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THE PMI VECTORLINK NIGERIA PROJECT

ANNUAL ENTOMOLOGY REPORT

OCTOBER 2020–SEPTEMBER 2021

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ACRONYMS

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

EXECUTIVE SUMMARY

Malaria vector surveillance and insecticide resistance monitoring activities provide 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 2021, vector surveillance was carried out in six sentinel sites while insecticide resistance monitoring was carried out in all 11 PMI focus states as well as three non-PMI focus states. From October 2020 to September 2021, 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 46,711 *Anopheles* mosquitoes were collected from six sentinel sites using human-baited CDC LTs (indoors/outdoors) and PSCs. *Anopheles gambiae* s.l. was the most abundant species across most sites, ranging from 20.9% in Akwa Ibom to 100% in Kebbi. Other *Anopheles* species identified in varying abundance were *An. marshallii* complex, *An. funestus, An. rufipes,* and *An. maculipalpis.* Other localized species observed were *An. squamosus, An. coustani, An. nili*, and *An. moucheti,* and *An. pharoensis.* Samples of mosquitoes from Akwa Ibom previously morphologically identified as *An. hargreavesi* were sent to University of Witwatersrand in South Africa for confirmation with sequencing. Sixteen percent (16%) of the mosquitoes were conclusively identified as *An. hargreavesi* based on key characteristics found while others were not and hence generally categorized as *An. marshallii* complex. Sequences generated from these specimens matched 84% of the shared sequence identity of *An. marshallii* isolate (MW257139.1) from Cameroon. Because no comparative sequencing exists for *An. hargreavesi*, these specimens have been categorized and reported as members of the *An. marshallii* complex by sequencing identification.

A total of 2,625 *An. gambiae* s.l. mosquitoes collected between October 2020 and September 2021 were subjected to species-specific PCR assays. Of these, 2,608 (99.4%) mosquito samples were amplified, while only 17 (0.6%) failed to amplify. Further results showed that 1,018 (39.0%) were identified as *An. gambiae* s.s.*,* 1,546 (59.3%) as *An. coluzzii,* 40 (1.5%) as *An. arabiensis*, and four (0.2%) were hybrid *An. gambiae*/*An. coluzzii.*

An. gambiae s.s. was the dominant vector species found both indoors and outdoors in Akwa Ibom and Plateau, compared to Ebonyi and Oyo, where it was found to be predominant indoors. *An. coluzzii* was predominant both indoors and outdoors in Sokoto. The highest proportion of *An. gambiae* s.s. was collected outdoors in Akwa Ibom (100%), while the highest indoor collection was in Plateau (60.1%). The highest proportion of *An. coluzzii* collected indoors (76.6%) and outdoors (70.1%) were from Sokoto. The highest proportion of *An. arabiensis* found indoors (0.7%) and outdoors (2.9%) were from Plateau. A total of 812 *An. funestus* mosquitoes collected by CDC LTs and PSCs in Ebonyi, Oyo, Plateau, and Sokoto were subjected to species-specific PCR assays. Of these, 365 (45.0%) mosquitoes were collected using CDC LTs, while 447 (55.0%) were collected using PSCs. *Anopheles funestus* s.s. were found to be the only species identified both indoors and/or outdoors in all sites except in Oyo where other unamplified samples were observed. For members of *An. funestus* group collected using PSCs, only *An. funestus* s.s. predominated in all four sites, except in Plateau where unamplified species (1.7%) and *An. leesoni* (0.9%) were found.

The mean indoor biting rates of *An. gambiae* s.l. peaked in July in Plateau (103.4 bites/person/night (b/p/n)), in September in Kebbi (37.6 b/p/n), and in April in Sokoto (26.5 b/p/n), with an earlier smaller peak in October in Oyo (21.6 b/p/n). Increased outdoor biting was recorded in Sokoto (22.3 b/p/n), Kebbi (20.7 b/p/n), and Oyo (11.2 b/p/n) in April, September, and October, respectively. The indoor resting density of *An. gambiae* s.l. mosquitoes varied across the sites and months, ranging from 0.2 mosquitoes/room/day in

Ebonyi in December 2020 and February 2021 to 52.4 mosquitoes/room/day in Sokoto in March 2021. In Sokoto, higher indoor resting densities were observed between October and November 2020 and from July to September 2021. The average number of *An. gambiae* s.l. mosquitoes collected per hour was generally higher indoors, ranging from one mosquito collected between 7-8 p.m. in Akwa Ibom to 49 mosquitoes collected between 12-1 a.m. in Kebbi. Biting peaked between 12-1 a.m. in Kebbi, Oyo, and Sokoto and between 3-4 a.m. in Ebonyi and Plateau. The mean indoor biting rates of *An. marshallii* peaked in the dry season; in September (26.6 b/p/n), in November 2020 (23.9 b/p/n) and December 2020 (10.1 b/p/n). Increased outdoor biting was recorded earlier during November 2020 (6.4 b/p/n) and later in September 2021 (6.3 b/p/n). Similarly, higher indoor resting densities were observed during the drier months in Akwa Ibom, in November 2020 (0.8 mosquitoes/room/day), December 2020 (0.7 mosquitoes/room/day), and September 2021 (0.7 mosquitoes/room/day). The biting rates of *An. funestus* clearly peaked within the dry season (November 2020 and September 2021) in Oyo, whereas in Plateau, smaller peaks were observed in January, July, and September.

Resistance to all three pyrethroids tested was recorded in all tested LGAs in Akwa Ibom, Bauchi, Bayelsa, Ebonyi, and Plateau. Mosquito susceptibility to alpha-cypermethrin was recorded in Benue, Cross River, Oyo, and Sokoto while resistance was observed in Akwa Ibom, Bauchi, and Ebonyi. Susceptibility to deltamethrin was recorded in *An. gambiae* s.l. populations in Cross River, FCT, Kebbi, Oyo, and Zamfara. Resistance to deltamethrin 1X was recorded in Akwa Ibom, Bauchi, Bayelsa, Ebonyi, Enugu, and Plateau. Permethrin resistance was recorded across all sentinel sites except in four LGAs in Sokoto. *An. gambiae* s.l. resistance to pirimiphos-methyl was observed in Cross River, Nasarawa, and Plateau. The intensity assays showed that the *An. gambiae* s.l. mosquitoes were susceptible to alpha-cypermethrin at 2X in all states with reported 1X resistance, except in Akwa Ibom, Nasarawa, and Plateau, where low resistance intensity to alpha-cypermethrin was observed. Similarly, all sites with deltamethrin 1X resistant vector populations showed full susceptibility to deltamethrin at 2X, except Plateau where low intensity (mortality between 98-100% at 5X) of deltamethrin resistance was observed. Low permethrin resistance intensity was observed in Bayelsa, Cross River, FCT, Kebbi, Oyo, Plateau, and Zamfara. Moderate permethrin resistance intensity was recorded in *An. gambiae* s.l. populations from Ebonyi, Enugu, and Nasarawa. High resistance intensity (mortality of less than 98% at 10X dosage) was recorded in Akwa Ibom and Enugu.

Pre-exposure of *An. gambiae* s.l. mosquitoes to the synergist piperonyl butoxide (PBO) resulted in increased mortality at varying degrees across sites when exposed to pyrethroids (alpha-cypermethrin, deltamethrin, and permethrin). Vector populations pre-exposed to PBO showed complete susceptibility to alpha-cypermethrin in Bauchi (one LGA), Bayelsa, Ebonyi, Enugu, FCT, Kebbi, Nasarawa, and Zamfara. Susceptibility of *An. gambiae* s.l. mosquitoes was not fully restored in Akwa Ibom, Bauchi (remaining five LGAs), and Plateau. Susceptibility of *An. gambiae* s.l. mosquitoes to deltamethrin were restored only in Akwa Ibom, Bayelsa, Benue, Ebonyi, Nasarawa, and Sokoto, but not in Bauchi, Enugu, or Plateau. PBO fully restored the susceptibility of *An. gambiae* s.l. populations to permethrin only in Bayelsa, Ebonyi, FCT, Sokoto, and Zamfara.

An. gambiae s.l. populations from all LGAs across all ecozones were susceptible to chlorfenapyr with 98-100% mortality at 72 hours except in one LGA each in Enugu (Enugu South, 81%) and FCT (AMAC, 97%). Mortality rates of *An. gambiae* s.l. 24 hours post-exposure to clothianidin were 99–100% in 12 out of 14 sites, 94-98% in Akwa Ibom, and 97-100% in Cross River.

I. INTRODUCTION

Malaria remains a major public health problem in sub-Saharan Africa. Of the six countries that accounted for about 55% of all cases globally, Nigeria alone accounts for 27% (World Malaria Report 2021). 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* complex (*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 six states and insecticide resistance monitoring only in an additional eight 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-focus states. Through VectorLink, each sentinel site recruits field staff comprising of technicians and mosquito collectors trained on entomological methods. VectorLink also provides basic equipment needed for entomological monitoring. Each sentinel site and insecticide resistance monitoring team worked in conjunction with the Malaria Control Program division of the State Ministry of Health and the Nigeria Institute for Medical Research (NIMR).

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

From October 2020 to September 2021, VectorLink Nigeria conducted longitudinal vector surveillance in six sites and insecticide resistance monitoring in 14 sites (11 PMI focus states and three non-PMI focus states), assessing species composition, density, feeding time, location (indoors or outdoors), seasonality, and insecticide susceptibility status of the major malaria vectors. The intensity and mechanism of insecticide resistance across the different ecozones of Nigeria were also determined. This report summarizes entomological monitoring activities completed from October 2020 to September 2021.

1.1 SENTINEL SITES AND COLLECTION AND ANALYTICAL METHODS

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

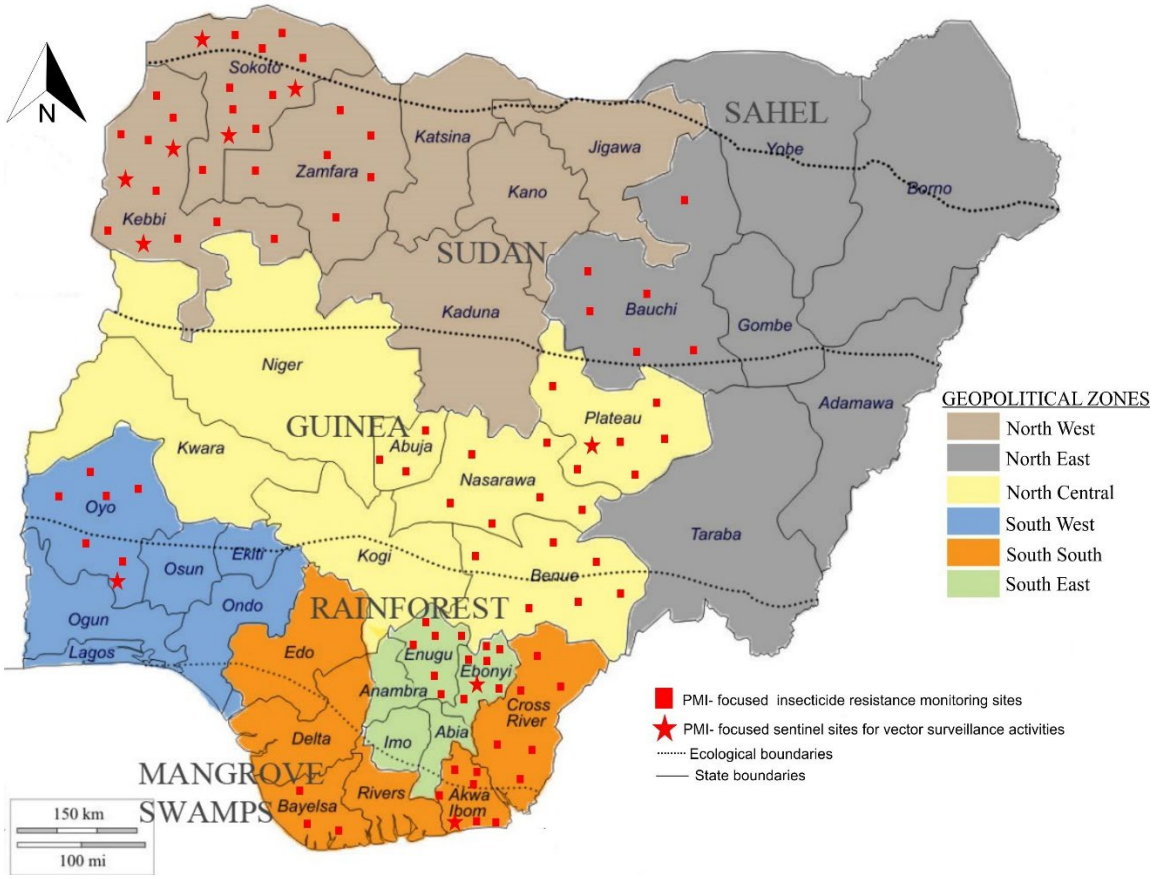
TABLE 1: LONGITUDINAL VECTOR SURVEILLANCE AND INSECTICIDE RESISTANCE MONITORING SITES AND AFFILIATED INSTITUTIONS

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

TABLE 2: ADDITIONAL INSECTICIDE RESISTANCE MONITORING SITES AND AFFILIATED INSTITUTIONS

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

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



From October 2020 to September 2021, *Anopheles* mosquitoes were collected monthly from six sentinel sites located in five ecozones of Nigeria (Figure 1). Mosquitoes were caught using human-baited CDC LTs indoors and outdoors, and PSCs. Details for each method are shown in Table 3. *Anopheles* larvae were collected using ladles and reared to adults for insecticide susceptibility tests. Data collected from longitudinal surveillance sites were collated and used to calculate the indicators in Table 4, which are also described in the sections on the respective mosquito collection methods 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.

1.4 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

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

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). The dissections were carried out on 20% of mosquitoes collected each month (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 multiplex PCR assay (Koekemoer, *et al.*, 2002) was used to determine members of the *An. funestus* group.

1.7 *Plasmodium* Sporozoite and Blood Meal Assays

To estimate the *Plasmodium* infection rate in the mosquito population, the team also performed enzyme-linked immunosorbent assays (ELISAs) for sporozoite antigen on about 20% 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). CS ELISA positives were not boiled according to Durnez et al. (2011). The blood meal index of the selected mosquitoes was also determined by ELISA testing of human, bovine, and goat blood sources of *Anopheles* mosquitoes (Beier *et al.,* 1988).

1.8 Insecticide Resistance Monitoring

Adult female *An. gambiae* s.l. mosquitoes (3-5 days old) reared from wild-caught larvae were exposed to pyrethroid (alpha-cypermethrin, deltamethrin, and permethrin) and organophosphate (pirimiphos-methyl) insecticides using CDC bottle bioassay methods (Brogdon and Chan, 2010). Resistance intensity assays were carried out with different doses (1X, 2X, 5X, and 10X) of pyrethroids to determine insecticide resistance intensity across all locations where pyrethroid resistance was detected. The test results were interpreted according to the WHO guideline (WHO, 2016). Susceptibility tests on chlorfenapyr (100 µg per bottle) and clothianidin (4 µg per bottle) using the CDC bottle assay were carried out on *An. gambiae* Kisumu strain mosquitoes (control) and wild-caught *An. gambiae* s.l. from all insecticide resistance monitoring sites.

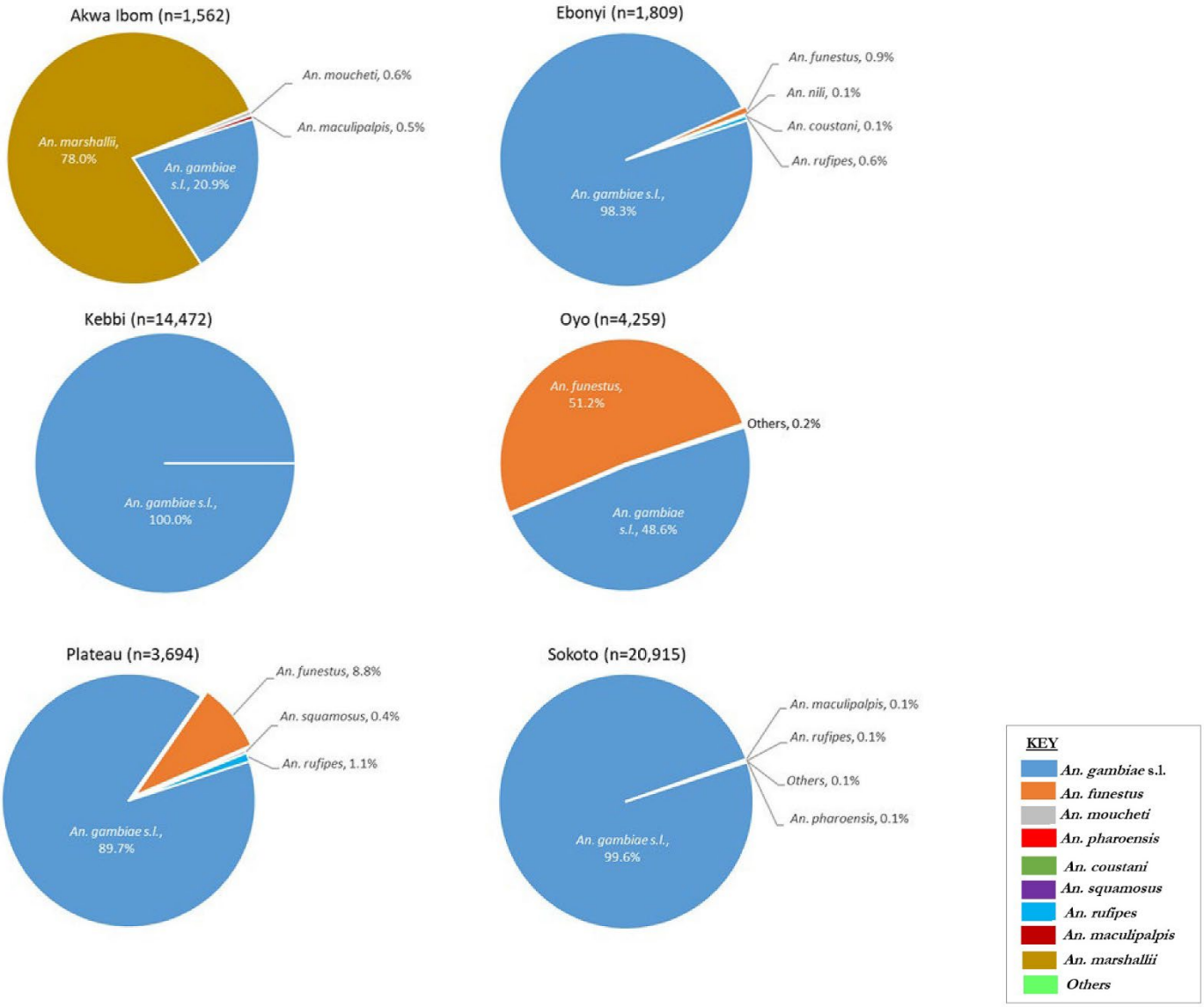
Synergist assays using PBO were also carried out using standard methods to determine mechanisms of resistance in the *An. gambiae* s.l. mosquitoes. The *kdr* genotype frequencies were determined among *An. gambiae* s.l. using allele-specific PCR assays. Surviving mosquitoes from intensity and synergist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

2. RESULTS

2.1 MOSQUITO ABUNDANCE AND SPECIES COMPOSITION

A total of 46,711 *Anopheles* mosquitoes were collected from six sentinel sites using human-baited CDC LTs (indoors/outdoors) and PSCs. *Anopheles gambiae* s.l. was the most abundant species across most sites, ranging from 20.9% in Akwa Ibom to 100% in Kebbi (Figure 2). Other *Anopheles* species identified in varying abundance were *An. marshallii* complex (Akwa Ibom only), *An. funestus, An. rufipes,* and *An. maculipalpis*. Other localized species observed were *An. squamosus, An. coustani, An. nili*, *An. moucheti,* and *An. pharoensis*. Annex 3 provides the number of each species collected by site and collection method (Figure 2).

FIGURE 2: *ANOPHELES* SPECIES COMPOSITION ACROSS SITES

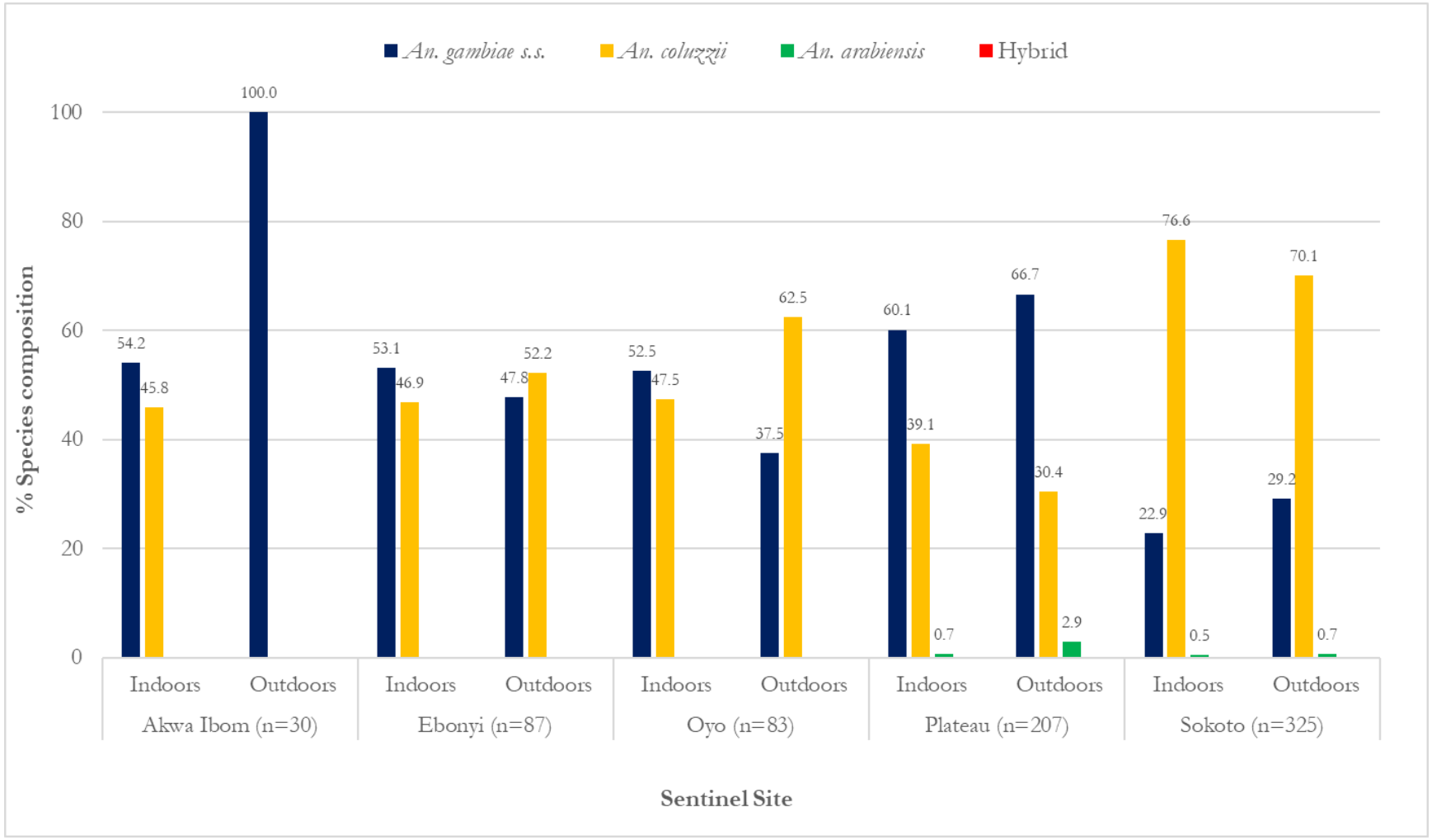


2.2 Molecular Identification of Members of the *An. gambiae* Complex and Determination of Sporozoite Rates

A total of 2,625 *An. gambiae* s.l. mosquitoes collected by PSCs and CDC LTs between October 2020 and September 2021 were subjected to species-specific PCR assays. Of these, 2,608 (99.4%) mosquito samples were amplified, (Annex 2) while 17 (0.6%) failed to amplify. Results showed that 1,018 (39.0%) were identified as *An. gambiae* s.s., 1,546 (59.3%) were *An. coluzzii,* 40 (1.5%) were *An. arabiensis*, and four (0.2%) were hybrid *An. gambiae*/*An. coluzzii* (Annex 4).

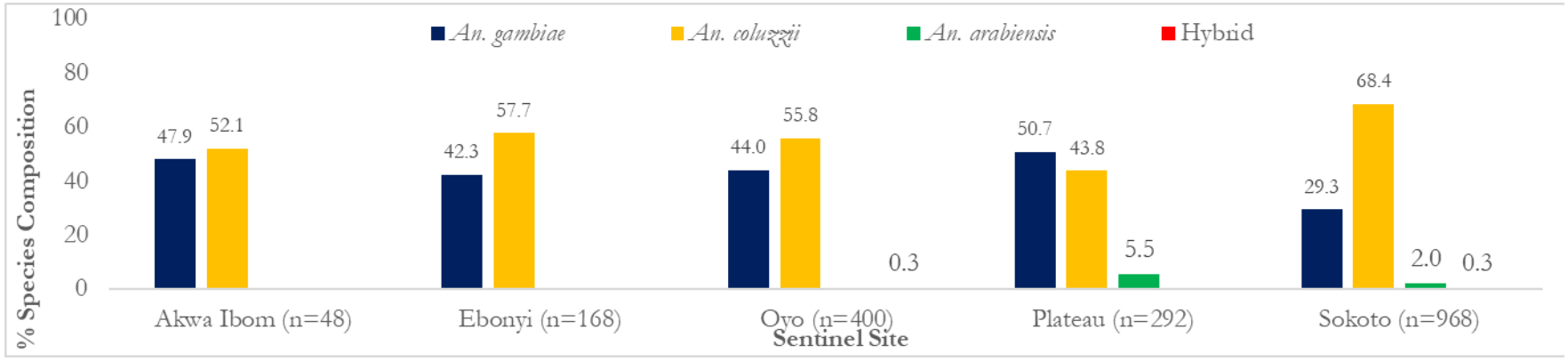
Anopheles gambiae s.s. was the dominant vector species found both indoors and outdoors in Akwa Ibom and Plateau compared to Ebonyi and Oyo, where it was found to be predominant indoors. The highest proportion of *An. gambiae* s.s. was collected outdoors in Akwa Ibom (100%), while the highest indoor collection was in Plateau (60.1%). *An. coluzzii* was predominant both indoors and outdoors in Sokoto (76.6% and 70.1%, respectively), as well as outdoors in Oyo. The proportion of *An. arabiensis* found indoors and outdoors were generally quite low, with the highest proportions reported from Plateau (0.7% and 2.9%, respectively) (Figure 3).

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



For mosquitoes caught using PSC method, *An. coluzzii* was the dominant vector species found in all sites except Plateau where *An. gambiae* s.s. predominated (50.7%). *An. arabiensis* was found only in Plateau (5.5%) and Sokoto (2.0%), while hybrid forms (*An. gambiae* s.s./*An. coluzzii*) were recorded in Oyo and Sokoto (both 0.3%) (Figure 4).

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



As shown in Table 5, *Plasmodium falciparum* sporozoite rates of *An. gambiae* s.s. indicate that the highest infection rates were recorded outdoors in Oyo (11.1%) followed by Plateau (2.2%), while for *An. coluzzii,* the highest infection rate was recorded outdoors also in Oyo (6.7%). Incidentally, no other site recorded infections outdoors. Indoors, the highest infection rates were recorded in Plateau (3.7%) followed by Oyo (3.6%), Ebonyi (3.3%), and Sokoto (0.7%). There was no sporozoite positivity recorded in *An. arabiensis* both 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*						*An. coluzzii*						*An. arabiensis*					
		Number identified (%)		No. Positive for Sporozoites		SPR (%)		Number identified (%)		No. Positive for Sporozoites		SPR (%)		Number identified (%)		No. Positive for Sporozoites		SPR (%)	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Akwa Ibom	30	13 (43.3)	6 (20)	0	0	0.0	0.0	11 (36.7)	0 (0)	0	0	0.0	0.0	0 (0)	0 (0)	0	0	0.0	0.0
Ebonyi	87	34 (39.1)	11 (12.6)	0	0	0.0	0.0	30 (34.5)	12 (13.8)	1	0	3.3	0.0	0 (0)	0 (0)	0	0	0.0	0.0
Oyo	83	31 (37.3)	9 (10.8)	0	1	0.0	11.1	28 (33.7)	15 (18.1)	1	1	3.6	6.7	0 (0)	0 (0)	0	0	0.0	0.0
Plateau	207	83 (40.1)	46 (22.2)	0	1	0.0	2.2	54 (26.1)	21 (10.1)	2	0	3.7	0.0	1 (0.5)	2 (1)	0	0	0.0	0.0
Sokoto	325	43 (13.2)	40 (12.3)	0	0	0.0	0.0	144 (44.3)	96 (29.5)	1	0	0.7	0.0	1 (0.3)	1 (0.3)	0	0	0.0	0.0
Total	**732**	**204 (27.9)**	**112 (15.3)**	**0**	**2**	**0.0**	**1.8**	**267 (36.5)**	**144 (19.7)**	**5**	**1**	**1.9**	**0.7**	**2 (0.3)**	**3 (0.4)**	**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 812 *An. funestus* mosquitoes collected by CDC LTs and PSCs in Ebonyi, Oyo, Plateau, and Sokoto were subjected to species-specific PCR assays. Of these, 365 (45.0%) mosquitoes were collected using CDC LTs (Figure 5), while 447 (55.0%) were collected using PSCs (Figure 6). *Anopheles funestus* s.s. were found to be the only species identified both indoors and/or outdoors in all sites except in Oyo where other unamplified samples were observed (Figure 5). For members of *An. funestus* group collected using PSCs, only *An. funestus* s.s. predominated in all four sites except in Plateau where unamplified species (1.7%) and *An. leesoni* (0.9%), were found (Figure 6).

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

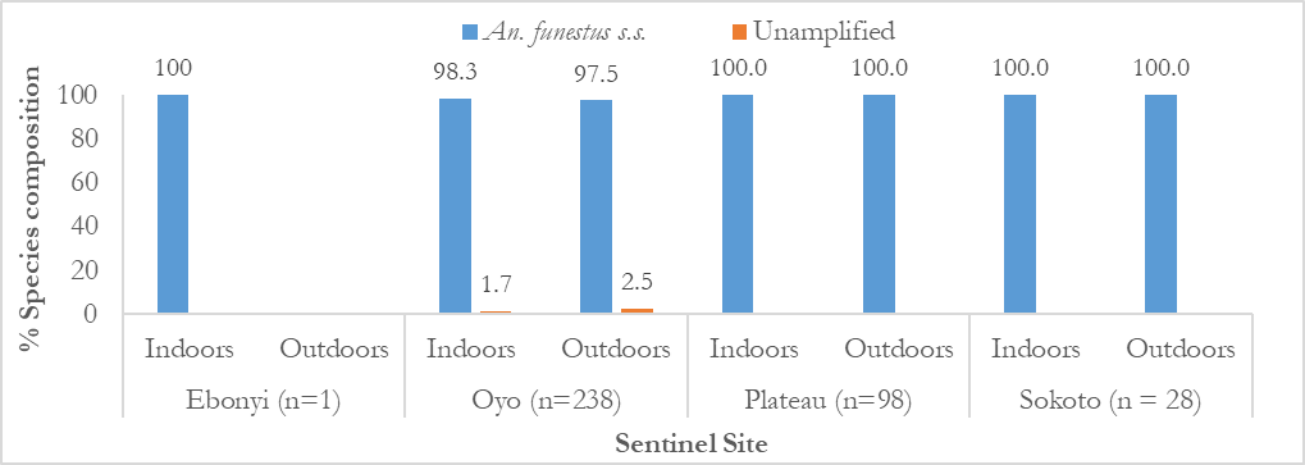
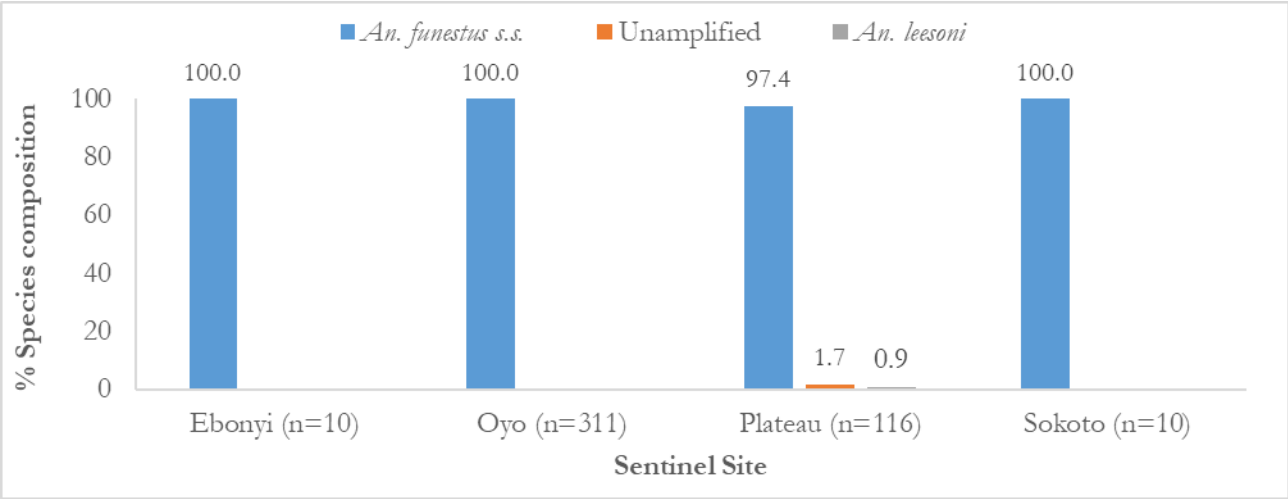


FIGURE 6: PROPORTION OF MEMBERS OF *AN. FUNESTUS* GROUP COLLECTED BY PSC ACROSS SENTINEL SITES



Plasmodium falciparum sporozoite rates in *An. funestus* s.s. collected indoors from CDC LTs ranged from 0.0% in Ebonyi and Sokoto to 2.8% in Plateau. Outdoors, sporozoite infection rates were 0.0% in Ebonyi, Plateau, and Sokoto and 6.0% in Oyo. No other unidentified members 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 Ebonyi and Sokoto to 5.1% in Oyo (Table 7). There was no sporozoite infection recorded in unidentified members of the *An. funestus* group. 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
Ebonyi	1	1 (100)	0 (0)	0	0	0.0	0.0	0 (0)	0 (0)	0	0	0.0	0.0
Oyo	238	116 (48.7)	117 (49.2)	3	7	2.6	6.0	2 (0.8)	3 (1.3)	0	0	0.0	0.0
Plateau	98	72 (73.5)	26 (26.5)	2	0	2.8	0.0	0 (0)	0 (0)	0	0	0.0	0.0
Sokoto	28	20 (71.4)	8 (28.6)	0	0	0.0	0.0	0 (0)	0 (0)	0	0	0.0	0.0
Total	**365**	**209 (57.3)**	**151 (41.4)**	**5**	**7**	**2.4**	**4.6**	**2 (0.5)**	**3 (0.8)**	**0**	**0**	**0.0**	**0.0**

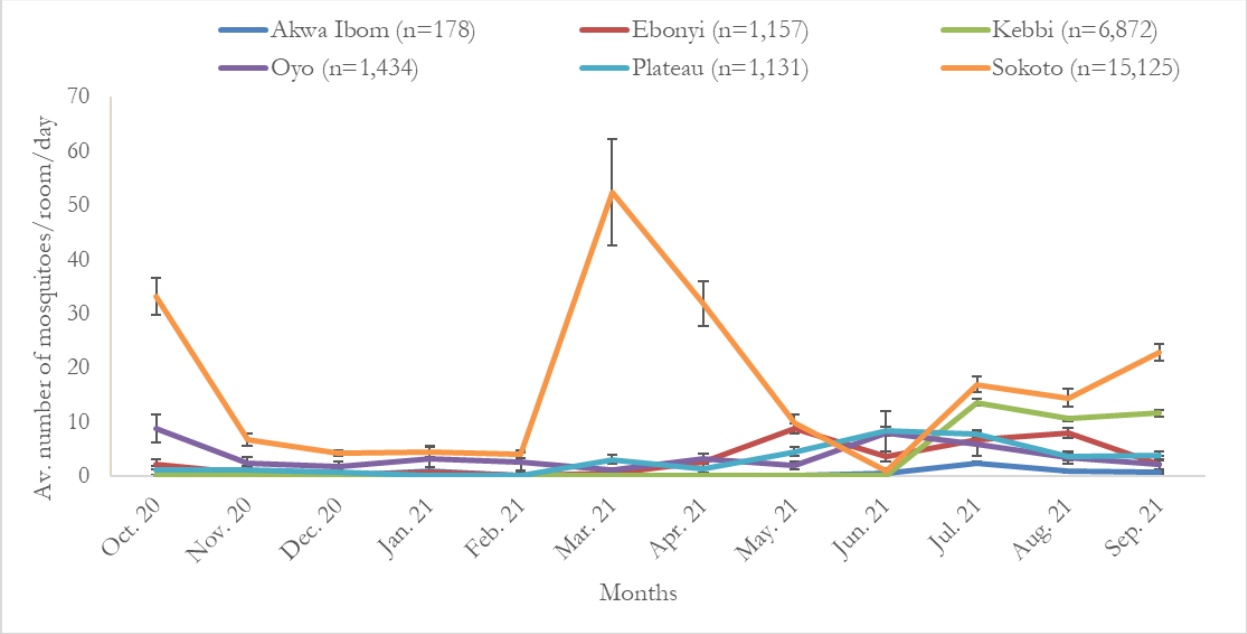
TABLE 7: SPOROZOITE POSITIVITY RATES OF *AN. FUNESTUS* MOSQUITOES COLLECTED BY PSC ACROSS SITES

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 (%)
Ebonyi	10	10 (100)	0	0.0	0 (0)	0	0.0	0 (0)	0	0.0
Oyo	311	311 (100)	16	5.1	0 (0)	0	0.0	0 (0)	0	0.0
Plateau	116	113 (97.4)	3	2.7	2 (1.7)	0	0.0	1 (0.9)	0	0.0
Sokoto	10	10 (100)	0	0.0	0 (0)	0	0.0	0 (0)	0	0.0
Total	**447**	**444 (99.3)**	**19**	**4.3**	**2 (0.5)**	**0**	**0.0**	**1 (0.2)**	**0**	**0.0**

2.4 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 0.2 mosquitoes/room/day in Ebonyi during December 2020 and February 2021 to 52.4 mosquitoes/room/day in Sokoto in March 2021 (Figure 7 and Annex 8). In Sokoto, higher indoor resting densities were also observed in October 2020 (33.2 mosquitoes/room/day) and between July and September 2021 (16.9 climbing to 22.8 mosquitoes/room/day, respectively).

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



2.5 *ANOPHELES MARSHALLII* COMPLEX

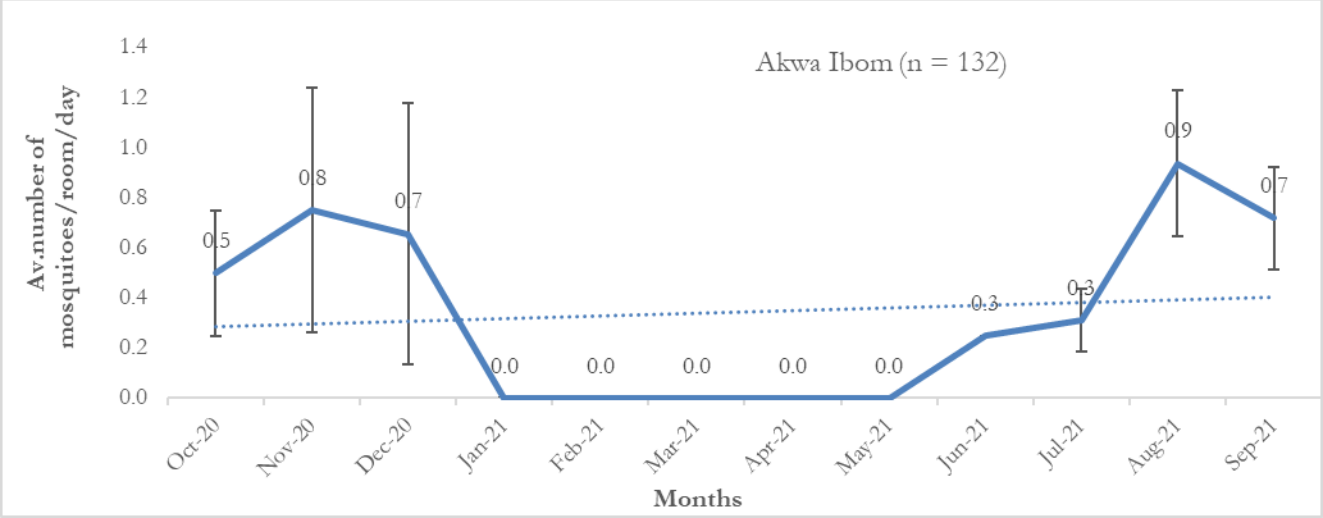
Following non-amplification of samples identified as *An. gambiae* s.l. from Akwa Ibom, the PMI VectorLink insectary in Keffi conducted a detailed re-identification exercise to confirm morphological identification. Results of this exercise indicated that these samples were possibly *An. hargreavesi*. Given the large number of mosquitoes that were misidentified, the team initiated preliminary steps to confirm these mosquitoes were not *An. gambiae* s.l. by sending randomly selected subsamples spread over several months to NIMR Lagos to be subjected to *An. gambiae* PCR analysis. All PCRs failed to amplify indicating that the samples were not members of the *An. gambiae* complex.

An initial subset of 50 freshly collected mosquitoes from Akwa Ibom were sent to University of Witwatersrand in South Africa in July 2021 for morphological confirmation and sequencing. Based on specimen condition, the morphological identification was inconclusive and a lack of comparable sequences on NCBI prevented identification with ITS2 sequences. A second subset of another 50 freshly collected mosquitoes were sent in December 2021, in an effort to improve the ability to morphologically identify the specimens and generate more sequences for analysis. The second batch were from hourly human-baited CDC light traps mounted indoors and outdoors in houses near coconut palms in Akwa Ibom. Morphological identification was performed immediately after collection to allow for better quality specimens with intact scale patterns. Among the second batch of samples sent, 16% were conclusively identified as *An. hargreavesi* based on key characteristics while others were not and hence generally categorized as *An. marshallii* complex. Sequences generated from the morphologically categorized *An. marshallii* specimens matched 84% of the shared sequence identity of the *An. marshallii* isolate (MW257139.1) from Cameroon. Because no comparative sequencing exists for *An. hargreavesi*, these specimens have also been categorized as *An. marshalli* complex by sequencing identification.

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

The indoor resting density of *An. marshallii* complex mosquitoes varied across the months, ranging from 0.0 mosquitoes/room/day from January through May to a maximum of 0.9 mosquitoes/room/day in August (Figure 7). Higher indoor resting densities were observed in the drier months of November 2020 (0.8), December 2020 (0.7), and September 2021 (0.7) (Figure 8).

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



2.7 HUMAN BITING RATES

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

The mean indoor biting rates of *An. gambiae* s.l. peaked in July in Plateau (103.4 bites/person/night (b/p/n)), in September in Kebbi (37.6 b/p/n) and in April in Sokoto (26.5 b/p/n) (Figure 9), with an earlier smaller peak in October (Oyo 21.6 b/p/n). Increased outdoor biting was recorded in Sokoto (22.3 b/p/n), Kebbi (20.7 b/p/n), and Oyo (11.2 b/p/n) in April, September, and October, respectively (Figure 10).

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

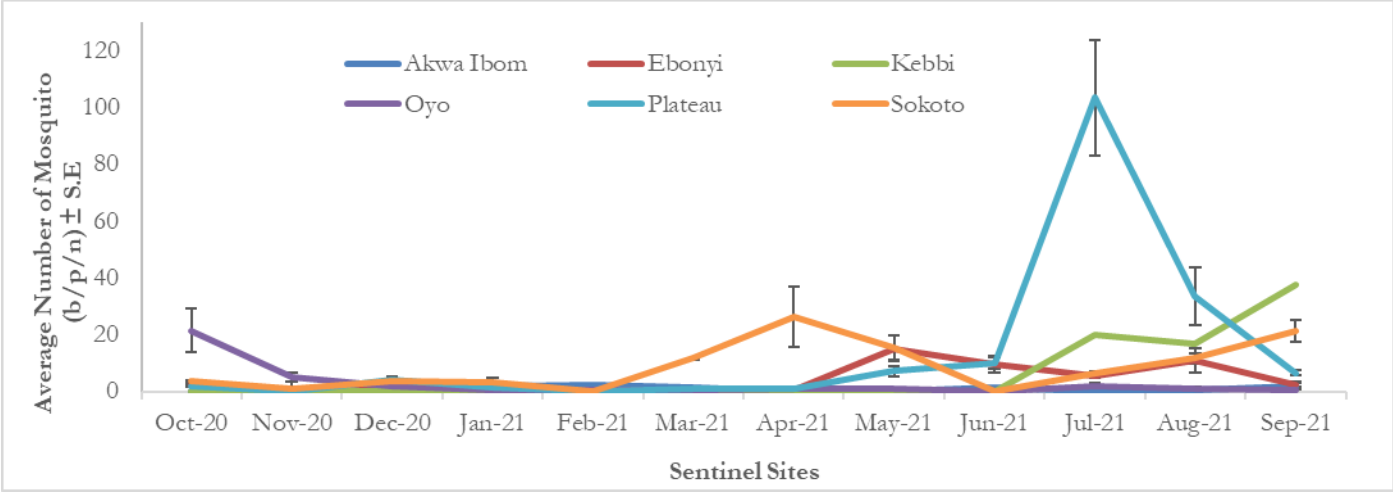
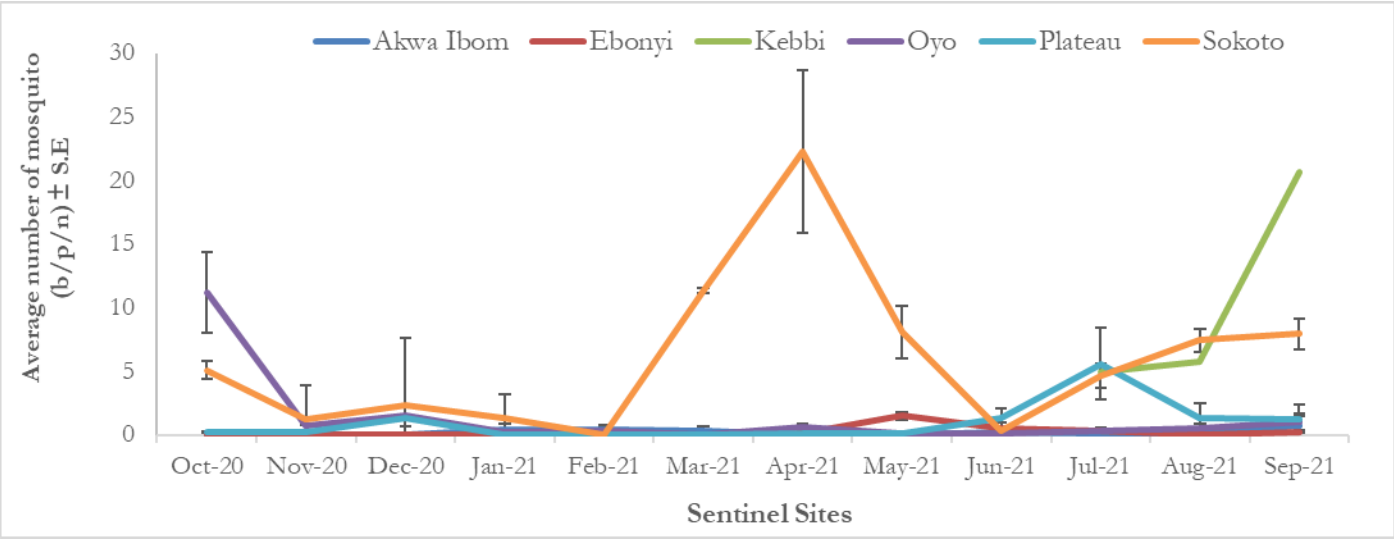


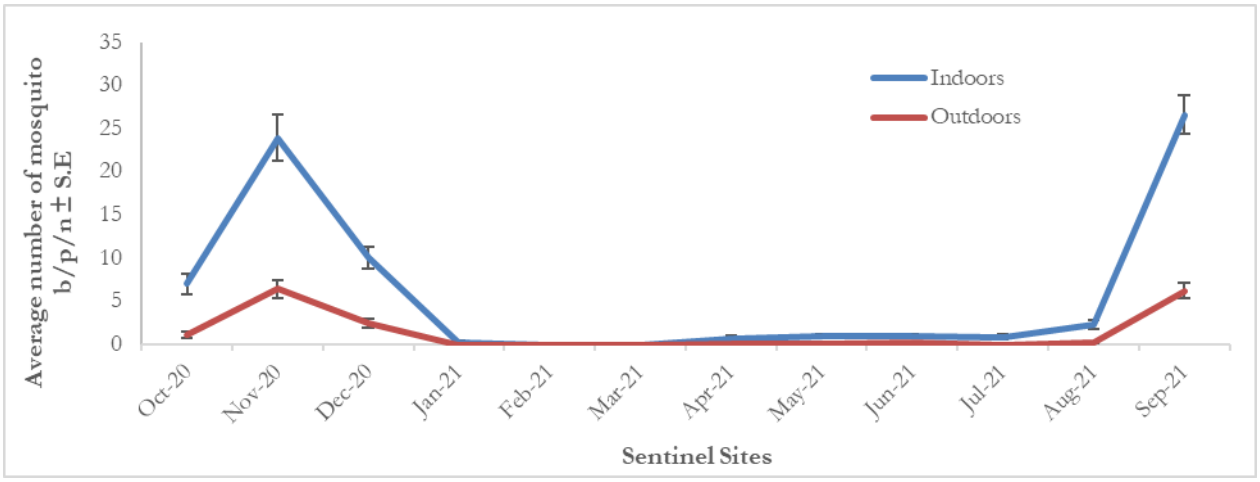
FIGURE 10: MONTHLY OUTDOOR HUMAN BITING RATES OF *AN. GAMBIAE* S.L. ACROSS SITES



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

The mean indoor biting rates of *An. marshallii* complex peaked in September (26.6 b/p/n) (Figure 11), with an earlier peak in November 2020 (23.9 b/p/n). Increased outdoor biting was recorded in the same months as increased indoor biting: November 2020 (6.4 b/p/n) and September 2021 (6.3 b/p/n).

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



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

The mean indoor biting rates of *An. funestus* were recorded in Oyo and Plateau. This peaked in November 2020 in Oyo (14.2 b/p/n), followed by another peak (13.4 b/p/n) observed in September 2021 (Figure 12). The highest indoor biting peaks of 1.7 and 1.6 b/p/n in Plateau were recorded in January and July 2021, respectively. Increased outdoor biting rates of 4.2 b/p/n and 6.1 b/p/n were recorded in Oyo during October 2020 and September 2021, respectively. In Plateau, outdoor biting peaked in November 2020 (1.0 b/p/n) (Figure 13).

FIGURE 12: MONTHLY INDOOR HUMAN BITING RATES OF *AN. FUNESTUS* IN OYO AND PLATEAU (OCTOBER 2020-SEPTEMBER 2021)

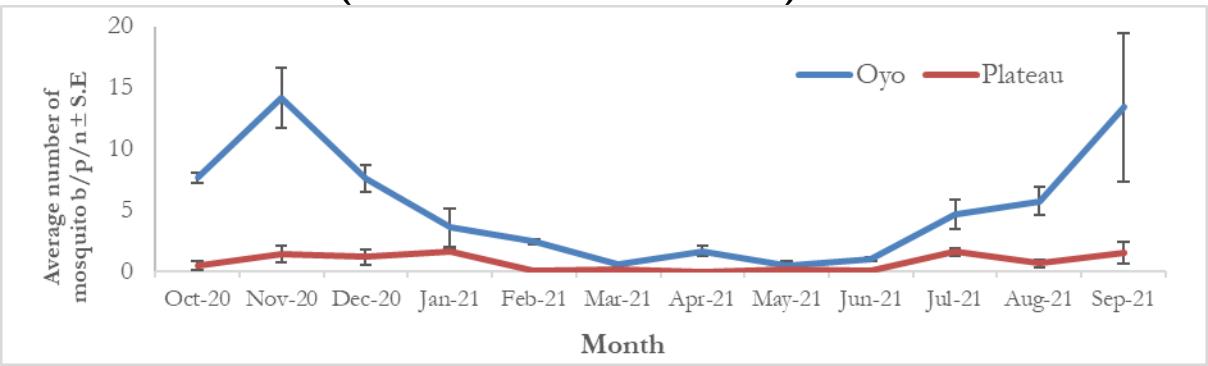
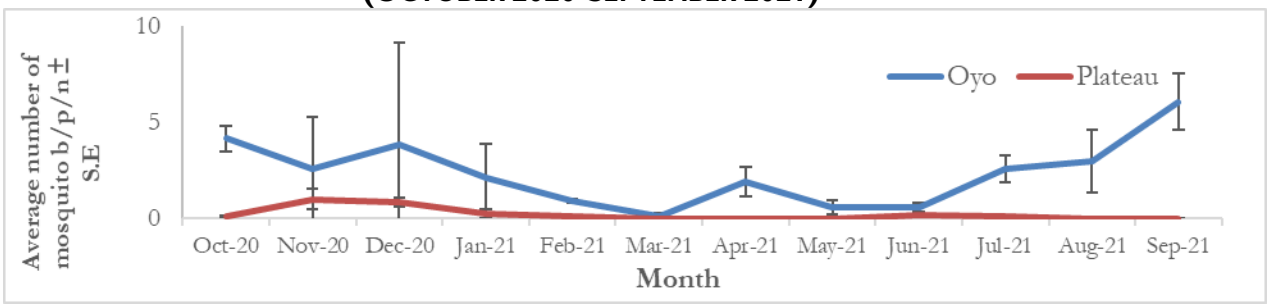


FIGURE 13: MONTHLY OUTDOOR HUMAN BITING RATES OF *AN. FUNESTUS* IN OYO AND PLATEAU (OCTOBER 2020-SEPTEMBER 2021)

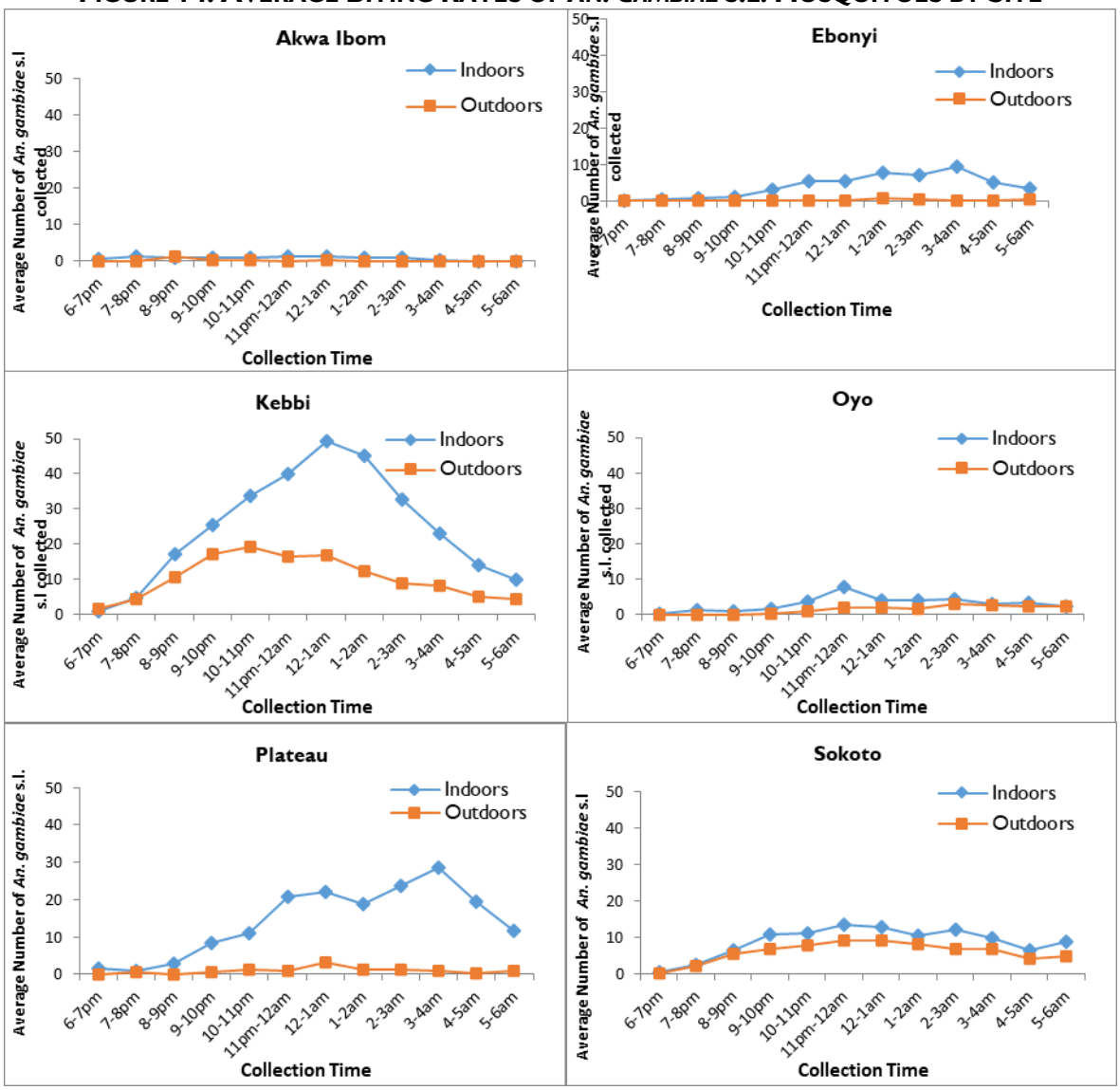


2.8 BITING TIME

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

The average number of mosquitoes caught biting per hour was generally higher indoors, ranging from one mosquito collected between 7-8 p.m. in Akwa Ibom to 49 mosquitoes collected between 12-1 a.m. in Kebbi. Outdoors, the hourly biting rates were lower in all sites, and peaked at 19 mosquitoes collected between 10-11 p.m. in Kebbi (Figure 14).

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



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

An. marshallii complex mosquitoes caught using PSC and CDC LT methods were screened for *Plasmodium falciparum* circumsporozoite antigens in Akwa Ibom. Results indicate that sporozoite infection was recorded in one mosquito caught using PSC method (0.6%). There was no sporozoite positivity recorded in *An. marshallii* complex mosquitoes caught using CDC LTs in Akwa Ibom (Table 8).

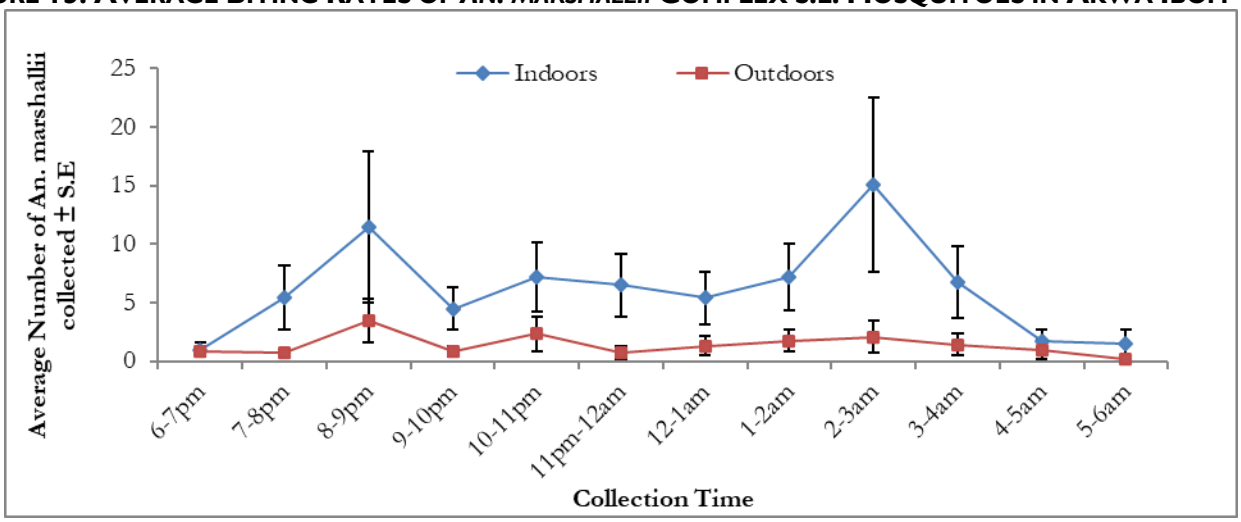
TABLE 8: SPOROZOITE POSITIVITY RATES OF *AN. MARSHALLII* MOSQUITOES COLLECTED BY CDC LT AND PSC AT AKWA IBOM SENTINEL SITE

Site	Method of Mosquito Collection	Total Analyzed	*An. marshallii.*		
			Number identified (%)	No. Positive for Sporozoites	SPR (%)
Akwa Ibom	CDC LT Indoors	50	50 (100)	0	0.0
	PSC	162	162 (100)	1	0.6

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

The average number of mosquitoes caught biting per hour was generally higher indoors, with two peaks, one between 2-3 a.m. (15 mosquitoes) and one between 8-9 p.m. (12 mosquitoes) (Figure 15).

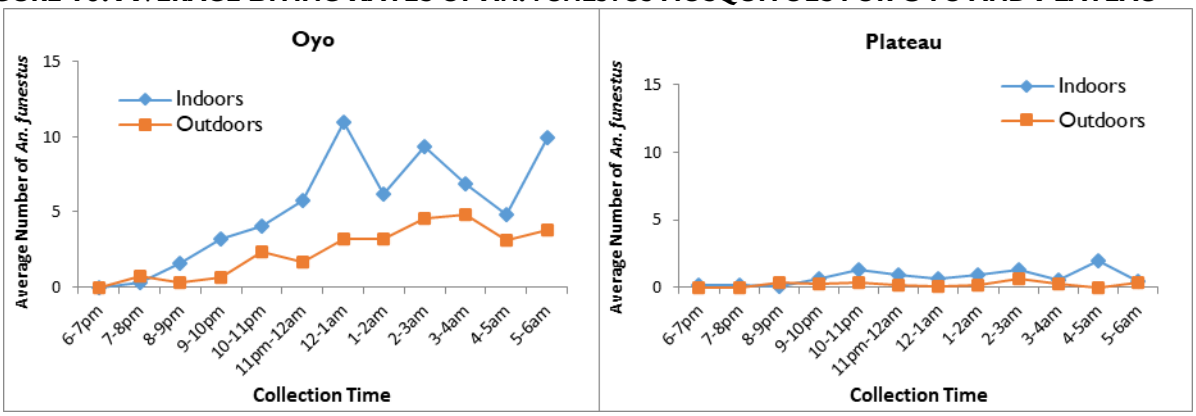
FIGURE 15: AVERAGE BITING RATES OF *AN. MARSHALLII* COMPLEX S.L. MOSQUITOES IN AKWA IBOM



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

The average number of *An. funestus* mosquitoes caught biting per hour was higher indoors compared to outdoors in Oyo and Plateau. The average hourly number of mosquito bites (11, 9, 10) in Oyo, peaked indoors at 12-1 a.m., 2-3 a.m., and 5-6 a.m., respectively. Steady biting was also observed outdoors between 10-11 p.m. through to 5-6 a.m., peaking at five mosquito bites per hour from 3-4 a.m. In Plateau, biting peaked indoors and outdoors at 4-5 a.m. and 2-3 a.m., respectively (Figure 16).

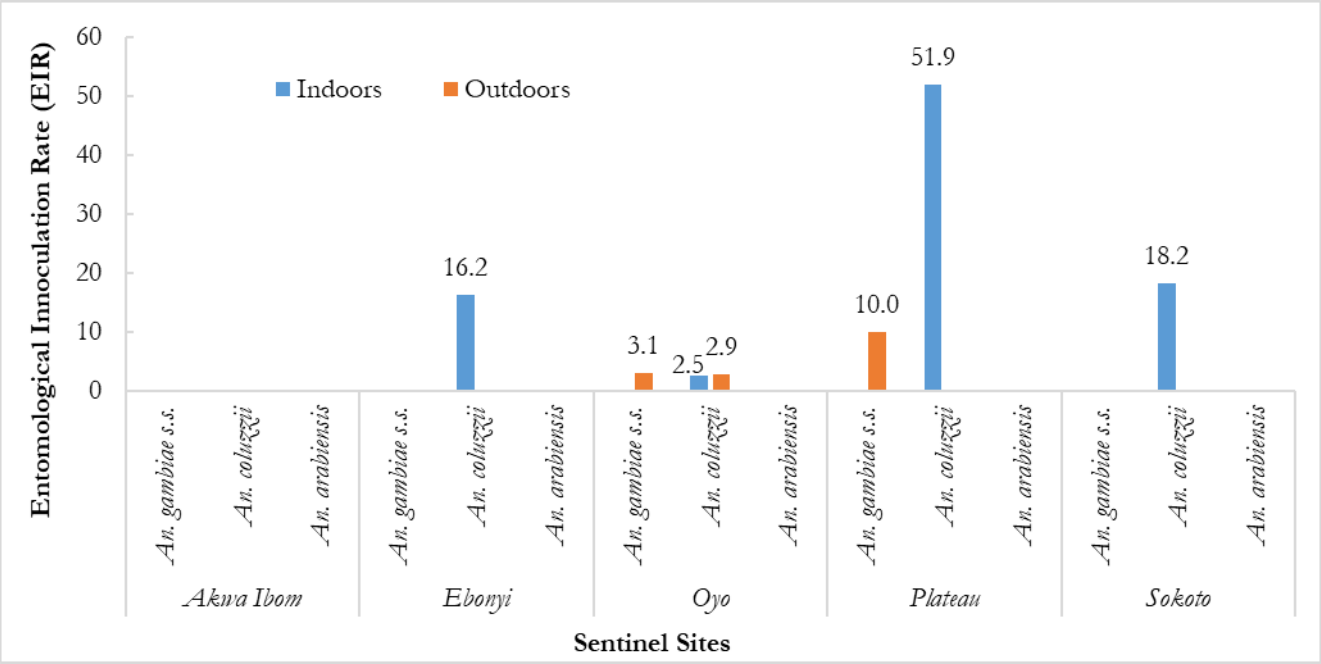
FIGURE 16: AVERAGE BITING RATES OF *AN. FUNESTUS* MOSQUITOES FOR OYO AND PLATEAU



2.10 ENTOMOLOGICAL INOCULATION RATES ACROSS SITES

EIRs were recorded indoors in four of the five sites among *An. coluzzii*. There were no infective bites recorded indoors among *An. gambiae* s.s. in any of the sentinel sites. The highest indoor EIR was recorded with *An. coluzzii* in Plateau (51.9 infective bites/person/year), Sokoto (18.2 infective bites/person/year), and Ebonyi (16.2 infective bites/person/year). The highest outdoor EIR was recorded among *An. gambiae* s.s. in Plateau (10.0 infective bites/person/year) followed by Oyo (3.1 infective bites/person/year), and *An. coluzzii* in Oyo (2.9 infective bites/person/year). There were no infective bites recorded among *An. arabiensis* indoors or outdoors in any of the sentinel sites (Figure 17 and Annex 7).

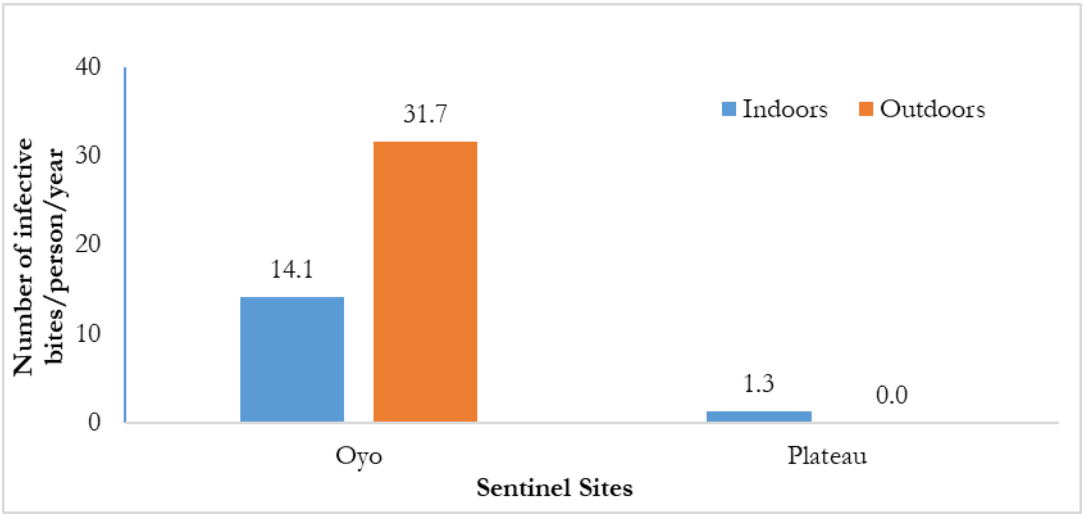
FIGURE 17: ANNUAL ENTOMOLOGICAL INOCULATION RATES ACROSS SITES



2.10.1 ENTOMOLOGICAL INOCULATION RATES IN *ANOPHELES FUNESTUS* S.S. ACROSS SITES

EIRs were recorded indoors for *An. funestus* s.s. in Oyo and Plateau. The highest EIR was recorded outdoors in Oyo (31.7 infective bites/person/year), followed by indoors EIR also in Oyo (14.1 infective bites/person/year). There was a statistical difference between outdoor and indoor EIR in Oyo (p=0.0004). There was only indoor EIR (1.3 infective bites/person/year) recorded in Plateau. There were no infective bites recorded among *An. funestus* s.s. outdoors in Plateau (Figure 18 and Annex 9).

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



2.11 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 19-21). The proportion varied by vector and site. The highest proportions of *An. gambiae* s.s. mosquitoes from CDC LTs that fed on human blood indoors were from Ebonyi (100%) and Oyo (100%), while the highest proportions for *An. coluzzii* were recorded indoors in Akwa Ibom (100%). For *An. arabiensis,* the highest proportions that fed on human blood was recorded from indoors in Sokoto (100%). *Anopheles gambiae* s.s. collected from CDC LTs indoors in Akwa Ibom fed on human and goat equally (50% each), while in Plateau, *An. coluzzii* fed on human and bovine equally as well (50% each) (Figure 19). Generally, human blood meal from CDC LT outdoors (50-100%) followed the same patterns as CDC LTs placed indoors (50-100%). In Ebonyi, Oyo, and Plateau, all *An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis* mosquitoes collected from CDC LTs outdoors fed on human blood. The percentage of mosquitoes collected by PSC (69-89%) that fed on humans was higher compared to those that fed on bovine (2-13%) and goat (6-23%) blood meals (Figure 21).

FIGURE 19: BLOOD MEAL SOURCES OF *AN. GAMBIAE* S.S., *AN. COLUZZII*, AND *AN. ARABIENSIS* FROM INDOOR CDC LIGHT TRAP COLLECTIONS ACROSS SITES (OCTOBER 2019-SEPTEMBER 2020)

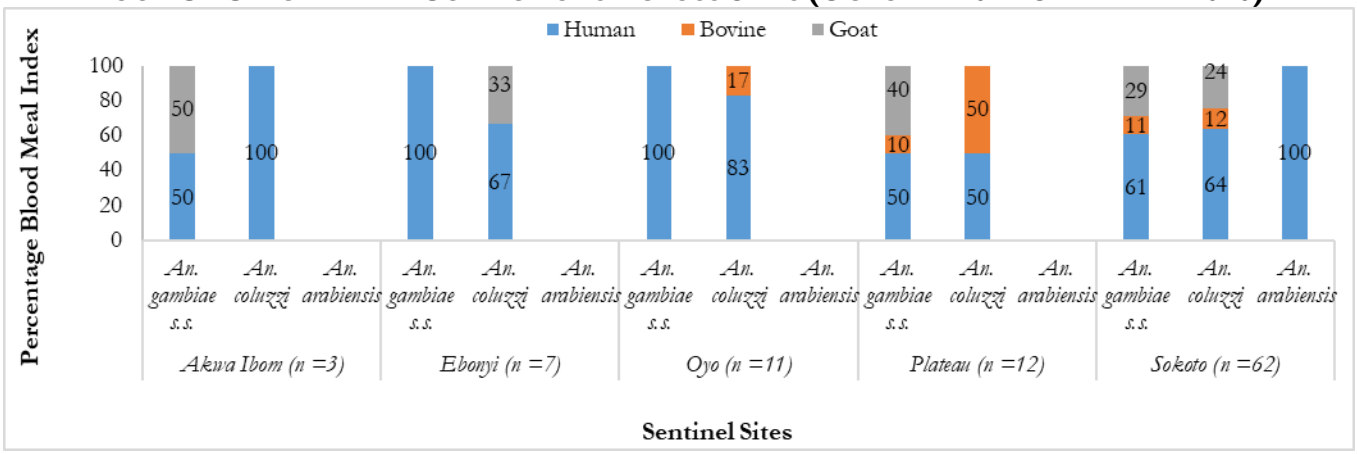


FIGURE 20: BLOOD MEAL SOURCES OF *AN. GAMBIAE* S.S., *AN. COLUZZII*, AND *AN. ARABIENSIS* FROM OUTDOOR CDC LIGHT TRAP COLLECTIONS ACROSS SITES (OCTOBER 2019-SEPTEMBER 2020)

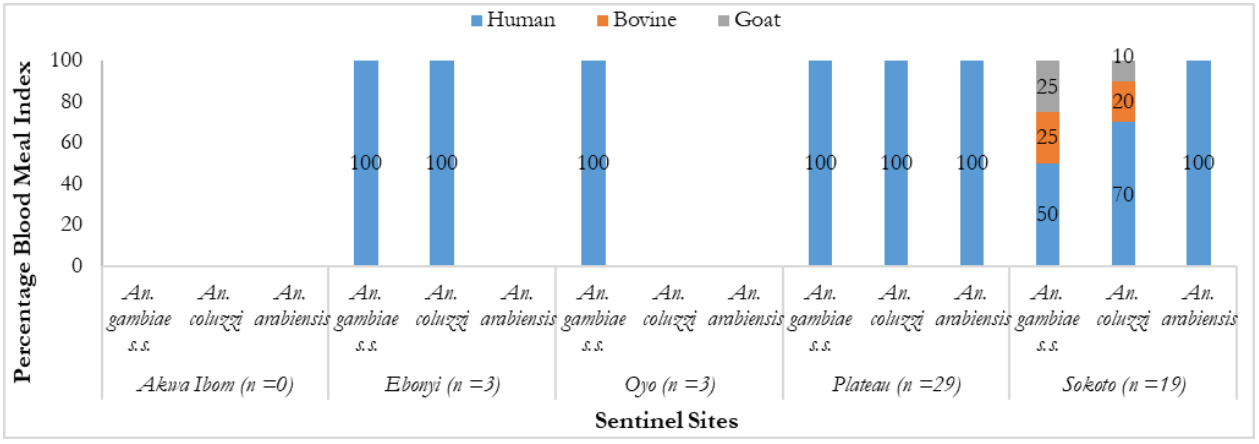
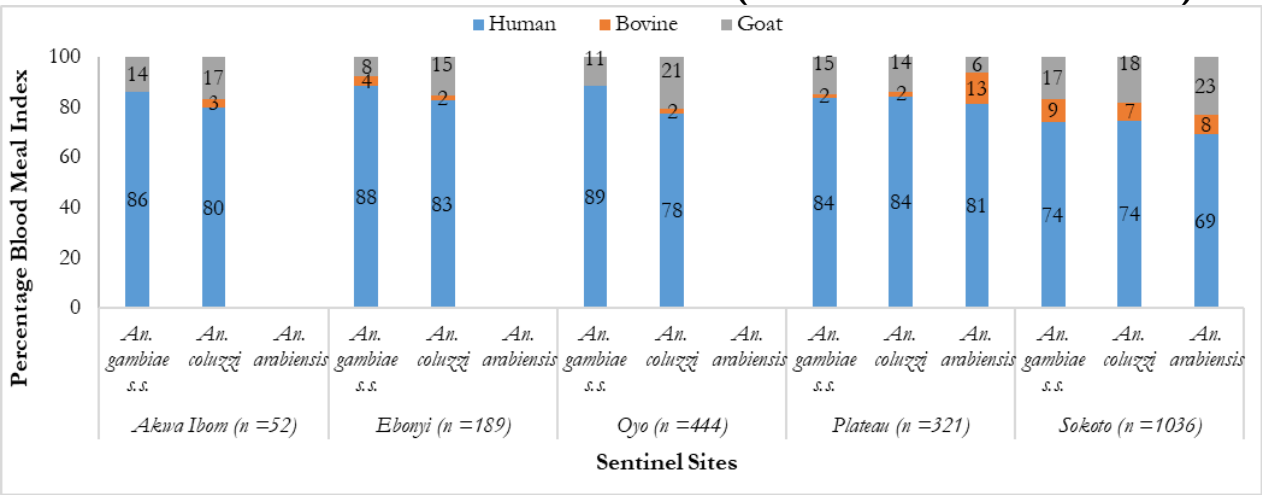


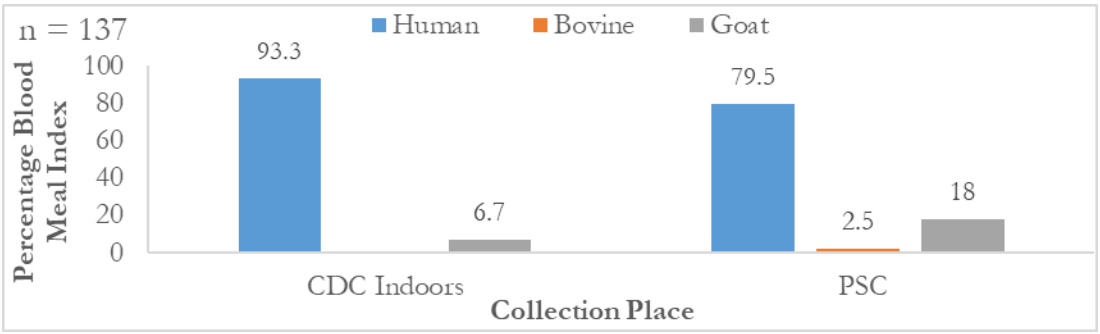
FIGURE 21: BLOOD MEAL SOURCES OF *AN. GAMBIAE* S.S., *AN. COLUZZII*, AND *AN. ARABIENSIS* FROM PYRETHRUM SPRAY CATCH COLLECTIONS ACROSS SITES (OCTOBER 2019-SEPTEMBER 2020)



2.12 BLOODMEAL SOURCES IN *AN. MARSHALLII* COMPLEX

Because of the preponderance of *An. marshallii* complex in Akwa Ibom, it became necessary to examine the blood meal preferences of the species and establish the level of human vector interactions in the area. Of the 137 blood fed mosquitoes identified as *An. marshallii*, 15 (11.0%) were from CDC LT indoors while 122 (89.0%) were collected from PSC. Fourteen (93.3%) mosquito samples collected from CDC LT fed on human blood, compared to one (6.7%) that fed on goat blood meal. Blood meal analysis of *An. marshallii* samples collected from PSC indicated that 97 (79.5%) fed on humans, compared to 3 (2.5%) and 22 (18.0%) that fed on bovine and goat blood meal, respectively. No bovine blood was detected in mosquitoes caught using CDC LT method (Figure 22).

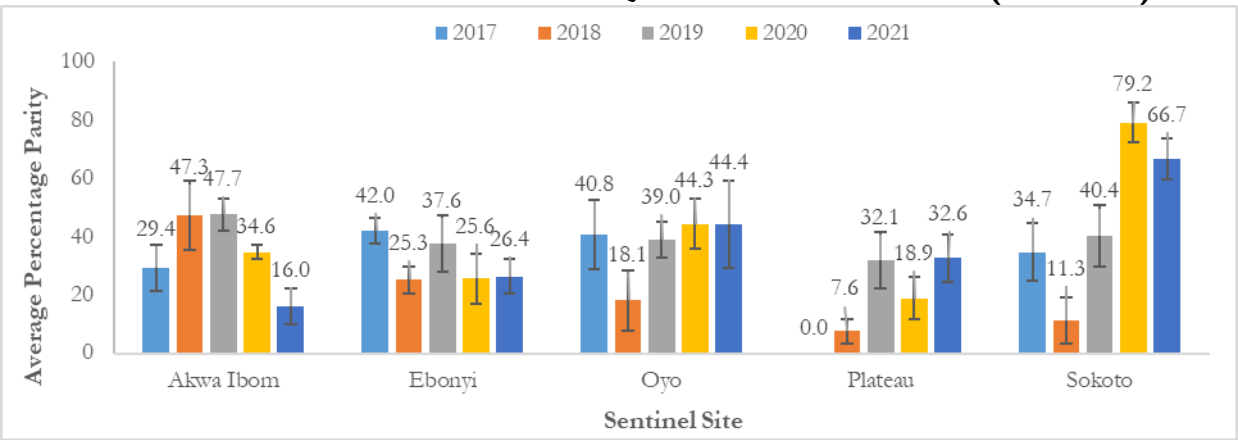
FIGURE 22: PERCENTAGE BLOOD MEAL INDEX FOR *AN. MARSHALLII*, OCTOBER 2020-SEPTEMBER 2021



2.13 PARITY RATE

Unfed, female *An. gambiae* s.l. specimens captured with human-baited CDC LTs were dissected across the sentinel sites to determine the parity rates of the mosquitoes. Average parity rates of *An. gambiae* s.l. mosquitoes from 2017-2021 were calculated and compared (Figure 23). Across the five years, the highest parity rates of 79.2% and 66.7% were recorded in Sokoto in 2020 and 2021, respectively. There was a significant difference in the average percentage of parous mosquitoes in Sokoto ($F_{4,46}$=0.7.2795, p=0.0001) and in Akwa Ibom ($F_{4,45}$=2.787, p=0.0376). The average percentage of parous mosquitoes recorded within five years in Oyo ($F_{4,46}$=0.776650, p=0.5461), Plateau ($F_{3,35}$=1.9167, p=0.1449), and Ebonyi ($F_{4,46}$=1.0604, p=0.3869) were not statistically significant (Figure 23).

FIGURE 23: PARITY RATES OF DISSECTED MOSQUITOES IN SENTINEL SITES (2017-2021)



2.14 INSECTICIDE SUSCEPTIBILITY AND MECHANISMS OF RESISTANCE

Insecticide susceptibility test results indicated that pyrethroid resistance was widespread in *An. gambiae* s.l. mosquitoes at all sentinel sites (Table 9). Pyrethroid resistance patterns varied within and among the states.

Full susceptibility of *An. gambiae* s.l. populations exposed to alpha-cypermethrin was recorded in all LGAs in Benue, Cross River, Oyo, and Sokoto. In contrast, susceptibility was only observed in *An. gambiae* s.l. mosquitoes from 6/9 LGAs in Kebbi, 2/3 LGAs in FCT, 1/4 LGAs in Enugu, and 4/6 LGAs in Zamfara. No susceptibility to alpha-cypermethrin was observed in Akwa Ibom, Bauchi, Bayelsa, Ebonyi, Nasarawa, and Plateau (Table 9).

Susceptibility of *An. gambiae* s.l. populations to deltamethrin was recorded in all LGAs in Cross River, FCT, Kebbi, Oyo, and Zamfara. Susceptibility to deltamethrin was observed in *An. gambiae* s.l. populations in 4/6 LGAs in Benue, 3/6 LGAs in Nasarawa, and 2/9 LGAs in Sokoto. No susceptibility to deltamethrin was observed in *An. gambiae* s.l. populations from Akwa Ibom, Bauchi, Bayelsa, Ebonyi, Enugu, or Plateau (Table 9). Resistance to permethrin was recorded in *An. gambiae* s.l. populations across all states except in 4/9 LGAs in Sokoto. Resistance to all three pyrethroids in all LGAs was recorded in Akwa Ibom, Bauchi, Bayelsa, Ebonyi, and Plateau (Table 9).

An. gambiae s.l. mosquitoes showed susceptibility to pirimiphos-methyl in all LGAs in Bayelsa (3/3), FCT (3/3), Kebbi (9/9), Oyo (6/6), Sokoto (9/9), and Zamfara (6/6). Susceptibility to pirimiphos-methyl was also observed in 2/6 LGAs in Akwa Ibom, 5/6 LGAs in Bauchi, 2/6 LGAs in Benue, 4/6 LGAs in Ebonyi, and 4/6 LGAs in Enugu. Resistance to pirimiphos-methyl was observed in all six LGAs from Cross River, Nasarawa, and Plateau (Table 9).

TABLE 9: CDC BOTTLE BIOASSAY TEST RESULTS FOR *AN. GAMBIAE* S.L. IN 2021

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	70%	R	88%	R	24%	R	96%	PR
	Itu	72%	R	84%	R	30%	R	96%	PR
	Mkpat Enin	68%	R	88%	R	20%	R	97%	PR
	Nsit Ubium	67%	R	86%	R	14%	R	99%	S
	Onna	63%	R	89%	R	13%	R	98%	S
	Ukanafun	70%	R	96%	PR	22%	R	94%	PR
Bauchi	Darazo	70%	R	74%	R	78%	R	93%	PR
	Dass	62%	R	76%	R	62%	R	98%	S
	Itas/Gadau	74%	R	66%	R	71%	R	93%	PR

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
	Katagun	78%	R	75%	R	72%	R	100%	S
	Ningi	77%	R	84%	R	77%	R	99%	S
	Toro	70%	R	81%	R	75%	R	99%	S
Bayelsa	Ogbia	89%	R	95%	PR	71%	R	100%	S
	Sagbama	95%	PR	96%	PR	94%	PR	98%	S
	Yenagoa	95%	PR	88%	R	86%	R	98%	S
Benue	Apa	100%	S	94%	PR	75%	R	81%	R
	Gwer	100%	S	98%	S	52%	R	99%	S
	Obi	100%	S	99%	S	48%	R	99%	S
	Tarkar	99%	S	98%	S	44%	R	86%	R
	Ukum	100%	S	95%	PR	37%	R	86%	R
	Vandeikya	99%	S	98%	S	36%	R	81%	R
Cross River	Abi	100%	S	100%	S	44%	R	79%	R
	Akamkpa	100%	S	100%	S	44%	R	33%	R
	Calabar Municipal	100%	S	100%	S	33%	R	43%	R
	Etung	100%	S	98%	S	36%	R	76%	R
	Obudu	98%	S	98%	S	45%	R	87%	R
	Ogoja	98%	S	98%	S	41%	R	90%	PR
Ebonyi	Abakaliki	43%	R	70%	R	45%	R	100%	S
	Ebonyi	41%	R	63%	R	30%	R	100%	S
	Ezza North	53%	R	59%	R	37%	R	100%	S
	Ezza South	52%	R	59%	R	41%	R	-	
	Izzi	42%	R	62%	R	37%	R	100%	S
	Ohaozara	56%	R	57%	R	33%	R	-	

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

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Alpha-cypermethrin		Deltamethrin		Permethrin		Pirimiphos-methyl	
Sentinel Site	LGA	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status
Enugu	Awgu	88%	R	88%	R	25%	R	98%	S
	Enugu South	100%	S	81%	R	5%	R	100%	S
	Ezeagu	-	-	-	-	-	-	-	-
	Igbo-Eze North	83%	R	94%	PR	45%	R	100%	S
	Isi-Uzo			97%	PR	11%	R		
	Nsukka	91%	PR	86%	R	40%	R	100%	S
FCT	AMAC	95%	PR	100%	S	75%	R	98%	S
	Gwagwalada	100%	S	100%	S	74%	R	100%	S
	Kwali	99%	S	98%	S	90%	PR	98%	S
Kebbi	Argungu	100%	S	100%	S	83%	R	100%	S
	Augie	100%	S	100%	S	80%	R	100%	S
	Bunza	100%	S	100%	S	85%	R	100%	S
	Fakai	90%	PR	100%	S	90%	PR	100%	S
	Gwandu	94%	PR	100%	S	88%	R	100%	S
	Jega	100%	S	100%	S	78%	R	100%	S
	Kalgo	100%	S	100%	S	83%	R	100%	S
	Maiyama	100%	S	100%	S	81%	R	100%	S
	Suru	96%	PR	100%	S	89%	R	100%	S
Nasarawa	Karu	92%	PR	96%	PR	34%	R	42%	R
	Keffi	97%	PR	97%	PR	43%	R	62%	R

Class of Insecticides		Pyrethroids						Organophosphate	
Insecticides		Alpha-cypermethrin		Deltamethrin		Permethrin		Pirimiphos-methyl	
Sentinel Site	LGA	% Mortality	Status	% Mortality	Status	% Mortality	Status	% Mortality	Status
	Kokona	86%	R	98%	S	42%	R	28%	R
	Nasarawa	73%	R	100%	S	7%	R	63%	R
	Nasarawa Eggon	93%	PR	98%	S	12%	R	59%	R
	Obi	94%	PR	96%	PR	25%	R	66%	R
Oyo	Akinyele	100%	S	100%	S	12%	R	100%	S
	Atiba	100%	S	100%	S	19%	R	100%	S
	Ibarapa North	100%	S	100%	S	40%	R	100%	S
	Itesiwaju	100%	S	100%	S	25%	R	100%	S
	Saki West	100%	S	100%	S	28%	R	100%	S
	Surulere	100%	S	100%	S	54%	R	100%	S
Plateau	Bassa	89%	R	87%	R	72%	R	84%	R
	Bokkos	87%	R	88%	R	65%	R	83%	R
	Jos-south	90%	PR	88%	R	68%	R	86%	R
	Kanam	90%	PR	83%	R	75%	R	84%	R
	Mangu	87%	R	86%	R	67%	R	89%	R
	Pankshin	85%	R	82%	R	77%	R	81%	R
Sokoto	Bodinga	100%	S	100%	S	80%	R	100%	S
	Gudu	100%	S	85%	R	100%	S	100%	S
	Kware	100%	S	92%	PR	89%	R	100%	S
	Rabah	100%	S	88%	R	78%	R	100%	S
	Shagari	100%	S	99%	S	100%	S	100%	S
	Sokoto North	100%	S	91%	PR	100%	S	100%	S
	Sokoto South	100%	S	94%	PR	100%	S	100%	S
	Tambuwal	100%	S	86%	R	82%	R	100%	S
	Wamakko	100%	S	84%	R	96%	PR	100%	S
Zamfara	Bakura	100%	S	100%	S	82%	R	100%	S
	Birnin Magaji	100%	S	100%	S	76%	R	100%	S
	Bungudu	90%	PR	100%	S	72%	R	100%	S
	Gummi	100%	S	100%	S	78%	R	100%	S
	Maradun	100%	S	100%	S	89%	R	100%	S
	Maru	91%	PR	100%	S	78%	R	100%	S

S = Susceptible, R = Resistant, PR = Possibly Resistant. Note: Diagnostic time is 30 minutes. A minimum of 100 mosquitoes were exposed each insecticide.

2.15 INSECTICIDE RESISTANCE INTENSITY

Insecticide resistance intensity in the vector populations were determined by subjecting *Anopheles* mosquitoes across the different ecozones to insecticide resistance test assays with different concentrations of three pyrethroids. *An. gambiae* s.l. was susceptible to 1X alpha-cypermethrin in 2/3 LGAs in FCT (Figure 31), 6/6 LGAs in Benue, Cross River, and Oyo, 6/9 LGAs in Kebbi, 9/9 LGAs in Sokoto (Figures 27, 28, 32, 34 and 36), and 4/6 LGAs in Zamfara (Figure 37). In a few LGAs where resistance at 1X (mortality less than 98%) was recorded, the intensity of resistance was low as *Anopheles gambiae* s.l. mosquitoes were susceptible to alpha-cypermethrin at 2X concentration. This was observed in one LGA in FCT, five LGAs in Bauchi, three LGAs in Bayelsa and Enugu, four LGAs in Ebonyi, five LGAs in Nasarawa, three LGAs in Kebbi, two LGAs in Zamfara (Figures 31, 25, 26, 30, 29, 33, 32, and 37). *An. gambiae* s.l. showed low resistance intensity (mortality between 98-100% at 5X dose) to alpha-cypermethrin in six LGAs each in Akwa Ibom and Plateau (Figures 24 and 35) and from one LGA in Nasarawa (Figure 33).

Complete susceptibility at 1X to deltamethrin was recorded in all LGAs in Cross River, FCT, Kebbi, Oyo, and Zamfara (Figures 28, 31, 32, 34, and 37). Susceptibility to deltamethrin at 2X concentration was observed in *An. gambiae* s.l. populations from all LGAs in Akwa Ibom, Bauchi, and Bayelsa (Figures 24, 25, and 26). *An. gambiae* s.l. from 2/6 LGAs in Benue, 5/6 LGAs in Ebonyi, 4/5 LGAs in Enugu, 3/6 LGAs in Nasarawa, and 7/9 LGAs in Sokoto showed complete susceptibility at 2X concentration. Low intensity of resistance (mortality

between 98-100% at 5X dose) to deltamethrin was only recorded in *An. gambiae* populations tested in six LGAs of Plateau (Figure 35).

An. gambiae s.l. populations from all field sites were resistant to permethrin except in four LGAs in Sokoto. Complete susceptibility to permethrin was however observed when the *An. gambiae* s.l. populations were exposed to 2X concentration test assays in one LGA each in Bayelsa and FCT, two LGAs in Ebonyi, three LGAs in Kebbi, five LGAs in Sokoto, and all LGAs in Bauchi and Benue, indicating low intensity of resistance (Figures 26, 31, 29, 32, 36, 25, and 27).

Low permethrin resistance intensity (mortality between 98–100% at 5X dose) was recorded in *An. gambiae* s.l. from two LGAs each in Bayelsa and FCT, six LGAs in Kebbi, and all LGAs in Cross River, Oyo, Plateau, and Zamfara (Figures 26, 31, 32, 28, 34, 35, and 37).

Moderate permethrin resistance intensity (mortality less than or equal to 98% at 10X dose) was observed in *An. gambiae* s.l. populations from three LGAs in Ebonyi, one LGA in Enugu, and six LGAs in Nasarawa (Figures 29, 30, and 33). High permethrin resistance intensity (mortality less than 98% at 10X dose) was recorded in six LGAs in Akwa Ibom and one LGA in Enugu (Figures 24 and 30).

FIGURE 24: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT AKWA IBOM

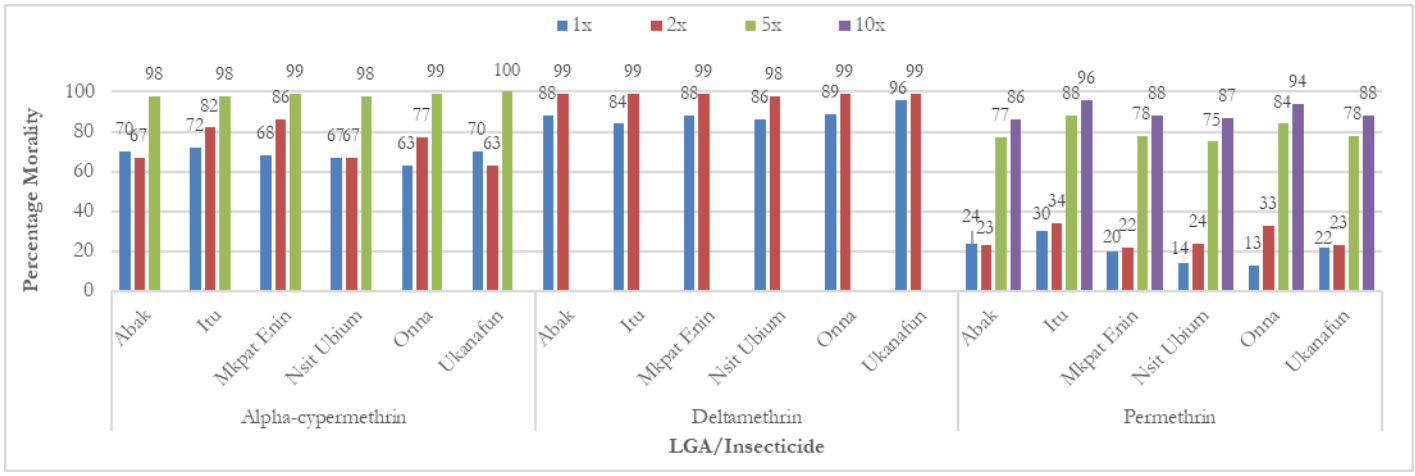


FIGURE 25: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT BAUCHI

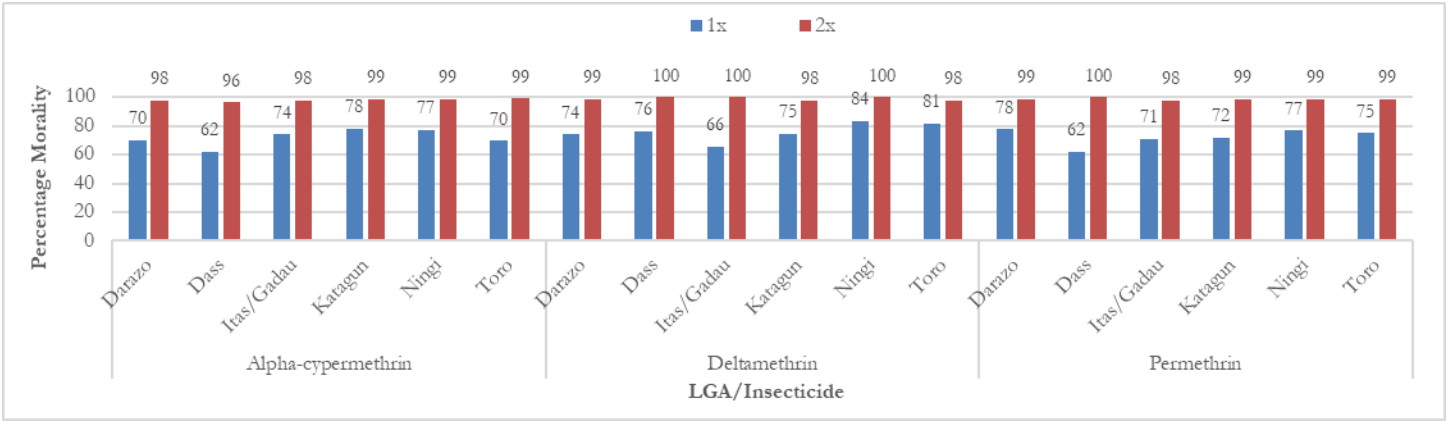


FIGURE 26: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT BAYELSA

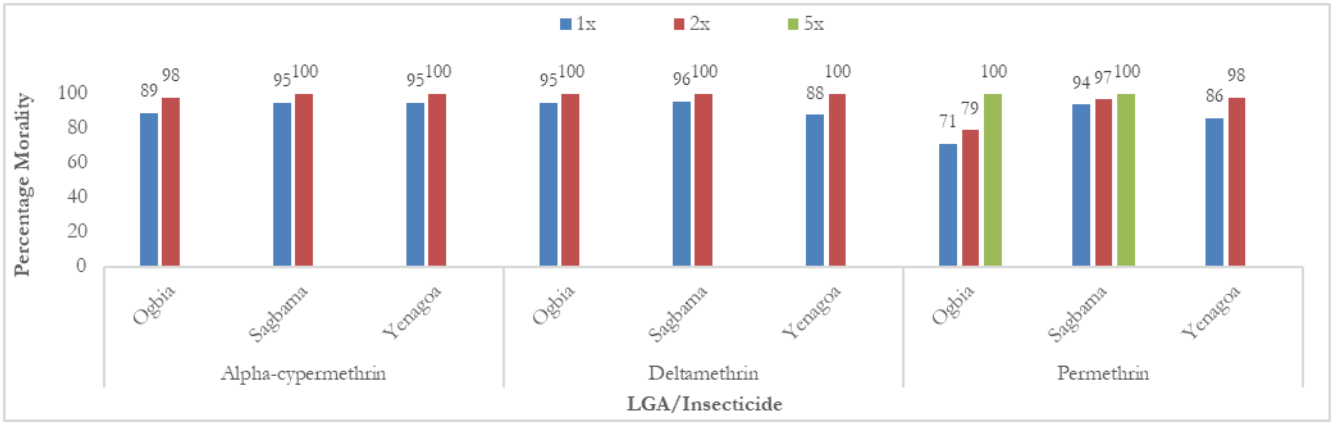


FIGURE 27: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT BENUE

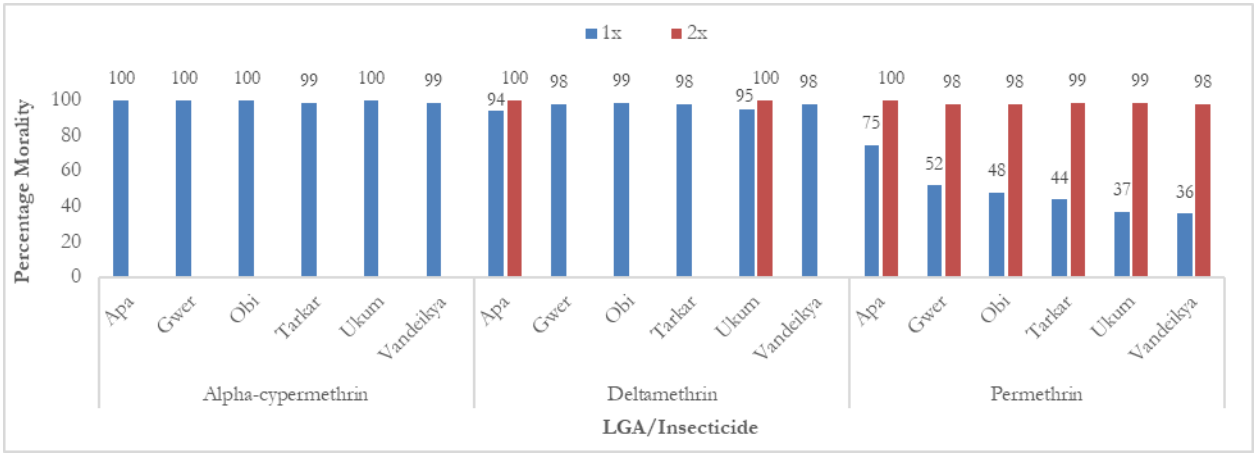


FIGURE 28: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT CROSS RIVER

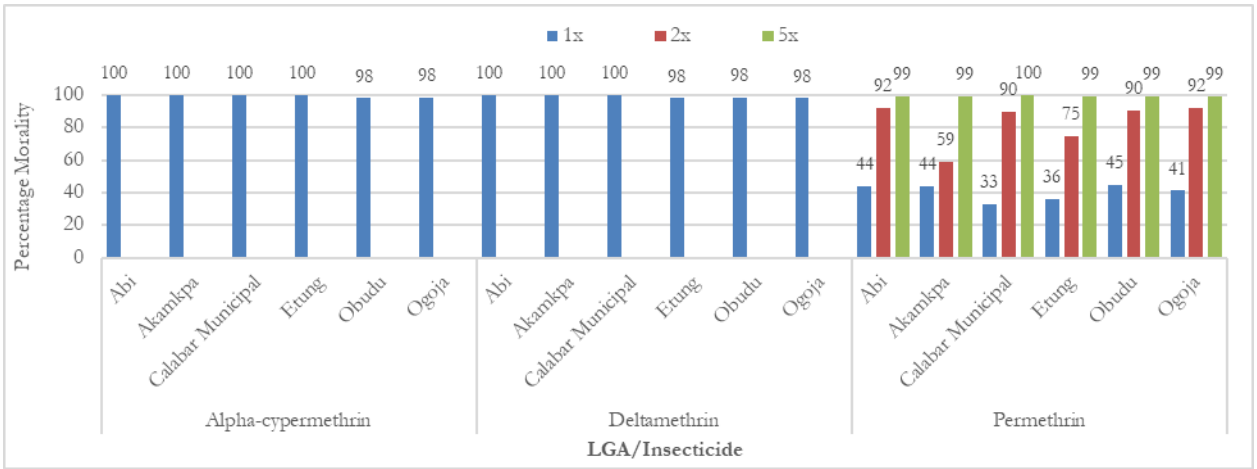


FIGURE 29: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT EBONYI

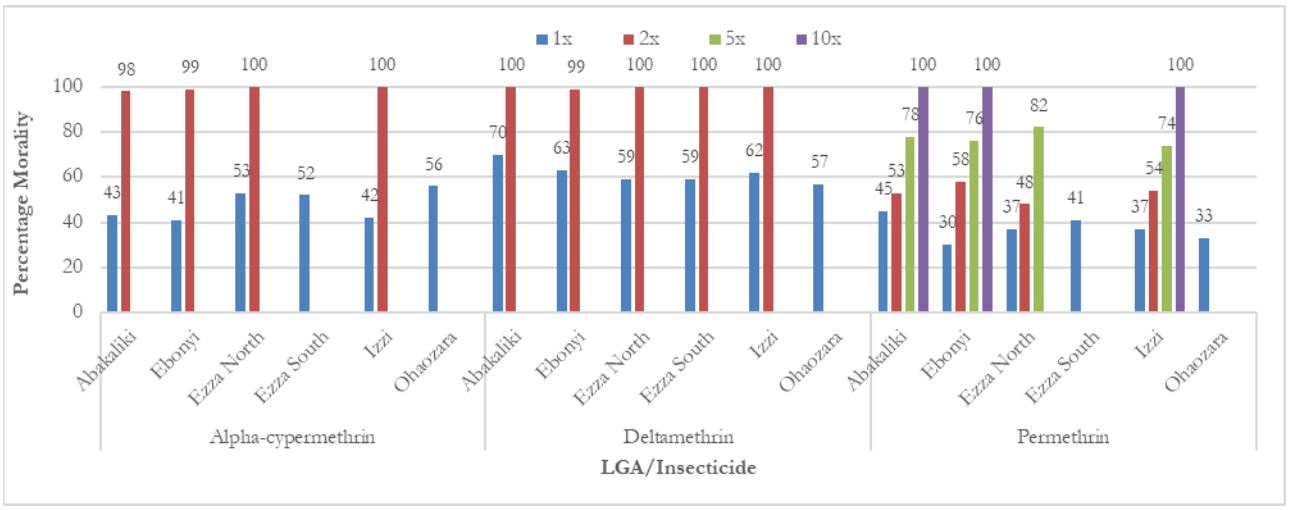


FIGURE 30: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT ENUGU

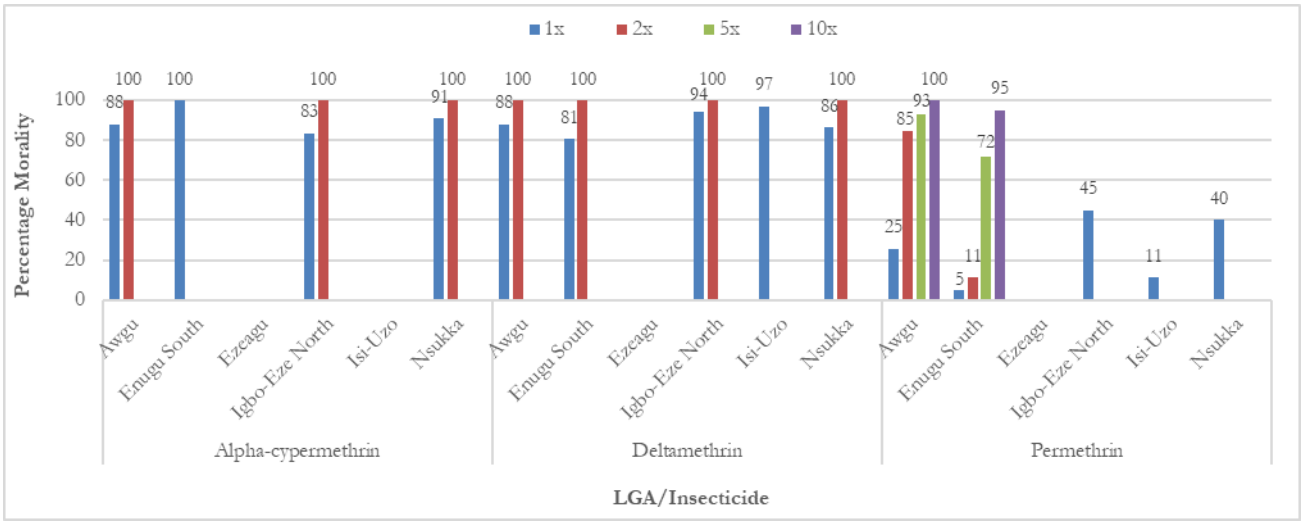


FIGURE 31: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT FCT

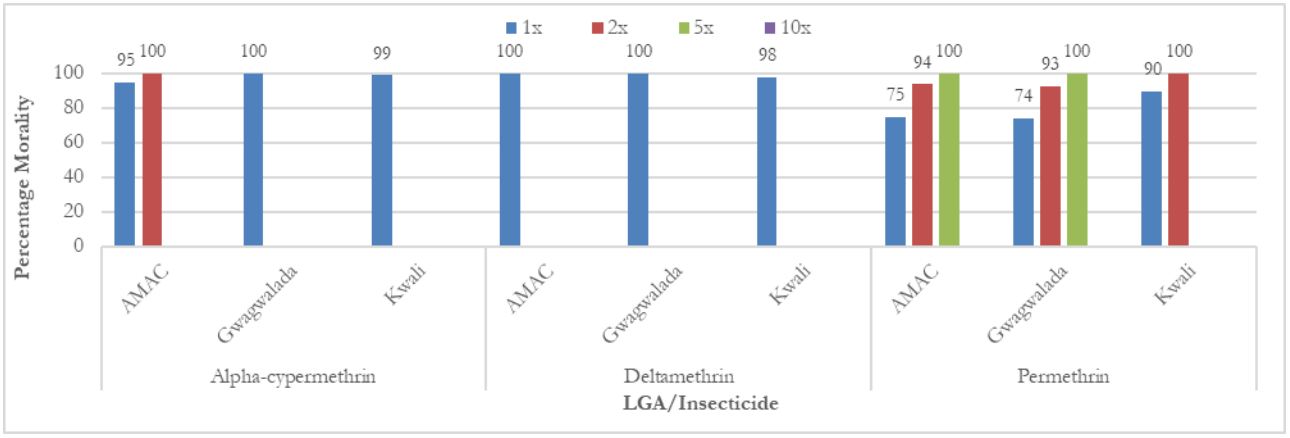


FIGURE 32: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT KEBBI

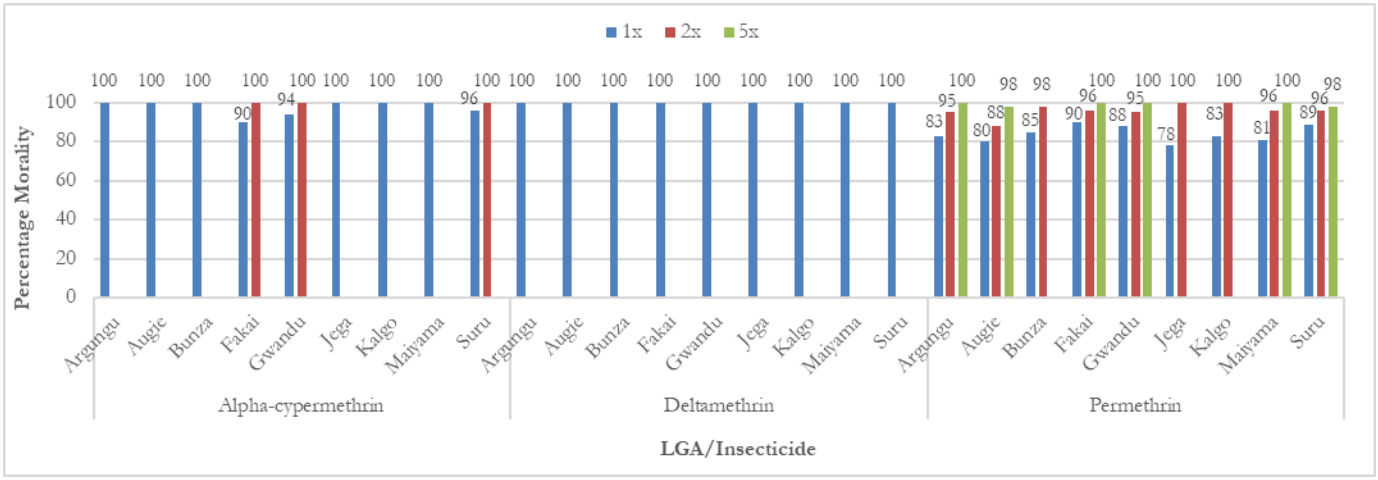


FIGURE 33: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT NASARAWA

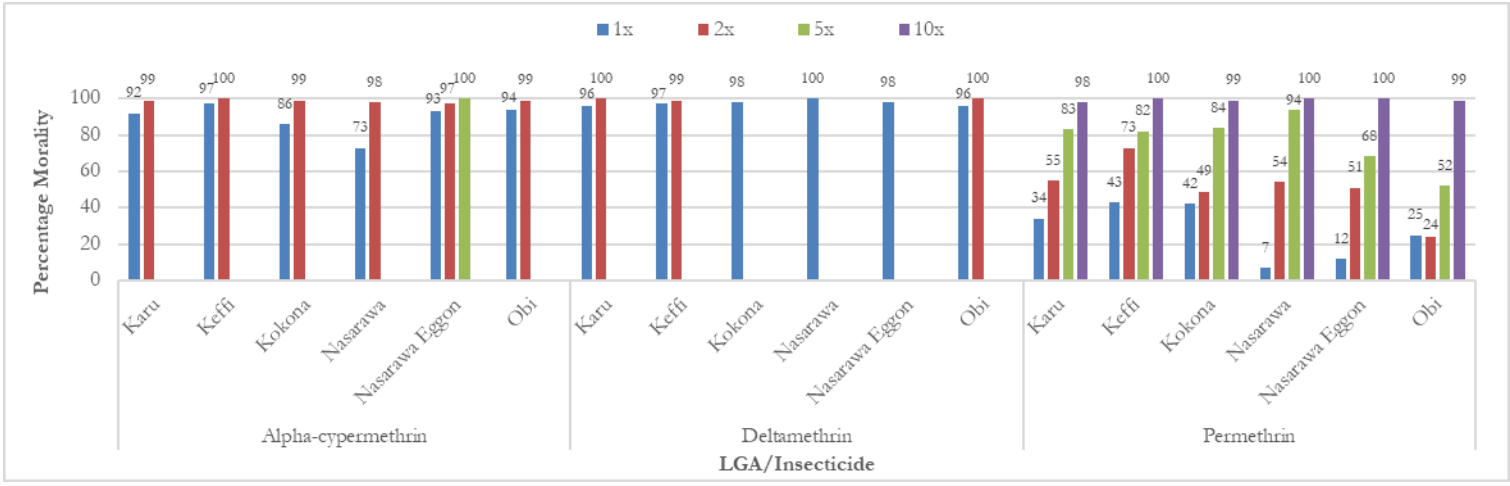


FIGURE 34: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT OYO

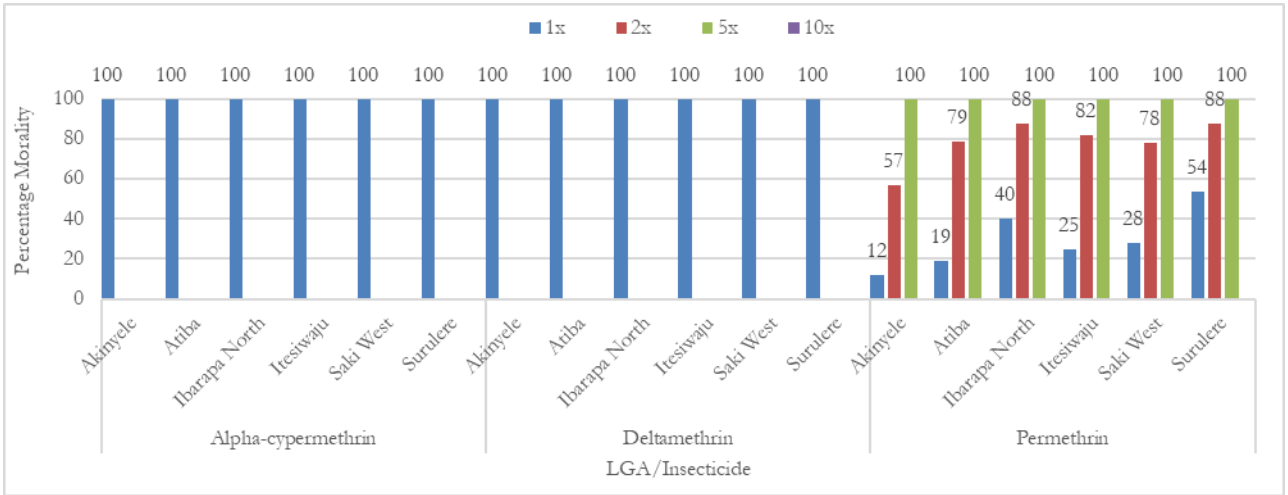


FIGURE 35: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT PLATEAU

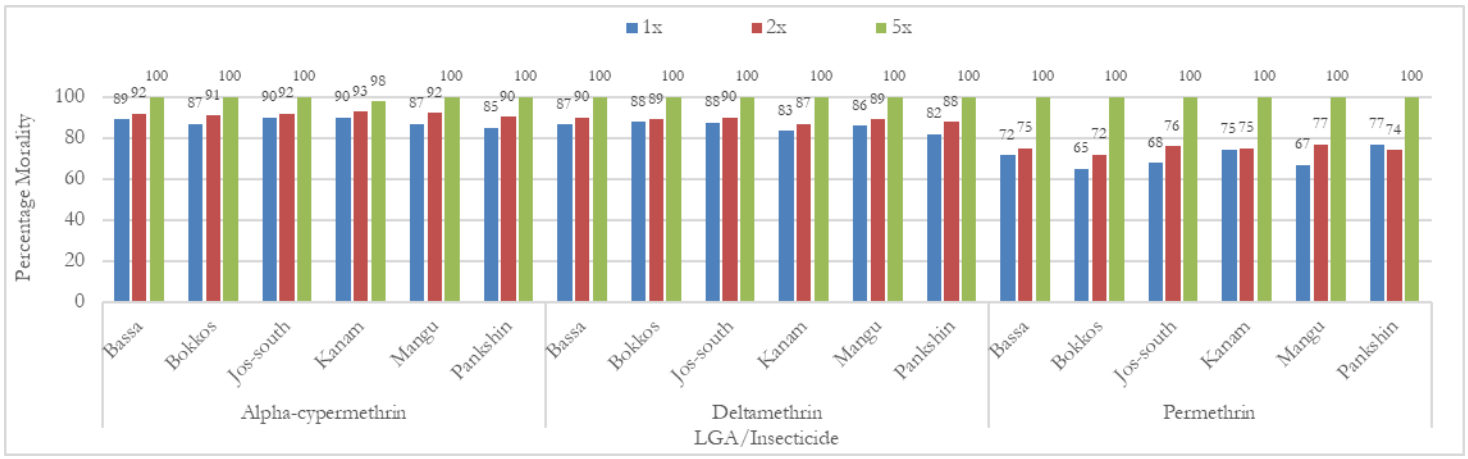


FIGURE 36: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT SOKOTO

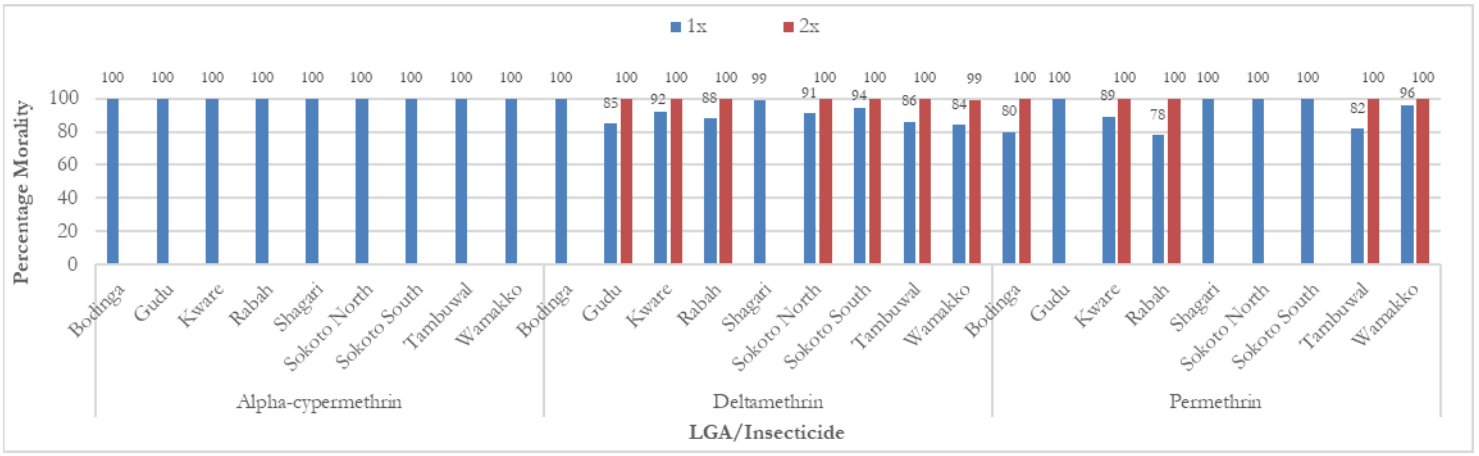
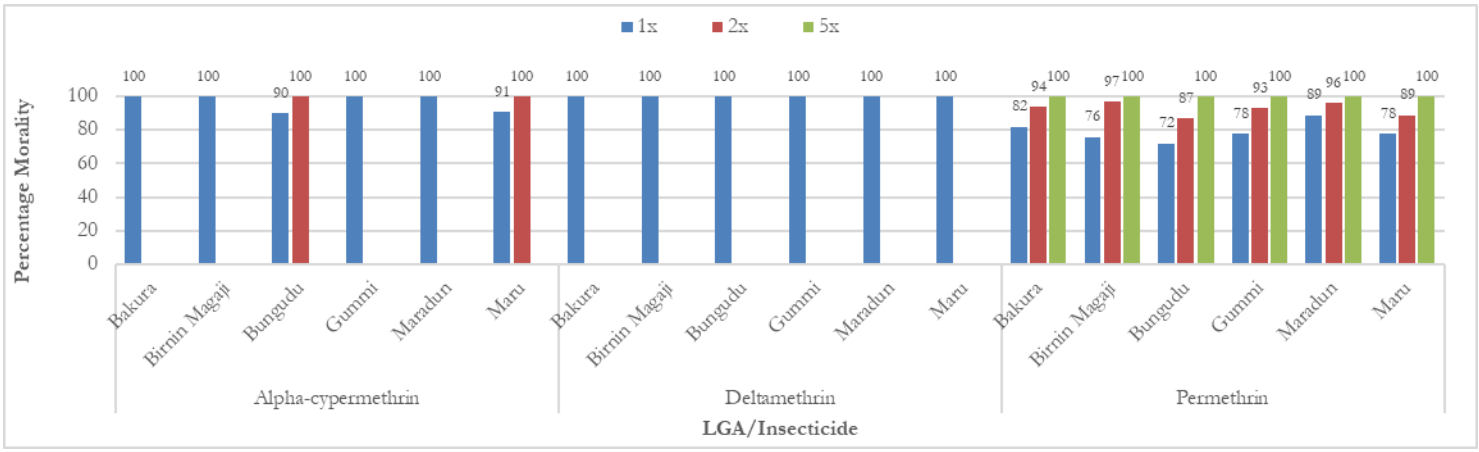


FIGURE 37: PYRETHROID RESISTANCE INTENSITY IN *AN. GAMBIAE* S.L. AT ZAMFARA



2.16 SYNERGIST ASSAYS

Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO synergist before exposure to pyrethroids (alpha-cypermethrin, deltamethrin, and permethrin) increased mortality at varying degrees across sites (Figures 38–51). In cases where full susceptibility (mortality greater than or equal to 98%) was not restored with PBO exposure, it suggests the existence of mechanisms unrelated to the activity of mixed function oxidases.

Where tested, susceptibility to alpha-cypermethrin was fully restored in *An. gambiae* s.l. mosquitoes pre-exposed to PBO in one LGA in Bauchi and all LGAs where tests were conducted in Bayelsa, Ebonyi, Enugu, FCT, Kebbi, Nasarawa, and Zamfara (Figures 39-40, 43-47, and 51). Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO did not restore susceptibility to alpha-cypermethrin in the six LGAs in Akwa Ibom, Plateau and in the remaining five LGAs in Bauchi (Figures 38, 39, and 47).

Where tested, susceptibility of *An. gambiae* s.l. mosquitoes to deltamethrin was restored in Akwa Ibom, Bayelsa, Benue, Ebonyi, Nasarawa, and Sokoto (Figures 38, 40, 41, 43, 47, and 50). Pre-exposure of *An. gambiae* s.l. mosquitoes to PBO did not restore susceptibility to deltamethrin in five LGAs in Bauchi, two LGAs in Enugu, and in all six LGAs in Plateau (Figures 39, 44, and 49).

Where tested, susceptibility to permethrin was fully restored in *An. gambiae* s.l. populations in all LGAs from Bayelsa Ebonyi and Sokoto, one out of the three LGAs in FCT, and four out of six LGAs in Zamfara (Figures 40, 45, and 43). For permethrin exposures, PBO did not fully restore susceptibility in *An. gambiae* s.l. populations in any LGAs in Akwa Ibom, Bauchi, Benue, Cross River, Enugu, Kebbi, Nasarawa, Oyo, and Plateau (Figures 38, 39, 41, 42, 44, and 46-49).

FIGURE 38: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM AKWA IBOM

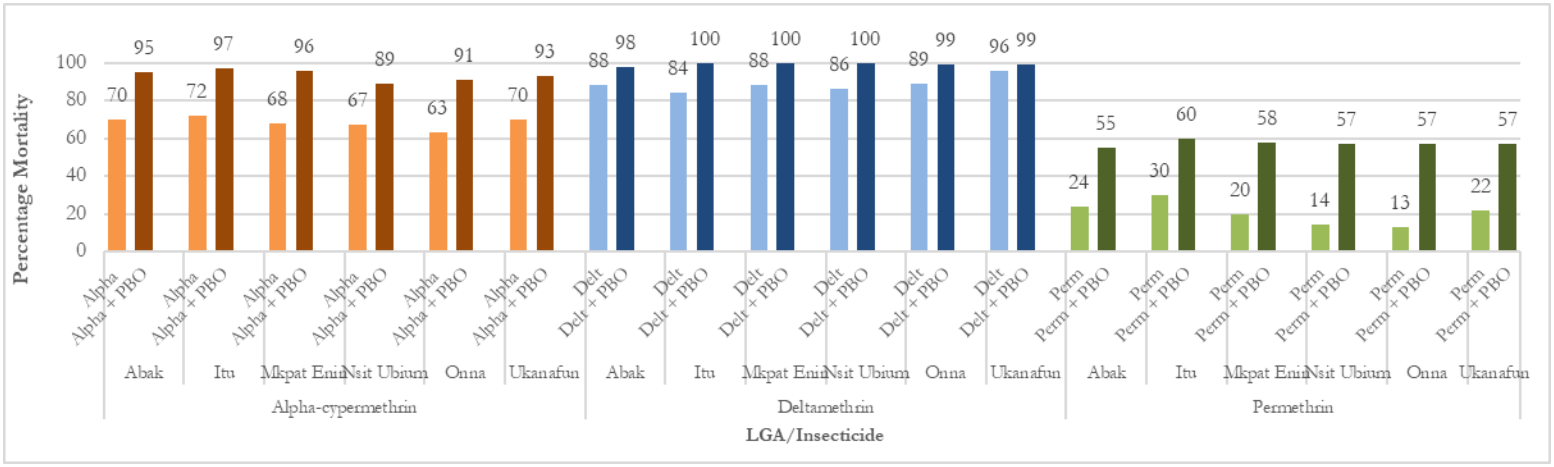


FIGURE 39: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM BAUCHI

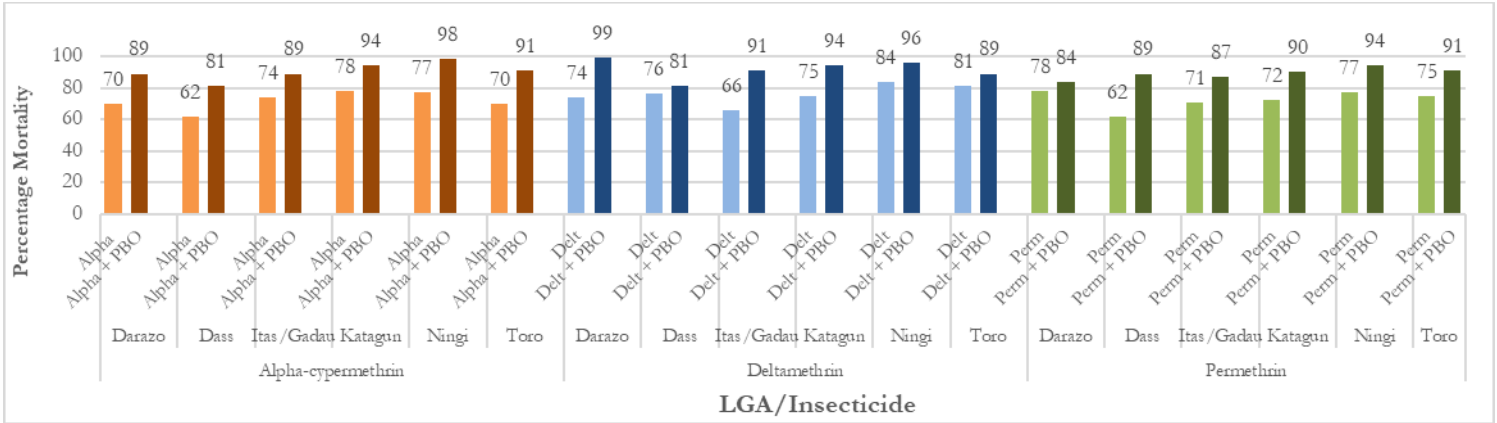


FIGURE 40: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM BAYELSA

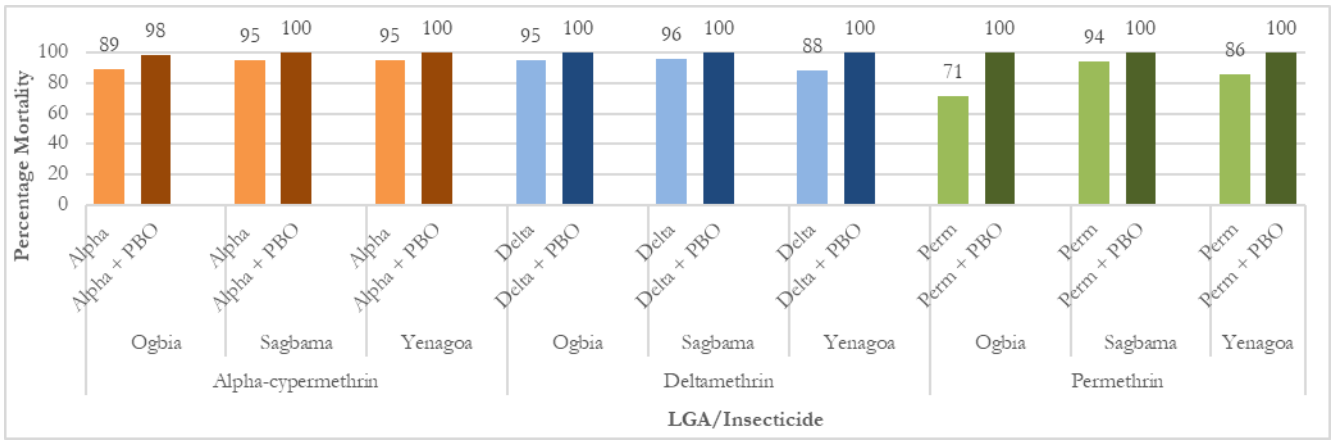


FIGURE 41: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM BENUE

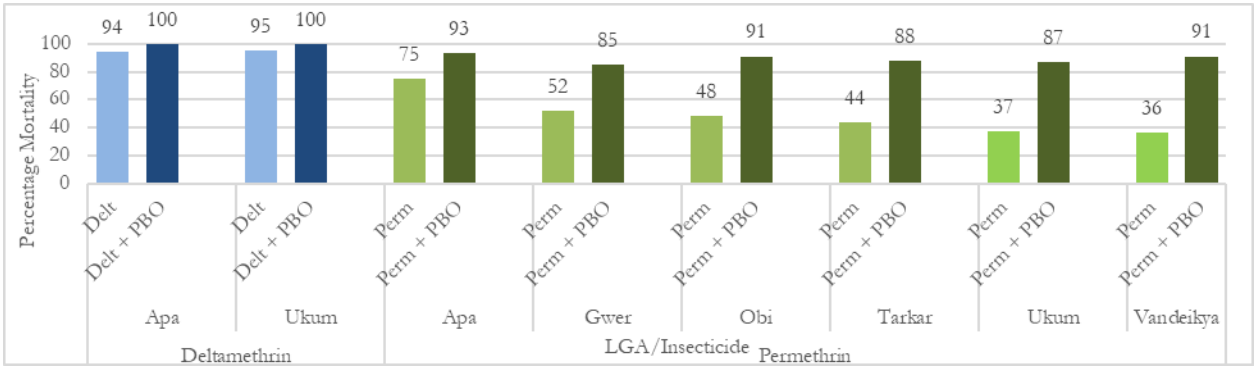


FIGURE 42: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM CROSS RIVER

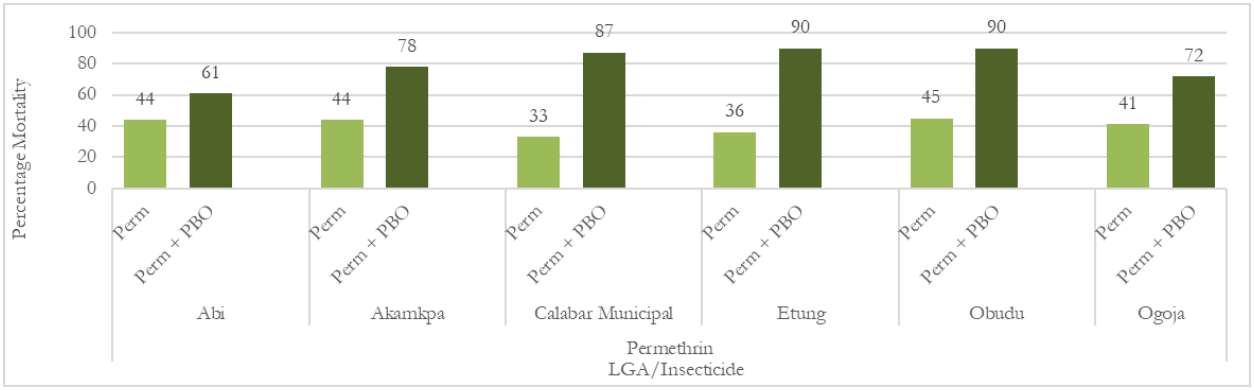


FIGURE 43: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM EBONYI

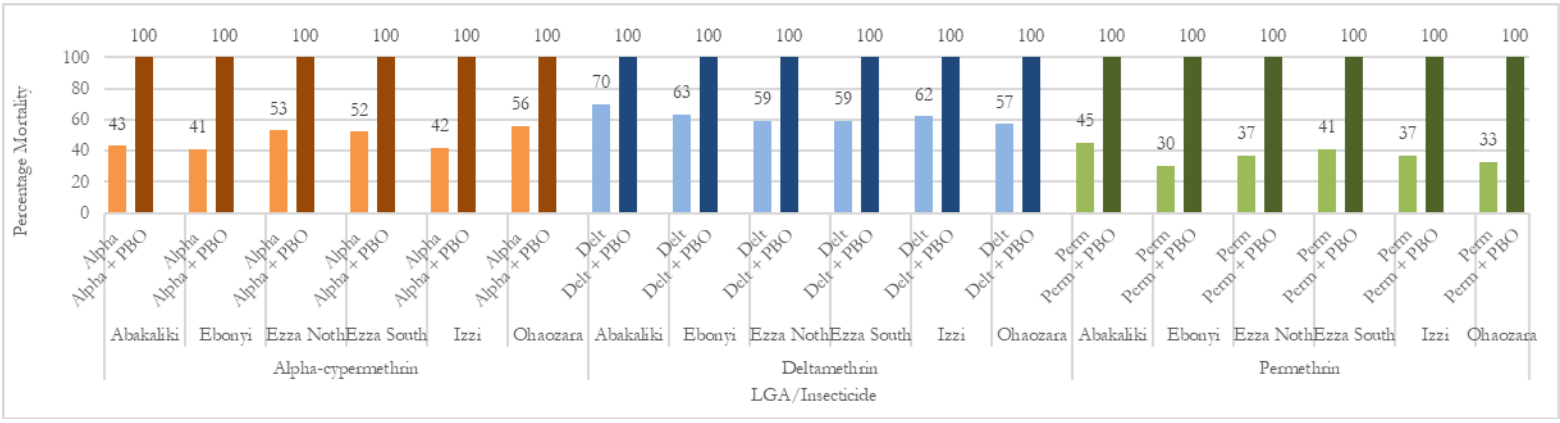


FIGURE 44: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM ENUGU

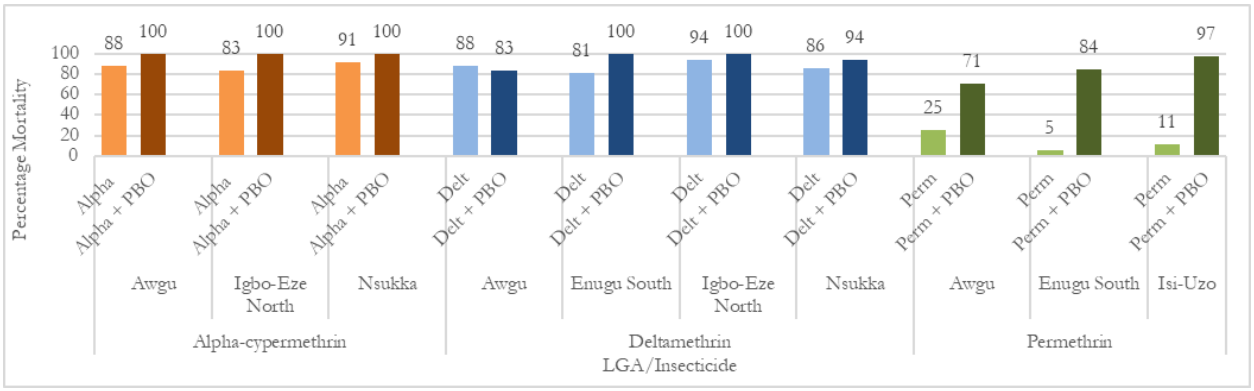


FIGURE 45: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM FCT

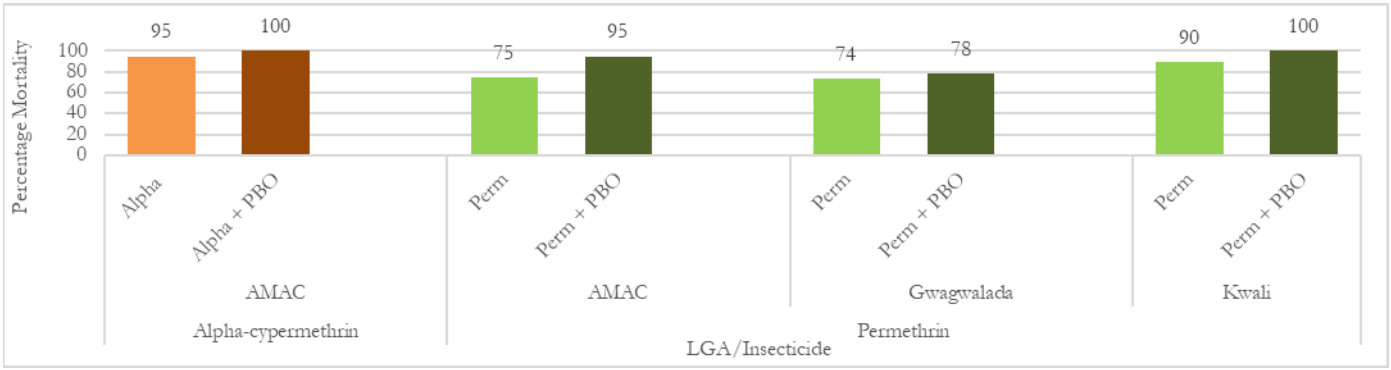


FIGURE 46: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM KEBBI

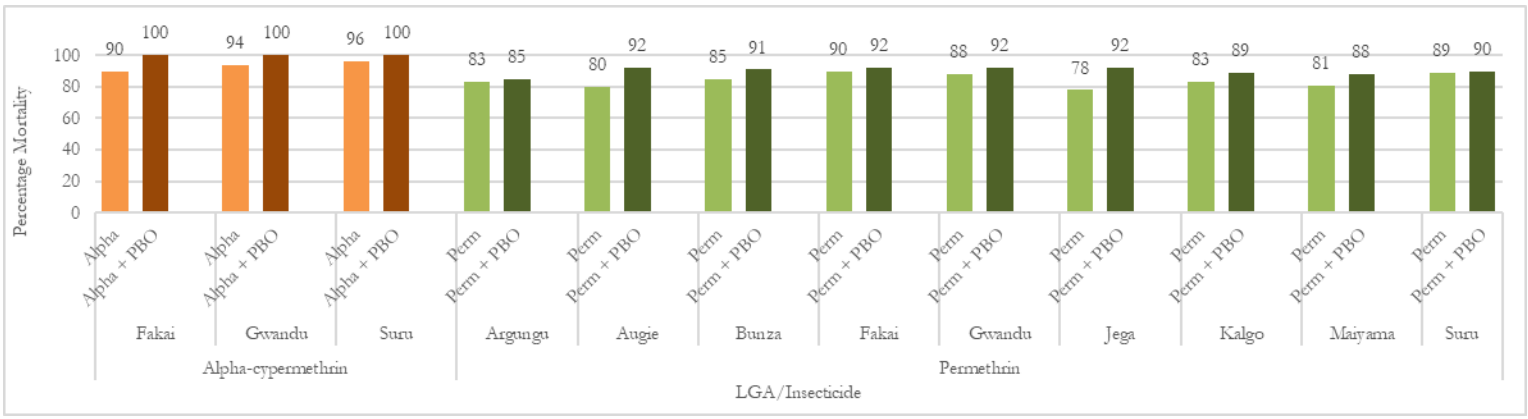


FIGURE 47: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM NASARAWA

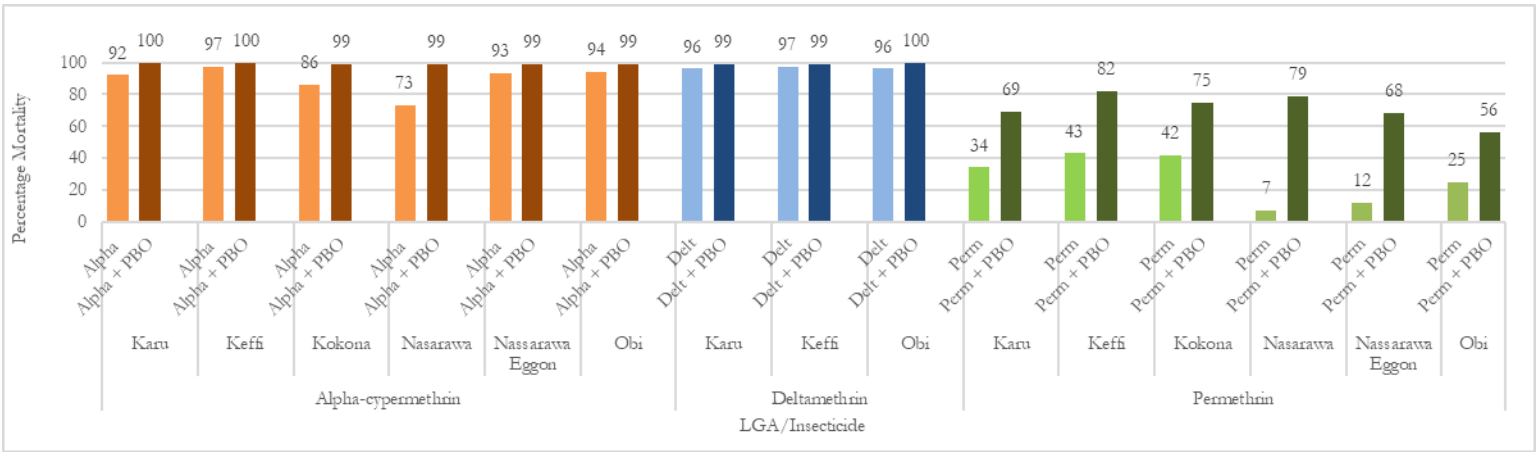


FIGURE 48: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM OYO

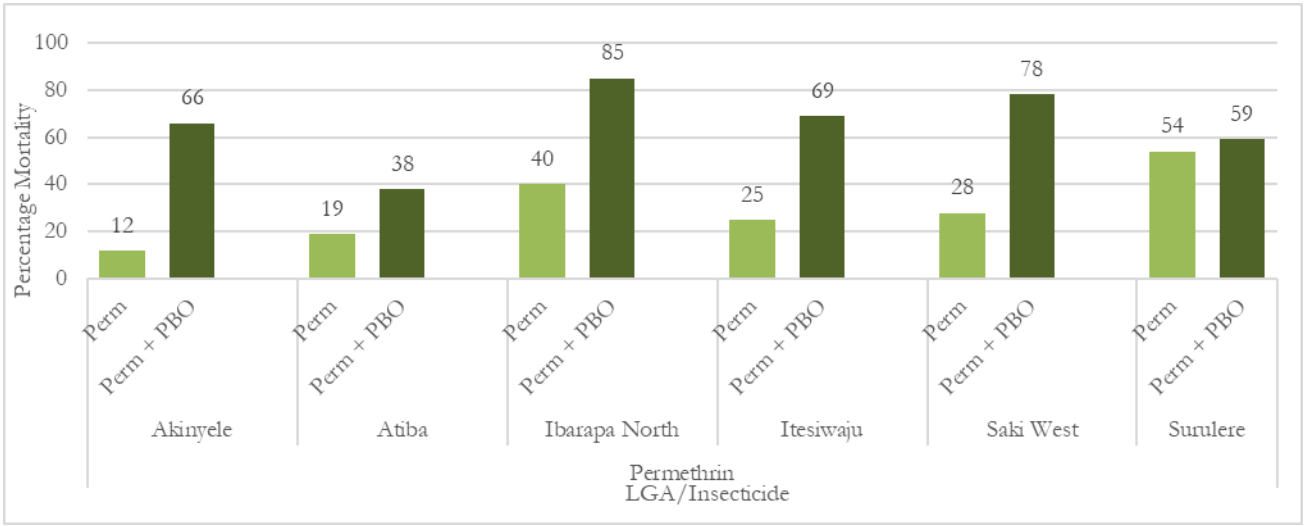


FIGURE 49: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM PLATEAU

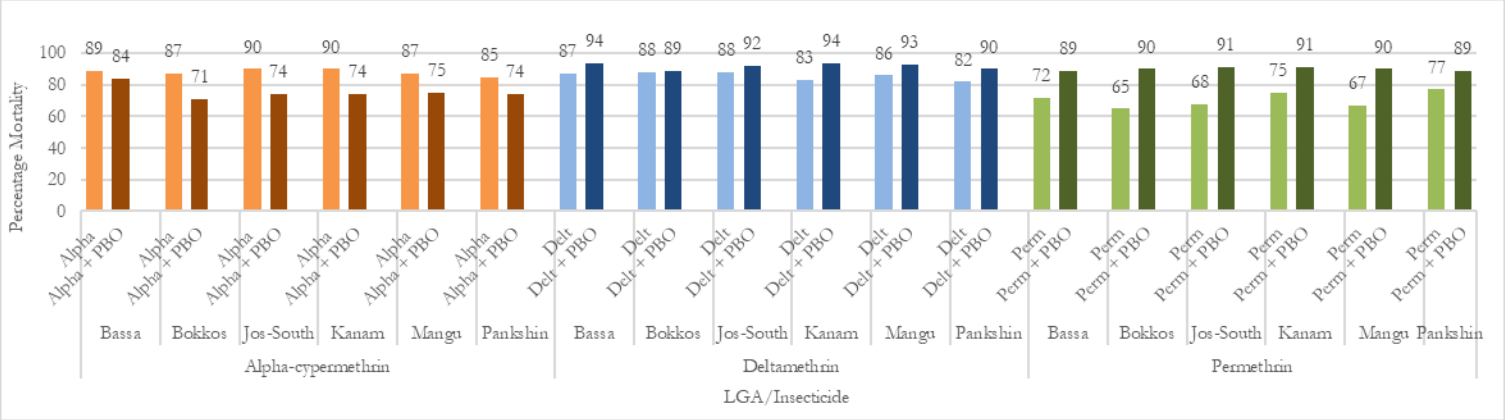


FIGURE 50: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM SOKOTO

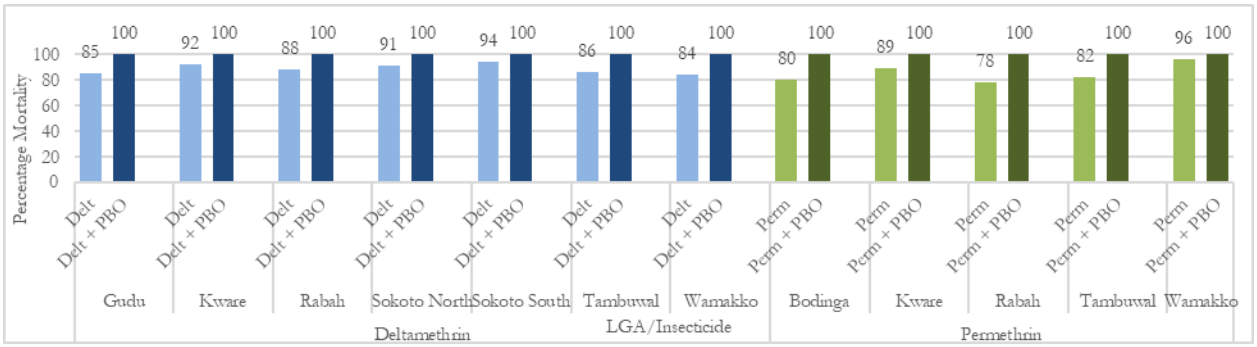
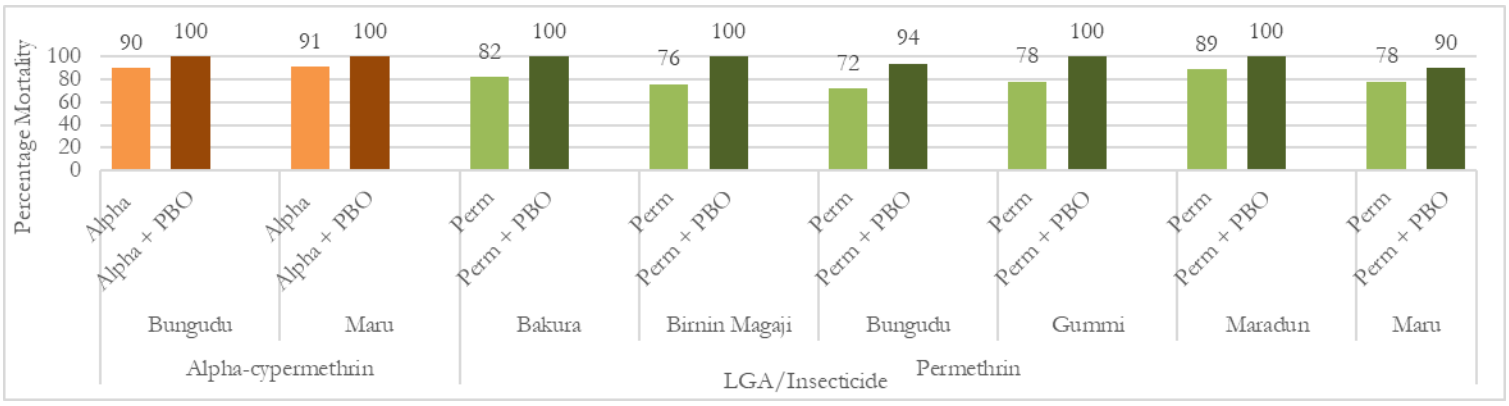


FIGURE 51: SYNERGIST BOTTLE ASSAY RESULTS FOR *AN. GAMBIAE* S.L. FROM ZAMFARA



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

The percentage knockdown of *An. gambiae* s.l. exposed to chlorfenapyr at 60 minutes varied across LGAs in Akwa Ibom (74–83%), Bauchi (98–100%), Bayelsa (92-100%), Benue (72–88%), Cross River (64–88%), Enugu (49-69%), Ebonyi (56-75%), Enugu (49-69%), FCT (62-84%), Kebbi (57–84%), Nasarawa (30–69%), Oyo (8–64%), Plateau (57–72%), Sokoto (34–52%), and Zamfara (54–68%).

The percentage mortality after the 24-hour holding period also varied in Akwa Ibom (87–91%), Bauchi (98–100%), Bayelsa (100%), Benue (100%), Cross River (94-100%), Ebonyi (86-91%), Enugu (70-78%), FCT (76-

94%), Kebbi (100%), Nasarawa (89-100%), Oyo (100%), Plateau (95-99%), Sokoto (81-97%), and Zamfara (93-100%) (Figures 50-63). Mortality rates in *An. gambiae* s.l. were between 98-100% after the 48-hour holding period in all LGAs in Bauchi, Bayelsa, Benue, Cross River, Ebonyi, Kebbi, Nasarawa, Oyo, Plateau, and Zamfara (Figures 53-54, 56-57, 60-63, and 65).

An. gambiae s.l. populations from all LGAs across all ecozones were susceptible to chlorfenapyr with 98-100% mortality at 72 hours (Figures 52-65), except in one LGA each in Enugu (Enugu South) and FCT (AMAC) (Figures 58 and 59).

FIGURE 52: 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

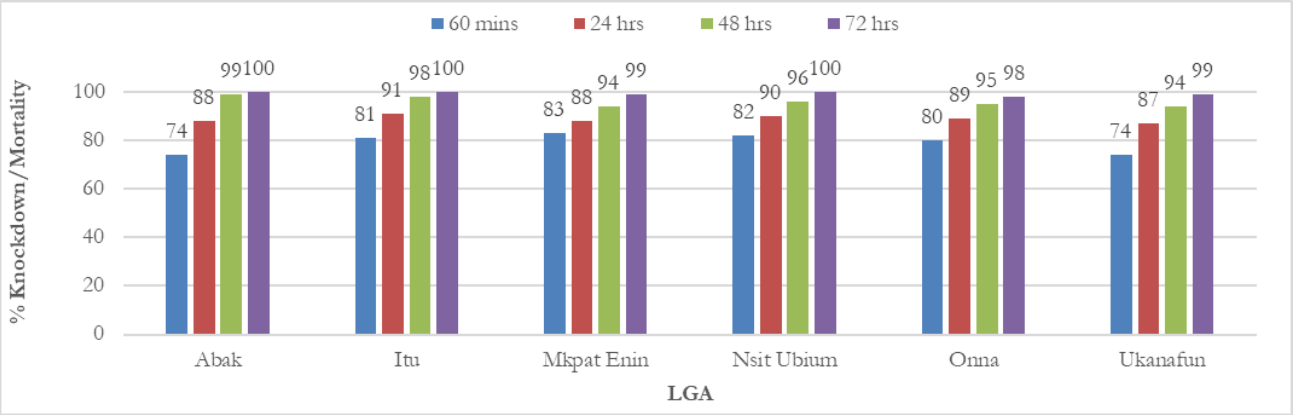


FIGURE 53: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT BAUCHI

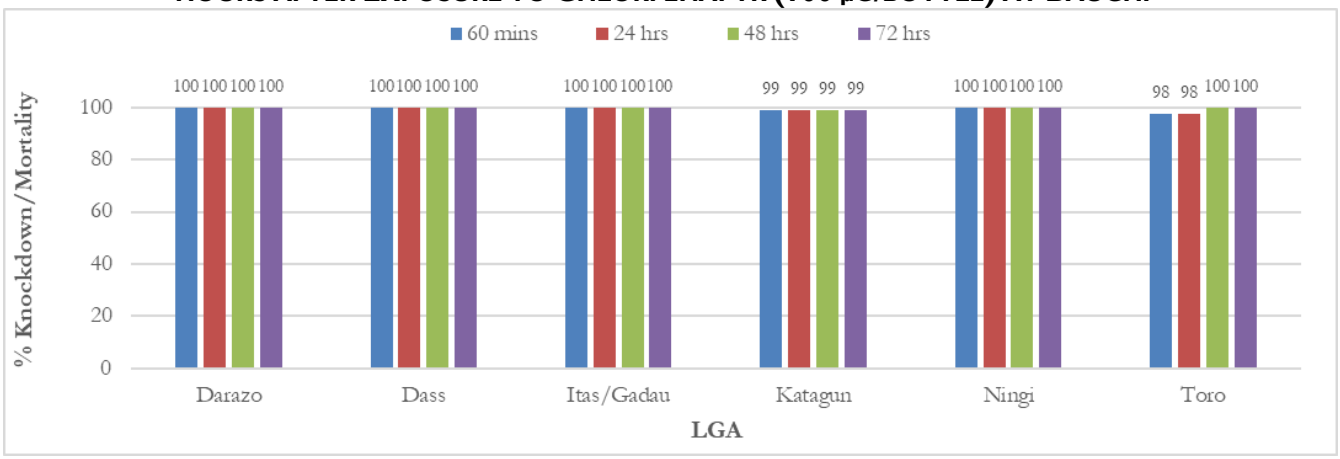


FIGURE 54: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT BAYELSA

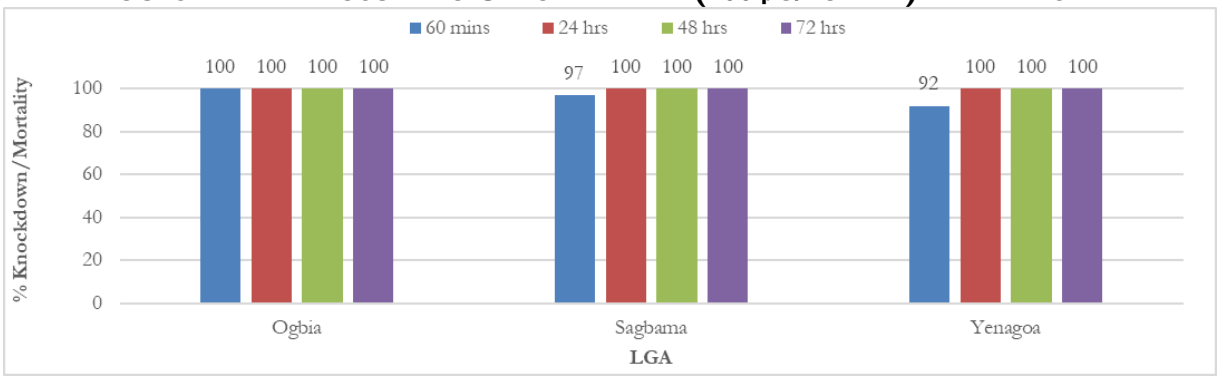


FIGURE 55: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT BENUE

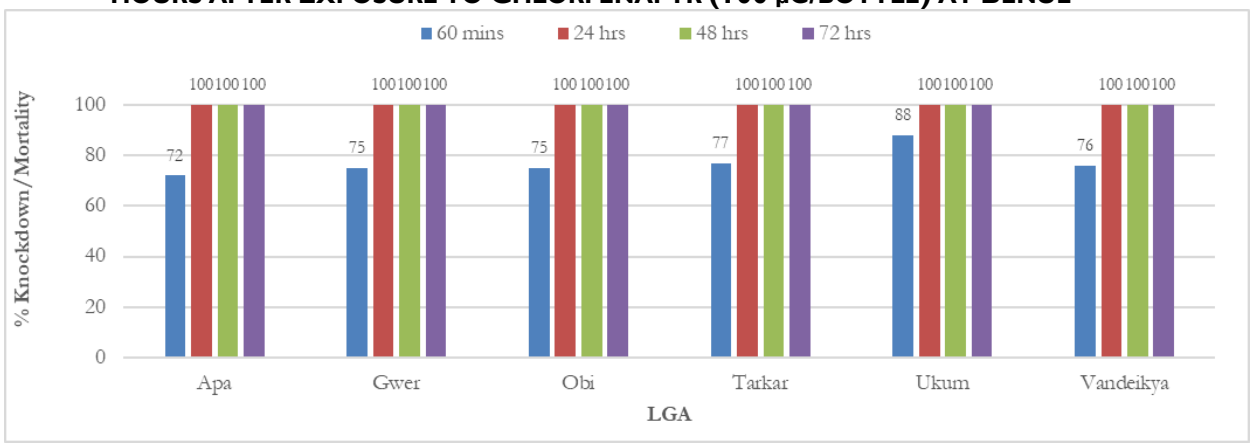


FIGURE 56: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT CROSS RIVER

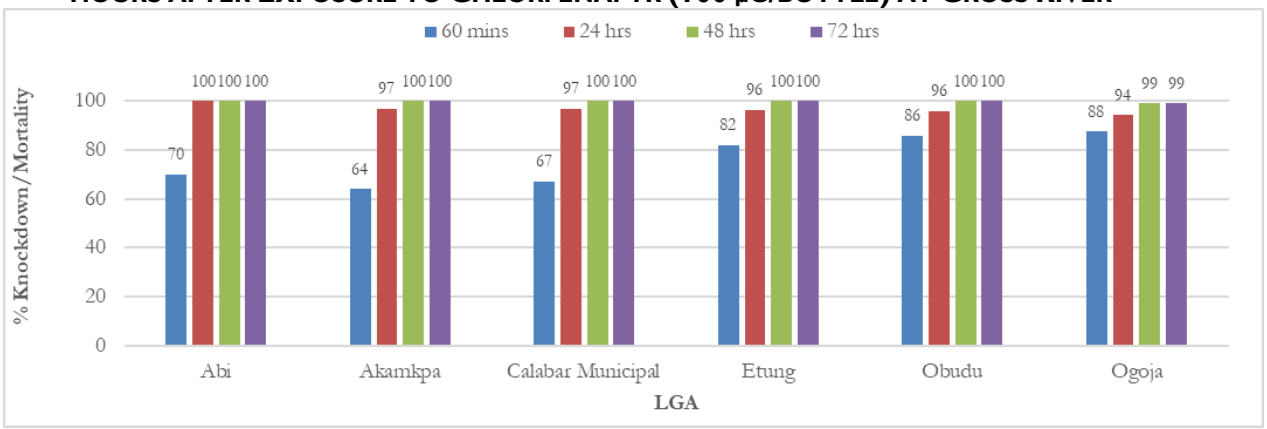


FIGURE 57: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT EBONYI

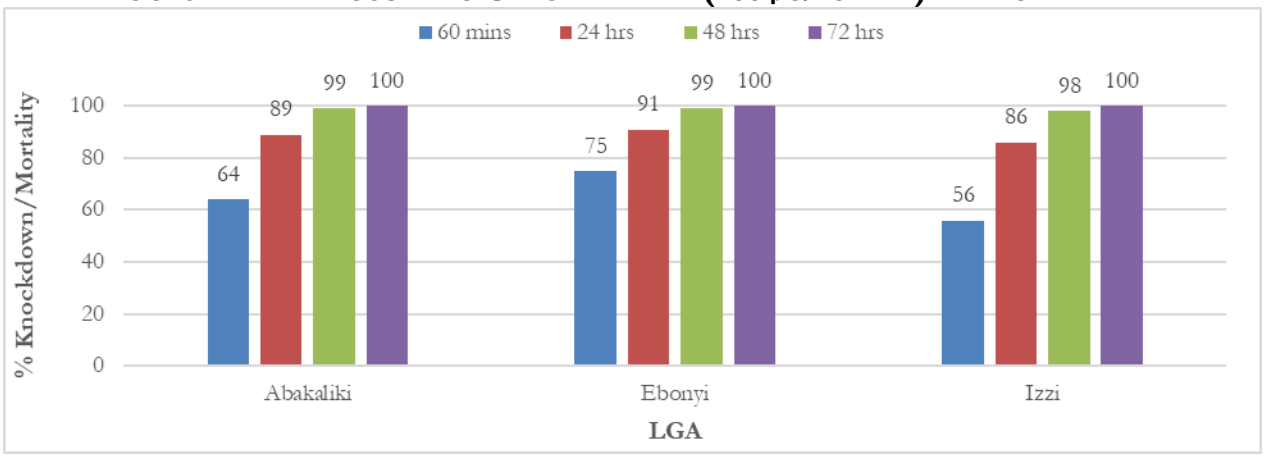


FIGURE 58: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT ENUGU

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

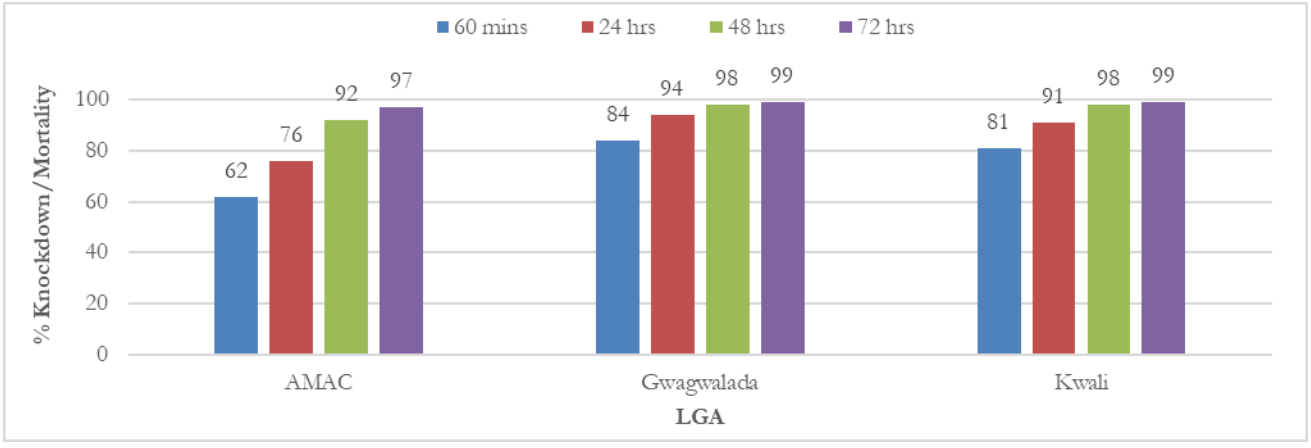


FIGURE 60: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT KEBBI

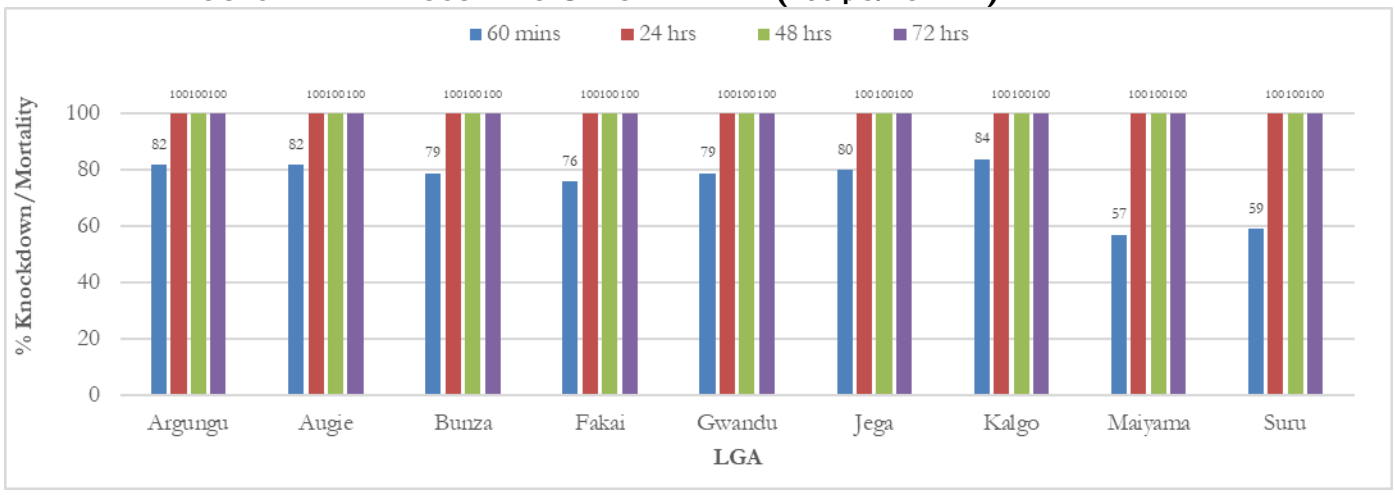


FIGURE 61: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT NASARAWA

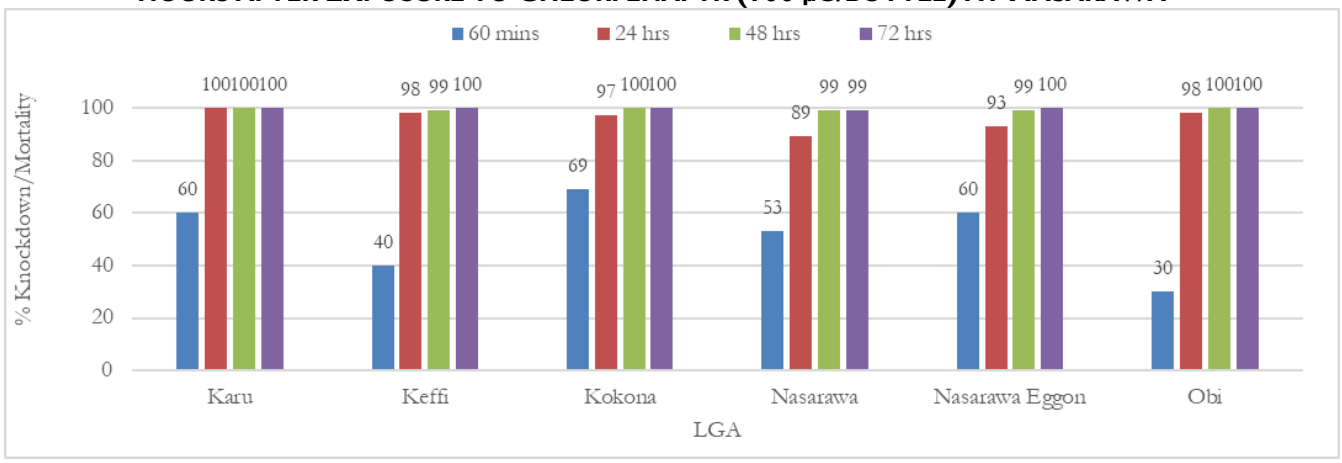


FIGURE 62: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT OYO

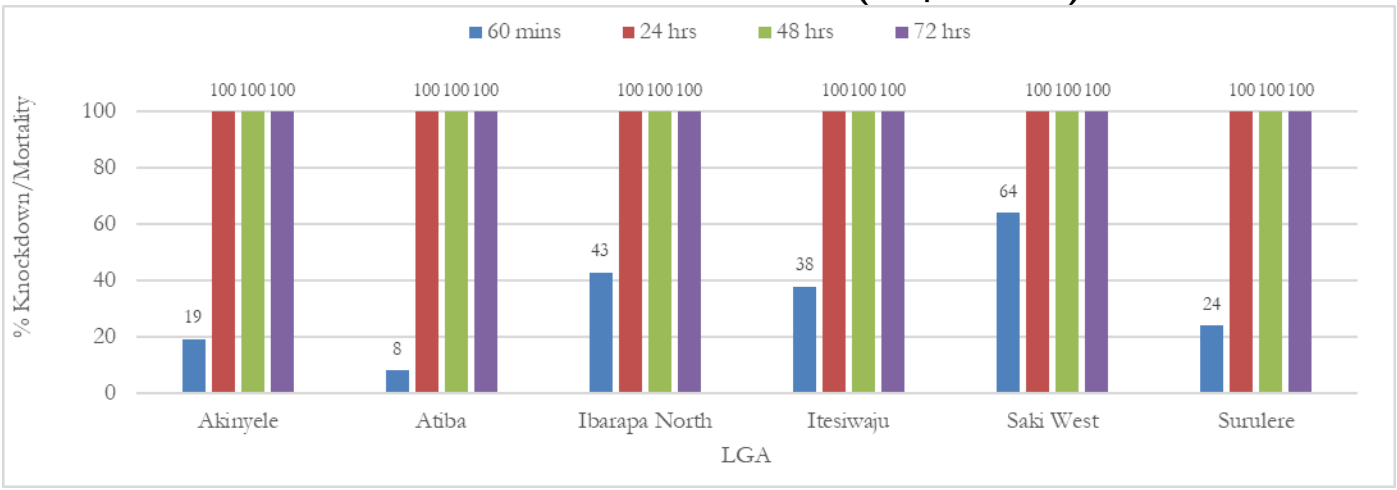


FIGURE 63: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) IN PLATEAU

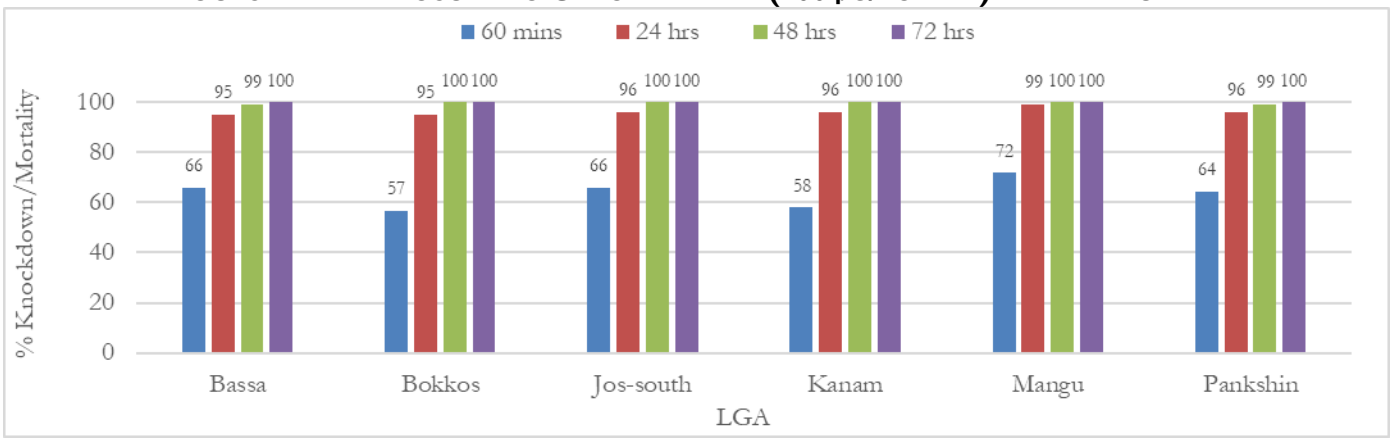


FIGURE 64: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT SOKOTO

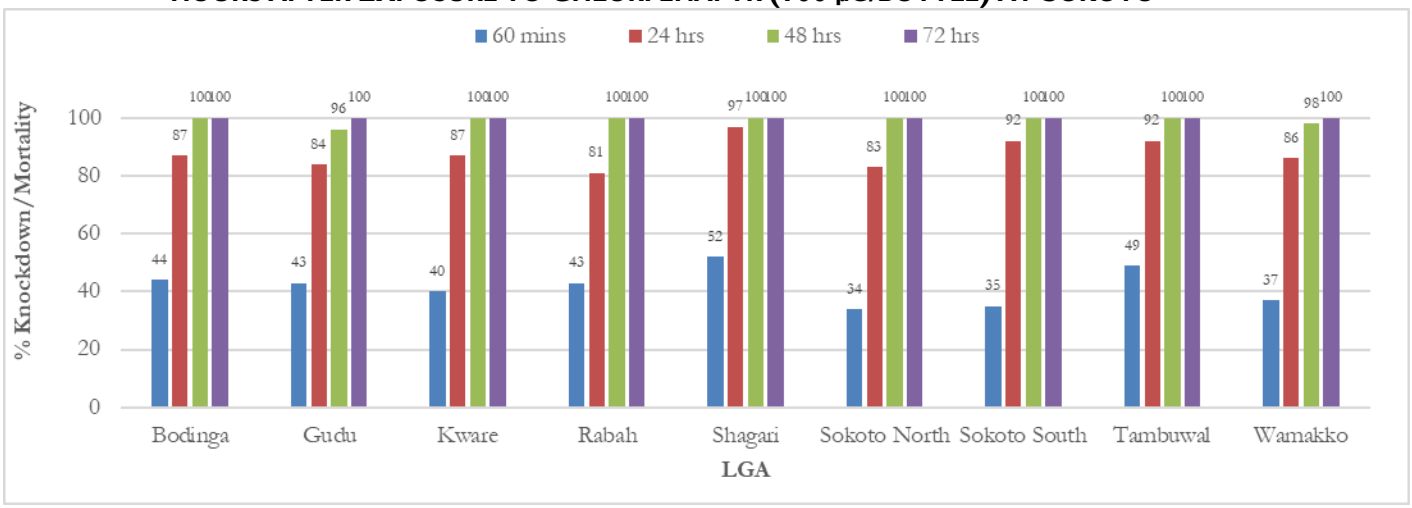
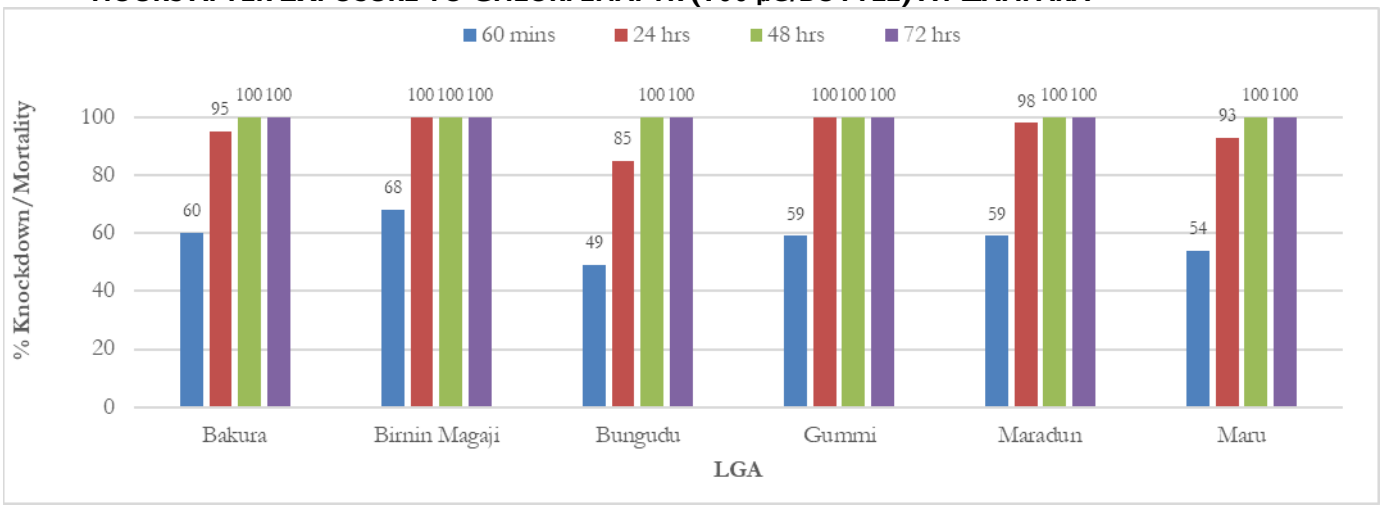


FIGURE 65: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. 60 MINUTES, 24 HOURS, 48 HOURS, AND 72 HOURS AFTER EXPOSURE TO CHLORFENAPYR (100 µG/BOTTLE) AT ZAMFARA



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

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

The percentage knockdown of *An. gambiae* s.l. mosquitoes at 30-minutes exposure to clothianidin varied across the sites: Akwa Ibom (48–64%), Bauchi (80–99%), Bayelsa (11-29%), Benue (76–84%), Cross River (51–80%), Ebonyi (26-58%), Enugu (100%), FCT (82-84%), Kebbi (18–26%), Nasarawa (63–80%), Oyo (22–81%), Plateau (88–91%), Sokoto (19–74%), and Zamfara (41–68%) (Table 10).

At 60 minutes, the percentage knockdown of *An. gambiae* s.l. mosquitoes exposed to clothianidin varied across the sites: Akwa Ibom (71–90%), Bauchi (100%), Bayelsa (88-97%), Benue (100%), Cross River (82–98%), Ebonyi (51-90%), Enugu (100%), FCT (89-94%), Kebbi (76–81%), Nasarawa (86-93%), Oyo (66–100%), Plateau (97–100%), Sokoto (73–92%), and Zamfara (54–88%) (Table 10).

Mortality rates of *An. gambiae* s.l. 24 hours post-exposure were 99–100% in 12 out of 14 sites, 94-98% in Akwa Ibom, and 97-100% in Cross River (Table 10).

TABLE 10: CDC BOTTLE BIOASSAY RESULTS (PERCENT MORTALITY AFTER 24 HOURS) FOR *AN. GAMBIAE* S.L. TO CLOTHIANIDIN

Sentinel Site	LGA	Number Tested	Percent Mortality at Various Test Times		
			30 mins	60 mins	24 hrs
Akwa Ibom	Abak	100	51	77	94
	Itu	100	55	78	96
	Mkpat Enin	100	53	79	96
	Nsit Ubium	100	61	87	98
	Onna	100	48	71	94
	Ukanafun	100	64	90	98
Bauchi	Darazo	101	97	100	100
	Dass	100	99	100	100
	Itas/Gadau	82	99	100	100
	Katagun	84	99	100	100
	Ningi	81	80	100	100
	Toro	81	99	100	100
Bayelsa	Ogbia	100	29	90	100
	Sagbama	100	12	88	100
	Yenagoa	100	11	97	100
Benue	Apa	100	84	100	100
	Gwer	100	81	100	100
	Obi	100	83	100	100
	Tarkar	100	76	100	100
	Ukum	100	76	100	100
	Vandeikya	100	83	100	100
Cross River	Abi	99	63	86	100
	Akamkpa	100	72	97	100
	Calabar Municipal	100	61	89	99
	Etung	100	51	82	97
	Obudu	106	80	90	100
	Ogoja	103	54	98	100
Ebonyi	Abakaliki	100	26	90	100
	Ebonyi	100	58	77	100
	Ezza North	100	49	78	100
	Ezza South	-	-	-	-
	Izzi	100	26	51	99
	Ohaozara	-	-	-	-

Sentinel Site	LGA	Number Tested	Percent Mortality at Various Test Times		
			30 mins	60 mins	24 hrs
Enugu	Awgu	106	100	100	100
	Enugu South	101	100	100	100
	Ezeagu	-	-	-	-
	Igbo-Eze North	-	-	-	-
	Isi-Uzo	-	-	-	-
	Nsukka	-	-	-	-
FCT	AMAC	100	83	94	100
	Gwagwalada	100	82	89	100
	Kwali	100	84	91	100
Kebbi	Argungu	100	18	79	100
	Augie	100	22	78	100
	Bunza	100	21	76	100
	Fakai	100	22	78	100
	Gwandu	100	22	81	100
	Jega	100	23	78	100
	Kalgo	100	24	77	100
	Maiyama	100	26	78	100
	Suru	100	26	76	100
Nasarawa	Karu	100	63	92	100
	Keffi	100	63	89	100
	Kokona	100	77	93	100
	Nasarawa	100	69	90	100
	Nasarawa Eggon	100	80	86	99
	Obi	100	73	90	100
Oyo	Akinyele	100	81	100	100
	Atiba	100	34	93	100
	Ibarapa North	100	80	99	100
	Itesiwaju	100	22	80	100
	Saki West	100	52	66	100
	Surulere	100	39	69	100
Plateau	Bassa	100	91	100	100
	Bokkos	100	89	97	100
	Jos-south	100	90	100	100
	Kanam	100	88	99	100
	Mangu	100	91	100	100
	Pankshin	100	91	98	100
Sokoto	Bodinga	100	19	75	100
	Gudu	100	19	73	100
	Kware	100	74	84	100
	Rabah	100	67	87	100
	Shagari	100	36	92	100
	Sokoto North	100	56	77	100
	Sokoto South	100	37	79	100
	Tambuwal	100	44	85	100
	Wamakko	100	43	91	100
Zamfara	Bakura	100	47	73	100
	Birnin Magaji	100	68	88	100
	Bungudu	100	44	61	100
	Gummi	100	59	78	100
	Maradun	100	46	60	100
	Maru	100	41	54	100

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

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

Assessment of *kdr* mutations in alpha-cypermethrin resistant *An. gambiae* s.l. indicated the presence of only *kdr-w* point mutations; no *kdr-e* point mutations were detected. The *kdr-w* gene frequencies in *An. coluzzii* ranged from 0.00 in FCT to 0.58 in Bayelsa, and from 0.42 in Akwa Ibom to 0.58 in Enugu for *An. gambiae* s.s. (Table 11). Although the overall *kdr-w* gene frequencies detected in *An. gambiae* s.s. were higher compared to *An. coluzzii* in Ebonyi, Enugu, Nasarawa, and Plateau, the reverse was the case in Akwa Ibom and Bauchi. These differences were however not statistically significant ($F_{1,15}$=0.1008, p=0.7552).

TABLE 11: FREQUENCY 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*	11	1	8	2	0.45	0	0	11	0.00
		An. gambiae s.s.	24	2	16	6	0.42	0	0	24	0.00
	Bauchi	*An. coluzzii*	22	2	17	3	0.48	0	0	22	0.00
		An. gambiae s.s.	14	3	7	4	0.46	0	0	14	0.00
	Bayelsa	*An. coluzzii*	13	3	9	1	0.58	0	0	13	0.00
	Cross River	*An. coluzzii*	2	1	0	1	0.50	0	0	2	0.00
		An. gambiae s.s.	2	0	2	0	0.50	0	0	2	0.00
	Ebonyi	*An. coluzzii*	32	3	24	5	0.47	0	0	32	0.00
		An. gambiae s.s.	4	0	4	0	0.50	0	0	4	0.00
	Enugu	*An. coluzzii*	12	1	11	0	0.54	0	0	12	0.00
		An. gambiae s.s.	6	1	5	0	0.58	0	0	6	0.00
	FCT	*An. coluzzii*	6	0	0	6	0.00	0	0	6	0.00
	Kebbi	*An. coluzzii*	11	0	10	1	0.45	0	0	11	0.00
		An. gambiae s.s.	1	0	1	0	0.50	0	0	1	0.00
	Nasarawa	*An. coluzzii*	7	0	6	1	0.43	0	0	7	0.00
		An. gambiae s.s.	28	0	25	3	0.45	0	0	28	0.00
	Plateau	*An. coluzzii*	35	1	28	6	0.43	0	0	35	0.00
		An. gambiae s.s.	1	0	1	0	0.50	0	0	1	0.00
	Sokoto	*An. coluzzii*	4	0	2	2	0.25	0	0	4	0.00
	Zamfara	*An. coluzzii*	14	1	12	1	0.50	0	0	14	0.00
Total			**249**	**19**	**188**	**42**	**0.45**	**0**	**0**	**249**	**0.00**

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

An assessment of *kdr* mutations in deltamethrin-resistant *An. gambiae* s.l. indicated the presence of only *kdr-w* point mutations. No *kdr-e* mutations were recorded across the sites. *Kdr-w* gene frequencies in *An. coluzzii* ranged from 0.00 in FCT to 0.54 in Bauchi and, in *An. gambiae* s.s., from 0.25 in Cross River to 0.67 in Sokoto. In general, *kdr-w* frequencies were higher in *An. coluzzii* than *An. gambiae* s.s. in Akwa Ibom, Bauchi, Cross River, Ebonyi, and Enugu (Table 12). Across all the sites, the difference in the frequency of *kdr-w* alleles between *An. coluzzii* and *An. gambiae* s.s. was not statistically significant ($F_{1,18}$=0.3569, p=0.5577).

TABLE 12: 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*	12	0	8	4	0.33	0	0	12	0.00
		An. gambiae s.s.	22	0	12	10	0.27	0	0	22	0.00
	Bauchi	*An. coluzzii*	24	3	20	1	0.54	0	0	24	0.00
		An. gambiae s.s.	12	0	8	4	0.33	0	0	12	0.00
	Bayelsa	*An. coluzzii*	10	1	8	1	0.50	0	0	10	0.00
		An. gambiae s.s.	2	0	2	0	0.50	0	0	2	0.00
	Benue	*An. coluzzii*	11	1	6	4	0.36	0	0	11	0.00
		An. gambiae s.s.	4	0	3	1	0.38	0	0	4	0.00
	Cross River	*An. coluzzii*	2	0	2	0	0.50	0	0	2	0.00
		An. gambiae s.s.	4	1	0	3	0.25	0	0	4	0.00
	Ebonyi	*An. coluzzii*	28	1	22	5	0.43	0	0	28	0.00
		An. gambiae	8	0	6	2	0.38	0	0	8	0.00
	Enugu	*An. coluzzii*	18	1	17	0	0.53	0	0	18	0.00
		An. gambiae s.s.	8	0	7	1	0.44	0	0	8	0.00
	FCT	*An. coluzzii*	2	0	0	2	0.00	0	0	2	0.00
	Nasarawa	*An. coluzzii*	3	0	3	0	0.50	0	0	3	0.00
		An. gambiae s.s.	13	2	11	0	0.58	0	0	13	0.00
	Plateau	*An. coluzzii*	34	3	21	10	0.40	0	0	34	0.00
		An. gambiae s.s.	2	0	2	0	0.50	0	0	2	0.00
	Sokoto	*An. coluzzii*	41	5	31	5	0.50	0	0	41	0.00
		An. gambiae s.s.	3	1	2	0	0.67	0	0	3	0.00
Total		**263**	**19**	**191**		**53**	**0.44**	**0**	**0**	**263**	**0.00**

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

An assessment of *kdr* mutations in permethrin-resistant *An. gambiae* s.l. indicated the presence of only *kdr-w* point mutations. *Kdr-w* gene frequencies in *An. coluzzii* ranged from 0.35 in Benue to 0.59 in Oyo, while *kdr-w* gene frequencies in *An. gambiae* s.s. ranged from 0.25 in FCT to 0.67 in Ebonyi and Kebbi. Higher *kdr-w* gene frequencies were observed in *An. gambiae* s.s. than *An. coluzzii* in Bauchi, Bayelsa, Benue, Cross River, Ebonyi, Enugu, Kebbi, Nasarawa, Plateau, Sokoto, and Zamfara (Table 13). However the difference in the frequency of the *kdr* allele recorded across sites in permethrin-resistant *An. coluzzii* and *An. gambiae* s.s. not vary significantly ($F_{1,26}$=2.2528, p=0.1454).

TABLE 13: 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*	12	2	7	3	0.46	0	0	12	0.00
		An. gambiae s.s.	24	4	14	6	0.46	0	0	24	0.00
	Bauchi	*An. coluzzii*	28	4	19	5	0.48	0	0	28	0.00
		An. gambiae s.s.	8	2	5	1	0.56	0	0	8	0.00
	Bayelsa	*An. coluzzii*	18	2	11	5	0.42	0	0	18	0.00
		An. gambiae s.s.	6	1	5	0	0.58	0	0	6	0.00
	Benue	*An. coluzzii*	26	0	18	8	0.35	0	0	26	0.00
		An. gambiae s.s.	7	0	6	1	0.43	0	0	7	0.00
	Cross River	*An. coluzzii*	27	1	18	8	0.37	0	0	27	0.00
		An. gambiae s.s.	9	0	7	2	0.39	0	0	9	0.00
	Ebonyi	*An. coluzzii*	32	5	24	3	0.53	0	0	32	0.00
		An. gambiae s.s.	3	1	2	0	0.67	0	0	3	0.00
	Enugu	*An. coluzzii*	23	1	22	0	0.52	0	0	23	0.00
		An. gambiae s.s.	7	2	5	0	0.64	0	0	7	0.00
	FCT	*An. coluzzii*	14	3	6	5	0.43	0	0	14	0.00
		An. gambiae s.s.	4	0	2	2	0.25	0	0	4	0.00
	Kebbi	*An. coluzzii*	46	3	35	8	0.45	0	0	46	0.00
		An. gambiae s.s.	3	1	2	0	0.67	0	0	3	0.00
	Nasarawa	*An. coluzzii*	3	0	3	0	0.50	0	0	3	0.00
		An. gambiae s.s.	32	7	21	4	0.55	0	0	32	0.00
	Oyo	*An. coluzzii*	23	5	17	1	0.59	0	0	23	0.00
		An. gambiae s.s.	8	0	7	1	0.44	0	0	8	0.00
	Plateau	*An. coluzzii*	28	1	23	4	0.45	0	0	28	0.00
		An. gambiae s.s.	8	2	5	1	0.56	0	0	8	0.00
	Sokoto	*An. coluzzii*	12	0	11	1	0.46	0	0	12	0.00
		An. gambiae s.s.	1	0	1	0	0.50	0	0	1	0.00
	Zamfara	*An. coluzzii*	30	3	21	6	0.45	0	0	30	0.00
		An. gambiae s.s.	6	1	4	1	0.50	0	0	6	0.00
Total			**448**	**51**	**321**	**76**	**0.47**	**0**	**0**	**448**	**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 most sites. Secondary malaria vectors such as *An. nili, An. moucheti, An. pharoensis,* and *An. coustani* were also found but with limited distribution and abundance. The percentage composition of *An. funestus* mosquitoes in 2018 (0.1-4.0%, four sites) increased in 2019 (1.0-16.9%, five sites) and 2020 (0.2-48.1%, four sites). This significantly increased in 2021 (0.9-51.2%) from three sites (Ebonyi, Plateau, and Oyo). *Anopheles funestus* became more abundant than *An. gambiae* s.l. in Oyo. Increased abundance of *An. funestus* (another major vector) indicates its potential to significantly contribute to malaria transmission, particularly in areas where suitable breeding conditions for the species are available. *Anopheles funestus* prefers to breed in water bodies that are stable, either permanent or semi-permanent and containing aquatic vegetation. This pattern of occurrence agrees with recent reports (AIRS Nigeria Final Entomology Report 2017, PMI VectorLink Nigeria Final Entomology Report 2018; PMI VectorLink Nigeria Final Entomology Report 2019 and PMI VectorLink Nigeria Final Entomology Report 2020). Investigations into failed amplifications from samples collected in Akwa Ibom have indicated the predominance (78.0%) of *An. marshallii* complex compared to *An. gambiae* (20.9%). All 1,218 *An. marshallii* complex mosquitoes caught during this period were from Akwa Ibom, a mangrove swamp area biting mainly indoors. This could contribute to malaria transmission in the area, hence the need to screen these species for sporozoites . Other localized species found included *An. squamosus, An. maculipalpis, An. longipalpis, An. rufipes*, and *An. pretoriensis*. A total of 13 *Anopheles* mosquito species were morphologically identified across the six sentinel sites. This is consistent with previous numbers of *Anopheles* species collected to date across the different ecological zones of Nigeria (PMI, 2017, 2018, 2019, and 2020), 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* complex (*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 molecularly identified were *An. funestus* s.s. and *An. leesoni* (Figures 5 and 6). However, the few unidentified from the PCR assay results suggest an improvement in molecular identification of *An. funestus* group.

3.2 HUMAN BITING RATE AND VECTOR BITING TIME

The mean indoor biting rates of *An. gambiae* s.l. peaked in July in Plateau (103.4 bites/person/night) and in April in Sokoto (26.5 bites/person/night), with an earlier smaller peak in October in Oyo (21.6 bites/person/night).

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 2020), biting rate did not increase much until July. Similarly, increased outdoor biting was equally recorded in April in Sokoto with an earlier smaller peak in October 2020 recorded in 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 2020). Delayed rainfall may be responsible for the shift in the monthly mosquito peak biting. This peak is usually the target of indoor residual spraying to reduce malaria transmission.

The average number of mosquitoes caught biting per hour was generally higher indoors, ranging from one mosquito collected between 7-8 p.m. in Akwa Ibom to 49 mosquitoes collected between 12-1 a.m. in Kebbi. This agrees with previous findings which show the peak biting time as after midnight in most sentinel sites (PMI VectorLink Annual Entomology Report 2019; 2020). Incidental shifts in peak biting time (10-11 p.m.) have been reported in Plateau in 2020 and may require further investigation of human activity at night and other environmental factors such as temperature and precipitation, which have been reported to influence the mosquito biting time (Dambach *et al.,* 2018). There was increased indoor biting for *An. gambiae* s.l. in Kebbi September (20.7 b/p/n). This agrees with the findings of Kabbale *et al.,* (2013) working in Budiope county of Uganda who found that although the abundance of *An. gambiae* s.l. was rainfall-dependent in some areas, both *An. gambiae* s.l. and *An. funestus* mosquitoes thrived all year round regardless of the amount of rainfall.

The average hourly number of mosquito bites (11, 9, 10) in Oyo, peaked indoors at 12-1 a.m., 2-3 a.m., and 5-6 a.m., respectively. This finding is in consonance with the report of Moiroux *et al.* (2012) working in Benin who reported significant changes in the host seeking behavior of the *An. funestus* population after scaling up universal coverage of ITNs in southern Benin. Due to the high rate of early morning biting by *An. funestus* in Oyo, extended morning collection by alternative non-LT method (such as HLC) may be considered.

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

3.3 SPOROZOITE INFECTION RATE

In this surveillance period, the highest infection rate was recorded outdoors among *An. gambiae* s.s. in Oyo (11.1%) followed by Plateau (2.2%). The highest infection rate among *An. coluzzii* was also recorded outdoors in Oyo (6.7%). This finding highlights increasing outdoor transmission in areas of high malaria endemicity, which could pose a challenge to malaria control and/or elimination efforts. Vector control efforts that target outdoor transmission should be encouraged. *Anopheles 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 2020, where sporozoite rate contributions were recorded in *An. gambiae* indoors only in Akwa Ibom (2.2%) and Ebonyi (1.3%), no *An. gambiae* collected indoors tested positive for sporozoites in this reporting period. Similarly diverging from last year's results where no infections were recorded among *An. coluzzii* both indoors and outdoors, infection rates in *An. coluzzii* indoors in this reporting period ranged from 0.7% in Sokoto to 3.7% in Plateau while infection rate of 6.7% was recorded outdoors only in Oyo. In 2019, sporozoites in *An. arabiensis* outdoors were recorded in Nasarawa (2.9%), Plateau (2.2%), and Sokoto (0.9%), while in 2021, no sporozoite infection was recorded among *An. arabiensis* in any other sites (PMI VectorLink Nigeria Annul Entomology Report 2019; 2020). The lack of sporozoite infection rate among *An. arabiensis* could be due to the small sample size analyzed. Overall, increased outdoor transmission recorded in this study suggests the need for interventions that target outdoor transmission.

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). Results indicate that sporozoite infection was recorded in *An. marshallii* mosquito caught using PSC method in Akwa Ibom (0.6%). This agrees with the work of Mustapha *et al.,* (2021) working in Kenya, who found the overall proportion of secondary vectors with *P. falciparum* sporozoite to be 0.63% in the Kisumu County of Kenya. Given their high densities and endophily equivalent to primary vectors and the recent incrimination with *Plasmodium* sporozoites, *An. marshallii* may contribute to malaria transmission in Akwa Ibom. Further morphological and molecular identification studies towards further characterization of this species is recommended. Continued monitoring is essential for understanding their temporal contributions to malaria transmission in the area.

3.4 Entomological Inoculation Rate

EIRs were recorded indoors in four of the five sites among *An. coluzzii*, with Plateau having the highest annual indoor EIR at 51.9 infective bites/person/year. There were no infective bites recorded indoors among *An. gambiae* s.s. in all sentinel sites. This contrasts with our previous findings where *An. gambiae* s.s. have contributed more EIRs both indoors and outdoors than other members of the *An. gambiae* complex. The highest outdoor EIR was recorded among *An. gambiae* s.s. in Plateau (10.0 infective bites/person/year) followed by Oyo (3.1 infective bites/person/year). This agrees with previous findings which indicated that *An. gambiae* s.s. contributed more to EIR outdoors in more LGAs compared to other members of the *An. gambiae* complex (PMI VectorLink Annual Entomology Report 2019; 2020). There were no infective bites recorded among *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; 2020). The highest EIR among *An. funestus* was recorded outdoors in Oyo (31.7 infective bites/person/year), followed by the indoor EIR also in Oyo (14.1 infective bites/person/year). This is in contrast with the findings of Degefa *et al.,* (2017) from Kenya where the EIR data showed that the majority of malaria transmission by *An. gambiae* s.s. and *An. funestus* occurred indoors, while *An. arabiensis* contributed almost equally to both outdoor and indoor transmission.

3.5 Blood Meal Sources

The blood meal analysis indicates that *An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis* collected using CDC LTs and PSCs varied in their preference for human blood meal. The co-occurrence of *An. gambiae* s.s. and *An. coluzzii,* and their preference for human blood meal in all sites attest to their strong anthropophilic behavior which makes them important malaria transmitters. The 100% preference for human blood meal in *An. arabiensis* both indoors and outdoors among mosquitoes collected using CDC LTs also indicates its behavior to fully utilize human blood meal indoors when zoophilic feeding is unavailable and thus aid transmission in 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; 2020). 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. *Anopheles gambiae* s.s. collected from CDC LT indoors in Akwa Ibom fed on human and goat equally (50% each) while in Plateau, *An. coluzzii* fed on human and bovine equally as well (50% each). This contrasts with our previous finding where a similar scenario was recorded outdoors only in Akwa Ibom among *An. gambiae* s.s. and *An. coluzzii* feeding on human and bovine blood (50% each). Compared to 2020, where there was higher feeding on animals recorded outdoors in Akwa Ibom (where 50% of *An. gambiae* s.s. and *An. coluzzii* blood meals were bovine) and Plateau (where 100% of *An. gambiae* s.s. blood meals outdoors were from goat), there was a higher preference for human blood among the three species of the *An. gambiae* complex during this reporting period across all sentinel sites (PMI VectorLink Annual Entomology Report 2020; Ogola *et al.,* 2017). Overall, despite the zoophilic nature of *An. arabiensis,* a significantly higher number of human blood meals ($p<0.0001$) were recorded than bovine and goat in those collected indoors using PSC method in Plateau and Sokoto. This agrees with the findings of Ogola *et al.,* (2017) from Kenya, which found a higher proportion of human blood meals among both *An. arabiensis* and *An. funestus* collected indoors. Generally, the prevalence of animal blood meal in human dwellings indicates that livestock live in close proximity to humans. This can

support higher malaria transmission by attracting infected mosquitoes to human habitations (Iwashita *et al.,* 2014). Overall, of the blood fed mosquitoes identified as *An. marshallii,* the majority (93.3% by indoor CDC LT, 79.5% by PSC) were positive for human blood, indicating a possible preference for human blood over animal blood, though bovine and goat blood meals were also present amongst the samples.

3.6 INSECTICIDE SUSCEPTIBILITY

Pyrethroid resistance patterns varied within and among the states. While permethrin resistance was observed in mosquito populations across all 14 states except in four out of nine LGAs of Sokoto, alpha-cypermethrin and deltamethrin resistance occurred in *Anopheles* populations in varying degrees across most sites. Resistance to all three pyrethroids was recorded in Akwa Ibom, Bauchi, Bayelsa, Ebonyi, and Plateau.

Susceptibility to alpha-cypermethrin was recorded in all six LGAs of Benue, Cross River, and Oyo, 1/4 LGAs in Enugu, 2/3 LGAs in FCT, 6/9 LGAs in Kebbi, all nine LGAs in Sokoto, and 4/6 LGAs of Zamfara. No susceptibility to alpha-cypermethrin was observed in Akwa Ibom, Bauchi, Bayelsa, Ebonyi, Nasarawa, or Plateau.

Susceptibility to deltamethrin was recorded in *An. gambiae* s.l. populations across all three LGAs in FCT, all six LGAs each in Cross River, Oyo, and Zamfara, and all nine LGAs in Kebbi. These results suggest possible use of these insecticides, especially for treating ITNs, under an insecticide resistance management plan. Deltamethrin resistance was recorded in *An. gambiae* s.l. in all six LGAs in Akwa Ibom, Bauchi, Bayelsa, Ebonyi Enugu, and Plateau. This accords with the findings across many countries that resistance to pyrethroids continues to be widespread (WHO, 2020, PMI VectorLink Final Entomology Report 2020).

The widespread resistance of vectors to permethrin at most of the sites strongly suggest that it is not the preferred insecticide choice for 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 three LGAs of Bayelsa and FCT, all six LGAs in Oyo and Zamfara, and all nine LGAs in Kebbi and Sokoto, but varied in other sites. This agrees with findings from WHO (2020) which reported low resistance of local *Anopheles* mosquitoes to pirimiphos-methyl in most countries, though resistance to pirimiphos-methyl was recorded in all six LGAs from Cross River, Nasarawa, and Plateau.

3.7 RESISTANCE INTENSITY

Low-intensity alpha-cypermethrin resistance was recorded in *An. gambiae* s.l. populations in six LGAs in Akwa Ibom and Plateau and one of six LGAs in Nasarawa. Deltamethrin resistance in *An. gambiae* s.l. mosquitoes attained low resistance intensity in all six LGAs only in Plateau. This agrees with findings from 2019 and 2020 (PMI VectorLink Final Entomology Report 2019; 2020) which found low resistance intensity in Plateau and Ebonyi but contrasted with findings in 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 VectorLink Final Entomology Report 2018). Deltamethrin resistance intensity results this year showed resistance only at 1X in all LGAs in Akwa Ibom, Bauchi, Bayelsa, and Ebonyi, three LGAs in Enugu, 2/6 LGAs in Benue, 3/6 LGAs in Nasarawa, and 7/9 LGAs in Sokoto. Permethrin resistance in *An. gambiae* at low level intensity was observed in all six LGAs in Cross River, Oyo, Plateau, and Zamfara, 6/9 LGAs of Kebbi, and 2/3 LGAs of Bayelsa and FCT, while moderate resistance intensity was observed in populations from all LGAs in Nasarawa and one LGA in Enugu, and high resistance intensity to permethrin was recorded in all six LGAs in Akwa Ibom and in one LGA in Enugu.

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 insecticides until novel tools based on new classes of insecticides are available (WHO, 2017).

3.8 SYNERGIST ASSAYS

A growing cause for concern is that 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, Bauchi, Benue, 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, 2020). Fluctuations in susceptibility outcomes in these states may require further observation over successive years or might need to consider ITNs comprising of chlorfenapyr. The restoration 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, 2015) but the partial restoration 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 may be a concern for the use of ITNs as the primary malaria vector control intervention in Nigeria, since most ITNs presently deployed in vector control are treated with pyrethroids only or in some combination with pyrethroids (Geleta and Ketema, 2016).

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

An. gambiae s.l. mosquitoes were susceptible (98-100% mortality) to chlorfenapyr at 72 hours across all LGAs except one in Enugu and one in FCT. This suggests the relevance of chlorfenapyr as a potential compound to manage the pyrethroid resistance observed at the monitoring sites and is consistent with several studies carried out elsewhere with chlorfenapyr. Several trials and studies have been conducted on the Interceptor® G2 net as described by Bayili *et al.,* (2017) working in Burkina Faso, Camara *et al.* (2018) working in Côte d'Ivoire, and Ngufor *et al.* (2011) working in Benin. These studies indicate that chlorfenapyr-treated nets evaluated in several areas with documented pyrethroid resistance have been proven to be effective for controlling pyrethroid-resistant malaria vectors and could contribute to insecticide resistance management in Nigeria.

3.10 DETERMINATION OF SUSCEPTIBILITY STATUS OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN

The percentage knockdown of *An. gambiae* s.l. mosquitoes after 30 and 60 minutes of exposure to clothianidin varied across the sites using the CDC bottle bioassay method. Complete susceptibility after the 24-hour holding period was recorded across all sites except in four of six LGAs in Akwa Ibom (Abak, Itu, Mkpat Enin, and Onna) and one LGA in Cross River (Etung).

The high degree of susceptibility of field populations of local mosquitoes to chlorfenapyr and clothianidin indicate a lack of cross-resistance with pyrethroid insecticides (Agumba *et al*., 2019). The target sites of both insecticides are different from that of pyrethroids, and the lack of cross-resistance indicates that enzymes involved in the metabolic detoxification of pyrethroids do not affect either chlorfenapyr or clothianidin. In addition, chlorfenapyr is considered a pro-insecticide that is activated by oxidase enzymes suggesting a potential for negative cross-resistance (Raghavendra *et al*., 2011). Possible resistance to clothianidin was recorded in some LGAs in Akwa Ibom and Cross River during this reporting period. Further tests are needed to confirm these outcomes. Resistance to chlorfenapyr and clothianidin have not yet been described in mosquitoes; but additional information on the molecular mechanisms of insecticide resistance may help to predict the potential for cross-resistance and to guide NMCPs in the selection of insecticides for ITNs and IRS.

3.11 *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 is consistent with our findings in the ecological zones in 2020, but contrasts with 2019 findings from the same locations, where both *kdr-w* and *kdr-e* point mutations were present (PMI VectorLink Annual Entomology Report 2019; 2020). Similar to the report in 2019, the *kdr-w* mutation was observed at all sites in all pyrethroid-resistant mosquitoes. The *kdr-e* allele, which was found only in two sites in 2019 (Ebonyi and Akwa Ibom), was not found in any of the sites this year. 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 2020). 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; 2007). Compared to the findings by Awolola *et al.* (2003, 2005, and 2007) when the *kdr* mutation was not recorded in the M form (*An. coluzzii*), the results in this report indicate that *kdr* mutations were recorded in *An. coluzzii*. The spread of *kdr* mutations in *An. coluzzii* across all ecozones (including the Sudan and Sahel savannah where it is not the predominant vector) may indicate an escalation of resistance in *An. coluzzii* as recorded by Ibrahim *et al.,* (2019) in northern Nigeria. It is also possible that there is an ongoing introgression from *An. gambiae* to *An. coluzzii* as suggested by other studies in West Africa (Hanemaaijer *et al.,* 2019), taking into consideration the *kdr* as a marker of introgression (Diabate *et al.,* 2003).

These findings are consistent with Ibrahim *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.

ANNEXES

ANNEX 1: GPS COORDINATES OF LONGITUDINAL SAMPLING SITE LOCATIONS

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

ANNEX 2: *ANOPHELES* MOSQUITOES COLLECTED BY DIFFERENT METHODS AND SUBJECTED TO PCR ACROSS SITES (OCTOBER 2020–SEPTEMBER 2021)

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	24	6	2	3	35	48	0	48	83
Ebonyi	64	23	0	1	88	168	1	169	257
Oyo	59	24	1	1	85	400	0	400	485
Plateau	138	69	0	1	208	292	0	292	500
Sokoto	188	137	0	3	328	968	4	972	1,300
Total	**473**	**259**	**3**	**9**	**744**	**1,876**	**5**	**1,881**	**2,625**

ANNEX 3: *ANOPHELES* CAUGHT BY SPECIES, METHOD, AND SITE (OCTOBER 2020–SEPTEMBER 2021)

Mosquito Species	Akwa Ibom			Ebonyi			Kebbi			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	In	Out	PSC				
An. gambiae s.l.	122	27	178	590	31	1,157	5,341	2,259	6,872	438	199	1,434	2,045	136	1,131	3,650	2,060	15,125	12,186	4,712	25,897	42,795
An. funestus	-	-	-	3	3	11	-	-	-	756	341	1,084	108	30	188	5	1	-	872	375	1,283	2,530
An. nili	-	-	-	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1
An. coustani	-	-	-	1	1	0	-	-	-	-	-	-	1	0	-	1	8	-	3	9	0	12
An. pharoensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	10	-	21	10	0	31
An. maculipalpis	8	0	0	-	-	-	-	-	-	-	-	-	0	1	-	5	10	-	13	11	0	24
An. moucheti	9	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	0	0	9
An. pretoriensis	-	-	-	-	-	-	-	-	-	0	0	1	-	-	-	1	4	1	1	4	2	7
An. rufipes	-	-	-	3	0	8	-	-	-	-	-	-	12	7	21	8	5	-	23	12	29	64
An. marshallii	885	201	132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	885	201	132	1,218
An. squamosus	-	-	-	-	-	-	-	-	-	-	-	-	6	8	-	-	-	-	6	8	0	14
An. longipalpis	-	-	-	-	-	-	-	-	-	0	0	6	-	-	-	-	-	-	0	0	6	6
An. flavicosta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
Grand Total	**1,024**	**228**	**310**	**598**	**35**	**1,176**	**5,341**	**2,259**	**6,872**	**1,194**	**540**	**2,525**	**2,172**	**182**	**1,340**	**3,691**	**2,098**	**15,126**	**14,020**	**5,342**	**27,349**	**46,711**

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

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

Sentinel Site	CDC Indoors					CDC Outdoors					PSC					Totals				
	No. PCR +ve	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*	No. PCR +ve	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*
Akwa Ibom	24	13	11	0	0	6	6	0	0	0	48	23	25	0	0	78	42	36	0	0
Ebonyi	64	34	30	0	0	23	11	12	0	0	168	71	97	0	0	255	116	139	0	0
Oyo	59	31	28	0	0	24	9	15	0	0	400	176	223	1	0	483	216	266	1	0
Plateau	138	83	54	0	1	69	46	21	0	2	292	148	128	0	16	499	277	203	0	19
Sokoto	188	43	144	0	1	137	40	96	0	1	968	284	662	3	19	1,293	367	902	3	21
Total	**473**	**204**	**267**	**0**	**2**	**259**	**112**	**144**	**0**	**3**	**1,876**	**702**	**1,135**	**4**	**35**	**2,608**	**1,018**	**1,546**	**4**	**40**

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

Sentinel Site	Overall Total Analyzed	Overall Totals			CDC LT Indoors				CDC Outdoors				PSC
		CDC LT Indoors	CDC LT Outdoors	PSC	*An. funestus* s.s.	*An. funestus* (Unamplified)	*An. leesoni*	*An. funestus* s.s.	*An. funestus* (Unamplified)	*An. leesoni*	*An. funestus* s.s.	*An. funestus* (Unamplified)	*An. leesoni*
Ebonyi	11	1	0	10	1	0	0	0	0	0	10	0	0
Oyo	549	118	120	311	116	2	0	117	3	0	311	0	0
Plateau	214	72	26	116	72	0	0	26	0	0	113	2	1
Sokoto	38	20	8	10	20	0	0	8	0	0	10	0	0
Total	**812**	**211**	**154**	**447**	**209**	**2**	**0**	**151**	**3**	**0**	**444**	**2**	**1**

ANNEX 6: INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES BY SITE

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		An. gambiae s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Akwa Ibom	Oct-20	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-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
	Dec-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
	Jan-21	0	0	23	0	0	0	0.00	0.00	1.92	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
	Feb-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-21	17	0	0	0	0	0	1.42	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
	Apr-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	May-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-21	8	2	8	0	0	0	0.67	0.17	0.67	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-21	0	0	4	0	0	0	0.00	0.00	0.33	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-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-21	10	9	16	0	0	0	0.87	0.75	1.30	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	66	27	56	0	0	0	5.51	2.25	4.66	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
Ebonyi	Oct-20	22	0	5	0	0	0	1.80	0.00	0.45	0.00	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-20	2	0	0	0	0	0	0.17	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
	Dec-20	3	0	8	0	0	0	0.21	0.00	0.63	0.00	0.00	0.00	0.000	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-21	6	0	6	0	0	0	0.46	0.00	0.46	0.00	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
	Feb-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-21	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
	Apr-21	6	1	3	0	0	0	0.50	0.08	0.25	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
	May-21	105	10	78	8	0	0	8.71	0.86	6.54	0.64	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-21	70	3	46	3	0	0	5.80	0.25	3.87	0.25	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-21	19	0	50	4	0	0	1.57	0.00	4.18	0.33	0.00	0.00	0.000	0.00	0.125	0.000	0.00	0.00	0.000	0.000	16.205	0.000	0.000	0.000
	Aug-21	105	0	26	0	0	0	8.73	0.00	2.18	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-21	10	1	19	1	0	0	0.81	0.08	1.61	0.08	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	313	15	277	16	0	0	26.12	1.24	23.05	1.35	0.00	0.00	0.000	0.000	0.033	0.000	0.00	0.00	0.000	0.000	16.205	0.000	0.000	0.000

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		An. gambiae s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Oyo	Oct-20	199	0	60	0	0	0	16.60	0.00	4.98	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-20	0	0	61	0	0	0	0.00	0.00	5.08	0.00	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-20	23	18	0	0	0	0	1.92	1.50	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-21	7	0	0	3	0	0	0.58	0.00	0.00	0.25	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
	Feb-21	0	3	0	1	0	0	0.00	0.22	0.00	0.11	0.00	0.00	0.00	0.500	0.00	0.000	0.00	0.00	0.000	3.111	0.000	0.000	0.000	0.000
	Mar-21	2	0	6	0	0	0	0.17	0.00	0.50	0.00	0.00	0.00	0.000	0.00	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Apr-21	5	2	8	5	0	0	0.42	0.19	0.67	0.39	0.00	0.00	0.000	0.000	0.125	0.250	0.00	0.00	0.000	0.000	2.500	2.917	0.000	0.000
	May-21	7	0	4	1	0	0	0.55	0.00	0.37	0.08	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
	Jun-21	2	0	0	1	0	0	0.17	0.00	0.00	0.08	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
	Jul-21	0	0	26	4	0	0	0.00	0.00	2.17	0.33	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-21	0	3	15	3	0	0	0.00	0.25	1.25	0.25	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Sep-21	0	4	6	8	0	0	0.00	0.33	0.50	0.67	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	TOTAL	230	75	208	124	0	0	19.18	6.22	17.32	10.36	0.00	0.00	0.000	0.111	0.036	0.067	0.00	0.00	0.000	3.111	2.500	2.917	0.000	0.000
Plateau	Oct-20	20	2	5	0	0	0	1.67	0.17	0.42	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-20	0	2	0	0	0	0	0.00	0.17	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
	Dec-20	40	13	10	3	0	0	3.33	1.09	0.83	0.21	0.00	0.04	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Jan-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Feb-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Mar-21	3	0	6	0	1	0	0.24	0.00	0.48	0.00	0.12	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Apr-21	9	0	2	0	0	0	0.73	0.00	0.18	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
	May-21	52	1	35	0	0	0	4.35	0.08	2.90	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-21	48	8	72	4	0	4	4.00	0.67	6.00	0.33	0.00	0.33	0.000	0.500	0.000	0.000	0.00	0.000	0.000	10.000	0.000	0.000	0.000	0.000
	Jul-21	621	34	621	34	0	0	51.71	2.79	51.71	2.79	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-21	211	6	191	10	0	0	17.59	0.53	15.91	0.80	0.00	0.00	0.000	0.000	0.105	0.000	0.00	0.00	0.000	0.000	51.925	0.000	0.000	0.000
	Sep-21	52	6	28	9	0	0	4.33	0.50	2.33	0.75	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	1230	91	800	41	15	4	102.50	7.56	66.68	3.45	1.23	0.33	0.000	0.022	0.037	0.000	0.000	0.000	0.000	10.000	51.925	0.000	0.000	0.000

Sentinel Site	Month	Number Identified						HBR						SPR						Monthly EIR					
		An. gambiae s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.		*An. coluzzii*		*An. arabiensis*	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Sokoto	Oct-20	30	12	15	43	0	6	2.50	1.02	1.25	3.56	0.00	0.51	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Nov-20	0	0	14	14	0	0	0.00	0.00	1.17	1.17	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Dec-20	11	9	27	19	5	0	0.90	0.78	2.24	1.56	0.45	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
	Jan-21	11	11	29	5	0	0	0.89	0.89	2.44	0.44	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
	Feb-21	1	0	0	0	0	0	0.08	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-21	42	20	99	116	0	0	3.53	1.70	8.23	9.63	0.00	0.00	0.000	0.000	0.071	0.000	0.00	0.00	0.000	0.000	18.213	0.000	0.000	0.000
	Apr-21	147	120	171	147	0	0	12.28	9.99	14.22	12.26	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
	May-21	87	22	99	75	0	0	7.23	1.80	8.27	6.29	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jun-21	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Jul-21	0	0	458	333	0	0	0.00	0.00	6.36	4.63	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
	Aug-21	17	0	836	0	0	0	0.23	0.00	11.61	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-21	103	0	1446	570	0	0	1.43	0.00	20.08	7.92	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
	TOTAL	835	601	2,796	1,444	19	15	69.57	50.12	232.98	120.29	1.62	1.25	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	18.213	0.000	0.000	0.000

ANNEX 7: ANNUAL EIR FOR ALL SENTINEL SITES

Sentinel Sites	Mosquito Species	Estimated EIR over 12 months	
		Indoors	Outdoors
Akwa Ibom	*An. gambiae* s.s.	0.0	0.0
	An. coluzzii	0.0	0.0
	An. arabiensis	0.0	0.0
Ebonyi	*An. gambiae* s.s.	0.0	0.0
	An. coluzzii	16.2	0.0
	An. arabiensis	0.0	0.0
Oyo	*An. gambiae* s.s.	0.0	3.1
	An. coluzzii	2.5	2.9
	An. arabiensis	0.0	0.0
Plateau	*An. gambiae* s.s.	0.0	10.0
	An. coluzzii	51.9	0.0
	An. arabiensis	0.0	0.0
Sokoto	*An. gambiae* s.s.	0.0	0.0
	An. coluzzii	18.2	0.0
	An. arabiensis	0.0	0.0

ANNEX 8: INDOOR RESTING DENSITY OF *ANOPHELES* BY SITE

Sentinel Sites	Month	# of Rooms	Total # of *Anopheles* Caught	Indoor Resting Density
Akwa Ibom	Oct-20	32	0	0.0
	Nov-20	32	0	0.0
	Dec-20	32	0	0.0
	Jan-21	32	27	0.8
	Feb-21	32	1	0.0
	Mar-21	32	5	0.2
	Apr-21	32	0	0.0
	May-21	32	2	0.1
	Jun-21	32	15	0.5
	Jul-21	32	79	2.5
	Aug-21	32	28	0.9
	Sep-21	32	21	0.7
			Mean	**0.5**
Ebonyi	Oct-20	32	71	2.2
	Nov-20	32	17	0.5
	Dec-20	32	6	0.2
	Jan-21	32	23	0.7
	Feb-21	32	6	0.2
	Mar-21	32	16	0.5
	Apr-21	32	80	2.5
	May-21	32	280	8.8
	Jun-21	32	117	3.7
	Jul-21	32	214	6.7
	Aug-21	32	257	8.0
	Sep-21	32	70	2.2
			Mean	**3.0**
Kebbi	Jul-21	192	2,613	13.6
	Aug-21	192	2,030	10.6
	Sep-21	192	2,229	11.6
			Mean	**11.9**
Oyo	Oct-20	32	281	8.8
	Nov-20	32	78	2.4
	Dec-20	32	58	1.8
	Jan-21	32	103	3.2
	Feb-21	32	82	2.6
	Mar-21	32	36	1.1
	Apr-21	32	105	3.3
	May-21	32	64	2.0
	Jun-21	32	255	8.0
	Jul-21	32	190	5.9
	Aug-21	32	110	3.4
	Sep-21	32	72	2.3
			Mean	**3.7**
Plateau	Oct-20	32	34	1.1
	Nov-20	32	34	1.1
	Dec-20	32	22	0.7
	Jan-21	32	6	0.2
	Feb-21	32	0	0.0
	Mar-21	32	97	3.0
	Apr-21	32	46	1.4

Sentinel Sites	Month	# of Rooms	Total # of *Anopheles* Caught	Indoor Resting Density
	May-21	32	143	4.5
	Jun-21	32	265	8.3
	Jul-21	32	248	7.8
	Aug-21	32	116	3.6
	Sep-21	32	120	3.8
			Mean	**2.9**
Sokoto	Oct-20	32	1,063	33.2
	Nov-20	32	216	6.8
	Dec-20	32	137	4.3
	Jan-21	32	143	4.5
	Feb-21	32	131	4.1
	Mar-21	32	1,677	52.4
	Apr-21	32	1,020	31.9
	May-21	32	317	9.9
	Jun-21	32	30	0.9
	Jul-21	192	3,242	16.9
	Aug-21	192	2,776	14.5
	Sep-21	192	4,373	22.8
			Mean	**16.8**

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

Sentinel Site	Month	Number Identified		HBR		SPR		Monthly EIR	
		In	Out	In	Out	In	Out	In	Out
Oyo	Oct-20	12	21	7.7	4.2	0.00	0.10	0.000	12.302
	Nov-20	22	12	14.2	2.6	0.00	0.08	0.000	6.458
	Dec-20	0	1	7.6	3.8	0.00	0.00	0.000	0.000
	Jan-21	19	21	3.6	2.1	0.00	0.14	0.000	9.226
	Feb-21	5	7	2.4	0.9	0.00	0.14	0.000	3.667
	Mar-21	12	7	0.6	0.1	0.17	0.00	3.014	0.000
	Apr-21	10	10	1.7	1.9	0.00	0.00	0.000	0.000
	May-21	6	5	0.5	0.6	0.00	0.00	0.000	0.000
	Jun-21	0	10	1.0	0.6	0.00	0.00	0.000	0.000
	Jul-21	13	15	4.7	2.6	0.08	0.00	11.128	0.000
	Aug-21	8	3	5.8	3.0	0.00	0.00	0.000	0.000
	Sep-21	9	5	13.4	6.1	0.00	0.00	0.000	0.000
		116	117	63.0	28.4	0.03	0.06	14.1	31.7
Plateau	Oct-20	0	0	0.5	0.1	0.00	0.00	0.000	0.000
	Nov-20	0	0	1.4	1.0	0.00	0.00	0.000	0.000
	Dec-20	2	0	1.2	0.8	0.00	0.00	0.000	0.000
	Jan-21	0	0	1.7	0.3	0.00	0.00	0.000	0.000
	Feb-21	3	2	0.1	0.1	0.00	0.00	0.000	0.000
	Mar-21	0	0	0.2	0.0	0.00	0.00	0.000	0.000
	Apr-21	17	1	0.0	0.0	0.00	0.00	0.000	0.000
	May-21	8	0	0.2	0.0	0.25	0.00	1.292	0.000
	Jun-21	10	0	0.1	0.2	0.00	0.00	0.000	0.000
	Jul-21	6	1	1.6	0.1	0.00	0.00	0.000	0.000
	Aug-21	13	12	0.7	0.0	0.00	0.00	0.000	0.000
	Sep-21	13	10	1.5	0.0	0.00	0.00	0.000	0.000
		72	26	9.0	2.5	0.03	0.00	1.3	0.0

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