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PMI VECTORLINK GHANA ANNUAL ENTOMOLOGICAL MONITORING REPORT FOR NORTHERN GHANA

MARCH 1–DECEMBER 31, 2022

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ACRONYMS

ABI	Animal Blood Index
Ace-1	Acetylcholinesterase 1
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
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
NMEP	National Malaria Elimination Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
P.	*Plasmodium*
PMI	President's Malaria Initiative
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 with the main objective assessing the impact of the 2022 indoor residual spray (IRS) campaign in northern Ghana. Monthly mosquito collections were performed from March through December 2022 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, Sagnerigu (SGD) and Tamale metropolis (TML). For vector bionomics monitoring, the project used indoor and outdoor human landing catches and Prokopack collections in sleeping rooms, animal shelters and pit traps. Insecticide resistance tests were also carried out in all IRS districts and Tolon District (TD), where IRS was withdrawn in 2013. CDC bottle assays and WHO tube tests were used to determine vector susceptibility to insecticides and the role of synergists. The project also performed wall cone assay tests to assess spray quality and residual efficacy of sprayed insecticide: SumiShield and Fludora Fusion. 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 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 Elimination Program (NMEP) 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 in partnership with Navrongo Health Research Center also collected vector bionomics data in six sites from six regions (two of these sites are project routine IRS sites) as a partner of the NMEP's National Entomological Surveillance Program. The results from the National Entomological Surveillance Program work will be shared with the NMEP 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, constituting 92.38% (19,983/21,632) and 87.79% (6,945/7,911) of all *Anopheles* collected in the IRS intervention and control sites, respectively. *An. gambiae* s.l. was the major species, comprising *An. gambiae* (82.2%), *An. coluzzii* (10.7%), and *An. arabiensis* (7.0%), as well as hybrids (0.1%). *An. gambiae* was the majority across all sites. The proportion of *An. arabiensis* in the IRS sentinel sites ranged from 0% to 10% and 3% to 25% in Control Sites; this was higher than the proportions observed from 2016 to 2019 but was not different from the last two years.

Human Biting Rate: The mean monthly HBRs of *An. gambiae* s.l. recorded for the IRS sites were similar to the mean HBR recorded for the control sites. The mean indoor HBR for *An. gambiae* s.l. was 20.5 b/p/n for IRS sites and 21.4 b/p/n for the control sites. The mean outdoor HBR for *An. gambiae* s.l. was 20.5 b/p/n for IRS sites and 19.9 b/p/n for the control sites.

Indoor and outdoor resting density: The mean indoor resting density of *An. gambiae* s.l. in sleeping rooms was 0.11 mosquitoes per room/day for the IRS sites and 0.06 mosquitoes per room/day for unsprayed sites. *An. gambiae* s.l. resting density in the animal shelters of control sites was higher (0.55 per shelter per day) than in IRS sites (0.22 per shelter per day). The number of mosquitoes collected resting indoors or outdoors was low to make any meaningful comparison between IRS and control sites or between daytime resting locations.

Parity rates: The mean proportion of parous females in the unsprayed sites (67.90%) was higher than the proportion collected from the IRS districts (51.89%) indicating the impact of IRS on vector longevity.

Entomological inoculation rate (EIR): 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. The overall EIR in the IRS and control sites was 84.85 and 43.35 infective bites/person/year, respectively. WMD had the highest monthly outdoor EIRs, which added up to 205.53 infective bites/person/year and EMD recorded the highest indoor EIRs, 179.8 infective bites/person/year. In 2022, higher EIRs were reported in some of the IRS sites than in the control sites. The average EIR in IRS sites is 84.85 and control sites is 43.35.

Spray quality and residual life of IRS insecticides: Wall bioassays on all surfaces (mud, cement, and wood) within seven days after spray indicated high-quality and uniform spraying 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 *An. gambiae* (Kisumu), *An. gambiae* (wild), and *An. gambiae (*Tiassalé).

Insecticide susceptibility: *An. gambiae* s.l. from Bandaya (GUD), Bunbuna (BND), Dimabi and Woribogu (TD), Kata/Banawa (WMD), Kulaa (SGD), Kunkwa (MMD), Namburugu (KAD), and Sanguli (TSD) were susceptible to 0.25% pirimiphos-methyl, and possible resistance was reported from Wundua (EMD) and Yagaba (MMD). Pyrethroid resistance was observed across all sites tested, and synergist assay results suggest that mono-oxygenases may play a significant role in the resistance to pyrethroids in these sites. *An. gambiae* s.l. from across all sites tested were susceptible to clothianidin (with 100% mortality two days post exposure) and chlorfenapyr (with 100% mortality three days post exposure).

Target Site Resistance: The frequency of the resistant alleles for the Ace-1 gene mutation ranged from 0.90 to 0.99 both in the IRS intervention and control sites. There was a slight increase in the frequency of Ace-1 resistant alleles in 2022 compared to 2021 when the frequency of Ace-1 alleles ranged from 0.73 to 1.00 in the IRS intervention sites. The frequency of kdr-w resistant alleles in samples analyzed was high across all sites. There is a slight increase in the frequency of kdr-w resistant alleles in most of the IRS sites compared to 2021. pesticides.

Blood Meal Source: The HBI for An. gambiae s.l. collected from animal shelters in the IRS sites was 20.7% and HBI from the control sites was 3.1% (Table 6). The HBI for An. gambiae s.l. collected from sleeping rooms in the IRS sites was 68.7% and 66.7% from the control sites. The ABI for An. gambiae s.l. in sleeping rooms (17.4%) was higher in the IRS sites than in the control sites (0%). The animal blood meal sources included bovine, chicken, dog, goat, pig, and other animals (undetermined).

CONCLUSION

HBR rates were similar in the IRS and control sites. However, slightly high sporozoites rate and EIRs were observed in the IRS sites compared to control sites. Possible explanations could be that the control sites are part of the sub-urban TML that is undergoing rapid urbanization, which may have affected biting and infectivity. The project may have to think of changing the control site to areas with similar characteristics to the IRS districts. The Ghana team collecting baseline data from two non-IRS districts during Year 1 Evolve and using them as controls starting Year 2 could be helpful in measuring the impact of IRS. The second reason for the low EIR in the control sites could be the ITN distribution campaign conducted in 2021 (for the first time with Piperonyl Butoxide (PBO) nets) which may also have affected vector infection rate and thus the EIR. . Parity rates showed significantly few old mosquitoes were collected in the sprayed sites than in the unsprayed sites which suggest that IRS is reducing mosquito longevity and possibly could impact malaria transmission in intervention sites.

Considering that vectors in most sites remain susceptible to clothianidin, SumiShield 50WG and Fludora Fusion remain appropriate options for the 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. The data also have shown a return of susceptible *An. gambiae* populations in most sites where resistance to pirimiphos-methyl was recorded in previous years. However, the frequency of the *Ace- 1* mutations remains very high with almost complete fixation.

I. INTRODUCTION

In 2022, 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 (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 2022: 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 these insecticides was based on results of insecticide susceptibility and residual efficacy tests from 2020 and 2021 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 KUD, WMD, KAD, and EMD in 2021. 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 (BND, KAD, MMD, WMD, and YND) and SumiShield 50WG was sprayed in four districts (EMD, GUD, KUD, and TSD) in 2022.

In 2022, the project continued spraying animal shelters in five districts 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 2022.

Specific objectives of the 2022 entomological surveys in Ghana were:

1. To monitor the species composition of malaria vectors in the target districts. The aim is to assess if there would be a change in species composition of vectors in response to vector control interventions over time.
2. To monitor the effect of IRS on vector densities, longevity and infectivity, and feeding and resting behavior.
3. To determine the seasonal trend of vectors to guide the timing of IRS.
4. To assess if spraying animal shelters had an effect on entomological indices of malaria transmission. Data on its impact on malaria case incidence will be reported in a separate manuscript.
5. To guide the selection of insecticide for IRS and other insecticide-based vector control products, based on the response of the vector to different insecticides and underlying mechanisms of resistance where resistance is detected.
6. To assess the quality of the IRS operations and identify early if any corrective measures or adjustments are needed and keep the quality in the remaining weeks of the campaign.
7. To monitor the residual efficacy of sprayed insecticides to generate data that can be used in the selection of the right product for IRS and timing of IRS.

The project also provided technical and financial support to the Noguchi Memorial Institute for Medical Research (NMIMR) to collect insecticide resistance data from 15 sentinel sites in 15 regions, through the National Insecticide Resistance Monitoring Partnership (NIRMOP).

The project in partnership with Navrongo Health Research Center also collected vector bionomics data in six sites in six regions as a partner to the NMEP'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 by VectorLink Ghana with the NMEP and all partners after one year of data collection by all partners is completed.

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 to conduct advanced molecular analyses of collected samples. This report presents findings and analyses of the entomological monitoring activities the project carried out in 2022.

2. METHODOLOGY

2.1 SENTINEL SITES

VectorLink Ghana conducted entomological data collection 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 Sagnerigu 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 Tolon District (TD), where IRS was withdrawn in 2013. Table 1 summarizes the spray history of each district from 2008 through 2022.

FIGURE 1: MAP OF PMI VECTORLINK GHANA DISTRICTS AND ENTOMOLOGICAL MONITORING SITES, 2022

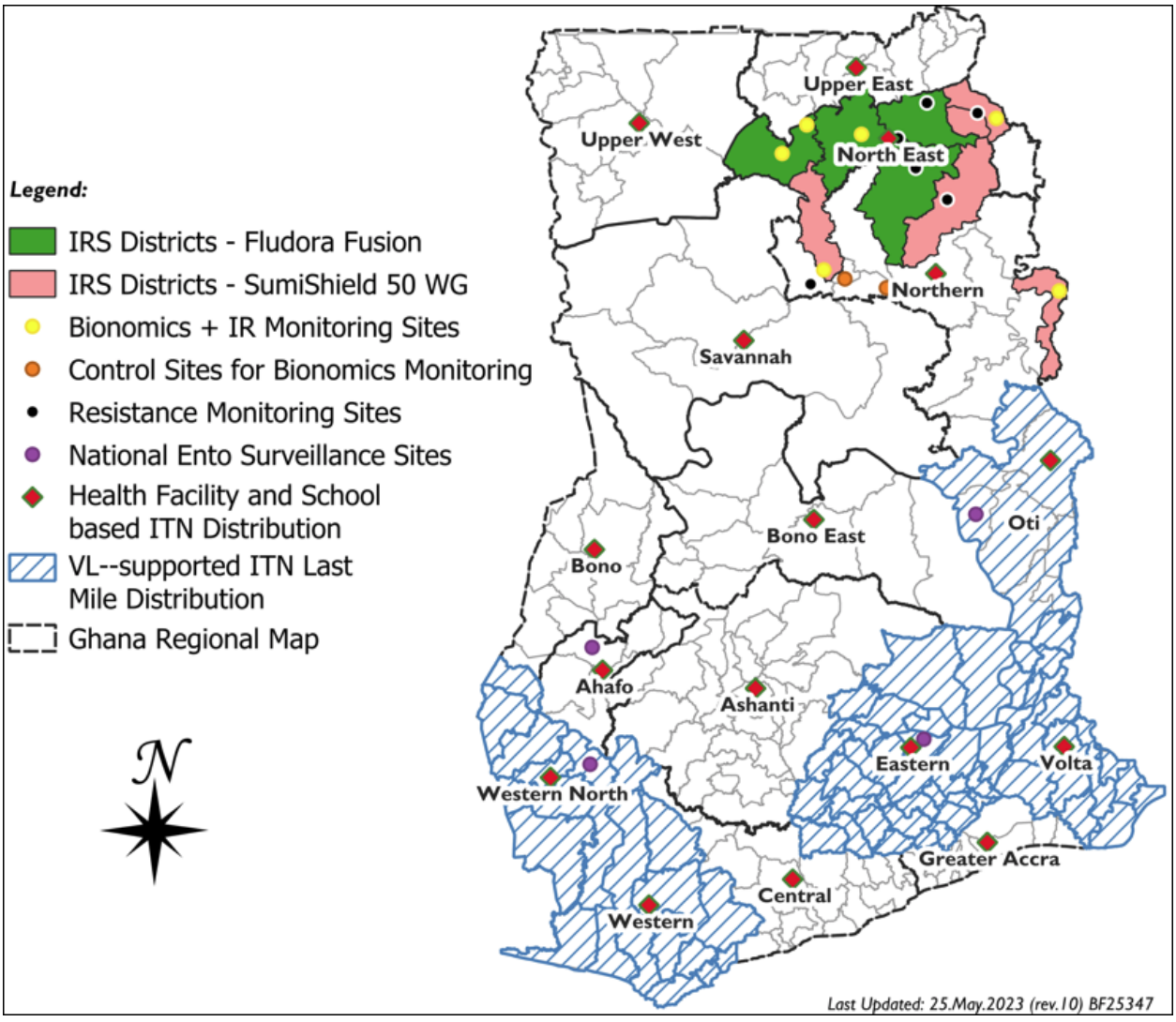


TABLE 1: ENTOMOLOGICAL MONITORING SITES, 2008–2022

District	Sentinel Site	Insecticide Spray History														
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Routine entomological and Insecticide resistance-monitoring sites																
GUD	Bandaya	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM	PM	PM	CLD+ DM	CLD	CLD
BND	Bunbuna	NSp	NSp	NSp	ACy	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD	CLD+ DM
KUD	Gbullung*‡	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM	PM	PM	PM	CLD+ DM	CLD+ DM	CLD
EMD	Zaratinga	NSp	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD+ DM	CLD	CLD
TSD	Sanguli and Njobilbo*	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	CLD+ DM	CLD+ DM	CLD
WMD	Kata/Banawa*‡	ACy	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD	CLD+ DM	CLD+ DM
SGD†	Kulaa	NSp	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	NSp
Insecticide resistance-monitoring only sites																
MMD	Yagaba and Kunkwa*	Acy	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	CLD	CLD	PM	CLD+ DM	CLD+ DM
EMD	Wundua	NSp	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD+ DM	CLD	CLD
KAD	Namburugu	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM	PM	PM	CLD	CLD+ DM	CLD+ DM
KUD	Kumbungu	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM	PM	PM	PM	CLD+ DM	CLD+ DM	CLD
TD≠	Dimabi and Woribugu	ACy	ACy	DM	ACy	ACy	NSp	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	CLD

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

***** = Bugyanga, Bunbuna, Gbullung, Kata Banawa, Sanguli, and Zarantinga for residual bioefficacy

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

‡ = National Entomological monitoring Site

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 *An. gambiae* s.s. Kisumu, *An. gambiae* Tiassalé (resistant lab colony) 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 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 aim of the quality assurance cone assays was to provide quick feedback (1–7 days after start of the IRS campaign) to the operation team on whether the performance of the spray operators and team was adequate, and the spray should continue or to identify if there are any concerns that needed to be addressed early and to keep the quality of the spray high in the remaining weeks.

The 2022 IRS campaign commenced on March 2, 2022, 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 week of the spray campaign in one community in each sprayed district. Six houses (two with cement walls and two with mud walls, which is the predominant surface type; and wood surfaces in windows and doors in two other houses 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)[1] to assess the quality of work done by the different spray teams in each district. The bioassays were conducted using *An. gambiae* s.s. Kisumu strain for all the nine districts. Also, wild *An. gambiae* s.l. and *An. gambiae* Tiassalé were used for SumiShield 50WG and Fludora Fusion-sprayed districts, respectively.

2.2.2 Residual Efficacy of Sprayed Insecticides

The questions to be addressed from the residual efficacy tests were:

- How long does the sprayed insecticide remain effective on the sprayed wall? Would it cover the entire peak malaria transmission season?
- Does type of surface affect the residual life of the insecticide?
- For SumiShield, do the wild and susceptible colonies (Kisumu) respond the same way and, for Fludora Fusion, is there a difference in response between a pyrethroid resistance lab colony (Tiassalé) and susceptible colony (Kisumu), given one of the components of Fludora Fusion is a pyrethroid (deltamethrin)?

Post-spray bioassays were conducted monthly from April 2022 through January 2023. The assays measured the residual bioefficacy of SumiShield 50WG sprayed in Gbullung (KUD), Sanguli (TSD), and Zarantinga (EMD) and of Fludora Fusion in Bugyanga (MMD), Bunbuna (BND), and Kata/Banawa (WMD). In each community, six houses (two with cement walls, two wooden surfaces, and two mud walls, except in Sanguli where no mud surfaces were tested as all the houses were constructed with cement and even the mud walls were plastered with cement.) Houses were purposefully selected to represent structures sprayed by different spray operators and spray teams.

[1] https://pmivectorlink.org/resources/tools-and-innovations/ SOP09/01

2.3 ADULT MOSQUITO COLLECTIONS

The project's entomology team collected mosquitoes from all eight sentinel sites for four consecutive days each month, for 10 months (March through December 2022). 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 6:00 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/household/ 5 households/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 entomological inoculation rates (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

The aim of the 2022 susceptibility tests was:

- To guide the project in the selection of the insecticide for the next IRS campaign.
- To generate and share data with the NMEP that can be used in the selection of insecticide-treated nets (ITNs) for the next distribution. The results of synergist assay with piperonyl butoxide (PBO) tests and chlorfenapyr tests were shared in the quarterly meeting with the NMEP and partners.

WHO tube tests (SOP06/01[2]) and CDC bottle assays (SOP04/01[3]) were performed to assess the susceptibility of local *An. gambiae* s.l. vector populations to insecticides used for IRS and ITNs. All sentinel sites have a history of ITN coverage either through mass distribution campaigns or school-based distribution and from health facility distribution.

2.4.1 WHO TUBE TESTS

WHO tube tests were conducted using WHO standardized insecticide papers: alpha-cypermethrin (0.05%), deltamethrin (0.05%), and pirimiphos-methyl (0.25%). Synergist assays were performed using alpha-cypermethrin and PBO on mosquitoes from selected sentinel sites according to the project's SOP for WHO tube tests.

[2] https://pmivectorlink.org/resources/tools-and-innovations/ SOP06/01

[3] https://pmivectorlink.org/resources/tools-and-innovations/ SOP04/01

2.4.2 CDC Bottle Assay

The CDC bottle assay method with recent modification was used to test susceptibility of *An. gambiae* s.l. to clothianidin[4] and chlorfenapyr.[5]

2.5 ELISA and Molecular Analyses

Enzyme-linked immunosorbent assay (ELISA): ELISA was used to determine sporozoite rates and calculate EIRs that were used to assess the impact of IRS. ELISA was also used to determine the blood meal source of blood-fed mosquitoes, which was used to indicate the host preference of the vector and resting patterns. ELISA was also performed to measure enhanced levels of detoxification enzymes (esterase and oxidases) that may be responsible for resistance to the different insecticide classes.

Polymerase chain reaction (PCR): PCR was used to identify members of the *An. gambiae* s.l. complex to species level. These data will be used to monitor if there is or there would be a shift in species composition over time, in response to control interventions or other factors. PCR was also performed to determine the frequency of knockdown resistance (*kdr)* and acetylcholinesterase 1 (*Ace-1)* genotypes and if these correlate to the prevalence of the phenotypic resistance. Moreover, the data will be used to understand if easing the selection pressure of certain types of insecticides would reverse or reduce the frequency of these mutations.

2.5.1 *P. falciparum* Sporozoite Rates

The heads and thoraxes of about 30% (average 100 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. These data were mainly used to calculate EIR, which in turn was used to assess the impact of IRS by comparing EIRs between IRS and non-IRS sites.

2.5.2 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-fed mosquitoes were selected by simple random sampling from the pool of blood-fed mosquitoes for testing. The Ghana team used these data to understand the feeding preference of the main vector, *An. gambiae* s.l., which also could give a clue as to the resting behavior of the vector.

- Do some of the vectors collected from sleeping rooms only feed on animal blood and have entered the rooms for resting only? Do they have both human and animal blood suggesting they can readily feed on available hosts either indoors or outdoors.
- Do the vectors collected outdoors or in animal shelters have human blood indicating the vector can bite humans outside or inside sleeping rooms but prefer to rest outdoors or animal shelters?

2.5.3 Species Identification

A sample of morphologically identified *An. gambiae* s.l. were further identified into sibling species, using ribosomal DNA-PCR (Scott, Brogdon and Collins, 1993). All samples tested for bloodmeal source detection, all dissected mosquitoes for parity, and about 25% of mosquitoes tested for sporozoite infection including all positive for *P. falciparum* were sent for species identification. However, in this report all the species ID results are disaggregated only by IRS and non-IRS site. With the recent implementation of the new labeling and barcoding system for all the samples going for lab analysis, moving forward, all lab processed mosquitoes will have unique ID and more disaggregated results will be presented. PCR-RFLP (restriction fragment length

[4] Protocol for conducting clothianidin bottle bioassay susceptibility tests at 4µg/bottle using wild *Anopheles* malaria vectors.

[5] Determining susceptibility status of *Anopheles* mosquitoes to a discriminating concentration of chlorfenapyr (100µg/bottle) in bottle bioassays.

polymorphism) was then used to further separate the *An. gambiae* s.s. into *An. gambiae* and *An. coluzzii* (Fanello, Santolamazza and Torre. 2002). In the long run, these data could be useful to determine if there is or would be a shift in species composition due to the control interventions or other factors.

2.5.4 *ACE-1* AND *KDR* GENOTYPING

Samples of live and dead mosquitoes (20–25 mosquitoes) from alpha-cypermethrin, deltamethrin and pirimiphos-methyl susceptibility tests were further analyzed, using the protocol described by Wilkins, Howell and Benedict, (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* and *kdr-e*. A few specific questions the Ghana team aimed to answer were:

- The project has been spraying organophosphate (2012-2020) but stopped due to the emergence of phenotypic resistance and buildup of the *Ace- 1* gene mutation. Given the organophosphate pressure has been stopped from IRS for 2-4 years now, would there be a change in the frequency of the *Ace- 1* mutation among the vector population or there are other drivers of selection pressure?
- Similarly, the pyrethroid pressure in the IRS sites is also now less, as IRS with pyrethroid has been withdrawn since 2013 and there was no mass distribution of ITNs for the last 4–5 years. Would this have an impact in reducing *kdr* frequency or there are other drivers of selection pressure for these mutations?

2.6 BIOCHEMICAL ASSAYS

One hundred *An. gambiae* s.l. from Bunbuna (BND) and Kata/Banawa (WMD) and 60 *An. gambiae* s.s. Kisumu strain was 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). These assays help to understand the underlying mechanisms of the phenotypic resistance to different insecticides observed in the vector. 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 an important role in organochlorine and pyrethroid resistance in malaria vectors.

2.7 PARITY DISSECTIONS

About fifty mosquitoes were dissected from each site per month. Mosquito dissection for parity determination involved immobilizing female mosquitoes and carefully dissecting their ovaries under a microscope. By examining the ovaries, the team was able to determine if the mosquitoes have laid eggs once in her lifetime (parity) or not (nulliparity).

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:

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

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 sites were calculated as:

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

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

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

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

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

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

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

- **EIR**: 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:

$$(HBR)\ per\ unit\ time\ reported\ \times sprozoite\ rate$$

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

$$Monthly\ =\ Monthly\ HBRs\ X\ monthly\ sporozoite\ rates$$

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

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

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

- **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).

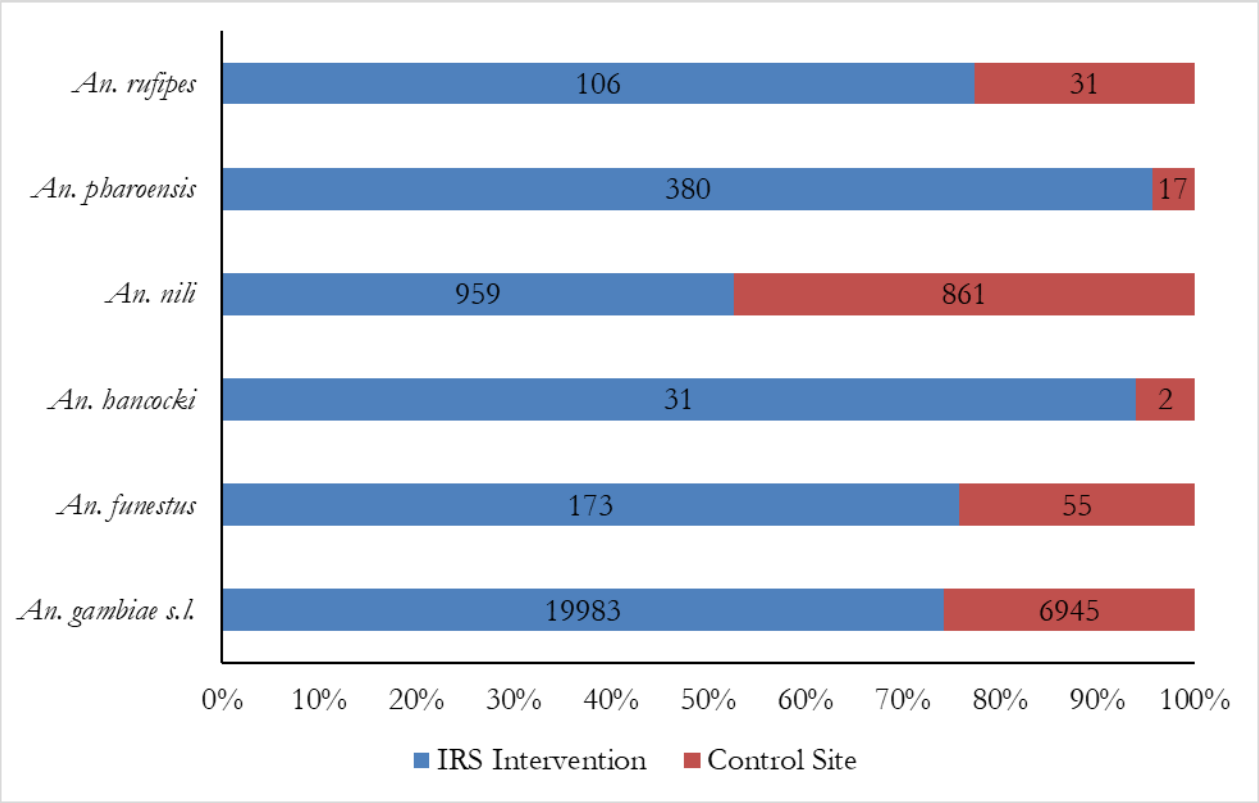
Differences in parity and sporozoite rates between the IRS versus control unsprayed sites were also compared through a z-test for differences in proportions. All tests were performed at 0.05 significance level, using Microsoft Excel®, STATA, and DHIS2-based VectorLink Collect.

3. RESULTS

3.1 VECTOR SPECIES COMPOSITION

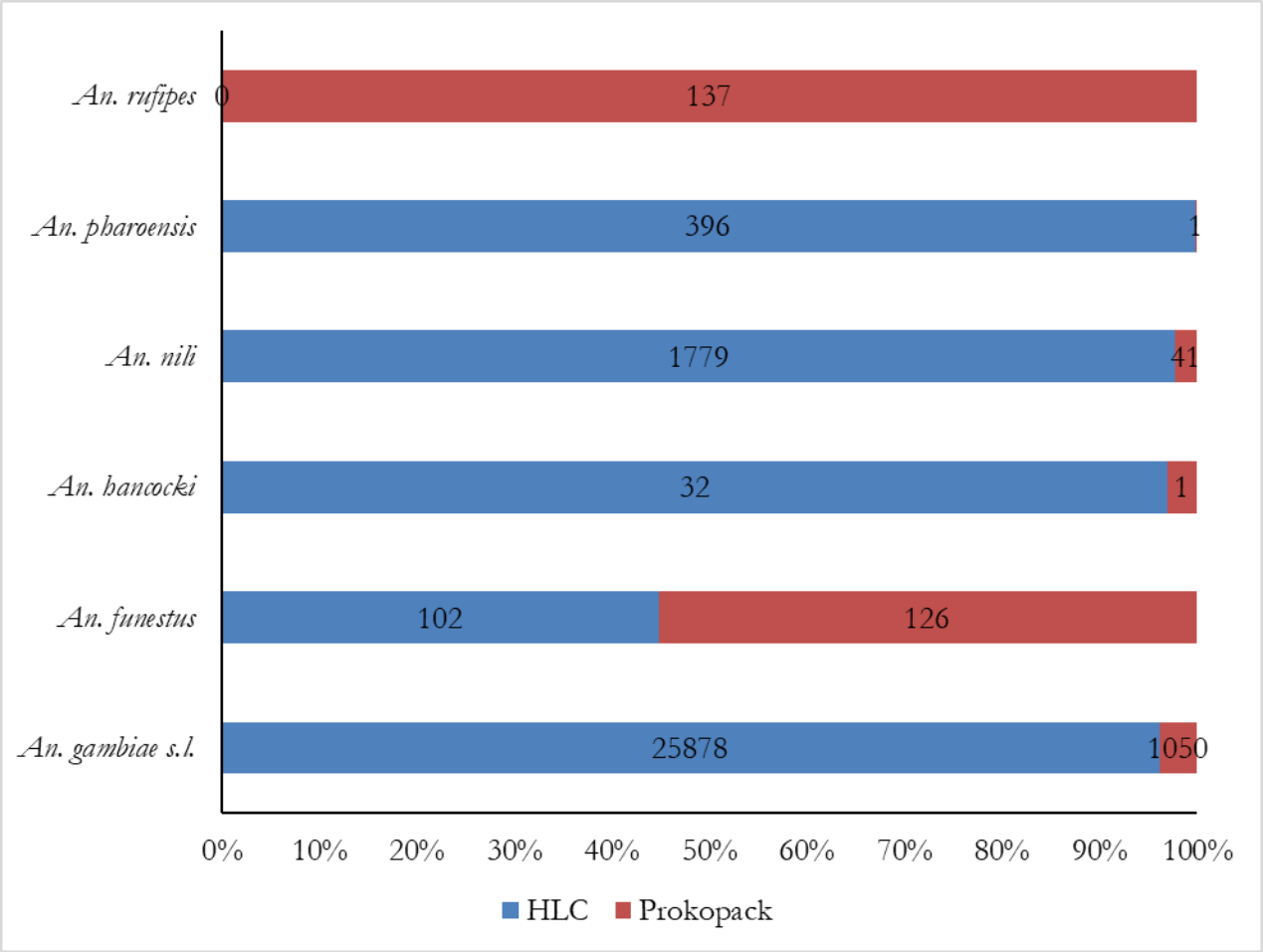
An. gambiae s.l. was the predominant species collected across all sites, constituting 92.38% (19,983/21,632) and 87.79% (6,945/7,911) of all *Anopheles* collected in the IRS intervention and control sites, respectively (Figure 2). *An. nili* was the second predominant species in most sites, constituting about 4.43% in intervention and 10.88% in control sites. This is an increase in *An. nili* proportions as compared to the 2% in IRS and 3% in control sites in 2021. 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-vector.

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



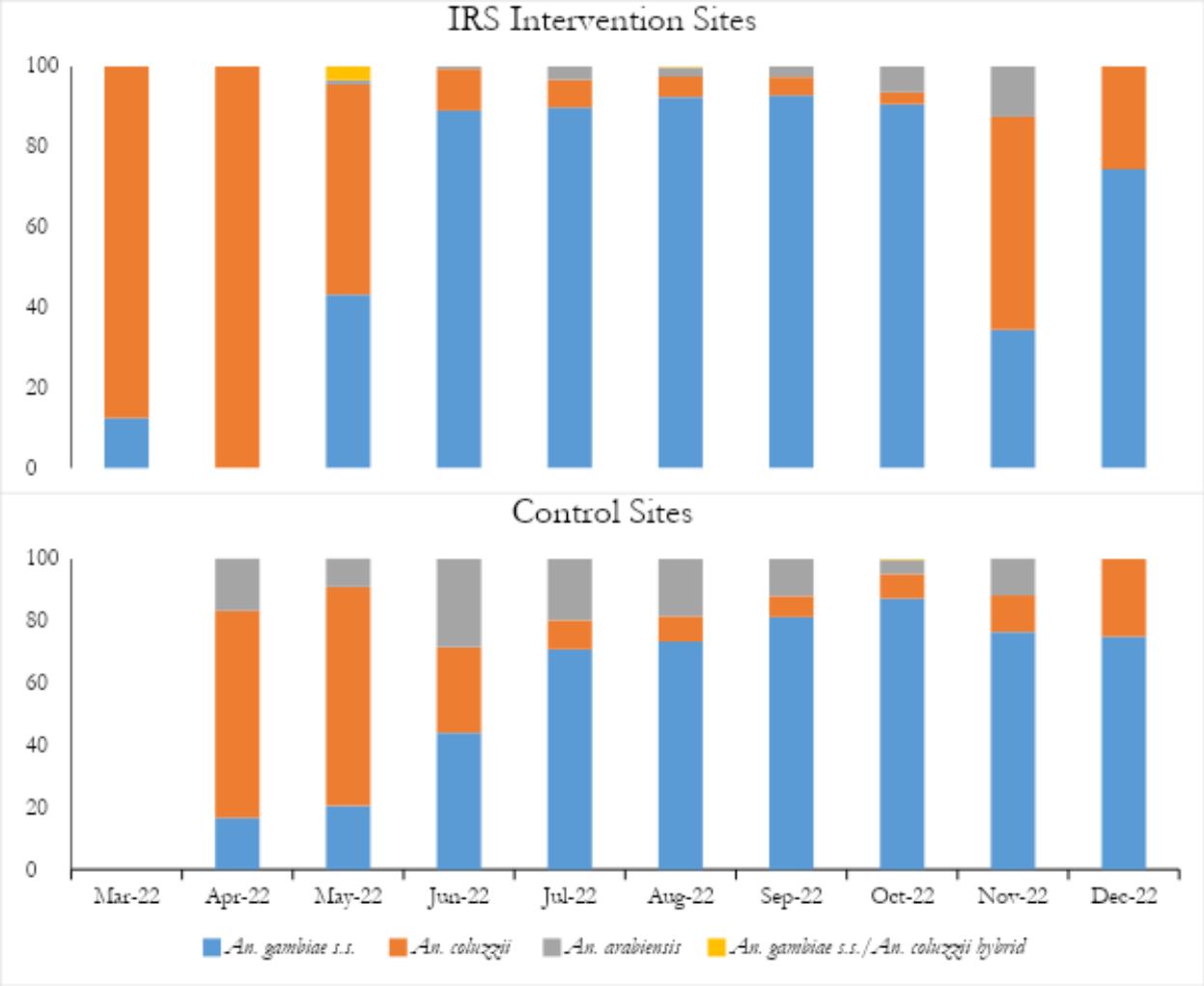
Of the total adult female *Anopheles* mosquitoes collected, 95.41% (28,187/29,543) were collected attempting to bite by HLC and 4.59% (1,356/29,543) were collected resting indoors and outdoors by Prokopack (Figure 3). The *Anopheles* species collected were predominantly *An. gambiae* s.l., which made up 91.81% and 77.43% of the HLC and Prokopack collections, respectively. The proportion of *An. gambiae* s.l., was higher in the HLC collections than the Prokopack collections that were performed in animal shelters and pit traps. None of the 137 *An. rufipes* were collected from HLC trying to bite humans. However, the higher proportion of *An. funestus* s.l. (9.29%) from the Prokopack collection than from the HLC collection (0.36%) was unexpected given *An. funestus* s.l., is more anthropophilic. Even if *An. funestus* s.l. may not be an important vector of malaria in northern Ghana due to its low numbers, identification of the complex to the species level could provide better understanding of this mosquito.

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



The molecular identification of 3,736 *An. gambiae* s.l. revealed three sibling species: *An. gambiae* (82.2%), *An. coluzzii* (10.7%), and *An. arabiensis* (7.0%), as well as hybrids of *An. coluzzii* and *An. gambiae* (0.1%). *An. gambiae* was the most abundant across most sites (Figure 4).

FIGURE 4: SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. ALL SENTINEL SITES, MARCH–DECEMBER 2022



3.2 HUMAN BITING RATES

The mean monthly HBRs of *An. gambiae* s.l. recorded for the IRS sites were similar to the mean HBR recorded for the control sites (Table 3). The mean indoor HBR for *An. gambiae* s.l. was 20.5 b/p/n for IRS sites and 21.4 b/p/n for the control sites. The mean outdoor HBR for *An. gambiae* s.l. was 20.5 b/p/n for IRS sites and 19.9 b/p/n for the control sites. *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.

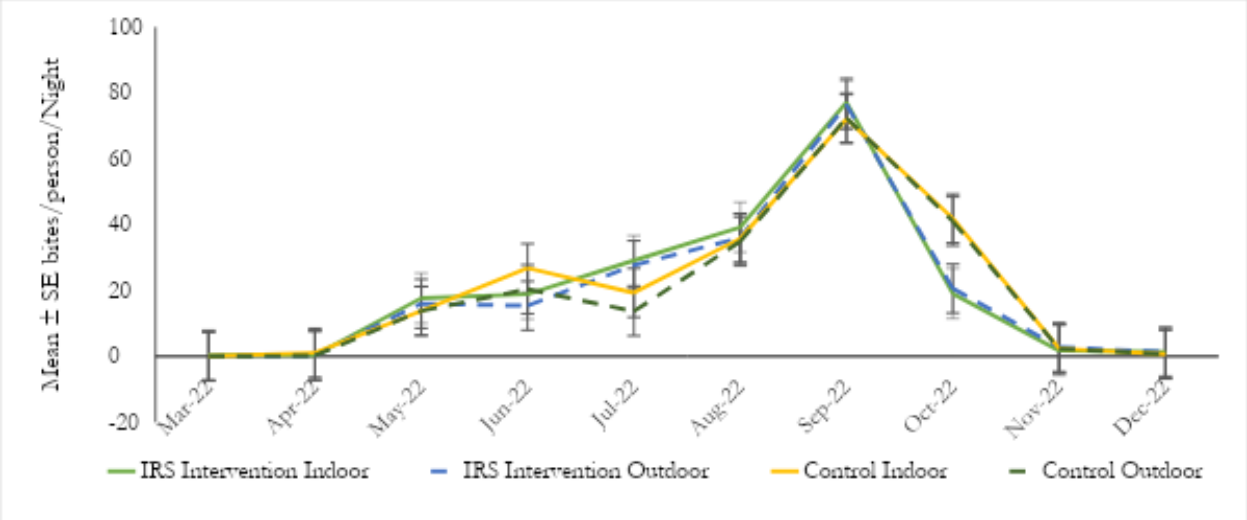
P-values on Table 3 indicate whether the in/out biting ratio for *An. gambiae* s.l. is significantly different from 50/50. Overall, the IRS recorded and endophagic/exophagic index of 0.51/0.49 and the control site recorded 0.52/0.48 both of which were significantly different from a 50/50 in/out ratio, at a p-value of 0.004. Tugu (TML) had an endophagic index of 0.51/0.49 however this was not significantly different from a 50/50 in/out biting ratio (p-Value=0.454). Similarly, Bandaya (GUD) recorded an in/out biting ratio of 0.51/0.49 but was not significantly different from a 50/50 in/out ratio (p-Value=0.178).

TABLE 3: MEAN INDOOR AND OUTDOOR HBR OF *AN. GAMBIAE* S.L., HLC, ALL SENTINEL SITES, MARCH–DECEMBER 2022

Sentinel Site	Number Collected Indoor	Number Collected Outdoor	Indoor Biting Rate (b/p/n)	Outdoor Biting Rate (b/p/n)	Endophagic Index	Exophagic Index	$\chi 2$	p-Value
SumiShield IRS sites								
Bandaya (GUD)	1983	1899	24.80	23.70	0.51	0.49	1.82	0.178
Gbullung (KUD)	1438	1015	18.00	12.70	0.59	0.41	72.94	0.000
Sanguli (TSD)	409	459	5.10	5.70	0.47	0.53	2.88	0.090
Zarantinga (EMD)	2103	1818	26.30	22.70	0.54	0.46	20.72	0.000
Fludora Fusion IRS sites								
Bunbuna (BND)	1485	1345	18.60	16.80	0.52	0.48	6.93	0.008
Kata/Banawa (WMD)	2415	2899	30.20	36.20	0.45	0.55	44.08	0.000
Control sites								
Kulaa (SGD)	1590	1403	19.90	17.50	0.53	0.47	11.68	0.001
Tugu (TML)	1831	1786	22.90	22.30	0.51	0.49	0.56	0.454
Overall								
Intervention	9833	9435	20.50	19.63	0.51	0.49	8.22	0.004
Control	3421	3189	21.40	19.90	0.52	0.48	8.14	0.004

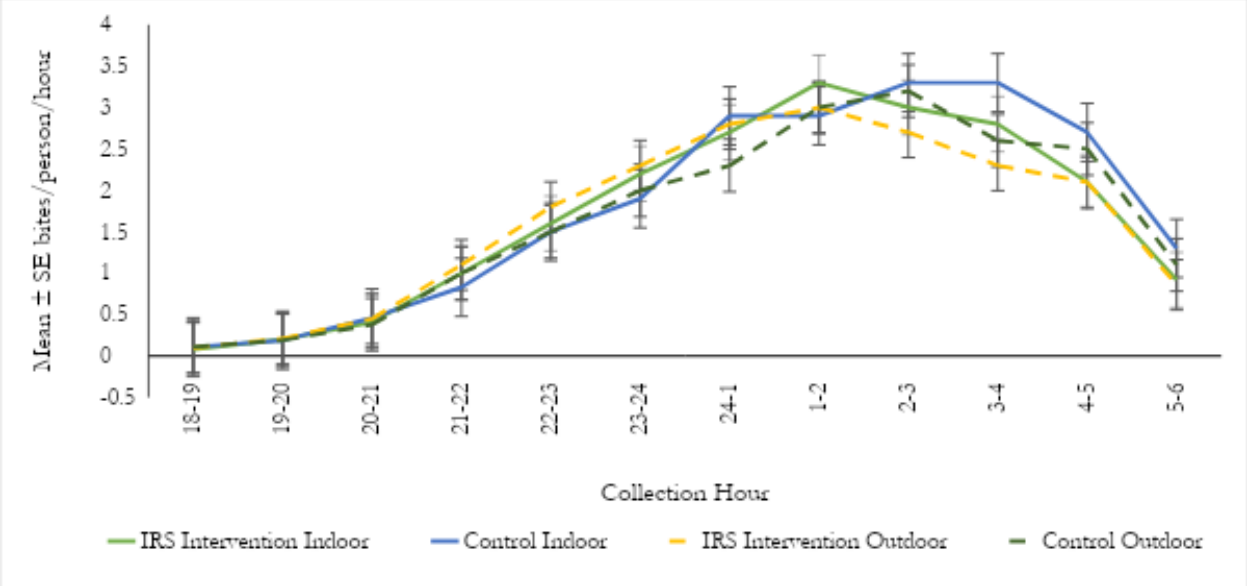
An. gambiae s.l. population densities, as measured by mean monthly HBRs, peaked in September for both IRS and control sites in 2022 (Figure 5). There was a dip in July in the control sites, possibly due to continuous rains and windy nights during collections.

FIGURE 5: MEAN DAILY INDOOR AND OUTDOOR HBR, *AN. GAMBIAE* S.L., SPRAYED AND UNSPRAYED SITES, MARCH–DECEMBER 2022



Indoor and outdoor biting activity of *An. gambiae* s.l. started at 6:00 pm and then gradually increased, with peak biting observed between 1:00 am and 4:00 am in both the IRS and control sites (Figure 6, Table A-2 and Table A-3). The pattern of mosquitoes biting during these peak times was similar between the control and IRS intervention sites.

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



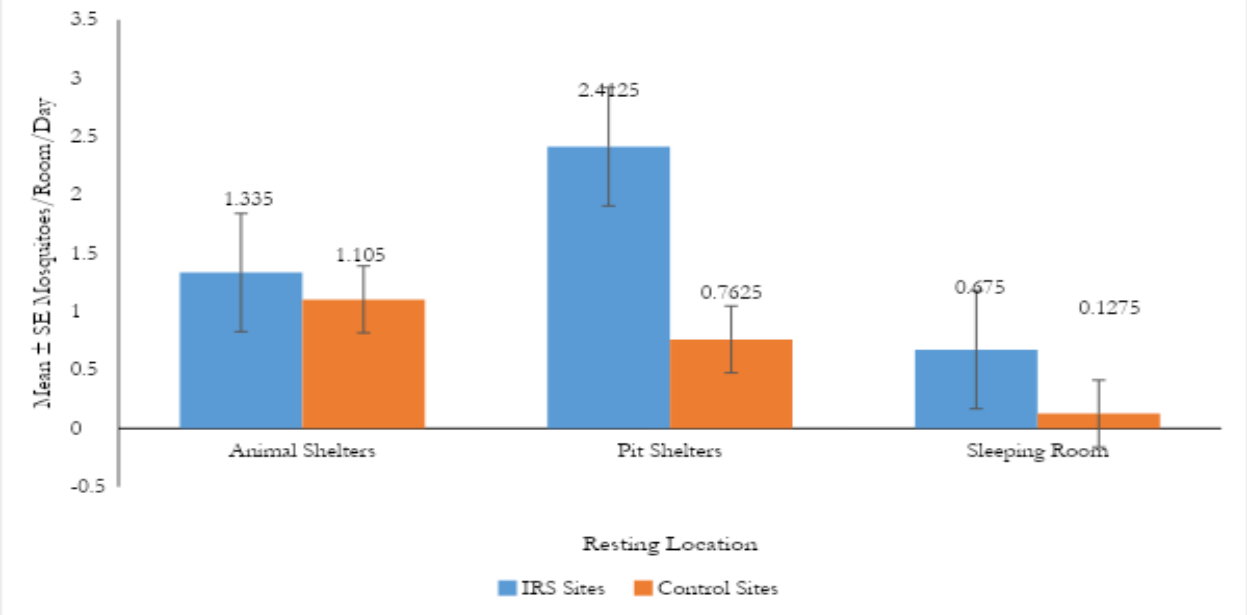
3.3 RESTING BEHAVIOR

The mean indoor resting density of *An. gambiae* s.l. in sleeping rooms was 0.18 mosquitoes per room/day for the IRS sites and 0.25 mosquitoes per room/day for unsprayed sites (Figure 7). The indoor resting density in IRS sites was slightly lower than in the control sites but the numbers found resting indoors in both cases were too low to make any meaningful comparison.

FIGURE 7: MEAN INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. IN SPRAYED AND UNSPRAYED SITES

As in 2021, VectorLink Ghana sprayed animal shelters in all districts to assess the entomological impact of spraying, with the aim of improving IRS efficacy. Though the overall numbers of *An. gambiae* s.l. collected resting in animal shelters was low, the density (0.55 per shelter per day) was higher in the control sites than in IRS sites (0.22 per shelter per day). The numbers collected resting in pit shelters located in the IRS sites (0.40 per shelter per day) were similar to the numbers collected from pit shelters in the intervention sites (0.38 per shelter per day). The resting density in sleeping rooms in the IRS sites was higher (0.11) compared to the control sites (0.06) (Figure 8).

FIGURE 8: MEAN RESTING DENSITY OF *AN. GAMBIAE* S.L. PER RESTING LOCATION.

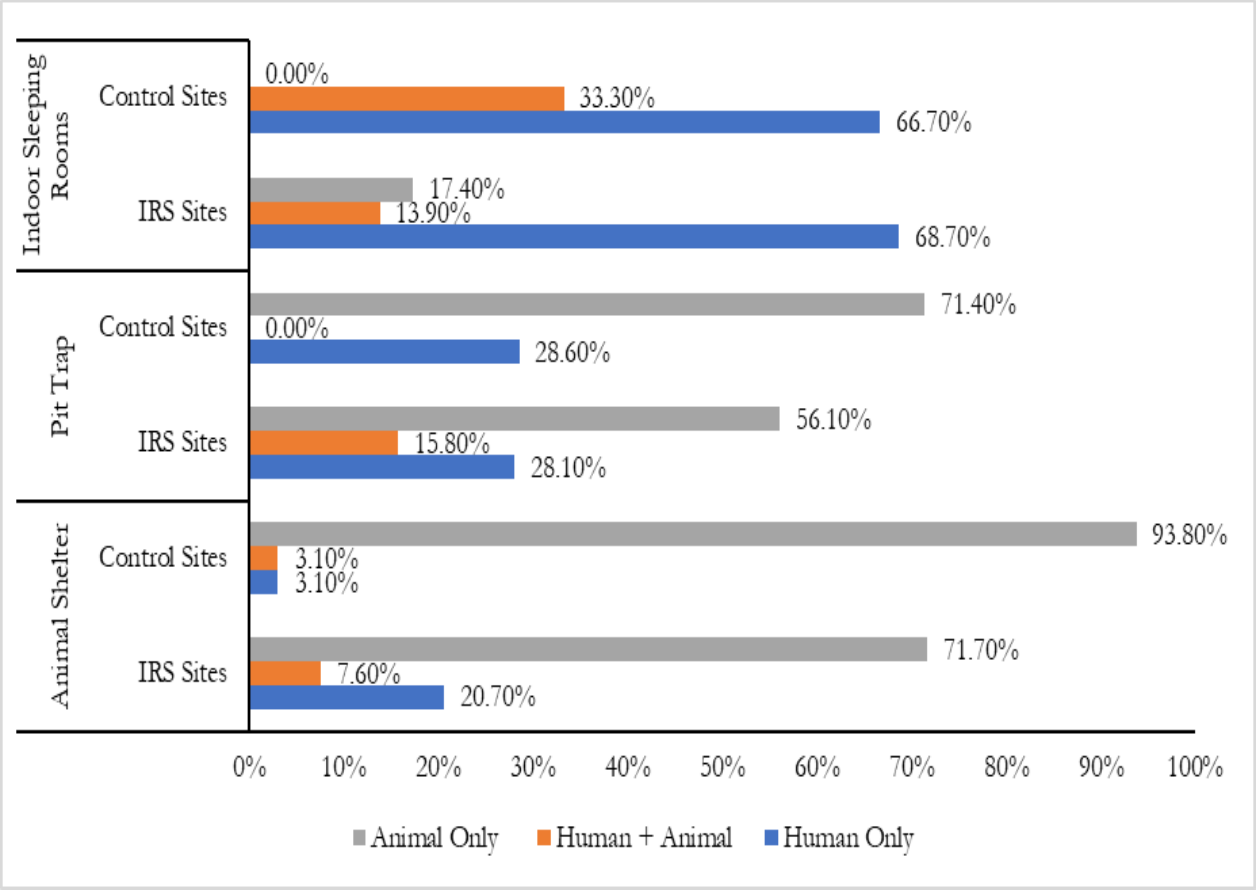


3.4 BLOOD MEAL SOURCE

For source of blood meal, 124 blood-fed *An. gambiae* s.l. from animal shelters, 64 from pit shelters, and 118 blood-fed *An. gambiae* s.l. from sleeping rooms were tested.

The HBI for *An. gambiae* s.l. collected from animal shelters in the IRS sites was 20.7% and HBI from the control sites was 3.1% (Figure 9). The HBI for *An. gambiae* s.l. collected from sleeping rooms in the IRS sites was 68.7% and 66.7% from the control sites. The ABI for *An. gambiae* s.l. in sleeping rooms (17.4%) was higher in the IRS sites than in the control sites (0%). The animal blood meal sources included bovine, chicken, dog, goat, pig, and other animals (undetermined). Of the 64 blood-fed mosquitoes collected from pit shelters by Prokopack aspiration in IRS sites, 28.1% had had a human-only blood meal. A considerable proportion (20.7%) of mosquitoes resting in animal shelters in the IRS sites had a human blood meal, indicating those mosquitoes had either taken their bite outdoors and rested in the animal shelters or had taken their blood meal indoors and exited to rest in the sheds. Similarly, 17.4% of the mosquitoes collected in sleeping rooms had animal-only blood and 13.9% mixed animal and human blood. Most of the mosquitoes with mixed blood meal were collected from pit shelters and sleeping rooms. Overall, the numbers processed were too small (as the numbers collected were also low) to make any meaningful comparison.

FIGURE 9: *AN. GAMBIAE* S.L. COLLECTED BY PROKOPACK AND THEIR SOURCE OF BLOOD MEAL, MARCH–DECEMBER 2022



3.5 PARITY RATES

Dissections of *An. gambiae* s.l. mosquitoes collected by HLC between March and December 2022 revealed that the proportion of parous females collected from the unsprayed sites in SGD and TML (67.9%) was significantly higher than the proportion collected from the IRS districts (52.0%) (Table 4), indicating IRS has impact on longevity of the vector. Parity rate was slightly higher in the sites sprayed with Fludora Fusion (53.7%) compared to the sites sprayed with SumiShield (50.7%), but the difference was not statistically significant (p=0.265).

TABLE 4: PROPORTION OF PAROUS FEMALES OF *AN. GAMBIAE* S.L. BY HLC, MARCH–DECEMBER 2022

Sentinel Site	#Dissected	Parous	% Parity	95% Confidence Interval	
				Lower Bound	Upper Bound
SumiShield IRS sites					
Bandaya (GUD)	263	120	45.6%	39.5%	51.9%
Gbullung (KUD)	186	92	49.5%	42.1%	56.9%
Sanguli (TSD)	117	45	38.5%	29.6%	47.991%
Zarantinga (EMD)	290	177	61.0%	55.2%	66.7%
Total SumiShield	856	434	50.70%	47.4%	54.0%
Fludora Fusion IRS sites					
Bunbuna (BND)	247	138	55.9%	49.4%	62.2%
Kata/Banawa (WMD)	329	171	52.0%	46.4%	57.5%
Total Fludora Fusion	576	309	53.7%	49.6%	57.7%
Control sites					
Kulaa (SGD)	254	169	66.5%	60.37%	72.3%
Tugu (TML)	260	180	69.2%	63.23%	74.8%
Total Control	514	349	67.9%	63.7%	71.9%
Overall					
Intervention	1432	743	52.0%	49.3%	54.5%
Control	514	349	67.9%	63.7%	71.9%

3.6 *P. FALCIPARUM* SPOROZOITE RATES

A total of 5,071 *An. gambiae* s.l. (about 30%) collected by HLC were assayed by ELISA to determine the presence of *P. falciparum* sporozoites. The sporozoite rate (SR) for Fludora Fusion-sprayed sites was 1.55% indoors and 1.97% outdoors, while for SumiShield-sprayed sites it was 1.54% indoors and 0.75% outdoors), and for control sites it was 0.77% indoors and 0.72% outdoors (Table 5). Overall, the SR in the intervention sites (1.34%) was higher than the SR in the control sites (0.75%) and the difference was marginally significant (p=0.046). While the difference in SR in the FF sprayed sites (1.78%) was significantly higher than the control sites (0.75%); p=0.020, the difference between the control and SS sprayed sites (0.75% vs 1.19%) was not significant (p=0.15). There was also no significant difference between the SRs of the FF and SS sprayed sites (p=0.21).

TABLE 5: *P. FALCIPARUM* SPOROZOITE INFECTIONS IN *AN. GAMBIAE* S.L. SAMPLED FROM ALL SENTINEL SITES, MARCH–DECEMBER 2022

Sentinel Site	Number Analyzed			No. Positive for *P. falciparum* sporozoite			Sporozoite Rate		
	In	Out	Total	In	Out	Total	In	Out	Total
SumiShield IRS sites									
Bandaya (GUD)	564	502	1,066	4	2	6	0.71%	0.40%	0.56%
Gbullung (KUD)	425	288	713	5	2	7	1.18%	0.69%	0.98%
Sanguli (TSD)	148	135	283	6	2	8	4.05%	1.48%	2.83%
Zarantinga (EMD)	160	138	298	5	2	7	3.13%	1.45%	2.35%
Total SumiShield	**1297**	**1063**	2,360	**20**	**8**	28	**1.54%**	**0.75%**	1.19%
Fludora Fusion IRS sites									
Bunbuna (BND)	160	125	285	4	3	7	2.50%	2.40%	2.46%
Kata/Banawa (WMD)	227	331	558	2	6	8	0.88%	1.81%	1.43%
Total Fludora Fusion	**387**	**456**	843	**6**	**9**	15	**1.55%**	**1.97%**	1.78%
Control sites									
Kulaa (SGD)	479	427	906	1	2	3	0.21%	0.47%	0.33%
Tugu (TML)	560	544	1,104	7	5	12	1.25%	0.92%	1.09%
Total Control	**1039**	**971**	2,010	**8**	**7**	15	**0.77%**	**0.72%**	0.75%
Overall									
Intervention	1684	1519	3,203	26	17	43	1.5%	1.1%	1.34%
Control	1039	971	2,010	8	7	15	0.8%	0.7%	0.75%

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. The overall average EIR in IRS sites was 84.85 infective bites/person/year (ib/p/yr.) and control sites was 43.35 ib/p/yr. The overall average indoor EIR was 70.49 and Outdoor EIR was 57.71. WMD had the highest monthly outdoor EIRs, which added up to 205.53 ib/p/yr. , and EMD, which recorded the highest indoor EIRs, 179.8 ib/p/yr. (Figure 8). The EIRs in control sites were lower than in some of the IRS sites due to their low rate of sporozoite infection. This seems to be a recent trend when some of the IRS sites in 2021 also started showing higher EIR than the controls. (Figure 10).

FIGURE 10: INDOOR AND OUTDOOR EIRS FOR *AN. GAMBIAE* S.L. IN IRS AND CONTROL SITES

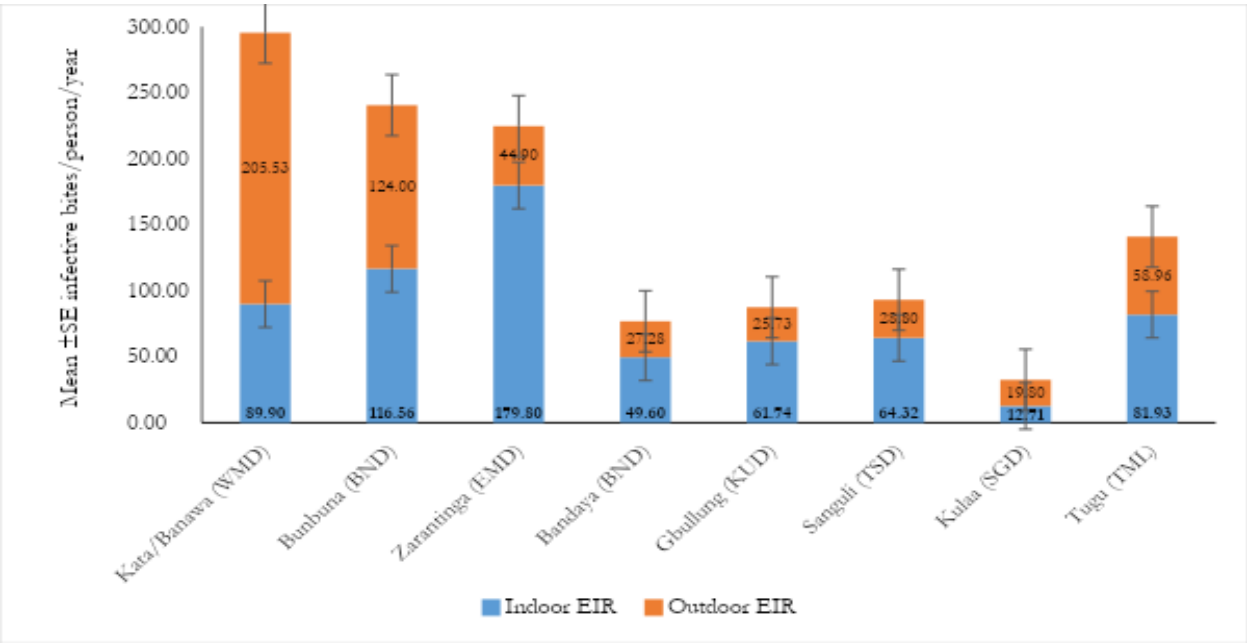
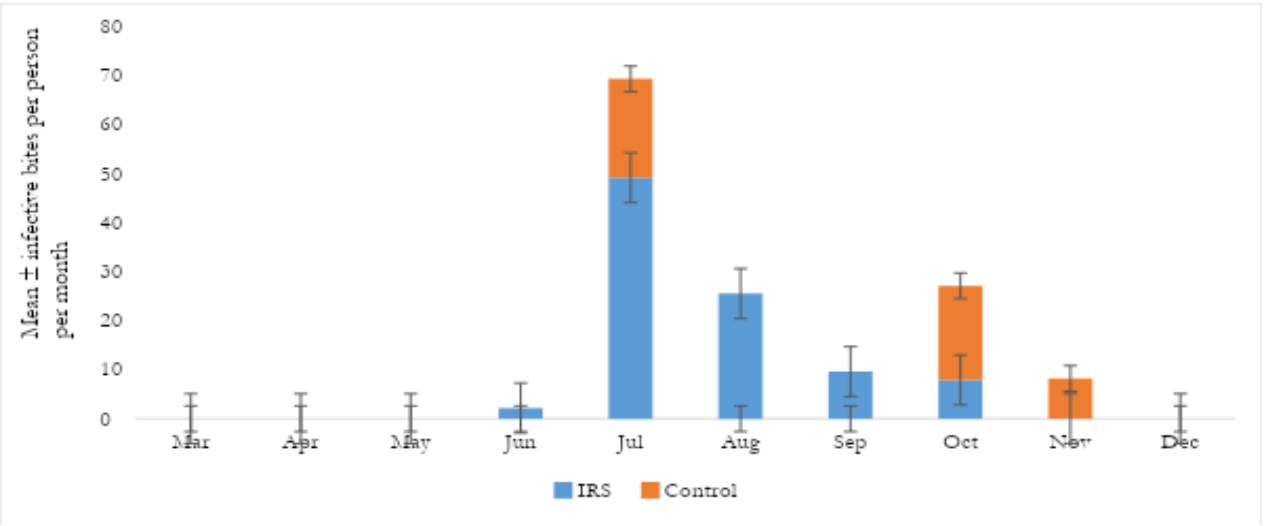


FIGURE 11: INDOOR AND OUTDOOR MONTHLY EIR TRENDS FOR *AN. GAMBIAE* S.L. IN IRS AND CONTROL SITES



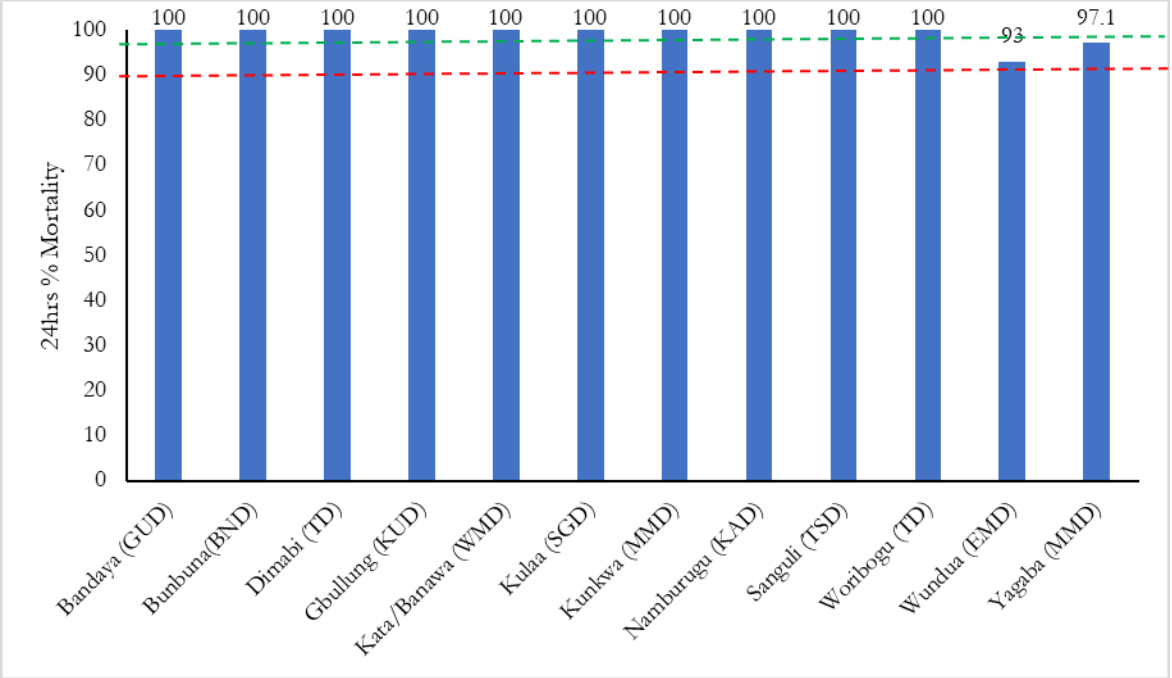
3.8 SPRAY QUALITY AND RESIDUAL EFFICACY

The wall bioassays of SumiShield 50WG and Fludora Fusion showed good residual efficacy (greater than 80%) up to at least 10 months post spray based on tests performed with Kisumu strain mosquitoes. Bioassays with *An. gambiae* (Kisumu), *An. gambiae* (wild), and a resistant colony of *An. gambiae* (Tiassalé) attained 100% mortality within five days in most months on walls sprayed with Fludora Fusion and SumiShield. SumiShield 50WG and Fludora Fusion killed the Kisumu, wild, and Tiassalé mosquitoes equally. Both SumiShield 50WG and Fludora Fusion showed long residual efficacy (up to at least 10 months post spray) and, based on the residual efficacy data. (Figure A-1, A-2, A-3, and A-4).

3.9 INSECTICIDE SUSCEPTIBILITY

Insecticide susceptibility results and synergist assays are shown in Figures 10–17 and Table A-1. *An. gambiae* s.l. from Bandaya (GUD), Bunbuna (BND), Dimabi and Woribogu (TD), Kata/Banawa (WMD), Kulaa (SGD), Kunkwa (MMD), Namburugu (KAD), and Sanguli (TSD) were susceptible to 0.25% pirimiphos-methyl, while Wundua (EMD) and Yagaba (MMD) reported possible resistance (Figure 12). This result is different from last year, when resistance or possible resistance was reported in 10 out of the 15 sites tested. Though this suggests susceptibility is coming back, it could be too early to shift to pirimiphos-methyl spray given the vector is still possibly resistant in two sites.

FIGURE 12: INSECTICIDE SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO PIRIMIPHOS-METHYL (0.25%), WHO TUBE TEST, TWELVE SITES



An. gambiae s.l. from all the sites tested were resistant to the 0.25% alpha-cypermethrin tested (Figure 12). *An. gambiae* s.l. from two sites tested were also resistant to 0.05% deltamethrin at the end of the 24-hour holding period (Figure 14). Based on these data, products with alpha-cypermethrin or deltamethrin only are not recommended.

FIGURE 13: INSECTICIDE SUSCEPTIBILITY OF *AN. GAMBIAE* S.L., TO ALPHA-CYPERMETHRIN (0.05%), WHO TUBE TEST, NINE SITES

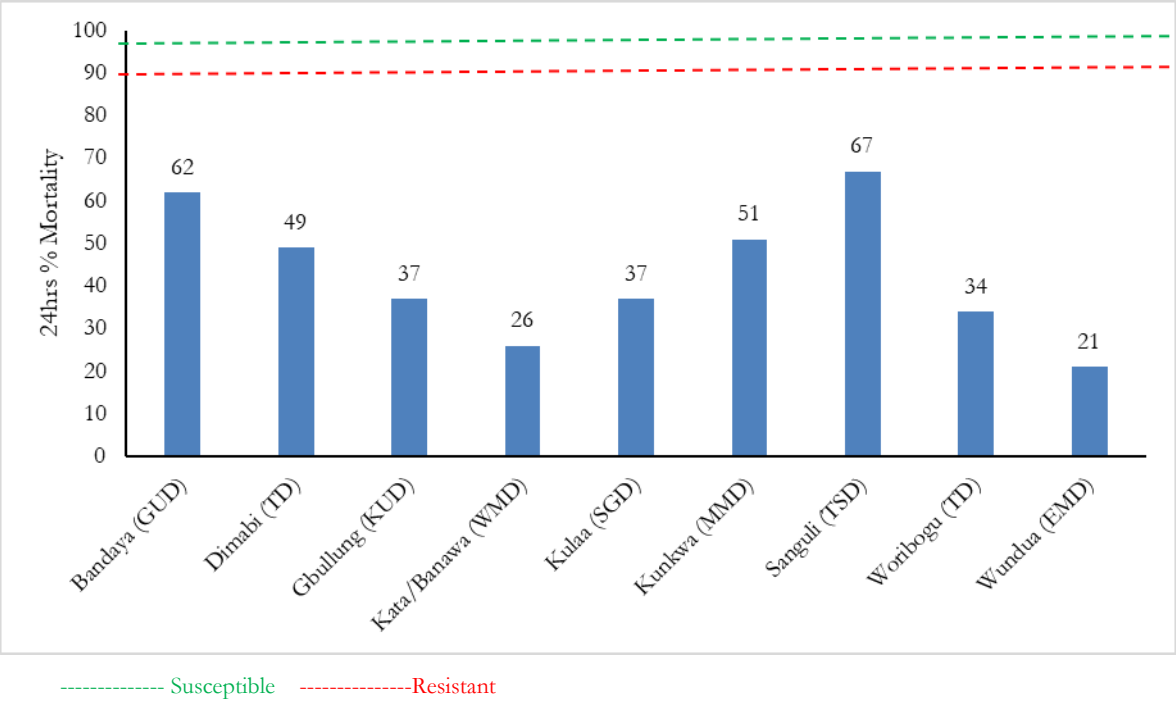
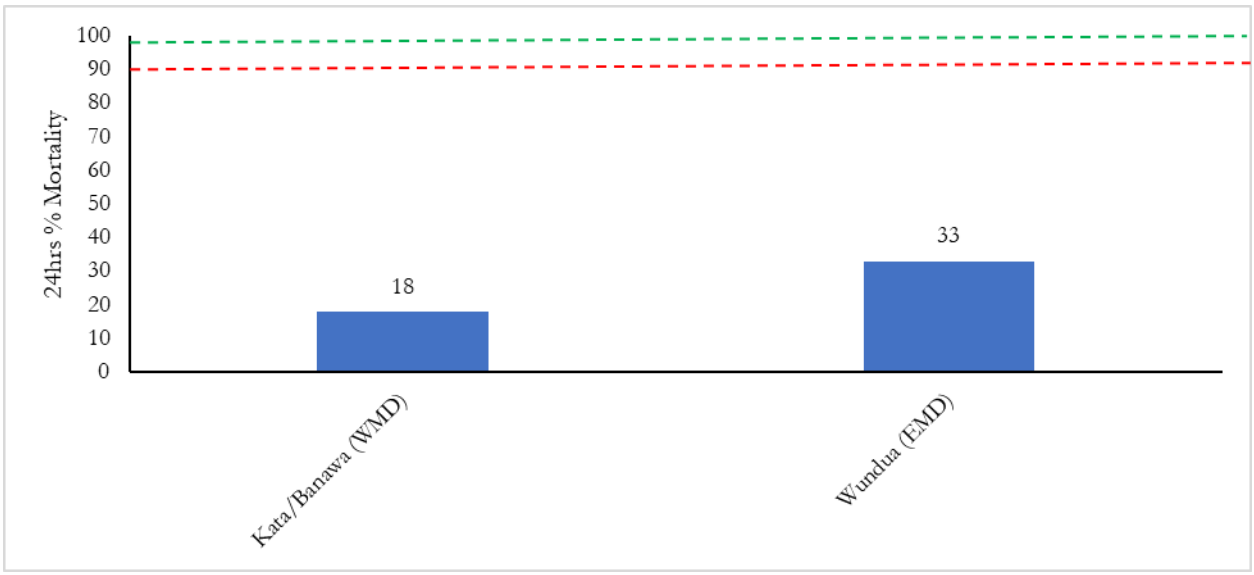


FIGURE 14: INSECTICIDE SUSCEPTIBILITY OF *AN. GAMBIAE* S.L., DELTAMETHRIN (0.05%), WHO TUBE TEST, TWO SITES



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 15). Results with

An. gambiae s.l. from six sites tested showed susceptibility to chlorfenapyr in six sites: Bunbuna (BND), (Kata/Banawa (WMD), Namburugu (KAD), Yagaba (MMD), and Zarantinga and Wundua (EMD) (Figure 16). The findings suggest products with chlorfenapyr could be effective against the vector in the project areas.

FIGURE 15: SUSCEPTIBILITY OF *AN. GAMBIAE* S.S. (KISUMU STRAIN), CHLORFENAPYR (100 µG/BOTTLE), CDC BOTTLE ASSAYS, SIX SITES

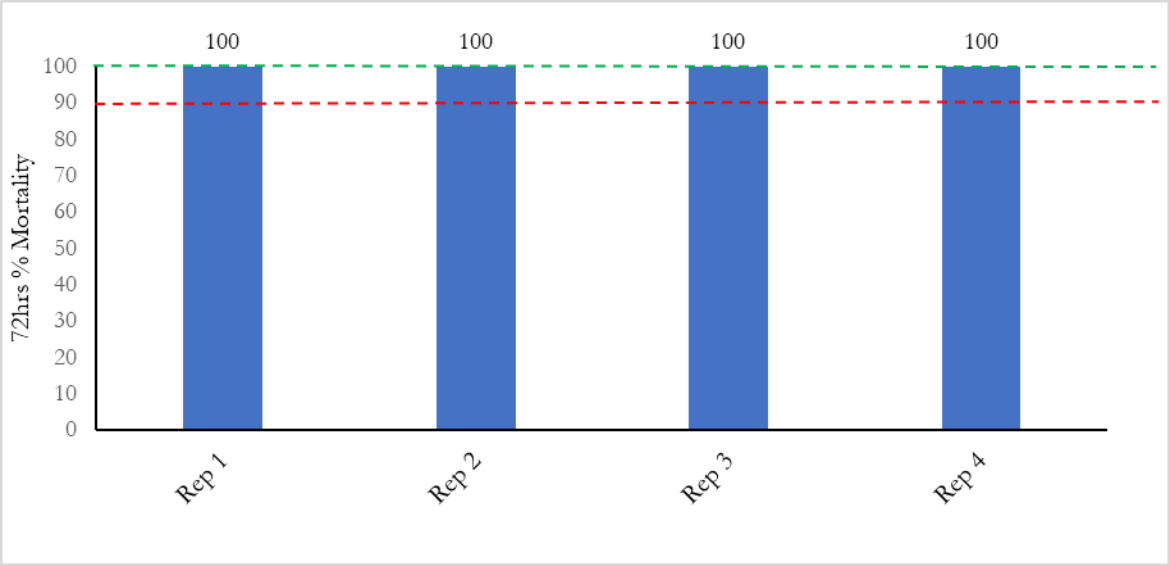
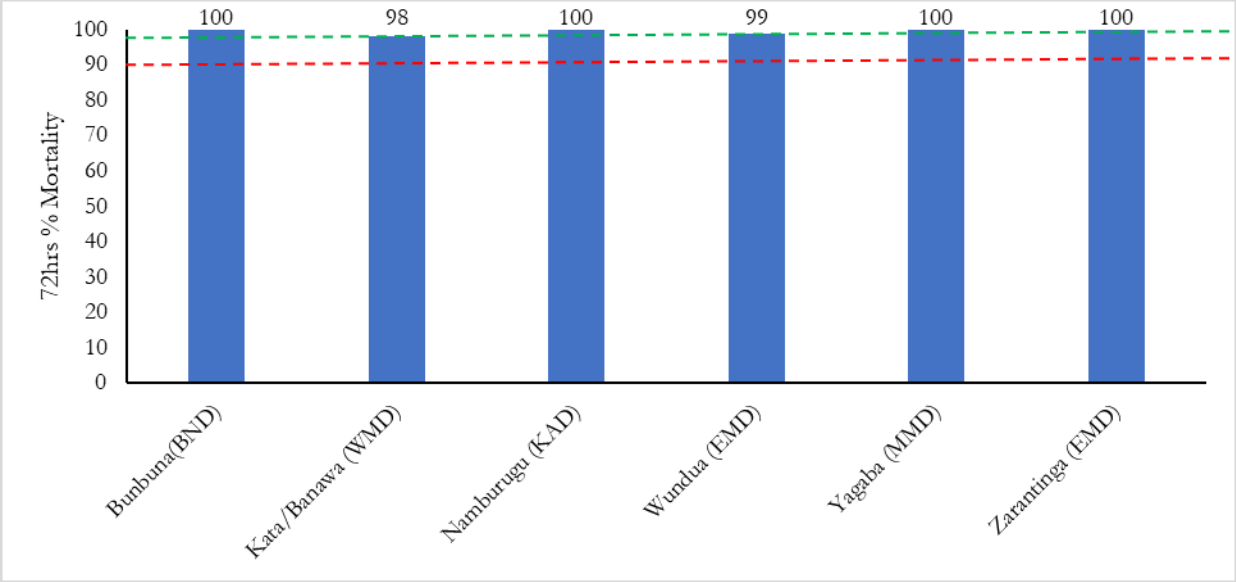
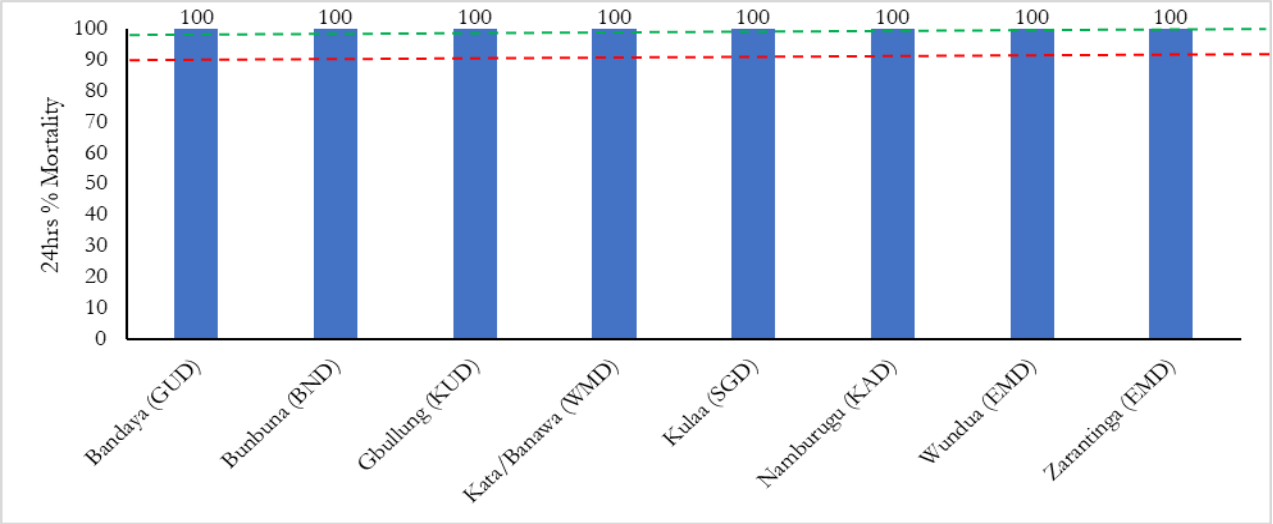


FIGURE 16: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L., CHLORFENAPYR (100 µG/BOTTLE), CDC BOTTLE ASSAY, SIX SITES



An. gambiae s.l. from all eight sites tested were fully susceptible to 4µg/bottle of clothianidin after the 24-hour holding period (Figure 17). The data suggest this insecticide is still effective in killing the vector and can continue to be used in IRS.

FIGURE 17: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L., CLOTHIANIDIN (4 µG/BOTTLE), CDC BOTTLE ASSAY, EIGHT SITES

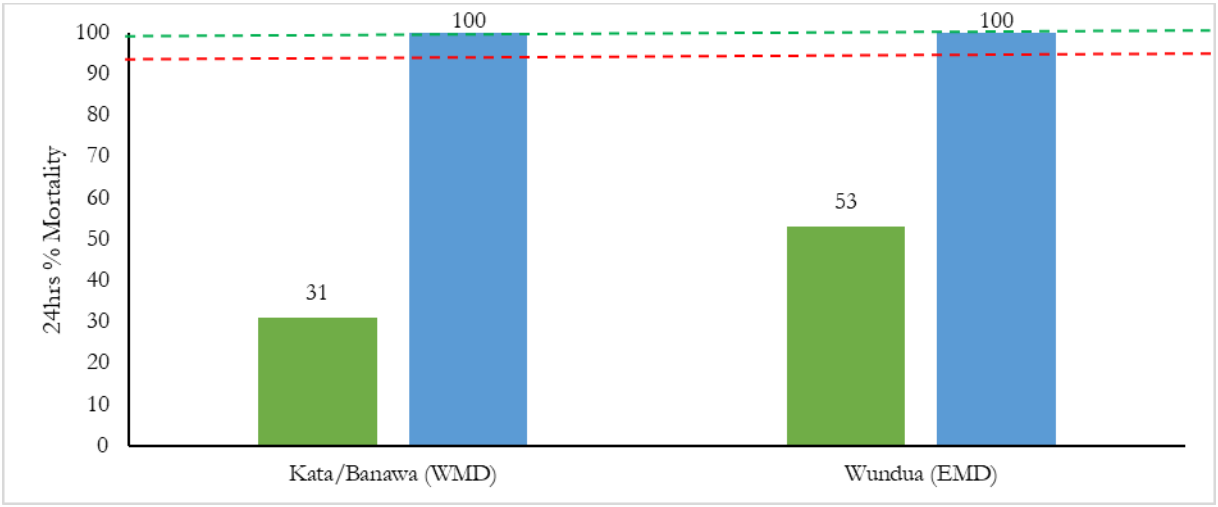


----------Susceptible ----------Resistant

3.10 SYNERGIST ASSAYS

An. gambiae s.l. from two 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 18). These results suggest products containing alpha-cypermethrin and PBO could be effective vector control tools in the area.

FIGURE 18: 24HR MORTALITY OF *AN. GAMBIAE* S.L. FROM TWO SITES POST EXPOSURE TO PYRETHROID (ALPHA-CYPERMETHRIN) AND PBO



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

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 0.90 to 0.99 both in the IRS intervention and control sites. There was a slight increase in the frequency of *Ace-1* resistant alleles in 2022 compared to 2021 when the frequency of *Ace-1* alleles ranged from 0.73 to 1.00 in the IRS intervention sites. Relatively high *Ace-1* frequency (0.99) was detected in Namburugu (KAD) (Table 6) where IRS was implemented with pirimiphos-methyl from 2017 to 2019. This result of high *Ace- 1* frequency was not expected given the phenotypic resistance to pirimiphos-methyl seems to get lower in 2022 compared to previous years. Though the phenotypic resistance seems to get lower, the very high frequency of *Ace- 1* indicates shifting to pirimiphos-methyl spray in any of the IRS districts may not be recommended at this stage. *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. There is a slight increase in the frequency of *kdr-w* resistant alleles in most of the IRS sites compared to 2021. The frequency of the resistant alleles ranged from 0.42 to 0.97 in the IRS intervention sites in 2022, compared to a range of 0.45 to 0.90 recorded in the IRS sites in 2021. About 33% of the mosquitoes analyzed were found to harbor both *kdr-w* and *kdr-e* gene mutations. The frequency of *kdr-e* resistance genotypes was high (0.68) in Kulaa (SGD), an unsprayed control site (Table 8). These data suggest that there is no indication of reduction in the *kdr* frequency and is also high in the control non-IRS sites, most likely due to the pyrethroid pressure from agriculture pesticides.

TABLE 6: DISTRIBUTION AND FREQUENCY OF *ACE-1* AND *KDR* ALLELES WITHIN *AN. GAMBIAE* S.L., IRS INTERVENTION AND CONTROL SITES, 2022

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 IRS sites															
Bandaya (GUD)	28	2	0	30	**0.97**	1	0	1	2	**0.50**	32	1	0	33	**0.98**
Gbullung (KUD)	28	26	12	66	**0.62**	15	0	22	37	**0.41**	53	13	0	66	**0.90**
Sanguli (TSD)	53	8	3	64	**0.89**	4	1	7	12	**0.38**	59	3	0	62	**0.98**
Wundua (EMD)	73	17	10	100	**0.82**	1	1	36	38	**0.04**	97	7	0	104	**0.97**
Fludora Fusion IRS sites															
Bunbuna (BND)	26	39	43	108	**0.42**	13	28	34	75	**0.36**	92	10	0	102	**0.95**
Kata/Banawa (WMD)	72	21	3	96	**0.86**	5	12	10	27	**0.41**	99	4	0	103	**0.98**
Kunkwa (MMD)	13	7	15	35	**0.47**	9	10	8	27	**0.52**	32	1	0	33	**0.98**
Yagaba (MMD)	25	20	26	71	**0.49**	8	18	17	43	**0.40**	58	7	0	65	**0.95**
Namburugu (KAD)	19		2	21	**0.90**	3	1	2	6	**0.58**	34	1	0	35	**0.99**
Withdrawn sites															
Dimabi (TD)	11	11	15	37	**0.45**	11	5	6	22	**0.61**	32	1	1	34	**0.96**
Woribogu (TD)	6	15	16	37	**0.36**	9	10	6	25	**0.56**	32	2	0	34	**0.97**
Control (Unsprayed) sites															
Kulaa (SGD)	29	19	19	67	**0.57**	22	10	8	40	**0.68**	56	4	0	60	**0.97**
Overall Total	**383**	**185**	**164**	**732**	**0.65**	**101**	**96**	**157**	**354**	**0.42**	**676**	**54**	**1**	**731**	**0.96**

3.12 BIOCHEMICAL ASSAYS

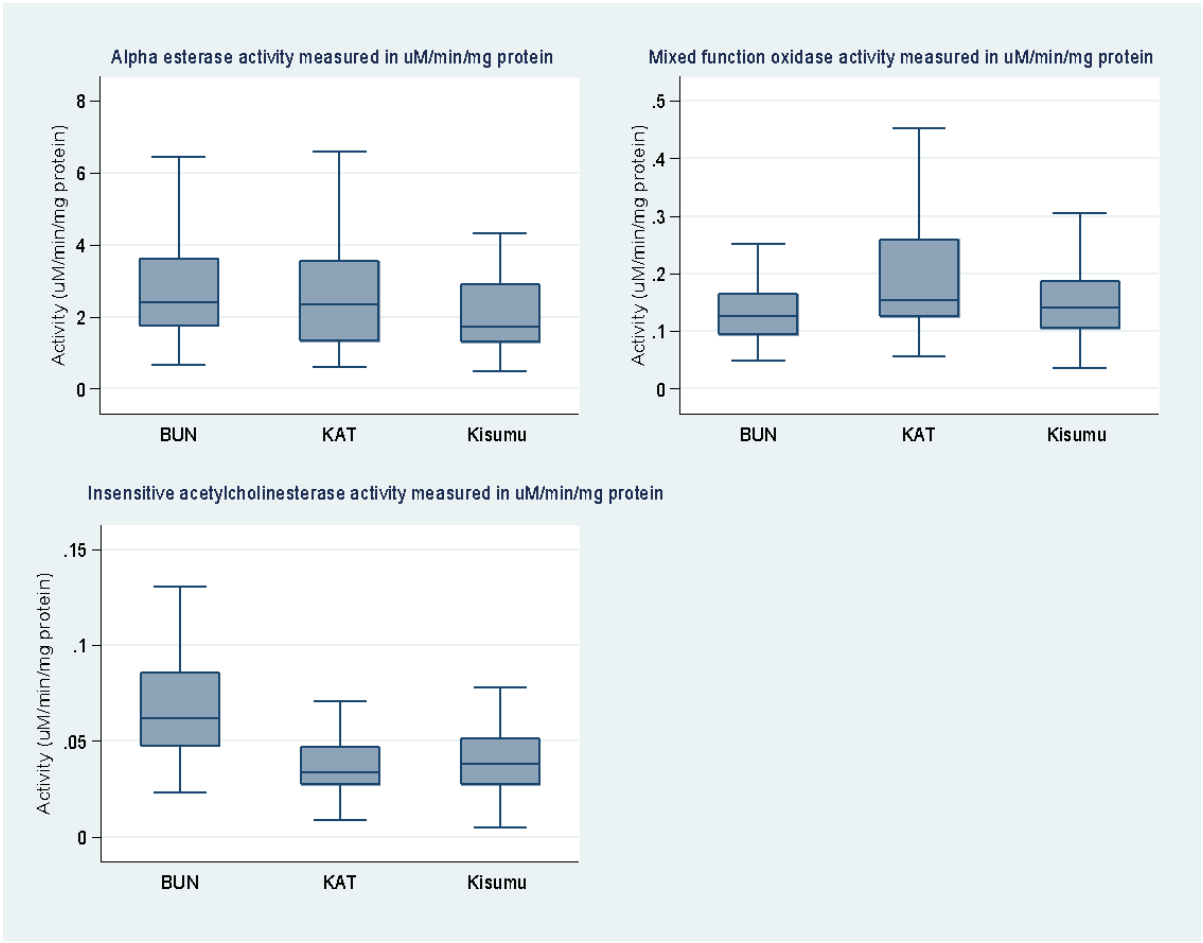
One hundred *An. gambiae* s.l. from Bunbuna (BND) and Kata/Banawa (WMD) and 60 *An. gambiae* s.s. Kisumu strain from the project's insectary were used in biochemical assay for three enzymes. Larval breeding areas in other sites were dry during the period of the test and therefore yielded no larvae. These 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 an important role in organochlorine and pyrethroid resistance in malaria vectors.

The data for all the enzymes did not pass the Shapiro Wilks test for normality and hence were analyzed with Wilcoxon Rank Sum and Mann Whitney test. No significant difference was observed for median alpha esterase activity among the three populations. This shows that alpha esterase enzymes involved in metabolism of carbamates and organophosphate was present in the three populations, though higher in the wild populations as compared to the reference strain (*An. gambiae* s.s Kisumu) but not significant The median mixed function oxidase activity recorded for the Kisumu is not statistically different from the activity in Bunbuna (BND) and Kata/Banawa (WMD) populations. The WMD population, however, recorded significantly higher activity of mixed function oxidases than the BND population (Table 7 and Figure 19). This shows that the activity of mixed function oxidase enzymes that metabolizes organochlorine and pyrethroid was higher in WMD than BND and Kisumu populations. Both WMD and Kisumu colonies recorded lower *Ace-1* activity compared to the BND population. However, there was no significant difference in the *Ace-1* activity in WMD and Kisumu populations. These data suggest that there is no straightforward correlation between activity of enzymes and phenotypic or target sites resistance of the vector in the sampling sites.

TABLE 7: OUTCOME OF WILCOXON RANK SUM (MANN WHITNEY) TEST

Enzyme	Colony	Mean	Median	Maximum	Minimum	p-Value
Alpha esterase	Bunbuna (BND)	2.98	2.39	12.78	0.65	0.1023
	Kata/Banawa (WMD)	2.89	2.33	10.4	0.59	0.3672
	Kisumu	2.25	1.71	9.30	0.49	
Mixed function oxidase	Bunbuna (BND)	0.15	0.13	0.58	0.05	0.2408
	Kata/Banawa (WMD)	0.21	0.15	0.72	0.06	0.5989
	Kisumu	0.19	0.14	1.72	0.04	
Insensitive acetylcholinesterase	Bunbuna (BND)	0.07	0.06	0.17	0.02	0.000
	Kata/Banawa (WMD)	0.04	0.03	0.08	0.01	0.6715
	Kisumu	0.04	0.04	0.09	0.005	

FIGURE 19: DISTRIBUTION OF ENZYME ACTIVITIES IN REFERENCE AND TEST POPULATIONS OF *AN. GAMBIAE* S.L., FROM BUNBUNA (BND) AND KATA/BANAWA (WMD)



4. Discussion, Conclusion, and Recommendations

The data from 2022 longitudinal entomological monitoring in northern Ghana indicate that *An. gambiae* is still the predominant vector and exists in sympatry with *An. coluzzii* and *An. arabiensis* in most sites. Data from 2016 to 2022 shows that *An. gambiae* s.l. consistently dominates the *Anopheles* mosquito population composition in the project sites (Table 8). However, among the complex, there seems to be an increase in the proportion of *An. arabiensis* over the years. The increase in the proportion of *An. arabiensis* does not seem to be related to IRS, as the proportion is often higher in the non-IRS sites than the IRS sites.

Table 8: Trends in *An. arabiensis* Composition Over the Years

Composition	2016	2017	2018	2019	2020	2021	2022
% *An. gambiae* s.l. (*An. gambiae* s.l./total *Anopheles)* All sites	98	96	88	93	95	96	91
% *An. arabiensis* (*An. arabiensis* / total *An. gambiae* s.l.) Range in sites	0	1.8	NA	2-11	4-35	0-29	0-25

While the HLC are conducted with doors open, the indoor resting collections are performed in houses that remained closed for the night. This could be a possible reason for the low numbers from indoor resting collections. The idea of performing HLC with closed doors was also previously discussed but was dropped to maintain comparability of the data over the years. However, this can also be discussed and piloted in the next workplan.

The 2022 HBRs were similar for the IRS and control sites, though historically the rates were higher in control sites. The reduction in HBR in the control sites could be attributed to the larviciding activity conducted and distribution of PBO nets targeting non-IRS districts only, including the control sites.

Indoor biting rates of *An. gambiae* s.l. were significantly higher than outdoor biting rates in all sites except Kata-Banawa (WMD) and Sanguli (TSD). 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. *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 some exophagy in the vector populations (Syme et al. 2021).

Per the Year 1 Evolve work plan, the Ghana team will look into the data from over the years to assess if there was any variation or shift in the composition of the species, including at the sibling species level, and if any of the variations or shift are also related to the vector control interventions.

Trends in Indoor and Outdoor HBR and Insecticides Used for IRS from 2015 to 2022 in Northern Ghana: Entomological monitoring has been conducted since the beginning of the PMI/IRS program in northern Ghana in 2008. CDC light traps were rarely used to collect mosquitoes as they were found not to be very efficient in the area and pyrethrum spray catch collections were also not productive. However, HLC data were consistently collected by a team that was well trained by the project and had team members who were more or less the same over the years. Given the relatively consistent data available from HLCs that were collected by a team of experienced collectors and supervised by field entomology technicians, we tried to create a simple descriptive table showing the trend in HBR over the years from 2015 to 2022. These HBR data are extracted from annual reports that have been reviewed and posted for public access over the years (Table 4).

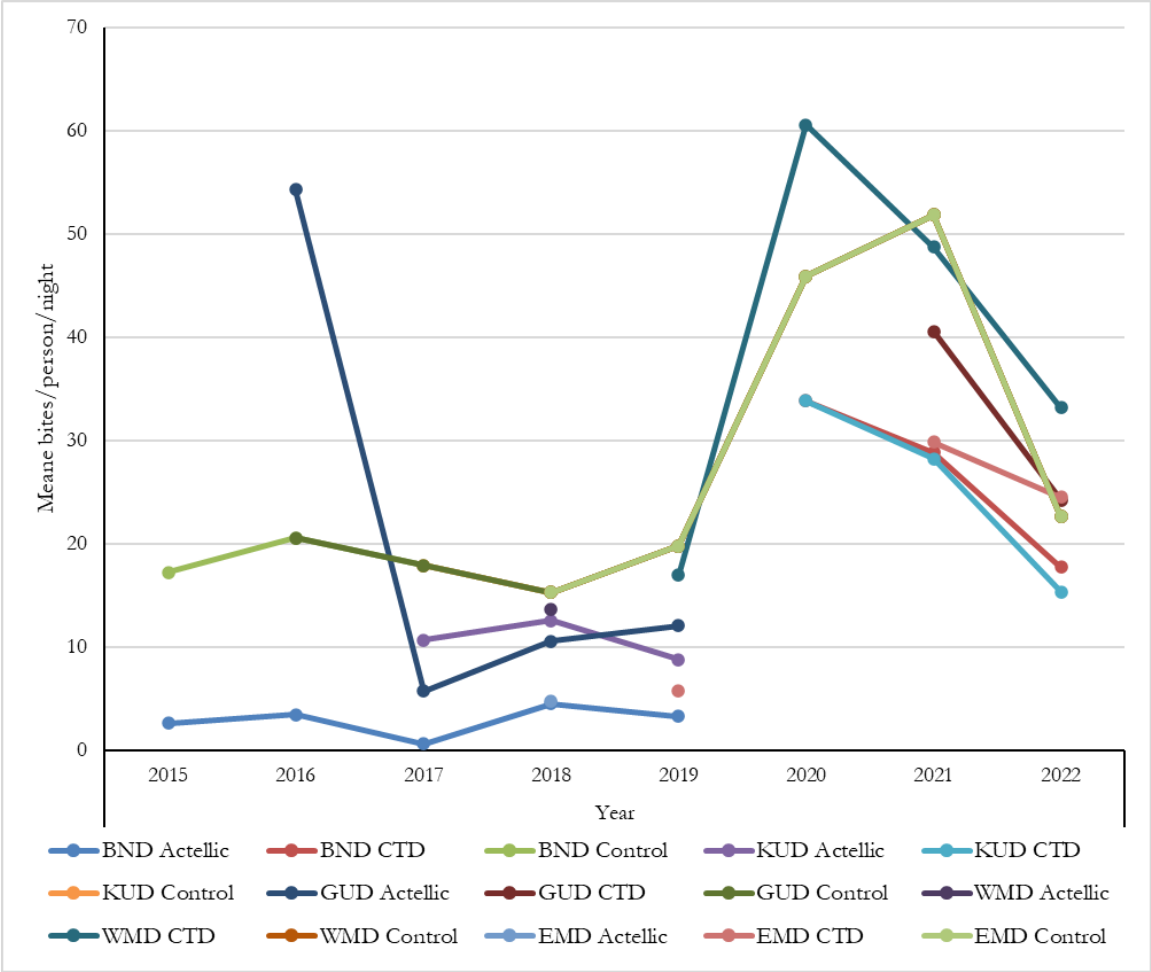
Our preliminary observation from Table A-4 shows:

- A sharp increase in HBR starting 2020 in most of the districts. For the IRS districts, it seems to coincide with the year that the districts shifted from Actellic spray to the spray of clothianidin-based insecticides.
- A similar increase can also be seen in the control district in 2020 and 2021, but the increase seems to be not as sharp as in the IRS districts.

This has raised the following questions:

- Could this be related to the efficacy of the clothianidin based insecticides?
  - Tests show that the vector is largely phenotypically susceptible to clothianidin. However, could the high kdr prevalence in the vector also confer resistance to clothianidin (Perrier et al. 2021)?
  - Could it then be that the current new test methods are not sensitive enough to detect phenotypic resistance to clothianidin?
- Could it be related to the slow-acting nature of the insecticides? Maybe the vector staying alive for a few days after exposure would allow egg laying and keeping the population at a high level. Could it then be that the impact of the clothianidin insecticides is more on reducing the longevity but not as much on HBR as the organophosphates? This was also demonstrated in the Ghana data that parity rate was lower in IRS sites compared to controls.
- Could this be related to changes in climatic factors (rainfall and humidity) as the increase in 2020 was also observed in the control district, though to a lesser extent when compared to IRS districts?
- Despite the consistent and relatively good-quality data, bias in data quality may not be fully excluded given that this was routine monitoring data and not systematic research.
- No definite conclusion can be made from this preliminary observation but:
  - Maybe it is worth looking into case data over these years?
  - Could it be worth looking into the experience from other countries also, given that there was a published concern from Uganda and unpublished concerns from the National Malaria Control Program of Malawi?

FIGURE 20: TRENDS IN INDOOR AND OUTDOOR HBR AND INSECTICIDES USED FOR IRS, 2015–2022



The control sites recorded lower EIRs than some of the IRS sites. This was also the case in some IRS sites in 2021 when some IRS sites started showing higher EIRs than the controls. One possible reason could be that the control sites are part of the sub-urban TML that is undergoing rapid urbanization, which may have affected biting and infectivity. The project may have to think of changing the control site to areas with similar characteristics to the IRS districts. The Ghana team collecting baseline data from two non-IRS districts during Year 1 Evolve and using them as controls starting Year 2 could be helpful in measuring the impact of IRS. The second reason for the low EIR in the control sites could be the ITN distribution campaign conducted in 2021 (for the first time with PBO nets) which may also have affected vector infection rate and thus the EIR.

Over 30% of *An. gambiae* s.l. collected in sleeping rooms had a blood meal from animals, while more than 30% collected from pit traps and over 20% collected from animal shelters had had a human blood meal. The mosquitoes collected by Prokopack in sleeping rooms that had animal blood meal may have bitten the animal outdoors before entering the house. This suggests 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, outdoors in pit traps, and in animal shelters were small.

Parity rates showed that a significant number of parous mosquitoes were collected in the sprayed sites than in the unsprayed sites. These data suggest that IRS had an impact in reducing mosquito longevity in intervention sites.

High outdoor sporozoite rates were observed in Kata/Banawa (WMD) compared to other IRS intervention sites. The monthly indoor and outdoor EIRs also revealed high outdoor transmission occurrs in WMD 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. But this should be based on data on human behavior and mosquito biting activities specific to these sites.

Overall, peak biting of An. gambiae vectors occurs after midnight even though there is a considerable amount of biting before midnight also.

An. gambiae s.l. remains resistant to the pyrethroids that were tested (deltamethrin and alpha-cypermethrin), possibly because of the selection pressure from several sources including agriculture. 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 six sites and was also susceptible to 4µg/bottle of clothianidin from all the 8 sites tested.

The data also show a return of susceptibility of *An. gambiae* s.l. populations to pirimiphos-methyl in most sites, whereas resistance was recorded in the previous years. However, there is still suspected pirimiphos-methyl resistance in 2 of the 12 sites tested. Moreover, the *Ace-1* gene mutation frequency is more than 90% in all sites. Though phenotypic resistance to pirimiphos seems to get lower, the very high frequency of *Ace- 1* mutation indicates, shifting to pirimiphos-methyl spray may not be recommended at this stage.

This data underscores the importance of implementing an insecticide rotation strategy in IRS campaigns. Considering that vectors in most sentinel sites currently remain susceptible to clothianidin, SumiShield 50WG and Fludora Fusion, they remain appropriate recommended insecticides for IRS campaigns in northern Ghana. However, continued close monitoring of resistance is necessary, nonetheless. Both insecticide formulations have demonstrated a residual efficacy that lasts beyond the malaria transmission season and can therefore remain in use for IRS.

The biochemical assays were not informative and of significant value and the test would be dropped in the subsequent work plans.

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ANNEX 2022 ENTOMOLOGICAL MONITORING RESULTS

TABLE A-1: SUMMARY OF WHO INSECTICIDE RESISTANCE TESTS OF *AN. GAMBIAE* S.L. TO SELECTED INSECTICIDES, 2022

Sentinel Site	Alpha-cypermethrin				Chlorfenapyr			Clothianidin		Deltamethrin		Pirimiphos-methyl	
	Total Tested	Mortality 24hrs	Insecticide + Synergist: Total Tested	Insecticide + Synergist Mortality 24hrs	Total Tested	Mortality 24hr	Mortality 72hr	Total Tested	Mortality 72hr	Total Tested	Mortality 24hrs	Total Tested	Mortality 24h
Bandaya								100	100			100	100
Bunbuna (BND)	100	62			100	79	100	100	100			100	100
Dimabi (TD)	100	49										100	100
Gbullung (KUD)	100	37						100	100			100	100
Kata/Banawa (WMD)	200	28.5	100	100	100	87	98	100	100	100	18	100	100
Kulaa (SGD)	100	37						100	100			100	100
Kunkwa (MMD)	100	51										100	100
Namburugu (KAD)_					100	94	100	100	100			100	100
Sanguli (TSD)	100	67										100	100
Woribogu (TD)	100	34										100	100
Wundua (EMD)	200	37	100	100	100	96	99	100	100	100	33	100	93
Yagaba (MMD)					100	98	100					100	97.1
Zarantinga (EMD)					100	93	100	100	100				

TABLE A-2: MONTHLY AND ANNUAL EIRS BY SITE (INDOOR)

Sites	Indicators	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	10 Months
GH-Bandaya Sentinel Site (IRS)	Sporozoite tested	0	0	40	80	108	79	188	69	0	0	
	Sporozoite Positive	0	0	0	0	4	0	0	0	0	0	
	SPR Pf (%)	0	0	0	0	3.7	0	0	0	0	0	
	Biting Rate	0	0	13.9	36.4	42.9	29	92.9	32.1	0.5	0.25	
	EIR (per person per night)	0	0	0	0	1.6	0	0	0			
	EIR (per person per month)	**0**	**0**	**0**	**0**	**49.6**	**0**	**0**	**0**	**0**	**0**	**49.6**
GH-Bunbuna (IRS)	Sporozoite tested	1	0	35	32	32	20	25	12	1	2	
	Sporozoite Positive	0	0	0	0	3	1	0	0	0	0	
	SPR Pf (%)	0	0	0	0	9.38	5	0	0	0	0	
	Biting Rate	0.38	0	42.8	21.4	30.4	19.3	65.8	4.5	0.25	1	
	EIR (per person per night)	0		0	0	2.8	0.96	0	0	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**86.8**	**29.76**	**0**	**0**	**0**	**0**	**116.56**
GH-Gbullung (IRS)	Sporozoite tested	1	2	10	34	41	103	204	23	5	2	
	Sporozoite Positive	0	0	0	1	1	2	1	0	0	0	
	SPR Pf (%)	0	0	0	2.94	2.44	1.94	0.49	0	0	0	
	Biting Rate	0.25	0.63	4	14.1	13.1	42.4	93.4	9.6	1.5	0.75	
	EIR (per person per night)	0	0	0	0.42	0.32	0.82	0.46	0	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**13.02**	**9.92**	**25.42**	**14.26**	**0**	**0**	**0**	**62.62**
GH-Kata/Banawa (IRS)	Sporozoite tested	4	2	25	33	40	25	25	28	21	24	
	Sporozoite Positive	0	0	0	0	2	0	0	0	0	0	
	SPR Pf (%)	0	0	0	0	5	0	0	0	0	0	
	Biting Rate	1.8	1.4	36.3	27.9	57.9	69	48.9	45	8	5.9	
	EIR (per person per night)	0	0	0	0	2.9	0	0	0	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**89.9**	**0**	**0**	**0**	**0**	**0**	**89.9**
GH-Kulaa (Control)	Sporozoite tested	0	3	20	81	37	87	152	91	6	2	
	Sporozoite Positive	0	0	0	0	0	0	0	1	0	0	
	SPR Pf (%)	0	0	0	0	0	0	0	1.1	0	0	
	Biting Rate	0	1.1	8.4	33.5	15.3	36.4	63.5	37.6	2.1	0.88	
	EIR (per person per night)	0	0	0	0	0	0	0	0.41	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12.71**	**0**	**0**	**12.71**
GH-Sanguli (IRS)	Sporozoite tested	0	0	1	15	22	8	75	26	1	0	
	Sporozoite Positive	0	0	0	0	0	1	3	2	0	0	
	SPR Pf (%)	0	0	0	0	0	12.5	4	7.69	0	0	
	Biting Rate	0	0	0.38	2.1	5.5	1.3	34.1	7.3	0.5	0	

Sites	Indicators	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	10 Months
	EIR (per person per night)	0	0	0	0	0	0.16	1.4	0.56	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**0**	**4.96**	**43.4**	**17.36**	**0**	**0**	**65.72**
GH-Tugu (Control)	Sporozoite tested	0	2	47	49	55	84	200	112	10	1	
	Sporozoite Positive	0	0	0	0	3	0	0	2	2	0	
	SPR Pf (%)	0	0	0	0	5.45	0	0	1.79	20	0	
	Biting Rate	0.25	1	19.1	20.1	23.3	35.1	80.6	46.3	2.6	0.5	
	EIR (per person per night)	0	0	0	0	1.3	0	0	0.83	0.53	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**40.3**	**0**	**0**	**25.73**	**16.43**	**0**	**82.46**
GH-Zarantinga (IRS)	Sporozoite tested	0	0	28	24	26	25	24	33	0	0	
	Sporozoite Positive	0	0	0	0	2	1	0	2	0	0	
	SPR Pf (%)	0	0	0	0	7.69	4	0	6.06	0	0	
	Biting Rate	0	0.13	8.8	11.4	25	74	127.4	16	0.25	0	
	EIR (per person per night)	0	0	0	0	1.9	3	0	0.97	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**58.9**	**93**	**0**	**30.07**	**0**	**0**	**181.97**

TABLE A-3: MONTHLY AND ANNUAL EIRS BY SITE (OUTDOOR)

Sites	Indicators	22-Mar	22-Apr	22-May	22-Jun	22-Jul	22-Aug	22-Sep	22-Oct	22-Nov	22-Dec	10 Months
GH-Bandaya Sentinel Site (IRS)	Sporozoite tested	0	0	12	71	98	58	196	63	4	0	
	Sporozoite Positive	0	0	0	0	1	0	0	1	0	0	
	SPR Pf (%)	0	0	0	0	1.02	0	0	1.59	0	0	
	Biting Rate	0	0	6	34.6	39.6	24.4	101	30	1.6	0.13	
	EIR (per person per night)	0	0	0	0	0.4	0	0	0.48	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**12.4**	**0**	**0**	**14.88**	**0**	**0**	**27.28**
GH-Bunbuna (IRS)	Sporozoite tested	0	0	15	19	18	30	25	9	1	8	
	Sporozoite Positive	0	0	0	0	3	0	0	0	0	0	
	SPR Pf (%)	0	0	0	0	16.67	0	0	0	0	0	
	Biting Rate	0	0	31	15.1	23.8	21	70.9	4	0.63	1.8	
	EIR (per person per night)	0	0	0	0	4	0	0	0	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**124**	**0**	**0**	**0**	**0**	**0**	**124**
GH-Gbullung (IRS)	Sporozoite tested	0	0	3	17	24	94	110	34	5	1	
	Sporozoite Positive	0	0	0	0	0	1	0	1	0	0	
	SPR Pf (%)	0	0	0	0	0	1.06	0	2.94	0	0	
	Biting Rate	0	0	1.6	7.3	8	38.9	54.8	14.3	1.8	0.38	
	EIR (per person per night)	0	0	0	0	0	0.41	0	0.42	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**0**	**12.71**	**0**	**13.02**	**0**	**0**	**25.73**
GH-Kata/Banawa (IRS)	Sporozoite tested	2	5	25	18	169	25	25	22	20	20	
	Sporozoite Positive	0	0	0	0	3	1	0	1	0	1	
	SPR Pf (%)	0	0	0	0	0.02	4	0	4.55	0	5	
	Biting Rate	0.75	1.6	50	24.6	64.4	73	79.5	50.5	12.1	5.9	
	EIR (per person per night)	0	0	0	0	1.14	2.9	0	2.3	0	0.29	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**35.44**	**89.9**	**0**	**71.3**	**0**	**8.99**	**205.63**
GH-Kulaa (Control)	Sporozoite tested	0	1	16	61	24	86	157	72	8	2	
	Sporozoite Positive	0	0	0	0	0	0	0	0	2	0	
	SPR Pf (%)	0	0	0	0	0	0	0	0	25	0	
	Biting Rate	0	0.25	6.4	25.8	9.9	35.4	64.3	30	2.6	0.88	
	EIR (per person per night)	0	0	0	0	0	0	0	0	0.66	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**20.46**	**0**	**20.46**
GH-Sanguli (IRS)	Sporozoite tested	0	0	2	6	19	12	78	18	0	0	
	Sporozoite Positive	0	0	0	0	0	0	2	0	0	0	
	SPR Pf (%)	0	0	0	0	0	0	2.56	0	0	0	
	Biting Rate	0	0	1.3	1.3	7.8	2.1	37.6	6.8	0.38	0.25	
	EIR (per person per night)	0	0	0	0	0	0	0.96	0	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**0**	**0**	**0**	**29.76**	**0**	**0**	**0**	**29.76**
GH-Tugu (Control)	Sporozoite tested	0	0	52.0	35.0	42.0	85.0	196.0	125.0	8.0	1.0	
	Sporozoite Positive	0	0	1.0	0	1.0	0	0	2.0	1.0	0	

Sites	Indicators	22-Mar	22-Apr	22-May	22-Jun	22-Jul	22-Aug	22-Sep	22-Oct	22-Nov	22-Dec	10 Months
	SPR Pf (%)	0	0	1.92		2.38			1.6	12.5	0	
	Biting Rate	0	0.25	21.1	15.0	17.5	34.4	80.6	52.0	2.0	0.38	
	EIR (per person per night)	0	0	0.41	0.0	0.42	0.0	0.0	0.83	0.25	0.0	
	EIR (per person per month)	**0**	**0**	**12.71**	**0**	**13.02**	**0**	**0**	**25.73**	**7.75**	**0**	**59.21**
GH-Tugu (Control)	Sporozoite tested	0	0	20.0	26.0	24.0	25.0	26.0	17.0	0	0	
	Sporozoite Positive	0	0	0	1.0	0	0	0	1.0	0	0	
	SPR Pf (%)	0	0	0	3.85	0	0	0	5.88	0	0	
	Biting Rate	0.0	0.13	5.9	9.3	22.5	56.6	114.6	17.9	0.38	0.0	
	EIR (per person per night)	0	0	0.0	0.36	0.0	0.0	0.0	1.1	0	0	
	EIR (per person per month)	**0**	**0**	**0**	**11.16**	**0**	**0**	**0**	**34.1**	**0**	**0**	**45.26**

Note for Figures A-1 to A-4: T0=Month of Spray (March), T1=First month after spray (April) T9=Ninth month after spray (December) T10=Tenth month after spray (January)

FIGURE A-1: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD 50WG REPRESENTED BY MORTALITY RATES OBSERVED IN EMD, KUD, AND TSD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, *AN. GAMBIAE* S.S. KISUMU STRAIN, MARCH 2022–JANUARY 2023

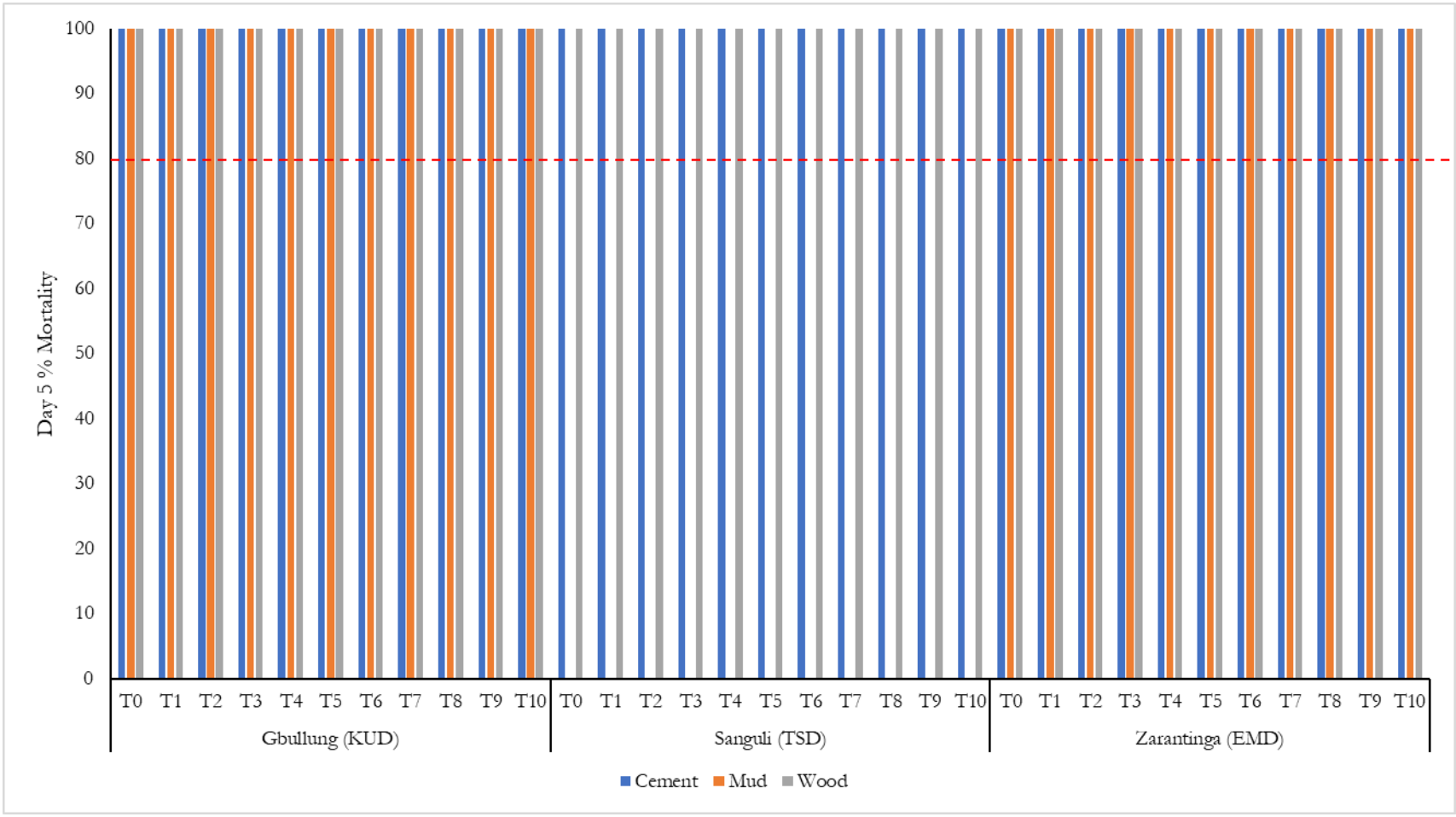

FIGURE A-2: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD 50WG REPRESENTED BY MORTALITY RATES OBSERVED IN EMD, KUD, AND TSD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, WILD *AN. GAMBIAE* S.L., MARCH 2022–JANUARY 2023

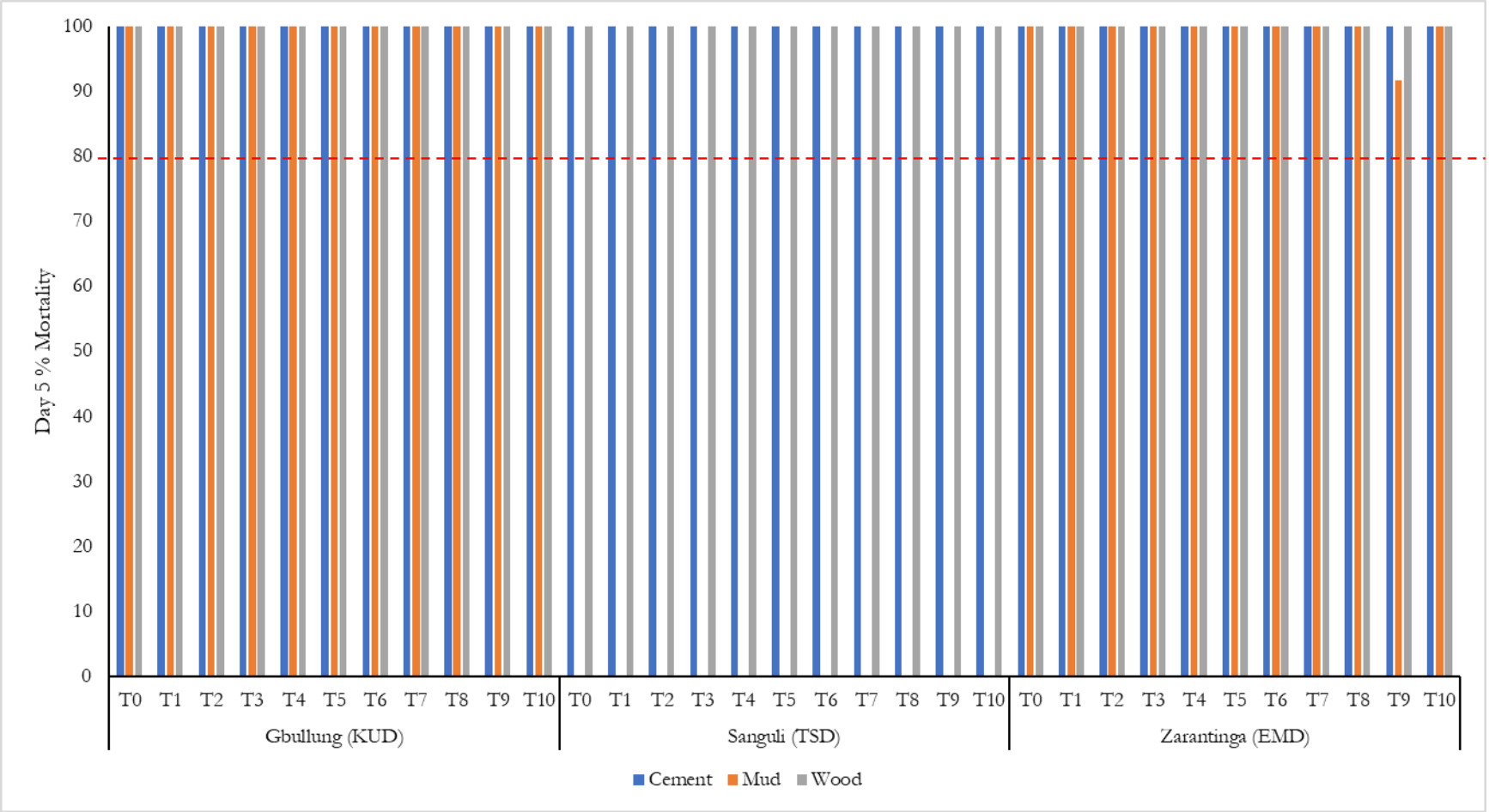

FIGURE A-3: SPRAY QUALITY AND RESIDUAL EFFICACY OF FLUDORA FUSION REPRESENTED BY MORTALITY RATES OBSERVED IN BND, MMD, AND WMD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, *AN. GAMBIAE* S.S. KISUMU STRAIN, MARCH 2022–JANUARY 2023

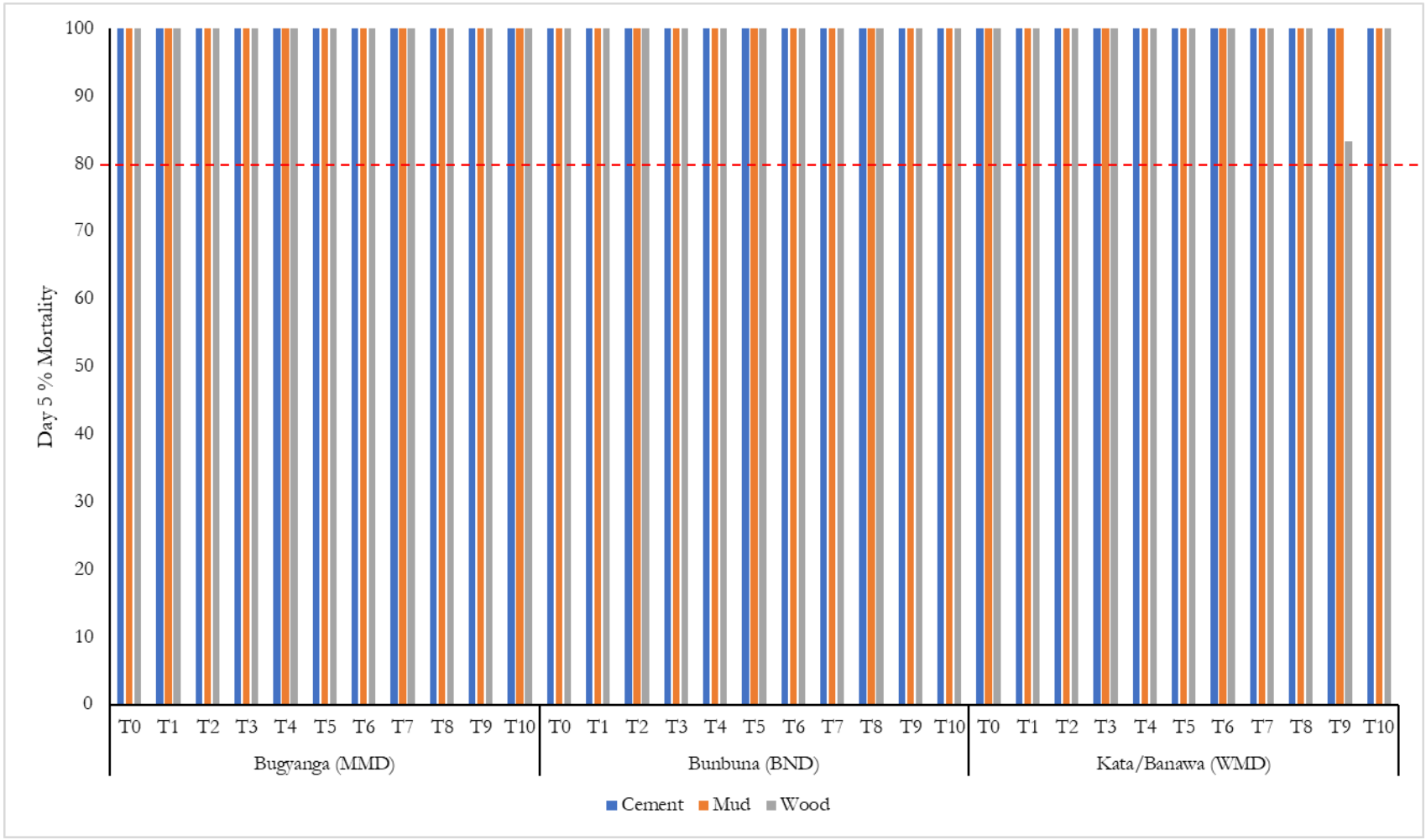

FIGURE A-4: SPRAY QUALITY AND RESIDUAL EFFICACY OF FLUDORA FUSION REPRESENTED BY MORTALITY RATES OBSERVED IN BND, MMD, AND WMD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, *AN. GAMBIAE* TIASSALÉ STRAIN, MARCH 2022–JANUARY 2023

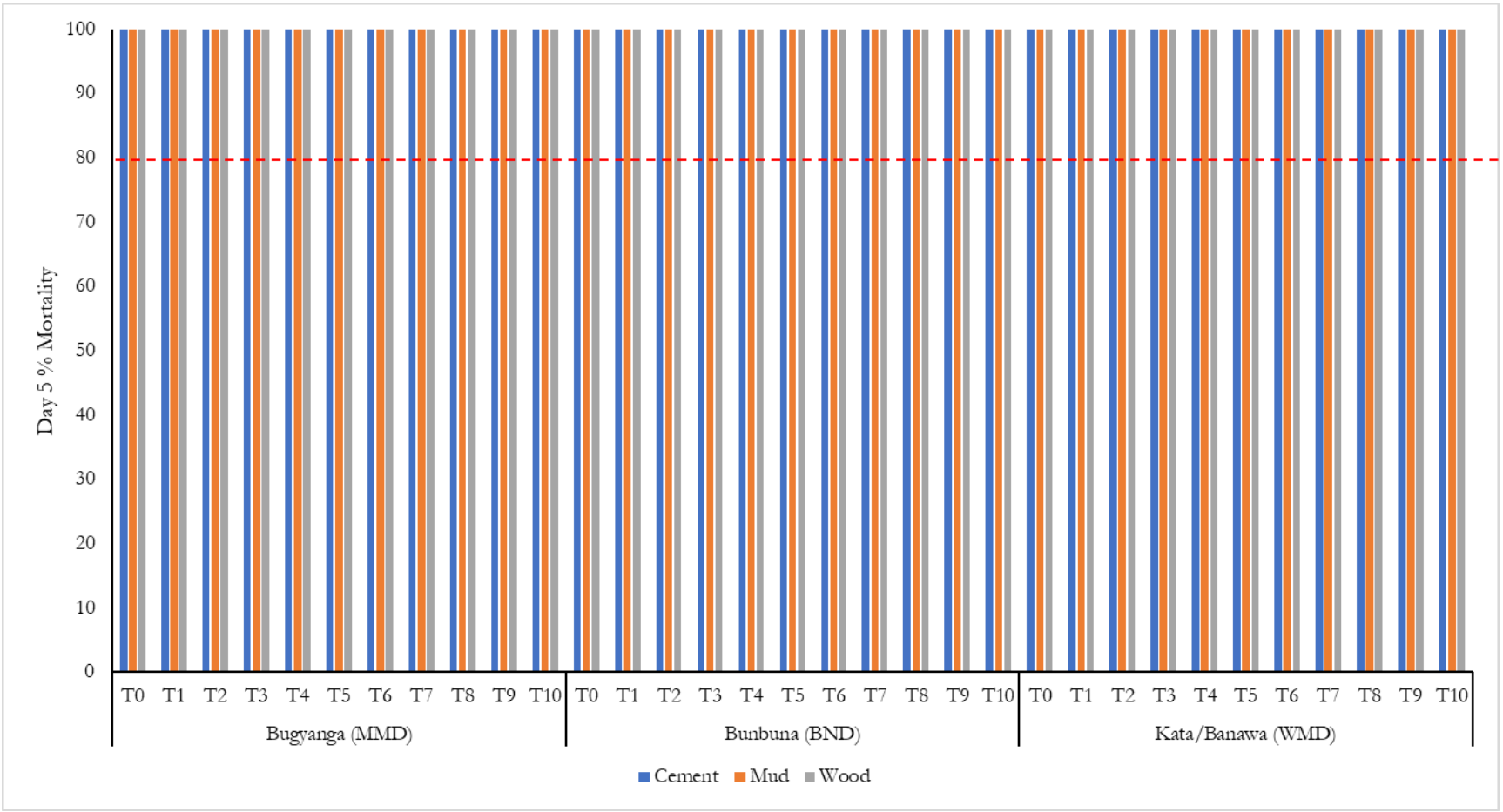

TABLE A-4 : TRENDS IN INDOOR AND OUTDOOR HBR AND INSECTICIDES USED FOR IRS, 2015–2022

Year	BYD		KUD		GUD		KAR		WMD		EMD		MMD		TSD		TML	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
2015	2.36	2.86	10.79	11.22													18.34	16.10
2016	3.3	3.56	6.57	7.55	55.8	52.8	31.8	32.3	*	*	*	*	*	*			21.7	19.47
2017	0.58	0.68	10.69	10.65	5.3	6.18	8.15	9.39	*	*	*	*	*	*			18.6	17.22
2018	4.31	4.7	11.74	13.32	9.34	11.79	15.62	16.92	11.87	15.48	4.54	5.03	*	*			16.65	13.91
2019	3.1	3.4	9.0	8.6	11.5	12.6	*	*	16.13	17.8	5.1	6.3	3.3	5.2			21.76	17.76
2020	35.89	31.81	28.63	39.08	*	*	*	*	59.39	61.78	*	*	17.9	25.4	30.39	22.34	45.55	46.23
2021	30.69	27.0	30.38	26.05	43.58	37.59	*	*	45.46	52.02	33.23	26.49	*	*	13.81	14.29	50.56	53.14
2022	18.6	16.8	18.0	12.7	24.8	23.7	*	*	30.2	36.2	26.3	22.7	*	*	5.1	5.7	22.9	22.3

Actellic	SumiShield	Fludora Fusion	IRS withdrawn but entomology data collected	Control (Never Sprayed	No IRS at the time

*Sprayed but were not entomology monitoring sites at the time