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THE PMI KINGA MALARIA PROJECT

KENYA ANNUAL ENTOMOLOGICAL MONITORING REPORT

AUGUST 2021-SEPTEMBER 2022

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ACRONYMS

CBS	Community-based (Entomological) Surveillance
CDC	Centers for Disease Control and Prevention
CDC-LT	CDC Light Trap
CHV	Community Health Volunteer
DNMP	Division of National Malaria Program
ELISA	Enzyme-linked Immunosorbent Assay
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Nets
kdr	knockdown resistance gene
ORP	Outdoor Resting Pot
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
PYR	Pyrethroid
SOP	Standard Operating Procedure
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

The PMI Kinga Malaria project conducted activities to evaluate the impact of the indoor residual spray (IRS) intervention of 2021/22 on entomological transmission indicators in western Kenya. Monthly entomological surveillance was carried out to monitor vector density, behavior, biting rates, biting times and location, resting sites, parity, and susceptibility of *An. gambiae* s.l. and *An. funestus* s.l. to insecticides used for public health. Resistance tests were performed using the discriminating doses, and intensity assays were performed for permethrin and deltamethrin. The project also conducted synergist assays for pyrethroid insecticides to assess if a metabolic mechanism of resistance is involved in sites where resistance to one or more insecticides is recorded. In the IRS counties of Homa Bay and Migori, the quality of spray operations and subsequent residual efficacy of the insecticide was assessed through wall cone bioassays. Monthly longitudinal monitoring was carried out in 18 sites across eight malaria endemic counties surrounding Lake Victoria in western Kenya, while insecticide resistance monitoring was done once a year in the eight counties.

The mean densities were highest in the non-IRS counties of Siaya and Kisumu. *An. funestus* s.l. was the primary vector across all sites combined, though the majority of this species was caught in Siaya. More than 95% of the catch in Siaya comprised *An. funestus* s.l., with *An. funestus* s.s. as the major sibling species. *An. leesoni,*a sibling species within the *An. funestus* group, was identified in three counties only: Busia, Homa Bay, and Kisumu. The predominant vector in the IRS counties of Homa Bay and Migori was *An. arabiensis* both before and after spraying. *An. gambiae* s.s. was the predominant vector in Bungoma, Busia, and Kakamega. The species composition of mosquito populations across the eight counties has remained consistent over the last 5 years. Most mosquitoes were captured using CDC light traps and pyrethrum spray catches, compared to outdoor collection methods, indicating that the vectors are predominantly endophilic.

Results from two rounds of community-based entomological surveillance (CBS) assessment demonstrate that Community Health Volunteers can be trained and gain skills in mosquito trapping, sample storage and transportation, vector identification and data management. Implementing CBS in additional sites could extend the coverage of existing entomological surveillance in high burden communities in Kenya.

No *Anopheles* mosquito was found with sporozoites in Migori county before or after the spray campaign of 2022, as in 2021. The sporozoite rate in Homa Bay was 2.2% (1/46) before the IRS campaign and dropped to 0.2% during the post-spray period. This is slightly higher compared to previous years (2019/20 and 2020/21) where no malaria mosquito captured in Homa Bay tested positive for sporozoites. However, the number of infected mosquitoes in Homa Bay remains lower than in non-IRS counties, indicating that IRS is still effective at controlling vector populations.

An. gambiae s.l. was resistant to the diagnostic doses of deltamethrin, permethrin, and alpha-cypermethrin in all eight counties, consistent with past observations across the counties. In all cases, pre-exposure to piperonyl butoxide resulted in higher mortality than exposure to the pyrethroids alone; there was full restoration in 7 cases (deltamethrin in Migori, Homa Bay, Kisumu, and Vihiga, permethrin in Migori and Homa Bay , and alpha-cypermethrin in Homa Bay) and partial restoration in 17 cases. The resistance intensity of *An. gambiae* s.l. to deltamethrin was low in Migori and Homa Bay and moderate in Kisumu, Siaya, Kakamega and Vihiga. Resistance intensity was high in Busia and Bungoma. Bioassay results showed that permethrin resistance was present in all eight sites, with less than 50% mortality at the diagnostic dose.

An. gambiae s.l. and *An. funestus* s.l., the main malaria vectors across all counties, are susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. Pirimiphos-methyl and clothianidin are the main active ingredients used in IRS products while chlorfenapyr is used in dual-active insecticide-treated nets (ITNs). Susceptibility of major vectors to chlorfenapyr suggests that ITNs treated with this insecticide, such as Interceptor G2, can be effective in Kenya. Continuous monitoring of these insecticides is crucial, as pirimiphos-methyl (Actellic 300CS) will be used in the 2023 and 2024 spray campaigns.

SumiShield and Fludora Fusion were used during the IRS campaign of 2022 in Homa Bay and Migori, respectively. The results show a high insecticidal effect against wild pyrethroid-resistant *An. funestus* mosquitoes and residual efficacy (>80% mortality) on mud and concrete wall substrates lasting between 5 and 6 months. In the previous campaign (2020/21), Fludora Fusion provided longer residual activity (9 months) on all surfaces than did Actellic 300CS, which lasted 4 to 6 months. Nevertheless, both insecticides are suitable for IRS to reduce mosquito densities and indoor transmission of malaria in Kenya as they offer protection for the length of the main transmission season from April to July. The major malaria mosquitoes across the 8 counties are susceptible to active ingredients used in IRS formulations (clothianidin and pirimiphos-methyl). In line with the Kenya Insecticide Resistance Management Plan, the two insecticide classes will be rotated, thus neonicotinoids will be sprayed in 2023 and organophosphates in the campaign of 2024.

I. INTRODUCTION

Malaria remains a major problem in Kenya, with the western part of the country bearing the highest burden. The country is divided into different epidemiological zones based on malaria endemicity. Counties in western Kenya region fall under the lake endemic zone where malaria prevalence was recently reported at 19% (DNMP 2021). Among the prevalent malaria cases identified during the 2020 Kenya Malaria Indicator Survey (KMIS), *P falciparum* was identified in 96% of all cases and *P. malariae* was identified in 23%, with mixed infections of *P. falciparum* and *P. malariae* representing 19% of the overall burden. The main malaria vectors in Kenya are *An. arabiensis*, *An. gambiae* s.s., and *An. funestus* s.s. (PMI VectorLink Kenya 2021).

The U.S. President's Malaria Initiative (PMI) Kinga Malaria Project is funded by the United States Agency for International Development, through PMI. PMI Kinga Malaria builds on the entomological surveillance activities implemented under the predecessor PMI VectorLink Kenya Project. PMI Kinga Malaria supports longitudinal vector surveillance and insecticide resistance activities in eight counties in western Kenya.

The current Kenya Malaria Strategy 2019–2023 is based on the vision of achieving a malaria-free Kenya with a goal of reducing malaria incidence and deaths by 75% of 2016 levels by 2023 (NMCP 2019). Efforts have been made to control malaria including provision of insecticide-treated nets (ITNs), indoor residual spraying (IRS), and larval source management (LSM) as a supplementary intervention. The Division of National Malaria Program (DNMP) implemented a mass net campaign in June 2021 in 27 counties, including all eight lake-endemic counties. Piperonyl butoxide (PBO)-pyrethroid ITNs were distributed for the first time in Kenya in three counties: Bungoma, Busia, and Kakamega. Other counties received standard pyrethroid-only ITNs. IRS has been implemented each year in Migori since 2017 and Homa Bay beginning in 2018. Following the insecticide resistance management strategy (DNMP, 2020), neonicotinoid-based insecticides were used in the IRS campaign implemented in March and April in 2022. In this campaign, Migori received Fludora Fusion and Homa Bay received SumiShield. Larval source management is a supplementary intervention implemented in January through to December 2022. The DNMP in collaboration with the Cuban government implemented the Cuba-Kenya Vector Control Collaboration Project for malaria in eight lake endemic counties in western Kenya. Two biolarvicides were used namely: Bactivec® (*Bacillus thuringiensis var israelensis* SH -14 266/2) and Griselesf® (*Bacillus sphaericus* strain 2362). The larvicides are produced by Labiofam Entrepreneurial Group of Cuba.

The project conducted monthly entomological surveillance to monitor vector density, behavior, biting rates, biting times and location, resting sites, parity, and susceptibility of *An. gambiae* s.l. and *An. funestus* s.l. to insecticides used for public health. Resistance tests were performed using the discriminating dose. Data generated from resistance tests inform the choice of insecticides for vector control in Kenya. Intensity assays were also performed for permethrin and deltamethrin. The project also performed synergist assays for pyrethroid insecticides to assess if a metabolic mechanism of resistance is involved in sites where resistance to one or more insecticides is recorded. In the IRS counties of Migori and Homa Bay, the quality of spray operations and subsequent residual efficacy of the insecticide was assessed through wall bioassays.

2. METHODOLOGY

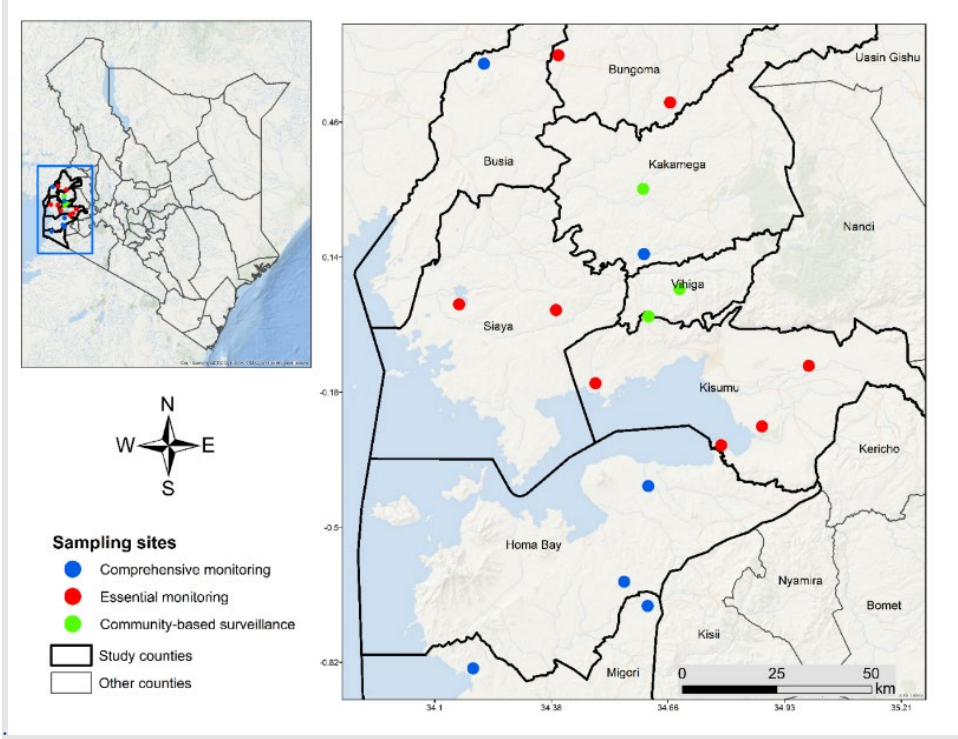
2.1 SURVEILLANCE SITES

Monthly entomological data were collected to monitor vector density species composition and behavior, and to evaluate the impact of IRS and ITN interventions on *Anopheles* mosquitoes. Entomological monitoring was carried out in 17 sites across the eight counties. Two counties (Migori and Homa Bay) have received IRS for the past 5 years. Neonicotinoids were used for the IRS in 2022. Bungoma, Busia, and Kakamega counties received PBO-pyrethroid nets during the mass net campaign of 2021. Kisumu, Siaya, and Vihiga received standard-pyrethroid ITNs in the most recent mass net campaign.

Comprehensive vector monitoring was implemented in four counties receiving either PBO ITNs or IRS: Migori (two sites), Homa Bay (two sites), Kakamega (one site), and Busia (one site) (Figure 1). Essential monitoring was conducted in the remaining four counties: Kisumu (four sites), Vihiga (two sites), Siaya (two sites), and Bungoma (two sites). Comprehensive monitoring included the collection of a suite of indicators including species composition, density, biting location (indoors or outdoors), resting location (indoors or outdoors), and parity rates, while essential monitoring includes species composition, human biting rate, and indoor resting density.

Three sites in two counties (two sites in Vihiga and one site in Kakamega) used a community-based entomological surveillance (CBS) approach. CBS was performed by county entomology teams under supervision of the PMI Kinga Malaria field technicians. Mosquito collections were performed using solar-powered CDC light traps (CDC-LTs) in selected houses in sentinel sites to monitor species composition and vector density as a proxy for the human biting rate (Figure 1).

Figure 1: Map of Kenya Showing Location of Sentinel Sites



2.2 LONGITUDINAL BIONOMICS

Adult mosquitoes were collected every month from December 2021 to September 2022. Three sampling methods were used: CDC-LTs, pyrethrum spray catches (PSCs), and outdoor resting pots (ORPs). All three methods were used in Migori, Homa Bay, Kakamega (Khwisero sub-county), and Busia. In Kisumu, Siaya, Bungoma, only CDC-LTs (indoors and outdoors) and PSCs were used. In Vihiga and Kakamega (Mumias East sub-county) only CDC-LTs set up indoors were used. Captured mosquitoes were identified to species/species complex based on morphological features using the anopheline key (Coetzee 2020).

Table 1: Entomology Surveillance Sites and Details of Data Collection

County	Sub-county	Sentinel Site	Intervention Status		Date Site Introduced	Data Collected Monthly	Data Collected Annually
			2021	2022			
Migori	Rongo	Sumba	IRS (Fludora Fusion) + PYR ITNs	IRS (Fludora Fusion) + PYR ITNs	December 2015	Monthly vector biting rates (indoors and outdoors), resting densities, species composition, sporozoite rates, cone bioassay	Insecticide resistance at Kowuoth and Nyochanda Riat
	Nyatike	Sori-Karungu	IRS (Fludora Fusion) + PYR ITNs	IRS (Fludora Fusion) + PYR ITNs	July 2016		
Homa Bay	Homa Bay	Imbo	IRS (Fludora Fusion) + PYR ITNs	IRS (SumiShield) + PYR ITNs	July 2016	Monthly vector biting rates (indoors and outdoors), resting densities, species composition, sporozoite rates, cone bioassay	Insecticide resistance at Nyambare and Otok Kamboya
	Rachuonyo North	Pap	IRS (Fludora Fusion) + PYR ITNs	IRS (SumiShield) + PYR ITNs	December 2017		
Kisumu	Nyando	Ahero	PYR ITNs	PYR ITNs	December 2017	Monthly vector biting rates (indoors and outdoors), resting densities, species composition, sporozoite rates	Insecticide resistance at Ahero
	Seme	Kirindo	PYR ITNs	PYR ITNs	December 2017		
	Nyakach	Sango Rota	PYR ITNs	PYR ITNs	December 2017		
	Mhoroni	Masogo	PYR ITNs	PYR ITNs	December 2017		
Siaya	Gem	Dienya	PYR ITNs	PYR ITNs	October 2018	Monthly vector biting rates (indoors and outdoors), resting densities, species composition, sporozoite rates	Insecticide resistance at Kadenge and Bondo
	Alego Usonga	Kadenge	PYR ITNs	PYR ITNs	October 2018		
Busia	Teso South	Odioi	PBO-PYR ITNs*	PBO-PYR ITNs*	October 2018	Monthly vector biting rates (indoors and outdoors), resting densities, species composition, sporozoite rates	Insecticide resistance at Odioi
Bungoma	Bumula	Kimaiti	PBO-PYR ITNs*	PBO-PYR ITNs*	October 2018	Monthly vector biting rates (indoors and	

County	Sub-county	Sentinel Site	Intervention Status		Date Site Introduced	Data Collected Monthly	Data Collected Annually
			2021	2022			
	Kanduyi	Mechimeru	PBO-PYR ITNNs*	PBO-PYR ITNNs*	July 2019	outdoors), resting densities, species composition, sporozoite rates	Insecticide resistance at Kimaiti
Kakamega	Khwisero	Buhili	PBO-PYR ITNs*	PBO-PYR ITNs*	April 2019	Monthly indoor vector biting rates (indoors and outdoors), resting densities, species composition, sporozoite rates	Insecticide resistance at Iguhu
	Mumias East	Eshiakulo	PBO-PYR ITNs*	PBO-PYR ITNs*	April 2019	Monthly CBS. Monthly indoor vector biting rates, species composition, sporozoite rates	
Vihiga	Vihiga	Busamo	PYR ITNs	PYR ITNs	April 2019	Monthly CBS. Monthly indoor vector biting rates, species composition, sporozoite rates	Insecticide resistance at Emakakh
	Luanda	Ebulakho	PYR ITNs	PYR ITNs	April 2019		

Note: PYR=pyrethroid
*PBO-pyrethroid ITNs were introduced during the mass net campaign in June 2021.

2.2.1 Monitoring Densities of Mosquitoes Resting Indoors

PSCs were performed in seven houses per site per month according to PMI VectorLink Standard Operating Procedure (SOP) 03/01 and using insecticidal spray containing 0.025% pyrethrum emulsifiable concentrate mixed with 0.1% PBO in kerosene.

2.2.2 Monitoring Densities of Mosquitoes Resting outdoors

Outdoor resting collections were performed using ORPs paired with the houses selected for PSCs (i.e., seven houses per month per site). Pots made of clay soil were sourced locally and placed in shaded areas within 5 meters of the house, in accordance with SOP 13/01. Each house received four clay pots. Mosquitoes were retrieved from the trap in the morning using mouth aspirators and transferred into paper cups.

2.2.3 Human Biting Rate Indoors

CDC-LT collections were carried out indoors in 10 houses per month per site following guidelines in the SOP 01/01.

2.2.4 Human Biting Rate Outdoors

Outdoor mosquito collections were performed monthly near one house at each site. One CDC-LT was hung outdoors approximately 10 meters from a house at 1.5 meters above the ground, next to a sleeper protected by an untreated bed net in accordance with SOP 01/01. This set-up was protected from the rain by a tarpaulin that was set up at 18:00h and from which mosquitoes were retrieved at 06:00h.

2.3 MONITORING FOR INSECTICIDE RESISTANCE

Insecticide susceptibility assays were carried out using wild-caught *An. gambiae* s.l. and *An. funestus* s.l. mosquitoes. *An. gambiae* s.l. larvae were collected from larval habitats in one site per county and reared to adults at the Kenya Medical Research Institute's insectary. Due to challenges associated with finding *An. funestus* s.l. larval habitats, adult *An. funestus* s.l. were sampled from houses using backpack aspirators in Siaya and Bungoma counties. The following insecticides were tested in tube tests, according to World Health Organization guidelines: permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), and pirimiphos-methyl (0.25%). CDC bottle assays were used to measure intensity of pyrethroid resistance (using 1×, 5×, and 10× the diagnostic concentrations), to measure susceptibility to chlorfenapyr (100µg/mL) and to clothianidin (4 µg/mL + 800ppm MERO). To identify a possible role of metabolic detoxification in pyrethroid resistance, synergist assays using a pre-exposure to PBO before exposure to pyrethroids were conducted in accordance with SOP 06/01.

At least 100 mosquitoes were exposed to each insecticide in four replicates of 25 mosquitoes each. Mosquitoes were then transferred to the holding tube (tube tests) or paper cups (bottle assays) with cotton wool soaked in sugar solution. Mortality was scored 24 hours after exposure except for chlorfenapyr where mortality was monitored up to three days and clothianidin where mortality was monitored up to 5 days).

2.4 QUALITY, RESIDUAL EFFICACY, AND FUMIGANT EFFECT OF IRS

2.4.1 QUALITY ASSURANCE AND INSECTICIDE RESIDUAL EFFICACY

Cone bioassays using wild *An. funestus* s.l. from Siaya were conducted from February (Migori) and from March (Homa Bay) to December 2021 to assess the residual efficacy of Actellic 300CS and Fludora Fusion in the 2021 IRS campaign.

Additionally, the quality, residual efficacy, and fumigant effect of IRS was assessed for the 2022 IRS campaign. The campaign began in March 2022 in Homa Bay and April in Migori county. Spray quality was assessed within the first week of the spray campaign in two sites in each sprayed county (T0 was March 2022 for Homa Bay and T0 was April for Migori). Cone bioassays were conducted in 20 eligible structures sprayed with Fludora Fusion in two villages, Kuna A and Nyochanda B (seven mud and three concrete houses in each village), in Migori county and another 20 eligible structures sprayed with SumiShield in two villages, Lower Kanyangwena and Kayoo, in Homa Bay county. Wall cone bioassays were conducted according to SOP 09/01) to assess the quality of work done by the different spray teams in each county.

Cone bioassays using wild *An. funestus* s.l. from Siaya were carried out from March to May 2022 to assess the residual efficacy of Fludora Fusion (Migori) and SumiShield (Homa Bay) in the 2022 IRS campaign. Residual efficacy of insecticide was determined monthly using the wall cone bioassays with wild *An. funestus* s.l. mosquitoes. The cone bioassays were performed on two main types of sprayed surfaces: mud walls and concrete walls. Bioassays were carried out following SOP 09/01, until mortality fell below 80% for two consecutive months.

Three cones were set up in each eligible structure on different walls at different heights: 0.5m, 1.0m, and 1.5m. Wild *An. funestus* s.l. mosquitoes were exposed to treated walls in batches of 10 mosquitoes in each cone for 30 minutes. In each structure, one control cone was set up approximately 5m outside the structure; the cone contained 10 mosquitoes exposed to an untreated surface made of cardboard. The cardboard was placed in a shaded spot. Knock-down was recorded at 30 minutes while mosquitoes were still in the cone. After exposure, mosquitoes were provided with sugar solution and held at 25±2°C temperature and 80±10% relative humidity during the observation period. Knockdown was recorded again 30 minutes later (KD60), and delayed mortality was recorded daily for up to 5 days post exposure.

2.4.2 FUMIGANT EFFECT

The fumigant effect of the Fludora Fusion and SumiShield on *An. funestus* s.l. was assessed within one week of spraying, using wire cages placed 10 cm away from the sprayed wall surfaces. Knockdown was observed and

recorded after 30 minutes and 60 minutes, and mortality recorded after 24 hours. Temperature and relative humidity were recorded for the duration of the experiment. Fumigant effect bioassays were carried out monthly until 24-hour mortality fell below 50% for 2 consecutive months.

2.5 Molecular Analysis

2.5.1 Molecular Species Identification

Legs and wings of morphologically identified *An. gambiae* s.l. mosquitoes were used for polymerase chain reaction (PCR) analyses to identify the sibling species of *An. gambiae* s.l. complex (primarily *An. gambiae* s.s. and *An. arabiensis*) and *An. funestus* s.l. group (*An. funestus*, *An. rivulorum*, *An. leesoni*, and *An. parensis*). PCR assays were performed following protocols developed by Scott (1993) for *An. gambiae* s.l. and Koekemoer (2002) for *An. funestus* s.l. group.

2.5.2 Detection of *Plasmodium* Sporozoites

Presence of sporozoites in female *Anopheles* mosquitoes captured using all trapping methods was determined by Enzyme-Linked Immunosorbent Assay (ELISA) on the head and thorax of individual mosquitoes following the MR4 protocol adapted from Wirtz et al. (1992).

2.5.3 Genotyping of Molecular Markers of Insecticide Resistance

Molecular assays were carried out to identify knockdown resistance (*kdr)* gene mutations in mosquitoes. *An. gambiae* s.l. mosquitoes were assayed to determine the presence of the West (L1014F) and East African (L1014S) *kdr* mutations.

2.6 Community-Based Surveillance Assessment

An assessment of CBS was carried out in three sites (Mumias East in Kakamega and Vihiga and Luanda in Vihiga). A total of six community health volunteers (two per site) underwent evaluations in June 2022 and September 2022. Both qualitative and quantitative methods were used in the assessment. Quality inspections of performance standards were conducted by the PMI Kinga Malaria entomology staff to ensure the health volunteers followed SOPs and set up CDC-LTs correctly following the trapping schedule.

In the quantitative assessments, the community health volunteers and the PMI Kinga Malaria project field team performed independent mosquito collections over six nights in the same sites using CDC-LT. Mosquito densities captured by the two teams were compared. The teams independently performed morphological identification on their mosquito samples. The PMI Kinga Malaria team cross-identified mosquitoes collected by community health volunteers to determine whether anophelines and culicines were correctly identified.

2.7 Data Management

All data were recorded in Open Data Kit (ODK) software installed on smartphones and subsequently uploaded to the project database. Individual mosquitoes were labelled with pre-printed barcodes and linked to the field data by house code and study number. The sporozoite rate was estimated as the proportion of mosquitoes positive for sporozoite ELISA of the total number of mosquitoes screened for sporozoites. Biting rate was estimated using CDC-LTs as the number of mosquitoes collected divided by the total number of trap nights. To estimate the entomologic inoculation rate, the biting rate was multiplied by the sporozoite rate.

3. RESULTS

3.1 MALARIA VECTOR SPECIES COMPOSITION

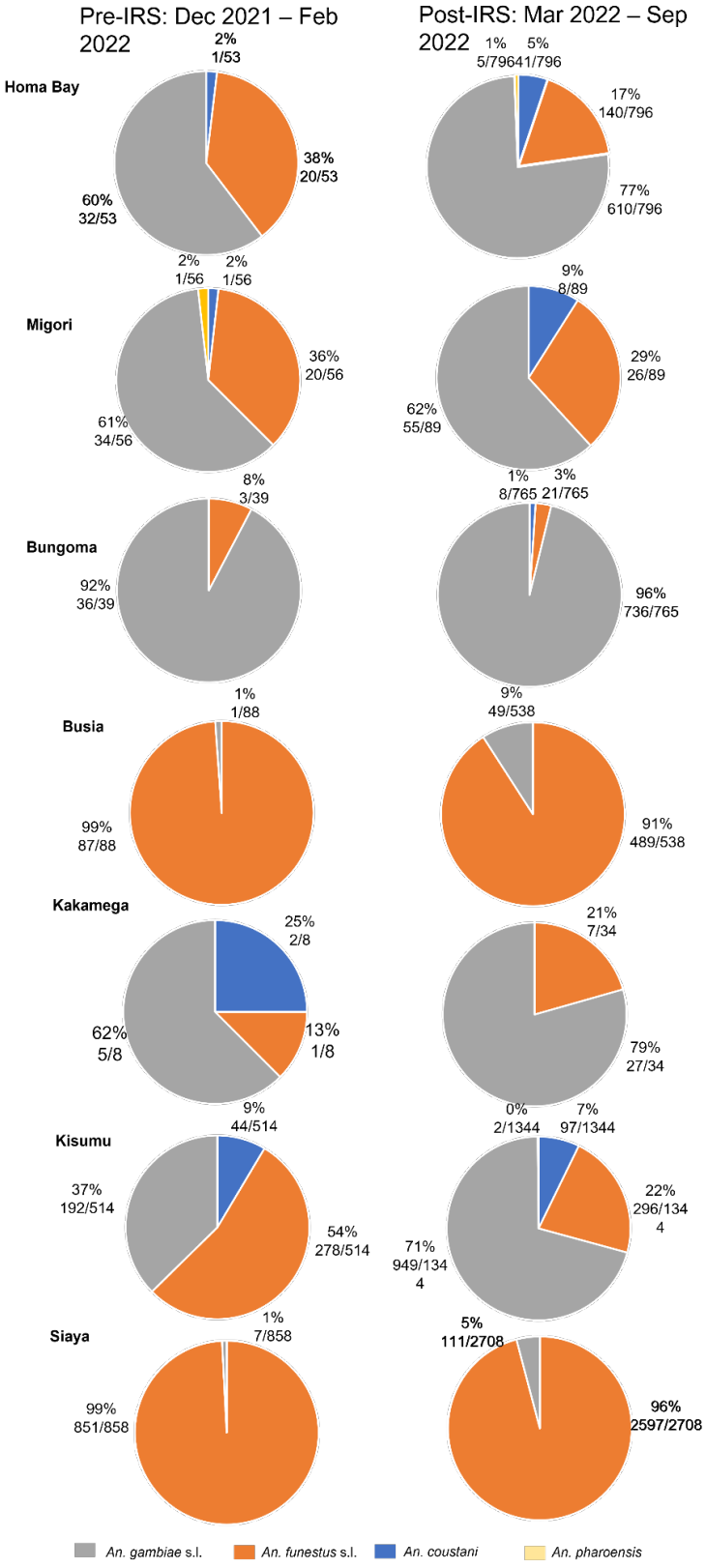
During the reporting period, a total of 7,890 mosquitoes from four species were collected using CDC-LTs, PSCs, and ORPs. *An. funestus* s.l. was the most abundant species (n=4,836, 61%), followed by *An. gambiae* s.l. (n=2,844, 36%) and small numbers of *An. coustani* (n=202, 2.6%) and *An. pharoensis* (n=8, 0%) (Figure 2). Most *An. funestus* s.l. (n=3448) were collected in Siaya county alone. The numbers are less than those reported in the previous annual entomological reports of 2019/20 and 2018/19.

To facilitate comparisons, data were pooled into two time periods: pre-IRS (December 2021 to February 2022) and post-IRS (March 2022 to September 2022). Of the 7,890 mosquitoes captured during the reporting period, 1,616 were captured before spray and 6,274 after spray.

The total number of mosquitoes captured in the IRS county of Migori was low during both pre- and post-spray periods. However, this trend was not the same in Homa Bay, as there was a marked increase in mosquito densities during the post-spray period. This result differs from previous reports that showed low densities before and after spray campaigns in both IRS counties.

Though mosquito densities varied across counties, higher mosquito numbers were always captured after the spray campaign compared to the pre-spray period. The relative proportions of anophelines changed in Kisumu during pre- and post- IRS periods. *An. gambiae* s.l. was the predominant vector during the pre-IRS period, while *An. funestus* s.l. was prevalent in the post-IRS period. This pattern is consistent with results of Kisumu in the previous year. Proportions of anophelines did not change in other counties.

Figure 2: Mosquito Species Composition of Combined Indoor CDC-LT and PSC Collections in Seven Counties, Pre- and Post-IRS 2022



A total of 3,850 samples were analyzed for identification of sibling species. Of these, 575 samples were from the pre-IRS period and 3,274 from the post-IRS period (Table 2). A total of 2,730 *An. gambiae* s.l. complex and 1,052 *An. funestus* s.l. group specimens were successfully amplified and identified to species using PCR. Seventy-seven (2%) samples did not amplify. Of the *An. gambiae* s.l. complex, *An. gambiae* s.s. was the predominant species in Bungoma, Busia, Kakamega, and Siaya, while *An. arabiensis* was prevalent in Homa Bay, Kisumu, and Migori. IRS targets indoor resting mosquitoes and seems to control *An. gambiae* s.s. compared to *An. arabiensis,* as evidenced by the high proportion of *An. arabiensis* in the IRS sites.

An. funestus s.l. were recorded in all sites, of which *An. funestus* s.s. were by far the most common. *An. leesoni* was less prevalent though found in all sites except Kakamega, Migori, and Siaya. Confirmatory repeat sample testing was performed on 55 *An. leesoni* mosquitoes. All samples retested were confirmed as *An. leesoni.* Samples identified as *An. leesoni* were tested with *An. gambiae* primers but did not amplify.

Table 2: PCR Identification of Members of the *An. gambiae* s.l. and *An. funestus* s.l. species Complexes in Seven Counties

Period	Species	Bungoma	Busia	Homa Bay	Kakamega	Kisumu	Migori	Siaya	Total
Pre-IRS	*An. arabiensis*	4 (15%)	1 (100%)	20 (100%)	2 (67%)	150 (99%)	28 (97%)	5 (71%)	210
	An. gambiae s.s.	23 (85%)	0	0	1	1 (1%)	1 (3%)	2 (29%)	28
	An. funestus s.s.	3	28	18	1	110	18	147	325
	An. leesoni	0	5	3	0	3	0	0	11
	Unidentified	0	0	0	0	0	1	0	1
Post-IRS	*An. arabiensis*	104 (15%)	21 (16%)	433 (82%)	5 (23%)	776 (85%)	28 (78%)	44 (32%)	1411
	An. gambiae s.s.	574 (85%)	158 (84%)	94 (18%)	17 (77%)	138 (15%)	8 (22%)	92 (68%)	1081
	An. funestus s.s.	8	110	72	1	175	9	301	676
	An. leesoni	0	38	0	0	2	0	0	40
	Unidentified	5	4	19	1	13	5	19	66

Note: Percentages in brackets were calculated using only specimens positively identified as *An. gambiae* s.l. as the denominator

3.2 SEASONAL VECTOR DENSITY

3.2.1 INDOOR DENSITIES (CDC-LT AND PSC)

The density of *An. gambiae* s.l. collected by CDC-LT peaked in the rainy season in May in both IRS (4.8 mosquitoes/house/night in Homa Bay) and non-IRS counties (25.0 mosquitoes/house/night in Bungoma). There was a peak in *An. funestus* s.l. numbers in June / July, most notably in Homa Bay and Siaya (Figure 3). Densities of *An. funestus* s.l. and *An. gambiae* s.l. in Homa Bay and Migori counties were lower (less than 4 mosquitoes/house/night) compared to non-IRS counties. The densities of *An. gambiae* s.l. and *An. funestus* s.l. were lower in the PBO ITN sites in Kakamega and Busia (less than 4 mosquitoes/house/night) compared to Kisumu where pyrethroid only ITNs were distributed.

Densities of *An. gambiae* s.l. remained low (less than 5.0 mosquitoes/house/night) before March 2022 across non-IRS counties (Figure 4). An increase in numbers was observed in March after the rains and a decline from June to September. Bungoma recorded the highest densities of *An. gambiae* s.l. mosquitoes with a peak in May 2022. The density of *An. funestus* s.l. in non-IRS counties was highest in Siaya, in June. *An. funestus* s.l. densities were low in other counties, at less than 6.0 mosquitoes/trap/night. Data presented in Figure 3 show results for all eight counties. Figure 4 shows results for IRS counties and has been re-scaled to allow visualization of the trends in densities pre- and post-IRS.

Figure 3: Mean Monthly Densities of *Anopheles* Mosquitoes Collected in all Counties Using CDC-LTs, December 2021–September 2022

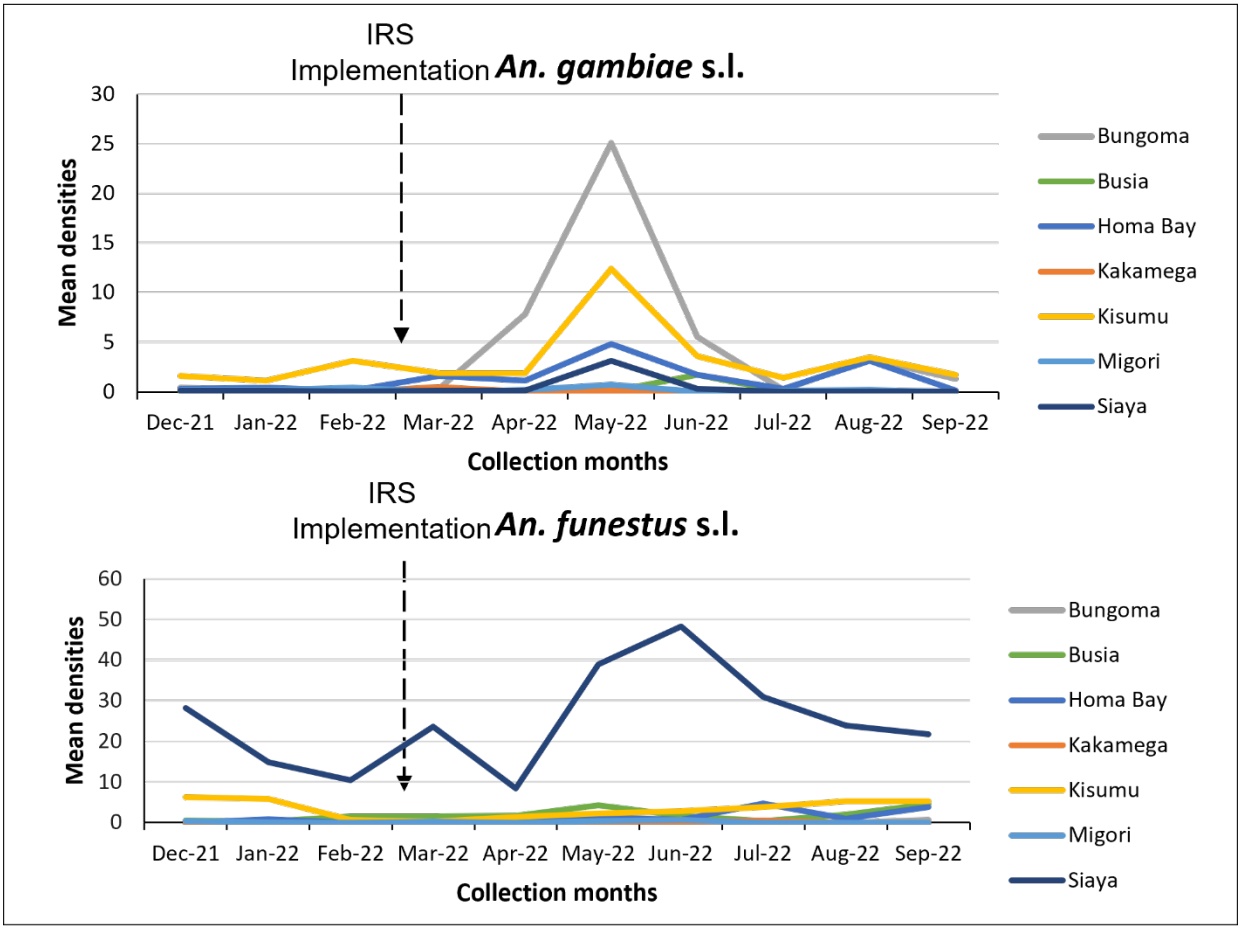
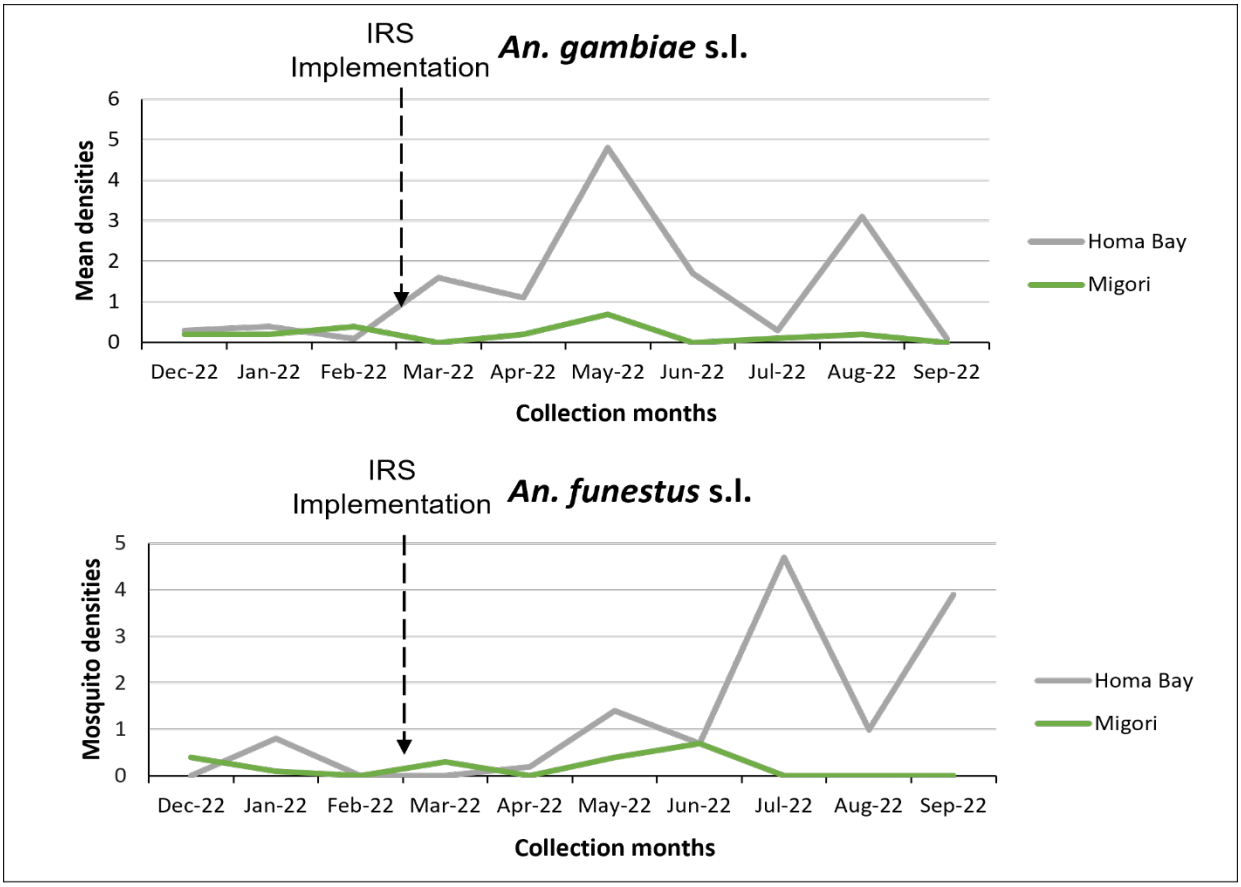


Figure 4: Mean Monthly Densities of *Anopheles* Mosquitoes Collected in IRS Counties of Migori and Homa Bay Using CDC-LTs, December 2021–September 2022



The indoor resting density for *An. gambiae* s.l. was highest in Kisumu in April 2022 (40 mosquitoes/house/night) followed by Bungoma (19 mosquitoes/house/night) (Figure 5). Mosquito densities remained low in other counties across the months. Two peaks in indoor resting density for *An. funestus* s.l. were observed in Siaya (February and July 2022) and Bungoma (January and July 2022). The indoor resting density of *An. gambiae* s.l. in Homa Bay county peaked in March 2022 (Figure 6).

Figure 5: Mean Monthly Densities of *Anopheles* Mosquitoes Collected in All Counties Using PSC, December 2021–September 2022

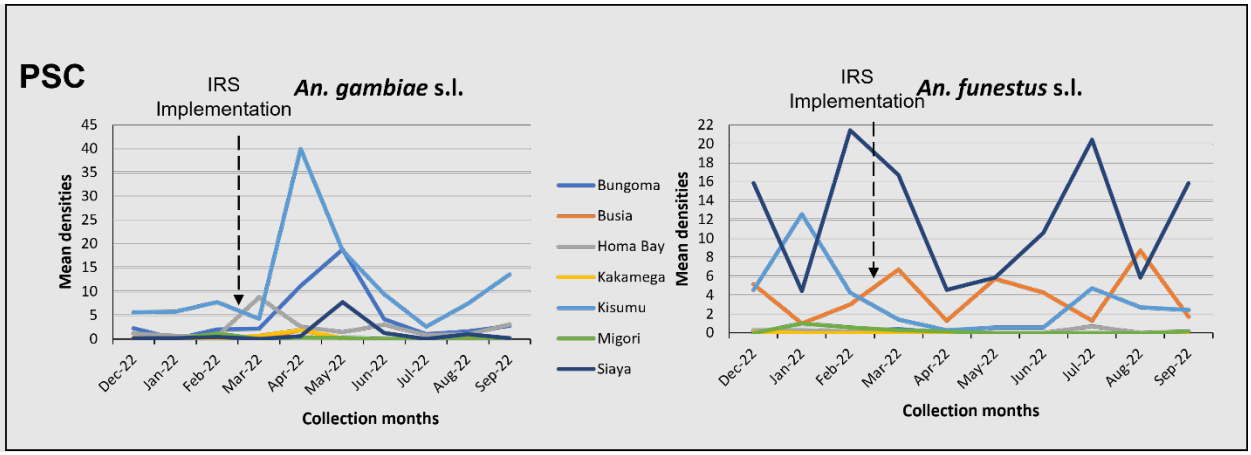
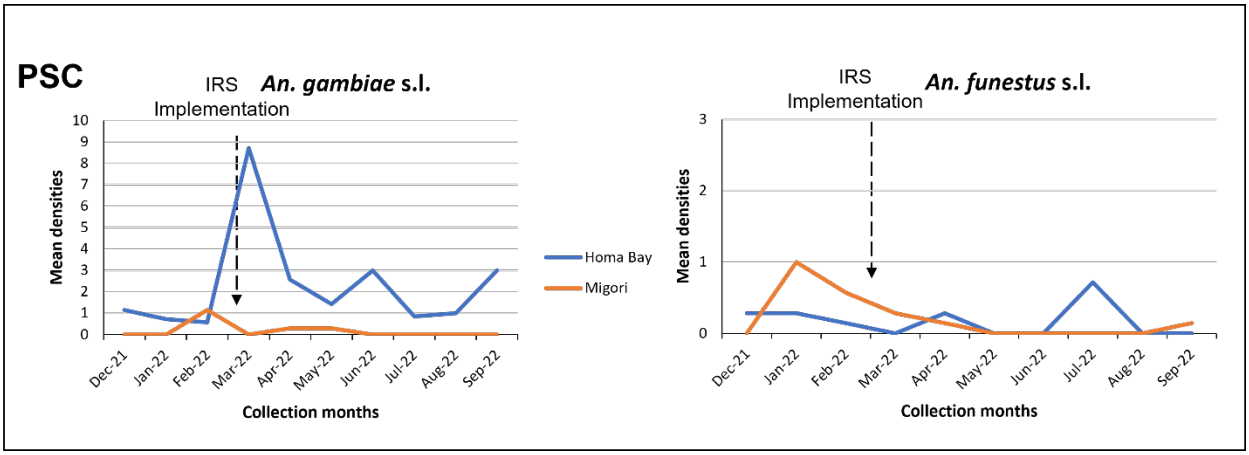


Figure 6: Mean Monthly Densities of *Anopheles* Mosquitoes Collected in IRS Counties Of Homa Bay and Migori Using PSC, December 2021–September 2022

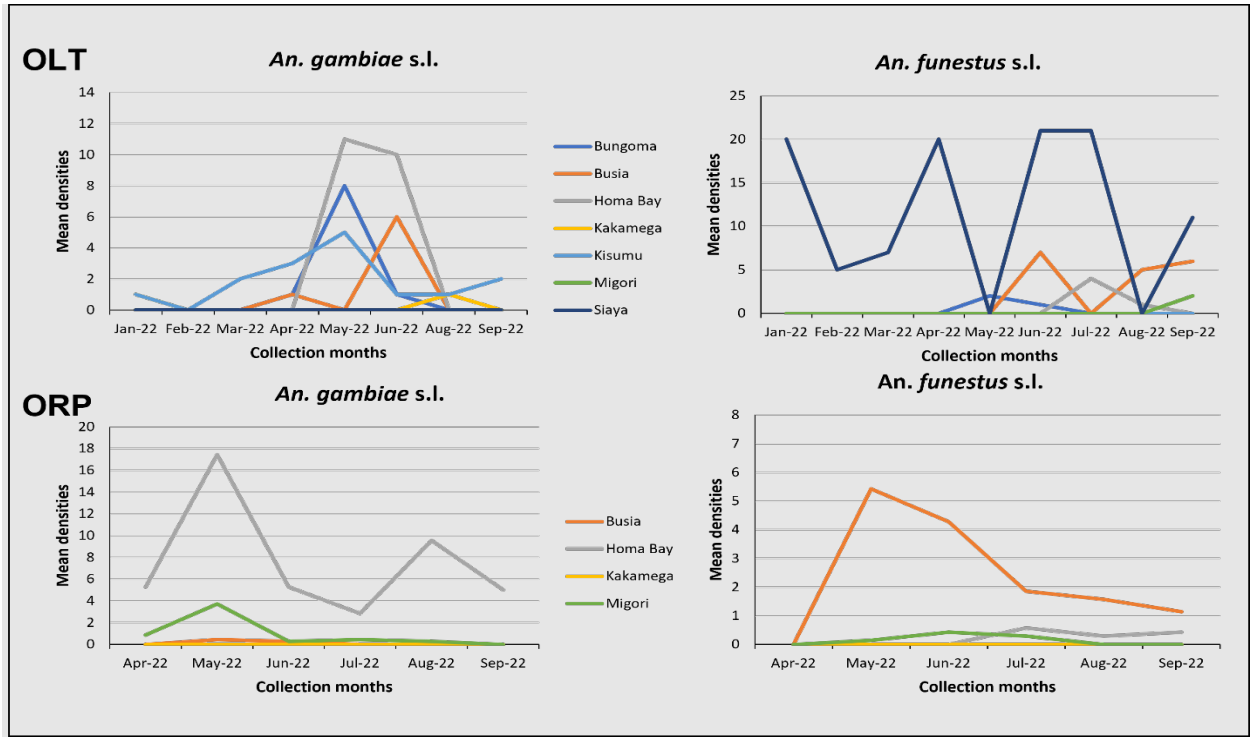


3.2.2 OUTDOOR DENSITIES

Homa Bay recorded the highest densities of *An. gambiae* s.l. (11 mosquitoes/house/trap) in May 2022. The highest number of *An. funestus* s.l. collected outdoors using CDC-LTs occurred in Siaya in June and July, with mean density of 21 mosquitoes/trap/night (Figure 7).

The density of female *Anopheles* mosquitoes captured outdoors varied between outdoor CDC-LTs and ORPs. Collections using ORPs were carried out between April and September 2022 in counties that received IRS and PBO nets. In Homa Bay, ORPs yielded the highest mean density of *An. gambiae* s.l. (17 mosquitoes/house/night) with a peak in May 2022 (Figure 7). The highest mean density of *An. funestus* s.l. was found in May in ORPs in Busia (5.5 mosquitoes/house/night).

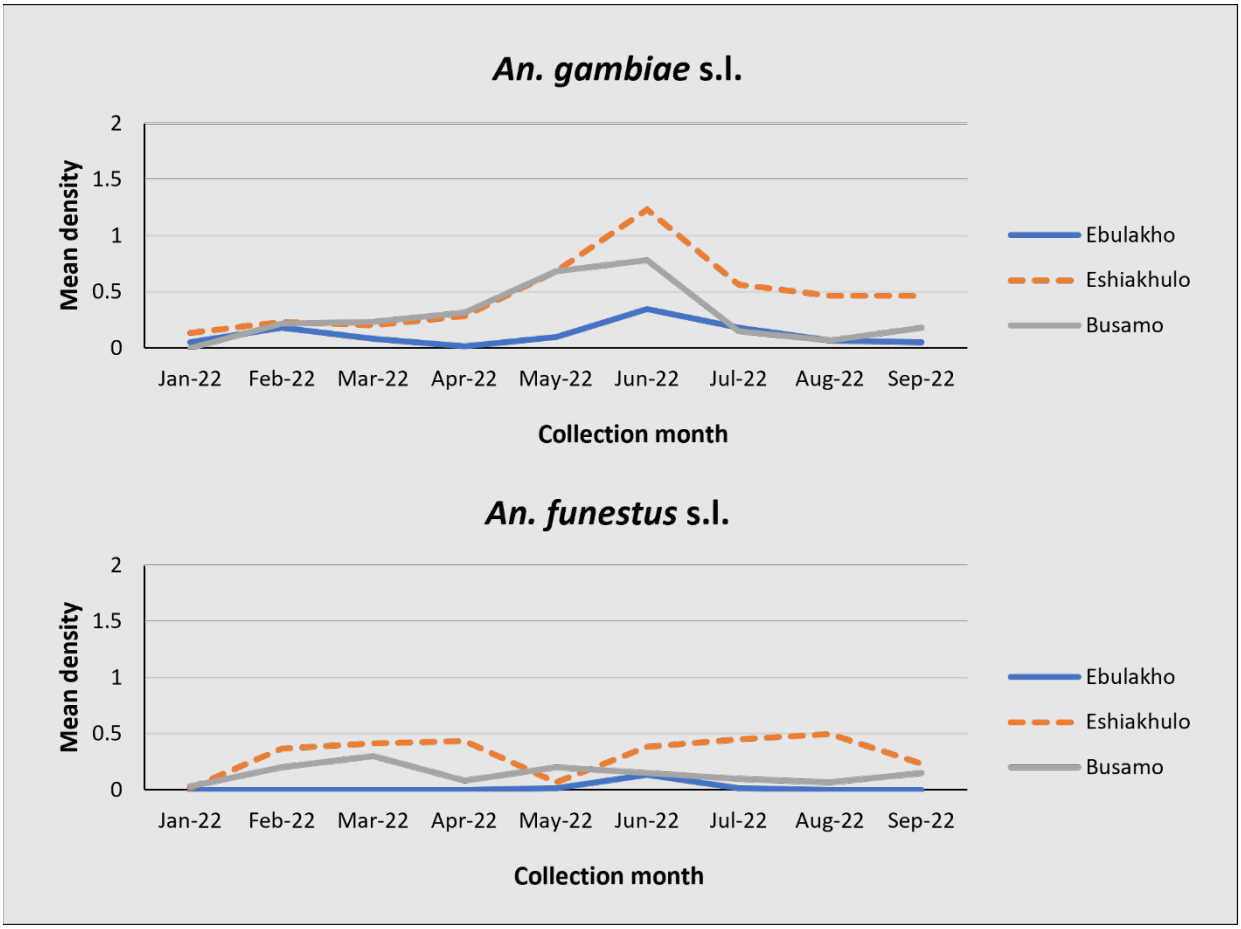
Figure 7: Mean Monthly Densities of *Anopheles* Mosquitoes Collected in IRS and non-IRS Counties Using Outdoor CDC-LTs and ORPs, December 2021–September 2022



3.2.3 Community-Based Vector Surveillance

Between January and September 2022, 741 *Anopheles* mosquitoes were collected in one site in Kakamega county and two sites in Vihiga county, using CDC-LTs. Of the 741, 479 were *An. gambiae* s.l., 259 were *An. funestus* s.l., and 3 were *An. coustani*. The highest density of malaria vectors was recorded in Kakamega (Eshiakulo site) (Figure 8).

Figure 8: Mean Indoor Density of *An. gambiae* s.l. and *An. funestus* s.l. Estimated by CDC-LTs in CBS in Kakamega (Eshiakulo) and Vihiga (Ebulako and Busamo) Counties



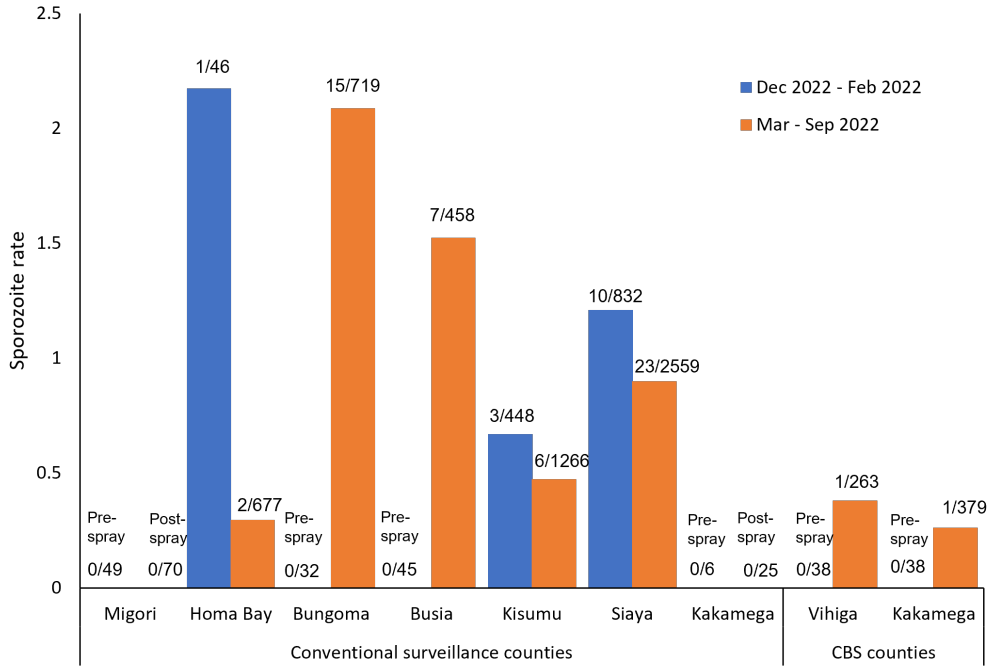
(Dashed lines represent Kakamega sites, solid lines represent Vihiga sites)

3.3 Malaria Vector Sporozoite Rates and Entomological Inoculation Rates

A total of 7,232 mosquitoes from longitudinal monitoring catches were screened for *P. falciparum* sporozoites, and 67 (0.9%) were positive. No mosquito was found with sporozoites in Migori county (Figure 8). This finding is consistent with that of the previous year. Sporozoites were not detected in mosquitoes captured in Buhili, Khwisero sub-county in Kakamega. Prior to IRS, the sporozoite rate in Homa Bay was 2.2% and after IRS decreased to 0.3% (Figure 9). The highest sporozoite rate was reported in Homa Bay (2.2%) and Bungoma (2.1%). The sporozoite rates for Bungoma, Busia, Kisumu and Siaya counties were higher during the post-IRS period compared to the pre-spray period.

A total of 707 *Anopheles* mosquitoes collected during CBS were tested for sporozoites. The highest sporozoite rates were recorded in Vihiga (0.38%) (Figure 9). No anopheline was found positive for sporozoites in Kakamega or Vihiga during the pre-spray period.

Figure 9 Sporozoite Rates of *Anopheles* Mosquitoes from All Collection Methods Across Eight Counties, December 2021–September 2022



Note: LM=longitudinal monitoring

3.4 INSECTICIDE RESISTANCE TESTING

3.4.1 PYRETHROID RESISTANCE, WITH OR WITHOUT PBO SYNERGIST

An. gambiae s.l. was resistant to the diagnostic doses of deltamethrin, permethrin, and alpha-cypermethrin in all eight counties. Pre-exposure of *An. gambiae* s.l. to PBO prior to exposure to discriminating doses of these insecticides restored the efficacy of the insecticides (Figures 10, 11, 12). There was full restoration in seven cases (deltamethrin in Migori, Homa Bay, Kisumu, and Vihiga, permethrin in Migori and Homa Bay, and alpha-cypermethrin in Homa Bay) and partial restoration in 17 cases.

An. funestus s.l. from Siaya was resistant to all pyrethroids tested (2–48% mortality at 24 hours). Pre-exposure to PBO partially restored the susceptibility of *An. funestus* s.l. to each pyrethroid.

Figure 10: Mortality of *An. gambiae* s.l. and *An. funestus* s.l. Tested with Deltamethrin Alone and After Pre-Exposure to PBO in Eight Counties

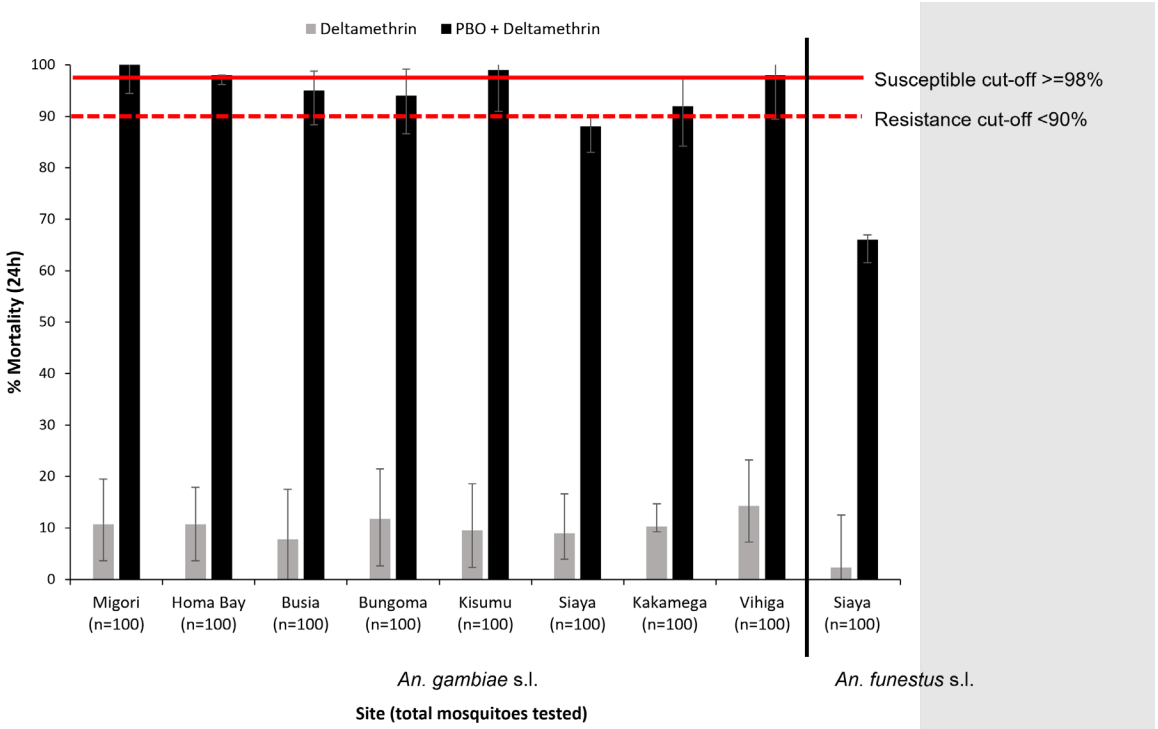


Figure 11: Mortality of *An. gambiae* s.l. and *An. funestus* s.l. Tested with Permethrin Alone and After Pre-Exposure to PBO in Eight Counties

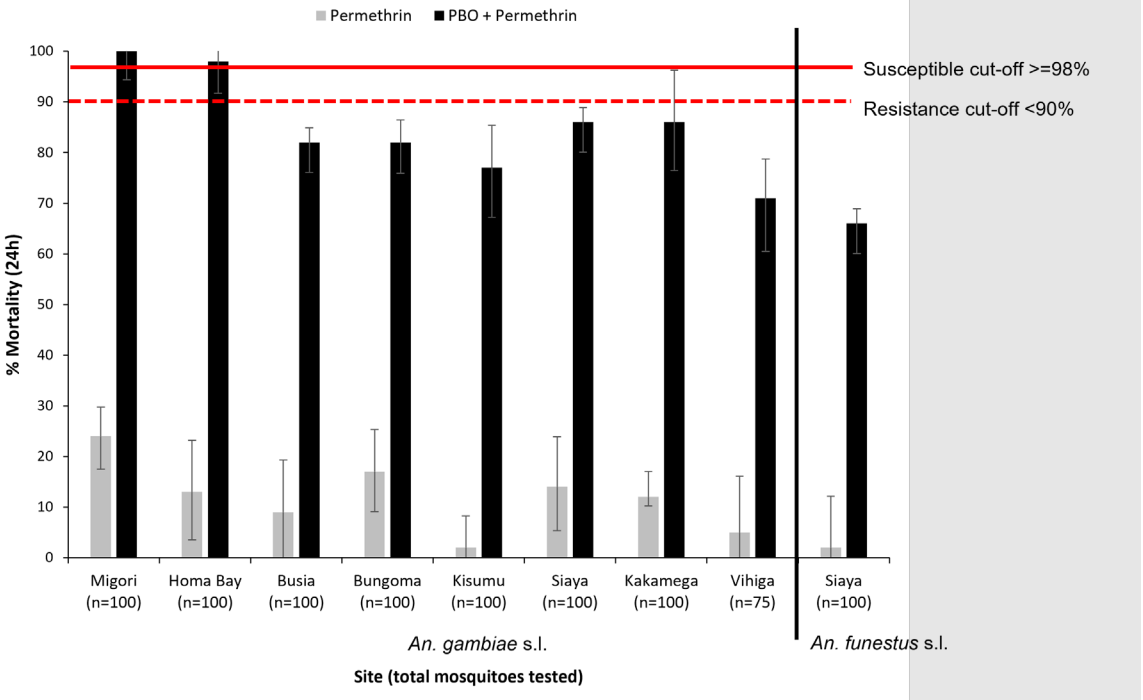
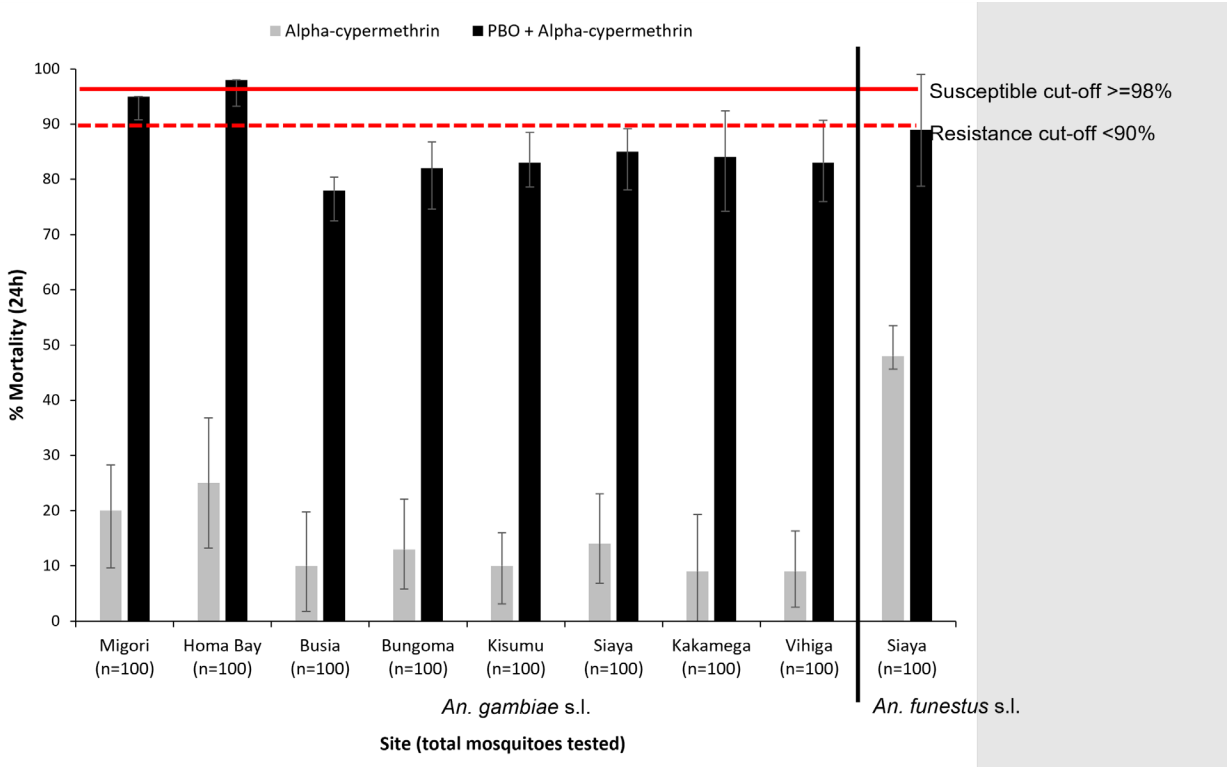


Figure 12: Mortality of *An. gambiae* s.l. and *An. funestus* s.l. Tested with Alpha-cypermethrin Alone and After Pre-Exposure to PBO in Eight Counties



3.4.2 INTENSITY OF PYRETHROID RESISTANCE

The resistance intensity of *An. gambiae* s.l. to deltamethrin was low (>98% mortality at 5× dose) in Migori and Homa Bay and moderate (>98% mortality at 10× dose) in Kisumu, Siaya, Kakamega, and Vihiga (Figure 13). Resistance intensity was high (<98% mortality at 10× dose) in Busia and Bungoma. Resistance intensity of *An. funestus* s.l. to deltamethrin was high in Bungoma and moderate in Siaya.

Bioassay results showed that *An. gambiae* s.l. resistance to permethrin was present in all eight sites, with less than 50% mortality at the diagnostic dose of 1× (Figure 14). Similar results were observed in *An. funestus* s.l. mosquitoes from Siaya. The team was unable to find sufficient adult *An. funestus* s.l. from Bungoma to perform permethrin intensity assays.

Mortality rates increased slightly with increased concentration, but high intensity to alpha-cypermethrin resistance was present in Busia, Bungoma, and Siaya with considerably less than 98% mortality at 10× the diagnostic concentration (Figure 15). There were not enough mosquitoes to test intensity of alpha-cypermethrin intensity at Kakamega (*An. gambiae* s.l.) or Bungoma (*An. funestus* s.l.)

Figure 13: Percent Mortality of *An. gambiae and An. funestus* to Diagnostic Concentrations of Deltamethrin

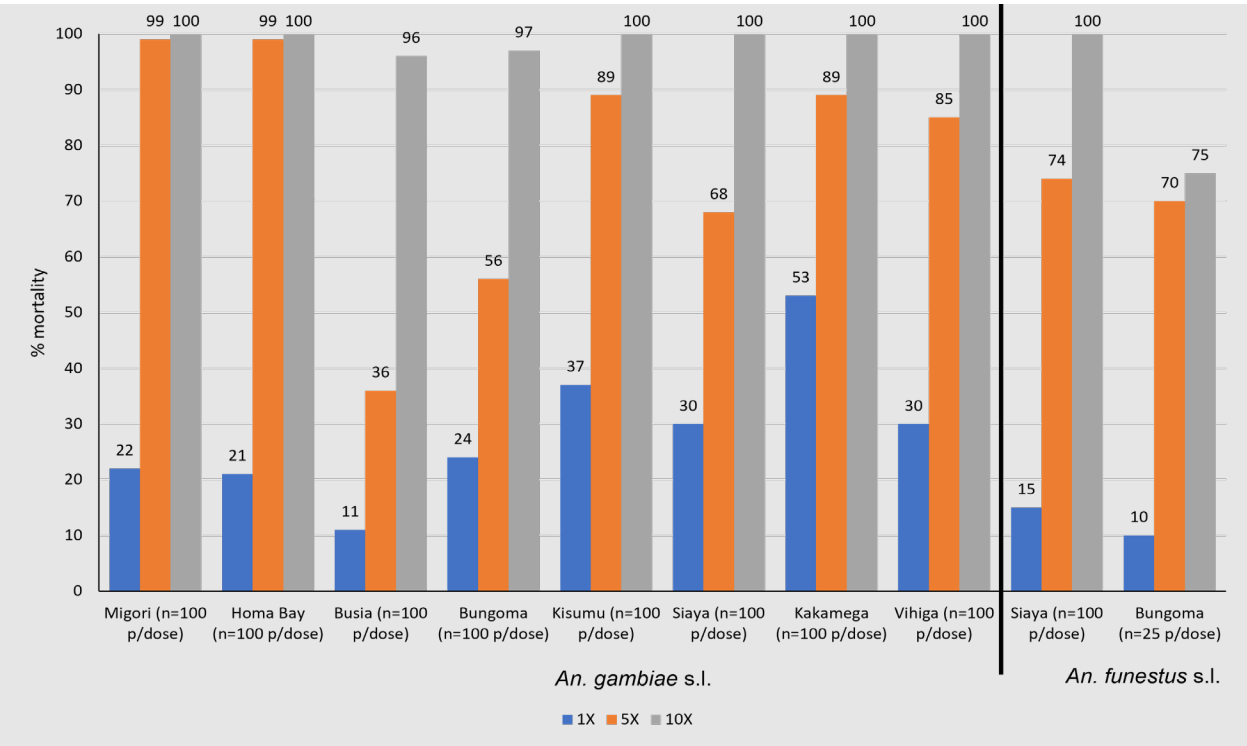


Figure 14: Percent Mortality of *An. gambiae* and *An. funestus* to Diagnostic Concentrations of Permethrin

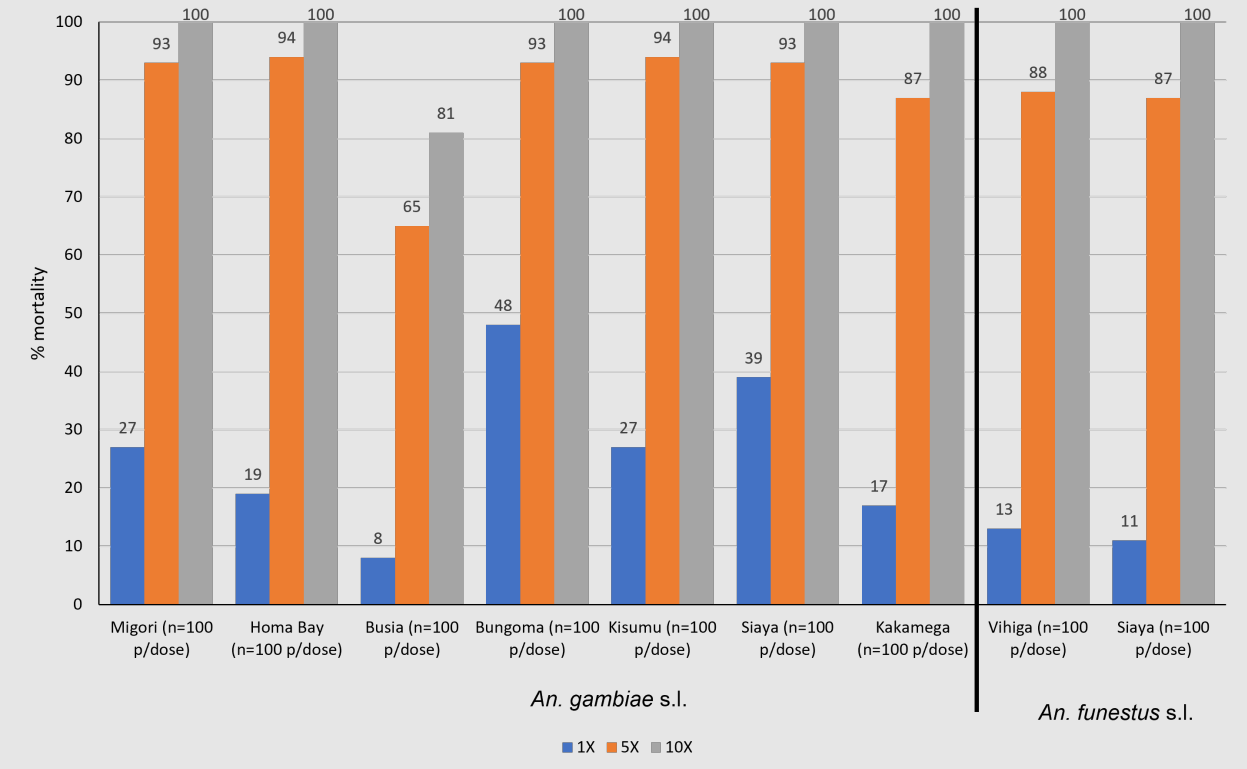
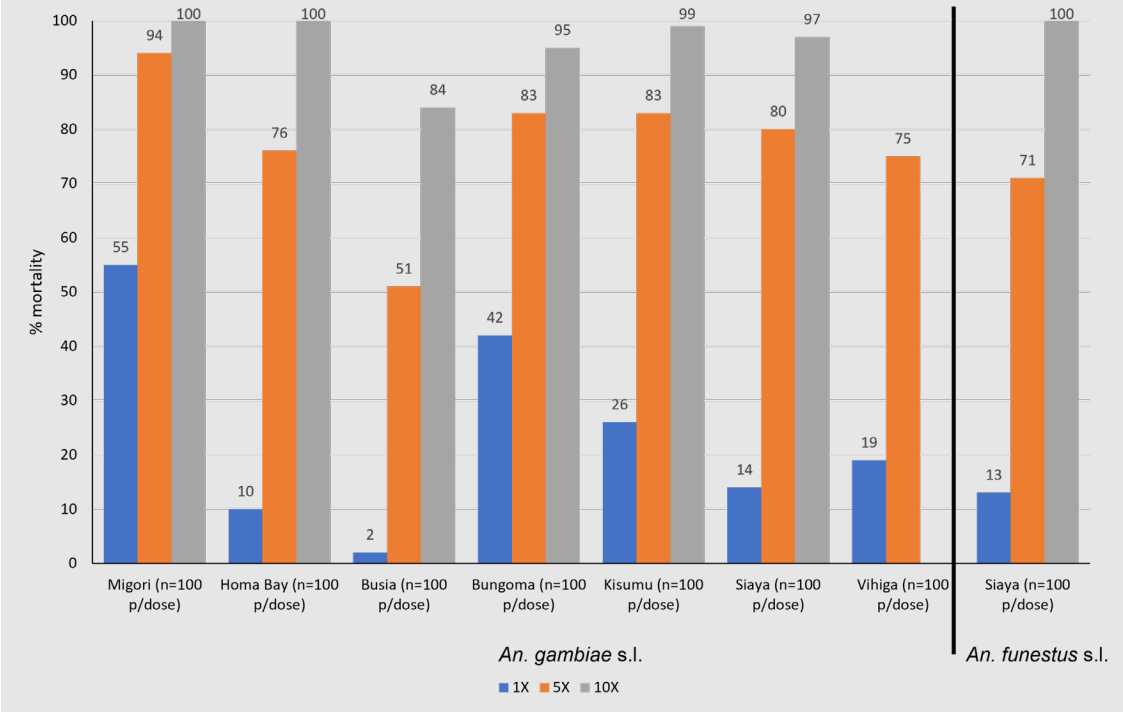


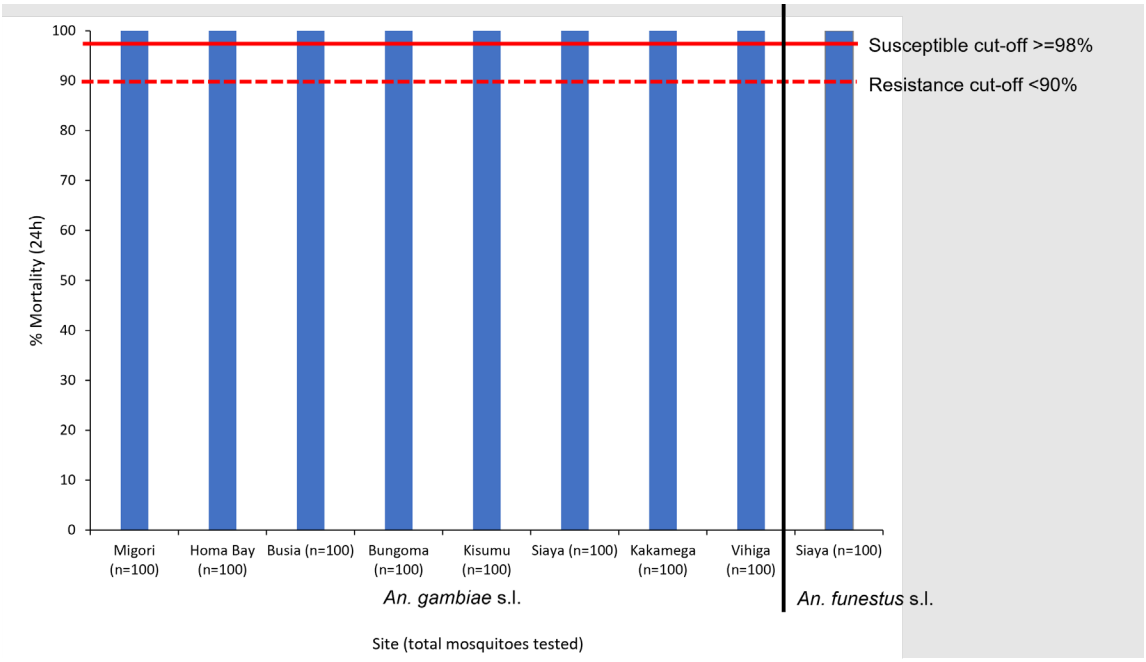
Figure 15: Percent Mortality of *An. gambiae* and *An. funestus* to Diagnostic Concentrations of Alpha-cypermethrin



3.4.3 INSECTICIDE RESISTANCE TO ORGANOPHOSPHATES

An. gambiae s.l. from all eight counties and *An. funestus* s.l. from Siaya were susceptible (100% mortality) to pirimiphos-methyl (Figure 16).

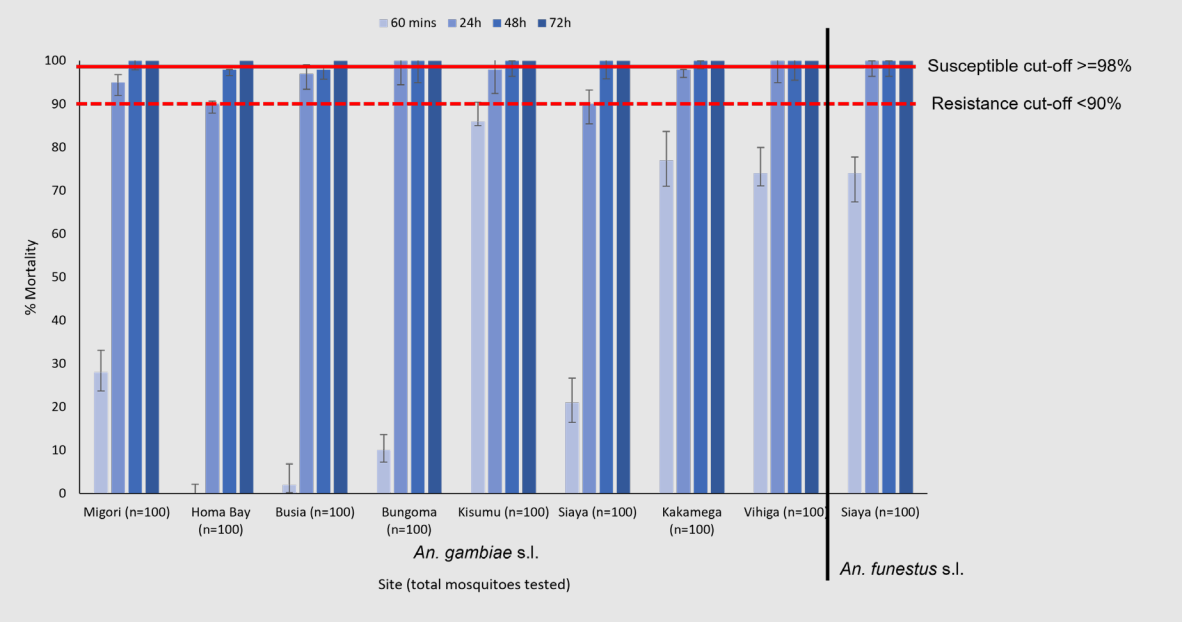
Figure 16: Mortality of *An. gambiae* s.l. and *An. funestus* s.l. Tested with Pirimiphos-methyl in Eight Counties



3.4.4 INSECTICIDE RESISTANCE TO CHLORFENAPYR AND CLOTHIANIDIN

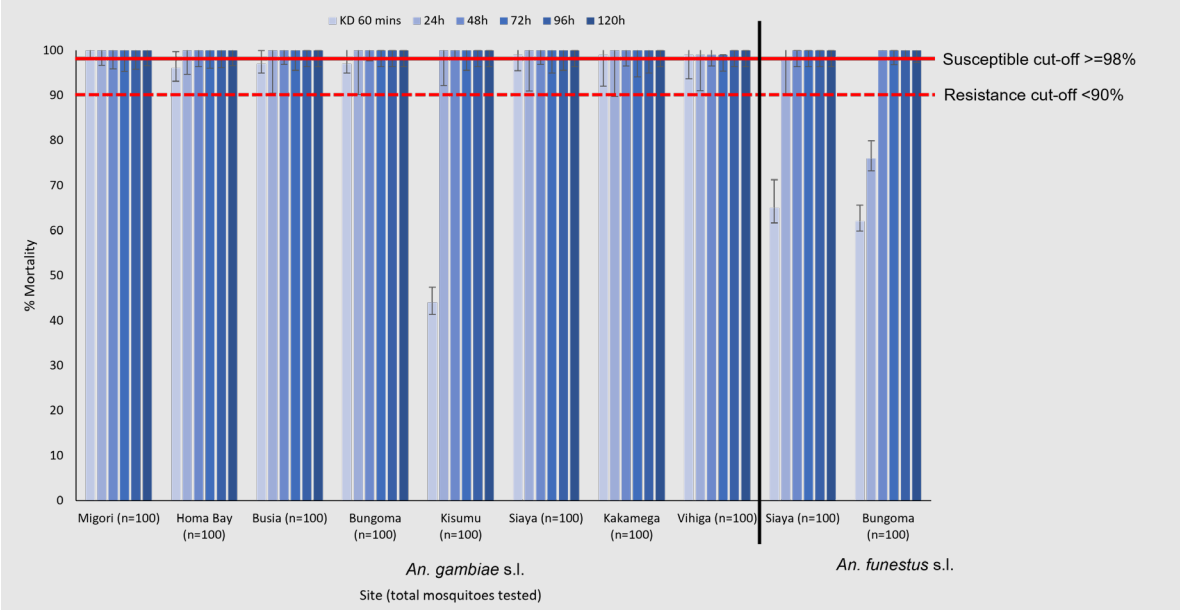
Full susceptibility to chlorfenapyr was observed in *An. gambiae* s.l. mosquitoes across all eight counties and *An. funestus* s.l. in Siaya (Figure 17). 100% mortality was seen within 48-72h post-exposure.

Figure 17: Mortality of *An. gambiae* s.l. and *An. funestus* s.l. Tested with Chlorfenapyr in Eight Counties



An. gambiae s.l. from all sites were susceptible to clothianidin (Figure 18). Complete mortality was observed within 48-72 hours of exposure. *An. funestus* s.l. populations from Siaya and Bungoma were similarly susceptible. This is the first-time susceptibility tests were carried out on *An. funestus* s.l. from Bungoma.

Figure 18: Mortality of *An. gambiae* s.l. and *An. funestus* s.l. Tested with Clothianidin in Eight Counties



3.4.5 Detection of L1014S and L1014F

kdr analysis was conducted on samples collected from all eight counties. A total of 802 female *An. gambiae* s.l. samples (504 *An. arabiensis* and 286 *An. gambiae* s.s.) were analyzed. Twelve samples failed to amplify. The allelic frequencies for *kdr*-E were generally varied across the eight counties; three counties did not record presence of the L1014S mutation, whereas for *An. gambiae* s.s. from Vihiga the frequency was as high as 90%. The frequencies for *kdr*-W were very low across all the sites, with the highest in Kisumu (18.75% in *An. arabiensis*). No homozygous resistant samples were detected for either species for either *kdr* mutation.

Table 3: Target Site Insecticide Resistance Mechanisms Across Eight Counties

County	Species	N	Genotype						Frequency	
			LL	LS	SS	LF	FF	SF	L1014S (East)	L1014F (West)
Bungoma	*An. gambiae* s.s.	42	20	2	18	1	0	1	0.46	0.02
	An. arabiensis	58	37	0	20	1	0	0	0.34	0.00
Busia	*An. gambiae* s.s.	59	21	0	34	0	0	4	0.61	0.03
	An. arabiensis	41	20	0	20	0	0	1	0.5	0.01
Homabay	*An. gambiae* s.s.	1	1	0	0	0	0	0	0	0
	An. arabiensis	98	94	0	0	4	0	0	0	0.02
Kakamega	*An. gambiae* s.s.	32	18	0	12	2	0	0	0.37	0.03
	An. arabiensis	66	37	0	24	5	0	0	0.36	0.03
Kisumu	*An. gambiae* s.s.	11	7	0	1	3	0	0	0.09	0.13
	An. arabiensis	80	52	0	1	24	3	0	0.01	0.18
Migori	*An. gambiae* s.s.	7	6	0	0	1	0	0	0	0.071
	An. arabiensis	93	91	0	2	0	0	0	0.021	0
Siaya	*An. gambiae* s.s.	48	16	0	29	3	0	0	0.60	0.03
	An. arabiensis	49	10	0	29	7	2	1	0.60	0.12
Vihiga	*An. gambiae* s.s.	83	6	4	73	0	0	0	0.90	0
	An. arabiensis	14	2	1	10	1	0	0	0.75	0.03

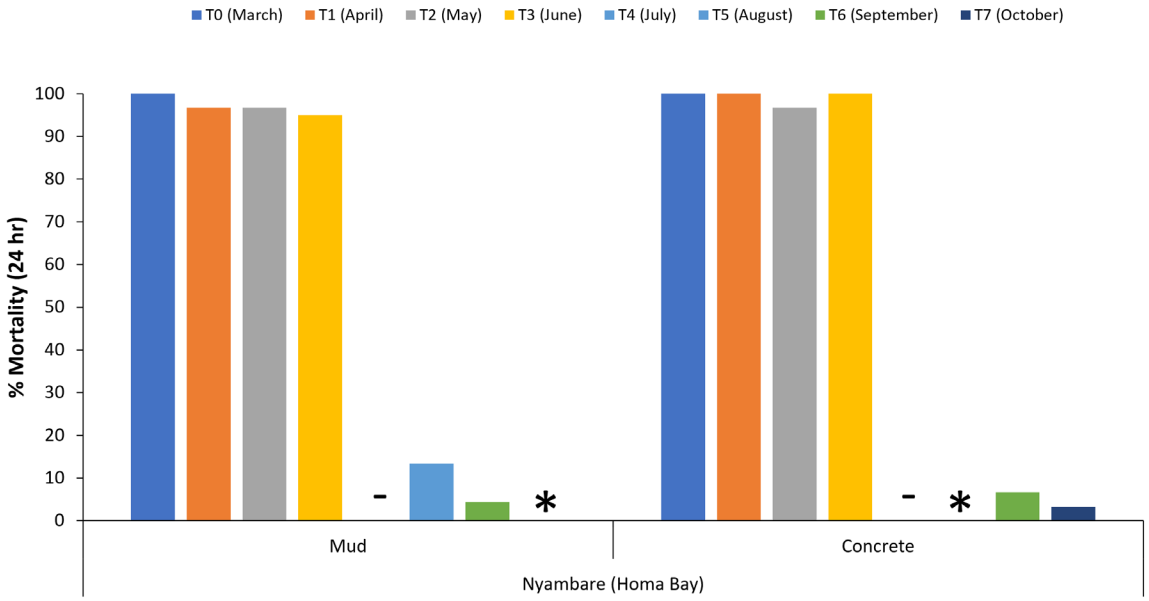
LL: fully susceptible individuals LS: heterozygote resistance (*kdr*-E) SS: homozygote resistance (*kdr*-E)
LF: heterozygote resistance (*kdr*-W) FF: homozygote resistance (*kdr*-W) SF: homozygote resistance(has both *kdr* east & west mutation;

3.5 Residual Efficacy And Fumigant Effect of IRS Insecticides

3.5.1 Residual Efficacy of Actellic in 2021

The wall cone bioassays showed that Actellic 300CS remained effective (mosquito mortality above the 80% threshold at 24 hours) up to 3 months post-IRS on mud and concrete walls in Homa Bay (Figure 19). The PMI VectorLink project in Kenya closed in June 2021, and thus, residual efficacy data for July (T4) were not collected. When wall cone assays resumed in mid-August 2021, at the start of the PMI Kinga Malaria project, mortality was very low, reflecting a rapid drop in efficacy. This finding was verified in September and October 2022, when mortality remained low.

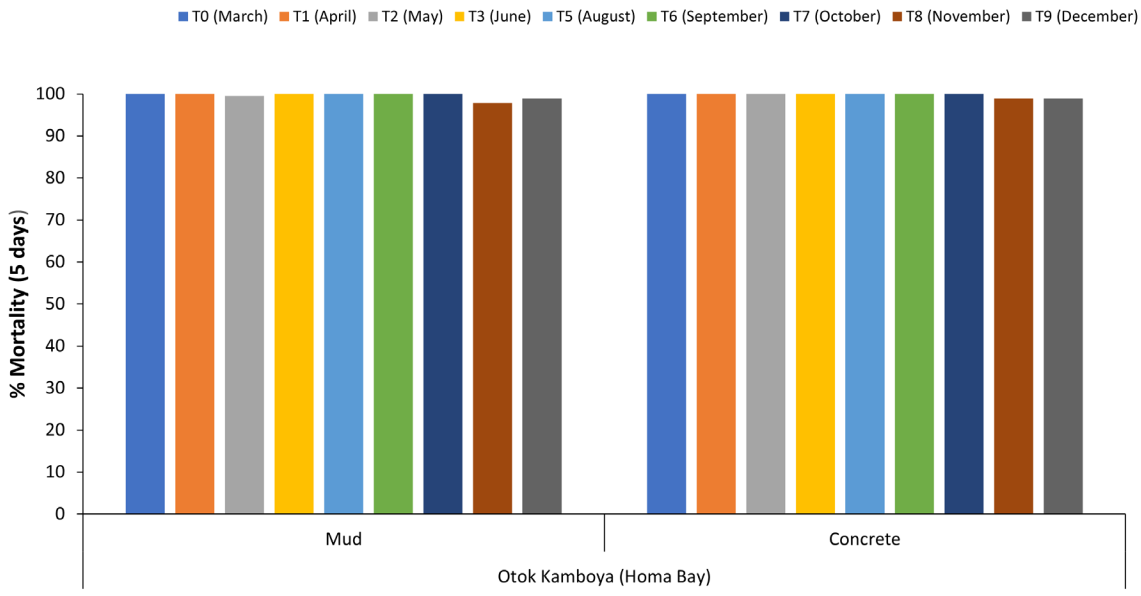
Figure 19: Residual Efficacy of Actellic 300CS on Mud and Concrete Walls in Nyambere, Homa Bay, 2021



3.5.2 Residual Efficacy of Fludora Fusion in Homa Bay in 2021

Fludora Fusion was effective in Otok Kamboya in Homa Bay county up to December 2021 (T9) with mortality rates of 98.9% (at 5 days) for wild *An. funestus* of unknown age on concrete and mud walls, respectively (Figure 20). Residual efficacy monitoring was stopped in Homa Bay in December 2021 due to budget restrictions. Residual efficacy for Fludora Fusion was at least 9 months.

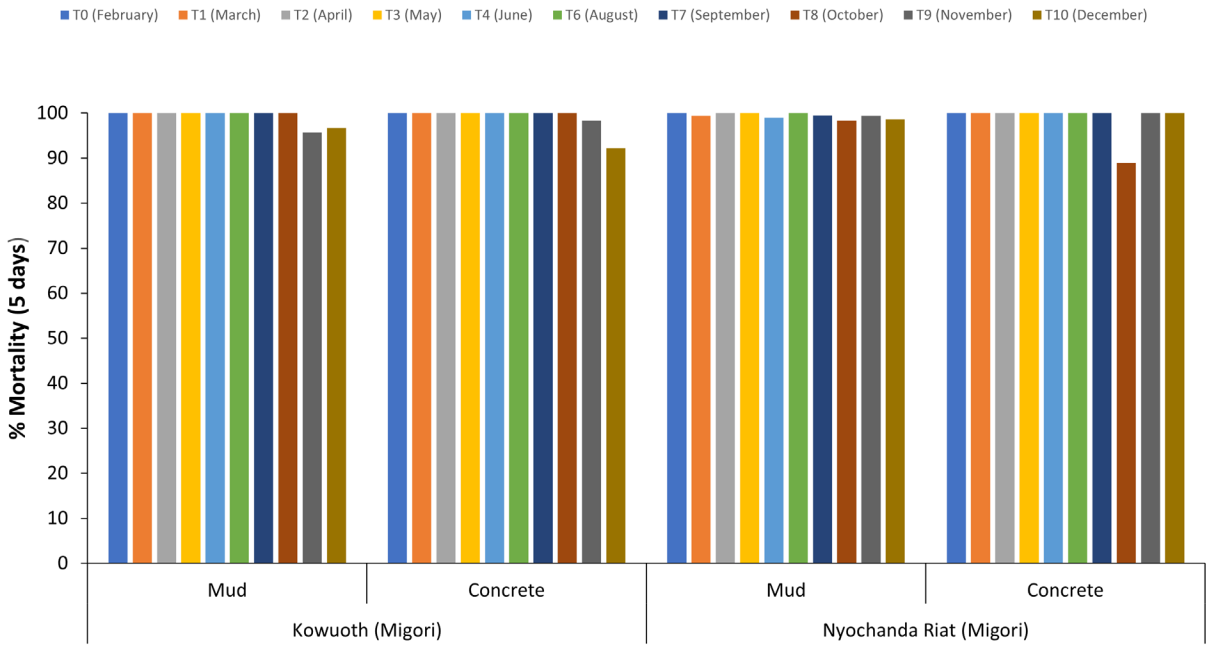
Figure 20: Residual Efficacy of Fludora Fusion on Mud and Concrete Walls in Otok Kamboya, Homa Bay, 2021



3.5.3 RESIDUAL EFFICACY OF FLUDORA FUSION IN MIGORI IN 2021

Fludora Fusion was effective in Kowuoth and Nyochanda Riat in Migori county up to December 2021 (T10), with mortality rates above the 80% threshold on both concrete and mud walls (Figure 21). Residual efficacy was not recorded for July (T5) because VectorLink Kenya had closed in June 2021 and July was the transition period to the PMI Kinga Malaria Project. Five-day mean mortality only dropped below 100% from October onward, and not in all cases. Residual efficacy monitoring was stopped in Migori in December 2021 due to budget restrictions.

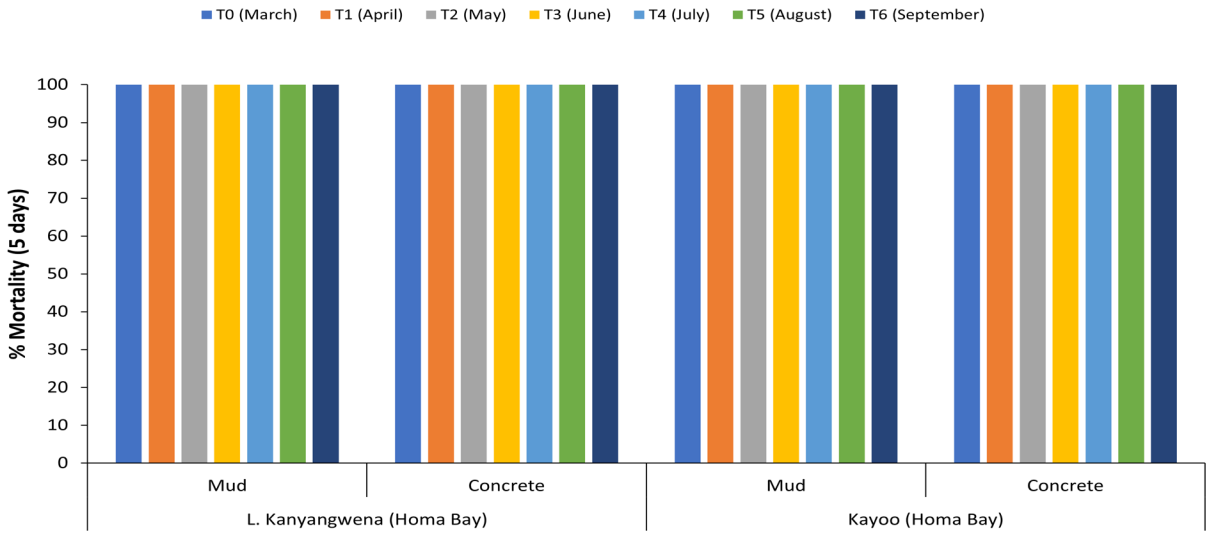
Figure 21: Residual Efficacy of Fludora Fusion on Mud and Concrete Walls in Migori, 2021



3.5.4 Residual Efficacy of SumiShield In 2022

Wall cone assays showed that SumiShield remained effective above the cut-off mortality level (80% after 5 days) for 6 months post-IRS on mud and concrete surfaces in Homa Bay (Figure 22). In both sites, 5-day mortality was reported as 100% at all timepoints T0-T6. Residual efficacy continues to be monitored beyond September 2022.

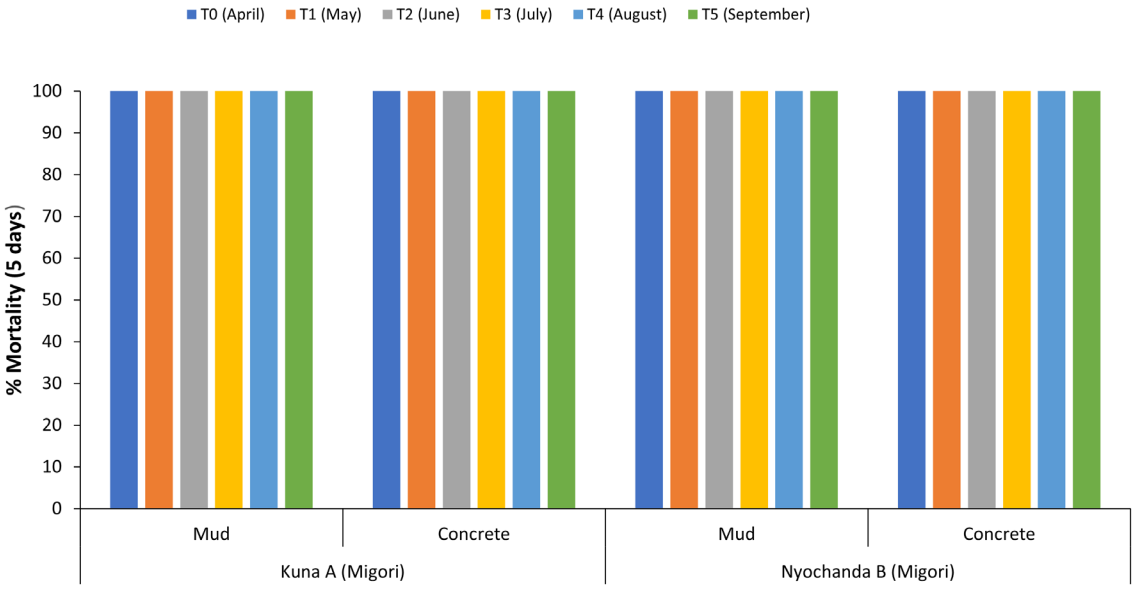
Figure 22: Residual Efficacy of SumiShield on Mud and Concrete Walls in Homa Bay, 2022



3.5.5 Residual Efficacy of Fludora Fusion in 2022

Fludora Fusion was effective 5 months after IRS on mud and concrete surfaces in Migori (Figure 23). For all surfaces in both sites, 5-day mortality was reported as 100% at all timepoints T0-T5.

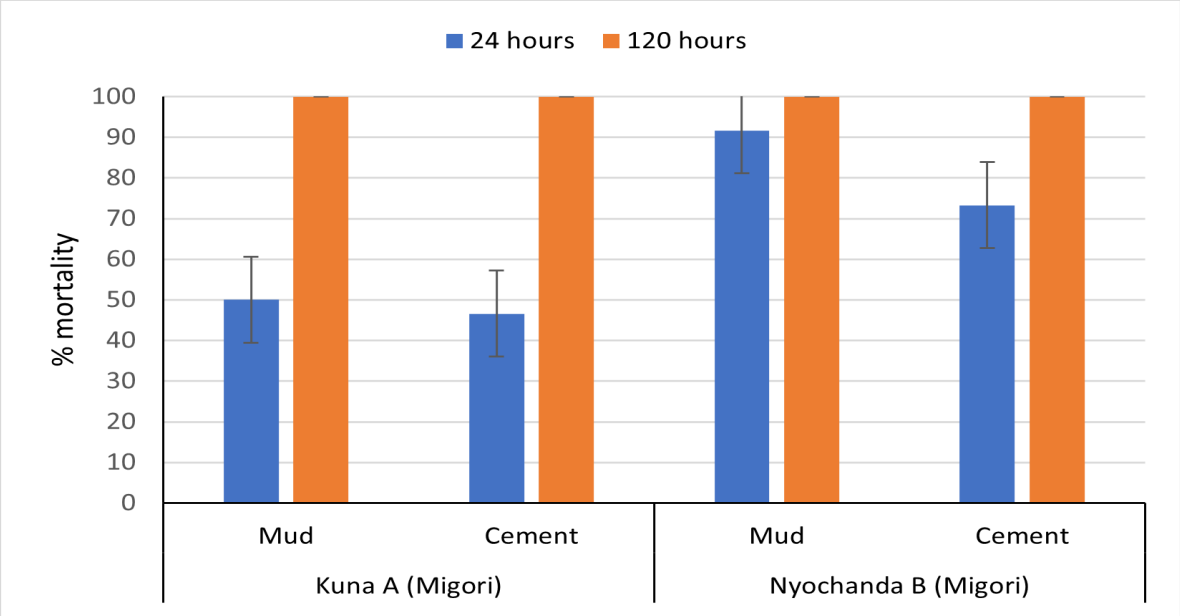
Figure 23: Residual Efficacy of Fludora Fusion on Mud and Concrete Walls in Migori, 2022



3.5.6 FUMIGANT EFFECT OF FLUDORA FUSION IN 2022

Fumigant assays showed a higher effect in Nyochanda B village with 91.7% and 73.3% mortality reported by 24 hours, in mud and concrete houses, respectively (Figure 24). 100% mortality was recorded in mud and concrete walls by 120 hours.

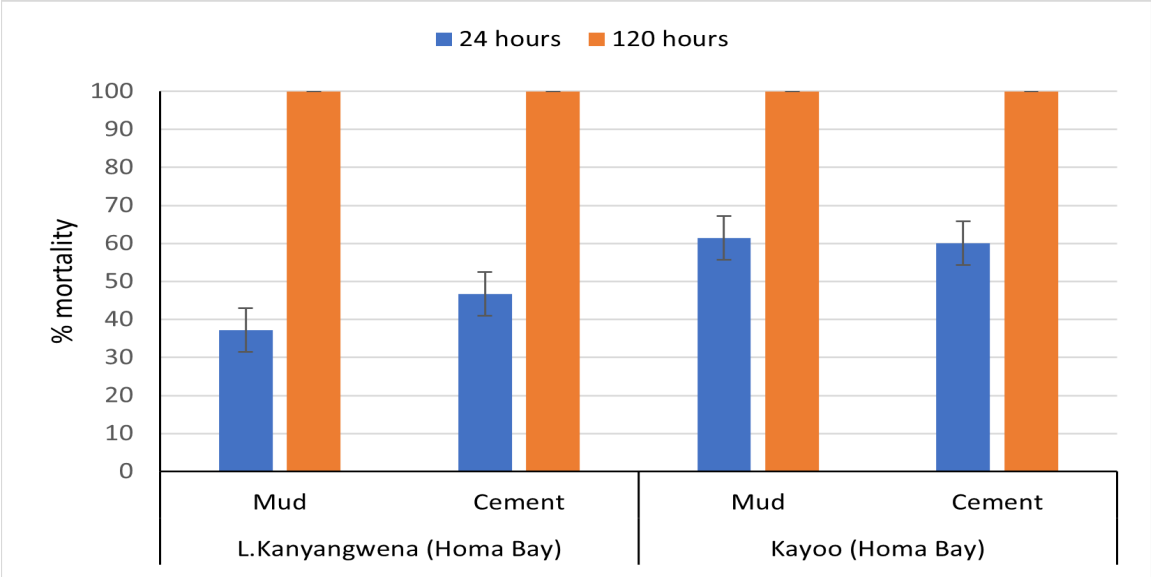
Figure 24: Fumigant Effect of Fludora Fusion in Migori with *An. funestus* s.l., April 2022



3.5.7 FUMIGANT EFFECT OF SUMISHIELD IN 2022

Fumigant assays showed a higher effect in Kayoo village with 61% and 60% mortality reported by 24 hours in mud and concrete houses, respectively (Figure 24). 100% mortality was recorded at 120 hours post-exposure to SumiShield to both wall types in both villages (Figure 25).

Figure 25: Fumigant Effect of SumiShield in Homa Bay with *An. funestus* s.l., March 2022



3.6 COMMUNITY-BASED SURVEILLANCE ASSESSMENT

Two rounds of CBS assessment were carried out in Kakamega and Vihiga during the reporting period, the first in July and second in September 2022. Each assessment round lasted for 6 days (3 days for the qualitative assessment and 3 days for the quantitative assessment). In total, six community health volunteers (CHVs) were evaluated, two each from Ebulakho and Busamo sites in Vihiga county and two from Eshiakhulo site in Kakamega county. CDC-LTs (quantitative study) were set up in a total of 60 houses per site per round (30 by CHVs and 30 by PMI Kinga Malaria team). Observations (qualitative study) of how CHVs set up CDC-LTs were performed in 18 houses per site per assessment round by the Kinga Malaria team.

3.6.1 QUANTITATIVE STUDY

The CHVs identified captured mosquitoes using morphological features. Collectively, the CHVs identified a total of 32 female mosquitoes, of which 18 were *An. gambiae* s.l. and 14 *An. funestus* s.l. The PMI Kinga Malaria team cross-checked the morphological ID of all 32 anophelines previously identified by CHVs.

The sensitivity (proportion of true positive identifications) and specificity (proportion of true negatives) of the samples identified by CHVs were 85.5% and 75.2%, respectively. This means that the CHVs were capable of correctly identifying malaria vectors in 28 out of 32 (85.5%) anophelines (Table 4). Specificity was lower than sensitivity and estimated at 75.2% which implied that CHVs can correctly identify mosquitoes that are non-anophelines.

Table 4: Total Anophelines Caught during CBS Assessment Rounds in Three Sites, July and September 2022

	CHVs			PMI Kinga Malaria Team		
Sites	*An. funestus* s.l.	*An. gambiae* s.l.	*Culex spp.*	*An. funestus* s.l.	*An. gambiae* s.l.	*Culex spp.*
Eshiakhulo	10	11	252	4	23	225
Busamo	4	7	76	10	4	101
Ebulakho	0	0	205	4	12	250
GRAND TOTAL	**14**	**18**	**533**	**18**	**39**	**576**

3.6.2 QUALITATIVE STUDY

The results of the qualitative study are presented under eight main themes: Household schedule; trapping preparations; consent; trapping activities; collection of traps and transportation of samples and equipment; sample identification; sample storage; and data management.

HOUSEHOLD SCHEDULE

All CHVs followed the household schedule correctly in both assessment rounds. The houses on the schedule were visited on the evening of observation.

TRAPPING PREPARATIONS

All CHVs ensured that the CDC-LT batteries were fully charged during morning hours after trap collection. All of the CHVs organized all materials required for the day's trapping activities. A majority of the CHVs ensured that the collection cups were clean and free from mosquitoes from the previous catch.

CONSENT

The interaction between CHVs and householders was above average. The CHVs explained the study well and the householders gave verbal consent before the CHV set up the CDC-LT.

TRAPPING ACTIVITIES

In general, all CHVs were able to set up the CDC-LT correctly. A majority of the CHVs were able to correctly set up the trap and gauge the battery performance by checking the speed of the fan and ensuring that the fan was sucking in air instead of blowing out on most trap nights. All CHVs ensured that trap collection cups were labeled correctly and included house code and date. All traps observed were set up between 5 and 6 pm, approximately 1.5 meters from the floor, hung at the foot end of the bed and next to an ITN.

COLLECTION OF TRAPS AND TRANSPORTATION OF SAMPLES AND EQUIPMENT

The majority of the CDC-LTs were retrieved from the households in the morning, between 6 and 7 am; one trap was removed after 7 am. Transportation of the collected mosquitoes was observed to be above average, with all CHVs transporting parts of the CDC-LT including trap assembly with light, motor and fan, lid, collection cup, power cable, and battery in cooler boxes.

SAMPLE IDENTIFICATION

The majority of the CHVs were able to correctly identify and group mosquitoes as *Anopheles*. Most CHVs were able to correctly identify and group Culex mosquitoes, and to differentiate male from female mosquitoes. The CHVs' skills in classifying mosquitoes according to their abdominal status was above average.

SAMPLE STORAGE

All CHVs correctly stored the mosquitoes in 1.5 ml sterile tubes containing ethanol. The majority of the CHVs usually correctly recorded the house and date information on the 1.5ml sterile tubes.

DATA MANAGEMENT

All CHVs understood the ODK application used for data collection. In addition to understanding the flow of the questionnaire in ODK, all CHVs were able to collect submit the collected data to the project server. Further, the CHVs were able to correctly fill the hard copy household mosquito forms.

4. DISCUSSION

4.1 VECTOR BIONOMICS

Mean densities were highest in the non-IRS counties of Siaya and Kisumu. For all sites combined, *An. funestus* s.l. was the primary vector, though this varied considerably at a county level, as most *An. funestus* were caught in Siaya. More than 95% of the catch in Siaya comprised *An. funestus* s.l. with *An. funestus* s.s. as the major sibling species. Within the *An. funestus* group, only *An. leesoni* was identified in Siaya, Busia, Homa Bay, and Kisumu. The predominant vector in IRS counties of Homa Bay and Migori was *An. arabiensis* both before and after spraying. *An. gambiae* s.s. was predominant in Bungoma, Busia, and Kakamega. The composition of mosquito populations across the eight counties has remained stable over recent years. Most mosquitoes were captured using CDC-LTs and PSCs, indicating that the vectors are endophilic.

4.2 EXTENSIVE RESISTANCE TO PYRETHROIDS BUT SUSCEPTIBILITY TO PIRIMIPHOS-METHYL AND CHLORFENAPYR

Resistance to deltamethrin, permethrin, and alpha-cypermethrin in *An. gambiae* s.l. in all study sites and to *An. funestus* s.l. in Siaya was extensive in all study counties. Past observations across the eight counties indicated resistance of malaria vectors to pyrethroids, and this has not changed in 2022. Pre-exposure of *An. gambiae* s.l. to PBO prior to exposure to discriminating doses of pyrethroids partially restored the efficacy of these insecticides. Across the study sites, PBO improved the killing effect on mosquitoes, but did not fully restore susceptibility to pyrethroids in some sites. PBO plus deltamethrin resulted in higher mosquito mortalities than PBO plus permethrin or alpha-cypermethrin. This trend was also observed in the PMI VectorLink report of 2019–2021. Pyrethroid-resistant mosquitoes may negatively affect the performance of pyrethroid-treated ITNs, especially where resistance intensity is high. Standard pyrethroid-only ITNs were distributed during the last mass net campaign of 2021 across the country, including in Siaya, Kisumu, Vihiga, Homa Bay, and Migori counties. PBO-pyrethroid nets were distributed for the first time in Kenya in Busia, Kakamega, and Bungoma counties. The efficacy of PBO nets is being monitored through a durability monitoring study coordinated by Kinga Malaria and implemented by the Pan-Africa Mosquito Control Association (PAMCA), following up ITNs in Busia and Kakamega. There is, however, the need for continuous monitoring of pyrethroids in lake endemic counties as pyrethroid resistance is widespread.

An. gambiae s.l. and *An. funestus* s.l., the main malaria vectors across all counties in which surveillance was conducted, are susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin diagnostic doses. Pirimiphos-methyl and clothianidin are the main active ingredients used in IRS products, while chlorfenapyr is used in two dual-active ITNs. Susceptibility of major vectors to chlorfenapyr suggests that ITNs treated with this insecticide, such as Interceptor G2, can be effective in Kenya, but first require registration trials in-country before they can be recommended. Continuous monitoring of these insecticides for which there is no reported resistance is crucial to IRM plans. Pirimiphos-methyl (Actellic 300CS) will be used in the 2023 and 2024 spray campaigns.

4.3 SUMISHIELD AND FLUDORA FUSION REMAIN EFFECTIVE THROUGH THE HIGH MALARIA TRANSMISSION SEASON

Clothianidin-based formulations showed marked persistence when applied on mud and concrete surfaces in Homa Bay and Migori. The residual efficacy of Fludora Fusion in 2021 was greater than 9 months, whereas Actellic 300CS lasted at least 5 months, by which time mortality was very low. Actellic 300CS was sprayed in the campaign of 2020 and recorded residual efficacy of 4 to 5 months. A similar observation has been reported

in Zambia and Malawi, where residual efficacy was also 4 to 5 months, though it is not clear what the underlying cause is for this relatively short residual efficacy.

The residual activity of SumiShield and Fludora Fusion was 5 and 6 months, respectively, during the reporting period in 2022. Clothianidin-based insecticides are good candidates for IRS, as they demonstrate high efficacy that last the high malaria transmission season. The fumigant effect was monitored for one month after spraying. A higher fumigant effect was observed within 24 hours in mud and concrete walls sprayed with Fludora Fusion in one village in Migori. This however does not mean that Fludora Fusion is better than SumiShield as both products yielded 100% mortality by 120 hours, the recommended diagnostic time.

4.4 IRS Effectively Reduced the Vectorial Capacity of Malaria Vectors in Migori and Homa Bay

No *Anopheles* mosquito was found with sporozoites in Migori county before and after the spray campaign of 2022. This finding is consistent with that of 2021. The sporozoite rate in Homa Bay was 2.2% before the IRS campaign, with 1 mosquito testing positive out of 46, and dropped to 0.2% in the post-spray period. This is a slight increase compared to previous years (2019/2020 and 2020/2021) where no malaria mosquito captured in Homa Bay tested positive for sporozoites. The number of infected mosquitoes are still lower in Homa Bay than in non-IRS counties, indicating that IRS is effective at suppressing vector populations.

4.5 Community-Based Surveillance Could Help Scale Up Entomological Surveillance

The CHVs continued to apply many of the skills on which they were trained during the refresher training in May 2022. The CBS assessment was to be implemented in four rounds of evaluation. Two rounds have been described in this report and the remaining two will be carried out in Year 2 of PMI Kinga Malaria.

To effectively implement CBS, it is crucial that local mosquito collectors are familiar with methods used to trap malaria vectors. The findings from these two rounds of the CBS assessment suggest that CHVs can be trained and gain skills in mosquito trapping, sample storage and transportation, vector identification, and data management. Given the lower specificity scores, it is important to continue with supportive supervision of CHVs.

Involving local communities in disease surveillance is crucial in the collection of data used for public health decisions. There is a need to build the capacity of CBS focal points in aspects of malaria vector surveillance to empower the community to identify malaria risk. CBS can provide the most benefit in rural, urban, peri-urban and hard-to-reach areas and can help fill specific malaria entomology gaps. The approach is also useful in low-resource settings.

Implementing CBS in some sites can extend the coverage of existing entomological surveillance in high burden communities. The use of software such as ODK for data management can result in lower costs and real-time reporting of data.

4.6 Conclusions

Residual efficacy of Actellic 300CS after the 2021 IRS campaign was at least 3 months, but not 5 months, by which time mortality was very low. Residual efficacy of Fludora Fusion in 2021 was greater than 9 months. SumiShield and Fludora Fusion sprayed in 2022 remained efficacious in the first 5 and 6 months, respectively, after spraying. The residual efficacy of both insecticides will continue to be monitored until mortality falls below 80% for 2 consecutive months. Both neonicotinoid and organophosphate insecticides resulted in high mortality in mosquitoes, indicating both are suitable for IRS to reduce mosquito densities and indoor transmission of malaria.

To date, pyrethroids are the most used insecticides for ITNs, and organophosphates and neonicotinoids for IRS for vector control in Kenya. Deltamethrin- and permethrin-impregnated ITNs are used extensively in

different counties after distribution through mass net campaigns. There is a need to continue monitoring the insecticide susceptibility status of malaria vectors against commonly used insecticides in public health control.

Interceptor G2 or any other chlorfenapyr-treated ITN could be considered a good alternative to PBO-pyrethroid ITNs for the control of malaria vectors in the country.

CHVs can be trained to carry out mosquito trapping and identify *An. gambiae* s.l. and *An. funestus* s.l. Additional evaluations have been planned for CBS thus full conclusions cannot be drawn using data collected during the reporting period.

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ANNEX

Table A1: Number of *An. gambiae* s.l. Collected by Indoor CDC-LTs, PSCs, Outdoor CDC-LTs, and ORPs, December 2021–September 2022

	Indoor CDC-LT		PSC		Outdoor CDC-LT		ORP	
County	Pre-IRS	Post-IRS	Pre-IRS	Post-IRS	Pre-IRS	Post-IRS	Pre-IRS	Post-IRS
Bungoma	6	435	30	291	0	10	0	0
Busia	1	19	0	18	0	7	0	5
Homa Bay	8	127	17	144	0	21	0	318
Kakamega	0	7	1	19	1	1	0	0
Kisumu	58	264	133	671	1	14	0	0
Migori	8	12	8	4	0	0	0	39
Siaya	2	36	5	75	0	0	0	0

Table A2: Number of *An. funestus* s.l. Collected by Indoor CDC-LTs, PSCs, Outdoor CDC-LTs, and ORP, December 2021–September 2022

	Indoor CDC-LT		PSC		Outdoor CDC-LT		ORP	
County	Pre-IRS	Post-IRS	Pre-IRS	Post-IRS	Pre-IRS	Post-IRS	Pre-IRS	Post-IRS
Bungoma	2	15	1	3	0	3	0	0
Busia	23	163	64	208	0	18	0	100
Homa Bay	8	119	5	7	0	5	0	9
Kakamega	0	7	0	0	0	0	0	0
Kisumu	128	207	150	89	0	0	0	0
Migori	5	14	11	4	0	2	0	6
Siaya	252	1958	185	559	25	80	0	0

Table A3: Mean Number of *An. gambiae* s.l. per Trap Night by Indoor CDC-LT Collected by CBS in Kakamega and Vihiga Counties, January–September 2022

Site (County)	Parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Eshiakulo (Kakamega)	N traps set	60	60	60	60	60	120	120	120	120
	N collected	1	22	25	26	4	23	27	30	14
	Mean density	0.02	0.34	0.42	0.43	0.1	0.19	0.23	0.25	0.12
Busamo (Vihiga)	N traps set	60	60	60	60	60	120	120	120	120
	N collected	2	12	18	5	12	9	6	4	9
	Mean density	0.03	0.2	0.3	0.08	0.2	0.07	0.05	0.03	0.08
Ebulakho (Vihiga)	N traps set	60	60	60	60	60	120	120	120	120
	N collected	0	0	0	0	1	8	1	0	0
	Mean density	0	0	0	0	0.01	0.07	0.01	0	0

Table A4: Sporozoite Rate by Vector Species and IRS Status, December 2021–September 2022

County	Species	Period	Positive	Total Analyzed	Sporozoite Rate
Bungoma	*An. arabiensis*	Post-IRS	0	104	0%
	An. arabienis	Pre-IRS	0	4	0%
	An. funestus s.s.	Post-IRS	0	8	0%
	An. funestus s.s.	Pre-IRS	0	3	1%
	An. gambiae s.s.	Post-IRS	14	574	1%
	An. gambiae s.s.	Pre-IRS	0	23	0%
Busia	*An. arabiensis*	Post-IRS	0	21	0%
	An. arabiensis	Pre-IRS	0	1	0%
	An. funestus s.s.	Post-IRS	4	110	0.03%
	An. funestus s.s.	Pre-IRS	0	28	0%
	An. gambiae s.s.	Post-IRS	0	158	0%
	An. leesoni	Post-IRS	2	38	0.0%
	An. leesoni	Pre-IRS	0	5	0%
Homa Bay	*An. arabiensis*	Post-IRS	1	432	1%
	An. arabiensis	Pre-IRS	0	20	0%
	An. funestus	Post-IRS	1	72	1%
	An. funestus	Pre-IRS	1	18	0.06%
	An. gambiae s.s.	Post-IRS	0	94	0%
	An. leesoni	Pre-IRS	0	3	0%
Kakamega	*An. arabiensis*	Post-IRS	0	5	0%
	An. arabiensis	Pre-IRS	0	2	0%
	An. funestus	Post-IRS	0	1	0%
	An. funestus	Pre-IRS	0	1	0%

County	Species	Period	Positive	Total Analyzed	Sporozoite Rate
	An. gambiae s.s.	Post-IRS	0	17	0%
	An. gambiae s.s.	Pre-IRS	0	1	0%
Kisumu	*An. arabiensis*	Post-IRS	1	776	0.0%
	An. arabiensis	Pre-IRS	0	150	0%
	An. funestus s.s.	Post-IRS	1	175	0.01%
	An. funestus s.s.	Pre-IRS	0	110	0%
	An. gambiae s.s.	Post-IRS	2	138	0.01%
	An. gambiae s.s.	Pre-IRS	0	1	0%
	An. leesoni	Post-IRS	0	2	0%
	An. leesoni	Pre-IRS	0	3	0%
Migori	*An. arabiensis*	Post-IRS	0	28	0%
	An. arabiensis	Pre-IRS	0	28	0%
	An. funestus s.s.	Post-IRS	0	9	0%
	An. funestus s.s.	Pre-IRS	1	18	0.06%
	An. gambiae s.s.	Post-IRS	0	8	0%
	An. gambiae s.s.	Pre-IRS	0	1	0%
Siaya	*An. arabiensis*	Post-IRS	1	44	0.02%
	An. arabiensis	Pre-IRS	0	5	0%
	An. arabiensis	Post-IRS	2	301	0.01%
	An. arabiensis	Pre-IRS	1	147	0.01%
	An. gambiae s.s.	Post-IRS	0	92	0%
	An. gambiae s.s.	Pre-IRS	0	2	0%

Table A5: Percentage Mortality of *An. gambiae* s.l. and *An. funestus* s.l. to Deltamethrin, Permethrin, or Alpha-cypermethrin Alone and Following Pre-exposure to PBO in WHO Tube Tests, 2022 (n=100)

Species/Site	% Mortality Deltamethrin	% Mortality Deltamethrin + PBO	% Mortality Permethrin	% Mortality Permethrin + PBO %	%Mortality Alpha-cypermethrin	% Mortality Alpha-cypermethrin + PBO
An. gambiae s.l.						
Migori	11	100	24	100	20	95
Homa Bay	11	98	13	98	25	98
Busia	8	95	9	82	10	78
Bungoma	12	94	17	82	13	82
Kisumu	10	99	2	77	10	83
Siaya	9	88	14	86	14	85
Kakamega	10	92	12	86	9	84
Vihiga	14	98	5	71	9	83
An. funestus s.l.						
Siaya	2	66	2	66	48	89
Bungoma	n/a	n/a	n/a	n/a	n/a	n/a

Table A6: Percentage Mortality of *An. gambiae* s.l. and *An. funestus* s.l. to Pirimiphos-methyl, Clothianidin, and Chlorfenapyr, 2022 (n=100)

Species/Site	% Mortality Pirimiphos-methyl (0.25%)	% Mortality Clothianidin (13.2 mg/paper)					% Mortality Chlorfenapyr (100 µg/bottle)	
An. gambiae s.l.	24h	24h	48h	72h	96h	120h	24h	48h
Migori	100	100	100	100	100	100	95	100
Homa Bay	100	100	100	100	100	100	90	100
Busia	100	100	100	100	100	100	97	100
Bungoma	100	100	100	100	100	100	100	100
Kisumu	100	100	100	100	100	100	98	100
Siaya	100	100	100	100	100	100	90	100
Kakamega	100	100	100	100	100	100	98	100
Vihiga	100	99	99	99	100	100	93	100
An. funestus s.l.								
Siaya	100	98	98	100	100	100	100	100
Bungoma	n/a	76	76	100	100	100	n/a	n/a

Table A7: Percentage Mortality of *An. gambiae* s.l. and *An. funestus* s.l. to Deltamethrin at 1×, 5×, and 10× the Diagnostic Concentration in CDC Bottle Bioassays, 2022 (n=100)

Species/Site	% Mortality Deltamethrin 1×	% Mortality Deltamethrin 5×	% Mortality Deltamethrin 10×
An. gambiae s.l.			
Migori	22	99	100
Homa Bay	21	99	96
Busia	11	36	97
Bungoma	24	56	100
Kisumu	37	89	100
Siaya	30	68	100
Kakamega	53	89	100
Vihiga	30	85	100
An. funestus s.l.			
Siaya	15	74	100
Bungoma	n/a	n/a	n/a

Table A8: Percentage Mortality of *An. gambiae* s.l. and *An. funestus* s.l. to Permethrin at 1×, 5×, and 10× the Diagnostic Concentration in CDC Bottle Bioassays, 2022 (n=100)

Species/Site	% Mortality Permethrin 1×	% Mortality Permethrin 5×	% Mortality Permethrin 10×
An. gambiae s.l.			
Migori	27	93	100
Homa Bay	19	94	100
Busia	8	65	81
Bungoma	48	93	100
Kisumu	27	94	100
Siaya	39	93	100
Kakamega	17	87	100
Vihiga	13	68	100
An. funestus s.l.			
Siaya	11	87	100
Bungoma	n/a	n/a	n/a

Table A9: Percentage Mortality of *An. gambiae* s.l. and *An. funestus* s.l. to Alpha-cypermethrin at 1×, 5×, and 10× the Diagnostic Concentration in CDC Bottle Bioassays, 2022 (n=100)

Species/Site	% Mortality Alpha-cypermethrin 1×	% Mortality Alpha-cypermethrin 5×	% Mortality Alpha-cypermethrin 10×
An. gambiae s.l.			
Migori	35	94	100
Homa Bay	10	76	100
Busia	2	51	84
Bungoma	42	83	95
Kisumu	26	83	99
Siaya	14	80	97
Kakamega	n/a	n/a	n/a
Vihiga	19	75	n/a
An. funestus s.l.			
Siaya	13	71	100
Bungoma	n/a	n/a	n/a