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Indoor Residual Spraying (IRS 2) Task Order Six

ENTOMOLOGICAL MONITORING OF THE PMI AIRS PROGRAM IN NORTHERN GHANA

2016 Annual Report

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ENTOMOLOGICAL MONITORING OF THE PMI AIRS PROGRAM IN NORTHERN GHANA

2016 ANNUAL REPORT

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

AIRS	Africa Indoor Residual Spraying
BYD	Bunkpurugu-Yunyoo District
DEF	S.S.S-tributlyphosphorotrithioate
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
EMD	East Mamprusi District
GST	Glutathione S-transferase
GUD	Gushegu District
HLC	Human Landing Catch
ib/p/n	Infective Bites/Person/Night
IRS	Indoor Residual Spraying
KAD	Karaga District
kdr	Knockdown Resistance
KUD	Kumbungu District
MBR	Man Biting Rate
NMIMR	Noguchi Memorial Institute for Medical Research
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SND	Savelugu-Nanton District
TD	Tolon District
TML	Tamale Metropolis
USAID	United States Agency for International Development
WHO	World Health Organization
WGD	West Gonja District
WMD	West Mamprusi District

EXECUTIVE SUMMARY

BACKGROUND AND METHODS

The Africa Indoor Residual Spraying (AIRS) Ghana project, funded by the United States Agency for International Development (USAID) through the President's Malaria Initiative (PMI), implemented indoor residual spraying (IRS) in five districts in Northern Ghana and carried out entomological monitoring in its target districts and beyond in 2016.

To assess the impact of IRS on entomological indices of malaria transmission across all sites, PMI AIRS carried out routine entomological surveys in eight sentinel districts. The districts were two IRS districts: Bunkpurugu-Yunyoo District (BYD) and Kumbungu District (KUD); four districts from which IRS was withdrawn: Gushegu District (GUD), Karaga District (KAD), Savelugu Nanton District (SND), and Tolon District (TD); and two districts that had never been sprayed: Tamale (TML) and West Gonja (WG) districts. The project used human landing, pyrethrum spray, and window exit trap collection methods to collect mosquitoes between March and November 2016 in all sites, except in GUD, KAD and WG. Mosquito collections in KAD took place in September–November 2016, while collections in GUD were done only in September. In WG, data collection took place between June and November 2016. World Health Organization (WHO) wall bioassay tests were performed to determine the decay rate of sprayed insecticide and tube tests for susceptibility. Finally, insecticide resistance intensity and synergist assays were performed using CDC bottle bioassay methods.

RESULTS AND DISCUSSION

Vector Species Composition and Seasonality: *An. gambiae* s.l. was the most abundant species in all the study sites, comprising 98% (44,780) of the total 45,668 *Anopheles* collected. *An. coluzzii* and *An. gambiae* were the main species identified by polymerase chain reaction (PCR). *An. coluzzii* was dominant in 4 out of 7 sites. . Mosquito populations peaked with the onset of the rains. High outdoor feeding behavior of *An. gambiae* s.l. was recorded from BYD, KUD, TD, and WG. In contrast, high indoor feeding tendencies were observed for *An. gambiae* s.l. in TML and SND.

Parity Rates: Ovary dissections showed lower longevity of *An. gambiae* s.l. in IRS districts (BYD and KUD) compared with the unsprayed districts (TD and TML). The mean proportion of parous *An. gambiae* s.l. for the two IRS districts (BYD 39.3% and KUD 54.2%) were significantly ($p<0.05$) different from the mean parity rates for TML (66.8%) and TD (69.4%). The parity rate for BYD was also significantly lower than the parity rate for IRS-withdrawn SND (57.2%).

Residual Life of Sprayed Insecticide: Monthly wall bioassays showed that insecticides remained efficacious in killing local vectors for an average of seven months. The sprayed insecticide lasted about seven months on cement and wooden surfaces (doors and windows) but lasted about 6 months on mud surfaces. The decay rate was monitored until percentage mortalities were below the 80% threshold for two consecutive months.

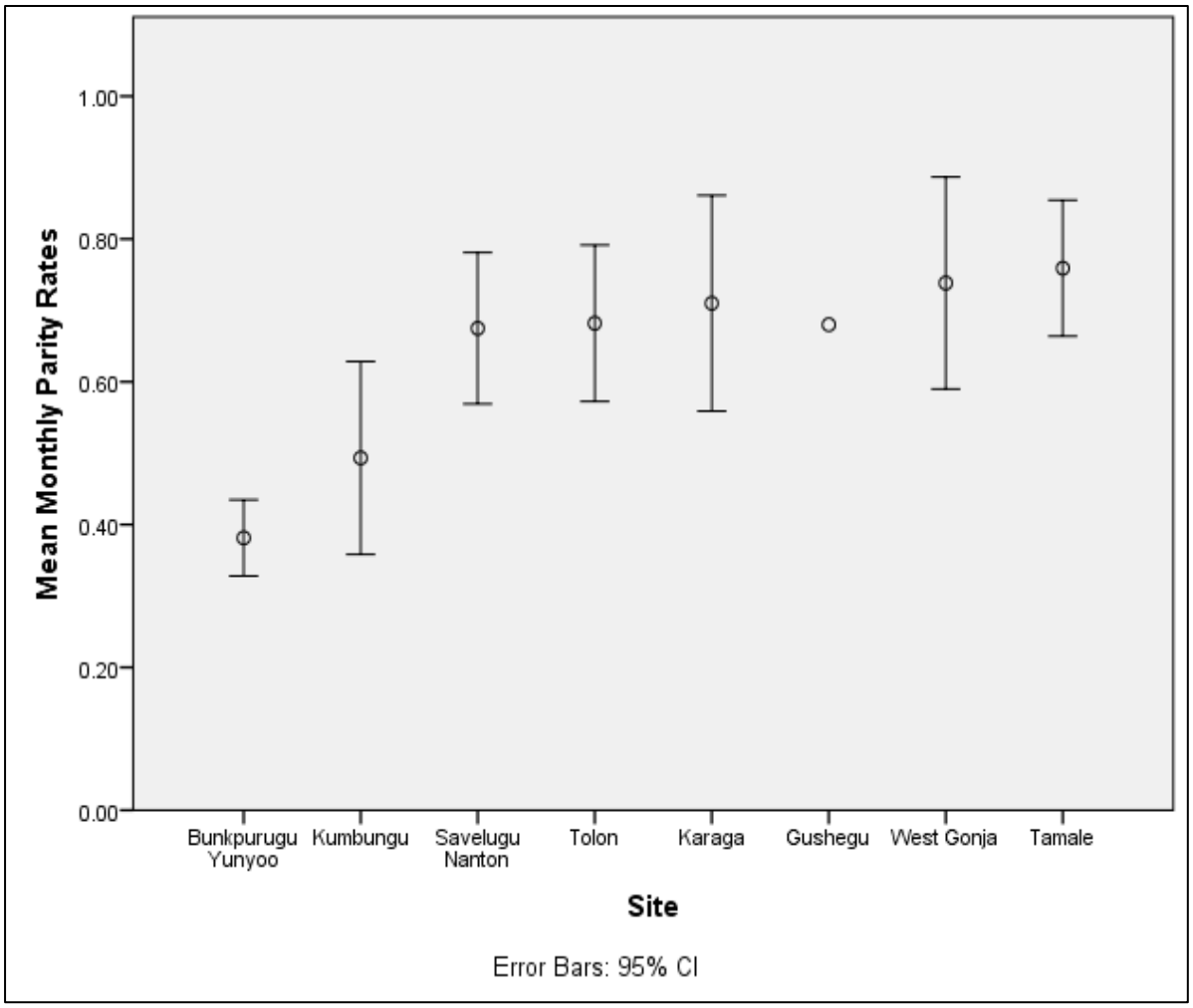
Insecticide Susceptibility/Resistance: WHO susceptibility tests indicate that *An. gambiae* s.l. mosquitoes from both IRS and non-IRS districts were susceptible to pirimiphos-methyl and fenitrothion (with mortalities between 98% and 100%) except in Dimabi, TD (non-IRS district), where the mosquitoes showed possible resistance to pirimiphos-methyl (97%). *An. gambiae* s.l. was resistant to DDT, deltamethrin, and alpha-cypermethrin across all the sites, but was susceptible to bendiocarb in all sites except Gbullung in Kumbungu District (IRS site), where it was resistant (89% mortality), and Nanton in Savelugu District (non-IRS district), where it was possibly resistant (92% mortality).

***Resistance Mechanisms*:** Biochemical assays to analyse the activity of oxidases, esterases (α and β), and glutathione S-transferase (GST) enzymes in over 170 *An. gambiae* s.l. revealed significant ($p<0.0001$) activity for all the enzymes (oxidases, α and β-esterases, and GSTs) in *An. gambiae* s.l. collected from Gbullung (IRS site). Results from the synergist assays also suggest that mono-oxygenases play a much more significant role than esterases in the resistance to the pyrethroids in *An. gambiae* s.l. from Gbullung.

Entomological Inoculation Rates (EIRs): The sums of monthly EIRs for the IRS areas (BYD 8.22ib/p/yr and KUD 11.32ib/p/yr) were lower than that for SND (16.80ib/p/yr), TD (14.36 ib/p/yr), and TML (55.13ib/p/yr). The baseline EIRs were 15.11 infective bites for the month of September 2016 in GUD and 61 infective bites from September to November for KAD. There was a 57% reduction in EIRs (26.2 ib/p/yr in 2015 vs 11.3 ib/p/yr in 2016) in KUD. Malaria transmission was highly seasonal, with transmission peaking in September and October.

Malaria transmission was generally higher outdoors than indoors for IRS areas (BYD and KUD) and IRS withdrawn sites (TD and SND). However, indoor EIRs in TML, and GUD, and KAD were higher than outdoor EIRs.

FIGURE ES-1: MEAN MONTHLY PARITY FOR *AN. GAMBIAE* S.L. COLLECTED FROM IRS INTERVENTION DISTRICTS AND UNSPRAYED DISTRICTS



CONCLUSION

IRS has significantly maintained parity rates at low levels in BYD. The reintroduction of spraying to KUD for the last two years seems to have contributed to suppressing parity rates in the district as well as further reducing EIRs compared with TML where there is no IRS, and TD, GUD, KAD, and SND, where IRS was withdrawn.

1. INTRODUCTION

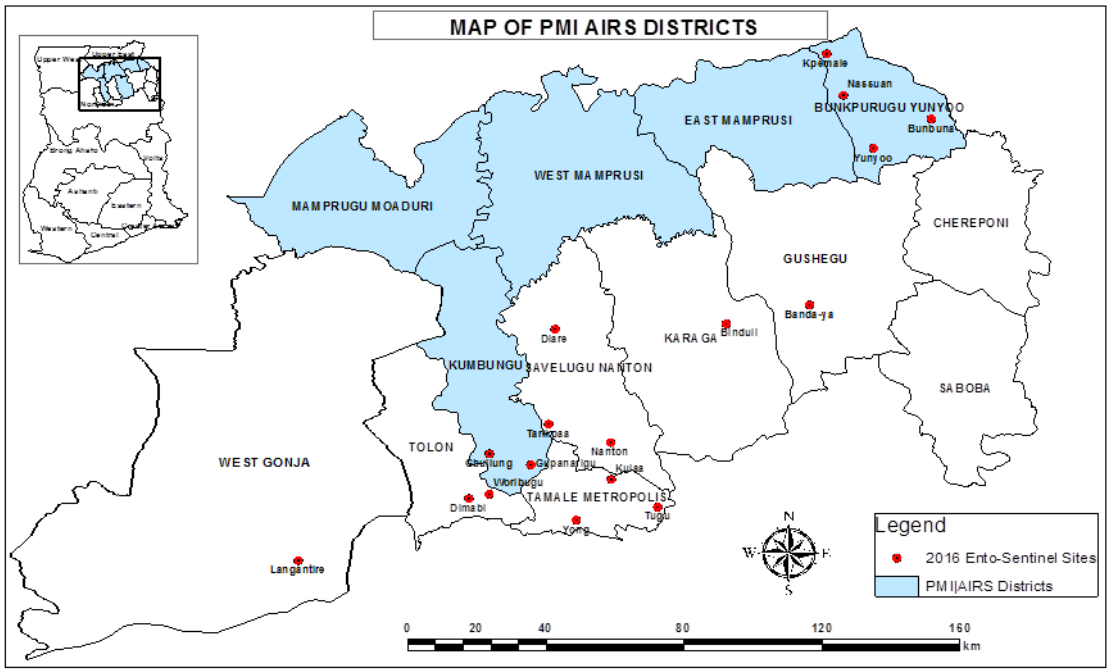
The Africa Indoor Residual Spraying (AIRS) Ghana project, funded by the United States Agency for International Development (USAID) through the President's Malaria Initiative (PMI), has since 2011 been implementing indoor residual spraying (IRS) in five districts in Northern Ghana as a primary means of controlling malaria. It also has carried out entomological monitoring in its target districts and beyond.

In 2016, AIRS Ghana carried out IRS in five districts: Bunkpurugu-Yunyoo District (BYD), East Mamprusi (EMD), Kumbungu District (KUD), Mamprugu Moaduri, and West Mamprusi (WMD) (Figure 1). To assess the impact of the IRS intervention on vector transmission indices, the project conducted entomological surveys from March through December. Specific objectives of the survey included:

1. Identifying the species of malaria vectors in the targeted districts;
2. Assessing vector density, behavior, and seasonality;
3. Determining the susceptibility of local vector species to the World Health Organization (WHO)-recommended insecticides for IRS and identifying mechanisms of resistance if resistance was detected;
4. Assessing the quality of the IRS operation and evaluating the residual efficacy of the sprayed Actellic 300CS formulation (pirimiphos-methyl CS, an organophosphate insecticide); and
5. Assessing change in malaria transmission indices in the sentinel sites.

The AIRS Ghana entomology team worked closely with the Ghana Health Service and District Assemblies to implement all planned field activities. AIRS Ghana also partnered with the Noguchi Memorial Institute for Medical Research (NMIMR) to support advanced molecular evaluations. This report focuses on the entomological monitoring activities the project carried out in 2016.

FIGURE 1: MAP OF PMI IRS DISTRICTS AND ENTOMOLOGICAL MONITORING SITES



2. METHODOLOGY

2.1 STUDY AREAS

In 2016, AIRS Ghana conducted entomological monitoring in eight districts. Seventeen communities in those districts were sentinel sites (including four control sites) for entomological monitoring activities (Figure 1).

Tribal conflict in Sanbiruk, a sentinel site in BYD, forced AIRS Ghana to change the site to Kpemale. Kpemale was an IRS sentinel site in 2011, but tribal conflict disrupted IRS operations there in 2012, 2013, and 2014; IRS resumed there in 2015.

Banda-ya in Gushegu District (GUD) and Binduli in Karaga District (KAD) were added as sentinel sites, to collect baseline data in anticipation of the reintroduction of IRS in these districts in 2017.

An additional sentinel site was established in West Gonja district (WG) in 2016 for use by both the AIRS Ghana team and AngloGold Malaria program. This partnership contributes to the standardization of entomological monitoring protocols as well as knowledge and experience sharing of baseline entomological data for this unsprayed district.

The project used the organophosphate pirimiphos-methyl (Actellic 300CS, at 1g/m^2) to spray all target districts in 2016, based on insecticide susceptibility and residual efficacy test results from the 2015 entomological monitoring. The IRS campaign ran from April 22–May 26, 2016.

The project continued entomological monitoring in Savelugu Nanton (SND) and Tolon district (TD) to observe changes in malaria transmission indices that could arise because the districts were not scheduled to be sprayed in 2016. The rural communities under the Tamale town (TML) selected as sentinel sites have never received IRS, so they continued to serve as control sites for comparison. The project obtained mean daily rainfall data from the Ghana Meteorological Services weather stations in SND and TML.

Table 1 provides information, including spray history from 2008-2016, on the districts and communities/sentinel sites.

TABLE 1: ENTOMOLOGICAL MONITORING SITES

Districts	Communities/ Sentinel Sites	Insecticide Spray History								
		2008	2009	2010	2011	2012	2013	2014	2015	2016
BYD	Bunbuna, Yunyoo, Nasuan, and Kpemale	NSp	NSp	NSp	ACy	ACy	PM	PM	PM	PM
KUD	Gbullung and Gupanerigu	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM
SND	Diare, Nanton, and Tarikpaa (IRS was withdrawn in 2015)	ACy	ACy	DM	ACy	PM	PM	PM	NSp	NSp
TD	Dimabi and Woribugu (IRS was withdrawn in 2013)	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp
GUD	Banda-ya	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp
AD	Binduli	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp
WG	Langantire (with no history of IRS)	Control	Control	Control	Control	Control	Control	Control	Control	Control
(TML)	Kulaa, Tugu, and Yong (comparison communities with no history of IRS)	Control	Control	Control	Control	Control	Control	Control	Control	Control

Note: NSp=not sprayed; DM=deltamethrin; ACy=alpha-cypermethrin; PM=pirimiphos-methyl;

2.2 ADULT MOSQUITO SURVEYS

Mosquito collections were performed pre- and post-spraying using the Human Landing Catch (HLC), Pyrethrum Spray Collection (PSC) methods (WHO 2013), and window exit trap collections to collect mosquitoes from sentinel sites to assess and understand species composition, density, exit behavior, longevity, and entomological inoculation rates of the local vectors. The data on collections were used to make comparisons between sprayed and unsprayed communities. In the 14 sites, the team conducted collections for four days each month, beginning in March 2016 and ending in November 2016; total nine months. Introduction of IRS to KAD in 2017 was anticipated and one site from KAD was added for baseline data late in the year. Mosquito collections for baseline in KAD took place in September–November 2016. When it became known that IRS will be introduced to GUD, collections for baseline data were done in GUD in September. In WG, an AIRS-AgaMal joint sentinel site, data collection started in June after the agreements were signed and ended in November 2016.

HLC: This was conducted using eight trained mosquito collectors in each community. The collectors worked in two teams of four persons, in two different houses each night simultaneously. In each house, two collectors worked indoors inside a sleeping room, while the other two worked outdoors in the open courtyard within the compound. A total of four nights were used to collect mosquitoes in eight compounds in each sentinel community per month.

PSC: This was performed the next morning after each HLC. PSCs determined indoor resting mosquito species and their densities. Collections took place between 6:00 a.m. and 7:00 a.m. in other rooms within the compound, different than those used for the HLCs the previous night. The team surveyed a total of eight rooms for each community every month.

Exit trap:

One mosquito window exit trap was installed in a room for four nights per community. The team surveyed a total of four rooms (one room per night) for four consecutive nights for each community

every month. The traps were fixed from 6.00pm to 6.00am the next morning. Mosquitoes were retrieved from the trap using a mouth aspirator.

The project team used taxonomic keys of Gillies and Coetzee (Gillies and Coetzee 1987) to identify mosquitoes collected from the HLCs, PSCs and exit trap collections. A proportion (approximately one-third) of unfed mosquitoes from the HLCs identified as *An. gambiae* s.l. were dissected to assess their parity rates by observing the degree of coiling in the ovarian tracheoles (Detinova 1962). The remaining specimens were preserved in 1.5 ml Eppendorf tubes with desiccant for further analyses at the NMIMR laboratory.

2.3 Assessment of Spray Quality and Residual Efficacy

AIRS Ghana carried out standard WHO cone bioassays tests (WHO 2013) to test the quality of work by the different spray teams and to evaluate the residual life of the sprayed insecticide (Actellic 300CS) using both the 'Kisumu' strain and wild *An. gambiae* s.l. reared from the field. The AIRS Ghana team conducted tests on the three main types of sprayed surfaces: mud in traditional houses, cement in modern houses, and wood in doors and window frames.

2.3.1 Quality Assurance of the IRS Program

Spray quality assessment was carried out in 10 sentinel communities:

- Cheyohi-1, Gbullung, and Yuni (KUD)
- Bunbuna, Kpemale, Kualik, Tuna-2, and Tusugu (BYD)
- Langbinsi (EMD)
- Kata-Banawa (WMD)

In most communities, four houses (two with cement wall surfaces and two with mud wall surfaces) were selected for the assessment of the quality of spray on the predominant surface types (cement and mud). In Langbinsi (EMD) and Kata-Banawa (WMD), the bioassays were conducted in only two houses each, since there weren't enough mosquitoes for four houses. Cone bioassays were conducted on the wooden doors or windows of each room selected for the test, to assess the performance of sprayed insecticide on a wood surface.

In Cheyohi-1, Gbullung, Kpemale, and Kualik, wall bioassays were conducted using both mosquitoes of the laboratory-raised Kisumu strain and wild female adults of *An. gambiae* s.l. reared from larvae collected in project districts. However, in Bunbuna, Tuna-2, Tusugu, Yuni, Langbinsi, and Kata-Banawa, the bioassays used Kisumu mosquitoes only, because the scarcity of breeding sites made it difficult to collect enough wild mosquito larvae from these areas and raise them for the bioassays. All mosquitoes used for the tests were 2–5 days old. To assess the spray quality on the different wall surfaces in each room, three spots in the room were tested; with assay cones fixed about 1.5m high on each wall. Additionally, one cone was fixed on the wooden door or window to test efficacy on the wood.

One control cone assay was done for every four bioassay tests, by fastening cardboard on unsprayed surfaces and exposing the control mosquitoes to the cardboard and to other conditions similar to exposed mosquitoes. To avoid control mortality increasing due to the airborne effect of the Actellic 300CS formulations, the control tests were set up in unsprayed structures with similar relative humidity and temperature to the sprayed rooms that were tested.

Each cone was fastened to the selected spot on the test wall with tape. Ten mosquitoes were introduced into the cone chamber and left exposed on the surface for 30 minutes. At the end of the exposure period, the mosquitoes were collected and transferred to paper cups. The number of mosquitoes that were knocked down at the end of each exposure period was recorded. Ten

mosquitoes were collected into paper cups and placed on a table in the center of the same sprayed rooms for 30 minutes to assess the airborne effect of the insecticide. Again, the number of mosquitoes knocked down at the end of 30 and 60 minutes was recorded.

Following the 30-minute exposure and transfer to clean paper cups, the mosquitoes were taken to the AIRS entomology laboratory, where the temperature and relative humidity were maintained between 25˚C and 29˚C and between 75 and 85%, respectively. The mosquitoes were given a 10% sugar solution on cotton pads during the 24-hour holding period. The dead and live mosquitoes were counted after 24 hours and the mortalities calculated. Mortalities were corrected using Abbot's formula (Abbott 1925) if the control mortalities were ≥ 5% and < 20%, but tests were discarded and repeated if control mortalities were ≥ 20%.

2.3.2 Residual Efficacy of Actellic 300CS

The project team conducted follow-up bioassays to assess the residual efficacy of pirimiphos-methyl in the following sentinel sites:

- Cheyohi-1, Gbullung, and Gupanerigu (KUD)
- Bunbuna, Kpemale, and Yunyoo (BYD)

The bioassays were conducted from May through December 2016 using susceptible Kisumu colonies from the AIRS insectary and the insectary of the Navrongo Health Research Center, as well as wild *An. gambiae* collected in Cheyohi, Gbullung, and Gupanerigu. Both the spray quality and residual efficacy were indirectly estimated from the percentage mortality of the exposed mosquitoes from the WHO cone bioassays on the different types of sprayed surfaces (mud, cement, and wood). The results for the tests are presented in the Results section below.

2.4 Insecticide Susceptibility Tests – WHO Tube Test

Susceptibility tests were performed using the WHO insecticide susceptibility test method in nine sentinel communities in five districts, all in the Northern region: Bunbuna (BYD); Kumbungu and Gbullung (KUD); Nanton and Tarikpaa (SND); Dimabi and Woribugu (TD); and Kulaa and Tugu (TML).

Larvae and pupae of *Anopheles* mosquitoes were collected from breeding sites in and around the sentinel communities and reared to adult for susceptibility tests. Mosquitoes were morphologically identified at adult stage and only *An. gambiae* s.l. were selected for the susceptibility test. WHO tube tests were conducted for the following insecticides: alpha-cypermethrin 0.5 %, deltamethrin 0.05 %, bendiocarb 0.1 %, propoxur 0.1 %, pirimiphos-methyl 0.25 %, fenithrothion 1.0 %, and DDT 4.0 %

After the 24-hour holding period, count of the number of dead mosquitoes in both the exposure and the control tubes was recorded. Mortalities were corrected using Abbot's formula (Abbott 1925) if the control mortalities were ≥ 5% and < 20%, but tests were discarded and repeated if control mortalities were ≥ 20%.

The susceptibility levels of *An. gambiae* s.l. were evaluated on the basis of the WHO criteria of test mortality (WHO 2013): 98–100% mortality after 24 hours indicates susceptibility. A mortality of less than 98% suggests the existence of resistance and further investigation is needed. If the observed mortality (corrected if necessary) is greater than 90% but less than 98%, the presence of resistant genes in the vector population must be confirmed; if mortality is less than 90% then the vector population is resistant.

2.5 Resistance Intensity Assay – CDC Bottle Assay

The intensity of pyrethroid resistance in *An. gambiae* s.l. from two sentinel sites (Gbullung and Kumbungu) was measured using a simplified version of the CDC bottle bioassay rapid diagnostic test (RDT) (Brogdon and Chan 2010). Four pre-measured vials provided by the U.S. Centers for Disease Control and Prevention (CDC), Atlanta, each containing deltamethrin at concentrations of 1x, 2x, 5x, and 10x were diluted in acetone and applied to 250 ml bottles.

Four replicates of 500 µl of acetone were added to each insecticide vial, and washed off into a 50ml graduated falcon tube. The falcon tube was topped up to the 50ml mark. The prepared insecticide solutions were stored in a refrigerator at 4°C until use. The control bottle was prepared by adding 1ml of acetone into a 250ml Wheaton bottle and coated as described by Brogdon and Chan (2010). Four test bottles were then coated with 1ml of different concentrations of the prepared insecticide solutions to get one bottle each of 1x, 2x, 5x, and 10x insecticide concentration. Between 20 and 25 mosquitoes

were introduced into the four replicates with different concentrations. A control bottle (coated with acetone only) was also run alongside the tests. The knockdown rate was recorded at 15-minute intervals until all mosquitoes were dead in each bottle.

2.6 Synergist Assays

An. gambiae s.l. populations from Gbullung, which showed resistance to deltamethrin were exposed to the effect of piperonyl butoxide (PBO) (100 µg/bottle) and S.S.S-tributlyphosphorotrithioate (DEF) (125 µg/bottle), synergists that have been found to inhibit oxidase and esterase activity, respectively.

Two bottles were prepared to run the synergist assays. One bottle was coated with 1 ml of acetone and served as a synergist-control bottle (without synergist); the second bottle was coated with 1ml of the synergist (PBO or DEF) stock solution and served as the synergist-exposure bottle. A batch of about 125 mosquitoes was introduced into the synergist-control bottle, and another 125 mosquitoes from the same population were introduced into the synergist-exposure bottle. Both setups were held for one hour. After the hour, the mosquitoes were transferred to two holding cartons, one for the synergist-control mosquitoes and another for the synergist-exposure mosquitoes.

CDC bottle bioassays were run using one set of insecticide-coated bottles (one control and four test bottles) for the synergist-control mosquitoes and another set (one control and four test bottles) for the synergist-exposed mosquitoes. The number of dead or alive mosquitoes was monitored at 15-minute intervals as per the CDC bottle bioassay protocol. Data for the two populations of test mosquitoes (mosquitoes exposed to synergist before test and mosquitoes not exposed) were then compared.

2.7 Analysis and Molecular Evaluations

NMIMR and AIRS Ghana agreed upon a scope of work to carry out molecular evaluations. The molecular evaluations are indicated below.

1. **Transmission indices:** sporozoite rates and entomological inoculation rates (EIRs)
2. **Identification to species:** (molecular identification): members of the *An. gambiae* complex identified to species and to molecular forms
3. **Detection of mechanisms of insecticide resistance:** use of molecular techniques to determine frequency of the knockdown resistance (*kdr)* gene and other mechanism of resistance

2.7.1 Species Identification

The mosquitoes were morphologically identified using the taxonomic keys of Gillies and Coetzee (1987). Samples of *An. gambiae* s.l. were then identified into sibling species using ribosomal DNA-polymerase chain reaction (PCR) (Scott *et al.* 1993) and into molecular forms following a PCR-RFLP (restriction fragment length polymorphism) procedure described by Fanello *et. al.* (2002).

2.7.2 Circumsporozoite-ELISA Evaluation

The head and thorax of all samples were sorted and tested for the presence of circumsporozoite antigens (CS) of *Plasmodium falciparum* using the enzyme-linked immunosorbent assay (ELISA) described by Wirtz *et al.* (1987). The ELISA tests were used to assess the parasite infection rate in the local mosquito vectors.

2.7.3 *KDR* AND *ACE-1* GENOTYPE TEST

The conventional PCR technique described by Martinez Torres *et al.* 1998 and real time PCR described by Bass et al. (2007) were used to detect the presence of the West Africa *kdr* and the *Ace-1* mutation in the local *An. gambiae* s.l. vectors using the protocol described by Weill *et al.* (2004).

2.7.4 BIOCHEMICAL ENZYME ASSAYS

Batches of 100 live female *An. gambiae* s.l. from IRS sentinel sites used for susceptibility tests were sent to Noguchi and kept frozen at -80°C until used for the assay. The nonspecific esterase and mixed function oxidase enzyme activities were determined and quantified according to the methodology described by Brogdon (1988). The susceptible Kisumu strain was used as a reference strain in the evaluation.

2.7.5 ANALYSIS OF DATA

The following parameters were estimated for the important *Anopheles* vector species (*An. gambiae* s.l. and *An. funestus* group):

- Man biting rate = the total number of vectors collected/number of collectors X number of nights of capture
- Sporozoite rates = the proportion of *Anopheles* found positive for the presence of circumsporozoite proteins
- Entomological inoculation rates – were calculated by the formula:

  EIR = daily human biting rates X sporozoite rates

  Annual EIR = sum of monthly EIRs

3. RESULTS

3.1 SPECIES COMPOSITION

3.1.1 MORPHOLOGICAL IDENTIFICATION

Morphologically, *An. gambiae* s.l., *An. funestus, An. nili, An. pharoensis,* and *An. rufipes* were identified in 2016. *An. gambiae* s.l. was the dominant species across all sites, constituting 98% of the total number (45,668) of *Anopheles* collected. *An. funestus, An. nili, An. pharoensis,* and *An. rufipes,* constituted 0.57%, 0.60%, 0.74%, and 0.04% of the collection, respectively. Most *An. funestus,* of the total 262 collected, were from TD (40.8%) and KAD (22.5%) in IRS withdrawn areas, and KUD (20.6%) and IRS district (Table 2 and 3).

Of the 45,668 adult female *Anopheles* mosquitoes collected, 97% (44,261/45,668) were collected attempting to bite (i.e., by HLC), 2% (985/45,668) were collected resting indoors, and 1% (422/45,668) were collected while exiting through windows. A higher percentage of *An. funestus* (17.2%) than *An. gambiae* (2%) were collected indoors (by PSC). The difference between collections of *An. gambiae* by PSC and window exit traps (3%) and HLC (97%) is high; possible reasons for this may have to be explored further (Table 2).

TABLE 2: NUMBER OF ANOPHELES SPECIES COLLECTED BY COLLECTION METHODS

Anopheles species	HLC		PSC		Window Exit Trap Collections		TOTAL	
	N	%	N	%	N	%	N	%
An. gambiae s.l.	43,424	*98.1%*	937	*95.1%*	419	*99.3%*	**44,780**	98.06%
An. funestus	216	*0.5%*	45	*4.6%*	1	*0.2%*	**262**	0.57%
An. nili	271	*0.6%*	1	*0.1%*	0	*0.0%*	**272**	0.60%
An. pharoensis	334	*0.8%*	1	*0.1%*	2	*0.5%*	**337**	0.74%
An. rufipes	16	*0.0%*	1	*0.1%*	0	*0.0%*	**17**	0.04%
Total	**44,261**		**985**		**422**		**45,668**	

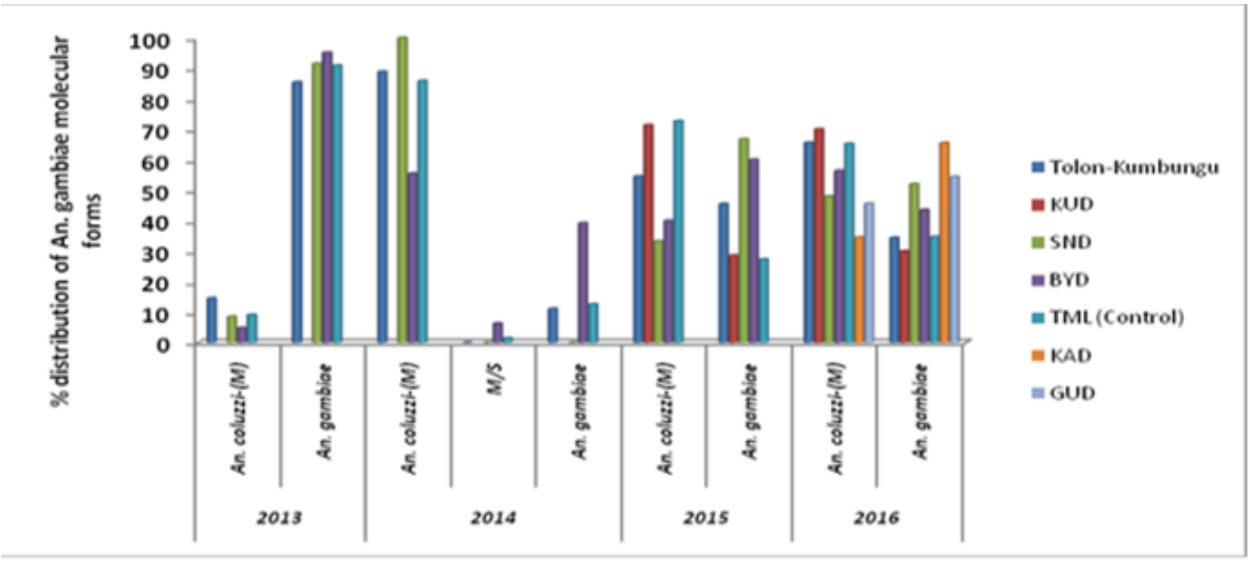
TABLE 3: NUMBER AND TYPE OF *ANOPHELES* SPECIES COLLECTED FROM SENTINEL DISTRICTS, USING HLC, PSC AND WINDOW EXIT TRAP COLLECTIONS

Anopheles species	BYD	KUD	SND	TD	GUD	KAD	TML	WG	TOTAL
An. gambiae s.l.	4,057	4,167	5,395	5,032	1,770	3,252	18,497	2,610	44,780
An. funestus	1	54	6	107	4	59	19	12	262
An. nili	1	23	7	23	7	14	187	10	272
An. pharoensis	2	29	125	25	3	4	148	1	337
An. rufipes	0	4	2	1	0	8	2	0	17
Total	**4,061**	**4,277**	**5,535**	**5,188**	**1,784**	**3,337**	**18,853**	**2,633**	**45,668**
	8.9%	*9.4%*	*12.1%*	*11.4%*	*3.9%*	*7.3%*	*41.3%*	*5.8%*	

3.1.2 PCR ANALYSIS

A total of 637 *An. gambiae* s.l. (80 from KUD, 78 from TD, 118 from SND, 164 from BYD, 118 from TML, 39 from GUD and 40 from KAD) representing about 6% of the samples examined by ELISA were analyzed by PCR for identification of sibling species of the *An. gambiae* complex. Figure 2 shows the yearly variation between populations of *An. coluzzii* and *An. gambiae* existing in sympatry in all sentinel sites from 2013 through 2016. There was an increase in the proportion of *An. coluzzii* between 2015 and 2016 in BYD, SND and TD. No hybrid was found in the samples analyzed in 2016.

FIGURE 2: DISTRIBUTION OF *AN. COLUZZII* AND *AN. GAMBIAE* IN IRS INTERVENTION, IRS WITHDRAWN, AND CONTROL DISTRICTS, 2013–2016



3.2 VECTOR SEASONALITY

The abundance of *An. gambiae* s.l. collected from BYD, KUD, SND, TD, KAD, WG, and TML were positively correlated to the mean rainfall (108.4mm) recorded from March through November 2016.

The coefficients of correlation were 0.704, 0.222, 0.277, 0.981, 0.667, and 0.357 for BYD, KUD, SND, TD, KAD, WG, and TML respectively (see Figure 3 in Section 3.3). The correlation was significant in BYD (p= 0.034).

3.3 BITING RATE

The mean man biting rates for *An. gambiae* s.l. – as has been noted, the predominant species collected from all sites – are presented in Figures 3 and 4.

Comparatively, the average monthly biting rates recorded for TML (control), GUD, and KAD were higher than those recorded for all other sites. The average man biting rates (MBRs) recorded for *An. gambiae* s.l. during the period are shown in Table 6 (in Section 3.5).

FIGURE 3: MEAN MAN BITING RATE OF *AN. GAMBIAE* S.L. FROM SENTINEL SITES AND MEAN RAINFALL, MARCH–NOVEMBER 2016FIGURE

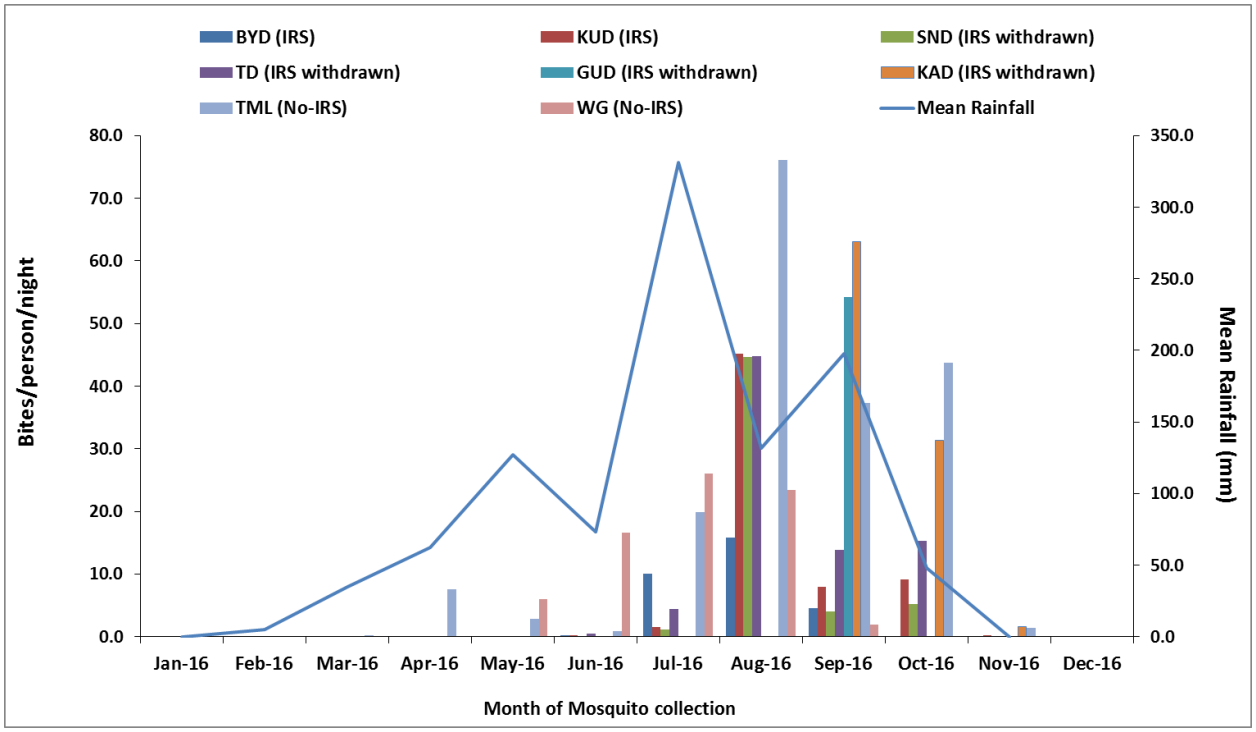
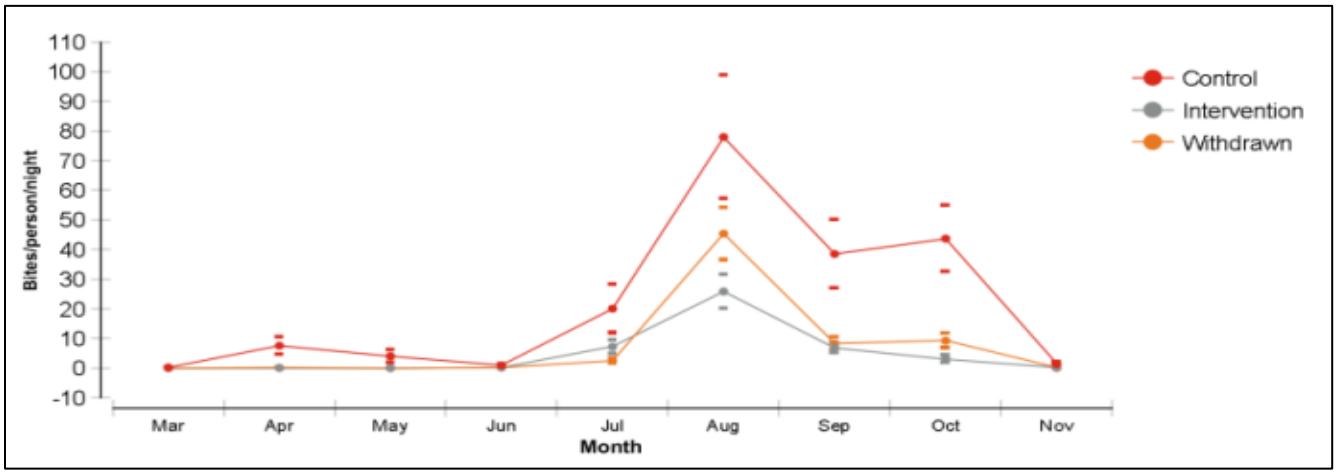


FIGURE 3[1]: MEAN MAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED BY HLC



3.4 SPOROZOITE RATES

A total of 10,635 *Anopheles* mosquitoes were tested using ELISA to determine the presence of sporozoites in their salivary glands (Table 4). The overall sporozoite rate for both *An. gambiae* s.l. and *An. funestus* in IRS districts was 0.74% (N=1,344) in KUD and 0.88% (N=1,812) in BYD. The sporozoite rate in non-IRS districts ranged from 0.68% (N=586) in KAD to 1.16% (N=1,984) in SND. Other species examined include *An. pharoensis* (15), but none were found positive for sporozoites.

A comparison of the indoor and outdoor sporozoite rates showed slightly higher outdoor sporozoite rates than indoor rates, in both IRS and non-IRS districts with the exception of TML, GUD, and KAD. Most of the sporozoite-positive samples were from samples collected between July and October 2016.

TABLE 4: SPOROZOITE INFECTIONS IN *AN. GAMBIAE AND AN. FUNESTUS* SAMPLED FROM ALL SENTINEL SITES

Sentinel Site	Number Examined ELISA	Number +ve for Sporozoite	Sporozoite Rate
IRS			
BYD (IRS)	1,812	16	0.88%
KUD (IRS)	1,344	10	0.74%
No-IRS			
SND (IRS withdrawn)	1,984	23	1.16%
TD (IRS withdrawn)	1,705	13	0.76%
GUD (IRS withdrawn)	432	4	0.93%
KAD (IRS withdrawn)	586	4	0.68%
TML (Non-IRS)	2,757	30	1.09%

[1] The figure represents the number of bites per person per night using both indoor and outdoor landing catch. The "control" series includes data from all control villages. The "intervention" series includes data from all intervention villages. Marks above and below each point represent 95% confidence intervals. Data from KAD, GUD and WG not included

3.5 RESTING AND EXITING BEHAVIOR OF VECTORS

The mean indoor resting densities of *An. gambiae* s.l. ranged from 0.19 mosquitoes per room in WG (non IRS) to 7.33 in KAD (IRS withdrawn district). Relatively low proportions of half-gravid and gravid females were caught resting indoors in sprayed rooms in the IRS districts (2.0% in BYD and 1.3% in KUD) compared with the proportion of gravid females collected from rooms in IRS withdrawn districts (9.8% in TD, 7.5% in SND, 6.9% in KAD, and 12.1% in GUD) and 13.4% in unsprayed TML (Table 5). All the mosquitoes collected in the rooms in WG were freshly blood-fed.

No gravid female *An. gambiae* s.l. was collected in the exit trap collections in BYD, KUD, and SND. However, high proportions of gravid females (50%) were collected in window exit traps in TD. With the exception of TD, relatively high proportions of unfed mosquitoes were collected from the exit traps in all the sites.

TABLE 5[2]: NUMBER OF *AN. GAMBIAE* S.L. COLLECTED BY PSC AND WINDOW EXIT TRAP COLLECTIONS

Site	% Unfed	% Freshly Fed	% Half Gravid and Gravid	Total *An. gambiae* s.l.	# of Rooms Sampled	Mosquitoes/Room
BYD (IRS)	10.9%	87.1%	2.0%	**101**	288	0.35
KUD (IRS)	14.5%	84.2%	1.3%	**76**	144	0.53
SND (IRS withdrawn)	32.8%	59.7%	7.5%	**67**	216	0.31
TD (IRS withdrawn)	13.1%	77.0%	9.8%	**61**	144	0.42
KAD (IRS withdrawn)†	47.1%	46.0%	6.9%	**176**	24	7.33
GUD (IRS withdrawn) *	6.1%	81.8%	12.1%	**33**	8	4.13
TML (Non-IRS)	11.9%	74.7%	13.4%	**411**	216	1.90
WG (Non-IRS)‡	0.0%	100.0%	0.0%	**12**	64	0.19
BYD (IRS)	75.0%	25.0%	0.0%	**4**	96	0.04
KUD (IRS)	90.9%	9.1%	0.0%	**22**	72	0.31
SND (IRS withdrawn)	94.3%	5.7%	0.0%	**35**	108	0.32
TD (IRS withdrawn)	33.3%	16.7%	50.0%	**6**	72	0.08
TML (Non-IRS)	96.9%	0.8%	2.3%	**130**	108	1.20
WG (Non-IRS)‡	54.1%	40.1%	5.9%	**222**	68	3.26

[2]*Mosquito collections in GUD was for only September

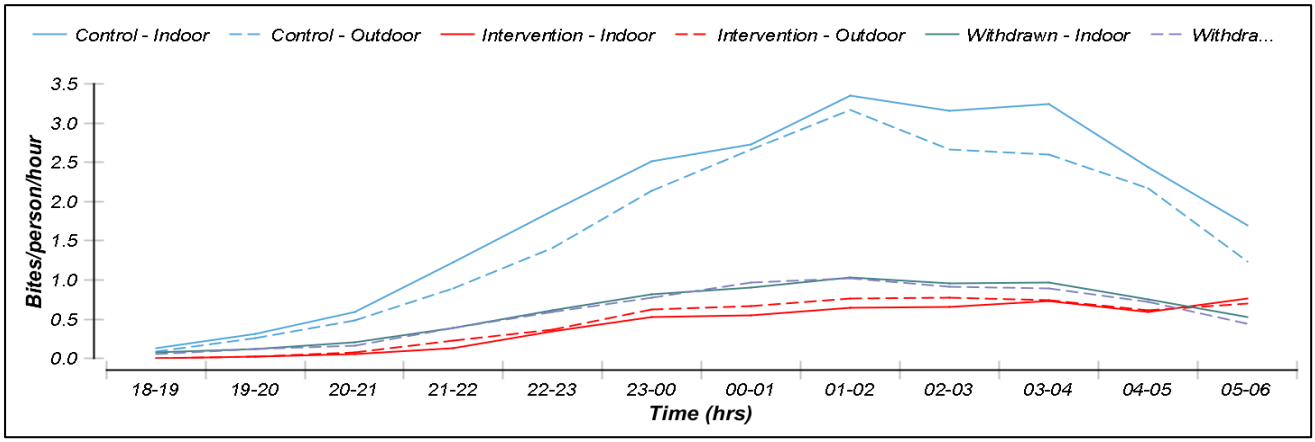
† Mosquito collections in KAD was carried out from September –November 2016

‡ Mosquito collections in WG was carried out from June –November 2016

3.6 FEEDING TIME/PATTERN

Figure 5 shows the biting cycle of *An. gambiae* s.l. (the dominant vector species) collected between March and November 2016. Indoor and outdoor biting activity started at 6:00 p.m. and then gradually increased at 8:00 p.m. in IRS intervention districts (BYD and KUD), IRS withdrawn districts (GUD, KAD, SND, and TD), and the unsprayed districts (TML and WG). Peak biting occurred around 11:00 p.m. and 5:00 a.m. The densities of mosquitoes biting during these peak times were higher in unsprayed districts than in IRS districts and IRS withdrawn districts.

FIGURE 4: HOST SEEKING BEHAVIOUR OF *AN. GAMBIAE* S.L. COLLECTED INSIDE AND OUTSIDE OF ROOMS



There were observed variations in indoor biting and outdoor biting densities of *An. gambiae* s.l. between the IRS and non-IRS sites. The differences in the mean man biting rates (MBR) indoor/outdoor were statistically significant at the 0.05 level for all the sites except in GUD and KAD (Table 6). The results again show high exophagic tendencies of *An. gambiae* s.l. in BYD, KUD, TD, and WG. In SND, GUD, and TML, *An. gambiae* s.l. showed more endophagic tendencies.

TABLE 6[3]: INDOOR AND OUTDOOR MBR (BITES/PERSON/NIGHT) OF *AN. GAMBIAE* S.L., HLC, ALL SENTINEL SITES, 2016

Sentinel Site	biting rate	biting rate	Endophagic index	Exophagic index	χ^2	P value
BYD (IRS)	3.30	3.56	0.48	0.52	5.39	0.020*
KUD (IRS)	6.57	7.55	0.47	0.53	19.4	0.000*
SND (IRS withdrawn)	6.54	5.71	0.53	0.47	24.3	0.000*
TD (IRS withdrawn)	8.38	8.87	0.49	0.51	4.10	0.044*
KAD (IRS withdrawn)	31.8	32.3	0.50	0.50	0.13	0.718
GUD (IRS withdrawn)	55.8	52.8	0.51	0.49	1.27	0.259
TML (Non-IRS)	21.70	19.47	0.54	0.46	107.0	0.000*
WG (Non-IRS)	8.40	12.81	0.39	0.60	102.71	0.000*

[3] * Differences in mean indoor/outdoor biting rates is statistically significant at 0.05 level

3.7 INSECTICIDE SUSCEPTIBILITY – WHO TUBE TEST RESULTS

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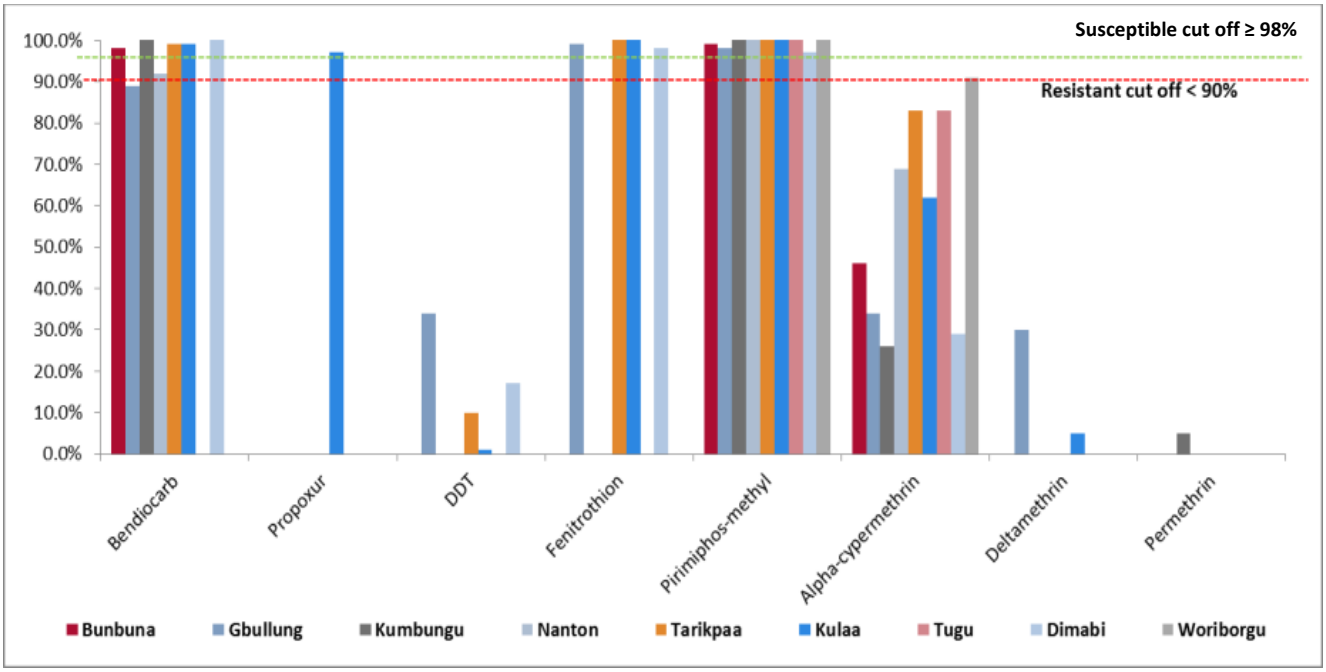
An. gambiae s.l. mosquitoes from both IRS and non-IRS districts were susceptible to pirimiphos-methyl and fenitrothion (with mortalities between 98% and 100%), except in Dimabi, where the mosquitoes showed possible resistance to pirimiphos-methyl (97%) (Figure 6). *An. gambiae* s.l. was resistant to DDT, deltamethrin, and alpha-cypermethrin in all sites. However, *An. gambiae* s.l. was susceptible to bendiocarb in most of the sites except Gbullung (KUD, an IRS site), where it was found to be resistant (89% mortality), and in Nanton (SND, no IRS), where it was found to be possibly resistant (92% mortality) (see Table A-1 in Annex).

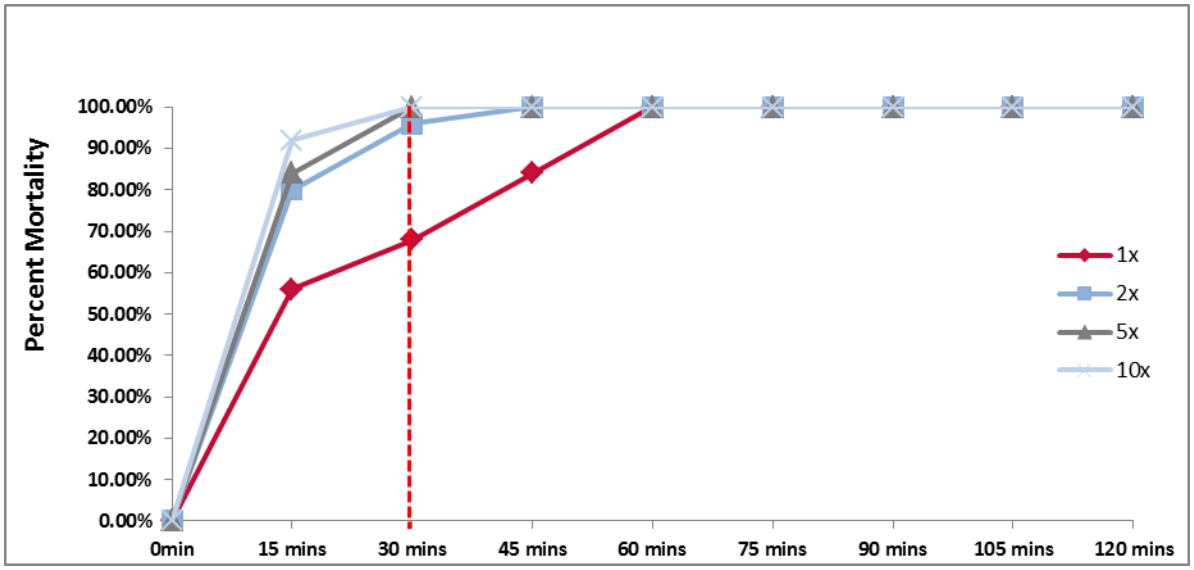
FIGURE 5: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TESTED AGAINST SELECTED WHO-RECOMMENDED INSECTICIDES FOR IRS



3.8 RESISTANCE INTENSITY ASSAY – CDC BOTTLE BIOASSAY

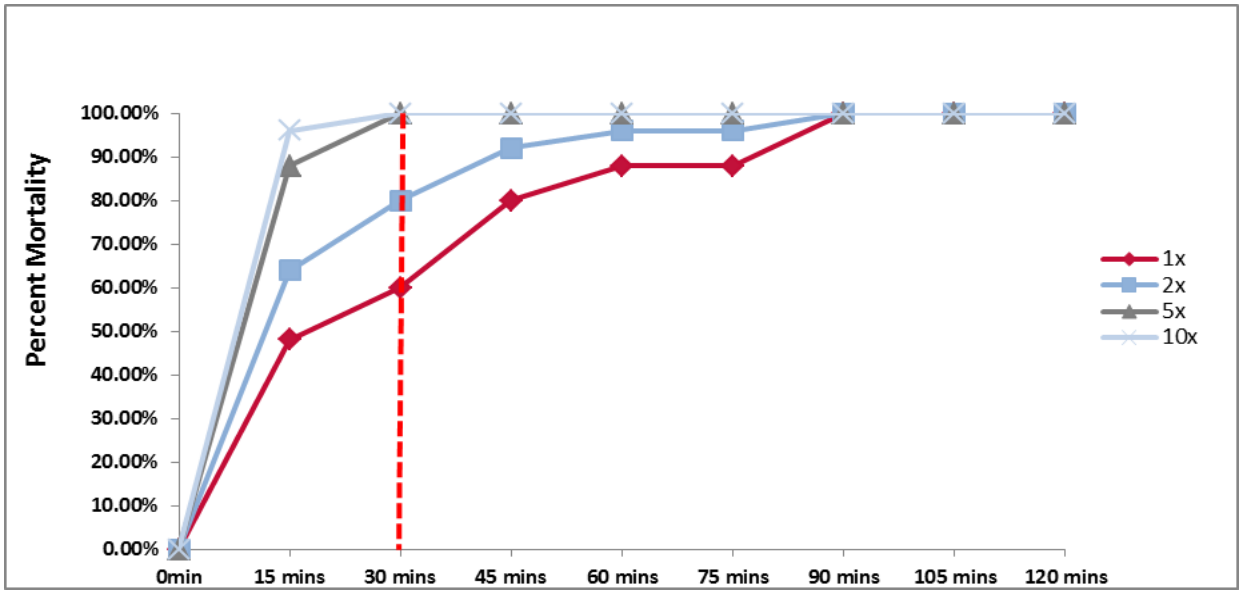
An. gambiae s.l. mosquitoes from Gbullung and Kumbungu were resistant to 1x and 2x diagnostic doses of deltamethrin, but susceptible to the 5X and 10x doses based on CDC bottle bioassay recommended thresholds (Figures 7 and 8).

FIGURE 7: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM GBULLUNG EXPOSED TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN, USING THE CDC-RESISTANCE INTENSITY RDT



Note: (red line indicate susceptible threshold)

FIGURE 8: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM KUMBUNGU EXPOSED TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN, USING THE CDC-RESISTANCE INTENSITY RDT

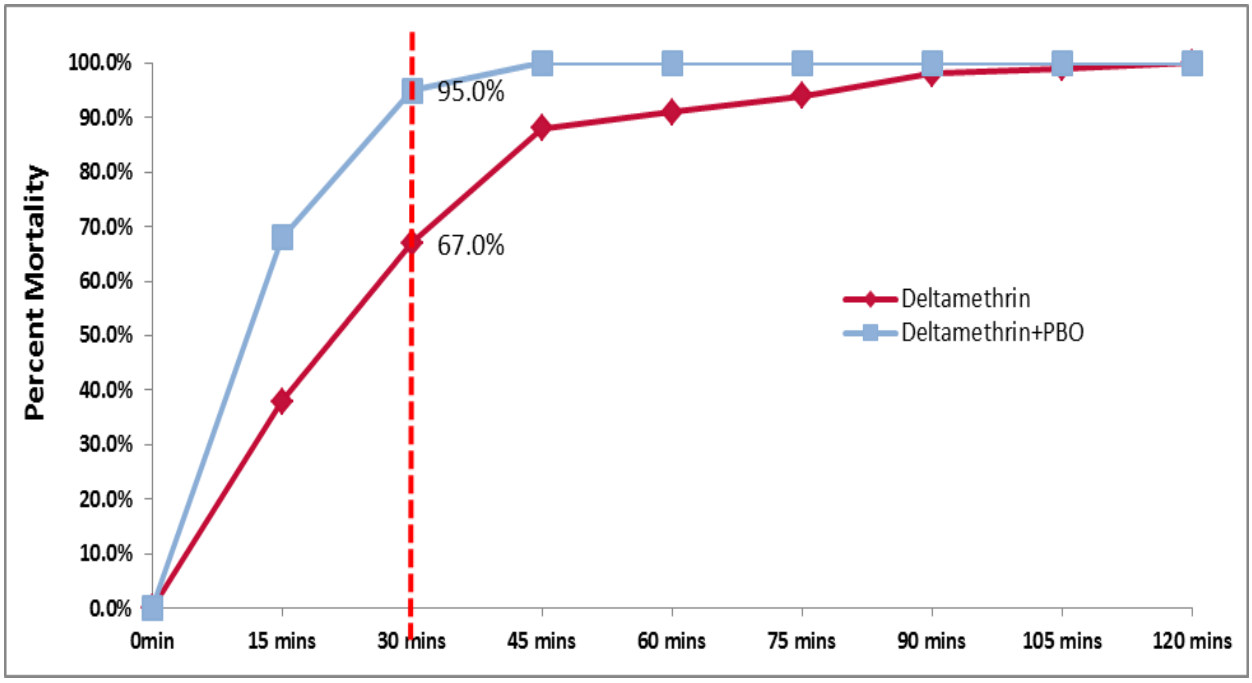


Note: (red line indicate susceptible threshold)

3.9 SYNERGIST ASSAYS

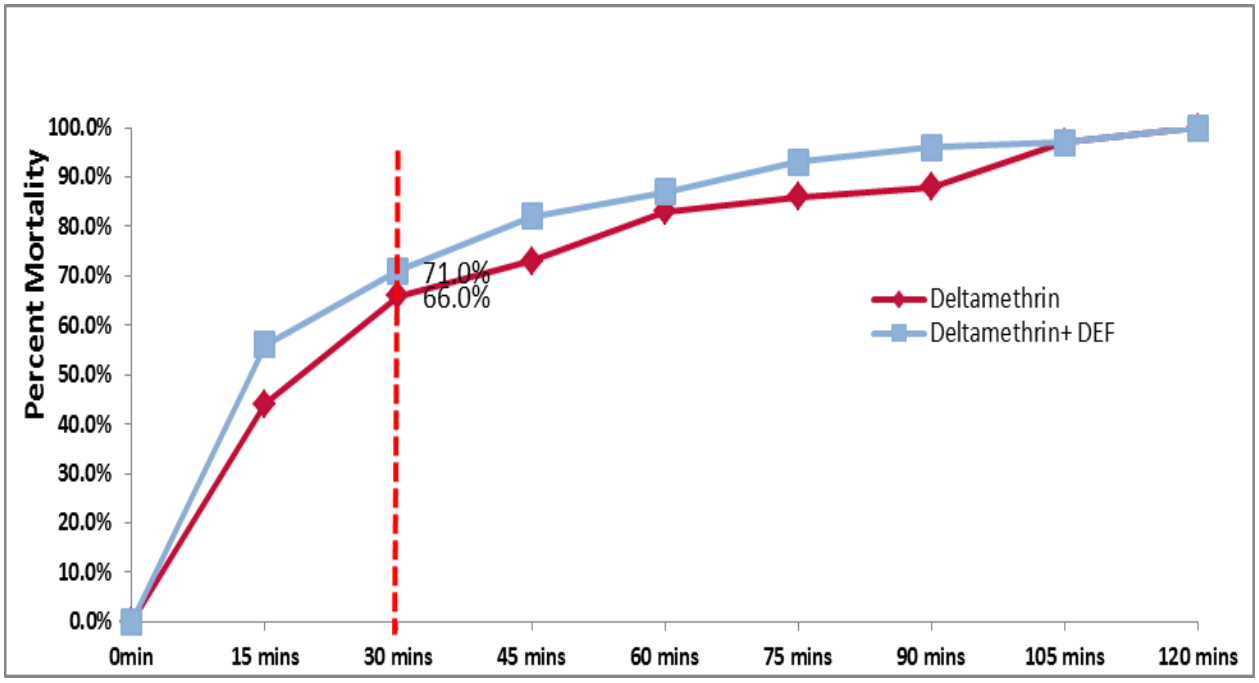
An. gambiae s.l. from Gbullung exposed to PBO before being tested against deltamethrin showed a significant increase in susceptibility to the insecticide; their mortality rate increased from about 67% to about 95% ($p<0.0001$) (Figure 10). However, exposing mosquitoes to DEF prior to testing them with deltamethrin increased mortality marginally, from 66% to 71% ($p= 0.447$) (Figure 11). This suggests that metabolic resistance mediated by mono-oxygenases might be the primary mechanism contributing to the high phenotypic resistance to pyrethroids observed in the area.

FIGURE 9: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM GBULLUNG EXPOSED TO DELTAMETHRIN AND DELTAMETHRIN + PBO



Note: (red line indicate susceptible threshold)

FIGURE 10: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM GBULLUNG EXPOSED TO DELTAMETHRIN AND DELTAMETHRIN + DEF



Note: (red line indicate susceptible threshold)

3.10 RESISTANCE MECHANISM

A total of 638 *An. gambiae* s.s. samples were analyzed for the presence of *kdr-w* and *Ace-1*.

3.10.1 KNOCKDOWN RESISTANCE (*KDR*-WEST)

The molecular analysis showed that the *kdr-w* homozygous resistant (RR) variant genotype was found in both *An. gambiae* s.s. and *An. coluzzii* from all the communities tested, with the homozygous resistant genotype dominant in *An. gambiae* s.s. (Table 7). Higher numbers of susceptible homozygote (SS) genotypes were found in the samples from TML, GUD, and KAD than from the other sites. The frequency of *kdr-w* ranged from 0.87 in GUD to 0.99 in KUD, TD, and BYD. Figure 11 shows the yearly trends in the distribution of *kdr-w* genotypes in molecular forms of *An. gambiae* s.l. from the IRS and non-IRS areas. Over 70% of the samples analyzed were homozygote resistant (RR).

3.10.2 *ACE-1* GENE

Ace-1 gene analysis showed that over 70% of the samples were homozygote susceptible (SS) – this was dominant in *An. gambiae* (Figure 12). The frequency of the *Ace-1* alleles ranged from 0 in KAD to 0.43 in BYD (Table 8). With the exception of Binduli, heterozygote (RS) resistant genotypes were found in samples from both the IRS and non-IRS areas. Homozygote resistant (RR) genotypes were also detected in samples from all the sites except in Gushegu and Karaga. These homozygote resistant (RR) genotypes were higher in samples from BYD and KUD.

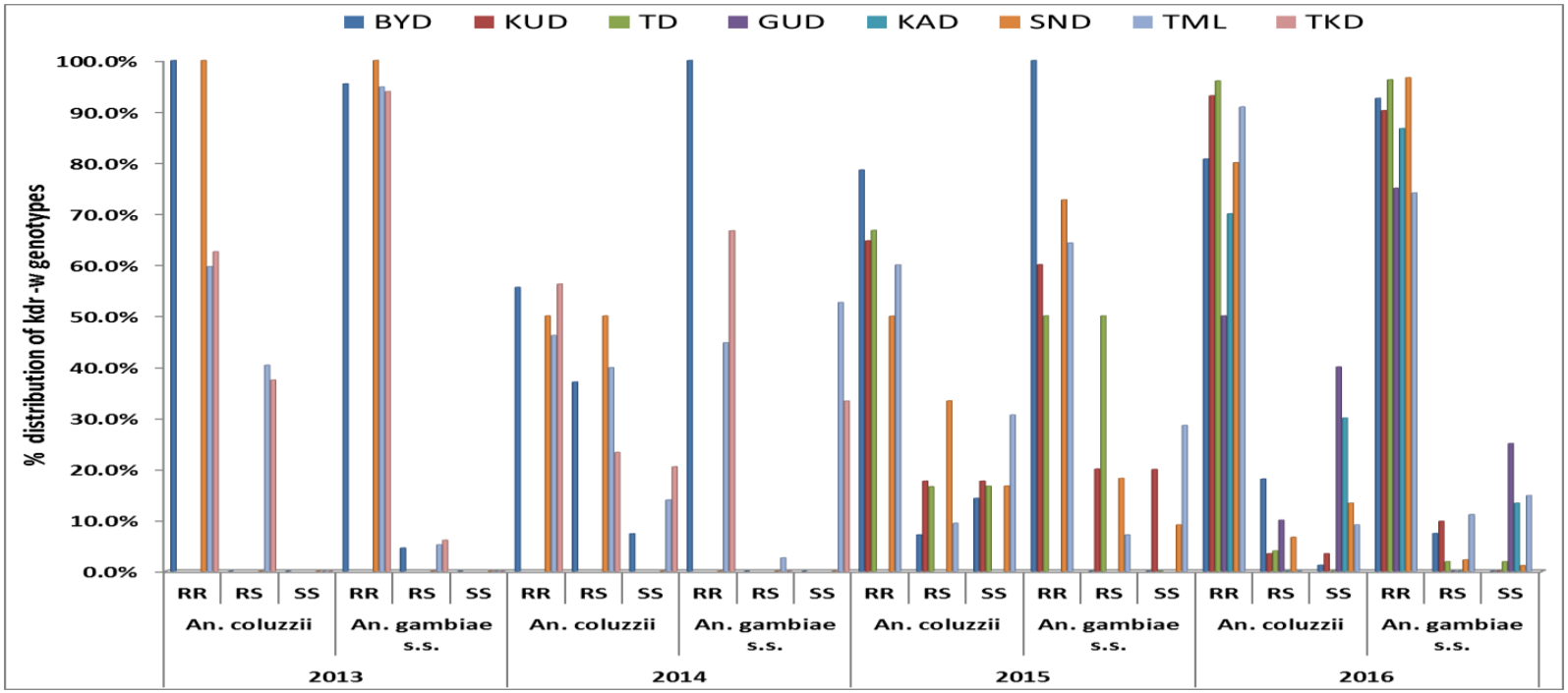
TABLE 7: DISTRIBUTION AND FREQUENCY OF *KDR-WEST* ALLELES IN *AN. GAMBIAE* S.S. FROM IRS AND NON-IRS AREAS, 2016

	Sentinel Site	Total Ex.	*An. gambiae s.s.*			*An. coluzzii*			*kdr w freq*
			RR	RS	SS	RR	RS	SS	
BYD	Nasuan	40	18	0	0	17	4	1	0.99
	Kpemale	41	21	1	0	11	8	0	
	Bunbuna	43	19	3	0	18	3	0	
	Yunyoo	40	17	2	0	21	0	0	
TD	Woribugu	40	24	0	1	14	1	0	0.99
	Dimabi	38	27	1	0	10	0	0	
SND	Nanton	39	29	2	0	7	0	1	0.97
	Diare	40	26	0	1	10	0	3	
	Tarikpaa	40	31	0	0	7	2	0	
KUD	Gupanerigu	40	21	0	0	19	0	0	0.99
	Gbullung	40	25	5	0	8	1	1	
GUD	Banda-ya	39	15	0	5	5	1	4	0.87
KAD	Binduli	40	26	0	4	7	0	3	0.90
TML	Tugu	40	14	5	3	5	0	3	0.90
	Yong	40	12	1	2	5	0	0	
	Kulaa	38	14	0	3	3	2	8	

TABLE 8: DISTRIBUTION AND FREQUENCY OF *ACE-1* ALLELES IN *AN. GAMBIAE* S.S. FROM IRS AND NON-IRS AREAS, 2016

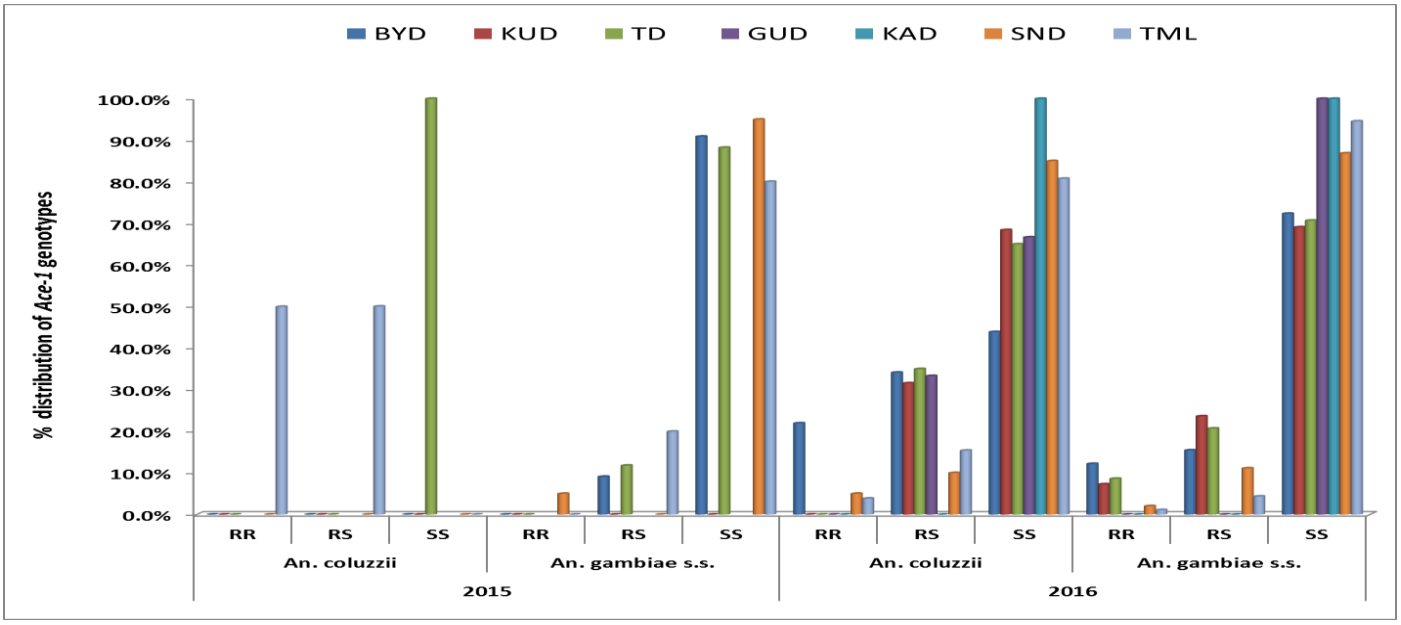
District	Sentinel site	Total Ex.	*An. gambiae s.s.*			*An. coluzzii*			Overall Ace 1 freq
			RR	RS	SS	RR	RS	SS	
BYD	Nasuan	40	6	4	20	3	3	4	0.43
	Kpemale	41	4	4	22	2	3	6	
	Bunbuna	43	3	5	25	2	3	5	
	Yunyoo	40	2	6	22	2	5	3	
KUD	Gupanerigu	40	2	5	20	0	2	5	0.41
	Gbullung	40	2	8	18	0	4	8	
SND	Nanton	39	0	2	32	1	0	4	0.16
	Diare	40	0	6	24	0	2	8	
	Tarikpaa	40	2	3	30	0	0	5	
TD	Woribugu	40	0	8	25	0	2	5	0.35
	Dimabi	38	5	4	16	0	5	8	
GUD	Banda-ya	39	0	0	24	0	5	10	0.13
KAD	Binduli	40	0	0	25	0	0	15	0.00
TML	Tugu	40	0	3	28	0	2	7	0.10
	Yong	40	0	1	30	1	0	8	
	Kulaa	38	1	0	29	0	2	6	

FIGURE 11[4]: YEARLY TRENDS IN THE DISTRIBUTION OF *KDR-W* GENOTYPES IN MOLECULAR FORMS OF *AN. GAMBIAE* S.S. AND *AN. COLUZZII* FROM IRS AND NON-IRS AREAS IN 2013–2016



[4] TKD was split into KUD and TD from 2015; no data available for TKD in 2015 and 2016. Data collection in GUD and KAD started in 2016

FIGURE 12: DISTRIBUTION OF *ACE-1* RESISTANT GENOTYPES IN *AN. GAMBIAE* S.S. AND *AN. COLUZZII* FROM IRS AND NON-IRS AREAS IN 2015 AND 2016



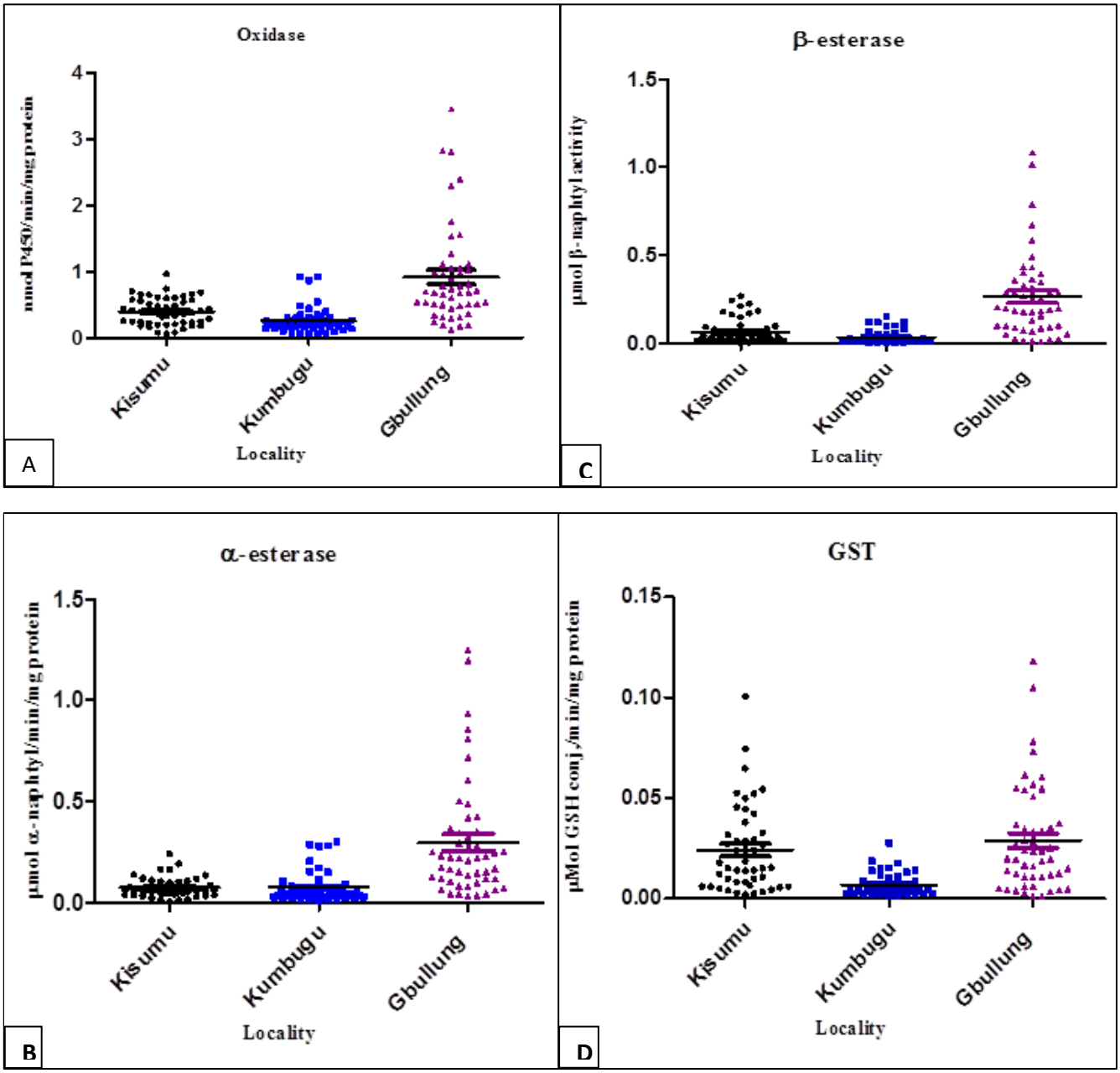
3.10.3 ENZYMATIC ACTIVITY IN *AN. GAMBIAE*

The activity of oxidases, esterases (α and β), and glutathione S-transferase (GST) enzymes were analyzed in 170 *An. gambiae* s.l. from Gbullung and Kumbungu (both IRS communities). The results showed that there was no increase in activity of any of the enzymes compared to Kisumu strain samples from Kumbungu. However, significant ($p<0.0001$) activity was observed for all the enzymes (oxidases, α and β-esterases) on samples collected from Gbullung (Table 9 and Figure 13).

TABLE 9: MEASUREMENT OF ENZYMATIC ACTIVITY WITHIN POPULATION OF *AN. GAMBIAE*, KUMBUNGU AND GBULLING SITES

Sites/strain	Oxidase (Mean P450 activity)	P value	α esterase (Mean α naphtyl activity)	P value	β esterase (Mean β naphtyl activity)	P value	GST (Mean GST activity)	P value
Kisumu	0.3959 ± 0.02887 N=50		0.07348 ± 0.006711 N=50		0.06216 ± 0.009777 N=51		0.02416 ± 0.003136 N=47	
Kumbungu	0.2560 ± 0.02765 N=50	0.0007	0.07347 ± 0.01092 N=49	0.0012	0.03357 ± 0.005340 N=48	0.0133	0.006877 ± 0.0008429 N=45	<0.0001
Gbullung	0.9140 ± 0.1071 N=48	<0.0001	0.2959 ± 0.04066 N=50	<0.0001	0.2617 ± 0.03483 N=48	<0.0001	0.02882 ± 0.003775 N=49	0.3469

FIGURE 13: ACTIVITY OF ENZYMES IN WILD *AN. GAMBIAE* POPULATIONS RELATIVE TO KISUMU REFERENCE STRAIN



3.11 IRS Spray Quality and Residual Efficacy

The 24-hour mortalities for all the tests conducted on all surfaces were 100% in all the communities evaluated. Control mortalities ranged between 0% and 5%. As a result, the project team did not calculate a correction for the mortalities recorded. The results for the spray quality tests are indicated as T0 in the test results presented in Figure A-2 in the Annex.

The results from the airborne effect tests showed that pirimiphos methyl has an airborne effect on mosquito mortality at T_o but follow-up bioassays showed no significant airborne effect after one month (Figure A-3), while mortality of mosquitoes exposed to the sprayed walls was still 100% (see decay rate data, Figure A-2 in the Annex).

The decay rate of the sprayed insecticide, Actellic 300CS, on different wall surfaces is presented as T_1–T_8. Based on the WHO Pesticide Evaluation Scheme-recommended threshold of 80%, the sprayed insecticide lasted between six and eight months depending on the type of sprayed surface.

3.12 Estimation of EIRs of Vectors

The risk of exposure to malaria (EIR) is estimated to be 0.03 infective bites/person/night (ib/p/n) for BYD, 0.05 ib/p/n for KUD, 0.07 ib/p/n for SND, 0.06 ib/p/n for TD, and 0.20 ib/p/n for TML (Table 10).

Monthly EIRs for the IRS areas (BYD: 8.22ib/p/yr and KUD: 11.32ib/p/yr) were lower than those for SND (16.80ib/p/yr), TD (14.36 ib/p/yr), and TML (55.13ib/p/yr).

The baseline EIR in GUD was about 15 infective bites (September 2016 only) and in KAD 61 infective bites (September–November 2016). Except in October, when KAD recorded the highest transmission intensity, 49.9ib/p/m, transmission remained higher in TML (the non-IRS control district) than in the other sites throughout the period (April–November 2016).

Malaria transmission was generally high outdoors than indoors for IRS areas (BYD and KUD) and IRS withdrawn sites (TD and SND) (Figure 14). However, indoor EIR in TML, GUD, and KAD was higher than outdoors. Figure 15 shows yearly trends in malaria transmission intensity from all sites.

Monthly trends of transmission showed that transmission was seasonal in both IRS and non-IRS districts, with transmission peaking in September and October (Figure 16).

TABLE 10[5]: ENTOMOLOGICAL PARAMETERS OF MALARIA TRANSMISSION, *AN. GAMBIAE* AND *AN. FUNESTUS*, ALL SENTINEL SITES, MARCH–NOVEMBER 2016

Sentinel Site	# Tested by ELISA	CS +ve	Sporozoite Rate	Mean Man Biting Rate (b/p/n)	EIR (ib/p/n)	Estimated Annual EIR (ib/p/yr)
IRS						
BYD	1,812	16	0.88%	3.43	**0.03**	**8.22**
KUD	1,344	10	0.74%	7.15	**0.05**	**11.32**
Non-IRS						
SND (IRS withdrawn)	1,984	23	1.16%	6.13	**0.07**	**16.80**
TD (IRS withdrawn)	1,705	13	0.76%	8.79	**0.07**	**14.36**
GUD (IRS withdrawn)	432	4	0.93%	54.41	**0.50**	**15.11**[*]
KAD (IRS withdrawn)	586	4	0.68%	32.52	**0.22**	**60.50**[†]
TML (Non-IRS)	2,757	30	1.09%	20.82	**0.20**	**55.13**

[5] Note: *Mosquito collection in GUD only in September; † Mosquito collections in KAD September–November 2016
‡ Mosquito collections in WG June–November 2016

FIGURE 14[6]: INDOOR AND OUTDOOR EIR, 2016

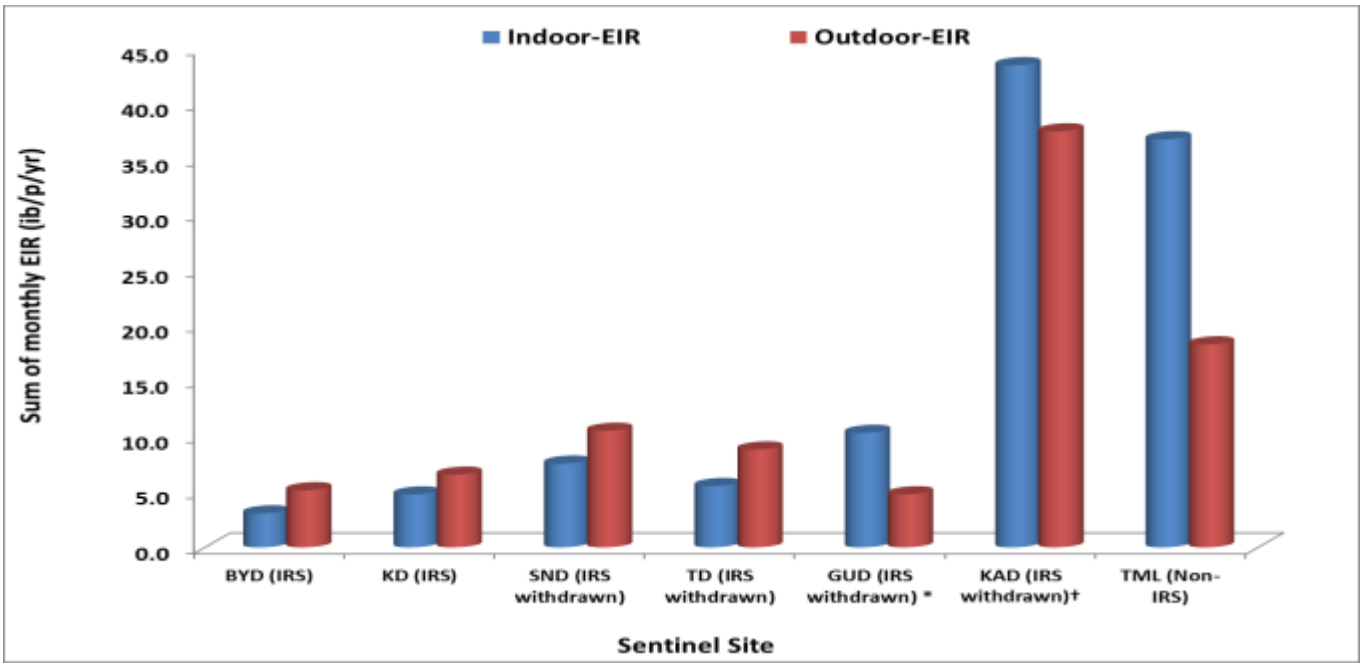
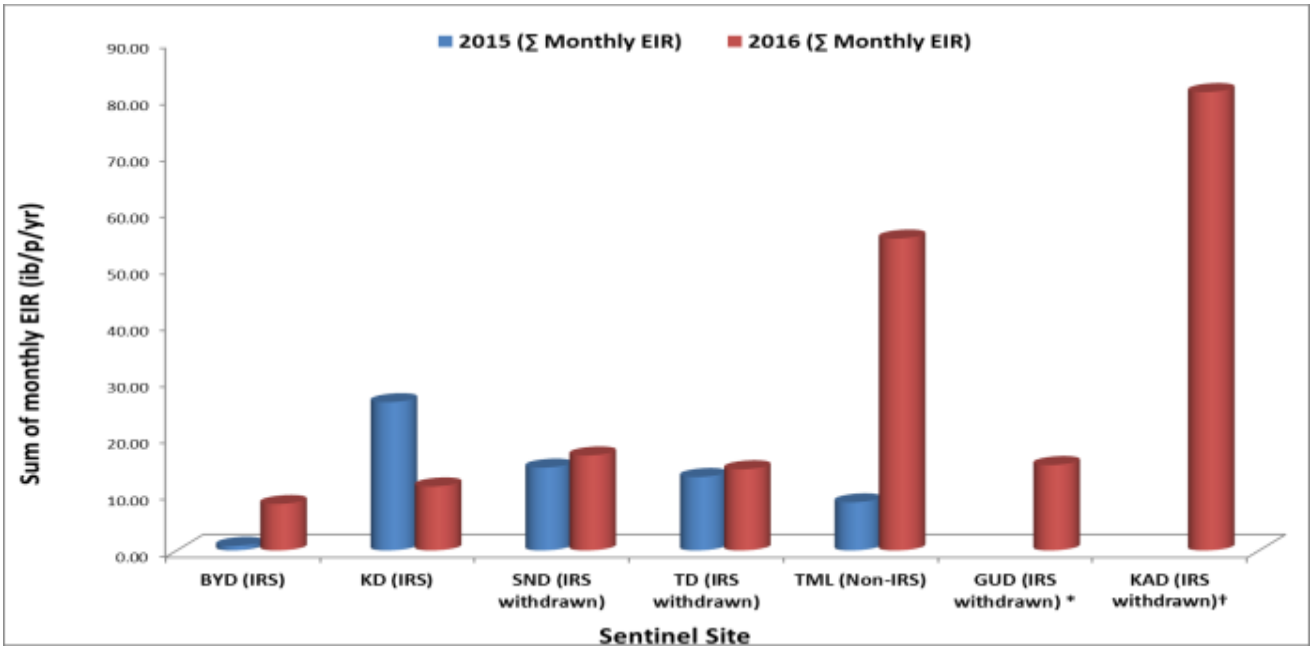
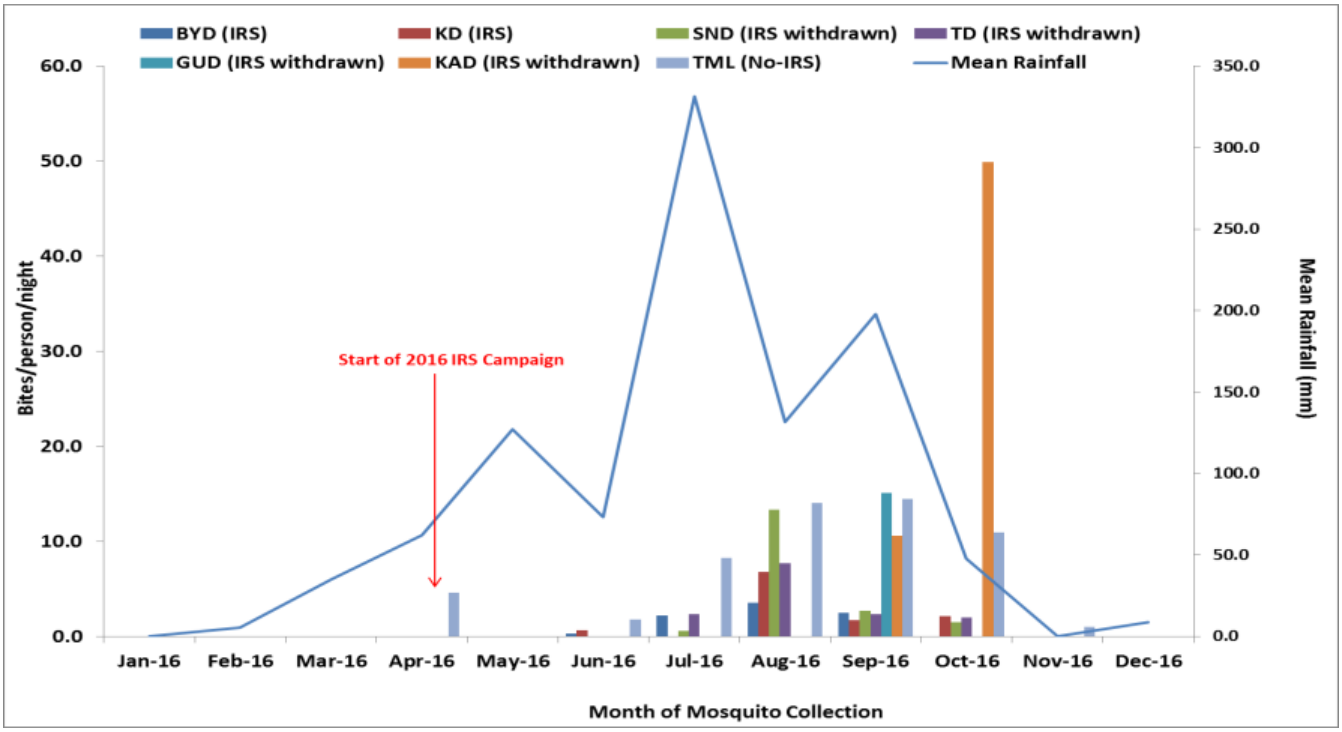


FIGURE 15[7]: COMPARISON OF EIR, 2015 AND 2016



[7 & 7] Comparison of Entomological Inoculation Rate (EIR) in Tolon (TD), Savelugu Nanton (SND), Kumbungu KD), Bunkpurugu-Yunyoo (BYD), Gushegu (GUD, Karaga (KAD) and Tamale (TML) districts, 2015 and 2016
*Mosquito collection in GUD only in September
† Mosquito collections in KAD September–November 2016

FIGURE 16: MONTHLY TRENDS IN EIR IN IRS AND NON-IRS SITES, 2016



3.13 PARITY RATES

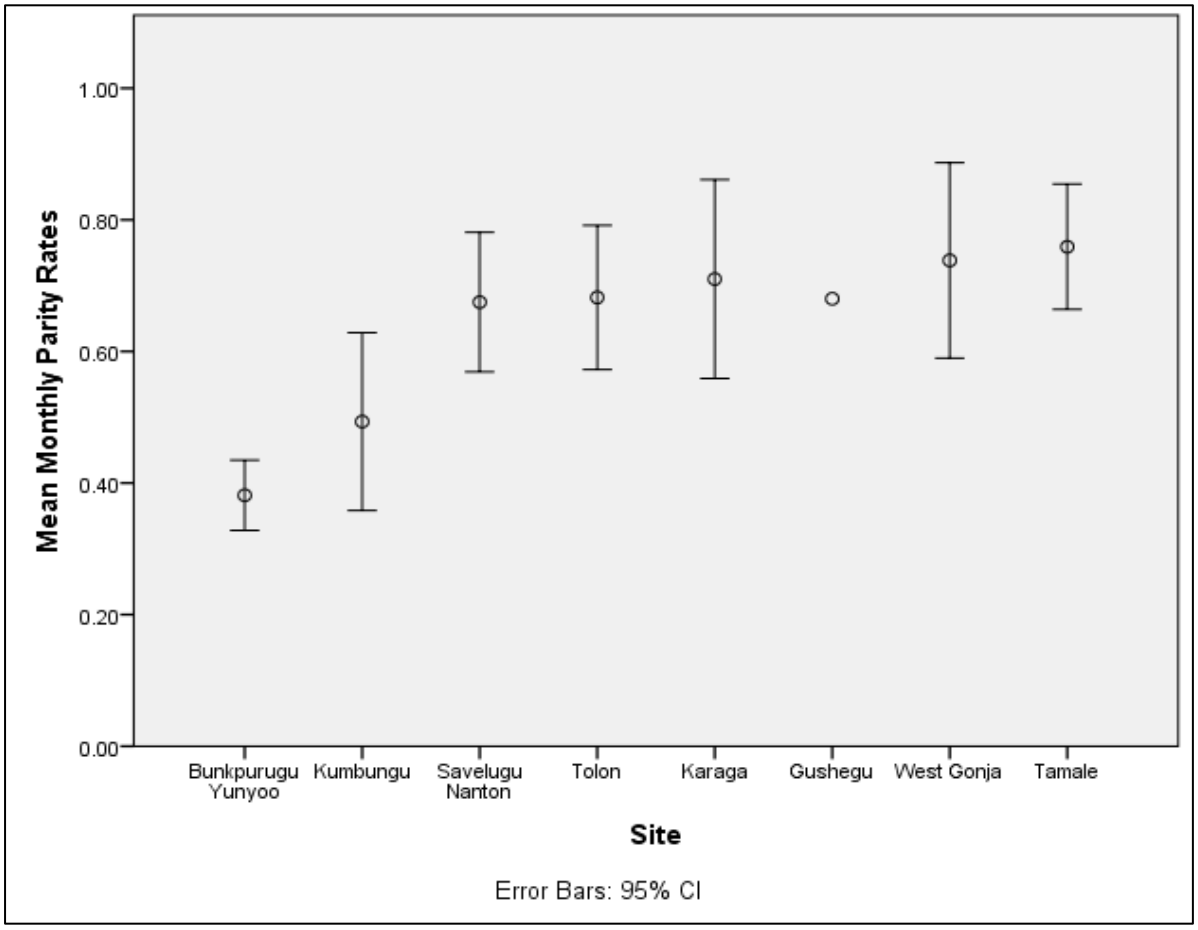
Dissections of *An. gambiae* s.l. mosquitoes collected from the study sites between March and November 2016 revealed that the proportion of parous females collected from TD, SND, and TML (unsprayed districts) as well as KUD (IRS district) was higher than the proportion collected from BYD alone. BYD recorded a mean parity rate of 39.3%, which is an increase in parity from 30.7 recorded in 2015. This increase trend in parity was observed across all sites, except TML, where a marginal decrease (68.3% to 66.8%) was observed, and this could be due to yearly fluctuations related to climate variability. However, the parity rate in BYD was significantly lower ($p<0.05$)[8] than the mean parity rates recorded for KUD (54.6%), SND (57.2%), TD (69.4%), GUD (68.3%), TML (66.8%), and WG (68.1%) (Table 11) (Similarly, the mean parity rate for KUD was significantly lower than the rates for TD ($z =-7.874$, $p<0.0001$), WG ($z=-5.73829$, $p<0.0001$), and TML ($z=-8.943$, $p<0.0001$), but not significantly different from rates for SND, where the team withdrew IRS in 2015 ($p=0.117$). The team found parity rates for SND were significantly lower than rates for TD ($p<0.0001$) and TML ($p<0.0001$) (Figure 17). There was no significant difference between parity rates recorded for TML and TD ($p=0.087$).

[8] BYD & KUD: $z = -8.339$, $p<0.0001$; BYD & SND: $z = -9.945$, $p<0.0001$; BYD & TD, $z = -16.0157$, $p<0.0001$; BYD & TML: $z = -19.8199$, $p<0.0001$; BYD & GUD, $Z=-7.79812$, $P<0.0001$; BYD & KAD, $z=-12.4516$, $p<0.0001$; BYD & WG, $z=-12.4843$, $P<0.0001$).

TABLE 11[9]: TOTAL NUMBER OF PAROUS FEMALE *AN. GAMBIAE* S.L., HLC, ALL SENTINEL SITES

District	#Dissected	Parous	%Parity	95% confidence interval	
				Lower Bound	Upper Bound
BYD (IRS)	1,842	723	39.3%	37.0%	41.5%
KUD (IRS)	1,345	735	54.6%	52.0%	57.3%
SND (IRS withdrawn)	1,450	829	57.2%	54.6%	59.7%
TD (IRS withdrawn)	1,198	832	69.4%	66.8%	72.1%
GUD (IRS withdrawn) *	202	138	68.3%	61.9%	74.7%
KAD (IRS withdrawn)†	549	384	69.9%	66.1%	73.8%
TML (Non-IRS)	4,375	2,924	66.8%	65.4%	68.2%
WG(Non-IRS)‡	655	446	68.1%	64.5%	71.7%

FIGURE 17: COMPARISON OF MEAN MONTHLY PARITY RATES FOR *AN. GAMBIAE* S.L.



[9] *Mosquito collection in GUD only in September
† Mosquito collections in KAD September–November 2016
‡ Mosquito collections in WG June–November 2016

4. DISCUSSION

The results indicate that *An. gambiae* s.l. remains the dominant *Anopheles* species in all the study sites, making up more than 98% of the total *Anopheles* species collected. PCR analysis showed that *An. coluzzii* and *An. gambiae* were present in sympatry at all five sites in varying proportions, with *An. coluzzii* dominating at 4 of 7 sites.

Biting rates of the vector species increased with the onset of the rains. However, there was about a month lag between the peak rainfall and mosquito densities.

Vector species in BYD, KUD, WG, and TD exhibited slightly more exophagic (outdoor feeding) behavior, possibly in response to the IRS and high coverage and use of long-lasting insecticide-treated bed nets. However, in SND, GUD, and TML, *An. gambiae* s.l. collected were slightly more endophagic (indoor feeding); this could be due to climatic or environmental factors or a difference in the behavior of local mosquitoes.

An. gambiae s.l. in the tested sites remain highly resistant to the pyrethroids tested (alpha-cypermethrin and deltamethrin), possibly a consequence of selection pressure maintained partly by the continuous distribution and use of deltamethrin-impregnated nets, as well as the use of pyrethrins in aerosol form and pyrethroids used for agriculture as found in the recent PMI pesticide market survey (unpublished) in northern Ghana. In contrast, the vector was susceptible to pirimiphos-methyl in all IRS communities. However, the use of organophosphate insecticides for agriculture in the study area threatens the future efficacy of pirimiphos-methyl used for IRS operations – it has been documented that mosquitoes exposed at the larval stages to sub lethal doses of pollutants, herbicides, or pesticides are more tolerant to insecticides as adults (Nkya *et al.* 2014). The detection of the homozygote resistant *Ace-1* genotype in mosquito samples from the IRS districts (BYD and KUD) suggests that the species could be becoming tolerant to pirimiphos-methyl – in BYD, pirimiphos-methyl has been used for IRS since 2013, and use of organophosphates in agriculture has been documented in both IRS and non-IRS areas (PMI AIRS Ghana pesticide market survey, unpublished).

The results from the biochemical as well as the synergist assays confirm the role of oxidases and esterases in contributing to resistance observed in the local vector species from Gbullung (an IRS area). Results from the synergist assays suggest that mono-oxygenases play a more significant role than esterases in the resistance of *An. gambiae* s.l. from Gbullung to the pyrethroids. Esterases have been found to be associated mainly with resistance to organophosphates and carbamates, but in some cases, high levels of these enzymes have also been involved in resistance to permethrin in *An. gambiae* (Vulule *et al.* 1999). The elevated level of detoxifying enzymes such as the esterases and mixed function oxidases in the populations of *An. gambiae* from Gbullung is worrying. Therefore, there is need for extensive insecticide susceptibility tests in IRS areas that practice intensive agriculture and also that have sprayed Actellic since 2013.

The observed increase in EIRs in BYD in 2016 as compared with 2015 could in part be attributed to the increased higher biting rate in 2016, the result of increased rainfall from 69.3mm in 2015 to 108.4mm in 2016. The rains might account for expanded breeding areas.

The relatively high outdoor transmission observed for most sites could be induced by the high net coverage and use as a result of the 2016 net distribution campaign between March and April 2016. This observation conforms to findings from other studies (e.g., Russell *et al.* 2011), suggesting that increasing net coverage results in a decline in the density of highly endophagic *An. gambiae* s.s., and this may select for vector species that express a preference toward outdoor feeding and biting (Durnez and Coosemans 2013).

Parity rates in BYD and unsprayed communities show that significantly fewer older mosquitoes were collected in BYD. The re-introduction of IRS in KUD could account for the suppression of the population of parous (older) females collected in 2016 compared with the proportion collected in 2015. In KUD, significant reduction in EIRs from 2015 levels indicates a reduction in malaria transmission risk. A 6–7 month residual life of the sprayed insecticide appears to have been adequate in offering the IRS communities the needed protection through the malaria transmission period, May to October. Lower EIRs were recorded in the IRS districts (BYD and KUD) than in unsprayed and IRS withdrawn communities such as SND (where IRS was withdrawn in 2013). The negative effect of IRS withdrawal is seen in the adjoining districts of TD and SND where IRS was withdrawn in 2013 and 2015, respectively. Malaria transmission indices increased significantly compared to the IRS districts.

5. CONCLUSIONS

IRS has maintained parity rates and EIR at low levels in BYD in comparison with unsprayed communities. The re-introduction of spraying in KUD seems to have contributed to maintaining low malaria transmission intensity in the district as compared with KAD, GUD, WG, TML, and TD, where no IRS is carried out.

Considering that pirimiphos-methyl has been used for IRS for close to five years, there is the need to intensify insecticide resistance monitoring in these areas to monitor for the early development of resistance to this insecticide. The impact of important vector behaviors (biting and resting) on transmission in both IRS and non-IRS areas should also be critically assessed. An operational research study is planned in 2017 to address important questions regarding outdoor and indoor vector biting and resting behaviors that relate to malaria transmission and their implications on the effectiveness of key vector control efforts (IRS/LLINs). The relationship between vector behaviors and human activities will also provide critical information for improved malaria control programs.

ANNEX A. WHO BIOASSAY TEST PROCEDURE

Sugar-fed, 2–5-day-old female *Anopheles gambiae* s.l. were used for the insecticide susceptibility tests by exposing them to WHO-approved diagnostic doses of selected insecticide-impregnated papers using the WHO tube method (WHO 2013). The following insecticides were tested.

- Pyrethroids: Alpha-cypermethrin 0.5% and deltamethrin 0.05%;
- Carbamates: Bendiocarb 0.1% and propoxur 0.1%;
- Organophosphate: Pirimiphos-methyl 0.25% and fenitrothion 1%
- Organochlorine: DDT 4%

Procedure:

- Four test replicates and two controls were set up for each insecticide tested, to assess the susceptibility of the local *An. gambiae* s.l.
- A total of 25 female *An. gambiae* s.l. mosquitoes were aspirated in batches of at most 10 from mosquito cages into the holding tubes (lined with clean white sheets) to give six replicate (four tests and two controls) samples. The mosquitoes were held for one hour before the test was started. Any damaged or weakened mosquito was removed at the end of the pre-exposure holding time.
- Mosquitoes were introduced into the exposure tubes lined with specific insecticide-impregnated test (as listed above) or oil-impregnated control papers for a period of one hour (60 minutes). Knockdown rates of the insecticides were scored at 10, 15, 20, 30, 40, 50, and 60 minutes during the one-hour exposure period. Whenever the observed knockdown rate was less than 80% after 60 minutes, another count at 80 minutes was made of the mosquitoes in the holding tube.
- At the end of the one-hour exposure period, the mosquitoes were transferred back to the holding tubes and a pad of cotton-wool soaked in 10% sugar solution placed on the mesh-screen end of the holding tubes.
- Mosquitoes were maintained in the holding tubes for 24 hours (the recovery period).
- Temperature and humidity during the exposure period and the recovery period for each test were maintained at 25°C ± 2°C and 80% ± 10% relative humidity.
- At the end of recovery period (i.e., 24 hours post-exposure), the number of dead mosquitoes were counted and recorded.
- Upon completion of the susceptibility test, mosquitoes were transferred to individual, clearly labeled tubes (separating dead and live mosquitoes into separate tubes) for preservation. Mosquitoes that survived after the 24-hour holding period were killed and immediately placed in cry-tubes, preserved in liquid nitrogen, and transported to NMIMR labs for further supplementary testing.

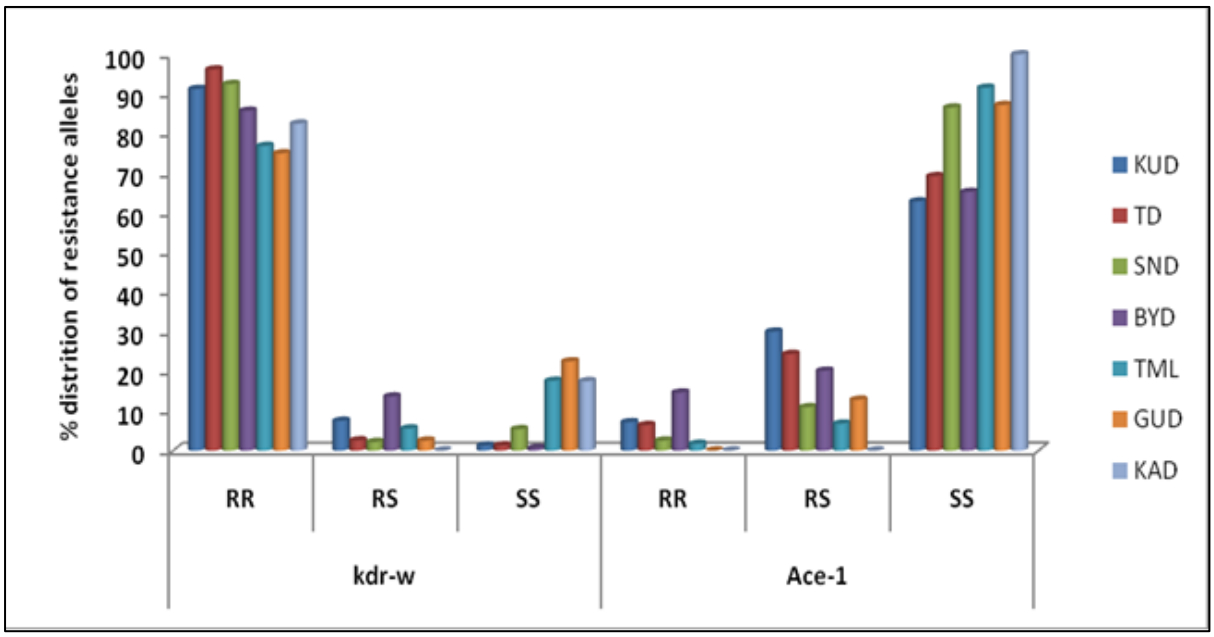
TABLE A-1: SUMMARY OF WHO INSECTICIDE RESISTANCE TEST RESULTS SHOWING INSECTICIDE RESISTANCE STATUS OF *AN. GAMBIAE* S.L. TESTED AGAINST SELECTED INSECTICIDES, 2016

District	Site	Carbamate		Organochlorine	Organophosphate		Pyrethroid		
		Bendiocarb	Propoxur	DDT	Fenitrothion	Pirimiphos methyl	Alpha cypermethrin	Deltamethrin	Permethrin
Bunkpurugu-Yunyoo	Bunbuna	98% (100)				99% (100)	46% (100)		
Kumbungu	Gbullung	89% (100)		34% (100)	99% (100)	98% (100)	34% (100)	30% (100)	
	Kumbungu	100% (100)				100% (100)	26% (100)	0% (100)	5% (100)
Savelugu Nanton	Nanton	92% (100)				100% (100)	69% (100)		
	Tarikpaa	99% (100)		10% (100)	100% (100)	100% (100)	83% (100)		
Tamale Metropolitan	Kulaa	99% (100)	97% (100)	1% (100)	100% (100)	100% (100)	62% (100)	5% (100)	
	Tugu					100% (100)	83% (100)		
Tolon	Dimabi	100% (100)		17% (100)	98% (100)	97% (100)	29% (100)		
	Woribugu					100% (100)	91% (100)		

Resistant Potentially resistant Susceptible

The top number in each cell is the corrected percentage mortality. The bottom number between parentheses is the number tested.

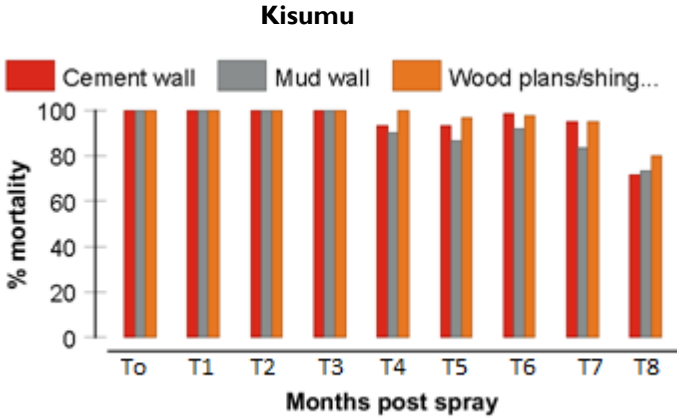
FIGURE A-1: DISTIRBUTION OF *KDR-W* AND *ACE*-1 GENE IN *AN. GAMBIAE* S.S. FROM IRS AND NON-IRS AREAS, 2016



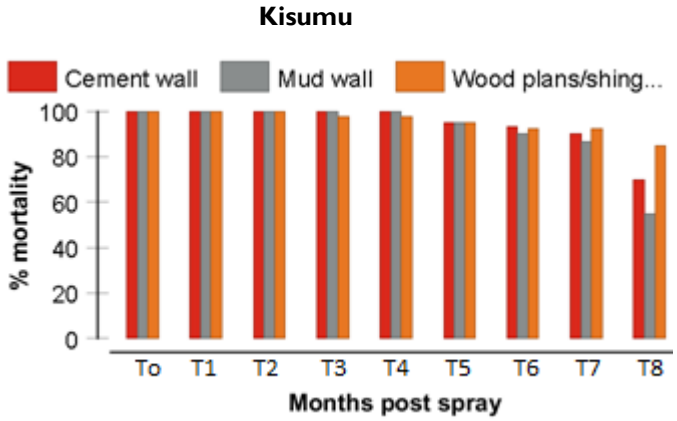
Note: RR=homozygote resistant allele; RS=heterozygote resistant allele; SS= homozygote susceptible allele

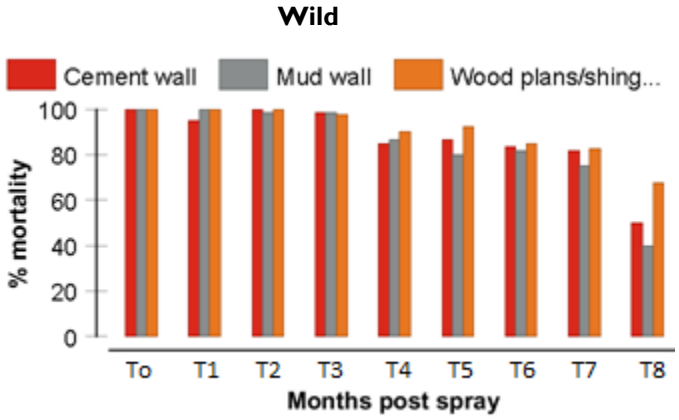
FIGURE A-2: IRS RESIDUAL EFFICACY USING KISUMU AND WILD *AN. GAMBIAE* S.L. MOSQUITOES

a. Bunbuna

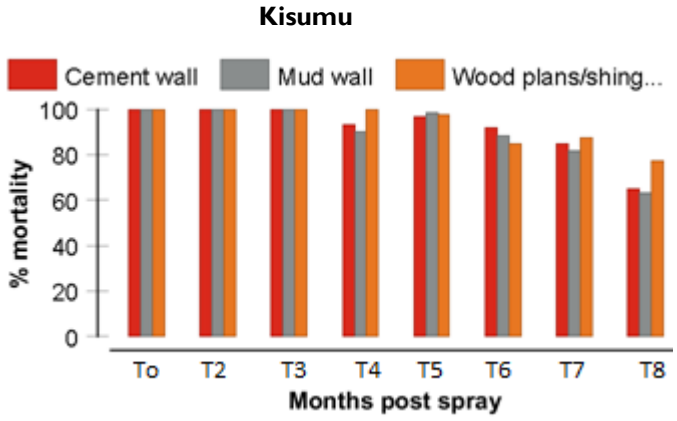


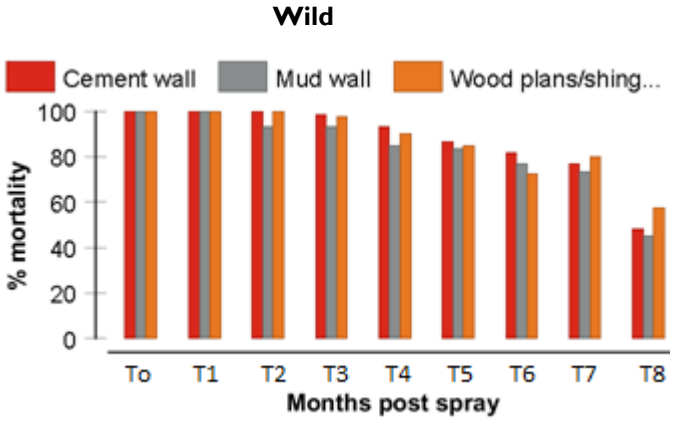
b. Cheyohi



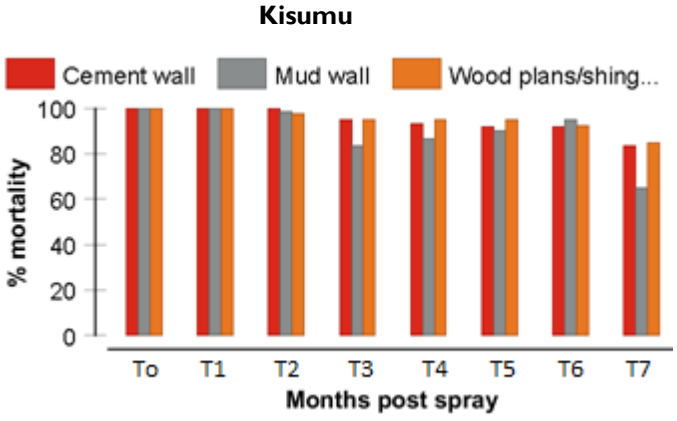


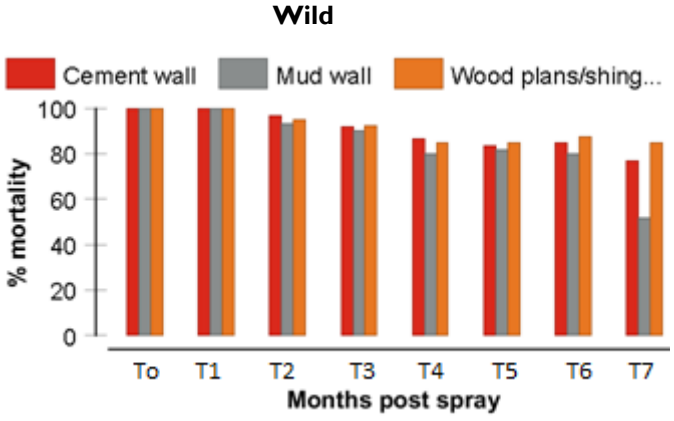
c. Gbullung



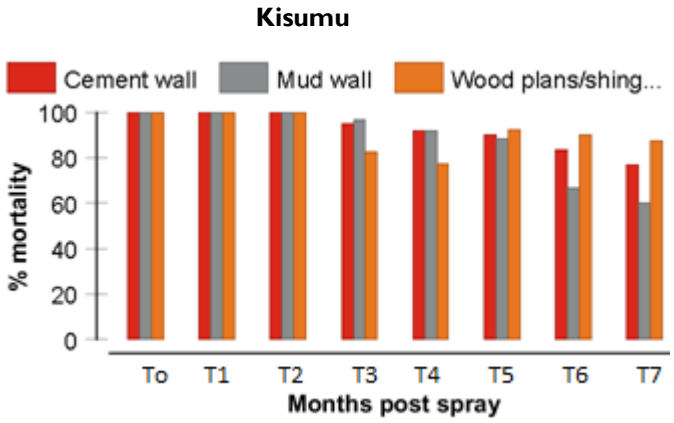


d. Gupanarigu





e. Kpemale



f. Yunyoo

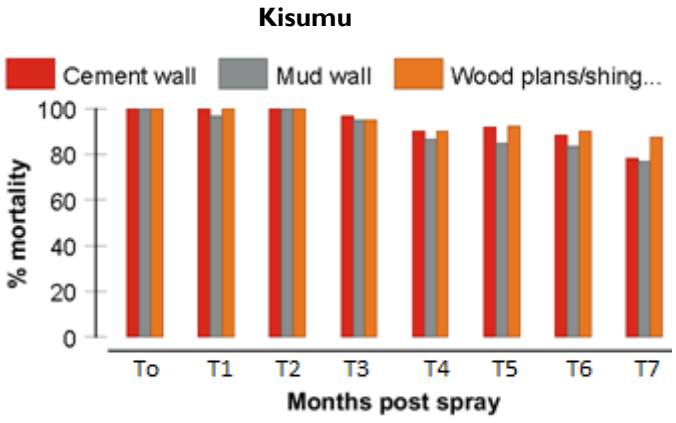
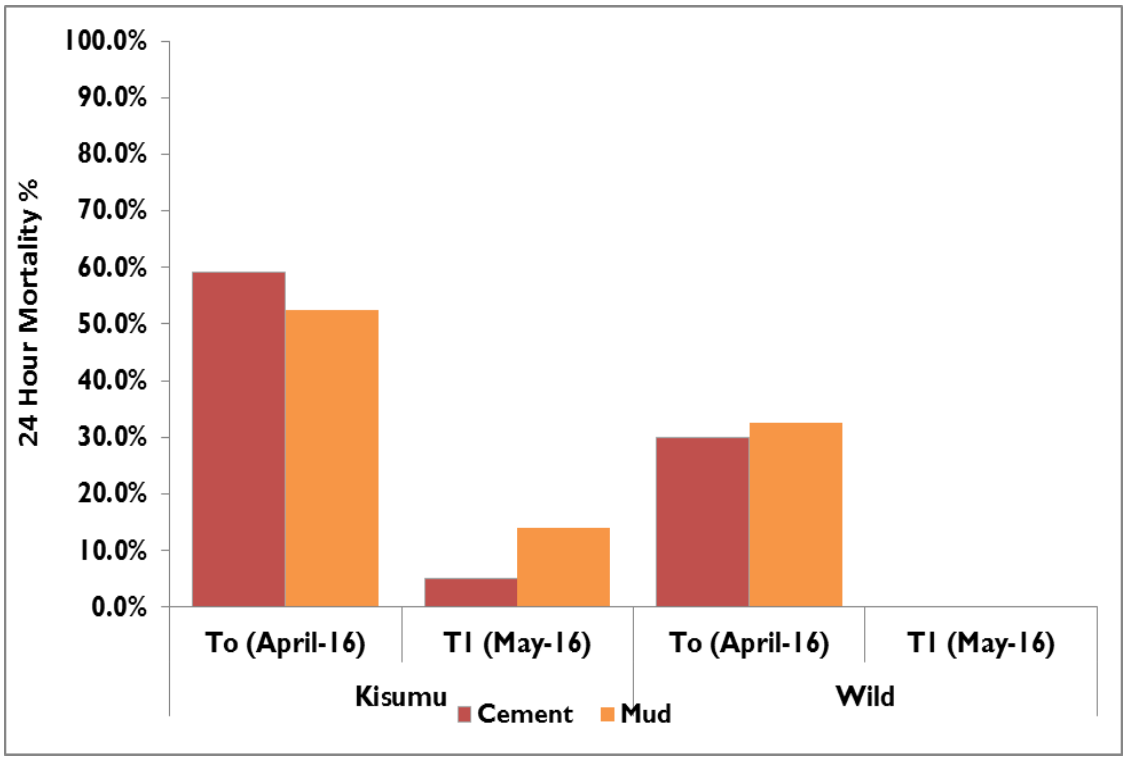


FIGURE A-3: AIRBONE EFFECT (% MORTALITY OF KISUMU AND WILD *AN. GAMBIAE* S.L. MOSQUITOES) OF ACTELLIC 300CS, IN CEMENT AND MUD PLASTERED ROOMS, 1 MONTH AFTER SPRAY, MAY 2016



ANNEX B. REFERENCES

Abbott, WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*. 18: 265:267.

Bass C, Williamson MS, Wilding CS, Donnelly MJ, Field LM. 2007. Identification of the main malaria vectors in the *Anopheles gambiae* species complex using a TaqMan real-time PCR assay. *Malar J*. 6:155.

Brogdon WG 1988. Microassay of acetylcholinesterase activity in small portions of single mosquito homogenates. *Comp Biochem Physiol C 90*: 145-150.

Brogdon WG, Hobbs JH, St. Jean Y, Jacques JR, Charles LB 1988. Microplate assay analysis of reduced fenitrothion susceptibility in Haitian *Anopheles albimanus*. *J Am Mosq Control Assoc* 1988a; 4:152-8.

Brogdon W, Chan A 2010. Guidelines for Evaluating Insecticide Resistance in Vectors using the CDC Bottle Bioassay/Methods in anopheles research. Second edition. CDC Atlanta USA: CDC technical report 343

Detinova TS 1962. Age grouping methods in Diptera of medical importance, with special reference to some vectors of malaria. World Health Organization Monographs series 47.

Durnez L and Coosemans M. 2013. Residual transmission of malaria: an old issue for new approaches. *Anopheles* mosquitoes - New insights into malaria vectors. In Manguin S. (Eds) (pp. 671-704). *http://www.intechopen.com/articles/show/title/residual-transmission-of-malaria-an-old-issue-for-new-approaches*,

Fanello, C., Santolamazza, F and Torre, A. D. (2002). Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. Medical and Veterinary Entomology. 16: 461-464.

Gillies MT and Coetzee M. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). *Publication of the South African Institute for Medical Research*, 55: 33–81.

Martinez-Torres D, Chandre F, Williamson MS et al. (1998) Molecular characterization of pyrethoid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. Insect Molecular Biology 7, 179–184.

Nkya TE, Poupardin R, Laporte F, Akhouayri I, Mosha F, Magesa S, et al. 2014. Impact of agriculture on the selection of insecticide resistance in the malaria vector *Anopheles gambiae*: a multigenerational study in controlled condition *Parasites & Vectors* 7:480 DOI: 10.1186/s13071-014-0480-z.

Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF. 2011. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar J*. 10:80 DOI: 10.1186/1475-2875-10-80.

Scott JA, Brogdon WG, Collins FH 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am J Trop Med Hyg* 49:520–529.

Scott J.G. 1996. CytochromeP450monooxygenase-mediated resistance to insecticides. *Journal of Pesticide Sciences* 21(2):241–245.

Vulule JM, Beach RF, Atieli FK, McAllister J, Brogdon WG, Roberts JM, Mwangi RW, Hawley WA. 1999. Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin-impregnated nets. *Med Vet Entomol* 13:239–244.

Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, Raymond, M. 2004. The unique mutation in ace-1^R giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Mol Biol.* 13:1–7.

Wirtz RD, Burkot TR, Graves PM. 1987. Field evaluation of enzyme-linked immunorsobent assays for Plasmodium falciparum and Plasmodium vivax sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *J. Med. Entomol* 24:433–437.

World Health Organization. 1975. *Manual on practical entomology in malaria.* Part II. Methods and Techniques World Health Organization Offset Publication. Geneva. Pp.13.

World Health Organization. 2006. Indoor residual spraying: Use of indoor residual spraying for scaling up global malaria control and elimination. WHO/HTM/MAL/2006. Pp.11–12.

World Health Organization. 2013. Global plan for insecticide resistance management in malaria vectors (GPIRM). World Health Organization Global Malaria Program.