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PMI VECTORLINK GHANA ANNUAL ENTOMOLOGICAL MONITORING REPORT FOR NORTHERN GHANA

MARCH 1 – DECEMBER 31, 2020

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

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ACRONYMS

ABI	Animal Blood Index
Ace-1	Acetylcholinesterase 1
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/m	Infective Bites/Person/Month
I-RDT	Intensity-Rapid Diagnostic Test
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Net
KAD	Karaga District
kdr	Knockdown Resistance
KUD	Kumbungu District
MMD	Mamprugu Moaduri District
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
P	*Plasmodium*
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
RFLD	Restriction Fragment Length Polymorphism
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 2020 indoor residual spray (IRS) campaign in northern Ghana. The entomological monitoring included tests to assess spray quality and residual efficacy of sprayed insecticide products such as Actellic, SumiShield, and Fludora Fusion as well as insecticide susceptibility tests and monitoring of malaria vector bionomics. Data collected from animal shelters is also presented in this report. Insecticide susceptibility testing and monthly mosquito collections were carried out between March and December 2020 in eight sentinel sites in seven districts: five IRS districts (Bunkpurugu-Nakpanduri, Kumbungu, Mamprugu Moaduri, Tatale-Sanguli, and West Mamprusi) and two districts that have never been sprayed (Sagnerigu district and Tamale metropolis). Human landing catches, pyrethrum spray collections, CDC light traps, and Prokopack aspirators were used to collect mosquitoes. 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. WHO tube tests were used to determine vector susceptibility to insecticides while insecticide resistance intensity and synergist assays were carried out using the CDC bottle bioassay. The ELISA protocol described by Wirtz et al. (1987) and Beier et al. (1988), tested for the presence of *Plasmodium falciparum* circumsporozoite proteins to determine 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.

The project also supported the National Malaria Control Program of the Ghana Health Service to collect insecticide resistance data from 13 sentinel sites in 13 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.

RESULTS AND DISCUSSION

Vector Species Composition and Behavior. *An. gambiae* s.l. was the predominant species collected across all sites. It constituted 95.6% (27,174/28,435) and 94.4% (12,455/13,199) of the total number of *Anopheles* collected in the IRS intervention and unsprayed sites, respectively. *An. gambiae* was the major species composition. Of the 2,112 *An. gambiae* s.l. analyzed further by polymerase chain reaction (PCR), the majority were identified as *An. gambiae*, with smaller numbers of *An. coluzzii* and *An. arabiensis* that varied between the sites. Significantly higher outdoor feeding behavior was observed in both IRS and unsprayed sites. The mean indoor resting density of *An. gambiae* s.l. in sleeping rooms was 2 mosquitoes per room/day for the IRS sites and 1 mosquito per room/day for unsprayed sites.

Parity Rates: The mean proportion of parous *An. gambiae* s.l. in IRS districts (38%) was significantly lower than that recorded in the unsprayed sites (52%) ($p=0.024$).

Entomological Inoculation Rates (EIR): The estimated risk of malaria transmission for the eight months (sum of monthly EIR for eight months) was higher in the unsprayed sites than in the sprayed sites. A mean EIR of about 105 infective bites/person/year (ib/p/yr) was recorded for the unsprayed districts (Sagnerigu and Tamale Metropolis), compared to a mean of 53 ib/p/yr recorded for the IRS sites. The mean indoor EIR recorded for the IRS sites was 26 ib/p/yr compared to 28 ib/p/yr recorded outdoor. In the unsprayed sites mean indoor and outdoor EIR recorded were 47ib/p/yr and 57 ib/p/yr respectively.

Spray Quality and Residual Life of IRS Insecticides: High-quality and uniform spray was observed across all the sites tested. Monthly wall bioassays conducted on all sprayed surfaces (mud, cement, and wood surfaces) to assess the residual efficacy of the sprayed insecticide showed that Actellic 300CS remained efficacious in killing susceptible mosquitoes up to 11 months. The results with local wild vectors averaged 9 to 10 months residual efficacy. The residual effect of Fludora Fusion and SumiShield 50 WG lasted at least 11 months on all types of surfaces sprayed in tests with Kisumu.

Insecticide Susceptibility: *An. gambiae* s.l. mosquitoes from both IRS and non-IRS districts were susceptible to pirimiphos-methyl (98–100% mortality) except in Zaratinga (East Mamprusi District), where the vectors were resistant and in Yunyoo where possible resistance (97%) was detected. 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 all sites tested were susceptible to both clothianidin (seven days post exposure) and chlorfenapyr (three days post exposure).

Spraying of animal shelters: The application of insecticide in animal shelters in 2020 led to about 63% lower the density of *An. gambiae* s.l. resting in sprayed shelters compared with unsprayed shelters.

CONCLUSION

Parity rates and EIRs were significantly low across most IRS sites in comparison with the unsprayed sites. The new IRS insecticide products used offered the desired protection from malaria for the IRS sites beyond the normal duration of the malaria transmission season.

I. INTRODUCTION

In 2020, 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 historically conducts IRS once annually, to coincide with the rainy season. The campaign is planned so that the spraying of houses is completed before the mosquito population peaks, which precedes the peak of the malaria transmission season.

Three insecticide products were sprayed in 2020: SumiShield 50WG (clothianidin at a rate of 300 mg/m^2), Fludora Fusion (clothianidin at a rate 200 mg/m^2 and deltamethrin at 25 mg/m^2), and Actellic 300CS (pirimiphos methyl CS formulation at 1g/m^2). The selection of insecticides was based on results of insecticide susceptibility and residual efficacy tests from the previous year (2019), and in accordance with the national insecticide resistance management strategy. Results from the 2019 insecticide susceptibility tests 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. This necessitated a switch to a different class of IRS insecticide to prevent further development of resistance to pirimiphos-methyl. Spraying of Actellic 300CS was restricted to MMD, while BND, KAD, and YND switched from Actellic 300CS (sprayed in 2019) to SumiShield 50WG, and EMD, GUD, KUD switched to Fludora Fusion. WMD continued to spray SumiShield 50WG in 2020. IRS operations in TSD, where IRS was first implemented in 2020, also were conducted with Fludora Fusion.

In 2020, the project began spraying animal shelters in five districts (BND, EMD, KUD, YND and MMD (Yizesi subdistrict only)) 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 in selected IRS sites has on malaria transmission indicators, the project compared data collected from the sites where animal shelters were sprayed with data from unsprayed sites and IRS sites where animal shelters were not sprayed.

To assess the impact of IRS on entomological indices of malaria transmission, VectorLink Ghana carried out routine entomological surveys in eight sites across seven districts (both sprayed and unsprayed) in Northern and North East regions of Ghana from March through December 2020, except for April–May due to COVID-19 restrictions.

Specific objectives of the 2020 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 and entomological inoculation rates) in sprayed and unsprayed sites
4. Monitoring the impact of spraying animal shelters on entomologocal indicators 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; and
6. Assessing the quality of the IRS operations across all nine districts and evaluating the residual efficacy of Actellic 300CS, Fludora Fusion and SumiShield 50WG.

The project also provided technical and financial support to the National Malaria Control Program of the Ghana Health Service to collect insecticide resistance data from 13 sentinel sites in 13 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 and Global Fund sponsored sites.

The VectorLink Ghana entomology team worked closely with the Ghana Health Service and District Assemblies to implement all planned field activities, and it partnered with AngloGold Ashanti Malaria Control Ltd (AGAMal) to support advanced molecular analyses of collected samples. This report presents findings and analyses of the entomological monitoring activities the project carried out in 2020.

2. METHODOLOGY

2.1 SENTINEL SITES

VectorLink Ghana conducted monitoring in eight sentinel sites located in five IRS districts (BND, KUD, MMD, WMD, and TSD), and in two districts that have never been sprayed (Tamale Metropolis (TML) and Sagnerigu District (SGD)), shown in Figure 1. Table 1 summarizes the spray history of each district from 2008 through 2020.

FIGURE 1: 2020 MAP OF PMI VECTORLINK GHANA DISTRICTS AND ENTOMOLOGICAL MONITORING SITES

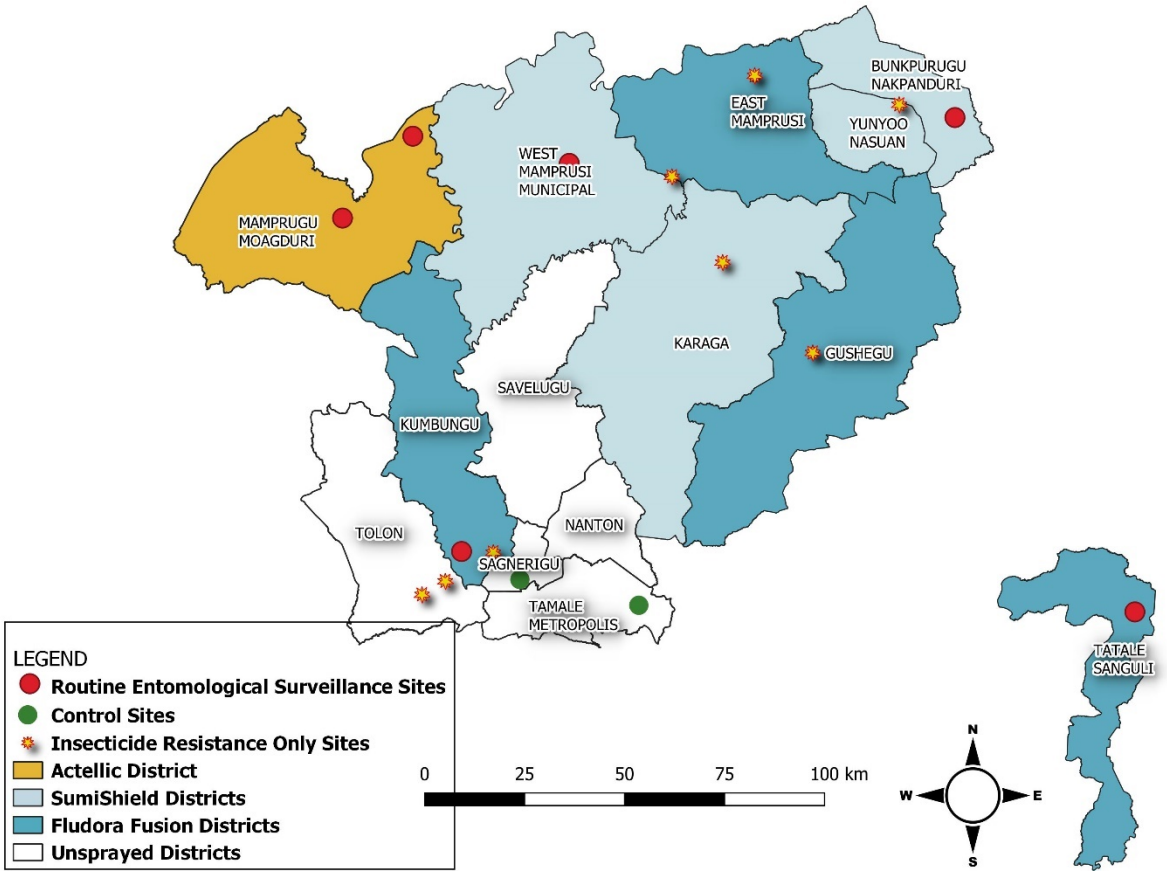


TABLE 1: ENTOMOLOGICAL MONITORING SITES

District	Sentinel Site	Insecticide Spray History												
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Routine Entomological Surveillance Sites														
BND	Bunbuna	NSp	NSp	NSp	ACy	ACy	PM	PM	PM	PM	PM	PM	PM	CLD
KUD	Gbullung and Cheyohi*	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM	PM	PM	PM	CLD+DM
MMD	Yagaba, Kunkwa and Bugyanga*	ACy	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	CLD	CLD	PM
TSD	Sanguli and Njobilbo*	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	CLD+DM
WMD	Kata-Banawa and Wulugu*	ACy	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD
SGD†	Kulaa	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
Only Insecticide Resistance-Monitoring Sites														
EMD	Wundua and Zaratinga	NSp	ACy	DM	ACy	PM	PM	PM	PM	PM	PM	PM	CLD	CLD+DM
GUD	Banda-ya	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM	PM	PM	CLD+DM
KAD	Namburugu	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	PM	PM	PM	CLD
KUD	Kumbungu	ACy	ACy	DM	ACy	ACy	NSp	NSp	PM	PM	PM	PM	PM	CLD+DM
TD≠	Dimabi and Woribugu	ACy	ACy	DM	ACy	ACy	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp
YND	Binkura	NSp	NSp	NSp	ACy	ACy	PM	PM	PM	PM	PM	PM	PM	CLD+DM

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* 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 for doors and windows. 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 2020 IRS campaign commenced on March 24, 2020, 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. Four houses (two with cement walls and two with mud walls, which is the predominant surface type) were purposefully selected in one selected community per district to represent structures sprayed by different spray operators and spray teams. Bioassays were also conducted on sprayed wood surfaces (from windows and doors) in sprayed rooms. 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 June through December 2020. Bioassays in the first two months post spray (April and May) were not conducted due to COVID restrictions. The assays measured the residual bioefficacy of Actellic 300CS, Fludora Fusion, and SumiShield 50WG in Bugyanga and Yagaba (MMD) (sprayed with Actellic), Bunbuna (BND) and Wulugu (WMD) (sprayed with SumiShield), and Cheyohi (KUD) and Njobilbo (TSD) (sprayed with Fludora Fusion). Six houses (three with cement walls and three with mud walls, except in Njobilbo, where no mud surfaces were tested) were purposefully selected in each community to represent structures sprayed by different spray operators and spray teams.

2.3 Adult Mosquito Collections

The project entomology team collected mosquitoes from all eight sentinel sites (Figure 1) for four days in each month per site, consecutively for 8 months (March, June through December 2020). Four mosquito collection methods were used, including human landing catches (HLCs), pyrethrum spray collections (PSCs), Prokopack aspiration, and CDC light traps (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)
PSC	6:00 am to 8:00 am	4 days per site per month	4 rooms per site per day
Prokopack	6:00 am to 9:00 am	4 days per site per month	1 animal shelter/house/site per day.
CDC light trap	6:00 pm to 5:50 am	4 nights per site per month	2 houses/site/night (1 trap per house).

The collected mosquitoes were analyzed based on species composition, resting density and preference, peak biting time, location, biting rate, *Plasmodium falciparum* sporozoite infection rates, parity rates, 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 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 either mass distribution campaigns and/or routine health facility- and school-based distributions.

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, 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. Mosquitoes were introduced in batches of 20–25 into each replicate (4 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 Resistance Intensity Assays

Using a simplified version of the CDC bottle bioassay resistance intensity test (Brogdon and Chan 2010), the team determined the intensity of deltamethrin resistance in *An. gambiae* s.l. from selected sentinel sites in BND, KUD, MMD, TD, and WMD. Four pre-measured vials provided by the U.S. Centers for Disease Control and Prevention, Atlanta, containing deltamethrin at concentrations of 1x, 2x, 5x, and 10x, were diluted in acetone and applied to 250ml bottles. Four replicates of 500µl of acetone were added to each insecticide vial and washed off into a 50ml graduated falcon tube. The falcon tube was topped up to the 50ml mark. The prepared insecticide solutions were stored in a refrigerator at 4°C until use. The control bottle was prepared by adding 1ml of acetone into a 250ml Wheaton bottle and coated as described by Brogdon and Chan (2010). Four test bottles were then coated with 1ml of different concentrations of the prepared deltamethrin solutions to get one bottle each of 1x, 2x, 5x, and 10x insecticide concentration. Between 20 and 25 mosquitoes were introduced into each of the four replicates. A control bottle (coated with acetone only) was run alongside the tests. The knockdown rate was recorded at 15-minute intervals until all mosquitoes were dead in each bottle.

Intensity assays (SOP06/01) were also conducted using the WHO tube tests for pirimiphos methyl in one site with 5x (1.25%) insecticide impregnated paper, because mortality was less than 90% with assays at 1x (0.25%) concentration.

2.6 Synergist Assays

Synergist assays were conducted using alpha-cypermethrin and deltamethrin with piperonyl butoxide (PBO) on mosquitoes from selected sentinel sites according to the project's SOP for CDC bottle assay (SOP04/01) and 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 diagnostic dose of deltamethrin and PBO.

2.7 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 AGAMal laboratory performed ELISA and molecular analyses of entomological samples to:

1. Determine blood meal source of all blood fed mosquitoes collected
2. Identify members of the *An. gambiae* s.l. complex to species
3. Determine the frequency of knockdown resistance (*kdr)* and acetylcholinesterase (*Ace -1)* genotypes

2.7.1 *P. falciparum* Sporozoite Rates

The heads and thoraxes of about 30% (averaged 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 parasite infection rate in the local vectors collected.

2.7.2 Host Blood Meal Identification

All blood-fed mosquitoes collected by PSCs and 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.

2.7.3 Species Identification

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

2.7.4 *ACE-1* AND *KDR* GENOTYPING

Samples of a live and dead mosquitoes (20–25 mosquitoes) from the insecticide susceptibility tests were further analyzed to determine presence of the *Ace-1* gene mutation using the protocol described by Wilkins et al. (2006) in the local *An. gambiae* s.l. vectors. The samples were also analyzed to determine the presence of the 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*.

2.8 INDICATORS AND DATA ANALYSIS

The following indicators were estimated for *An. gambiae* s.l. and *An. funestus* group, where samples collected were sufficient to allow for analysis:

- **Human Biting Rate (HBR):** The number of mosquito bites people in the area receive per unit 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 for IRS vs non-IRS were calculated as:

$$\frac{\text{Number of mosquitoes species collected resting indoors from PSC or animal structures from Prokopack per site per period of collection}}{\text{Total number of rooms or animal structures surveyed per site per period of collection}}$$

- **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 sites 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 time 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:

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

- **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).

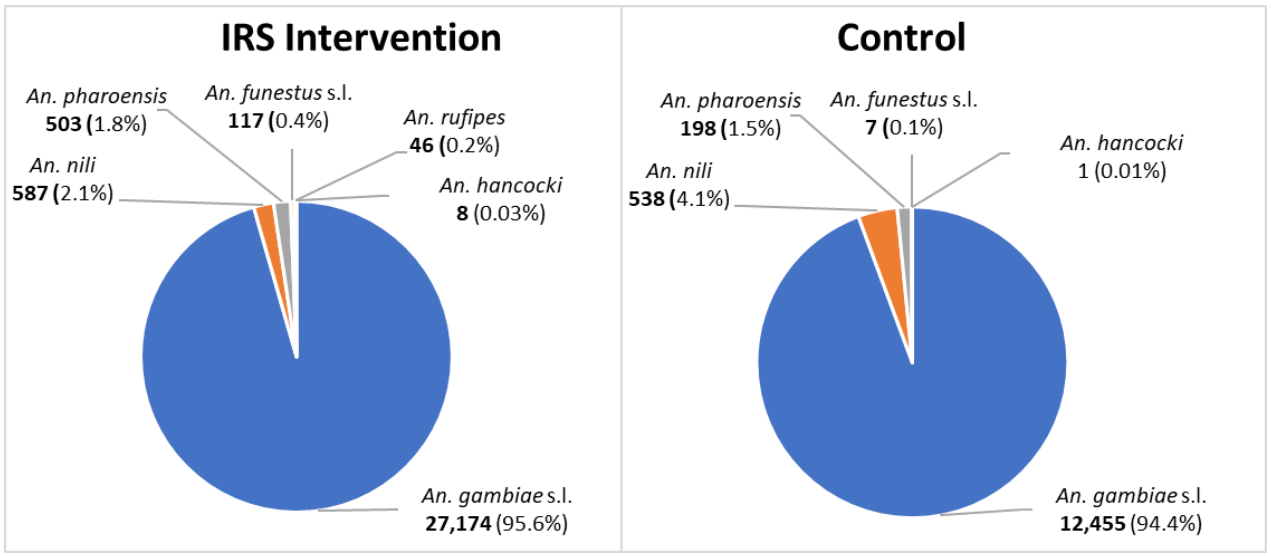
Differences in parity and sporozoite rates between the IRS versus control unsprayed sites were also compared through a z-test for differences in proportions. All tests were performed at 0.05 significance level, using Microsoft Excel® and STATA.

3. RESULTS

3.1 VECTOR SPECIES COMPOSITION

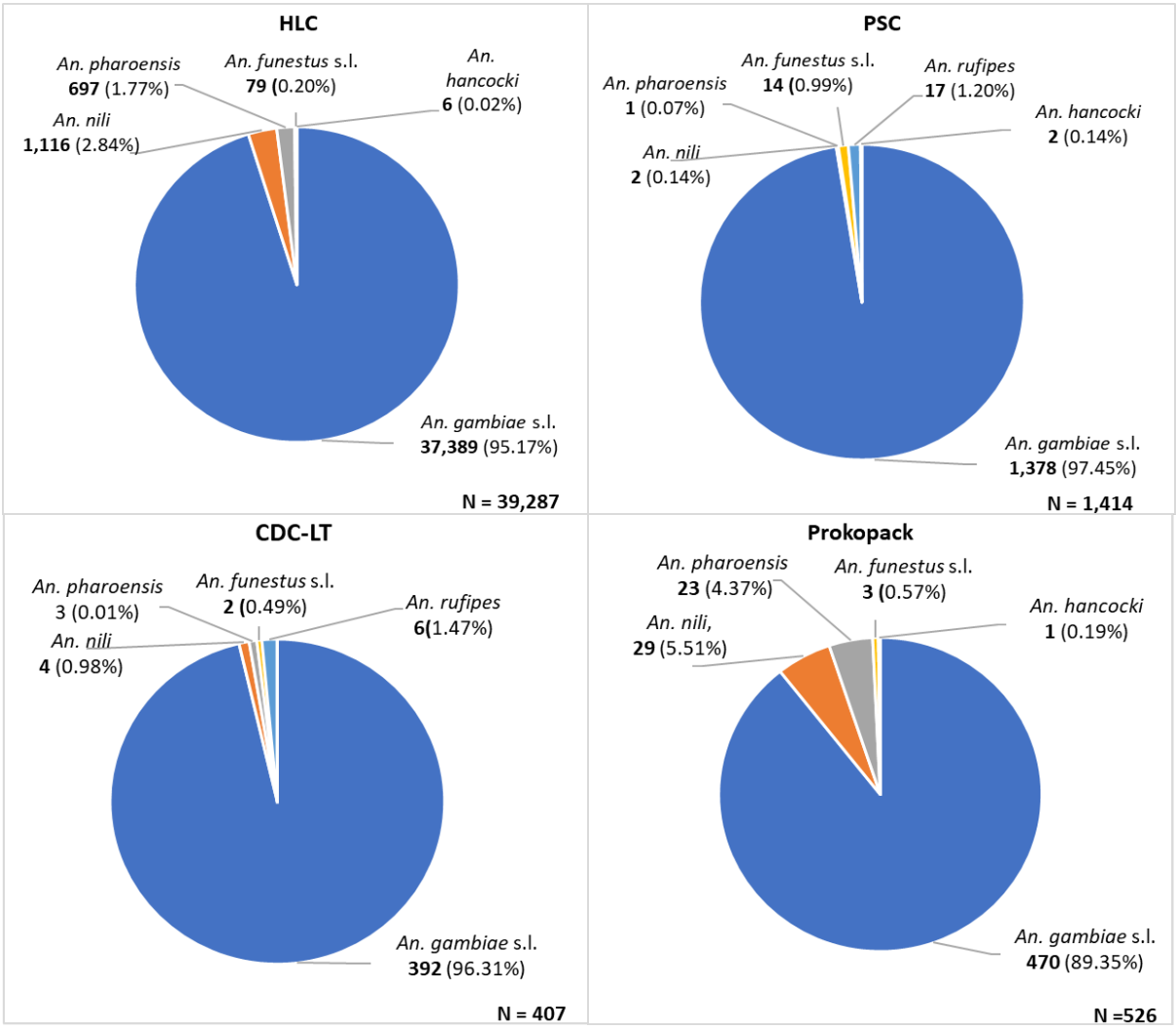
An. gambiae s.l. was the predominant species collected across all sites, constituting 95.6% (27,174/28,435) and 94.4% (12,455/13,199) of all *Anopheles* collected in the IRS intervention and control sites, respectively (Figure 2). *An. nili* was the second most predominant species in most sites, constituting about 2.1% in intervention and 4.1% 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, PSC, PROKOPACK, AND CDC LIGHT TRAP METHODS, IRS INTERVENTION AND CONTROL DISTRICTS



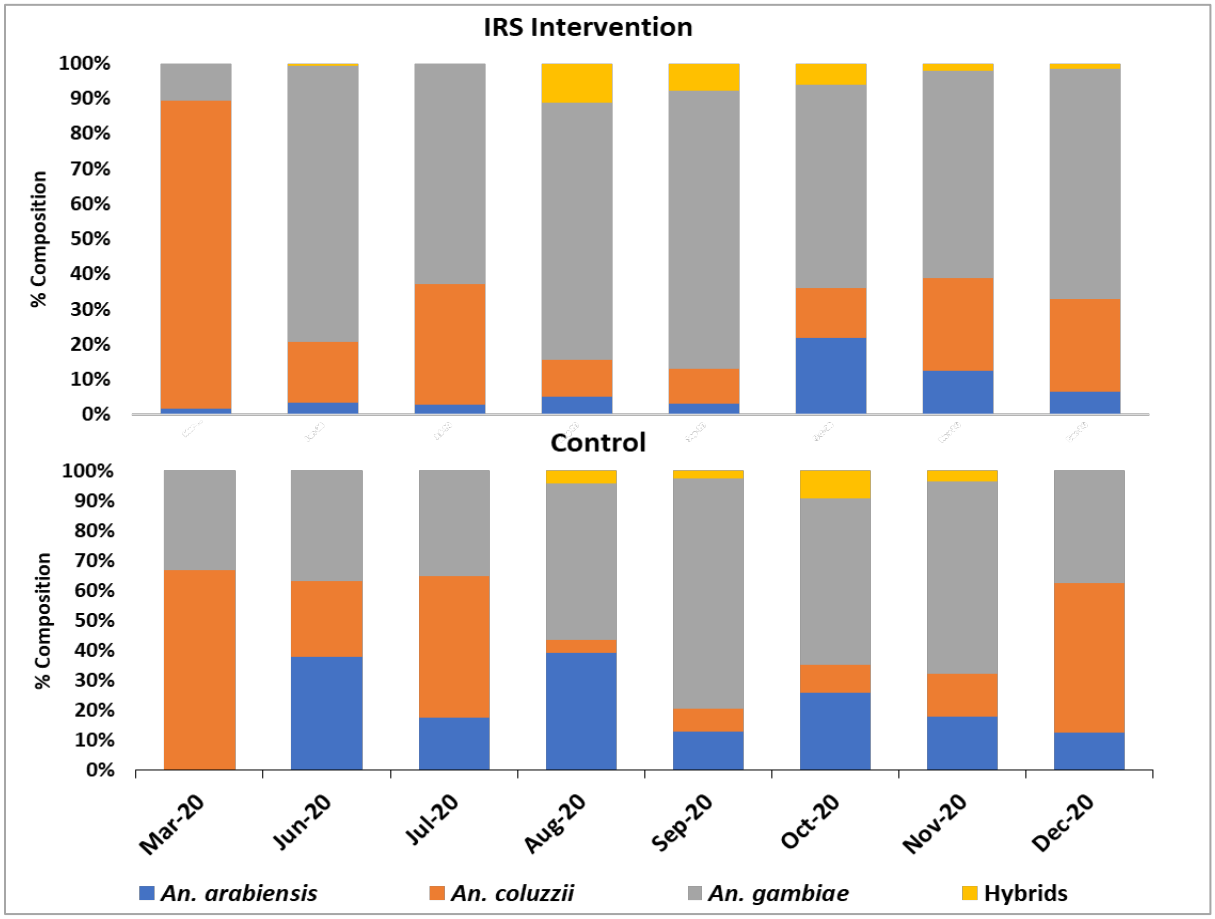
Of the total adult female *Anopheles* mosquitoes collected, 94.0% (39,287/41,634) were collected attempting to bite (i.e., by HLC), 3.4% (1,414/41,634) were collected resting indoors (PSC), 1% and 1.3% were collected from the CDC light trap and by Prokopack respectively (Figure 3). The *Anopheles* species collected were predominantly *An. gambiae* s.l., which made up 95%, 98%, 96%, and 89% of the HLC, PSC, CDC light trap, and Prokopack collections, respectively.

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



Molecular identification of 2,112 *An. gambiae* s.l. revealed three sibling species: *An. gambiae* (60%), *An. coluzzii* (26%), and *An. arabiensis* (11%). *An. gambiae* was the majority across all sites (Figure 4), ranging from 93% in TSD to 50% in TML. Compared to 2019, there is a slight decline in the proportion of *An. gambiae* which ranged between 76% and 99% in 2019. In contrast the proportion of *An. coluzzii* has increased from a range of 1%-16% recorded in 2019 to 16%- 35% in 2020. Similarly, the proportion of *An. arabiensis* increased from a range of 2%-11% in 2019 to 4% -35% in 2020. Hybrids of *An. coluzzii* and *An. gambiae* (3%) were also identified, in both IRS intervention and control sites.

FIGURE 4: SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. ALL SENTINEL SITES, MARCH–DECEMBER 2020



3.2 HUMAN BITING RATES

The mean monthly HBR of *An. gambiae* s.l. in the control sites (47 bites per person per night (b/p/n)) was significantly higher than the mean HBR (33 b/p/n) recorded for the IRS sites (p=0.012). Variations were observed between indoor and outdoor HBRs for *An. gambiae* s.l. in both the IRS intervention and control sites. The mean indoor HBR for *An. gambiae* s.l. from the IRS sites was 32 b/p/n whereas the mean outdoor HBR was 34 b/p/n. In the control sites a mean indoor HBR of 48 b/p/n and compared to mean outdoor HBR of 46 b/p/n (Table 3). *An. gambiae* s.l. from two IRS sites, KUD and MMD, showed a slight preference for exophagy. In contrast, *An. gambiae* s.l. from TSD and BND (sprayed) as well as SGD (unsprayed) showed endophagic tendencies. *An. gambiae* s.l. from TML and WMD showed an almost equal preference for feeding indoors and outdoors.

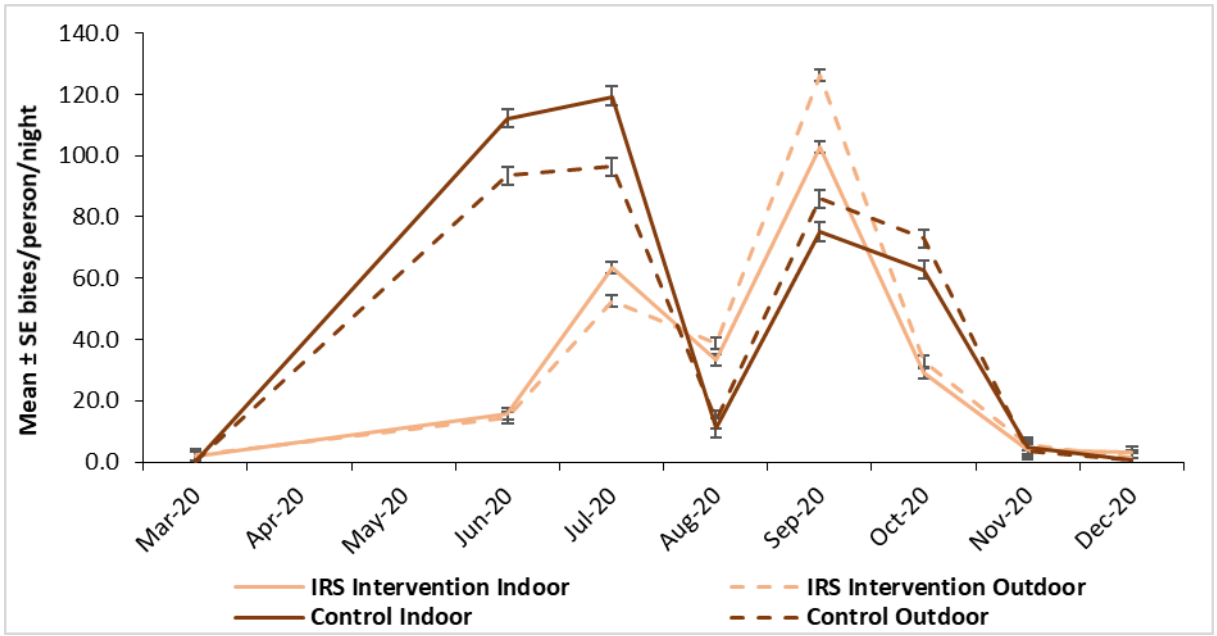
TABLE 3: MEAN INDOOR AND OUTDOOR HBR OF *AN. GAMBIAE* S.L., AS DETERMINED FROM HLC, ALL SENTINEL SITES, MARCH–DECEMBER 2020

Sentinel Site	Indoor Biting Rate (b/p/n)	Outdoor Biting Rate (b/p/n)	Endophagic Index	Exophagic Index	χ^2	P-value
IRS_Intervention						
Fludora Fusion districts						
KUD	28.63	39.08	0.42	0.58	103.29	<0.001*
TSD	30.39	22.34	0.58	0.42	78.59	<0.001*
SumiShield districts						
WMD	59.39	61.78	0.49	0.51	3.02	0.082
BND	35.89	31.81	0.53	0.47	15.72	0.001*
Actellic CS districts						
MMD	17.9	25.4	0.41	0.59	167.82	<0.001*
Control						
SGD	51.86	45.55	0.53	0.47	26.18	<0.001*
TML	44.55	46.23	0.49	0.51	2.01	0.157
Overall						
IRS Intervention	31.7	34.3	0.48	0.52	40.32	<0.001*
Control	48.2	45.9	0.51	0.49	7.27	0.007*

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

An. gambiae s.l. population densities, as measured by mean monthly HBRs, peaked in September in the IRS intervention sites whereas the HBR for *An. gambiae* s.l. from the control sites peaked in June and July 2020 (Figure 5).

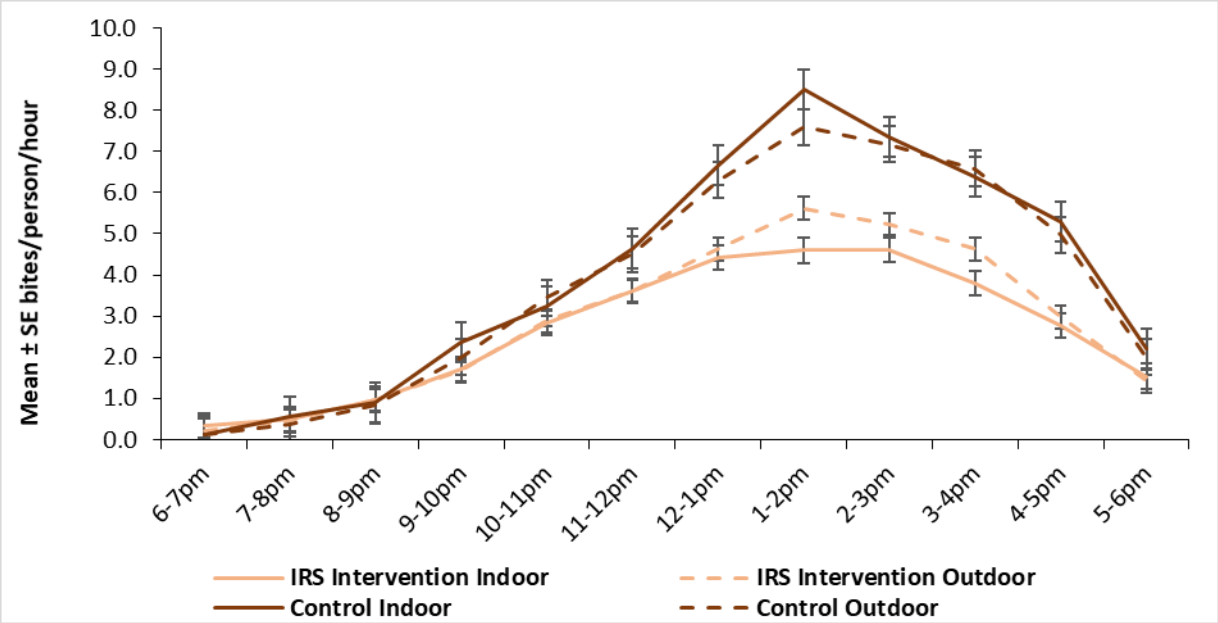
FIGURE 5: MEAN DAILY INDOOR AND OUTDOOR HBR, *AN. GAMBIAE* S.L., SPRAYED AND UNSPRAYED SITES, MARCH–DECEMBER 2020



Note: No mosquito collections in April and May 2020, due to COVID restrictions

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, however the number of mosquitoes biting during these peak times was higher in the control sites than in the IRS intervention sites (Figure 6).

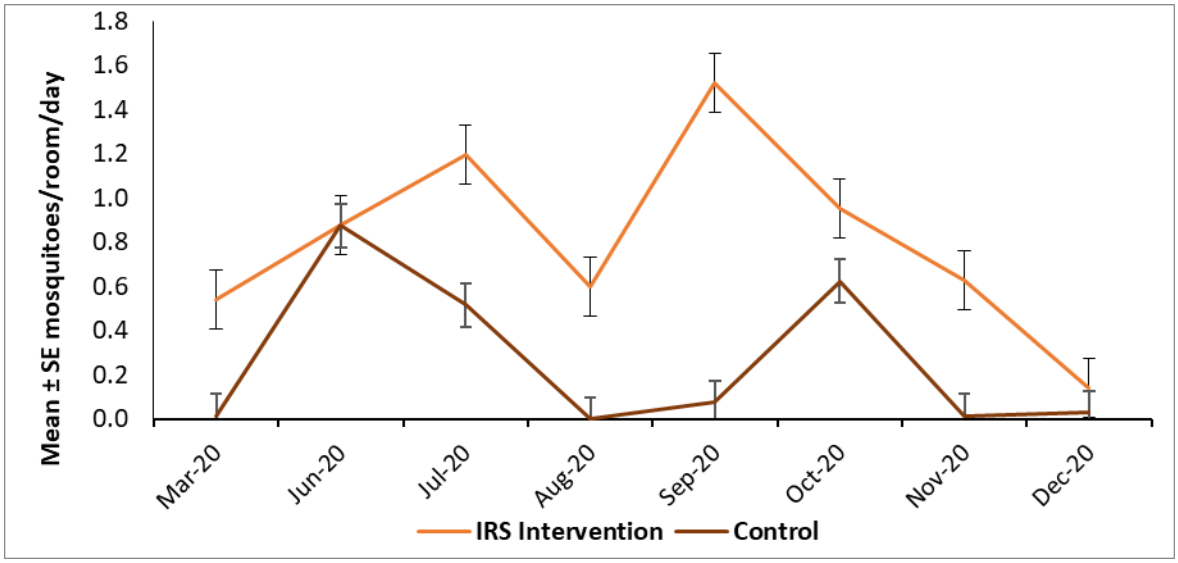
FIGURE 6: INDOOR AND OUTDOOR HOURLY BITING ACTIVITY, *AN. GAMBIAE* S.L., SPRAYED AND UNSPRAYED SITES, MARCH–DECEMBER 2020



3.3 RESTING BEHAVIOR

Overall, the mean indoor resting density of *An. gambiae* s.l. in sleeping rooms was 2.0 mosquitoes per room/day for the IRS sites and 1.0 mosquito 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 2020



3.3.1 ANIMAL SHELTERS

Based on 2019 *An. gambaie* s.l. feeding and resting behaviour data collected from select sites, VectorLink Ghana piloted spraying of animal shelters in select districts to assess the entomological impact of spraying animal shelters in 2020 with the aim of improving IRS efficacy. In the selected districts, all eligible animal shelters in the villages of the entire district were sprayed. Results of the Prokopack aspirations showed approximately 63% lower *An. gambiae* s.l. resting densities in sprayed shelters compared with unsprayed shelters in the control sites (Table 4).

TABLE 4: MEAN RESTING DENSITY OF *AN. GAMBIAE* S.L. IN SPRAYED AND UNSPRAYED ANIMAL SHELTERS, ALL SENTINEL SITES, MARCH–DECEMBER 2020

Sentinel Sites, and Insecticide Sprayed	Number Collected	Mean Resting Density	Unfed	Blood Fed	Half Gravid	Gravid
IRS Intervention						
Fludora Fusion districts						
Gbullung (KUD)†	36	0.50	1.43	2.43	1.00	0.57
Sanguli (TSD)	25	0.20	0.43	3.00	0.00	0.14
SumiShield districts						
Bunbuna (BND)†	9	0.15	0.71	1.14	0.43	0.29
Kata-Banawa (WMD)	36	0.93	5.29	3.57	1.00	0.00
Actellic 300CS districts						
Yagaba (MMD)	19	0.29	1.00	0.86	1.14	0.14
Kunkwa (MMD)†	58	1.62	7.71	3.57	0.57	1.14
Control						
Kulaa (SGD)	203	3.36	7.00	16.43	3.29	1.57
Tugu (TML)	59	0.74	2.14	3.86	2.00	0.43
Overall						
IRS Sprayed Animal Shelters	103	0.76	3.28	2.38	0.67	0.67
IRS Unsprayed Animal Shelters	80	0.47	2.24	2.48	0.71	0.09
Control Unsprayed Animal Shelter	262	2.05	4.57	10.14	2.64	1.00

† *Animal shelters in Bunbuna (BND), Gbullung (KUD), and Kunkua (MMD) were sprayed* Note: No mosquito collections in April and May 2020, due to COVID restrictions

3.4 BLOOD MEAL SOURCE

The overall HBI for *An. gambiae* s.l. collected from sleeping rooms in the IRS intervention sites was 87%, compared with 85% in the control sites (Table 5). The ABI for *An. gambiae* s.l. was slightly lower in the IRS intervention sites (13%) than in the control sites (15%). The animal blood meal sources included cattle, goat, chicken, pig, and dog blood.

The HBI recorded from Prokopack aspirations in the animal shelters in IRS intervention (55%) and control sites (27%) was lower than the HBI recorded from PSCs in sleeping rooms for the intervention (87%) and control (85%). Nevertheless, a majority of the mosquitoes collected from the animal shelters in the IRS intervention sites had fed on human blood (55%) compared to 27% from the unsprayed sites. The proportion of mosquitoes collected from animal shelters that had fed on cattle was 36% in the sprayed sites and 45% in the unsprayed sites.

TABLE 5: *AN. GAMBIAE* S.L. COLLECTED BY PSC AND THEIR SOURCE OF BLOOD MEAL, MARCH–DECEMBER 2020

Treatment	No Analyzed	Human	Cattle	Goat	Chicken	Pig	Dog
PSC (Sleeping Rooms)							
IRS intervention	169	87%	4%	4%	0%	1%	4%
Control	364	85%	7%	2%	1%	2%	3%
Prokopack (Animal Shelters)							
IRS intervention	48	55%	36%	0%	0%	9%	0%
Control	106	27%	45%	8%	1%	14%	5%

3.5 PARITY RATES

Dissections of *An. gambiae* s.l. mosquitoes collected by HLC between March and December 2020 revealed that the proportion of parous females collected from the unsprayed sites in SGD and TML (52%) was significantly higher than the proportion (38%) collected from the IRS districts ($(F_{(1,43)}=5.49$, $p=0.024$) (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					
Fludora Fusion districts					
KUD	430	176	40.9%	36.3%	45.6%
TSD	333	139	41.7%	36.4%	47.0%
SumiShield districts					
WMD	571	212	37.1%	33.2%	41.1%
BND	819	297	36.3%	33.0%	39.6%
Actellic 300CS districts					
MMD	775	297	38.3%	34.9%	41.7%
Control					
SGD	553	293	53%	48.8%	57.1%
TML	493	254	52%	47.1%	55.9%

3.6 *P. FALCIPARUM* SPOROZOITE RATES

A total of 7,565 *An. gambiae* s.l. (about 22%) collected by HLC were assayed by ELISA to determine the presence of *P. falciparum* sporozoites. The mean *An. gambiae* s.l. sporozoite rate (0.39%) in the IRS intervention sites (0.41%) was comparable to that in the control districts (0.44%) (Table 7). The sporozoite rate recorded for TSD, which started receiving IRS in 2020, was significantly higher than the rate recorded for all other sites. IRS intervention districts that have received at least three rounds of IRS recorded a lower mean sporozoite rate (0.26%) than did the control sites (0.44%).

TABLE 7: *P. FALCIPARUM* SPOROZOITE INFECTIONS IN *AN. GAMBIAE* S.L. SAMPLED FROM ALL SENTINEL SITES, MARCH TO DECEMBER 2020

Study Site	Number Examined by ELISA	Mosquitoes Positive for *P. falciparum* Sporozoites					Sporozoite Rate
IRS Intervention		Number	Species of Infected mosquitoes				
			An. gambiae	*An. coluzzii*	Hybrid	An. arabiensis	
BND	933	**3**	3	0	0	0	**0.32%**
KUD	557	**2**	1	0	1	0	**0.36%**
MMD	1,064	**3**	2	0	0	1	**0.28%**
TSD	856	**6**	5	0	1	0	**0.70%**
WMD	1,198	**4**	2	1	0	1	**0.33%**
Control Sites							
SGD	1,497	**6**	4	0	2	0	**0.40%**
TML	1,460	**7**	4	1	2	0	**0.48%**
IRS Intervention	4608	**19**	14	1	2	2	**0.41%**
Control Sites	2957	**13**	8	1	4	0	**0.44%**

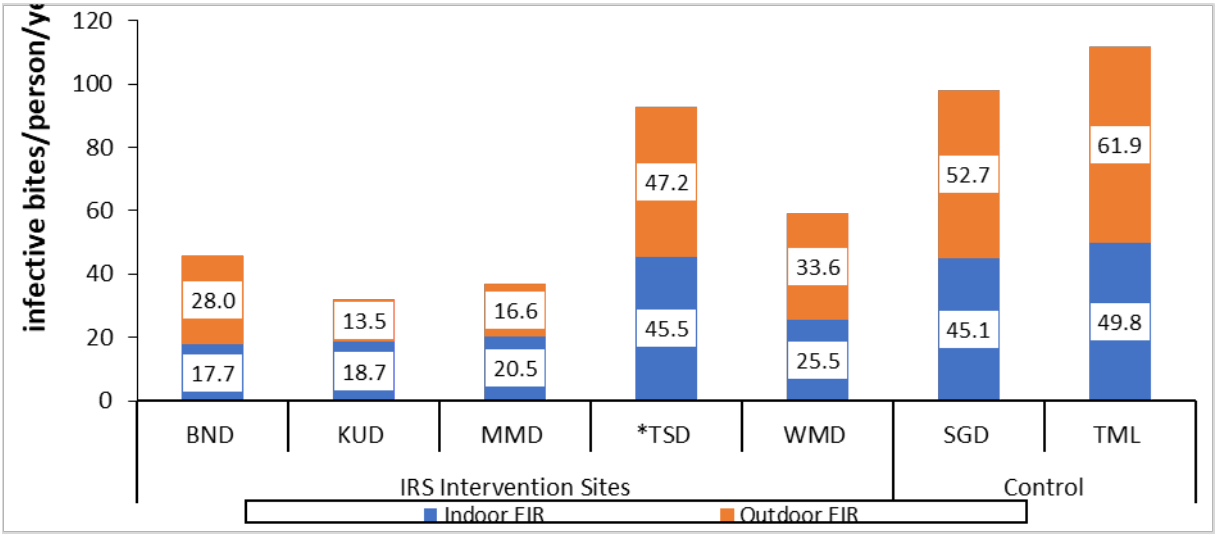
Note: No mosquito collections in April and May 2020, due to COVID restrictions

3.7 ESTIMATION OF EIRS

The annual EIR was estimated from the sum of monthly EIRs between March and December. The sum of monthly EIRs calculated was highest in the two control districts, SGD and TML, which recorded 97.8 infective bites/person/year (ib/b/yr) and 111.7 ib/b/yr, respectively. TSD, which was sprayed for the first time in 2020, also recorded a very high EIR of 92.7 ib/b/yr. The lowest EIR was recorded in KUD (32.2 ib/p/yr). (See Table A-1 in the Annex.)

A comparison of the sum of monthly indoor and outdoor EIRs reveals higher outdoor transmission occurring in TSD, SGD, and TML (Figure 8 and Table A-1), in contrast to what was noted in 2019, when most transmission occurred indoors. Similarly, higher outdoor transmission was recorded in the IRS sites in BND and WMD.

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

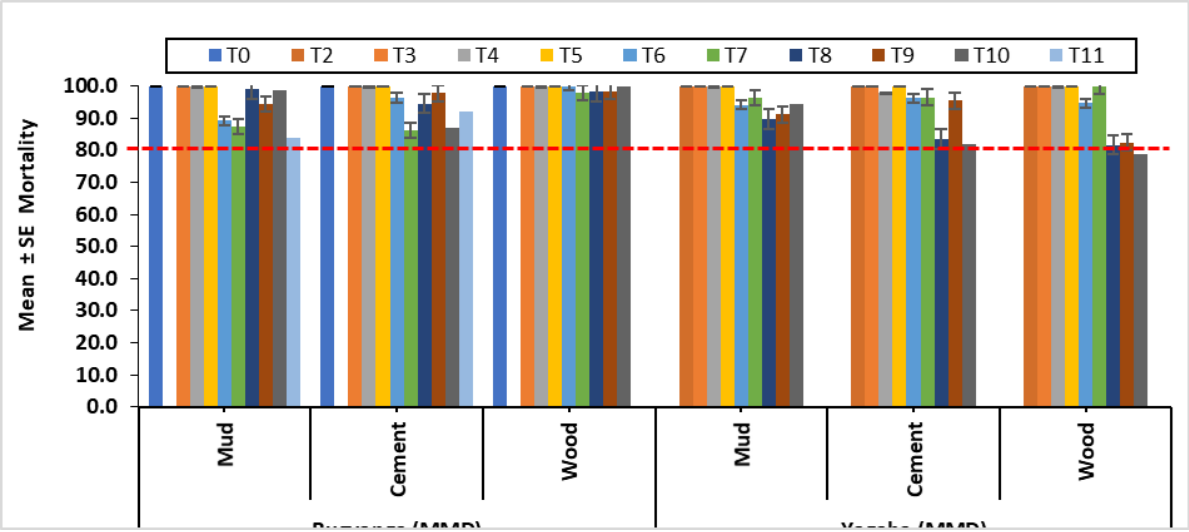


**TSD has received only one round of IRS (March 2020)*

3.8 Spray Quality and Residual Efficacy

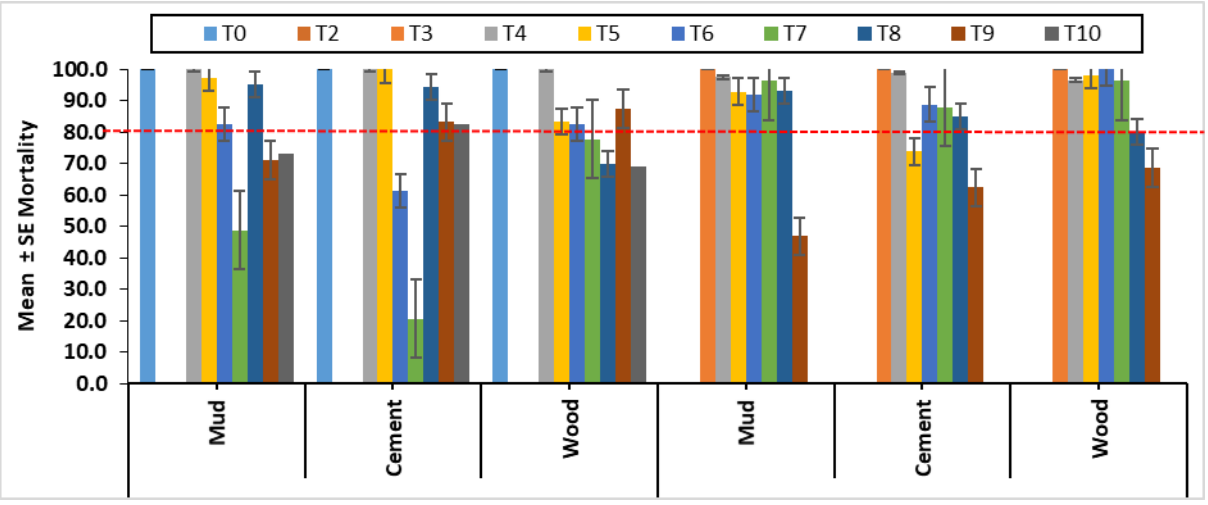
The wall bioassays showed that the Actellic 300CS remained effective above the cut-off mortality level (80% 24-hour mortality) up to 11 months post-IRS on most surfaces based on tests conducted with the Kisumu strain (Figure 9), whereas tests conducted with wild *An. gambiae* s.l. suggest that the insecticide remains effective up to 10 months, depending on the surface sprayed (Figure 10). Testing with wild *An. gambiae* s.l. could not continue beyond January 2021(month 10) due to difficulty in getting enough larvae to rear to adult for the tests in February.

FIGURE 9: SPRAY QUALITY AND RESIDUAL EFFICACY OF ACTELLIC 300CS BY CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES IN MMD, KISUMU MOSQUITOES, MARCH 2020–FEBRUARY 2021



Note: Bugyanga was sprayed in March, and Yagaba was sprayed in late April. Bioassays in Yagaba started after T2. No T1 and T2 data due to COVID-19 restrictions. No T11 tests for Yagaba

FIGURE 10: SPRAY QUALITY AND RESIDUAL EFFICACY OF ACTELLIC 300CS REPRESENTED BY MEAN MORTALITY RATES OBSERVED FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES IN MMD, WILD *AN. GAMBIAE* S.L. MOSQUITOES, MARCH 2020–JANUARY 2021



Note: Bugyanga sprayed in March 2020 while Yagaba was sprayed in late April. Bioassays in Yagaba started after T2. Tests with wild mosquitoes up to T10 in Bugyanga and T9 in Yagaba.

SumiShield 50WG (Figures 11 to 14) and Fludora Fusion (Figures 15 to 18) also showed a residual efficacy of at least 11 months post spray based on tests performed with Kisumu strain mosquitoes. Tests with wild *An. gambiae* s.l. could not be done for some months in the dry season (January and February 2021) in both SumiShield sprayed and Fludora Fusion sprayed districts, due to difficulty in getting enough larvae to rear to adult for the tests. Tests with Kisumu strain attained 100% mortality within 48 hours in most months on walls sprayed with Fludora Fusion, while tests from the SumiShield-sprayed communities averaged about 72 hours before 100% mortality was achieved. Figures A-1 through A-9 in the Annex present results for mean percentage knock downs and mortalities of Kisumu strain of *An. gambiae* and wild *An. gambiae* s.l. from spray quality cone wall bioassays conducted on all sprayed surfaces in nine districts. Figure A-10 provides results of same knock down and mortality tests from animal shelters in in EMD, KUD AND MMD.

FIGURE 11: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD 50WG REPRESENTED BY MORTALITY RATES OBSERVED IN BND FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, *AN. GAMBIAE* S.S. KISUMU STRAIN, MARCH 2020–FEBRUARY 2021

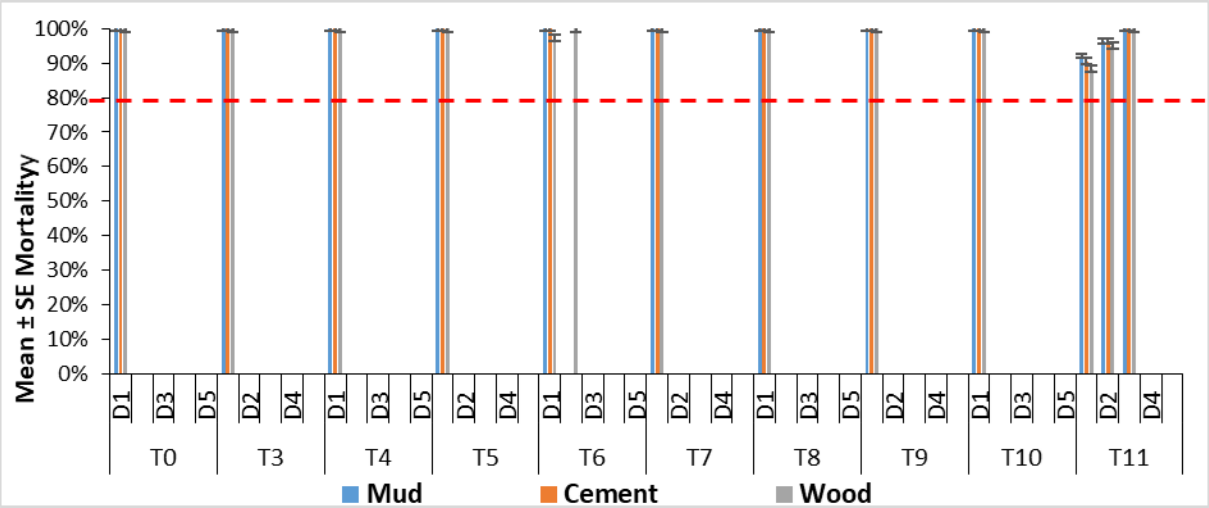
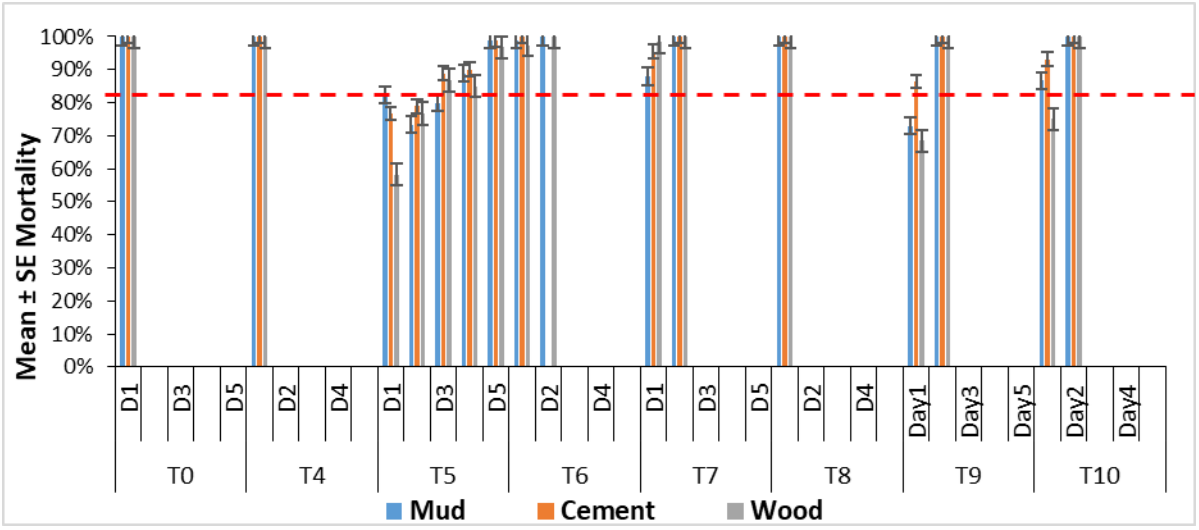


FIGURE 12: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD 50WG REPRESENTED BY MORTALITY RATES OBSERVED IN BND FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, WILD *AN. GAMBIAE* S.L., MARCH 2020–JANUARY 2021



Note: T3 test not done for wild

FIGURE 13: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD 50WG REPRESENTED BY MORTALITY RATES OBSERVED IN WMD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, *AN. GAMBIAE* S.S. KISUMU STRAIN, MARCH 2020–FEBRUARY 2021

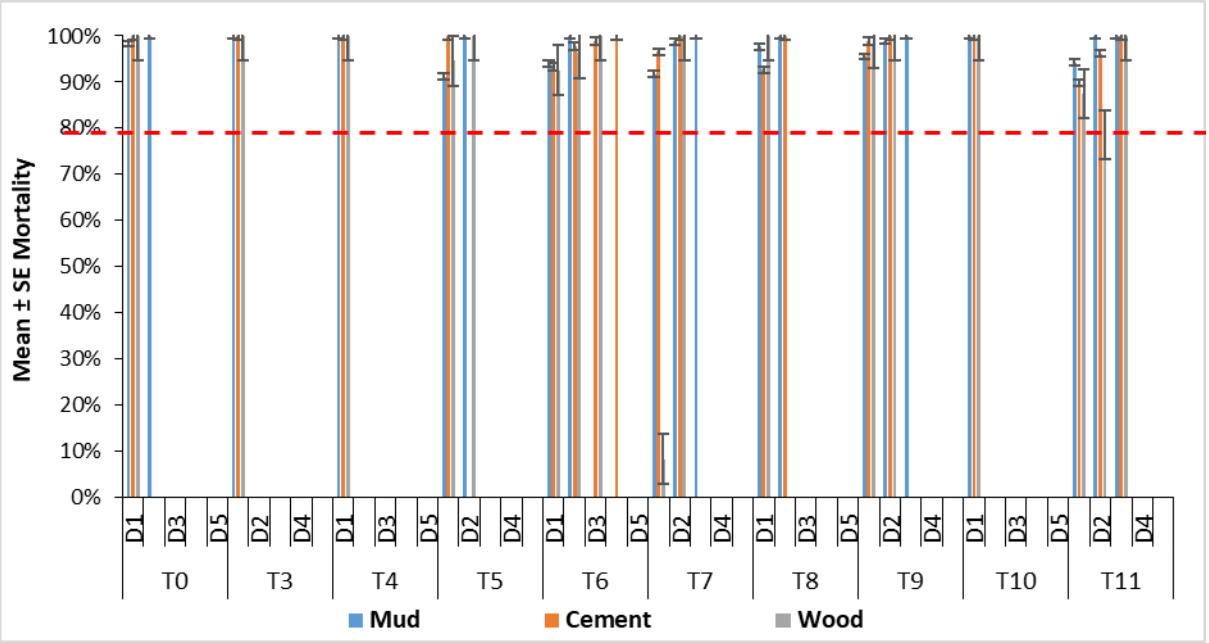
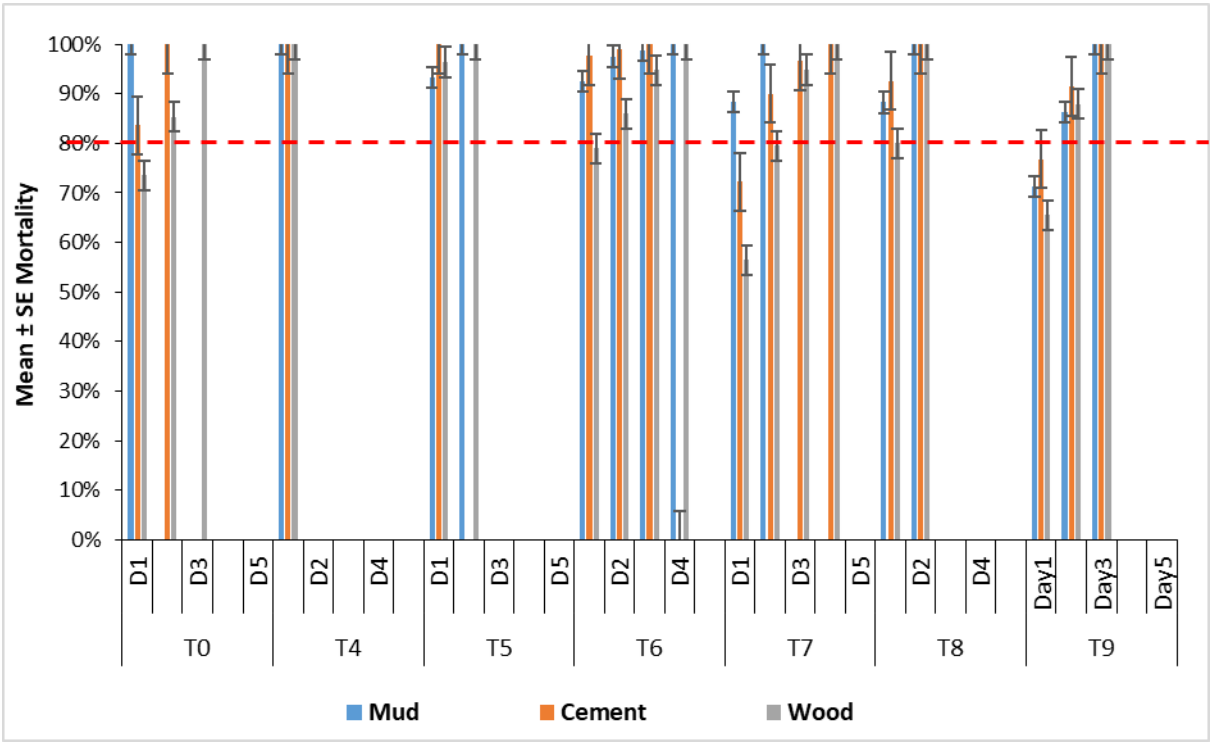


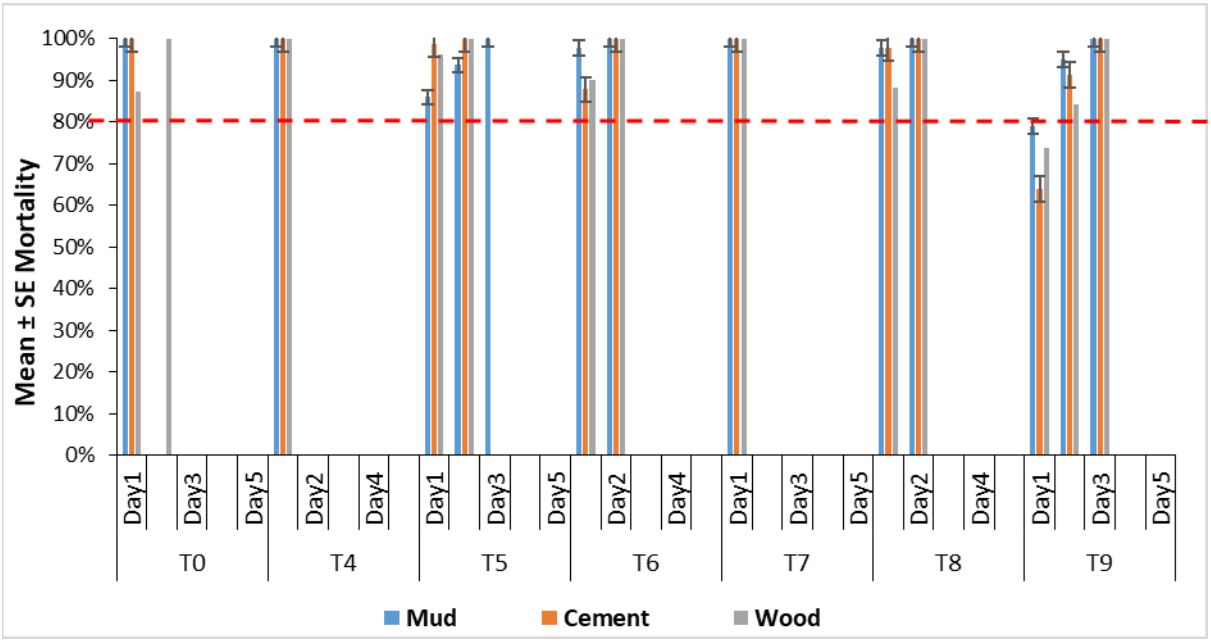
FIGURE 14: SPRAY QUALITY AND RESIDUAL EFFICACY OF SUMISHIELD 50WG REPRESENTED BY MORTALITY RATES OBSERVED IN WMD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, WILD *AN. GAMBIAE* S.L, MARCH–DECEMBER 2020



Note: *T3 test not done for wild

FIGURE 15: SPRAY QUALITY AND RESIDUAL EFFICACY OF FLUDORA FUSION REPRESENTED BY MORTALITY RATES OBSERVED IN KUD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, *AN. GAMBIAE* S.S. KISUMU STRAIN, MARCH 2020–FEBRUARY 2021

FIGURE 16: SPRAY QUALITY AND RESIDUAL EFFICACY OF FLUDORA FUSION REPRESENTED BY MORTALITY RATES OBSERVED IN KUD FOLLOWING CONE BIOASSAYS ON CEMENT, MUD, AND WOOD SURFACES, WILD *AN. GAMBIAE* S.L, MARCH–DECEMBER 2020



Note: *T3 test not done for wild

FIGURE 17: SPRAY QUALITY AND RESIDUAL EFFICACY OF FLUDORA FUSION REPRESENTED BY MORTALITY RATES OBSERVED IN TSD FOLLOWING CONE BIOASSAYS ON CEMENT AND WOOD SURFACES, *AN. GAMBIAE* S.S. KISUMU STRAIN, MARCH 2020–FEBRUARY 2021

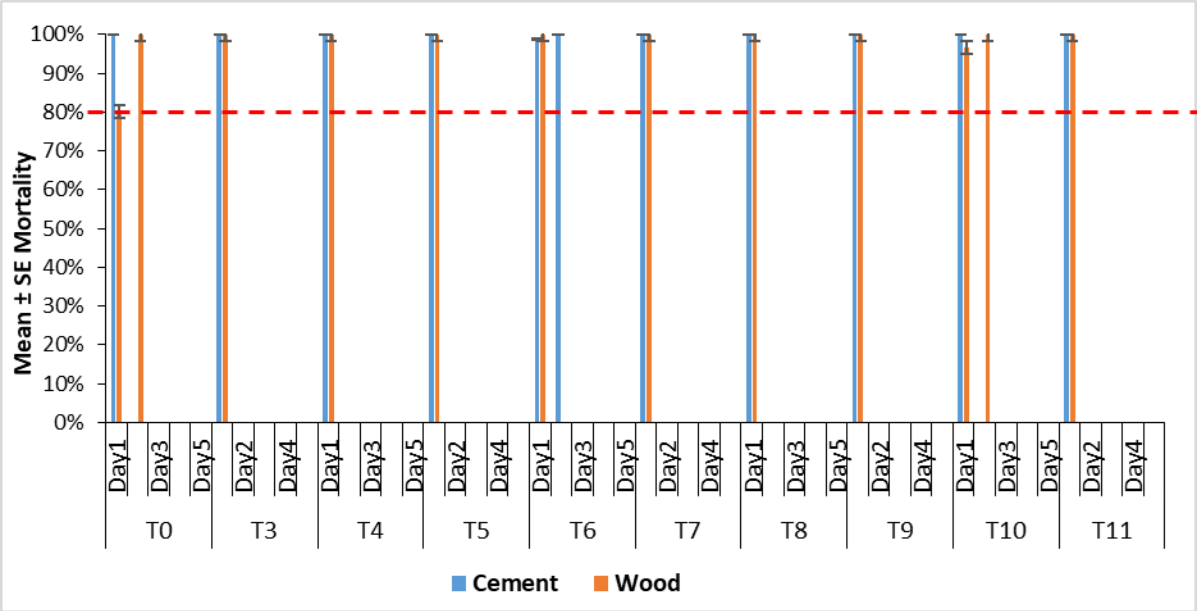
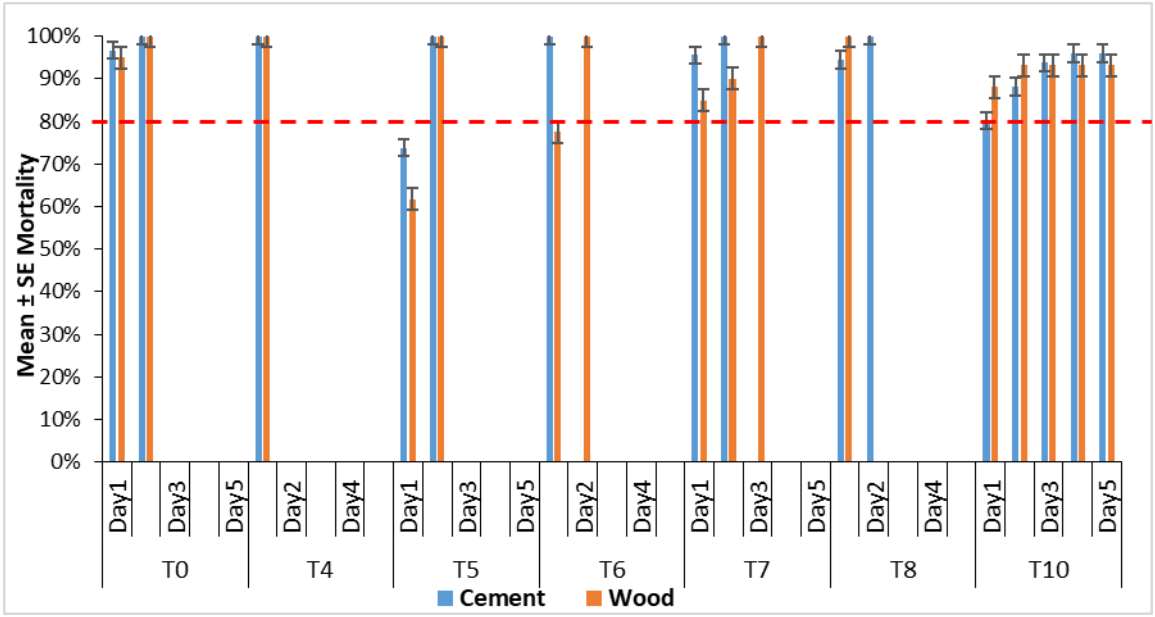


FIGURE 18: SPRAY QUALITY AND RESIDUAL EFFICACY OF FLUDORA FUSION REPRESENTED BY MORTALITY RATES OBSERVED IN TSD FOLLOWING CONE BIOASSAYS ON CEMENT AND WOOD SURFACES, WILD *AN. GAMBIAE* S.L, MARCH 2020–JANUARY 2021

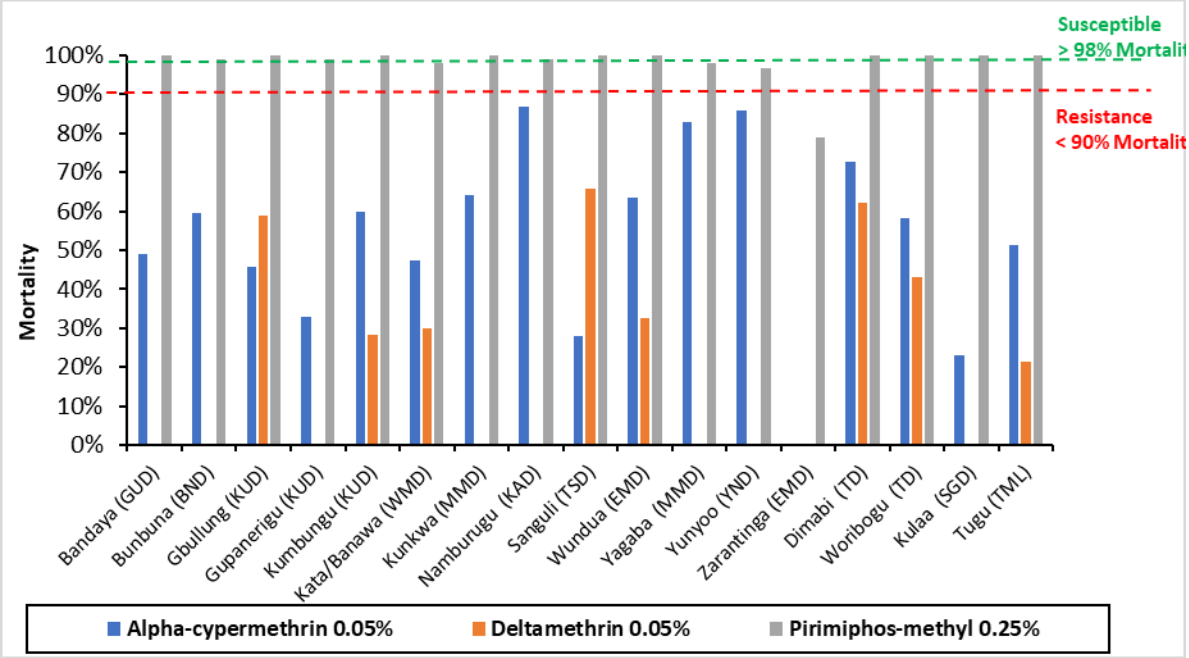


Note: T3 and T9 test not done for wild.

3.9 INSECTICIDE SUSCEPTIBILITY

The number of mosquitoes tested and percentage mortality after exposure for all sites are provided in Table A-2 of the Annex. WHO susceptibility tests indicate that *An. gambiae* s.l. mosquitoes are susceptible to pirimiphos-methyl 0.25% in all sites tested except in Zaratinga (EMD), where resistance to pirimiphos-methyl 0.25% was confirmed (79%) and in Yunyoo where possible resistance (97%) was detected (Figure 19) *An. gambiae* s.l. across all sites tested were resistant to the pyrethroids, alpha-cypermethrin (0.05%) and deltamethrin (0.5%). However, exposure to PBO before alphacypermethrin resulted in a significant increase in 24-hour mortality compared to test with alphacypermethrin alone, among *An. gambiae* s.l. tested across all sites.

FIGURE 19: INSECTICIDE SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. FROM ALL SITES IN 2020 BY WHO TUBE TEST



An. gambiae s.l. from all sites tested were fully susceptible to 2% clothianidin (13.2mg/paper) and chlorfenapyr (100µg/bottle) at 7 days (Figure 20) and 3 days (Figure 21) post-exposure, respectively. An insectary strain was used to standardize the clothianidin papers and as a control for the chlorfenapyr tests.

FIGURE 20: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. FROM SELECTED SITES IN NORTHERN GHANA TO CHLOTHIANIDIN:WHO TUBE TEST, 2020

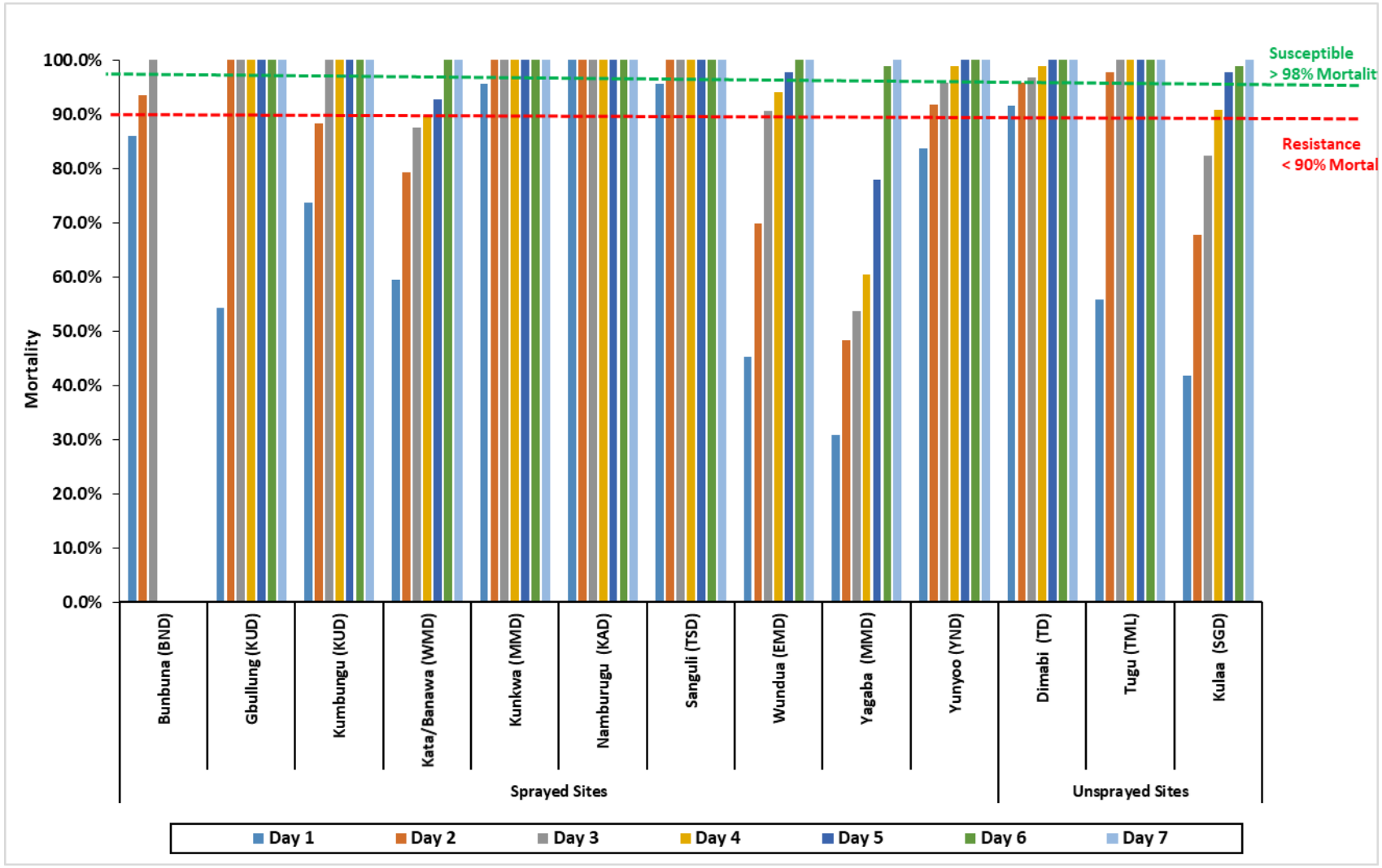
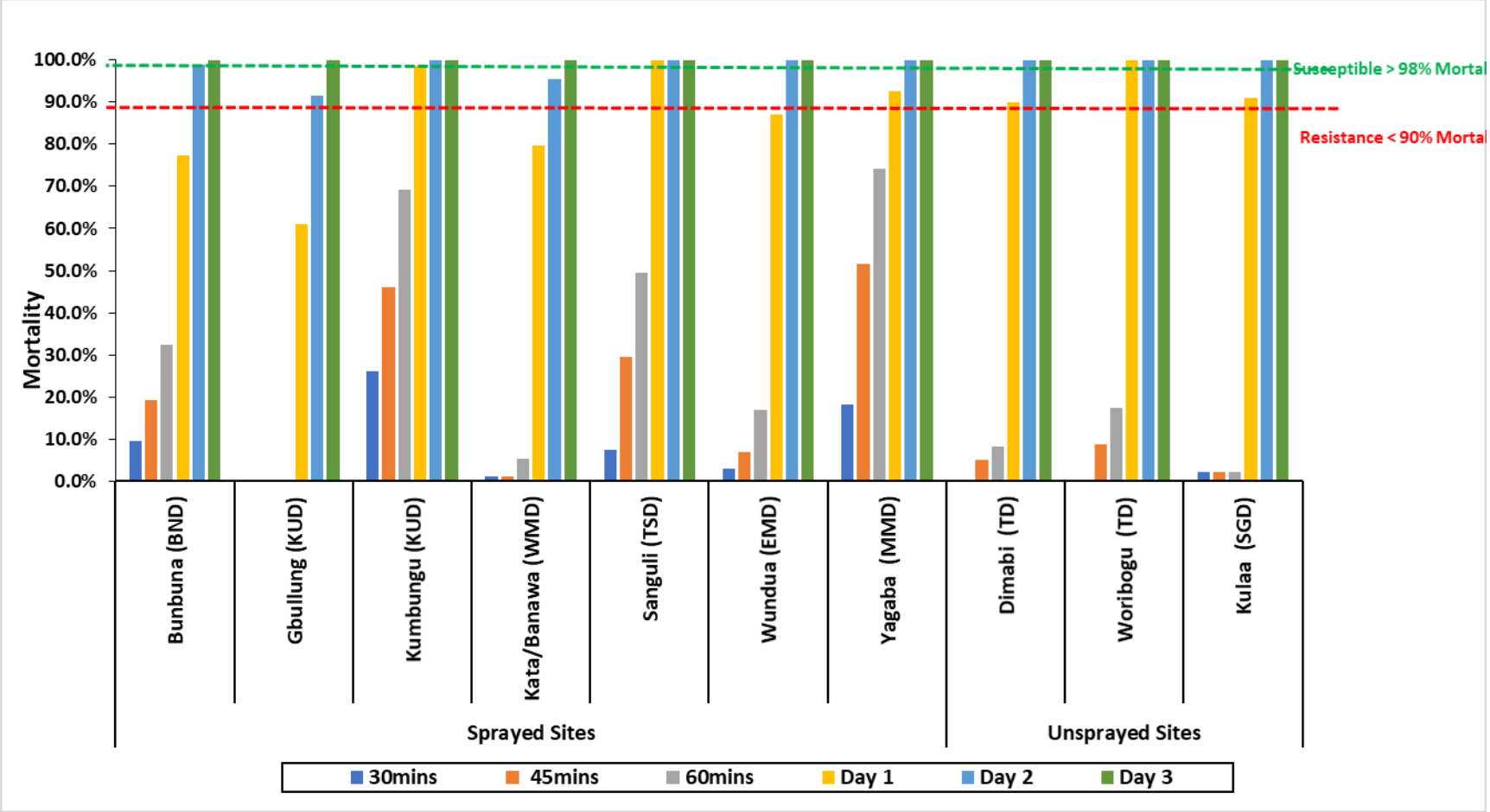


FIGURE 21: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. FROM SELECTED SITES IN NORTHERN GHANA TO 100 µG/BOTTLE CHLORFENAPYR: CDC BOTTLE ASSAYS, 2020

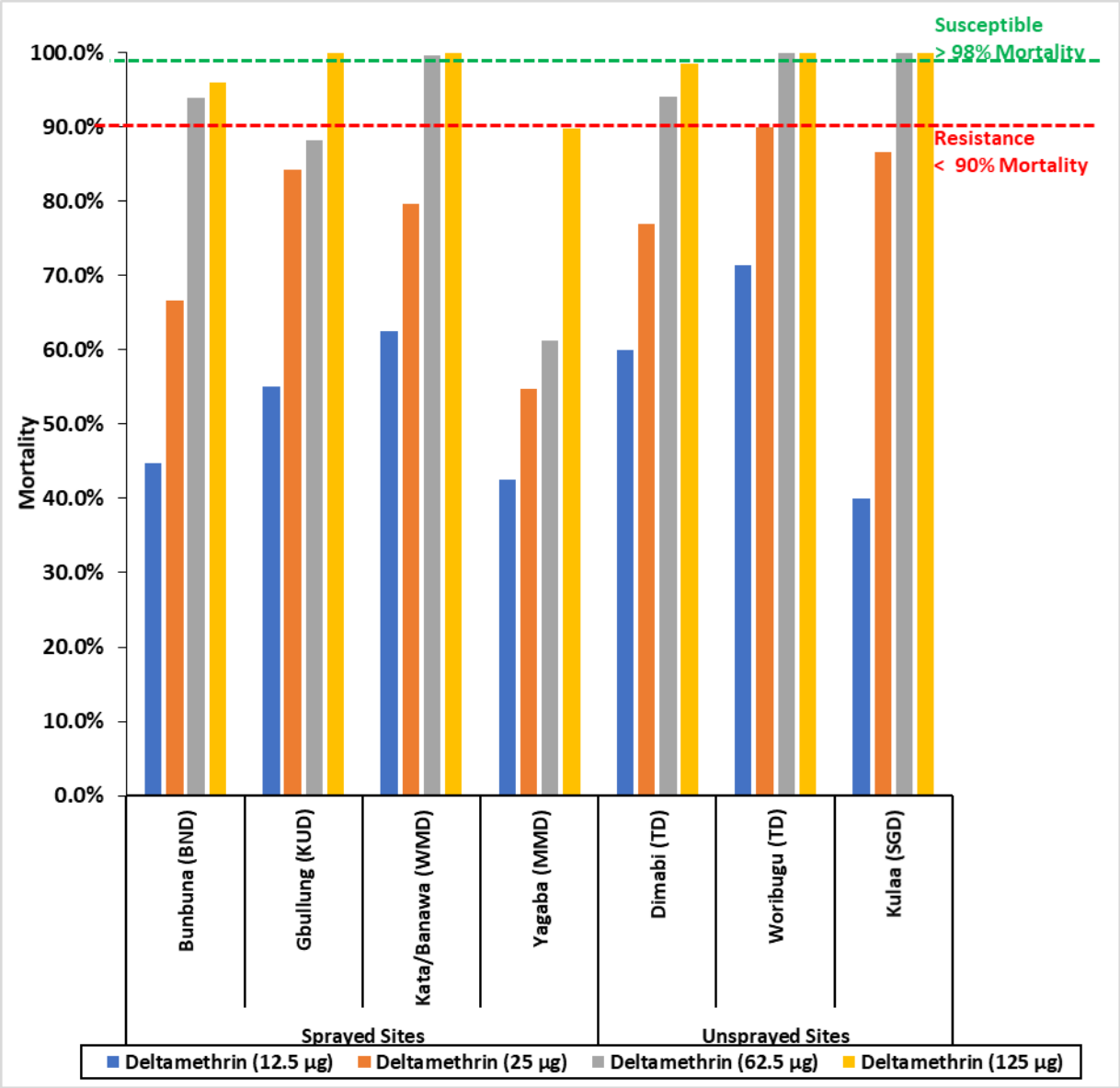


3.10 RESISTANCE INTENSITY

An. gambiae s.l. mosquitoes from all sites tested were resistant to the 25 µg/bottle (2x the diagnostic dose) of deltamethrin based on the CDC bottle bioassay-recommended thresholds (Figure 22). Resistance to the 62.5 µg/bottle (5x of the diagnostic dose) concentration was also observed in KUD and MMD. High deltamethrin resistance intensity was noted in MMD, where the vector was also resistant to the 125 µg/bottle concentration of deltamethrin.

Mortality after 24 hours in the follow up pirimiphos methyl resistance intensity assay in Zaratinga using the WHO tube method, was 100%, when mosquitoes were exposed to 1.25% (5x the diagnostic dose) pirimiphos-methyl in resistance intensity assays.

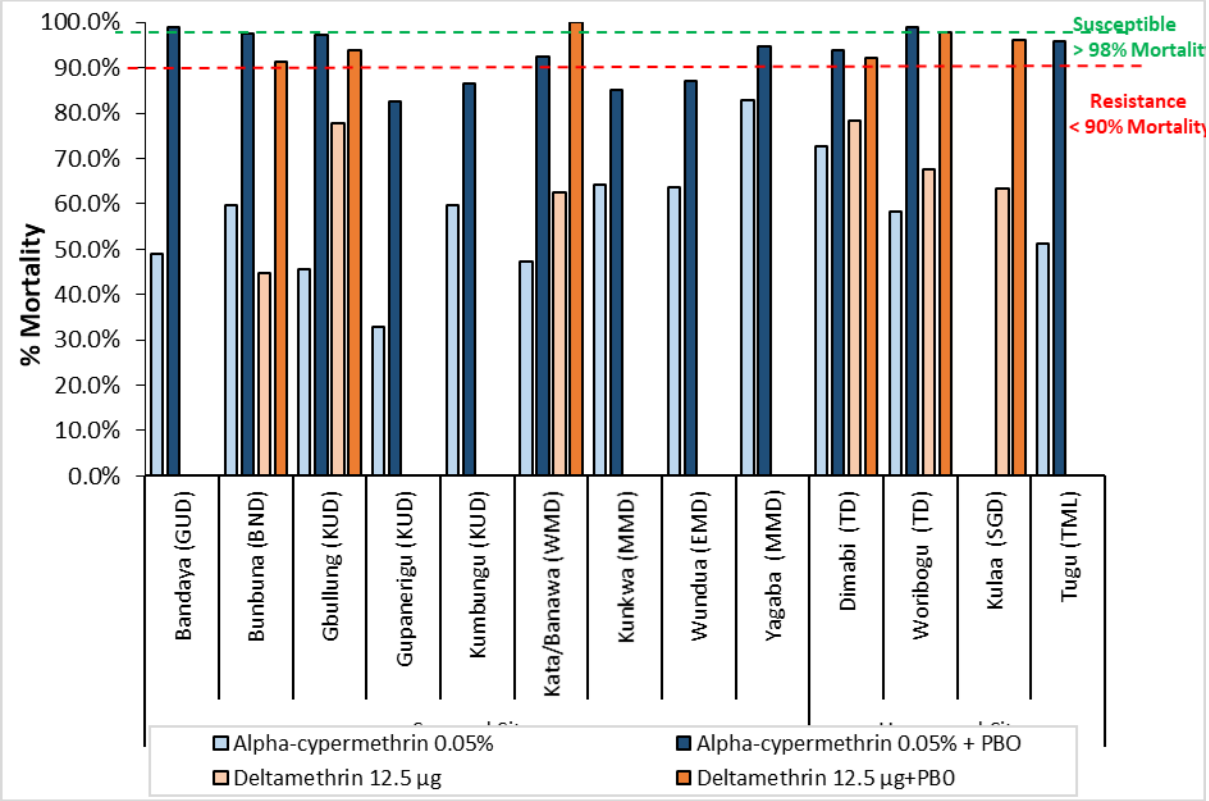
FIGURE 22: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM SPRAYED AND UNSPRAYED SITES EXPOSED TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN, USING CDC RESISTANCE I-RDT ASSAYS



3.11 SYNERGIST ASSAYS

An. gambiae s.l. from 6 sites where mosquitoes were pre-exposed to PBO showed higher mortalities than those with no pre-exposure to the synergist (Figure 23).

FIGURE 23: TIME MORTALITY FOR *AN. GAMBIAE* S.L. FROM SPRAYED AND UNSPRAYED SITES TESTED AFTER EXPOSURE TO PYRETHROIDS AND PBO



Note: Alphacypermethrin tested using WHO tube method; Deltamethrin tested using CDC Bottle bioassay method

3.12 TARGET SITE RESISTANCE

3.12.1 *ACE-1* GENE MUTATION

The *ace-1* gene mutation has been reported to confer cross-resistance to carbamates and organophosphates in mosquito species, whereas *kdr* gene mutation confers resistance to pyrethroids and DDT. The frequency of the resistant alleles ranged from 0.57 to 0.91 in the IRS intervention sites. There was a general increase in the frequency of *ace-1* resistant alleles compared to 2019 when the frequency of *ace-1* alleles ranged from 0.28 to 0.48 in the IRS intervention sites. Relatively high *ace-1* frequency (0.91) was detected in Kunkua in MMD (Table 8) where IRS was implemented with pirimiphos-methyl from 2012 to 2018 and in 2020.

3.12.2 *KDR* MUTATION

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 2019. The frequency of the resistant alleles ranged from 0.61 to 0.91 in the IRS intervention sites in 2019, compared to a range of 0.52-0.81 recorded in the IRS sites in 2020. About 18% 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 Kulaa, an unsprayed site.

TABLE 8: DISTRIBUTION AND FREQUENCY OF *ACE-1* AND *KDR* ALLELES WITHIN *AN. GAMBIAE* S.L., IRS INTERVENTION AND CONTROL SITES, 2020

Sentinel site		*Ace-1*					*kdr-w*					*kdr-e*			
		Total Examined	SS	RS	RR	*f(R)*	Total Examined *kdr-W and kdr-E*	SS	RS	RR	*f(R)*	SS	RS	RR	*f(R)*
IRS Intervention															
BND	Bunbuna	49	10	9	30	**0.70**	52	5	10	37	**0.81**	52	0	0	**0.00**
GUD	Gushegu	20	3	5	12	**0.73**	29	6	7	16	**0.67**	15	1	13	**0.47**
KAD	Karaga	20	1	8	11	**0.75**	30	6	8	16	**0.67**	21	4	5	**0.23**
MMD	Kunkua	21	1	1	19	**0.93**	36	6	9	21	**0.71**	28	4	4	**0.17**
KUD	Gbullung	29	3	17	9	**0.60**	50	11	5	34	**0.73**	50	0	0	**0.00**
WMD	Banawa	25	1	2	22	**0.92**	25	7	10	8	**0.52**	20	1	4	**0.18**
YND	Yunyoo	29	1	8	20	**0.83**	34	8	4	22	**0.71**	28	2	4	**0.15**
Control															
TD	Woribugu	34	13	10	11	**0.47**	49	2	3	44	**0.93**	48	1	0	**0.01**
SGD	Kulaa	23	3	8	12	**0.70**	44	4	5	35	**0.85**	16	1	27	**0.63**
TML	Tugu	34	10	10	14	**0.56**	35	2	4	29	**0.89**	35	0	0	**0.00**

4. Discussion and Conclusion/ Recommendation

The data from 2020 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. Despite a marginal increase in the proportion of *An. coluzzii* and *An. arabiensis* in 2020, *An. gambiae* remains the most abundant vector species collected across all sites, possibly because of its adaptability to the temporary breeding environments in the area (Diabate et al. 2003). The increase in the proportions of *An. coluzzii* and *An. arabiensis* in 2020 as compared to 2019 is not well understood. However, a recent study in Burkina Faso (Perugini et al. 2020) found that *An. coluzzii* and *An. arabiensis* exhibit some behavioral plasticity that allows them to evade indoor targeted interventions which may account for their high numbers. Nonetheless, it may be necessary to collect additional data to definitively understand if this is the trend.

In general, PSC and CDC light trap collections from sleeping rooms recorded lower mosquito densities than HLCs. It is likely that improved housing (screening of doors and windows) made the entry of mosquitoes into sleeping rooms difficult. Studies have noted that fewer mosquitoes are collected from houses with tightly sealed entrances or screened doors and windows (Nguela et al. 2020).

Similar indoor and outdoor biting rates of *An. gambiae* s.l. in TML and WMD may be due to behavioral plasticity, that has been observed in some *An. gambiae* s.l. populations, which allows the species to feed based on available host (Lefèvre et al., 2009). *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 may induce exophagy in the vector populations (Syme et al. 2021). The marked outdoor feeding preferences noted in KUD and MMD could be due to the irritant effect from deltamethrin (in Fludora Fusion) sprayed in KUD and pirimiphos-methyl (in Actellic 300CS) sprayed in MMD. In contrast, clothianidin (in SumiShield) did not induce as much excito-repellency and could in part explain the endophagic behavior observed in BND, and an almost similar endophagic and exophagy tendencies of *An. gambaie* s.l. in WMD. The slightly higher proportion of *An. coluzzii* and *An. arabiensis* in WMD than in BND may in part account for the slightly higher exophagic tendencies noted in WMD in comparison with BND. The significant outdoor biting preference of *An. gambiae* in Tugu, which is an unsprayed site, could be due to high coverage and noted use of ITNs in the area. Given IRS started in TSD in 2020, it may be too early to see marked changes in entomological parameters.

An. gambiae s.l. remains anthrophilic, with a high HBI observed in all sites despite the relative increase in outdoor feeding. This may increase the risk of exposure to individuals who engage in night-time recreational outdoor activities (such as watching videos, storytelling and playing games) without adequate personal protection. Collections from animal shelters suggest increased zoophagy, possibly due to the presence of IRS or, in the unsprayed communities, use of ITNs in houses. The detection of mosquito samples with animal blood in PSCs suggests that even though mosquitoes may seek alternative (animal) hosts, some still prefer resting indoors. Data should be interpretated with caution since the sample size from PSCs analysed were small, and solely from indoor resting mosquitoes.

The detection of mosquitoes that had fed on animals as well as humans in the animal shelters indicate that mosquitoes in the area also have the tendency to enter animal shelters not only for animal blood meal but also for resting after biting humans elsewhere. This makes targeting animal shelters with IRS a reasonable approach. Additionally, data collected from the animal shelters in Bunbuna (BND), Gbullung (KUD), and Kunkua (MMD) showed significantly lower numbers of *An. gambiae* s.l. collected from sprayed shelters compared with unsprayed shelters in the control site. This furthermore suggests that spraying such structures in addition to human dwellings could have significant impact on vector density and the vector survival rate, and likely result in further reductions in malaria transmission. The data however suggests that utilization of animal shelters as a

resting place for mosquitoes is not uniform across all districts. Before widely adapting this strategy however, additional data is needed to properly guide this decision.

Analysis of parity rates showed that significantly fewer older mosquitoes were collected in the sprayed sites than in the unsprayed sites. The annual EIR for *An. gambiae* s.l. in the sprayed sites was also significantly lower than that in the unsprayed sites. These data suggest that IRS is effectively reducing mosquito longevity—and thus malaria transmission—in these areas. All sites except KUD and MMD recorded relatively high outdoor EIRs in comparison with EIRs recorded indoors. This suggests that interventions that target outdoor transmission may be required as an add-on to IRS and ITNs in certain areas where outdoor transmission is high. Sleeping outdoors during the malaria peak season is however uncommon in the area. The high EIRs recorded for TSD could mean that the district will require additional rounds of IRS implementation to see a significant impact on sporozoite infections and EIRs.

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 results from synergist assays suggest that oxidases could be contributing to resistance observed in the local vector species from most sites and PBO ITNs may be appropriate vector control tools in the region. In all sites, *An. gambaie* s.l were susceptible to chlorfenapyr, which indicates that new types of dual active ingredients in vector control tools such as ITNs may be appropriate for deployment in this region of Ghana. The detection of *kdr-e* mutation for the first time 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), that suggest a rapid spread in the region of the *kdr-e* mutation, originally found in East Africa.

The spread of the *ace-1* resistant allele represents a threat for the use of pirimiphos-methyl in these areas for IRS. These data underscore the importance of implementing a rotation strategy in IRS campaigns. Considering that vectors in the area remain susceptible to clothianidin, SumiShield 50WG and Fludora Fusion remain plausible alternatives for future IRS campaigns in northern Ghana. Both insecticide formulations have demonstrated a residual efficacy that lasts beyond the malaria transmission season and can therefore remain in use for IRS.

ANNEX: 2020 ENTOMOLOGICAL MONITORING RESULTS

TABLE A-1: SUMMARY OF WHO INSECTICIDE RESISTANCE TEST OF *AN. GAMBIAE* S.L. TO SELECTED INSECTICIDES, 2020

Treatment		Alpha-cypermethrin 0.05%		Alpha-cypermethrin 0.05% + PBO		Deltamethrin 0.05%		Pirimiphos-methyl 0.25%		Clothianidin 2%	
Sprayed Sites	Bandaya (GUD)	49.0%	(98)	98.9%	(91)			100.0%	(98)		
	Bunbuna (BND)	59.6%	(95)	97.8%	(93)			99.0%	(97)	100.0%	(93)
	Gbullung (KUD)	45.7%	(86)	97.2%	(79)	58.9%	(90)	100.0%	(100)	100.0%	(92)
	Gupanerigu (KUD)	33.0%	(91)	82.4%	(99)			99.0%	(100)		
	Kumbungu (KUD)	59.8%	(95)	86.7%	(98)	28.2%	(86)	100.0%	(96)	100.0%	(95)
	Kata/Banawa (WMD)	47.4%	(94)	92.5%	(94)	29.8%	(80)	98.0%	(98)	100.0%	(96)
	Kunkwa (MMD)	64.1%	(92)	85.1%	(94)			100.0%	(92)	100.0%	(90)
	Namburugu (KAD)	86.7%	(90)					99.0%	(96)	100.0%	(90)
	Sanguli (TSD)	28.3%	(99)			65.6%	(79)	100.0%	(98)	100.0%	(91)
	Wundua (EMD)	63.6%	(92)	87.0%	(96)	32.5%	(99)	100.0%	(95)	100.0%	(86)
	Yagaba (MMD)	83.0%	(94)	94.8%	(96)			98.0%	(100)	100.0%	(98)
	Yunyoo (YND)	85.7%	(91)					96.7%	(91)		
	Zarantinga (EMD)							78.9%	(71)		
Unsprayed Sites	Dimabi (TD)	72.6%	(95)	93.6%	(78)	43.0%	(77)	100.0%	(99)	100.0%	(95)
	Woribugu (TD)	58.3%	(96)	98.9%	(95)	62.0%	(96)	100.0%	(100)	100.0%	(95)
	Kulaa (SGD)	22.9%	(96)					100.0%	(97)	100.0%	(96)
	Tugu (TML)	51.3%	(92)	95.8%	(77)	21.4%	(98)	100.0%	(94)	100.0%	(93)

TABLE A-2: MONTHLY ENTOMOLOGICAL PARAMETERS OF *AN. GAMBIAE*, ALL SENTINEL DISTRICTS, MARCH–DECEMBER 2020

Treatment	Districts	Month	Indoor_ HBR	Number Examined by ELISA	Number +ve for sporozoite	SPZ Rate	Monthly EIR	Outdoor_ HBR	Number Examined by ELISA	Number +ve for sporozoite	SPZ Rate	Monthly EIR	Total
Intervention	BND	Mar-20	4.6	10	0	0.00%	0.0	1.8	3	0	0.00%	0.0	**0.0**
		Jun-20	24.6	74	0	0.00%	0.0	15.3	44	1	2.27%	10.4	**10.4**
		Jul-20	30.4	47	0	0.00%	0.0	26.6	41	0	0.00%	0.0	**0.0**
		Aug-20	54.4	91	0	0.00%	0.0	45.6	78	1	1.28%	17.5	**17.5**
		Sep-20	137.3	233	1	0.43%	17.7	135.5	227	0	0.00%	0.0	**17.7**
		Oct-20	31.0	8	0	0.00%	0.0	26.1	64	0	0.00%	0.0	**0.0**
		Nov-20	2.8	8	0	0.00%	0.0	1.2	5	0	0.00%	0.0	**0.0**
		Dec-20	7.6	0	0	0.00%	0.0	5.3	0	0	0.00%	0.0	**0.0**
	BND Total		**36.6**	**471**	**1**	**0.21%**	**17.7**	**32.2**	**462**	**2**	**0.43%**	**28.0**	**45.6**
	KUD	Mar-20	2.0	2	0	0.00%	0.0	0.5	1	0	0.00%	0.0	**0.0**
		Jun-20	21.9	47	0	0.00%	0.0	19.4	43	1	2.33%	13.5	**13.5**
		Jul-20	75.3	125	1	0.80%	18.7	116.5	174	0	0.00%	0.0	**18.7**
		Aug-20	25.9	44	0	0.00%	0.0	43.0	74	0	0.00%	0.0	**0.0**
		Sep-20	68.1	8	0	0.00%	0.0	105.8	8	0	0.00%	0.0	**0.0**
		Oct-20	34.3	8	0	0.00%	0.0	24.3	8	0	0.00%	0.0	**0.0**
		Nov-20	2.9	8	0	0.00%	0.0	3.0	7	0	0.00%	0.0	**0.0**
		Dec-20	0.3	0	0	0.00%	0.0	0.6	0	0	0.00%		**0.0**
	KUD Total		**28.8**	**242**	**1**	**0.41%**	**18.7**	**39.1**	**315**	**1**	**0.32%**	**13.5**	**32.2**
	MMD	Mar-20	0.7	3	0	0.00%	0.0	0.4	2	0	0.00%	0.0	**0.0**
		Jun-20	0.1	0	0	0.00%	0.0	0.2	0	0	0.00%		**0.0**
		Jul-20	4.7	18	0	0.00%	0.0	9.1	36	0	0.00%	0.0	**0.0**
		Aug-20	30.0	90	0	0.00%	0.0	38.8	118	1	0.85%	9.9	**9.9**
		Sep-20	87.4	296	0	0.00%	0.0	120.5	403	0	0.00%	0.0	**0.0**
		Oct-20	22.5	34	1	2.94%	20.5	34.7	16	0	0.00%	0.0	**20.5**
		Nov-20	2.9	18	0	0.00%	0.0	6.7	30	1	3.33%	6.7	**6.7**
		Dec-20	0.3	0	0	0.00%	0.0	0.5	0	0	0.00%		**0.0**
	MMD Total		**18.6**	**459**	**1**	**0.37%**	**20.5**	**26.3**	**605**	**2**	**0.33%**	**16.6**	**37.0**
	TSD	Mar-20	0.0	0	0	0.00%	0.0	0.0	0	0	0.00%	0.0	**0.0**
		Jun-20	30.0	75	0	0.00%	0.0	35.6	78	1	1.28%	14.1	**14.1**
		Jul-20	132.5	276	2	0.72%	28.8	57.4	116	1	0.86%	14.8	**43.6**
		Aug-20	7.8	14	1	7.14%	16.7	14.4	26	0	0.00%	0.0	**16.7**
		Sep-20	51.3	88	0	0.00%	0.0	53.5	91	1	1.10%	18.2	**18.2**
		Oct-20	11.3	28	0	0.00%	0.0	15.9	48	0	0.00%	0.0	**0.0**
		Nov-20	3.4	8	0	0.00%	0.0	3.2	8	0	0.00%	0.0	**0.0**
		Dec-20	8.3	0	0	0.00%		0.0	0	0	0.00%		**0.0**
	TSD Total		**30.6**	**489**	**3**	**0.61%**	**45.5**	**22.5**	**367**	**3**	**0.82%**	**47.2**	**92.7**

Treatment	Districts	Month	Indoor_ HBR	Number Examined by ELISA	Number +ve for sporozoite	SPZ Rate	Monthly EIR	Outdoor_ HBR	Number Examined by ELISA	Number +ve for sporozoite	SPZ Rate	Monthly EIR	Total
	WMD	Mar-20	7.9	19	0	0.00%	0.0	12.6	28	0	0.00%	0.0	**0.0**
		Jun-20	27.5	45	0	0.00%	0.0	24.5	48	1	2.08%	15.3	**15.3**
		Jul-20	134.3	364	0	0.00%	0.0	99.5	252	0	0.00%	0.0	**0.0**
		Aug-20	58.6	138	2	1.45%	25.5	61.4	101	1	0.99%	18.2	**43.7**
		Sep-20	185.8	81	0	0.00%	0.0	222.9	59	0	0.00%	0.0	**0.0**
		Oct-20	52.4	8	0	0.00%	0.0	62.3	8	0	0.00%	0.0	**0.0**
		Nov-20	13.0	22	0	0.00%	0.0	16.1	25	0	0.00%	0.0	**0.0**
		Dec-20	3.0	0	0	0.00%		1.3	0	0	0.00%		**0.0**
	WMD Total		**60.3**	**677**	**2**	**0.30%**	**25.5**	**62.6**	**521**	**2**	**0.38%**	**33.6**	**59.0**
Control	SGD	Mar-20	0.7	2	0	0.00%	0.0	0.3	2	0	0.00%	0.0	**0.0**
		Jun-20	110.4	250	1	0.40%	13.2	75.8	175	0	0.00%	0.0	**13.2**
		Jul-20	151.4	276	1	0.36%	17.0	116.0	184	1	0.54%	19.5	**36.5**
		Aug-20	12.0	21	0	0.00%	0.0	15.8	27	1	3.70%	17.6	**17.6**
		Sep-20	67.3	136	1	0.74%	14.8	72.5	139	1	0.72%	15.6	**30.5**
		Oct-20	65.3	108	0	0.00%	0.0	77.5	155	0	0.00%	0.0	**0.0**
		Nov-20	7.5	8	0	0.00%	0.0	5.8	8	0	0.00%	0.0	**0.0**
		Dec-20	0.9	6	0	0.00%	0.0	1.0	0	0	0.00%		**0.0**
	SGD Total		**51.9**	**807**	**3**	**0.19%**	**45.1**	**45.6**	**690**	**3**	**0.43%**	**52.7**	**97.8**
	TML	Mar-20	0.3	1	0	0.00%	0.0	1.7	3	0	0.00%	0.0	**0.0**
		Jun-20	113.8	198	0	0.00%	0.0	111.1	303	1	0.33%	11.0	**11.0**
		Jul-20	87.3	161	1	0.62%	16.8	76.8	140	2	1.43%	34.0	**50.8**
		Aug-20	10.0	17	1	5.88%	17.6	11.8	21	1	4.76%	16.9	**34.5**
		Sep-20	82.8	153	0	0.00%	0.0	99.3	191	0	0.00%	0.0	**0.0**
		Oct-20	60.0	121	1	0.83%	15.4	68.3	137	0	0.00%	0.0	**15.4**
		Nov-20	1.9	5	0	0.00%	0.0	1.8	6	0	0.00%	0.0	**0.0**
		Dec-20	0.6	4	0	0.00%	0.0	0.4	0	0	0.00%		**0.0**
	TML Total		**44.6**	**660**	**3**	**0.45%**	**49.8**	**46.4**	**800**	**4**	**0.50%**	**61.9**	**111.7**

FIGURE A-1: MEAN PERCENTAGE KNOCK DOWN 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 IN BUGYANGA, MMD, ACTELLIC 300CS, MARCH 2020

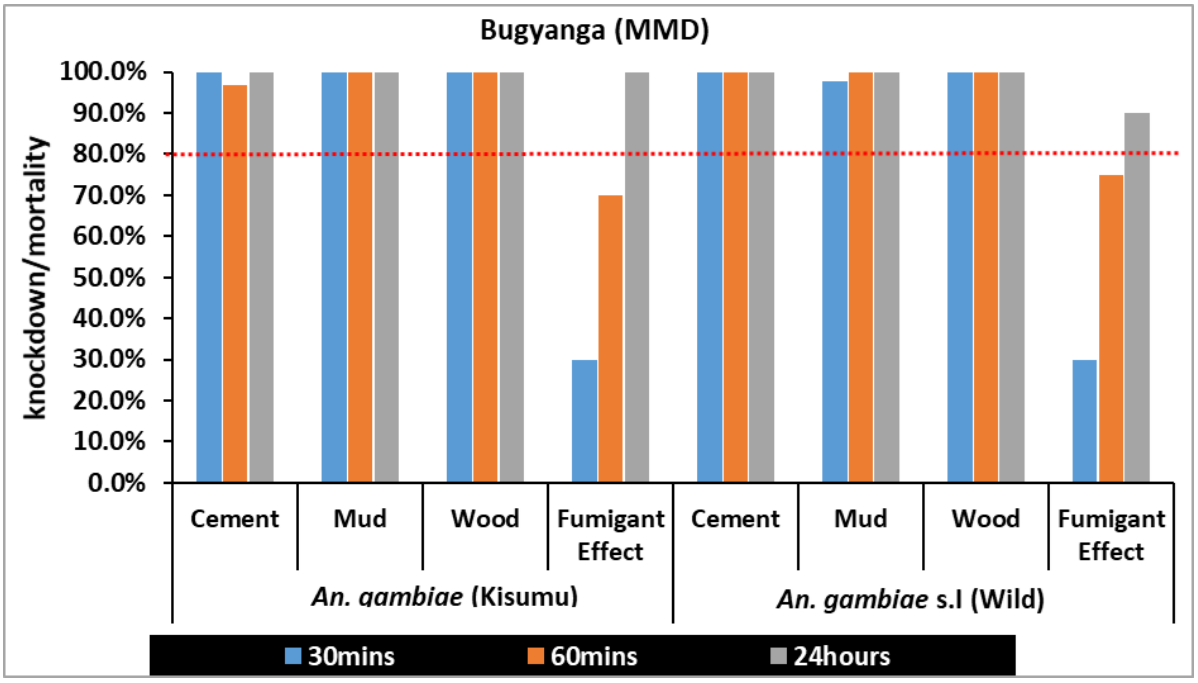


FIGURE A-2: MEAN PERCENTAGE KNOCK DOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* AND WILD *AN. GAMBIAE* S.L. FROM SPRAY QUALITY CONE WALL BIOASSAYS CONDUCTED ON ALL SPRAYED SURFACES IN YAPALA, EMD, FLUDORA FUSION, MARCH 2020

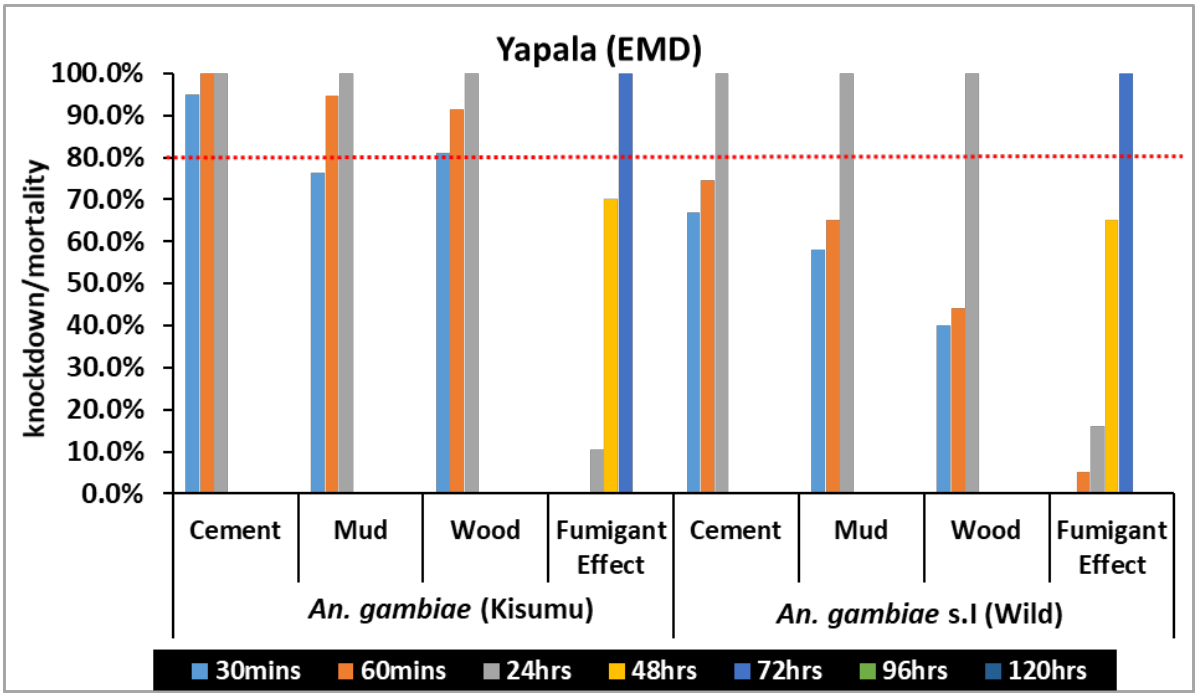


FIGURE A-3: MEAN PERCENTAGE KNOCK DOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* AND WILD *AN. GAMBIAE* S.L. FROM SPRAY QUALITY CONE WALL BIOASSAYS CONDUCTED ON ALL SPRAYED SURFACES IN GARICHE, GUD, FLUDORA FUSION, MARCH 2020

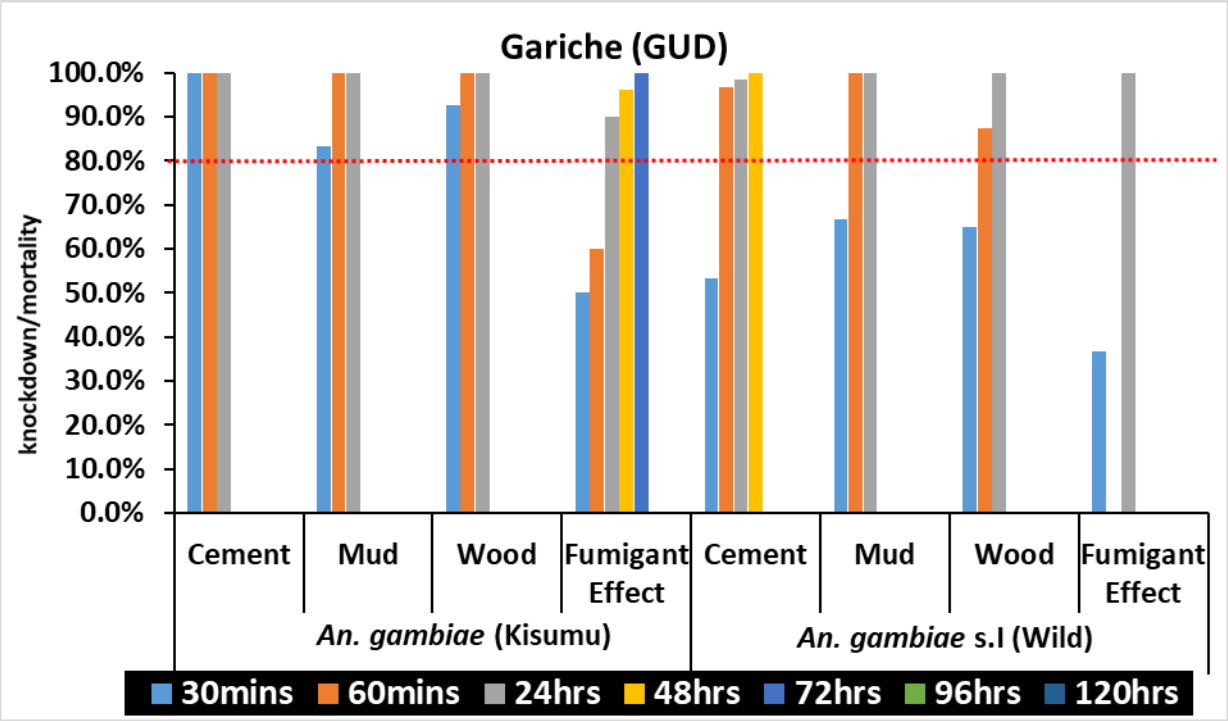


FIGURE A-4: MEAN PERCENTAGE KNOCK DOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* AND WILD *AN. GAMBIAE* S.L. FROM SPRAY QUALITY CONE WALL BIOASSAYS CONDUCTED ON ALL SPRAYED SURFACES IN CHEYOHI, KUD, FLUDORA FUSION, MARCH 2020

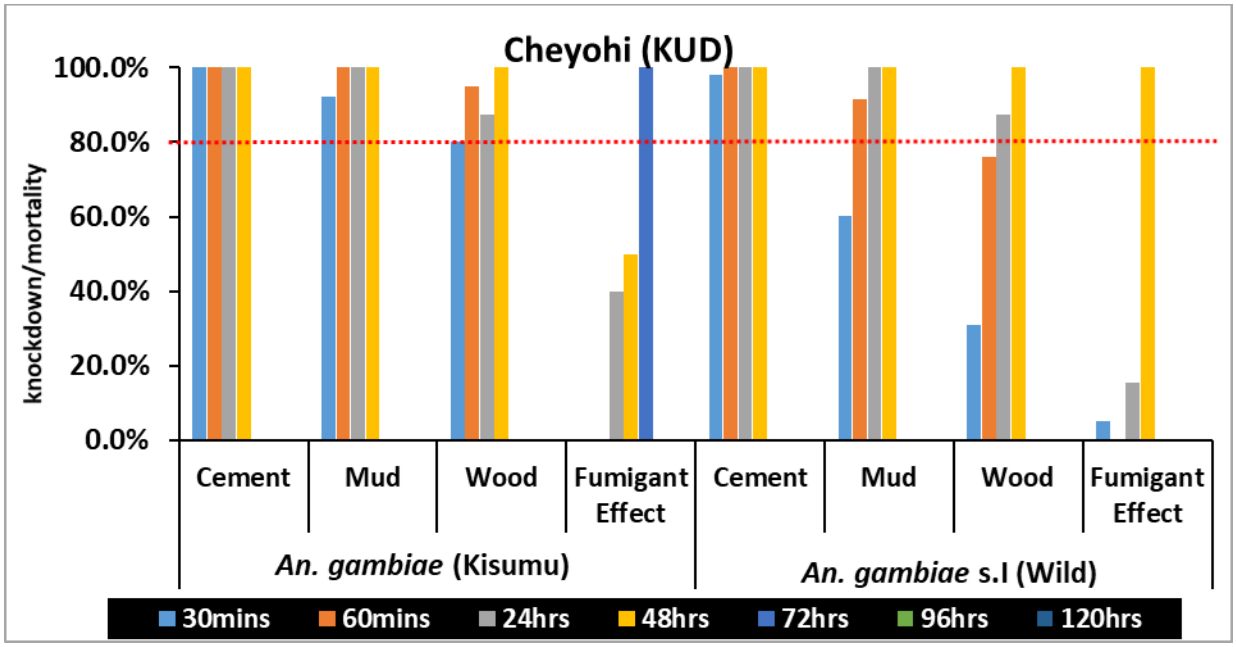


FIGURE A-5[1]: MEAN PERCENTAGE KNOCK DOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* AND WILD *AN. GAMBIAE* S.L. FROM SPRAY QUALITY CONE WALL BIOASSAYS CONDUCTED ON ALL SPRAYED SURFACES IN NJOBILBO, TSD, FLUDORA FUSION, MARCH 2020

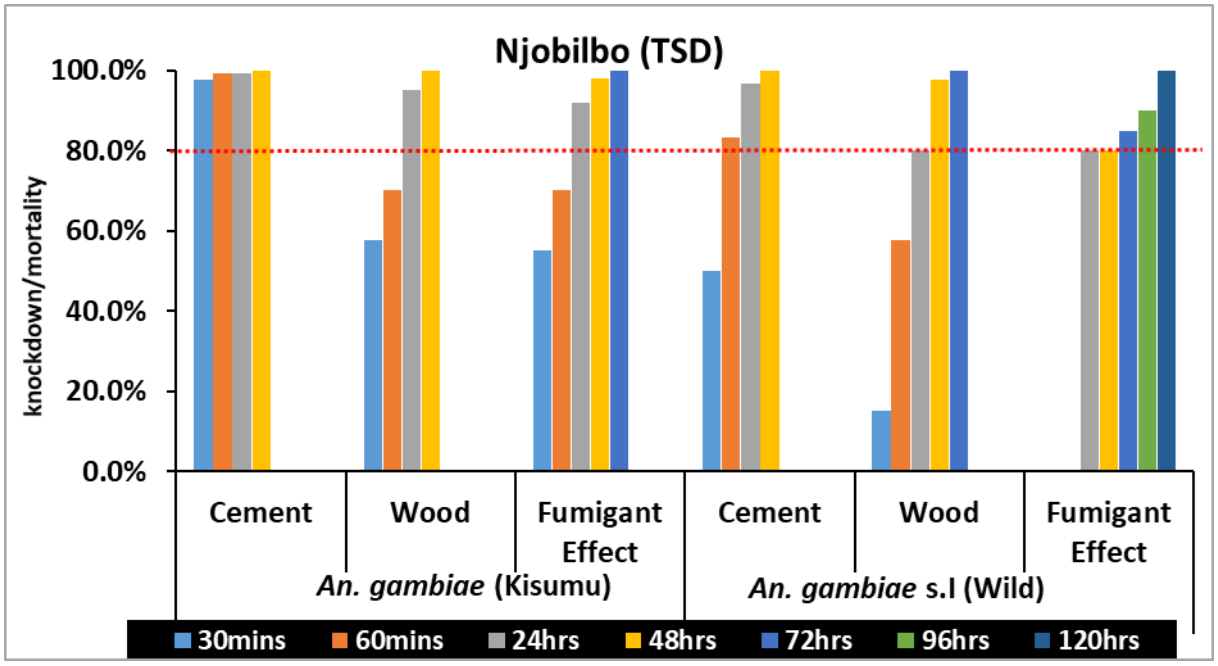
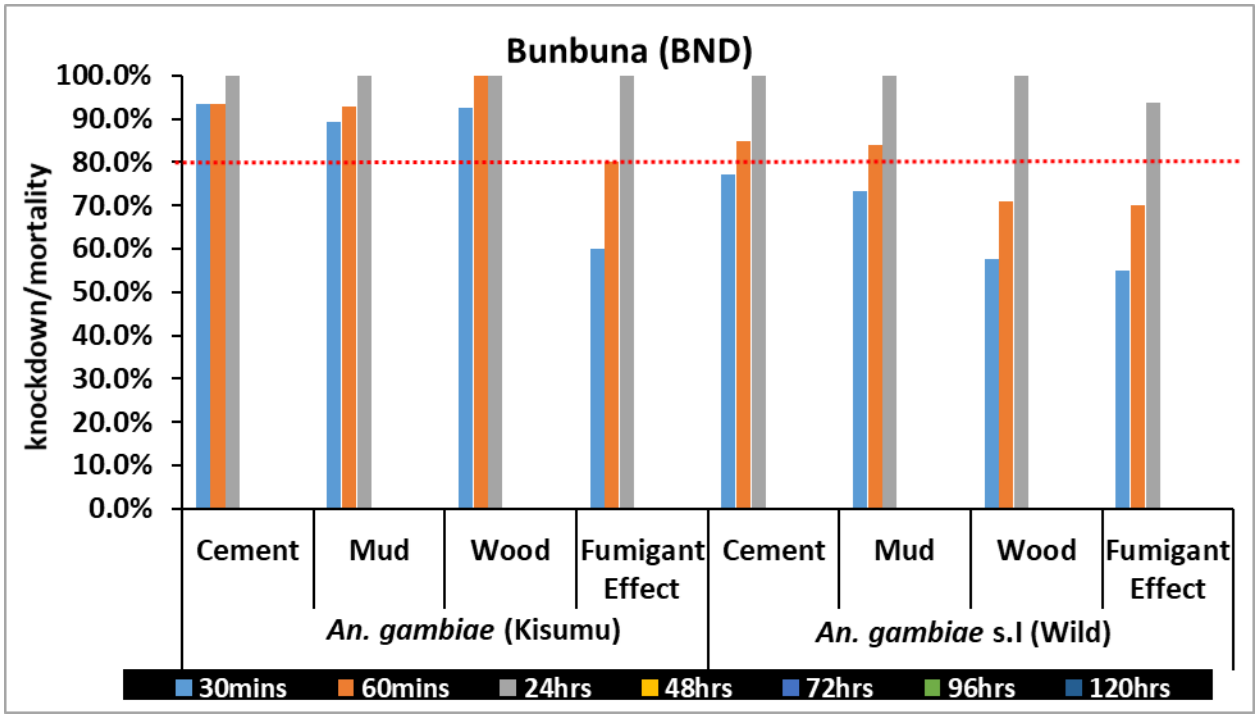


FIGURE A-6: MEAN PERCENTAGE KNOCK DOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* AND WILD *AN. GAMBIAE* S.L. FROM SPRAY QUALITY CONE WALL BIOASSAYS CONDUCTED ON ALL SPRAYED SURFACES IN BUNBUNA, BND, SUMISHIELD, MARCH 2020



[1] Community tested did not have mud surfaces

FIGURE A-7[2]: MEAN PERCENTAGE KNOCK DOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* FROM SPRAY QUALITY CONE WALL BIOASSAYS CONDUCTED ON ALL SPRAYED SURFACES IN BUNBUNA-NASUAN, YND, SUMISHIELD, MARCH 2020

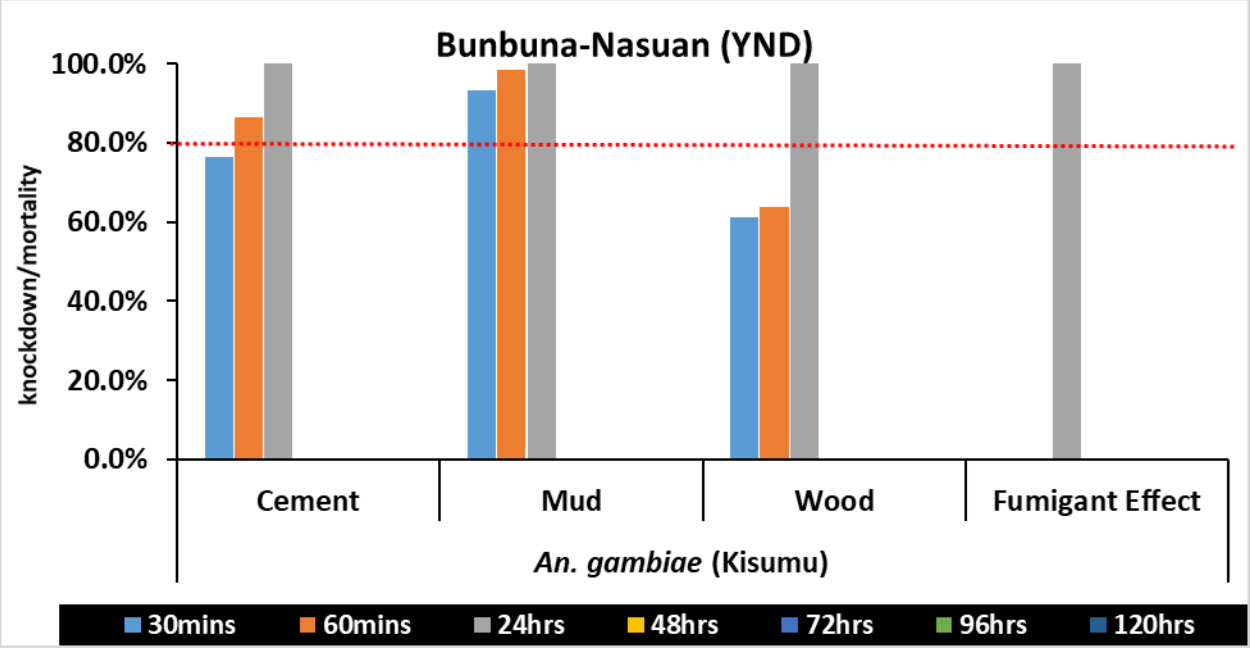
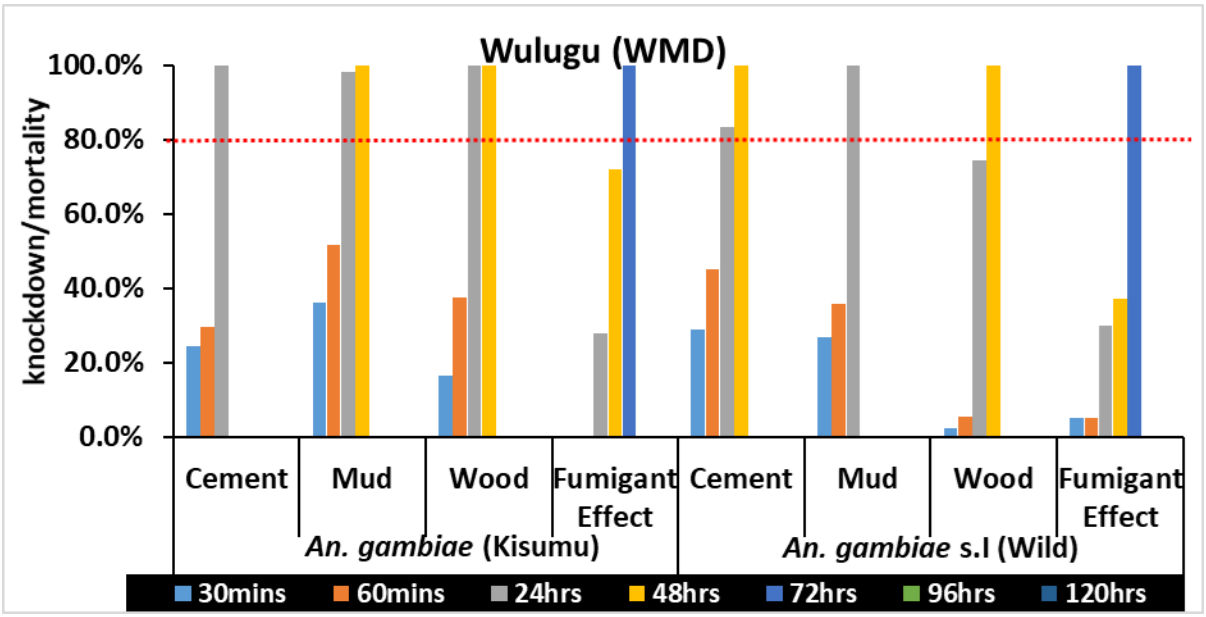


FIGURE A-8: MEAN PERCENTAGE KNOCK DOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* AND WILD *AN. GAMBIAE* S.L. FROM SPRAY QUALITY CONE WALL BIOASSAYS CONDUCTED ON ALL SPRAYED SURFACES IN WULUGU, WMD, SUMISHIELD, MARCH 2020



[2] Only Kisumu strain tested

FIGURE A-9: MEAN PERCENTAGE KNOCK DOWN 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 IN YIPILI NAA FON, KAD, SUMISHIELD, MARCH 2020

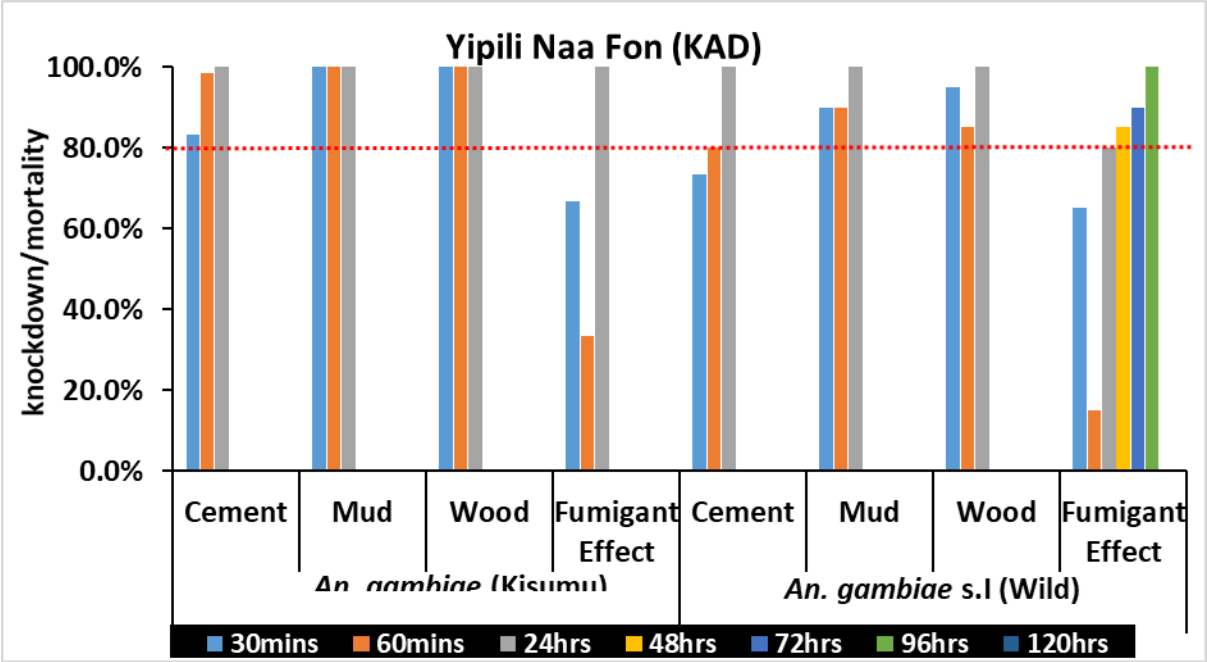
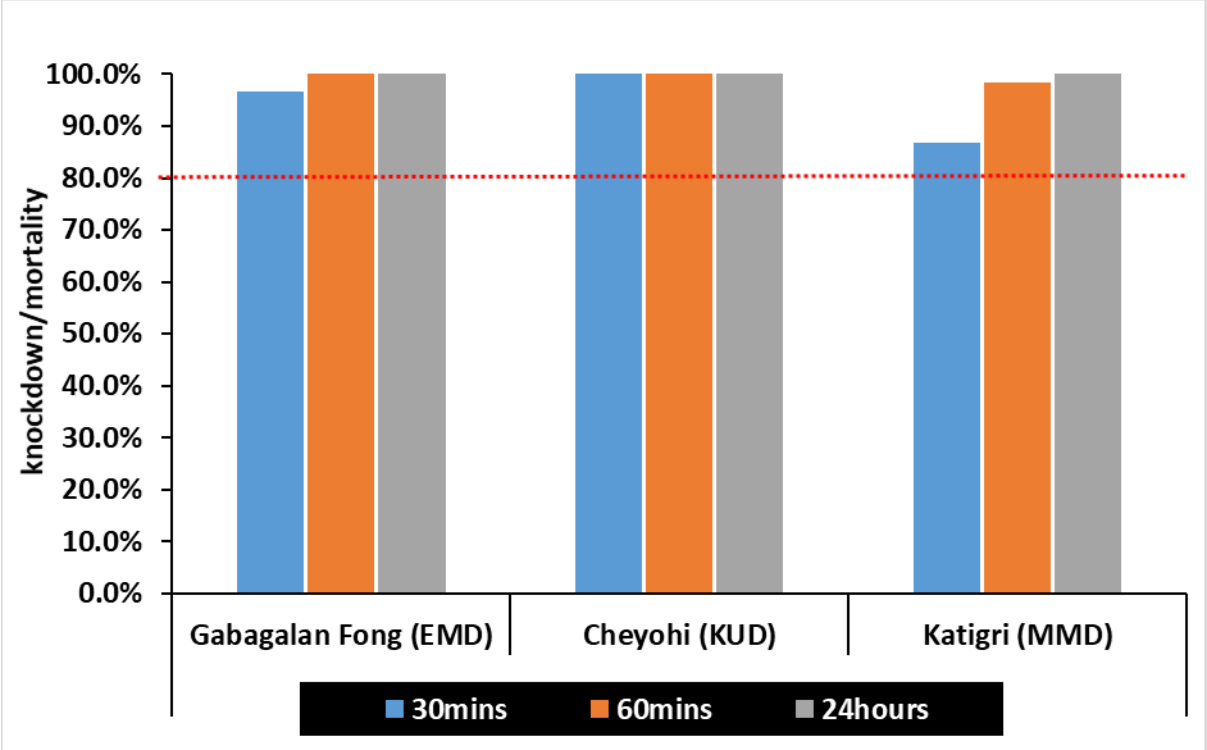


FIGURE A-10: MEAN PERCENTAGE KNOCK DOWN AND MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* FROM SPRAY QUALITY CONE WALL BIOASSAYS ON SPRAYED SURFACES IN ANIMAL SHELTERS IN EMD, KUD AND MMD, MARCH 2020



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