0	19.06	0.00	10.09	0.00	0.22	0.00	0.25	0.00	0.03	0.00	0.00	0.00	147.71	0.00	8.23	0.00	0.00	0.00	0.00	
	Aug-18	35	5	9	0	0	35	8.73	1.18	2.29	0.11	0.00	8.73	0.00	0.00	0.00	1.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	3.55	0.00	33.82
	Sep-18	7	0	15	0	0	0	1.76	0.00	3.72	0.00	0.07	0.00	0.00	0.00	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		218	31	104	13	4	16	54.60	7.76	26.08	3.36	0.99	4.02	0.06	0.00	0.03	0.08	0.00	0.08	147.71	0.00	23.83	3.55	0.00	33.82		
Sokoto	Apr-18	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	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-18	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.29	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jun-18	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	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jul-18	4	0	62	0	0	0	1.12	0.00	15.44	0.00	0.08	0.00	0.00	0.00	0.16	0.00	0.00	0.08	0.00	0.00	75.56	0.00	0.00	0.00	0.00	
	Aug-18	0	21	0	7	0	1	0.00	5.25	0.00	1.63	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Sep-18	0	11	0	1	0	11	0.00	2.63	0.00	0.33	0.00	2.63	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		14	19	421	62	0	1	3.58	4.86	105.19	15.58	0.03	0.35	0.00	0.00	0.08	0.00	0.09	0.07	0.00	0.00	75.56	0.00	0.00	0.00	0.00	
																				383.86	0.00	197.27	17.22	13.71	33.82		

HBR=Human Biting Rate, SPR=Sporozoite Positive Rate
In=Indoor CDC Light Trap, Out=Outdoor CDC Light Trap, PSC=Pyrethrum Spray Catch

ANNEX F: INDOOR RESTING DENSITY OF ANOPHELINE MOSQUITOES BY SITE

Sentinel Sites	Month	# of Rooms	Total # of <i>Anopheles</i> Caught	Indoor Resting Density
Akwa Ibom	Apr-18	32	2	0.1
	May-18	32	14	0.4
	Jun-18	32	4	0.1
	Jul-18	32	48	1.5
	Aug-18	32	118	3.7
	Sep-18	32	111	3.5
Mean				1.5
Bauchi	Apr-18	32	20	0.6
	May-18	32	49	1.5
	Jun-18	32	67	2.1
	Jul-18	32	73	2.3
	Aug-18	32	31	1.0
	Sep-18	32	154	4.8
Mean				2.1
Ebonyi	Apr-18	32	73	2.3
	May-18	32	134	4.2
	Jun-18	32	143	4.5
	Jul-18	32	196	6.1
	Aug-18	32	127	4.0
	Sep-18	32	224	7.0
Mean				4.7

Sentinel Sites	Month	# of Rooms	Total # of <i>Anopheles</i> Caught	Indoor Resting Density
Nasarawa (Doma)	Apr-18	32	33	1.0
	May-18	32	52	1.6
	Jun-18	32	79	2.5
	Jul-18	32	203	6.3
	Aug-18	32	80	2.5
	Sep-18	32	229	7.2
Mean				3.5
Nasarawa (N/Eggon)	Apr-18	32	149	4.7
	May-18	32	38	1.2
	Jun-18	32	48	1.5
	Jul-18	32	181	5.7
	Aug-18	32	91	2.8
	Sep-18	32	89	2.8
Mean				3.1
Oyo	Apr-18	32	47	1.5
	May-18	32	84	2.6
	Jun-18	32	150	4.7
	Jul-18	32	95	3.0
	Aug-18	32	96	3.0
	Sep-18	32	60	1.9
Mean				2.8

Sentinel Sites	Month	# of Rooms	Total # of <i>Anopheles</i> Caught	Indoor Resting Density
Plateau	May-18	32	730	22.8
	Jun-18	32	463	14.5
	Jul-18	32	519	16.2
	Aug-18	32	323	10.1
	Sep-18	32	274	8.6
Mean				14.4
Sokoto	Apr-18	32	625	19.5
	May-18	32	225	7.0
	Jun-18	32	62	1.9
	Jul-18	32	1239	38.7
	Aug-18	32	2181	68.2
	Sep-18	32	2088	65.3
Mean				33.4

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