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Indoor Residual Spraying (IRS 2) Task Order Six

ENTOMOLOGICAL MONITORING OF THE PMI IRS PROGRAM IN NORTHERN GHANA

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Prepared by: Abt Associates Inc.

Abt Associates Inc. | 6130 Executive Blvd.
Rockville, MD 20852 | Suite 800 North | T. 301.347.5000 | F. 301.913.9061 | www.abtassociates.com

ENTOMOLOGICAL MONITORING OF THE PMI AIRS PROGRAM IN NORTHERN GHANA

2017 ANNUAL REPORT

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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
HBR	Human 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
RDT	Rapid Diagnostic Test
SND	Savelugu-Nanton District
TD	Tolon District
TML	Tamale Metropolis
USAID	United States Agency for International Development
WHO	World Health Organization
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 seven districts in Northern Ghana and continued entomological monitoring in its target districts and beyond in 2017.

To assess the impact of IRS on entomological indices of malaria transmission across all sites, PMI AIRS carried out routine entomological surveys in seven sentinel districts. The districts included four IRS districts: Bunkpurugu-Yunyoo District (BYD), Gushegu District (GUD), Karaga District (KAD) and Kumbungu District (KUD); two districts from which IRS was withdrawn: Savelugu Nanton District (SND), and Tolon District (TD); and one district that has never been sprayed: Tamale (TML) metropolis. The project used human landing, pyrethrum spray and window exit trap collection methods to collect mosquitoes between March and December 2017 in all sites. 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 both the CDC bottle bioassay and WHO Tube methods.

RESULTS AND DISCUSSION

Vector Species Composition and Seasonality: *An. gambiae* s.l. was the most abundant species in all the study sites, comprising about 98% (40,482) of the total 41,131 *Anopheles* collected. *An. coluzzii* and *An. gambiae* existed in sympatry in all sentinel sites, with *An. coluzzii* predominating at most sites except in GUD and KAD. *An. coluzzii* made up about 58% of the *An. gambiae* s.l. collected. *An. arabiensis* made up only 1.8% of the *An. gambiae* s.l. collected. Mosquito populations peaked between July and September. *An. gambiae* s.l. from all sites showed higher outdoor biting rates except in the control sites (TML), where the vectors exhibited relatively high indoor feeding tendencies.

Parity Rates: Ovary dissections showed lower longevity of *An. gambiae* s.l. in IRS districts compared with the unsprayed districts. The mean proportion of parous *An. gambiae* s.l. for the four IRS districts (BYD 41%; GUD 50%; KAD 44%; and KUD 40%) were significantly ($p<0.05$) lower from the mean parity rates for SND (66.4%), TD (71.8%) and TML (75.6%).

Residual Life of Sprayed Insecticide: Monthly wall bioassays showed that insecticides remained efficacious in killing local vectors for an average of seven months. 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 clothianidin (with mortalities between 98% and 100%) except in Kumbungu site (KUD), where the mosquitoes showed possible resistance to clothianidin (96%). *An. gambiae* s.l. was resistant to DDT, deltamethrin and alpha-cypermethrin across all the sites, but was susceptible to bendiocarb in Tarikpaa site (KUD).

***Resistance Mechanisms*:** Results from the synergist assays also suggest that mono-oxygenases and esterases could be playing a role in the resistance to the pyrethroids in *An. gambiae* s.l. from the sites tested. An increase in frequency of the *Ace-1* genotype was observed in vectors from two IRS districts (BYD and KUD).

Entomological Inoculation Rates (EIRs): Malaria transmission was highly seasonal, with transmission occurring between June and October, 2017. A general decline of about 86% in EIR was observed in 2017 when compared to 2016. However the sum of monthly EIRs for the IRS areas

(GUD: 4.4 infective bites/person/year (ib/p/yr) and KUD: 3.19 ib/p/yr) were lower than that for the unsprayed district TML (12.06 ib/p/yr). No infected mosquitoes were detected in tested samples for BYD and KAD (IRS districts) or in SND where IRS was withdrawn in 2015.

CONCLUSION

IRS continues to suppress the parity rates in IRS areas in comparison with unsprayed communities and IRS withdrawn communities. The re-introduction of spraying in GUD and KUD seems to have contributed to reduced longevity and reduction in malaria transmission intensity in the main vector, as shown in the very low EIRs recorded in the IRS areas compared to the unsprayed district.

1. INTRODUCTION

In 2017, Africa Indoor Residual Spraying (AIRS) Ghana project, funded by the United States Agency for International Development (USAID) through the President's Malaria Initiative (PMI) carried out IRS in seven districts: Bunkpurugu-Yunyoo District (BYD), East Mamprusi (EMD), Gushegu District (GUD), Karaga District (KAD), Kumbungu District (KUD), Mamprugu Moaduri, and West Mamprusi (WMD).

To assess the impact of IRS on entomological indices of malaria transmission, the PMI AIRS Ghana project carried out routine entomological surveys in seven sentinel districts, from March through December 2017. Specific objectives of the surveys 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 2017.

2. METHODOLOGY

2.1 STUDY AREAS

The sentinel districts included four IRS districts: BYD, GUD, KAD and KUD; two districts from which IRS was withdrawn: Savelugu Nanton District (SND), and Tolon District (TD); and 1 district that has never been sprayed: Tamale (TML) metropolis (Figure 1). Table 1 provides information, including spray history from 2008-2017, on the districts and communities/sentinel sites.

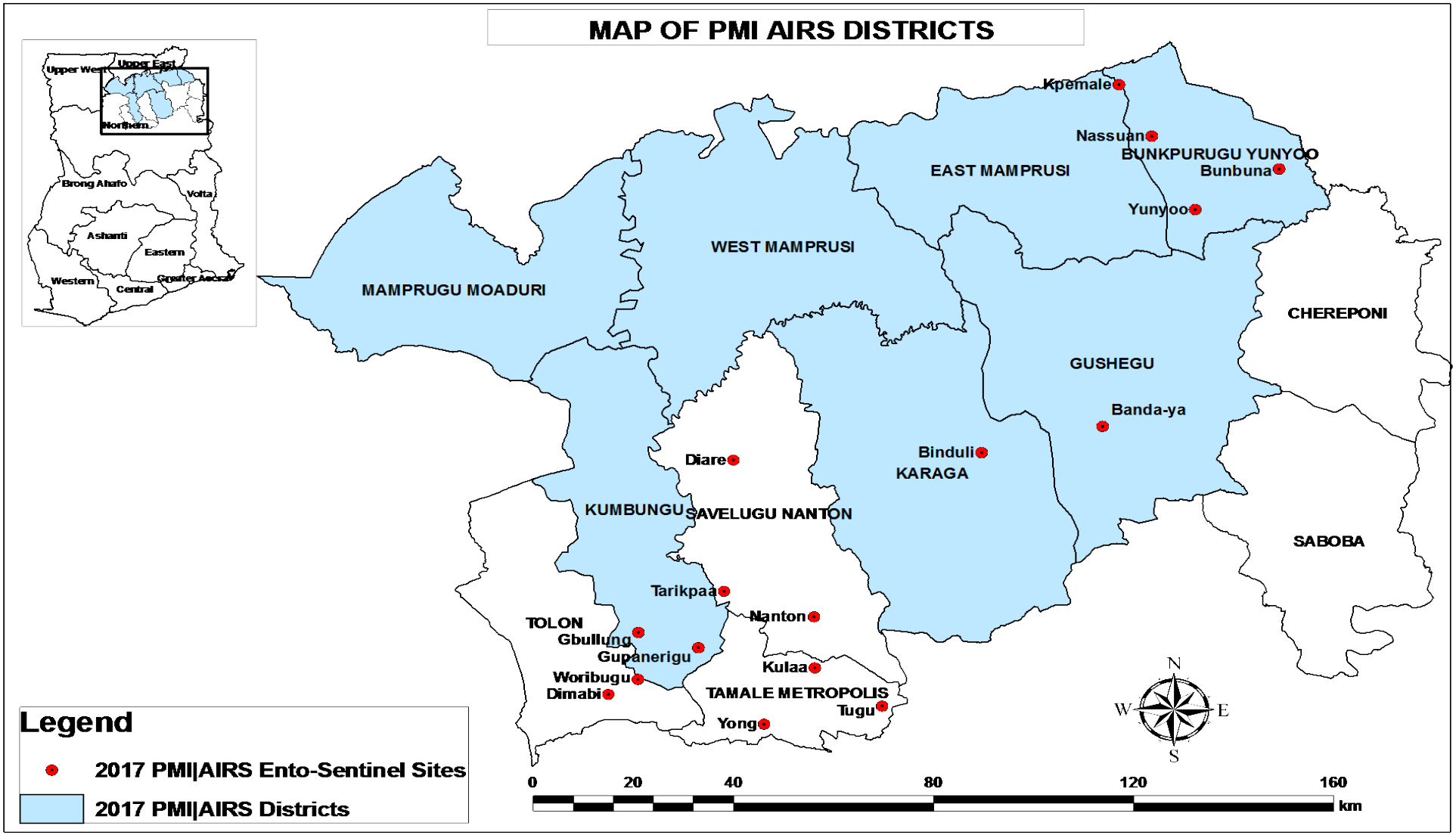
Based on insecticide susceptibility and residual efficacy test results from the previous year, the project used the organophosphate, pirimiphos-methyl (Actellic 300CS, at 1g/m^2) to spray all target districts in 2017. The IRS campaign ran from April 25 to May 30, 2017.

TABLE 1: ENTOMOLOGICAL MONITORING SITES

Districts	Communities/ Sentinel Sites	Insecticide Spray History									
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
BYD	Bunbuna, Yunyoo, Nasuan, and Kpemale	NSp	NSp	NSp	ACy	ACy	PM	PM	PM	PM	PM
KUD	Gbullung and Gupanarigu	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM	PM
SND	Diare, Nanton, and Tarikpaa (IRS was withdrawn in 2015)	ACy	ACy	DM	ACy	PM	PM	PM	NSp	NSp	NSp
TD	Dimabi and Woribugu (IRS was withdrawn in 2013)	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	NSp
GUD	Banda-ya	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM
KAD	Binduli	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM
TML	Kulaa, Tugu, and Yong (comparison communities with no history of IRS)	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control

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

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



2.2 Adult Mosquito Surveys

Pre- and post-spraying mosquito collections were performed using the Human Landing Catches (HLCs), Pyrethrum Spray Collections (PSCs) (WHO 2013), and window exit trap collections to collect mosquitoes from sentinel sites to assess and understand vector species composition, density, number and type (unfed, fed, gravid) of mosquitoes exiting rooms, longevity, and entomological inoculation rates (EIRs). The data collected were used to make comparisons between sprayed and unsprayed communities. In the 16 sites, the team conducted collections for four days each month, beginning in March 2017 and ending in December 2017 (10 months).

HLCs were performed for a total of four nights (collecting mosquitoes hourly from 6pm to 6am) in eight compounds in each sentinel community per month. The collections were 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.

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

Exit traps were utilized to catch mosquitoes exiting from houses as unfed, freshly fed or gravid in order to estimate their resting preference between indoors and outdoors. 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) in four separate compounds 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 (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 (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 *An. gambiae* 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 used for doors/windows in sprayed rooms.

2.3.1 Quality Assurance of the IRS Program

Selection of Community/Houses: One community was selected from each district for the spray quality assessment, using the spray plans of the district except for KUD where three communities were selected, two of which are within the area where an operational research is being conducted.

The communities were: Bunbuna and Kpemale (BYD); Dabari (EMD); Cheyohi, Gbullung and Gupanarigu (KUD); Kariminga (WMD); Kuuba (MMD); Bilsinaayili (GUD); and Timtishe (KAD).

The quality of IRS was assessed in communities that were sprayed during the first 1-3 days of the campaign and houses were systematically sampled to represent different spray teams and spray operators.

In each community, 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).

Test Procedure: The WHO cone bioassay method was used to assess the spray quality using both laboratory raised *An. gambiae* Kisumu strain and wild female *An. gambiae* s.l. adult mosquitoes reared from larvae collected in the project districts.

Three (3) cone assays were carried out on the walls in any one house together with 1 assay on the wooden door or window using 10 adult female mosquitoes per cone. One control cone assay was done for every 4 bio-assay tests, by fastening cardboard on unsprayed surfaces and exposing the control mosquitoes to the cardboard but also to conditions similar to exposed mosquitoes. To avoid the possibility of the control mortality increasing due to the fumigant effect of the Actellic 300CS formulation, the control tests were setup in unsprayed structures with fairly similar conditions (relative humidity and temperature) as the rooms being tested.

The cone exposure chamber was fastened to selected spot on the wall surface at three different heights (1.5m, 1.0m, and 0.5m) with tape. Batches of ten (10) 2-5 day old non-blood fed mosquitoes were introduced into the chamber and left exposed on the surface for 30 minutes. At the end of the exposure period, the mosquitoes were collected and transferred to holding cups. The number of mosquitoes that were knocked down at the end of the exposure period (30 mins) and at 60 mins was recorded. A third setup was mounted in the sprayed rooms for 30 mins to assess the fumigant effect of the sprayed insecticides at the time of the assays. Ten (10) mosquitoes were introduced into holding cups and placed on table which was kept 10cm from the sprayed wall surfaces. The number of mosquitoes that were knocked down at the end of 30 mins and at 60 mins were recorded as was done for the tests. The mosquitoes were all brought to the AIRS entomology laboratory and maintained at temperature (25◦C-29◦C) and relative humidity (75% - 85%). The mosquitoes were given a 10% sugar solution in cotton pads during the 24 hour holding period. The dead and alive mosquitoes were counted after 24 hours and the mortalities calculated. Mortalities were corrected using Abbot's formula if the control mortalities were between 5% and 20%, but tests were discarded and repeated if control mortalities exceeded 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: Bunbuna (BYD), Dabari (EMD), Gupanarigu (KUD), Kariminga (WMD), Kuuba (MMD), Binduli (KAD) and Banda-ya (GUD)

The bioassays were conducted using susceptible *An. gambiae* Kisumu strain mosquitoes from the AIRS insectary and the insectary of the Navrongo Health Research Center, as well as wild *An. gambiae* reared from larvae collected from the field. The procedure for the bioassays was done as described in 2.3.1 above.

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

Insecticide susceptibility tests were performed using the WHO tube test method in nine sentinel communities: Bunbuna (BYD) Kumbungu and Gbullung (KUD) which were sprayed in 2017; Dimabi and Woribugu (TD), and Nanton and Tarikpaa (SND) where IRS was withdrawn in 2012 and 2015 respectively; and Kulaa and Tugu (TML) which have never been sprayed.

Larvae and pupae of *Anopheles* mosquitoes were collected from breeding sites in and around the sentinel communities and reared to adults for susceptibility tests. Mosquitoes were morphologically identified at

adult stage and only *An. gambiae* s.l. were used for the susceptibility tests. WHO tube tests were conducted for the following insecticides: alpha-cypermethrin 0.05%, bendiocarb 0.1%, pirimiphos-methyl 0.25 %, and DDT 4.0 %.

Additionally, baseline susceptibility status of wild *An. gambiae* s.l. to 13.2mg (per one impregnated paper, 15x12cm) of clothianidin (a neonicotinoid) was determined using papers that were impregnated in-country for WHO tube tests.

After the 24-hour holding period, 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 Assays

2.5.1 WHO Tube Test method

Intensity of resistance to pyrethroids and carbamates was tested using 5x concentrations of alpha-cypermethrin 0.25% and bendiocarb 0.5%, and 10x concentration of alpha-cypermethrin 0.5% using the WHO tube method if resistance was detected from the initial susceptibility tests with 1x diagnostic dose.

2.5.2 CDC Bottle Assay

Using a simplified version of the U.S. Centers for Disease Control and Prevention (CDC) bottle bioassay rapid diagnostic test (RDT) (Brogdon and Chan 2010), the intensity of pyrethroid resistance in *An. gambiae* s.l. from two sentinel sites (Gbullung Township and Kumbungu Township) was measured. These sites have a long history of agriculture activities, and have been the most active vector breeding sites among the IRS sentinel sites making it easy to monitor changes over the years. They have had the longest use of pyrethroids for IRS (2008-2012). Four pre-measured vials provided by the 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

2.6.1 WHO TUBE TEST METHOD

An. gambiae s.l. populations from selected sites, were pre-exposed to piperonyl butoxide (PBO) impregnated papers for 1 hour, before testing them against, alphacypermethrin (0.05%), using the WHO method outlined in Annex A.

After the 24-hour holding period, 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%.

2.6.2 CDC BOTTLE ASSAY

An. gambiae s.l. populations from Gbullung, which showed resistance to deltamethrin were exposed to the effect of PBO (100 µg/bottle) and S.S.S-tributyl phosphorotrithioate 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 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 the molecular analyses indicated below:

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

2.7.1 SPECIES IDENTIFICATION

Morphologically identified *An. gambiae* s.l., were further identified into sibling species, using ribosomal DNA-polymerase chain reaction (PCR) (Scott *et al*, 1993). PCR-RFLP (restriction fragment length polymorphism) was then used to further distinguish *An. gambiae* s.s. and *An. coluzzi* (Fanello *et. al.* 2002).

2.7.2 CIRCUMSPOROZOITE-ELISA EVALUATION

The head and thorax of a proportion of *An. gambiae* s.l. and *An. funestus* were sorted and tested for the presence of circumsporozoite antigens (CS) of *Plasmodium falciparum* sporozoites using enzyme-linked immunosorbent assays (ELISA) described by Wirtz *et al.* (1985). The ELISAs were used to assess the parasite infection rate in the local vectors collected.

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

Samples of surviving and dead mosquitoes from the insecticide susceptibility tests were further analyzed to determine presence of *kdr-w* and *Ace-1* genotypes. The conventional PCR technique described by Martinez Torres *et al*, 1998 and real time PCR described by Chris Bass *et al*, 2007 were used to detect the presence of West Africa *kdr* (Knockdown resistance gene) and the *Ace-1* mutation using the protocol described by Weill *et al*, 2003 in the local *An. gambiae* s.l. vectors.

2.7.4 ANALYSIS OF DATA

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

- Human 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, An. rufipes* and *An. hancocki* were identified in 2017. *An. gambiae* s.l. was the predominant species across all sites, constituting about 96% of the total number (39,439) of *Anopheles* collected. *An. funestus, An. nili, An. pharoensis, An. rufipes and An. hancocki* constituted 0.68%, 2.15%, 1.19%, 0.09%, and 0.01% of the mosquitoes collected, respectively.

Of the 41,131 adult female *Anopheles* mosquitoes collected, 98.4% (40,482/41,131) were collected attempting to bite (i.e., by HLC), 1.4% (594/41,131) were collected resting indoors, and 0.1% (55/41,131) were collected while exiting through windows (Table 2). Most *An. funestus,* of the total 281 collected, were from KUD (52.7%), an IRS district, and TD (31.8%), a district from which IRS was withdrawn.

The majority of *Anopheles* collected was from TML (48%) and KUD (20%). Relatively low numbers of *Anopheles* mosquitoes were collected from all other sites: BYD (2%), GUD (5%), KAD (8%), SND (7%) and TD (10%) (Table 3).

TABLE 2: NUMBER AND TYPE OF *ANOPHELES* SPECIES COLLECTED BY COLLECTION METHOD

Anopheles species	HLC		PSC		Window Exit Trap Collections		TOTAL	
	N	%	N	%	N	%	N	%
An. gambiae s.l.	38,833	*95.93%*	551	*92.76%*	55	*100.00%*	39,439	*95.89%*
An. nili	880	*2.17%*	3	*0.51%*	0	*0.00%*	883	*2.15%*
An. pharoensis	490	*1.21%*	-	*0.00%*	0	*0.00%*	490	*1.19%*
An. funestus s.l.	271	*0.67%*	10	*1.68%*	0	*0.00%*	281	*0.68%*
An. rufipes	5	*0.01%*	30	*5.05%*	0	*0.00%*	35	*0.09%*
An. hancocki	3	*0.01%*	-	*0.00%*	0	*0.00%*	3	*0.01%*
	40,482		**594**		**55**		**41,131**	

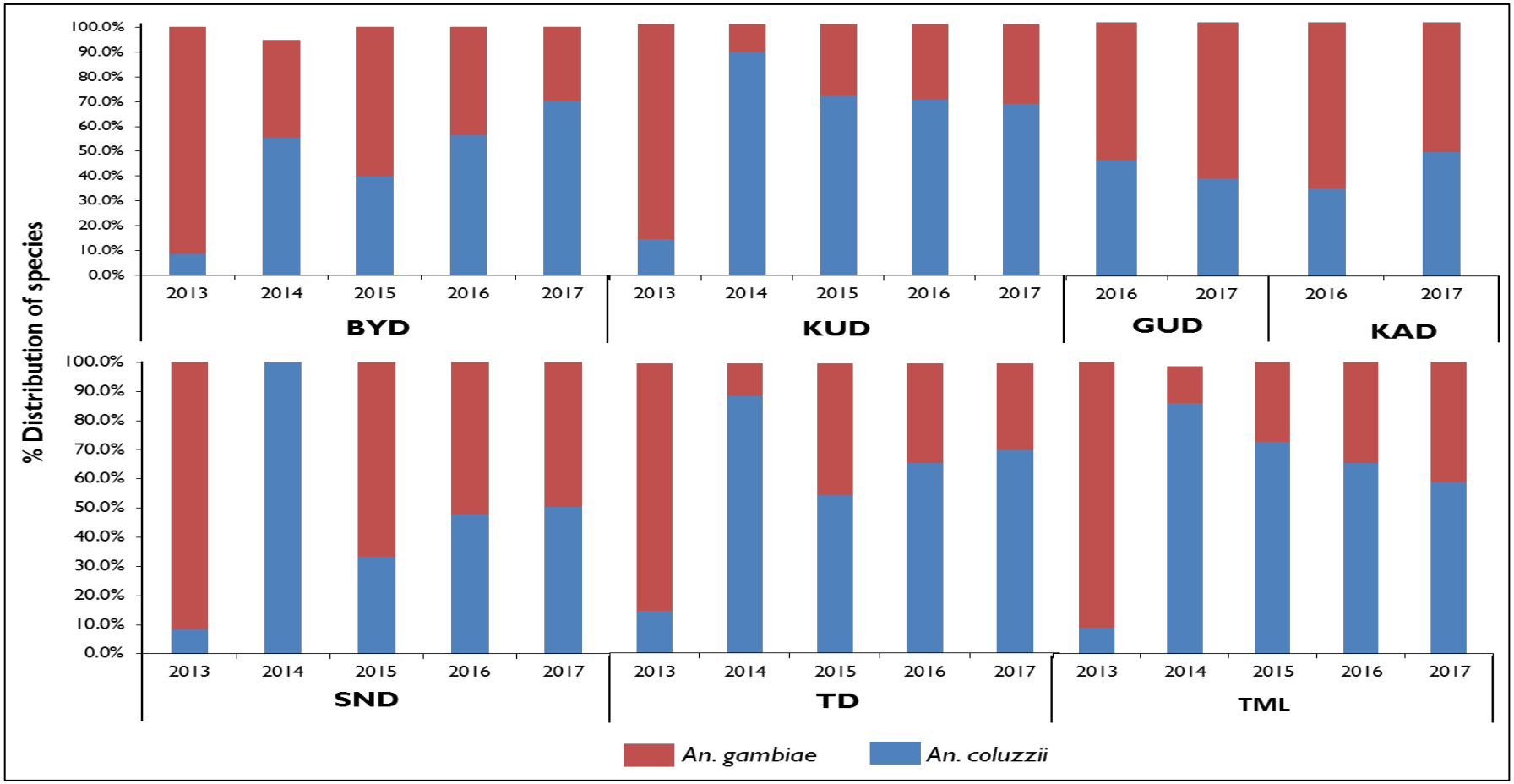
TABLE 3: NUMBER AND TYPE OF *ANOPHELES* SPECIES COLLECTED USING HLC, PSC AND WINDOW EXIT TRAP COLLECTIONS, BY SENTINEL DISTRICT

Anopheles species	BYD	GUD	KAD	KUD	SND	TML	TD	TOTAL
An. gambiae s.l.	828	1885	2904	7914	2699	19108	4101	**39439**
An. nili	21	245	106	90	8	392	21	**883**
An. pharoensis	59	88	42	27	61	192	21	**490**
An. funestus s.l.	19	2	12	148	1	11	88	**281**
An. rufipes	24	2	4	0	0	1	4	**35**
An. hancocki	0	2	0	0	0	0	1	**3**
Total	**951**	**2,224**	**3,068**	**8,179**	**2,769**	**19,704**	**4,236**	**41,131**
	2%	*5%*	*7%*	*20%*	*7%*	*48%*	*10%*	

3.1.2 MOLECULAR IDENTIFICATION

A total of 1,600 mosquitoes (21% of ELISA samples) from all the study areas were analysed by PCR for identification of sibling species of the *An. gambiae* complex. The results indicated that both *An. coluzzii* and *An. gambiae* existed in sympatry in all sentinel sites, with *An. coluzzii* predominating (58%) at most sites except in GUD and KAD. *An. arabiensis* made up only 1.8% of the *An. gambiae* s.l. collected, all of which were collected from Binduli (KAD). Figure 2 shows the yearly variation in populations of *An. coluzzii* and *An. gambiae*. Of the 637 *An. gambiae* s.l. analysed in 2016, 44.9% were *An. gambiae* and 55.1% *An. coluzzii,* whilst in 2017 *An. gambiae* and *An. coluzzii* made up 40.7% and 57.5% respectively of the 1,600 *An. gambiae* s.l. analysed. There was no significant difference (p= 0.068) in the diversity of both species relative to the 2016 populations, although the proportion of *An. coluzzii* has increased over the years in all the study areas.

FIGURE 2[1]: DISTRIBUTION OF *AN. COLUZZII* AND *AN. GAMBIAE* IN IRS INTERVENTION, IRS WITHDRAWN, AND CONTROL DISTRICTS, 2013–2017



[1] Tolon and Kumbungu districts were joined as Tolon Kumbungu district between 2013 and 2014. The district was split since 2015 into Tolon district (TD) and Kumbungu district (KUD). Data Collections in GUD and KAD started in 2016.

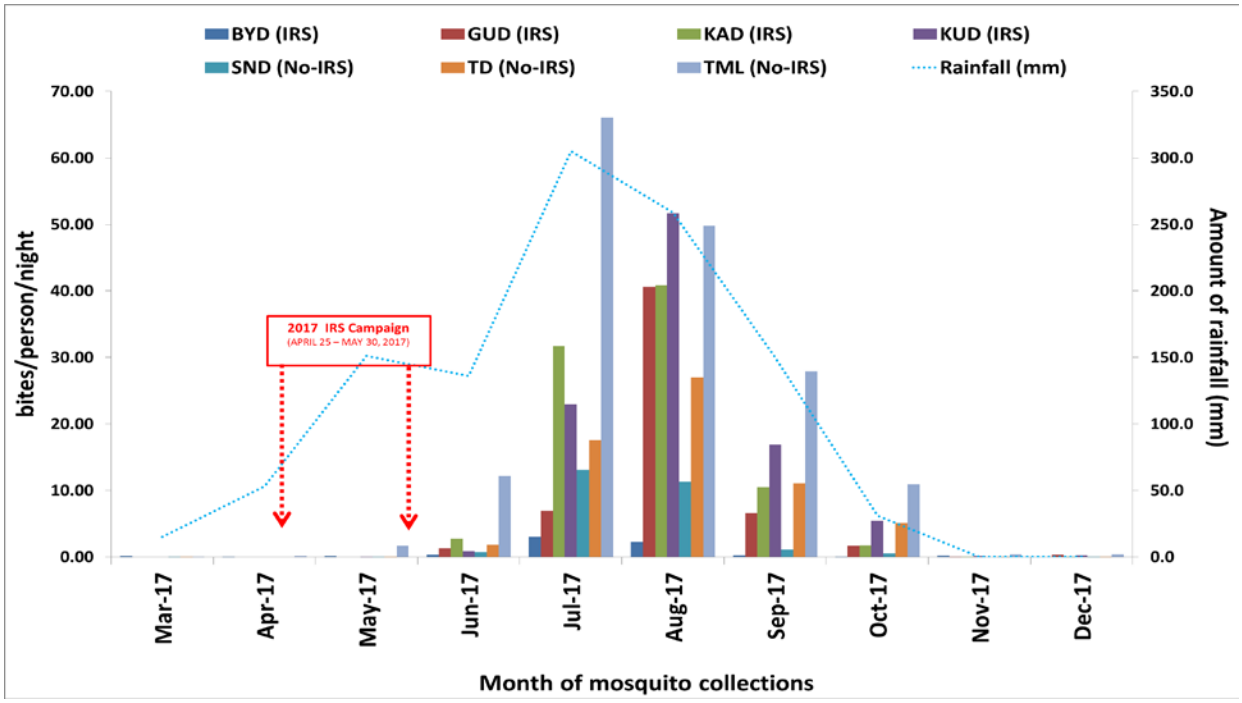
3.2 VECTOR SEASONALITY

The abundance of *An. gambiae* s.l. collected from all sites was positively correlated with the mean rainfall (91.67 mm) recorded in 2017 (Figure 3). The coefficients of correlation were 0.857, 0.613, 0.857, 0.764, 0.860, 0.816 and 0.900 for BYD, GUD, KAD, KUD, SND, TD, and TML, respectively. With the exception of GUD, the correlation was significant for all sites ($p<0.05$).

3.3 BITING RATE

The mean man biting rates for *An. gambiae* s.l., the predominant species collected from all sites, are presented in Figures 3. Comparatively, the average monthly biting rates recorded for TML (control), GUD, and KAD were higher than those recorded for all other sites. The average human biting rates (HBRs) recorded for *An. gambiae* s.l. during the period is shown in Table 4 (in Section 3.4).

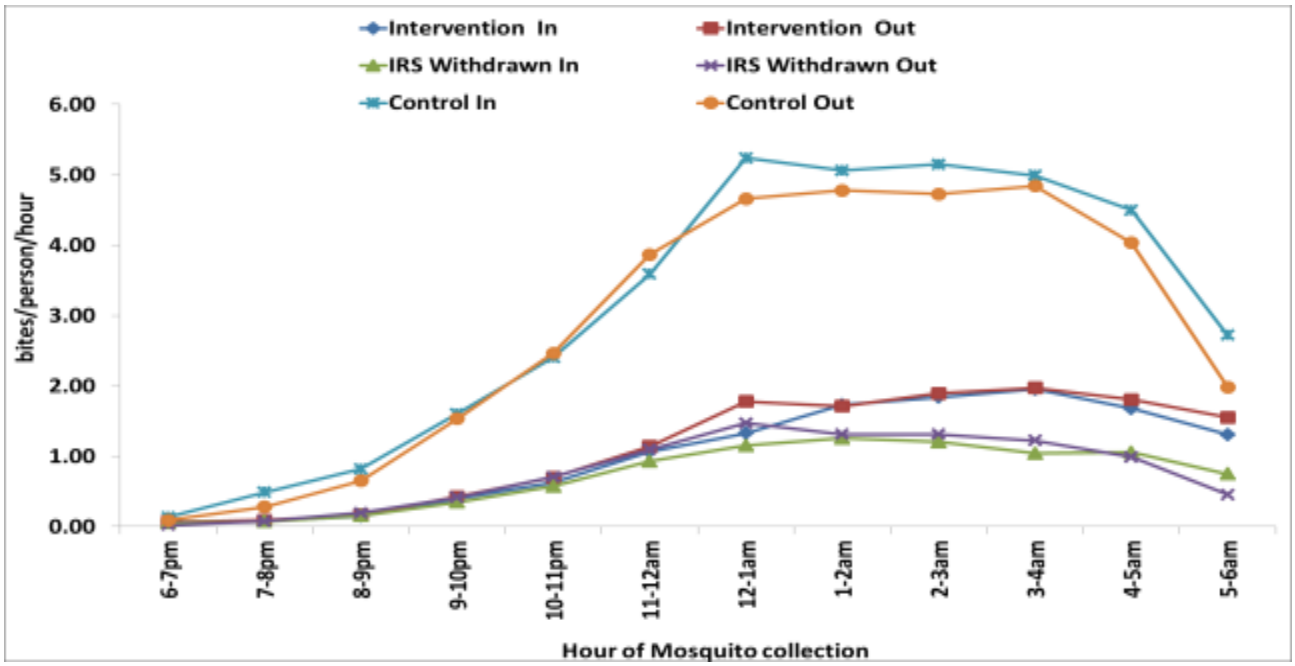
FIGURE 3: MEAN HUMAN BITING RATE OF *AN. GAMBIAE* S.L. FROM SENTINEL SITES AND MEAN RAINFALL, MARCH–DECEMBER 2017



3.4 FEEDING TIME/PATTERN

Figure 4 shows the biting cycle of *An. gambiae* s.l. (the predominant vector species) collected between March and December 2017. 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 district (TML). Peak biting occurred from around 11:00 p.m. to 5:00 a.m. The densities of mosquitoes biting during these peak times were higher in the unsprayed district than in the IRS and IRS withdrawn districts.

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



Variations in indoor biting and outdoor biting densities of *An. gambiae* s.l. between the IRS and non-IRS sites were observed. The differences in the mean indoor and outdoor HBRs were statistically significant at the 0.05 level for all the sites except in KUD. The results show exophagic tendencies of *An. gambiae* s.l. in BYD, GUD, KAD, SND, and TD. In TML, *An. gambiae* s.l. showed more endophagic tendencies (Table 4).

TABLE 4[2]: INDOOR AND OUTDOOR HBR OF *AN. GAMBIAE* S.L., HLC, ALL SENTINEL SITES, 2017

Sentinel Site	Indoor biting rate (b/p/n)	Outdoor biting rate (b/p/n)	Endophagic index	Exophagic index	χ^2	P-value
BYD (IRS)	0.58	0.68	0.46	0.54	4.47	0.0346*
GUD (IRS)	5.30	6.18	0.46	0.54	19.40	0.0011*
KAD (IRS)	8.15	9.39	0.46	0.54	14.11	0.0002*
KUD (IRS)	10.69	10.65	0.50	0.50	0.03	0.8656
SND (IRS withdrawn)	2.58	2.80	0.48	0.52	4.60	0.0320*
TD (IRS withdrawn)	6.04	6.50	0.48	0.52	5.53	0.0187*
Tamale (No-IRS)	18.64	17.22	0.52	0.48	29.62	0.0000*

3.5 RESTING AND EXITING BEHAVIOR OF VECTORS

The mean indoor resting densities of *An. gambiae* s.l. ranged from 0.07 mosquitoes per room in BYD to 1.13 in KAD. Relatively low proportions of half-gravid and gravid females were caught resting indoors in sprayed rooms in the IRS districts (9.1% in BYD, 10.6% in GUD, 9.9% in KAD and 16.2% in KUD) compared with the proportion of gravid females collected from rooms in IRS withdrawn districts (45% in SND, 36% in TD) and 30% in unsprayed TML (Table 5).

[2] * Differences in mean indoor/outdoor biting rates is statistically significant at 0.05 level;
b/p/n = bites/person/night

Very low mosquito numbers were collected using exit traps. However, the mean numbers of mosquitoes exiting the rooms were slightly higher for the IRS areas (0.15 and 0.11 mosquitoes/trap for KAD and KUD, respectively) compared to the unsprayed areas, which ranged from 0.01 to 0.07 mosquitoes/trap. The proportion of gravid female *An. gambiae* s.l. collected from the exit trap collections in KAD (16.7%) and KUD (7.7%) was much lower than the high proportions of gravid females from the TML (52.9%).

TABLE 5[3]: 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
Pyrethrum Spray Collections						
BYD (IRS)	13.6%	77.3%	9.1%	22	320	0.07
GUD (IRS)	12.8%	76.6%	10.6%	47	80	0.59
KAD (IRS)	8.8%	81.3%	9.9%	91	80	1.14
KUD (IRS)	18.9%	64.9%	16.2%	37	160	0.23
SND (IRS withdrawn)	6.1%	49.1%	44.7%	114	240	0.48
TD (IRS withdrawn)	2.3%	61.6%	36.1%	86	160	0.54
Tamale (No-IRS)	5.2%	64.3%	30.5%	154	240	0.64
Total				**551**		
Window Exit Trap Collections						
BYD (IRS)*	-	-	-	0	120	0.00
GUD (IRS)	100.0%	0.0%	0.0%	2	40	0.05
KAD (IRS)	50.0%	33.3%	16.7%	6	40	0.15
KUD (IRS)	11.5%	80.8%	7.7%	26	240	0.11
SND (IRS withdrawn)	0.0%	50.0%	50.0%	2	80	0.03
TD (IRS withdrawn)	0.0%	100.0%	0.0%	2	80	0.01
Tamale (No-IRS)	17.6%	29.4%	52.9%	17	240	0.07
Total				**55**		

[3]*No mosquitoes collected in exit traps in BYD

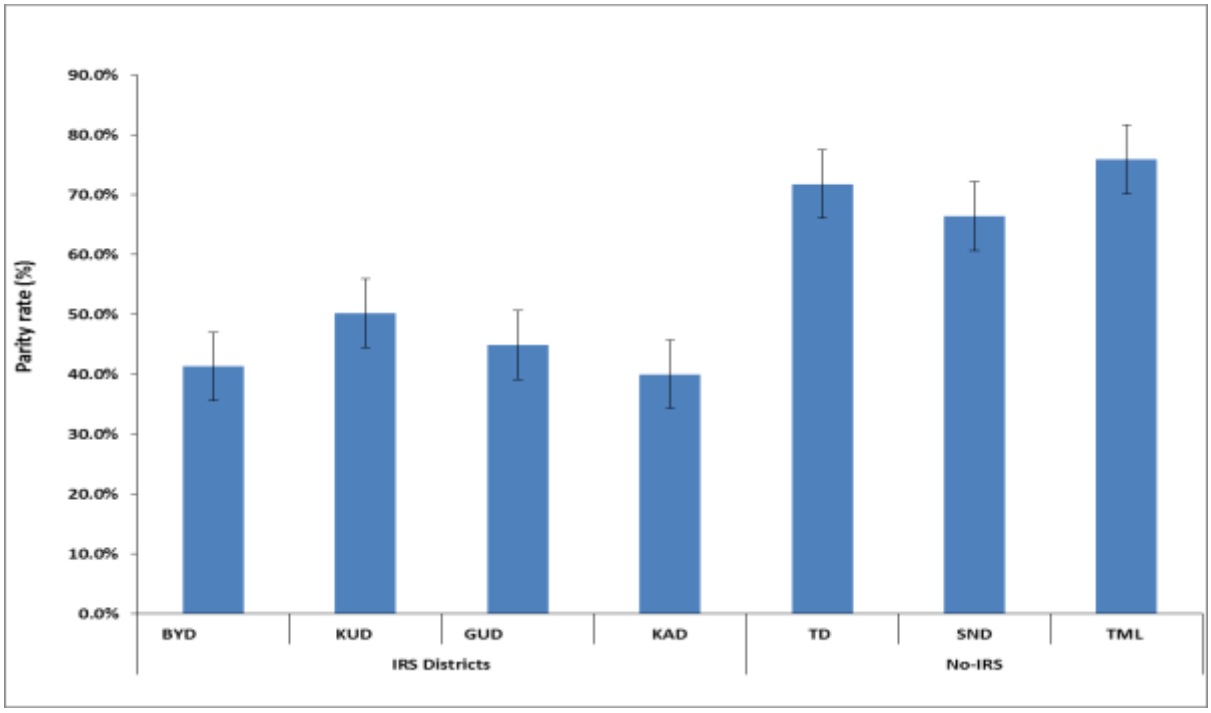
3.6 PARITY RATES

Dissections of *An. gambiae* s.l. mosquitoes collected from the study sites between March and December 2017 revealed that the proportion of parous females collected from TD, SND, and TML (unsprayed districts) was higher than the proportion collected from the IRS districts (BYD, KUD, KAD, and GUD) (Table 6). The differences in parity between the IRS districts of BYD, KUD, and the unsprayed districts of TD and TML were significant ($p<0.0001$) (Figure 5).

TABLE 6: 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)	223	92	41.30%	34.8%	47.7%
GUD (IRS)	241	121	50.21%	43.9%	56.5%
KAD (IRS)	401	180	44.90%	40.0%	49.8%
KUD (IRS)	894	358	40.00%	36.8%	43.3%
SND (IRS withdrawn)	446	296	66.40%	62.0%	70.8%
TD (IRS withdrawn)	586	421	71.80%	68.2%	75.5%
Tamale (No-IRS)	2,067	1568	75.90%	74.0%	77.7%

FIGURE 5: COMPARISON OF MEAN MONTHLY PARITY RATES FOR *AN. GAMBIAE* S.L.



3.7 SPOROZOITE RATES

A total of 7,771 *Anopheles* mosquitoes were assayed by ELISA in 2017 to determine the presence of *Plasmodium falciparum* sporozoites in their salivary glands. *An. gambiae* s.l. constituted over 99% of the numbers examined, followed by *An. funestus*. The sporozoite rates (SR%) of mosquitoes from all the sentinel communities are shown in Table 7. Generally, there was a significant reduction in the sporozoite rates in all the sites compared to 2016. The highest sporozoite rates (0.40%) were detected in Tamale, the control district. GUD and KUD recorded sporozoite rates of 0.16% and

0.17%, respectively. No sporozoites were detected in samples from the other IRS districts (BYD and KAD) or from SND where IRS was withdrawn in 2015.

Most of the sporozoite positive mosquitoes were from samples collected between the rainy months of June and October 2018.

TABLE 7: SPOROZOITE INFECTIONS IN *AN. GAMBIAE AND AN. FUNESTUS* SAMPLED FROM ALL SENTINEL SITES

Sentinel Site	Number Examined by ELISA	Number +ve for Sporozoites	Sporozoite Rate
IRS			
BYD (IRS)	602	0	0.00%
GUD (IRS)	645	1	0.16%
KAD (IRS)	465	0	0.00%
KUD (IRS)	1,209	2	0.17%
No-IRS			
SND (IRS withdrawn)	1,190	0	0.00%
TD (IRS withdrawn)	1,151	1	0.09%
TML (Non-IRS)	2,509	10	0.40%

3.8 ESTIMATION OF EIRS OF VECTORS

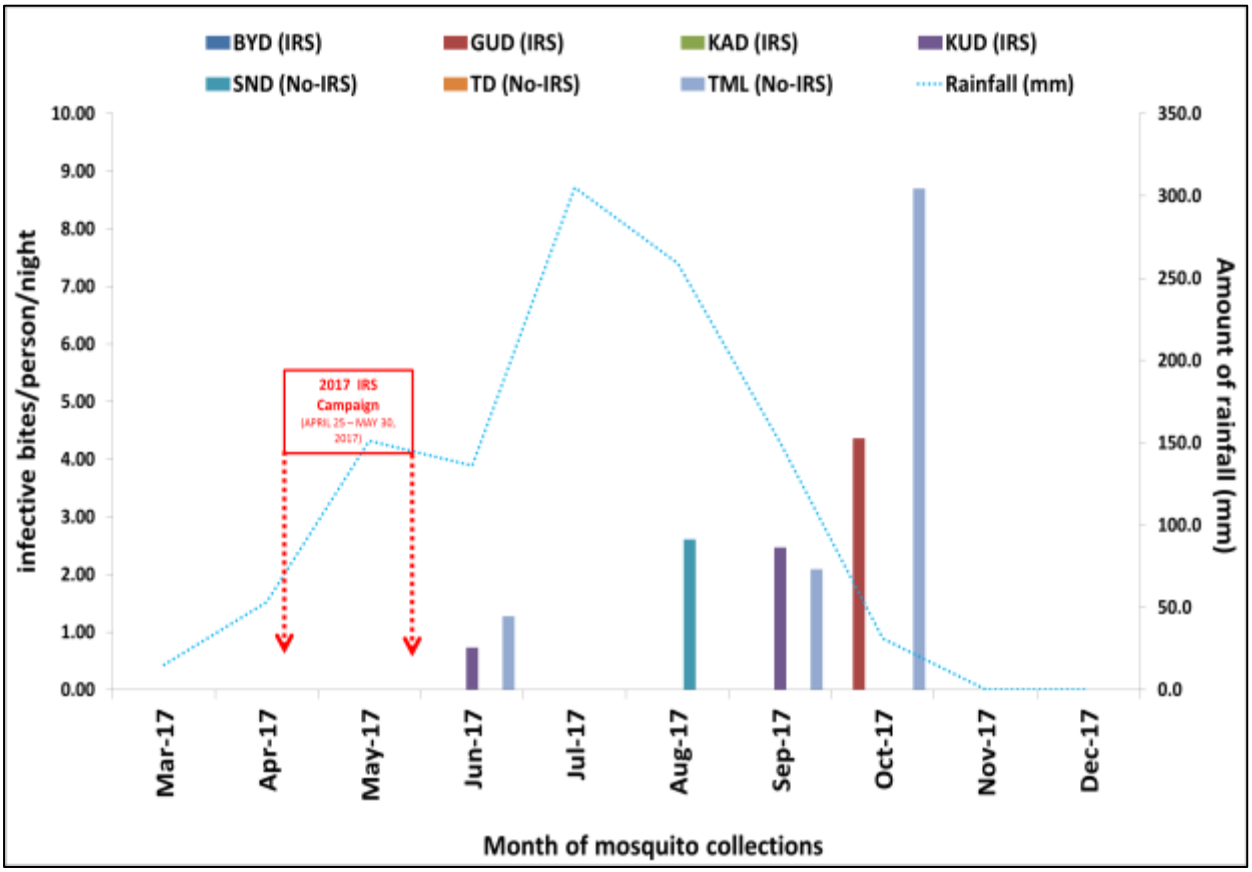
The mean risk of exposure to malaria (EIR) is estimated to be 0.01infective bites/person/night (ib/p/n) for GUD, 0.02 ib/p/n for KUD, 0.01 ib/p/n for TD, and 0.08 ib/p/n for TML. A general decline (about 86%) in EIR was observed in 2017 when compared to 2016. The sum of monthly EIRs (calculated for the months that sporozoites were detected) was 4.4 infective bites/person/year (ib/p/yr) for GUD, 3.19 ib/p/yr for KUD, 2.61 ib/p/yr for TD, and 12.06 ib/p/yr for TML (Table 8).

Monthly trends showed that transmission was seasonal in both IRS and non-IRS districts, with transmission occurring primarily between June and October (Figure 6).

TABLE 8: ENTOMOLOGICAL PARAMETERS OF MALARIA TRANSMISSION, *AN. GAMBIAE* S.L. AND *AN. FUNESTUS*, ALL SENTINEL SITES, MARCH–DECEMBER 2017

Sentinel Site	# Tested by ELISA	CS +ve	Sporozoite Rate	Mean Man Biting Rate (b/p/n)	Nightly EIR (ib/p/n)	Estimated (Annual EIR=∑ monthly EIRs) (ib/p/yr)
IRS						
BYD (IRS)	602	0	0.00%	0.64	**0.00**	**0.00**
GUD (IRS)	645	1	0.16%	5.74	**0.01**	**4.40**
KAD (IRS)	465	0	0.00%	8.81	**0.00**	**0.00**
KUD (IRS)	1,209	2	0.17%	12.50	**0.02**	**3.19**
Non-IRS						
SND (IRS withdrawn)	1,190	0	0.00%	2.69	**0.00**	**0.00**
TD (IRS withdrawn)	1,151	1	0.09%	6.40	**0.01**	**2.61**
TML (Non-IRS)	2,509	10	0.40%	19.74	**0.08**	**12.06**

FIGURE 6: MONTHLY TRENDS IN EIR IN IRS AND NON-IRS SITES, MARCH–DECEMBER 2017



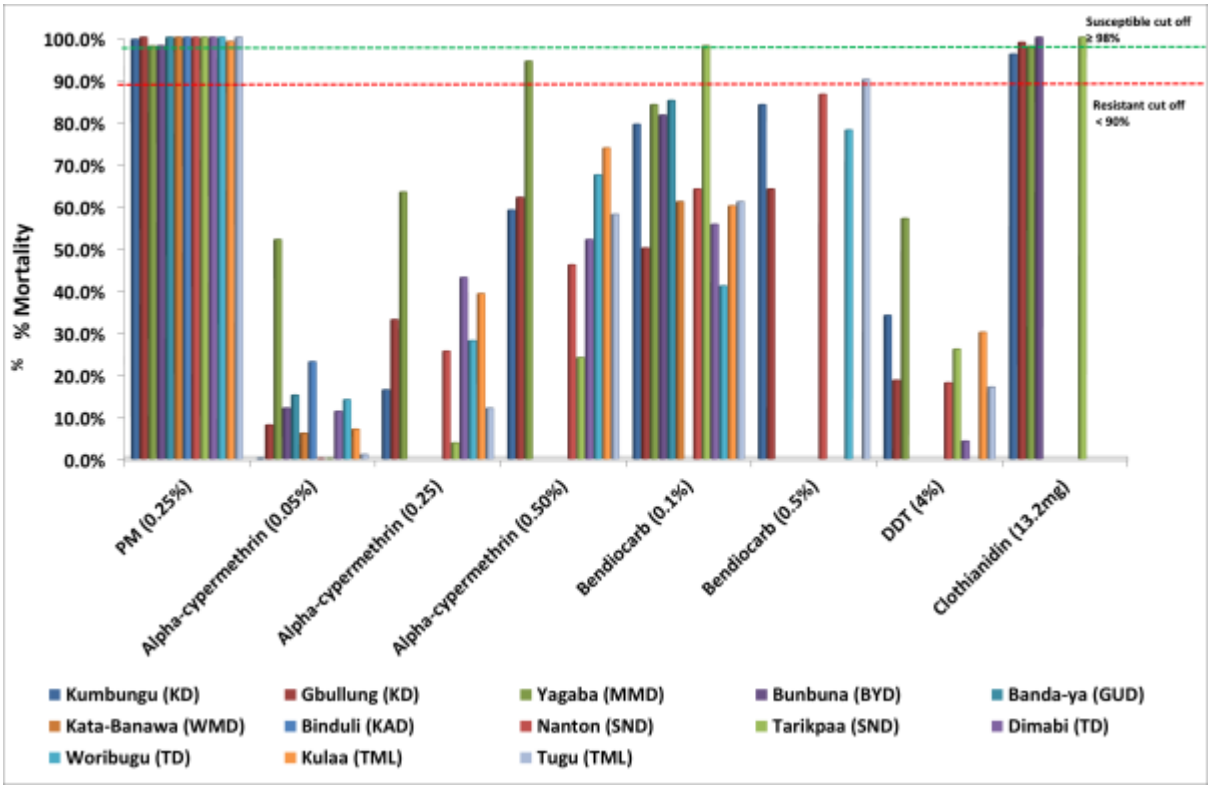
3.9 SPRAY QUALITY TESTS AND RESIDUAL EFFICACY OF ACTELLIC 300CS

Results for the spray quality tests and decay rate of the sprayed pirimiphos-methyl insecticide, Actellic 300CS, on different wall surfaces are presented in Figure A-1, Annex A. The results for the bioassays show the insecticide remains effective above the cut-off mortality level (80 percent 24-hour mortality) on all surfaces in the communities tested 7-8 months post-IRS, depending on the type of surface sprayed.

3.10 INSECTICIDE SUSCEPTIBILITY – WHO TUBE TEST RESULTS

An. gambiae s.l. mosquitoes from both IRS and non-IRS districts were susceptible to pirimiphos-methyl with mortalities between 98% and 100%. Similarly, the vectors from selected sites (Yagaba (MMD), Gbullung (KUD), Bunbuna (BYD) were susceptible to clothianidin (13.2mg). However, mosquitoes from Kumbungu showed possible resistance to clothianidin (13.2mg). The mosquitoes tested from all sites showed resistance to DDT and 0.05%, 0.25% and 0.50%x concentrations of alphacypermethrin, except in Yagaba where the vectors showed possible resistance to 0.05% concentration of alphacypermethrin. *An. gambiae* s.l. was resistant to 0. 1% and 0.5% concentrations of bendiocarb in most of the sites except Tarikpaa where *An. gambiae* s.l. was susceptible (98%) to bendiocarb 0.1%, (see Figure 7; Table A-1 in Annex A).

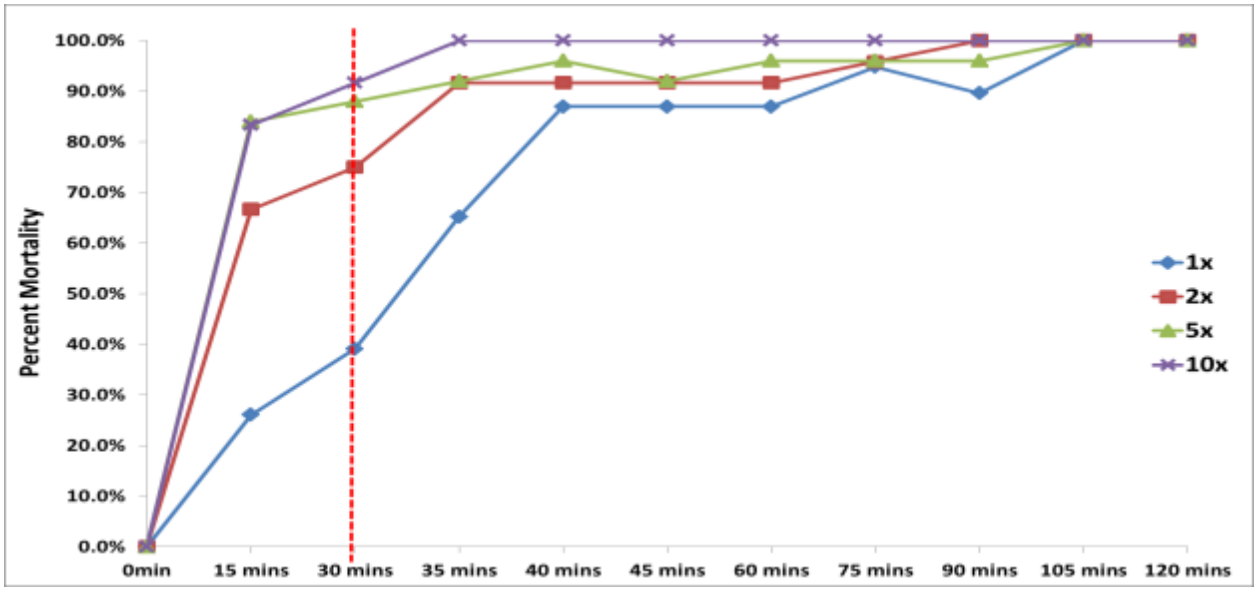
FIGURE 7: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. AGAINST SELECTED WHO-RECOMMENDED INSECTICIDES FOR IRS



3.11 RESISTANCE INTENSITY ASSAYS – CDC BOTTLE BIOASSAYS

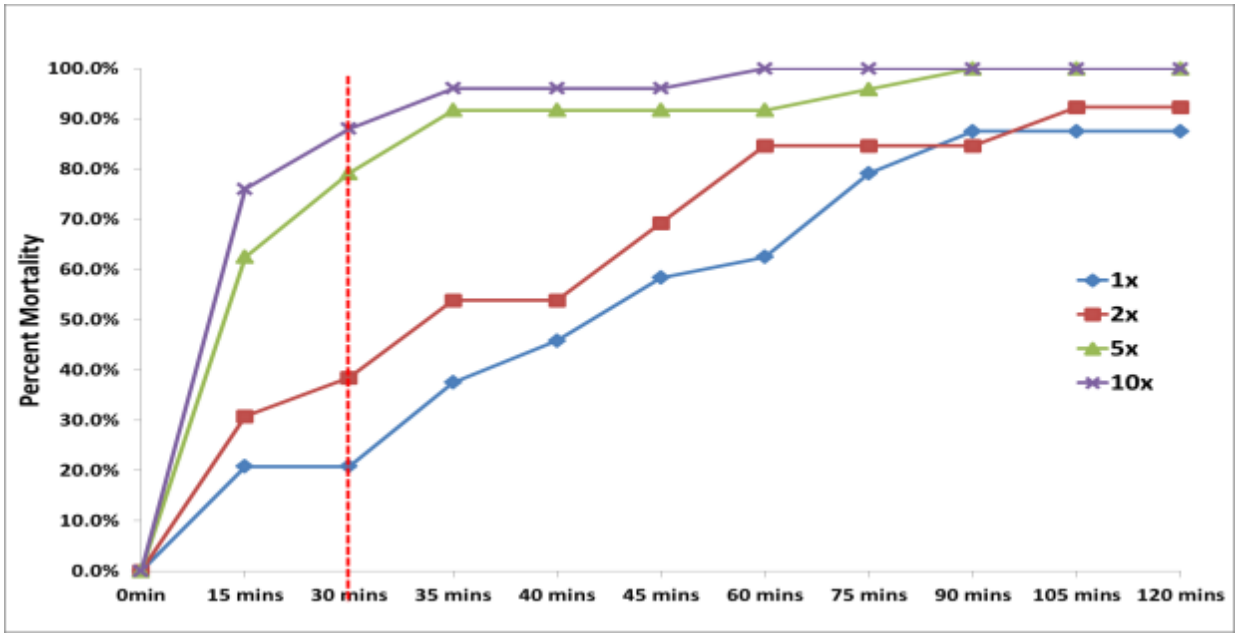
An. gambiae s.l. mosquitoes from Gbullung and Kumbungu were resistant to 1x, 2x, 5x and 10x diagnostic doses of deltamethrin and bendiocarb, based on the CDC bottle bioassay recommended thresholds (Figures 8-11).

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



Note: red line indicates susceptibility threshold

FIGURE 9: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM KUMBUNGU EXPOSED TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN, USING THE CDC-RESISTANCE INTENSITY RDT



Note: red line indicates susceptibility threshold

FIGURE 10: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM GBULLUNG EXPOSED TO DIFFERENT CONCENTRATIONS OF BENDIOCARB, USING THE CDC-RESISTANCE INTENSITY RDT

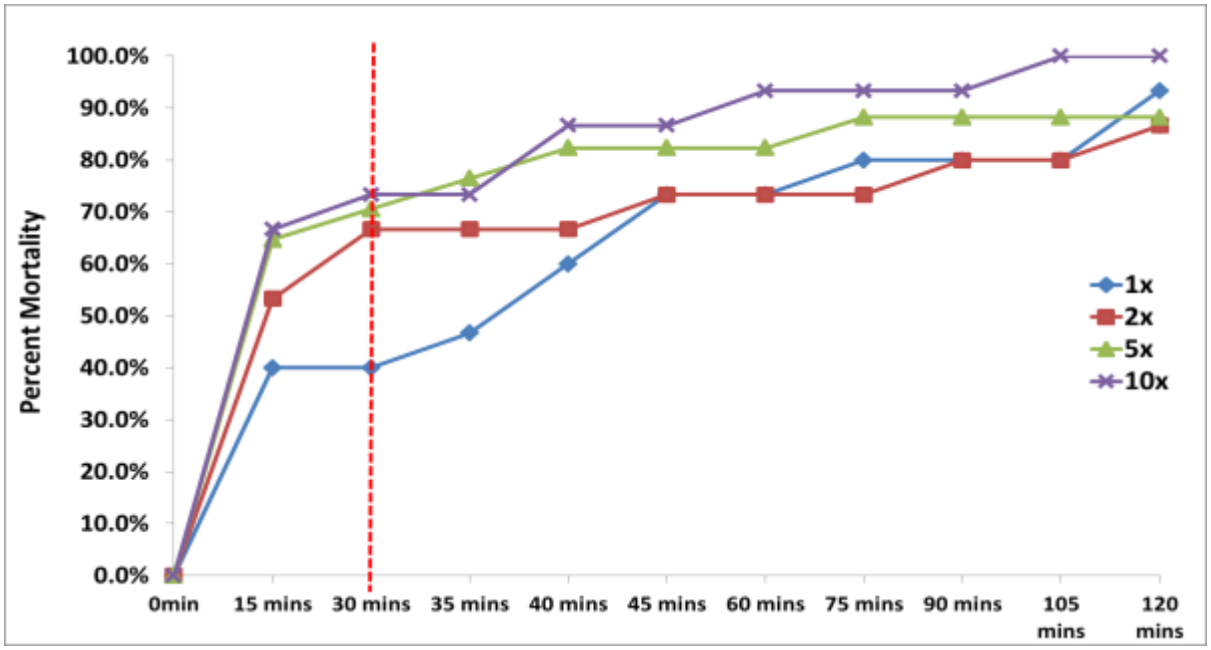
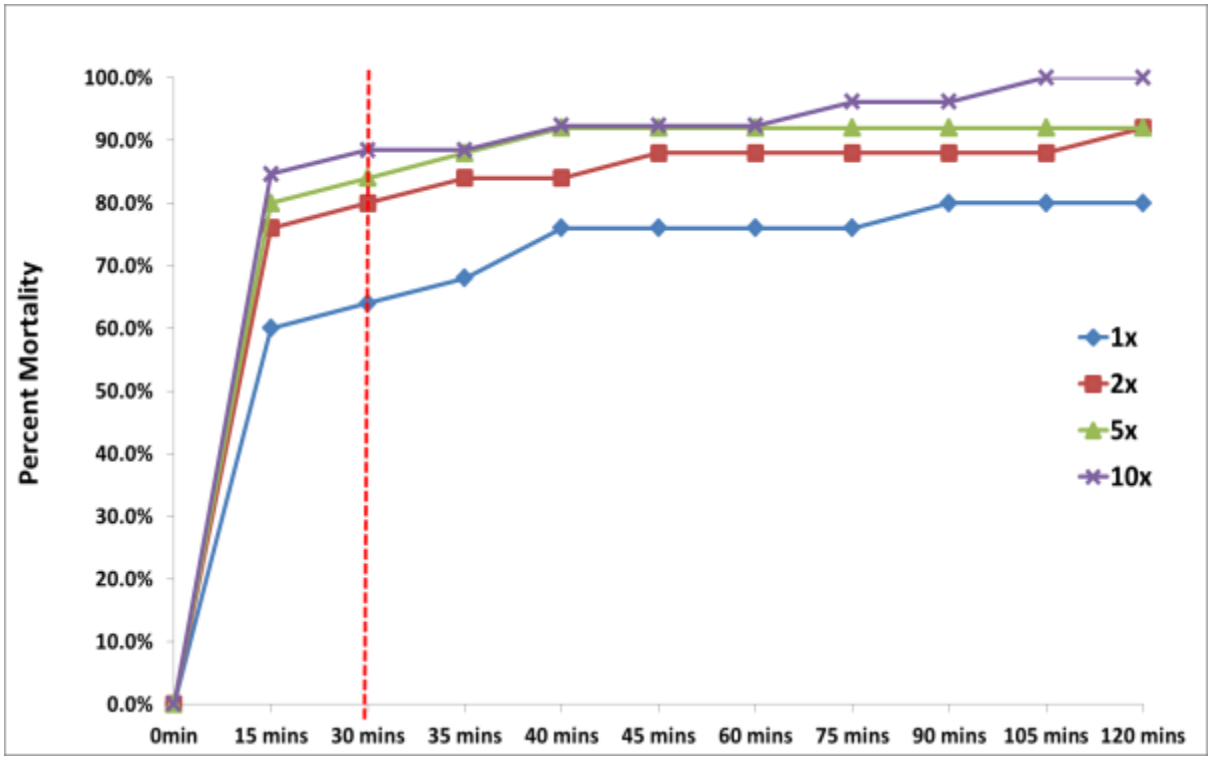


FIGURE 11: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM KUMBUNGU EXPOSED TO DIFFERENT CONCENTRATIONS OF BENDIOCARB, USING THE CDC-RESISTANCE INTENSITY RDT

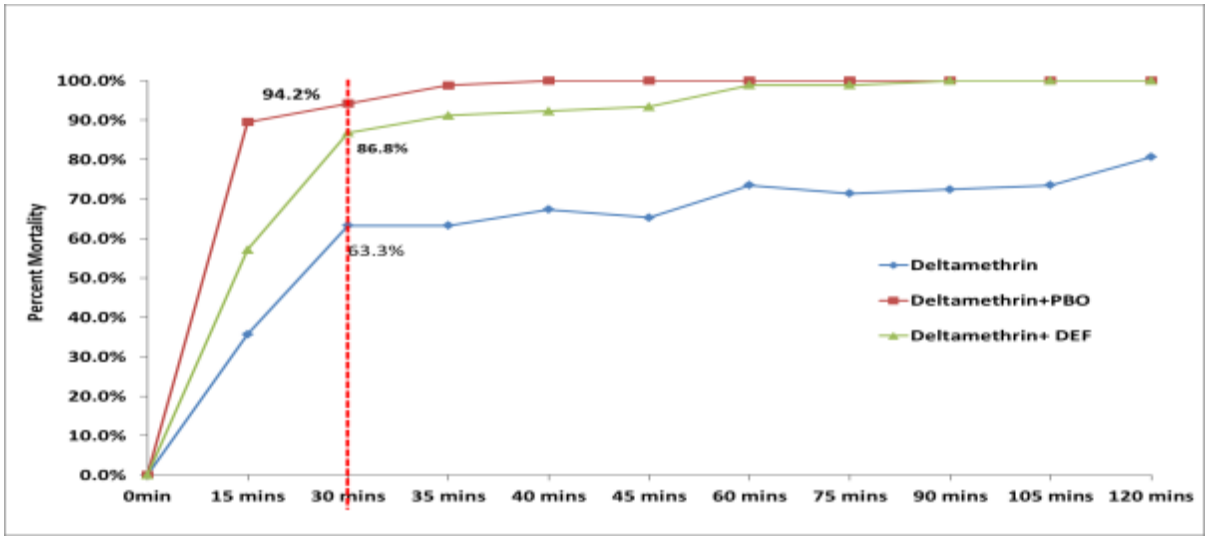


3.12 SYNERGIST ASSAYS

An. gambiae s.l. from Kumbungu exposed to synergist (PBO or DEF) before being tested against deltamethrin showed high mortalities (94% for PBO tests; 86.8% for DEF tests) compared to those with no prior exposure to the synergists (63%) ($p<0.05$). Similarly, exposing mosquitoes from Gbullung to synergist prior to testing them with deltamethrin showed significantly higher mortality (86.9% for PBO tests; 69.7% for DEF tests) compared to mortalities recorded for test without any prior exposure to synergist 41.0% (Figures12 and13).

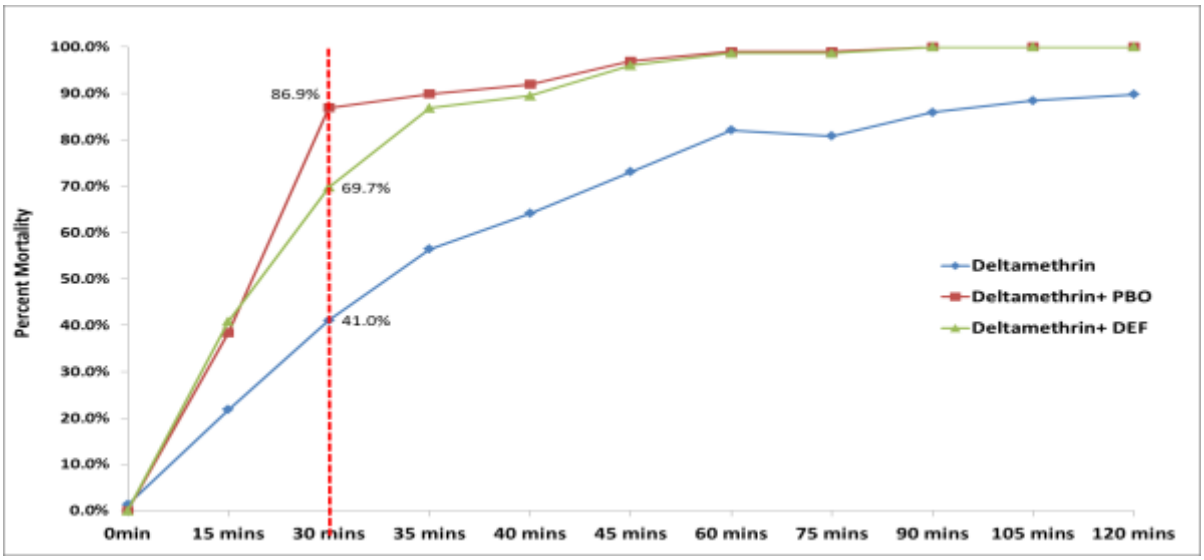
The WHO synergist assays also showed that prior exposure to PBO increased the susceptibility of *An. gambiae* s.l. to alphacypermethrin. In Kulaa, prior exposure to PBO resulted in susceptibility (99%) to alphacypermethrin 0.05% as compared to previous test with the 1x concentration (0.05%) which showed mortalities as low as 7.0% (Figure 14). However, the effect of PBO on the 24 hour mortalities of *An. gambiae* s.l. from Tarikpaa was reduced.

FIGURE 12: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM KUMBUNGU EXPOSED TO DELTAMETHRIN, DELTAMETHRIN + PBO AND DELTAMETHRIN + DEF, CDC METHOD



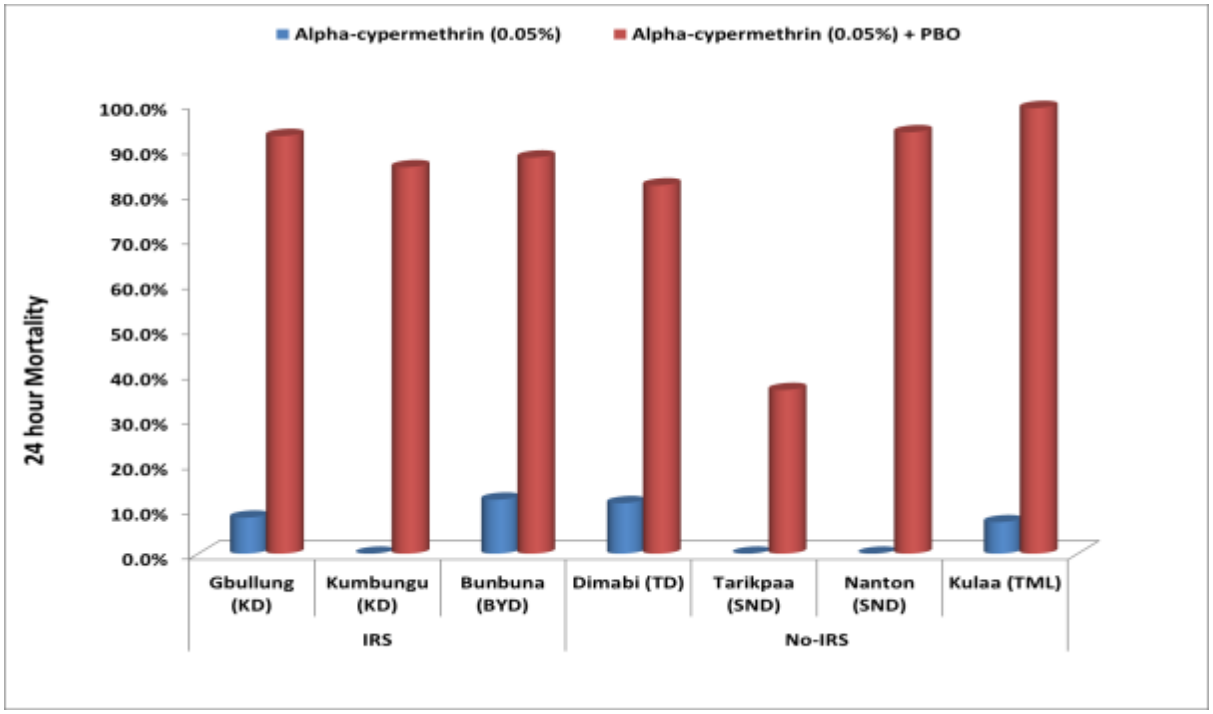
Note: red line indicates susceptibility threshold

FIGURE 13: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM GBULLUNG EXPOSED TO DELTAMETHRIN, DELTAMETHRIN + PBO AND DELTAMETHRIN + DEF , CDC METHOD



Note: red line indicates susceptibility threshold

FIGURE 14: MORTALITY FOR *AN. GAMBIAE* S.L. FROM IRS AND NON-IRS SITES EXPOSED TO ALPHA-CYPERMETHRIN AND ALPHA-CYPERMETHRIN + PBO, WHO TUBE METHOD.



3.13 RESISTANCE MECHANISMS

Two target-site gene mutations were investigated in *An. gambiae s*.l. mosquitoes. The first, which consists of a leucine–phenylalanine substitution at amino acid position 1014, is widespread in West Africa (hitherto called *kdr-w*) and is responsible for pyrethroid and DDT resistance. On the other hand, *Ace-1* gene is responsible for organophosphate and carbamate resistance. A total of 570 *An. gambiae* s.l. samples were analysed for the presence of *kdr-w* and *Ace-1*.

3.13.1 KNOCKDOWN RESISTANCE (*KDR*-WEST)

Molecular analyses showed that the *kdr-w* homozygous resistant (RR) variant genotype was predominant among both *An. gambiae* and *An. coluzzii* from all the communities tested (Table 9). The frequency of the *kdr-w* ranged from 0.91 to 1.0 in all the study sites. Figure 15 shows the trends in the distribution of *kdr-w* genotypes in molecular forms of *An. gambiae* s.l. from the IRS and non-IRS areas in 2016 and 2017. There was a relative decline in the frequency of *kdr-w* in the population of *An. gambiae* in 2017 compared to 2016 in some sites.

3.13.2 *ACE-1* GENE

The results of the *Ace-1* gene analysis showed that over 71% (406) of the mosquitoes possess the homozygous susceptible alleles (SS) and this was predominant in *An. gambiae* s.l. (Figure 16). However, the number of homozygote resistant (RR) genotypes has increased in the population compared to 2016. The frequency of the alleles ranged from 0.10 in Banda-ya (GUD) to 0.58 in Kpemale (BYD) Table10.

TABLE 9: DISTIBUITION AND FREQUENCY OF *KDR-WEST* GENOTYPES IN *AN. GAMBIAE* AND *AN. COLUZZII* FROM IRS AND NON-IRS AREA, 2017

District	Sentinel Site	Total Ex.	*An. gambiae*			*An. coluzzii*			Overall *kdr-w freq*
			RR	RS	SS	RR	RS	SS	
BYD	Nasuan	30	10	3	1	13	1	2	0.94
	Kpemale	40	13	7	0	15	1	4	0.94
	Bunbuna	30	8	4	2	12	3	1	0.94
	Yunyoo	40	16	10	2	8	2	2	0.94
GUD	Banda-ya	40	20	3	1	15	1	0	0.99
KAD	Binduli	40	18	8	0	12	2	0	1.00
KUD	Gupanarigu	45	17	7	2	15	4	0	0.97
	Gbullung	35	15	5	0	10	4	1	0.98
SND	Nanton	30	16	3	0	7	2	2	0.96
	Diare	35	10	6	0	12	4	3	0.95
	Tarikpaa	30	12	5	0	10	2	1	0.98
TD	Woribugu	30	12	8	1	7	2	0	0.98
	Dimabi	30	14	5	0	8	2	1	0.98
TML	Tugu	40	18	5	3	10	1	3	0.91
	Yong	38	15	4	4	11	2	2	0.91
	Kulaa	37	18	5	0	10	3	1	0.98

TABLE 10: DISTRIBUTION AND FREQUENCY OF *ACE-1* (G119S) GENOTYPES IN *AN. GAMBIAE* AND AN. COLUZZII FROM IRS AND NON-IRS AREAS, 2017

District	Sentinel Site	Total Ex.	*An. gambiae*			*An. coluzzii*			Overall *Ace-1 freq*
			RR	RS	SS	RR	RS	SS	
BYD	Nasuan	30	6	3	14	2	1	4	0.53
	Kpemale	40	5	7	8	3	5	12	0.58
	Bunbuna	30	4	1	13	2	3	7	0.44
	Yunyoo	40	5	4	10	4	3	14	0.51
GUD	Banda-ya	40	0	1	18	0	3	18	0.10
KAD	Binduli	40	1	3	16	1	2	17	0.21
KUD	Gupanarigu	45	2	5	20	0	4	14	0.28
	Gbullung	35	0	5	17	1	4	8	0.31
SND	Nanton	30	0	3	13	3	2	9	0.33
	Diare	35	1	2	20	3	0	9	0.26
	Tarikpaa	30	0	4	10	1	3	12	0.29
TD	Woribugu	30	1	6	7	0	2	14	0.32
	Dimabi	30	6	3	12	1	0	8	0.46
TML	Tugu	40	2	7	15	3	1	12	0.40
	Yong	38	3	3	11	3	2	16	0.39
	Kulaa	37	1	3	16	2	3	12	0.30

FIGURE 15[4]: YEARLY TRENDS IN THE DISTRIBUTION OF *KDR-W* GENOTYPES IN *AN. GAMBIAE* S.L. FROM IRS AND NON-IRS AREAS, 2016 AND 2017

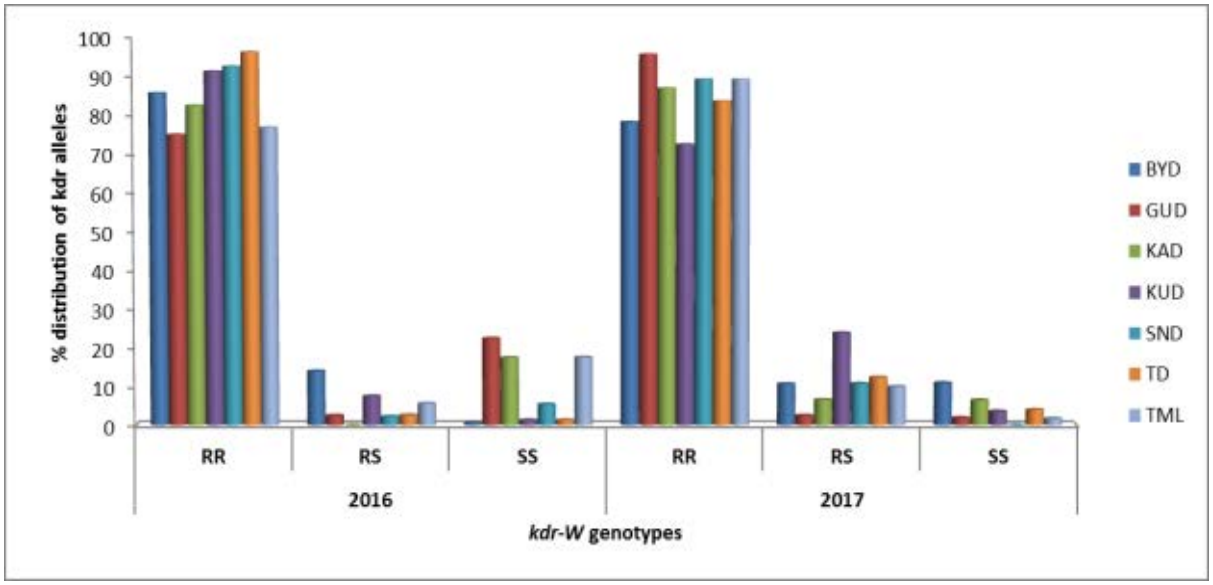
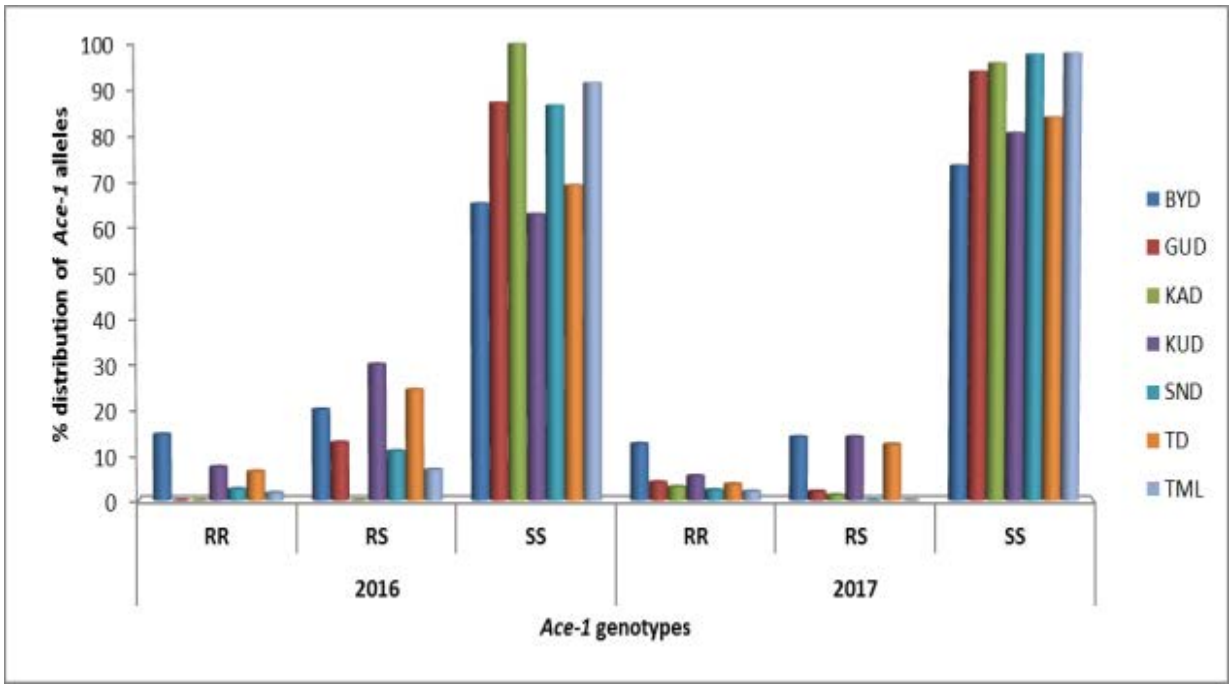


FIGURE 16: YEARLY TRENDS IN THE DISTRIBUTION OF *ACE-1* GENOTYPES IN *AN. GAMBIAE* S.L. FROM IRS AND NON-IRS AREAS, 2016 AND 2017



[4] Data collection in GUD and KAD started in 2016

4. DISCUSSION

Entomological monitoring results indicate that *An. gambiae* s.l. remains the predominant *Anopheles* vector species in all the study sites, making up more than 98% of the total *Anopheles* species collected. *An. coluzzii* and *An. gambiae* were present in sympatry at all the sites in varying proportions, as has been observed in previous years. Relatively low numbers *of An. arabiensis* (1.8% of all samples analysed by PCR) were found in Binduli, Karaga district. Compared to 2016, an increase in the proportion of *An. coluzzii* populations was found in all sites in 2017. More than 98% of the *Anopheles* species collected were from HLC. The very low indoor resting density of *An. gambiae* s.l. is not clearly understood but could be due to a number of factors, including the fumigant effect of the IRS insecticide, repellent activity of pyrethroids in LLINs, and the design of houses in the region, which have no open eves and doors that are closed tightly during the night.

Biting rates of the vector species increased with the onset of the rains as has been observed during previous years. Mosquito populations begin to increase soon after the rains and peak towards the end of the rainy season. This seems different from some ecology where mosquito densities peak immediately after the rains, but is in agreement with the predominance of *An. gambiae* s.l., which prefers areas with temporary rain pools more than permanent water bodies.

The results show exophagic tendencies of *An. gambiae* s.l. in BYD, GUD, KAD, SND, and TD. In TML, *An. gambiae* s.l. showed more endophagic tendencies. *An. gambiae* s.l. from KUD showed equal preference for host either outdoor or indoor. This outdoor behavior could possibly be in response to the IRS and high coverage and use of long-lasting insecticide-treated bed nets.

An. gambiae s.l. in the tested sites remain highly resistant to the pyrethroids (alpha-cypermethrin and deltamethrin) tested, possibly resulting from selection pressure maintained by the continuous distribution and use of pyrethroid-impregnated nets, as well as the use of pyrethroids in aerosol form. The detection of resistance to bendiocard in most sites and possible resistance to clothianidin in Kumbungu could be due to the long history of pesticide use for agriculture purposes in the study area. However, vectors from most sites are susceptible to pirimiphos-methyl and clothianidin.

The detection of relatively high frequency of homozygote resistant *Ace-1* genotype in mosquito samples from the IRS districts (BYD and KUD) suggests that the vector species (*An. gambiae* and *An. coluzzii*) could be building up some tolerance 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). This could also account for the general resistance of *An. gambiae* s.l. to 1x and 5x concentrations of bendiocarb in most of the sites (except in Tarikpaa).

The result from the synergist assays suggests that oxidases and esterases could be contributing to resistance observed in the local vector species from Gbullung and Kumbungu (IRS areas). 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).

Parity rates in the IRS areas show that significantly fewer older mosquitoes were collected in the sprayed communities. The re-introduction of IRS in GUD and KAD could account for the suppression of the population of parous (older) females in 2017 compared with the unsprayed districts.

There was a marked decline in malaria transmission intensity (EIR) in all study sites as compared to 2016. However, the sum of monthly EIRs was relatively higher in the unsprayed district than the IRS districts. Generally, malaria transmission also declined in the non-IRS areas such as Tamale, the control district, and Tolon, where IRS was withdrawn in 2012. This reduction may have been

facilitated by the presence of other interventions such as LLINs which are known to still provide protection even in the presence of pyrethroid resistance; as well as improved housing and other traditional methods adapted to prevent mosquito entry into rooms. There was a mass LLIN distribution campaign before the transmission season and 2016 spray campaign in the northern region covering all IRS and non-IRS districts. In all study areas, malaria transmission occurred mostly within the rainy season (June - October). A 7-8 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.

The general decline or relative reductions in transmission intensity in TD and TML, and no detection of infected mosquitoes in SND (which was not sprayed) shows that factors other than varying IRS acceptance and coverages, such as rainfall, ITN usage and improved housing (door and window screening) observed in most sites in recent times, could have affected transmission indices.

5. Conclusion and Recommendation

IRS continues to suppress the EIR and parity rates of mosquito vectors in IRS areas in comparison to unsprayed communities and IRS withdrawn communities. The re-introduction of spraying in GUD and KUD seems to have contributed to reduced longevity and reduction in malaria transmission intensity in the main vector in the sprayed communities.

It is recommended to raise the number of samples to be analyzed for sporozoite infections to increase the chance of detecting sporozoite infections in the subsequent years work. It is also recommend expanding susceptibility testing for clothianidin and chlorfenapyr in preparation for implementing IRS insecticide rotation strategies and availability of next gen LLINs, respectively.

ANNEX A. WHO BIOASSAY TESTS

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 0.05%, 0.25%, 0.5%;
- Carbamates: bendiocarb 0.1%;
- Organophosphate: pirimiphos-methyl 0.25%
- Organochlorine: DDT 4%
- Neonicotinoid: clothianidin 13.2mg

Steps:

- 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.

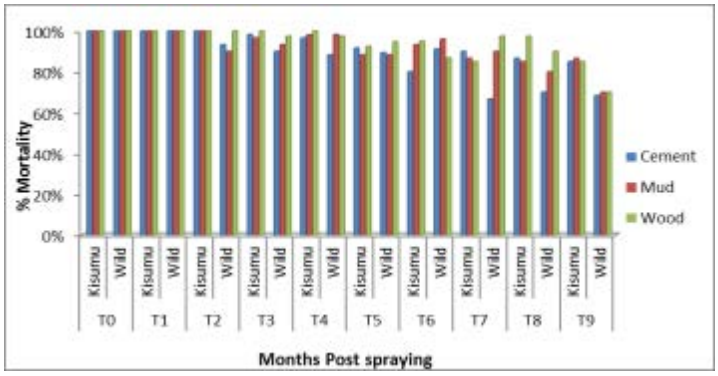
TABLE A-1: SUMMARY OF WHO INSECTICIDE RESISTANCE TEST RESULTS SHOWING INSECTICIDE RESISTANCE STATUS OF *AN. GAMBIAE* S.L. TESTED AGAINST SELECTED INSECTICIDES, 2017

Sentinel Site	Pirimiphos-methyl (0.25%)	Alpha-cypermethrin (0.05%)	Alpha-cypermethrin (0.25%)	Alpha-cypermethrin (0.50%)	Alpha-cypermethrin + PBO	Bendiocarb (0.1%)	Bendiocarb (0.5%)	DDT (4%)	Clothianidin (13.2mg)
IRS Sites									
Kumbungu	200 *(99.0%)*	100 *(0.0%)*	100 *(18.0%)*	100 *(59.0%)*	99 *(85.9%)*	92 *(79.3%)*	100 *(84.0%)*	100 *(34.0%)*	100 *(96.0%)*
Gbullung	100 *(100.0%)*	100 *(8.0%)*	100 *(33.0%)*	100 *(62.0%)*	97 *(92.8%)*	100 *(50.0%)*	100 *(64.0%)*	100 *(22.0%)*	100 *(98.8%)*
Yagaba	96 *(97.9%)*	100 *(52.0%)*	98 *(63.3%)*	88 *(94.3%)*		100 *(84.0%)*		100 *(57.0%)*	96 *(97.90%)*
Bunbuna	100 *(98.0%)*	100 *(12.0%)*			100 *(88.0%)*	96 *(81.5%)*			100 *(100.0%)*
Banda-ya	100 *(100.0%)*	96 *(15.1%)*				100 *(85.0%)*			
Kata-Banawa	97 *(100.0%)*	100 *(6.0%)*				100 *(61.0%)*			
Binduli	100 *(100.0%)*	100 *(23.0%)*							
Non-IRS									
Nanton	100 *(100.0%)*	100 *(0.0%)*	98 *(25.5%)*	100 *(46.0%)*	94 *(93.6%*	100 *(64.0%)*	96 *(86.5%)*	100 *(18.0%)*	
Tarikpaa	198 *(100.0%)*	100 *(0.0%)*	80 *(3.8%)*	100 *(24.0%)*	99 *(36.4%*	100 *(98.0%)*		100 *(26.0%)*	94 *(100.0%)*
Woribugu	100 *(100.0%)*	100 *(14.0%)*	100 *(28.0%)*	98 *(67.3%)*		100 *(41.0%)*	100 *(78.0%)*		
Dimabi	100 *(100.0%)*	98 *(11.2%)*	100 *(43.0%)*	100 *(52.0%)*	99 *(81.8%)*	92 *(55.6%)*		80 *(4.2%)*	
Kulaa	100 *(99.0%)*	100 *(7.0%)*	97 *(39.2%)*	99 *(73.7%)*	100 *(99.0%)*	100 *(60.0%)*		100 *(30.0%)*	
Tugu	100 *(100.0%)*	100 *(1.0%)*	100 *(12.0%)*	100 *(58.0%)*		100 *(61.0%)*	100 *(90.0%)*	100 *(17.0%)*	

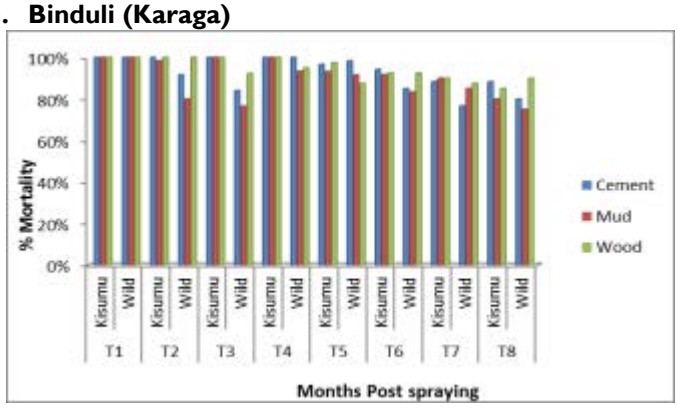
Resistant Potentially resistant Susceptible

FIGURE A-1: IRS RESIDUAL EFFICACY USING KISUMU AND WILD *AN. GAMBIAE* S.L. MOSQUITOES

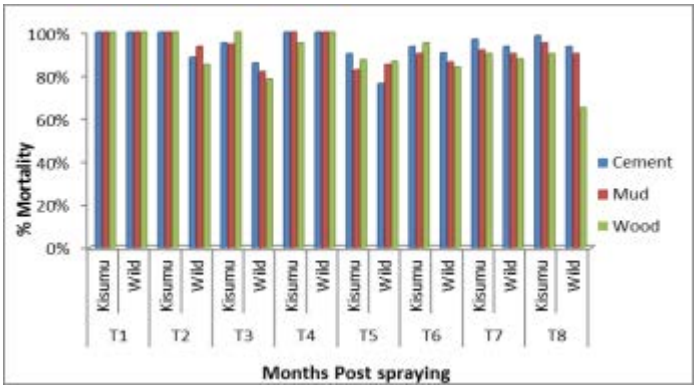
F. Bunbuna(Bunkpurugu Yunyoo)



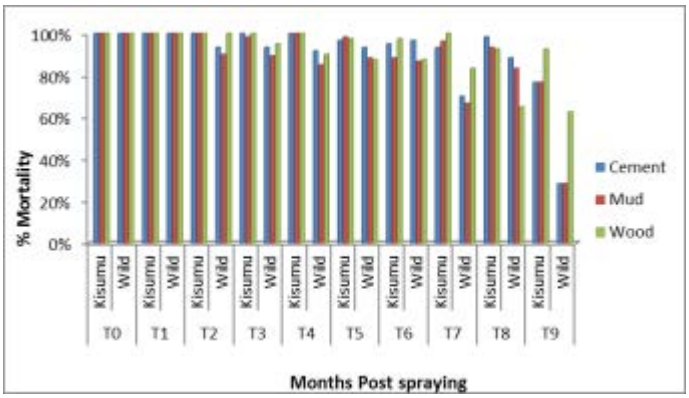
G. Binduli (Karaga)



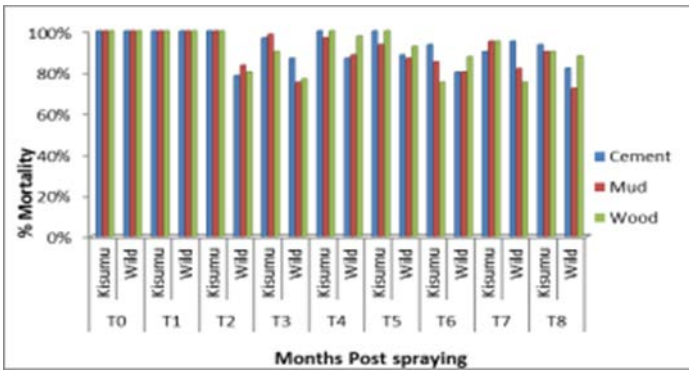
A. Bandaya (Gushegu)



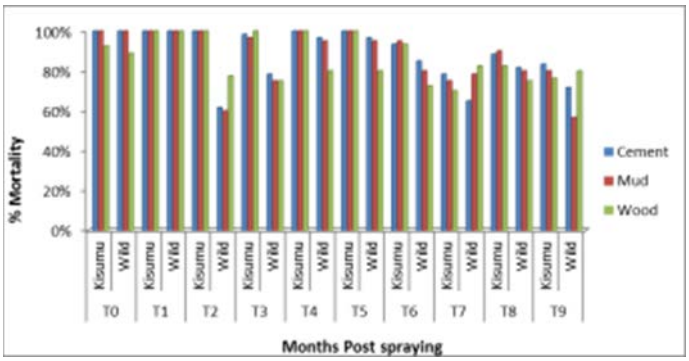
B. Dabari (East Mamprusi)



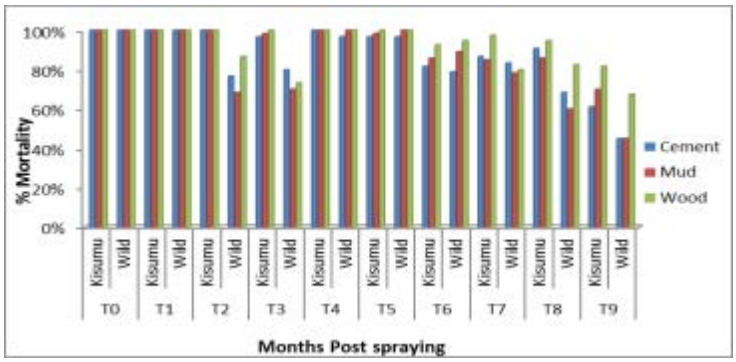
C. Gupanarigu (Kumbungu)



D. Kariminga (West Mamprusi)



E. Kuuba (Mamprugu Moaduri)



ANNEX B. REFERENCES

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