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PMI VECTORLINK GHANA ANNUAL ENTOMOLOGICAL MONITORING REPORT FOR NORTHERN GHANA MARCH 1 – DECEMBER 31, 2018

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PMI VECTORLINK GHANA ANNUAL ENTOMOLOGICAL MONITORING REPORT FOR NORTHERN GHANA

MARCH 1-DECEMBER 31, 2018

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ACRONYMS

b/p/n	Bites/Person/Night
BYD	Bunkpurugu-Yunyoo District
CDC	U.S. Centers for Disease Control and Prevention
DEF	S,S,S-tributyl phosphorotrithioate
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
EMD	East Mamprusi District
EA	Ethacrynic Acid
GHS	Ghana Health Service
GUD	Gushegu District
GST	Glutathione transferase
HBR	Human Biting Rate
HLC	Human Landing Catch
ib/p/n	Infective Bites/Person/Night
ib/p/yr	Infective Bites/Person/Year
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
KAD	Karaga District
kdr	Knockdown Resistance
Pf	*Plasmodium falciparum*
KUD	Kumbungu District
LLIN	Long-lasting Insecticidal Net
MMD	Mamprugu Moaduri District
NMIMR	Noguchi Memorial Institute for Medical Research
PBO	Piperonyl Butoxide
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
RDT	Rapid Diagnostic Test
SND	Savelugu-Nanton District
SS	SumiShield 50WG
TD	Tolon District
TG	Technical Grade
TML	Tamale Metropolis
USAID	United States Agency for International Development
WHO	World Health Organization
WMD	West Mamprusi District

EXECUTIVE SUMMARY

BACKGROUND AND METHODS

In the 2018, the VectorLink Ghana Project, funded by the United States Agency for International Development through the President's Malaria Initiative, worked in partnership with the Ghana Health Service (GHS) through the National Malaria Control Program (NMCP), and the District Assemblies to conduct indoor residual spray (IRS) operations in seven districts of the Northern region: Bunkpurugu-Yunyoo (BYD), East Mamprusi (EMD), Gushegu (GUD), Karaga (KAD), Kumbungu (KUD), Mamprugu Moaduri (MMD), and West Mamprusi (WMD). With the support of the GHS, the project piloted a new insecticide product, SumiShield® 50WG (clothianidin; SS), in MMD in 2018. Although the project had not detected pirimiphos-methyl resistance in that district, the trial was held to demonstrate feasibility of a rotation strategy when more than one insecticide option is available. Actellic® 300CS (pirimiphos-methyl) was sprayed in all other districts.

To assess the impact of IRS on entomological indices of malaria transmission, VectorLink Ghana carried out routine entomological surveys in 20 sentinel sites across 10 districts in the Northern Region. The districts included all seven IRS districts, two districts: Tolon District (TD) and Savelugu-Nanton District (SND) from which IRS was withdrawn in 2013 and 2015 respectively) and one district that has never been sprayed (Tamale metropolis (TML)). The project used human landing catch, pyrethrum spray collection, and window exit trap methods to collect mosquitoes monthly between March and December 2018 in all sites. January and February are driest months of the year and mosquito collections are usually unproductive. World Health Organization (WHO) wall bioassay tests were performed to determine the decay rate of sprayed insecticide and tube tests were performed to monitor insecticide susceptibility. Finally, insecticide resistance intensity and synergist assays were performed using the Centers for Disease Control and Prevention (CDC) bottle bioassay method.

RESULTS AND DISCUSSION

Vector Species Composition and Seasonality: *An. gambiae* s.l. was the most abundant species in all study sites, comprising about 88 percent of the total 79,744 *Anopheles* collected. Mosquito populations peaked between July and September. *An. gambiae* s.l. from all sites showed higher outdoor biting rates except in WMD (an IRS site) and the control site (TML), where the vectors exhibited relatively high indoor feeding tendencies. The differences in the mean indoor and outdoor HBRs were statistically significant at the 0.05 level for most sites, except in SND and WMD.

Parity Rates: Ovary dissections showed decreased longevity of *An. gambiae* s.l. in IRS districts compared with the unsprayed districts. The mean proportion of parous *An. gambiae* s.l. in both Actellic-sprayed districts (42 percent) and SS-sprayed districts (40 percent were significantly ($p<0.05$) lower than the mean parity rates for IRS-withdrawn districts (64 percent) and the control site (67 percent).

Sporozoite infectivity: No sporozoite infection was detected in mosquitoes collected from BYD. The sporozoite rates for the other sprayed sites ranged between 0.27 percent and 0.86 percent. The sporozoite rates for *An. gambiae* s.l. was highest in MMD and EMD. Most sporozoite infections occurred in the rainy months (June to October 2018).

Entomological Inoculation Rate (EIR): Malaria transmission was seasonal, with the peak transmission period occurring between June and October 2018. The sum of monthly EIRs (March to December 2018) was highest in WMD, 26.2 infective bites/person/year (ib/p/yr), MMD at 23.2 ib/p/yr, and TML at 20.1 ib/p/yr. A general increase in EIR was observed across all sites in 2018 when compared to 2017. In the IRS sites EIRs increased from 0 to 18.4 ib/p/yr in KAD; 4.4 to 16.2 ib/p/yr in GUD, and from 3.2 to 6.6 ib/p/yr in KUD. Similar trends were also observed in the unsprayed districts (TD, SND and TML). The lowest was in BYD where no infections have been detected for two consecutive years. The relatively high EIRs recorded in the IRS districts (MMD, WMD, KAD, GUD and EMD) compared to unsprayed

(SND and TD) could be a consequence of an increase in biting densities that resulted from rains and flooding in August and September 2018, that affected these areas.

Residual Life of Sprayed Insecticides: Monthly wall bioassays showed SS had a residual efficacy above the WHO cut-off mortality level (80 percent mortality) on all surfaces eight months after spraying. This seems longer than the 6–7months of residual efficacy of Actellic® 300CS.

Insecticide Resistance Monitoring: WHO susceptibility tests indicated that *An. gambiae* s.l. mosquitoes from both IRS and non-IRS districts were susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr (with mortalities between 98 percent and 100 percent) except in Nanton (SND), a non-IRS site, where the mosquitoes showed possible resistance to pirimiphos-methyl (94.6 percent mortality). *An. gambiae* s.l. was resistant to alpha-cypermethrin and bendiocarb across all sites. High intensity of resistance to deltamethrin was observed across all sites. *An. gambiae* s.l. was found to be resistant to 1x, 2x, 5x, and 10x deltamethrin across all sites. Pre-exposure to the synergists PBO, DEF and EA increased mortality of vectors to deltamethrin by about 3-fold across the sites where synergists assays were conducted. This suggest that mono-oxygenase, esterase, and glutathione transferase could be playing a role in the resistance to the pyrethroids in *An. gambiae* s.l. from the sites tested.

CONCLUSION

IRS continues to suppress the parity rates in IRS areas in comparison with unsprayed and IRS-withdrawn sites, despite the high EIRs recorded. SS exhibits a residual life of at least eight-months, compared with the six to seven-month residual life of Actellic® 300CS.

I. INTRODUCTION

In 2018, the VectorLink Ghana project, funded by the United States Agency for International Development through the President's Malaria Initiative (PMI), carried out indoor residual spraying (IRS) in seven districts in the Northern Region: Bunkpurugu-Yunyoo District (BYD), East Mamprusi District (EMD), Gushegu District (GUD), Karaga District (KAD), Kumbungu District (KUD), Mamprugu Moaduri District (MMD), and West Mamprusi District (WMD). In 2017, the project piloted spraying SumiShield® 50WG (SS), a WHO prequalified product that contains clothianidin (a neonicotinoid) in one district (MMD). The project sprayed the other six districts with Actellic® 300CS (Actellic), the active ingredient of which is pirimiphos-methyl, an organophosphate insecticide. The IRS campaign ran from April 24 through May 29 in six districts sprayed with Actellic and from May 7 through June 9 in one district (MMD) sprayed with SS.

To assess the impact of IRS on entomological indices of malaria transmission, VectorLink Ghana carried out routine entomological surveys in 20 sentinel sites across 10 districts in Northern Region from March through December 2018. Additionally, insecticide susceptibility data was collected from Chereponi district in preparation for proposed IRS implementation in 2019. VL also supported the NMCP/GHS to collect insecticide resistance data from 10 PMI funded sentinel sites, through the National Insecticide Resistance Monitoring Partnership. Specific objectives of the surveys included:

1. Identifying the species of malaria vectors in the target districts;
2. Assessing vector density, behavior, and seasonality;
3. Determining the susceptibility of local vector species to the WHO-recommended insecticides for malaria vector control 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 and SS; and
5. Assessing changes in malaria transmission indices in the sentinel sites.

The VectorLink Ghana entomology team worked closely with the GHS and District Assemblies to implement all planned field activities. VectorLink Ghana also partnered with the Noguchi Memorial Institute for Medical Research (NMIMR) to support advanced molecular analyses. This report focuses on the entomological monitoring activities the project carried out in 2018.

2. METHODOLOGY

2.1 STUDY AREAS

Monitoring was conducted at two sites in each of 10 districts, including the seven IRS districts: BYD, EMD, GUD, KAD, KUD, MMD, and WMD; two districts from which IRS was withdrawn: Savelugu-Nanton District (SND) and Tolon District (TD); and one district that has never been sprayed: Tamale metropolis (TML) (Figure 1). Mean daily rainfall data was obtained from the Ghana Meteorological Services Department weather stations located in Savelugu, Tamale, Walewale and Savannah Agricultural Research Institute weather station in Tolon. Table 1 summarizes the spray history of each district from 2008 through 2018.

FIGURE 1: 2018 MAP OF PMI VECTORLINK GHANA DISTRICTS AND ENTOMOLOGICAL MONITORING SITES

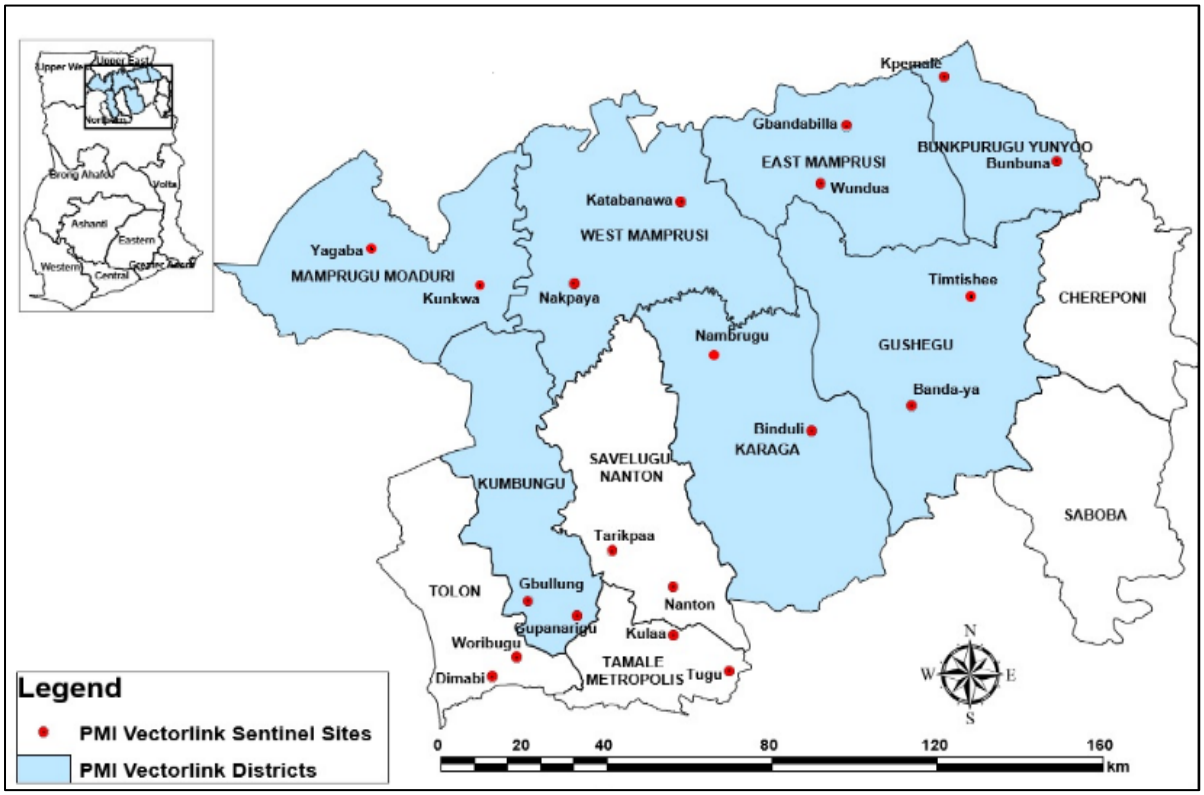


TABLE 1: ENTOMOLOGICAL MONITORING SITES

		Insecticide Spray History										
		2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
BYD	Bunbuna and Kpemale	NSp	NSp	NSp	ACy	ACy	PM	PM	PM	PM	PM	PM
EMD	Wundua and Gbandabila	NSp	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM
GUD	Banda-ya and Tum-Tu zie	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM	PM
KAD	Binduli and Nakpaya	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM	PM
KUD	Gbullung and Gupanarigu	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM	PM	PM
MMD	Yagaba and Kunkwa	ACy	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	CLD
WMD	Kata-Banawa and Nakpaya	ACy	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM
SND	Nanton and Tarikpaa (IRS was withdrawn in 2015)	ACy	ACy	DM	ACy	PM	PM	PM	NSp	NSp	NSp	NSp
TD	Dimabi and Woribugu (IRS was withdrawn in 2013)	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	NSp	NSp
TML	Kulaa and Tugu (comparison sites with no history of IRS)	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control

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

2.2 Adult Mosquito Surveys

The project entomology team collected mosquitoes in all 20 sentinel sites for four days each month for 10 months from March through December. Pre- and post-spraying mosquito collections included human landing catches (HLCs), pyrethrum spray collections (PSCs) (WHO 2013), and window exit trap collections. Mosquitoes collected were used to assess vector species composition, density, resting preference, peak biting time and location, biting rate, infectivity, parity, and entomological inoculation rates (EIRs). The data collected from the sentinel sites in the sprayed districts were compared with the data from the unsprayed district and IRS withdrawn districts.

Monthly HLCs were performed for a total of four nights (collecting mosquitoes hourly from 6:00 pm to 5:50 am) in eight compounds in each sentinel site. Two teams of four trained collectors performed HLCs, in two different houses each night, simultaneously. In each house, two persons collected indoors inside a sleeping room, while the other two collected mosquitoes outdoors in the open courtyard within the compound.

PSCs were performed the morning after each HLC to determine indoor resting mosquito species and densities. Collections took place between 6:00 a.m. and 8:00 a.m. in rooms in the same house other than those that had been used for the HLCs the previous night. The team surveyed a total of eight rooms (one in each of the eight compounds) for each site every month.

Window exit traps were used to catch mosquitoes exiting from one room in each of four houses in separate compounds from 6:00 pm to 5:50 am for four consecutive nights, for a total of four room-night collections in each site over 10 month period. Mosquitoes were retrieved from the traps each morning using a mouth aspirator, and were identified as unfed, freshly fed, or gravid in order to estimate their resting preference between indoors and outdoors.

A taxonomic key (Gillies and Coetzee 1987) was used to morphologically identify all *Anopheles* mosquitoes collected by each method. About one-third (on average 50–60 mosquitoes per month) of unfed mosquitoes from HLCs identified as *An. gambiae* s.l. were dissected to assess parity by observing the degree of coiling in the ovarian tracheoles (Detinova 1962). The remaining specimens (all mosquitoes collected) were preserved in 1.5ml Eppendorf tubes with desiccant for further analysis at the NMIMR laboratories.

2.3 Assessment of Spray Quality and Residual Efficacy

Standard WHO cone bioassays (WHO 2013) were conducted to assess spray quality and evaluate the residual life of the sprayed insecticides monthly, using both the *An. gambiae* Kisumu strain and wild *An. gambiae* s.l. reared from the field. The cone bioassays were performed on three main types of sprayed surfaces: mud walls (in traditional houses), cement walls (in modern houses), and wood, used for doors and windows.

2.3.1 Quality Assurance of the IRS Program

As noted above, one objective of the bioassays was to assess the quality of work by the different spray teams and spray operators in a sample of sites.

Selection of Community/Houses: The bioassays were done in selected communities that were sprayed during the first 1–3 days of the campaign. The first tests were conducted in Actellic-sprayed communities: Arigu (WMD); Bunbuna (BYD); Gbullung (KUD); Gushegu (GUD); Karaga (KAD); and Yapala (EMD). Tests for SS were carried out in two communities in MMD, Dabozesi and Licha. Four to six rooms in different houses were systematically sampled to assess spray quality.

Test Procedure: Three cone assays were conducted on the walls in one randomly selected house and one assay on the wooden door or window using 10 adult female mosquitoes per cone. The cones were fixed at different heights (0.5m, 1.0m, and 1.5m) on sprayed walls in the selected rooms. One control cone assay was conducted for every four bioassay tests by fastening a cone to an unsprayed surface (cardboard) and

exposing the control mosquitoes to the cardboard. The control test took place in an unsprayed room (food store/hut) with conditions similar to the sprayed room being tested.

Batches of 10 2–5-day-old non-blood-fed mosquitoes were introduced into the cone chamber and left exposed on the surface for 30 minutes. In addition, to assess the fumigant effect of the sprayed insecticides, 20 mosquitoes (10 Kisumu and 10 wild *An. gambiae* s.l.) were introduced into holding cups and placed on a table that was 10cm from the sprayed wall surfaces. At the end of the 30-minute exposure period, the mosquitoes were collected and transferred to holding cups. The numbers of mosquitoes that were knocked down at the end of the 30 minutes and at 60 minutes were recorded. For the Actellic-sprayed communities, the dead and live mosquitoes were counted after a 24-hour holding period and the mortalities calculated. For the SS-sprayed communities, if any mosquitoes were alive after 24 hours, observation continued until all mosquitoes had died (1–5 days).

2.3.2 Residual Efficacy of Actellic® 300CS and SumiShield® 50WG

The project team conducted follow-up bioassays monthly to assess the residual efficacy of Actellic in the 6 sentinel sites: Banda-ya (GUD), Binduli (KAD), Bunbuna (BYD), Gbullung (KUD), Kata-Banawa (WMD), and Wundua (EMD). In MMD, the team conducted follow-up bioassays to assess the residual efficacy of SS in Yagaba.

The bioassays were conducted using susceptible *An. gambiae* Kisumu strain mosquitoes from the project insectary and Navrongo Health Research Center insectary, and wild *An. gambiae* reared from larvae collected from the field. The bioassays were carried out as described in 2.3.1 above.

Both spray quality and residual efficacy were 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). Test results are presented in Chapter 3, Results.

2.4 Insecticide Susceptibility Tests

WHO tube tests and U.S. Centers for Disease Control and Prevention (CDC) bottle assays were performed to assess the susceptibility of local vector populations to insecticides used for IRS and long lasting insecticidal nets (LLINs). All the sentinel sites have long history of LLIN coverage which has been maintained over the years through mass distribution campaigns, as well as routine health facility and school based distributions. Methodologies are described below.

2.4.1 WHO Tube Tests

The project team performed insecticide susceptibility tests using the WHO tube test method, as described in Annex A, in selected sentinel sites in sprayed and unsprayed communities. Larvae and pupae of *Anopheles* mosquitoes were collected from breeding sites in and around the sentinel sites 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 WHO standardized insecticide papers: alpha-cypermethrin (0.05 percent), bendiocarb (0.1 percent), and pirimiphos-methyl (0.25 percent). Additionally, susceptibility status of wild *An. gambiae* s.l. from selected sites to 13.2mg (per one impregnated paper, 15x12cm) of clothianidin (SumiShield® 50 WG) was determined using papers that were impregnated in country. After the 24-hour holding period, the number of dead mosquitoes in both the exposure and the control tubes were recorded. Mortalities were corrected using Abbott's formula if the control mortalities were ≥ 5 percent and < 20 percent, but tests were discarded and repeated if control mortalities were ≥ 20 percent. For clothianidin, knockdown was recorded after 30 and 60 minutes and mortalities recorded at 1, 2, 3, 4, 5, 6, 7 days post exposure.

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

2.4.2 CDC Bottle Assays

The CDC bottle assay method was also used to test for the vector susceptibility to clothianidin and chlorfenapyr. The clothianidin test was conducted using a method developed by Bayer in which 250ml Wheaton bottles are coated with insecticide solution prepared by the protocol described in Annex B. To coat a negative control bottle, 1ml of acetone/mero solution was used as described by Brogdon and Chan (2010). The treated bottles resulting from this method had 90µg of clothianidin per bottle. *An. gambiae* s.l. were exposed to treated bottles. Between 20 and 25 mosquitoes were introduced into the four replicates of the test bottles and one control bottle (coated with acetone/mero only). Knockdown was recorded every five minutes for a maximum of 60 minutes (1hr) or until 100 percent knockdown was reached.

The CDC bottle assay with chlorfenapyr was conducted using a method described by Brogdon and Chan (2010) with some modifications (60 minutes exposure time). Diagnostic concentrations of 12.5µg, 25µg, 50µg, 100µg, and 200µg/bottle were tested. *An. gambiae* s.l. reared from larvae were exposed to 250ml Wheaton bottles treated with different concentrations. Tests with *An. gambiae* Kisumu were run in parallel as controls. Mosquitoes were introduced in batches of 20–25 in the one replicate of each concentration. After the exposure period, mosquitoes were released into clean cages and then gently aspirated into labeled paper cups covered with untreated netting and provided with 10 percent sugar solution. Knockdown was recorded 60 minutes after the start of the test, while mosquitoes were still in the bottle. Mortality was recorded one, two, and three days after the end of exposure. A negative control was tested at the same time and mortality recorded at one, two, and three days so that corrected mortality could be calculated.

2.5 Resistance Intensity Assays

Using a simplified version of CDC bottle bioassay resistance Intensity Rapid Diagnostic Test (I-RDT) (Brogdon and Chan 2010), the team determined the intensity of deltamethrin resistance in *An. gambiae* s.l. from selected sentinel sites in BYD, EMD, KUD, TD, and WMD. Four pre-measured vials provided by the CDC, Atlanta, containing deltamethrin at concentrations of 1x, 2x, 5x, and 10x, were diluted in acetone and applied to 250ml bottles. Four replicates of 500µl 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 each of the four replicates. A control bottle (coated with acetone only) was 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 Dimabi (TD), Kumbungu (KUD) and Wundua (EMD), which showed resistance to deltamethrin, were exposed to the effect of piperonyl butoxide (PBO) (100 µg/bottle), S,S,S-tributyl phosphorotrithioate (DEF) (125µg/bottle), and Ethacrynic acid (ETAA) (8 µg/bottle), synergists that have been found to inhibit oxidase, esterase, and glutathione transferases (GST) activity, respectively. *An. gambiae* s.l. populations from Bunbuna (BYD), which showed resistance to deltamethrin, were only exposed to PBO.

Two separate bottles (synergist exposure and control) were prepared to run each synergist assays. One bottle was coated with 1ml of acetone and served as a synergist-control bottle (without synergist); the second bottle was coated with 1ml of the synergist (PBO, DEF, or ETAA) stock solution and served as the synergist-exposure bottle. Mosquitoes were pre-exposed to the synergist coated and acetone coated bottles for an hour before testing them against insecticide-coated bottles. A batch of 100–125 mosquitoes was introduced into the synergist-control bottle, and another 100–125 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 cages, one for the synergist-control mosquitoes and another for the synergist-exposed mosquitoes.

CDC bottle bioassays were then 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 were then compared.

2.7 Analysis and Molecular Evaluations

NMIMR performed molecular analyses to:

1. Determine sporozoite rates and calculate EIRs;
2. Identify members of the *An. gambiae* s.l. complex to species; and
3. Determine the frequency of knockdown resistance (*kdr)* genes and other molecular markers of insecticide resistance.

2.7.1 Circumsporozoite-ELISA Evaluation

The heads and thoraxes of about 15% of the *An. gambiae* s.l. and all *An. funestus* collected monthly were sorted and tested for the presence of circumsporozoite antigens of *Plasmodium falciparum* (Pf) sporozoites using enzyme-linked immunosorbent assays (ELISA) described by Wirtz *et al.* (1987). The ELISAs were used to assess the parasite infection rate in the local vectors collected.

2.7.2 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).

2.7.3 *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 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 *An. gambiae* s.l. and *An. funestus* group:

- Human biting rate (HBR) = the total number of vectors collected/number of collectors/ number of nights of capture (reported as bites/person/night)
- Endophagic / Exophagic index = $\frac{\textit{Number of mosquitoes species collected (either indoors or outdoors)}}{\textit{Total Number of Mosquitoes collected indoors and outdoors}}$
- Sporozoite rate = the proportion of *Anopheles* found positive for the presence of *Plasmodium falciparum* circumsporozoite proteins
- Entomological Inoculation Rate = number of infectious bites/per person/per unit time. Monthly and annual EIRs were estimated for each site as follows:

  Monthly EIR = daily HBRs X sporozoite rates X # of days in the month

  Annual EIR = sum of monthly EIRs.

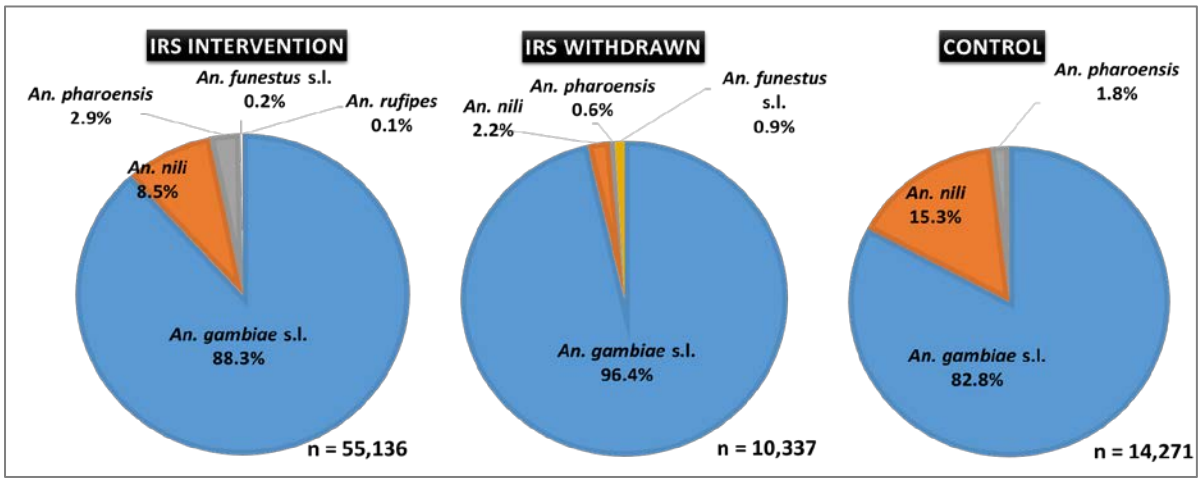
Variations in indoor and outdoor biting rates for the vector species collected from IRS intervention, IRS withdrawn and unsprayed districts were compared using the Chi-square goodness of fit test. Differences in parity and sporozoite rates between the sites were also compared through a z-test for differences in proportions. Pearson's correlation was used to assess the relationship between rainfall and biting rates. All tests were performed at 0.05 significance level, using SPSS version 20 and Microsoft Excel®.

3. RESULTS

3.1 SPECIES COMPOSITION

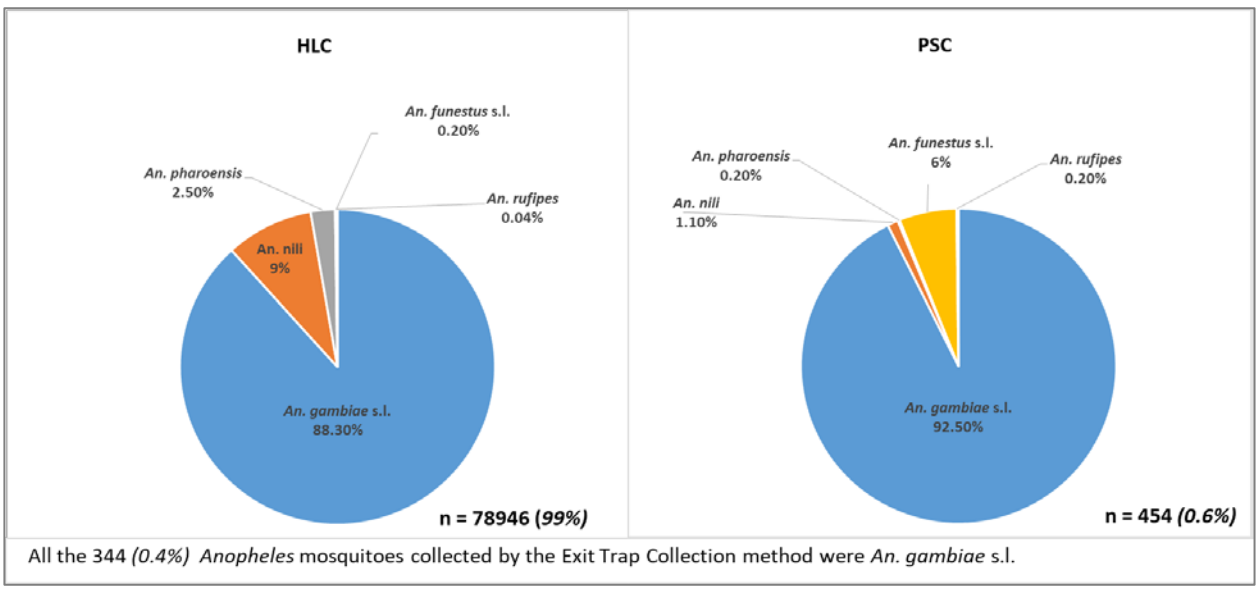
An. gambiae s.l. was the predominant species collected across all sites, constituting between 83 percent and 97 percent of the total number (79,744) of *Anopheles* collected (Figures 2). *An. nili*, a non-vector species, was the second most predominant species in GUD, KAD, TML, BYD, KUD, EMD, SND, and TD, making up 2–18 percent of the total *Anopheles* mosquitoes collected. Other *Anopheles* collected included *An. funestus* (vector), *An. pharoensis*, and *An. rufipes* (both non-vector species). The distribution of the different *Anopheles* species by district is presented in Annex C, Figures C- 1.

FIGURE 2: TYPE OF *ANOPHELES* SPECIES COLLECTED USING HLC, PSC, AND WINDOW EXIT TRAP COLLECTIONS, IN IRS INTERVENTION, IRS WITHDRAWN AND CONTROL DISTRICTS



Of the total adult female *Anopheles* mosquitoes collected, 99 percent (78,946/79,744) were collected attempting to bite (i.e., by HLC), 0.6 percent (454/79,744) were collected resting indoors, and 0.4 percent (344/79,744) were collected exiting through windows (Figure 3). The *Anopheles* species collected were predominantly *An. gambiae* s.l.; making up 88.3 percent and 92.5 percent of the HLC and PSC collections respectively. All the mosquitoes collected by the Exit trap were *An. gambiae* s.l.. The majority of *Anopheles* were collected in TML (18 percent) followed by KAD (16 percent), WMD (14 percent), KUD (13 percent) and GUD (11 percent).

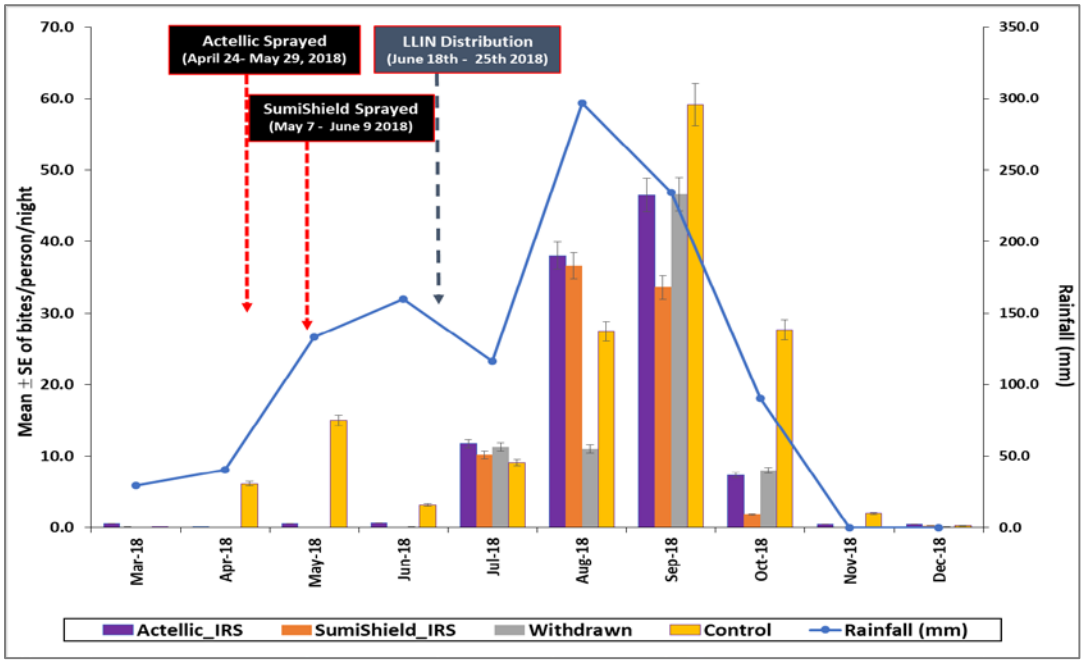
FIGURE 3: NUMBER AND TYPE OF *ANOPHELES* SPECIES COLLECTED, BY COLLECTION METHOD



3.2 VECTOR SEASONALITY

The abundance and HBR of *An. gambiae* s.l. collected from all sites positively correlated ($p<0.05$) with the mean rainfall (94.7mm) recorded in 2018 (Figure 4). The coefficients of correlation for the IRS sites were 0.761 (BYD), 0.741 (EMD), 0.768 (GUD), 0.8.46 (KAD), 0.727 (KUD), 0.852 (MMD), and 0.761(WMD). Except TD, where a significant correlation (0.749) was seen between rainfall and HBR, the correlation for the non-IRS sites; SND (0.443) and TML (0.545); was not significant. There was about 25% increase in the cumulative rainfall (910.1mm) recorded in 2017 compared to the cumulative rainfall (1,136mm) recorded in 2018. The peak densities (measured by HBR) were in August and September 2019.

FIGURE 4: MEAN HBR OF *AN. GAMBIAE* S.L. FROM SENTINEL SITES AND MEAN RAINFALL, MARCH–DECEMBER 2018



3.3 BITING RATE / BEHAVIOR AND FEEDING TIME

Comparatively, the average monthly HBRs recorded for the control sites (TML) were higher than those for most IRS and IRS-withdrawn sites, but the differences were not significant ($p>0.05$). However the peak HBR in the sprayed and IRS withdrawn districts occurred over a shorter period of time as compared to the controls The mean rates were 10.4 bites/person/night (b/p/n) for the IRS intervention districts , 7.7 b/p/n for IRS-withdrawn sites, and 15.3 b/p/n for the unsprayed sites. A notable increase in HBRs was observed in 2018 as compared to 2017 in both IRS and IRS withdrawn sites: 7-fold increase for BYD, 2-fold increase in both GUD and KAD, and 1.2-fold in KUD, all IRS districts; in the non-IRS sites, it was 3-fold increase in SND and 1.4-fold increase in TD. However, the biting rates in TML declined marginally in 2018 when compared to 2017 HBR. This trend was observed in both indoor and outdoor biting rates across all sites (Table C-2).

There were variations in indoor and outdoor HBRs for *An. gambiae* s.l. between the IRS and non-IRS sites. The differences in the mean indoor and outdoor HBRs were statistically significant at the 0.05 level for most sites (Table 2), except in SND and WMD. The results show exophagic tendencies of *An. gambiae* s.l. in all districts except in WMD and TML (control), where *An. gambiae* s.l. showed more endophagic tendencies (Table 2). Similar trends have been observed across most IRS sites with some yearly variations (Figure C-6).

Indoor and outdoor biting activity of *An. gambiae* s.l. started at 6:00 p.m. and then gradually increased with peak biting observed between 11:00 p.m. and 4:00 a.m (Figure 5). The number of mosquitoes biting during these peak times was higher in the unsprayed district than in the IRS and IRS- withdrawn districts.

FIGURE 5: INDOOR AND OUTDOOR HOURLY BITING ACTIVITY OF *AN. GAMBIAE* S.L. IN SPRAYED AND UNSPRAYED SITES, MARCH –DECEMBER 2018

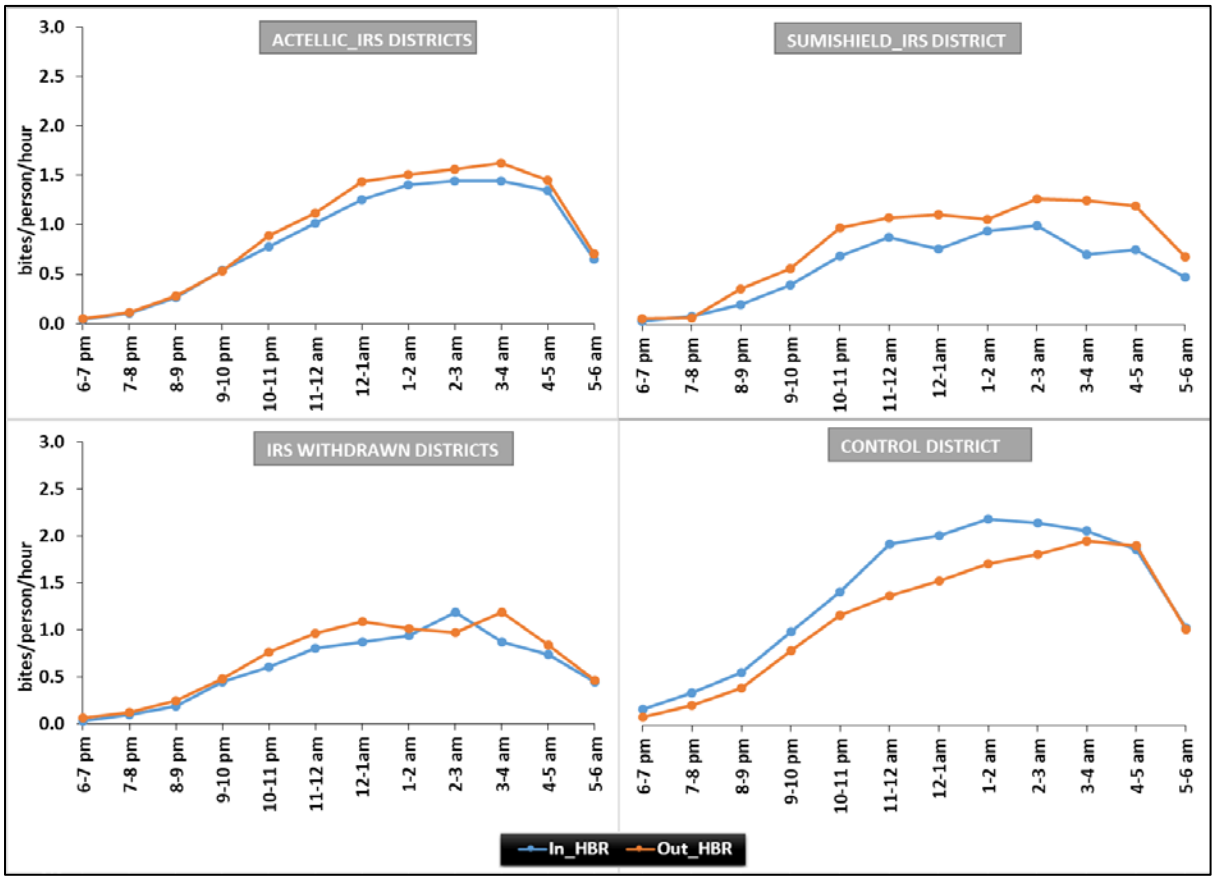


TABLE 2: INDOOR AND OUTDOOR HBRS OF *AN. GAMBIAE* S.L., AS DETERMINED FROM HLCS, ALL SENTINEL SITES, MARCH – DECEMBER 2018

Sentinel Site	Indoor Biting Rate (b/p/n)	Outdoor Biting Rate (b/p/n)	Endophagic Index	Exophagic Index	χ^2	P-value
Actellic IRS						
BYD	4.31	4.70	0.48	0.52	5.51	0.019*
EMD	4.54	5.03	0.47	0.53	8.15	0.004*
GUD	9.34	11.79	0.44	0.56	91.36	0.000*
KAD	15.62	16.92	0.48	0.52	16.70	0.000*
KUD	11.74	13.32	0.47	0.53	38.03	0.000*
WMD	15.87	15.48	0.51	0.49	1.53	0.216
SumiShield IRS						
MMD	6.89	9.65	0.42	0.58	147.00	0.00*
Withdrawn						
SND	6.56	6.94	0.49	0.51	3.45	0.063
TD	7.89	9.48	0.45	0.55	46.45	0.000*
Control						
TML	16.65	13.91	0.54	0.46	94.28	0.000*

Note: b/p/n = bites/person/night

* Differences in mean indoor/outdoor biting rates is statistically significant at 0.05 level.

3.4 RESTING BEHAVIOR

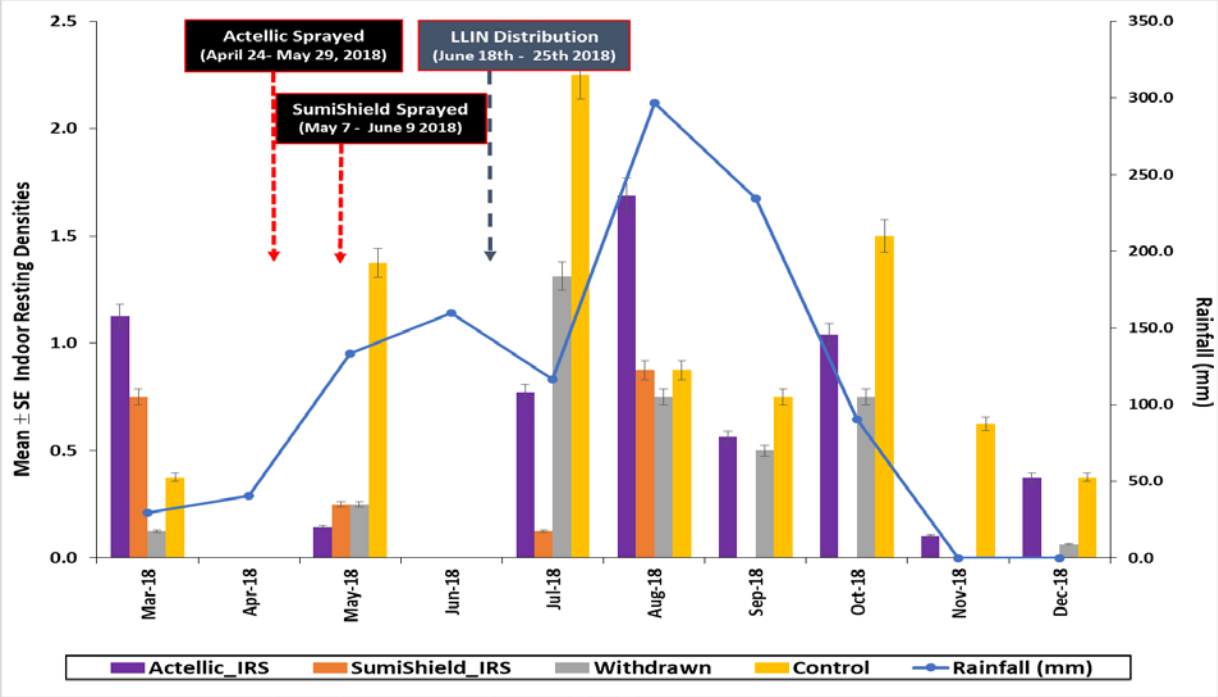
The mean IRD of *An. gambiae* s.l. were relatively low compared to the biting rates. IRD of *An. gambiae* s.l. from the IRS intervention sites were 0.29 mosquito per room/day and 0.10 mosquito per room/day for Actellic sprayed and SS-sprayed sites respectively. The IRD of 0.10 recorded for MMD and 0.19 (60 mosquitoes from 320 room-days) for IRS-withdrawn districts (SND and TD) were lower than the IRD of 0.41 of *An. gambiae* s.l. recorded for the never-sprayed control (TML). (65 mosquitoes from 160 room-days). Year on comparison between 2017 and 2018, show a decline in IRD for most sites except in TML where IRDs increased from 0.64 in 2017 to 1.67 in 2018 (Figure C-7). Relatively low proportions of half-gravid and gravid females were caught resting indoors in sprayed rooms in the IRS districts (22–25 percent) compared with the proportions of gravid females collected indoors in IRS-withdrawn districts (38 percent) and the control district (51 percent) (Table 3).

TABLE 3: *AN. GAMBIAE* S.L. COLLECTED BY PYRETHRUM SPRAY COLLECTIONS AND THEIR ABDOMINAL CONDITION, MARCH–DECEMBER 2018

Treatment	Unfed	Freshly Fed	Half gravid + Gravid	Total # Caught Resting in Sleeping Rooms	Mean # Caught Resting in a Room/Day
IRS - Actellic	14%	65%	22%	279	0.29
IRS - SumiShield	31%	44%	25%	16	0.10
IRS Withdrawn	7%	55%	38%	60	0.19
Control	12%	37%	51%	65	0.41

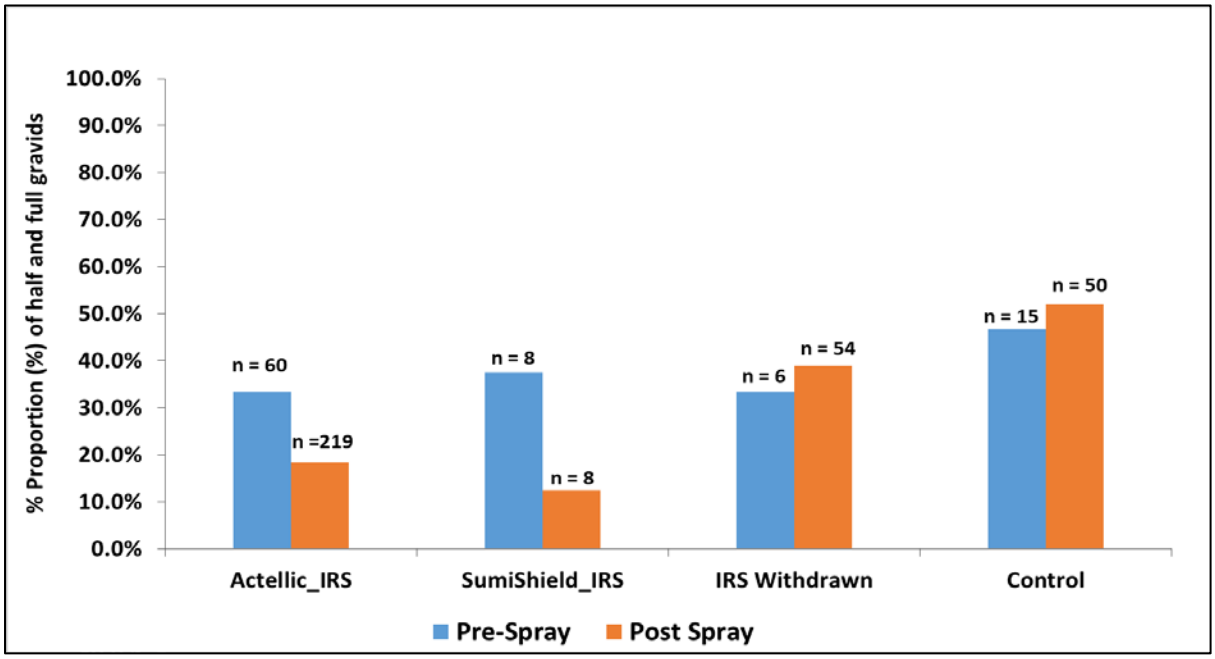
Monthly variations were observed in the mean indoor resting density (IRD) of *An. gambiae* s.l. for all sites (Figure 6). However the number of mosquitoes collected were very low. As was observed for HBRs, IRDs also increased with the onset of the rains.

FIGURE 6: MEAN MONTHLY IRDS OF *AN. GAMBIAE* S.L. AS DETERMINED BY PSC IN IRS AND UNSPRAYED DISTRICTS, MARCH–DECEMBER 2018



The proportion of half-gravid and gravid *An. gambiae* s.l. in the IRS sites declined significantly in the months after IRS (June–December) compared with the proportion recorded before the IRS campaign (March–April/May for Actellic districts and March–June for the SS district), though the numbers collected were few. However, the proportion of gravids in the unsprayed districts (IRS withdrawn and control) remained unchanged (Figure 7).

FIGURE 7: PRE- AND POST-IRS ABDOMINAL CONDITION OF 420 *AN. GAMBIAE* S.L. COLLECTED FROM IRS AND UNSPRAYED DISTRICTS, MARCH–DECEMBER 2018



3.5 EXITING BEHAVIOR

A low number of mosquitoes (n=344 *An. gambiae* s.l.) was collected from window exit traps as compared with HLC and PSC collections. In Actellic-sprayed districts, the mean numbers of mosquitoes exiting sprayed rooms were 0.34 mosquitoes/trap/room; the number was 1.19 in the SS-sprayed district. The numbers in IRS districts were higher as compared with 0.17 mosquitoes/trap/room in IRS-withdrawn districts and 0.10 in the control district (Table 4).

Lower proportions of gravid female *An. gambiae* s.l. were collected from the window exit traps in the sprayed districts than unsprayed districts. Gravid and half-gravid females made up 6 percent (13/206) and 17 percent (16/95) of *An. gambiae* s.l. collected from Actellic- and SS-sprayed districts, respectively (Table 4). The proportion of gravid females collected in window exit traps in the unsprayed district was 41 percent (11/27) for the IRS-withdrawn districts and 69 percent (11/16) for the control district.

TABLE 4: *AN. GAMBIAE* S.L. COLLECTED BY WINDOW EXIT TRAP COLLECTIONS AND THEIR ABDOMINAL CONDITION, MARCH–DECEMBER 2018

Treatment	Unfed	Freshly Fed	Half gravid + Gravid	Total # Caught Exiting Rooms	Mean # Caught Exiting Room/Day
IRS - Actellic	66.0%	27.7%	6.3%	206	0.34
IRS - SumiShield	11.6%	71.6%	16.8%	95	1.19
IRS Withdrawn	18.5%	40.7%	40.7%	27	0.17
Control	12.5%	18.8%	68.8%	16	0.10

3.6 PARITY RATES

Dissections of *An. gambiae* s.l. mosquitoes collected between March and December 2018 revealed that the proportion of parous females collected from the unsprayed districts of TD, SND, and TML was higher than the proportion collected from the IRS districts (Table 5).

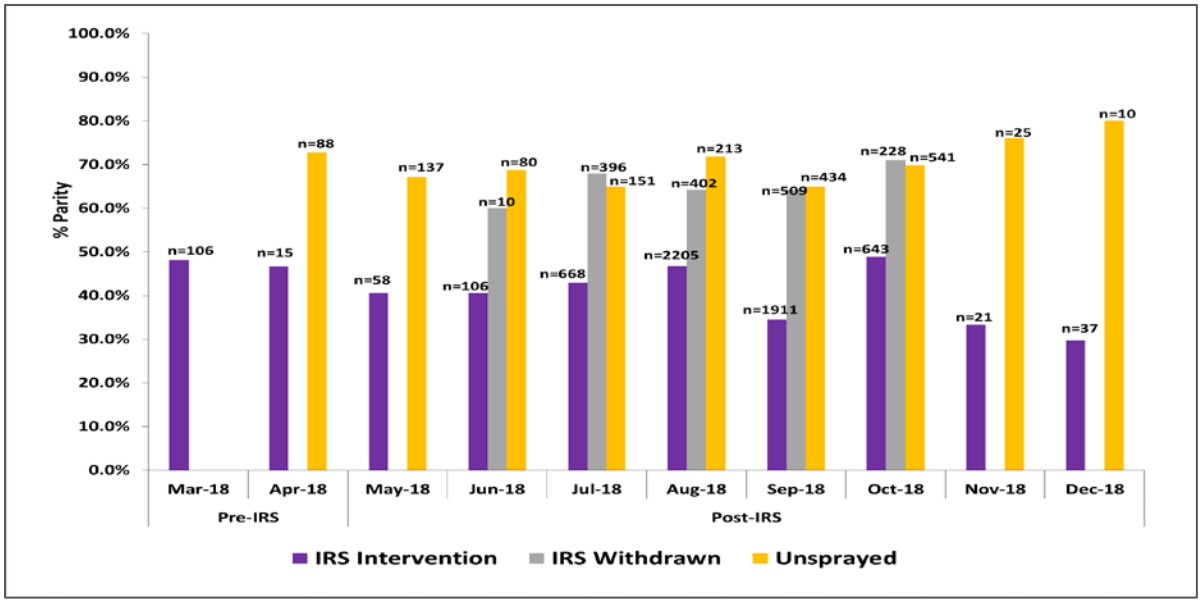
TABLE 5: PROPORTION OF PAROUS FEMALES OF *AN. GAMBIAE* S.L. BY HLC

District	#Dissected	Parous	% Parity	95% Confidence Interval	
				Lower Bound	Upper Bound
IRS - Actellic					
BYD	565	204	36.1%	32.1%	40.1%
EMD	399	179	44.9%	40.0%	49.7%
GUD	913	423	46.3%	43.1%	49.6%
KAD	1061	450	42.4%	39.4%	45.4%
KUD	1435	583	40.6%	38.1%	43.2%
WMD	834	362	43.4%	40.0%	46.8%
IRS - SumiShield					
MMD	563	226	40.1%	36.1%	44.2%
IRS withdrawn					
SND	787	494	62.8%	59.4%	66.1%
TD	759	489	64.4%	61.0%	67.8%
Control					
TML	1682	1133	67.4%	65.1%	69.6%

The differences in parity between the IRS districts and the three unsprayed districts were significant ($p<0.0001$). The post-spray parity rates were lower in most IRS sites as compared to the pre-spray parity rates (Figure 8). However, the number of mosquitoes dissected in KAD was less than 20, whereas only 5 and 3 mosquitoes were dissected in BYD and KUD, respectively, during the pre-spray period. No

dissection was done for MMD, SND, and TD during the pre-spray months (March–May 2018) due to the very low numbers of mosquitoes. The parity rates in TML remained unchanged with a marginal increase observed during the post-spray period (June–December).

FIGURE 8: COMPARISON OF MEAN MONTHLY PARITY RATES PRE- AND POST-IRS FOR *AN. GAMBIAE* S.L.



3.7 SPOROZOITE RATES FOR PF

A total of 9,976 (14.3 percent) *Anopheles gambiae* s.l. and all 187 *An. funestus* collected by HLC were assayed by ELISA in 2018 to determine the presence of Pf sporozoites in their salivary glands. No sporozoite infections were found in BYD. The sporozoite rates for the other sprayed sites ranged between 0.27 percent and 0.86 percent (Table 6). The sporozoite rates for *An. gambiae* s.l. was highest in MMD and EMD (Table 6 and Table C-3).

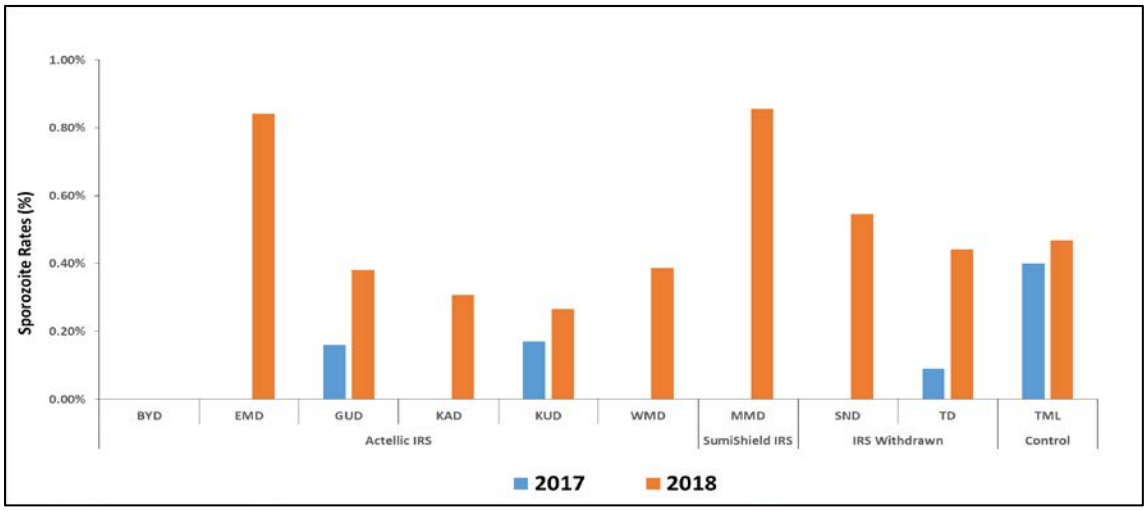
TABLE 6: PF. SPOROZOITE INFECTIONS IN *AN. GAMBIAE* S.L. SAMPLED FROM ALL SENTINEL SITES, 2018

Sentinel Site	Number Examined by ELISA	Number Positive for Pf. Sporozoites	Sporozoite Rate
IRS – Actellic			
BYD	855	0	0.00%
EMD	951	8	0.84%
GUD	786	3	0.38%
KAD	974	3	0.35%
KUD	1128	3	0.27%
WMD	775	3	0.39%
IRS - SumiShield			
MMD	701	6	0.86%
IRS withdrawn			
SND	550	3	0.55%
TD	905	4	0.44%
Control			
TML	2351	11	0.47%

Comparison of 2017 and 2018 *An. gambiae* s.l. sporozoite rates shows a general increase in sporozoite infections in most sites where infections were detected in 2017 (Figure 9). Only one out of 64 *An. funestus* from Gbullung (KUD) analysed was positive for sporozoites.

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

FIGURE 9: COMPARISON OF SPOROZOITE RATES IN 2017 AND 2018 FOR *AN. GAMBIAE* S.L. COLLECTED FROM IRS AND NON-IRS SENTINEL SITES*

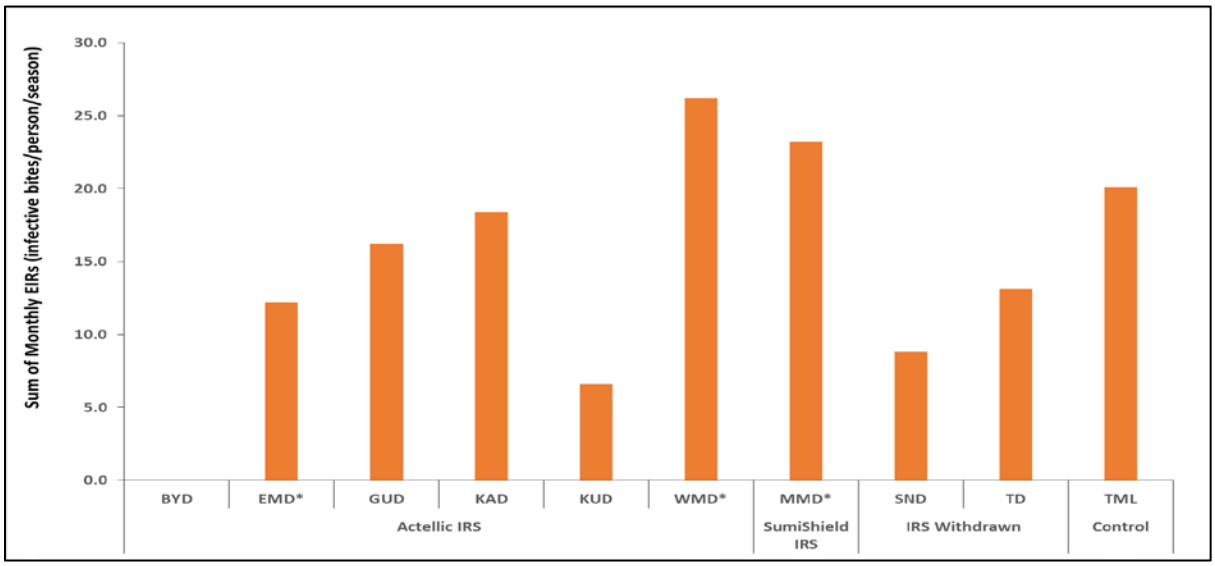


**2017 sporozoite rate data not available for EMD, WMD, and MMD. Data collections began in 2018.*

3.8 ESTIMATION OF EIRS

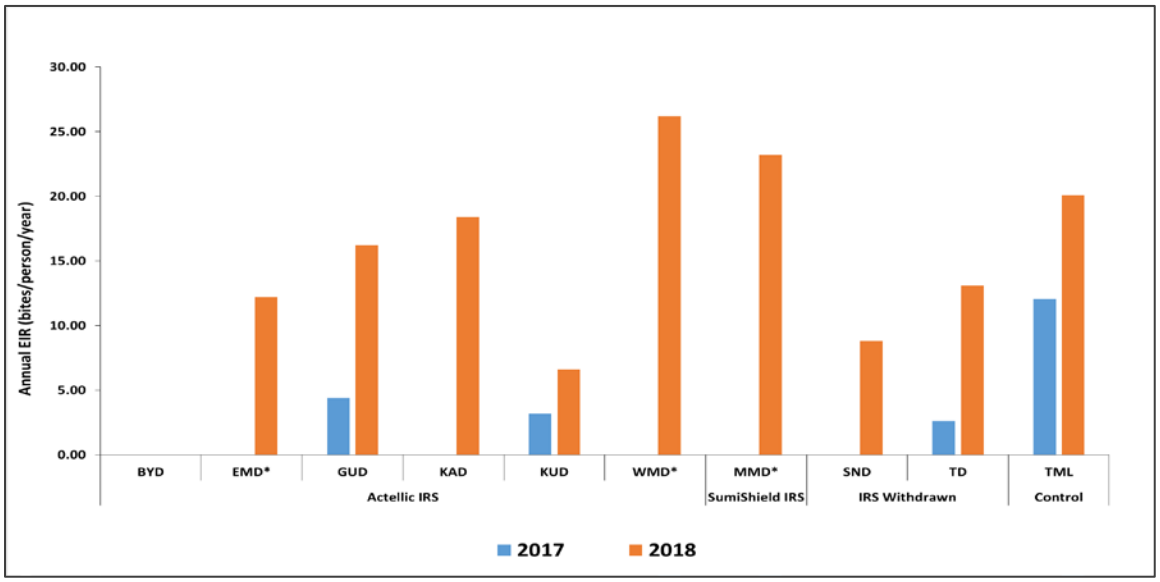
The annual EIR was estimated from the sum of monthly EIRs between March and December. The 10 months data is considered representative of the annual EIR because previous repeated data has shown that mosquitoes are rarely collected in January and February (dry season) and EIR is considered zero at this time. The sum of monthly EIRs (calculated for the months that sporozoite were detected) was highest in MMD (23.2 ib/p/yr), WMD (26.2 ib/p/yr), and TML (20.1 ib/p/yr). The lowest EIR, BYD where no infections were detected (Figure 10 and Table C-3).

FIGURE 10: SUM OF MONTHLY EIR FOR *AN. GAMBIAE* S.L. COLLECTED FROM IRS AND NON-IRS SENTINEL SITES, MARCH TO DECEMBER 2018



Comparison of the sum of monthly EIRs recorded in 2017 and 2018 also shows a general increase in EIRs across all sites in 2018 (Figure 11).

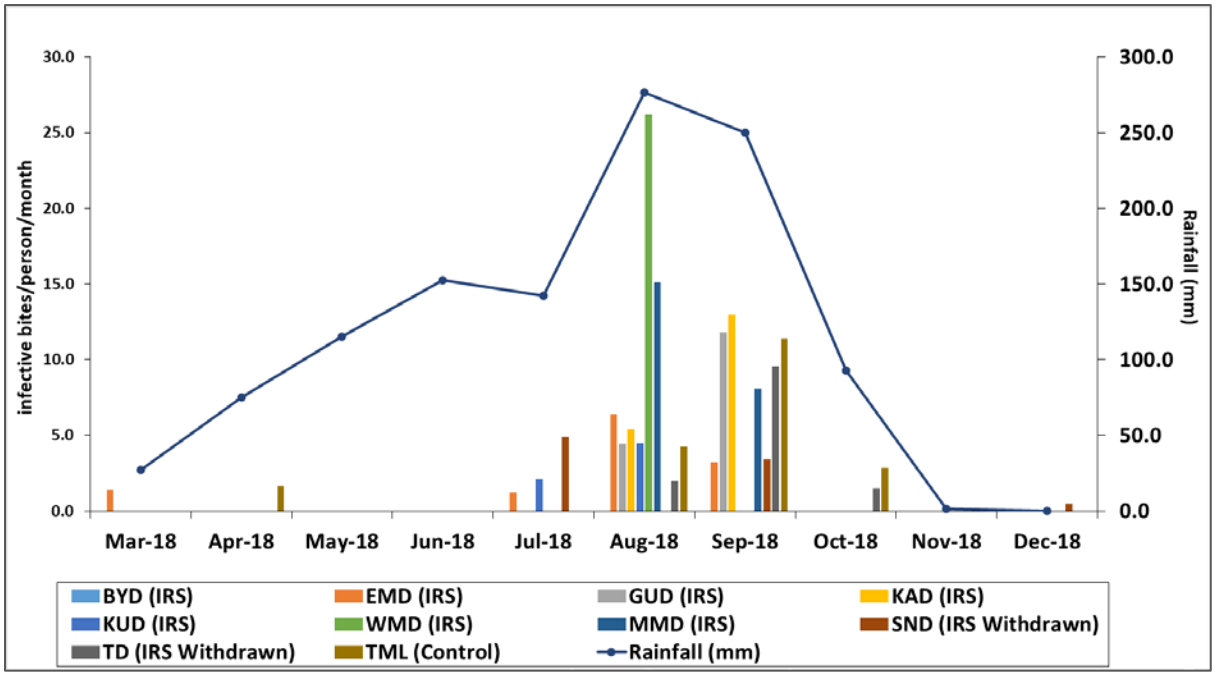
FIGURE 11: COMPARISON OF EIRS FROM MARCH TO DECEMBER IN 2017 AND 2018 FOR *AN. GAMBIAE* S.L. COLLECTED FROM IRS AND NON-IRS SENTINEL SITES*



* 2017 EIR data not available for EMD, WMD, AND MMD. Data collections started in 2018.

Monthly trends showed that transmission was seasonal in both IRS and non-IRS districts, with a greater proportion of transmission occurring primarily between June and October (Figure 12). The highest EIRs were recorded in the IRS sites in August. WMD accounted for the highest EIR followed by MMD and EMD. KAD and GUD also accounted for the highest EIRs in September 2018.

FIGURE12: MONTHLY EIRS IN IRS AND NON-IRS SITES, MARCH–DECEMBER 2018



3.9 SPRAY QUALITY AND RESIDUAL EFFICACY OF ACTELLIC® 300CS AND SUMISHIELD® 50WG

The bioassays for Actellic showed the insecticide remains effective above the cut-off mortality level (80 percent 24-hour mortality) on all surfaces in the communities tested for 6–7 months post-IRS, depending on the type of surface sprayed and the species of mosquitoes tested. SS was found to be effective, resulting in up to 90 percent mortality when mosquitoes were held for up to 120 hours (five days), at eight months post-spray. Spray quality tests and decay rate data for the two insecticides on different wall surfaces are presented in Annex C, Figures C-2, C-3, C-4 and C-5.

3.10 INSECTICIDE SUSCEPTIBILITY

The number of mosquitoes tested and percentage of mortalities after exposure for all sites tested are provided in Annex C, Table C-1. WHO susceptibility tests indicate that *An. gambiae* s.l. mosquitoes from all sites were susceptible to pirimiphos-methyl (with mortalities ranging between 98 percent and 100 percent), except in Nanton (KUD), where the mosquitoes showed possible resistance (94.6 percent). *An. gambiae* s.l. was resistant to alpha-cypermethrin and bendiocarb across all the sites (Figure 13). *An. gambiae* s.l. from across all sites tested were susceptible to clothianidin within seven days post-exposure (Figure 14).

FIGURE13: INSECTICIDE SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. FROM SENTINEL SITES IN NORTHERN REGION, GHANA IN 2018, BY WHO TUBE TEST

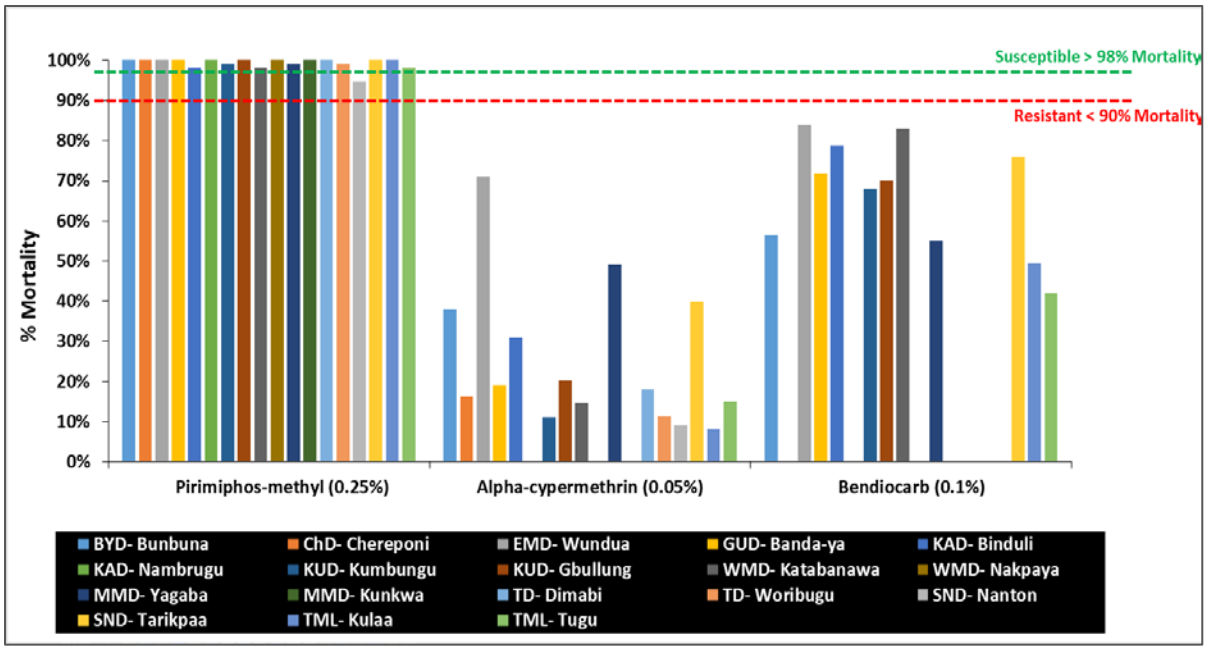
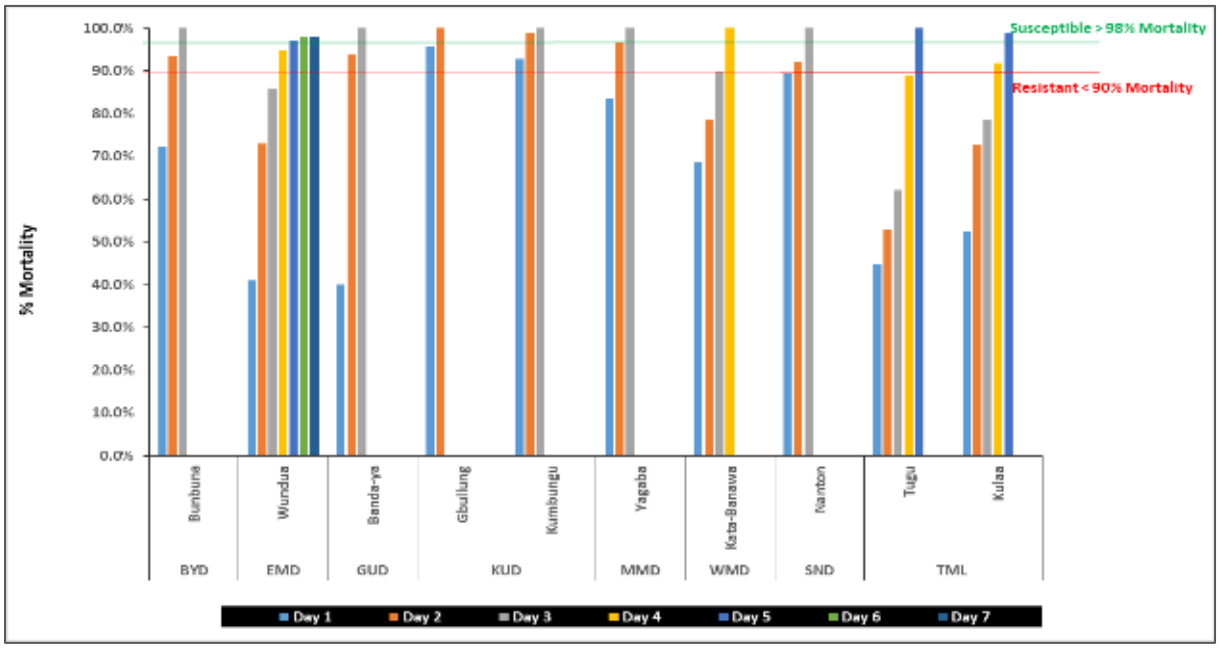
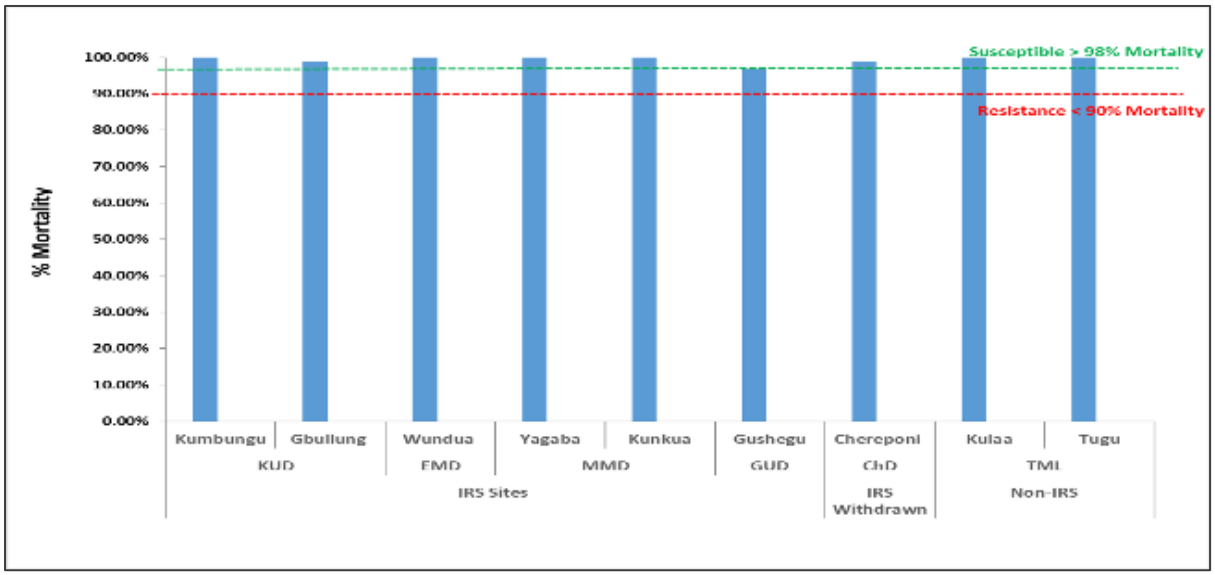


FIGURE 14: CLOTHIANIDIN SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. FROM SENTINEL SITES IN NORTHERN REGION, GHANA IN 2018, BY WHO TUBE TEST



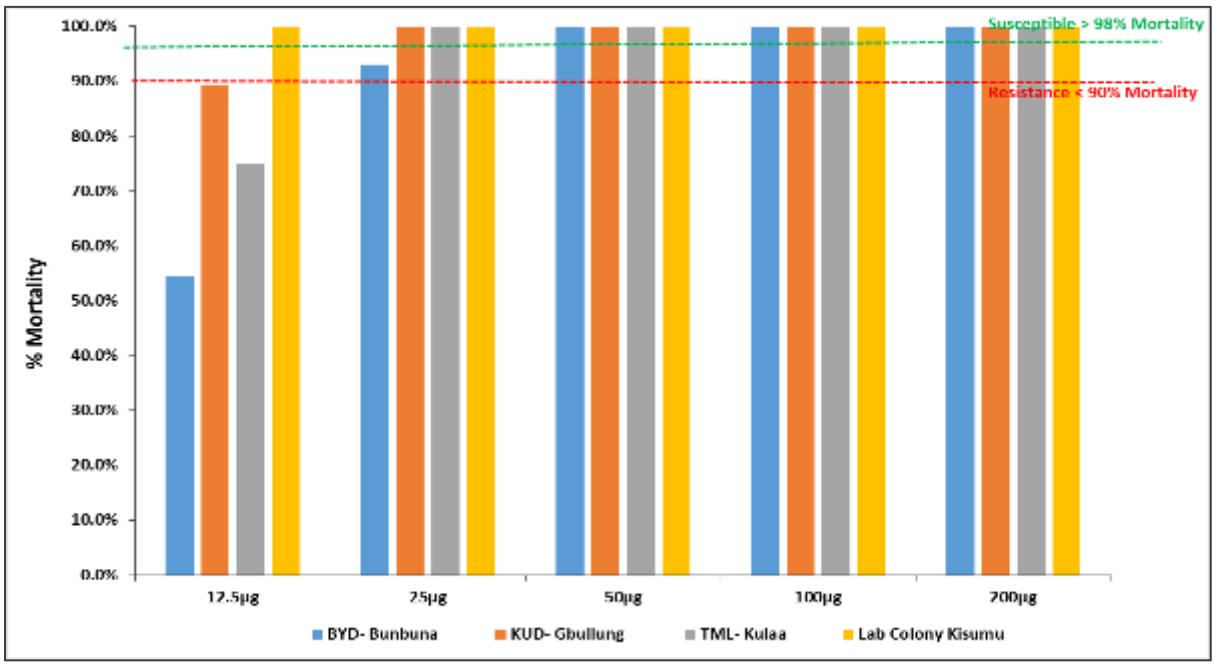
CDC bottle assays also showed that *An. gambiae* s.l. from all sites were susceptible (> 98 percent mortality) to clothianidin, with the exception of Gushegu, where vectors were potentially resistant (97 percent mortality) (Figure 15).

FIGURE 15: CLOTHIANIDIN SUSCEPTIBILITY *OF AN. GAMBIAE* S.L. FROM SENTINEL SITES IN NORTHERN REGION, GHANA IN 2018, BY CDC BOTTLE ASSAYS



An. gambiae s.l. from all three sites tested were fully susceptibility to chlorfenapyr (50 µg and 100µg/bottle) at 72 hours (three days) post-exposure. An insectary strain was used as a control (Figure 16).

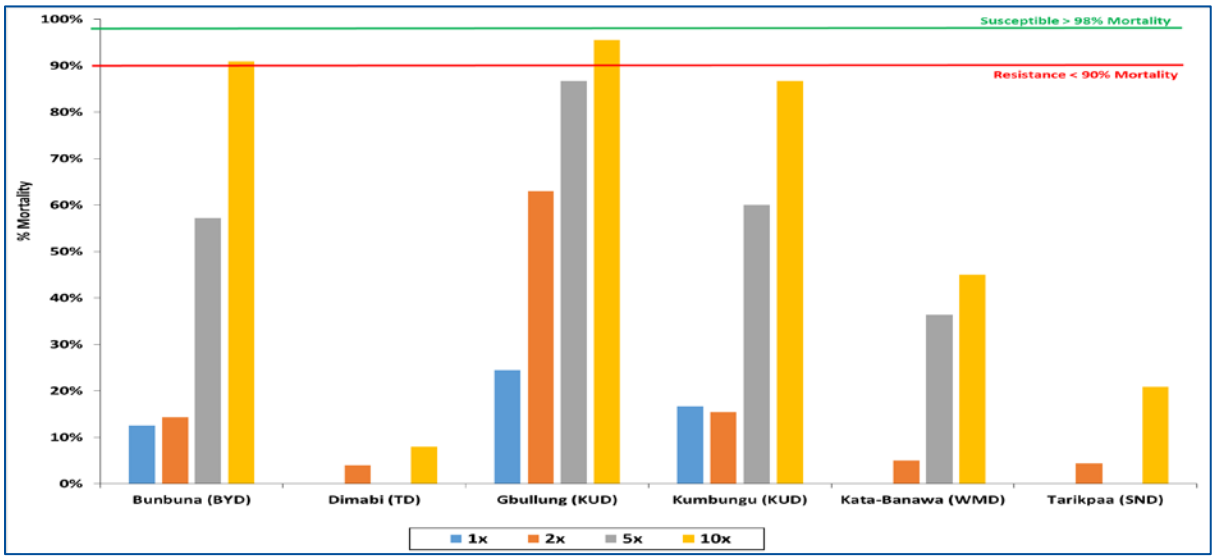
FIGURE 16: CHLORFENAPYR SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. FROM SELECTED SITES IN NORTHERN GHANA TO VARIOUS CONCENTRATIONS OF CHLORFENAPYR (µG/BOTTLE) AT 72 HOURS POST-EPOSURE IN CDC BOTTLE ASSAYS, 2018



3.11 RESISTANCE INTENSITY

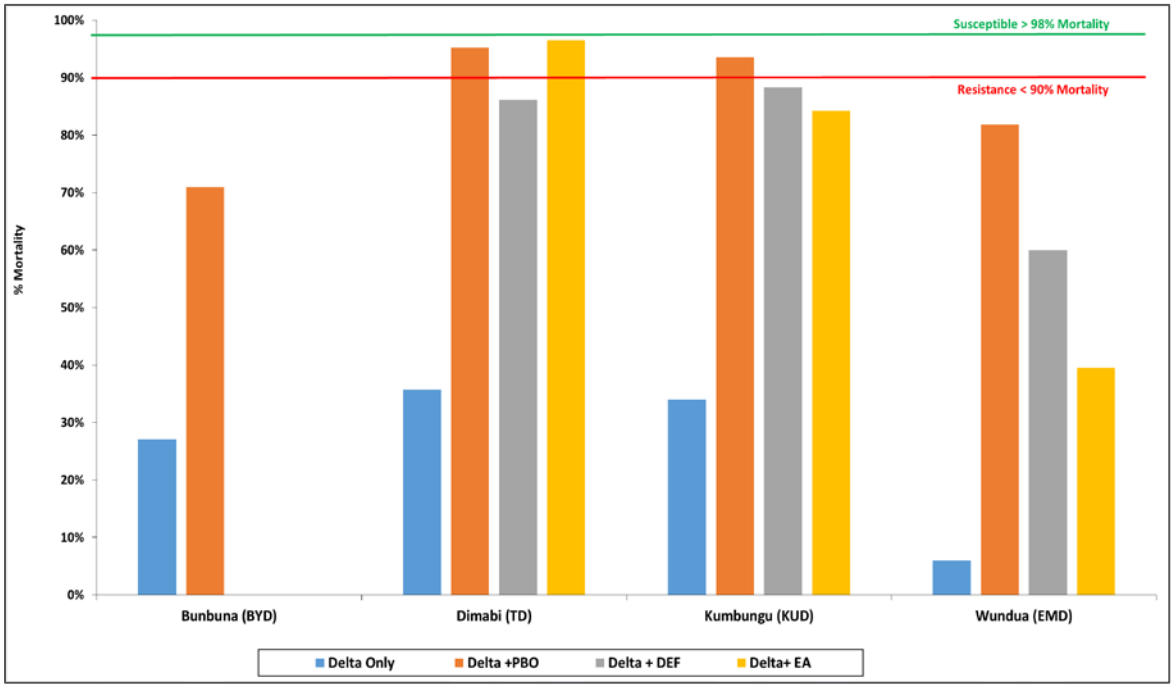
An. gambiae s.l. mosquitoes from sentinel sites in BYD, GUD, KUD, SND, TD, and WMD were resistant to 1x, 2x, 5x, and 10x diagnostic doses of deltamethrin based on the CDC bottle bioassay recommended thresholds. *An. gambiae* s.l. from Tarikpaa (SND) and Dimabi (TD) were highly resistant to high doses of the insecticide (Figures 17).

FIGURE 17: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM SPRAYED AND UNSPRAYED SITES EXPOSED TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN, USING THE CDC-RESISTANCE INTENSITY-RDT SYNERGIST ASSAYS



An. gambiae s.l. from all the sites that were pre-exposed to synergists (PBO or DEF or EA) before deltamethrin showed high mortalities compared to those with no prior exposure to the synergists ($p<0.05$). Mortalities increased by about 3 fold in the local vectors. (Figures 18).

FIGURE 18: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM SPRAYED AND UNSPRAYED SITES TESTED AGAINST DELTAMETHRIN, DELTAMETHRIN + PBO, DELTAMETHRIN + DEF, AND DELTAMETHRIN + EA, CDC METHOD



4. Discussion And Conclusion/Recommendation

Entomological monitoring results indicate that *An. gambiae* s.l. remains the predominant *Anopheles* vector species in all sites, making up more than 98 percent of the total *Anopheles* collected.

An. gambiae s.l. abundance seemed to have been influenced by the rains, as seen in the increased HBRs and IRDs of the vector species with the onset of the rains. The very low IRD of *An. gambiae* s.l. is not clearly understood but could be due to a number of factors, including the fumigant or killing effect of the IRS insecticide, repellent activity of pyrethroids in long-lasting insecticidal bed nets (LLINs), and the design of houses in the region, which have no open eaves, screened doors and windows and have doors that are closed tightly during the night. However, it is less likely to be due only to the killing effect of the insecticide, as the IRD is also low in the non-sprayed sites. The repellent effect of LLINs and housing type may have an impact on IRDs. A recommendation is possibly to modify collection methods to determine the cause for the very low density of mosquitoes collected indoors and also include outdoor resting collections in the routine entomological monitoring. HLCs account for more than 99 percent of all mosquitoes caught and more than 40 percent are collected trying to bite indoors. HLCs are done in houses whose doors are open for the night whereas PSCs are done in houses whose doors are closed. Conducting PSC in the houses where people sleep under LLINs but with doors remaining open would likely provide more clues as to whether house design is a factor in the low IRD. The results show exophagic tendencies of *An. gambiae* s.l. across all sites except in TML, where *An. gambiae* s.l. showed a more endophagic behavior. This outdoor biting preference could be a response to the high IRS coverage and use of LLINs.

Although very low numbers of mosquitoes were collected resting indoors as compared to the HLCs, the IRD of *An. gambiae* s.l. was significantly affected during the post-IRS period in both SS- and Actellic-sprayed districts. Similarly, the proportion of gravid females also declined during the post-IRS period. This could mean that sprayed insecticides could either be killing vectors or preventing them from resting at least 48 hours in the sprayed rooms to develop their eggs. Parity rates in the IRS areas show that significantly fewer older mosquitoes were collected in the sprayed sites.

Although the total number of mosquitoes collected in window exit traps was low, the relatively high proportion of them being collected in the IRS districts suggests that the insecticides could be affecting the resting preference of the mosquitoes. However due to the low numbers collected not much conclusions can be drawn on the resting preference of the vectors.

The general increase in EIR could be the result of increased rainfall in 2018 and associated increase in biting rates. There was a about 3-fold increase in the biting rates of *An. gambaie* s.l. in GUD, KAD and SND, in 2018 when compared to 2017. The relatively high EIRs recorded in MMD, WMD, GUD, KAD and EMD could be a consequence of an increase in biting densities that resulted from flooding that affected most IRS districts in August and September 2018 (Duodu and Fugu 2018; IFRC, 2018; Naatogmah, 2018. & News Ghana, 2018). Bivariate Pearson's correlation analyses suggests that the rains in the non-IRS sites (SND and TML) did not impact biting rates significantly as it did in the IRS districts. The relatively low EIR in KUD as compared to TD (which has similar characteristics as KUD) suggest that IRS could be suppressing transmission in the KUD despite the effect of the rains.

The NMCP also conducted a highly organized mass distribution of LLINs in June just before the peak malaria season in 2018. This campaign excludes all IRS districts but covers all comparison non IRS communities. The impact of this fresh distribution of nets, also could affect comparisons of EIR between 2017 and IRS and non-IRS districts.

An. gambiae s.l. in the tested sites remain resistant to the pyrethroids, possibly resulting from selection pressure maintained by the continuous distribution (under the Health Facility-based Continuous

Distribution of ITNs) and use of the pyrethroid-impregnated bed nets, as well as the use of pyrethroids in aerosol form or in agriculture. This could also account for the high deltamethrin resistance intensity (1x, 2x, 5x, and 10x) observed in *An. gambiae* s.l. across all sentinel sites tested.

Vectors from most sites are susceptible to pirimiphos-methyl and clothianidin. However, the 97 percent mortality recorded for clothianidin in the CDC bottle assay is not conclusive and tests will be repeated to confirm. Vectors in Gbullung and Bunbuna (IRS sites) and Tugu (unsprayed site) were also susceptible to chlorfenapyr. Results from synergist assays suggest that oxidases, esterases and GSTs could be contributing to resistance observed in the local vector species from most sites.

SS exhibited a residual efficacy on all surfaces eight months after spraying, which seems longer than the 6–7months of residual efficacy of Actellic. The general susceptibility of vectors to clothianidin and residual life of SS piloted in the 2018 IRS campaign suggests that the use of SS could be expanded to other PMI IRS districts that have a long history of Actellic use as a rotation strategy to delay resistance development. In 2018, this insecticide has been sprayed in one district by the PMI/VL Ghana project and in 12 districts by the Global Fund supported AGAMal project. This product has also received a WHO/PQ approval. Since only one year of entomological data have been collected from MMD, it may be difficult to assess how the insecticide has affected malaria transmission as compared to the previous year. Continued data collection in the second year is needed to fully understand how SS is affecting malaria transmission indices.

IRS continues to suppress the parity rates and proportion of gravid female mosquito vectors in IRS communities in comparison to unsprayed communities and IRS withdrawn communities. However, there was a general increase in malaria transmission across all sites, which could be a result of the increased biting rates across all sites. The relatively high EIRs recorded in the IRS districts (MMD, WMD, KAD, GUD and EMD) compared to unsprayed (SND and TD) could be a consequence of the increase in biting densities that resulting from heavy rains and flooding in August and September 2018 in most of the IRS sentinel sites. The observed eight-month residual life of SS makes SS a possible option to be widely used for spraying as part of rotation plan in subsequent IRS campaigns.

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 percent
- Carbamates: bendiocarb 0.1 percent
- Organophosphate: pirimiphos-methyl 0.25 percent
- 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 samples (four tests and two controls). 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 percent 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 percent 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 percent ± 10 percent relative humidity.
- At the end of recovery period (i.e., 24 hours post-exposure), the number of dead mosquitoes was 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.

ANNEX B. PREPARATION OF SOLUTION FOR CDC BOTTLE BIOASSAY FOR CLOTHIANIDIN (BAYER PROTOCOL)

Preparation of solutions

- Measure 100ml of acetone using a measuring cylinder and transfer into solution bottle labelled 'A.' As acetone evaporates quickly, be sure to seal the lid of the solution bottle quickly.
- Decant about 5-10ml of Mero ((81 percent rapeseed oil methyl ester, supplied by Bayer) into a small container (do not pipette directly from the bottle because that risks contaminating the whole stock).
- Measure 170µl (0.17ml) Mero using a 200µl micropipette with barrier tip and transfer into solution bottle A with the acetone. As Mero is viscous, some may remain in the pipette tip.
- Use the acetone/Mero solution to rinse the pipette tip. As acetone evaporates quickly, be sure to seal the lid of the solution bottle quickly.
- Seal lid before shaking to thoroughly mix the acetone and Mero solution.
- Transfer 42ml of the acetone/Mero solution into solution bottle labelled 'B.'
- Weigh 3,780µg (equivalent to 3.78mg) of technical grade clothianidin powder using an accurate balance and clean spatula and measuring boat. Carefully check the units of the balance.
- If your balance measures to less than 4 decimal places, you will need to multiply the amount of clothianidin to make the weighing accurate. You will also need to adjust the amount of acetone/Mero solution.
- Transfer 3,780µg clothianidin Technical Grade (TG) (supplied by Bayer) into solution bottle B and thoroughly mix with the 42ml acetone/Mero solution.
- Shake the mixture by hand until fully dissolved. Keep the bottle sealed to prevent evaporation of acetone. Make sure the clothianidin TG is fully dissolved. A magnetic stirrer may be used to aid dissolving.

ANNEX C: 2018 ENTOMOLOGICAL MONITORING RESULTS

FIGURE C-1: COMPOSITION OF *ANOPHELES* SPECIES COLLECTED BY HLC, PSC, AND WINDOW EXIT TRAP COLLECTIONS, IN BYD AND EMD

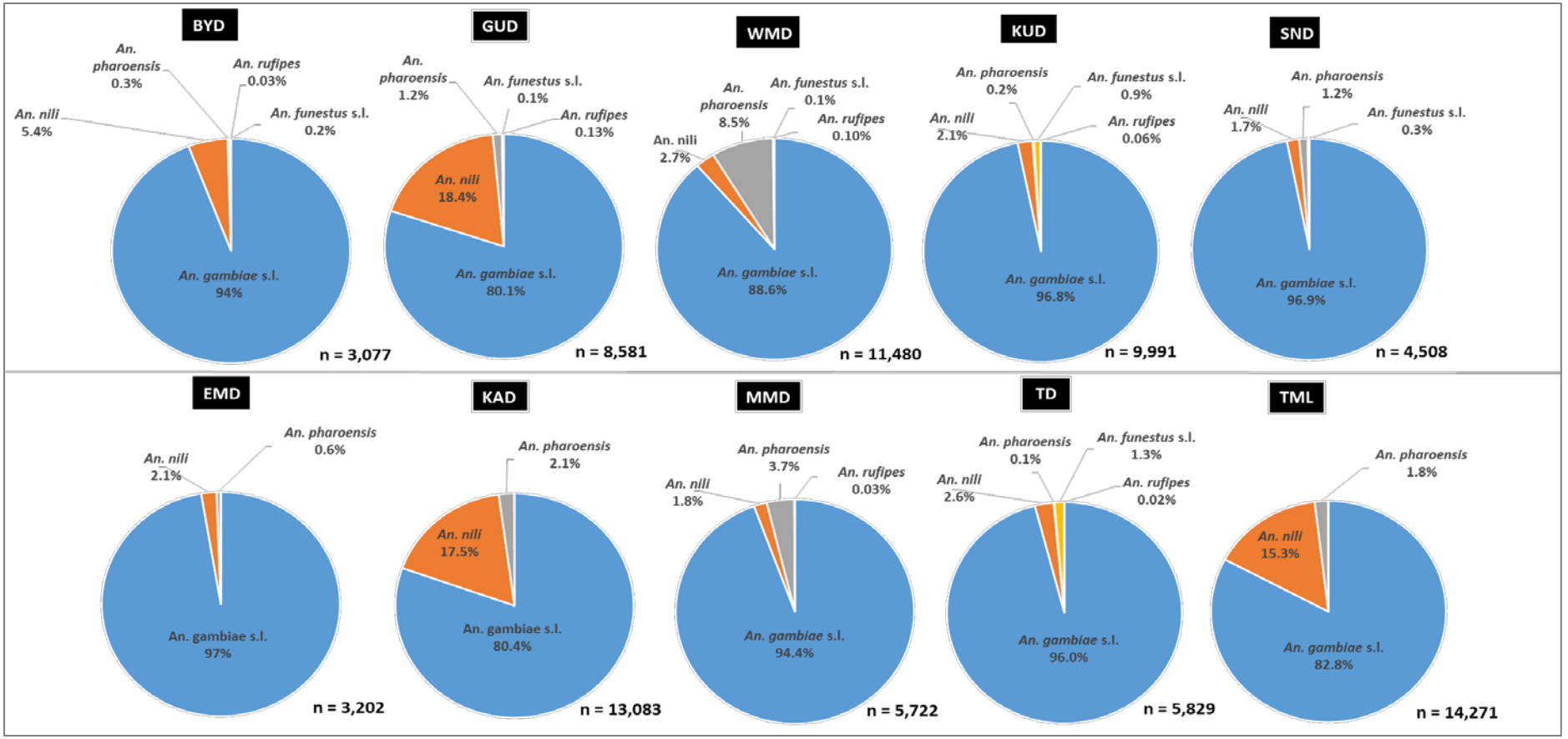


FIGURE C-2: SPRAY QUALITY AND RESIDUAL EFFICACY OF ACTELLIC® 300CS REPRESENTED BY MORTALITY RATES OBSERVED FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES USING KISUMU MOSQUITOES, 2018

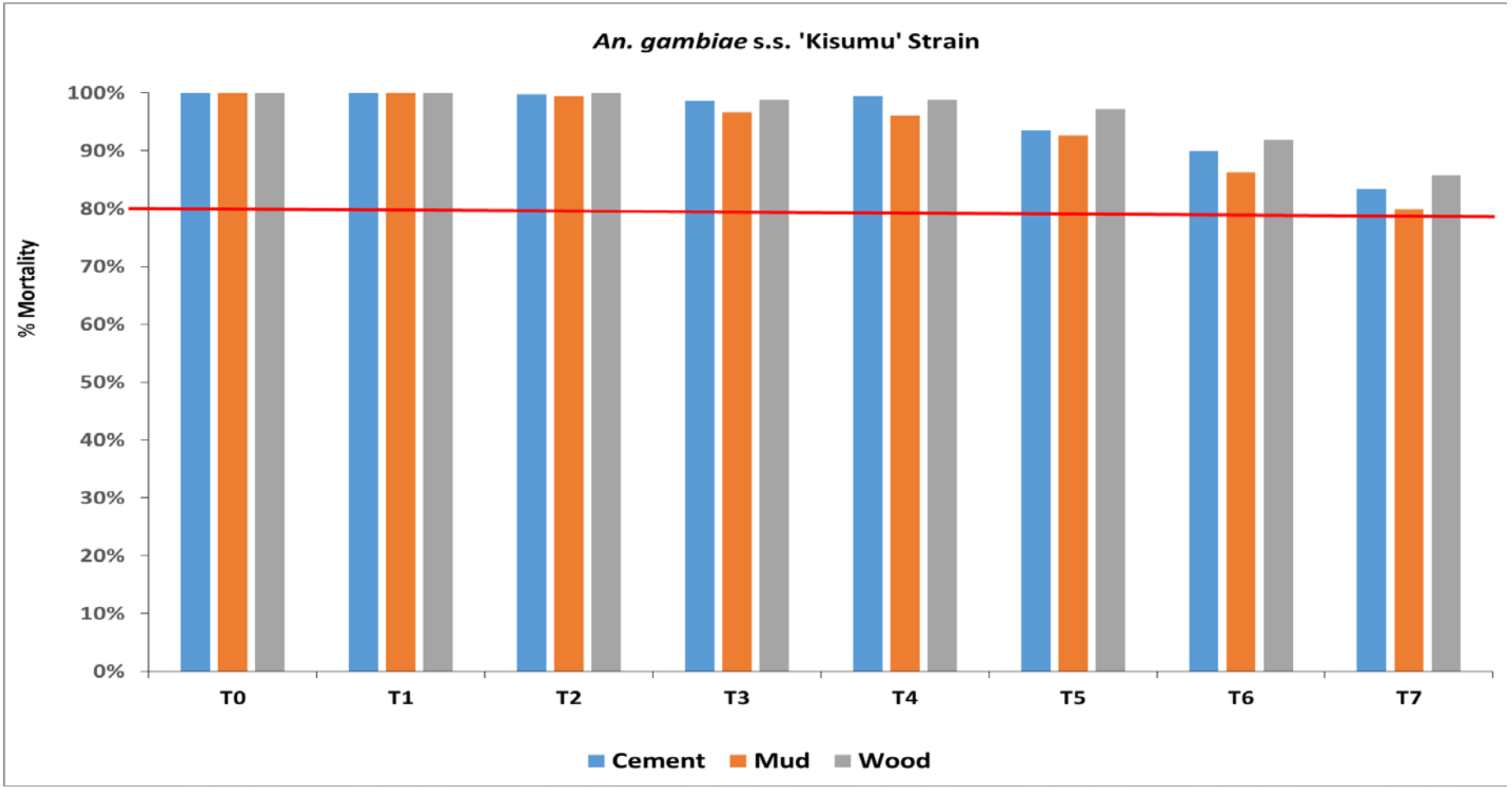


FIGURE C-3: SPRAY QUALITY AND RESIDUAL EFFICACY OF ACTELLIC® 300CS REPRESENTED BY MORTALITY RATES OBSERVED FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES USING WILD *AN. GAMBIAE* S.L. MOSQUITOES, 2018

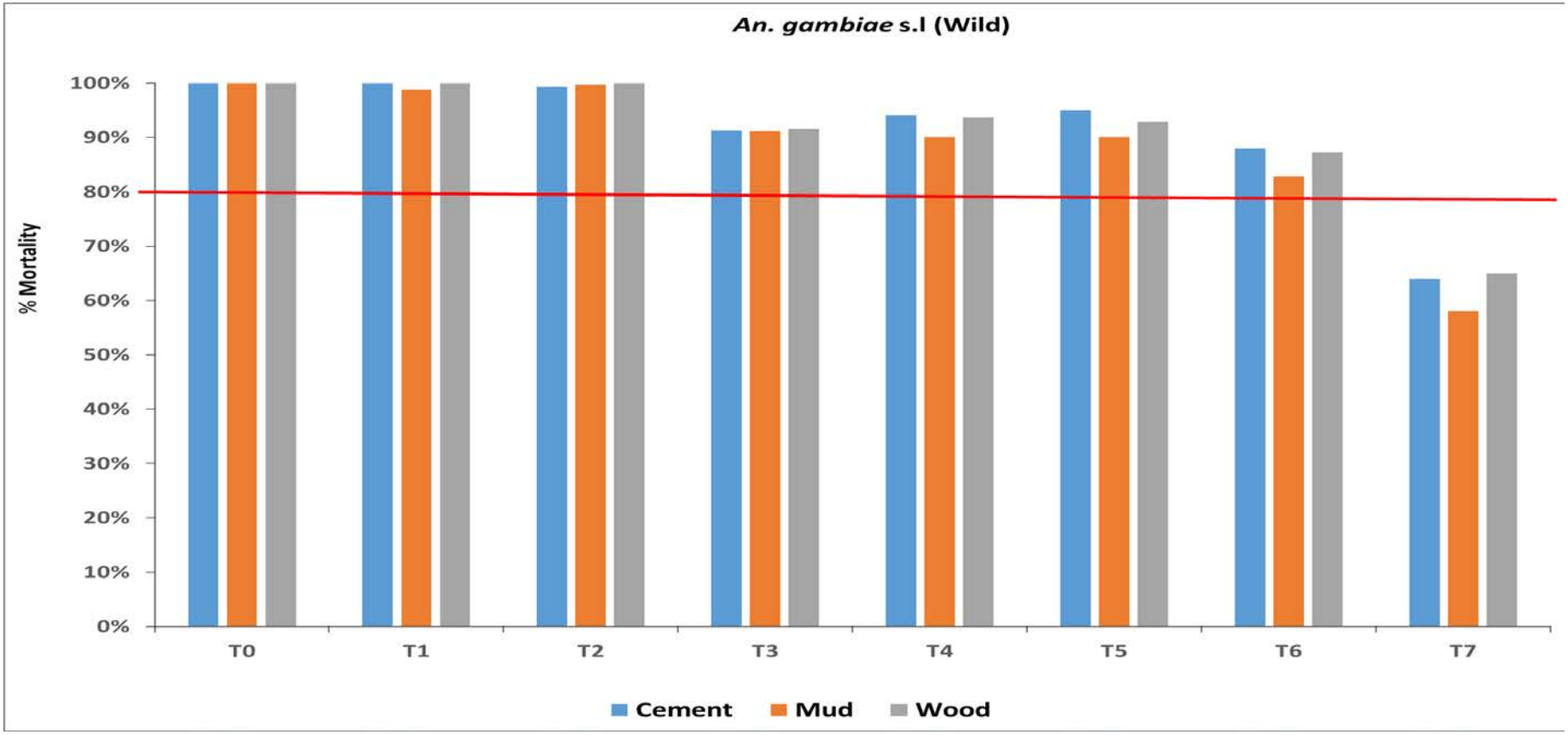


FIGURE C-4: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD® 50WG REPRESENTED BY MORTALITY RATES OBSERVED FOLLOWING CONE BIOASSAYS ON CEMENT SURFACES USING *AN. GAMBIAE* S.S. KISUMU STRAIN, YAGABA (MMD), 2018

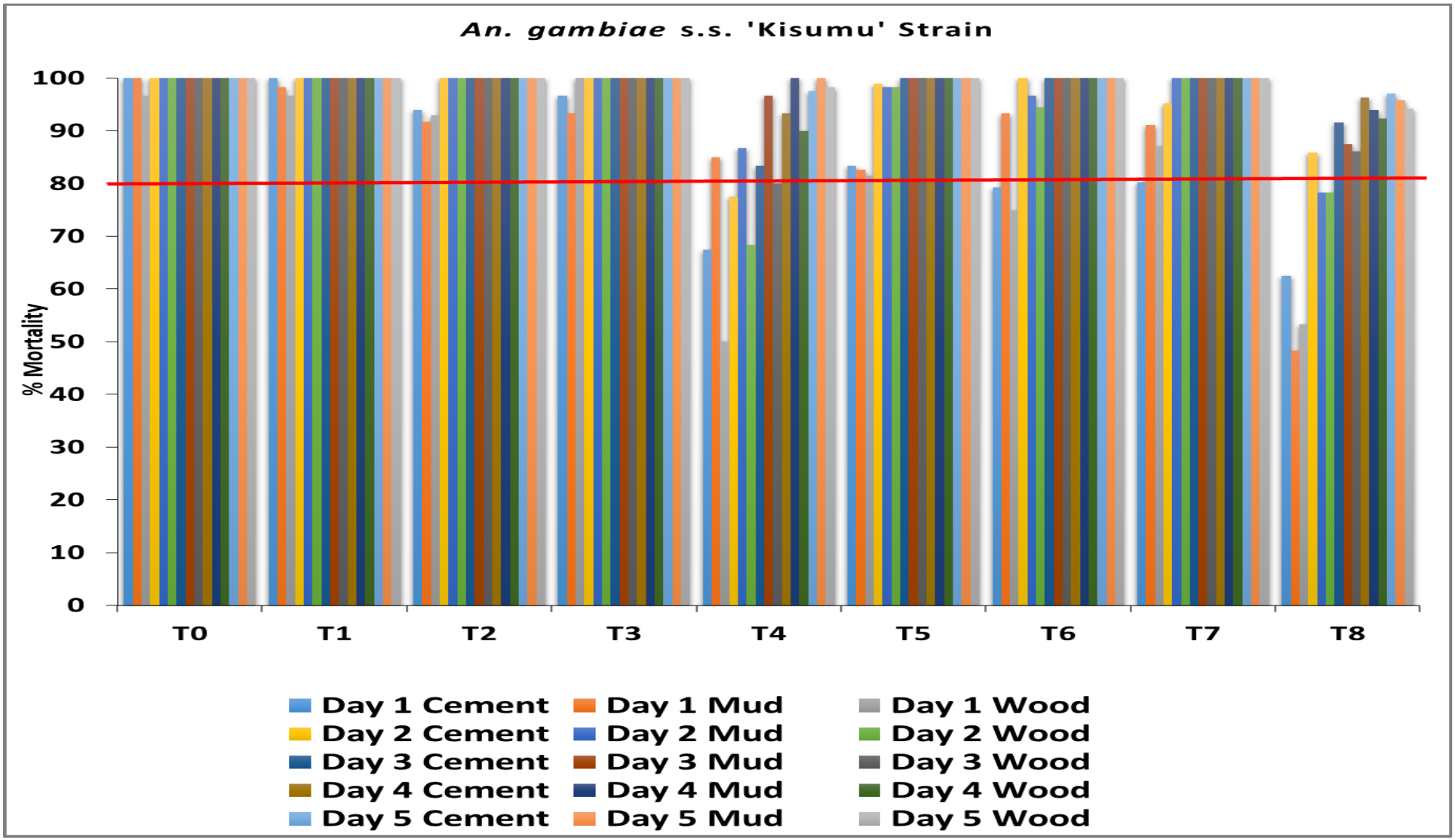


FIGURE C-5 SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD® 50WG REPRESENTED BY MORTALITY RATES OBSERVED FOLLOWING CONE BIOASSAYS ON CEMENT SURFACES USING WILD *AN. GAMBIAE* S.L. MOSQUITOES, YAGABA (MMD), 2018

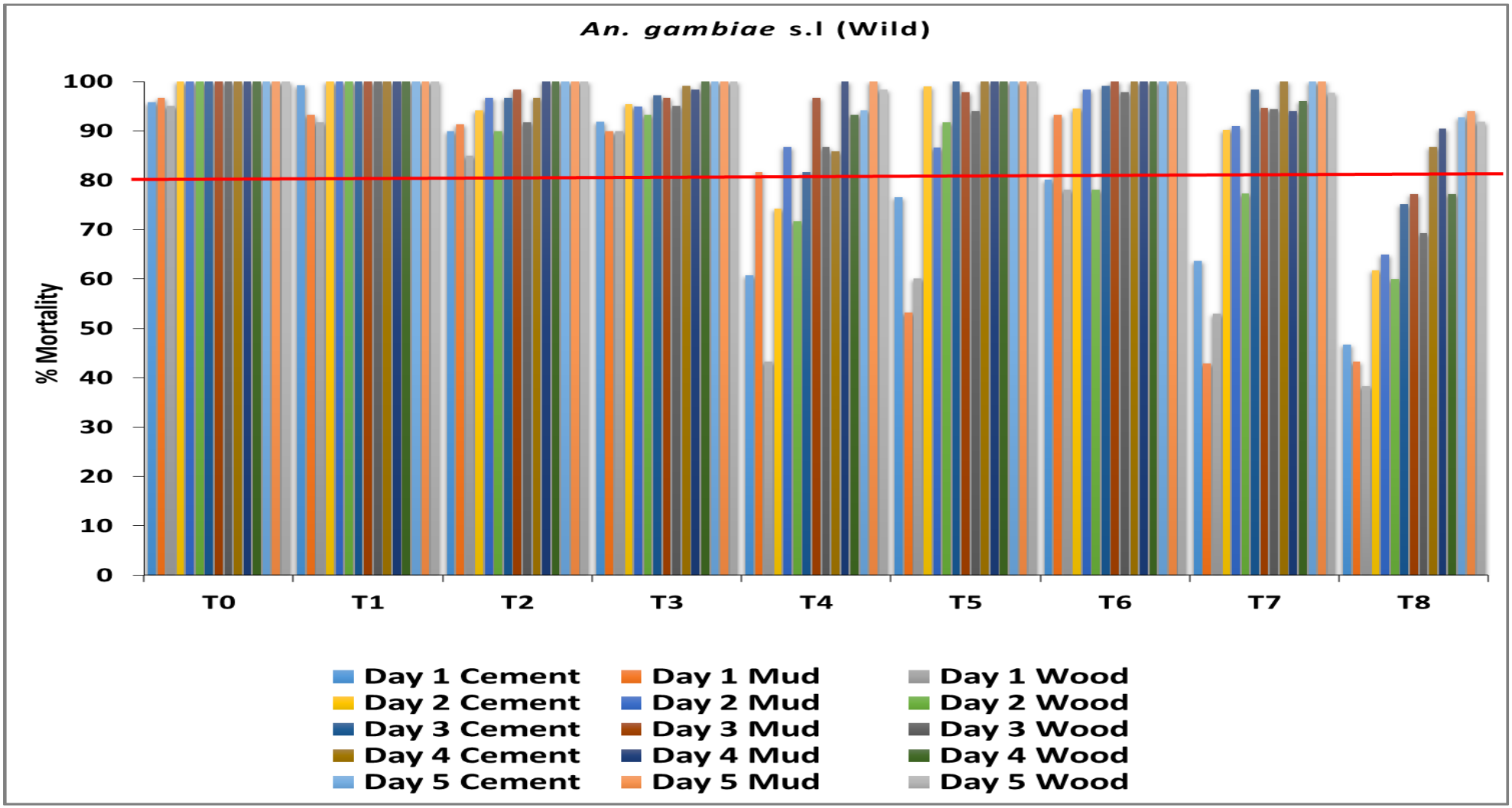


FIGURE C-6: TRENDS IN INDOOR AND OUTDOOR BITING PROPORTIONS OF *AN. GAMBIAE* S.L COLLECTED BY HLC, 2010 TO 2018

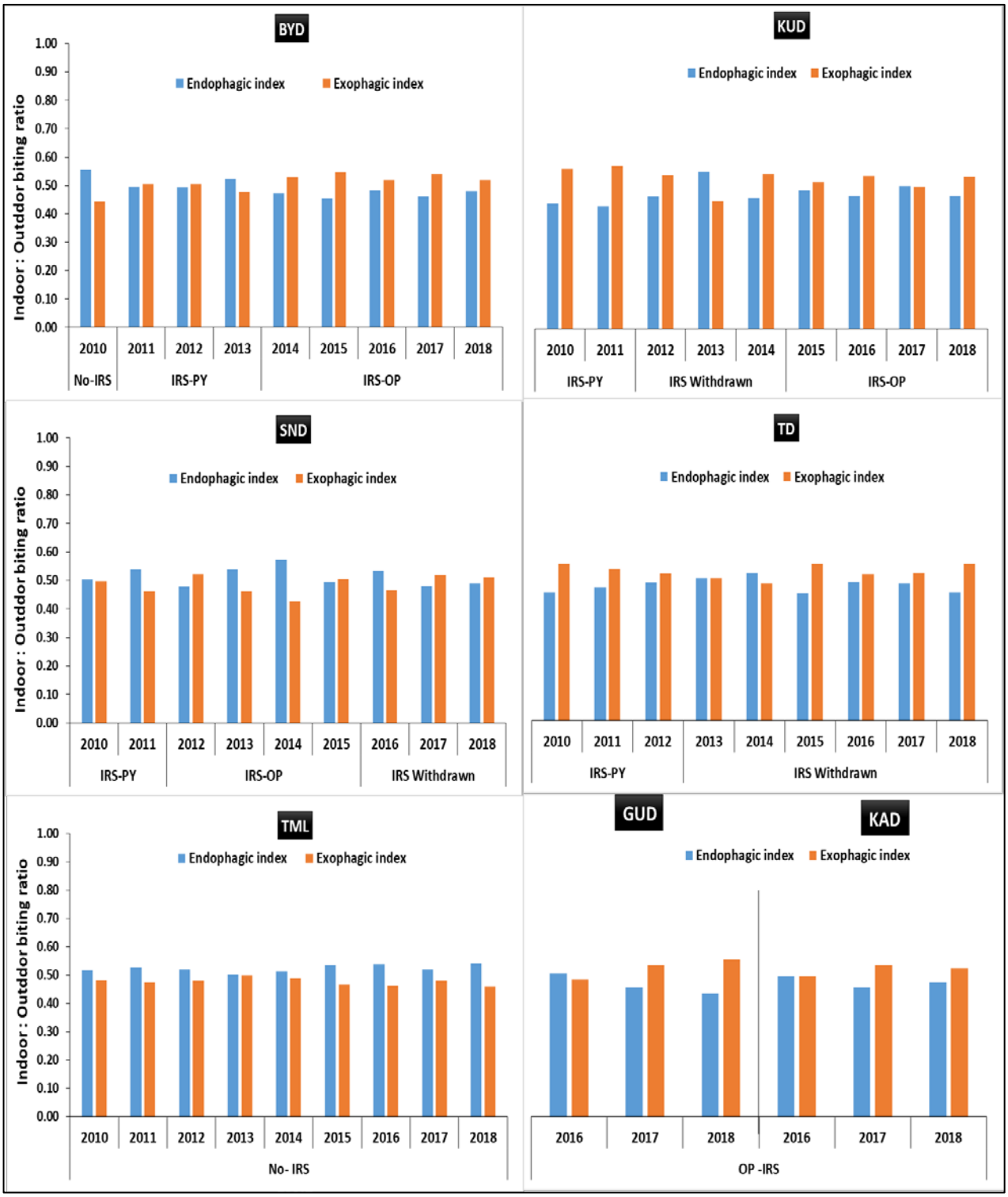
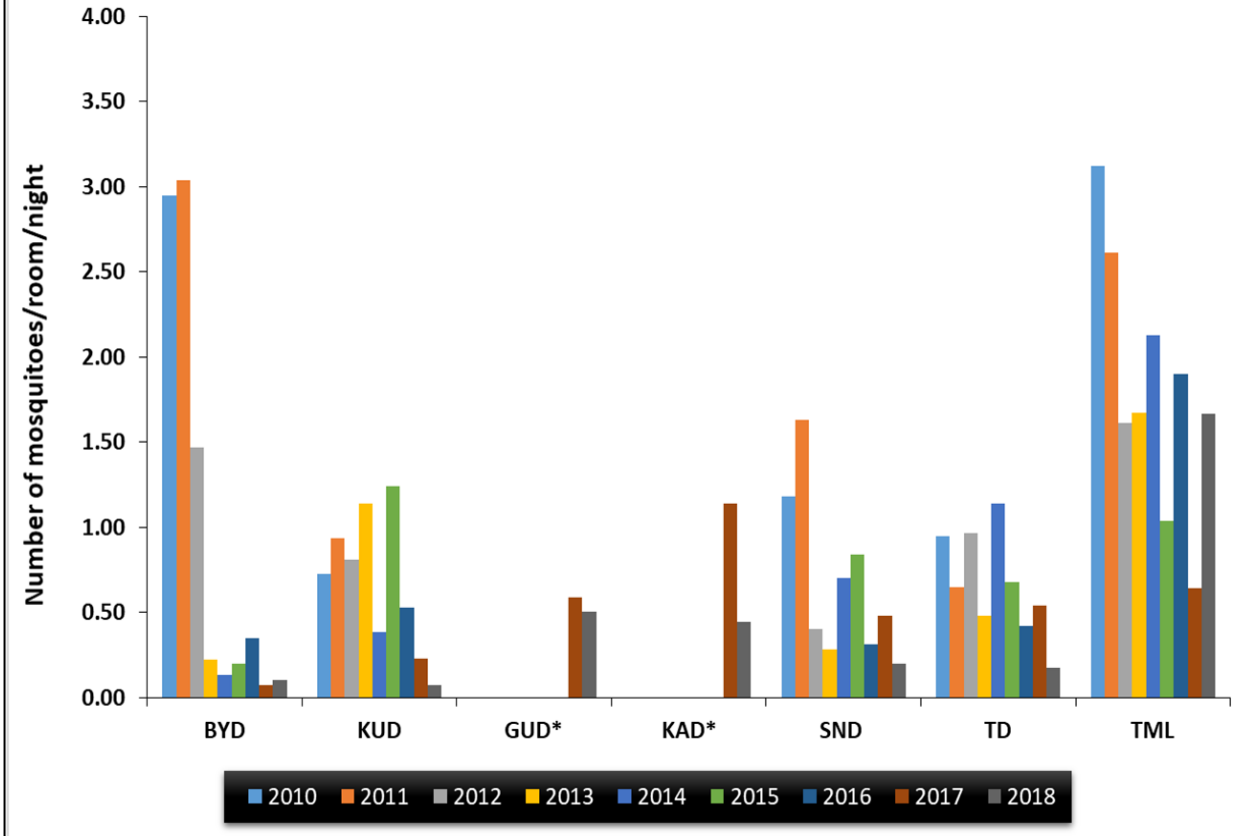


FIGURE C-7: TRENDS IN INDOOR RESTING DENSITIES OF *AN. GAMBIAE* S.L. COLLECTED BY PSC, 2010 TO 2018.



* Data collection in GUD and KAD started in 2016. Mosquito collections in GUD was for only September; mosquito collections in KAD was carried out from September –November 2016. IRDs were 4.13 for GUD and 7.33 for KAD.
IRS was withdrawn in 2013 in TD and KUD, but was reintroduced in KUD in 2015. TD has remained unsprayed since 2013.
IRS was withdrawn in SND in 2015.
TML has never been sprayed.

TABLE C-1: SUMMARY OF WHO INSECTICIDE RESISTANCE TEST RESULTS SHOWING MORTALITY OF *AN. GAMBIAE* S.L. TO SELECTED INSECTICIDES, 2018

Sentinel Site		Pirimiphos-methyl (0.25%)		Alpha-cypermethrin (0.05%)		Bendiocarb (0.1%)		Clothianidin (13.2mg)	
		n	%	n	%	n	%	n	%
BYD	Bunbuna	91	*(100.0%)*	100	*(38.0%)*	100	*(56.5%)*	94	*(100.0%)*
CHD	Chereponi	93	*(100.0%)*	98	*(16.2%)*				
EMD	Wundua	100	*(100.0%)*	97	*(71.1%)*	100	*(81.0%)*	100	*(98.0%)*
GUD	Banda-ya	95	*(100.0%)*	100	*(19.0%)*	99	*(71.7%)*	80	*(100.0%)*
KAD	Binduli	100	*(98.0%)*	100	*(31.0%)*	80	*(78.8%)*		
	Nambrugu	97	*(100.0%)*						
KUD	Kumbungu	100	*(99.0%)*	100	*(11.0%)*	100	*(68.0%)*	97	*(100.0%)*
	Gbullung	96	*(100.0%)*	99	*(20.2%)*	100	*(70.0%)*	94	*(100.0%)*
WMD	Katabanawa	100	*(98.0%)*	96	*(14.6%)*	100	*(83.0%)*	80	*(100.0%)*
	Nakpaya	100	*(100.0%)*						
MMD	Yagaba	100	*(99.0%)*	100	*(49.0%)*	100	*(55.0%)*	92	*(100.0%)*
	Kunkwa	98	*(100.0%)*						
TD	Dimabi	88	*(100.0%)*	100	*(18.0%)*				
	Woribugu	100	*(99.0%)*	80	*(11.3%)*				
SND	Nanton	92	*(94.6%)*	100	*(9.0%)*			98	*(100.0%)*
	Tarikpaa	100	*(100.0%)*	100	*(40.0%)*	100	*(76.0%)*		
TML	Kulaa	100	*(100.0%)*	99	*(8.1%)*	97	*(49.5%)*	99	*(99.0%)*
	Tugu	80	*(98.0%)*	100	*(15.0%)*	100	*(42.0%)*	98	*(100.0%)*

Resistant Potentially resistant Susceptible

TABLE C-2: A COMPARISON OF 2017 AND 2018 INDOOR AND OUTDOOR HBRS OF *AN. GAMBIAE* S.L., AS DETERMINED FROM HLCS

Districts	2017		2018		Fold increase between 2017 and 2018	
	Indoor biting rate (b/p/n)	Outdoor biting rate (b/p/n)	Indoor biting rate (b/p/n)	Outdoor biting rate (b/p/n)	Indoor biting rate (b/p/n)	Outdoor biting rate (b/p/n)
BYD (IRS)	0.58	0.68	4.31	4.7	7.4	6.9
GUD (IRS)	5.3	6.18	9.34	11.79	1.8	1.9
KAD (IRS)	8.15	9.39	15.62	16.92	1.9	1.8
KUD (IRS)	10.69	10.65	11.74	13.32	1.1	1.3
SND (IRS withdrawn)	2.58	2.80	6.56	6.94	2.5	2.5
TD (IRS withdrawn)	6.04	6.50	7.89	9.48	1.3	1.5
Tamale (No-IRS)	18.64	17.22	16.65	13.91	0.9	0.8

TABLE C-3: OVERALL ENTOMOLOGICAL PARAMETERS OF MONTHLY MALARIA TRANSMISSION FOR *ANOPHELES GAMBIAE* COLLECTED FROM ALL SENTINEL DISTRICTS FROM MARCH TO DECEMBER2018

BYD						
Month	HBR	# Examined by ELISA	Number +ve for sporozoite	SPZ rate	Nightly EIR	Monthly EIR
March	0.08	2	0	0.00	0.00	0.0
April	0.00	0	0	0.00	0.00	0.0
May	0.00	0	0	0.00	0.00	0.0
June	0.00	0	0	0.00	0.00	0.0
July	1.06	50	0	0.00	0.00	0.0
August	23.36	350	0	0.00	0.00	0.0
September	17.83	361	0	0.00	0.00	0.0
October	0.38	23	0	0.00	0.00	0.0
November	0.56	32	0	0.00	0.00	0.0
December	1.77	37	0	0.00	0.00	0.0
					Sum of EIR	0.0
EMD						
Month	HBR	Number ex ELISA	Number +ve for sporozoite	SPZ rate	Nightly EIR	Monthly EIR
March	0.67	15	1	0.07	0.04	1.4
April	0.00	0	0	0.00	0.00	0.0
May	0.00	0	0	0.00	0.00	0.0
June	0.00	0	0	0.00	0.00	0.0
July	2.36	61	1	0.02	0.04	1.2
August	29.52	429	3	0.01	0.21	6.4
September	14.38	400	3	0.01	0.11	3.2
October	0.69	42	0	0.00	0.00	0.0
November	0.13	0	0	0.00	0.00	0.0
December	0.14	4	0	0.00	0.00	0.0
					Sum of EIR	12.2

GUD						
Month	HBR	Number ex ELISA	Number +ve for sporozoite	SPZ rate	Nightly EIR	Monthly EIR
March	0.00	0	0	0.00	0.00	0.0
April	0.14	0	0	0.00	0.00	0.0
May	3.38	50	0	0.00	0.00	0.0
June	0.00	0	0	0.00	0.00	0.0
July	5.03	110	0	0.00	0.00	0.0
August	28.36	198	1	0.01	0.14	4.4
September	64.11	326	2	0.01	0.39	11.8
October	4.38	100	0	0.00	0.00	0.0
November	0.14	2	0	0.00	0.00	0.0
December	0.13	0	0	0.00	0.00	0.0
					Sum of EIR	**16.2**

KAD						
Month	HBR	Number ex ELISA	Number +ve for sporozoite	SPZ rate	Nightly EIR	Monthly EIR
March	0.23	5	0	0.00	0.00	0.0
April	0.06	0	0	0.00	0.00	0.0
May	0.00	0	0	0.00	0.00	0.0
June	0.00	0	0	0.00	0.00	0.0
July	39.25	270	0	0.00	0.00	0.0
August	37.95	227	1	0.00	0.17	5.4
September	67.05	300	2	0.01	0.43	13.0
October	16.98	144	0	0.00	0.00	0.0
November	0.66	18	0	0.00	0.00	0.0
December	0.48	10	0	0.00	0.00	0.0
					Sum of EIR	**18.4**

KUD						
Month	HBR	Number ex ELISA	Number +ve for sporozoite	SPZ rate	Nightly EIR	Monthly EIR
March	0.00	0	0	0.00	0.00	0.0
April	0.03	0	0	0.00	0.00	0.0
May	0.00	0	0	0.00	0.00	0.0
June	3.20	64	0	0.00	0.00	0.0
July	6.73	100	1	0.01	0.07	2.1
August	15.25	210	2	0.01	0.15	4.5
September	60.05	491	0	0.00	0.00	0.0
October	14.76	244	0	0.00	0.00	0.0
November	0.08	5	0	0.00	0.00	0.0
December	0.19	14	0	0.00	0.00	0.0
					Sum of EIR	**6.6**

MMD						
Month	HBR	Number ex ELISA	Number +ve for sporozoite	SPZ rate	Nightly EIR	Monthly EIR
March	0.06	0	0	0.00	0.00	0.0
April	0.00	0	0	0.00	0.00	0.0
May	0.00	0	0	0.00	0.00	0.0
June	0.00	0	0	0.00	0.00	0.0
July	10.19	121	0	0.00	0.00	0.0
August	36.63	300	4	0.01	0.49	15.1
September	33.61	250	2	0.01	0.27	8.1
October	1.89	21	0	0.00	0.00	0.0
November	0.05	1	0	0.00	0.00	0.0
December	0.27	8	0	0.00	0.00	0.0
					Sum of EIR	**23.2**

WMD						
Month	HBR	Number ex ELISA	Number +ve for sporozoite	SPZ rate	Nightly EIR	Monthly EIR
March	2.34	46	0	0.00	0.00	0.0
April	0.00	0	0	0.00	0.00	0.0
May	0.00	0	0	0.00	0.00	0.0
June	0.00	0	0	0.00	0.00	0.0
July	14.63	130	0	0.00	0.00	0.0
August	90.81	322	3	0.01	0.85	26.2
September	43.66	202	0	0.00	0.00	0.0
October	4.02	57	0	0.00	0.00	0.0
November	1.20	15	0	0.00	0.00	0.0
December	0.06	3	0	0.00	0.00	0.0
					Sum of EIR	**26.2**

SND						
Month	HBR	Number ex ELISA	Number +ve for sporozoite	SPZ rate	Nightly EIR	Monthly EIR
March	0.00	0	0	0.00	0.00	0.0
April	0.00	0	0	0.00	0.00	0.0
May	0.00	0	0	0.00	0.00	0.0
June	0.00	0	0	0.00	0.00	0.0
July	15.86	100	1	0.01	0.16	4.9
August	6.31	114	0	0.00	0.00	0.0
September	37.36	328	1	0.00	0.11	3.4
October	7.81	0	0	0.00	0.00	0.0
November	0.02	1	0	0.00	0.00	0.0
December	0.11	7	1	0.14	0.02	0.5
					Sum of EIR	**8.8**

TD						
Month	**HBR**	**Number ex ELISA**	**Number +ve for sporozoite**	**SPZ rate**	**Nightly EIR**	**Monthly EIR**
March	0.00	0	0	0.00	0.00	0.0
April	0.02	0	0	0.00	0.00	0.0
May	0.00	0	0	0.00	0.00	0.0
June	0.16	4	0	0.00	0.00	0.0
July	6.72	130	0	0.00	0.00	0.0
August	15.73	247	1	0.00	0.06	2.0
September	55.88	350	2	0.01	0.32	9.6
October	8.17	167	1	0.01	0.05	1.5
November	0.06	3	0	0.00	0.00	0.0
December	0.08	4	0	0.00	0.00	0.0
					Sum of EIR	**13.1**

TML						
Month	**HBR**	**Number ex ELISA**	**Number +ve for sporozoite**	**SPZ rate**	**Nightly EIR**	**Monthly EIR**
March	0.03	0	0	0.00	0.00	0.0
April	5.13	95	1	0.01	0.05	1.6
May	15.03	200	0	0.00	0.00	0.0
June	2.69	156	0	0.00	0.00	0.0
July	7.59	200	0	0.00	0.00	0.0
August	22.91	500	3	0.01	0.14	4.3
September	49.30	650	5	0.01	0.38	11.4
October	23.05	500	2	0.00	0.09	2.9
November	2.02	35	0	0.00	0.00	0.0
December	0.21	15	0	0.00	0.00	0.0
					Sum of EIR	**20.1**

ANNEX D. REFERENCES

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