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PMI VECTORLINK GHANA ANNUAL ENTOMOLOGICAL MONITORING REPORT FOR NORTHERN GHANA

MARCH 1 – DECEMBER 31, 2021

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ACRONYMS

ABI	Animal Blood Index
Ace-1	Acetylcholinesterase 1
AChE	Acetylcholinesterase
AGAMal	AngloGold Ashanti Malaria Control Ltd
b/p/n	Bites/Person/Night
BND	Bunkpurugu-Nakpanduri District
CDC	U.S. Centers for Disease Control and Prevention
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
EMD	East Mamprusi District
GUD	Gushegu District
HBI	Human Blood Index
HBR	Human Biting Rate
HLC	Human Landing Catch
ib/p/yr	Infective Bites/Person/Year
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Net
KAD	Karaga District
kdr	Knockdown Resistance
KUD	Kumbungu District
MMD	Mamprugu Moaduri District
NIRMOP	National Insecticide Resistance Monitoring Partnership
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
P.	*Plasmodium*
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SGD	Sagnerigu District
SOP	Standard Operating Procedure
TD	Tolon District
TML	Tamale Metropolis
TSD	Tatale-Sanguli District
USAID	United States Agency for International Development
WHO	World Health Organization
WMD	West Mamprusi District
YND	Yunyoo-Nasuan District

EXECUTIVE SUMMARY

BACKGROUND AND METHODS

The President's Malaria Initiative (PMI) VectorLink Project conducted entomological monitoring of the 2021 indoor residual spray (IRS) campaign in northern Ghana. The entomological monitoring included tests to assess spray quality and residual efficacy of sprayed insecticide products SumiShield and Fludora Fusion. In 2021, the project continued insecticide susceptibility tests, routine monitoring of malaria vector bionomics, and data collection from animal shelters. Insecticide susceptibility testing and monthly mosquito collections were performed from March through December 2021 in eight sentinel sites in eight districts: six IRS districts (Bunkpurugu-Nakpanduri (BND), Kumbungu (KUD), East Mamprusi (EMD), Gushegu (GUD), Tatale-Sanguli (TSD), and West Mamprusi (WMD)) and two districts that have never been sprayed (Sagnarigu (SGD) and Tamale metropolis (TML)). Insecticide resistance tests only were also carried out in Tolon District (TD), where IRS was withdrawn in 2013. For routine monitoring, the project used indoor and outdoor human landing catches and Prokopack collections in sleeping rooms (for indoor) and animal shelters and pit traps (for outdoor). The project followed the World Health Organization (WHO) and PMI VectorLink Project standard operating procedure (SOP05/01) for wall bioassay tests to determine the spray quality and decay rate of the sprayed insecticides. CDC bottle assays, WHO tube tests, and synergist assays were used to determine vector susceptibility to insecticides. The ELISA (enzyme-linked immunosorbent assay) protocols described by Wirtz et al. (1987) and Beier et al. (1988) were used to test for the presence of *Plasmodium falciparum* circumsporozoite proteins to determine the parasite infection rate and blood meal source, respectively. The frequency of acetylcholinesterase-1 (*Ace-1)* and knockdown resistance (*kdr)* genotypes in *An. gambiae* s.l. populations across the sentinel sites were determined by polymerase chain reaction (PCR) using Wilkins et al. (2006) and Martinez-Torres et al. (1998) protocols, respectively. Biochemical assays were also performed to determine the activity levels of detoxification enzymes in *An. gambiae* s.l. using the protocol described by Leong et al. (2019).

The project supported the National Malaria Control Program (NMCP) of the Ghana Health Service to collect insecticide resistance data from 15 sentinel sites in 15 regions, through the National Insecticide Resistance Monitoring Partnership (NIRMOP) managed by the Noguchi Memorial Institute for Medical Research. The results from the NIRMOP activities will be submitted in a separate report that combines data from all PMI-sponsored sites as well as Global Fund-supported sites. The project also collected vector bionomics data in six sites in six regions (two of these sites are project routine IRS sites) as a partner of the NMCP's National Entomological Surveillance Program. The results from the National Entomological Surveillance Program work will be shared with the NMCP and partners after one year of data collection by all partners.

RESULTS AND DISCUSSION

Vector species composition and behavior. *An. gambiae* s.l. was the predominant species collected across all sites. It constituted 96.2% (31,238/32,481) and 94.8% (15,405/16,247) of all *Anopheles* collected in the IRS intervention and unsprayed sites, respectively. *An. gambiae* was the major species comprising 61.3% of the 1,864 *An. gambiae* s.l. analyzed further by PCR, An. *coluzzii* and *An. arabiensis* making 25.4% and 9.4%, respectively. The rest were *An. gambiae*/*An. coluzzii* hybrid. Higher indoor human biting rates were observed in both IRS and unsprayed sites as compared to outdoor biting rates. The mean indoor resting densities of *An. gambiae* s.l. were generally low, 0.61 mosquitoes per room per day for the IRS sites and 0.55 mosquito per room per day for unsprayed sites.

Parity rates: The mean proportion of parous *An. gambiae* s.l. in IRS districts (42%) was significantly lower than that recorded in the unsprayed sites (54%) (p=0.024).

Entomological inoculation rate (EIR): The estimated risk of malaria transmission for the 10 months was estimated from the sum of monthly EIRs in sites from March through December. The sum of monthly EIRs

calculated was highest in a SumiShield-sprayed community in BND, which recorded 5.22 outdoor and 6.07 indoor infective bites/person/year (ib/p/yr). TML, an unsprayed site, recorded the highest outdoor EIR of 5.70 ib/p/yr. The sum of monthly indoor EIRs was highest in BND (6.07 ib/p/yr), followed by EMD (3.42 ib/p/yr).

Spray quality and residual life of IRS insecticides: Monthly wall bioassays conducted on all sprayed surfaces (mud, cement, and wood) indicate high-quality and uniform spraying was observed across all sites tested. The residual effect of Fludora Fusion and SumiShield 50 WG lasted at least 10 months on all types of surfaces sprayed when tests were performed with Kisumu strain *An. gambiae*.

Insecticide susceptibility: *An. gambiae* s.l. mosquitoes from both IRS and non-IRS districts were resistant to pirimiphos-methyl (<90% mortality) except in Kunkwa (MMD) and Dimabi (TD), where mosquitoes were susceptible (98–100% mortality). Bunbuna (BND) and Kulaa (SGD), where mortality was between 90 and 97%, had possible resistance. Moderate to high pyrethroid resistance intensity was observed across most sites, and synergist assay results suggest that mono-oxygenases may play a significant role in this resistance in most sites. *An. gambiae* s.l. from across most sites tested were susceptible to clothianidin (two days post exposure) and chlorfenapyr (three days post exposure).

Spraying of animal shelters: Mosquito resting density in IRS intervention sites where animal shelters were sprayed was lower (0.69, 1.03, and 0.14 per day per animal shed, pit shelter, and sleeping room, respectively) than in IRS intervention sites where animal shelters were not sprayed (1.17, 2.7, and 0.39 per day per animal shed, pit shelter, and sleeping room, respectively) and in control sites (3.23, 2.49, and 0.55 per day per animal shed, pit shelter, and sleeping room, respectively). This suggests that spraying animal shelters had an impact on mosquito resting density.

CONCLUSION

Analysis of parity rates showed that significantly fewer older mosquitoes were collected in the sprayed sites than in the unsprayed sites. These data suggest that IRS is reducing mosquito longevity—and thus malaria transmission—in intervention sites.

Considering that vectors in most sites remain susceptible to clothianidin, SumiShield 50WG and Fludora Fusion indicate that these insecticides remain plausible alternatives for future IRS campaigns in northern Ghana; however, close monitoring of the resistance is necessary to continue. Both insecticide formulations have demonstrated a residual efficacy that lasts beyond the malaria transmission season and can therefore remain in use for IRS.

I. INTRODUCTION

In 2021, the President's Malaria Initiative (PMI) VectorLink Ghana project implemented indoor residual spraying (IRS) in nine districts in northern Ghana: Bunkpurugu-Nakpanduri (BND), East Mamprusi (EMD), Gushegu (GUD), Karaga (KAD), Kumbungu (KUD), Mamprugu Moaduri (MMD), Tatale-Sanguli District (TSD), West Mamprusi (WMD), and Yunyoo-Nasuan (YND). The project has historically conducted IRS once a year, just before the beginning of the rainy season. The campaign is planned so that the spraying of houses is completed before the mosquito population peaks (shortly after the rains start), which precedes the peak of the malaria transmission season.

Two insecticide products were sprayed in 2021: SumiShield 50WG (clothianidin at a rate of 300 mg/m^2) and Fludora Fusion (clothianidin at a rate 200 mg/m^2 and deltamethrin at 25 mg/m^2). The selection of insecticides was based on results of insecticide susceptibility and residual efficacy tests from 2019 and 2020 and in accordance with the National Insecticide Resistance Management Strategy. Test results indicated that vectors from all sites were susceptible to clothianidin, an active ingredient in SumiShield 50WG and Fludora Fusion. However, resistance to pirimiphos-methyl, the active ingredient in Actellic 300CS, was detected in EMD in 2019. Preventing further development of resistance to pirimiphos-methyl necessitated a switch to a different class of IRS insecticide. Fludora Fusion was sprayed in five districts (KAD, KUD, MMD, TSD, and WMD) and SumiShield 50WG was sprayed in four districts (BND, EMD, GUD, and YND) in 2021.

In 2021, the project continued spraying animal shelters in five districts (BND, EMD, KUD, MMD, and YND) based on the results from an operational research study conducted between 2017 and 2019 that identified animal shelters as important resting places for the predominant malaria vectors in the study area. To monitor the impact of spraying animal shelters on malaria transmission indicators, the project compared data collected from the sites where animal shelters were sprayed with data from unsprayed sites.

To assess the impact of IRS on entomological indices of malaria transmission, VectorLink Ghana carried out routine entomological surveys in eight sites in eight districts (both sprayed and unsprayed) across the Northern and Northeast regions of Ghana from March through December 2021.

Specific objectives of the 2021 entomological surveys were:

1. Monitoring the species composition of malaria vectors in the target districts
2. Monitoring vector densities, behavior, and seasonality
3. Estimating and comparing malaria transmission indices (parity, entomological inoculation rate (EIR), and blood meal source) in sprayed and unsprayed sites
4. Monitoring the impact of spraying animal shelters on entomologocal indices of malaria transmission
5. Determining the susceptibility of local vector species to relevant IRS and insecticide-treated net (ITN) insecticides for malaria vector control, and identifying mechanisms of resistance where resistance was detected
6. Assessing the quality of the IRS operations across all nine districts and evaluating the residual efficacy of SumiShield 50WG and Fludora Fusion

The project also provided technical and financial support to the National Malaria Control Program (NMCP) of the Ghana Health Service to collect insecticide resistance data from 15 sentinel sites in 15 regions, through the National Insecticide Resistance Monitoring Partnership (NIRMOP) managed by the Noguchi Memorial Institute for Medical Research. The project also collected vector bionomics data in six sites in six regions as a partner to the NMCP's National Entomological Surveillance Program. The results from the NIRMOP activities will be submitted in a separate report that combines data from all PMI- and Global Fund-sponsored sites. The results from the National Entomological Surveillance Program work will be shared with the NMCP and partners after one year of data collection by all partners.

The VectorLink Ghana entomology team worked closely with the Ghana Health Service and District Assemblies to implement all planned field activities and partnered with AngloGold Ashanti Malaria Control Ltd (AGAMal) to conduct advanced molecular analyses of collected samples. This report presents findings and analyses of the entomological monitoring activities the project carried out in 2021.

2. METHODOLOGY

2.1 SENTINEL SITES

VectorLink Ghana conducted monitoring in eight sentinel sites located in six IRS districts (BND, EMD, GUD, KUD, TSD, and WMD) and in two districts that have never been sprayed (Tamale Metropolis (TML) and Sagnarigu District (SGD), shown in Figure 1. Insecticide resistance tests were performed in eight sites (six are in the IRS districts of EMD, GUD, KAD, KUD, and YND) and two sites in TD, where IRS was withdrawn in 2013. Table 1 summarizes the spray history of each district from 2008 through 2021.

FIGURE 1: MAP OF PMI VECTORLINK GHANA DISTRICTS AND ENTOMOLOGICAL MONITORING SITES, 2021

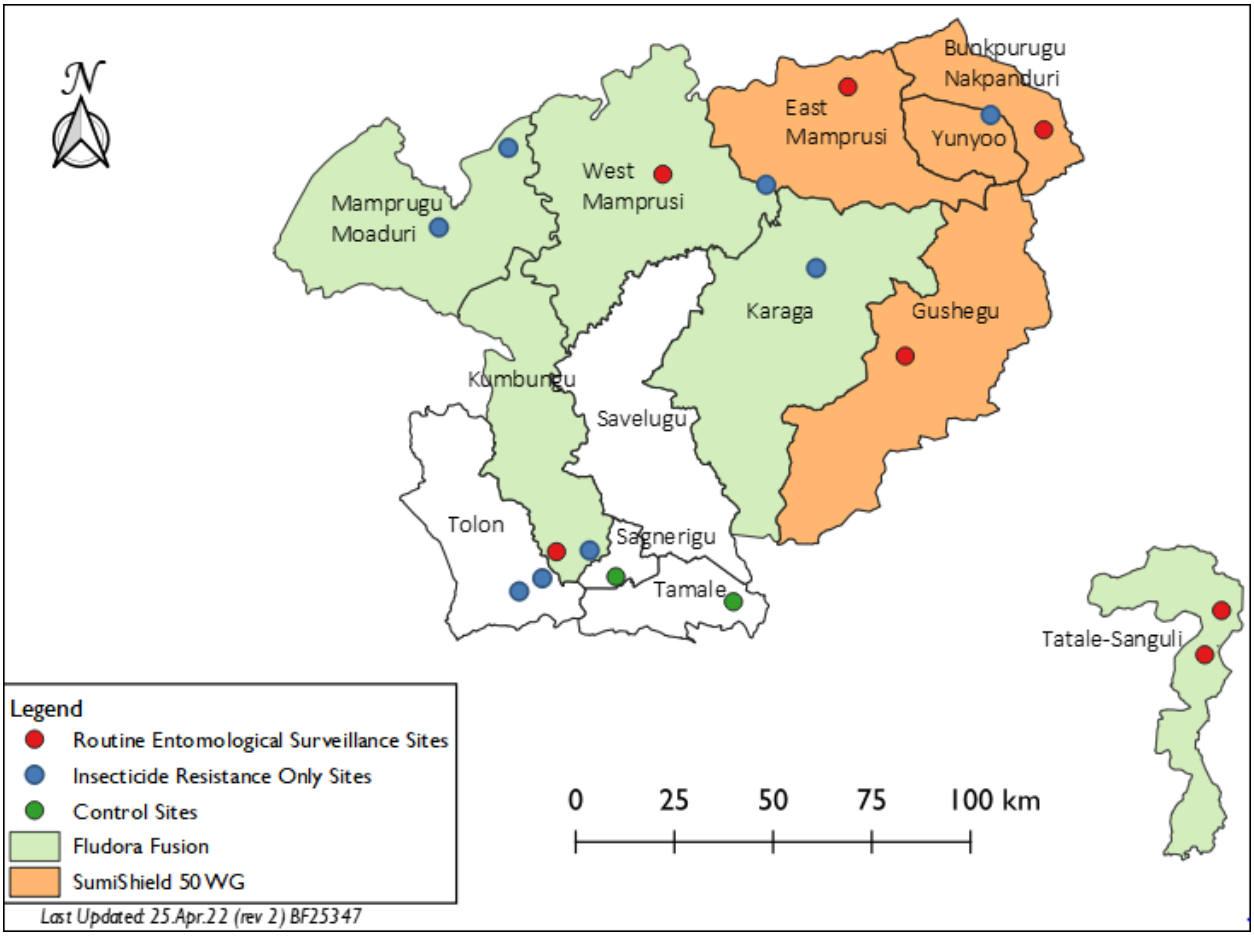


TABLE 1: ENTOMOLOGICAL MONITORING SITES, 2008–2021

District	Sentinel Site	Insecticide Spray History													
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Routine entomological surveillance sites															
GUD	Bandaya	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM	PM	PM	CLD+DM	CLD
BND	Bunbuna	NSp	NSp	NSp	ACy	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD
KUD	Gbullung*	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM	PM	PM	PM	CLD+DM	CLD+DM
EMD	Zaratinga	NSp	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD+DM	CLD
TSD	Sanguli and Njobilbo*	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	CLD+DM	CLD+DM
WMD	Kata/Banawa*	ACy	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD	CLD+DM
SGD†	Kulaa	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp
TML†	Tugu	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp
Only insecticide resistance-monitoring sites															
MMD	Yagaba and Kunkwa*	Acy	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	CLD	CLD	PM	CLD+DM
EMD	Wundua	NSp	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD+DM	CLD
KAD	Namburugu	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM	PM	PM	CLD	CLD+DM
KUD	Kumbungu	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM	PM	PM	PM	CLD+DM	CLD+DM
TD≠	Dimabi and Woribugu	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp
YND	Binkura	NSp	NSp	NSp	ACy	ACy	PM	PM	PM	PM	PM	PM	PM	CLD+DM	CLD

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

† = comparison sites with no history of IRS; ≠ =IRS withdrawn in 2013.

* = Bugyanga, Cheyohi, Njobilbo, and Wulugu sites for residual bioefficacy

2.2 Assessment of Spray Quality and Residual Efficacy

Standard World Health Organization (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* s.s. Kisumu strain and wild *An. gambiae* s.l. reared from larvae (dependent on the availability of wild larvae). 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 in the doors and window frames. 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.

2.2.1 Quality Assurance of the IRS Program

The 2021 IRS campaign commenced on March 23, 2021, across all nine targeted districts. In line with the project's objective of implementing high-quality IRS operations, the project entomology team carried out spray quality tests within the first three days of the spray campaign in one community in each sprayed district. Six houses (three with cement walls and three with mud walls, which is the predominant surface type; and wood surfaces in windows and doors) were purposefully selected in one community per district to represent structures sprayed by different spray operators and spray teams. Standard WHO wall cone bioassays were conducted according to the project's Standard Operating Procedure (SOP) for cone wall-bioassays (SOP009/01) to assess the quality of work done by the different spray teams in each district. The bioassays were conducted using both *An. gambiae* s.s. Kisumu strain and wild *An. gambiae* s.l.

2.2.2 Residual Efficacy of Sprayed Insecticides

Post-spray bioassays were conducted monthly from April through December 2021. The assays measured the residual bioefficacy of SumiShield 50WG sprayed in Bunbuna (BND), Bandaya (GUD), and Zarantinga (EMD) and of Fludora Fusion in Gbullung (KUD), Kata/Banawa (WMD), and Njobilbo (TSD). In each community, six houses (three with cement walls and three with mud walls, except in Njobilbo, where no mud surfaces were tested; and their wooden doors and windows) were purposefully selected to represent structures sprayed by different spray operators and spray teams.

2.3 Adult Mosquito Collections

The project's entomology team collected mosquitoes from all eight sentinel sites for four days in each month, consecutively, for 10 months (March through December 2021). Two mosquito collection methods were used, human landing catches (HLCs) and Prokopack aspiration (Table 2).

TABLE 2: ADULT MOSQUITO COLLECTION METHODS

Collection Method	Time	Frequency	Sampling
HLC	6:00 pm to 5:50 am	4 nights per site per month	2 houses/site/night using 4 collectors (2 indoor, 2 outdoor)
Prokopack	6:00 am to 9:00 am	4 days per site per month	5 animal shelter/site /day 2 sleeping rooms/house/site in 5 houses/day 2 pit traps/site/day

The collected mosquitoes were analyzed based on species composition, resting density and preference, peak biting time, location, biting rate, *Plasmodium (P.) falciparum* sporozoite infection rates, parity rates, blood meal source, and EIRs. Indicators for the sprayed districts were compared with those from the unsprayed districts.

A taxonomic key (Coetzee 2020)) was used to morphologically identify all *Anopheles* mosquitoes collected by each method. An average of 50–60 unfed *An. gambiae* s.l. mosquitoes collected by HLC per site per month were dissected to assess parity by observing the degree of coiling in the ovarian tracheoles (Detinova et al. 1962). The remaining specimens (all mosquitoes collected) were preserved in 1.5ml Eppendorf tubes with desiccant (blue silica gel) for further laboratory analysis as described below.

2.4 Insecticide Susceptibility Tests

WHO tube tests (SOP06/01) and CDC bottle assays (SOP04/01) were performed to assess the susceptibility of local *An. gambiae* vector populations to insecticides used for IRS and ITNs. All sentinel sites have a history of ITN coverage through mass distribution campaigns, school-based distribution, and/or health facility distribution.

2.4.1 WHO Tube Tests

Insecticide susceptibility tests were performed with wild mosquitoes collected from selected sentinel sites in sprayed and unsprayed communities, using the WHO tube test method. Larvae and pupae of *Anopheles* mosquitoes were collected from breeding sites in and around the sentinel sites and reared to adults. Mosquitoes were morphologically identified at the adult stage, and only *An. gambiae* s.l. were used for the susceptibility tests. WHO tube tests were conducted using WHO standardized insecticide papers: alpha-cypermethrin (0.05%), deltamethrin (0.05%), and pirimiphos-methyl (0.25%). Susceptibility of *An. gambiae* s.l. to clothianidin was also tested using papers that were impregnated at a concentration of 13.2mg (2%) (per one impregnated paper, 15x12cm) of clothianidin (SumiShield 50WG), and standardized using *An. gambiae* s.s. Kisumu strain.

After a 24-hour holding period following exposure to impregnated papers, the numbers 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% and <20%, but tests were discarded and repeated if control mortalities were ≥20%. For clothianidin, knockdown was recorded after 60 minutes and mortalities recorded at 1, 2, 3, 4, 5, 6, and 7-days post exposure.

The susceptibility levels of *An. gambiae* s.l. were evaluated based on the WHO criteria of test mortality (WHO 2013): 98–100% mortality after 24 hours indicates susceptibility. 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%, the vector population is resistant.

2.4.2 CDC Bottle Assays

The CDC bottle assay method was used to test for vector susceptibility to chlorfenapyr and clothianidin with some modifications (60 minutes exposure time). *An. gambiae* s.l. reared from larvae were exposed to 250ml Wheaton bottles treated with 100µg of chlorfenapyr or 4µg of clothianidin. Mosquitoes were introduced in batches of 20–25 into each replicate (four replicates in total). 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% 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. An insectary strain was used as a positive control. A negative control (i.e., 250ml glass bottle treated with 1ml of acetone only) was tested using *An. gambiae* s.l. reared from the field at the same time and mortality recorded at one, two, and three days so that corrected mortality could be calculated.

2.5 Synergist Assays

Synergist assays were conducted using alpha-cypermethrin and piperonyl butoxide (PBO) on mosquitoes from selected sentinel sites according to the project's SOP for WHO tube tests (SOP06/01), to determine the role of monooxygenases in the pyrethroid resistance that was detected. *An. gambiae* s.l. populations, which showed resistance to deltamethrin, were again exposed to a diagnostic dose of deltamethrin and PBO.

2.6 MOLECULAR ANALYSES

In a newly established enzyme-linked immunosorbent assay (ELISA) laboratory, VectorLink Ghana analyzed mosquito samples collected from the sentinel sites, to determine sporozoite rates and calculate EIRs. The blood meal source of all blood-fed mosquitoes collected was also determined using ELISA. Biochemical assays were carried out in the VectorLink Ghana ELISA laboratory, to measure enhanced levels of detoxification enzymes (esterase and oxidases) that may be responsible for resistance to the different insecticide classes. The AGAMal laboratory performed molecular analyses of entomological samples to:

1. Identify members of the *An. gambiae* s.l. complex to species
2. Determine the frequency of knockdown resistance (*kdr)* and acetylcholinesterase (*Ace-1)* genotypes

2.6.1 *P. FALCIPARUM* SPOROZOITE RATES

The heads and thoraxes of about 30% (average 50 per site/month) of the *An. gambiae* s.l. collected from the monthly HLCs were sorted and tested for the presence of *P. falciparum* sporozoite circumsporozoite antigens using ELISA as described by Wirtz et al. (1987) to determine the parasite infection rate in the local vectors collected.

2.6.2 HOST BLOOD MEAL IDENTIFICATION

Blood-fed mosquitoes collected by Prokopack aspiration were analyzed by ELISA using the Beier et al. (1988) method to determine what portion of mosquito blood meals are taken from humans versus animals. Blood feed mosquitoes were selected by simple random sampling from the pool of blood fed mosquitoes for testing.

2.6.3 SPECIES IDENTIFICATION

A sample of 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 separate the *An. gambiae* s.s. into *An. gambiae* and *An. coluzzii* (Fanello et al. 2002).

2.6.4 *ACE-1* AND *KDR* GENOTYPING

Samples of live and dead mosquitoes (20–25 mosquitoes) from the insecticide susceptibility tests were further analyzed, using the protocol described by Wilkins et al. (2006), to determine the presence of the *Ace-1* gene mutation in the local *An. gambiae* s.l. vectors. The samples were also analyzed to determine the presence of West Africa knockdown resistance gene (*kdr-w*) and East Africa knockdown resistance gene (*kdr-e*) mutations. The conventional PCR technique described by Martinez-Torres et al. (1998) was used to detect the presence of *kdr-w*. The method described by Ranson et al. (2000) was used to detect *kdr-e*.

2.7 BIOCHEMICAL ASSAYS

One hundred *An. gambiae* s.l. from Wundua (EMD) and 60 *An. gambiae* s.s. Kisumu strain were used in biochemical assay for three enzymes. Larval sites in other sites were dry during the period of the test and therefore yielded no larvae. The assay measures the levels of non-specific α-esterases, oxidase, and insensitive acetylcholinesterase (AChE) present in the sample using the protocol described by Leong et al. (2019).

2.8 INDICATORS AND DATA ANALYSIS

The following indicators were estimated for *An. gambiae* s.l. when samples collected were sufficient to allow for analysis:

- **Human biting rate (HBR):** The number of mosquito bites people in the area receive per unit of time reported as bites/person/night was estimated as:

$$\frac{\text{Total number of mosquitoes collected by HLC}}{\text{Total number of collectors/ Number of nights of capture}}$$

Mean indoor and outdoor HBRs were calculated both hourly and monthly for IRS and non-IRS sites.

- **Resting density:** Mean monthly indoor and outdoor resting densities per site for IRS vs non-IRS site were calculated as:

$$\frac{\text{Number of mosquitoes species collected resting indoors (sleeping rooms) from Prokopack per site per period of collection}}{\text{Total number of rooms surveyed per site per period of collection}}$$

- **Endophagic / Exophagic index:** The proportion of females of a given species that bite either indoors or outdoors (monthly) were estimated as:

$$\frac{\text{Number of mosquitoes species collected (either indoors or outdoors)}}{\text{Total number of mosquitoes collected indoors and outdoors}}$$

- **Parity rates:** Parity rates were estimated for the collection period for each site and as IRS and non-IRS sites as:

$$\frac{\text{Number of parous female mosquitoes}}{\text{Total number of female mosquitoes dissected}}$$

- **Sporozoite rates:** This was estimated monthly for each site and for IRS and non-IRS sites were estimated as:

$$\frac{\text{Number of mosquitoes postive for } P.\ falciparum \text{ circumsporozoite proteins}}{\text{Total number of mosquitoes tested per period per site}}$$

- **Entomological inoculation rate**: This describes the number of infectious bites an individual in a study area is exposed to in a given period (typically a year or transmission season), expressed as number of infectious bites/per person/per unit time. This was estimated as:

$$\text{(HBR) per unit time reported} \times \text{Sprozoite Rate}$$

Monthly and annual EIRs were estimated for each site for indoor and outdoor collections as follows:

$$\text{Monthly} = \text{monthly HBRs X monthly sporozoite rates}$$

$$\text{Annual EIR (for March} - \text{December only)} = \text{Sum of monthly EIRs}$$

- **Human or animal blood index (HBI or ABI):** The HBI or ABI was estimated per resting collection method across the whole sampling period as:

$$\frac{\text{Number of mosquitoes which fed on humans or animal}}{\text{Total number of mosquitoes whose blood meals were identified}}$$

- **Insecticide resistance allele frequencies:**

$$f\ (R) = \frac{2\ (RR) + RS}{2(RR + RS + SS)}$$

where R = *resistant allele and S represents susceptible allele*

Variations in indoor and outdoor biting rates for the vector species collected from IRS intervention and unsprayed districts were compared using the Chi-square goodness of fit test.

Linear hierarchical regression was used to calculate average differences in biting rates between IRS and control districts. In the linear hierarchical regression, type of treatment (sprayed versus unsprayed district) was included as the main outcome of interest, month of data collection as a fixed effect, and community, household, and place of collection (indoor/outdoor) as random effects. Robust standard errors were used to account for any non-normality in the error term (due to, for example, truncation of the error term at zero bites).

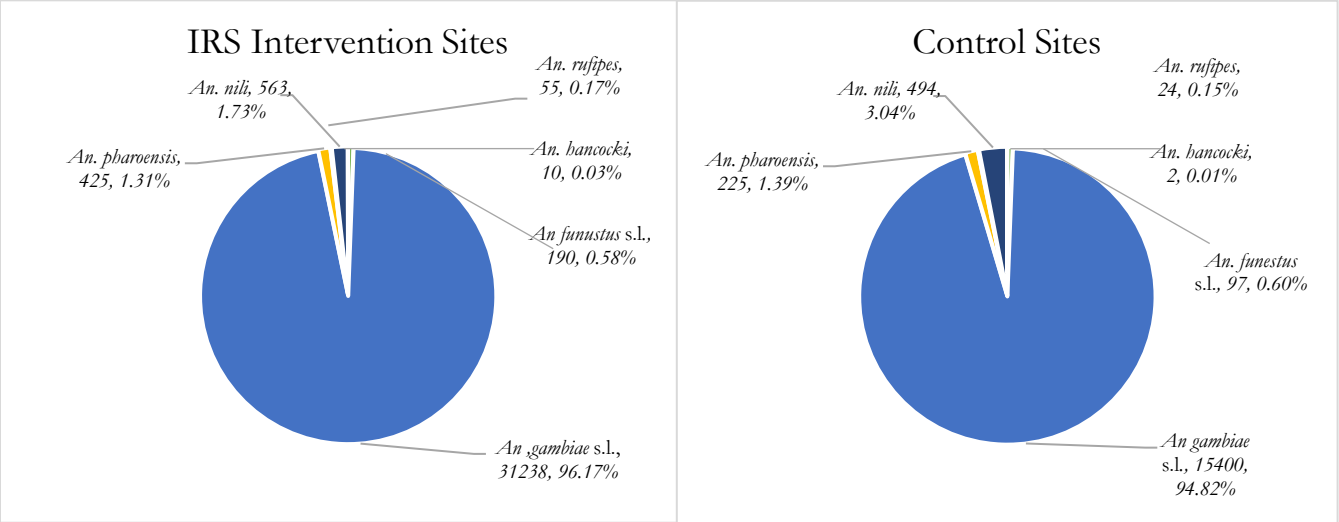
Differences in parity and sporozoite rates between the IRS versus control unsprayed sites were also compared through a z-test for differences in proportions. All tests were performed at 0.05 significance level, using Microsoft Excel®, STATA, and DHIS2-based VectorLink Collect.

3. RESULTS

3.1 VECTOR SPECIES COMPOSITION

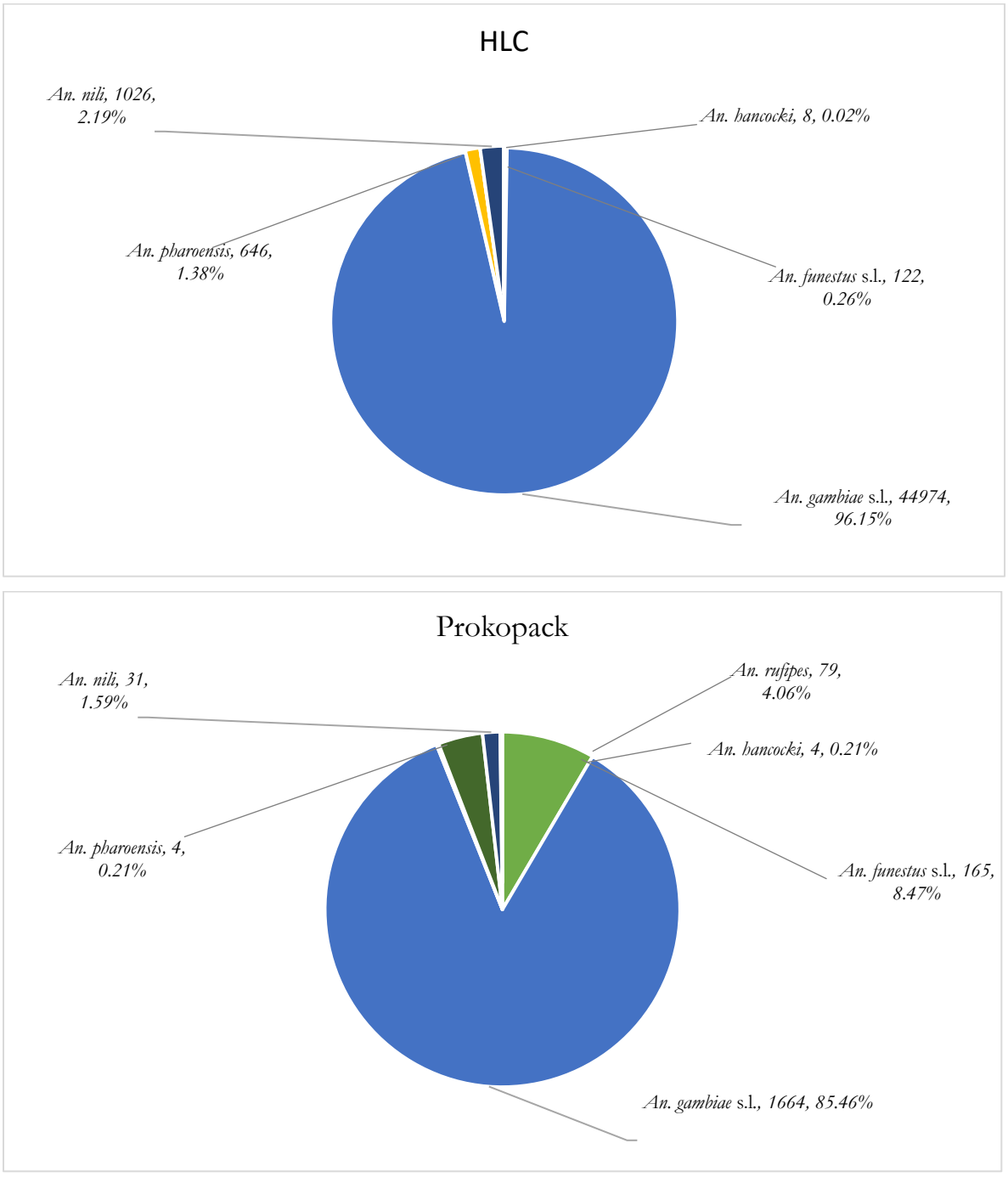
An. gambiae s.l. was the predominant species collected across all sites, constituting 96% (31,238/32,481) and 95% (15,405/16,247) of all *Anopheles* collected in the IRS intervention and control sites, respectively (Figure 2). *An. nili* was the second most predominant species in most sites, constituting about 2% in intervention and 3% in control sites. Other *Anopheles* collected included *An. funestus* s.l., *An. pharoensis*, *An. rufipes*, and *An. hancocki*. *An. gambiae* s.l. and *An. funestus* are the only species incriminated in malaria transmission in Ghana. *An. nili, An. pharoensis,* and *An. rufipes* are non-vectors (Baffoe-Wilmot et al. 2001).

FIGURE 2: TYPE OF *ANOPHELES* SPECIES COLLECTED USING HLC AND PROKOPACK METHODS, IRS INTERVENTION AND CONTROL SITES



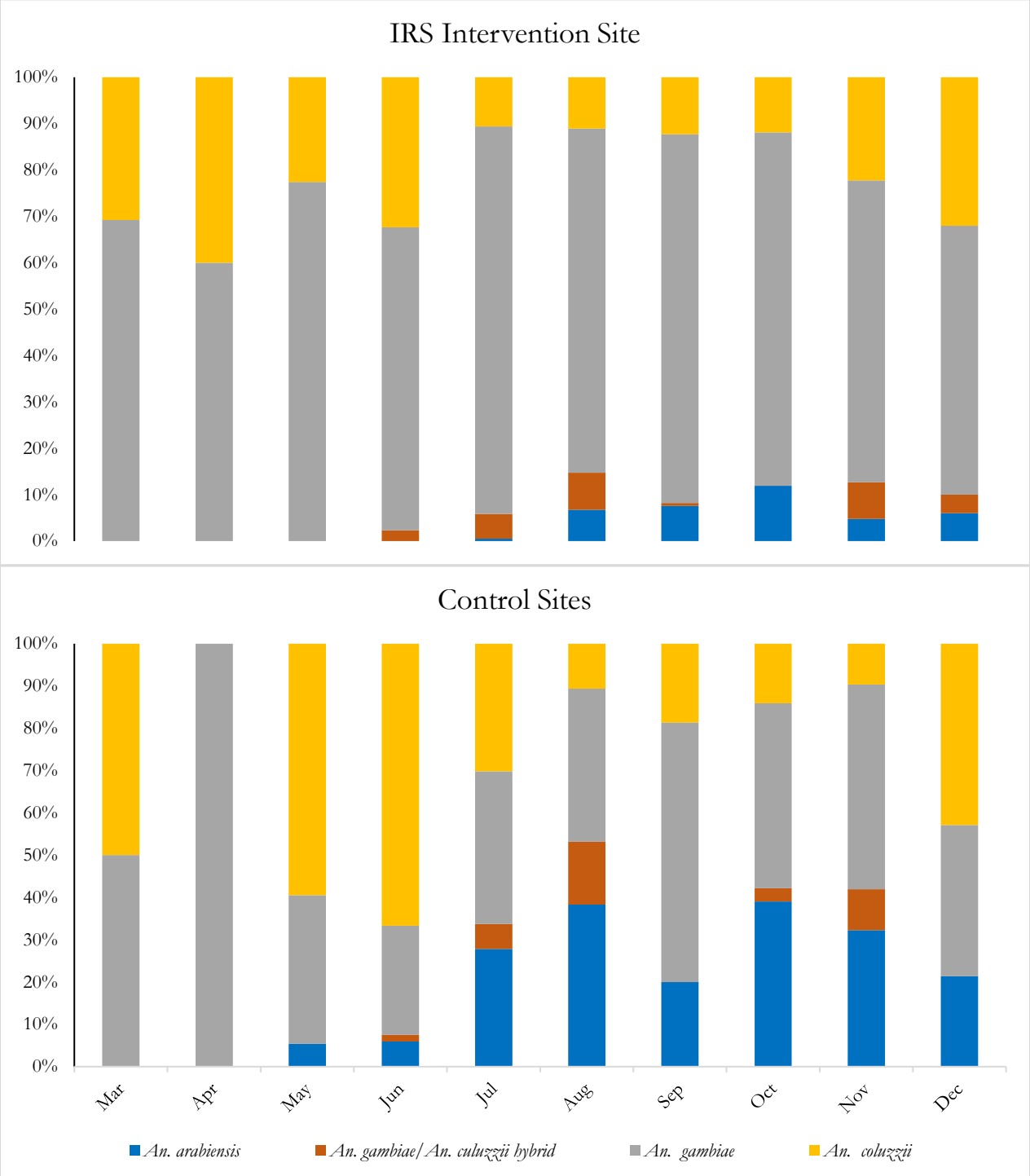
Of the total adult female *Anopheles* mosquitoes collected, 96.0% (46,776/48,723) were collected attempting to bite by HLC and 4% (1,947/48,723) were collected resting indoors and outdoors by Prokopack. The *Anopheles* species collected were predominantly *An. gambiae* s.l., which made up 96.1% and 85.5% of the HLC and Prokopack collections, respectively.

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



Molecular identification of 1,864 *An. gambiae* s.l. revealed three sibling species: *An. gambiae* (61.26%), *An. coluzzii* (25.38%), and *An. arabiensis* (9.39%), as well as hybrids (3.97%). *An. gambiae* was the majority across all sites. Hybrids of *An. coluzzii* and *An. gambiae* (3.97%) were also identified, in both IRS intervention and control sites (Figure 4).

FIGURE 4: SPECIES COMPOSITION OF *AN. GAMBIAE S.L.* ALL SENTINEL SITES, MARCH–DECEMBER 2021



3.2 HUMAN BITING RATES

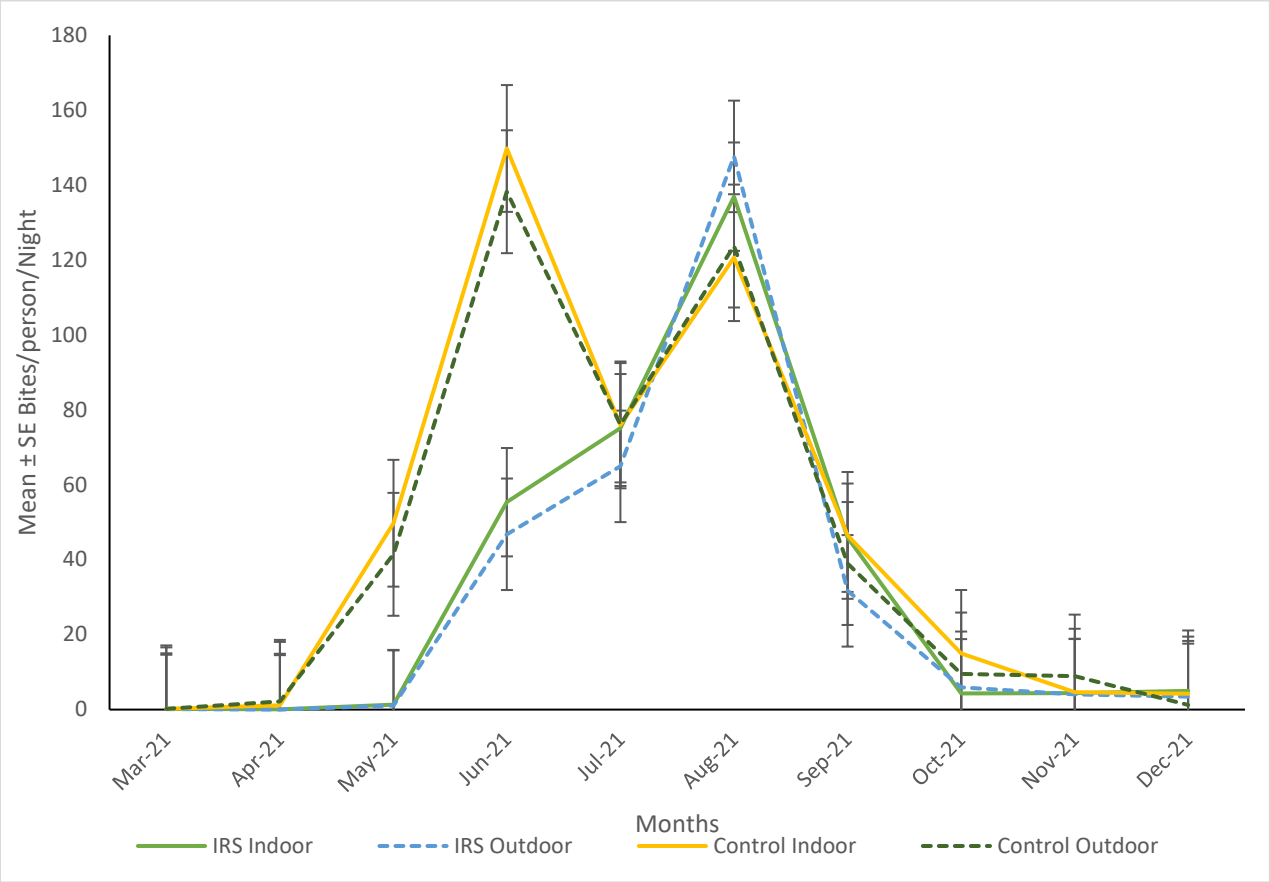
The mean monthly HBR of *An. gambiae* s.l. recorded for the control sites (45 bites per person per night (b/p/n)) was significantly higher than the mean HBR (33 b/p/n) recorded for the IRS sites (Table 3). Variations were observed between indoor and outdoor HBRs for *An. gambiae* s.l. in the IRS intervention and control sites. The mean indoor HBR for *An. gambiae* s.l. from the IRS sites was 33 b/p/n, whereas the mean outdoor HBR was 31 b/p/n. In the control sites, a mean indoor HBR was 47 b/p/n and mean outdoor HBR was 44 b/p/n. *An. gambiae* s.l. from two IRS sites, WMD and TSD, showed a slight preference for exophagy. In all other districts, *An. gambiae* s.l. showed endophagic tendencies.

TABLE 3: MEAN INDOOR AND OUTDOOR HBR OF *AN. GAMBIAE* S.L., HLC, ALL SENTINEL SITES, MARCH–DECEMBER 2021

Sentinel Site	Indoor Biting Rate (b/p/n)	Outdoor Biting Rate (b/p/n)	Endophagic Index	Exophagic Index	χ^2	p-Value
IRS_Intervention						
SumiShield sites						
Bandaya (GUD)	43.58	37.59	0.54	0.46	35.19	0.001*
Bunbuna (BND)	30.69	27.00	0.53	0.47	18.87	0.001*
Zarantinga (EMD)	33.23	26.49	0.56	0.44	61.06	0.001*
Fludora Fusion sites						
Gbullung (KUD)	30.38	26.05	0.54	0.46	26.83	0.001*
Kata/Banawa (WMD)	45.46	52.02	0.47	0.53	35.35	0.001*
Sanguli (TSD)	13.81	14.29	0.49	0.51	0.64	0.42287
Control sites						
Kulaa (SGD)	43.02	34.98	0.55	0.45	66.27	0.001*
Tugu (TML)	50.56	53.14	0.49	0.51	5.17	0.0231
Overall						
IRS intervention sites	32.86	30.57	0.52	0.48	39.61	0.001*
Control sites	46.79	44.06	0.51	0.49	13.08	0.00029

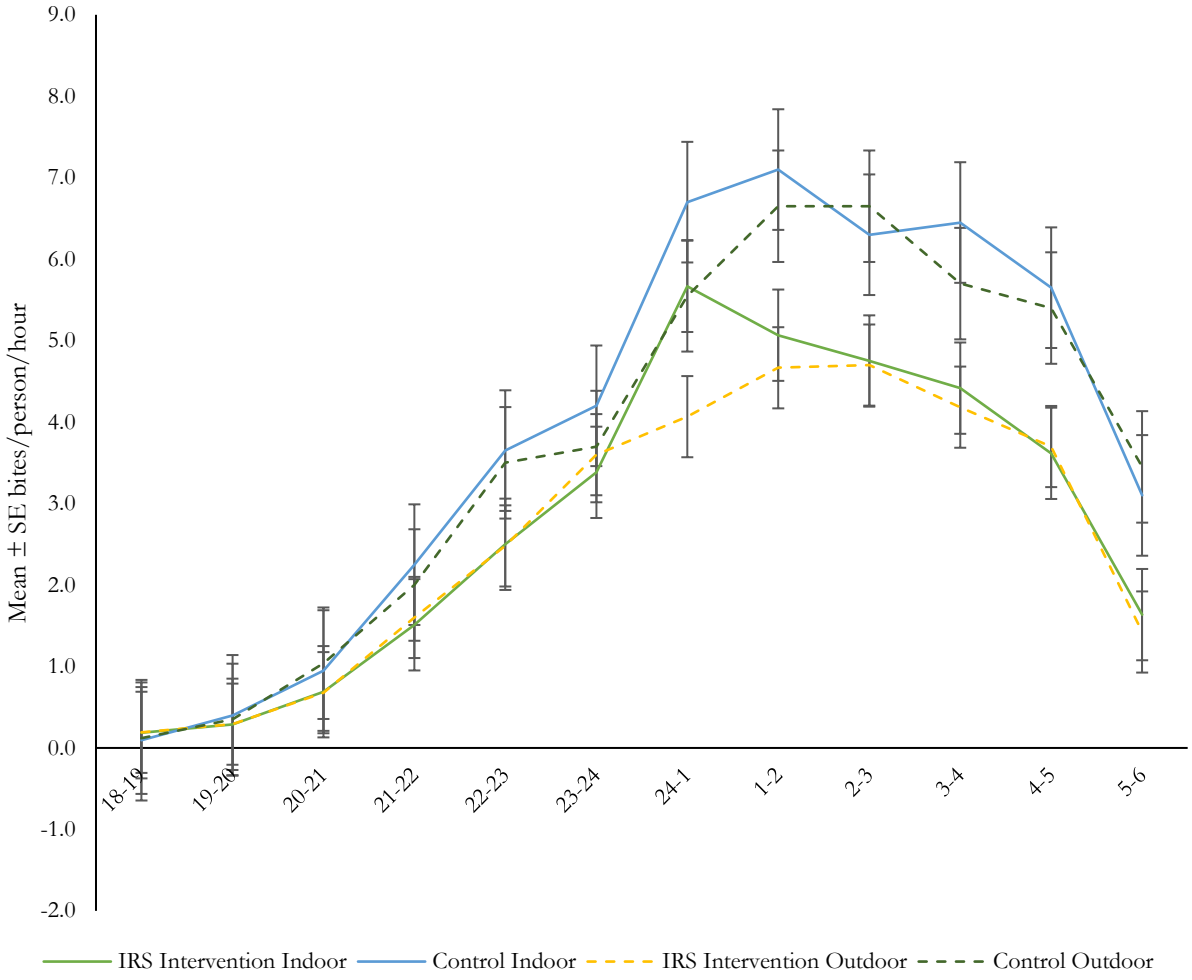
An. gambiae s.l. population densities, as measured by mean monthly HBRs, peaked in August in the IRS intervention sites and in June 2021 in the control sites (Figure 5). There was a dip in July possibly due to continuous rains and windy nights.

FIGURE 5: MEAN DAILY INDOOR AND OUTDOOR HBR, *AN. GAMBIAE* S.L., SPRAYED AND UNSPRAYED SITES, MARCH–DECEMBER 2021



Indoor and outdoor biting activity of *An. gambiae* s.l. started at 6:00 pm and then gradually increased, with peak biting observed between 11:00 pm and 4:00 am in both the IRS and control sites (Figure 6, Annex Table A-1). The number of mosquitoes biting during these peak times was higher in the control sites than in the IRS intervention sites. The highest indoor biting occurred in Bandaya (GUD) between 1:00 and 2:00 am, at about 13 bites (not shown in Figure 6).

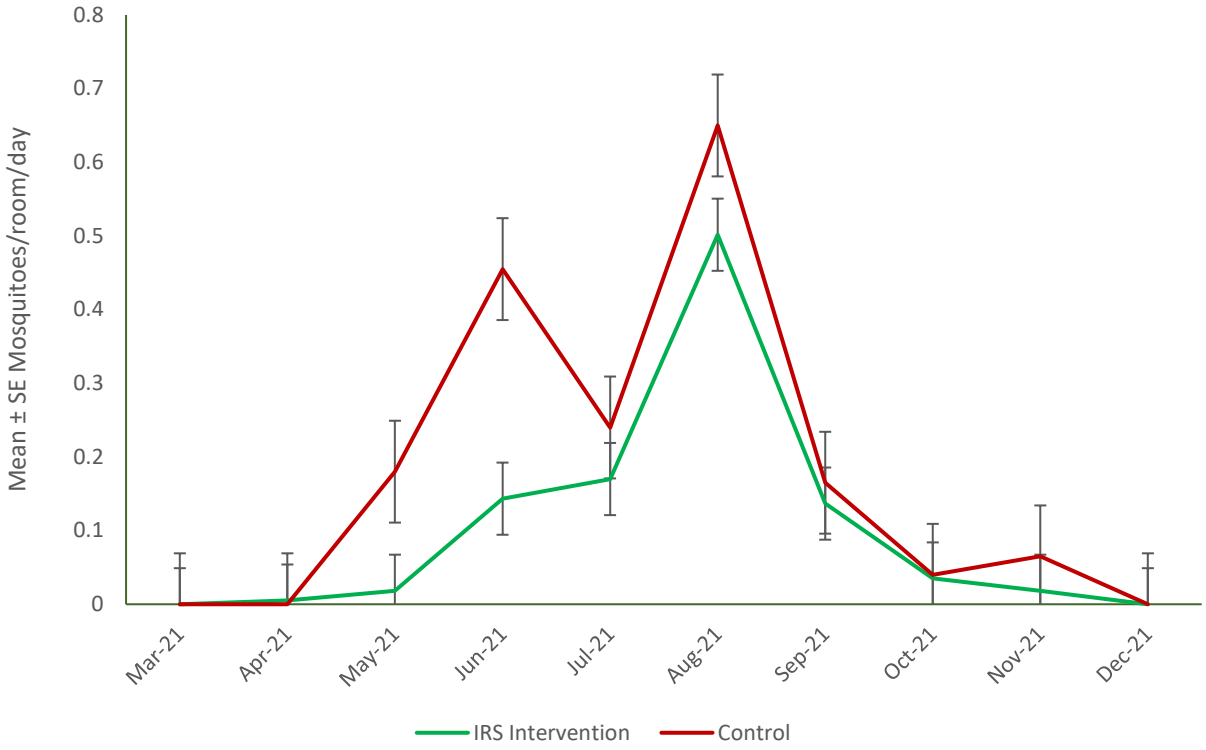
FIGURE 6: INDOOR AND OUTDOOR HOURLY BITING ACTIVITY, *AN. GAMBIAE* S.L., SPRAYED AND UNSPRAYED SITES, MARCH–DECEMBER 2021



3.3 RESTING BEHAVIOR

The mean indoor resting density of *An. gambiae* s.l. in sleeping rooms was 0.1 mosquitoes per room/day for the IRS sites and 0.2 mosquitoes per room/day for unsprayed sites (Figure 7).

FIGURE 7: MEAN INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. IN SPRAYED AND UNSPRAYED SITES, MARCH–DECEMBER 2021



3.3.1 PROKOPACK COLLECTIONS IN ANIMAL SHELTERS, PIT SHELTERS AND SLEEPING ROOMS.

As in 2020, in 2021 VectorLink Ghana sprayed animal shelters in selected districts to assess the entomological impact of spraying the shelters with the aim of improving IRS efficacy. All eligible animal shelters throughout each selected district were sprayed. *An. gambiae* s.l. resting density in the animal shelters of control sites was higher (3.23 per shelter per day) than in IRS sites where animal shelters were not sprayed (1.17 per shelter per day) and in IRS sites where shelters sprayed (0.69 per shelter per day) (Table 4).

TABLE 4: MEAN RESTING DENSITY OF *AN. GAMBIAE S.L.* IN SPRAYED AND UNSPRAYED SENTINEL SITES (PROKOPACK COLLECTION), MARCH–DECEMBER 2021

Sentinel Site	Resting Density		
	Animal Shelters	Pit Shelters	Sleeping Room
	An. gambiae s.l. **/shelter/day**	*An. gambiae* s.l. **/pit/day**	*An. gambiae* s.l. **/room/day**
SumiShield sites			
Bandaya (GUD)	0.13 (26)	0.25 (20)	0.13 (52)
Bunbuna (BND) †	0.28 (55)	0.65 (52)	0.10 (39)
Zarantinga (EMD)	0.59 (118)	2.01 (161)	0.17 (68)
Fludora Fusion sites			
Gbullung (KUD) †	0.42 (83)	0.38 (30)	0.04 (17)
Kata/Banawa (WMD)	0.39 (77)	0.68 (54)	0.16 (64)
Sanguli (TSD)	0.04 (7)	0.06 (5)	0.05 (19)
Control sites			
Kulaa (SGD)	2.84 (568)	1.60 (128)	0.45 (179)
Tugu (TML)	0.39 (78)	0.89 (71)	0.10 (40)
Overall			
IRS intervention sites with animal shelters sprayed	0.69 (138)	1.03 (82)	0.14 (56)
IRS intervention sites with animal shelters not sprayed	1.17 (234)	2.70 (216)	0.39 (156)
Control sites	3.23 (646)	2.49 (199)	0.55 (219)

Note: Number in bracket is total number of mosquitoes collected.

† *Animal shelters in Bunbuna (BND)* and *Gbullung (KUD) were sprayed.*

3.4 BLOOD MEAL SOURCE

For source of blood meal, 120 blood-fed *An. gambiae* s.l. from sleeping rooms and 95 from pit shelters were tested. The HBI for *An. gambiae* s.l. collected from sleeping rooms in the IRS sites was 60.7% compared to 50.0% from the control sites (Table 5). The ABI for *An. gambiae* s.l. in sleeping rooms (39.3%) was lower in the IRS sites than in the control sites (50.0%). Of the 95 blood-fed mosquitoes collected from pit shelters by Prokopack aspiration, 13.7% recorded an HBI. The animal blood meal sources included bovine, goat, and other animals.

TABLE 5: *AN. GAMBIAE* S.L. COLLECTED BY PROKOPACK AND THEIR SOURCE OF BLOOD MEAL, MARCH–DECEMBER 2021

Sentinel Site	Pit Trap					Indoor Sleeping Rooms				
	Number Analyzed	Human	Bovine	Goat	Other	Number Analyzed	Human	Goat	Bovine	Other
SumiShield sites										
Bandaya (GUD)	2 (20)	50.00%	0.00%	0.00%	50.00%	2 (52)	100.00%	0.00%	0.00%	0.00%
Bunbuna (BND)	5 (52)	40.00%	0.00%	20.00%	40.00%	27 (39)	88.90%	3.70%	0.00%	7.40%
Zaratinga (EMD)	32 (161)	12.50%	0.00%	25.00%	62.50%	33 (68)	60.60%	6.10%	21.20%	12.10%
Fludora Fusion sites										
Kata/Banawa (WMD)	2 (54)	50.00%	0.00%	50.00%	0.00%	2 (64)	50.00%	50.00%	0.00%	0.00%
Gbullung (KUD)	4 (30)	0.00%	0.00%	50.00%	50.00%	2 (17)	50.00%	50.00%	0.00%	0.00%
Sanguli (TSD)	1 (5)	0.00%	0.00%	100.00%	0.00%	2 (19)	100.00%	0.00%	0.00%	0.00%
Control sites										
Tugu (TML)	16 (71)	18.80%	0.00%	37.50%	43.80%	5 (40)	40.00%	20.00%	0.00%	40.00%
Kulaa (SGD)	33 (128)	6.10%	0.00%	39.40%	54.50%	3 (179)	66.70%	0.00%	0.00%	33.30%
Overall										
IRS intervention sites	46 (322)	17.40%	0.00%	28.30%	54.30%	68 (259)	60.70%	8.00%	12.50%	18.80%
Control sites	49 (199)	10.20%	0.00%	38.80%	51.00%	8 (219)	50.00%	12.50%	0.00%	37.50%
Total	**95 (521)**	**13.70%**	**0.00%**	**33.70%**	**52.60%**	**76 (478)**	**60.00%**	**8.30%**	**11.70%**	**20.00%**

Note: Number in bracket is total number of mosquitoes collected.

3.5 PARITY RATES

Dissections of *An. gambiae* s.l. mosquitoes collected by HLC between March and December 2021 revealed that the proportion of parous females collected from the unsprayed sites in SGD and TML (54.26%) was slightly higher than the proportion collected from the IRS districts (41.95%) (Table 6).

TABLE 6: PROPORTION OF PAROUS FEMALES OF *AN. GAMBIAE* S.L. BY HLC

District	#Dissected	Parous	% Parity	95% Confidence Interval	
				Lower Bound	Upper Bound
IRS Intervention					
SumiShield districts					
GUD	1581	754	47.69%	46.98%	48.41%
BND	1357	542	39.94%	39.15%	40.73%
EMD	1251	540	43.17%	42.42%	43.91%
Fludora Fusion districts					
KUD	1262	572	45.32%	44.59%	46.06%
WMD	2174	931	42.82%	42.07%	43.58%
TSD	681	223	32.75%	31.88%	33.62%
Control *districts*					
TML	2050	1099	53.61%	52.94%	54.28%
SGD	1626	893	54.92%	54.25%	55.59%

3.6 *P. FALCIPARUM* SPOROZOITE RATES

A total of 8,181 *An. gambiae* s.l. (about 25%) collected by HLC were assayed by ELISA to determine the presence of *P. falciparum* sporozoites. The overall mean *An. gambiae* s.l. sporozoite rate was 0.85%. The sporozoite rate in Fludora Fusion-sprayed sites (0.58% indoors and 0.56% outdoors) was lower than in SumiShield-sprayed sites (1.07% indoors and 1.29% outdoors) and control sites (0.09% indoors and 0.44% outdoors) (Table 7). However, the rates are too low to make any meaningful comparison between sites.

TABLE 7: *P. FALCIPARUM* SPOROZOITE INFECTIONS IN *AN. GAMBIAE* S.L. SAMPLED FROM ALL SENTINEL SITES, MARCH–DECEMBER 2021

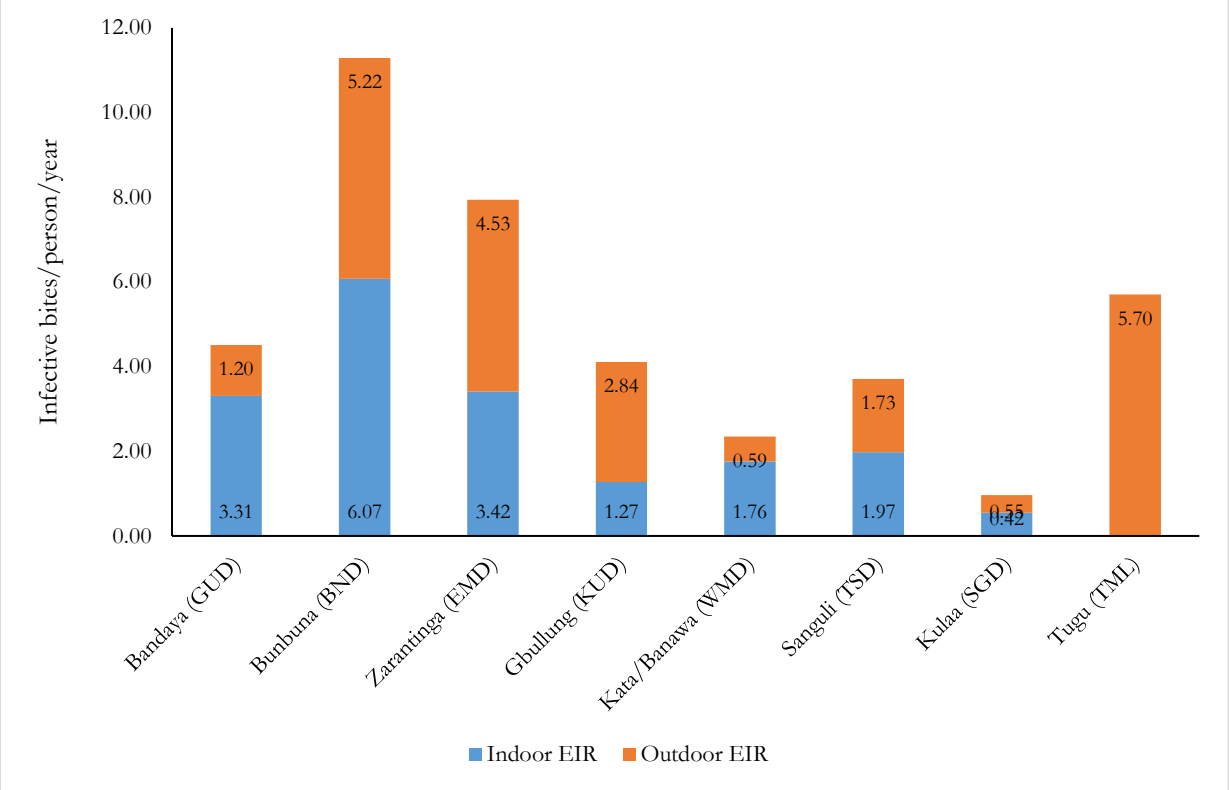
Sentinel Site	Number Analyzed		No. Positive for *P. falciparum* Sporozoites		Sporozoite Rate	
	Indoor	*Outdoor*	*Indoor*	*Outdoor*	*Indoor*	*Outdoor*
SumiShield sites						
Bandaya (GUD)	586	612	2	2	0.34%	0.33%
Bunbuna (BND)	483	431	10	10	2.07%	2.32%
Zaratinga (EMD)	519	435	5	7	0.96%	1.61%
Total SumiShield	1588	1478	17	19	**1.07%**	**1.29%**
Fludora Fusion sites						
Gbullung (KUD)	525	438	2	4	0.38%	0.91%

Sentinel Site	Number Analyzed		No. Positive for *P. falciparum* Sporozoites		Sporozoite Rate	
	Indoor	*Outdoor*	*Indoor*	*Outdoor*	*Indoor*	*Outdoor*
Kata/Banawa (WMD)	628	737	3	1	0.48%	0.14%
Sanguli (TSD)	231	241	3	3	1.30%	1.24%
Total Fludora Fusion	**1384**	**1416**	**8**	**8**	**0.58%**	**0.56%**
Control sites						
Kulaa (SGD)	593	494	1	1	0.17%	0.20%
Tugu (TML)	583	645	0	4	0.00%	0.62%
Total control	**1176**	**1139**	**1**	**5**	**0.09%**	**0.44%**

3.7 ESTIMATION OF EIRS

The estimated risk of malaria transmission for the 10-month period from March through December was calculated from the sum of the 10 monthly EIRs. By district, BND (a SumiShield-sprayed site) had the highest monthly EIRs, which added up to 5.22 outdoor and 6.07 indoor infective bites/person/year (ib/p/yr) (Figure 8). TML, which is an unsprayed site, recorded the highest outdoor EIR of 5.70 ib/p/yr. The highest indoor transmission occurred in BND and EMD.

FIGURE 8: INDOOR AND OUTDOOR EIR FOR *AN. GAMBIAE* S.L.



3.8 SPRAY QUALITY AND RESIDUAL EFFICACY

The wall bioassays of SumiShield 50WG (Figures 9 and 10) and Fludora Fusion (Figures 11 and 12) showed good residual efficacy (greater than 80%) up to at least 10 months post spray based on tests performed with Kisumu strain mosquitoes. Tests with wild *An. gambiae* s.l. could not be done in Njobilbo in July 2021 because larval breeding sites dried up and were not able to produce enough larvae to rear to adult stage for the test. Bioassays with Kisumu strain attained 100% mortality within five days in most months on walls sprayed with Fludora Fusion and SumiShield (Annex Table A-2).

FIGURE 9: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD 50WG REPRESENTED BY MORTALITY RATES OBSERVED IN BND, GUD, AND EMD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, *AN. GAMBIAE* S.S. KISUMU STRAIN, MARCH 2021–FEBRUARY 2022

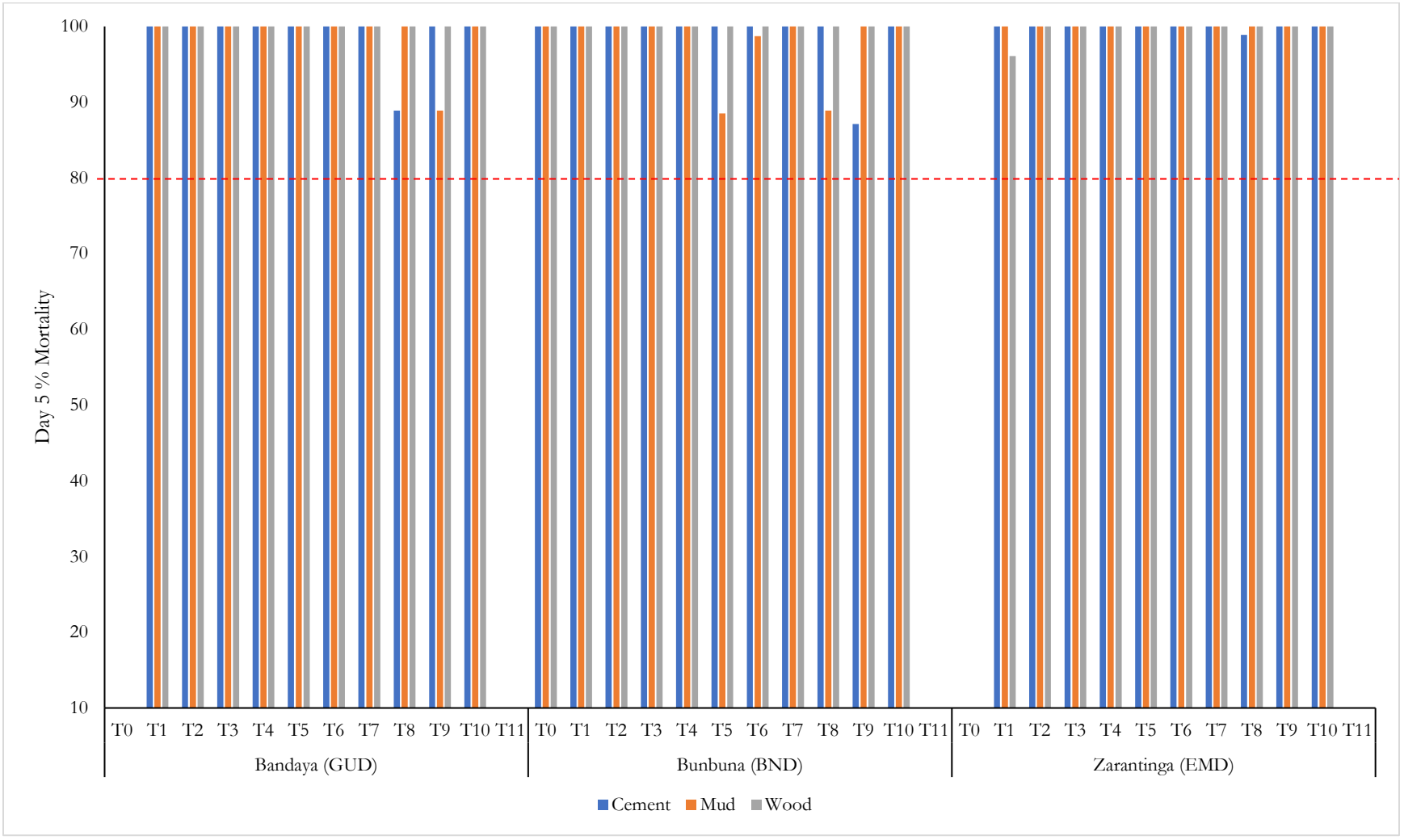


FIGURE 10: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD 50WG REPRESENTED BY MORTALITY RATES OBSERVED IN BND, GUD, AND EMD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, WILD *AN. GAMBIAE* S.L., MARCH 2021–JANUARY 2022

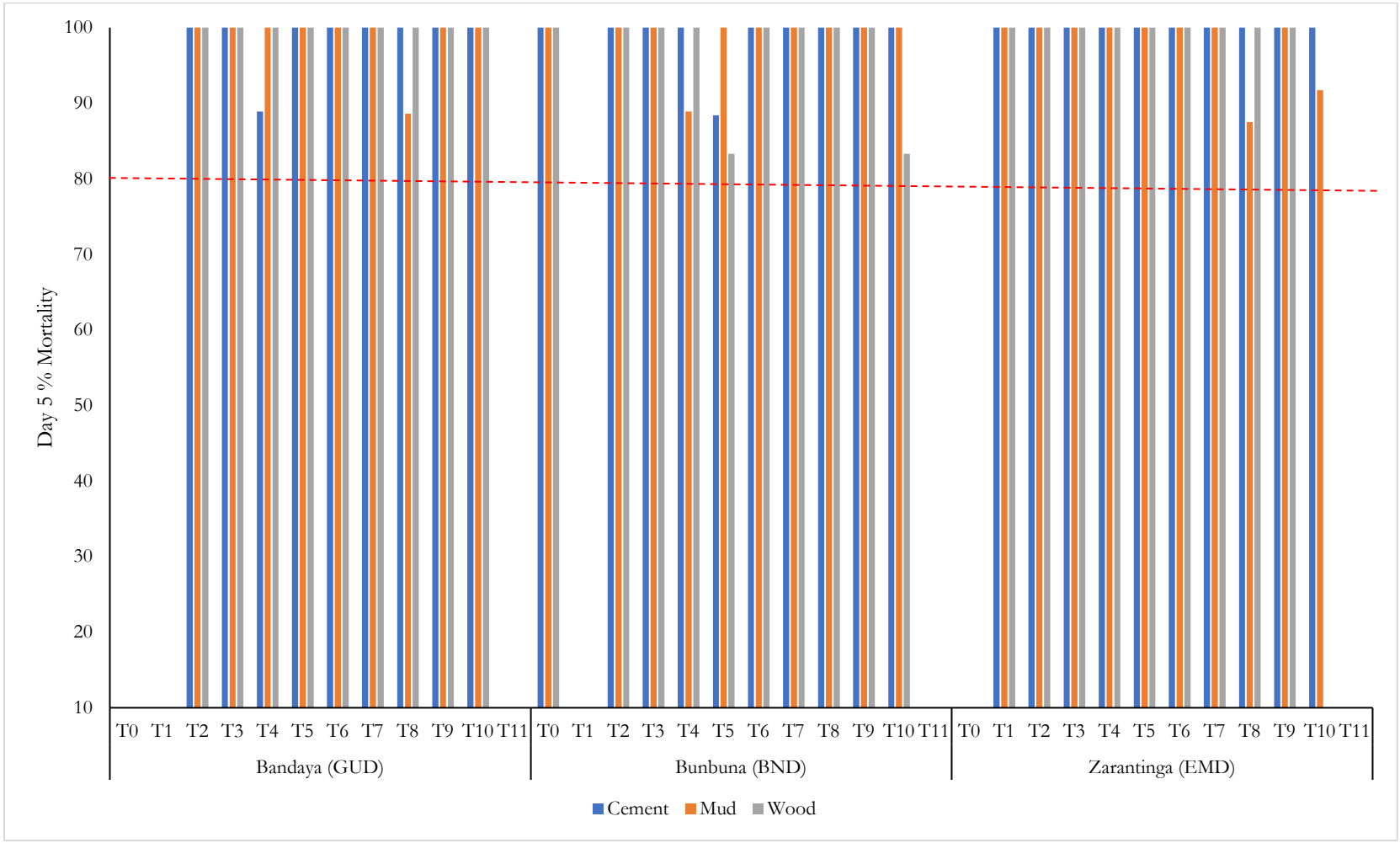


FIGURE 11: SPRAY QUALITY AND RESIDUAL EFFICACY OF FLUDORA FUSION REPRESENTED BY MORTALITY RATES OBSERVED IN KUD, WMD, AND TSD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, *AN. GAMBIAE* S.S. KISUMU STRAIN, MARCH 2021–JANUARY 2022

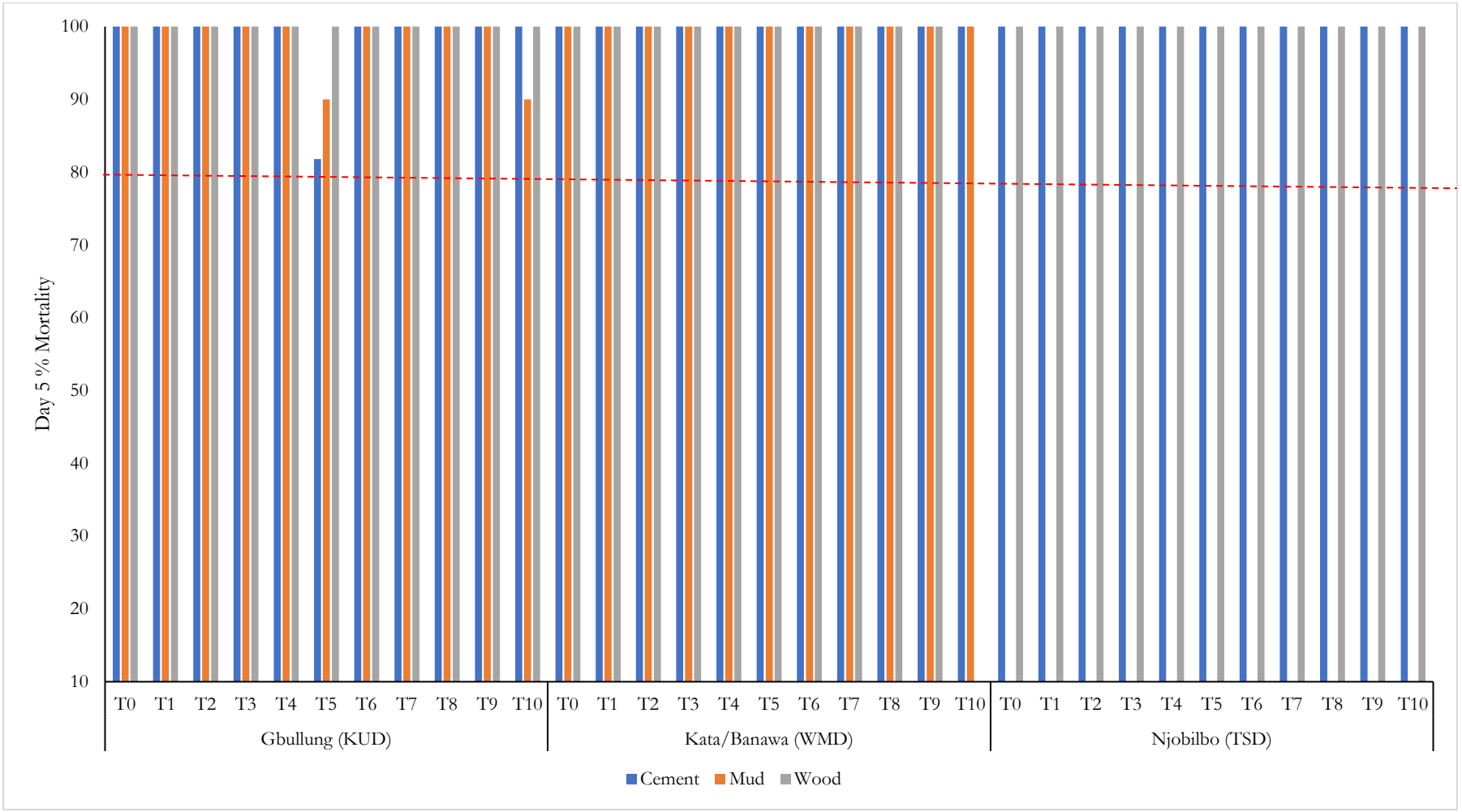
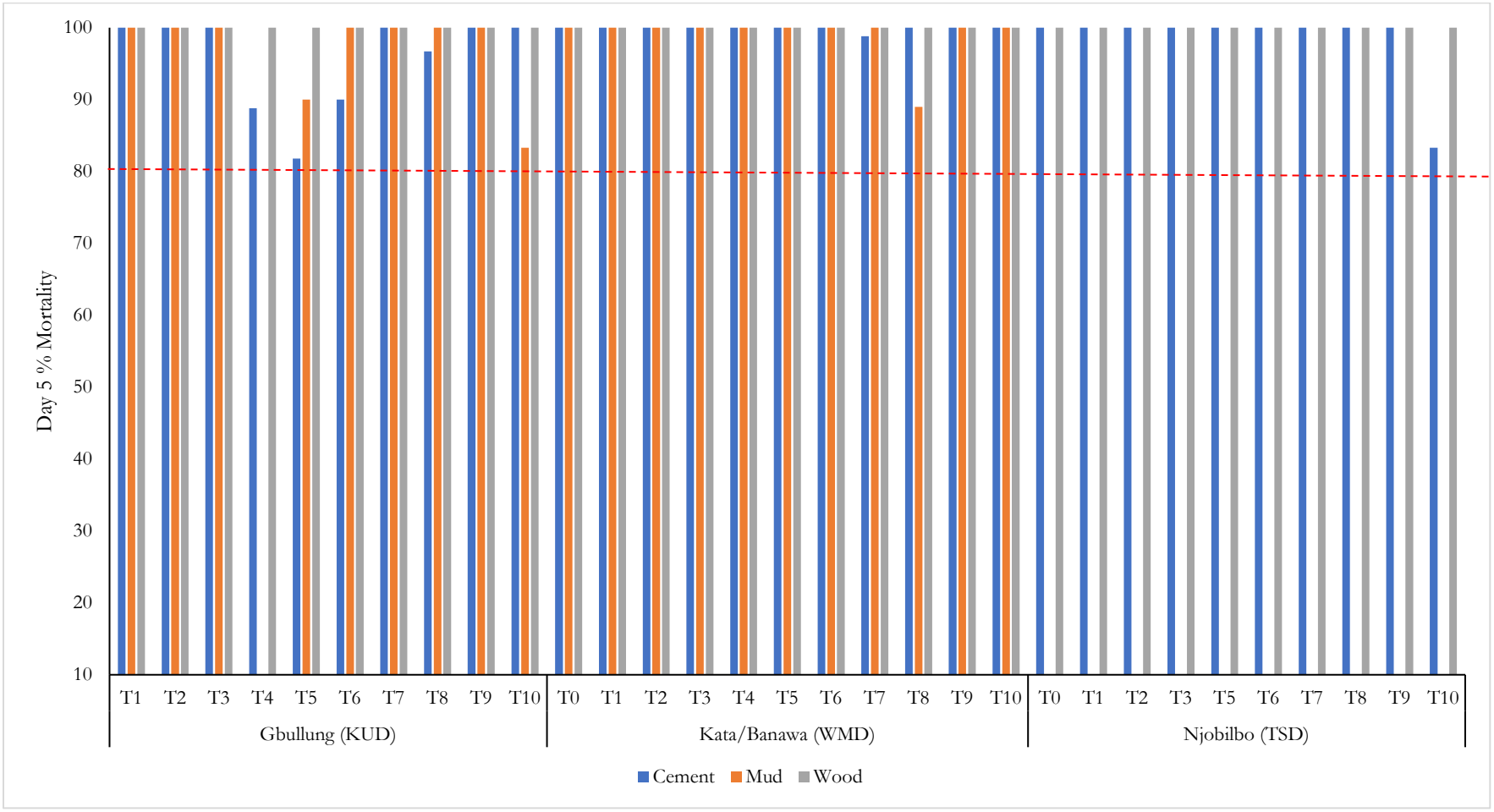


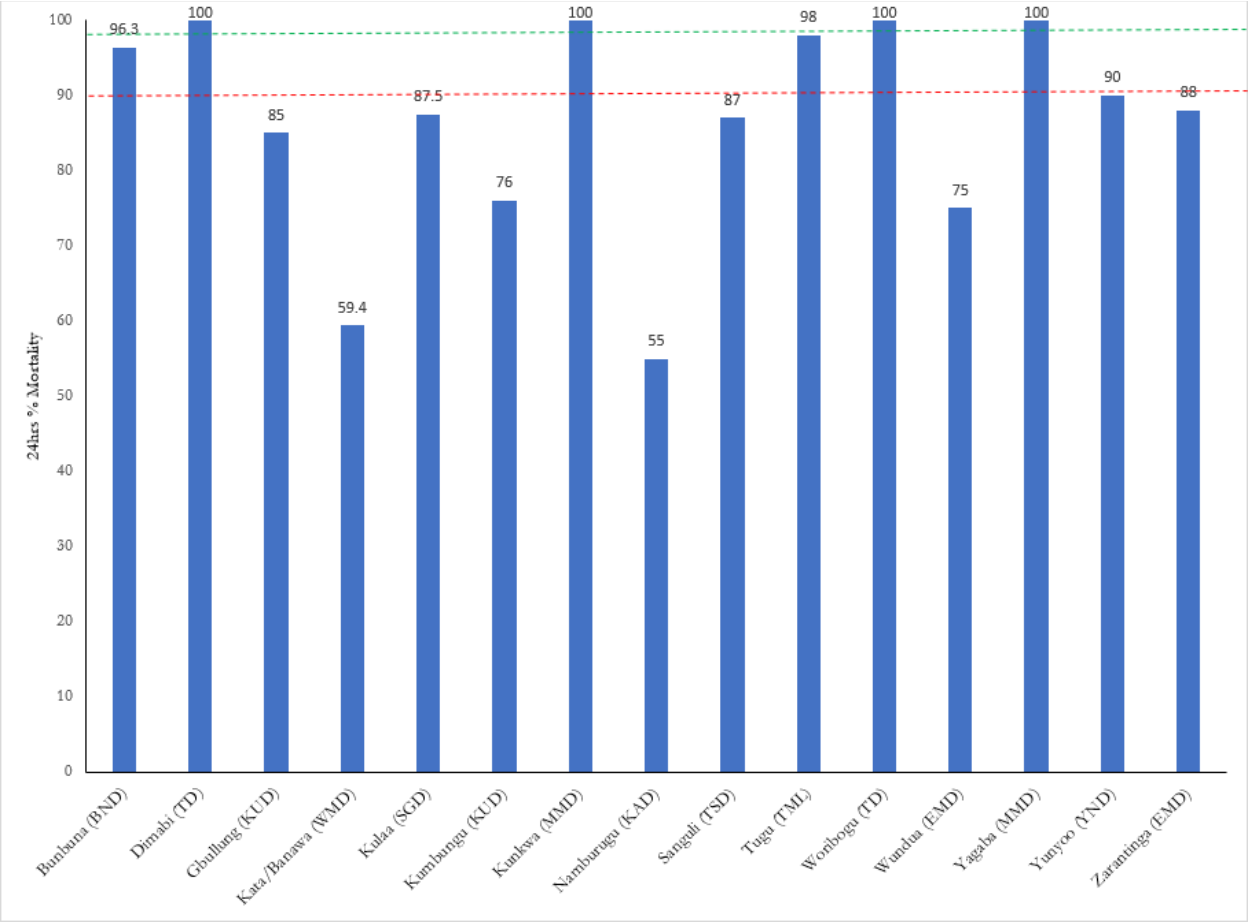
FIGURE 12: SPRAY QUALITY AND RESIDUAL EFFICACY OF FLUDORA FUSION REPRESENTED BY MORTALITY RATES OBSERVED IN KUD, WMD, AND TSD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, WILD *AN. GAMBIAE* S.L., MARCH 2021–JANUARY 2022



3.9 INSECTICIDE SUSCEPTIBILITY

Insecticide susceptibility results and synergist assays are shown in Figures 13–21 and Annex Table A-3. *An. gambiae* s.l. from Dimabi and Woribogu (TD), Kunkwa and Yagaba (MMD), and Tugu (TML) that were tested were susceptible to 0.25% pirimiphos-methyl and possible resistance was reported from Bunbuna (BND) and Yunyoo (YND) (Figure 13.) *An. gambiae* s.l. from all other sites tested were resistant to the 0.25% pirimiphos-methyl.

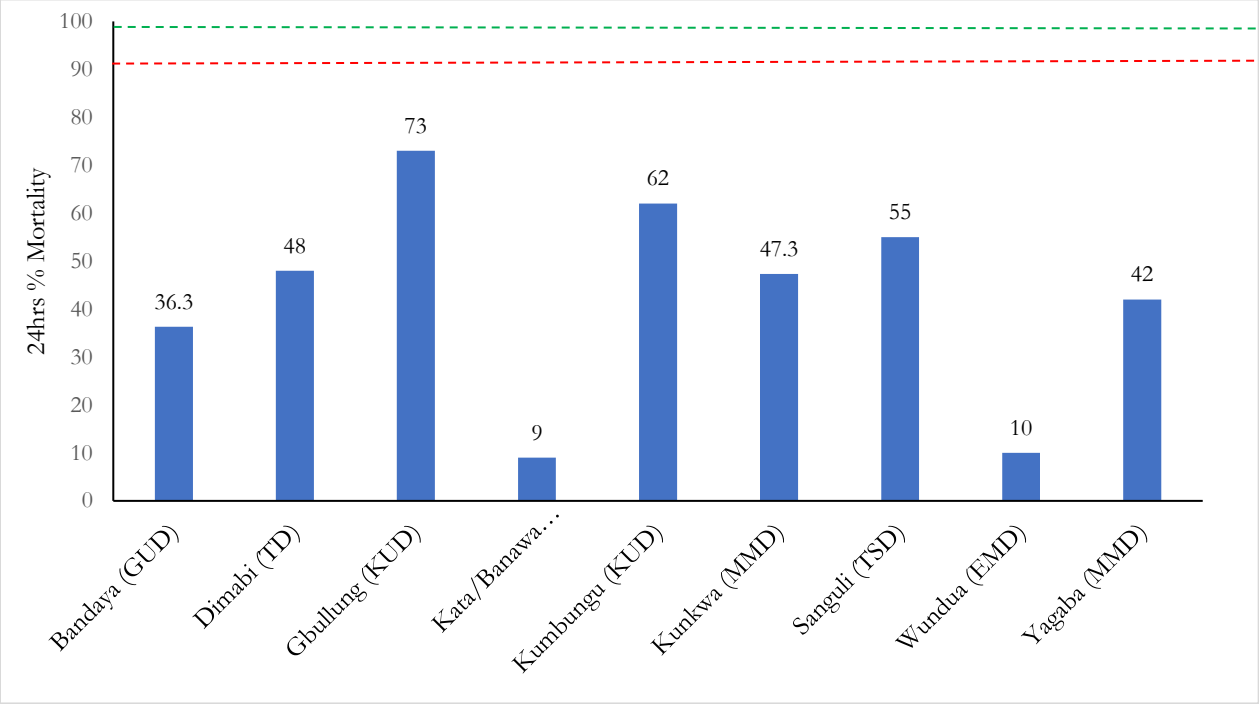
FIGURE 13: INSECTICIDE SUSCEPTIBILITY OF *AN. GAMBIAE S.L.*, 0.25% PIRIMIPHOS-METHYL, WHO TUBE TEST, FIFTEEN SITES



---------- Susceptible
----------Resistant

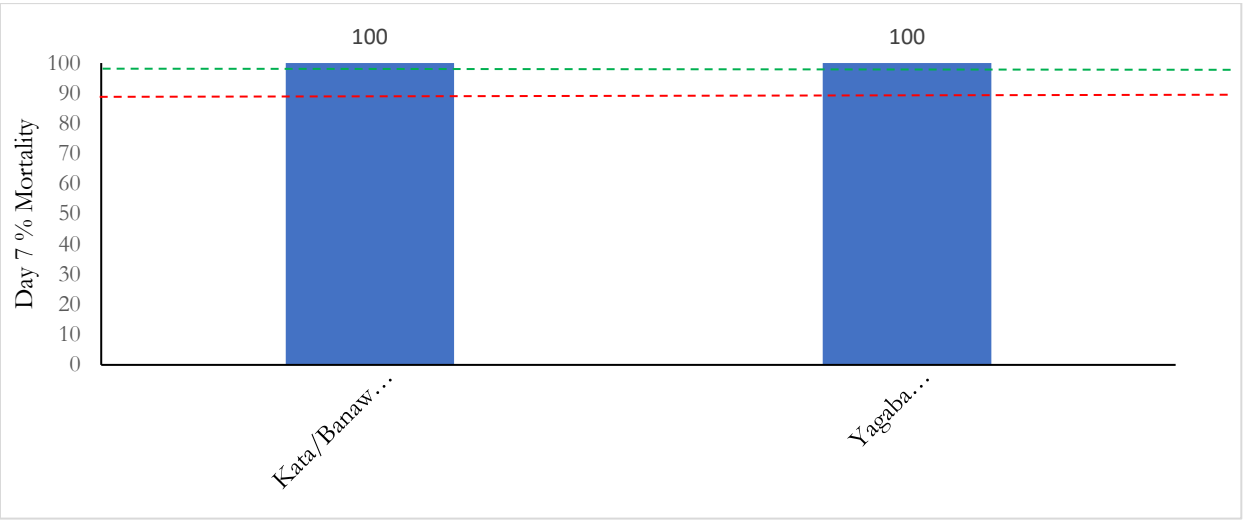
An. gambiae s.l. from all the sites tested were resistant to the 0.25% alpha-cypermethrin tested (Figure 14).

FIGURE 14: INSECTICIDE SUSCEPTIBILITY OF *AN. GAMBIAE* S.L., 0.25% ALPHA-CYPERMETHRIN, WHO TUBE TEST, NINE SITES



An. gambiae s.l. from Kata/Banawa (WMD) and Yagaba (MMD) were tested against 13.2mg/paper clothianidin by WHO tube assay and were susceptible to the insecticide (Figure 15).

FIGURE 15: SUSCEPTIBILITY OF *AN. GAMBIAE* S.S., 13.2MG/PAPER CHLOTHIANIDIN, WHO TUBE TEST, TWO SITES



An. gambiae s.s. (Kisumu strain) were tested in parallel to *An. gambiae* s.l. against 100µg/bottle chlorfenapyr under standard laboratory conditions of temperature (25 ± 2°C) and relative humidity (80 ± 10%). All four replicates with the Kisumu strain recorded 100% mortalities at 72 hours post exposure (Figure 16). Results with *An. gambiae* s.l. from seven sites tested showed possible resistance to chlorfenapyr in three sites (Kata/Banawa (WMD), and Gbullung and Kumbungu (KUD)), resistance in two sites (Namburugu (KAD) and Woribugu (TD)), and susceptibility in two sites (Wundua and Zarantinga (EMD)) (Figure 17). These tests will be repeated and, if the results are confirmed, vectors will be tested against 200µg/bottle chlorfenapyr.

FIGURE 16: SUSCEPTIBILITY OF AN. GAMBIAE S.S. (KISUMU STRAIN), 100 µG/BOTTLE CHLORFENAPYR, CDC BOTTLE ASSAYS, SEVEN SITES

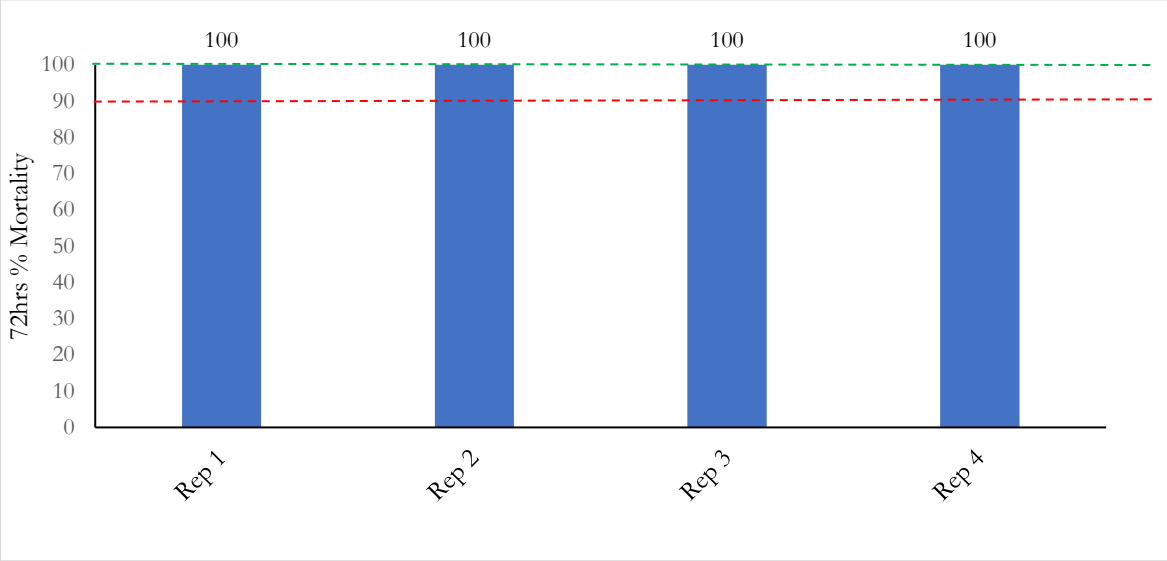
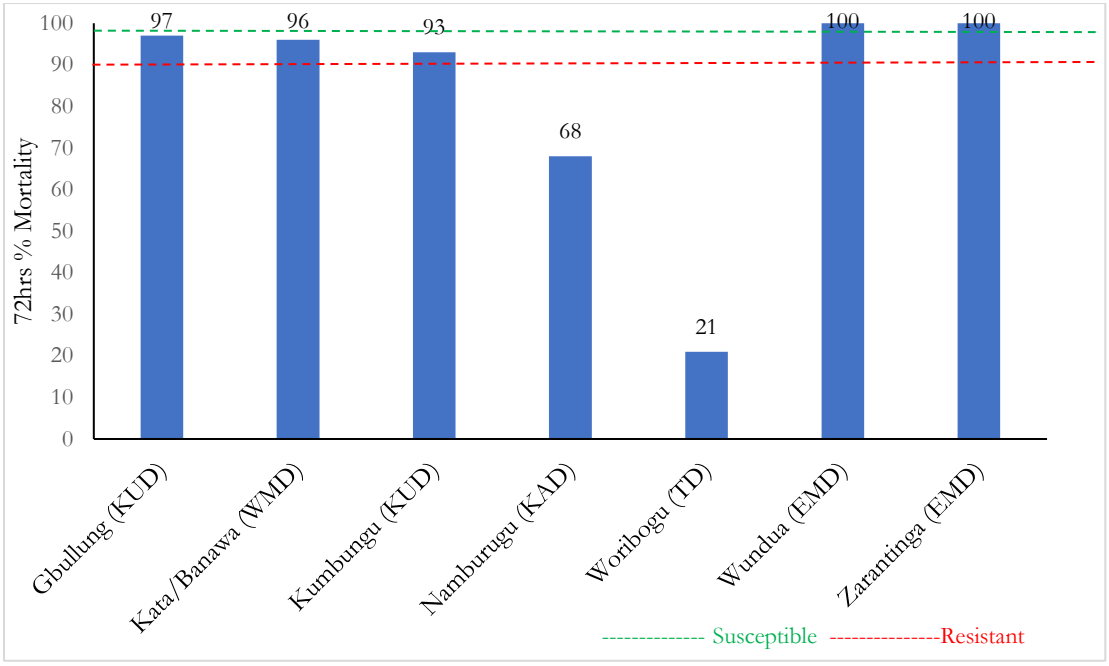
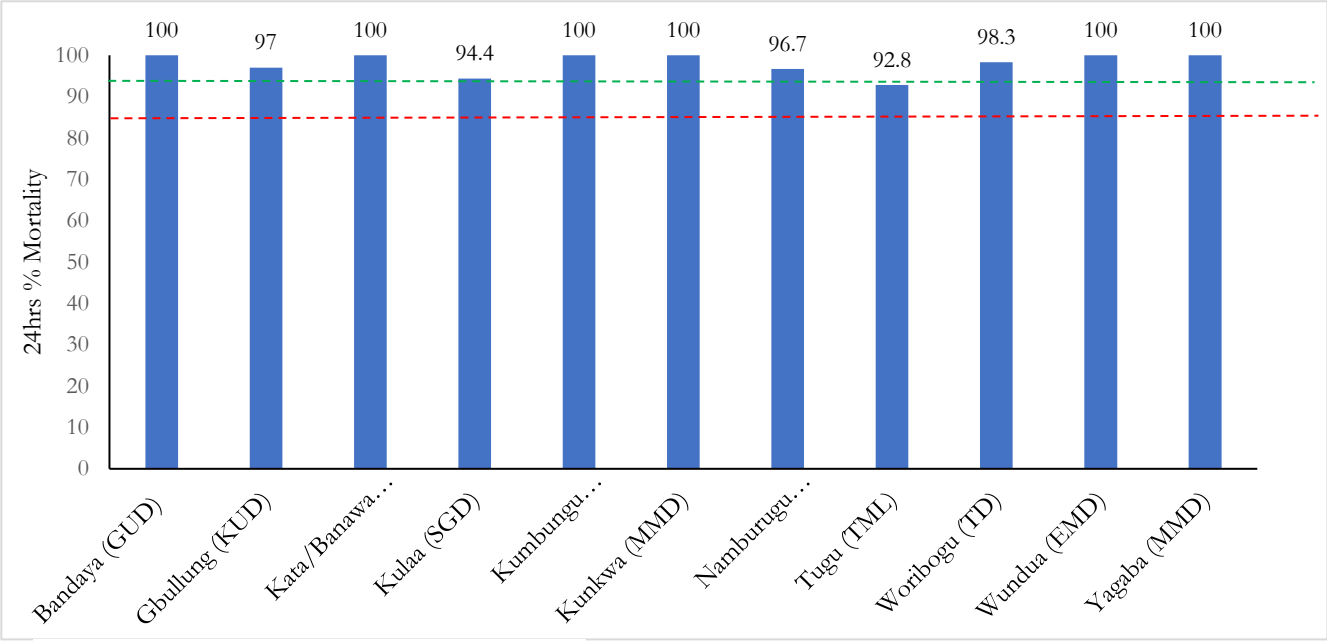


FIGURE 17: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L., 100 µG/BOTTLE CHLORFENAPYR, CDC BOTTLE ASSAY, SEVEN SITES



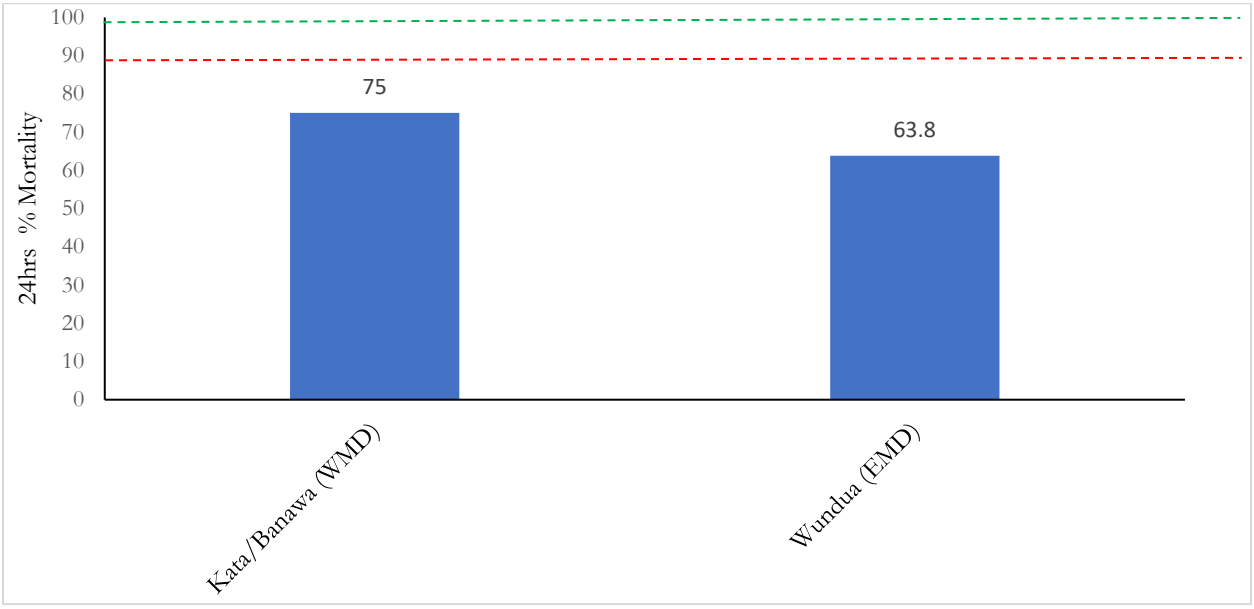
An. gambiae s.l. from seven of the 11 sites tested were fully susceptible to 4µg/bottle of clothianidin after the 24-hour holding period; possible resistance was recorded in the *An. gambiae* s.l. from the four other sites (Figure 18).

FIGURE 18: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L., 4 µG/BOTTLE CLOTHIANIDIN, CDC BOTTLE ASSAY, 11 SITES



An. gambiae s.l. from two sites tested were resistant to 0.05% deltamethrin at the end of the 24-hour holding period (Figure 19).

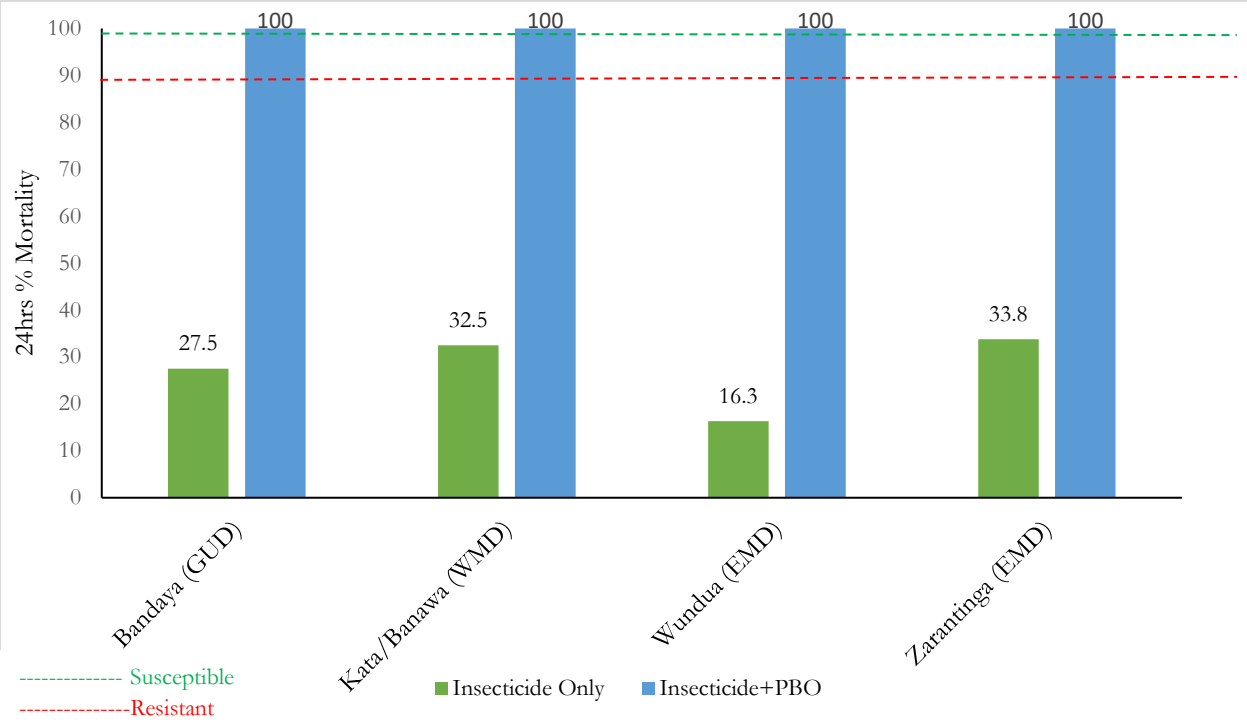
FIGURE 19: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L.,0.05% DELTAMETHRIN, WHO TUBE ASSAY, TWO SITES



3.10 SYNERGIST ASSAYS

An. gambiae s.l. from four sites where mosquitoes were pre-exposed to PBO showed higher mortalities than those with no pre-exposure to the synergist. PBO seems to fully restore susceptibility, suggesting that mono-oxygenases may play a significant role in the resistance mechanism of *An. gambiae* s.l. in these sites (Figure 20).

FIGURE 20: 24HR MORTALITY OF *AN. GAMBIAE* S.L. FROM SPRAYED AND UNSPRAYED SITES POST EXPOSURE TO PYRETHROID (ALPHA-CYPERMETHRIN) AND PBO



3.11 TARGET SITE RESISTANCE

3.11.1 *ACE-1* GENE MUTATION

The *Ace-1* gene mutation has been reported to confer cross-resistance to carbamates and organophosphates in mosquito species. The frequency of the resistant alleles ranged from 0.73 to 1.00 in the IRS intervention sites. There was a general increase in the frequency of *Ace-1* resistant alleles compared to 2020 when the frequency of *Ace-1* alleles ranged from 0.57 to 0.91 in the IRS intervention sites. Relatively high *Ace-1* frequency (0.91) was detected in Kunkwa (MMD) (Table 8) where IRS was implemented with pirimiphos-methyl from 2012 to 2018 and in 2020.

3.11.2 *KDR* MUTATION

The *kdr* gene mutation confers resistance to pyrethroids and DDT. The frequency of *kdr-w* resistant alleles in samples analyzed was high across all sites. However, there appears to be a slight decline in the frequency of *kdr-w* resistant alleles in most of the IRS sites compared to 2020. The frequency of the resistant alleles ranged from 0.44 to 0.80 in the IRS intervention sites in 2021, compared to a range of 0.52 to 0.81 recorded in the IRS sites in 2020. About 45% of the mosquitoes analyzed were found to harbor both *kdr-w* and *kdr-e* gene mutations. The frequency of *kdr-e* resistance genotypes was highest in Binkura (YND) (Table 8).

TABLE 8: DISTRIBUTION AND FREQUENCY OF *ACE-1* AND *KDR* ALLELES WITHIN *AN. GAMBIAE* S.L., IRS INTERVENTION AND CONTROL SITES, 2021

Sentinel Site	*kdr-W*			*Number Examined*	*f (R)*	*kdr-E*			*Number Examined*	*f (R)*	*Ace-1*			*Number Examined*	*f (R)*
	RR	*RS*	*SS*			*RR*	*RS*	*SS*			*RR*	*RS*	*SS*		
SumiShield sites															
Bandaya (GUD)	3	5	2	10	**0.55**	5	5	0	10	**0.75**	21	1	0	22	**0.98**
Binkura (YND)	2	9		11	**0.59**	10	1	0	11	**0.95**	14	10	0	24	**0.79**
Bunbuna (BND)	4	6	2	12	**0.58**	8	4	0	12	**0.83**	21	2	3	26	**0.85**
Zaratinga (EMD)	8	3		11	**0.86**	9	1	0	10	**0.95**	21	2	1	24	**0.92**
Wundua (EMD)	7	2	1	10	**0.80**	5	5	0	10	**0.75**	18	4	0	22	**0.91**
Fludora Fusion sites															
Gbullung (KUD)	6	0	4	10	**0.60**	3	7	0	10	**0.65**	22	0	0	22	**1.00**
Kata/Banawa (WMD)	8	1	1	10	**0.85**	9	1	0	10	**0.95**	18	4	0	22	**0.91**
Kumbungu (KUD)	8	2		10	**0.90**	4	6	0	10	**0.70**	17	5	0	22	**0.89**
Namburugu (KAD)	3	4	4	11	**0.45**	4	7	0	11	**0.68**	22	2	0	24	**0.96**
Sanguli (TSD)	8	1	1	10	**0.85**	6	4	0	10	**0.80**	21	1	0	22	**0.98**
Control sites															
Kulaa (SGD)	1	4	5	10	**0.30**	1	8	0	9	**0.56**	15	7	0	22	**0.84**
Tugu (TML)	5	2	4	11	**0.55**	3	8	0	11	**0.64**	23	2	0	25	**0.96**
Woribogu (TD)	9	0	1	10	**0.90**	5	5	0	10	**0.75**	18	4	0	22	**0.91**
Grand Total	**72**	**39**	**25**	**136**	**0.67**	**72**	**62**	**0**	**134**	**0.77**	**251**	**44**	**4**	**299**	**0.91**

3.12 BIOCHEMICAL ASSAYS

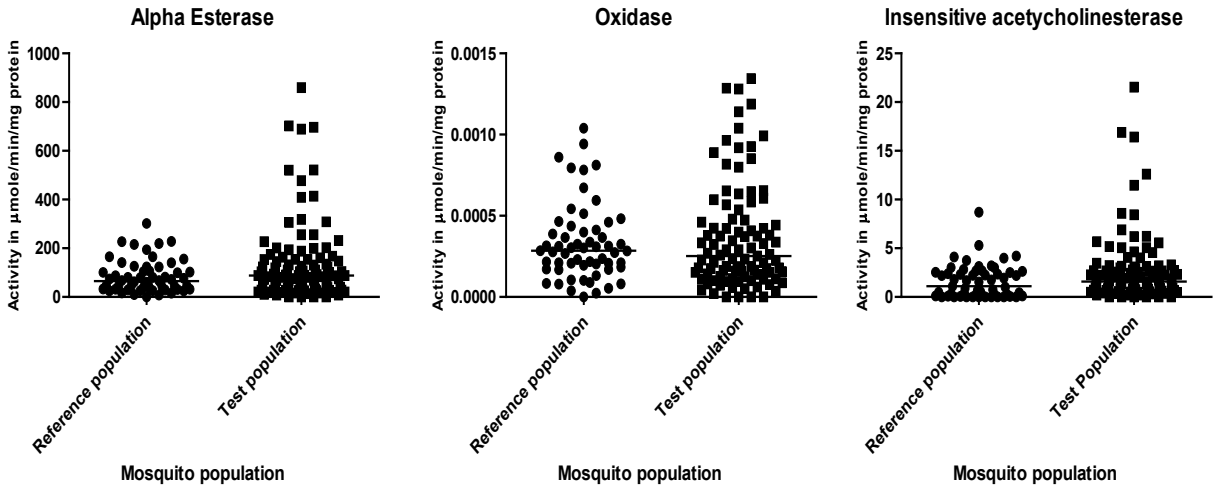
One hundred *An. gambiae* s.l. from Wundua (EMD) and 60 *An. gambiae* s.s. Kisumu strain from the project insectary were used in biochemical assay for three enzymes. Larval breeding areas in other sites dried up during the period of the test, thus no larvae were available for testing. These assays detect any increase in the activity of enzymes typically involved in insecticide metabolism. Elevated levels of esterase are related to carbamate and organophosphate resistance and Mixed Function Oxidase plays important role in organochlorine and pyrethroid resistance in malaria vectors. The activities of the three enzymes failed the Shapiro-Wilk W test for normal data, hence the choice of non-parametric text (Wilcoxon Rank Sum). Using the normal quintile plot, there was no observed normal distribution of any of the three enzyme activities (Annex Figure A-1).

Table 9 shows the outcome of the Mann Whitney test on the two populations. The alpha esterase activity in the test population recorded a higher rank sum than the reference population. However, the difference in the median alpha esterase activity between the two populations is not statistically significant (Z=-1.42, p=0.1549). The median mixed function oxidase activity was higher in the test population than the reference but there was no significant difference in the median activities (Z=0.09, p=0.9281). The difference in the median activity of insensitive AChE in the two population is statistically insignificant (Z=-145, p=0.1461). The median activity, however, was higher in the test population than in the reference population. Figure 21 shows the distribution of the enzyme activities in the two populations, with the median line indicated on the graph. Some individual mosquitoes from the test population recorded very high activities for alpha esterase and the insensitive AChE compared to the reference.

TABLE 9: OUTCOME OF WILCOXON RANK SUM (MANN WHITNEY) TEST

Enzyme	Sample	Observation	Rank Sum	Expected	Adjusted Variance	Z	P|z|
Alpha	Reference	59.00	4216.00	4602.00	73632	-1.42	0.1549
	Test population	96.00	7874.00	7488.00			
Oxidase	Reference	59.00	4626.50	4602.00	73632	0.09	0.9281
	Test population	96.00	7463.50	7488.00			
AChE	Reference	51.00	3372.00	3723.00	58327	-1.45	0.1461
	Test population	94.00	7213.00	6862.00			

FIGURE 21: DISTRIBUTION OF ENZYME ACTIVITIES IN REFERENCE AND TEST POPULATIONS OF *AN. GAMBIAE* S.L., WUNDUA (EMD)



4. Discussion, Conclusion, and Recommendations

The data from 2021 longitudinal entomological monitoring in northern Ghana indicate that *An. gambiae* is the predominant vector and exists in sympatry with *An. coluzzii* and *An. arabiensis* in most sites.

Indoor and outdoor human biting rates (per person per night) were significantly higher in the control sites than in the intervention sites. *An. gambiae* has historically been found to be primarily endophagic and endophilic (Reddy et al. 2011); however, the prolonged implementation of IRS and/or the use of ITNs over many years might have induced exophagy in the vector populations (Syme et al. 2021). Indoor biting rates of *An. gambiae* s.l. were significantly higher than outdoor biting rates in all sites except Kata-Banawa (WMD), Sanguli (TSD), and Tugu (TML). Significant outdoor biting rates observed in Kata/Banawa may be the result of sustained pressure from IRS, which has been implemented in this site since 2008, while those observed in Tugu (TML), an unsprayed site, could be due to the recent mass campaign of ITN distribution in the area. The high proportion of *An. coluzzii* and *An. arabiensis* in TML, the control site, as compared to IRS sites may also contributed to the high exophagic tendencies observed in TML.

Forty percent of *An. gambiae* s.l. collected in sleeping rooms had a blood meal from animals and 13.7% collected from pit traps had a human blood meal. The mosquitoes collected by Prokopack in sleeping rooms that had an animal blood meal may have bitten the animal outdoors before entering. This suggests, therefore, that even though mosquitoes may seek alternative (animal) hosts outdoors, some still prefer resting indoors. Mosquitoes collected with human blood in the pit traps may have fed on humans who were outdoors or may have taken their bite from humans indoors and left the house to rest outdoors. These findings, both from IRS and control sites, should be interpreted with caution since the sample sizes from the Prokopack aspirations of resting mosquitoes in sleeping rooms and outdoors in pit traps and the numbers tested for blood meal source were small.

Prokopack aspirations of resting mosquitoes from animal shelters, pit traps, and sleeping rooms collected fewer mosquitoes in sites where animal shelters were also sprayed than where they were not. The resting densities in sleeping rooms and pit traps of IRS sites where animal shelters were sprayed were also lower than in the IRS sites where animal shelters were not sprayed and in control sites. This suggests that spraying animal shelters in addition to human dwellings could have a significant effect on vector density and vector survival rates, and likely further reduce malaria transmission.

Analysis of parity rates showed significantly fewer older mosquitoes were collected in the sprayed sites than in the unsprayed sites. These data suggest that IRS is reducing mosquito longevity—and thus malaria transmission—in intervention sites. A comparison of the sum of monthly indoor and outdoor EIRs revealed higher outdoor transmission occurring in TML and BND. This suggests that interventions that target outdoor transmission may be required as an add-on to IRS and ITNs where outdoor transmission is high. Higher sporozoite rates and EIRs were observed in Bunbuna (BND) than other IRS intervention and control sites. However, the EIRs in 2021 were generally much lower than in 2020 in both IRS and control sites. The recent mass distribution of ITNs conducted in 2021 might have affected the EIR in the control sites. The EIRs were also generally too low to make any meaningful comparisons between sites.

An. gambiae s.l. remain resistant to the pyrethroids that were tested (deltamethrin and alpha-cypermethrin), possibly because of selection pressure from several sources including agriculture activities. The detection of *kdr-e* mutation in the study area conforms with findings in other West African countries such as Burkina Faso (Hanemaaijer et al. 2019) and Côte d'Ivoire (Mouhamadou et al. 2019) and suggests a rapid spread in the West Africa region of the *kdr-e* mutation, originally found in East Africa. Complete restoration of pyrethroid

susceptibility, evidenced by 100% mosquito mortality at 24 hours post exposure to the synergist PBO, suggests that oxidases could be the main contributing factor to resistance observed in the local vector species. Therefore, PBO ITNs may be an appropriate vector control tool in the region. *An. gambiae* s.l. was susceptible to 100µg/bottle chlorfenapyr in two sites, resistant in two sites, and possibly resistant in three sites. These tests will be repeated and, if the earlier results are confirmed, test will be performed with a higher dose of the insecticide (200µg/bottle). There is the need for large-scale testing, such as the NIRMOP activity, of chlorfenapyr before ITNs containing this active ingredient can be recommended as appropriate for deployment in these regions of Ghana.

The confirmed pirimiphos-methyl resistance in six of the ten sites is a concern because the insecticide may not be a suitable option and therefore less likely to be part of insecticides included in the rotation strategy as was originally anticipated. The spread of the *Ace-1* resistant allele seems to confirm the spread of phenotypic resistance observed in 2021 and represents a threat for the use of pirimiphos-methyl in these areas for IRS. Vectors from eight out of the 12 sites were fully susceptible to clothianidin.

These data underscore the importance of implementing an insecticide rotation strategy in IRS campaigns. Considering that vectors in most sites remain susceptible to clothianidin, SumiShield 50WG and Fludora Fusion remain plausible alternatives for future IRS campaigns in northern Ghana; nonetheless, continued close monitoring of resistance is necessary. Both insecticide formulations have demonstrated a residual efficacy that lasts beyond the malaria transmission season and can therefore remain in use for IRS.

ANNEX: 2021 ENTOMOLOGICAL MONITORING RESULTS

TABLE A-1: MONTHLY MEAN NUMBER OF MOSQUITOES COLLECTED PER PERSON PER HOUR, *AN. GAMBIAE*, HLC, ALL SENTINEL SITES, MARCH–DECEMBER 2021

Ghana									
		Bandaya Sentinel Site (Intervention)	Bunbuna Sentinel Site (Intervention)	Gbullung Sentinel Site (Intervention)	Kata/Banawa Sentinel Site (Intervention)	Sanguli Sentinel Site (Intervention)	Tugu Sentinel Site (Control)	Zarantinga Sentinel Site (Intervention)	Kulaa Sentinel Site (Control)
HLC 18-19: *An. gambiae* s.l.- In	Mar-21	0	0	0		0	0	0	0
	Apr-21	0	0	0	0	0	0	0	0
	May-21	0.13	0	0	0	0	0	0	0
	Jun-21	0	0	0	0	0	0.5	0.75	0.5
	Jul-21	0.88	0	0	0.5	0.13	0	0.38	0
	Aug-21	3.4	0	0.38	1.5	0.5	0.13	0.5	0.38
	Sep-21	1	0	0	0.13	0	0	0.13	0.25
	Oct-21	0	0	0	0.13	0	0	0	0
	Nov-21	0	0	0	0.13	0	0	0	0.13
	Dec-21	0.25	0	0	0.13	0	0	0	0
HLC 19-20: *An. gambiae* s.l.- In	Mar-21	0	0	0		0	0	0	0
	Apr-21	0	0	0	0	0	0	0	0
	May-21	0	0	0	0	0	0.5	0.13	0
	Jun-21	0.25	0	0	3.4	0	0.13	0.88	3.1
	Jul-21	0.75	0.13	0	0.38	0	1.4	0.13	0.63
	Aug-21	2	0.13	0.38	2.4	0.88	1	0.5	0.88
	Sep-21	0.5	0.38	0	0.75	0.88	0	0.25	0.25
	Oct-21	0.13	0.13	0	0.13	0	0.13	0	0
	Nov-21	0.13	0.38	0	0.13	0	0	0	0
	Dec-21	0.13	0.13	0	0.13	0	0	0	0
HLC 20-21: *An. gambiae* s.l.- In	Mar-21	0	0.25	0		0	0	0	0
	Apr-21	0	0	0	0	0	0	0	0

Ghana									
		Bandaya Sentinel Site (Intervention)	Bunbuna Sentinel Site (Intervention)	Gbullung Sentinel Site (Intervention)	Kata/Banawa Sentinel Site (Intervention)	Sanguli Sentinel Site (Intervention)	Tugu Sentinel Site (Control)	Zarantinga Sentinel Site (Intervention)	Kulaa Sentinel Site (Control)
	May-21	0	0	0	0	0	2.1	0.38	0.25
	Jun-21	0.88	1	0	4	0	3	1.3	5.6
	Jul-21	1.5	1.4	0.13	1.8	0.13	2.5	1.1	0.38
	Aug-21	4.1	1.4	1.8	8.1	1	2	1.5	2.1
	Sep-21	1.3	0.88	0.13	1.3	1	0.38	0.75	0.38
	Oct-21	0.25	0	0	0.5	0	0.13	0.13	0.13
	Nov-21	0.25	0.13	0	0.75	0	0	0.13	0.13
	Dec-21	0.25	0.5	0	0.25	0	0.13	0	0
HLC 21-22: *An. gambiae* s.l.- In	Mar-21	0	0	0		0	0.13	0	0
	Apr-21	0	0	0	0	0	0	0	0.38
	May-21	0.13	0	0	0.25	0	3.8	0.13	0.75
	Jun-21	0.75	2.6	0.75	9.5	0.5	9.8	3.6	9.5
	Jul-21	3.4	3.1	0.5	2.6	1.5	4.4	3.1	1.9
	Aug-21	6.4	5.1	7.1	13.6	1.6	4.6	2.9	5
	Sep-21	1.4	1.5	0.5	1.5	1.8	2.1	1.9	0.75
	Oct-21	0.38	0	0.25	1	0	1	0.5	0.38
	Nov-21	0.63	1	0	1.9	0	0.13	0	0.38
	Dec-21	0.25	2.3	0.13	0.75	0	0.13	0	0.38
HLC 22-23: *An. gambiae* s.l.- In	Mar-21	0	0.25	0		0	0	0	0

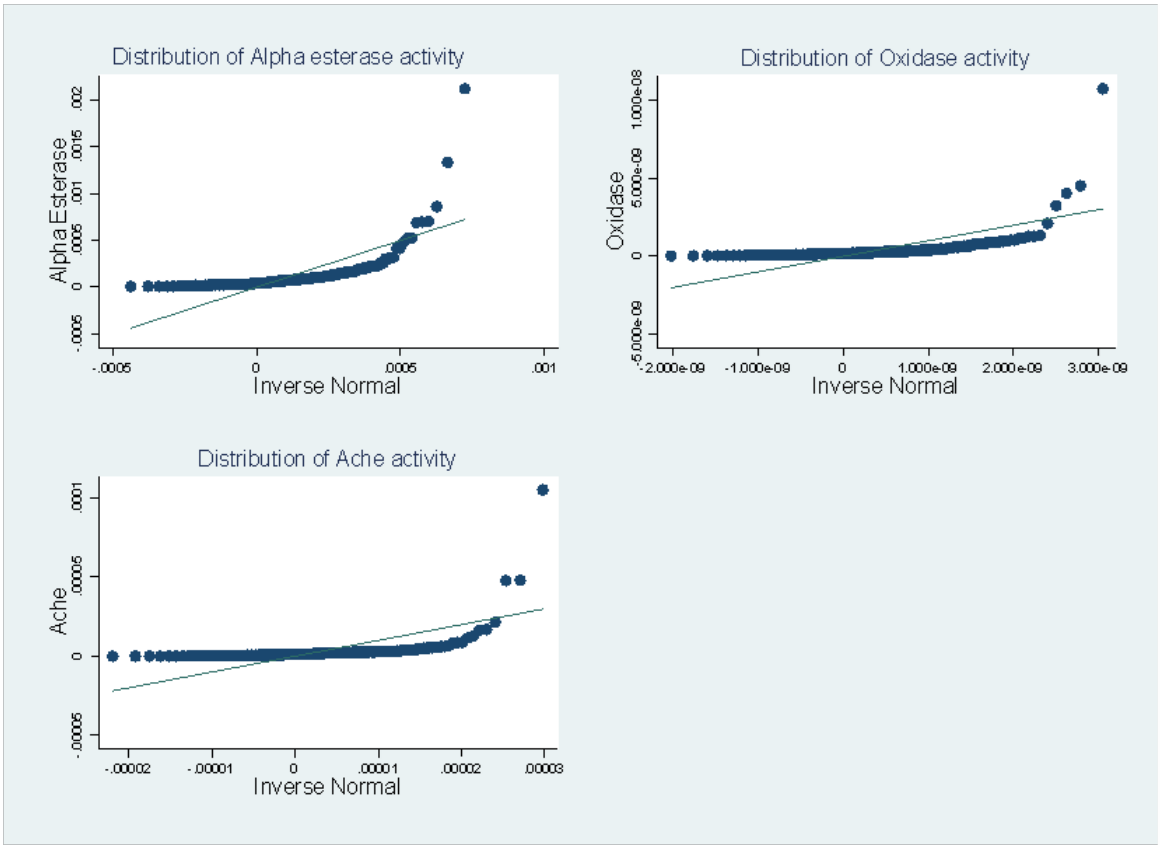
TABLE A-2: MEAN PERCENTAGE KNOCKDOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* AND WILD *AN. GAMBIAE* S.L. FROM SPRAY QUALITY CONE WALL BIOASSAYS CONDUCTED ON ALL SPRAYED SURFACES, ALL SITES, MARCH 2021–JANUARY 2022

WHO cone bioassay mortality by wall type by month												
SumiShield and Fludora Fusion, Ghana 2021												
			T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Bandaya Sentinel Site	Cement	Clothianidin (SumiShield)		100	100	100	94.4	100	100	100	94.4	100	100
	Mud	Clothianidin (SumiShield)		100	100	100	100	100	100	100	94.3	94.4	100
	Wood	Clothianidin (SumiShield)		100	100	100	100	100	100	100	100	100	100
Bunbuna Sentinel Site	Cement	Clothianidin (SumiShield)	100	100	100	100	100	94.3	100	100	100	93.7	100
	Mud	Clothianidin (SumiShield)	100	100	100	100	94.3	94.3	99.4	100	94.4	100	100
	Wood	Clothianidin (SumiShield)	100	100	100	100	100	91.6	100	100	100	100	90.9
Zarantinga Sentinel Site	Cement	Clothianidin (SumiShield)		100	100	100	100	100	100	100	99.4	100	100
	Mud	Clothianidin (SumiShield)		100	100	100	100	100	100	100	93.9	100	95
	Wood	Clothianidin (SumiShield)		97.7	100	100	100	100	100	100	100	100	100
Gbullung Sentinel Site	Cement	Clothianidin + Deltamethrin (Fludora Fusion)	100	100	100	100	93.2	81.8	95	100	98	100	100
	Mud	Clothianidin + Deltamethrin (Fludora Fusion)	100	100	100	100	100	90	100	100	100	100	86.4
	Wood	Clothianidin + Deltamethrin (Fludora Fusion)	100	100	100	100	100	100	100	100	100	100	100
Kata/Banawa Sentinel Site		Clothianidin + Deltamethrin (Fludora Fusion)	100	100	100	100	100	100	100	99.4	100	100	100
	Mud	Clothianidin + Deltamethrin (Fludora Fusion)	100	100	100	100	100	100	100	100	94.6	100	100
	Wood	Clothianidin + Deltamethrin (Fludora Fusion)	100	100	100	100	100	100	100	100	100	100	100
Njobilbo Sentinel Site	Cement	Clothianidin + Deltamethrin (Fludora Fusion)	100	100	100	100	100	100	100	100	100	100	100
	Wood	Clothianidin + Deltamethrin (Fludora Fusion)	100	100	100	100	100	100	100	100	100	100	100

TABLE A-3: SUMMARY OF WHO INSECTICIDE RESISTANCE TESTS OF *AN. GAMBIAE* S.L. TO SELECTED INSECTICIDES, 2021

	Alpha-cypermethrin (0.05%)		Alpha-cypermethrin 0.05% + PBO		Deltamethrin (0.05%)		Pirimiphos-methyl (0.25%)		Clothianidin (2%)			Chlorfenapyr 100µg/Bottle		Clothianidin 4µg/Bottle	
	24hrs Mortality	# Tested	24hrs Mortality	# Tested	24hrs Mortality	# Tested	24hrs Mortality	# Tested	24hrs Mortality	Day 7 Mortality	# Tested	72hrs Mortality	# Tested	24hrs Mortality	# Tested
Bandaya (GUD)	31.90%	160	100%	80			52.50%	160						100%	100
Bunbuna (BND)							96.30%	80							
Gbullung (KUD)	73%	100					85%	80				97%	100	97%	100
Kata/Banawa (WMD)	19.40%	180	100%	80	75%	80	59.40%	180	66.70%	100%	87	96%	100	100%	80
Kumbungu (KUD)	62%	100					76%	100				93%	100	100%	100
Kunkwa (MMD)	47.30%	91					100%	100						100%	100
Namburugu (KAD)							55%	100				68%	100	96.70%	180
Sanguli (TSD)	55%	80					87%	100							
Wundua (EMD)	12.80%	180	100%	80	63.80%	80	75%	100				100%	100	100%	80
Yagaba (MMD)	42%	100					100%	100	60.20%	100%	93			100%	100
Yunyoo (YND)							90%	80							
Zarantinga (EMD)	45%	160	100%	80			88%	100				100%	100		
Dimabi (TD)	14%	100					100%	100						100%	100
Kulaa (SGD)							87.50%	80						94.40%	180
Tugu (TML)							98%	100						92.80%	180
Woribogu (TD)							100%	100				21%	100	98.30%	180

FIGURE A-1: NORMAL QUANTILE PLOT OF KISUMU STRAIN OF *AN. GAMBIAE* AND WILD *AN. GAMBIAE* S.L., WUNDUA (EMD)



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