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THE PMI VECTORLINK NIGERIA PROJECT

ANNUAL ENTOMOLOGY REPORT

OCTOBER 2019–SEPTEMBER 2020

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Abt Associates | 6130 Executive Blvd | Rockville, Maryland 20852
T. 301.347.5000
abtassociates.com

THE PMI VECTORLINK NIGERIA PROJECT ANNUAL ENTOMOLOGY REPORT

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ACRONYMS

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

EXECUTIVE SUMMARY

Malaria vector surveillance and insecticide resistance monitoring activities provide 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 2020, vector surveillance was carried out in five sentinel sites while insecticide resistance monitoring was carried out in all 11 PMI focus states. From October 2019 to September 2020, pyrethrum spray catches (PSCs) and human-baited U.S. Centers for Disease Control and Prevention Light Traps (CDC LTs) were used to collect mosquitoes both indoors and outdoors to determine species composition, behavior, seasonality, biting rates, infectivity rates, blood meal sources, and entomological inoculation rates (EIRs) of malaria vectors across sentinel sites. CDC bottle bioassays were used to determine the insecticide resistance status, intensity, and underlying resistance mechanisms.

A total of 23,028 *Anopheles* mosquitoes were collected from five sentinel sites using human-baited CDC LTs (indoors/outdoors) and PSCs. *An. gambiae* s.l. was the most abundant species across all sites ranging from 51.7% in Oyo to 99.4% in Ebonyi. A total of 1,815 *An. gambiae* s.l. mosquitoes collected by CDC LTs and PSCs were identified by species-specific polymerase chain reaction (PCR) assays. Of these, 1,771 (97.6%) mosquito samples successfully amplified while 44 (2.4%) failed to amplify. A total of 1,189 (67.1%) across all sites were identified as *An. gambiae* s.s., 567 (32.0%) were *An. coluzzii,* 14 (0.8%) were *An. arabiensis*, and 1 (0.1%) was hybrid *An. gambiae*/*An. coluzzii*.

An. gambiae s.s. was the dominant vector species found both indoors and outdoors in all sentinel sites, followed by *An. coluzzii*. The highest proportion of *An. gambiae* collected outdoors was in Oyo (94.1%), while the highest indoor collections were from Plateau (70.8%). The highest indoor collections of *An. coluzzii* were also in Oyo (39.6%), while the highest proportion outdoors was from Akwa Ibom (38.4%). Hybrid forms were recorded indoors only in Plateau (0.4%). *An. arabiensis* was found indoors in Ebonyi, Plateau, and Sokoto (0.9% in each), while the highest occurrence recorded outdoors was in Plateau (3.1%). *Plasmodium falciparum* sporozoite rates of *An. gambiae* s.s. collected indoors ranged from 0.0% in Oyo, Plateau, and Sokoto to 2.2% in Akwa Ibom. All *An. coluzzii* and *An. arabiensis* mosquitoes sampled from indoor and outdoor collections across the various ecozones tested negative for the *Plasmodium* circumsporozoite antigens.

In Plateau and Sokoto, the mean indoor and outdoor biting rates of *An. gambiae* s.l. peaked in July 2020 and August 2020, respectively. An earlier but smaller peak was observed in December 2019 in Akwa Ibom and Oyo. Indoor and outdoor biting rates in the other sites were generally low. The indoor resting density of *An. gambiae* s.l. mosquitoes varied across the sites and months, ranging from 0.1 mosquitoes/room/day in Plateau in February 2020 to 71.9 mosquitoes/room/day in Sokoto in August 2020, which is the peak of rainfall. The average number of mosquitoes caught biting per unit time was generally higher indoors versus outdoors and ranged from 8 mosquitoes collected between 2-3 a.m. in Ebonyi to 41 mosquitoes collected between 10-11 p.m. in Plateau. In Plateau, two biting peaks were observed, one at 10-11 p.m. and a second at 1-2 a.m. EIRs were recorded only among *An. gambiae* s.s. collected indoors in Ebonyi (4.6 infective bites/person/year) and indoors in Akwa Ibom (3.0 infective bites/person/year).

Human blood index (HBI) analysis showed that, across all sites, human blood meals were detected in varying proportions in *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* collected using both PSC and CDC LT methods. The proportion varied by vector and site. The highest proportions of *An. gambiae* mosquitoes from CDC LTs that fed on human blood were from Akwa Ibom (100%), Ebonyi (100%), and Oyo (100%) while the highest proportions of human-bloodfed *An. coluzzii* were recorded indoors in Akwa Ibom and Ebonyi (both 100%). HBI in mosquitoes collected by PSC (94-100%) was higher compared to both indoor (70-100%) and outdoor (50-100%) CDC LT collections.

Average parity rates of An. gambiae s.l. mosquitoes for 2017, 2018, 2019, and 2020 were calculated and compared. The all-time highest parity rate (79.2%) was recorded in Sokoto in 2020.

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 continues to be widespread in *An. gambiae* s.l. mosquitoes at all sentinel sites.

Pyrethroid resistance was detected in *An. gambiae* s.l. from all 11 states, but patterns varied within and among states. Resistance to all three pyrethroids was recorded in Akwa Ibom. Deltamethrin and permethrin resistance was recorded in all six LGAs in Plateau. Out of the 69 sites where *An. gambiae* s.l. mosquitoes were tested for susceptibility to permethrin, 65 indicated the presence of resistance, one indicated the presence of possible resistance, and three (all in Sokoto) indicated susceptibility.

In six LGAs each in Akwa Ibom, Nasarawa, and Sokoto, five LGAs in Bauchi, one LGA in Oyo, seven LGAs in Ebonyi, and four LGAs in Zamfara, *An. gambiae* s.l. populations previously resistant at 1X the diagnostic dose were susceptible to deltamethrin at 2X concentration. Low resistance intensity to deltamethrin was recorded in *An. gambiae* s.l. in Bauchi, Ebonyi, and Plateau. *Anopheles gambiae* s.l. were susceptible to alpha-cypermethrin at the diagnostic dose (1X) across six LGAs in Benue, Cross River, Oyo, and Sokoto. Some *An. gambiae* s.l. collected in Akwa Ibom, Bauchi, Ebonyi, Kebbi, Nasarawa, and Zamfara that were initially resistant at 1X alpha-cypermethrin concentration later showed susceptibility at 2X the diagnostic dose. Low-intensity of resistance to alpha-cypermethrin (mortality between 98–100% at 5X the diagnostic dose) was recorded in *An. gambiae* s.l. populations from four LGAs in Akwa Ibom, one LGA in Bauchi, one LGA in Ebonyi, and all six LGAs in Plateau. Susceptibility of *An. gambiae* s.l. mosquitoes to permethrin at 2X the diagnostic dose was observed in four LGAs in Bauchi, six LGAs in Benue, and three LGAs in Sokoto.

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

Susceptibility of *An. gambiae* s.l. mosquitoes to deltamethrin was restored in Akwa Ibom, Ebonyi, Nasarawa, Oyo, Sokoto, and Zamfara. Pre-exposure to PBO synergist did not restore susceptibility to deltamethrin in *An. gambiae* s.l. mosquitoes from two out of the six LGAs in Bauchi. In Plateau, susceptibility to deltamethrin was not restored in *An. gambiae* s.l. mosquitoes from all six LGAs. Susceptibility to alpha-cypermethrin was restored in *An. gambiae* s.l. mosquitoes across all LGAs in Ebonyi, Nasarawa, Zamfara, and in two LGAs in Kebbi. Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO did not restore susceptibility to alpha-cypermethrin in the six LGAs in Bauchi or Plateau. For permethrin, PBO did not fully restore susceptibility in *An. gambiae* s.l. populations from LGAs in Akwa Ibom, Cross River, Kebbi, Nasarawa, and Plateau as well as in five of six LGAs in Oyo and Zamfara, respectively.

1. INTRODUCTION

The malaria burden has remained high in Nigeria, with the country contributing the highest proportion of malaria cases (25%) and deaths (24%) in the world (World Malaria Report, 2020). The country has five diverse geo-ecological zones, 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. (*An. gambiae* s.s. , *An. coluzzii,* and *An. arabiensis)* and *An. funestus.* Secondary malaria vectors in the country include *An. nili, An. moucheti, An. pharoensis, An. coustani,* and *An. longipalpis* (PMI VectorLink Annual Entomology Report, 2019).

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 VectorLink Project in 2017, the number of entomological monitoring sites was increased to seven across seven states and two insecticide resistance monitoring sites were added in two states, bringing the total to nine states. 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 entomological 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 October 2019 to September 2020, VectorLink Nigeria conducted routine vector surveillance in five sites and insecticide resistance monitoring in all 11 PMI focus states, assessing species composition, density, feeding time, location (indoors or outdoors), seasonality, and insecticide susceptibility status of the major malaria vectors. The intensity and mechanism of insecticide resistance across the different ecozones of Nigeria were also determined. This report summarizes entomological monitoring activities completed from October 2019 to September 2020.

1.1 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 five sentinel sites and insecticide resistance monitoring only in six additional sites (Tables 1 and 2). Annex A contains GPS coordinates of sampling site locations.

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

Geopolitical Zone	State/Institution	Local Government Areas (LGA)/Sentinel Site	Ecozone(s)
South West	Oyo/University of Ibadan	Akinyele/Elekuru	Rainforest/Guinea Savannah
South East	Ebonyi/State Univ Abakaliki	Ezaa North/Umuaghara, Enyim Izzi/Ndiechi Onuebonyu, Ndukabi Ohaukwu/Okwerike, Umuebe	Rainforest
South South	Akwa Ibom/University of Uyo	Mpat Enin/Ibekwe Akpannya	Mangrove swamps/Rainforest
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

Geopolitical Zone	State/Institution	LGA/Insecticide Resistance Monitoring Site	Ecozone(s)
North East	Bauchi/Abubakar Tafawa Balewa University	Ningi, Misau, Shira, Dass, Bauchi, Toro	Sudan Savannah
North Central	Benue/Federal University of Agriculture Makurdi	Apa, Gwer, Obi, Tarkaa, Ukum, Vandeikya	Guinea Savannah
South South	Cross River/University of Calabar	Abi, Akampka, Calabar Municipal, Etung, Ogoja, Obudu	Rainforest/Mangrove swamps
North West	Zamfara/Federal University Gusau	Bakura, Birnin Magaji, Bungudu, Gummi, Maradun, Maru	Sahel Savannah
North West	Kebbi/Federal University Birin Kebbi	Augie, Maiyama, Shanga, Suru, Gwandu, Fakai	Sahel Savannah
North Central	Nasarawa/Nasarawa State University Keffi	Keana, Keffi, Kokona, Obi, Toto, Wamba	Guinea Savannah

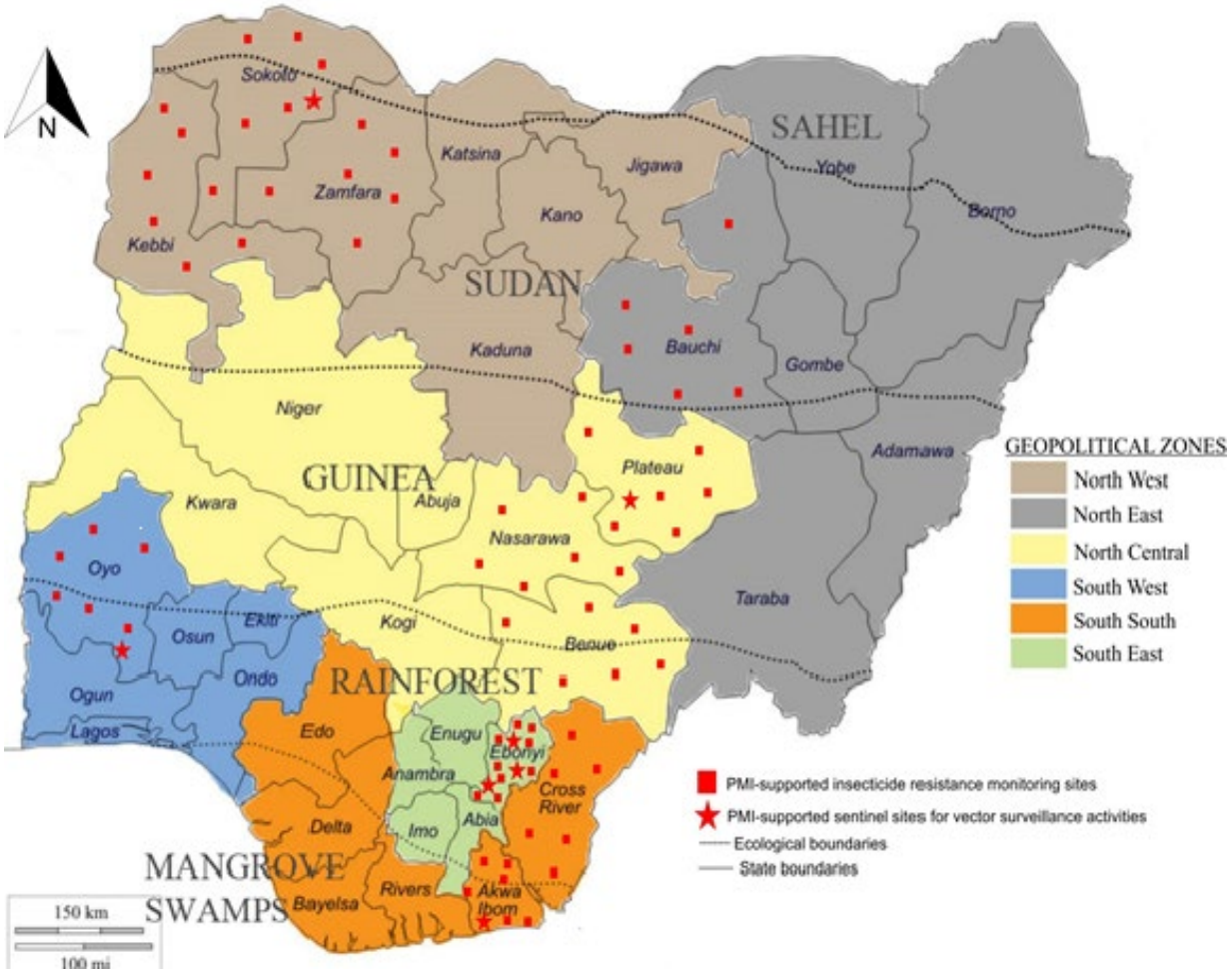

Figure 1: Map of Nigeria Showing the Sentinel Sites and Insecticide Resistance Monitoring Sites

From October 2019 to September 2020, *Anopheles* mosquitoes were collected monthly from five 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 adulthood 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 below.

Table 3: Longitudinal Monitoring Adult Mosquito Collection Methods

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

Table 4: Entomological Surveillance Indicators

Indicator	Definition
Indoor resting density	Number of adult female vectors collected indoors per room per day. This was estimated from PSC collections.
Human biting rate	Number of female Anopheles vectors attempting to feed or freshly fed, per person per unit time. This was estimated from CDC-LT collections.
Parity rate	Proportion of adult female vectors that laid eggs. This was estimated through ovary dissection.
Sporozoite rate	Proportion of adult female vectors harboring sporozoites in their salivary glands. This was estimated using PCR method.
Human blood index	Proportion of blood-fed adult female vectors that fed on humans. This was determined with PCR method.
Entomological inoculation rate	Number of infectious bites by adult female vectors per person per unit time. Calculated as the product of human biting rate and sporozoite rate.
Resistance status	Classification of adult female vectors as confirmed resistant, 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 two human-baited CDC LTs—one indoors and one outdoors—at four houses per sentinel site for three nights each month to measure mosquito biting time and location. Collection cups were changed hourly throughout the night. The teams followed the methods outlined by Yohannes and Boelee (2012) and VectorLink Standard Operating Procedure (SOP) #1[1]. The teams sent all samples collected from the field to the centrally-located insectary at Nasarawa State University Keffi for further processing and later sent to NIMR for analyses to identify sibling species and determine sporozoite rate and bloodmeal source. The mean indoor and outdoor human biting rate (HBR) was calculated as the number of mosquitoes collected per human-baited CDC LT per night. The 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 (SOP #3). The teams sent all samples collected from the field to the centrally-located insectary at Nasarawa State University Keffi and later sent to NIMR 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] Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/

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), Kent (2006) and Coetzee (2020). All *Anopheles* specimens collected were labelled and stored individually over silica gel in Eppendorf tubes for further processing. All samples collected were sent to the centrally-located insectary at Nasarawa State University Keffi where samples were verified for accuracy of morphological identification and 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 and *An. funestus* Group

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

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 female *An. gambiae* s.l. mosquitoes (3-5 days old) reared from wild-caught larvae were exposed to pyrethroid (alpha-cypermethrin, deltamethrin, and permethrin) and organophosphate (pirimiphos-methyl) insecticides using CDC bottle bioassay methods (Brogdon and Chan, 2010; 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 *kdr* genotype frequencies were determined among *An. gambiae* s.l. using allele-specific PCR assays. Surviving mosquitoes from intensity and synergist assays across all sites were analyzed for *kdr* alleles.

2. RESULTS

2.1 MOSQUITO ABUNDANCE AND SPECIES COMPOSITION

A total of 23,028 *Anopheles* mosquitoes were collected from five sentinel sites using human-baited CDC LTs (indoors/outdoors) and PSCs. *An. gambiae* s.l. was the most abundant species across the sites, ranging from 51.7% in Oyo to 99.4% in Ebonyi (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 C provides the number of each species collected by site and collection method.

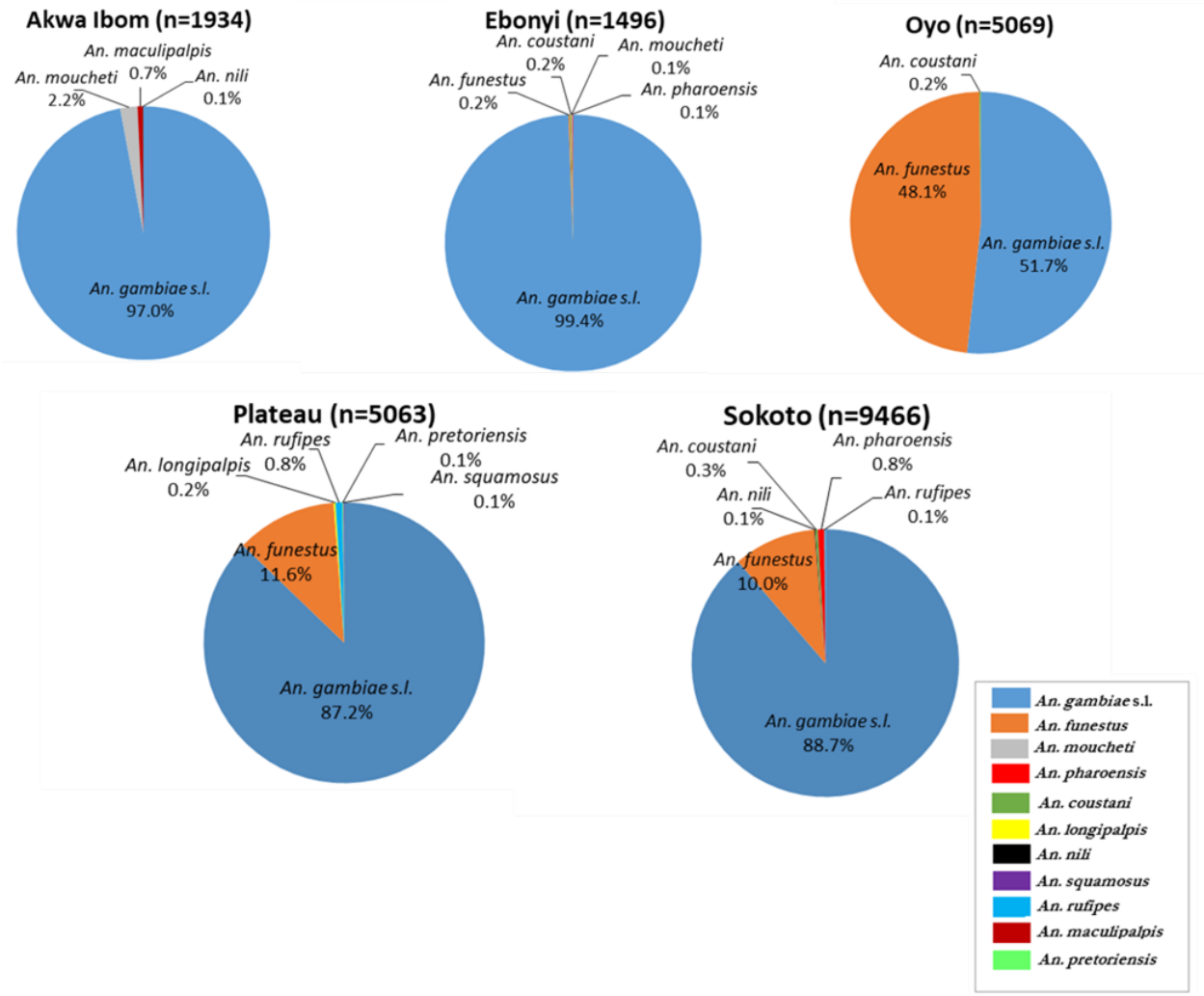

Figure 2: *Anopheles* Species Composition Across Sites

2.2 Molecular Identification of Members of the *An. gambiae* Complex and Determination of Sporozoite Rates

A total of 1,815 *An. gambiae* s.l. mosquitoes collected by PSCs and CDC LTs between October 2019 and September 2020 were subjected to species-specific PCR assays. Of these, 1,771 (97.6%) mosquito samples were amplified, (Annex B) while 44 (2.4%) failed to amplify. A total of 1,189 (67.1%) were identified as *An. gambiae* s.s.*,* 567 (32.0%) were *An. coluzzii,* 14 (0.8%) were *An. arabiensis*, and 1 (0.1%) was hybrid *An. gambiae*/*An. coluzzii* (Annex B).

An. gambiae s.s. was the dominant vector species found both indoors and outdoors in all sentinel sites, followed by *An. coluzzii*. The highest proportion of *An. gambiae* s.s. was collected outdoors in Oyo (94.1%), while the highest indoor collection was in Plateau (70.8%). The highest proportion of *An. coluzzii* collected indoors was from Oyo (39.6%), while Akwa Ibom had the highest proportion collected outdoors (38.4%). Hybrid forms were recorded indoors only in Plateau (0.4%). The proportion of *An. arabiensis* found indoors was 0.9% in three states (Ebonyi, Plateau, and Sokoto), while the highest occurrence outdoors was recorded in Plateau (3.1%) (Figure 3).

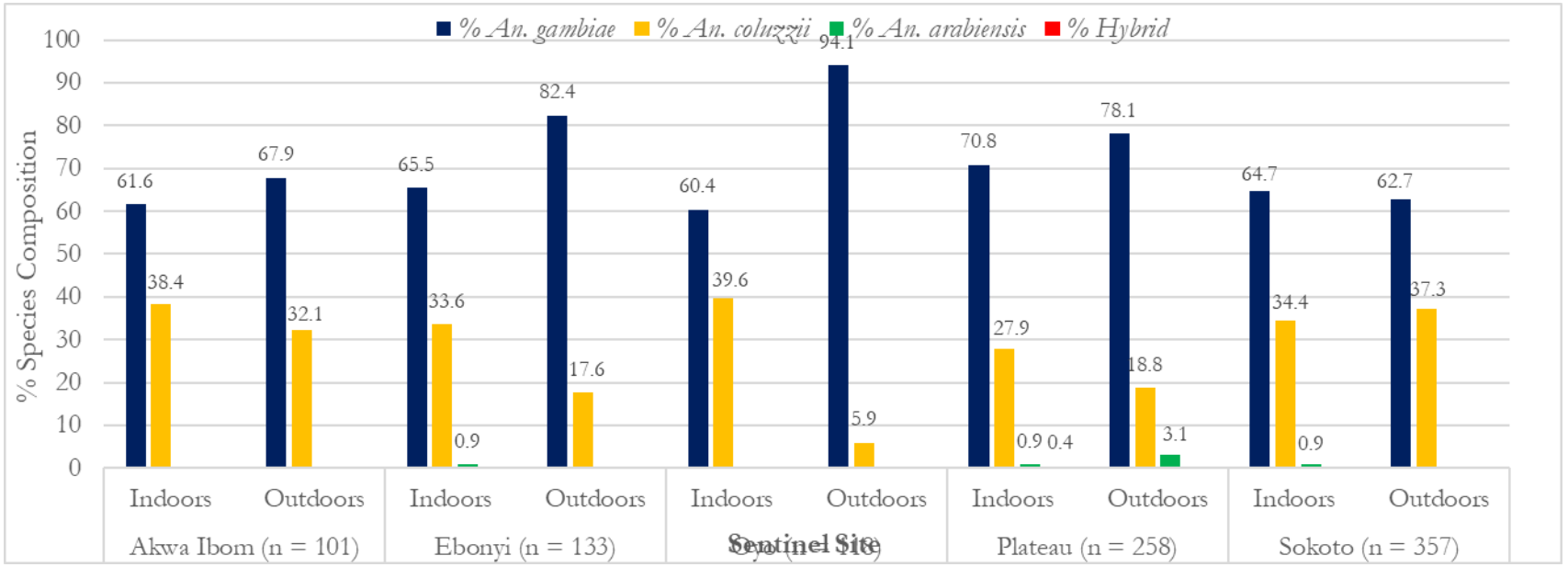

Figure 3: Proportion of *An. gambiae* s.s., *An. coluzzii*, *An. arabiensis*, and Hybrid Species Collected Indoors and Outdoors Across Sentinel Sites

As shown in Table 5, *Plasmodium falciparum* sporozoite rates of *An. gambiae* s.s. collected indoors ranged from 0.0% in Oyo, Plateau, and Sokoto to 2.2% in Akwa Ibom, while there was no sporozoite positivity recorded outdoors from any sites. There was no sporozoite infection in *An. coluzzii* and *An. arabiensis* collected indoors and outdoors across the various ecozones.

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

Site	Total Analyzed	*An. gambiae* s.s.						*An. coluzzii*						*An. arabiensis*					
		Number of *An. gambiae* s.s. (%)		No. Positive for Sporozoites		SPR (%)		Number of *An. coluzzii* (%)		No. Positive for Sporozoites		SPR (%)		Number of *An. arabiensis* (%)		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	107	45 (42.1)	19 (17.8)	1	0	2.2	0.0	28 (26.2)	45 (42.1)	0.0	0.0	0.0	0.0	0 (0.0)	0 (0.0)	0	0	0.0	0.0
Ebonyi	133	76 (57.1)	14 (10.5)	1	0	1.3	0.0	39 (29.3)	76 (57.1)	0.0	0.0	0.0	0.0	1 (0.8)	0 (0.0)	0	0	0.0	0.0
Oyo	121	61 (50.4)	16 (13.2)	0	0	0.0	0.0	40 (33.1)	61 (50.4)	0.0	0.0	0.0	0.0	0 (0.0)	0 (0.0)	0	0	0.0	0.0
Plateau	259	160 (61.8)	25 (9.7)	0	0	0.0	0.0	63 (24.3)	160 (61.8)	0.0	0.0	0.0	0.0	2 (0.8)	1 (0.4)	0	0	0.0	0.0
Sokoto	360	139 (38.6)	89 (24.7)	0	0	0.0	0.0	74 (20.6)	139 (38.6)	0.0	0.0	0.0	0.0	2 (0.6)	0 (0.0)	0	0	0.0	0.0
Total	**980**	**481 (49.1)**	**163 (16.6)**	**2**	**0**	**0.4**	**0.0**	**244 (24.9)**	**481 (49.1)**	**0**	**0**	**0.0**	**0.0**	**5 (0.5)**	**1 (0.0)**	**0**	**0**	**0**	**0.0**

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

2.3 Molecular Identification of Members of the *An. funestus* Group and Determination of Sporozoite Rates

A total of 1,015 *An. funestus* mosquitoes collected by CDC LTs and PSCs in Oyo, Plateau, and Sokoto were subjected to species-specific PCR assays. Of these, 542 (53.4%) mosquitoes were collected using CDC LTs, while 473 (46.6%) were collected using PSCs (Figures 4 and 5). *Anopheles funestus* s.s. were found to be the predominant species both indoors and outdoors in Oyo, while other unidentified *An. funestus* species predominated indoors and outdoors in Plateau (Figure 4). In Sokoto, *An. funestus* s.s. predominated indoors and an unidentified *An. funestus* species predominated outdoors (Figure 4). For members of *An. funestus* group collected using PSCs, *An. funestus* s.s. predominated in all three sites followed by unidentified species. *An. leesoni* was recorded in all sites, with the highest proportion of *An. leesoni* recorded in Plateau (5.2%) (Figure 5). Only two members of the *An. funestus* group amplified, likely due to optimization problems with the primers.

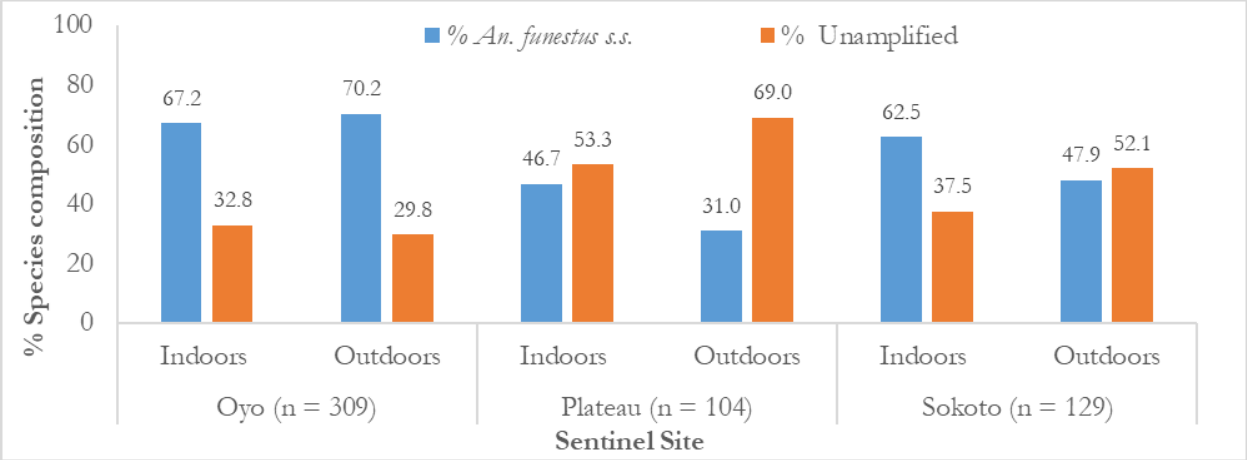

Figure 4: Proportion of Members of *An. funestus* Group Collected by CDC Light Traps Indoors and Outdoors Across Sentinel Sites

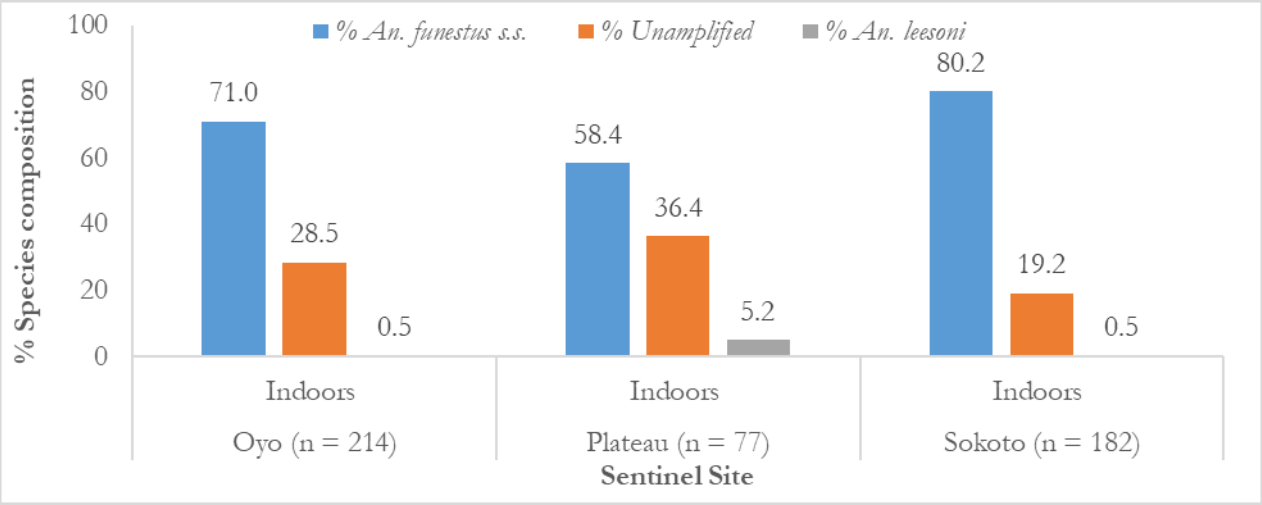

Figure 5: Proportion of Members of *An. funestus* Group Collected by PSC Across Sentinel Sites

Plasmodium falciparum sporozoite rates in *An. funestus* s.s. collected indoors from CDC LTs ranged from 0.0% in Plateau to 5.7% in Sokoto. Outdoors, sporozoite infection rates ranged from 0.0% in Oyo and Plateau to 2.9% in Sokoto. None of the other unidentified species of *An. funestus* group tested positive (Table 6). For *An. funestus* s.s. collected by PSC methods, sporozoite positivity rates ranged from 0.0% in Plateau to 1.4% in Sokoto (Table 7). Sporozoite infection rates in unidentified members of the *An. funestus* group ranged from 0.0% in Plateau to 3.3% in Oyo. None of the mosquitoes collected by PSC and identified as *An. leesoni* tested positive for the *P. falciparum* circumsporozoite antigen (Table 7).

Table 6: Sporozoite Positivity Rates of *An. funestus* Mosquitoes Collected by CDC Light Trap Indoors and Outdoors Across Sites

Site	Total Analyzed	*An. funestus* s.s.						Unamplified					
		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
Oyo	309	131 (42.4)	80 (25.9)	1	0	0.8	0.0	64 (20.7)	34 (11.0)	0.0	0.0	0.0	0.0
Plateau	104	35 (33.7)	9 (8.7)	0	0	0.0	0.0	40 (38.5)	20 (19.2)	0.0	0.0	0.0	0.0
Sokoto	129	35 (27.1)	35 (27.1)	2	1	5.7	2.9	21 (16.3)	38 (29.5)	0.0	0.0	0.0	0.0
Total	**542**	**201 (37.1)**	**124 (22.9)**	**3**	**1**	**1.5**	**0.8**	**125 (23.1)**	**92 (17.0)**	**0.0**	**0.0**	**0.0**	**0.0**

Table 7: Sporozoite Positivity Rates of *An. funestus* Mosquitoes Collected by PSC Across Sites

Site	Total Analyzed	*An. funestus* s.s.			Unamplified			*An. leesoni*		
		Number identified (%)	No. Positive for Sporozoites	SPR	Number identified (%)	No. Positive for Sporozoites	SPR	Number identified	No. Positive for Sporozoites	SPR
Oyo	54	152 (71.0)	2	1.3	61 (28.5)	2	3.3	1	0	0.0
Plateau	214	45 (58.4)	0	0.0	28 (36.4)	0	0.0	4	0	0.0
Sokoto	145	146 (80.2)	2	1.4	35 (19.2)	1	2.9	1	0	0.0
Total	**473**	**343 (72.5)**	**4**	**1.2**	**124 (26.2)**	**3**	**2.4**	**6**	**0**	**0.0**

2.4 HUMAN BITING RATES

The mean indoor biting rates of *An. gambiae* s.l. peaked in July in Plateau (103.8 bites/person/night) and in August in Sokoto (98.6 bites/person/night) (Figure 6), with an earlier smaller peak in December 2019 (Oyo 12.7 bites/person/night and Akwa Ibom 8.9 bites/person/night) . Increased outdoor biting was recorded during both July and August in Plateau (16.5 bites/person/night) and Sokoto (35.3 bites/person/night), respectively, with an earlier smaller peak in December 2019 recorded in Sokoto (9.3 bites/person/night) and Oyo (6.3 bites/person/night) (Figure 6). Indoor and outdoor biting rates in the other sites were generally low. Biting occurred both outdoors and indoors at all sites, increasing during the late rainy season (July-August).

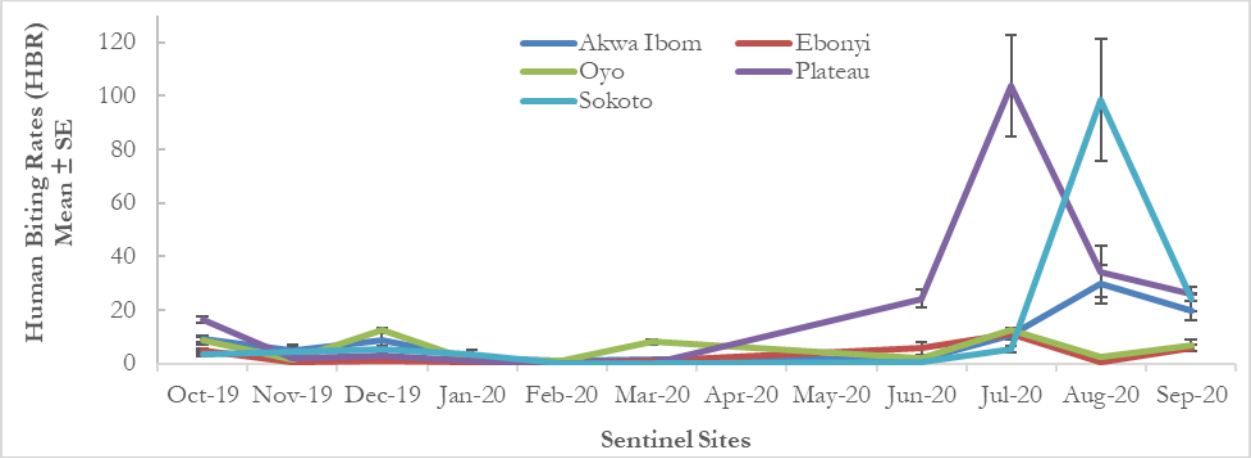

Figure 6: Monthly Indoor Human Biting Rates of *An. gambiae* s.l. Across Sites

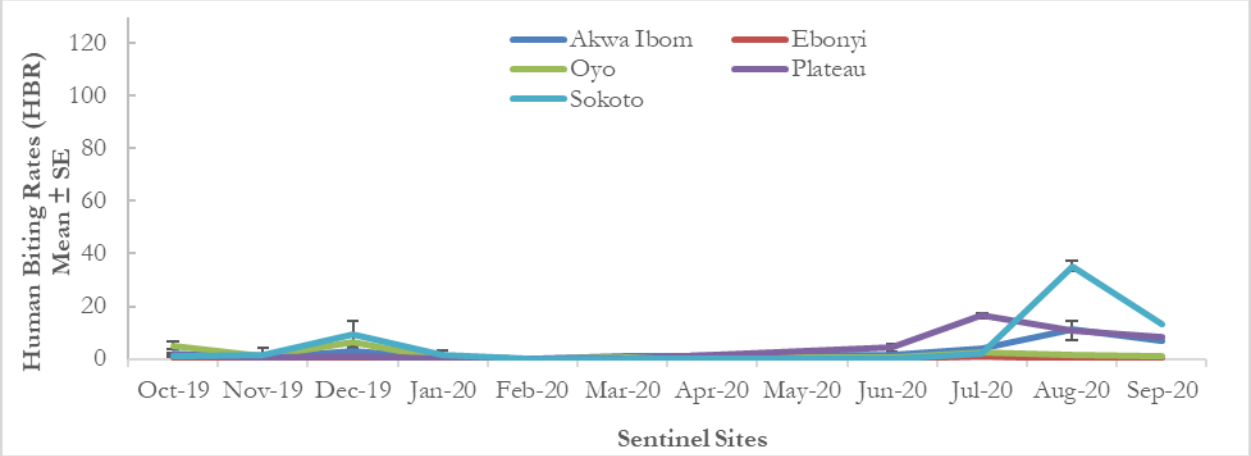

Figure 7: Monthly Outdoor Human Biting Rates of *An. gambiae* s.l. Across Sites

2.5 Monthly Indoor Resting Density of *An. gambiae* s.l.

The indoor resting density of *An. gambiae* s.l. mosquitoes varied across the sites and months, ranging from 0.1 mosquitoes/room/day in Plateau in February to 71.9 mosquitoes/room/day in Sokoto in August 2020, which is the peak of rainfall (Figure 8 and Annex G). In Sokoto, higher indoor resting densities were observed between November and December 2019, with the peak at the height of the rainy season in August 2020.

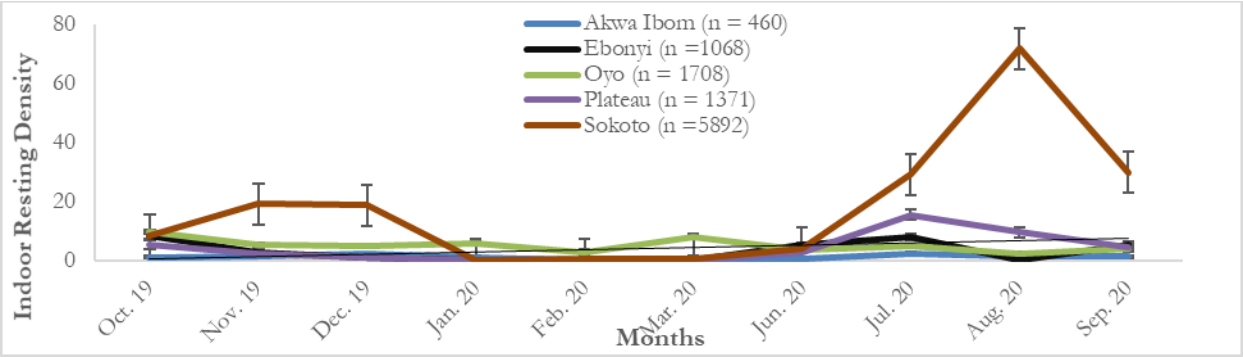

Figure 8: Monthly Outdoor Resting Density of *An. gambiae* s.l. Across Sites

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

The average number of mosquitoes caught biting per hour was generally higher indoors, ranging from 8 mosquitoes collected between 2-3 a.m. in Ebonyi to 41 mosquitoes collected between 10-11 p.m. in Plateau. Biting peaked at 10-11 p.m. and then again at 1-2 a.m. in Plateau. Biting peaked in Sokoto between 12-1 a.m. and 2-3 a.m. Outdoors, the numbers of mosquitoes caught per hour ranged from 1 mosquito collected between 10-11 p.m. in Ebonyi to 16 mosquitoes between 12-1 a.m. in Sokoto (Figure 9).

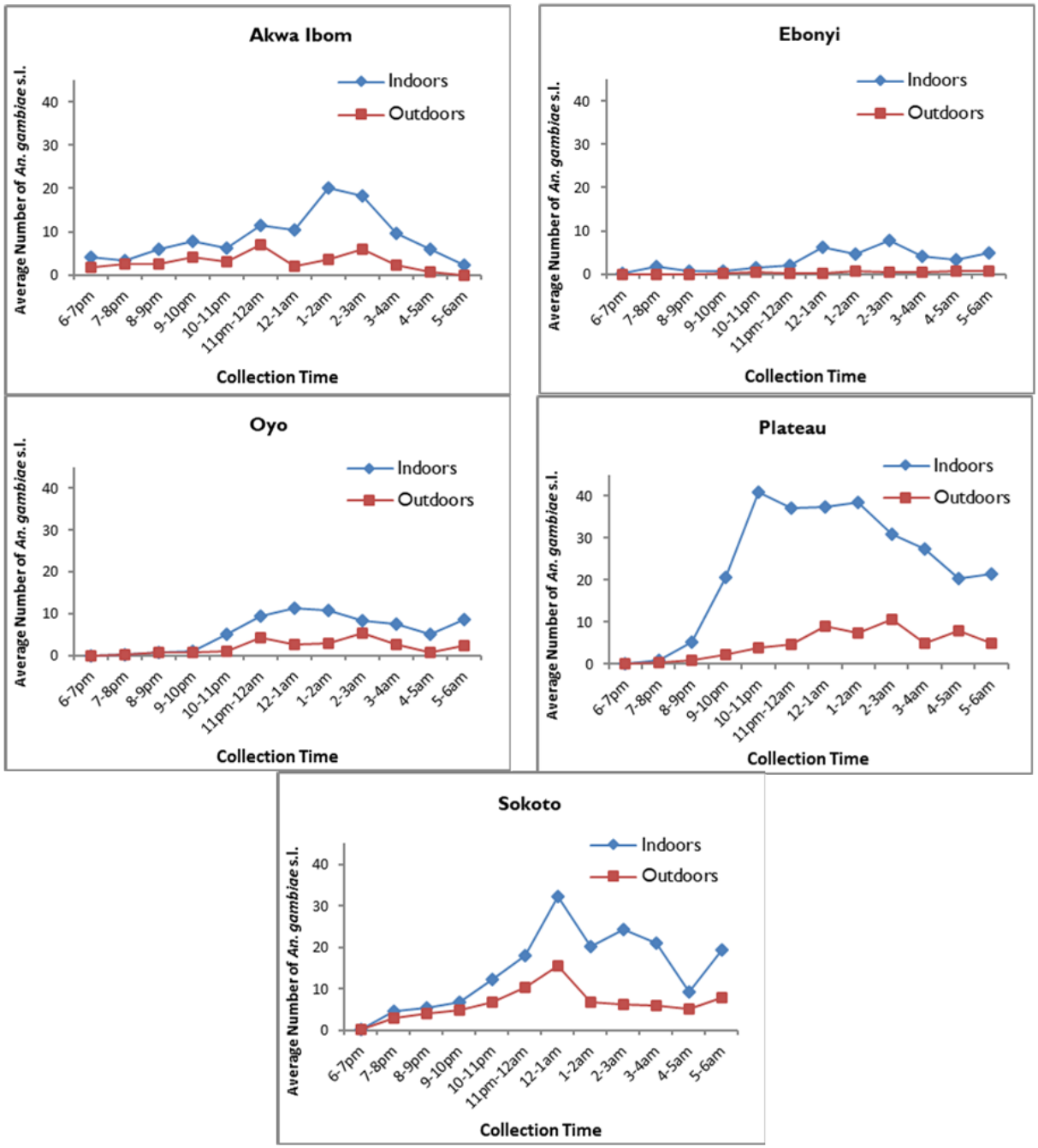

Figure 9: Average Biting Rate of *An. gambiae* s.l. Mosquitoes by Site

2.7 ENTOMOLOGICAL INOCULATION RATES ACROSS SITES

The only EIRs recorded indoors were in Ebonyi among *An. gambiae* (4.6 infective bites/person/year) and Akwa Ibom (3.0 infective bites/person/year). There were no infective bites/person/year recorded among *An. coluzzii* or *An. arabiensis* indoors or outdoors across the sites (Figure 10, and Annexes E-F), since there was no sporozoite positive sample found in these species.

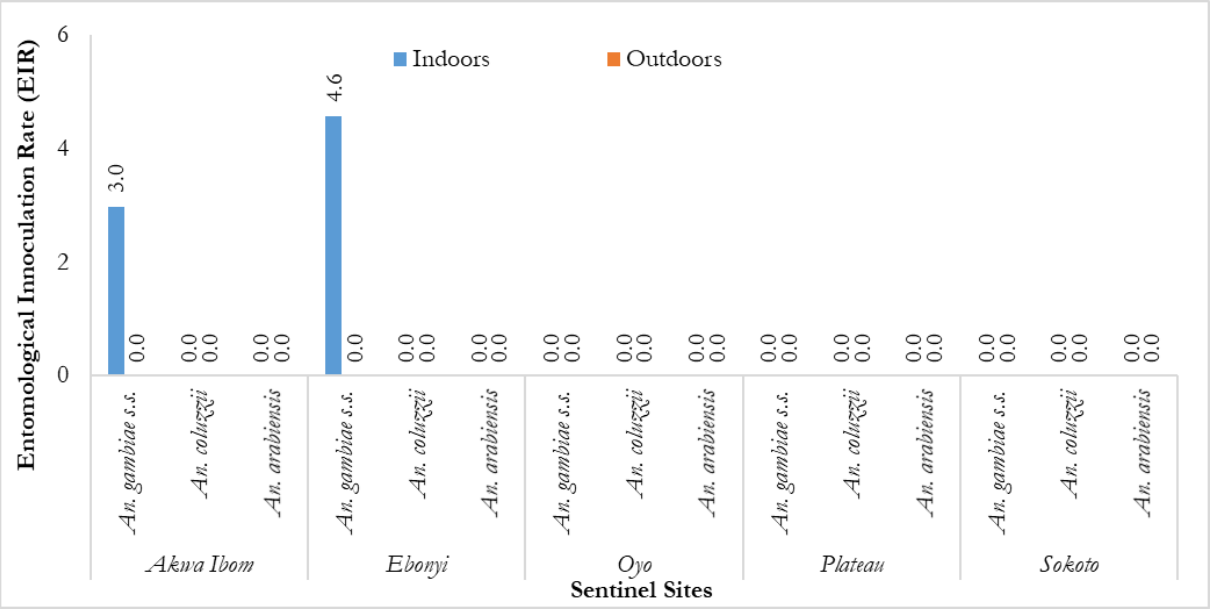

Figure 10: Annual Entomological Inoculation Rates Across Sites

2.8 HUMAN BLOOD INDEX

Across the sites, human blood index (HBI) analysis detected human blood meals in varying proportions in *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* collected using CDC LT and PSC methods (Figures 11-13). The proportion varied by vector and site. The highest proportions of *An. gambiae* mosquitoes from CDC LT that fed on human blood were from Akwa Ibom (100%), Ebonyi (100%), and Oyo (100%), while the highest proportions for *An. coluzzii* were recorded indoors in Akwa Ibom and Ebonyi (both 100%). Generally, human blood meal from CDC LT indoors (70-100%) was higher than CDC LTs placed outdoors (50-100%). HBI in mosquitoes collected by PSC (94-100%) was higher compared to both indoor and outdoor CDC LT collections (50-100%). *An. gambiae* s.s. and *An. coluzzii* collected from CDC LT outdoors in Akwa Ibom fed on human and bovine equally (50% apiece) (Figure 12). In Ebonyi, all *An. gambiae* s.s. mosquitoes collected from CDC LT outdoors fed on human blood, while all *An. coluzzii* fed on bovine blood. The single *An. gambiae* s.s. collected from CDC LT outdoors in Plateau was found to have fed on goat blood. Fewer numbers of mosquitoes from both indoor and outdoor CDC LT collections were found to have fed on human blood across the sites compared to the PSC method. The few bloodfed mosquitoes collected from CDC LTs and analyzed by PCR for blood meal source might not conclusively indicate the feeding behavior of these mosquitoes in the various sites.

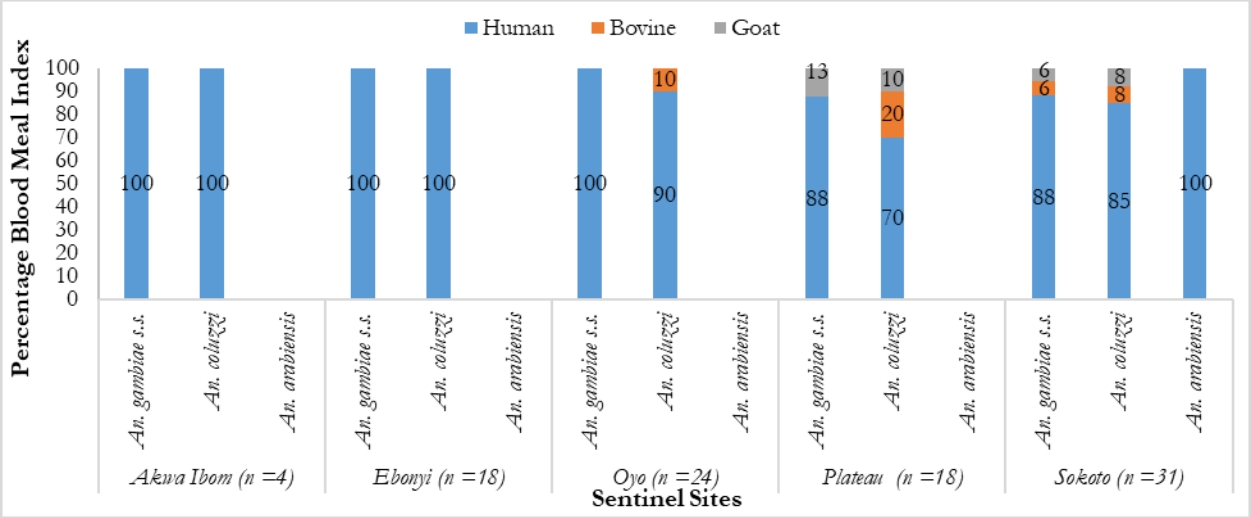

Figure 11: Blood Meal Sources of *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* from Indoor CDC Light Trap Collections Across Sites (October 2019 to September 2020)

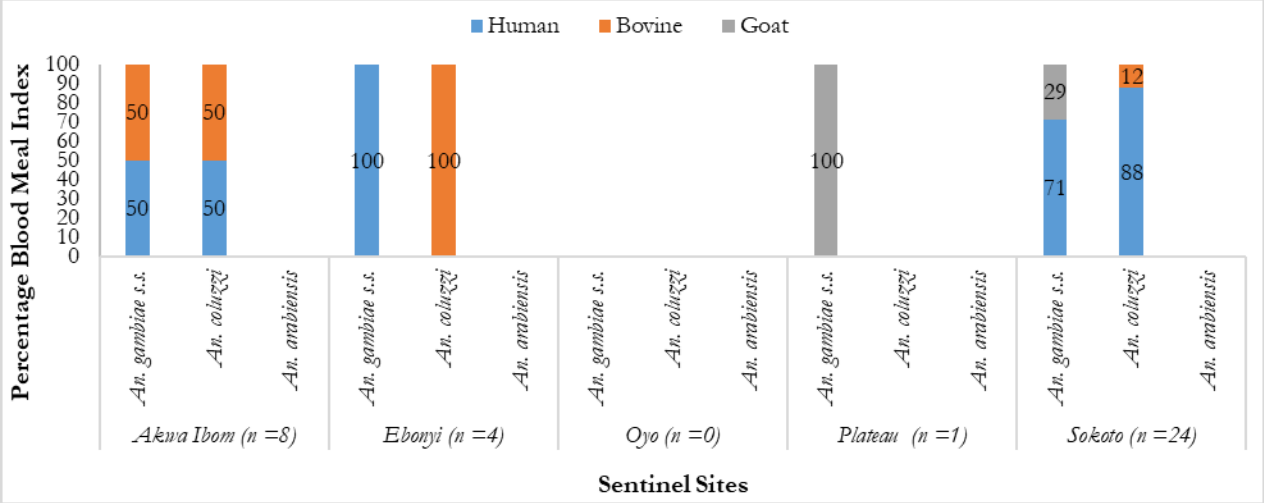

Figure 12: Blood Meal Sources of *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* from Outdoor CDC Light Trap Collections Across Sites (October 2019 to September 2020)[2]

[2] Mosquitoes analyzed for blood meal analysis were only blood fed mosquitoes selected from a pool of mosquitoes from June to September 2020 only.

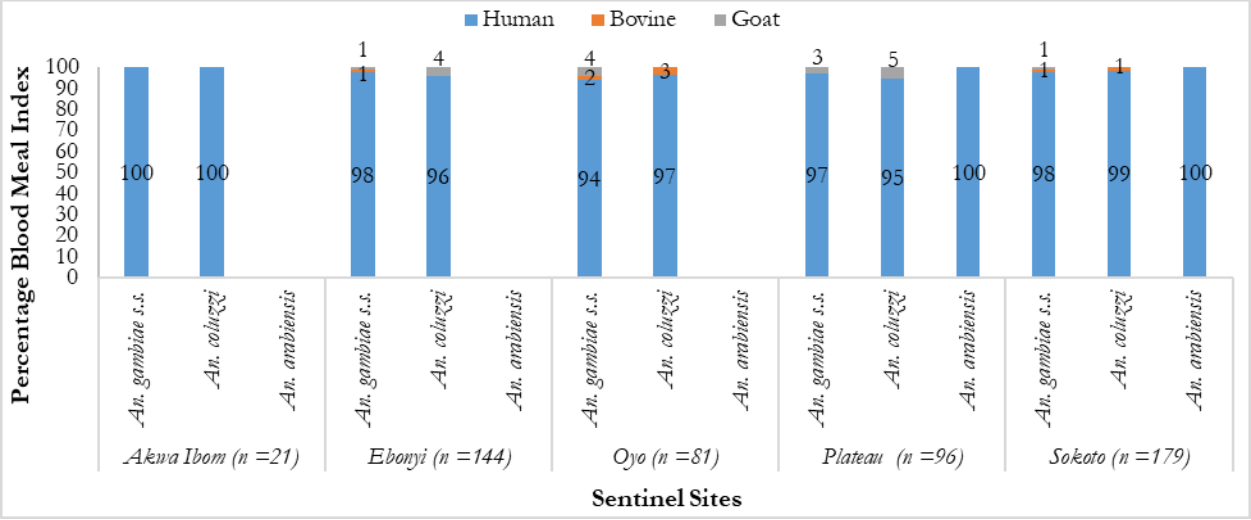

Figure 13: Blood Meal Sources of *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* from Pyrethrum Spray Catch Collections Across Sites (October 2019 to September 2020)

2.9 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, 2019, and 2020 were calculated and compared (Figure 14). Across the four years, the highest parity rate (79.2%) was recorded in Sokoto in 2020. There was no consistent reduction in the average percentage number of parous mosquitoes across the sites over the years (Figure 14).

Figure 14: Party Rates of Dissected Mosquitoes in Sentinel Sites (2017-2020)

2.10 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 sentinel sites (Table 8). Pyrethroid resistance patterns varied within and among the states. Mosquito susceptibility to alpha-cypermethrin was recorded in six LGAs each in Benue, Cross River, Oyo, and Sokoto, and four LGAs in Kebbi. Susceptibility to deltamethrin was recorded in *An. gambiae* s.l. populations in six LGAs each in Benue, Cross River, and Kebbi, and five LGAs in Oyo. Resistance to all three pyrethroids was recorded in Akwa Ibom. Deltamethrin and permethrin resistance was recorded in all six LGAs in Plateau. Out of the 69 sites where *An. gambiae* s.l. mosquitoes were tested for permethrin susceptibility, 65 indicated the presence of resistance, one indicated the presence of possible resistance, and three (all in Sokoto) indicated susceptibility.

An. gambiae s.l. susceptibility to pirimiphos-methyl was observed in six LGAs each in Nasarawa, Oyo, Sokoto, and Zamfara. *An. gambiae* s.l. mosquitoes were susceptible to pirimiphos-methyl in two of six LGAs in Akwa Ibom, four out of six LGAs in Bauchi, and five out of nine LGAs in Ebonyi (Table 8). Resistance to pirimiphos-methyl was observed in all six LGAs from Benue, Cross River, Plateau and four LGAs in Kebbi.

Table 8: CDC Bottle Bioassay Test Results for *An. gambiae* s.l. in 2020

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Alpha-cypermethrin		Deltamethrin		Permethrin		Pirimiphos-methyl	
Sentinel Site	LGA	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status
Akwa Ibom	Abak	67%	R	86%	R	29%	R	100%	S
	Itu	63%	R	87%	R	52%	R	97%	PR
	Mkpat Enin	65%	R	81%	R	24%	R	97%	PR
	Nsit Ubium	68%	R	89%	R	27%	R	98%	S
	Onna	58%	R	84%	R	30%	R	92%	PR
	Ukanafun	59%	R	83%	R	28%	R	94%	PR
Bauchi	Darazo	88%	R	78%	R	76%	R	99%	S
	Dass	81%	R	78%	R	83%	R	95%	PR
	Itas/Gadau	88%	R	96%	PR	81%	R	100%	S
	Katagun	98%	S	96%	PR	83%	R	99%	S
	Ningi	84%	R	86%	R	82%	R	98%	S
	Toro	74%	R	78%	R	69%	R	97%	PR
Benue	Apa	99%	S	99%	S	90%	PR	49%	R
	Gwer	98%	S	100%	S	81%	R	35%	R
	Obi	99%	S	98%	S	89%	R	66%	R
	Tarkaa	98%	S	100%	S	89%	R	57%	R
	Ukum	98%	S	98%	S	56%	R	33%	R
	Vandeikya	98%	S	98%	S	84%	R	85%	R
Cross River	Abi	100%	S	100%	S	68%	R	30%	R
	Akamkpa	100%	S	100%	S	51%	R	39%	R
	Calabar Municipality	100%	S	100%	S	78%	R	38%	R
	Etung	100%	S	100%	S	73%	R	57%	R
	Obudu	100%	S	100%	S	61%	R	29%	R
	Ogoja	100%	S	100%	S	70%	R	36%	R

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Alpha-cypermethrin		Deltamethrin		Permethrin		Pirimiphos-methyl	
Sentinel Site	LGA	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status	Percentage Mortality	Status
Ebonyi	Abakaliki	95%	PR	92%	PR	26%	R	100%	S
	Ebonyi	90%	PR	91%	PR	76%	R	100%	S
	Ezza North	88%	R	81%	R	37%	R	95%	PR
	Ezza South	91%	PR	85%	R	19%	R	99%	S
	*Ishielu	87%	R	82%	R	65%	R	69%	R
	Izzi	84%	R	86%	R	78%	R	100%	S
	*Ohaozara	86%	R	84%	R	76%	R	90%	PR
	Ohaukwu	89%	R	80%	R	45%	R	99%	S
	*Onicha	84%	R	85%	R	27%	R	90%	PR
Kebbi	Augie	100%	S	100%	S	72%	R	83%	R
	Fakai	100%	S	100%	S	86%	R	70%	R
	Gwandu	93%	PR	100%	S	65%	R	95%	PR
	Maiyama	100%	S	100%	S	79%	R	96%	PR
	Shanga	100%	S	100%	S	39%	R	88%	R
	Suru	97%	PR	100%	S	88%	R	87%	R
Nasarawa	Keana	83%	R	90%	PR	56%	R	98%	S
	Keffi	92%	PR	92%	PR	82%	R	98%	S
	Kokona	94%	PR	95%	PR	47%	R	99%	S
	Obi	88%	R	86%	R	60%	R	99%	S
	Toto	74%	R	92%	PR	72%	R	100%	S
	Wamba	83%	R	80%	R	63%	R	99%	S
Oyo	Akinyele	100%	S	97%	PR	53%	R	100%	S
	Ibarapa North	100%	S	100%	S	70%	R	100%	S
	Itesiwaju	100%	S	98%	S	9%	R	100%	S
	Orelope	100%	S	100%	S	26%	R	100%	S
	Saki West	100%	S	100%	S	30%	R	100%	S
	Surulere	100%	S	100%	S	20%	R	100%	S
Plateau	Bassa	90%	PR	87%	R	70%	R	82%	R
	Bokkos	86%	R	89%	R	64%	R	81%	R
	Jos-south	89%	R	87%	R	67%	R	84%	R
	Kanam	90%	PR	89%	R	69%	R	83%	R
	Mangu	90%	PR	88%	R	65%	R	82%	R
	Pankshin	86%	R	83%	R	65%	R	79%	R
Sokoto	Bodinga	100%	S	88%	R	75%	R	100%	S
	Gudu	100%	S	80%	R	98%	S	100%	S
	Kware	100%	S	95%	PR	87%	R	100%	S
	Sokoto South	100%	S	94%	PR	100%	S	100%	S
	Tambuwal	100%	S	86%	R	81%	R	100%	S
	Wamakko	100%	S	81%	R	100%	S	100%	S
Zamfara	Bakura	94%	PR	96%	PR	41%	R	100%	S
	Birnin Magaji	100%	S	100%	S	63%	R	100%	S
	Bungudu	99%	S	100%	S	54%	R	100%	S
	Gummi	89%	R	94%	PR	45%	R	98%	S
	Maradun	89%	R	94%	PR	45%	R	100%	S
	Maru	87%	R	90%	PR	39%	R	98%	S

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

Note: Diagnostic time is 30 minutes. A minimum of 100 mosquitoes were exposed each insecticide.

*Indicates expanded PBO net monitoring site. Pirimiphos-methyl tests conducted with less than 100 mosquitoes.

2.11 INSECTICIDE RESISTANCE INTENSITY

Insecticide resistance intensity in the vector population were determined by subjecting the *Anopheles* mosquitoes across the different ecozones to insecticide resistance test assays with different concentrations of three pyrethroids.

Anopheles gambiae s.l. was susceptible to alpha-cypermethrin at 1X across six LGAs in Benue, Cross River, Oyo, and Sokoto (Figures 17, 18, 22, and 24). Some of the *An. gambiae* s.l. populations in Akwa Ibom, Bauchi, Ebonyi, Kebbi, Nasarawa, and Zamfara that were initially resistant at 1X alpha-cypermethrin concentration later showed susceptibility at 2X (Figures 15, 16, 19, 20, and 21). Low-intensity alpha-cypermethrin resistance (mortality between 98–100% at 5X dose) was recorded in *An. gambiae* s.l. populations from four LGAs in Akwa Ibom, one LGA in Bauchi, one LGA in Ebonyi, and all six LGAs in Plateau (Figures 15, 16, 19 and 23).

In six LGAs each in Akwa Ibom, Nasarawa, and Sokoto, five LGAs in Bauchi, one LGA in Oyo, seven LGAs in Ebonyi, and four LGAs in Zamfara *An. gambiae* s.l. populations previously resistant at 1X concentration were susceptible to deltamethrin at 2X concentration (Figures 15, 16, 19, 21, 22, 24, and 25). Low resistance intensity to deltamethrin was recorded in *An. gambiae* s.l. in one LGA in Bauchi, two LGAs in Ebonyi, and all six LGAs in Plateau (Figures 16, 19, and 23, respectively).

Susceptibility of *An. gambiae* s.l. mosquitoes to 2X permethrin was observed in four LGAs in Bauchi, six LGAs in Benue, and three LGAs in Sokoto (Figures 16, 17, and 24). Low resistance intensity to permethrin was observed in Bauchi (Darazo and Toro LGAs), Cross River (Abi, Etung, Obudu, and Ogoja LGAs), Kebbi (Fakai, Gwandu, Maiyama, and Suru LGAs), Nasarawa (Keana, Keffi, Kokona, Obi, and Toto LGAs) and Oyo (Ibarapa North, Itesiwaju, Orelope, Saki West and Surulere LGAs) (Figures 16, 18, 19, 20, 21, and 22). Similarly, complete low permethrin resistance intensity was observed in *An. gambiae* populations across six LGAs each in Plateau and Zamfara (Figures 23 and 25). Moderate resistance (mortality less than 98% at 5X dose) to permethrin was observed in *An. gambiae* s.l. populations from Ohaozara LGA in Ebonyi and Akinyele LGA in Oyo (Figures 19 and 22, respectively). High resistance intensity to permethrin (less than 98% at 10X dose) was recorded in five LGAs in Akwa Ibom and in four LGAs in Ebonyi (Figures 15 and 19, respectively).

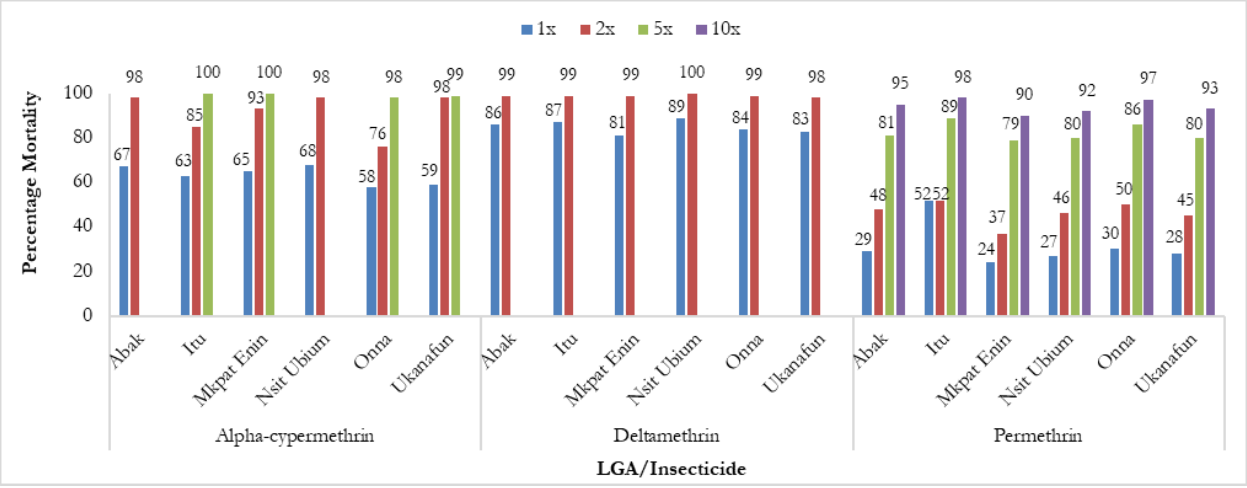

Figure 15: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Akwa Ibom

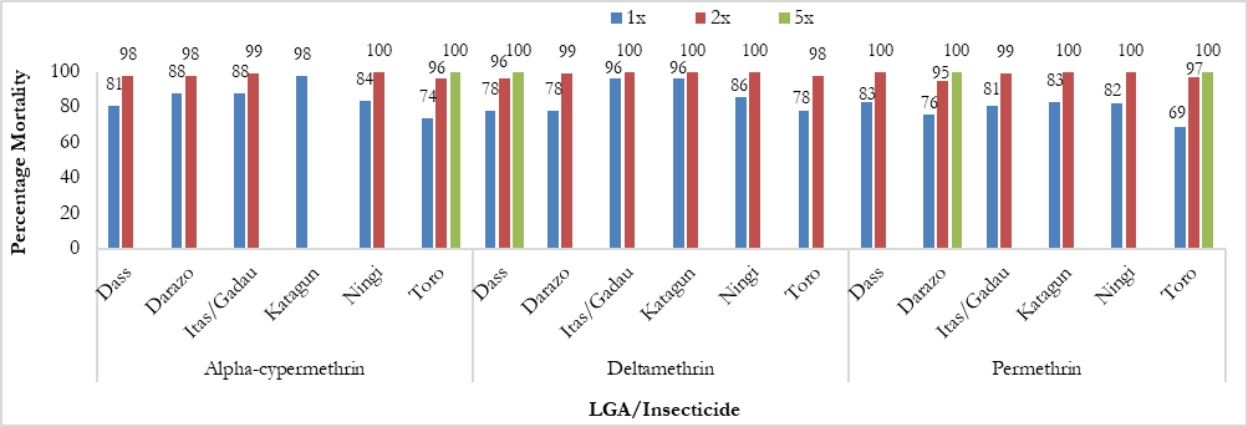

Figure 16: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Bauchi

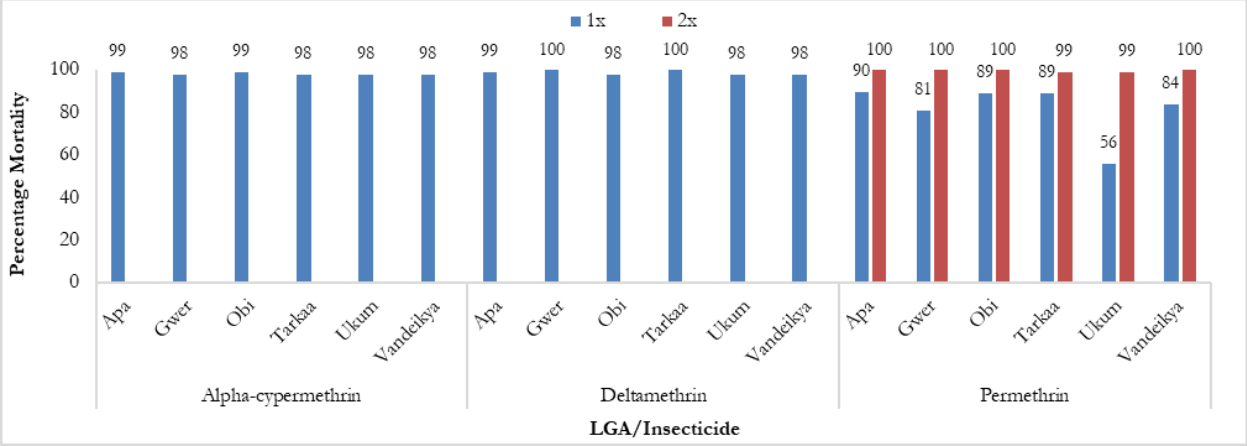

Figure 17: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Benue

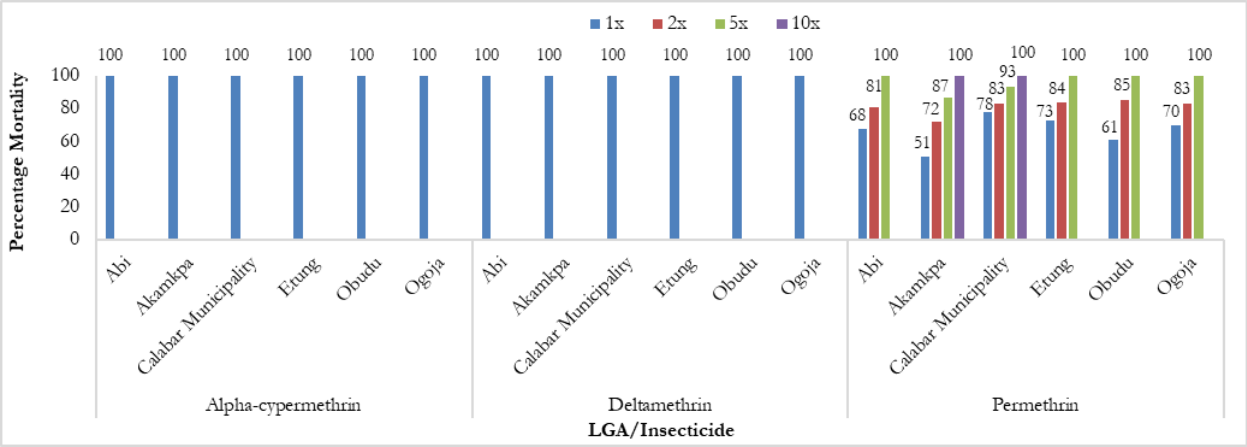

Figure 18: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Cross River

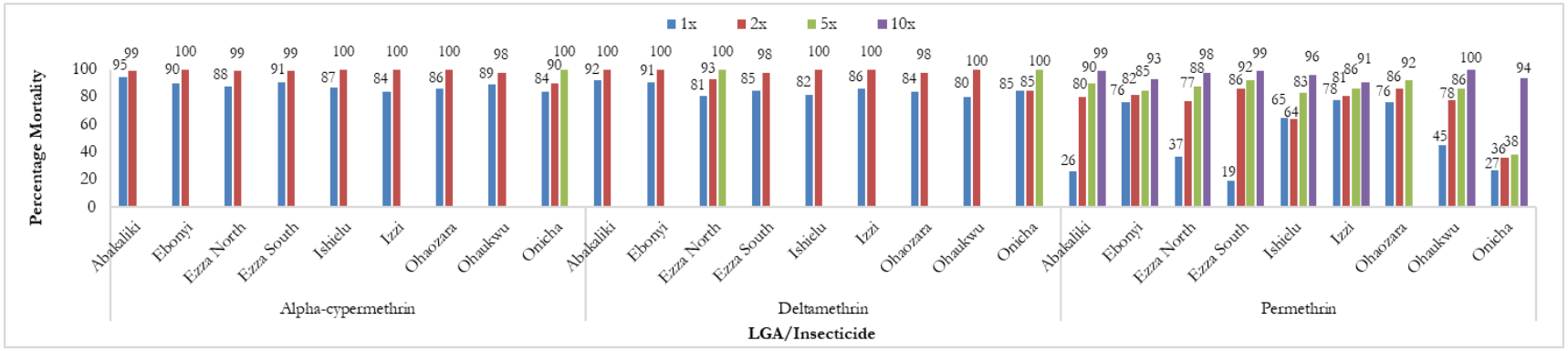

Figure 19: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Ebonyi

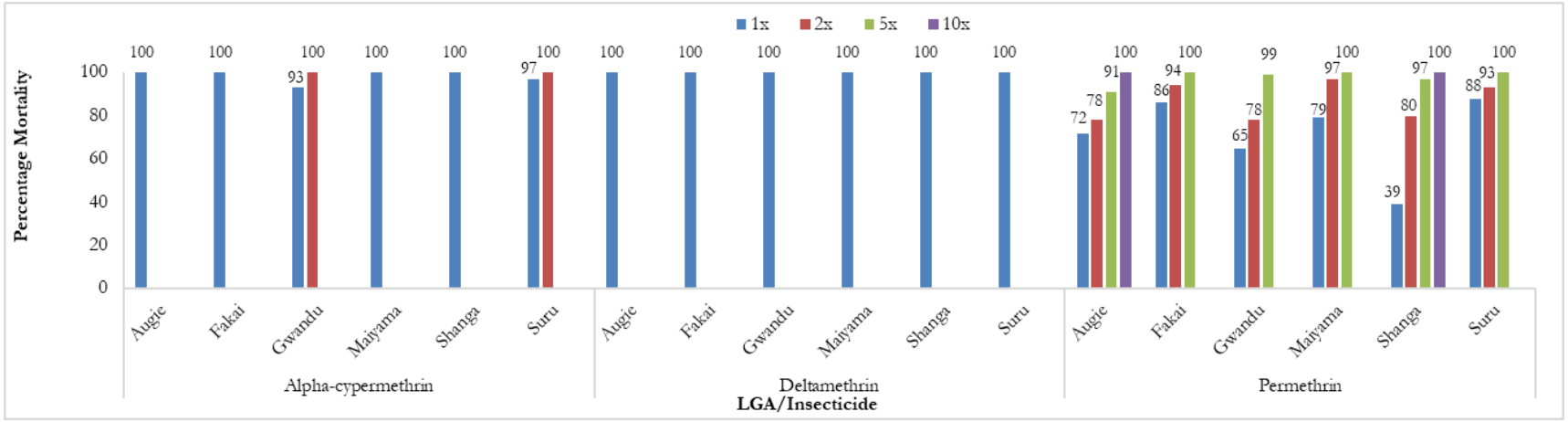

Figure 20: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Kebbi

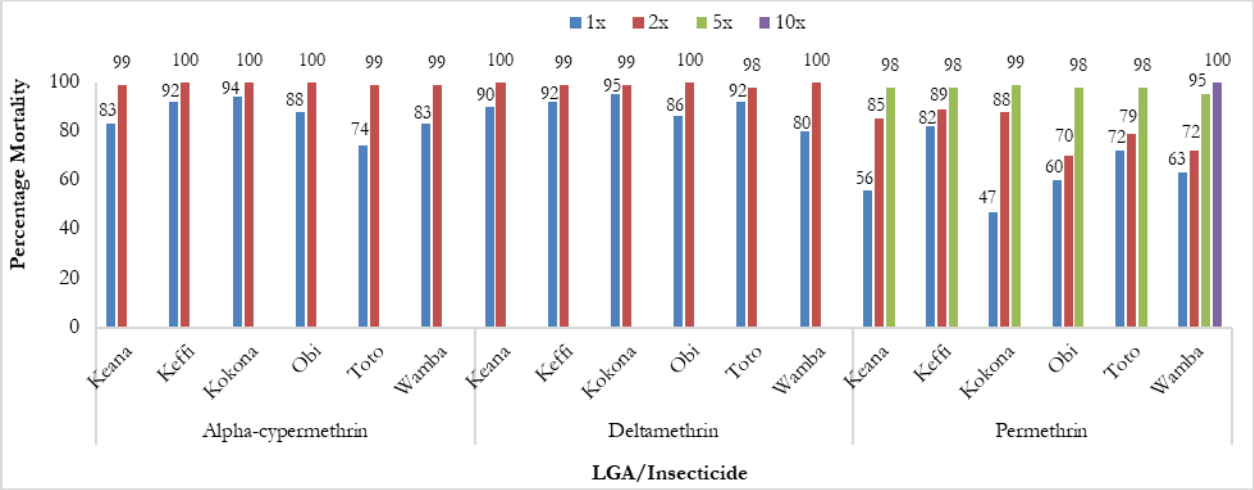

Figure 21: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Nasarawa

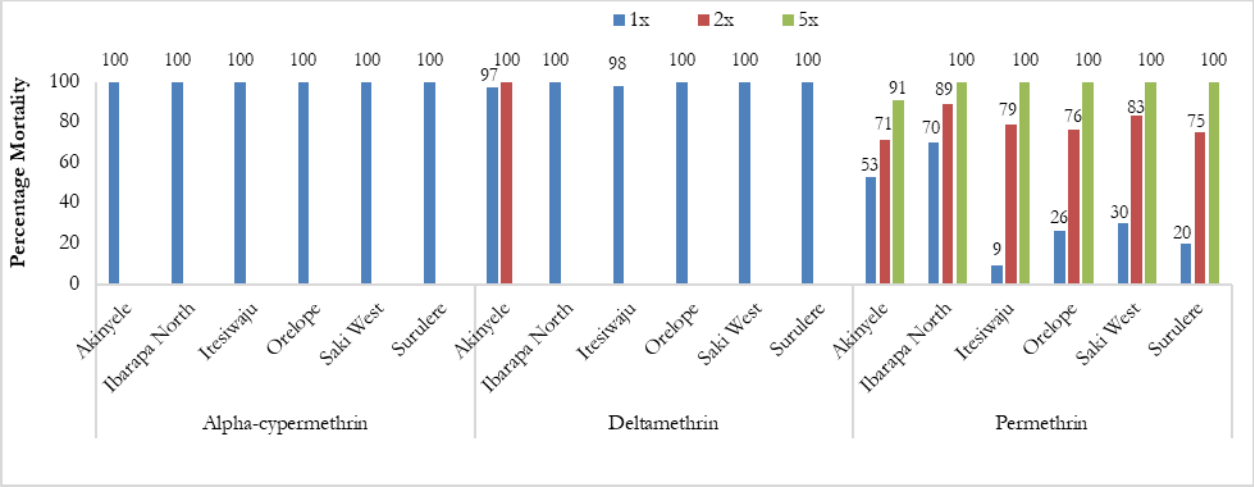

Figure 22: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Oyo

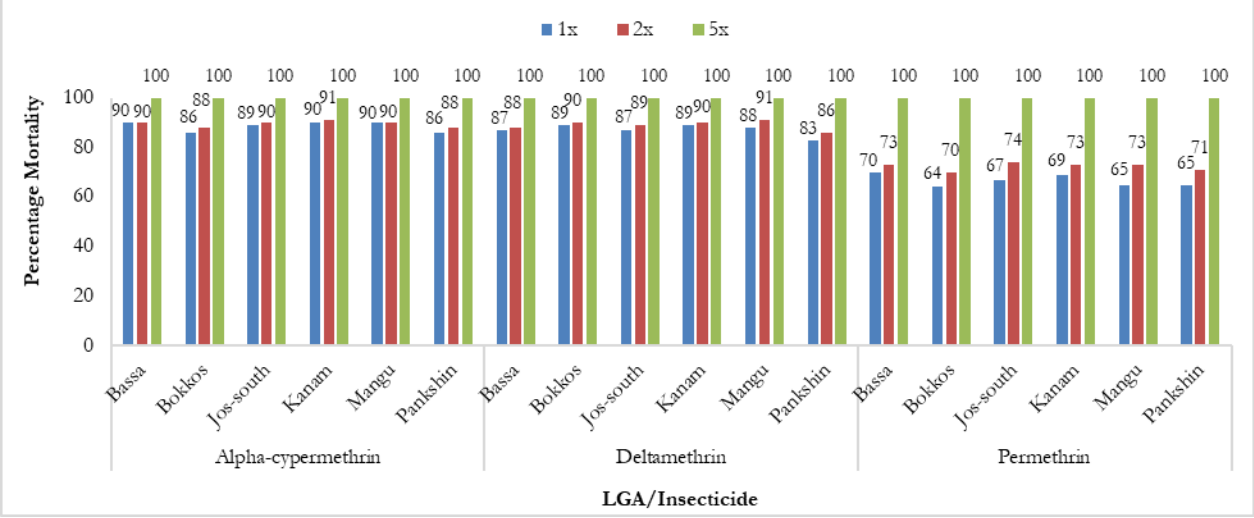

Figure 23: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Plateau

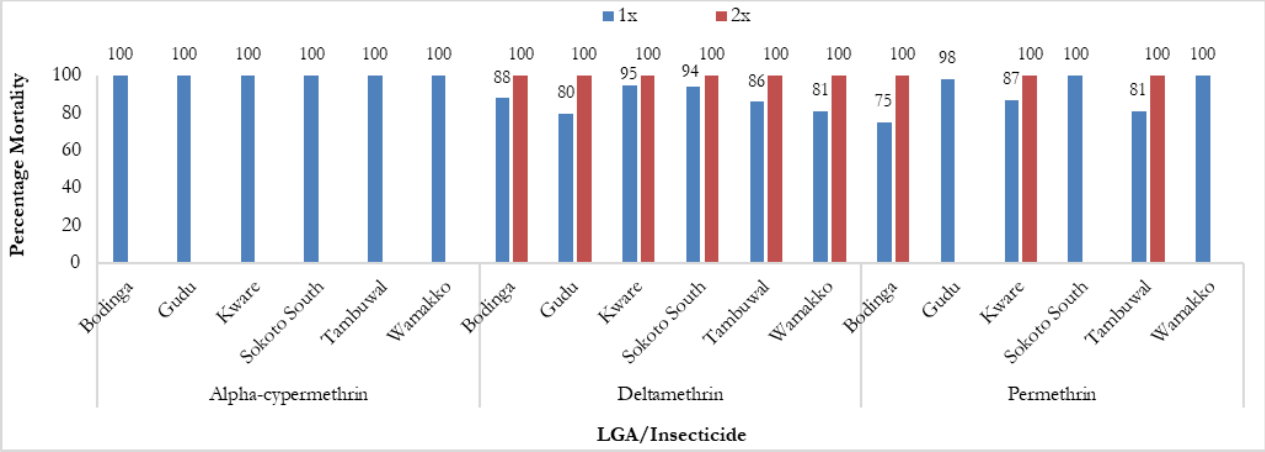

Figure 24: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Sokoto

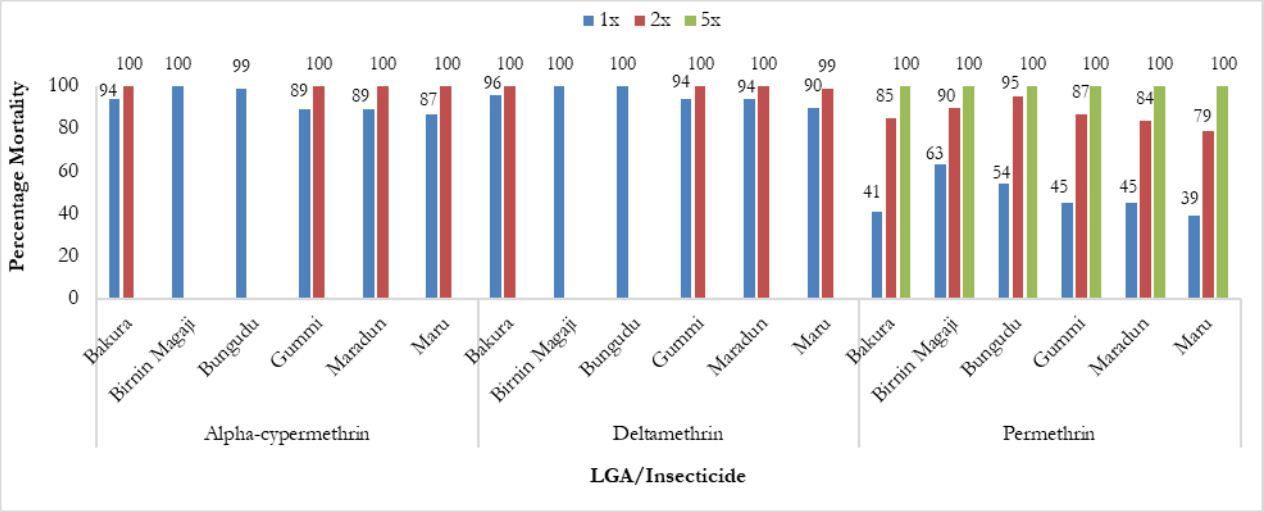

Figure 25: Pyrethroid Resistance Intensity in *An. gambiae* s.l. at Zamfara

2.12 SYNERGIST ASSAYS

Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO synergist before exposure to pyrethroids (alpha-cypermethrin, deltamethrin, and permethrin) increased mortality at varying degrees across sites (Figures 26–38). In cases where full susceptibility (mortality greater than or equal to 98%) was not restored with PBO exposure, it suggests the existence of mechanisms unrelated to the activity of mixed function oxidases.

Complete susceptibility to alpha-cypermethrin was restored in *An. gambiae* s.l. mosquitoes across all LGAs in Ebonyi (Figure 30), Nasarawa (Figure 34), Zamfara (Figure 38), and in two LGAs in Kebbi (Figure 33). Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO did not restore susceptibility to alpha-cypermethrin in the six LGAs in Bauchi and Plateau (Figures 27 and 36), and in five LGAs in Akwa Ibom (Figure 26). Susceptibility of *An. gambiae* s.l. mosquitoes to deltamethrin was restored in Akwa Ibom, Ebonyi, Nasarawa, Oyo, Sokoto, and Zamfara (Figures 26, 31, 34, 35, 37, and 38). Susceptibility to deltamethrin was not restored in *An. gambiae* s.l. mosquitoes from two out of the six LGAs in Bauchi pre-exposed to PBO synergist (Figure 27). In Plateau, susceptibility to deltamethrin was not restored in *An. gambiae* s.l. mosquitoes from the six LGAs (Figure 36). For permethrin exposures, PBO did not fully restore susceptibility in *An. gambiae* s.l. populations from all six LGAs in Akwa Ibom, Cross River, Kebbi, Nasarawa, and Plateau (Figures 26, 29, 33, 34, and 36), in five of six LGAs in Oyo and Zamfara (Figures 35 and 38), in four LGAs in Bauchi (Figure 27), and in three LGAs in Benue (Figure 28). However, pre-exposed *An. gambiae* s.l. mosquitoes tested from all LGAs in Ebonyi and Sokoto (Figures 32 and 37) became fully susceptible to permethrin.

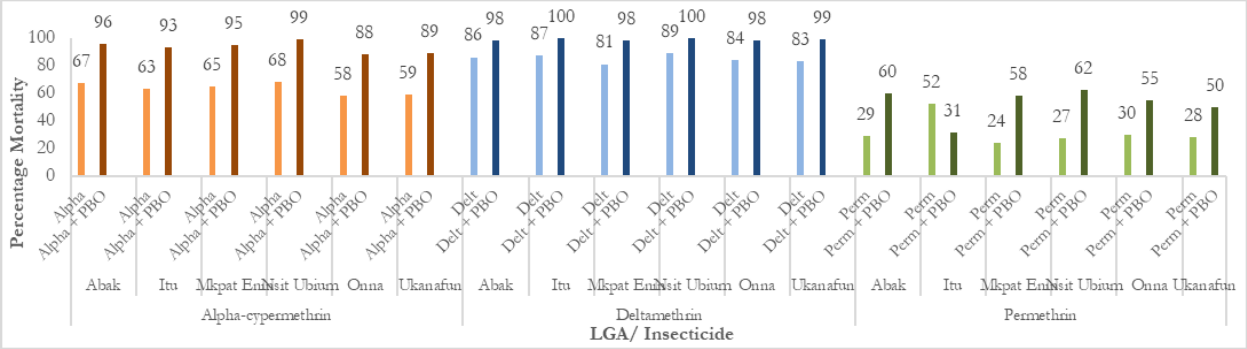

Figure 26: Synergist Bottle Assay Results for *An. gambiae* s.l. from Akwa Ibom

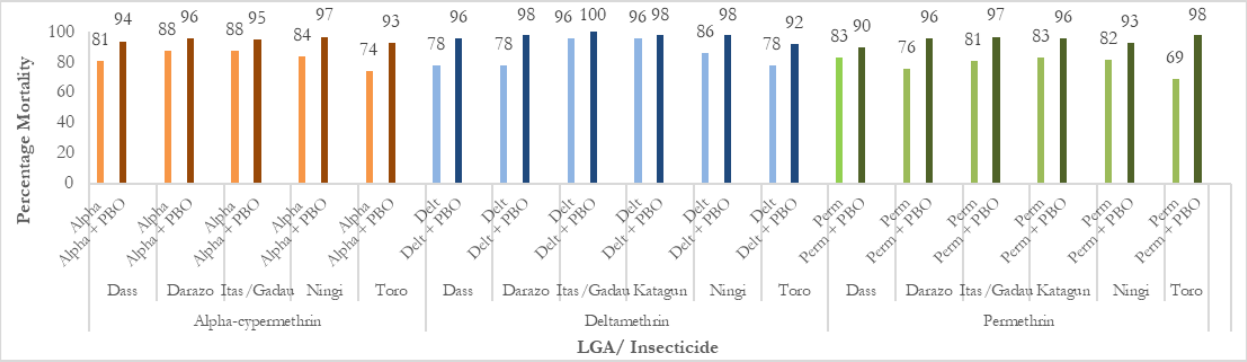

Figure 27: Synergist Bottle Assay Results for *An. gambiae* s.l. at Bauchi

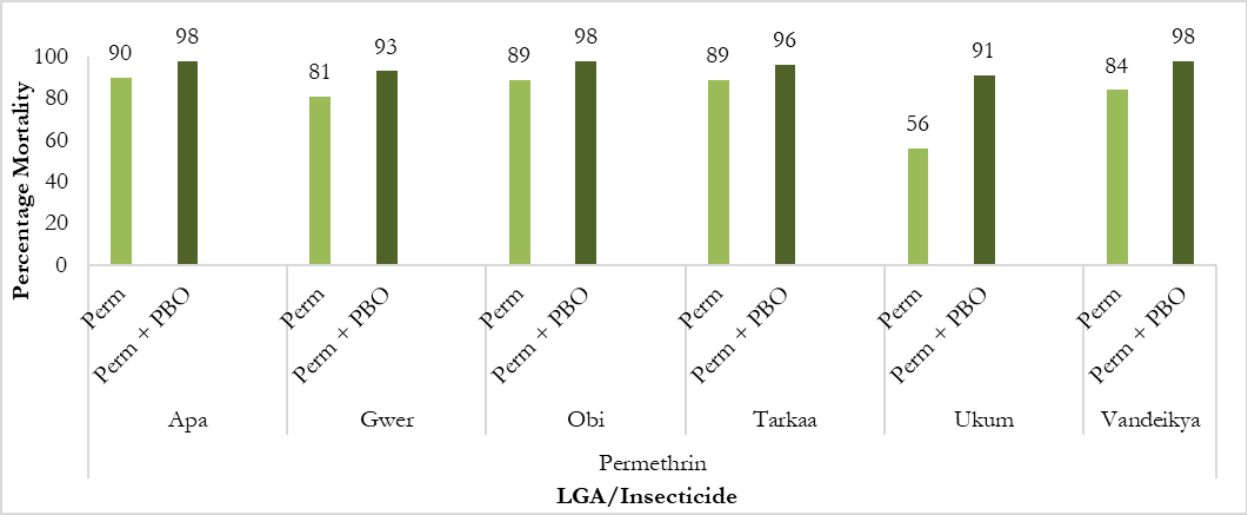

Figure 28: Synergist Bottle Assay Results for *An. gambiae* s.l. at Benue

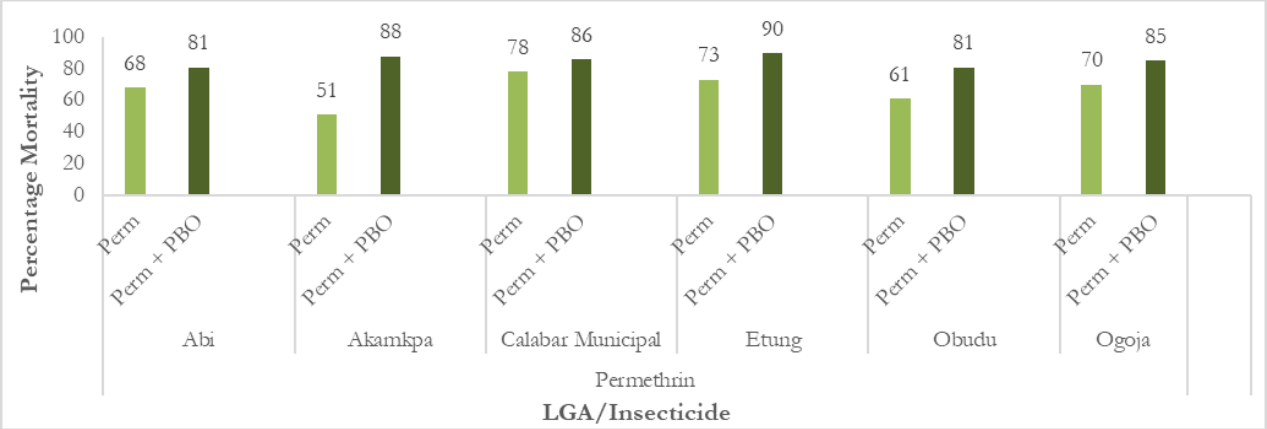

Figure 29: Synergist Bottle Assay Results for *An. gambiae* s.l. at Cross River

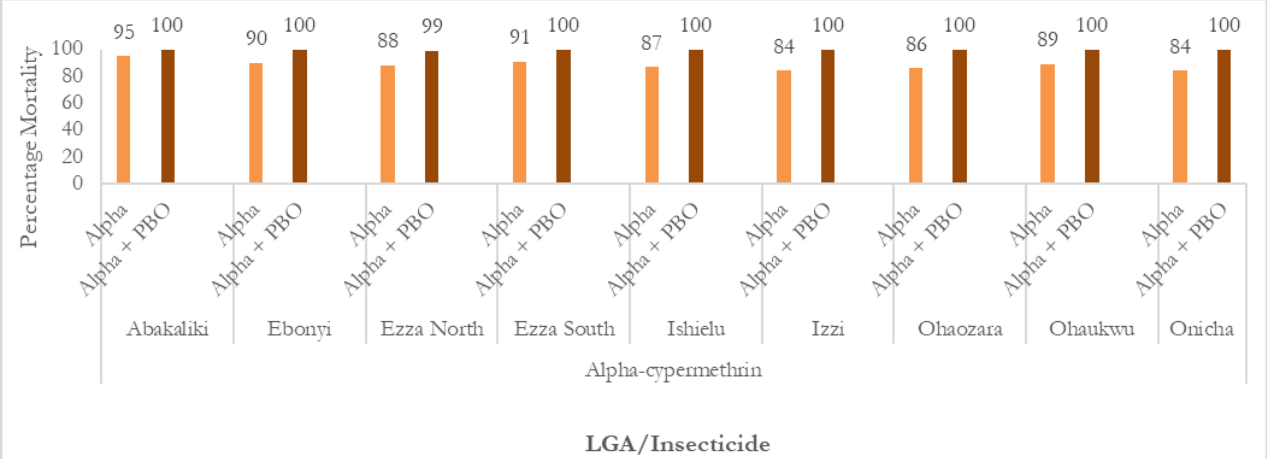

Figure 30: Synergist Bottle Assay Results for *An. gambiae* s.l. at Ebonyi (Alpha-Cypermethrin)

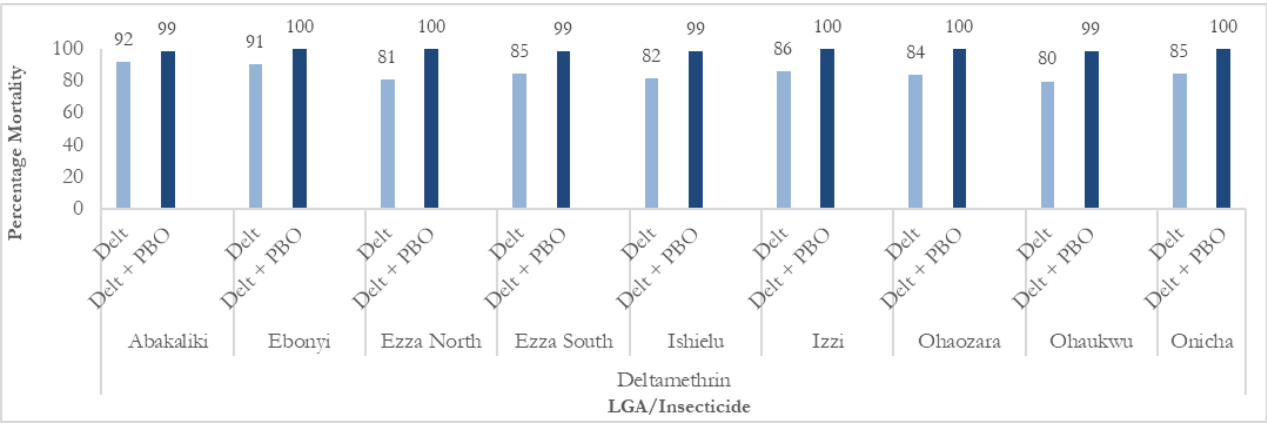

Figure 31: Synergist Bottle Assay Results for *An. gambiae* s.l. at Ebonyi (Deltamethrin)

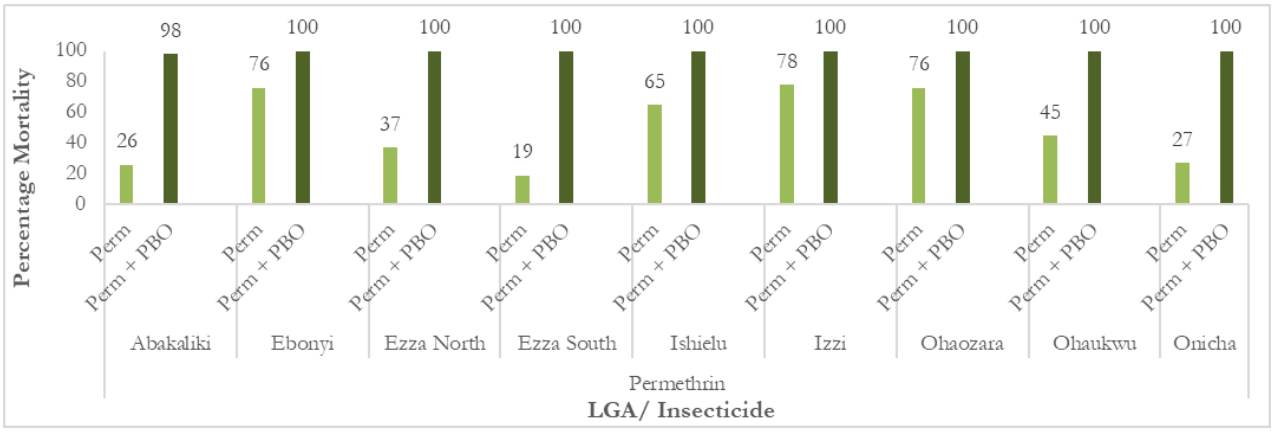

Figure 32: Synergist Bottle Assay Results for *An. gambiae* s.l. at Ebonyi (Permethrin)

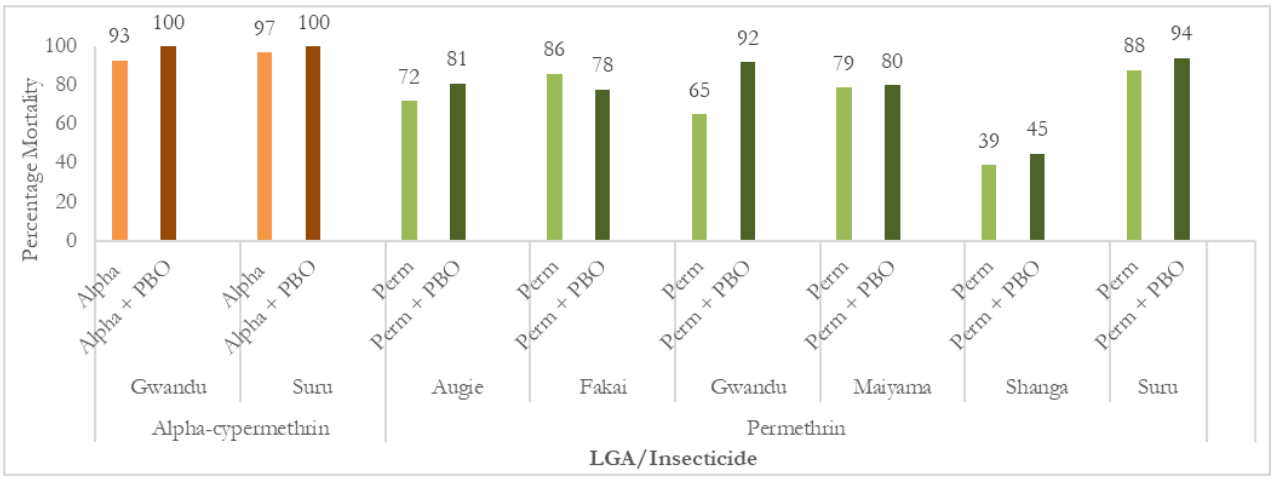

Figure 33: Synergist Bottle Assay Results for *An. gambiae* s.l. at Kebbi

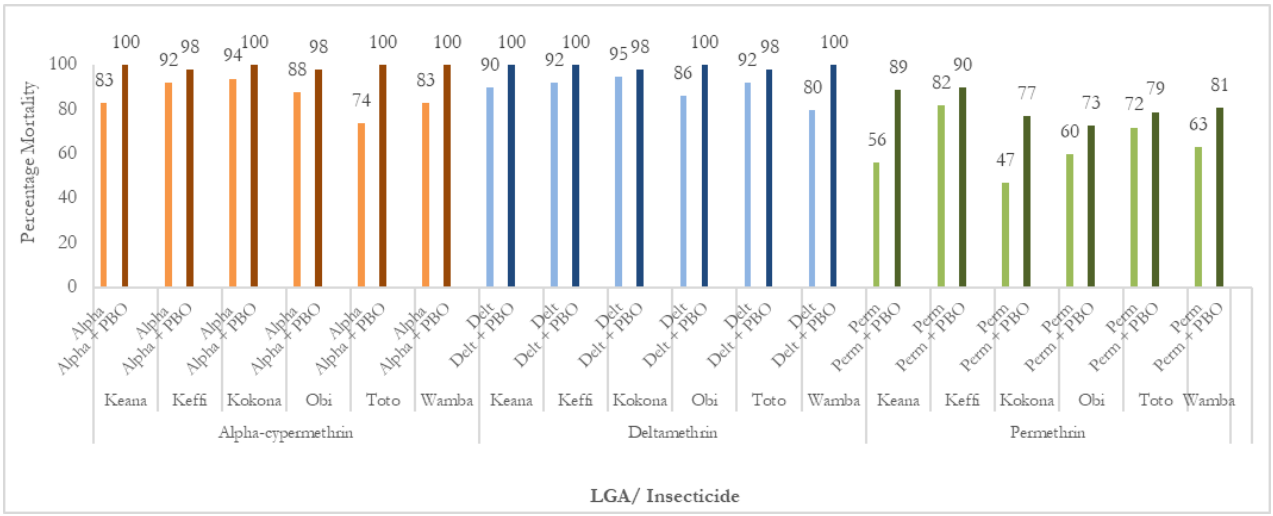

Figure 34: Synergist Bottle Assay Results for *An. gambiae* s.l. at Nasarawa

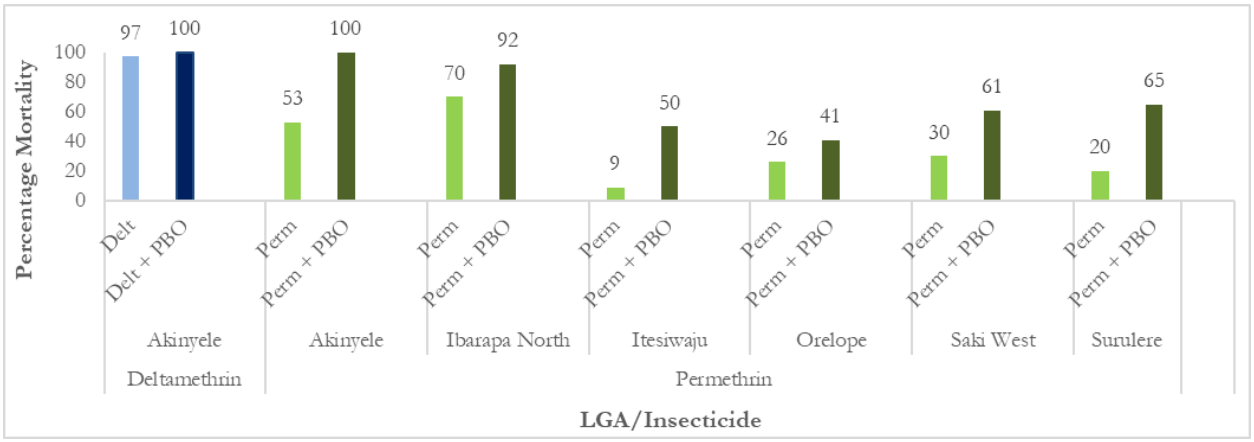

Figure 35: Synergist Bottle Assay Results for *An. gambiae* s.l. at Oyo

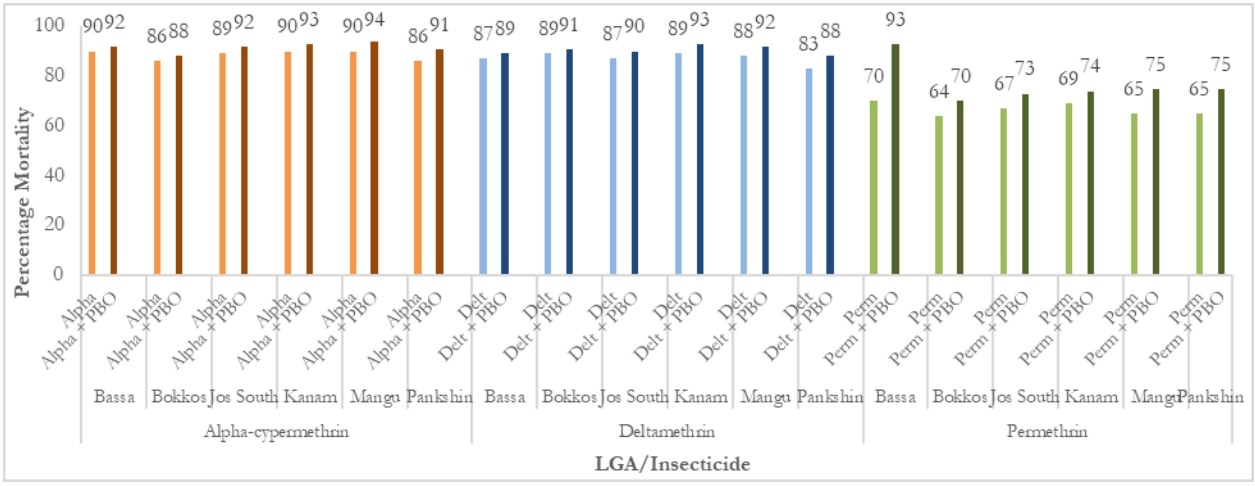

Figure 36: Synergist Bottle Assay Results for *An. gambiae* s.l. at Plateau

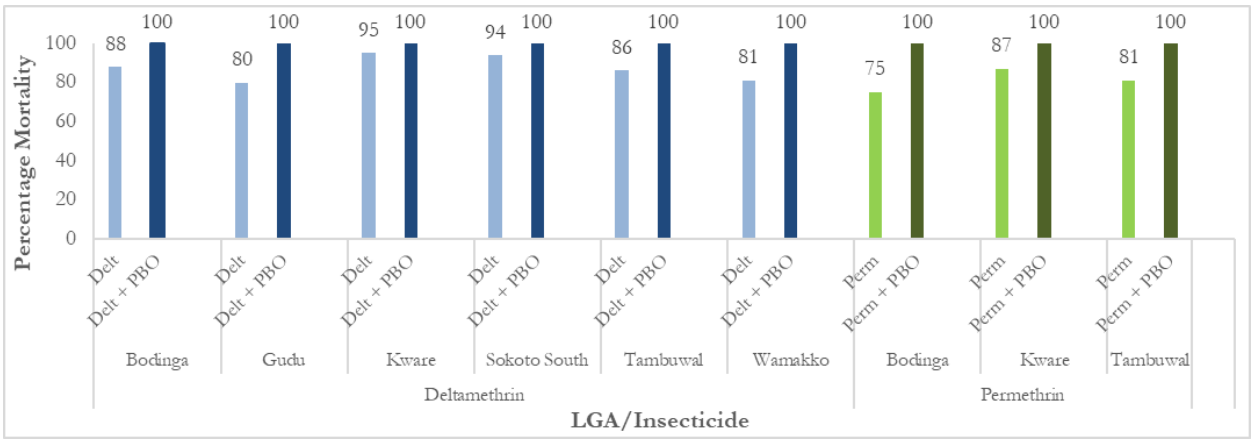

Figure 37: Synergist Bottle Assay Results for *An. gambiae* s.l. at Sokoto

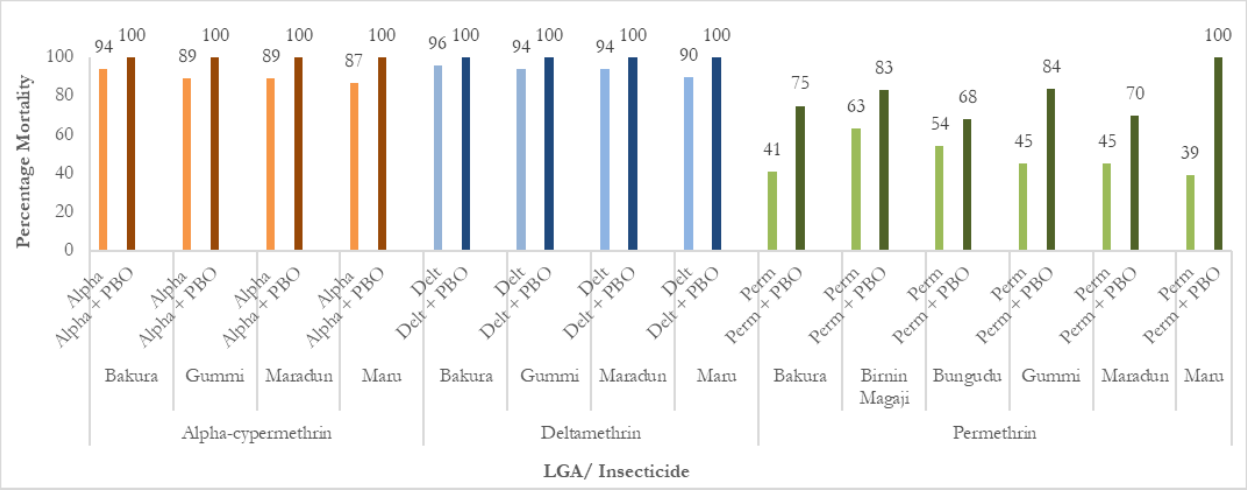

Figure 38: Synergist Bottle Assay Results for *An. gambiae* s.l. at Zamfara

2.13 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 (79–93%), Bauchi (81–97%), Benue (64–84%), Cross River (72–86%), Ebonyi (35-73%), Kebbi (73–93%), Nasarawa (52–66%), Oyo (16–49%), Plateau (42–49%), Sokoto (71–86%), and Zamfara (69–90%).

The percentage mortality after the 24-hour holding period also varied in Akwa Ibom (89–100%), Bauchi (94–98%), Benue (99-100%), Cross River (92–100%), Ebonyi (77-100%), Kebbi (100%), Nasarawa (92–100%), Oyo (100%), Plateau (93–97%), Sokoto (86–100%) and Zamfara (92-100%) (Figures 39-49).

Mortality rates in *An. gambiae* s.l. were 100% after the 24-hour holding period in all LGAs in Kebbi and Oyo (Figures 44 and 46) and after 48 hours in Benue, Oyo, Kebbi, and Zamfara (Figures 41, 44, 46, and 49). *An. gambiae* s.l. populations from all LGAs in across all ecozones were susceptible to chlorfenapyr with 100% mortality at 72 hours (Figures 39-49).

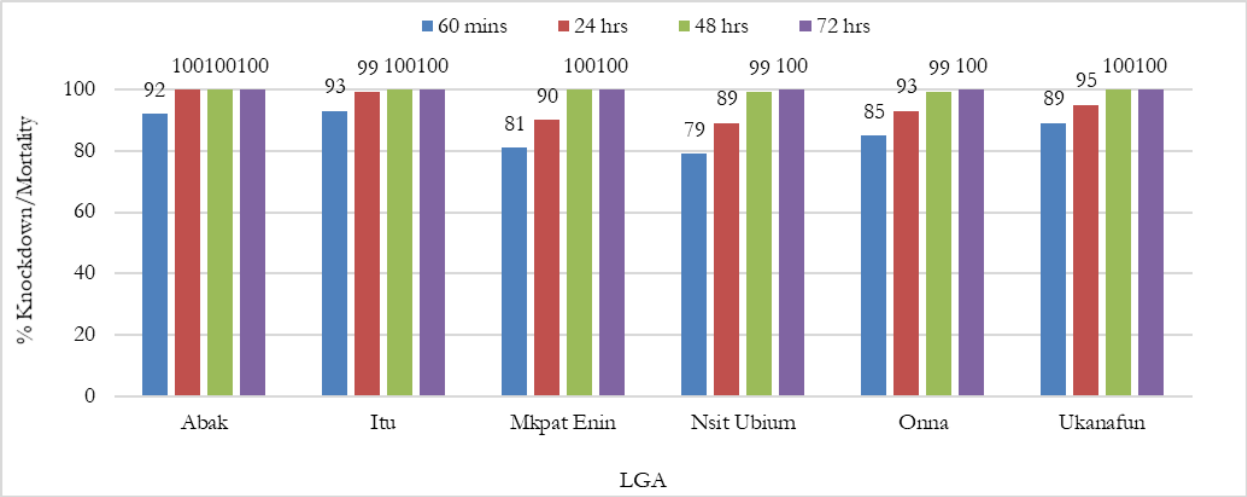

Figure 39: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Akwa Ibom

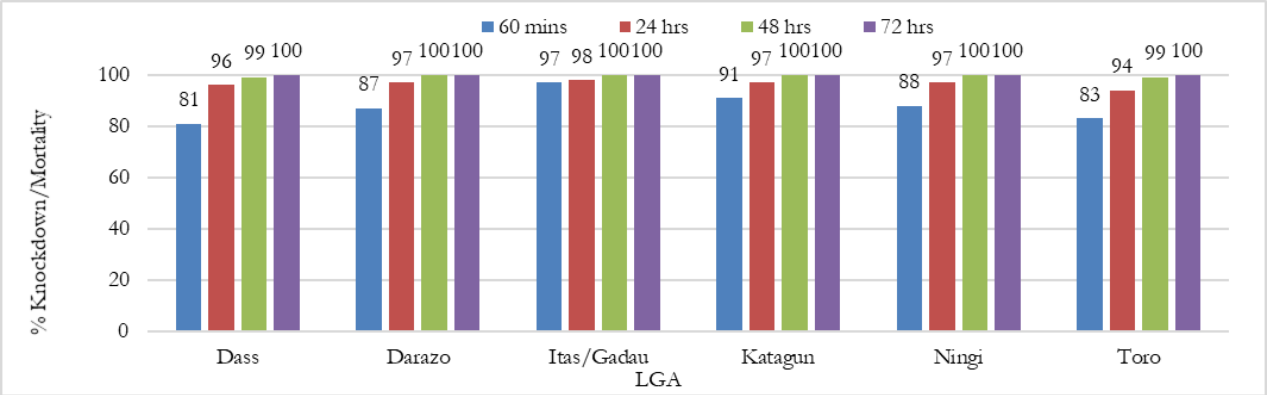

Figure 40: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Bauchi

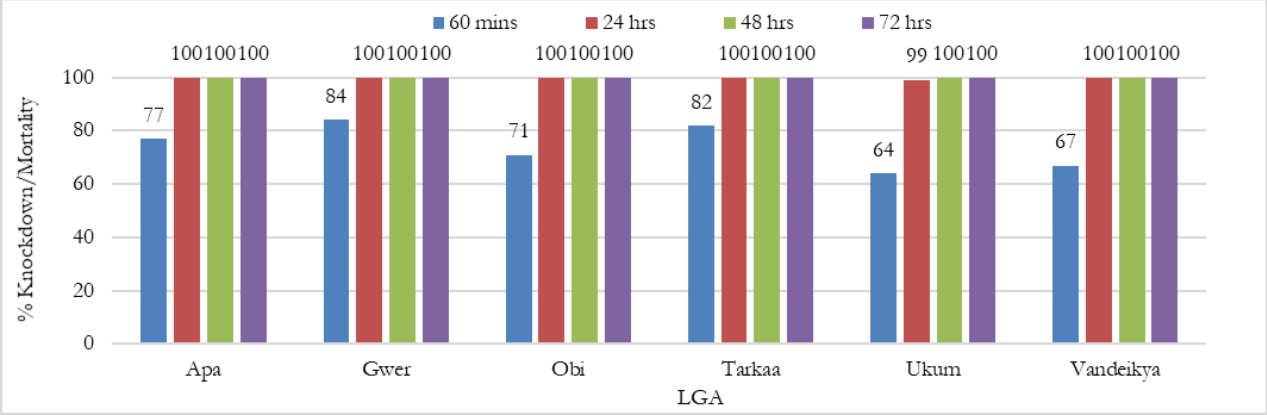

Figure 41: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Benue

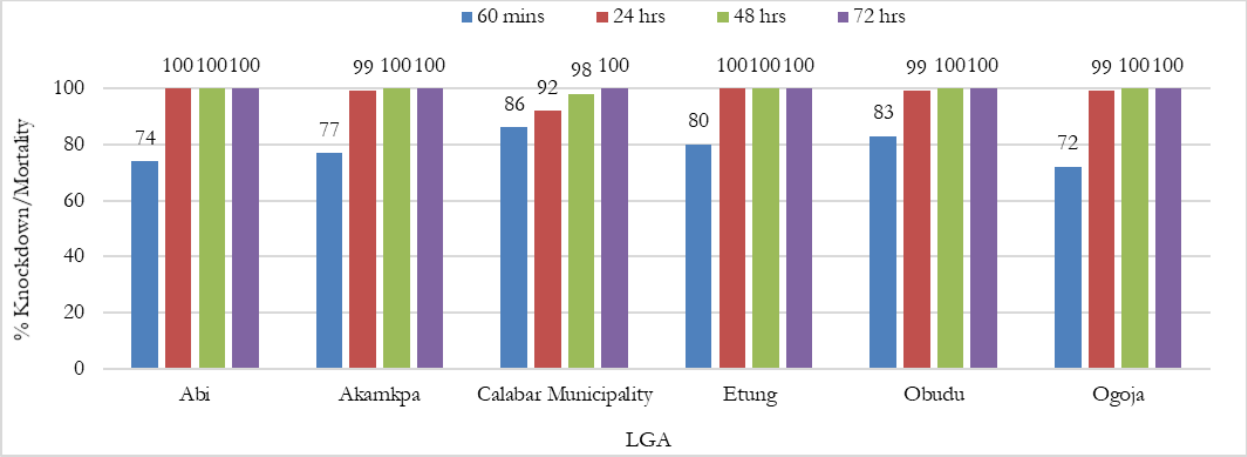

Figure 42: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Cross River

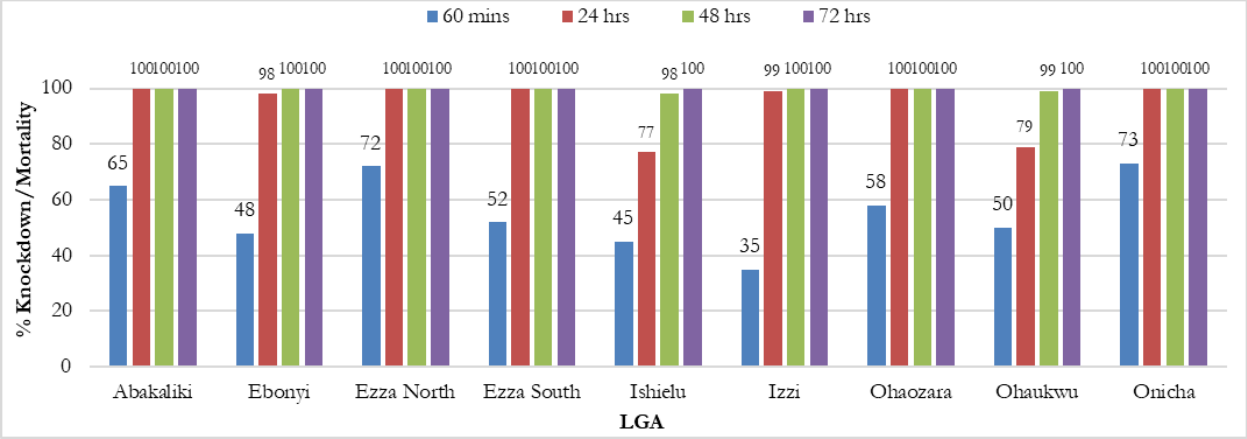

Figure 43: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Ebonyi

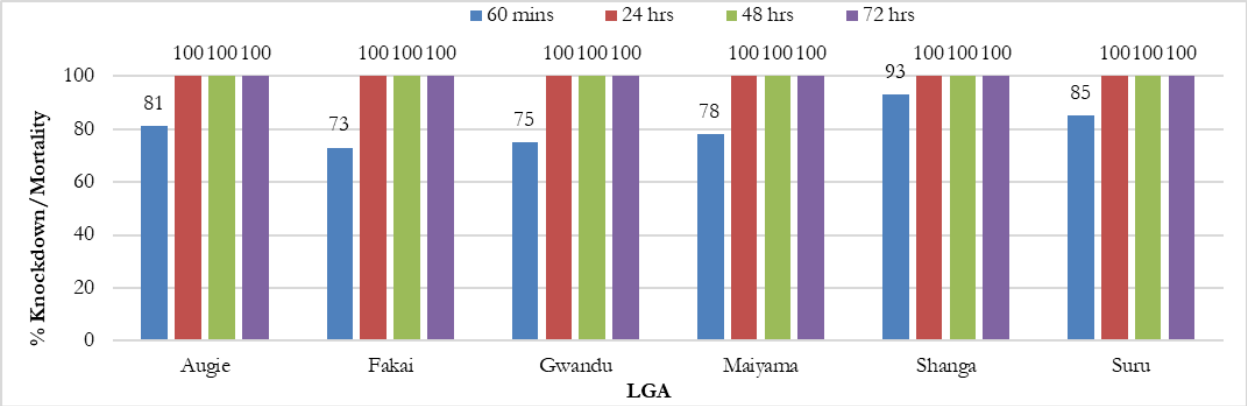

Figure 44: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Kebbi

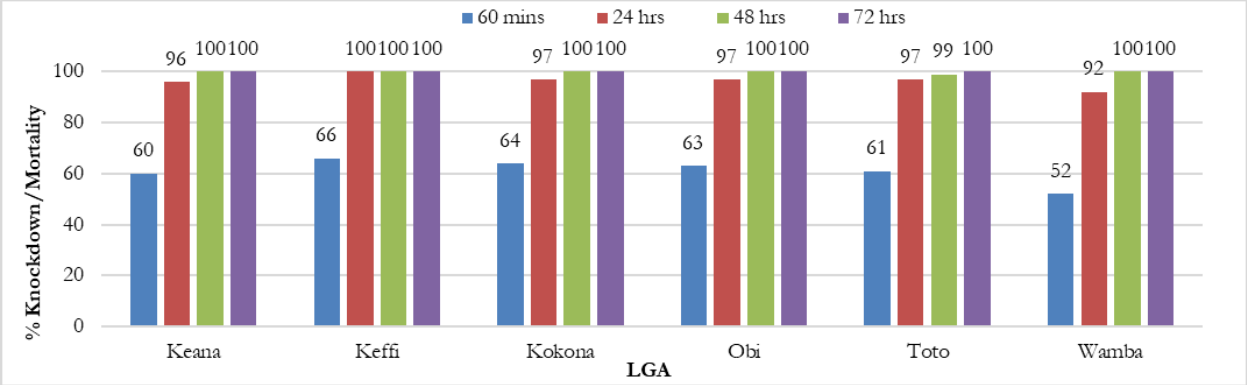

Figure 45: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Nasarawa

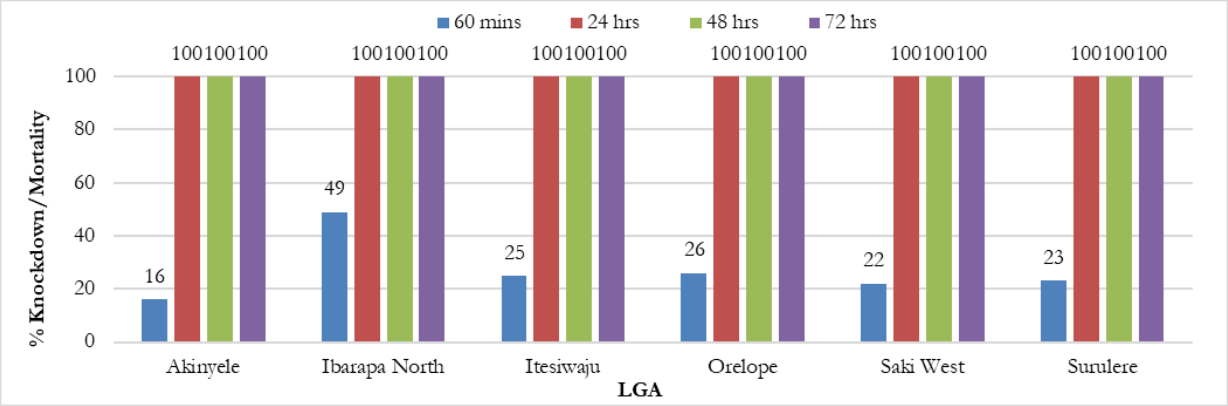

Figure 46: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Oyo

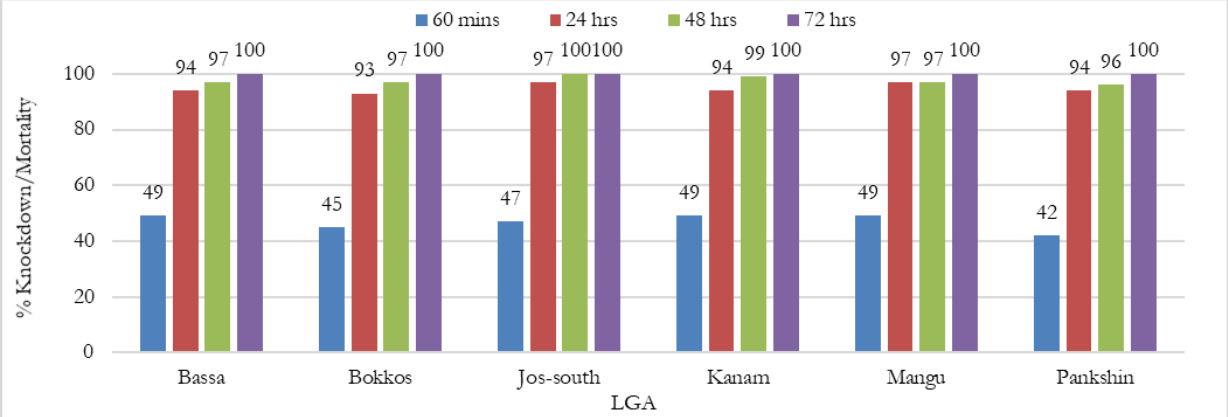

Figure 47: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) in Plateau

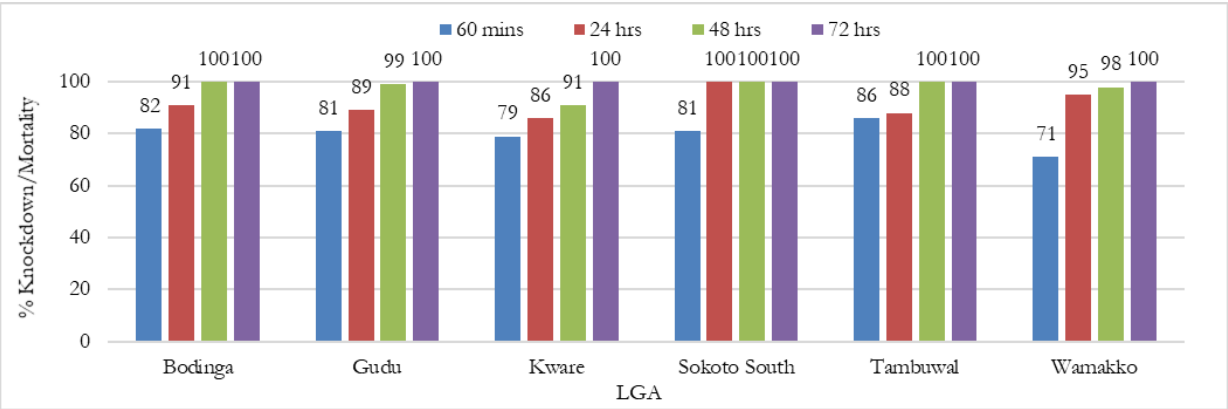

Figure 48: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Sokoto

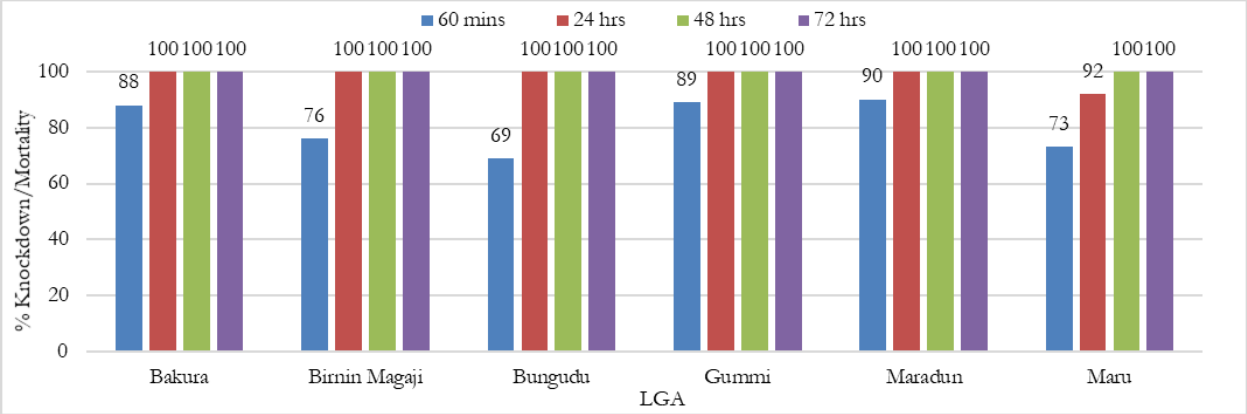

Figure 49: Percentage Mortality of *An. gambiae* s.l. 60 minutes, 24 hours, 48 hours, and 72 hours after Exposure to Chlorfenapyr (100 µg/bottle) at Zamfara

2.14 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: Akwa Ibom (19–28%), Bauchi (30–43%), Benue (51–62%), Cross River (44–75%), Ebonyi (36-100%), Kebbi (73–83%), Nasarawa (49–89%), Oyo (11–39%), Plateau (76–83%), Sokoto (42–71%), and Zamfara (55–83%).

An. gambiae s.l. mosquitoes from Ishielu, Ohaozara, and Onicha in Ebonyi were fully knocked down (100%) after just 60-minutes exposure. After 24 hours (Day 1) of exposure, susceptibility to clothianidin (98–100% mortality) was observed in *An. gambiae* s.l. across all six sites in Benue and Cross River, four LGAs in Kebbi, five LGAs in Nasarawa, one LGA in Plateau, and two LGAs in Zamfara. At Day 2, susceptibility to clothianidin (98-100% mortality) was observed in *An. gambiae* s.l. across all six LGAs in Benue, Cross River, Kebbi, Nasarawa, Oyo and Zamfara, five LGAs in Plateau, four LGAs in Akwa Ibom, and three LGAs in each Ebonyi and Sokoto. In Bauchi, no site recorded susceptibility of clothianidin at Day 2. At Day 3, with the exception of Sokoto South LGA in Sokoto and Ishielu LGA in Ebonyi, complete susceptibility was recorded in all LGAs. By Day 5, complete susceptibility across all LGAs was recorded (Table 9).

Table 9: WHO Tube Test Results (Percent Mortality after 7 days) for *An. gambiae* s.l.

Sentinel Site	LGA	Test Time/% Mortality							
		60 mins	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Akwa Ibom	Abak	21	85	100	100	100	100	100	100
	Itu	21	83	100	100	100	100	100	100
	Mkpat Enin	19	78	97	100	100	100	100	100
	Nsit Ubium	28	92	100	100	100	100	100	100
	Onna	21	79	97	100	100	100	100	100
	Ukanafun	23	89	99	100	100	100	100	100
Bauchi	Dass	34	64	94	100	100	100	100	100
	Darazo	32	66	86	99	100	100	100	100
	Itas/Gadau	33	67	94	100	100	100	100	100
	Katagun	43	77	95	100	100	100	100	100
	Ningi	30	67	95	100	100	100	100	100
	Toro	30	64	93	100	100	100	100	100
Benue	Apa	61	100	100	100	100	100	100	100
	Gwer	57	100	100	100	100	100	100	100
	Obi	51	100	100	100	100	100	100	100
	Tarkaa	62	100	100	100	100	100	100	100

Sentinel Site	LGA	Test Time/% Mortality							
		60 mins	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	Ukum	54	100	100	100	100	100	100	100
	Vandeikya	62	100	100	100	100	100	100	100
Cross River	Abi	56	99	100	100	100	100	100	100
	Akamkpa	75	100	100	100	100	100	100	100
	Calabar Municipality	75	100	100	100	100	100	100	100
	Etung	63	100	100	100	100	100	100	100
	Obudu	44	99	100	100	100	100	100	100
	Ogoja	69	100	100	100	100	100	100	100
Ebonyi	Abakaliki	72	95	99	99	100	100	100	100
	Ebonyi	63	78	90	100	100	100	100	100
	Ezza North	39	91	100	100	100	100	100	100
	Ezza South	36	82	97	100	100	100	100	100
	Ishielu	100	46	80	94	98	100	100	100
	Izzi	38	81	92	100	100	100	100	100
	Ohaozara	100	49	97	100	100	100	100	100
	Ohaukwu	55	91	100	100	100	100	100	100
	Onicha	100	33	86	100	100	100	100	100
Kebbi	Augie	80	100	100	100	100	100	100	100
	Fakai	77	100	100	100	100	100	100	100
	Gwandu	83	100	100	100	100	100	100	100
	Maiyama	79	100	100	100	100	100	100	100
	Shanga	73	87	100	100	100	100	100	100
	Suru	76	80	100	100	100	100	100	100
Nasarawa	Keana	89	100	100	100	100	100	100	100
	Keffi	49	99	100	100	100	100	100	100
	Kokona	68	98	100	100	100	100	100	100
	Obi	81	97	100	100	100	100	100	100
	Toto	78	100	100	100	100	100	100	100
	Wamba	66	98	99	100	100	100	100	100
Oyo	Akinyele	12	92	100	100	100	100	100	100
	Ibarapa North	39	93	100	100	100	100	100	100
	Itesiwaju	11	78	100	100	100	100	100	100
	Orelope	11	77	100	100	100	100	100	100
	Saki West	12	91	100	100	100	100	100	100
	Surulere	32	94	100	100	100	100	100	100
Plateau	Bassa	80	95	98	100	100	100	100	100
	Bokkos	79	96	99	100	100	100	100	100
	Jos-south	83	98	100	100	100	100	100	100
	Kanam	83	96	98	100	100	100	100	100
	Mangu	81	96	98	100	100	100	100	100
	Pankshin	76	93	97	100	100	100	100	100
Sokoto	Bodinga	71	97	98	100	100	100	100	100
	Gudu	45	83	95	100	100	100	100	100
	Kware	50	97	98	100	100	100	100	100
	Sokoto South	63	70	73	93	96	100	100	100
	Tambuwal	69	81	100	100	100	100	100	100
	Wamakko	42	69	93	98	100	100	100	100
Zamfara	Bakura	75	91	100	100	100	100	100	100
	Birnin Magaji	60	100	100	100	100	100	100	100
	Bungudu	73	100	100	100	100	100	100	100
	Gummi	55	83	100	100	100	100	100	100
	Maradun	83	92	100	100	100	100	100	100
	Maru	64	79	100	100	100	100	100	100

A minimum of 100 mosquitoes were exposed in each site. Clothianidin and chlorfenapyr were done with less than 100 mosquitoes in three LGAs (Ishielu, Ohaozara, and Onicha).

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

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

Assessment of *kdr* mutations in alpha-cypermethrin resistant *An. gambiae* s.l. also indicated the presence of only *kdr-w* point mutations; no *kdr-e* point mutations were detected. *Kdr-w* gene frequencies in *An. coluzzii* ranged from 0.50 in Akwa Ibom, Nasarawa, Plateau, and Zamfara to 0.67 in Kebbi. *Kdr-w* gene frequencies in *An. gambiae* s.s. ranged from 0.42 in Ebonyi to 0.69 in Nasarawa. Higher *kdr-w* gene frequencies were detected in *An. coluzzii* than *An. gambiae* s.s. in Benue (0.58 vs. 0.50) (p=0.44) and Kebbi (0.67 vs. 0.50) (p=0.11), but these did not vary significantly (Table 10).

Table 10: Frequently of *kdr* Genes in Alpha-cypermethrin Resistant *An. coluzzii* and *An. gambiae* s.s. Across Sites

Insecticide	State	Species Identified	Alpha-cypermethrin Resistant								
			Number Tested for *kdr*	*kdr-w*				*kdr-e*			
				RR	Rr	rr	*kdr* frequency	RR	Rr	rr	*kdr* frequency
Alpha-cypermethrin	Akwa Ibom	*An. coluzzii*	9	2	5	2	0.50	0	0	9	0.00
		An. gambiae s.s.	9	3	6	0	0.67	0	0	9	0.00
	Bauchi	*An. coluzzii*	8	2	6	0	0.63	0	0	8	0.00
		An. gambiae s.s.	6	1	5	0	0.58	0	0	6	0.00
	Benue	*An. coluzzii*	6	1	5	0	0.58	0	0	6	0.00
		An. gambiae s.s.	1	0	1	0	0.50	0	0	1	0.00
	Cross River	*An. coluzzii*	-	-	-	-	-	-	-	-	-
		An. gambiae s.s.	1	0	1	0	0.50	0	0	1	0.00
	[2]Ebonyi	*An. coluzzii*	27	9	11	7	0.54	0	0	27	0.00
		An. gambiae s.s.	64	14	26	24	0.42	0	0	64	0.00
	Kebbi	*An. coluzzii*	3	1	2	0	0.67	0	0	3	0.00
		An. gambiae s.s.	3	0	3	0	0.50	0	0	3	0.00
	Nasarawa	*An. coluzzii*	8	0	8	0	0.50	0	0	8	0.00
		An. gambiae s.s.	8	4	3	1	0.69	0	0	8	0.00
	[1]Oyo	*An. coluzzii*	0	-	-	-	-	-	-	-	-
		An. gambiae s.s.	0	-	-	-	-	-	-	-	-
	Plateau	*An. coluzzii*	11	1	9	1	0.50	0	0	11	0.00
		An. gambiae s.s.	6	1	5	0	0.58	0	0	6	0.00
	[1] Sokoto	*An. coluzzii*	0	-	-	-	-	-	-	-	-
		An. gambiae s.s.	0	-	-	-	-	-	-	-	-
	Zamfara	*An. coluzzii*	5	0	5	0	0.50	0	0	5	0.00
		An. gambiae s.s.	7	2	5	0	0.64	0	0	7	0.00
Total			**91**	**18**	**69**	**4**	**0.58**	**0**	**0**	**91**	**0.00**

[1] All mosquitoes exposed to alpha-cypermethrin insecticides were susceptible

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

An assessment of *kdr* mutations in pyrethroid-resistant *An. gambiae* s.l. indicated the presence of only *kdr-w* point mutations. No *kdr-e* mutations were recorded across the sites. The *kdr-w* gene frequencies varied by ecozone, types of pyrethroids and vector composition. *Kdr-w* gene frequencies in *An. coluzzii* ranged from 0.27 in Ebonyi to 0.75 in Benue and, in *An. gambiae* s.s., from 0.25 in Ebonyi to 0.70 in Plateau. In general, *kdr-w* frequencies were higher in *An, gambiae* s.s. versus *An, coluzzii*. Significantly higher *kdr-w* mutations were recorded in *An. coluzzii* than *An. gambiae* s.s. in Benue (0.75 vs 0.33) (p=0.0001). This did not vary significantly in Zamfara (0.60 vs 0.50) (p=0.22) (Table 11).

Table 11: Frequency of *kdr* Genes in Deltamethrin Resistant *An. coluzzii* and *An. gambiae* s.s. Across Sites

Insecticide	State	Species Identified	Deltamethrin Resistant								
			Number Tested for *kdr*	*kdr*-w				*kdr*-e			
				RR	Rr	rr	*kdr* frequency	RR	Rr	rr	*kdr* frequency
Deltamethrin	Akwa Ibom	*An. coluzzii*	4	0	3	1	0.38	0	0	4	0.00
		An. gambiae s.s.	7	2	4	1	0.57	0	0	7	0.00
	Bauchi	*An. coluzzii*	8	0	7	1	0.44	0	0	8	0.00
		An. gambiae s.s.	10	1	8	1	0.50	0	0	10	0.00
	Benue	*An. coluzzii*	2	1	1	0	0.75	0	0	2	0.00
		An. gambiae s.s.	3	0	2	1	0.33	0	0	3	0.00
	[1]Cross River	*An. coluzzii*	0	-	-	-	-	-	-	-	-
		An. gambiae s.s.	0	-	-	-	-	-	-	-	-
	[2]Ebonyi	*An. coluzzii*	11	1	4	6	0.27	0	0	11	0.00
		An. gambiae s.s.	57	7	14	36	0.25	0	0	57	0.00
	[1]Kebbi	*An. coluzzii*	0	-	-	-	-	-	-	-	-
		An. gambiae s.s.	0	-	-	-	-	-	-	-	-
	Nasarawa	*An. coluzzii*	7	3	2	2	0.57	0	0	7	0.00
		An. gambiae s.s.	10	5	3	2	0.65	0	0	10	0.00
	Oyo	*An. coluzzii*	1	0	1	0	0.50	0	0	1	0.00
		An. gambiae s.s.	3	1	2	0	0.67	0	0	3	0.00
	Plateau	*An. coluzzii*	12	1	11	0	0.54	0	0	12	0.00
		An. gambiae s.s.	5	2	3	0	0.70	0	0	5	0.00
	Sokoto	*An. coluzzii*	4	1	3	0	0.63	0	0	4	0.00
		An. gambiae s.s.	8	3	5	0	0.69	0	0	8	0.00
	Zamfara	*An. coluzzii*	5	1	4	0	0.60	0	0	5	0.00
		An. gambiae s.s.	5	0	5	0	0.50	0	0	5	0.00
		Total	**162**	**29**	**82**	**51**	**-**	**0**	**0**	**94**	**0.00**

[1] All mosquitoes exposed to deltamethrin insecticides were susceptible

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

Kdr-w gene frequencies in *An. coluzzii* ranged from 0.36 in Ebonyi to 0.75 in Kebbi while *kdr-w* gene frequencies in *An. gambiae* s.s. ranged from 0.30 in Plateau to 0.67 in Zamfara. Higher *kdr-w* gene frequencies were observed in *An. coluzzii* than *An. gambiae* in Kebbi (0.75 vs. 0.65) (p=0.398) and Plateau (0.46 vs 0.30) (p=0.06), but these did not vary significantly (Table 12). The *kdr-e* mutations were not recorded in any of the sites.

Table 12: Frequency of *kdr* Genes in Permethrin Resistant *An. coluzzii* and *An. gambiae* s.s. Across Sites

Insecticide	State	Species Identified	Permethrin Resistant								
			Number Tested for *kdr*	*kdr-w*				*kdr-e*			
				RR	Rr	rr	*kdr* frequency	RR	Rr	rr	*kdr* frequency
Permethrin	Akwa Ibom	*An. coluzzii*	8	1	7	0	0.56	0	0	8	0.00
		An. gambiae s.s.	10	1	8	1	0.50	0	0	10	0.00
	Bauchi	*An. coluzzii*	8	1	7	0	0.56	0	0	8	0.00
		An. gambiae s.s.	9	2	7	0	0.61	0	0	9	0.00
	Benue	*An. coluzzii*	12	3	8	1	0.58	0	0	12	0.00
		An. gambiae s.s.	4	0	4	0	0.50	0	0	4	0.00
	Cross River	*An. coluzzii*	7	0	7	0	0.50	0	0	7	0.00
		An. gambiae s.s.	11	2	9	0	0.59	0	0	11	0.00
	[2]Ebonyi	*An. coluzzii*	11	2	4	5	0.36	0	0	11	0.00
		An. gambiae s.s.	65	11	30	24	0.40	0	0	65	0.00
	Kebbi	*An. coluzzii*	4	2	2	0	0.75	0	0	4	0.00
		An. gambiae s.s.	13	5	7	1	0.65	0	0	13	0.00
	Nasarawa	*An. coluzzii*	5	0	5	0	0.50	0	0	5	0.00
		An. gambiae s.s.	11	3	8	0	0.64	0	0	11	0.00
	Oyo	*An. coluzzii*	12	0	10	2	0.42	0	0	12	0.00
		An. gambiae s.s.	4	1	2	1	0.50	0	0	4	0.00
	Plateau	*An. coluzzii*	13	0	12	1	0.46	0	0	13	0.00
		An. gambiae s.s.	5	0	3	2	0.30	0	0	5	0.00
	Sokoto	*An. coluzzii*	7	3	4	0	0.71	0	0	7	0.00
		An. gambiae s.s.	2	0	2	0	0.50	0	0	2	0.00
	Zamfara	*An. coluzzii*	8	0	8	0	0.50	0	0	8	0.00
		An. gambiae s.s.	9	3	6	0	0.67	0	0	9	0.00
		Total	**238**	**40**	**160**	**38**	**-**	**0**	**0**	**238**	**0.00**

3. Discussion and Conclusion

3.1 Species Composition

An. gambiae s.l. remained the most abundant major malaria vector with widespread distribution across all sites. Secondary malaria vectors such as *An. nili, An. moucheti, An. pharoensis, An. coustani* were also found but with limited distribution and abundance. The percentage composition of *An. funestus* mosquitoes in 2018 (0.1-4.0%) reported from four sites increased in 2019 (1.0-16.9%) from five sites. Increased abundance of *An. funestus* (another major vector) indicates its potential to significantly contribute to malaria transmission, particularly in areas where breeding site conditions are suitable such as in Oyo, Plateau, and Sokoto. This pattern of occurrence agrees with recent reports (AIRS Nigeria Final Entomology Report 2017, PMI VectorLink Nigeria Final Entomology Report, 2018; 2019). Other localized species found included *An. squamosus, An. maculipalpis, An. longipalpis, An. rufipes*, and *An. pretoriensis*. A total of eleven *Anopheles* mosquito species were identified in all the five sentinel sites. This is consistent with previous numbers of *Anopheles* species collected by PMI to date across the different ecological zones of Nigeria (PMI, 2017, 2018 and 2019), as well as from collections reported from the rural communities in the Guinea and Savannah transitional forest zone of Nigeria (Oduola et al., 2013). The ability of *An. gambiae* s.l. to utilize different breeding habitats, coupled with secondary and localized vectors that leverage specific habitats and seasonal conditions, accounts for variation in occurrence, predominance and abundance across the different ecological zones in Nigeria. The collective or individual roles of these vectors during both the rainy and dry seasons may be responsible for sustaining malaria transmission all year round. All three members of the *An. gambiae* s.l. species (*An. gambiae* s.s.*, An. coluzzii,* and *An. arabiensis*) were found at varying proportion in each sentinel site. The only members of the *An. funestus* group identified were *An. funestus* s.s. and *An. leesoni* (Figures 4 and 5). However, the significant proportions unidentified from the PCR assay results suggest the need to carry out further analysis on reasons behind the unamplified members of the *An. funestus* group.

3.2 Human Biting Rate and Vector Biting Time

The mean indoor biting rates of *An. gambiae* s.l. peaked in July in Plateau and in August in Sokoto. This was expected as biting rate has been found to be largely dependent on mosquito abundance which is influenced by rainfall patterns. In contrast to our previous observations (PMI VectorLink Annual Entomology Report 2019), biting rate did not increase much until July. Similarly, increased outdoor biting was equally recorded between July and August also in Plateau and Sokoto, with an earlier smaller peak in December 2019 recorded in Sokoto and Oyo, which stresses the need for those who sleep outdoors to protect themselves by sleeping under nets. Both indoor and outdoor biting rates in the other sites were generally low. Biting rate activities for most of the surveillance sites occurred both outdoors and indoors, increasing during the late rainy season (July – August) compared to previous findings where peak biting rates were observed during the early rainy season (April –June) (PMI VectorLink Annual Entomology Report 2019). Delayed rainfall may be responsible for the shift in the monthly mosquito peak biting. This peak is usually the target of indoor residual spraying in order to reduce malaria transmission.

The average number of mosquitoes caught biting per hour was generally higher indoors, ranging from 8 mosquitoes collected between 2-3 a.m. in Ebonyi to 41 mosquitoes collected between 10-11 p.m. in Plateau. This agrees with previous findings which show the peak biting time as after midnight in most sentinel sites (PMI VectorLink Annual Entomology Report 2019). Incidental shifts in peak biting time (10-11 p.m. as found in Plateau) may require further investigation of human activity at night and other environmental factors such as temperature and precipitation, which have been reported to influence the mosquito biting time (Dambach et al., 2018).

3.3 SPOROZOITE INFECTION RATE

In this surveillance period, only *An. gambiae* s.s. from Akwa Ibom and Ebonyi tested positive for the *P. falciparum* circumsporozoite protein. No sporozoite infection was recorded among *An. coluzzii* and *An. arabiensis* collected indoors or outdoors in any site. *An. gambiae* s.s. still remained the major malaria vector with higher sporozoite rate and vector densities. Previous reports from different sites have already indicated *An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis* as vectors of malaria in Nigeria (PMI VectorLink Nigeria Annual Entomology Report 2019). Recent studies have also indicated that *An. gambiae* s.s. and *An. coluzzii* have similar potentials to transmit *P. falciparum* (Akogbeto *et al.,* 2018). This year's outcome greatly contrasts with our previous findings of higher sporozoite infection rates in *An. gambiae* s.s. and *An. coluzzii* across the sentinel sites. Compared to 2019, where higher sporozoite rate contributions were recorded in *An. gambiae* indoors in Bauchi (7.4%) and outdoors in Ebonyi (8.3%), only *An. gambiae* collected indoors tested positive for sporozoites (1.3%-2.2%) in 2020. Similarly diverging from last year's results, infection rates in *An. coluzzii* indoors in 2019 ranged from 1.5-2.8% compared to 0% in 2020. In 2019, sporozoites in *An. arabiensis* outdoors were recorded in Nasarawa (2.9%), Plateau (2.2%), and Sokoto (0.9%) (PMI VectorLink Nigeria Annul Entomology Report 2019). The lack of sporozoite infection rate among *An. coluzzii* and *An. arabiensis*, as well as the absence of outdoor transmission, could be due to the small sample size analyzed. Overall, there were generally higher sporozoite rates recorded across the sentinel sites in 2017, 2018, and 2019, compared to 2020.

Positivity for *P. falciparum* circumsporozoites among *An. funestus* s.s. collected indoors and outdoors further establishes its role as a major malaria vector. Though *An. funestus* is less studied in Nigeria compared to *An. gambiae* s.l., its role in malaria transmission has been reported (Awolola *et al*., 2003). Some of the other unidentified species of *An. funestus* group tested positive for the circumsporozoites antigen. There is need to conduct further investigations to clarify the identity of the unamplified samples.

3.4 ENTOMOLOGICAL INOCULATION RATE

EIRs among *An. gambiae* s.s. were recorded indoors in Ebonyi (4.6 infective bites/person/year) and Akwa Ibom (3.0 infective bites/person/year). This is in consonance with our previous findings which indicated that *An. gambiae* s.s. contributed more to EIR indoors and outdoors in more LGAs compared to other members of the *An. gambiae* complex (PMI VectorLink Annual Entomology Report 2019). There were no infective bites recorded among *An. coluzzii* or *An. arabiensis* (indoors or outdoors) across the sites. This could be due to sample size and contrasts with earlier findings where both *An. coluzzii* and *An. arabiensis* have been found to be efficient contributors to EIR both indoors and outdoors (PMI VectorLink Annual Entomology Report 2019).

3.5 BLOOD MEAL SOURCES

The blood meal analysis indicates that *An. gambiae* s.s.*, An. coluzzii,* and *An. arabiensis* collected using PSCs had higher preference for human blood meal. These are predominantly indoor resting mosquitoes that have fed on humans possibly the previous night. The co-occurrence of *An. gambiae* s.s. and *An. coluzzii,* and their preference for human blood meal in Akwa Ibom, Ebonyi, and Oyo, attest to their strong anthropophilic behavior which makes them important malaria transmitters. The inclusion of *An. arabiensis* as another competent malaria vector species with preference for animal blood meal in Plateau and Sokoto further potentiates the transmission risk indices of malaria transmission in these localities. The 100% preference for human blood meal in *An. arabiensis* also indicates its behavior to fully utilize human blood meal indoors when zoophilic feeding is unavailable and thus aid transmission in Plateau and Sokoto. The ability of these three vector species to utilize human, bovine, and goat blood meal as observed in this report agrees with previous findings (PMI VectorLink Annual Entomology Report 2019). An understanding of the feeding behavior of these vectors will ensure proper planning and consideration of all factors that may potentiate malaria risks. Caution should be exercised in interpreting the blood meal preferences of mosquitoes collected from CDC light traps, as only few blood-fed mosquitoes collected in CDC light traps were analyzed. There was also higher feeding on animals as recorded outdoors in Akwa Ibom, where 50% of blood meals of *An. gambiae* s.s.

and *An. coluzzii* were bovine, and in Plateau, where 100% of blood meals in *An. gambiae* s.s. outdoors were from goat. This zoophilic behavior of *An. gambiae* s.s. and *An. coluzzii* in Akwa Ibom, Ebonyi, and Plateau might affect the maintenance of residual malaria transmission in those sites (WHO, 2019). Overall, despite the zoophilic nature of *An. arabiensis,* no blood meal was found in those collected indoors in four of five sites, and no blood meal was detected in outdoor collected *An. arabiensis* from any of the sites. Generally, the prevalence of animal blood meal in human dwellings indicates that livestock live in close proximity to humans. This can support higher malaria transmission by attracting infected mosquitoes to human habitations (Iwashita *et al.,* 2014).

3.6 Insecticide Susceptibility

Pyrethroid resistance patterns varied within and among the states. While permethrin resistance was observed in mosquito populations across all 11 states, deltamethrin and alpha-cypermethrin resistance occurred in *Anopheles* populations from six states. Resistance to all three pyrethroids was recorded in Akwa Ibom, Bauchi, and Ebonyi. Deltamethrin resistance was recorded in *An. gambiae* s.l. in all six LGAs in Akwa Ibom and Plateau. This accords with the findings across many countries that resistance to pyrethroids continues to be widespread (WHO, 2020).

Mosquito susceptibility to alpha-cypermethrin was also recorded in all six LGAs of Benue, Cross River, Oyo, and Sokoto and four LGAs in Kebbi. Susceptibility to deltamethrin was recorded in *An. gambiae* s.l. populations across all six LGAs of Benue, Cross River, and Kebbi, and five LGAs in Oyo. These results suggest possible use of these insecticides, especially for treating ITNs, under an insecticide resistance management plan. The widespread resistance of vectors to permethrin at most of the sites strongly suggest that it is not the preferred insecticide choice for standard ITNs in most of the country. These variations in resistance could be an indication of local selective pressure from intensive use of pyrethroids for agricultural purposes. It has become a common practice in these areas to use pesticides meant for outdoor agricultural purposes, indoors as a preferred method for controlling mosquitoes and other insects, with little or no consideration of the consequences (Ononamadu *et al,* 2020).

An. gambiae s.l. susceptibility to pirimiphos-methyl was observed in all six LGAs in Nasarawa, Oyo, Sokoto, and Zamfara, but varied in other sites. This agrees with findings of WHO (2020) which reported low resistance of local *Anopheles* mosquitoes to pirimiphos-methyl in most countries.

3.7 Resistance Intensity

Low-intensity alpha-cypermethrin resistance was recorded in *An. gambiae* s.l. populations from Akwa Ibom, Bauchi, Ebonyi, and Plateau. Deltamethrin resistance in *An. gambiae* s.l. mosquitoes attained low resistance intensity in all six LGAs only in Plateau, one LGA in Bauchi, and two LGAs in Ebonyi. This agrees with findings from 2019 (PMI 2019) where low resistance intensity was recorded in Plateau and Ebonyi but contrasted with findings from 2018 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, Bauchi, Ebonyi, Nasarawa, Oyo, Sokoto, and Zamfara. Permethrin resistance in *An. gambiae* at low level intensity was observed in Bauchi, Cross River, Kebbi, Nasarawa, Oyo, Plateau, and Zamfara, moderate resistance intensity was observed in populations from one LGA each in Ebonyi and Oyo, and high resistance intensity to permethrin was recorded in five LGAs in Akwa Ibom and in four LGAs in Ebonyi.

Insecticide resistance intensity recorded in *An. gambiae* s.l. mosquitoes across the different ecozones reveals that different resistance management options are needed. These results indicate that the increasing resistance intensity being selected in field populations of mosquitoes will reduce the efficacy of pyrethroid-based interventions. Therefore, proactive approaches need to be adopted to delay the spread of resistance and preserve the effectiveness of current

3.8 SYNERGIST ASSAYS

A growing cause for concern, PBO has shown inability to restore alpha-cypermethrin, deltamethrin, and permethrin susceptibility in *An. gambiae* s.l. in all LGAs in Plateau and an increasing number of LGAs in Akwa Ibom, Cross River, Kebbi, Oyo, and Nasarawa. 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 (PMI, 2018). Fluctuations in susceptibility outcomes in these states may require further observation over successive years or might need to consider nets comprising of chlorfenapyr. The recovery of susceptibility to permethrin and other pyrethroids with pre-exposure to PBO (cytochrome P450 inhibitor) implicated the involvement of detoxification enzyme, cytochrome P450 oxidase system (metabolic resistance) to the resistance of permethrin. This is in line with previous studies (Hemingway, *et al.,* 2014, Djouaka, *et al*, 2011; Liu, 20115) but the low recovery of susceptibility also suggests that other mechanisms aside from the expression of cytochrome P450 oxidase system may be involved as reported by Riveron *et al.*, (2019) in a study in Mozambique. This is of serious consequence to ITN use for malaria vector control in Nigeria, since most nets presently deployed in vector control are treated with pyrethroids only (Geleta and Ketema, 2016).

3.9 CHLORFENAPYR AND CLOTHIANIDIN

An. gambiae s.l. mosquitoes were susceptible (98-100% mortality) to chlorfenapyr at 48 hours across all sites, except in Plateau (four LGAs) and Sokoto (one LGA). This contrasts with previous findings from 2019, where chlorfenapyr resistance at 48 hours holding period was recorded in Akwa Ibom and Sokoto only. This suggests the relevance of chlorfenapyr as a potential compound to manage the pyrethroid resistance observed at the monitoring sites. However, the vector was not susceptible at 100 µg/bottle after 48 hours in Plateau (Bassa, Bokkos, Mangu, and Pankshin LGAs), and further tests are needed at a higher concentration.

The percentage knockdown of *An. gambiae* s.l. mosquitoes after 60-minutes exposure to clothianidin and over the seven-day holding period varied across the sites. At Day 4, susceptibility was recorded (greater than 98% mortality) for wild *An. gambiae* s.l. at all 11 sites, except in Sokoto South LGA where there was 96% mortality at Day 4 and 100% on Day 5. Generally, more than 90% of clothianidin-induced mortality occurred within 72 hours of exposure, however, five days was required to reach 100% mortality at all sites.

3.10 *KDR* GENE FREQUENCIES

Assessing the knockdown (*kdr)* mutations, an important mechanism associated with pyrethroid resistant *An. gambiae* s.l., indicated the presence of only *kdr-w* (1014F) point mutations in alpha-cypermethrin, deltamethrin, and permethrin tested across the sites. No *kdr-e* (1014S) mutations were recorded in any of the mosquitoes exposed to the three pyrethroids tested. This contrasts with previous findings in the same locations where both *kdr-w* and *kdr-e* point mutations were present (PMI VectorLink Annual Entomology Report 2019). Where metabolic resistance is ruled out, mutations in the binding site of insecticides are often involved. Though it is not evident that the presence of this resistance allele alone is sufficient to result in control failure, the *kdr-w* allelic frequencies in mosquitoes resistant to alpha-cypermethrin, deltamethrin, and permethrin increased this year compared to last year (PMI VectorLink Annual Entomology Report 2019). There is need for continued monitoring of the spread and gene frequencies of these mutations in *An. gambiae* s.l. populations. Analysis of the dynamics and trends over time may indicate the presence of selection pressure among the mosquito population. The observed higher frequency of *kdr* mutation in *An. coluzzii* in many sites is contrary to previous findings in southwest Nigeria (Awolola *et al.,* 2003; 2005). This could suggest variations in some physico-chemical parameters of the water, such as seen in Cameroon where increased larval tolerance was observed due to increased ability to dissolve oxygen and ammonia (Fossog *et al.,* 2012), that may contribute in different measures to the insecticide resistance and adaptation of *An. coluzzii* across the various ecozones (Ononamadu *et al.,* 2020). These findings are consistent with Ibrfahim *et al. (*2019) who reported an escalation of pyrethroid resistance in *An. coluzzii* from three sites in the Sudan-Sahel ecozone of Nigeria. Overall, the pattern of resistance and *kdr* mutant allele distribution suggests that target site mutations in association with other mechanisms may account for the observed resistance to pyrethroids in the study.

ANNEX A: GPS COORDINATES OF SAMPLING SITE LOCATIONS

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

ANNEX B: *ANOPHELES* MOSQUITOES COLLECTED BY DIFFERENT METHODS AND SUBJECTED TO PCR ACROSS SITES (OCTOBER 2019-SEPTEMBER 2020)

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	73	28	3	3	107	49	5	54	161
Ebonyi	116	17	0	0	133	207	7	214	347
Oyo	101	17	0	3	121	142	3	145	266
Plateau	226	32	1	0	259	159	0	159	418
Sokoto	215	142	1	2	360	247	16	263	623
Total	**731**	**236**	**5**	**8**	**980**	**804**	**31**	**835**	**1,815**

ANNEX C:
ANOPHELES CAUGHT BY SPECIES, METHOD, AND SITE (OCTOBER 2019–SEPTEMBER 2020)

Mosquito Species	Akwa Ibom			Ebonyi			Oyo			Plateau			Sokoto			Total (In)	Total (Out)	Total (PSC)	Overall
	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC	In	Out	PSC				
An. gambiae s.l.	1,059	358	460	379	40	1,068	682	233	1,708	2,533	512	1,371	1,738	766	5,892	6,391	1,909	10,499	18,799
An. funestus	-	-	-	2	0	1	1,211	451	774	241	60	284	61	114	770	1,515	625	1,829	3,969
An. nili	1	0	0	-	-	-	-	-	-	-	-	-	0	12	0	1	12	0	13
An. coustani	-	-	-	2	1	0	4	5	0	0	1	0	6	19	0	12	26	0	38
An. pharoensis	-	-	-	1	0	0	-	-	-	-	-	-	31	43	0	32	43	0	75
An. maculipalpis	13	0	1	-	-	-	-	-	-	-	-	-	1	0	0	14	0	1	15
An. moucheti	25	6	11	1	0	1	-	-	-	-	-	-	-	-	-	26	6	12	44
An. pretoriensis	-	-	-	-	-	-	1	0	0	0	0	3	-	-	-	1	0	3	4
An. rufipes	-	-	-	-	-	-	-	-	-	4	2	32	6	7	0	10	9	32	51
An. squamosus	-	-	-	-	-	-	-	-	-	6	2	0	-	-	-	6	2	0	8
An. longipalpis	-	-	-	-	-	-	-	-	-	0	0	12	-	-	-	0	0	12	12
An. flavicosta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
Grand Total	**1,098**	**364**	**472**	**385**	**41**	**1,070**	**1,898**	**689**	**2,482**	**2,784**	**577**	**1,702**	**1,843**	**961**	**6,662**	**8,008**	**2,632**	**12,388**	**23,028**

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

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

Sentinel Site	CDC LT Indoors					CDC LT Outdoors					PSC					Totals				
	No. PCR +ve	*An. gambiae* s.s.	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae* s.s.	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae* s.s.	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae* s.s.	*An. coluzzii*	Hybrid	*An. arabiensis*
Akwa Ibom	73	45	28	0	0	28	19	9	0	0	49	29	20	0	0	150	93	57	0	0
Ebonyi	116	76	39	0	1	17	14	3	0	0	207	140	67	0	0	340	230	109	0	1
Oyo	101	61	40	0	0	17	16	1	0	0	142	94	48	0	0	260	171	89	0	0
Plateau	226	160	63	1	2	32	25	6	0	1	159	124	30	0	5	417	309	99	1	8
Sokoto	215	139	74	0	2	142	89	53	0	0	247	158	86	0	3	604	386	213	0	5
Total	**731**	**481**	**244**	**1**	**5**	**236**	**163**	**72**	**0**	**1**	**804**	**545**	**251**	**0**	**8**	**1,771**	**1,189**	**567**	**1**	**14**

ANNEX E:
INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES BY SITE

Sentinel Site	Month-Year	Number Identified						HBR						SPR						Monthly EIR					
		An. gambiae s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Akwa Ibom	Oct-19	48	18	60	6	0	0	4.00	1.50	5.00	0.50	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-19	0	0	0	9	0	0	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-19	71	8	36	25	0	0	5.94	0.69	2.97	2.06	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-20	23	0	0	0	0	0	1.92	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-20	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-20	14	10	4	0	0	0	1.15	0.83	0.35	0.00	0.00	0.00	0.077	0.000	0.000	0.00	0.00	0.00	2.735	0.000	0.000	0.000	0.000	0.000
	Jun-20	6	10	13	6	0	0	0.53	0.80	1.06	0.53	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-20	63	27	63	18	0	0	5.25	2.25	5.25	1.50	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-20	355	0	0	0	0	0	29.58	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-20	237	81	0	0	0	0	19.75	6.75	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Total		653	243	406	115	0	0	54.40	20.24	33.85	9.59	0.00	0.00	0.022	0.000	0.000	0.000	0.00	0.00	2.735	0.000	0.000	0.000	0.000	0.000
Bauchi	Oct-19	60	0	0	9	0	0	5.00	0.00	0.00	0.75	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-19	9	3	0	0	0	0	0.75	0.25	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-19	2	0	9	5	0	0	0.18	0.00	0.73	0.42	0.00	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-20	4	5	0	0	0	0	0.33	0.42	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-20	3	0	0	0	0	0	0.25	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-20	10	0	3	0	0	0	0.81	0.00	0.27	0.00	0.00	0.00	0.167	0.00	0.000	0.00	0.00	0.00	4.198	0.000	0.000	0.000	0.000	0.000
	Jun-20	39	2	29	0	1	0	3.23	0.17	2.43	0.00	0.09	0.00	0.000	0.000	0.000	0.00	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-20	53	0	79	11	0	0	4.40	0.00	6.60	0.92	0.00	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-20	7	0	1	0	0	0	0.62	0.00	0.05	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-20	42	4	28	0	0	0	3.50	0.33	2.33	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Total		248	33	127	7	3	0	20.69	2.75	10.62	0.59	0.27	0.00	0.013	0.000	0.000	0.000	0.000	0.00	4.198	0.000	0.000	0.000	0.000	0.000

Sentinel Site	Month-Year	Number Identified						HBR						SPR						Monthly EIR					
		An. gambiae s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Oyo	Oct-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	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-19	11	0	4	0	0	0	0.89	0.00	0.36	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-19	152	75	0	0	0	0	12.67	6.25	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-20	13	0	1	0	0	0	1.05	0.00	0.12	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-20	9	0	2	0	0	0	0.73	0.00	0.18	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-20	55	6	43	0	0	0	4.59	0.50	3.57	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-20	6	9	18	0	0	0	0.50	0.75	1.50	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-20	0	0	151	0	0	0	0.00	0.00	12.58	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-20	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-20	27	13	54	3	0	0	2.25	1.04	4.50	0.21	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Total		412	219	270	14	0	0	34.33	18.27	22.51	1.14	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Plateau	Oct-19	162	8	35	8	0	0	13.52	0.67	2.90	0.67	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-19	20	4	7	0	0	0	1.63	0.33	0.54	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-19	24	12	9	0	0	0	2.00	1.00	0.75	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-20	12	0	0	0	2	0	0.97	0.00	0.00	0.00	0.19	0.00	0.000	0.00	0.00	0.00	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-20	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-20	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-20	172	51	119	0	0	0	14.30	4.25	9.95	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-20	987	113	258	57	0	28	82.28	9.43	21.47	4.71	0.00	2.36	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-20	286	0	126	0	0	0	23.84	0.00	10.49	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-20	262	63	42	38	10	0	21.81	5.21	3.49	3.13	0.87	0.00	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Total		1,801	400	709	96	23	16	150.10	33.33	59.10	8.00	1.88	1.33	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sokoto	Oct-19	28	11	11	0	0	0	2.37	0.92	0.93	0.00	0.03	0.00	0.000	0.000	0.000	0.00	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-19	40	17	12	0	0	0	3.30	1.42	1.04	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-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	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-20	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-20	0	1	0	0	0	0	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-20	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-20	3	0	3	2	0	0	0.25	0.00	0.25	0.17	0.00	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-20	34	9	30	17	0	0	2.82	0.72	2.51	1.44	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-20	611	227	534	197	38	0	50.88	18.92	44.52	16.42	3.18	0.00	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-20	117	0	170	0	0	0	9.78	0.00	14.13	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Total		1,124	480	598	286	16	0	93.64	40.01	49.85	23.83	1.35	0.00	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000

ANNEX F: ANNUAL EIR FOR ALL SENTINEL SITES

Sentinel Sites	Mosquito Species	EIR over 10 months		Estimated EIR over 12 months	
		Indoors	Outdoors	Indoors	Outdoors
Akwa Ibom	*An. gambiae* s.s.	2.7	0.0	3.0	0.0
	An. coluzzii	0.0	0.0	0.0	0.0
	An. arabiensis	0.0	0.0	0.0	0.0
Ebonyi	*An. gambiae* s.s.	4.2	0.0	4.6	0.0
	An. coluzzii	0.0	0.0	0.0	0.0
	An. arabiensis	0.0	0.0	0.0	0.0
Oyo	*An. gambiae* s.s.	0.0	0.0	0.0	0.0
	An. coluzzii	0.0	0.0	0.0	0.0
	An. arabiensis	0.0	0.0	0.0	0.0
Plateau	*An. gambiae* s.s.	0.0	0.0	0.0	0.0
	An. coluzzii	0.0	0.0	0.0	0.0
	An. arabiensis	0.0	0.0	0.0	0.0
Sokoto	*An. gambiae* s.s.	0.0	0.0	0.0	0.0
	An. coluzzii	0.0	0.0	0.0	0.0
	An. arabiensis	0.0	0.0	0.0	0.0

ANNEX G:
INDOOR RESTING DENSITY OF *ANOPHELES* MOSQUITOES BY SITE

Sentinel Sites	Month-Year	# of Rooms	Total # of Anopheles Caught	Indoor Resting Density
Akwa Ibom	**Oct-19**	32	42	1.3
	Nov-19	32	54	1.7
	Dec-19	32	79	2.5
	Jan-20	32	35	1.1
	Feb-20	32	13	0.4
	Mar-20	32	24	0.8
	Jun-20	32	25	0.8
	Jul-20	32	87	2.7
	Aug-20	32	55	1.7
	Sep-20	32	46	1.4
			Mean	**1.4**
Ebonyi	**Oct-19**	32	271	8.5
	Nov-19	32	101	3.2
	Dec-19	32	44	1.4
	Jan-20	32	5	0.2
	Feb-20	32	12	0.4
	Mar-20	32	13	0.4
	Jun-20	32	171	5.3
	Jul-20	32	257	8.0
	Aug-20	32	17	0.5
	Sep-20	32	177	5.5
			Mean	**3.3**
Oyo	**Oct-19**	32	314	9.8
	Nov-19	32	172	5.4
	Dec-19	32	160	5.0
	Jan-20	32	188	5.9
	Feb-20	32	101	3.2
	Mar-20	32	266	8.3

Sentinel Sites	Month-Year	# of Rooms	Total # of Anopheles Caught	Indoor Resting Density
	Jun-20	196	115	3.6
	Jul-20	196	165	5.2
	Aug-20	196	85	2.7
	Sep-20	196	142	4.4
			Mean	**5.3**
Plateau	**Oct-19**	32	176	5.5
	Nov-19	32	83	2.6
	Dec-19	32	41	1.3
	Jan-20	32	6	0.2
	Feb-20	32	2	0.1
	Mar-20	32	8	0.3
	Jun-20	32	91	2.8
	Jul-20	32	502	15.7
	Aug-20	32	311	9.7
	Sep-20	32	151	4.7
			Mean	**4.3**
Sokoto	**Oct-19**	32	277	8.7
	Nov-19	32	619	19.3
	Dec-19	32	603	18.8
	Jan-20	32	15	0.5
	Feb-20	32	19	0.6
	Mar-20	32	22	0.7
	Jun-20	32	138	4.3
	Jul-20	32	937	29.3
	Aug-20	32	2,301	71.9
	Sep-20	32	961	30.0
			Mean	**18.4**

ANNEX H: BIBLIOGRAPHY

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