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THE PMI VECTORLINK NIGERIA PROJECT

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ACRONYMS

CDC	U.S. Centers for Disease Control and Prevention
CDC LT	CDC Light Trap
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
HF	Health Facility
HBR	Human Biting Rate
ITN	Insecticide-treated Net
<i>Kdr</i>	Knock down resistance
LGA	Local Government Area
NMEP	National Malaria Elimination Program
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SPR	Sporozoite rate
WHO	World Health Organization

EXECUTIVE SUMMARY

Vector surveillance and insecticide resistance monitoring activities provide malaria control stakeholders with data that can inform 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. In 2019, insecticide resistance monitoring in Cross River and Kebbi began, joining nine other states involved in vector surveillance and insecticide resistance monitoring activities. From November 2018 to September 2019, pyrethrum spray catches (PSCs) and human-baited U.S. Centers for Disease Control and Prevention Light Traps (CDC LTs) both indoors and outdoors were used to collect mosquitoes and determine the species composition, behavior, seasonality, biting rates, infectivity rates, blood meal sources, and entomological inoculation rates (EIRs) of malaria vectors across sentinel sites. CDC bottle bioassays were used to determine the insecticide resistance status, intensity, and underlying resistance mechanisms.

A total of 29,174 *Anopheles* mosquitoes were collected from seven sentinel sites over an 11 month period. *Anopheles gambiae* s.l. was the most abundant species across the sites, ranging from 81.1% in Plateau to 98.2% in Akwa Ibom. Other *Anopheles* species identified with limited distribution were *An. funestus*, *An. coustani*, *An. moucheti*, *An. nili*, *An. pharoensis*, *An. squamosus*, *An. maculipalpis*, *An. longipalpis*, *An. rufipes*, and *An. pretoriensis*. *An. gambiae*, a member of the *An. gambiae* s.l. complex, was found to be the dominant species both indoors and outdoors in Bauchi, Nasarawa, Oyo, and Plateau, while in Akwa Ibom, Ebonyi, and Sokoto, its predominance was limited to indoors (in Akwa Ibom, *An. coluzzii* predominated outdoors, while in Ebonyi and Sokoto, *An. arabiensis* predominated outdoors). The highest proportion of *An. coluzzii* indoors was recorded in Ebonyi (39.4%), while the highest outdoor collections of *An. coluzzii* were in Akwa Ibom (40%). Hybrid forms were recorded indoors only in Akwa Ibom (2.0%), Ebonyi (1.2%), Nasarawa, (0.8%), Plateau (2.9%), and Sokoto (1.9%). *An. arabiensis* was found in all sites, with the highest indoor occurrence in Sokoto (43.5%) and the lowest in Ebonyi (3.6%).

Mosquito availability and abundance in Nigeria is both seasonal and rainfall-dependent. The highest mean indoor resting density was observed in Sokoto at the start of the rainy season in April (26.3 mosquitoes/room/day) and in September (peak of rainfall) (67.5 mosquitoes/room/day). EIRs varied by month, vector species, and location (indoor/outdoor). Indoor EIRs for *An. gambiae* ranged from 7.9 infective bites/person/year in Akwa Ibom to 60.2 infective bites/person/year in Bauchi, while outdoor EIRs ranged from 10.3 infective bites/person/year in Ebonyi to 134.9 infective bites/person/year in Nasarawa. Indoor EIRs for *An. coluzzii* ranged from 20.4 infective bites/person/year in Nasarawa to 48.2 infective bites/person/year in Plateau. No outdoor EIR for *An. coluzzii* was recorded at any of the sites. Also, there was no record of indoor EIR for *An. arabiensis* across all sites. However, outdoor EIRs for *An. arabiensis* ranged from 2.7 infective bites/person/year in Plateau to 22.2 infective bites/person/year in Nasarawa.

The proportion of human blood meal detected varied according to vector and ecozones. Blood meal analysis revealed that *An. coluzzii* fed predominantly on humans. The highest proportions of *An. coluzzii* mosquitoes which fed on human blood were from Ebonyi (95%) and Akwa Ibom (91%). There was also evidence of mosquitoes feeding on goats and bovines. The proportion of *An. gambiae* that fed on human blood meal varied from 22% in Sokoto to 93% in Akwa Ibom. Bovine blood meals were also detected in *An. gambiae* across all sites. The proportions of bovine blood meals in *An. gambiae* ranged from 7% in Akwa Ibom to 62% in Sokoto, while bovine blood meals in *An. coluzzii* ranged from 5% in Ebonyi to 67% in Oyo. The proportion of *An. arabiensis* that fed on human blood was highest indoors in Sokoto (89%), Akwa Ibom (100%), Nasarawa (100%), and Plateau (100%). The proportions of bovine blood meals in *An. arabiensis* ranged from 20% in Ebonyi to 76% in Sokoto from mosquitoes collected outdoors.

Insecticide susceptibility test results indicated that pyrethroid resistance was widespread in *An. gambiae* s.l. mosquitoes at all sentinel sites across all ecozones. Resistance patterns of *An. gambiae* to deltamethrin were similar across all Local Government Areas (LGAs) in Akwa Ibom, Bauchi, Nasarawa, Sokoto, and Zamfara

(resistant to deltamethrin at 1X but susceptible at 2X). Low-intensity deltamethrin resistance was recorded in *An. gambiae* s.l. populations from five of nine LGAs in Ebonyi, three of six LGAs in Nasarawa, and all six LGAs in Plateau. Moderate deltamethrin resistance intensity was observed only in Ishielu LGA in Ebonyi. In Plateau, low resistance intensity was observed in *An. gambiae* s.l. populations exposed to permethrin. Other outcomes showed that high permethrin resistance intensity exists in mosquito populations from Makurdi LGA (in Benue state) and all LGAs in Akwa Ibom and Ebonyi.

Exposure of *An. gambiae* s.l. to piperonyl butoxide (PBO) synergist before exposure to the three pyrethroids increased mortality to varying degrees across all sentinel sites. Full susceptibility was restored in *An. gambiae* s.l. populations exposed to deltamethrin and PBO in Akwa Ibom, Ebonyi, Oyo, Sokoto, and Zamfara. In contrast, *An. gambiae* s.l. populations from Bauchi and Nasarawa only restored full susceptibility in three LGAs. PBO did not fully restore permethrin susceptibility in *An. gambiae* populations in most of the LGAs in six states (Akwa Ibom, Bauchi, Benue, Cross River, Nasarawa, and Plateau), suggesting the existence of mechanisms unrelated to the activity of mixed function oxidases. These data suggest that the use of new vector control products such as PBO and dual-active ingredient nets will be useful tools in the management of widespread pyrethroid resistance in Nigeria.

I. INTRODUCTION

The burden of malaria remains high in Nigeria with the country contributing the highest proportion of malaria cases (25%) and deaths (24%) in the world (World Malaria Report, 2019). The country has five diverse geo-ecological zones with each supporting a variety of *Anopheles* species involved in malaria transmission. 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*. Secondary malaria vectors in the country include *An. nili*, *An. moucheti*, *An. pharoensis*, *An. constani*, and *An. longipalpis*. (PMI, 2018)

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 to the PMI VectorLink Project in 2017, the number of entomological monitoring sites was increased to seven and two insecticide resistance monitoring sites were added. Currently, VectorLink is supporting longitudinal vector surveillance and insecticide resistance monitoring in five states and insecticide resistance monitoring only in an additional six states.

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

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 also 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 November 2018 to September 2019, VectorLink Nigeria conducted vector surveillance and insecticide resistance monitoring in 11 sites and assessed 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. VectorLink Nigeria also initiated entomological and epidemiological analysis in Ebonyi to assess the impact of piperonyl butoxide (PBO)-treated ITNs distributed in November 2019. This report summarizes entomological monitoring activities completed between November 2018 and September 2019.

I.1 SENTINEL SITES AND COLLECTION AND ANALYTICAL METHODS

During the period covered by this report, VectorLink Nigeria implemented both vector surveillance and insecticide resistance monitoring in seven sentinel sites and insecticide resistance monitoring only in four additional sites (Tables 1 and 2).

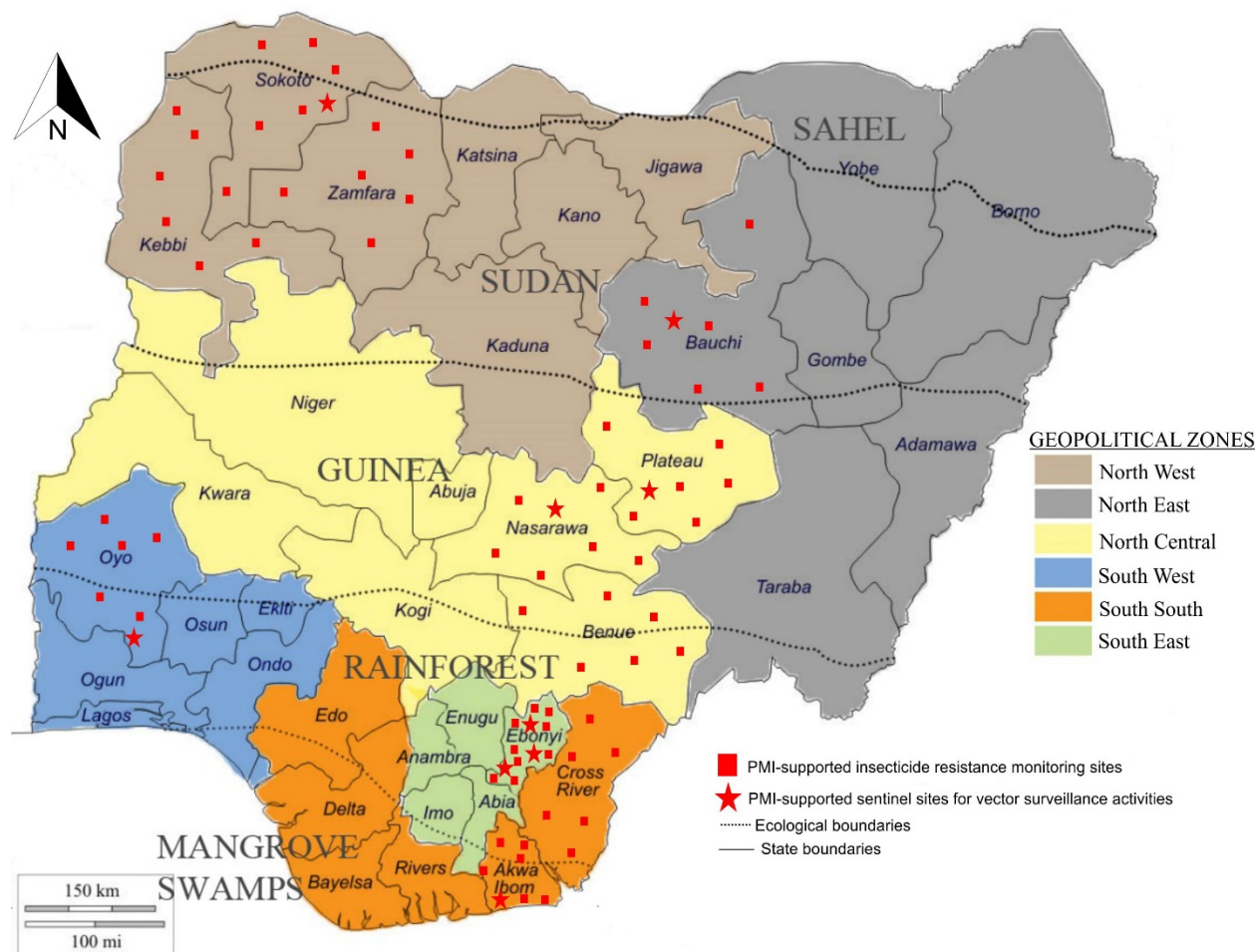
Table 1: Longitudinal Vector Surveillance and Insecticide Resistance Monitoring Sites and Affiliated Institutions

Geopolitical Zone	State/Institution	Local Government Areas (LGA)/Sentinel Site	Ecozone(s)
South West	Oyo/University of Ibadan	Akinyele/Olorisaoko	Rainforest/Guinea Savannah
South East	Ebonyi/State University Abakaliki	Ezaa North/ Umuaghara	Rainforest
South	Akwa Ibom/University of Uyo	Mpat Enin/Ibekwe Akpannya	Mangrove swamps/Rainforest
North East	Bauchi/Abubakar Tafawa Balewa University	Dass/Gwantar	Sudan Savannah
North Central	Nasarawa/State University Keffi	Doma/Alagye	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	LGA/Insecticide Resistance Monitoring Site	Ecozone(s)
North Central	Benue/Federal University of Agriculture Makurdi	Makurdi, Gboko, Katsina-Ala, Kwande, Otukpo, and Oju	Guinea Savannah
South	Cross River/University of Calabar	Calabar Municipal, Odukpani, Yakurr, Ikom, Ogoja, Obudu	Rainforest/Mangrove swamps
North West	Zamfara/Usmanu Danfodiyo University Sokoto	Gusau, Chafe, Anka, Kaura Namoda Talata-Mafara and Maru,	Sahel Savannah
North West	Kebbi/Federal University Birnin Kebbi	Argungu, Jega, Birin-Kebbi	Sahel Savannah

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



From November 2018 to September 2019, *Anopheles* mosquitoes were collected monthly from seven sentinel sites located in five ecozones of Nigeria (Figure 1). Mosquitoes were caught using human-baited CDC LTs indoors and outdoors, and PSCs. Details for each method are shown in Table 3. *Anopheles* larvae were collected using ladles and reared to adults for insecticide susceptibility tests. 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 described below.

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)

Table 4: Entomological Surveillance Indicators

Indicator	Definition
Indoor resting density	Number of adult female vectors collected indoor per room per day. This was estimated from the PSC.
Human biting rate	Number of female <i>Anopheles</i> vectors attempted 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 to determine proportion of parous mosquitoes.
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 ELISA method.
Entomological inoculation rate	Number of infectious bites by adult female vectors per person per unit time. It was calculated as the product of human biting rate and sporozoite rate.
Resistance status	Classification of adult female vectors as confirmed resistant, possible resistant, or susceptible following bioassay tests.
Resistance intensity	Classification of adult female vector populations as having high, moderate, or low resistance following bioassay tests at different concentrations.

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 to identify sibling species and determine sporozoite rate and bloodmeal source. The mean indoor and outdoor human biting rates (HBR) were calculated as the number of mosquitoes collected per human-baited CDC LT per night. The 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 one year.

1.3 PYRETHRUM SPRAY CATCHES

The team randomly sampled 32 houses per sentinel site per month using the PSC method (WHO 1975) to collect 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 to identify sibling species and determine sporozoite rate and blood meal source. 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 Gillies and De Meillon (1968), Gillet (1972), Gillies and Coetzee (1987), and Kent (2006). The teams labeled all *Anopheles* specimens and stored them individually over silica gel in Eppendorf tubes for further processing. All samples collected from the field teams were sent to the centrally-located insectary at Nasarawa State University Keffi where samples were verified for accuracy of morphological identification and later sorted for shipment to NIMR in Lagos for molecular analysis.

1.5 DETERMINATION OF PARITY RATE

To determine parity rate, the team dissected ovaries from 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). 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 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 extracted DNA using the *An. gambiae* species-specific multiplex PCR (Scott *et al.* 1993; Fanello *et al.* 2002).

1.7 *PLASMODIUM* SPOROZOITE AND BLOOD MEAL ASSAYS

To estimate the *Plasmodium* infection rate in the mosquito population, the team also performed enzyme-linked immunosorbent assays (ELISAs) for sporozoite antigen on a proportion of randomly-selected mosquitoes collected from the field using PSC and CDC LT methods. These were carried out according to methods described by Burkot *et al.* (1984). The blood meal index of the selected mosquitoes was also determined by ELISA testing of animal blood sources of *Anopheles* mosquitoes (Beier *et al.*, 1988).

1.8 INSECTICIDE RESISTANCE MONITORING

Adult *An. gambiae* s.l. mosquitoes (3–5 days-old) caught from the wild or reared from wild-caught larvae were exposed to pyrethroid (deltamethrin, permethrin, lambda-cyhalothrin and alpha-cypermethrin) and organophosphate (pirimiphos-methyl) insecticides using CDC bottle bioassay methods (Brogdon and Chan, 2010; WHO, 2013). 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 the WHO guideline (WHO, 2016). Susceptibility tests on chlorfenapyr (100 µg per bottle) and clothianidin using the CDC bottle assay and WHO tube bioassays, respectively, were carried out on *An. gambiae* Kisumu strain mosquitoes (control) and wild-caught *An. gambiae* s.l. from all 11 insecticide resistance monitoring sites.

Synergist assays using PBO were also carried out using standard methods to determine mechanisms of resistance in the *An. gambiae* s.l. mosquitoes. The *ldr* 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 *ldr* alleles.

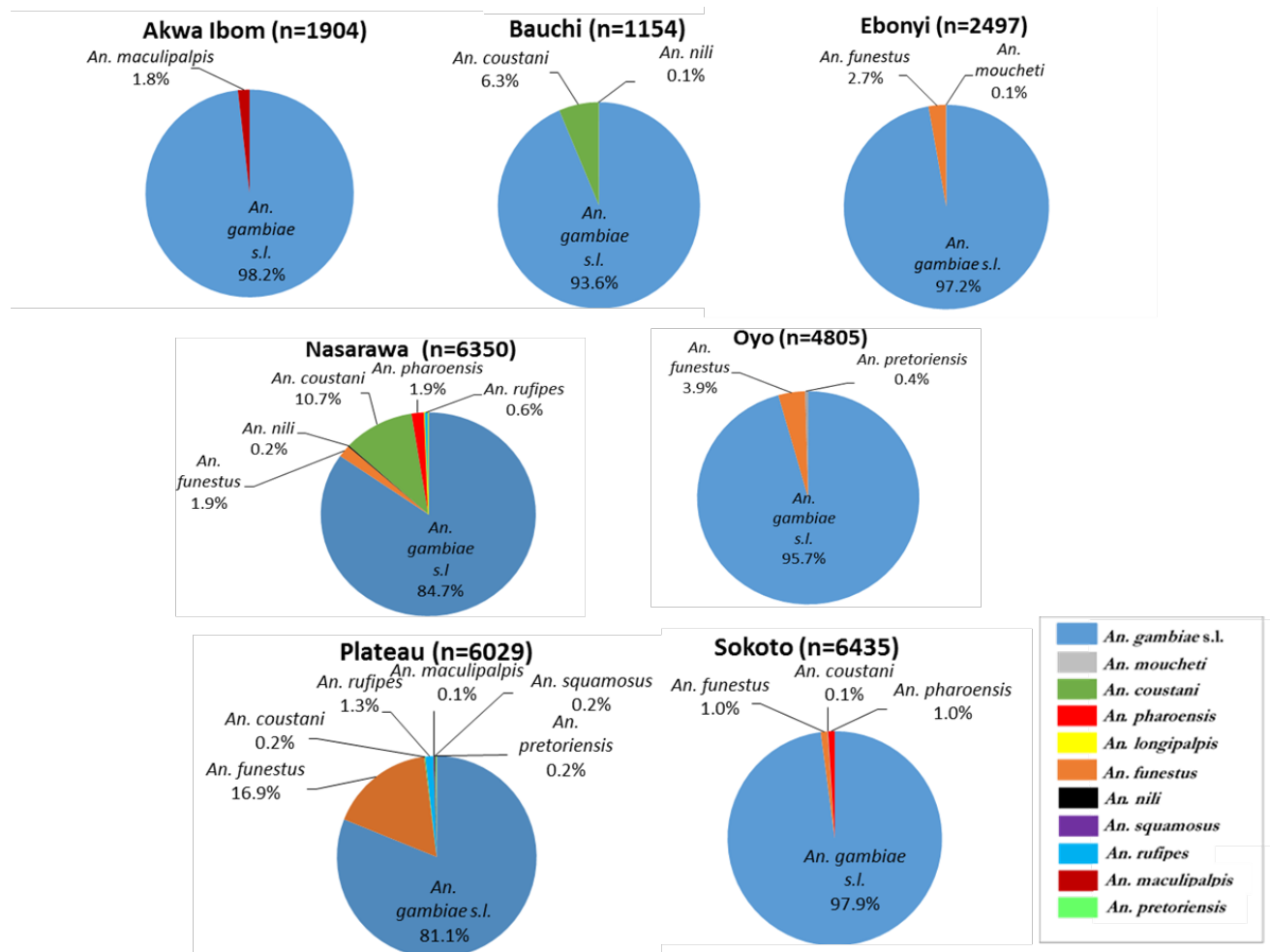
2. RESULTS

2.1 MOSQUITO ABUNDANCE AND SPECIES COMPOSITION

A total of 29,174 *Anopheles* mosquitoes were collected from seven sentinel sites using human-baited CDC LTs (indoors/outdoors) and PSCs (Annex 1).

An. gambiae s.l. was the most abundant species across all the sites ranging from 81.1% in Plateau to 98.2% in Akwa Ibom (Figure 2). Other *Anopheles* species identified in varying abundance were *An. funestus*, *An. coustani*, *An. moucheti*, *An. nili*, and *An. pharoensis*. Other localized species observed were *An. squamosus*, *An. maculipalpis*, *An. longipalpis*, *An. rufipes*, and *An. pretoriensis*. Annex 1 provides the number of each species collected by site and collection method.

Figure 2: Species Composition across Sites (November 2018 to September 2019)



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

A total of 4,192 *An. gambiae* s.l. mosquitoes collected by PSCs and CDC LTs between November 2018 and September 2019 were identified by species-specific PCR assays. Of these, 4,044 (96.5%) mosquitoes successfully amplified (Annex 2a), while 148 (3.5%) failed to amplify. A total of 2,620 (64.9%) were identified as *An. gambiae*, 647 (16.0%) were *An. coluzzii*, 752 (18.6%) were *An. arabiensis*, and 25 (0.6%) were hybrid *An. gambiae/An. coluzzii* (Annex 2b). Of the 4,044 mosquitoes identified, 2,049 (50.7%) were from CDC LT collections. *An. gambiae* (1214, 59.3%) was the predominant species compared to *An. coluzzii* (338, 16.5%), *An. arabiensis* (476, 23.2%), and hybrid forms of *An. gambiae/An. coluzzii* (21, 1.0%) (Annex 2b).

An. gambiae was the dominant species both indoors and outdoors in Bauchi, Nasarawa, Oyo, and Plateau while in Akwa Ibom, Ebonyi, and Sokoto, its predominance was limited to indoors. *An. coluzzii* predominated outdoors in Akwa Ibom, while *An. arabiensis* predominated outdoors in Ebonyi and Sokoto, respectively. The highest proportion of *An. coluzzii* indoors was recorded in Ebonyi (39.4%), while the highest outdoor collections of *An. coluzzii* was in Akwa Ibom (40%). Hybrid forms were recorded indoors only in Akwa Ibom (2.0%), Ebonyi (1.2%), Nasarawa (0.8%), Plateau (2.9%), and Sokoto (1.9%). *An. arabiensis* was found in all sites, with the highest indoor occurrence recorded in Sokoto (43.5%) and the lowest recorded in Ebonyi (3.6%). *An. arabiensis* was predominant outdoors in Sokoto (64.5%) followed by Ebonyi (50.0%) (Figure 3).

The number of *Plasmodium falciparum*-infected *An. funestus* caught by PSC was 1 (25%) in Nasarawa and 13 (7.4%) in Oyo. *An. coustani* in Nasarawa from indoor CDC LT collections had an infection rate of 1.2% (Table 5). As shown in Table 6, *P. falciparum* sporozoite rates of *An. gambiae* collected indoors ranged from 0.5% in Plateau to 7.4% in Bauchi (*An. gambiae* collected indoors in Sokoto were non-reactive). Outdoors, the sporozoite rates of *An. gambiae* ranged from 1.8% in Sokoto to 8.3% in Ebonyi. *An. gambiae* analyzed from outdoor collections in Akwa Ibom, Bauchi, and Oyo were not reactive. Sporozoite infection rates in *An. coluzzii* collected indoors varied from 1.5% in Ebonyi to 2.8% in Plateau. Nasarawa had the highest rate of *An. arabiensis* collected outdoors that tested positive for *P. falciparum* (2.9%), followed by Plateau (2.2%) and Sokoto (0.9%). No *An. arabiensis* collected indoors tested positive for sporozoites.

Table 5: Sporozoite Positivity Rates of *An. coustani* and *An. funestus* for Nasarawa and Oyo

Site	CDC LT							PSC		
	Total Analyzed	<i>An. coustani</i>						Total Analyzed	<i>An. funestus</i>	
		Number identified (%)		No. Positive for Sporozoites		SPR (%)			No. Positive for Sporozoites	SPR (%)
		In	Out	In	Out	In	Out		In	In
Nasarawa	446	129 (30.9)	317 (69.1)	2	0	1.2	0.0	4	1	25.0
Oyo	-	-	-	-	-	-	-	176	13	7.4

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

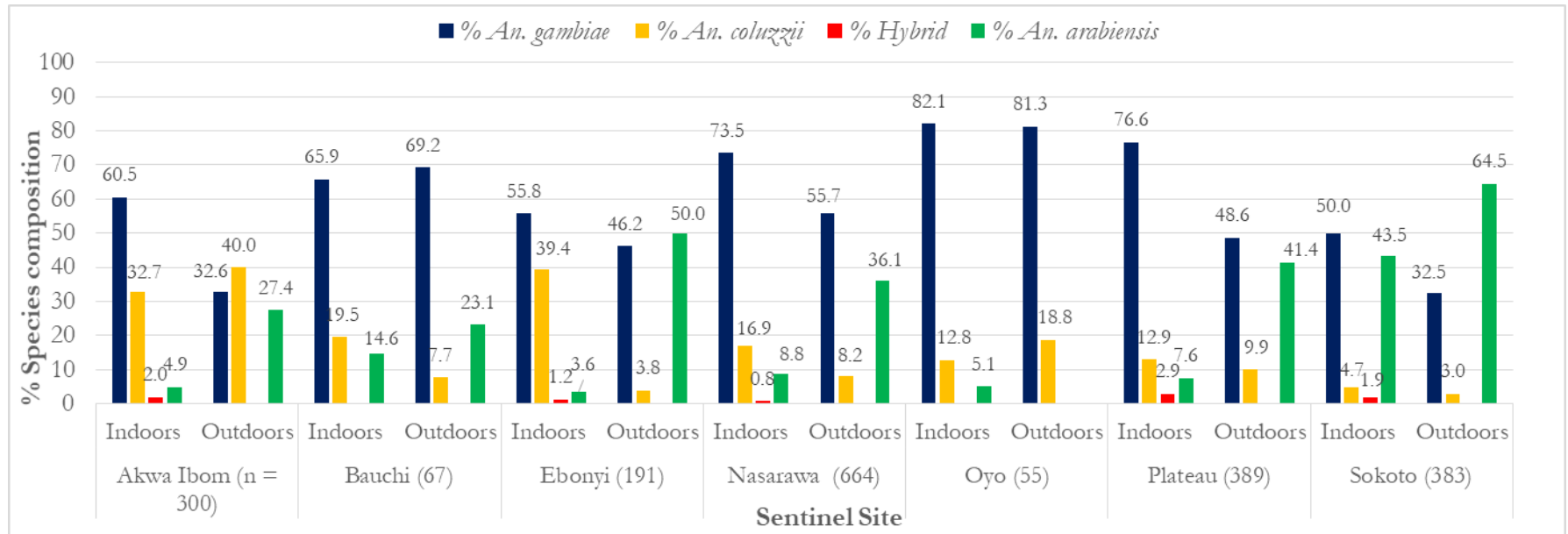


Table 6: Sporozoite Positivity Rates of *An. gambiae*, *An. coluzzii*, and *An. arabiensis* Mosquitoes across Sites

Site	Total Analyzed	<i>An. gambiae</i>						<i>An. coluzzii</i>						<i>An. arabiensis</i>					
		Number of <i>An. gambiae</i> (%)		No. Positive for Sporozoites		SPR (%)		Number of <i>An. coluzzii</i> (%)		No. Positive for Sporozoites		SPR (%)		Number of <i>An. arabiensis</i> (%)		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	327	124 (37.9)	31 (9.5)	2	0	1.6	0.0	67 (20.5)	38 (11.6)	0.0	0.0	0.0	0.0	10 (3.1)	26 (8.0)	0	0	0.0	0.0
Bauchi	69	27 (39.1)	18 (26.1)	2	0	7.4	0.0	8 (11.6)	2 (2.9)	0.0	0.0	0.0	0.0	6 (8.7)	6 (8.7)	0	0	0.0	0.0
Ebonyi	213	92 (43.2)	12 (5.6)	1	1	1.1	8.3	65 (30.5)	1 (0.5)	1.0	0.0	1.5	0.0	6 (2.8)	13 (6.1)	0	0	0.0	0.0
Nasarawa	686	274 (39.9)	162 (23.6)	3	5	1.1	3.1	63 (9.2)	24 (3.5)	1.0	0.0	1.6	0.0	33 (4.8)	105 (15.3)	0	3	0.0	2.9
Oyo	55	32 (58.2)	13 (23.6)	2	0	6.3	0.0	5 (9.1)	3 (5.5)	0.0	0.0	0.0	0.0	2 (3.6)	0 (0.0)	0	0	0.0	0.0
Plateau	400	213 (53.3)	54 (13.5)	1	0	0.5	0.0	36 (9.0)	11 (2.8)	1.0	0.0	2.8	0.0	21 (5.3)	46 (11.5)	0	1	0.0	2.2
Sokoto	401	107 (23.7)	55 (13.7)	0	1	0.0	1.8	10 (2.5)	5 (1.2)	0.0	0.0	0.0	0.0	93 (23.2)	109 (27.2)	0	1	0.0	0.9
Total	2151	869 (40.4)	345 (16.0)	11	7	1.3	2.0	254 (11.8)	84 (3.9)	3	0	1.2	0.0	171 (7.9)	305 (14.2)	0	5	0	1.6

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

2.3 HUMAN BITING RATES

The mean indoor biting rates of *An. gambiae* s.l. peaked in November and December in Akwa Ibom, in February and June in Nasarawa, in July and August in Plateau, in July in Oyo, and September in Sokoto (Figure 4). Increased outdoor biting was recorded between January and August, with a peak in February in Nasarawa. Outdoor biting rate increased between July and August in Oyo (Figure 5). The 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 rainy season (April–September) compared to isolated peak biting rates observed indoors (Nasarawa and Akwa Ibom) and outdoors (Nasarawa, Akwa Ibom, and Sokoto) during the dry season.

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

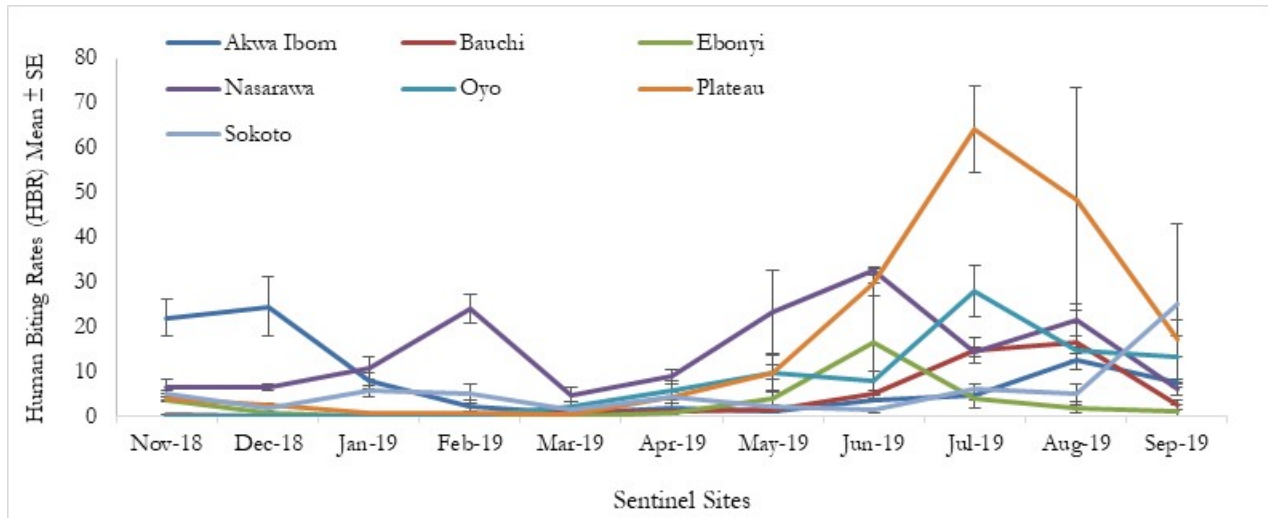
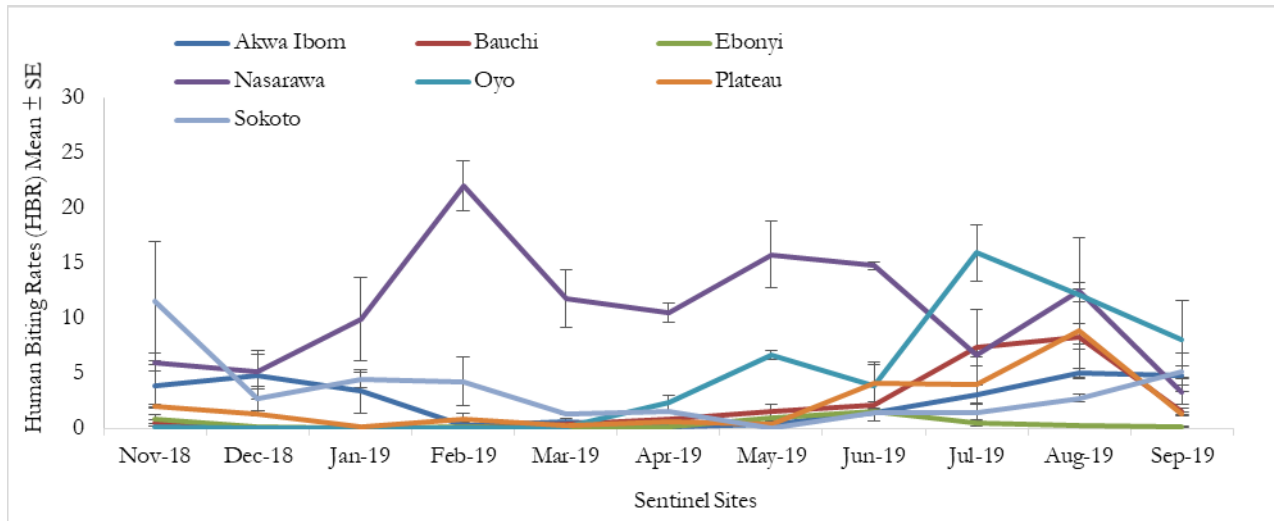


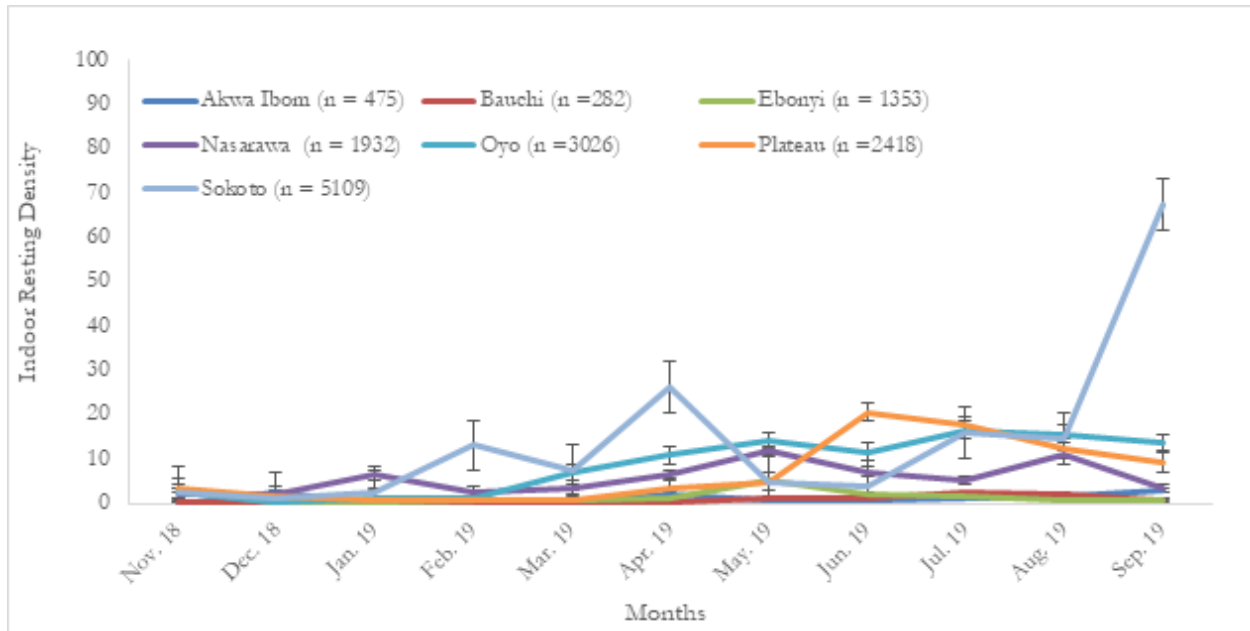
Figure 5: Monthly Outdoor Human Biting Rates of *An. gambiae* s.l. by Site



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

Indoor resting density varied across the sites and months, ranging from 0 mosquitoes per room per day in Ebonyi, Oyo, and Sokoto in December 2018 to 67.5 mosquitoes per room per day in Sokoto in September 2019, which is the peak of rainfall (Figure 6). In general, higher indoor resting densities were observed between April and September, with the peak at the height of the rainy season in September in Sokoto.

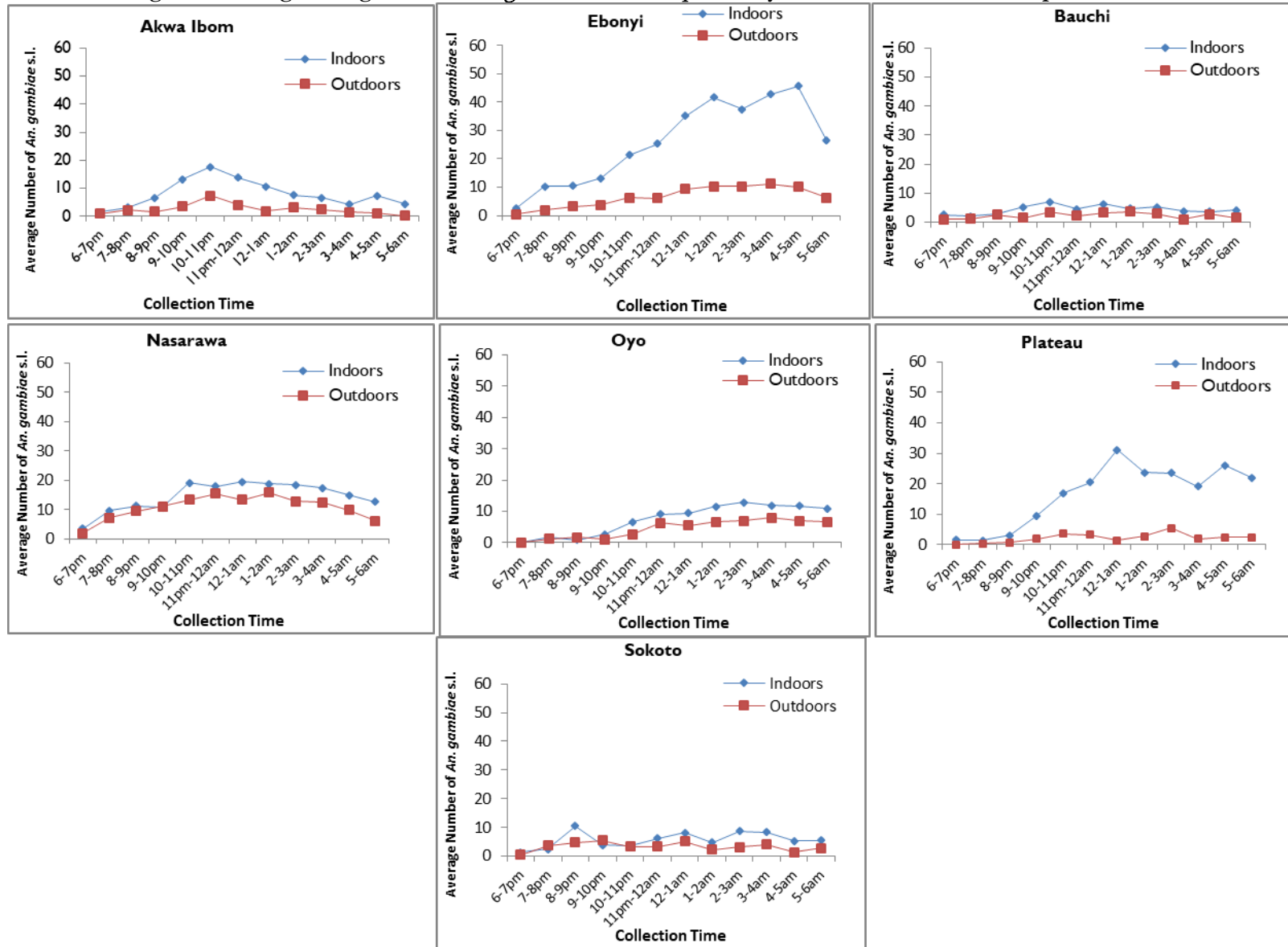
Figure 6: Indoor Resting Density by Site



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

The average number of mosquitoes caught biting per unit time was generally higher indoors, ranging from 11 mosquitoes collected between 8-9 p.m. in Sokoto to 46 mosquitoes collected between 4-5 a.m. in Ebonyi. Biting peaked at 1-2 a.m. and then again at 4-5 a.m. in Ebonyi. Similarly, biting peaked in Plateau between 12-1 a.m. and 4-5 a.m. Outdoors, the numbers of mosquitoes caught per hour ranged from 3 mosquitoes collected between 10-11 p.m. in Bauchi to 16 mosquitoes collected between 1-2 a.m. in Nasarawa (Figure 7).

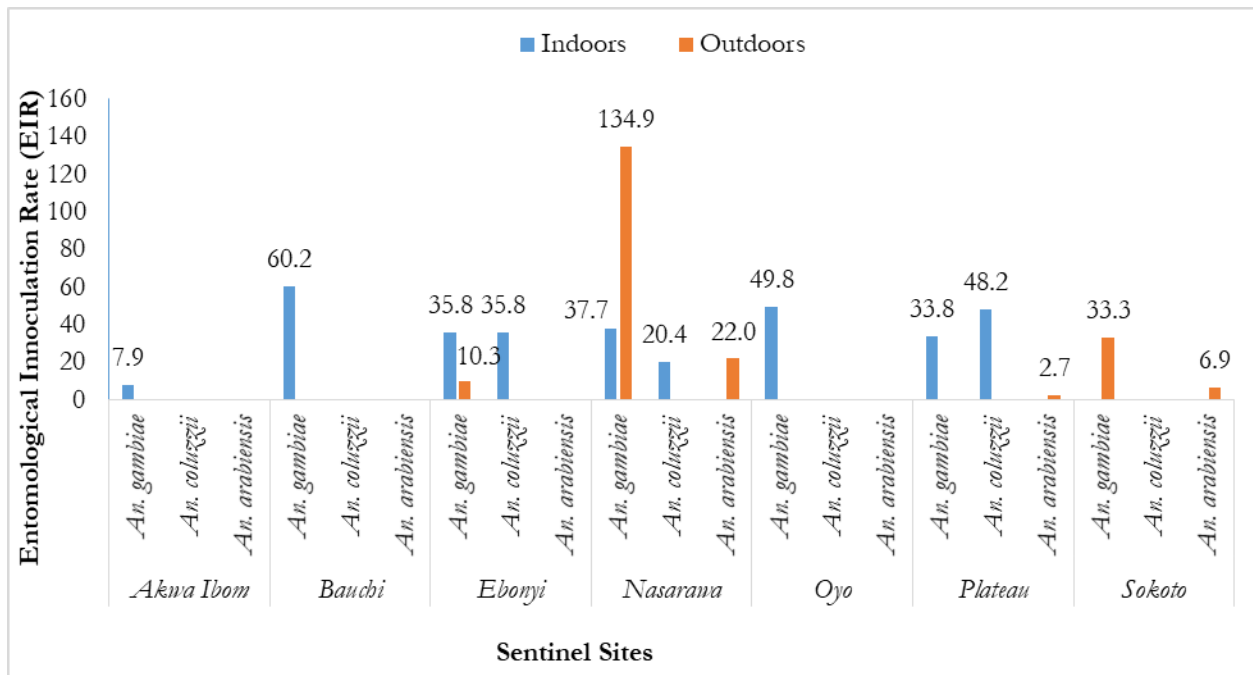
Figure 7: Average Biting Rates of *An. gambiae* s.l. Mosquitoes by Site, November 2018 to September 2019



2.6 ENTOMOLOGICAL INOCULATION RATES ACROSS SITES

Entomological inoculation rates varied by month, vector species, and location (indoor/outdoor). Indoor EIR for *An. gambiae* ranged from 7.9 infective bites/person/year in Akwa Ibom to 60.2 infective bites/person/year in Bauchi, while outdoor EIR ranged between 10.3 infective bites/person/year in Ebonyi to 134.9 infective bites/person/year observed in Nasarawa. Indoor EIR for *An. coluzzii* ranged from 0.0 infective bites/person/year in Akwa Ibom, Bauchi, Oyo, and Sokoto States to 48.2 infective bites/person/year recorded in Plateau with no outdoor EIR for *An. coluzzii* recorded at any of the sites. Whereas there was no indoor EIR recorded for *An. arabiensis* across all the sites, outdoor EIR ranged 0.0 infective bites/person/year in Akwa Ibom, Bauchi, Ebonyi, and Oyo to 22.2 infective bites/person/year recorded in Nasarawa (Figure 8 and Annex 4 and 5).

Figure 8: Annual Entomological Inoculation Rates across Sites



2.7 HUMAN BLOOD INDEX

Overall across all sites human blood meals were detected in varying proportions in *An. gambiae*, *An. coluzzii*, and *An. arabiensis* collected using both PSC and CDC LT methods (Figures 9-11). The proportion varied by vector and site. The highest proportions of *An. coluzzii* mosquitoes from CDC LT that fed on human blood were from Ebonyi (97%) and Akwa Ibom (94%). Generally, human blood meal from CDC LT indoors (32-100%) was higher than CDC LTs placed outdoors (4-85%). Human blood meal index in mosquitoes collected by PSC (76-100%) was higher compared to both indoor and outdoor CDC LT collections. *An. gambiae*, *An. coluzzii*, and *An. arabiensis* collected from CDC LT outdoors fed more on bovine blood meal in Sokoto (50-76%), Plateau (50-88%), and Nasarawa (57-89%). In Bauchi, 100% of *An. coluzzii* mosquitoes fed on goat blood. However, only very few samples (n=22) were analyzed from Bauchi and this might not conclusively indicate the feeding behavior of *An. coluzzii* in the area. Other results further indicated that all *An. gambiae* analyzed had fed on human blood across all ecozones (Figure 9-11).

Figure 9: Blood Meal Sources of *An. gambiae*, *An. coluzzii*, and *An. arabiensis* from Indoor CDC Light Trap Collections across Sites (November 2018 to September 2019)

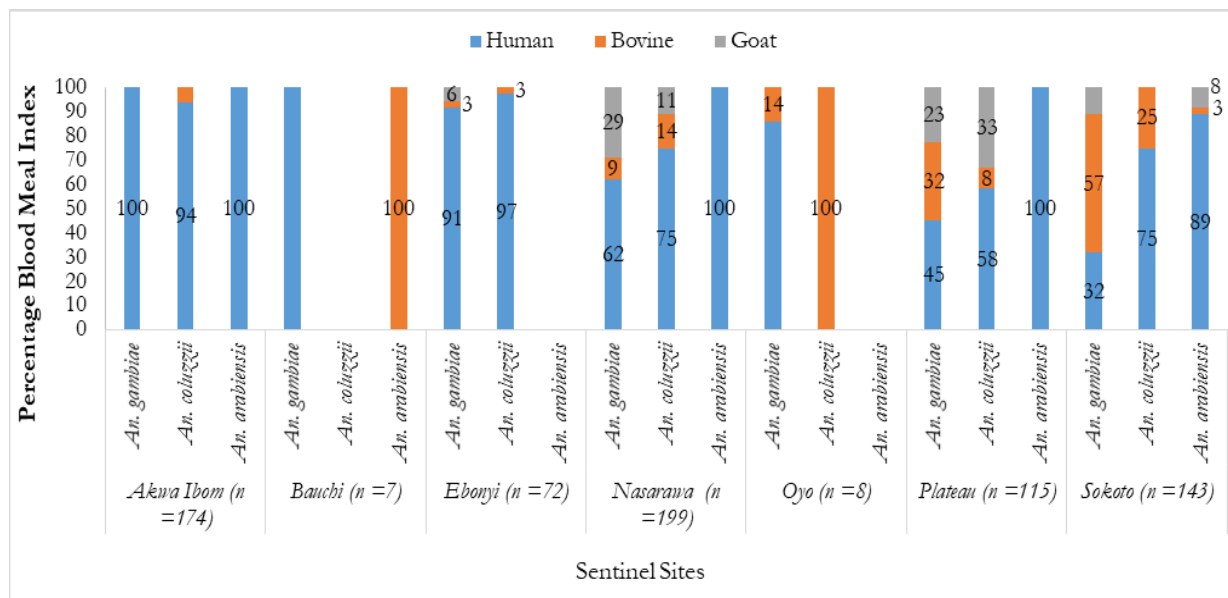


Figure 10: Blood Meal Sources of *An. gambiae*, *An. coluzzii*, and *An. arabiensis* from Outdoor CDC Light Trap Collections across Sites (November 2018 to September 2019)

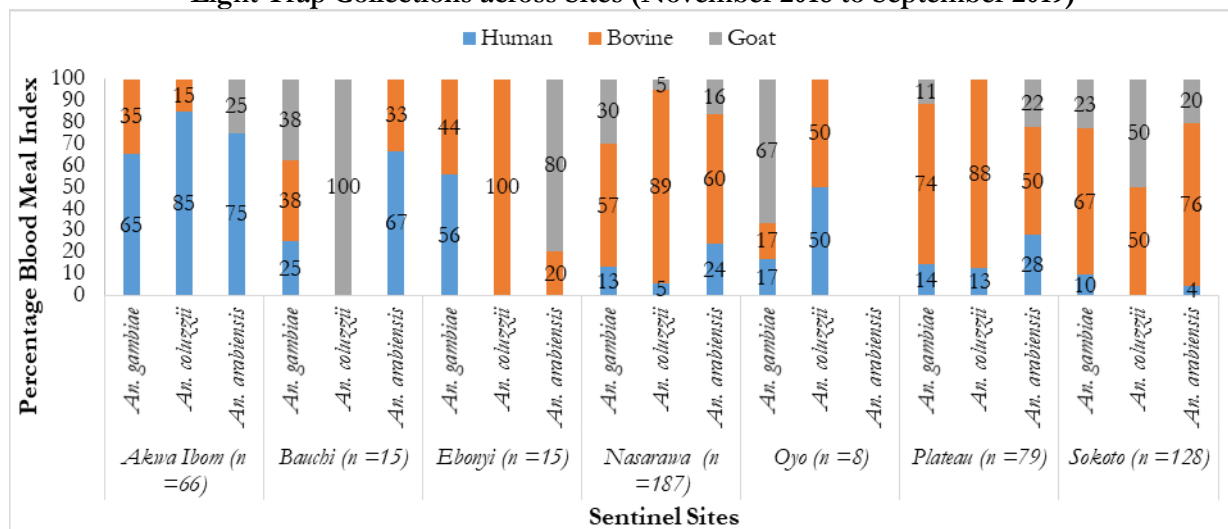
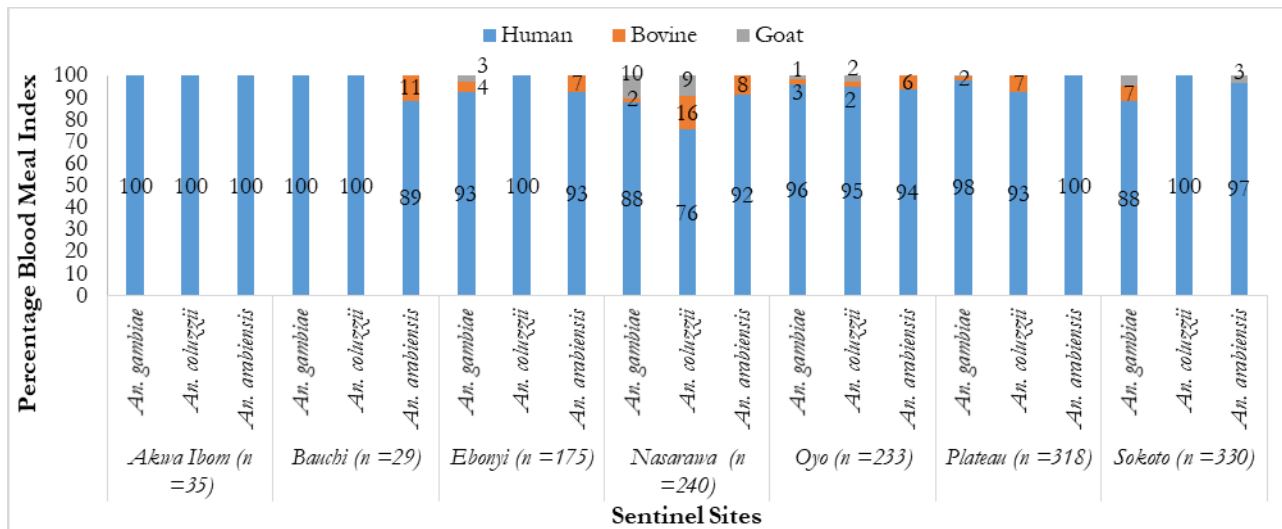


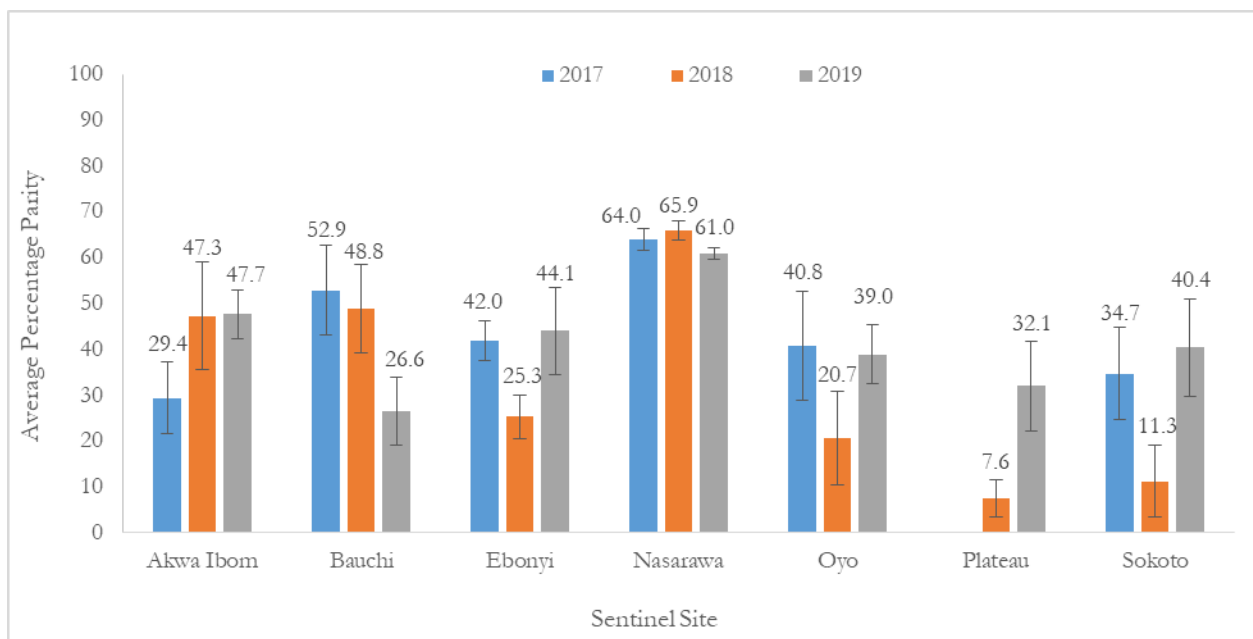
Figure 11: Blood Meal Sources of *An. gambiae*, *An. coluzzii*, and *An. arabiensis* from Pyrethrum Spray Catch Collections across Sites (November 2018 to September 2019)



2.8 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 for 2017, 2018, and 2019 were calculated and compared (Figure 12). Results from Bauchi alone indicated a consistent reduction in the average percentage number of parous mosquitoes over the years. Similar trend of reduction of average percentage parous mosquitoes in 2018 when compared to 2017 was observed in Ebonyi (25.3%), Oyo (20.7%), and Sokoto (11.3%). This increased in 2019 to 44.1%, 39.0% and 40.4% respectively. In Akwa Ibom, the average percentage of parous mosquitoes in 2018 and 2019 was higher than 2017.

Figure 12: Parity Rates of Dissected Mosquitoes in Sentinel Sites (2017-2019)



2.9 INSECTICIDE SUSCEPTIBILITY AND MECHANISMS OF RESISTANCE

CDC bottle bioassays were used to determine the susceptibility of vector populations to insecticides 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.

Pyrethroid resistance was detected in *An. gambiae* s.l. from all eight states, but patterns varied within and among states. Susceptibility to deltamethrin was recorded in *An. gambiae* s.l. populations across all six LGAs of Benue, Kebbi and five LGAs in Cross River, four LGAs in Oyo while resistance was recorded across eight LGAs in Ebonyi, six LGAs in Plateau, five LGAs in Nasarawa, and four out of six LGAs each in Akwa Ibom and Zamfara. Deltamethrin resistance was also recorded in *An. gambiae* s.l. from two LGAs in Bauchi, while possible resistance was recorded in the remaining four LGAs. Possible resistance was recorded in four LGAs in Bauchi two LGAs each in Sokoto, Zamfara, and one LGA each in Akwa Ibom, Ebonyi, Nasarawa, and Oyo. The other four LGAs recorded resistance. Out of the 69 sites tested for permethrin susceptibility in *An. gambiae* s.l., 65 indicated the presence of resistance. Susceptibility was reported in mosquitoes from two sites in Sokoto (Gudu and Wamakko), and possible resistance was observed in one LGA each in Benue (Oju) and Kebbi (Kalgo) (Table 7).

Complete susceptibility to alpha-cypermethrin was recorded in all six LGAs of Benue and in five out of six LGAs in Cross River. Four out of six LGAs in Sokoto and Kebbi also recorded susceptibility while complete resistance was recorded across all six LGAs of Nasarawa, Plateau, and Akwa Ibom. *An. gambiae* s.l. mosquitoes were susceptible to alpha-cypermethrin in five out of six LGAs of Cross River and four out of six LGAs of Sokoto and Kebbi.

An. gambiae s.l. susceptibility to pirimiphos-methyl was observed in all six LGAs in Akwa Ibom, Bauchi, Oyo, Kebbi, and Zamfara. In Ebonyi, *An. gambiae* s.l. mosquitoes in eight out of nine LGAs were susceptible to pirimiphos-methyl, while resistance detected across all six LGAs in Cross River and Plateau. Possible resistance to pirimiphos-methyl was also observed in mosquito populations in four of six LGAs in Nasarawa and two out of six LGAs in Sokoto. Out of nine LGAs in Ebonyi, only *An. gambiae* s.l. from Ebonyi LGA showed possible resistance to pirimiphos-methyl (Table 7).

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

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Deltamethrin		Permethrin		Alpha-cypermethrin		Pirimiphos-methyl	
Sentinel Site	LGA	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status
Akwa Ibom	Abak	89%	R	14%	R	65%	R	100%	S
	Ikot Ekpene	91%	PR	15%	R	66%	R	100%	S
	Itu	80%	R	24%	R	67%	R	99%	S
	Mkpat Enin	83%	R	15%	R	62%	R	100%	S
	Nsit Ubium	87%	R	12%	R	63%	R	100%	S
	Oron	90%	PR	22%	R	68%	R	100%	S
Bauchi	Bauchi	90%	PR	85%	R	92%	PR	100%	S
	Dass	81%	R	80%	R	83%	R	100%	S
	Misau	91%	PR	89%	R	87%	R	100%	S
	Ningi	93%	PR	82%	R	90%	PR	100%	S
	Shira	94%	PR	85%	R	100%	S	100%	S
	Toro	83%	R	79%	R	78%	R	100%	S
Benue	Gboko	98%	S	86%	R	98%	S	93%	PR
	Buruku	99%	S	86%	R	98%	S	93%	PR
	Kwande	98%	S	88%	R	99%	S	93%	PR
	Makurdi	98%	S	82%	R	98%	S	91%	PR
	Oju	99%	S	93%	PR	100%	S	98%	S
	Otukpo	98%	S	89%	R	99%	S	94%	PR
Cross River	Calabar Municipality	83%	R	76%	R	90%	PR	52%	R
	Ikom	100%	S	71%	R	100%	S	68%	R
	Obudu	100%	S	52%	R	100%	S	67%	R
	Odukpani	99%	S	52%	R	100%	S	60%	R
	Ogoja	100%	S	49%	R	100%	S	50%	R
	Yakur	100%	S	85%	R	100%	S	83%	R
Ebonyi	Abakaliki*	66%	R	19%	R	94%	PR	98%	S
	Ebonyi	85%	R	27%	R	59%	R	96%	PR
	Ezza North	80%	R	21%	R	88%	R	99%	S
	Ezza South	80%	R	16%	R	91%	PR	100%	S
	Ishielu*	86%	R	45%	R	95%	PR	98%	S
	Izzi	92%	PR	15%	R	96%	PR	98%	S
	Ohaozara	69%	R	13%	R	97%	PR	99%	S
	Ohaukwu	86%	R	20%	R	96%	PR	100%	S
	Onicha*	83%	R	18%	R	86%	R	100%	S

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

Note: Diagnostic time is 30 minutes for all except pirimiphos-methyl, which is 60 minutes.

A minimum of 100 mosquitoes were exposed to each of the insecticides

*Expanded PBO nets monitoring sites

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Deltamethrin		Permethrin		Alpha-cypermethrin		Pirimiphos methyl	
Sentinel Site	LGA	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status
Kebbi	Argungu	100%	S	74%	R	98%	S	100%	S
	Augie	100%	S	56%	R	96%	PR	100%	S
	Birnin Kebbi	100%	S	79%	R	77%	R	100%	S
	Bunza	100%	S	77%	R	100%	S	98%	S
	Jega	100%	S	64%	R	100%	S	100%	S
	Kalgo	100%	S	93%	PR	100%	S	100%	S
Nasarawa	Doma	85%	R	60%	R	80%	R	93%	PR
	Karu	88%	R	48%	R	78%	R	91%	PR
	Keffi	80%	R	50%	R	80%	R	77%	R
	Lafia	78%	R	73%	R	86%	R	94%	PR
	Nasarawa	74%	R	49%	R	82%	R	84%	R
	Nasarawa Eggon	94%	PR	58%	R	84%	R	96%	PR
Oyo	Afijio	97%	PR	88%	R	67%	R	100%	S
	Akinyele	100%	S	84%	R	90%	PR	100%	S
	Atiba	100%	S	77%	R	100%	S	100%	S
	Egbeda	81%	R	28%	R	86%	R	100%	S
	Ibarapa East	100%	S	66%	R	100%	S	100%	S
	Oluyole	100%	S	82%	R	100%	S	100%	S
Plateau	Langtang North	68%	R	57%	R	74%	R	69%	R
	Langtang South	58%	R	30%	R	67%	R	61%	R
	Mikang	72%	R	42%	R	76%	R	61%	R
	Quanpan	64%	R	38%	R	75%	R	68%	R
	Shendam	70%	R	26%	R	70%	R	73%	R
	Wase	69%	R	62%	R	77%	R	71%	R
Sokoto	Bodinga	90%	PR	80%	R	100%	S	83%	R
	Gudu	93%	PR	100%	S	100%	S	100%	S
	Illela	77%	R	77%	R	81%	R	90%	PR
	Rabah	81%	R	80%	R	86%	R	97%	PR
	Tambawal	80%	R	79%	R	100%	S	100%	S
	Wamakko	76%	R	100%	S	100%	S	100%	S
Zamfara	Anka	59%	R	64%	R	79%	R	100%	S
	Chafe	93%	PR	48%	R	98%	S	100%	S
	Gusau	92%	PR	54%	R	99%	S	100%	S
	Kaura								
	Namoda	68%	R	70%	R	79%	R	100%	S
	Maru	74%	R	75%	R	83%	R	100%	S
Talata Mafara	85%	R	0%	R	75%	R	100%	S	

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

Note: Diagnostic time is 30 minutes for all except pirimiphos-methyl, which is 60 minutes.

A minimum of 100 mosquitoes were exposed to each of the insecticides

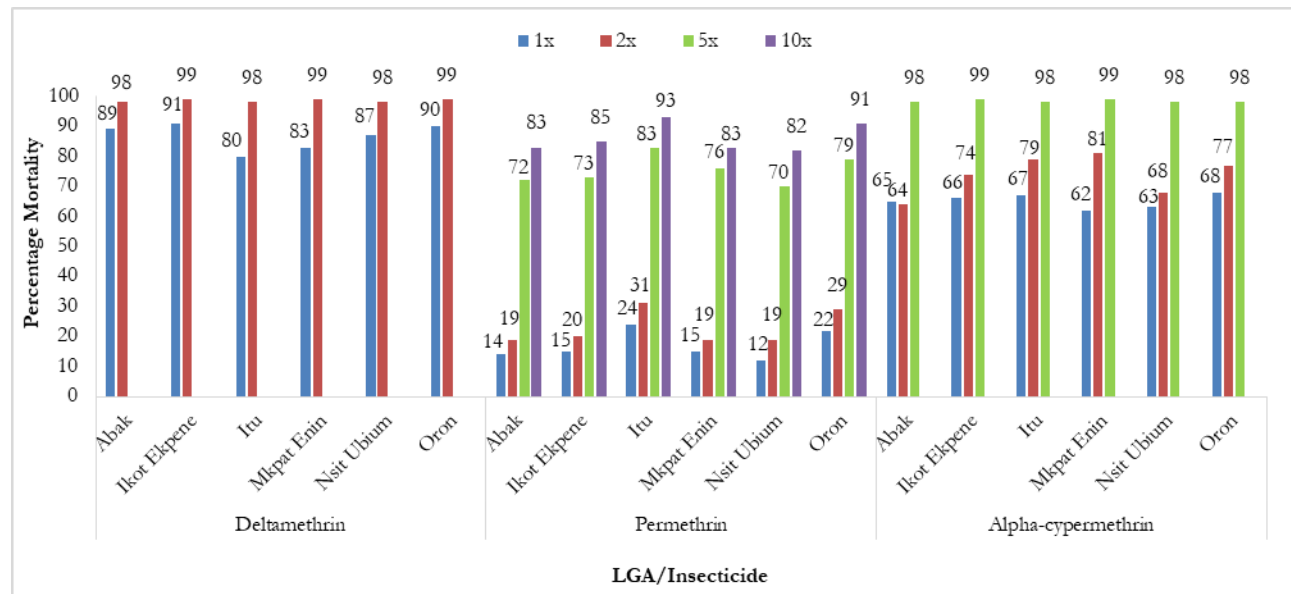
2.10 INSECTICIDE RESISTANCE INTENSITY

Insecticide resistance intensity assays were carried out for the three pyrethroids across the different ecozones. Resistance patterns of *An. gambiae* to deltamethrin at 1X was similar across all LGAs in Akwa-Ibom, Bauchi, Nasarawa, Sokoto, and Zamfara. Mosquito populations across these sites were resistant to deltamethrin at 1X but were susceptible to the 2X dose assays. In two LGAs in Oyo (Afijio and Egbeda) and one LGA in Cross River (Calabar Municipality), 100% mosquito mortality was recorded at exposure to deltamethrin at 2X.

Low-intensity deltamethrin resistance (mortality between 98-100% at 5X dose) was recorded in *An. gambiae* s.l. populations from five LGAs in Ebonyi (Ezza South, Ishielu¹, Izzi, Ohaozara, and Onicha¹), three LGAs in Nasarawa (Karu, Nasarawa, and Nasarawa Eggon), and all six LGAs in Plateau (Figures 17, 19, and 21). Moderate deltamethrin resistance intensity (mortality less than 98% at 5X dose) was observed only in Ishielu¹ LGA in Ebonyi. In Plateau, low resistance intensity was observed in *An. gambiae* s.l. populations exposed to permethrin. Other outcomes showed that high permethrin resistance intensity (less than 98% mortality at 10X dosage) exists in mosquito populations from Makurdi LGA (Benue) and all LGAs in Akwa Ibom and Ebonyi (Figures 13, 15, and 17).

For alpha-cypermethrin, moderate resistance intensity was observed in *An. gambiae* s.l. tested from every LGA in Akwa Ibom, Nasarawa, and Plateau (Figures 13, 19, and 21). *An. gambiae* s.l. mosquitoes in most sites in Bauchi, Ebonyi, Nasarawa, and Zamfara (Figures 14, 17, 19, and 23) became susceptible to alpha-cypermethrin insecticides at 2X dose. Mosquitoes in only two LGAs in Kebbi and Sokoto and three LGAs in Oyo (Figures 18, 20, and 22) were susceptible to alpha-cypermethrin at 2X dose intensity assays. Evidence of the vector's susceptibility to alpha-cypermethrin at the diagnostic dose (1X) was observed in all LGAs in Benue, five LGAs in Cross River, four LGAs in both Kebbi and Sokoto, three LGAs in Oyo, and two LGAs in Zamfara (Figures 15, 16, 18, 20, 22, and 23).

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



¹ Additional sites selected for the PBO net monitoring activities.

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

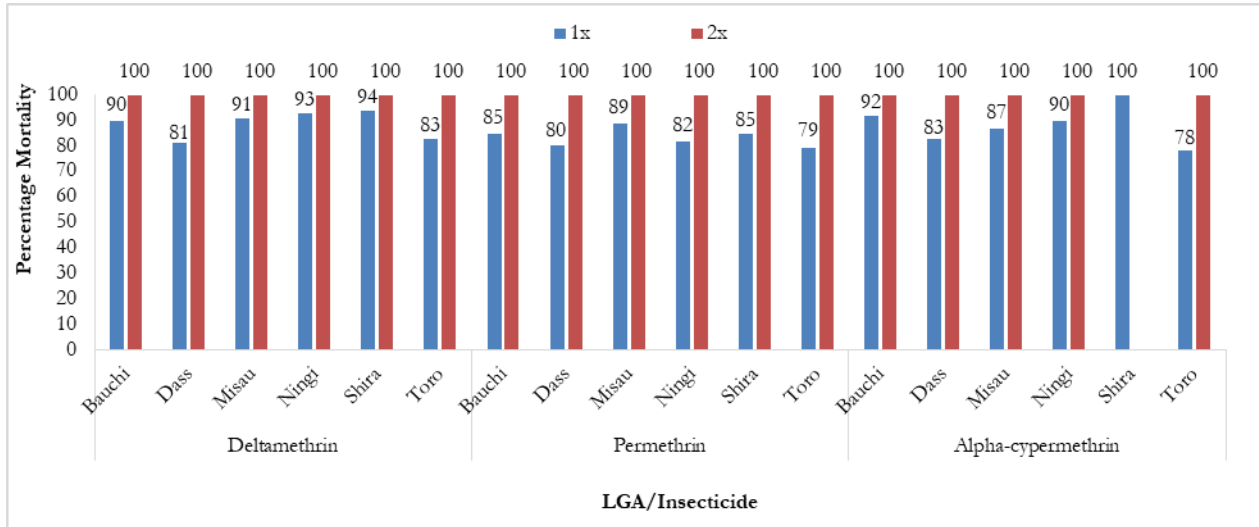


Figure 15: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Benue

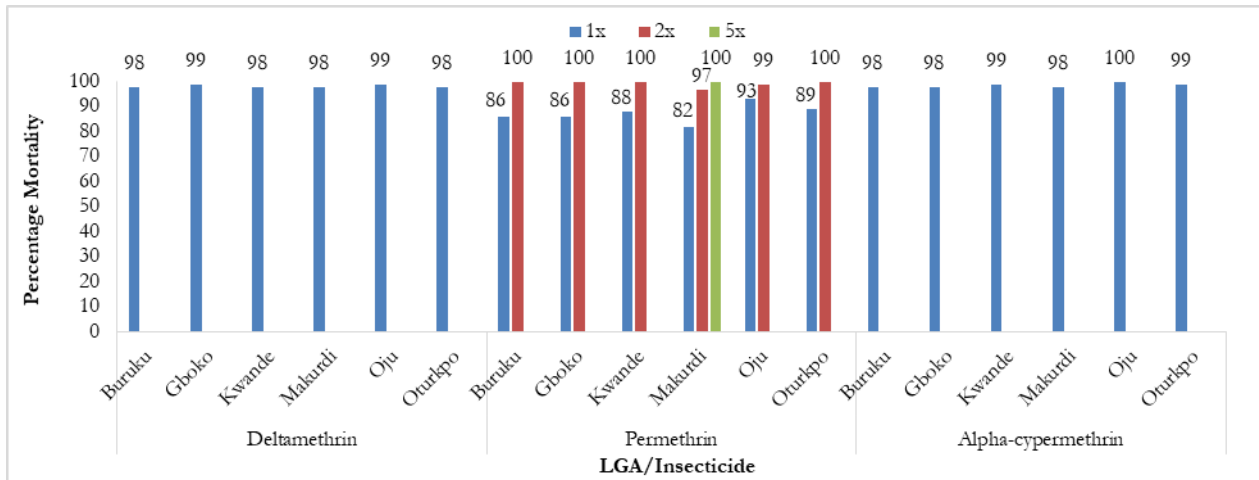


Figure 16: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Cross River

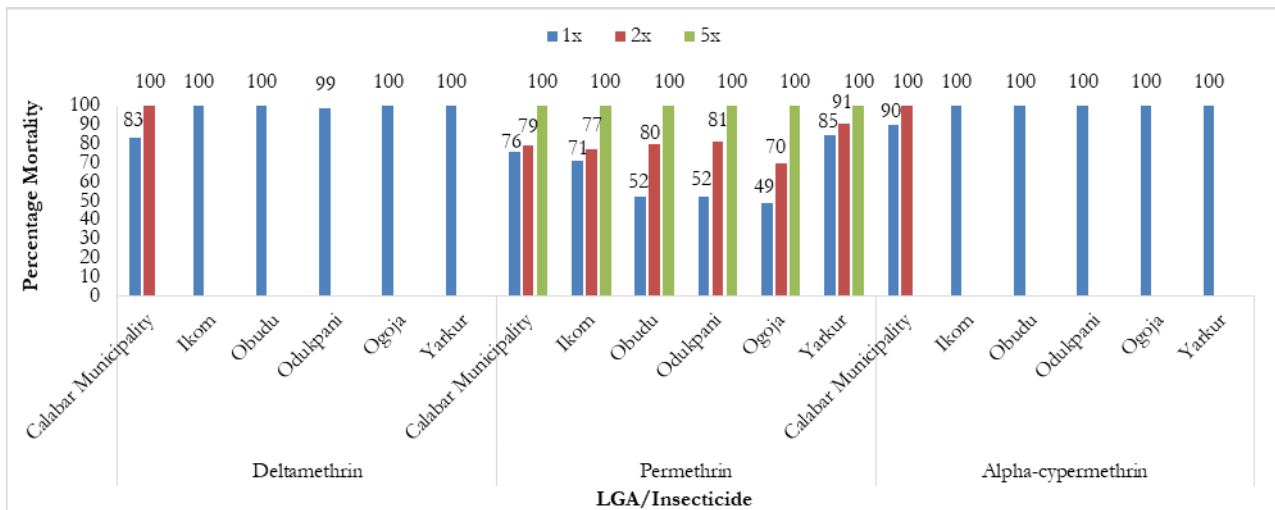


Figure 17: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Ebonyi

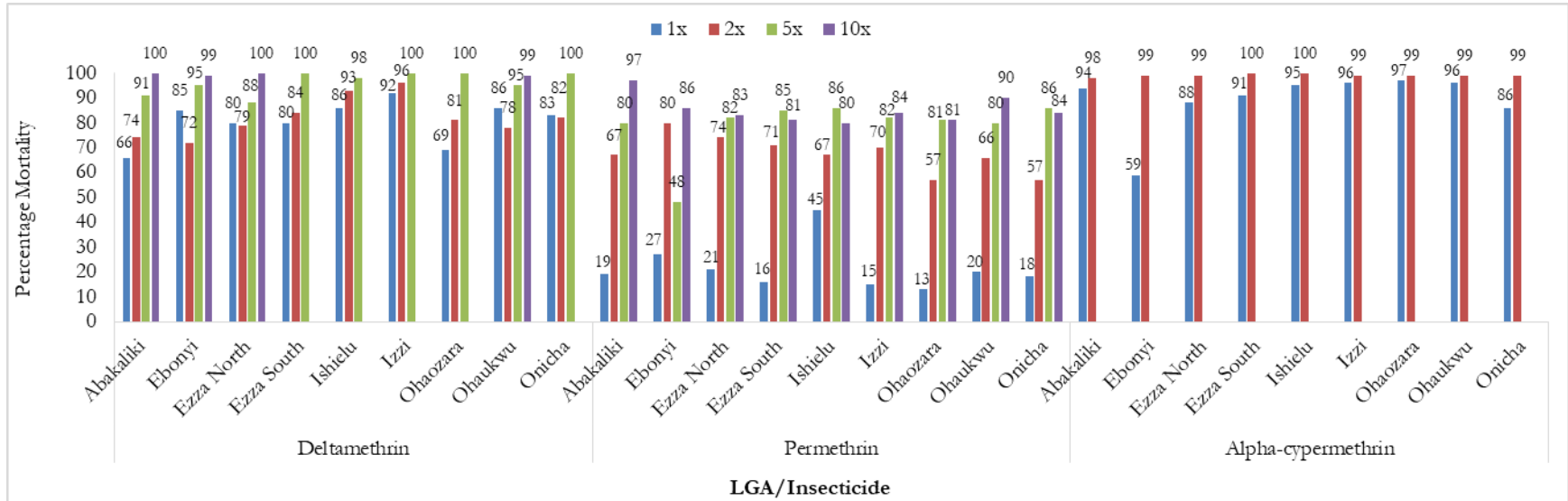


Figure 18: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Kebbi

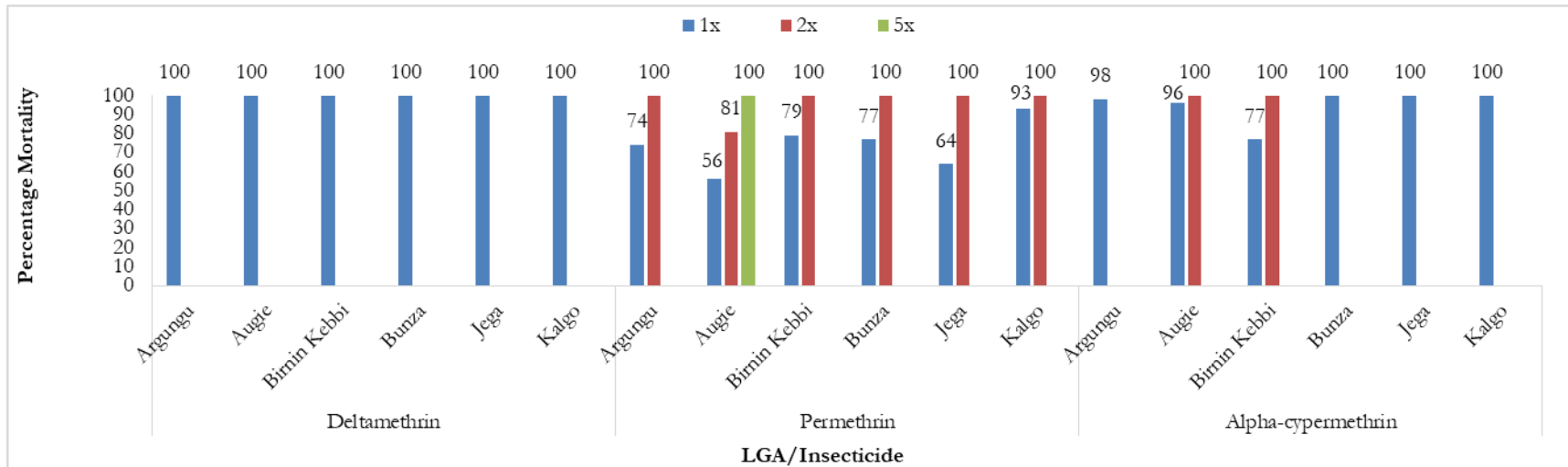


Figure 19: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Nasarawa

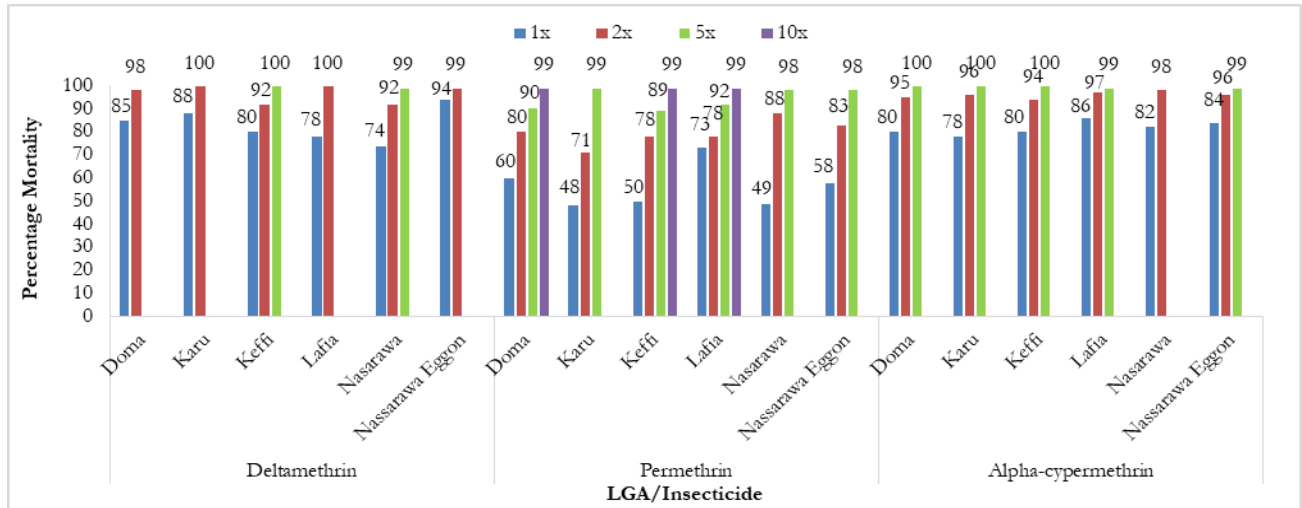


Figure 20: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Oyo

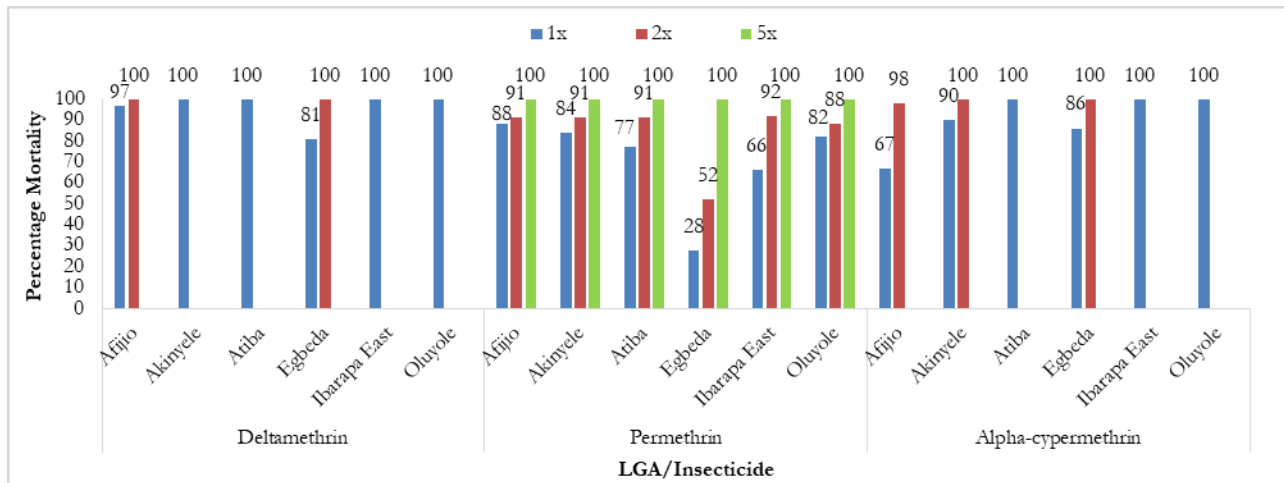


Figure 21: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Plateau

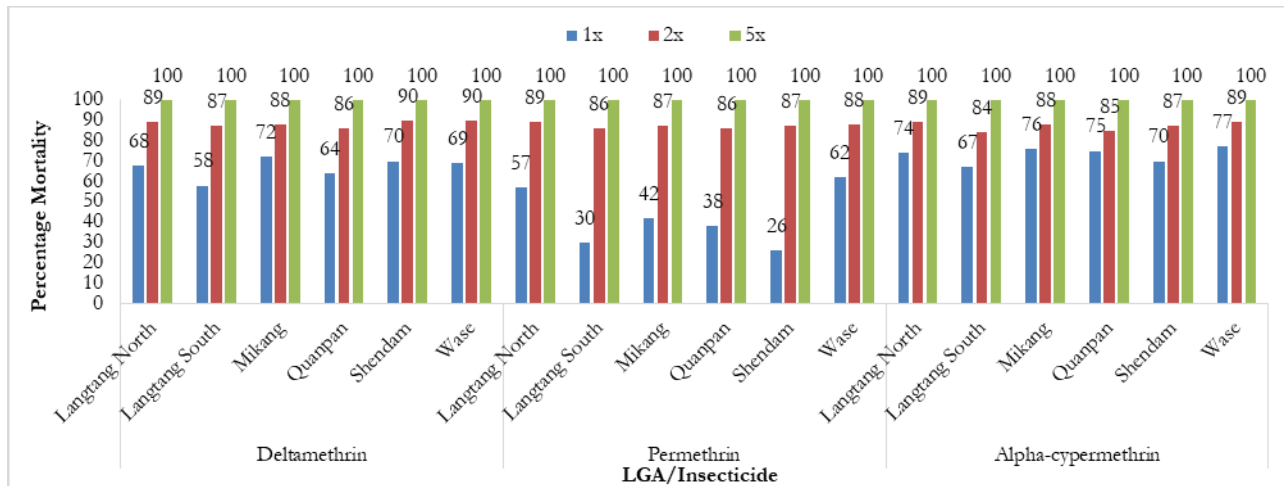


Figure 22: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Sokoto

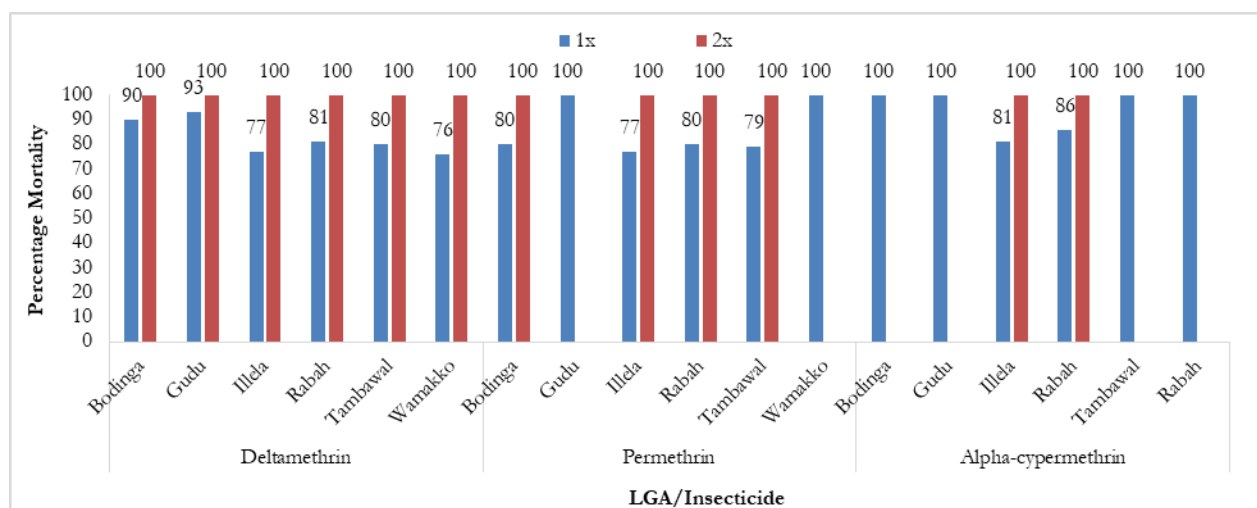
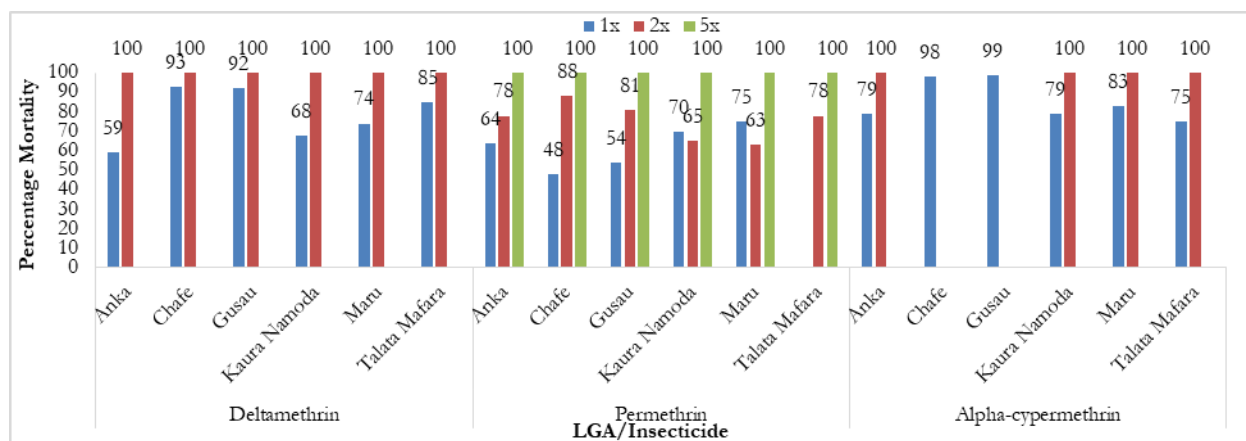


Figure 23: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Zamfara



2.11 SYNERGIST ASSAYS

Pre-exposure of *An. gambiae* s.l. mosquitoes to the synergist PBO before exposure to pyrethroids (deltamethrin, alpha-cypermethrin, and permethrin) increased mortality to varying degrees across all sentinel sites (Figures 24-36). In most cases, full susceptibility (mortality greater than or equal to 98%) was not restored with PBO exposure, suggesting the existence of mechanisms unrelated to the activity of mixed function oxidases.

Full susceptibility was restored in *An. gambiae* s.l. populations exposed to deltamethrin and PBO in Akwa Ibom, Ebonyi, Oyo, Sokoto, and Zamfara. In contrast, *An. gambiae* s.l. populations from Bauchi and Nasarawa only restored full susceptibility in three LGAs (Figures 24, 28, 30, 33, 34, and 36).

PBO did not fully restore permethrin susceptibility in *An. gambiae* populations in most of the LGAs in six states (Akwa Ibom, Bauchi, Benue, Cross River, Nasarawa, and Plateau) (Figures 24, 25, 26, 27, 32, 34). Full susceptibility to permethrin was restored in *An. gambiae* s.l. mosquitoes across all LGAs in Kebbi, Oyo, and Sokoto (Figures 30, 32, and 34). Out of the 10 states that conducted alpha-cypermethrin synergist assays, full susceptibility was only recorded in *An. gambiae* s.l. in Cross River (tested in one LGA), Ebonyi, Oyo, Sokoto, and Zamfara (Figures 29, 32, 34, and 35), while partial restoration of alpha-cypermethrin susceptibility was recorded in Akwa Ibom, Bauchi, Benue, Kebbi, Plateau, and Nasarawa (Figures 23, 24, 25, 30, 31, and 33).

Figure 24: Synergist Bottle Assay Results for *An. gambiae* s.l. from Akwa Ibom

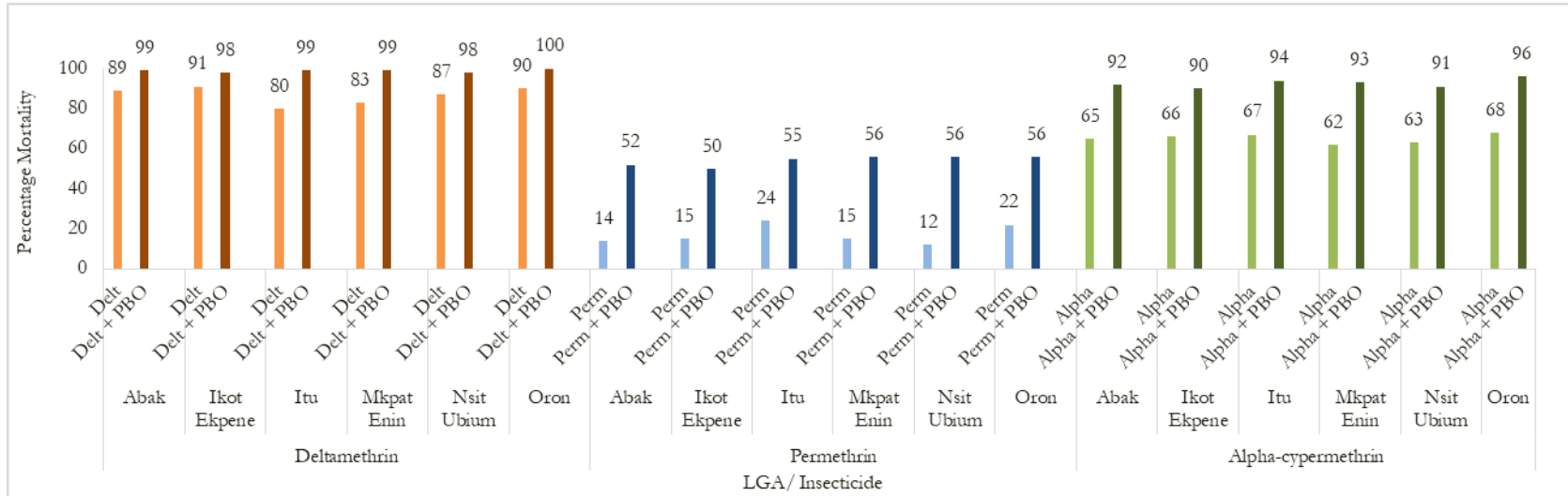


Figure 25: Synergist Bottle Assay Results for *An. gambiae* s.l. at Bauchi

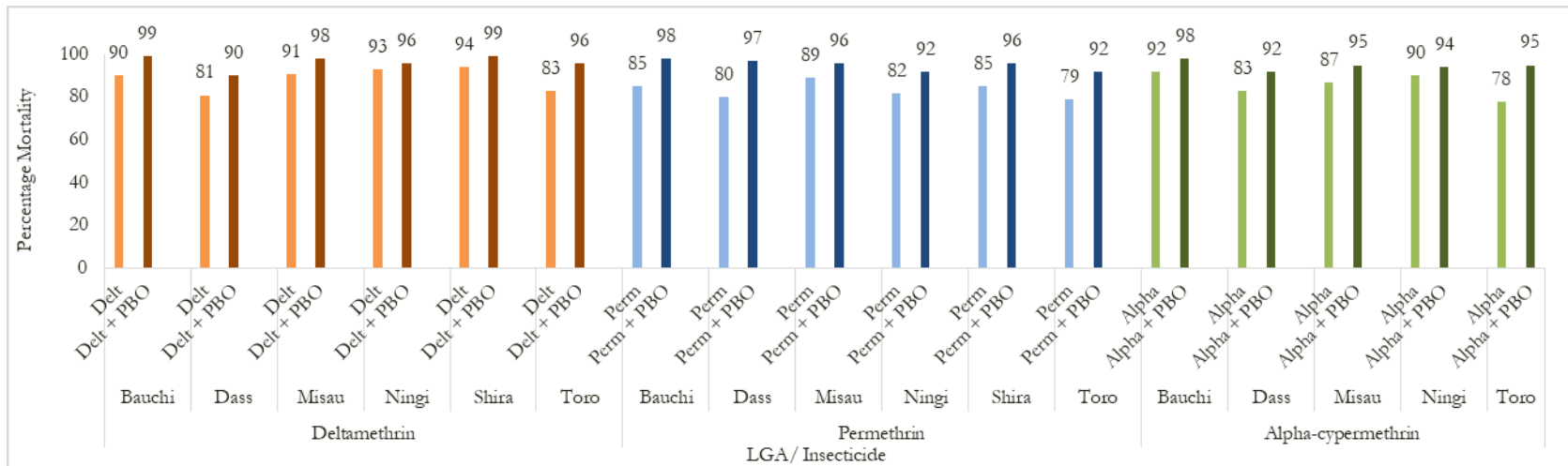


Figure 26: Synergist Bottle Assay Results for *An. gambiae* s.l. at Benue

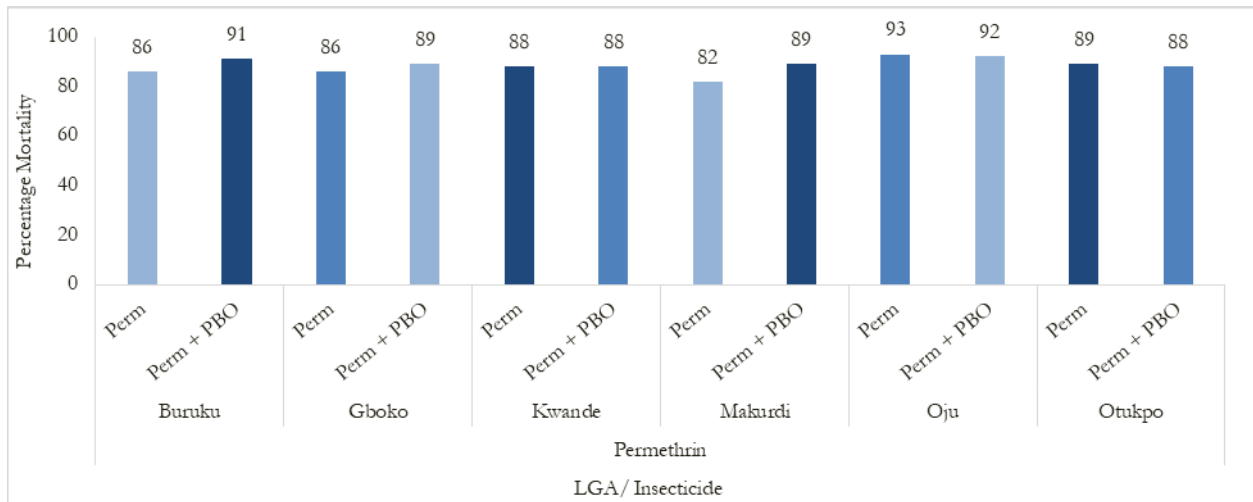


Figure 27: Synergist Bottle Assay Results for *An. gambiae* s.l. at Cross River

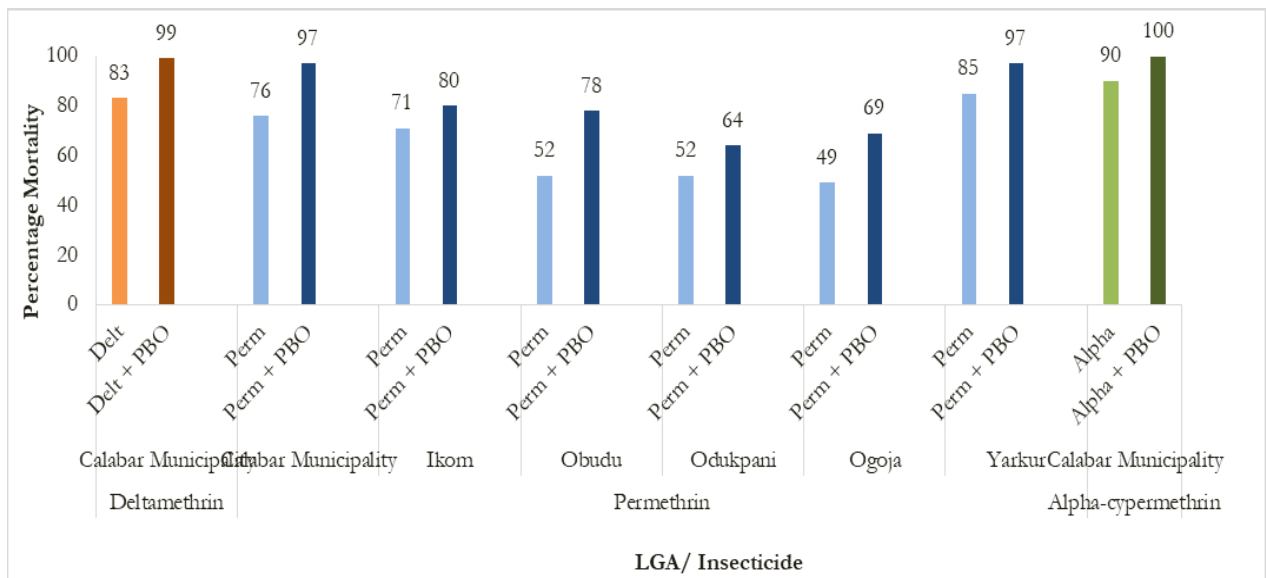


Figure 28: Deltamethrin Synergist Bottle Assay Results for *An. gambiae* s.l. at Ebonyi

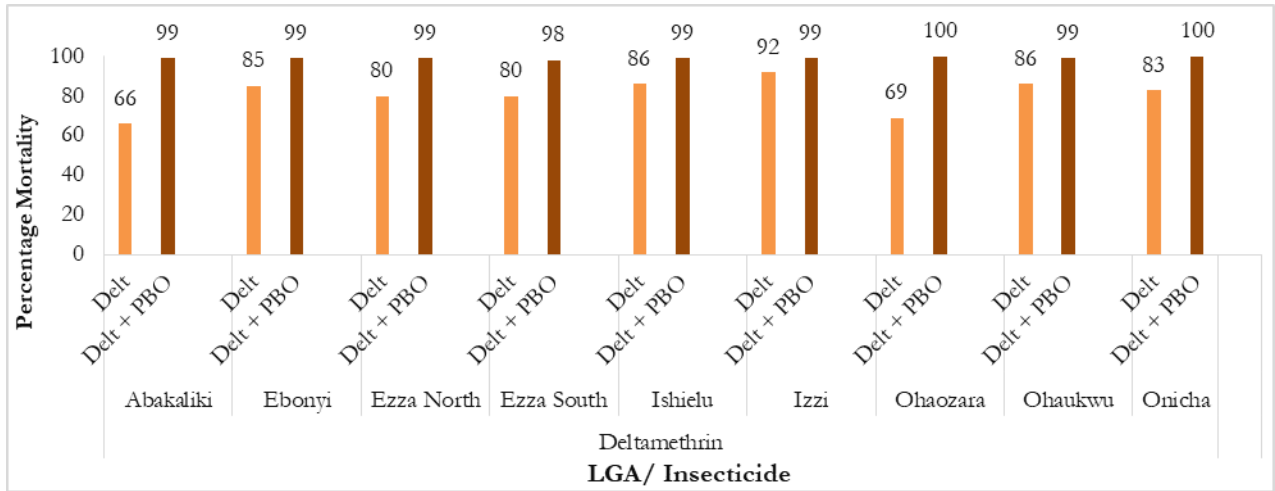


Figure 29: Permethrin Synergist Bottle Assay Results for *An. gambiae* s.l. at Ebonyi

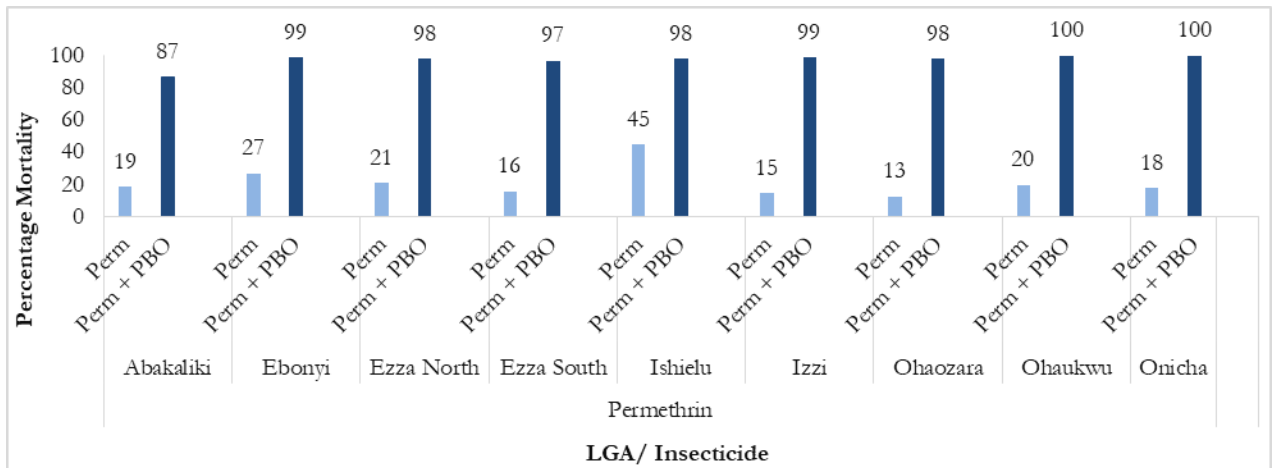


Figure 30: Alpha-cypermethrin Synergist Bottle Assay Results for *An. gambiae* s.l. at Ebonyi

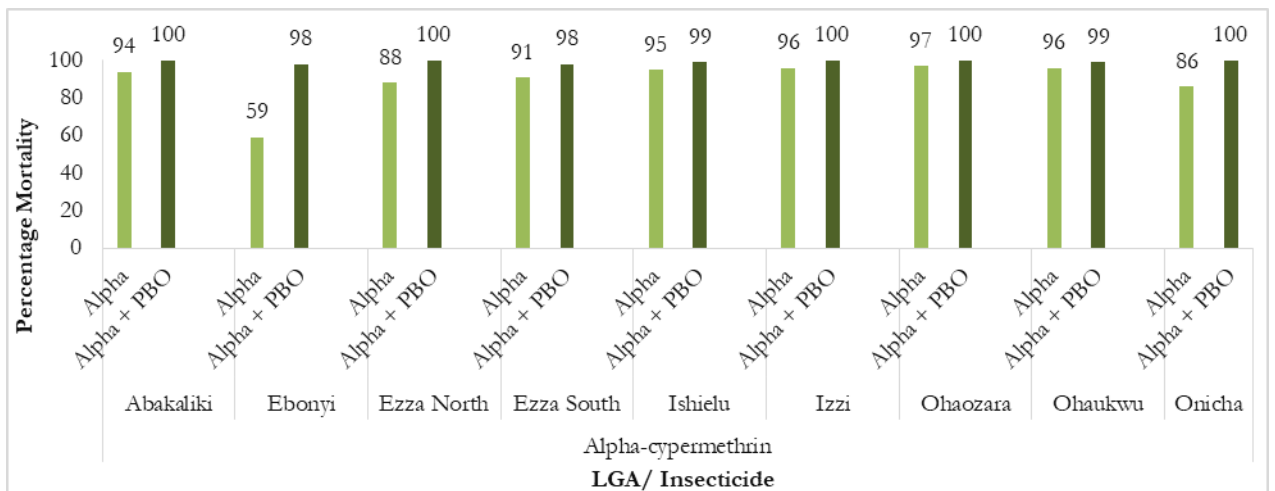


Figure 31: Synergist Bottle Assay Results for *An. gambiae* s.l. at Kebbi

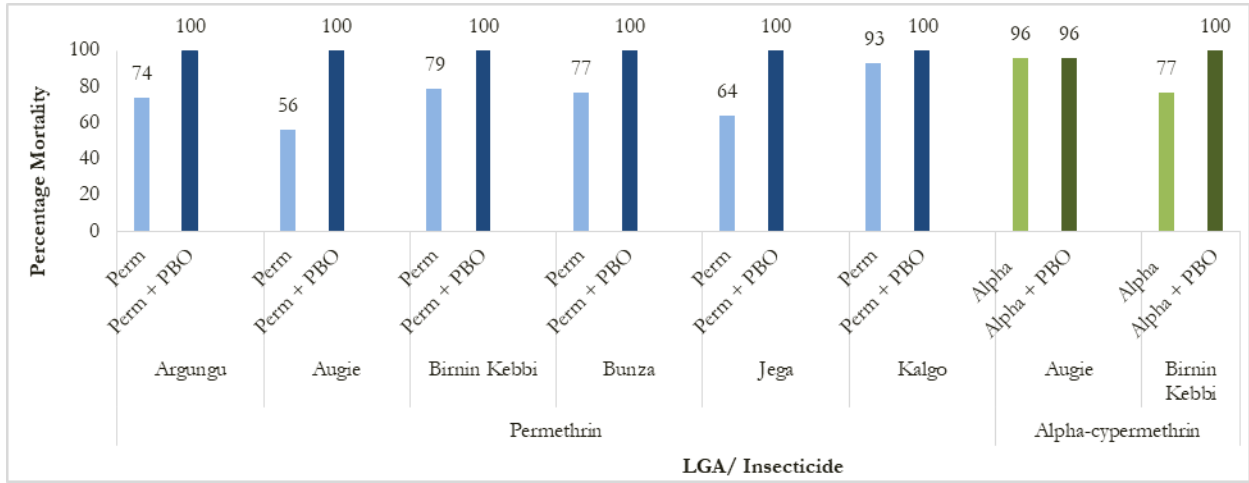


Figure 32: Synergist Bottle Assay Results for *An. gambiae* s.l. at Nasarawa

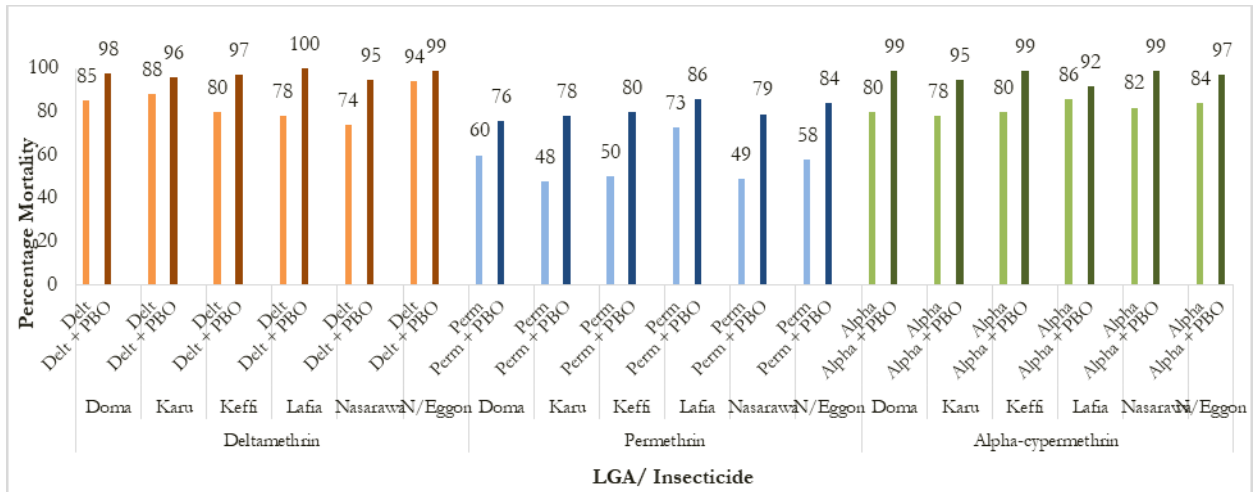


Figure 33: Synergist Bottle Assay Results for *An. gambiae* s.l. at Oyo

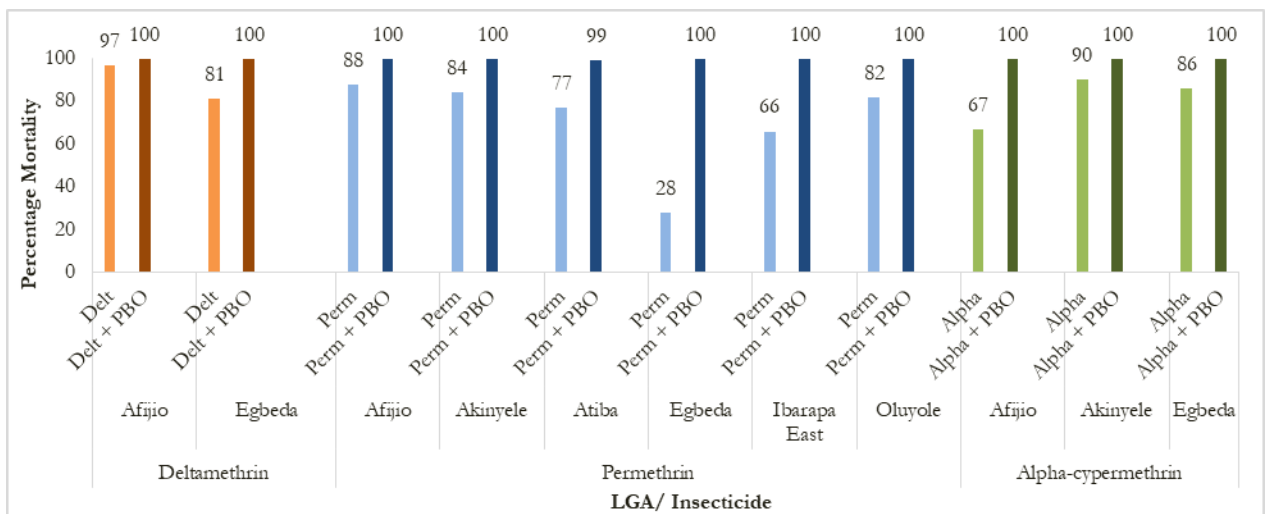


Figure 34: Synergist Bottle Assay Results for *An. gambiae* s.l. at Plateau

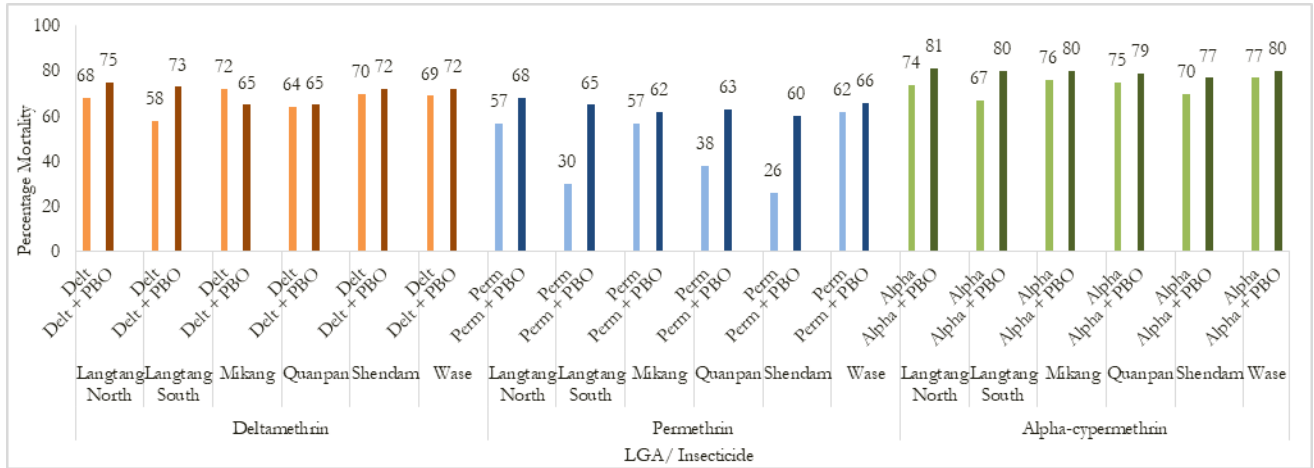


Figure 35: Synergist Bottle Assay Results for *An. gambiae* s.l. at Sokoto

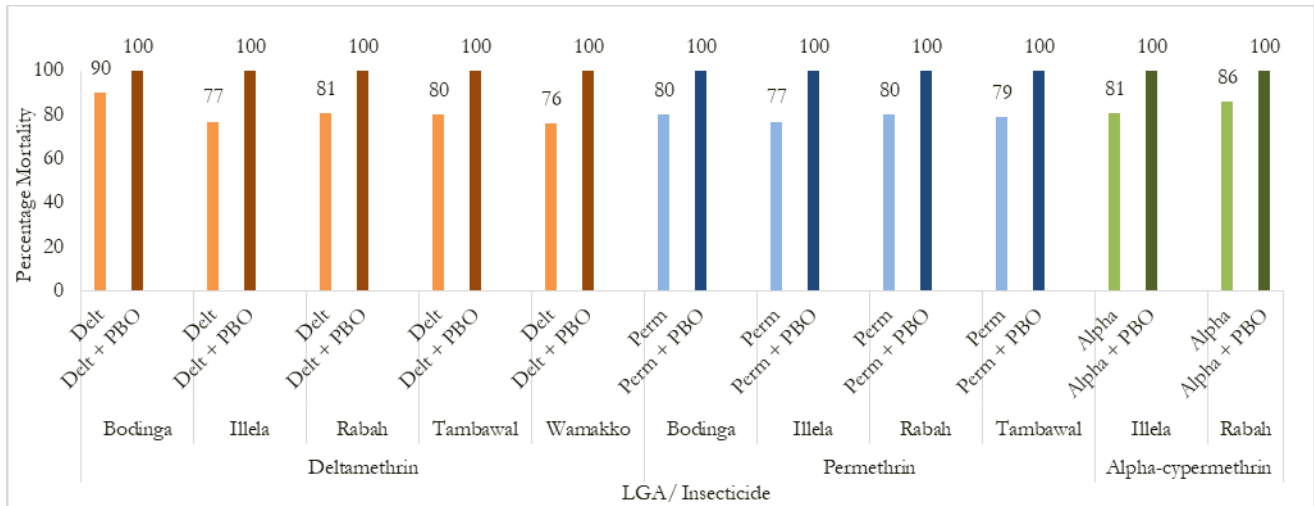
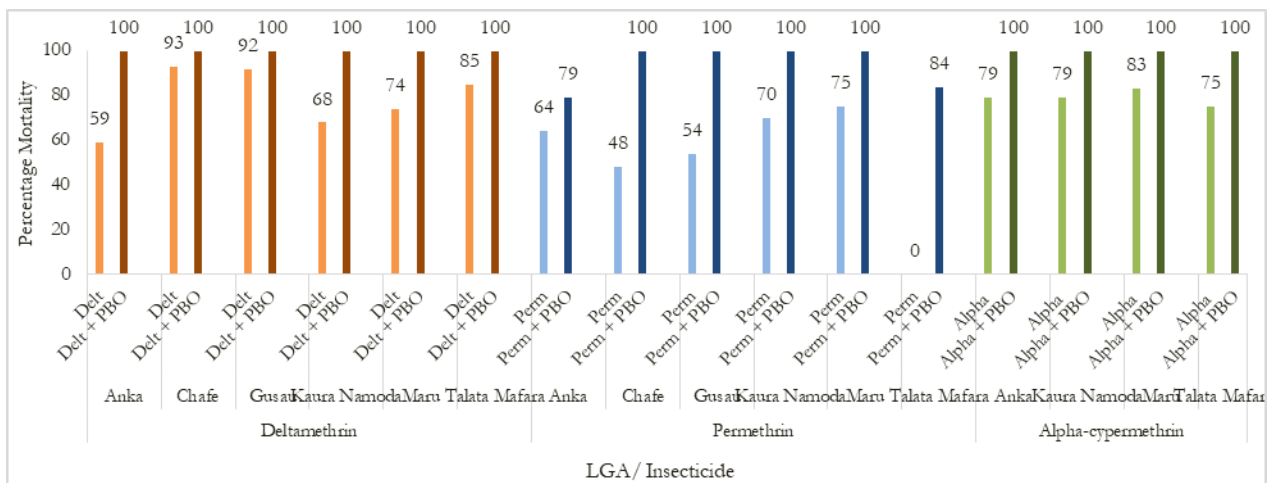


Figure 36: Synergist Bottle Assay Results for *An. gambiae* s.l. at Zamfara



2.12 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 (62-71%), Bauchi (64-89%), Benue (49-73%), Cross River (26-72%), Ebonyi (0-74%), Kebbi (43-94%), Nasarawa (27-73%), Oyo (13-63%), Plateau (42-54%), Sokoto (76-94%), and Zamfara (27-45%). The percentage mortality after the 24-hour holding period also varied in Akwa Ibom (70-77%), Bauchi (91-97%), Benue (100%), Cross River (64-99%), Ebonyi (88-100%), Kebbi (75-100%), Nasarawa (65-98%), Oyo (100%), Plateau (98-100%), Sokoto (89-100%) and Zamfara (100%) (Figures 37-47).

Mortality rates in *An. gambiae* s.l. were between 98-100% after the 24-hour holding period in all LGAs in Benue, Oyo, Plateau, and Zamfara (Figures 39, 44, 45, and 47) and after 48 hours in Bauchi, Cross River (except in Calabar municipality), Ebonyi (except in Abakaliki), Kebbi, and Nasarawa (Figures 38, 40, 41, 42, and 43). *An. gambiae* s.l. populations from all LGAs in Plateau and Sokoto showed 100% mortality at 72 hours (Figures 45 and 46). In all LGAs of Akwa Ibom, mortality rates of *An. gambiae* s.l. populations exposed to chlorfenapyr (100 µg/bottle) did not exceed 80% after 72 hours (Figure 37). The team will repeat the test at 100 µg/bottle and also conduct the assay at a higher dose of 200 µg/bottle (per the standard protocol) with the susceptible colony and *An. gambiae* s.l. collected during the 2020 rainy season when larvae are prevalent.

Figure 37: 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

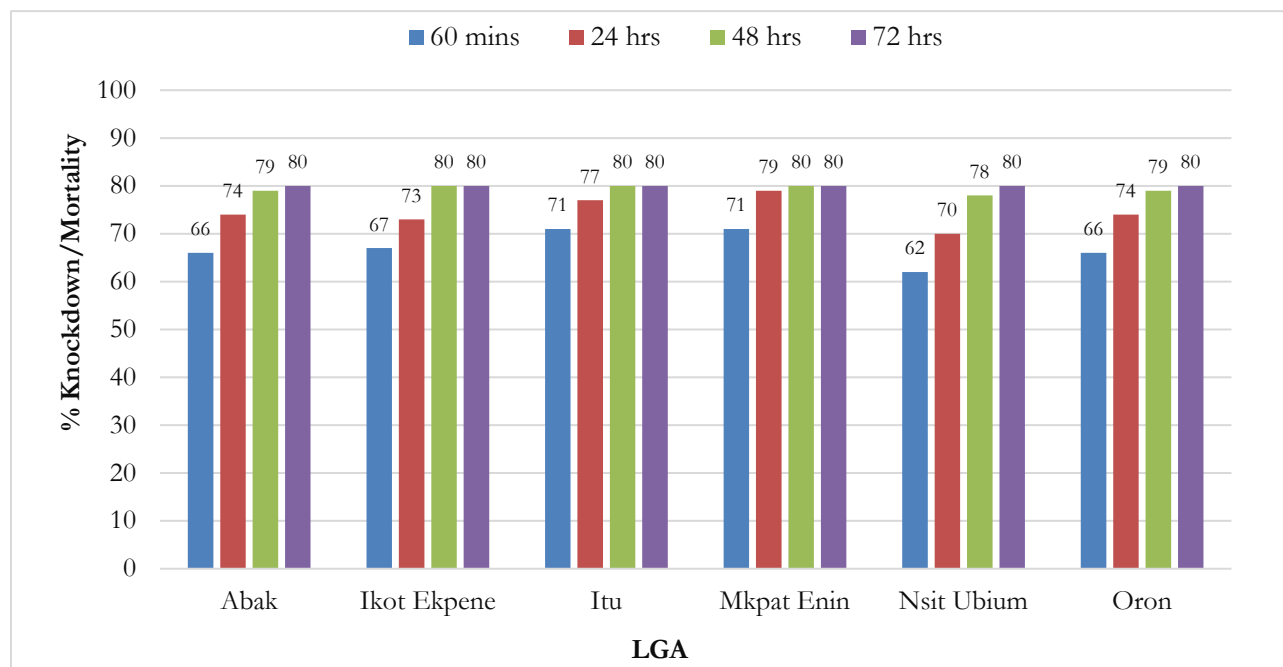


Figure 38: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Bauchi

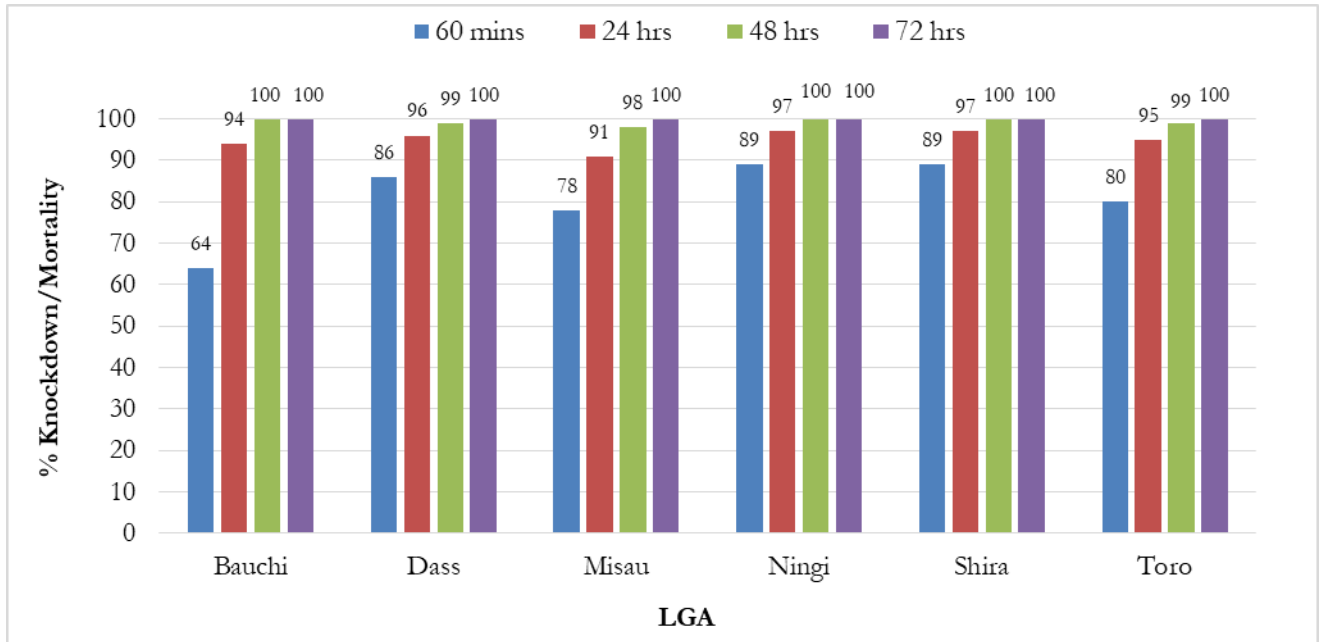


Figure 39: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Benue

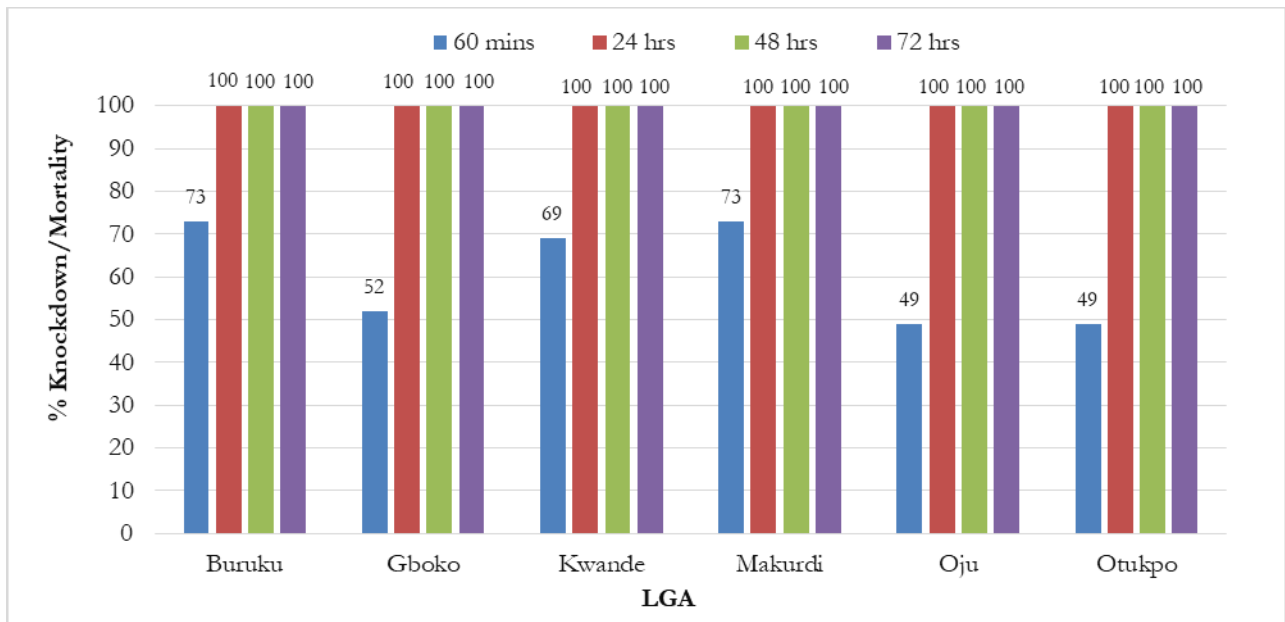


Figure 40: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Cross River

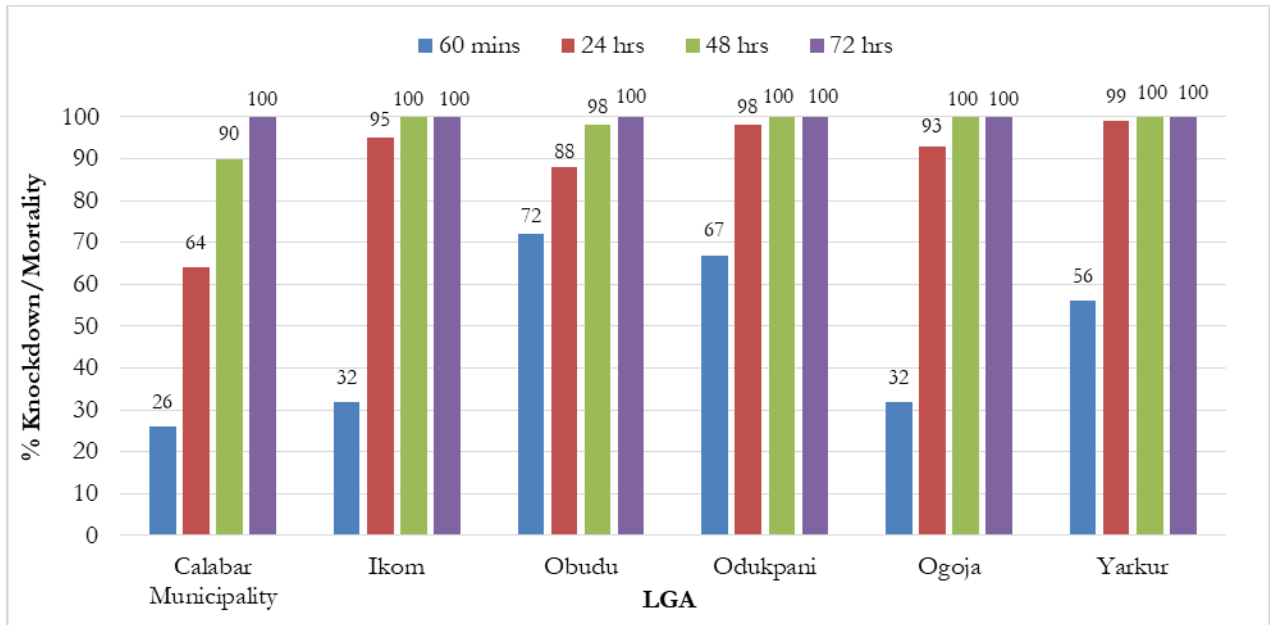


Figure 41: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Ebonyi

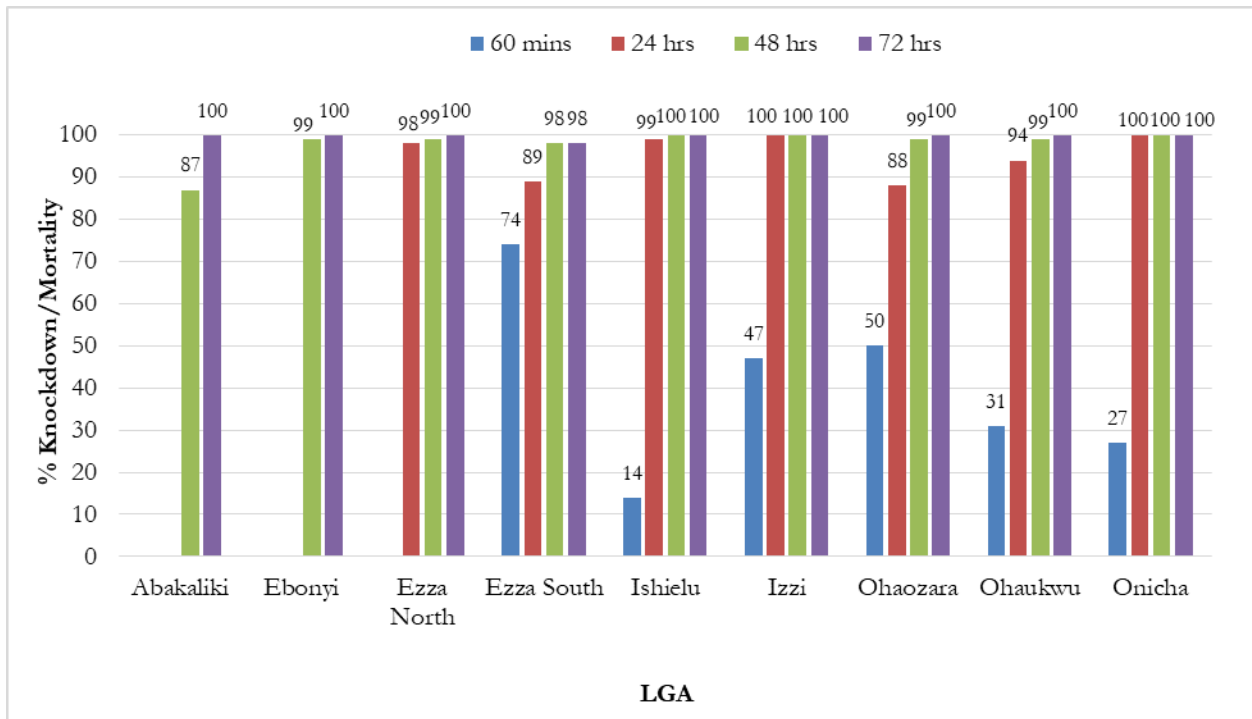


Figure 42: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Kebbi

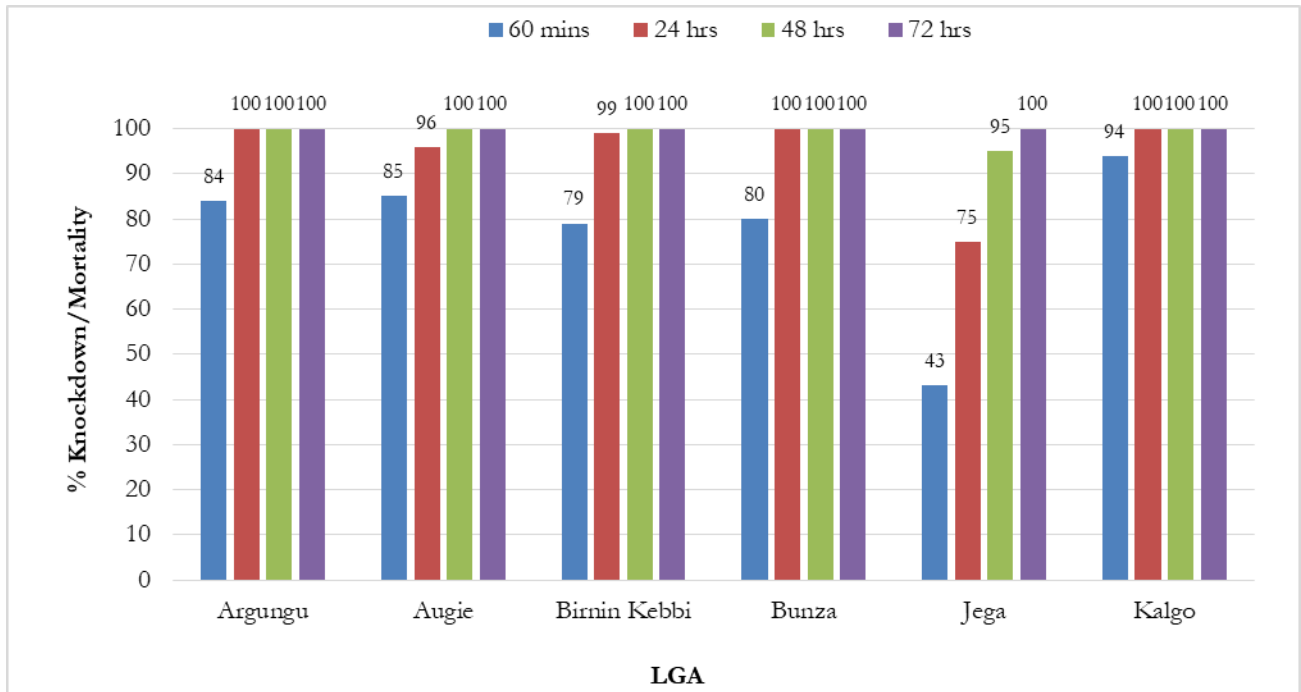


Figure 43: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Nasarawa

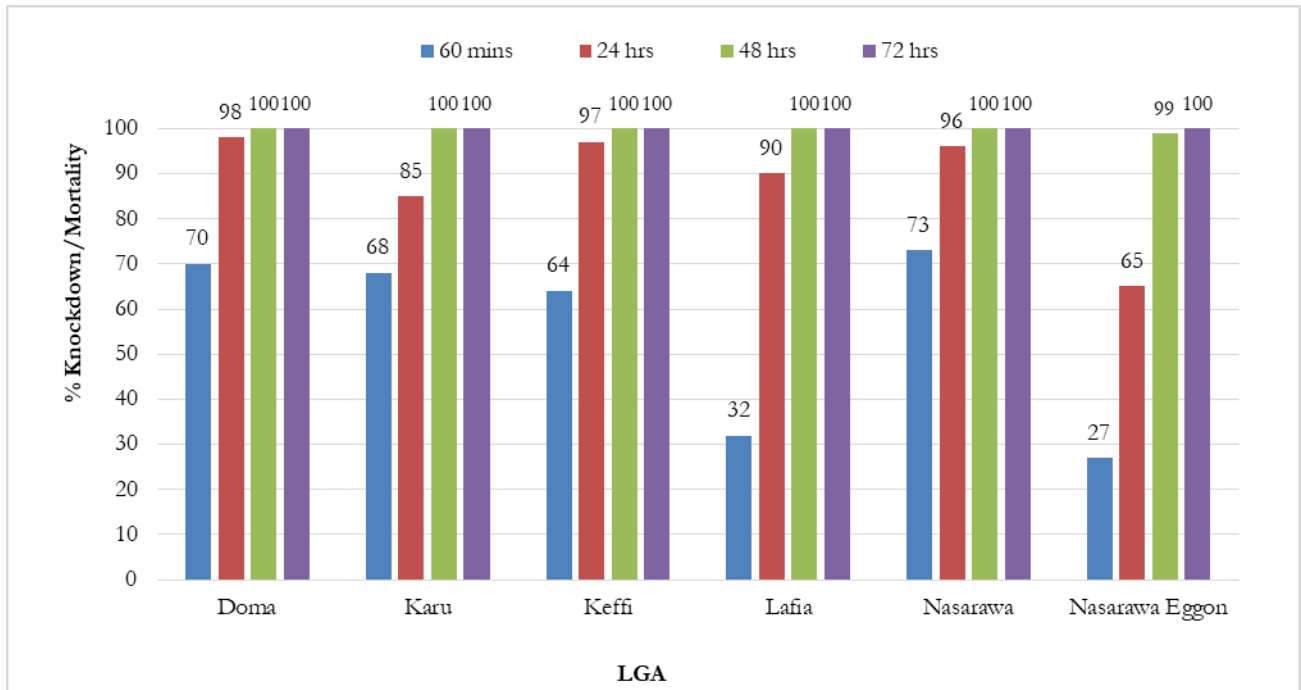


Figure 44: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Oyo

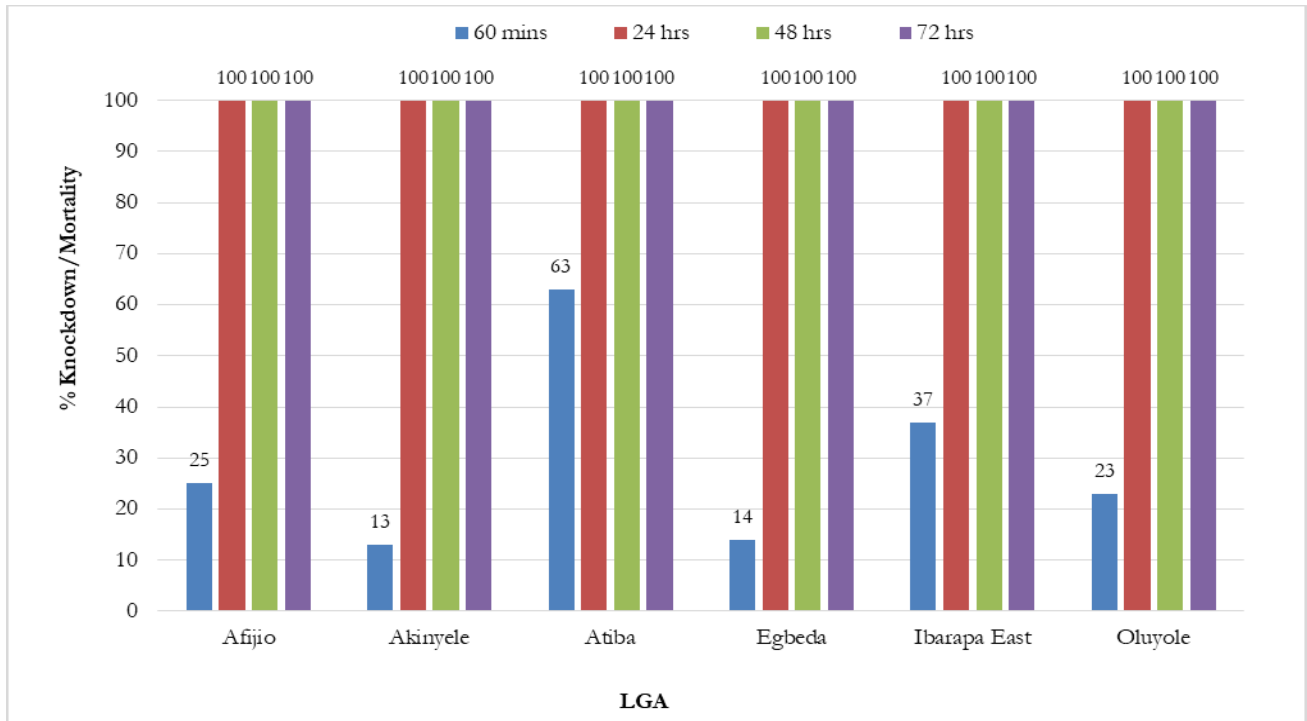


Figure 45: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) in Plateau

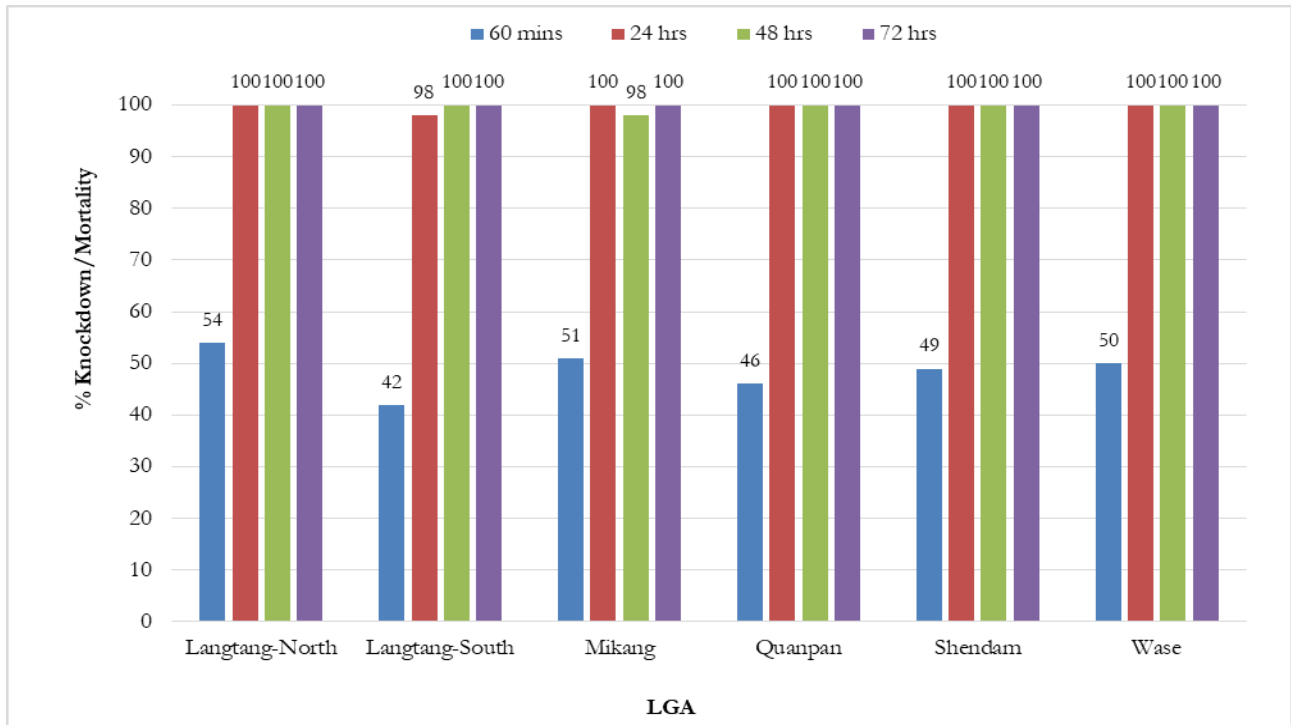


Figure 46: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Sokoto

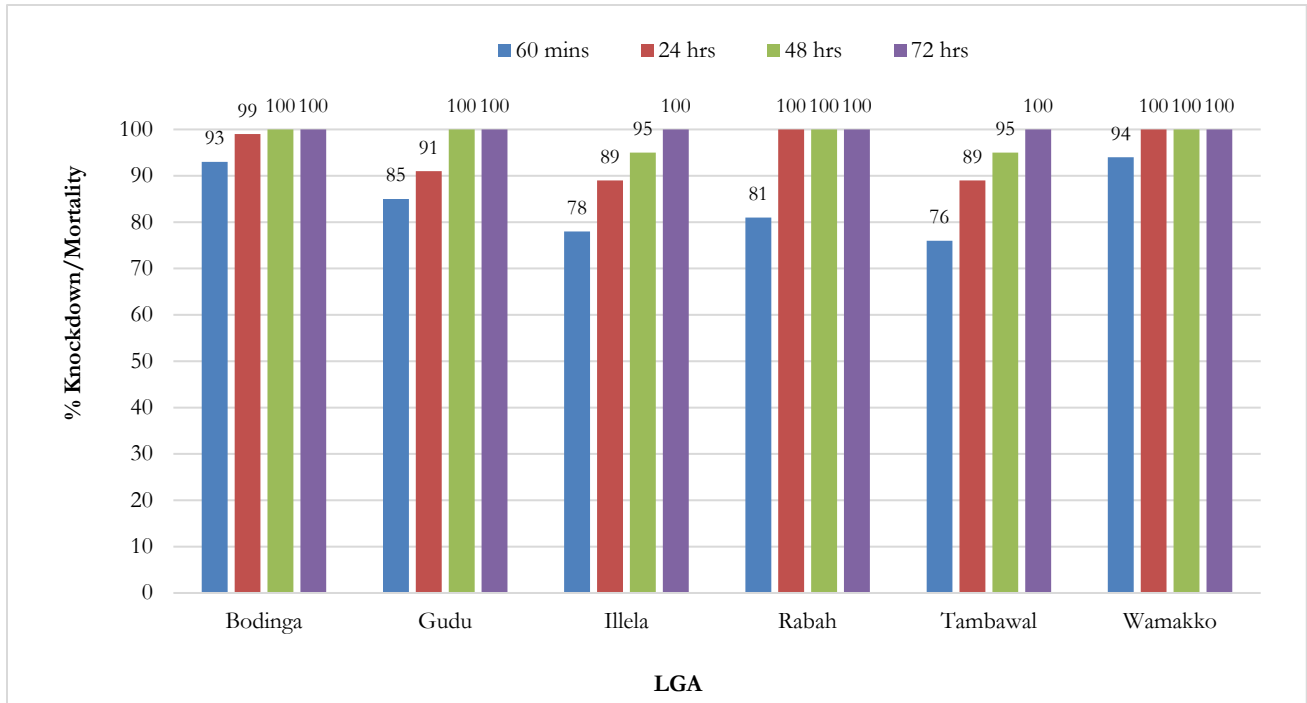
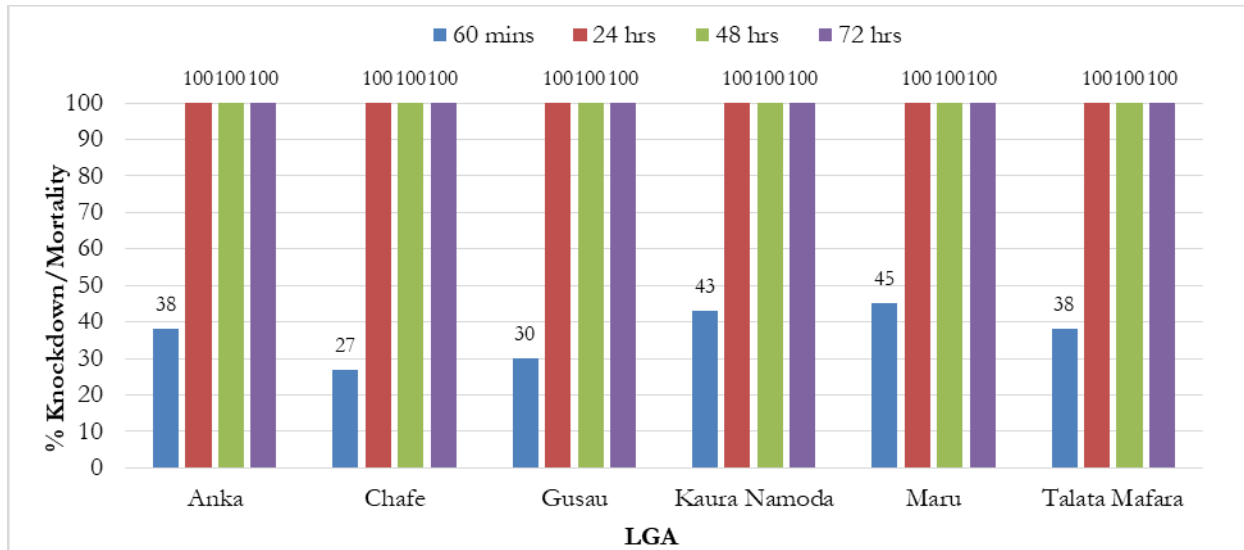


Figure 47: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Zamfara



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

2.13 DETERMINATION OF SUSCEPTIBILITY STATUS OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN USING WHO TUBE TEST

The percentage knockdown of *An. gambiae* s.l. mosquitoes at 60-minutes exposure to clothianidin varied across the sites in Akwa Ibom (19-28%), Bauchi (65-74%), Benue (3-21%), Cross River (33-86%), Ebonyi (6-18%), Kebbi (20-49%), Nasarawa (54-78%), Oyo (5-25%), Plateau (99-100%), Sokoto (3-39%), and Zamfara (50-

75%). Compared to other sites where lower knockdown rates were observed, *An. gambiae* s.l. populations from Plateau showed 100% knockdown after just 60 minutes of exposure. After 24 hours (Day 1), susceptibility to clothianidin (98–100% mortality) was observed in *An. gambiae* s.l. in one LGA each in Benue (Gboko) and Nasarawa (Doma), and two LGAs each in Ebonyi (Ishielu and Izzi) and Oyo (Afijio and Ibarapa East). At Day 2, Day 3, Day 4, Day 5, and Day 6, mosquitoes in all LGAs across four, seven, eight, ten and eleven states, respectively, were susceptible to clothianidin (Table 8).

Table 8: WHO Tube Test Results (Percent Mortality after 7 days) for *An. gambiae* s.l.

Sentinel Site	LGA	Test Time							
		60 mins	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Akwa Ibom	Abak	19	81	100	100	100	100	100	100
	Ikot Ekpene	24	89	100	100	100	100	100	100
	Itu	22	85	100	100	100	100	100	100
	Mkpat Enin	20	79	99	100	100	100	100	100
	Nsit Ubium	21	82	100	100	100	100	100	100
	Oron	28	91	100	100	100	100	100	100
Bauchi	Bauchi	67	84	97	100	100	100	100	100
	Dass	74	88	98	100	100	100	100	100
	Misau	66	79	90	100	100	100	100	100
	Ningi	70	82	92	99	100	100	100	100
	Shira	71	83	93	99	100	100	100	100
	Toro	65	76	91	96	100	100	100	100
Benue	Buruku	8	91	100	100	100	100	100	100
	Gboko	21	100	100	100	100	100	100	100
	Kwande	12	95	100	100	100	100	100	100
	Makurdi	6	97	97	99	99	99	99	100
	Oju	3	75	95	99	100	100	100	100
	Otukpo	8	84	95	98	100	100	100	100
Cross River	Calabar Municipality	86	100	100	100	100	100	100	100
	Ikom	79	100	100	100	100	100	100	100
	Obudu	33	100	100	100	100	100	100	100
	Odukpani	84	92	98	98	100	100	100	100
	Ogoja	84	92	98	98	100	100	100	100
	Yarkur	61	93	100	100	100	100	100	100
Ebonyi	Abakaliki	10	68	97	99	100	100	100	100
	Ebonyi	9	89	98	98	98	100	100	100
	Ezza North	6	80	87	89	96	100	100	100
	Ezza South	6	20	71	82	96	100	100	100
	Ishielu	18	99	100	100	100	100	100	100
	Izzi	9	100	100	100	100	100	100	100
	Ohaozara	16	86	99	100	100	100	100	100
	Ohaukwu	11	85	100	100	100	100	100	100
Onicha	8	88	92	95	100	100	100	100	
Kebbi	Argungu	43	56	70	94	100	100	100	100
	Augie	28	38	64	75	91	96	100	100
	Birnin Kebbi	49	55	79	91	98	100	100	100

Sentinel Site	LGA	Test Time							
		60 mins	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	Bunza	30	50	71	86	95	98	100	100
	Jega	20	38	61	71	90	100	100	100
	Kalgo	31	56	69	86	96	99	100	100
Nasarawa	Doma	64	100	100	100	100	100	100	100
	Karu	78	93	100	100	100	100	100	100
	Keffi	54	93	100	100	100	100	100	100
	Lafia	62	77	93	98	100	100	100	100
	Nasarawa	70	93	97	100	100	100	100	100
	Nasarawa Eggon	56	91	100	100	100	100	100	100
Oyo	Afijio	21	99	100	100	100	100	100	100
	Akinyele	12	78	100	100	100	100	100	100
	Atiba	23	91	98	99	100	100	100	100
	Egbeda	10	78	91	98	100	100	100	100
	Ibarapa East	25	98	100	100	100	100	100	100
	Oluyole	5	73	98	100	100	100	100	100
Plateau	Langtang-North	100	100	100	100	100	100	100	100
	Langtang-South	99	100	100	100	100	100	100	100
	Mikang	100	100	100	100	100	100	100	100
	Quanpan	99	100	100	100	100	100	100	100
	Shendam	100	100	100	100	100	100	100	100
	Wase	100	100	100	100	100	100	100	100
Sokoto	Bodinga	26	48	74	88	98	100	100	100
	Gudu	3	29	51	85	100	100	100	100
	Illela	38	50	71	86	94	99	99	100
	Rabah	39	58	79	95	100	100	100	100
	Tambawal	14	44	70	95	99	100	100	100
	Wamakko	29	46	65	84	95	100	100	100
Zamfara	Anka	65	87	100	100	100	100	100	100
	Chafe	73	93	100	100	100	100	100	100
	Gusau	65	85	100	100	100	100	100	100
	Kaura Namoda	50	90	100	100	100	100	100	100
	Maru	65	89	100	100	100	100	100	100
	Talata Mafara	61	97	100	100	100	100	100	100

A minimum of 100 mosquitoes were exposed in each site.

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

Assessment of *ldr* mutations in pyrethroid-resistant *An. gambiae* s.l. indicated the presence of both *ldr-w* and *ldr-e* point mutations. Three deltamethrin-resistant mosquitoes from Ebonyi tested positive for heterozygous *ldr-e* allele compared to one positive sample found in permethrin resistant mosquitoes in Akwa Ibom (Tables 8 and 9). The frequency of the *ldr-w* mutations in deltamethrin-resistant *An. gambiae* s.l. mosquitoes was generally low, ranging from 0.01 in Kebbi to 0.44 in Plateau. A low *ldr-e* frequency (0.01) was observed in

Ebonyi (Table 9). The *kdr-w* gene frequency was also low in permethrin-exposed *An. gambiae* s.l. ranging from 0.12 in Benue to 0.46 in Plateau (Table 9).

Table 9: 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	69	4	5	60	0.09	0	0	69	0.00
	Bauchi	66	0	2	64	0.02	0	0	66	0.00
	Benue	101	28	4	69	0.30	0	0	101	0.00
	Cross River	77	6	1	70	0.08	0	0	77	0.00
	Ebonyi	232	46	37	149	0.28	0	3	229	0.01
	Kebbi	54	0	1	53	0.01	0	0	54	0.00
	Nasarawa	134	19	20	95	0.22	0	0	134	0.00
	Oyo	66	0	2	64	0.02	0	0	66	0.00
	Plateau	120	46	14	60	0.44	0	0	120	0.00
	Sokoto	49	0	2	47	0.02	0	0	49	0.00
Zamfara	118	18	9	91	0.19	0	0	118	0.00	

Table 10: Frequency of *kdr* genes in Permethrin-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
Permethrin	Akwa Ibom	115	30	22	63	0.36	0	1	114	0.00
	Benue	69	7	2	60	0.12	0	0	69	0.00
	Cross River	132	34	5	93	0.28	0	0	132	0.00
	Ebonyi	456	89	53	314	0.25	0	0	456	0.00
	Kebbi	106	12	5	89	0.14	0	0	106	0.00
	Plateau	141	58	14	69	0.46	0	0	141	0.00

3. DISCUSSION AND CONCLUSION

3.1 SPECIES COMPOSITION

An. gambiae s.l. remained the most abundant major malaria vector in Nigeria with widespread distribution across all sites. Secondary malaria vectors such as *An. funestus*, *An. nili*, *An. moucheti*, *An. pharoensis*, and *An. constani* were also found but with limited distribution and abundance. The percent composition of *An. funestus* mosquitoes in 2018 (0.1-4.0%) from four sites increased in 2019 (1.0-16.9%) to five sites. This pattern of occurrence agrees with our previous reports in recent years (AIRS Nigeria Final Entomology Report 2017, PMI VectorLink Nigeria Final Entomology Report, 2018). Other localized species found included *An. squamosus*, *An. maculipalpis*, *An. longipalpis*, *An. rufipes*, and *An. pretoriensis*. 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 and abundance of vectors 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 year-round.

All three members of the *An. gambiae* s.l. species (*An. gambiae*, *An. coluzzii*, and *An. arabiensis*) were found in varying degrees at each sentinel site. In most sites, these important malaria vector species were found both indoors and outdoors. Previous reports also observed *An. coluzzii* and *An. arabiensis* activity indoors and outdoors, though with greater abundance outdoors in most of the sentinel sites (PMI 2017, 2018). The highest occurrence of *An. coluzzii* was recorded outdoors (40.0%) in Akwa Ibom and indoors (39.4%) in Ebonyi. In addition, low numbers of hybrid *An. gambiae/An. coluzzii* species were found in Akwa Ibom, Ebonyi, Nasarawa, Plateau, and Sokoto. The co-occurrence of these three species across all sites and their ability to switch indoors and outdoors has been fully documented (PMI 2017, 2018). The behavioral adaptations of these mosquitoes to overlap with human activities contribute to their role in malaria transmission. The flexibility in behavior of these three species across the diverse ecozones of Nigeria is a challenge to the effectiveness of ITNs, which are deployed indoors for malaria control across the country.

3.2 VECTOR BITING RATE AND BITING TIME

Except for isolated cases of increased indoor and outdoor biting rates of *An. gambiae* s.l. in Akwa Ibom and Nasarawa during the peak dry season (November to March), biting rate is largely dependent on mosquito abundance which is influenced by rainfall patterns. In most sentinel sites, biting of *An. gambiae* s.l. increased during the rainy season (April to September), suggesting rainfall is a strong factor. In Akwa Ibom and Nasarawa, other factors such as flooding, irrigated farming, or open pools near the sites may support mosquito breeding. Outdoor biting activity was highest at Nasarawa, which stresses the important role of behavior change communication there for those who sleep outdoors without nets.

3.3 SPOROZOITE INFECTION RATE

Plasmodium falciparum sporozoite rates in *An. gambiae*, *An. coluzzii*, and *An. arabiensis* indoors and outdoors varied across the different ecological zones. Although recent studies have indicated that *An. gambiae* and *An. coluzzii* have similar sporozoitic indices (Akogbeto *et al.*, 2018), *An. gambiae* still remained the major malaria vector with higher sporozoite rate and vector density. The highest sporozoite rate indoors was recorded among *An. gambiae* in Bauchi (7.4%), whereas an earlier report indicated a higher infection rate among *An. coluzzii* also in Bauchi (12.5%) (2018 PMI VectorLink Nigeria Annual Entomology Report). The highest infection rate of *An. arabiensis* outdoors (2.9%) was recorded in Plateau; in 2018, the highest infection rate for this species (both indoors and outdoors) was recorded in Sokoto (8.5% and 6.8%, respectively) (2018 PMI VectorLink Nigeria Annual Entomology Report). The high level of outdoor transmission calls for interventions targeting outdoor-biting

mosquitoes. Overall, there were generally lower sporozoite rates recorded across the sentinel sites in 2017 and 2018 than in 2019. *An. funestus* group is another major malaria vector, second only to *An. gambiae* s.s. in terms of vectorial capacity. Its role in malaria transmission has been reported in Nigeria (Awolola et al., 2003). *P. falciparum* sporozoite rates recorded indoors in *An. coustani* (1.2%) in Nasarawa further corroborated previous reports on sporozoite-positive *An. coustani* both indoors and outdoors (1.8% and 0.8%, respectively) using CDC LT in the same location. (Inyama et al., 2017).

3.4 ENTOMOLOGICAL INOCULATION RATE

Across LGAs, *An. gambiae* contributed more to EIR indoors and outdoors than other members of the *An. gambiae* s.l. complex. Notably, *An. gambiae* also contributed the highest EIR outdoors in Nasarawa, followed by Sokoto and Ebonyi. This result provides evidence of the outdoor malaria transmission potential of *An. gambiae*. The ability of *An. gambiae* to contribute to malaria transmission both indoors and outdoors establishes its role as a major vector across the sentinel sites. However, the lower contributions of *An. coluzzii* to EIR this year compared to previous years demonstrates that both *An. gambiae* and *An. coluzzii* are efficient vectors of malaria (PMI, 2018, Carnavale et al., 2015; Akogbeto et al., 2018). The outdoor EIR of *An. arabiensis* in Nasarawa, Sokoto, and Plateau confirmed its role in outdoor malaria transmission. Overall, the comparative contributions of EIRs between members of *An. gambiae* s.l. varied across months, vectors, and ecozones (Annex 4). The highest outdoor EIR of *An. gambiae* in Nasarawa is contrary to earlier findings which reported that the highest outdoor EIR was contributed by *An. arabiensis* in Plateau (PMI VectorLink Nigeria Report 2018).

3.5 BLOOD MEAL SOURCES

In four out of seven sites, *An. coluzzii* had higher human blood indices than *An. gambiae*. In addition, higher numbers of mosquitoes fed on humans in Akwa Ibom and Ebonyi compared to all other sites. Increased ITN usage in Akwa Ibom and Ebonyi will hopefully reverse this trend and reduce the number of mosquitoes feeding on humans. There was also higher feeding on animals recorded in Bauchi where 100% of blood meals of *An. coluzzii* were from goats. Given very few samples (n=22) were analyzed from this site, this finding might not conclusively indicate the feeding behavior of *An. coluzzii* in the area. However, this zoophilic behavior of *An. coluzzii* in Bauchi could affect the maintenance of residual malaria in those sites (WHO, 2019). Overall across all sites, despite the zoophilic nature of *An. arabiensis*, human blood meals were detected in varying proportions in the vector. Bovine blood meals were also detected in *An. gambiae* across all sites. Generally, the prevalence of animal blood meal in human dwellings indicates that livestock live in close proximity to humans, which can lead to higher malaria transmission by attracting infected mosquitoes to human habitations (Iwashita et al., 2014).

3.6 INSECTICIDE SUSCEPTIBILITY

An. gambiae were susceptible to deltamethrin in Benue, Cross River, and Kebbi, and to alpha-cypermethrin in Kebbi, Oyo, and Sokoto, suggesting possible use of these insecticides, especially for treating ITNs, under an insecticide resistance management plan. The results also indicated that *An. gambiae* s.l. is susceptible to pirimiphos-methyl in most locations. The widespread resistance of vectors to permethrin at most of the sites showed that it is not the preferred insecticide choice for standard ITNs in these areas. PMI data from 2017 and 2018 demonstrated widespread permethrin, lambda-cyhalothrin, deltamethrin, and alpha-cypermethrin resistance across all ecozones in Nigeria. *An. gambiae* s.l. mosquitoes were susceptible (98-100% mortality) to chlorfenapyr at 48 hours across all sites, except in Akwa Ibom (all LGAs) and Sokoto (one LGA). This suggests the relevance of chlorfenapyr as a potential compound to manage the pyrethroid resistance observed at the monitoring sites. However, the vector is not susceptible at 100 µg/bottle in Akwa Ibom, and further tests are needed at a higher concentration.

An. gambiae s.l. susceptibility to clothianidin in 11 states also supports the potential use of clothianidin-based insecticides as part of the resistance management strategy for vector control in the country.

3.7 RESISTANCE INTENSITY AND MECHANISMS

Insecticide resistance intensity recorded in *An. gambiae* s.l. mosquitoes across the different ecozones reveals different resistance management options. Deltamethrin resistance in *An. gambiae* s.l. mosquitoes attained low resistance intensity only in Plateau and Ebonyi. This contrasted with the previous year's report where low deltamethrin resistance intensity was observed in *An. gambiae* s.l. mosquitoes from Akwa Ibom, Benue, and Oyo but not in Plateau (PMI, 2018). Deltamethrin resistance intensity results this year showed resistance only at 1X in Akwa Ibom and Oyo. These results suggest that deltamethrin+PBO ITNs may adequately manage the deltamethrin resistance in Akwa Ibom, Oyo, Sokoto, and Zamfara. However, the inability of PBO to restore deltamethrin, permethrin, and alpha-cypermethrin susceptibility in *An. gambiae* s.l. in an increasing number of LGAs in Bauchi, Nasarawa, and Plateau is worrisome. The contiguous position of these states may be responsible for the similar resistance pattern observed. Previous reports showed that pre-exposure of *An. gambiae* s.l. to PBO did restore susceptibility to deltamethrin in all LGAs in Nasarawa and Plateau but not in Bauchi. Fluctuations in susceptibility outcomes in these three states may require further observation over successive years or might need to consider Interceptor G2 nets comprising of chlorfenapyr.

3.8 KDR GENE FREQUENCIES

The knockdown (*kdr*) point mutation is another important mechanism associated with pyrethroid resistance. Two types of these point mutations; the 1014F (*kdr-w*) and 1014S (*kdr-e*) were observed in deltamethrin- and permethrin-resistant mosquitoes. The *kdr-w* mutation was observed at all sites in both permethrin- and deltamethrin-resistant mosquitoes. The *kdr-e* allele, which is still very rare in Nigeria, was identified from the same sites (Akwa Ibom and Ebonyi) reported last year. Where metabolic resistance is ruled out, mutations in the binding site of insecticides are often involved. Though the presence of this resistance allele alone may not lead to control failure, the *kdr-w* allelic frequencies in both deltamethrin- and permethrin-resistant mosquitoes increased this year compared to last year. There is need to continually monitor 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.

ANNEXES

ANNEX 1: GPS COORDINATES OF 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		
	Ikot Ekpene	5.190752	7.72.633		
	Oron	4.797047	8.238453		
	Mkpat Enin	4.7708499,	7.735482	Primary Health Care Mkpat Enin	4.784669, 7.731115
Bauchi	Ningi	11.044710	9.572530		
	Misau	11.305730	10.474310		
	Shira	11.428900	9.963100		
	Dass	10.083360	9.633120	Gwantar Health Facility	9.994724, 9.525576
	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	6.338803, 8.060135
	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		

State	Location of Sampling Sites	Latitude	Longitude	Name of Nearest Health Facility	GPS Coordinate of Health Facility
Nasarawa	Kokona	8.847000	7.893000		
	Lafia	8.594000	8.557000		
	Nasarawa	8.536000	7.674000		
	Nasarawa Eggon	8.775000	8.531000		
	Obi	8.502000	8.543000		
	Toto	8.436000	7.283000		
	Wamba	8.956000	8.608000		
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	Nyuun Primary Health Center	569248.5, 977431.8
	Mikang	9.016930	9.539600		
	Qua'an pan	8.977620	9.264100		
	Shendam	8.825520	9.459720		
	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		

ANNEX 2: ANOPHELES MOSQUITOES COLLECTED BY DIFFERENT METHODS AND SUBJECTED TO PCR ACROSS SITES (NOVEMBER 2018–SEPTEMBER 2019)

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	205	95	16	11	327	40	1	41	368
Bauchi	41	26	2	0	69	33	2	35	104
Ebonyi	165	26	20	2	213	238	12	250	463
Nasarawa	373	291	15	7	686	385	5	390	1,076
Oyo	39	16	0	0	55	357	5	362	417
Plateau	278	111	7	4	400	411	10	421	821
Sokoto	214	169	9	9	401	531	11	542	943
Total	1,315	734	69	33	2,151	1,995	46	2,041	4,192

ANNEX 3: ANOPHELES CAUGHT BY SPECIES, METHOD, AND SITE (NOVEMBER 2018–SEPTEMBER 2019)

Mosquito Species	Nasarawa			Ebonyi			Bauchi			Akwa Ibom			Oyo			Sokoto			Plateau			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				
<i>An. gambiae</i> s.l.	1,913	1,420	2,047	962	111	1,353	528	270	282	1,063	332	475	982	589	3,026	754	436	5,111	2,185	284	2,418	8,387	3,442	14,712	26,541
<i>An. funestus</i>	52	69	2	8	3	57	-	-	-	-	-	-	9	3	176	46	8	13	293	127	599	408	210	847	1,465
<i>An. nili</i>	3	8	0	-	-	-	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	4	8	0	12
<i>An. coustani</i>	208	469	0	-	-	-	15	38	20	-	-	-	-	-	-	3	1	0	6	4	0	232	512	20	764
<i>An. pharoensis</i>	64	54	1	-	-	-	-	-	-	-	-	-	-	-	-	25	38	0	-	-	-	89	92	1	182
<i>An. maculipalpis</i>	-	-	-	-	-	-	-	-	-	18	16	0	-	-	-	-	-	-	0	1	5	18	17	5	40
<i>An. moucbeti</i>	-	-	-	0	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	2	3
<i>An. pretoriensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	8	1	9	-	-	-	5	6	3	13	7	12	32
<i>An. rufipes</i>	10	28	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	15	32	42	43	34	119
<i>An. squamosus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	5	3	5	5	3	13
<i>An. longipalpis</i>	-	-	-	-	-	-	-	-	-	-	-	-	0	0	2	-	-	-	0	0	1	0	0	3	3
<i>An. flavicosta</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
Grand Total	2,250	2,048	2,052	970	115	1,412	544	308	302	1,081	348	475	999	593	3,213	828	483	5,124	2,526	442	3,061	9,198	4,337	15,639	29,174

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 LT Indoors					CDC LT Outdoors					PSC					Totals				
	No. PCR +ve	<i>An. gambiae</i>	<i>An. coluzzii</i>	Hybrid	<i>An. arabiensis</i>	No. PCR +ve	<i>An. gambiae</i>	<i>An. coluzzii</i>	Hybrid	<i>An. arabiensis</i>	No. PCR +ve	<i>An. gambiae</i>	<i>An. coluzzii</i>	Hybrid	<i>An. arabiensis</i>	No. PCR +ve	<i>An. gambiae</i>	<i>An. coluzzii</i>	Hybrid	<i>An. arabiensis</i>
Akwa Ibom	205	124	67	4	10	95	31	38	0	26	40	9	26	0	5	340	164	131	4	41
Bauchi	41	27	8	0	6	26	18	2	0	6	33	19	4	0	10	100	64	14	0	22
Ebonyi	165	92	65	2	6	26	12	1	0	13	238	120	101	0	17	429	224	167	2	36
Nasarawa	373	274	63	3	33	291	162	24	0	105	385	271	62	0	52	1,049	707	149	3	190
Oyo	39	32	5	0	2	16	13	3	0	0	357	245	59	0	53	412	290	67	0	55
Plateau	278	213	36	8	21	111	54	11	0	46	411	338	30	2	41	800	605	77	10	108
Sokoto	214	107	10	4	93	169	55	5	0	109	531	404	27	2	98	914	566	42	6	300
Total	1,315	869	254	21	171	734	345	84	0	305	1,995	1,406	309	4	276	4,044	2,620	647	25	752

ANNEX 5: INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES BY SITE

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		<i>An. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</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	Nov-18	156	14	92	19	14	14	13.01	1.13	7.71	1.57	1.20	1.13	0.019	0.000	0.000	0.000	0.000	0.000	7.23	0.00	0.00	0.00	0.00	0.00
	Dec-18	195	0	98	58	0	0	16.28	0.00	8.14	4.83	0.00	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jan-19	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
	Feb-19	20	0	7	0	0	0	1.69	0.00	0.56	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mar-19	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
	Apr-19	20	1	0	0	0	0	1.67	0.08	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-19	12	5	0	0	0	0	1.00	0.42	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jun-19	43	0	0	17	0	0	3.58	0.00	0.00	1.42	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jul-19	38	37	19	0	0	0	3.17	3.08	1.58	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aug-19	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-19	0	58	0	0	0	0	0.00	4.83	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
		656	108	354	133	53	91	54.65	9.03	29.53	11.07	4.41	7.57	0.016	0.000	0.000	0.000	0.000	0.000	7.2	0.00	0.00	0.00	0.00	0.00
Bauchi	Nov-18	0	2	0	0	3	4	0.00	0.20	0.00	0.00	0.25	0.30	0.00	0.000	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00
	Dec-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
	Jan-19	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
	Feb-19	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
	Mar-19	0	5	0	0	0	0	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Apr-19	14	9	0	0	0	0	1.17	0.75	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-19	18	9	0	9	0	0	1.50	0.75	0.00	0.75	0.00	0.00	0.000	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jun-19	59	13	0	13	0	0	4.92	1.04	0.00	1.04	0.00	0.00	0.000	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jul-19	106	88	70	0	0	0	8.80	7.33	5.87	0.00	0.00	0.00	0.167	0.000	0.000	0.00	0.00	0.00	45.47	0.00	0.00	0.00	0.00	0.00
	Aug-19	125	100	75	0	0	0	10.42	8.33	6.25	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sep-19	27	18	4	0	0	0	2.26	1.50	0.32	0.00	0.00	0.00	0.143	0.000	0.000	0.00	0.00	0.00	9.69	0.00	0.00	0.00	0.00	0.00	
		338	187	100	21	75	62	28.21	15.58	8.36	1.73	6.27	5.19	0.074	0.000	0.000	0.000	0.000	0.000	55.15	0.00	0.00	0.00	0.00	0.00
Ebonyi	Nov-18	14	3	28	0	3	7	1.16	0.28	2.32	0.00	0.27	0.56	0.000	0.000	0.000	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00
	Dec-18	4	0	6	0	0	1	0.31	0.00	0.52	0.00	0.00	0.08	0.000	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00
	Jan-19	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Feb-19	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
	Mar-19	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
	Apr-19	8	0	0	0	0	0	0.67	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-19	45	11	4	0	0	0	3.74	0.92	0.34	0.00	0.00	0.00	0.000	0.333	0.000	0.00	0.00	0.00	0.00	9.47	0.00	0.00	0.00	0.00
	Jun-19	524	54	66	0	0	0	43.70	4.50	5.46	0.00	0.00	0.00	0.025	0.000	0.200	0.00	0.00	0.00	32.78	0.00	32.78	0.00	0.00	0.00
	Jul-19	0	0	141	0	0	0	0.00	0.00	11.75	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aug-19	71	0	0	10	0	0	5.92	0.00	0.00	0.83	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sep-19	41	0	0	0	0	0	3.42	0.00	0.00	0.00	0.00	0.00	0.000	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. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</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
		543	51	384	4	35	56	45.25	4.27	31.97	0.36	2.95	4.63	0.011	0.083	0.015	0.000	0.000	0.000	32.78	9.47	32.78	0.00	0.00	0.00
	Nov-18	58	28	14	7	7	37	4.85	2.35	1.15	0.59	0.58	3.06	0.000	0.000	0.000	0.000	0.000	0.038	0.00	0.00	0.00	0.00	0.00	3.53
	Dec-18	59	27	8	2	11	33	4.94	2.24	0.71	0.17	0.94	2.76	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00
	Jan-19	75	71	40	24	15	24	6.25	5.95	3.33	1.98	1.25	1.98	0.067	0.056	0.000	0.000	0.000	0.000	12.92	10.25	0.00	0.00	0.00	0.00
	Feb-19	161	128	72	14	56	121	13.38	10.70	6.02	1.19	4.68	10.11	0.050	0.000	0.111	0.000	0.000	0.059	18.73	0.00	18.73	0.00	0.00	16.65
	Mar-19	36	63	12	10	10	68	2.97	5.22	1.02	0.87	0.84	5.66	0.031	0.000	0.000	0.000	0.000	0.000	2.88	0.00	0.00	0.00	0.00	0.00
Doma	Apr-19	106	126	0	0	0	0	8.83	10.50	0.00	0.00	0.00	0.00	0.000	0.050	0.00	0.00	0.00	0.00	0.00	15.75	0.00	0.00	0.00	0.00
	May-19	278	189	0	0	0	0	23.17	15.75	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jun-19	390	177	0	0	0	0	32.50	14.75	0.00	0.00	0.00	0.00	0.000	0.059	0.00	0.00	0.00	0.00	0.00	26.03	0.00	0.00	0.00	0.00
	Jul-19	108	80	65	0	0	0	8.96	6.67	5.38	0.00	0.00	0.00	0.000	0.250	0.000	0.00	0.00	0.00	0.00	51.67	0.00	0.00	0.00	0.00
	Aug-19	118	60	141	60	0	30	9.81	5.00	11.77	5.00	0.00	2.50	0.000	0.000	0.000	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00
	Sep-19	42	32	31	8	0	0	3.48	2.67	2.61	0.67	0.00	0.00	0.000	0.250	0.000	0.000	0.00	0.00	0.00	20.00	0.00	0.00	0.00	0.00
		1,431	791	326	117	171	512	118.05	65.88	27.14	9.76	14.22	42.70	0.011	0.031	0.016	0.000	0.000	0.029	34.53	123.69	18.73	0.00	0.00	20.18
	Nov-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
	Dec-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
	Jan-19	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
	Feb-19	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mar-19	19	0	0	0	9	0	1.56	0.00	0.00	0.00	0.78	0.00	0.250	0.00	0.00	0.000	0.00	0.00	12.06	0.00	0.00	0.00	0.00	0.00
Oyo	Apr-19	70	28	0	0	0	0	5.83	2.33	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-19	117	80	0	0	0	0	9.75	6.67	0.00	0.00	0.00	0.00	0.111	0.000	0.00	0.00	0.00	0.00	33.58	0.00	0.00	0.00	0.00	0.00
	Jun-19	95	46	0	0	0	0	7.92	3.83	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jul-19	0	64	0	127	0	0	0.00	5.31	0.00	10.61	0.00	0.00	0.000	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aug-19	0	0	176	0	0	0	0.00	0.00	14.67	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Sep-19	79	0	79	0	0	0	6.54	0.00	6.54	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		806	479	126	110	50	0	67.15	39.88	10.49	9.20	4.20	0.00	0.063	0.000	0.000	0.000	0.000	0.00	45.64	0.00	0.00	0.00	0.00	0.00
	Nov-18	34	9	7	2	6	13	2.85	0.76	0.62	0.16	0.54	1.08	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00
	Dec-18	20	9	3	1	8	6	1.63	0.78	0.27	0.08	0.68	0.47	0.000	0.000	0.000	0.000	0.000	0.167	0.00	0.00	0.00	0.00	0.00	2.43
	Jan-19	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
	Feb-19	10	0	0	0	0	0	0.83	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mar-19	3	1	0	2	1	0	0.25	0.08	0.00	0.17	0.08	0.00	0.000	0.000	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Plateau	Apr-19	50	7	0	0	0	0	4.17	0.58	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-19	116	3	0	3	0	0	9.67	0.21	0.00	0.21	0.00	0.00	0.000	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jun-19	359	33	0	16	0	0	29.92	2.72	0.00	1.36	0.00	0.00	0.034	0.000	0.00	0.000	0.00	0.00	30.95	0.00	0.00	0.00	0.00	0.00
	Jul-19	524	47	216	0	31	0	43.63	3.92	17.97	0.00	2.57	0.00	0.000	0.000	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aug-19	496	106	86	0	0	0	41.37	8.83	7.13	0.00	0.00	0.00	0.000	0.000	0.200	0.00	0.00	0.00	0.00	0.00	44.22	0.00	0.00	0.00
	Sep-19	167	15	40	0	0	0	13.91	1.25	3.34	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		1,779	138	291	28	170	118	143.64	11.51	24.28	2.35	14.16	9.81	0.005	0.000	0.028	0.000	0.000	0.022	30.95	0.00	44.22	0.00	0.00	2.43

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		<i>An. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</i>		<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</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
Sokoto	Nov-18	18	46	3	0	40	92	1.51	3.83	0.28	0.00	3.29	7.67	0.000	0.000	0.000	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00
	Dec-18	7	0	0	0	15	32	0.61	0.00	0.00	0.00	1.22	2.67	0.000	0.00	0.00	0.00	0.000	0.077	0.00	0.00	0.00	0.00	0.00	6.36
	Jan-19	51	0	2	0	14	53	4.27	0.00	0.16	0.00	1.15	4.42	0.000	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00
	Feb-19	39	0	3	0	21	0	3.23	0.00	0.22	0.00	1.72	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mar-19	0	7	0	1	16	8	0.00	0.59	0.00	0.11	1.33	0.64	0.00	0.000	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00
	Apr-19	51	18	0	0	0	0	4.25	1.50	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	May-19	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jun-19	16	17	0	0	0	0	1.33	1.42	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jul-19	62	17	10	0	0	0	5.14	1.42	0.86	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aug-19	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-19	261	24	20	37	20	0	21.74	2.03	1.67	3.05	1.67	0.00	0.000	0.500	0.000	0.000	0.000	0.00	0.00	0.00	30.50	0.00	0.00	0.00	
		384	142	36	13	334	281	32.02	11.82	2.99	1.07	27.83	23.43	0.000	0.018	0.000	0.000	0.000	0.009	0.00	30.50	0.00	0.00	0.00	6.36

ANNEX 6: ANNUAL EIR FOR ALL SENTINEL SITES

Sentinel Sites	Mosquito species	EIR over 11 months		Estimated EIR over 12 months	
		Indoors	Outdoors	Indoors	Outdoors
Akwa Ibom	<i>An. gambiae</i>	7.2	0.0	7.9	0.0
	<i>An. coluzzii</i>	0.0	0.0	0.0	0.0
	<i>An. arabiensis</i>	0.0	0.0	0.0	0.0
Bauchi	<i>An. gambiae</i>	55.2	0.0	60.2	0.0
	<i>An. coluzzii</i>	0.0	0.0	0.0	0.0
	<i>An. arabiensis</i>	0.0	0.0	0.0	0.0
Ebonyi	<i>An. gambiae</i>	32.8	9.5	35.8	10.3
	<i>An. coluzzii</i>	32.8	0.0	35.8	0.0
	<i>An. arabiensis</i>	0.0	0.0	0.0	0.0
Nasarawa	<i>An. gambiae</i>	34.5	123.7	37.7	134.9
	<i>An. coluzzii</i>	18.7	0.0	20.4	0.0
	<i>An. arabiensis</i>	0.0	20.2	0.0	22.0
Oyo	<i>An. gambiae</i>	45.6	0.0	49.8	0.0
	<i>An. coluzzii</i>	0.0	0.0	0.0	0.0
	<i>An. arabiensis</i>	0.0	0.0	0.0	0.0
Plateau	<i>An. gambiae</i>	30.9	0.0	33.8	0.0
	<i>An. coluzzii</i>	44.2	0.0	48.2	0.0
	<i>An. arabiensis</i>	0.0	2.4	0.0	2.7
Sokoto	<i>An. gambiae</i>	0.0	30.5	0.0	33.3
	<i>An. coluzzii</i>	0.0	0.0	0.0	0.0
	<i>An. arabiensis</i>	0.0	6.4	0.0	6.9

ANNEX 7: INDOOR RESTING DENSITY OF ANOPHELINE MOSQUITOES BY SITE

Sentinel Sites	Month	# of Rooms	Total # of <i>Anopheles</i> Caught	Indoor Resting Density
Akwa Ibom	Nov-18	32	88	0.0
	Dec-18	32	75	2.3
	Jan-19	32	13	0.4
	Feb-19	32	16	0.5
	Mar-19	32	5	0.2
	Apr-19	32	66	2.1
	May-19	32	20	0.6
	Jun-19	32	20	0.6
	Jul-19	32	30	0.9
	Aug-19	32	49	1.5
	Sep-19	32	93	2.9
			Mean	1.1
Bauchi	Nov-18	32	11	0.3
	Dec-18	32	1	0.0
	Jan-19	32	0	0.0
	Feb-19	32	0	0.0
	Mar-19	32	11	0.3
	Apr-19	32	12	0.4
	May-19	32	42	1.3
	Jun-19	32	34	1.1
	Jul-19	32	73	2.3
	Aug-19	32	70	2.2
	Sep-19	32	28	0.9
			Mean	0.8
Ebonyi	Nov-18	32	96	3.0
	Dec-18	32	13	0.4
	Jan-19	32	13	0.4
	Feb-19	32	17	0.5
	Mar-19	32	18	0.6
	Apr-19	32	36	1.1
	May-19	32	168	5.3
	Jun-19	196	403	2.1
	Jul-19	196	291	1.5
	Aug-19	196	130	0.7
	Sep-19	196	168	0.9
			Mean	1.5
Nasarawa	Nov-18	32	30	0.9
	Dec-18	32	72	2.3
	Jan-19	32	202	6.3
	Feb-19	32	86	2.7
	Mar-19	32	103	3.2
	Apr-19	32	204	6.4
	May-19	32	380	11.9
	Jun-19	32	229	7.2
Nasarawa	Jul-19	32	165	5.2
	Aug-19	32	354	11.1
	Sep-19	32	107	3.3

Sentinel Sites	Month	# of Rooms	Total # of <i>Anopheles</i> Caught	Indoor Resting Density
			Mean	5.6
Oyo	Nov-18	32	74	2.3
	Dec-18	32	14	0.4
	Jan-19	32	42	1.3
	Feb-19	32	35	1.1
	Mar-19	32	220	6.9
	Apr-19	32	351	11.0
	May-19	32	449	14.0
	Jun-19	32	373	11.7
	Jul-19	32	531	16.6
	Aug-19	32	498	15.6
	Sep-19	32	439	13.7
			Mean	9.1
Plateau	Nov-18	32	106	3.3
	Dec-18	32	51	1.6
	Jan-19	32	22	0.7
	Feb-19	32	25	0.8
	Mar-19	32	24	0.8
	Apr-19	32	114	3.6
	May-19	32	157	4.9
	Jun-19	32	660	20.6
	Jul-19	32	561	17.5
	Aug-19	32	399	12.5
	Sep-19	32	299	9.3
			Mean	6.9
Sokoto	Nov-18	32	86	2.7
	Dec-18	32	35	1.1
	Jan-19	32	80	2.5
	Feb-19	32	417	13.0
	Mar-19	32	238	7.4
	Apr-19	32	843	26.3
	May-19	32	150	4.7
	Jun-19	32	121	3.8
	Jul-19	32	515	16.1
	Aug-19	32	465	14.5
	Sep-19	32	2,159	67.5
			Mean	14.5

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