USAID
FROM THE AMERICAN PEOPLE

U.S. PRESIDENT'S MALARIA INITIATIVE

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

U.S. PRESIDENT'S MALARIA INITIATIVE
vectorlink
INTELLIGENT › INNOVATIVE › INTEGRATED

# PMI VECTORLINK MALAWI ANNUAL ENTOMOLOGICAL MONITORING REPORT

# JULY 1, 2020 – JUNE 30, 2021

**Recommended Citation:** The PMI VectorLink Project. September 2021. *Malawi Annual Entomological Monitoring Report, July 1, 2020 –June 30, 2021*. Rockville, MD: USA: Abt Associates Inc.

**Contract:** AID-OAA-17-00008

**Task Order:** AID-OAA-TO-17-00027

**Submitted to:** United States Agency for International Development/PMI

**Submitted on**: September 30, 2021

**Approved on:** November 26, 2021

**Prepared by:** Abt Associates Inc.



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

# TABLE OF CONTENTS

## List of Tables

## List of Figures

# ACRONYMS

| | |
|---|---|
| ***Ace-1*** | Acetylcholinesterase 1 |
| **b/p/n** | bites/person/night |
| **CDC** | Centers for Disease Control and Prevention |
| **EIR** | Entomological Inoculation Rate |
| **$F_0$** | Filial generation 0 |
| **$F_1$** | Filial generation 1 |
| **HBR** | Human Biting Rate |
| **HLC** | Human Landing Catch |
| **ib/p/m (y)** | infective bites/person/month (year) |
| **IMP** | Intentional Mismatched Primer |
| **IRD** | Indoor Resting Density |
| **IRS** | Indoor Residual Spraying |
| *kdr* | Knockdown Resistance |
| **LT** | Light Trap |
| **MR4** | Malaria Research and Reference Reagent Resource Center |
| **PBO** | Piperonyl Butoxide |
| **IG2** | **Interceptor G2** |
| **PCR** | Polymerase Chain Reaction |
| ***Pf*** | *Plasmodium falciparum* |
| **PMI** | President's Malaria Initiative |
| **PSC** | Pyrethrum Spray Catch |
| **s.l.** | sensu lato |
| **SR** | Sporozoite Rate |
| **s.s.** | sensu stricto |
| **USAID** | United States Agency for International Development |
| **WHO** | World Health Organization |

# EXECUTIVE SUMMARY

**Introduction:** Malaria is the main cause of mortality and illness in Malawi, mainly affecting children under the age of 5 and pregnant women. Long-lasting insecticidal nets have been used nationwide as a vector control intervention, with indoor residual spraying (IRS) currently deployed in four districts (Nkotakhota, Nkhata Bay, Balaka and Mangochi) where the intervention is expected to have an impact on case burden.

The U.S. President's Malaria Initiative VectorLink Project in Malawi in collaboration with the Malaria Alert Centre (MAC) conducted monthly monitoring from July 2020 to June 2021 in 13 sentinel sites in six districts (Karonga, Nkhata Bay, Nkhotakota, Kasungu, Salima and Chikwawa) to assess malaria vector bionomics. Susceptibility of the main malaria vectors to insecticides in use for public health was also assessed in six sites from six districts.

**Vector Bionomics**: A total of 32,042 female *Anopheles* mosquitoes were collected from 13 sentinel sites in all six monitoring districts from July 2020 to June 2021. Out of these, 19,537 were collected using pyrethrum spray catches (PSCs) (61%), 10,203 using Centers for Disease Control light traps (CDC-LTs) (31.8%), and 2,302 (7.2%) using human landing catches (HLCs). Although the total number of *Anopheles* mosquitoes collected from PSCs was higher than from the other two collection methods, the mean difference per collection (PSCs=8.3 mosquitoes/house/day and CDC-LTs=6.5 mosquitoes/house/night) was not statistically significant ($p>0.05$).

Overall, 55.6% (n=17,816) of the *Anopheles* collected were identified morphologically as *An. gambiae* s.l.; 41.3% (n=13,247) were *An. funestus* s.l. and 3.1% (n=979) were *An. coustani*. However, species composition varied by sentinel site. *An. gambiae* s.l. was predominant in Mwenimambwe and Mwakanyamale sites (Karonga District), Chilungo site (Salima District), and Nyamphota site (Chikwawa District). *An. funestus* s.l. was predominant in Vwawa, Chimkwende, and Ngalauka sites (Nkhotakota District), Kachokolo and Nyalubwe sites (Kasungu District), Sanga and Kande sites (Nkhata Bay), Cholokoto site (Salima District), and Ntwana site (Chikwawa District). Species composition also varied based on indoor or outdoor location. Overall, the highest number of *An. funestus* s.l. were collected indoors (68.9% n=676). The highest number of *An. gambiae* s.l. and *An. coustani* were collected outdoors, 58.4% (n=808) and 60.0% (n=767) respectively

A total of 57,314 other mosquito species were collected during the monitoring period using the three methods. *Anopheles* mosquitoes collected include: *An. maculipalpis* (n=5), *An. pharoensis* (n=48), *An. pretoriensis* (n=23), *An. rufipes* (n=7), *An. tenebrosus* (n=186), and *An. squamosus* (n=1). Other mosquito species collected included 19,060 *Culex*, 37, 906 *Mansonia,* and 79 *Aedes*. A total of 1041 *An. gambiae* s.l. were randomly sampled and identified to species-specific level by polymerase chain reaction (PCR). From the processed samples, 64.6 were collected through PSCs (n=672), 17.2% through CDC-LTs (n=179), 1.3% through HLCs (n=14) and 16.9% through prokopack aspirator (n=176). Out of the 1041 *An. gambiae* s.l. collected, 895 (86.0%) were identified as *An. arabiensis* and 141(14.0%) as *An. gambiae* s.s. *An. arabiensis* and *An. gambiae* s.s. were identified across all six districts. A total of 594*An. funestus* s.l. were identified to species-specific level using PCR; all were identified as *An. funestus* s.s.

**Human Biting Rate (HBR) and Location:** Overall, the HBRs for *An. funestus* s.l. were 3.7 bites per person per night (b/p/n) indoors and 1.7 b/p/n outdoors. The HBRs for *An. gambiae* s.l. were 2.6 b/p/n indoors and 3.7 b/p/n outdoors and for *An. coustani* 3.1 indoors and 4.4 b/p/n outdoors. The highest indoor biting activity of *An. funestus* s.l. occurred in June from Salima District with 16.3 b/p/n. The highest outdoor biting activity of this species was observed in Nkhotakota District in June (7.8 b/p/n). The highest indoor biting activity of *An. gambiae* s.l. was observed in both Kasungu and Nkhotakota districts in March (11.7 b/p/n). The highest outdoor biting activity of *An. gambiae* s.l. was observed in March in Kasungu District (17.5 b/p/n), during the rainy season. The highest human biting activity of *An. coustani* was observed in Nkhata Bay District with HBRs of 21.0 b/p/n and 25.0 b/p/n indoors and outdoors respectively. Both *An. funestus* s.l. and *An. gambiae* s.l. exhibited similar preference for biting location, indoors and outdoors. The biting activity of both *An. funestus* s.l. and *An. gambiae* s.l. occurred from dawn to dusk

in all the four districts with a greater proportion (>70%) occurring when people were asleep. Lower levels of morning/daytime biting were also observed, after 5 am until 11 am, when people were awake.

**Blood Meal Source**: *An. funestus* s.l. predominantly fed on human blood (89.3%), whereas *An. gambiae* s.l. mainly fed on cow blood (61.6%) in all the six districts.

**Resistance Mechanism:** The absence of Acetylcholinesterase (*Ace-1)* resistant allele is consistent with the absence of phenotypic resistance to pirimiphos-methyl (organophosphate) in *An. arabiensis* and *An. gambiae* s.s. in Malawi. There appears to be knockdown resistance (*kdr)-east* resistance in *An. gambiae* s.s. and in *An. arabiensis* populations in Malawi. However, these results need to be verified further to ascertain its validity.

**Parity Rate:** Overall, the proportions of parous females for *An. funestus* s.l. and *An. gambiae* s.l. were similar, 61% and 57% respectively. The highest *An. funestus* s.l. parity rate was observed in Salima (70%), followed by Chikwawa (64%); the lowest parity rate was recorded in Nkhata Bay (54%). The highest parity rate for *An. gambiae* s.l. was observed in Nkhata Bay (66%), followed by Karonga (64%), and the lowest parity rate was recorded in Chikwawa (40%). The proportion of both parous *An. funestus* s.l. and *An. gambiae* s.l. was higher before IRS than after spraying in both Nkhata Bay and Nkhotakota. In non-IRS districts (Karonga, Salima, and Chikwawa), higher proportions of parous *An. gambiae* s.l. were collected during the dry season than during the rainy season; the opposite was true in Kasungu District. In Kasungu District, high parity rates of *An. funestus* s.l. were observed in the dry season as compared to the rainy season. Conversely, in Chikwawa District, higher *An. funestus* s.l. were observed during the rainy season than in the dry season.

**Infection Detection:** A total of 2215 *An. funestus* s.l. collected using Human Landing Catches, Pyrethrum Spray Catches and CDC-Light traps from the four districts of Nkhata Bay, Nkhotakota, Kasungu and Salima were screened for sporozoite infection. The overall sporozoite infection rate (SR) was 3.5%.

A total of 2,971 *An. gambiae* s.l. from all the three collection methods were tested for *Plasmodium falciparum* (*Pf)* and the overall SR was 1.2%. In IRS districts, high *An. gambiae* s.l. SRs were recorded after spraying both indoors, 0.6% (n=156) and outdoors, 1.1% (n=276). No sporozoite-positive *An. gambiae* s.l. were found before spray both indoors (n =49) and outdoors (n=99). In non-IRS districts, high *An. gambiae* s.l. SRs were recorded indoors, 6.7% (n=45) and no sporozoite-positive *An. gambiae* s.l. were found outdoor (n=49).

In IRS districts, high *An. funestus* s.l. SRs were recorded before spraying, 3.5% (n=85) indoors and no sporozoite-positive *An. funestus* s.l. were found indoors (n=13). No sporozoite *An. funestus* s.l. SRs were recorded after spraying both indoors (n =34) and outdoors (n=8). In non-IRS districts, high *funestus* s.l. SRs were recorded indoors, 6.7% (n=30) and no sporozoite-positive *An. gambiae* s.l. were found outdoor (n=37).

The annual entomological inoculation rate (EIR) was estimated from CDC-LT collections. The annual EIR (12 months)

was highest in Chikwawa District, 75.7 infective bites/person/year (ib/p/yr) (*An. gambiae* s.l. = 27.3 ib/p/yr; *An. funestus* s.l. = 48.4 ib/p/yr). The second highest EIR was recorded in Nkhotakota District (71.0 ib/p/yr total; 57.0 ib/p/yr from *An. gambiae* s.l. and 14.0 ib/p/yr from *An. funestus* s.l.) followed by Nkhata Bay District (47.3 ib/p/yr total; *An. gambiae* s.l. = 3.1 ib/p/yr and *An. funestus* s.l. = 44.2 ib/p/yr), Karonga District (25.6 ib/p/yr total; *An. gambiae* s.l. = 25.6 ib/p/yr and *An. funestus* s.l. = no infective bites), Salima (23.6 ib/p/yr; *An. gambiae* s.l. = 9.3 ib/p/yr and *An. funestus* s.l. = 14.4 ib/p/yr), and Kasungu District (20.9 ib/p/yr; *An. gambiae* s.l. = 4.6 ib/p/yr and *An. funestus* s.l. = 16.3 ib/p/yr).

In the IRS districts, the estimated risk of malaria transmission over a 12-month period was higher in Nkhotakota District (71.0 ib/p/yr) than in Nkhata Bay (47.3 ib/p/yr). In Nkhotakota, the highest monthly EIRs from *An. funestus* s.l. were observed a month after spray (18.4 ib/p/m recorded in November) though it was also high during the month of spray in October (15.4 ib/p/m). EIR remained between 0 and 5.8 ib/p/m for the months December to June. Similarly, in Nkhata Bay District, highest monthly EIRs from *An. funestus* s.l. were observed eight months after spray (16.5 ib/p/m recorded in June).

In non-IRS districts, there was variation in the monthly EIRs of *Anopheles* mosquitoes. The highest estimated risk of malaria transmission over a 12-month period was observed in Chikwawa, a standard net distribution district (75.7 ib/p/yr), followed by the PBO net distribution districts of Karonga (25.6 ib/p/yr) and Salima (23.6 ib/p/yr). Kasungu, a standard net district, had the lowest annual EIR, 20.9 ib/p/yr.

**Residual Life of Sprayed Insecticide:** Spray quality was satisfactory in both IRS districts: Nkhotakota and Nkhata Bay. The residual efficacy of Actellic 300CS ranged from 2 to 3 months in Nkhotakota (approximately 2000 households) and Nkhata Bay districts. SumiShield 50WG is still effective 8 months after spraying in Nkhotakota District, with above 80% mortality at 1–4 days' holding period.

**Insecticide Resistance:** Both *An. funestus* s.l. and *An. gambiae* s.l. are fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. Both species are highly resistant to the pyrethroids deltamethrin, permethrin, and alpha-cypermethrin. Pre-exposure of *An. funestus* s.l. and *An. gambiae* s.l. to 4% PBO restored their susceptibility to pyrethroids.

***Conclusions:*** *An. gambiae* s.l. was the most abundant vector. *An. arabiensis* was the predominant member identified to the species-specific level. Only *An. funestus* s.s. was identified to the species-specific level from the *An. funestus* group in all six districts. *An. gambiae* s.l. predominantly fed on cattle whereas *An. funestus* s.l. fed mainly on humans in all six districts. *An. funestus* s.s. is the most important malaria vector in the six districts, with an overall 3.5% sporozoite infection rate. Both *An. funestus* s.l. and *An. gambiae* s.l. exhibited no specific preference for indoor or outdoor biting. The biting activity of *An. funestus* s.l. and *An. gambiae* s.l. occurred from dawn to dusk in all four districts.

Spray quality was satisfactory in the two IRS districts where monitoring was conducted, Nkhotakota and Nkhata Bay. The residual efficacy of Actellic 300CS ranged from 2 to 3 months in both districts. SumiShield 50WG is still effective 8 months after spraying in Nkhotakota District. *An. funestus* s.l., and *An. gambiae* s.l. were susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin and highly resistant to the three pyrethroids tested. Pre-exposure to 4% PBO followed by pyrethroids greatly improved the efficacy of pyrethroids.

The absence of *Ace-1* resistant allele is consistent with the absence of phenotypic resistance to pirimiphos-methyl (organophosphate) in *An. arabiensis* and *An. gambiae* s.s. in Malawi. There appears to be *kdr-east* resistance in *An. gambiae* s.s. and *An. arabiensis* populations in Malawi. However, these results need to be verified further to ascertain their validity.

***Recommendations:***

Despite shorter residual efficacy of Actellic 300CS than SumiShield 50WG, IRS with both insecticides seem to have similar impact on entomological and epidemiological indicators of malaria. Given this impact and the absence of phenotypic or genetic resistance to organophosphates, pirimiphos-methyl is recommended for IRS as part of the rotation strategy for insecticide resistance management. Further investigation is recommended into the causes for the short residual efficacy of Actellic despite.

Based on suscetability testing results, IRS with clothianidin and ITNs with the active ingredient chlorfenapyr (e.g., IG2) can be used as part the malaria vector control interventions in Malawi. Furthermore, ITNs with PBO (alphacypermethrin and PBO) and can also be recommended for distribution in Malawi.

# I. INTRODUCTION

In October 2020, VectorLink Malawi carried out indoor residual spraying (IRS) in Nkhotakota District in the central region of Malawi. SumiShield 50WG, a clothianidin formulation and Actellic 300CS, a microencapsulated suspension formulation of pirimiphos-methyl, an organophosphate, were used to spray structures in Nkhotakota District. In the same year, the Malawian Government's National Malaria Control Program, supported by World Vision International with funding from the Global Fund, conducted IRS in the southern region's Mangochi and Balaka districts and Nkhata Bay in the northern region. Nkhata Bay and Balaka districts were sprayed with Actellic 300CS and SumiShield 50WG, respectively, while Mangochi was sprayed with both Actellic 300CS and SumiShield 50WG. The U.S. President's Malaria Initiative (PMI) VectorLink Malawi project, in collaboration with the Malaria Alert Centre of the Kamuzu University of Health Sciences, conducted spray quality assessment and monitoring of residual lifespans in Nkhotakota and Nkhata Bay districts. In addition, comprehensive longitudinal entomological monitoring was also conducted in thirteen sentinel sites in six districts (Karonga, Nkhata Bay, Nkhotakota, Salima, Kasungu, and Chikwawa) across the country to assess vector bionomics (vector density, composition, distribution, and behavior), species identification, infection rates, and insecticide resistance. Resistance mechanism tests were also conducted on major malaria vectors in Malawi.

This report summarizes the key findings of the longitudinal entomological monitoring for species composition, vector abundance, biting behavior and rates, blood meal analysis and infectivity rates of malaria vectors as well as residual efficacy of Actellic 300CS and SumiShield 50WG, and the susceptibility status of malaria vectors to different insecticides, across Malawi.

# 2. METHODOLOGY

## 2.1 LONGITUDINAL MONITORING

The VectorLink Malawi team sampled adult mosquitoes from July 2020 to June 2021 in six districts, located across Malawi (Figure 1). Four sites are in the northern region, seven in the central region, and two in the southern region (Table 1).

Figure 1: Map of Malawi Showing Entomological Monitoring Sites

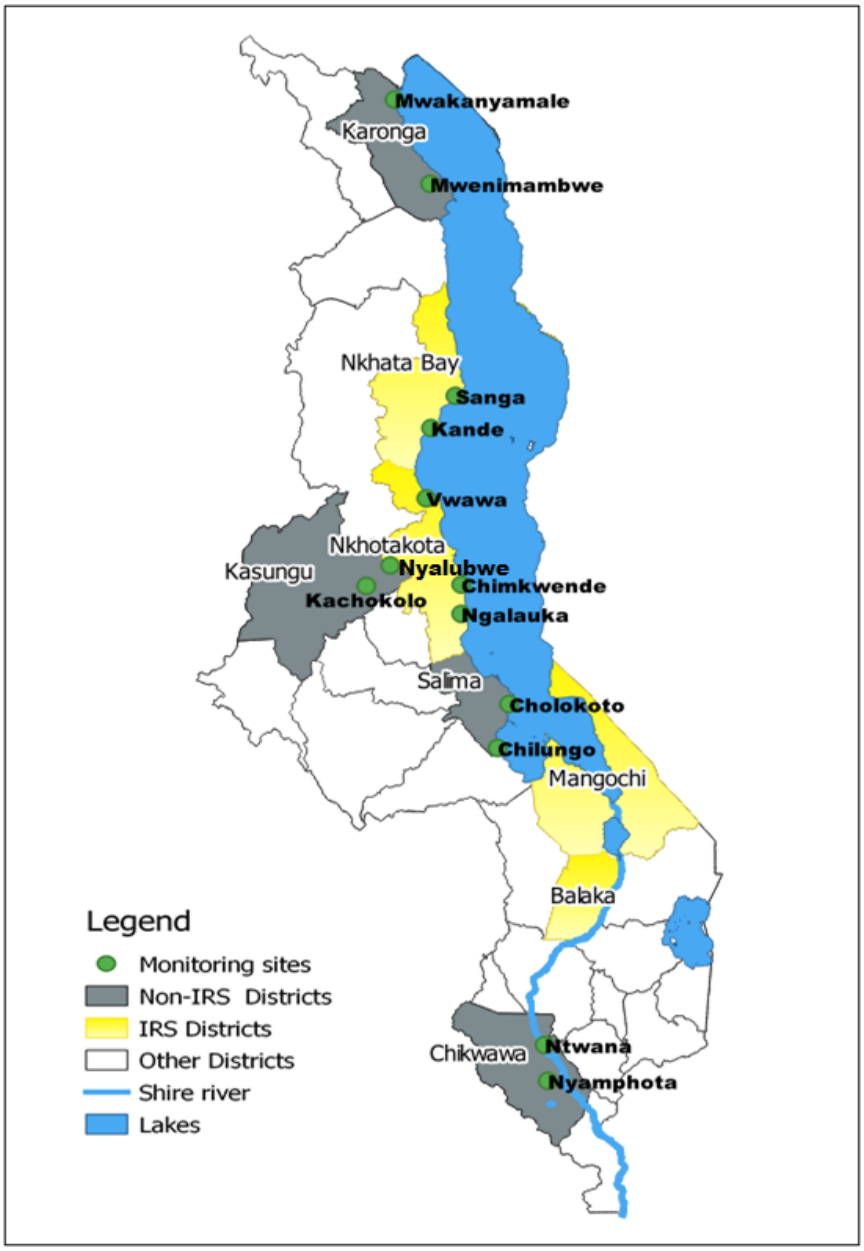


Table 1: Sentinel Sites for Entomological Monitoring in Malawi

| Region | District | Sentinel Sites | Latitude and longitude | Collections | Malaria Control Interventions |
|---|---|---|---|---|---|
| Northern | Karonga | Mwakanyamale | S 9° 47' 1.7"; E 33° 53' 34.36" | PSC, CDC-LT | PBO nets |
| | | Mwenimambwe | S 10° 20' 24.14"; E 34° 6' 41.62" | PSC, CDC-LT | |
| | Nkhata Bay | Kande | S 11° 57' 3.3"; E 34° 7' 1.2" | PSC, CDC-LT | Standard nets and IRS (all sites sprayed with Actellic 300CS) |
| | | Sanga | S 11° 44' 18.58"; E 34° 16' 5.04" | HLC, PSC, CDC-LT | |
| Central | Salima | Chilungo | S 14° 3' 44.41"; E 34° 31' 42.08" | HLC, PSC, CDC-LT | PBO nets |
| | | Cholokoto | S 13° 46' 20.77"; E 34° 35' 57.51" | PSC, CDC-LT | |
| | Nkhotakota | Vwawa | S 12° 24' 54.4"; E 34° 5' 16.44" | HLC, PSC, CDC-LT | IRS (sprayed with SumiShield) |
| | | Chimkwende | S 12° 59' 3.49"; E 34° 18' 13.15" | PSC, CDC-LT | IRS (sprayed with SumiShield) |
| | | Ngalauka | S 13° 10' 38.52"; E 34° 18' 12.84" | HLC, PSC, CDC-LT | IRS (sprayed with SumiShield) |
| | Kasungu | Kachokolo | S 12° 59' 35.09"; E 33° 43' 4.19" | HLC, PSC, CDC-LT | Standard nets |
| | | Nyalubwe | S 12° 51' 26.44"; E 33° 51' 57.79" | PSC, CDC-LT | |
| Southern | Chikwawa | Nyamphota | S 16° 15' 31.71"; E 34° 50' 17" | PSC, CDC-LT | Standard nets |
| | | Ntwana | S 16° 1' 18.05"; E 34° 49' 7.16" | PSC, CDC-LT | |

*Note: PBO=Piperonyl butoxide*

The team used pyrethrum spray catches (PSCs) (SOP03/01) and Centers for Disease Control and Prevention (CDC) miniature light traps (LTs) (SOP01/01) monthly in 13 sentinel sites in the six districts. Adult mosquitoes were also sampled from July 2020 to June 2021 using human landing catches (HLC) on a quarterly basis from five sentinel sites in four districts: Sanga (Nkhata Bay), Vwawa and Ngalauka (Nkhotakota), Kachokolo (Kasungu), and Chilungo (Salima) (Table 2). Six households from each village were selected for a single night of collection per quarter. Collection was done from 5:00 pm to 11:00 am. A total of six volunteers, divided into groups of two, sat one indoor the other outside a house for 6-hour shifts per night. Mosquitoes collected every hour were held in labeled resting cups, provided with 10% sugar solution, and stored in a cooler box. The next morning, ovary dissections were done on a percentage of female *Anopheles* collected per night to check their parity status. Samples were then morphologically identified using the method described by (Gillies and Coetzee 1987) and, finally, sent to the laboratory for further analyses.

Table 2: Longitudinal Monitoring of Adult Mosquitoes in Sentinel Sites

| Collection Method | Time | Frequency | Sample |
|---|---|---|---|
| PSCs | 6:00 am to 8:00 am | 1 day per site per month | 15 houses per site/same house every month |
| CDC-LTs | 6:00 pm to 6:30 am | 1 night per site per month | 10 houses per site/same house every month |
| HLCs | 5:00 pm to 11:00 am | 1 night per house every three months | 6 houses per site/same house every quarter |

## 2.2 Insecticide Resistance Monitoring

### 2.2.1 Insecticide Susceptibility Tests And Intensity Assays

To determine the frequency and intensity of insecticide resistance, larval and adult malaria vectors were collected from one sentinel site in each of the six districts. Additional sites were visited to collect adult malaria vectors when low numbers were encountered at the planned sentinel site. *An. funestus* s.l. is the predominant vector species in many areas and, due to the difficulty of finding larval stages of this species, mosquitoes were

collected as adults, allowed to lay eggs, and reared to the adult stage for subsequent testing. *An. gambiae* s.l. (predominantly *An. arabiensis*) is the primary vector in Karonga District. Adult and/or larvae of this species were collected from larval habitats and reared to adult stage for testing.

### 2.2.2 MOSQUITO COLLECTIONS

Adult *Anopheles* mosquitoes were sampled from a single sentinel site each in three districts (Karonga, Nkhata Bay, and Kasungu) and two sites each in Nkhotakota and Chikwawa to determine the frequency and intensity of insecticide resistance. Mosquitoes were collected as adults, allowed to lay eggs, and reared to $F_1$ generation and subsequently tested using the World Health Organization (WHO) tube and the CDC bottle assays. In addition, some samples were collected as larvae from their natural habitats in Lisuli Oxbow (Chikwawa District), Lifizi and Chilungo (Salima District), Chimkwende (Nkhotakota District), and Mwenimambwe (Karonga District). The collected larvae were reared to adults and subsequently tested for susceptibility to different insecticides. All mosquito-rearing activities were carried out in the insectary at the Malaria Alert Centre.

### 2.2.3 INSECTICIDES TESTED

Table 3 summarizes the sentinel sites and insecticides tested. The CDC bottle assay was used to determine intensity of resistance in the main malaria vectors by exposing adult mosquitoes to 1x and 5x deltamethrin, permethrin, and alpha-cypermethrin. Tests for chlorfenapyr (pyrrole) and clothianidin (neonicotinoid) were performed using newly developed bottle or paper-based assay protocols.

Table 3: Anopheles Species and Insecticides Tested in Six Sentinel Sites

| District | Sentinel Site | Source | Species Tested | Tests |
|---|---|---|---|---|
| Chikwawa | Lisuli Oxbow | Larvae | *An. gambiae* s. l. | Deltamethrin 0.05% |
| | | | | Permethrin 0.75% |
| | | | | Pirimiphos-methyl 0.25% |
| | | | | Chlorfenapyr 100µg/bottle |
| | | | | Clothianidin 2%(13.2mg/paper) |
| | Chakanira | Adults | *An. funestus* s.l. | Deltamethrin 0.25% |
| | | | | Permethrin 3.75% |
| | Ntwana | Adults | *An. funestus* s.l. | Deltamethrin 0.05% |
| | | | | 4%PBO +Deltamethrin 0.05% |
| | | | | Deltamethrin 0.25% |
| | | | | Deltamethrin 0.5% |
| | | | | Permethrin 0.75% |
| | | | | 4% PBO + Permethrin 0.75% |
| | | | | Permethrin 3.75% |
| | | | | Alpha-cypermethrin 0.05% |
| | | | | 4% PBO + Alpha-cypermethrin 0.75% |
| | | | | Pirimiphos-methyl 0.25% |
| | | | | Chlorfenapyr 100µg/bottle |
| | | Adults | *An. gambiae* s.l. | Alpha-cypermethrin 0.05% |
| | | | | 4%PBO +Deltamethrin 0.05% |
| | | | | 4% PBO + Permethrin 0.75% |
| | | | | 4% PBO + Alpha-cypermethrin 0.05% |
| | | | | Pirimiphos-methyl 0.25% |
| Salima | Lifizi | Larvae | *An. gambiae* s.l. | Alpha-cypermethrin 0.05% |
| | | | | Pirimiphos-methyl 0.25% |
| | Chilungo | Larvae | *An. gambiae* s.l. | Deltamethrin 0.05% |

| District | Sentinel Site | Source | Species Tested | Tests |
|---|---|---|---|---|
| | | | | 4%PBO +Deltamethrin 0.05% |
| | | | | Permethrin 0.75% |
| | | | | 4% PBO + Alpha-cypermethrin 0.05% |
| | | | | Alpha-cypermethrin 0.05% |
| | | | | 4% PBO + Alpha-cypermethrin 0.05% |
| | | | | Chlorfenapyr100µg/bottle |
| Kasungu | Kachokolo | Adults | *An. funestus* s.l. | Deltamethrin 0.05% |
| | | | | 4%PBO +Deltamethrin 0.05% |
| | | | | Permethrin 0.75% |
| | | | | 4% PBO + Permethrin 0.75% |
| | | | | Pirimiphos-methyl 0.25% |
| | | | | Chlorfenapyr100µg/bottle |
| | | | | Clothianidin 2% (13.2mg/paper) |
| Nkhotakota | Chimkwende | Adult | *An. gambiae* s.l. | Deltamethrin 0.05% |
| | | Adult | *An. funestus* s.l. | Permethrin 0.75% |
| | | Larvae | *An. funestus* s.l. | Alpha-cypermethrin 0.05% |
| | | Adult | *An. funestus* s. l. | Clothianidin 2%(13.2mg/paper) |
| | Vwawa | Adults | *An. funestus* s.l. | Deltamethrin 62.5µg/bottle |
| | | | *An. funestus* s.l. | Pirimiphos-methyl 0.25% |
| | | | | Chlorfenapyr100µg/bottle |
| Nkhata Bay | Mazembe | Adults | *An. funestus* s.l. | Deltamethrin 0.05% |
| | | | | 4%PBO+ Deltamethrin 0.05% |
| | | | | Permethrin 0.75% |
| | | | | 4%PBO + Permethrin 0.75% |
| | | | | 4%PBO+ Alpha-cypermethrin 0.75% |
| | | | | Pirimiphos-methyl 0.25% |
| | | | | Clothianidin 2% (13.2mg/paper) |
| | | | | Chlorfenapyr 100µg/bottle |
| | | | | Deltamethrin 62.5µg/bottle |
| | | | | Permethrin 107.5 µg/bottle |
| Karonga | Mwenimambwe | Larvae | *An. gambiae* s.l. | Deltamethrin 0.05% |
| | | | | Permethrin 0.75% |
| | | Adults | | Permethrin 107.5 µg/bottle |
| | | | | Alpha-cypermethrin 0.05% |
| | | | | Pirimiphos-methyl 0.25% |
| | | | | Clothianidin 2% (13.2mg/paper) |
| | | | | Chlorfenapyr 100µg/bottle |

### 2.2.4 WHO TUBE ASSAYS

Tests were performed according to standard WHO procedures (WHO 2016) and SOP06/01. Both $F_1$ and those collected as larvae from their aquatic habitats were raised to adults and females aged 2–5 days old were used for susceptibility tests by exposing them to WHO-recommended diagnostic doses. All assays were conducted using WHO tube assays except for chlorfenapyr in all the districts, deltamethrin 5x in Nkhotakota, deltamethrin 5x and permethrin 5x in Nkhata-Bay districts which used CDC bottle bioassays.

*Procedure*: Four test replicates and two controls were set up for each insecticide that was tested with the few exceptions when a single control tube was used due to a limited number of mosquito samples. A total of 20–25 female *An. funestus* s.l., *An. arabiensis,* and *An. gambiae* s.l. were aspirated into the holding tubes lined with untreated white sheets to give six replicates (four test and two controls). Mosquitoes were exposed in the tubes for a period of one hour. Knockdown rate was scored at 60 minutes. At the end of the hour, mosquitoes were

transferred back to the holding tubes. Cotton wool soaked in 10% sugar solution was placed on top of the holding tubes. Thereafter, the tubes were placed in a cool box with a wet towel inside, to avoid mortality due to desiccation of the mosquitoes. Mosquitoes were maintained in the holding tubes for 24 hours and up to five days for slow-acting insecticides (clothianidin). Relative humidity and temperature were recorded during exposure and recovery periods. At the end of the recovery period, the numbers of dead and alive mosquitoes were counted and recorded. Each mosquito was placed in an individual tube, which was placed in a Ziploc bag with desiccants in it and clearly labeled with the assay date, mosquito species, dead or alive after exposure, insecticide used, and location. A susceptible strain of *An. gambiae* (Kisumu) was also tested as a control to confirm the quality of insecticide-treated papers and bottles.

### 2.2.5 CDC Bottle Assays

The CDC bottle bioassay method (Brogdon and Chan 2010) with modifications (on knockdown effect observation and holding period of mosquitoes exposed to chlorfenapyr) was also used to test for the susceptibility of malaria vectors (*An. funestus* s.l. and *An. gambiae* s.l.). Four Wheaton bottles (250mls) with caps were coated with 1ml of an insecticide by rolling and inverting the bottles. In addition, two control bottles were coated with 1ml of acetone. The lids were removed, and the coated bottles were then placed in a drawer covered with paper towel and left overnight to dry completely in the dark. The next morning, mosquitoes were exposed for 60 minutes, after which they were placed in recovery cups covered with untreated netting material and provided with 10% sugar solution. Knockdown effect was observed every 10 minutes up to 60 minutes (original protocol states that knockdown be observed after 60) and mortality at 24, 48, and 72 hours after exposure to chlorfenapyr. When mortality was less than 100% on Day 3 for chlorfenapyr, the observation period was extended to 5 days after exposure (for original protocol, holding period is 3 days). Mosquitoes exposed to pyrethroids were observed for 30 minutes, after which they were transferred into vials containing RNA for further analysis at CDC.

### 2.2.6 Interpretation of Results

Susceptibility of *An. funestus* s.l. and *An. gambiae* s.l. was evaluated based on the WHO criteria of test mortality (WHO 2016): 98–100% mortality indicates susceptibility. Mortality of equal to or more than 90% but less than 98% suggests the existence of resistance and the need for confirmation. If mortality is less than 90%, then the population is resistant. When control mortality was greater than or equal to 5% but less than 20%, the observed mortality was corrected using Abbott's formula (Abbott 1925). If the control mortality was above 20%, the test results were discarded.

## 2.3 Molecular Detection of Resistance Markers

### 2.3.1 Detection of *Acetylcholinesterase (Ace-1)*

The protocol used in the detection of *Acetylcholinesterase (Ace-1)* mutation in dead and alive mosquitoes from susceptibility bioassays, LTs, and PSCs were adopted from the Malaria Research and Reference Reagent Resource (MR4) Center Manual (2016). DNA was extracted from the head and thorax of female *An. gambiae* s.l. mosquitoes using Livak grinding buffer; samples were incubated for 30 minutes at 65°C. Potassium acetate (14µL) was added then incubated on ice for 30 minutes, centrifuged, and the supernatant was transferred into a new tube. 200µl ethanol was then added, vortexed, and spun, the supernatant was discarded, and the pellet rinsed in 100µl ice-cold 70% ethanol. Tubes were then left to dry and reconstituted with 100µl TE and then incubated for 10 minutes at 65°C. The extracted DNA was analyzed using an Intentional Mismatched Primer-Polymerase Chain Reaction (IMP-PCR) assay to detect *Ace-1* gene mutation in *An. gambiae* s.l. Mastermix was prepared by adding the following reagents: sterile water, 5xGoTaqPCR buffer, dNTP (2.5mM mix), $MgCl_2$ (25mM), CDCACEF(8pmol/µl) [GGT GGA CGT GTG TGG CTC], CDCACER(8pmol/µl) [CTA CCG TAG CGC AAG GTT C], CDCWT (10pmol/µl) [TGT GGA TCT TCG GCG TCG], CDCG119SR (25pmol/µl) [CGG TCG CGG AGT AGA ATCT], Taq DNA polymerase (5U/ µl). Then samples were run on 1.5% agarose gel.

### 2.3.2 DETECTION OF *KDR*

Female *An. gambiae* s.s. and *An. arabiensis* from susceptibility tests were analyzed for knockdown resistance (*kdr*)-east by using a protocol adopted from MR4, 2015 with minor modifications. Mosquito DNA was extracted using Livak method. Then PCR Master Mix was prepared by adding the following primers and reagents; sterile water, Taq 5x PCR buffer (containing 15mM $Mgcl_2$), dNTPs (2.0mM), $MgCl_2$, IPCF:(F, 2.5pmol/µl) [GATAATGTGGATAGATTCCCCGACCATG], AltRev: (R,2.5 pmol/µl) [TGCCGTTGGTGCAGACAAGGATG], WT: R, (5.0 pmol/µl) [GGTCCATGTTAATTTGCATTACTTACGAaTA], East: F, (2.5pmol/µl) [CTTGGCCACTGTAGTGATAGGAAAaTC] and GoTaq DNA polymerase (5 U/ µl). Thereafter, 2µl of DNA template was added to 23µl Master Mix. The PCR products were visualized by electrophoresis on 2% agarose with 3 µl Redsafe gel.

## 2.4 WALL CONE BIOASSAYS

### 2.4.1 SPRAY QUALITY ASSESSMENT

The 2020 spray campaign commenced on October 26, 2020, in Nkhotakota District and in November in Nkhata Bay District. VectorLink Malawi, in collaboration with the Malaria Alert Centre, conducted wall bioassay tests for spray quality checks in six villages (Vwawa, Chamba 1, Chimkwende, Ngalauka, Mtachi 3, and Kalungama) (Table 4), selected to represent the three entomological sites in Nkhotakota, as well as a sampling of operations sites and a village sprayed with Actellic 300CS. The tests were carried out on October 27–November 3, 2020. The catchment areas covering five operations sites (Dwangwa, Boma, Chididi, Mkaika, and Benga) were sprayed with SumiShield 50WG and one operations site (Bua) was sprayed with Actellic 300CS. In Nkhata Bay District, no spray quality assessment was conducted due to non-availability of mosquitoes at the start of the spraying period.

Table 4: Spray Quality and Residual Life Assessment Villages in Nkhotakota District

| District | Villages | Operations Site | Latitude and Longitude | Insecticide |
|---|---|---|---|---|
| Nkhotakota | Vwawa*† | Dwangwa | S 12° 24' 46"; E 34° 5' 1" | SumiShield 50WG |
| | Chimkwende† | Boma | S 12° 59' 3"; E 34° 18' 12" | SumiShield 50WG |
| | Chamba 1 | Chididi | S 13° 1' 15"; E 34° 19' 54" | SumiShield 50WG |
| | Ngalauka† | Mkaika | S 13° 10' 28"; E 34° 18' 6" | SumiShield 50WG |
| | Mtachi 3 | Benga | S 13° 17' 17"; E 34° 16' 53" | SumiShield 50WG |
| | Kalungama† | Bua | S 12°46' 6"; E 34° 13' 30" | Actellic 300 CS |

** Positive control site † Residual life monitoring sites*

Cone bioassay tests were performed 24 hours after spray in all the villages. In five villages (Vwawa, Chamba 1, Ngalauka, Mtachi 3, and Kalungama), six structures made of different wall surfaces (brick, cement-plastered, and mud walls) were randomly selected at each site to conduct wall bioassay tests for spray quality assessment. However, at Chimkwende and Mtachi 3, only brick and mud houses were tested due to the absence of cement-plastered structures. At Vwawa Village, 12 structures were randomly selected; six were sprayed under close supervision of VectorLink staff to serve as a positive control and the remaining six structures were sprayed without close supervision (normal spraying). Kisumu strain of *An. gambiae* s.s. (2–5 days old) reared at the Malaria Alert Centre insectary were used for the wall cone bioassays.

The results of the spray quality assessment and the fumigation effect of the two insecticides mentioned above are presented in this report.

### 2.4.2 MONITORING RESIDUAL EFFICACY

The Malaria Alert Centre, as a PMI VectorLink Malawi partner, conducted wall bioassay tests in Nkhotakota and Nkhata Bay districts to assess the decay rate of the sprayed insecticides. In Nkhotakota District, the assessment was performed in four villages: Vwawa, Chimkwende and Ngalauka (SumiShield sites) and

Kalungama (Actellic 300CS site). In Nkhata Bay District, residual life monitoring of Actellic 300CS also was conducted in four villages: Kande, Sanga, Kavuzi, and Musawanika.

Six structures made of the different wall surfaces (burned brick, cement-plastered, and mud walls) were randomly selected for the wall bioassay tests in each village.

## 2.5 LABORATORY ANALYSIS

PCR was used to identify members of *An. gambiae* s.l. and *An. funestus* s.l. to the species level as described by Benedict (2007). The heads and thoraxes of a sample of the *An. gambiae* s.l. and *An. funestus* s.l. were sorted and tested for the presence of circumsporozoite antigens of *Plasmodium falciparum* (*Pf*) using enzyme-linked immunosorbent assays (ELISA) described by Wirtz et al. (1987) to determine sporozoite rate (SR) and subsequently calculate Entomological Inoculation Rates (EIRs). Blood meal conventional PCR as described in the MR4 2016 Manual was used to determine the source of blood in female blood-fed *Anopheles* mosquitoes.

## 2.6 DATA ANALYSIS

The following parameters were calculated:

- Indoor resting density (IRD) = number of adult *Anopheles*/house/day
- Human biting rate (HBR) = total number of vectors collected divided by number of collectors per hour or night
- The proportion of vector bites occurring indoors for an unprotected individual ($\pi I, u$)
- The proportion of vector bites occurring indoors during sleeping hours, for an unprotected individual ($\pi I, s$)
- SR = *Anopheles* found positive for the presence of circumsporozoite proteins (CSP) / total number tested*100)
- EIRs = number of infectious bites/per person /per unit time
- Nightly EIR = Daily HBRs * SRs
- Monthly EIR = Nightly EIR * No. of days per month
- Annual EIR = $\sum$Monthly EIRs

# 3. RESULTS & DISCUSSION

## 3.1 LONGITUDINAL MONITORING

### 3.1.1 SPECIES COMPOSITION

A total of 32,042 female *Anopheles* mosquitoes were collected from 13 sentinel sites in all the six monitoring districts from July 2020 to June 2021. Out of these, 19,537 were collected using PSCs (61%), 10,203 using CDC-LTs (31.8%), and 2,302 (7.2%) using HLCs (Figures 2–4). Although the total number of *Anopheles* mosquitoes collected from PSCs was higher than from the other two collection methods, the mean per collection (PSCs=8.3 mosquitoes/house/day and CDC-LTs=6.5 mosquitoes/house/night) was not statistically significant ($p>0.05$). The mean HBR from HLC was 1.4 bites per person per night (b/p/n).

Overall, 55.6% (n=17,816) of the *Anopheles* collected were identified morphologically as *An. gambiae* s.l.; 41.3% (n=13,247) were *An. funestus* s.l. and 3.1% (n=979) were *An. coustani*. However, species composition varied by sentinel site. *An. gambiae* s.l. was predominant in Mwenimambwe and Mwakanyamale sites (Karonga District), Chilungo site (Salima District), and Nyamphota site (Chikwawa District). *An. funestus* s.l. was predominant in Vwawa, Chimkwende, and Ngalauka sites (Nkhotakota District), Kachokolo and Nyalubwe sites (Kasungu District), Sanga and Kande sites (Nkhata Bay), Cholokoto site (Salima District), and Ntwana site (Chikwawa District) (Figures 2–4). Species composition also varied based on indoor or outdoor location. Overall, the more *An. funestus* s.l. were collected indoors (68.9% n=676) and *An. gambiae* s.l. and *An. coustani* were collected outdoors, 58.4% (n=808) and 60.0% (n=767) respectively (Figure 4). Furthermore, a total of 57,314 other mosquito species were collected during the monitoring period using the three methods. Among *Anopheles* mosquitoes collected were: *An. maculipalpis* (n=5), *An. pharoensis* (n=48), *An. pretoriensis* (n=23), *An. rufipes* (n=7), *An. tenebrosus* (n=186), and *An. squamosus* (n=1). Other mosquito genera collected were: 19, 060 *Culex sp*, 37, 906 *Mansonia sp* and 79 *Aedes sp*.

Figure 2: *Anopheles* Composition, by Sentinel Site Across All Six Monitoring Districts, from PSC Collection

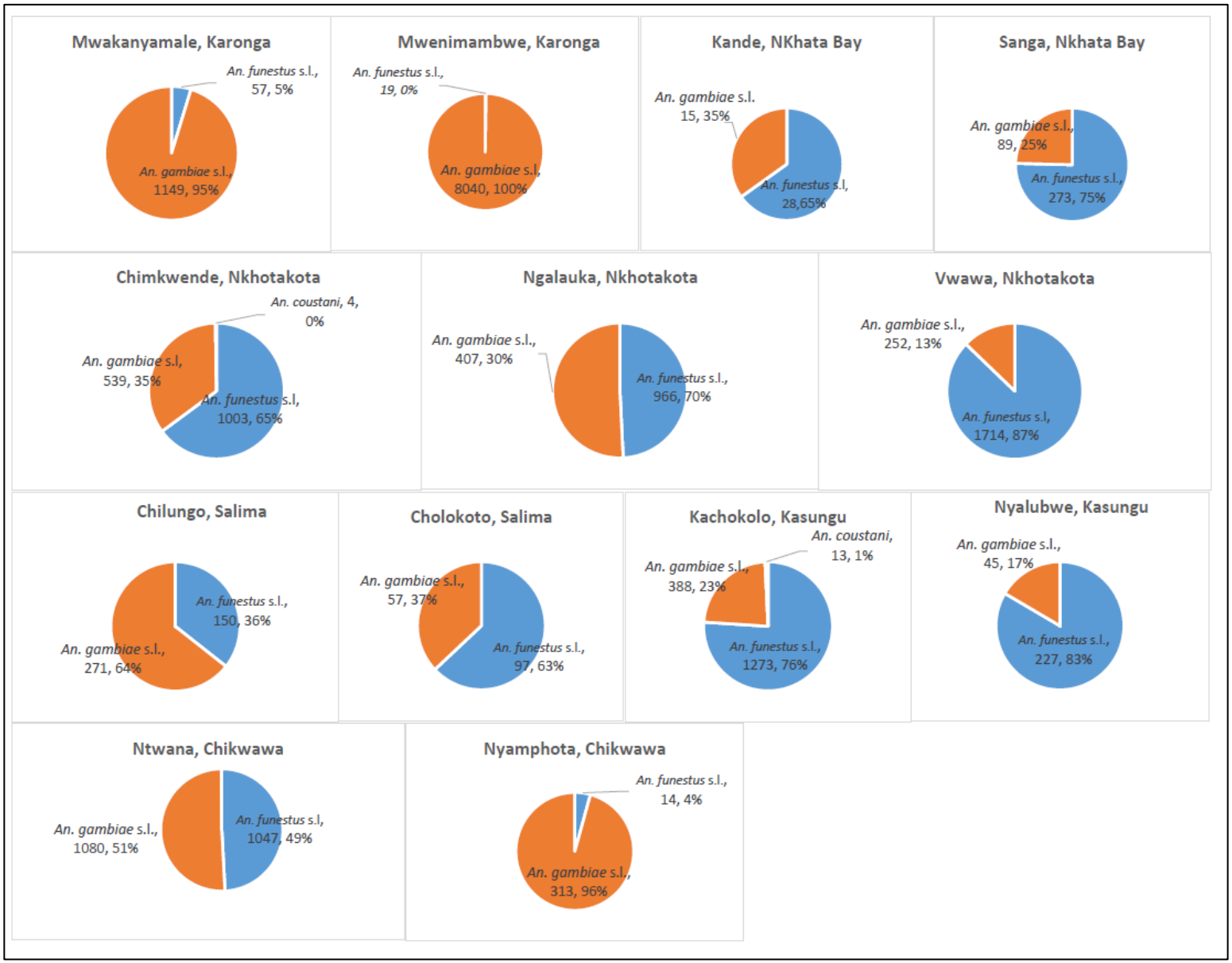


Figure 3: *Anopheles* Composition, by Sentinel Site Across All Six Monitoring Districts, from CDC-LT Collection

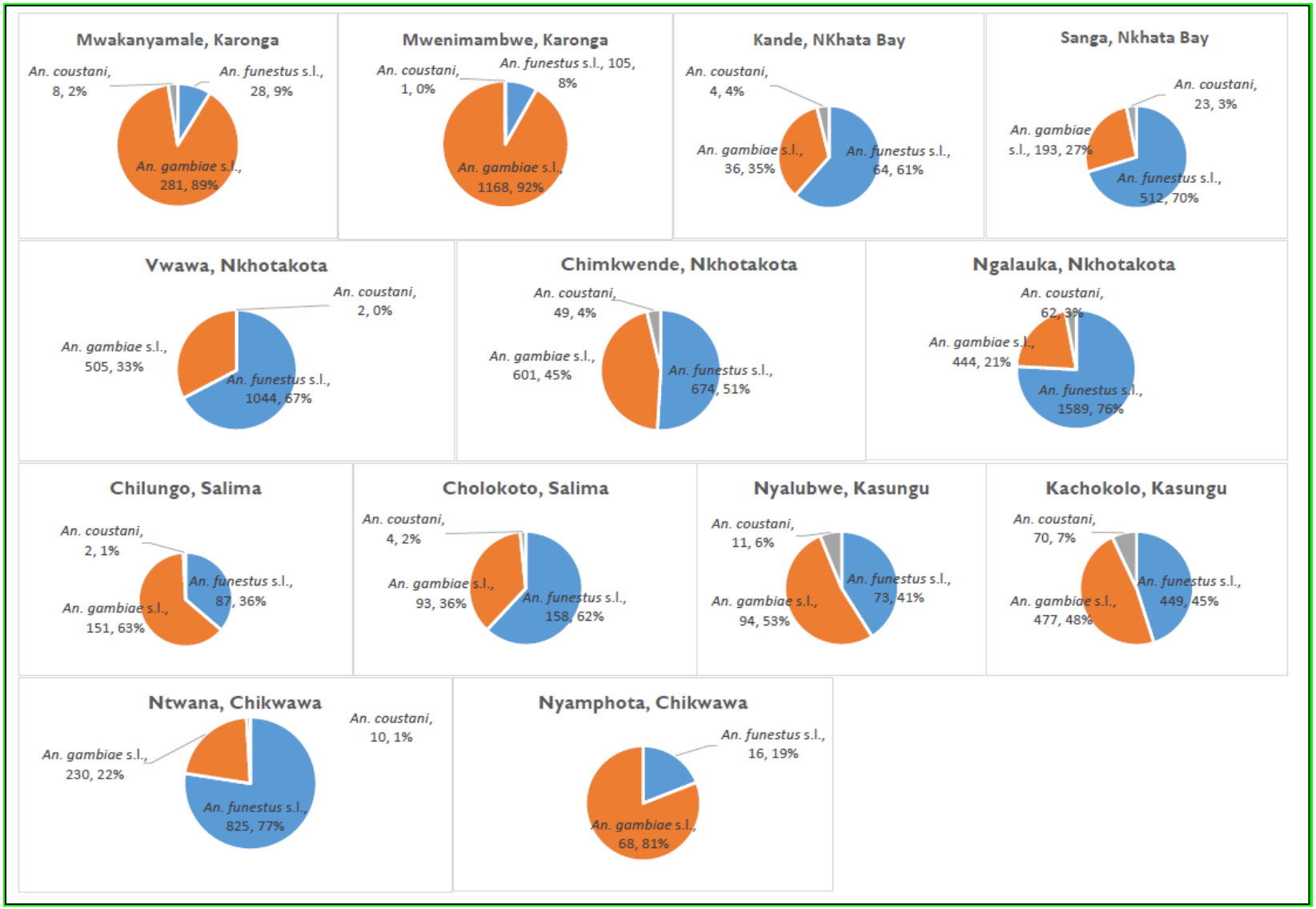
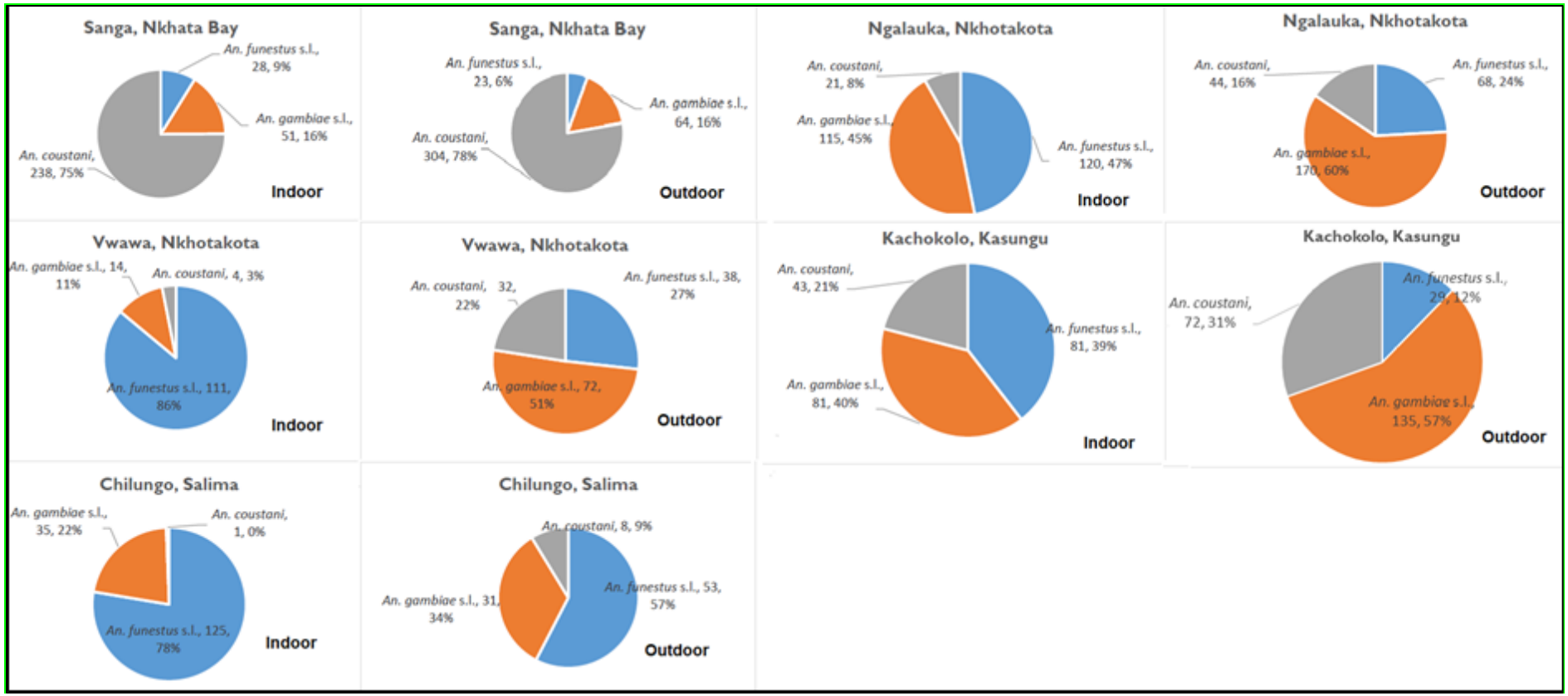


Figure 4: *Anopheles* Composition, by Sentinel Site Across All Four Monitoring Districts, from HLC Collection



### 3.1.2 INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY PSC

#### *DENSITY OF AN. FUNESTUS* S.L.

The overall IRD across all 13 sentinel sites from July 2020 to June 2021 was 2.9 *An. funestus* s.l. per house per day. The highest mean IRD of *An. funestus* s.l. from PSC collections was observed in May 2021, with a mean catch of 7.5 *An. funestus* s.l. per house per day. The lowest was observed in December 2020 (<1.0 mosquito/house/day) (Figure 5).

Figure 5: Mean IRD ± SE of *An. funestus* s.l. Collected by PSCs Across Six Districts (13 Sentinel Sites), July 2020–June 2021

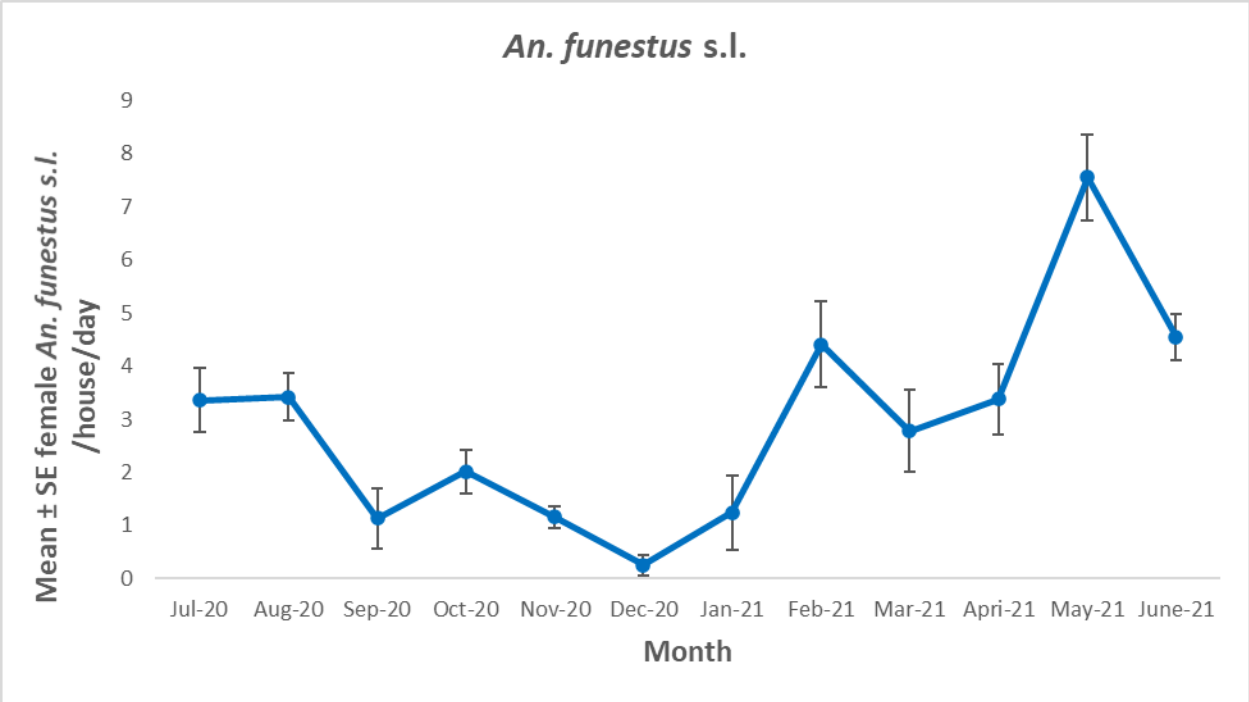
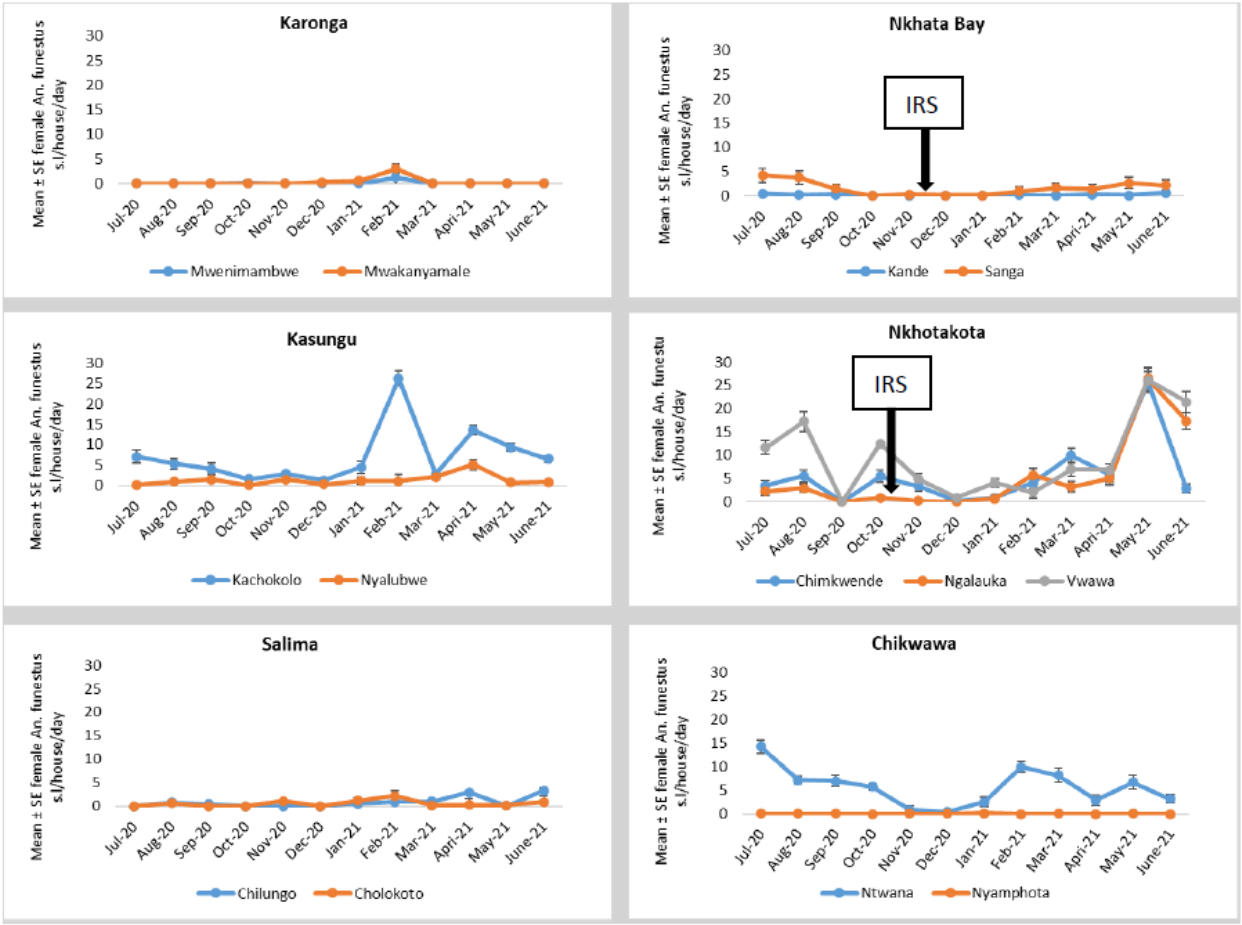



Figure 6 summarizes the mean IRD of *An. funestus* s.l. in all the six districts. The highest mean IRD of *An. funestus* s.l. from PSC collections was observed in May 2021 at Ngalauka site (Nkhotakota District) with a mean catch of 26.4 mosquitoes/house/day. The mean IRD of *An. funestus* s.l. remained low (<5.0 mosquitoes/house/day) throughout the monitoring period in six sites: Mwenimambwe and Mwakanyamale (Karonga), Cholokoto and Chilungo (Salima), Nyalubwe (Kasungu), and Nyamphota (Chikwawa). The IRD of *An. funestus* s.l. in Kachokolo site (Kasungu) was low during the dry season and peaked during the rainy season, with the highest mean catch observed in February (26.3 mosquitoes/house/day). In Ntwana (Chikwawa), the IRD of *An. funestus* s.l. was relatively high in July (15.0 mosquitoes/house/day), then steadily declined to <1.0 mosquito/house/day in December and rose again in the rainy season with the highest catch in February (10 mosquitoes/house/day).

The IRD of *An. funestus* s.l. in Sanga and Kande (Nkhata Bay IRS district) was low (<1.0 mosquito/house/day) throughout the sampling period; before spray (July–October) and after spray (December–June). However, in Nkhotakota IRS district, the IRD of *An. funestus* s.l. was relatively high before spraying (July–August) and declined in September. After spraying in October, the density was low (<5 mosquitoes/house/day) from

November through April, then rose in May–June 2021. This trend was observed in all the three sites of Vwawa, Chimkwende, and Ngalauka (Figure 6).

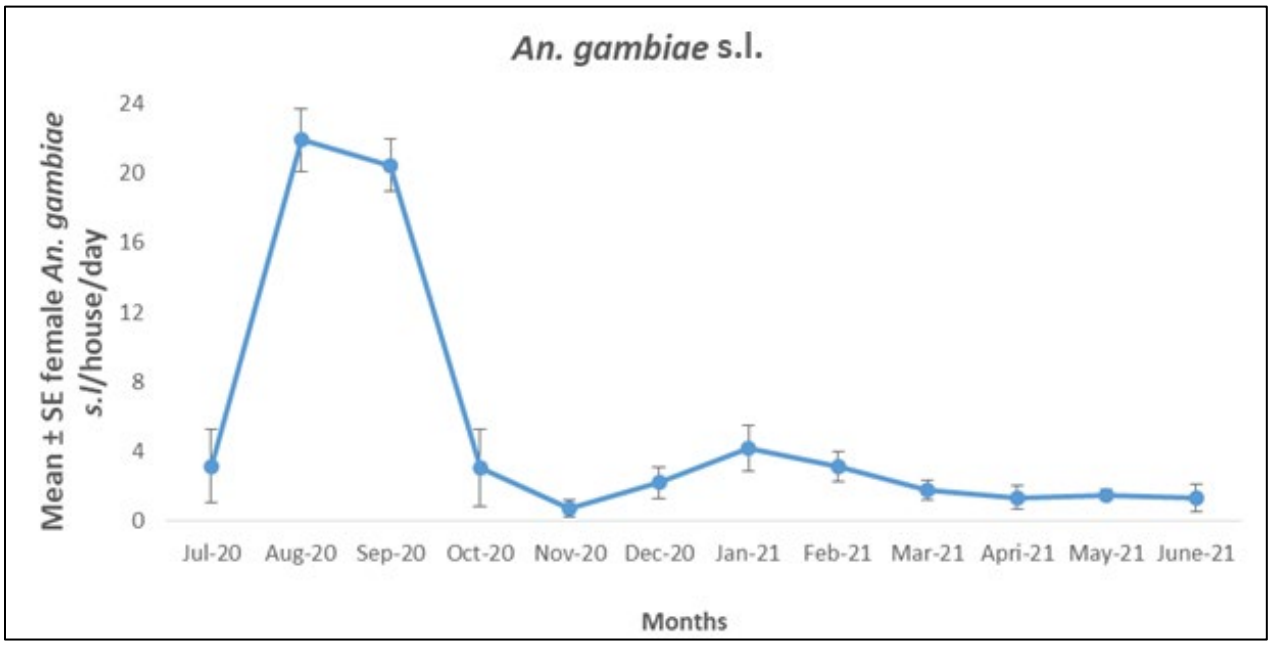
Figure 6: Mean IRD ± SE of *An. funestus* s.l. Collected by PSCs in the Six Districts, July 2020–June 2021




## *DENSITY OF AN. GAMBIAE*

The overall IRD of *An. gambiae* s.l. was 5.4 mosquitoes per house per day across all 13 sentinel sites. The highest mean IRD of *An. gambiae* s.l. from PSC collections was observed in August 2020 (21.9 *An. gambiae* s.l. collected per house per day). The lowest IRD was in November 2020 (<1.0 mosquitoes/house/day) (Figure 7).

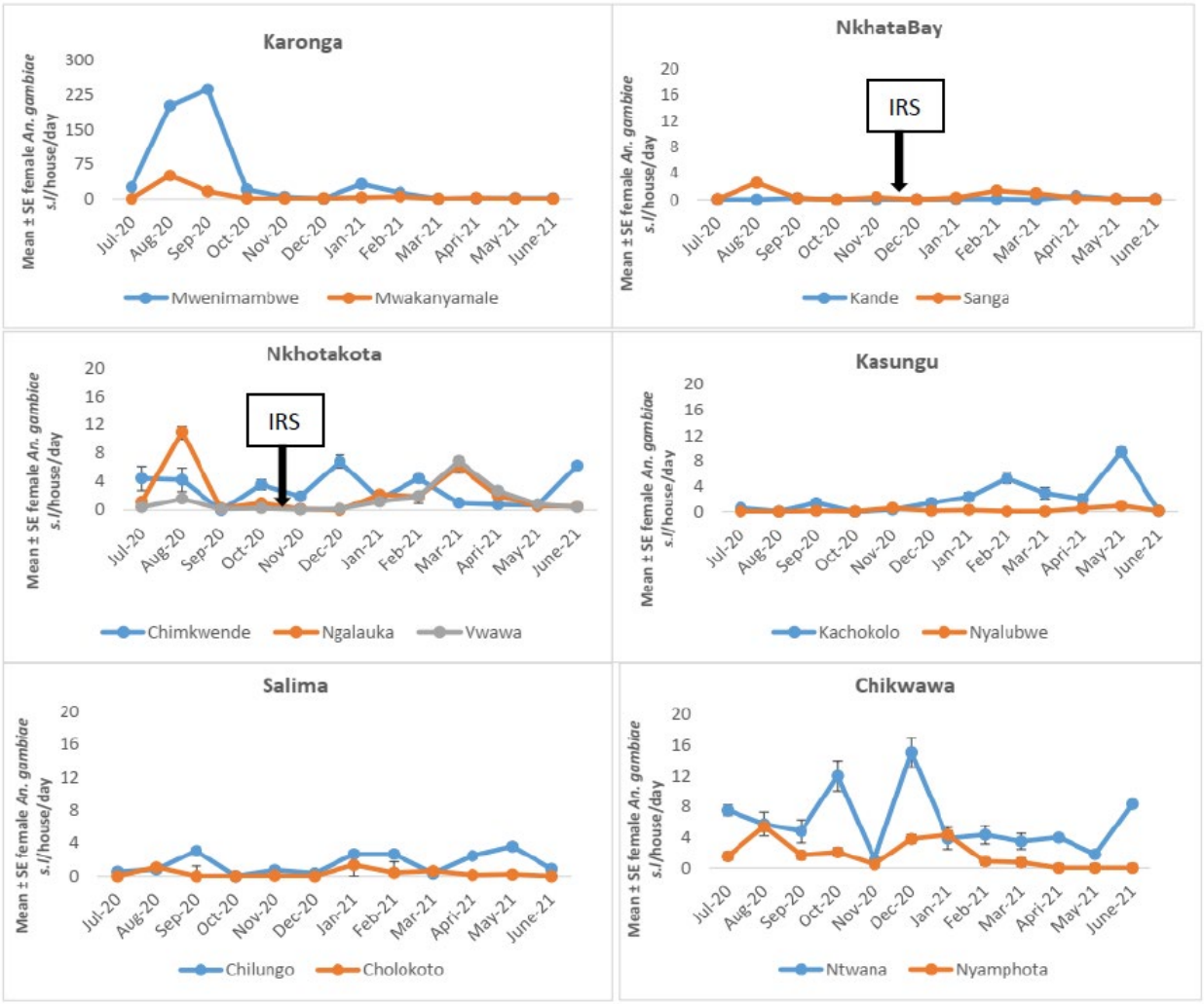
Figure 7: IRD ± SE of *An. gambiae* s.l. Collected by PSCs Across Six Districts (13 Sentinel Sites), July 2020–June 2021




Mwenimambwe site in Karonga recorded the highest IRD in September with a mean *An. gambiae* s.l. IRD of 238.1 mosquitoes per house per day (Figure 8). The IRD of *An. gambiae* s.l. remained low throughout the sampling period in three sites (<5.0 mosquitoes/house/day): Chilungo and Cholokoto (Salima), and Nyalubwe (Kasungu) (Figure 8). However, in Ntwana (Chikwawa), the IRD of *An. gambiae* s.l. was relatively low in July–September (<8 mosquitoes/house/day); it peaked in October (12 mosquitoes/house/day), drastically dropped in November (<2 mosquitoes/house/day), and then rose again in December, when the highest catch of 15.07 mosquitoes/house/day was recorded. In Kachokolo (Kasungu), the IRD of *An. gambiae* s.l. was low during the dry season but slightly peaked during the rainy season with the highest catch observed in May (9.4 mosquitoes/house/day).

In Nkhata Bay District (IRS), the IRD of *An. gambiae* s.l. remained very low (<3.0 mosquitoes/house/day) throughout the monitoring period before spray (July–October) and after spray (December–June). In Nkhotakota IRS district, there was a drop in *An. gambiae* s.l. density just after spray in November–December in Vwawa and Ngalauka sites; however, the density slightly increased during the rainy season (March 2021). While in Chimkwende site the IRD density of *An. gambiae* s.l. was relatively high in July–August (before spray), it dropped in September (<1.0 mosquitoes/house/day). After spray in October, the IRD of *An. gambiae* s.l. was relatively high from November to February, before it dropped in March and rose again in June 2021 (Figure 8).

Figure 8: Mean IRD ± SE of *An. gambiae* s.l. Collected by PSCs in Six Districts, July 2020–June 2021




## 3.1.3 GONOTROPHIC STATUS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY PSC

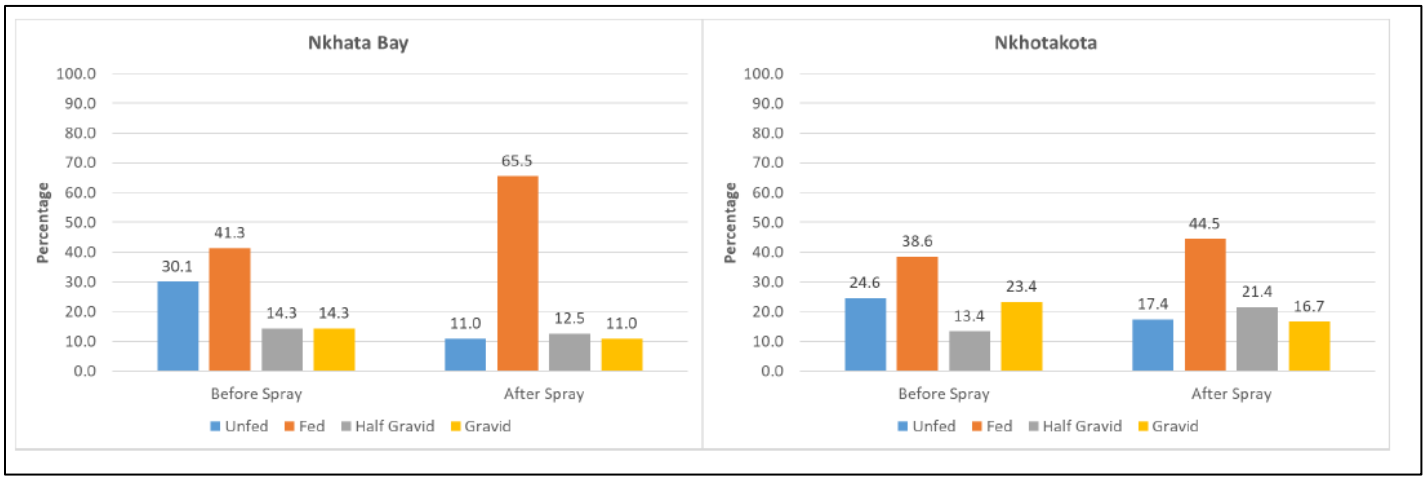
A total 19,537 female *Anopheles* mosquitoes were collected using PSCs. Of these mosquitoes, 19, 514 were *An. funestus* s.l. and *An. gambiae* s.l. Overall, the highest proportion of the female *Anopheles* mosquitoes collected were blood fed (n= 9,979, 51.1%), 1,740 (8.9 %) were gravid, 2,410 (12.4%) were half gravid, and 5,324 (27.3%) were unfed. The gonotrophic status for 61 (0.3%) samples could not be determined because their abdomens were damaged (Table 5). The proportion of gravid mosquitoes was higher among *An. funestus* s.l. (18.8%) than among *An. gambiae* s.l. (3.6%), indicating the more endophilic behavior of *An. funestus* s.l.

Table 5: Gonotrophic Status of *An. funestus* s.l. and *An. gambiae* s.l. Sampled by PSCs in All 13 Sentinel Sites

| Species | Gonotrophic Status | | | | | |
|---|---|---|---|---|---|---|
| | Unfed | Fed | Half Gravid | Gravid | Undetermined | Grand Total |
| ***An. funestus* s.l.** | 1185 (17.3%) | 3154 (45.9%) | 1212 (17.6%) | 1291 (18.8%) | 26 (0.4%) | 6868 |
| ***An. gambiae* s.l.** | 4139 (32.7%) | 6825 (54%) | 1198 (9.5%) | 449 (3.6%) | 35 (0.2%) | 12646 |
| **Grand Total** | 5324 (27.3%) | 9979 (51.1%) | 2410 (12.4%) | 1740 (8.9%) | 61(0.3%) | 19514 |

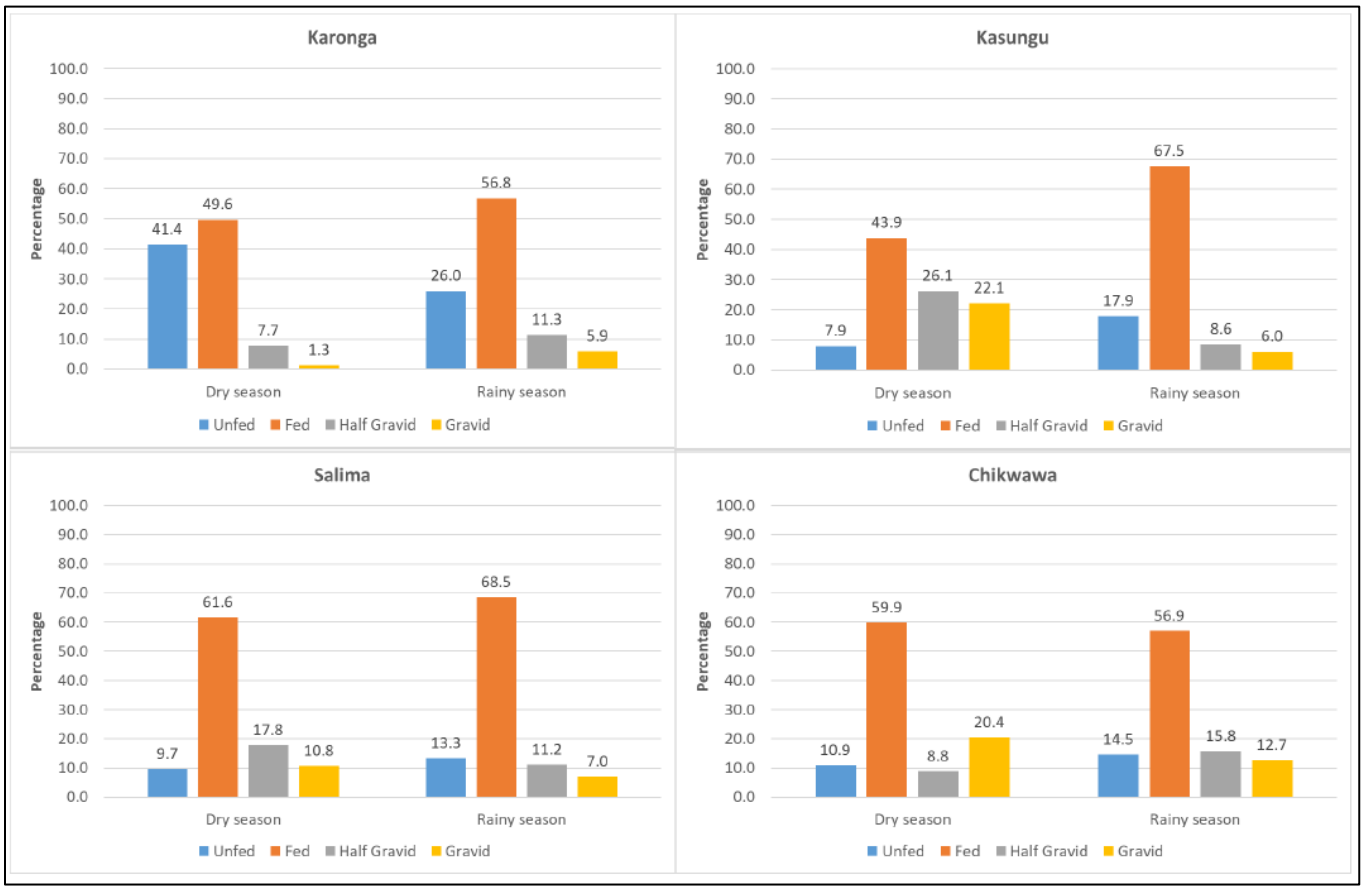
In Nkhotakota IRS district, the proportion of blood-fed mosquitoes was high after spray (November–June) compared to before spray (July–September). The proportion of gravid was lower after spray (November–June) than before spray (July–August). In Nkhata Bay IRS district, the proportion of blood-fed mosquitoes was higher after spray (December–June) than before spray (July–October). The proportion of gravid mosquitoes was relatively high before spray (July–November) but decreased after spray (Figure 9).

Figure 9: Gonotrophic Status of Female *Anopheles* Mosquitoes Collected by PSCs in the Two IRS Districts




In Karonga, Kasungu, and Salima, the proportion of blood-fed *Anopheles* mosquitoes was higher during the rainy season (November–May) than during the dry season (June–October). The opposite was observed in Chikwawa; a higher proportion of blood fed was collected in the dry season than in the rainy season (Figure 10).

Figure 10: Gonotrophic Status of Female *Anopheles* Mosquitoes Collected by PSCs in the Four Monitoring Districts

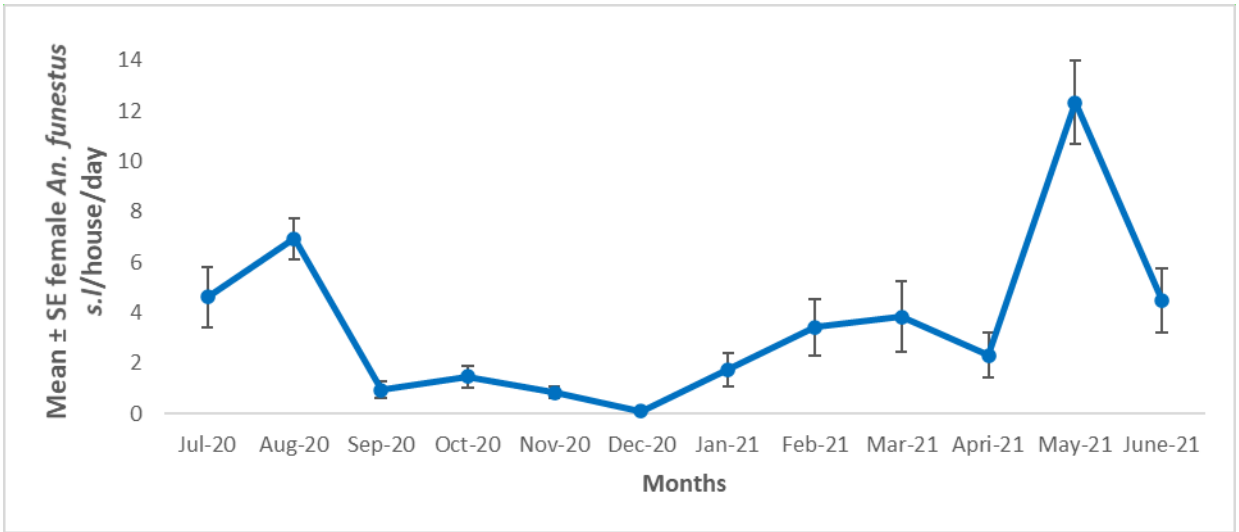



## 3.2 NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY CDC-LT INDOORS

### 3.2.1 *AN. FUNESTUS* S.L. NUMBERS COLLECTED BY CDC-LT

The density of *An. funestus* s.l. collected indoors by CDC-LT in all 13 sentinel sites is presented in Figure 11. The overall mean density of *An. funestus* s.l. over the period was 3.6 mosquitoes/trap/night. The highest mean number of *An. funestus* s.l. was collected in May 2021 (12.3 mosquitoes/trap/night) and the lowest was recorded in December 2020 (<1.0 mosquitoes/trap/night).

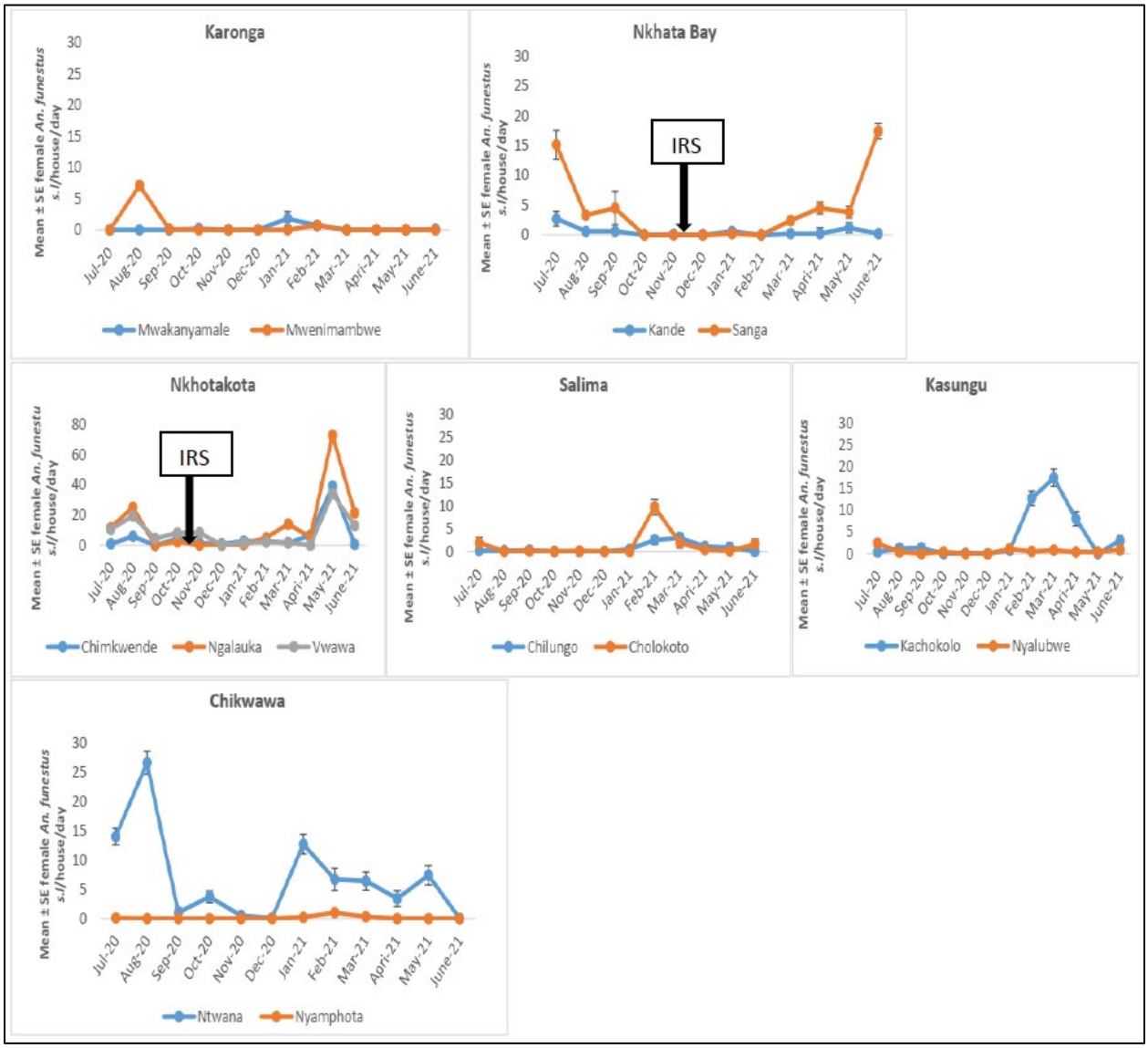
Figure 11: Mean Number of *An. funestus* s.l. Collected by CDC-LT Across Six Districts (13 Sentinel Sites), July 2020–June 2021




The highest mean numbers of *An. funestus* s.l. were collected from Nkhotakota IRS district in May 2021 (34.4, 39.2, and 72.9 mosquitoes/trap/night) in Vwawa, Chimkwende, and Ngalauka sites, respectively. In Nkhata Bay IRS district, the mean density of *An. funestus* s.l. was high (15.1 mosquitoes/trap/night) in July (before spray) and steadily declined in October to <1.0 mosquitoes/trap/night. After spraying in November, *An. funestus* s.l. density remained low from December to February (<1.0 mosquitoes/trap/night); it then began to rise in March, during the rainy season, and the highest catch was recorded in June with a mean catch of 17.4 mosquitoes/trap/night (Figure 12).

The mean number of *An. funestus* s.l. remained low (<10.0 mosquitoes/trap/night) throughout the sampling period in six sites: Mwenimambwe and Mwakanyamale (Karonga), Cholokoto and Chilungo (Salima), Nyalubwe (Kasungu), and Nyamphota (Chikwawa). However, in Ntwana (Chikwawa), the density of *An. funestus* s.l. was high in July–August, with a highest peak catch of 26.6 mosquitoes/trap/night in August; it then dropped from September–December before rising again during the rainy season (January–May), and eventually declined in June. In Kachokolo (Kasungu) *An. funestus* s.l. was very low during the dry season, but it increased during the rainy season with a highest catch recorded in March (17.4 mosquitoes/trap/night) (Figure 12).

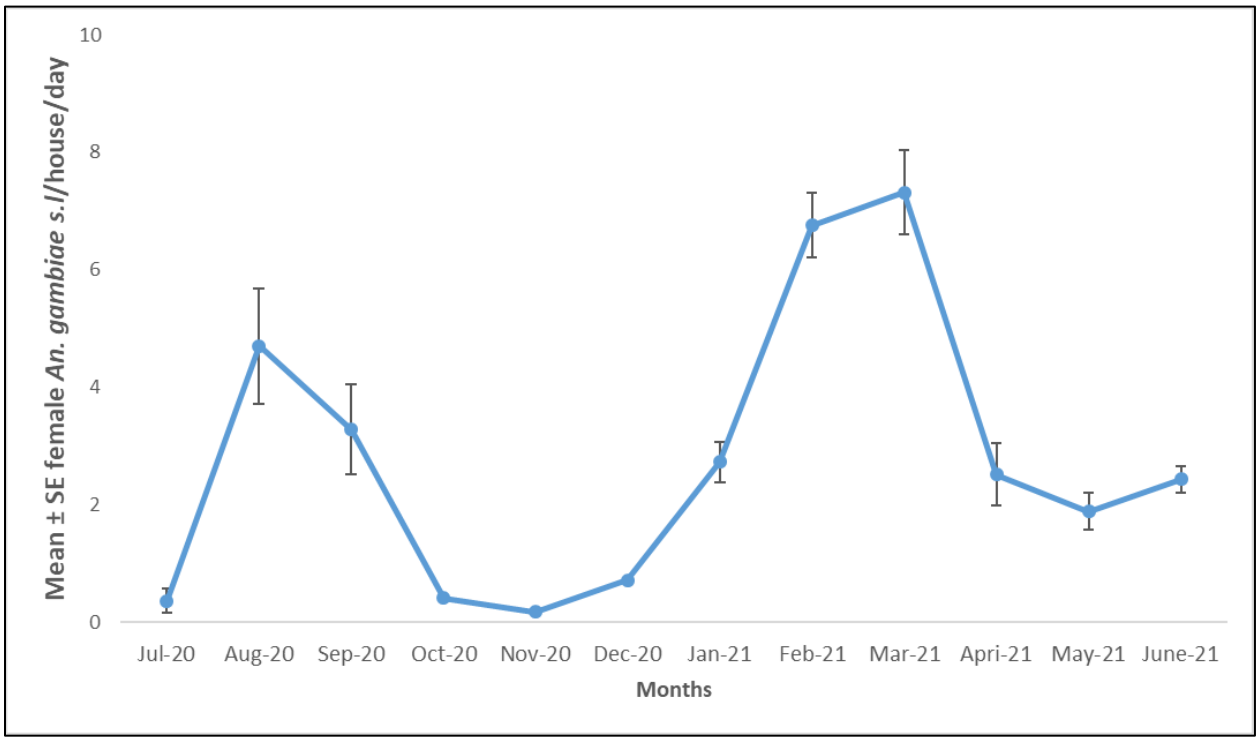
Figure 12: Mean Number of *An. funestus* s.l. Collected by CDC-LT, July 2020–June 2021




## 3.2.2 *AN. GAMBIAE* S.L. NUMBERS COLLECTED BY CDC-LT

The number of *An. gambiae* s.l. collected indoors using CDC-LTs in the 13 sentinel sites is presented in Figure 13. The overall mean density of *An. gambiae* s.l. was 2.8 mosquitoes per trap per night. The highest density of *An. gambiae* s.l. was captured in March 2021 (7.3 mosquitoes/trap/night), the lowest in November (<1.0 mosquitoes/trap/night).

Figure 13: Indoor Density of *An. gambiae* s.l. Collected by CDC-LT Across Six Districts (13 Sentinel Sites), July 2020–June 2021

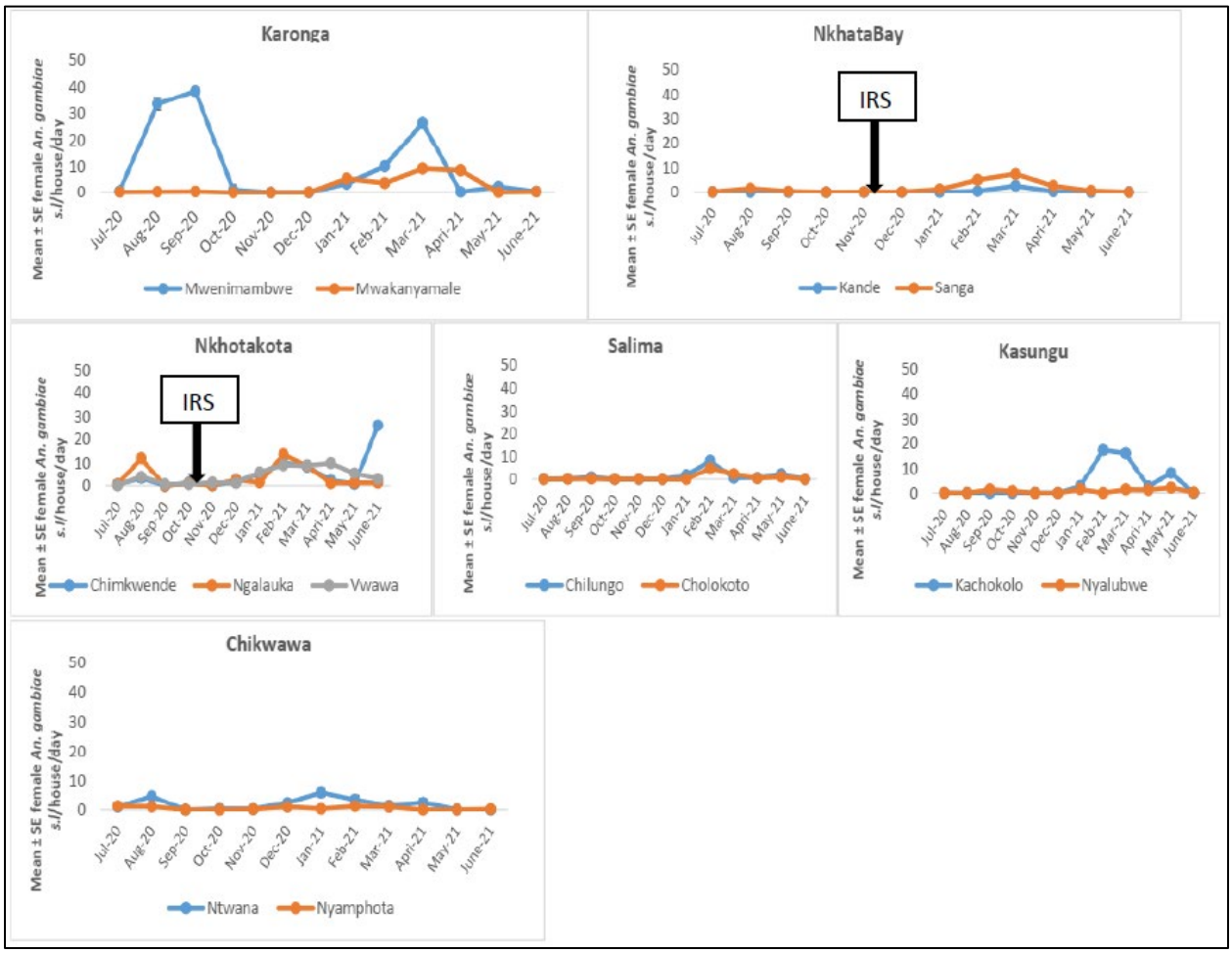



The highest number of *An. gambiae* s.l. was captured at Mwenimambwe site in Karonga District (38.6 mosquitoes/trap/night) in September. The lowest densities were observed in Nyalubwe (Kasungu) and Nyamphota (Chikwawa) (<1.0 mosquito/trap/night). In Kachokolo (Kasungu), the density of *An. gambiae* s.l. was very low (<2.0 mosquitoes/trap/night) during the dry season (July–November), but it then peaked during the rainy season (February–March) with the highest catch observed in February (17.5 mosquitoes/trap/night) (Figure 14).

In Nkhata Bay IRS district, the number of *An. gambiae* s.l. was very low (2.0 mosquitoes/trap/night) before spray (July–October). The numbers remained very low after spray; however, there was a slight peak observed in Sanga (7.5 mosquitoes/trap/night) during the rainy season in March.

In Nkhotakota IRS district, the number of *An. gambiae* s.l. at Vwawa site was very low (<5.0 mosquitoes/trap/night) before spray (July–September). After spray in October, the numbers remained low (<2 mosquitoes/trap/night) from November to December; it then peaked in January and remained relatively high from January to April, before it dropped in May–June. In Chimkwende, the number of *An. gambiae* s.l. was very low before spray (<4 mosquitoes/trap/night) (July–September) and remained low (<2 mosquitoes/trap/night) after spray from November to December, but increased in January–March, then declined in April–May before it peaked again in June, when the highest catch of 26.1 mosquitoes/trap/night was recorded. In Ngalauka, the number was low in July, peaked in August (11.0 mosquitoes/trap/night), then dropped in September. After spray in October, it remained low from November to January, peaked February to March, then dropped in April to June.

Figure 14: Mean Indoor Numbers of *An. gambiae* s.l. Collected by CDC-LT, by Sentinel Site, July 2020–June 2021




## 3.2.3 GONOTROPHIC STATUS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY CDC-LTS

A total of 10,203 female *Anopheles* mosquitoes were collected using CDC-LTs. Out of these mosquitoes, 9,957 were *An. funestus* s.l. and *An. gambiae* s.l. Overall, the highest proportion of the female *Anopheles* mosquitoes collected were unfed (n=7,685,77%), 944(9%) were fed, 312 (3%) were half-gravid, and 752 (8%) were gravid (Table 6). The gonotrophic status of 264 (3%) samples could not be determined because their abdomens were damaged. CDC-LTs captured more host-seeking (unfed) females than the unfed females captured by PSCs.

In Nkhotakota IRS district, the proportion of unfed mosquitoes was higher before spray (July–September) than after spray (November–June). In Nkhata Bay IRS district, the proportion of unfed mosquitoes was higher before spray (July–October) than after spray (December–June) (Figure 15). In Karonga, Kasungu, and Chikwawa, the proportion of unfed mosquitoes was higher during the dry season (June–October) than during the rainy season (November–May). In Salima, a higher proportion of unfed mosquitoes was collected during the rainy season than during the dry season (Figure 16).

Table 6: Gonotrophic Status of *An. funestus* s.l. and *An. gambiae* s.l. Sampled by CDC-LT in All 13 Sentinel Sites

| Species | Gonotrophic Status | | | | | |
|---|---|---|---|---|---|---|
| | Unfed | Fed | Half Gravid | Gravid | Undetermined | Grand Total |
| *An. funestus s.l.* | 4530 (81%) | 372 (7%) | 101 (2%) | 481 (9%) | 140 (3%) | 5624 |
| *An. gambiae s.l.* | 3155 (73%) | 380 (9%) | 211 (5%) | 463 (11%) | 124 (3%) | 4333 |
| **Grand Total** | 7685 (77%) | 752 (8%) | 312 (3%) | 944 (9%) | 264 (3%) | 9957 |

Figure 15: Gonotrophic Status of Female *Anopheles* Mosquitoes Collected by CDC-LTs in the Two IRS Districts

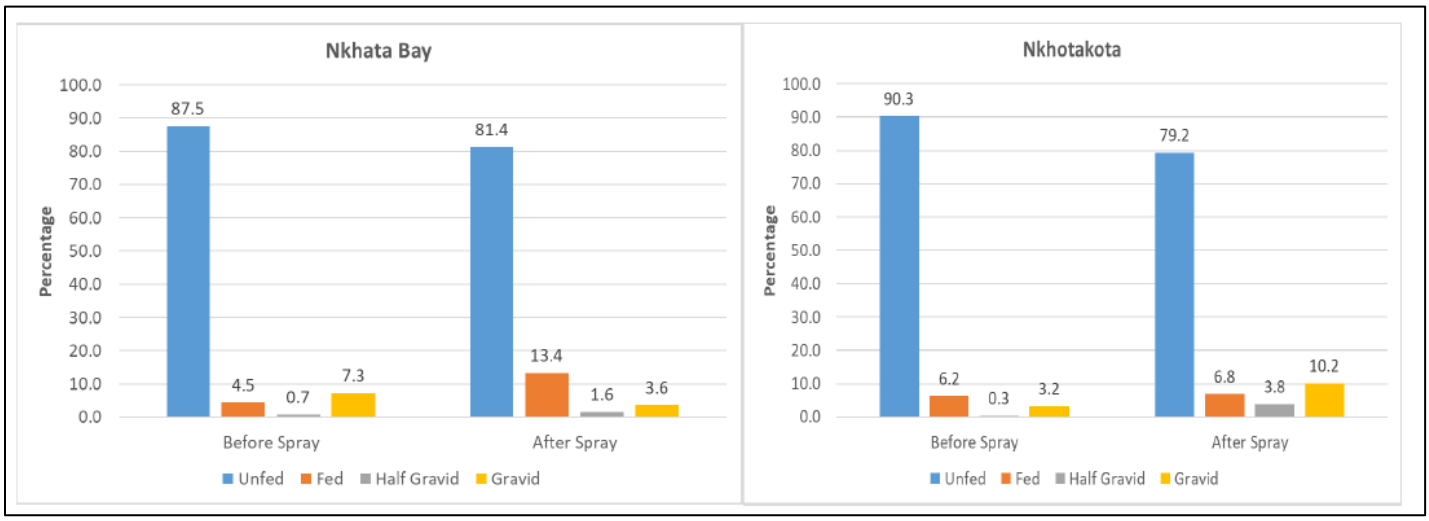
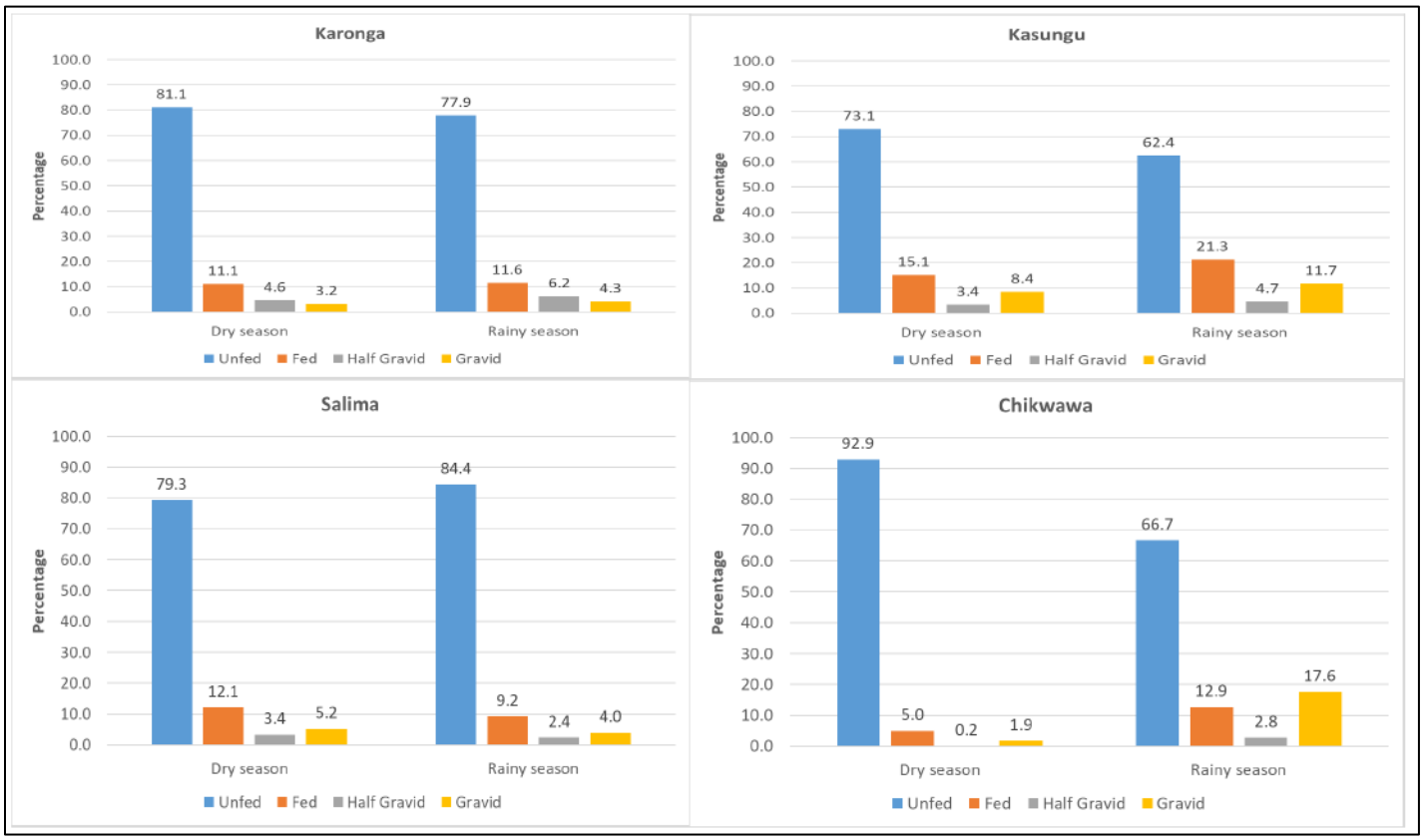


Figure 16: Gonotrophic Status of Female *Anopheles* Mosquitoes Collected by CDC-LTs in the Four non-IRS Districts




## 3.3 LABORATORY ANALYSIS

### 3.3.1 SPECIES IDENTIFICATION

*A) An. gambiae* s.l.

A total of 1,041 *An. gambiae s.l.* were randomly sampled and identified to species-specific level by PCR. Mosquito samples were collected using PSCs (n=672, 64.6%), CDC-LTs (n=179, 17.2%), HLC (n=14, 1.3%), and Prokopack aspirator (n=176, 16.9%). Out of the 1,041 *An. gambiae* s.l., 895 (86%) were identified as *An. arabiensis* and 146 (14%) as *An. gambiae* s.s. Both species were found in all the six districts but *An. arabiensis* was the predominant member of the *An. gambiae* complex in all the six districts except Salima (Table 7).

## Table 7: Number and Percentage of *An. gambiae* s.l. Identified to Species Specific Level by District, Sentinel Site, and Collection Method

| District | Site | Species Identification by PCR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CDC-LTs | | PSCs | | HLCs | Prokopack aspirator | | Total | |
| | | *An. arabiensis* N (%) | *An. gambiae* s.s. N (%) | *An. arabiensis* N (%) | *An. gambiae* s.s. N (%) | *An. arabiensis* N (%) | *An. arabiensis* N (%) | *An. gambiae* s.s. N (%) | *An. arabiensis* N (%) | *An. gambiae* s.s. N (%) |
| **Karonga** | Mwakanyamale | 1 (100) | - | 64 (100) | 0 | * | 51 (94.4) | 3 (5.6) | 116 (97.5) | 3 (2.5) |
| | Mwenimambwe | 30 (94) | 2 (6) | 226 (97) | 7 (3) | * | - | - | 256 (96.6) | 9 (3.4) |
| **Nkhata Bay** | Kande | 1 (100) | - | 5 (100) | - | * | - | -- | 6 (100) | - |
| | Sanga | 6 (86) | 1 (14) | 23 (100) | - | 2 (100) | - | - | 31 (96.9) | 1 (3.1) |
| **Nkhotakota** | Chimkwende | 11 (85) | 2 (15) | 86 (97) | 3 (3) | * | - | - | 97 (95.3) | 5 (4.7) |
| | Ngalauka | 27 (90) | 3 (10) | 10 (100) | 0 (0) | 7 (100) | - | - | 44 (93.6) | 3 (6.4) |
| | Vwawa | 42 (84) | 8 (16) | 18 (69) | 8 (31) | - | 45 (97.5) | 2(4.3) | 105 (85.4) | 18 (14.6) |
| **Kasungu** | Kachokolo | 15 (83) | 3 (17) | 10 (91) | 1 (9) | 1 (100) | -- | -- | 26 (86.7) | 4 (13.3) |
| | Nyalubwe | 3 (75) | 1 (25) | 4 (67) | 2 (33) | * | - | - | 7 (53.5) | 3 (46.5) |
| **Salima** | Chilungo | 4 (67) | 2 (33) | 33 (49) | 35 (51) | 4 (100) | 8 (14.8) | 46(85.2) | 49 (37.1) | 83 (62.9) |
| | Cholokoto | 6 (86) | 1 (14) | 13 (87) | 2 (13) | * | - | - | 19 (86.4) | 3 (13.6) |
| **Chikwawa** | Ntwana | 4 (57) | 3 (50) | 49 (92) | 4 (8) | * | 17 (81) | 4 (19) | 70 (86.4) | 12 (13.6) |
| | Nyamphota | 1 (50) | 1 (50) | 68 (99) | 1 (1) | * | - | - | 69 (97.2) | 2 (2.8) |
| **Total** | | **151 (84)** | **28 (16)** | **609 (91)** | **63 (9)** | **14 (100)** | **121 (71.9)** | **55 (28.1)** | **895 (86.0)** | **146 (14.0)** |

*Sentinel sites where HLCs are not conducted

### *B) An. funestus* s.l.

A total of 594 *An. funestus* s.l. were identified to species-specific level using PCR collected from PSCs (n =323, 54.4%), CDC-LTs (n = 42, 7.1%), HLCs (n=12, 2.0%), and Prokopack aspirators (n=217, 36.5%). All 594 *An. funestus* s.l. were identified as *An. funestus* s.s. This species occurred in all six districts (Table 8).

**Table 8: Number and Percentage of *An. funestus* s.l. Identified to Species-Specific Level by District, Sentinel Site, and Collection Method**

| District | Site | Species Identified by PCR | | | | |
|---|---|---|---|---|---|---|
| | | CDC-LTs | PSCs | HLCs | Prokopack aspirator | Total |
| | | *An. funestus* s.s. N (%) | *An. funestus* s.s. N (%) | *An. funestus* s.s. N (%) | *An. funestus* s.s. N (%) | *An. funestus* s.s. N (%) |
| **Karonga** | Mwakanyamale | 0 (0) | 1 (100) | * | - | 1 (100) |
| | Mwenimambwe | 2 (100) | 76 (100) | * | - | 78 (100) |
| **Nkhata Bay** | Kande | 1(100) | 4 (100) | * | 42 (100) | 47 (100) |
| | Sanga | 9 (100) | 18 (100) | * | - | 27 (100) |
| **Nkhotakota** | Chimkwende | 7 (100) | 29 (100) | * | 80 (100) | 116 (100) |
| | Ngalauka | 4 (100) | 11 (100) | 10 (100) | - | 25 (100) |
| | Vwawa | 4 (100) | 56 (100) | * | - | 60 (100) |
| **Salima** | Chilungo | 3 (100) | 30 (100) | 1 (100) | 8 (100) | 42 (100) |
| | Cholokoto | 3 (100) | 6 (100) | * | - | 9 (100) |
| **Kasungu** | Kachokolo | 1 (100) | 38 (100) | 1 (100) | 55 (100) | 95 (100) |
| | Nyalumbwe | 0 (0) | 27 (100) | * | - | 27 (100) |
| **Chikwawa** | Ntwana | 7 (100) | 15 (100) | * | 32 (100) | 54 (100) |
| | Nyamphota | 1 (100) | 12 (100) | * | - | 13 (100) |
| **Grand Total** | | **42 (100)** | **323 (100)** | **12 (100)** | **217 (57.4)** | **594 (100)** |

*Sentinel sites where HLCs are not conducted

## 3.3.2 BLOOD MEAL ANALYSIS

A total of 9979 blood-fed *An. funestus* s.l. mosquitoes were collected from all the six districts. Out of these, 233 (2.3%) were randomly selected and tested for bloodmeal analysis using PCR to identify the source of their blood meal. Human blood was the predominant source: 208 *An. funestus* s.l. (89.3%) were found to have fed on humans followed by cows (n=22, 9.4%), goats (n=2, 0.9%), and dog (n=1, 0.4) (Table 9). A total of 294 blood-fed *An. gambiae* s.l. mosquitoes from all six districts were tested to identify the source of their blood meal. Cow blood was the predominant source: 181 (61.6%) had fed on cows followed by humans (n=84, 28.6%), goats (n=18, 6.1%), dogs (n=6, 2.0%), and pigs (n=5, 1.7%).

Table 9: Number and Proportion of Female *An. funestus* s.l. and *An. gambiae* s.l. Tested to Determine Blood Meal Source

| District | Site | *An. funestus s.l.* Blood Meal Source | | | | | | *An. gambiae s.l.* Blood Meal Source | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Human N (%) | Cow N (%) | Dog N (%) | Goat N (%) | Pig N (%) | Total Tested N | Human N (%) | Cow | Dog N (%) | Goat N (%) | Pig N (%) | Total Tested N |
| Karonga | Mwenimambwe | 31 (96.9) | 1 (3.1) | 0 | 0 | 0 | 32 | 9 (15.5) | 44 (75.9) | 1 (1.7) | 1 (1.7) | 3 (5.2) | 58 |
| | Mwakanyamale | 0 | 1 (100) | 0 | 0 | 0 | 1 | 2 (5.0) | 38 (95.0) | 0 | 0 | 0 | 40 |
| Nkhata Bay | Kande | 4 (100) | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sanga | 21(87.5) | 1 (4.2) | 1 (4.2) | 1 (4.2) | 0 | 24 | 5 (29.4) | 1 (5.9) | 1 (5.9) | 9 (52.9) | 1(5.9) | 17 |
| Nkhotakota | Vwawa | 20 (90.9) | 1 (4.6) | 0 | 1 (4.6) | 0 | 22 | 13 (86.7) | 1 (6.7) | 1 (6.7) | 0 | 0 | 15 |
| | Chimkwende | 7 (77.8) | 2 (22.2) | 0 | 0 | 0 | 9 | 7 (35) | 11 (55.0) | 1 (5.0) | 1 (5.0) | 0 | 20 |
| | Ngalauka | 13 (100) | 0 | 0 | 0 | 0 | 13 | 11 (84.6) | 2 (15.4) | 0 | 0 | 0 | 13 |
| Salima | Chilungo | 7 (77.8) | 2 (22.2) | 0 | 0 | 0 | 9 | 11 (39.3) | 11 (39.3) | 0 | 6 (21.4) | 0 | 28 |
| | Cholokoto | 5 (83.3) | 1 (16.7) | 0 | 0 | 0 | 6 | 4 (40.0) | 5 (50.0) | 0 | 1 (10.0) | 0 | 10 |
| Kasungu | Kachokolo | 52 (100) | 0 | 0 | 0 | 0 | 52 | 3 (100) | 0 | 0 | 0 | 0 | 3 |
| | Nyalubwe | 25 (96.1) | 1 (3.9) | 0 | 0 | 0 | 26 | 4 (100) | 0 | 0 | 0 | 0 | 4 |
| Chikwawa | Nyamphota | 3 (37.5) | 5 (62.5) | 0 | 0 | 0 | 8 | 1 (2.2) | 42 (93.3) | 2 (4.4) | 0 | 0 | 45 |
| | Ntwana | 20 (74.1) | 7 (25.9) | 0 | 0 | 0 | 27 | 14 (34.2) | 26 (63.4) | 0 | 0 | 0 | 41 |
| **Total** | | **208 (89.3)** | **22 (9.4)** | **1 (0.4)** | **2 (0.9)** | **0** | **233** | **84 (28.6)** | **181 (61.6)** | **6 (2.0)** | **18 (6.1)** | **5 (1.7)** | **294** |

## 3.4 BITING RATES OF MALARIA VECTORS

### 3.4.1 BITING RATES OF MALARIA VECTORS BY DISTRICT FROM HLC COLLECTIONS

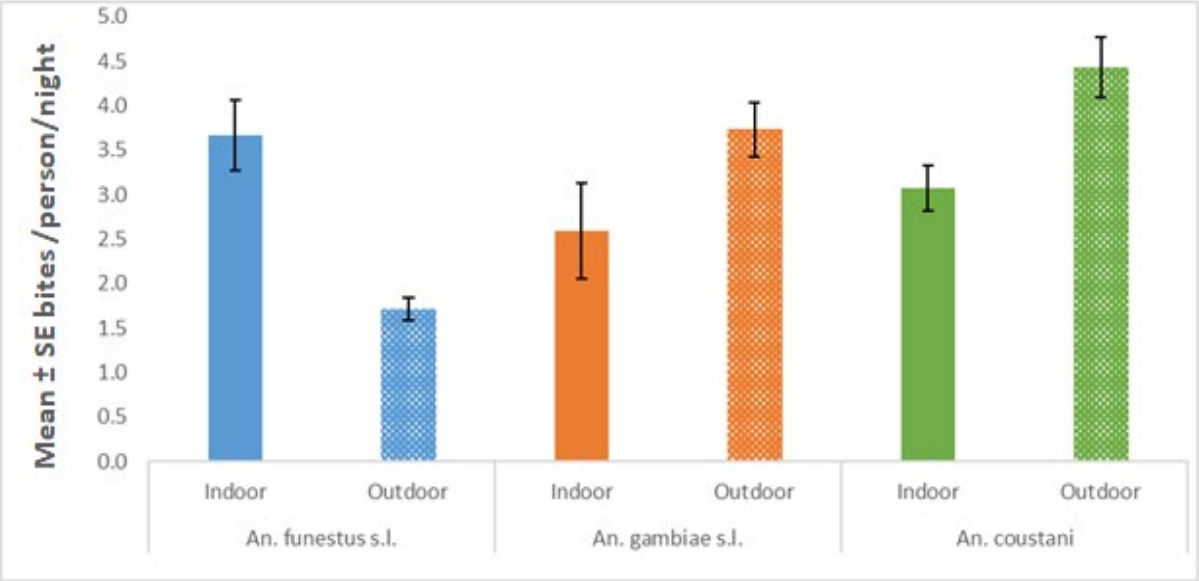
HBR was measured quarterly in four districts: Nkhata Bay, Nkhotakota, Salima, and Kasungu. Overall, the HBRs for *An. funestus* s.l. were 3.7 b/p/n indoors and 1.7 b/p/n outdoors. The HBRs for *An. gambiae s.l.* were 2.6 b/p/n indoors and 3.7 b/p/n outdoors and for *An. coustani* 3.1 indoors and 4.4 b/p/n outdoors (Table 10 and Figure 17).

Table 10: HBRs of *Anopheles* Mosquitoes from Five Sentinel Sites, July 2020–June 2021

| Site | Month | *An. funestus s.l.* | | *An. gambiae s.l.* | | *An. coustani* | |
|---|---|---|---|---|---|---|---|
| | | Indoor | Outdoor | Indoor | Outdoor | Indoor | Outdoor |
| **Nkhata Bay** | September | 0.00 | 0.17 | 0.67 | 0.50 | 2.83 | 3.33 |
| | December | 0.00 | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 |
| | March | 0.17 | 0.33 | 7.67 | 10.17 | 15.83 | 25.00 |
| | June | 4.83 | 4.33 | 0.00 | 0.00 | 21.00 | 22.83 |
| | | | | | | | |
| **Kasungu** | September | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | December | 1.83 | 0.67 | 1.17 | 5.00 | 0.00 | 0.00 |
| | March | 2.00 | 2.83 | 11.67 | 17.50 | 7.17 | 12.00 |
| | June | 9.67 | 1.33 | 0.33 | 0.00 | 0.00 | 0.00 |
| | | | | | | | |
| **Nkhotakota** | September | 1.58 | 0.50 | 1.92 | 3.08 | 0.17 | 0.00 |
| | December | 0.08 | 0.00 | 0.33 | 0.75 | 0.00 | 0.42 |
| | March | 2.50 | 0.67 | 11.67 | 16.08 | 1.25 | 2.58 |
| | June | 15.00 | 7.75 | 0.08 | 1.50 | 0.75 | 3.33 |
| | | | | | | | |
| **Salima** | September | 0.17 | 0.83 | 1.33 | 2.67 | 0.17 | 0.33 |
| | December | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | March | 4.33 | 5.33 | 4.50 | 2.50 | 0.00 | 1.00 |
| | June | 16.33 | 2.67 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | | | |
| **Overall** | | **3.67** | **1.71** | **2.59** | **3.73** | **3.07** | **4.43** |

The highest indoor biting activity of *An. funestus* s.l. in Salima district was recorded in June,16.3 b/p/n. The highest outdoor biting activity was observed in Nkhotakota District in June (7.8 b/p/n). The highest indoor biting activity of this *An. gambiae* s.l. was observed in both Kasungu and Nkhotakota districts in March (11.7 b/p/n). The highest outdoor biting activity was observed in March in Kasungu District (17.5 b/p/n), during the rainy season. The highest human biting activity of *An. coustani* was observed in Nkhata Bay District with HBRs of 21.0 b/p/n indoors in June and 25.0 b/p/n outdoors in March (Table 10).

Figure 17: Average Bites of *Anopheles* Mosquitoes per Person per Night in Five Sentinel Sites




### 3.4.2 Biting Rates of Malaria Vectors by District from CDC-LT Collections

CDC-LT collections were performed monthly for one night in 20 houses, except in Nkhotakota, where the collection was done in 30 houses. Table 11 shows nightly HBR, which is equivalent to numbers collected per trap per night.

Table 11: Estimate of HBRs of *Anopheles* Mosquitoes from CDC-LT Collection in Five Sentinel Sites, July 2020–June 2021

| Species | Indicator | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Karonga** | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total collected by CDC-LTs | 8 | 339 | 390 | 11 | 0 | 1 | 89 | 137 | 356 | 87 | 24 | 7 |
| | HBR/night | 0.4 | 16.95 | 19.5 | 0.55 | 0 | 0.05 | 4.45 | 6.85 | 17.8 | 4.35 | 1.2 | 0.35 |
| *An. funestus* s.l. | Total collected by CDC-LTs | 0 | 97 | 1 | 2 | 0 | 0 | 18 | 14 | 0 | 0 | 0 | 1 |
| | HBR/night | 0 | 4.85 | 0.05 | 0.1 | 0 | 0 | 0.9 | 0.7 | 0 | 0 | 0 | 0.05 |
| **Nkhata Bay** | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total collected by CDC-LTs | 2 | 16 | 3 | 0 | 1 | 1 | 12 | 55 | 102 | 30 | 6 | 1 |
| | HBR/night | 0.1 | 0.8 | 0.15 | 0 | 0.05 | 0.05 | 0.6 | 2.75 | 5.1 | 1.5 | 0.3 | 0.05 |
| *An. funestus* s.l. | Total collected by CDC-LTs | 178 | 39 | 51 | 0 | 1 | 0 | 8 | 0 | 26 | 47 | 50 | 176 |
| | HBR/night | 8.9 | 1.95 | 2.55 | 0 | 0.05 | 0 | 0.4 | 0 | 1.3 | 2.35 | 2.5 | 8.8 |
| **Nkhotakota** | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total collected by CDC-LTs | 12 | 194 | 7 | 30 | 17 | 56 | 126 | 329 | 260 | 134 | 76 | 304 |
| | HBR/night | 0.4 | 6.47 | 0.23 | 1 | 0.57 | 1.87 | 4.2 | 10.97 | 8.67 | 4.47 | 2.53 | 10.13 |
| *An. funestus* s.l. | Total collected by CDC-LTs | 232 | 506 | 45 | 150 | 101 | 12 | 50 | 100 | 176 | 121 | 1463 | 351 |
| | HBR/night | 7.73 | 16.87 | 1.5 | 5 | 3.37 | 0.4 | 1.67 | 3.33 | 5.87 | 4.03 | 48.77 | 11.7 |
| **Salima** | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total collected by CDC-LTs | 1 | 3 | 12 | | 1 | | 19 | 132 | 30 | 11 | 35 | 0 |
| | HBR/night | 0.05 | 0.15 | 0.6 | 0 | 0.05 | 0 | 0.95 | 6.6 | 1.5 | 0.55 | 1.75 | 0 |
| *An. funestus* s.l. | Total collected by CDC-LTs | 21 | 2 | 4 | 0 | 1 | | 5 | 122 | 49 | 15 | 10 | 16 |
| | HBR/night | 1.05 | 0.1 | 0.2 | 0 | 0.05 | 0 | 0.25 | 6.1 | 2.45 | 0.75 | 0.5 | 0.8 |
| **Kasungu** | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total collected by CDC-LTs | 1 | 0 | 15 | 9 | 0 | 0 | 44 | 176 | 177 | 39 | 104 | 3 |
| | HBR/night | 0.1 | 0 | 0.8 | 0.5 | 0 | 0 | 2.2 | 8.8 | 8.9 | 2 | 5.2 | 0.2 |
| *An. funestus* s.l. | Total No. CDC-LT | 28 | 16 | 13 | 4 | 1 | | 19 | 132 | 182 | 84 | 4 | 39 |
| | HBR/night | 1.4 | 0.8 | 0.65 | 0.2 | 0.05 | 0 | 0.95 | 6.6 | 9.1 | 4.2 | 0.2 | 1.95 |
| **Chikwawa** | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total collected by CDC-LTs | 48 | 612 | 427 | 54 | 24 | 94 | 355 | 878 | 951 | 327 | 246 | 317 |
| | HBR/night | 2.4 | 30.6 | 21.35 | 2.7 | 1.2 | 4.7 | 17.75 | 43.9 | 47.55 | 16.35 | 12.3 | 15.85 |
| *An. funestus* s.l. | Total collected by CDC-LTs | 141 | 266 | 10 | 37 | 5 | 1 | 129 | 77 | 67 | 34 | 74 | 0 |
| | HBR/night | 7.05 | 13.3 | 0.5 | 1.85 | 0.25 | 0.05 | 6.45 | 3.85 | 3.35 | 1.7 | 3.7 | 0 |

## 3.5 INFECTION DETECTION

### *A) Sporozoite Rates from HLC Collections (July 2019–March 2020)*

Annex A1 and A2 summarize the sporozoite infections of *An. gambiae* s.l. and *An. funestus* s.l. captured by HLCs. Very low SRs were recorded from all six districts during the sampling period.

The overall *An. gambiae* s.l. SR in the four districts where HLCs were conducted was 1.1% (n=654): 1.6% (n=249) indoors and 0.7% (n=415) outdoors. In IRS districts, high *An. gambiae* s.l. SRs were recorded after spraying both indoors, 0.6% (n=156) and outdoors, 1.1% (n=276). No sporozoite-positive *An. gambiae* s.l. were found before spray both indoors (n =49) and outdoors (n=99). In non-IRS districts, high *An. gambiae* s.l. SRs were recorded indoors, 6.7% (n=45) and no sporozoite-positive *An. gambiae* s.l. were found outdoor (n=49).

The overall *An. funestus* s.l. SR from the four districts was 3.4% (n=207), 3.4% (n=149) indoors and 3.4% (n=58) outdoors. In IRS districts, high *An. funestus* s.l. SRs were recorded before spraying, 3.5% (n=85) indoors and no sporozoite-positive *An. funestus* s.l. were found indoors (n=13). No sporozoite *An. funestus* s.l. SRs were recorded after spraying both indoors (n =34) and outdoors (n=8). In non-IRS districts, high *funestus* s.l. SRs were recorded indoors, 6.7% (n=30) and no sporozoite-positive *An. gambiae* s.l. were found outdoor (n=37).

A total of 80 *An. coustani* (42% from indoor and 38% from outdoor collections) were tested for infection and no sporozoite-positive *An. coustani* was found in any of the four districts.

### *B) Sporozoite Rates from PSC and CDC-LT Collections (July 2019–March 2020)*

#### *i) From PSC collections (July 2020–June 2021):*

***An. gambiae* s.l.:** Annex A3 summarizes the SR of *An. gambiae* s.l. collected from PSCs. A total of 52 *An. gambiae* s.l. were tested for *Pf* infection. No sporozoite-positive *An. gambiae* s.l. was detected in any of the six districts.

***An. funestus* s.l.:** Annex A4 summarizes the SR of *An. funestus* s.l. collected from PSCs. A total of 40 *An. funestus* s.l. were tested for *Pf* infection. The overall *An. funestus* s.l. SR from the six districts was 10.0%. High *An. funestus* s.l. SRs were recorded in Kasungu (16.7%) and Chikwawa (11.5%). No sporozoite-positive *An. funestus* s.l. was recorded in Karonga, Nkhotakota, Nkhata Bay, and Salima districts.

#### *ii) From CDC-LT collections (July 2020–June 2021):*

***An. gambiae* s.l.:** Annex A5 summarizes the SR of *An. gambiae* s.l. collected from CDC-LTs. A total of 2,265 *An. gambiae* s.l. were tested for *Pf* infection. The overall *An. gambiae* s.l. SR from all six districts was 1.3%. Generally, *An. gambiae* s.l. SRs were low in all the districts; the highest was recorded in Nkhotakota and Salima districts (1.9%).

***An. funestus* s.l.:** Annex A6 summarizes the SR of *An. funestus* s.l. collected from CDC-LTs. A total of 1,968 *An. funestus* s.l. were tested for *Pf* infection. The overall *An. funestus* s.l. SR from the six districts was 3.4%. High sporozoite rates among *An. funestus* s.l. were recorded in Nkhata Bay (4.8%) and Nkhotakota (4.2%). No sporozoite-positive *An. funestus* s.l. was recorded in Karonga District. All 19 *An. coustani* collected from CDC-LTs tested negative for *Pf* infection.

## 3.6 EIRS OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. ESTIMATED FROM CDC-LT COLLECTIONS (JULY 2020–JUNE 2021)

Monthly trends in HBR were estimated from CDC-LT collections that were conducted monthly.[1] The number of vectors collected per trap per night was considered equivalent to the number of bites per person per night. The HBRs from CDC-LT collections were then used to estimate monthly and annual EIRS. Table 12 summarizes the SRs and monthly EIRs of *An. gambiae* s.l. and *An. funestus* s.l. estimated from CDC-LTs collections over a 12-month period in the six districts. Annex B summarizes SRs and monthly EIRs of *An. gambiae* s.l. and *An. funestus* s.l. from each sentinel site.

The estimated risk of malaria transmission for the 12 months (annual EIR) was highest in Chikwawa District, 75.7 infective bites/person/year (ib/p/yr) (*An. gambiae* s.l. = 27.3 ib/p/yr; *An. funestus* s.l. = 48.4 ib/p/yr). The second highest EIR was recorded in Nkhotakota District (71.0 ib/p/yr; 57.0 ib/p/yr from *An. gambiae* s.l. and 14.0 ib/p/yr from *An. funestus* s.l.) followed by Nkhata Bay District (47.3 ib/p/yr; *An. gambiae* s.l. = 3.1 ib/p/yr and *An. funestus* s.l. = 44.2 ib/p/yr), Karonga District (25.6 ib/p/yr; *An. gambiae* s.l. = 25.6 ib/p/yr and *An. funestus* s.l. = no infective bites), Salima (23.6 ib/p/yr; *An. gambiae* s.l. = 9.3 ib/p/yr and *An. funestus* s.l. = 14.4 ib/p/yr) and Kasungu District (20.9 ib/p/yr; *An. gambiae* s.l. = 4.6 ib/p/yr and *An. funestus* s.l. = 16.3 ib/p/yr).

In the IRS districts, the estimated risk of malaria transmission over a 12-month period was higher in Nkhotakota District (71.0 ib/p/yr) than in Nkhata Bay (47.3 ib/p/yr). In Nkhotakota, the highest monthly EIR from *An. funestus* s.l. was observed one month after spray (18.4 ib/p/m recorded in November). Similarly, in Nkhata Bay

[1] HLC collections were performed quarterly and were not used to estimate trends in monthly HBR and EIR. They were used to show biting location and biting time preference.

District, highest monthly EIRs from *An. funestus* s.l. was observed eight months after spray (16.5 ib/p/m recorded in June).

In non-IRS districts, there was variation in the monthly EIRs of *Anopheles* mosquitoes. The highest estimated risk of malaria transmission over a 12-month period was observed in Chikwawa, a standard net distribution district, (75.7 ib/p/yr) followed by Karonga (25.6 ib/p/yr) and Salima (23.6 ib/p/yr) where piperonyl butoxide (PBO) nets were distributed in late 2018. Kasungu, a standard net district, had the lowest annual EIR, 20.9 ib/p/yr.

## Table 12: SRs and EIRs of *An. gambiae* s.l. and *An. funestus* s.l. from CDC-LT Collections by District, July 2020–June 2021

| Species | Indicator | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Karonga** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 106 | 194 | 15 | 0 | 1 | 42 | 60 | 231 | 77 | 19 | 2 | |
| | No. sporozoite positive | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | |
| | Sporozoite rate | 0 | 0.02 | 0.01 | 0 | 0 | 0 | 0 | 0.02 | 0.01 | 0.01 | 0 | 0 | |
| | Total collected by CDC-LTs | 8 | 339 | 390 | 11 | 0 | 1 | 89 | 137 | 356 | 87 | 24 | 7 | |
| | HBR/night | 0.4 | 16.95 | 19.5 | 0.55 | 0 | 0.05 | 4.45 | 6.85 | 17.8 | 4.35 | 1.2 | 0.35 | |
| | Nightly EIR | 0 | 0.32 | 0.2 | 0 | 0 | 0 | 0 | 0.11 | 0.15 | 0.06 | 0 | 0 | |
| | Monthly EIR | 0 | 9.91 | 6.03 | 0 | 0 | 0 | 0 | 3.2 | 4.78 | 1.69 | 0 | 0 | 25.61 |
| *An. funestus* s.l. | Total No. tested | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Total collected by CDC-LTs | 0 | 97 | 1 | 2 | 0 | 0 | 18 | 14 | 0 | 0 | 0 | 1 | |
| | HBR/night | 0 | 4.85 | 0.05 | 0.1 | 0 | 0 | 0.9 | 0.7 | 0 | 0 | 0 | 0.05 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total Monthly EIR | 0 | 9.91 | 6.03 | 0 | 0 | 0 | 0 | 3.2 | 4.78 | 1.69 | 0 | 0 | 25.61 |
| **Nkhata Bay** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 16 | 11 | 0 | 1 | 2 | 11 | 35 | 127 | 20 | 3 | 1 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | |
| | Sporozoite rate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.33 | 0 | |
| | Total collected by CDC-LTs | 2 | 16 | 3 | 0 | 1 | 1 | 12 | 55 | 102 | 30 | 6 | 1 | |
| | HBR/night | 0.1 | 0.8 | 0.15 | 0 | 0.05 | 0.05 | 0.6 | 2.75 | 5.1 | 1.5 | 0.3 | 0.05 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.1 | 0 | 3.1 |
| *An. funestus* s.l. | Total No. tested | 135 | 31 | 50 | 0 | 1 | 6 | 5 | 0 | 10 | 23 | 32 | 48 | |
| | No. sporozoite positive | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | |
| | Sporozoite rate | 0.06 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.04 | 0.03 | 0.06 | |
| | Total collected by CDC-LTs | 178 | 39 | 51 | 0 | 1 | 0 | 8 | 0 | 26 | 47 | 50 | 176 | |
| | HBR/night | 8.9 | 1.95 | 2.55 | 0 | 0.05 | 0 | 0.4 | 0 | 1.3 | 2.35 | 2.5 | 8.8 | |
| | Nightly EIR | 0.53 | 0.19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.08 | 0.55 | |
| | Monthly EIR | 16.3 | 5.85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.065 | 2.42 | 16.5 | 44.19 |
| | Total Monthly EIR | 16.35 | 5.85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.07 | 5.52 | 16.5 | 47.29 |

| Species | Indicator | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nkhotakota** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 72 | 141 | 43 | 21 | 50 | 120 | 142 | 444 | 56 | 1 | 0 | |
| | No. sporozoite positive | 0 | 0 | 2 | 5 | 0 | 1 | 2 | 1 | 4 | 1 | 0 | 0 | |
| | Sporozoite rate | 0 | 0 | 0.01 | 0.12 | 0 | 0.02 | 0.02 | 0.01 | 0.01 | 0.02 | 0 | 0 | |
| | Total collected by CDC-LTs | 12 | 194 | 7 | 30 | 17 | 56 | 126 | 329 | 260 | 134 | 76 | 304 | |
| | HBR/night | 0.4 | 6.47 | 0.23 | 1 | 0.57 | 1.87 | 4.2 | 10.97 | 8.67 | 4.47 | 2.53 | 10.13 | |
| | Nightly EIR | 0 | 0 | 0 | 0.12 | 0 | 0.04 | 0.07 | 0.08 | 0.08 | 0.08 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0.1 | 3.605 | 0 | 1.16 | 2.17 | 2.16 | 2.42 | 2.39 | 0 | 0 | 14.01 |
| *An. funestus* s.l. | Total No. tested | 65 | 118 | 237 | 79 | 33 | 11 | 30 | 49 | 67 | 72 | 2 | 0 | |
| | No. sporozoite positive | 2 | 2 | 8 | 6 | 6 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | |
| | Sporozoite rate | 0.03 | 0.02 | 0.03 | 0.08 | 0.18 | 0.09 | 0 | 0.02 | 0.01 | 0.03 | 0 | 0 | |
| | Total collected by CDC-LTs | 232 | 506 | 45 | 150 | 101 | 12 | 50 | 100 | 176 | 121 | 1463 | 351 | |
| | HBR/night | 7.73 | 16.87 | 1.5 | 5 | 3.37 | 0.4 | 1.67 | 3.33 | 5.87 | 4.03 | 48.77 | 11.7 | |
| | Nightly EIR | 0.24 | 0.29 | 0.05 | 0.38 | 0.61 | 0.04 | 0 | 0.07 | 0.09 | 0.11 | 0 | 0 | |
| | Monthly EIR | 7.38 | 8.862 | 1.519 | 11.77 | 18.4 | 1.13 | 0 | 1.9 | 2.714 | 3.361 | 0 | 0 | 57 |
| | Total Monthly EIR | 7.38 | 8.86 | 1.62 | 15.38 | 18.36 | 2.28 | 2.17 | 4.07 | 5.13 | 5.75 | 0 | 0 | 71.01 |
| **Salima** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 1 | 33 | 0 | 1 | 0 | 22 | 126 | 38 | 8 | 30 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 4 | 0 | 0 | 0 | |
| | Sporozoite rate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.02 | 0.11 | 0 | 0 | 0 | |
| | Total collected by CDC-LTs | 1 | 3 | 12 | | 1 | | 19 | 132 | 30 | 11 | 35 | 0 | |
| | HBR/night | 0.05 | 0.15 | 0.6 | 0 | 0.05 | 0 | 0.95 | 6.6 | 1.5 | 0.55 | 1.75 | 0 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.16 | 0.16 | 0 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.4 | 4.895 | 0 | 0 | 0 | 9.29 |
| *An. funestus* s.l. | Total No. tested | 5 | 0 | 68 | 0 | 1 | 0 | 6 | 110 | 73 | 14 | 9 | 10 | |
| | No. sporozoite positive | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 5 | 5 | 0 | 0 | 0 | |
| | Sporozoite rate | 0 | 0 | 0.01 | 0 | 0 | 0 | 0.17 | 0.05 | 0.07 | 0 | 0 | 0 | |
| | Total collected by CDC-LTs | 21 | 2 | 4 | 0 | 1 | | 5 | 122 | 49 | 15 | 10 | 16 | |
| | HBR/night | 1.05 | 0.1 | 0.2 | 0 | 0.05 | 0 | 0.25 | 6.1 | 2.45 | 0.75 | 0.5 | 0.8 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0.04 | 0.28 | 0.17 | 0 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0.09 | 0 | 0 | 0 | 1.29 | 7.76 | 5.2 | 0 | 0 | 0 | 14.35 |
| | Total Monthly EIR | 0 | 0 | 0.09 | 0 | 0 | 0 | 1.29 | 12.16 | 10.1 | 0 | 0 | 0 | 23.64 |

| Species | Indicator | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Kasungu** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 0 | 10 | 9 | 1 | 37 | 38 | 146 | 143 | 38 | 23 | 2 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | |
| | Sporozoite rate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.08 | 0 | 0 | |
| | Total collected by CDC-LTs | 1 | 0 | 15 | 9 | 0 | 0 | 44 | 176 | 177 | 39 | 104 | 3 | |
| | HBR/night | 0.1 | 0 | 0.8 | 0.5 | 0 | 0 | 2.2 | 8.8 | 8.9 | 2 | 5.2 | 0.2 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.15 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.618 | 0 | 0 | 4.62 |
| *An. funestus* s.l. | Total No. tested | 13 | 16 | 12 | 4 | 6 | 15 | 18 | 111 | 133 | 59 | 3 | 26 | |
| | No. sporozoite positive | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.08 | 0 | 0 | 0 | 0.17 | 0 | 0 | 0 | 0.04 | 0.02 | 0 | 0 | |
| | Total No. CDC-LT | 28 | 16 | 13 | 4 | 1 | | 19 | 132 | 182 | 84 | 4 | 39 | |
| | HBR/night | 1.4 | 0.8 | 0.65 | 0.2 | 0.05 | 0 | 0.95 | 6.6 | 9.1 | 4.2 | 0.2 | 1.95 | |
| | Nightly EIR | 0.11 | 0 | 0 | 0 | 0.01 | 0 | 0 | 0 | 0.34 | 0.07 | 0 | 0 | |
| | Monthly EIR | 3.34 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0 | 10.61 | 2.136 | 0 | 0 | 16.33 |
| | Total Monthly EIR | 3.34 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0 | 10.61 | 6.75 | 0 | 0 | 20.95 |
| **Chikwawa** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 13 | 11 | 31 | 12 | 31 | 37 | 35 | 21 | 18 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | |
| | Sporozoite rate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.06 | 0 | 0 | |
| | Total collected by CDC-LTs | 48 | 612 | 427 | 54 | 24 | 94 | 355 | 878 | 951 | 327 | 246 | 317 | |
| | HBR/night | 2.4 | 30.6 | 21.35 | 2.7 | 1.2 | 4.7 | 17.75 | 43.9 | 47.55 | 16.35 | 12.3 | 15.85 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.91 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27.25 | 0 | 0 | 27.25 |
| *An. funestus* s.l. | Total No. tested | 0 | 34 | 55 | 43 | 6 | 1 | 94 | 47 | 43 | 19 | 51 | 0 | |
| | No. sporozoite positive | 0 | 3 | 3 | 3 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | |
| | Sporozoite rate | 0 | 0.09 | 0.05 | 0.07 | 0 | 0 | 0.01 | 0 | 0.02 | 0.05 | 0 | 0 | |
| | Total collected by CDC-LTs | 141 | 266 | 10 | 37 | 5 | 1 | 129 | 77 | 67 | 34 | 74 | 0 | |
| | HBR/night | 7.05 | 13.3 | 0.5 | 1.85 | 0.25 | 0.05 | 6.45 | 3.85 | 3.35 | 1.7 | 3.7 | 0 | |
| | Nightly EIR | 0 | 1.17 | 0.03 | 0.13 | 0 | 0 | 0.07 | 0 | 0.08 | 0.09 | 0 | 0 | |
| | Monthly EIR | 0 | 36.38 | 0.82 | 4.001 | 0 | 0 | 2.13 | 0 | 2.42 | 2.684 | 0 | 0 | 48.43 |
| | Total Monthly EIR | 0 | 36.38 | 0.82 | 4 | 0 | 0 | 2.13 | 0 | 2.42 | 29.93 | 0 | 0 | 75.68 |

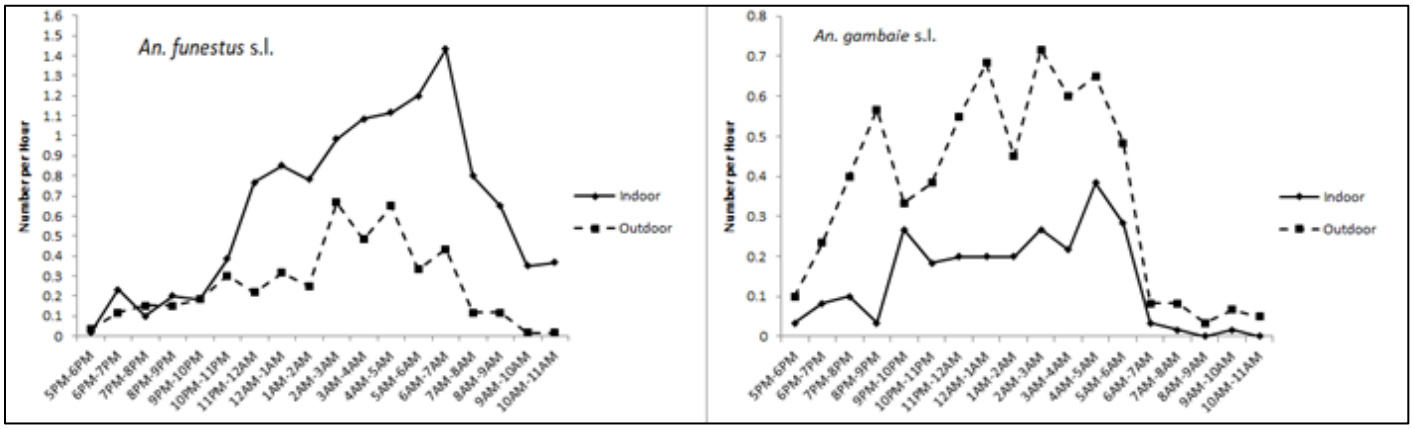
Note. A total of 20 CDC-LTs (10 at each site) were used to collect mosquitoes in all the six districts except Nkhotakota, where 30 traps were used (10 traps in each of the 3 sites).

*HBR/Person/Night = Number of female An. gambiae* s.l. *or An. funestus* s.l. */trap/night*

### 3.6.1 TIME AND LOCATION OF BITING OF MALARIA VECTORS

The overall biting activity of *An. funestus* s.l. and *An. gambiae* s.l. from the four districts of Nkhata Bay, Nkhotakota, Kasungu, and Salima are presented in Figure 18 and in Annex C, Tables C1 and C2. *An. funestus* s.l. and *An. gambiae* s.l. exhibited both endophagic (predominant indoor feeding) and exophagic behavior (predominant outdoor feeding). The biting activity of *An. funestus* s.l. occurred throughout the night 5 pm to 5 am both indoors and outdoors. There was a rise in indoor biting of this species from 3 am reaching its peak at 6 am before rapidly declining after 6 am until 11 am. The biting activity of *An. gambiae* s.l. showed a similar pattern. However, the outdoor biting activity of this species was observed between 8 pm and 5 am before rapidly declining until 11 am.

Figure 18: Average Hourly Indoor and Outdoor Biting Rates by Time of Night for *An. funestus* s.l. and *An. gambiae* s.l. from the Four Districts, July 2020–June 2021




The biting pattern of *An. funestus* s.l. and *An. gambiae* s.l. in the four districts of Nkhata Bay, Nkhotakota (two sites), Salima, and Kasungu collected quarterly from July 2020 to June 2021 is summarized in Figure 19.

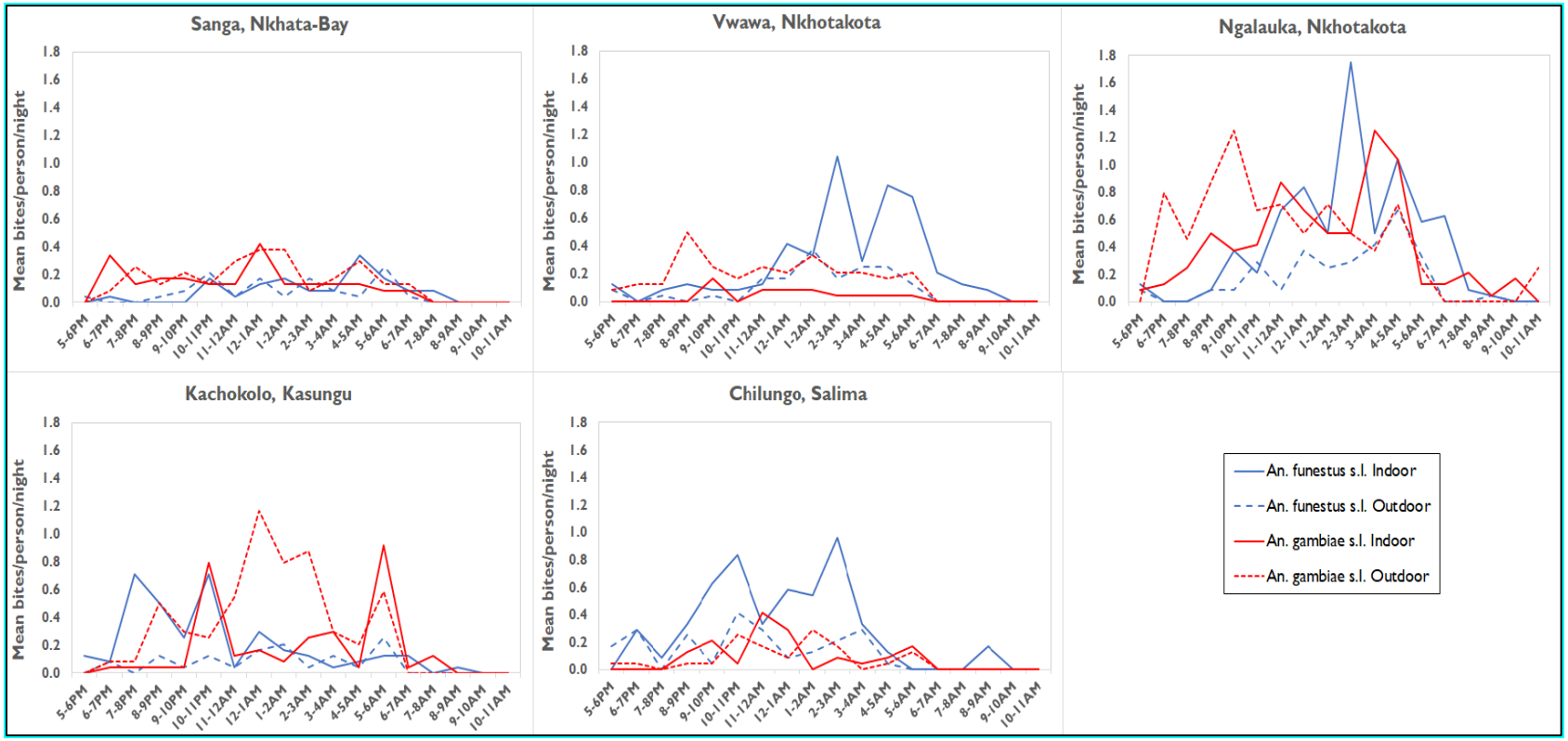
The indoor and outdoor biting activity of *An. funestus* s.l. and *An. gambiae* s.l. in all the four districts occurred from dusk to dawn (5 pm throughout the night to 11am in the morning).

In Nkhata Bay District, the human biting activity (indoor and outdoor) of *An. gambiae* s.l. and *An. funestus* s.l. was very low throughout the night. In Nkhotakota District, the highest *An. funestus* s.l. biting activity occurred during the night to early in the morning mainly indoors between 1 am and 3 am. The outdoor biting activity of *An. funestus* s.l. in this district was very low. A similar pattern was observed for *An. gambiae* s.l. and the highest biting activity of this species occurred from 9 pm to 10 pm (outdoors) and 4 am to 5 am (indoors).

In Kasungu District, the highest indoor biting of *An. funestus* s.l. was observed from 7 pm to 8 pm and was generally low throughout the night until in the morning. The highest biting activity of *An. gambiae* s.l. was observed from 10 pm to 11 pm and from 5 am to 6 am (indoors) and from 12 am to 3 am (outdoors).

In Salima District, the biting activity of *An. funestus* s.l. occurred indoors from 9 pm to 10 pm and 2 am to 3 am. The outdoor biting activity of this species was very low throughout the night till morning. Similarly, the indoor and outdoor biting activity of *An. gambiae* s.l. in this district was very low throughout the night till the morning.

Figure 19: Average Hourly Indoor and Outdoor Biting Rates by Time of Night for *An. funestus* s.l. and *An. gambiae* s.l. from Each of the Four Districts, July 2020–June 2021



### 3.6.2 INTERSECTION BETWEEN MOSQUITO AND HUMAN BEHAVIOR

In human behavior surveys conducted in the four districts of Nkhata Bay, Nkhotakota, Kasungu, and Salima, 89.9% of people were inside their houses by 10 pm and 88.0% were in bed and asleep by 10 pm. We estimated the exposure of persons according to their behavior and the time and location of mosquito biting using models described by Monroe et al. (2020).

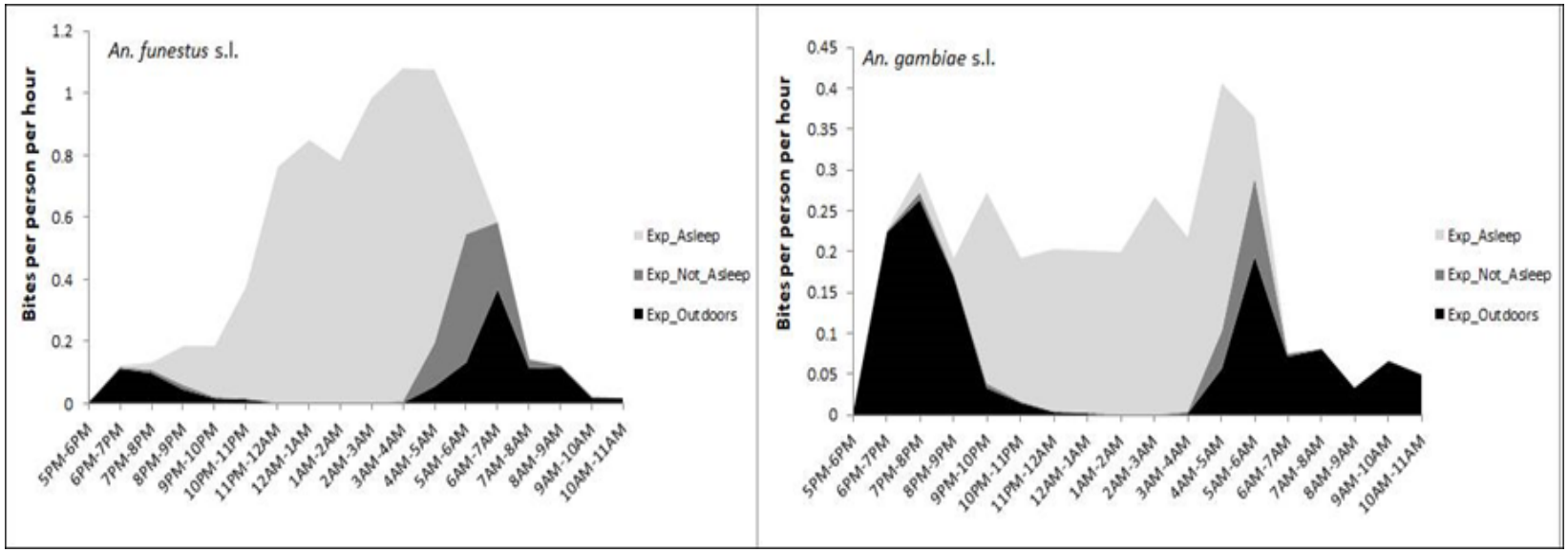
*An. funestus* s.l. biting occurred equally between indoors and outdoors (50%). However, a small proportion of indoor *An. funestus* s.l. bites were observed after 5 am (16.9%), 6 am (8.0%), and 7 am (2.8%), when people were waking up. Similarly, a lower percentage of *An. funestus* s.l. bites occurred outdoors after 5 am (14.7%), 6 am (1.3%), and 7 am (0.4%). When mapped with human behavior, 70% of *An. funestus* s.l. bites occurred when people were indoors ($\pi_{i,i}$) and 59% occurred when people were sleeping ($\pi_{i,s}$) (Table 13 and Figure 20).

*An. gambiae* s.l. biting equally occurred both indoors and outdoors (50%). Morning biting of *An. gambiae* s.l. was also observed after 5 am: 14.7% and 8.1% indoors and outdoors, respectively. When mapped with human behavior, 78% of *An. gambiae* s.l. bites occurred when people were indoors ($\pi_{i,i}$) and 67% occurred when people were sleeping ($\pi_{i,s}$) (Table 13 and Figure 20).

Table 13: Summary of Interaction Between Mosquito and Human Behavior by Species

| Parameter | *An. funestus* s.l. | *An. gambiae* s.l. |
|---|---|---|
| Proportion of human exposure occurring indoors ($\pi_{i,i}$): | 0.70 | 0.78 |
| Proportion of human exposure occurring while asleep ($\pi_{i,s}$): | 0.59 | 0.67 |
| | | |
| % Biting indoors | 50 | 50 |
| Indoor/Outdoor ratio | 1 | 1 |
| | | |
| % Biting before 9pm (Indoor) | 10.4 | 12.1 |
| % Biting before 10pm (Indoor) | 13.9 | 18.8 |
| | | |
| % Biting before 9pm (outdoor) | 11.2 | 20.0 |
| % Biting before 10pm (outdoor) | 13.8 | 29.9 |
| | | |
| % Biting after 5am (indoor) | 16.9 | 14.7 |
| % Biting after 6am (indoor) | 8.0 | 5.5 |
| % Biting after 7am (indoor) | 2.8 | 3.8 |
| | | |
| % Biting after 5am (outdoor) | 14.7 | 8.1 |
| % Biting after 6am (outdoor) | 1.3 | 1.8 |
| % Biting after 7am (outdoor) | 0.4 | 1.2 |

Figure 20: Profiles of Biting by *An. funestus* s.l. and *An. gambiae* s.l. Experienced by the Human Population in the Four Districts



### 3.6.3 PARITY RATES

Mosquito dissections of *An. funestus* s.l. and *An. gambiae* s.l. from the CDC-LTs and HLCs are presented in Tables 14 and 15; see also Annex C, Tables C1 and C2. The total number of mosquitoes dissected depended on the number collected. All mosquitoes that were fresh/not brittle were dissected at each sentinel site. Moist mutton cloth was placed in CDC-LT collection cups to maintain mosquito freshness. No female *An. funestus* s.l. captured from Karonga District were dissected to examine their parity status.

Overall, the proportions of parous females for *An. funestus* s.l. and *An. gambiae* s.l. were similar, 61% and 57% respectively. The highest *An. funestus* s.l. parity rate was observed in Chikwawa (74.5%), followed by Kasungu (60.5%), and the lowest parity rate was recorded in Nkhata Bay (55.1%). The highest parity rate for *An. gambiae* s.l. was observed in Salima (81.6%) followed by Nkhata Bay (64.7%), and the lowest parity rate was recorded in Chikwawa (43.8%).

Figure 21 and Annex C3 summarizes the proportion of parous female *An. funestus* s.l. and *An. gambiae* s.l.: 1) before spray vs after spraying in IRS districts, and 2) in the dry vs the rainy season in non-IRS districts.

In Nkhotakota IRS district, the proportion of parous *An. funestus* s.l. was slightly higher before spraying, July - September (63.8%) than after spraying, November - June (59.7). Similarly, the *An. gambiae* s.l. parity rate was higher before spraying (68.8%) than after spraying (44%). In Nkhata Bay IRS district, a similar trend was observed in *An. gambiae* s.l. where the parity rate was 100% before spraying and dropped to 65.1% after spraying. No clear trend was observed for *An. funestus* s.l. due to low numbers collected before spraying.

In the non-IRS district of Kasungu, high proportions of parous *An. funestus* s.l. were collected in the dry season, July - October (62.5%) compared to the rainy season, November – May (53.8%). Conversely, in Chikwawa District, higher parity rates were observed in *An. funestus* s.l. during the rainy season (87.5%) compared to the dry season (60%). No clear trends were observed in Karonga and Salima because no mosquitoes were dissected during the dry season due to the low numbers collected. Few mosquitoes collected during the dry season were brittle and could not be dissected. More parous *An. gambiae* s.l. were collected during the dry season than the rainy season in Karonga (64% vs 50%), Salima (69% vs 31%), and Chikwawa (44% vs 30%). The opposite was observed in Kasungu District, where a higher proportion of parous *An. gambiae* s.l. was collected during the rainy season (100%) than the dry season (62%). However, some of the parity results should be interpreted with caution as the numbers dissected were small.

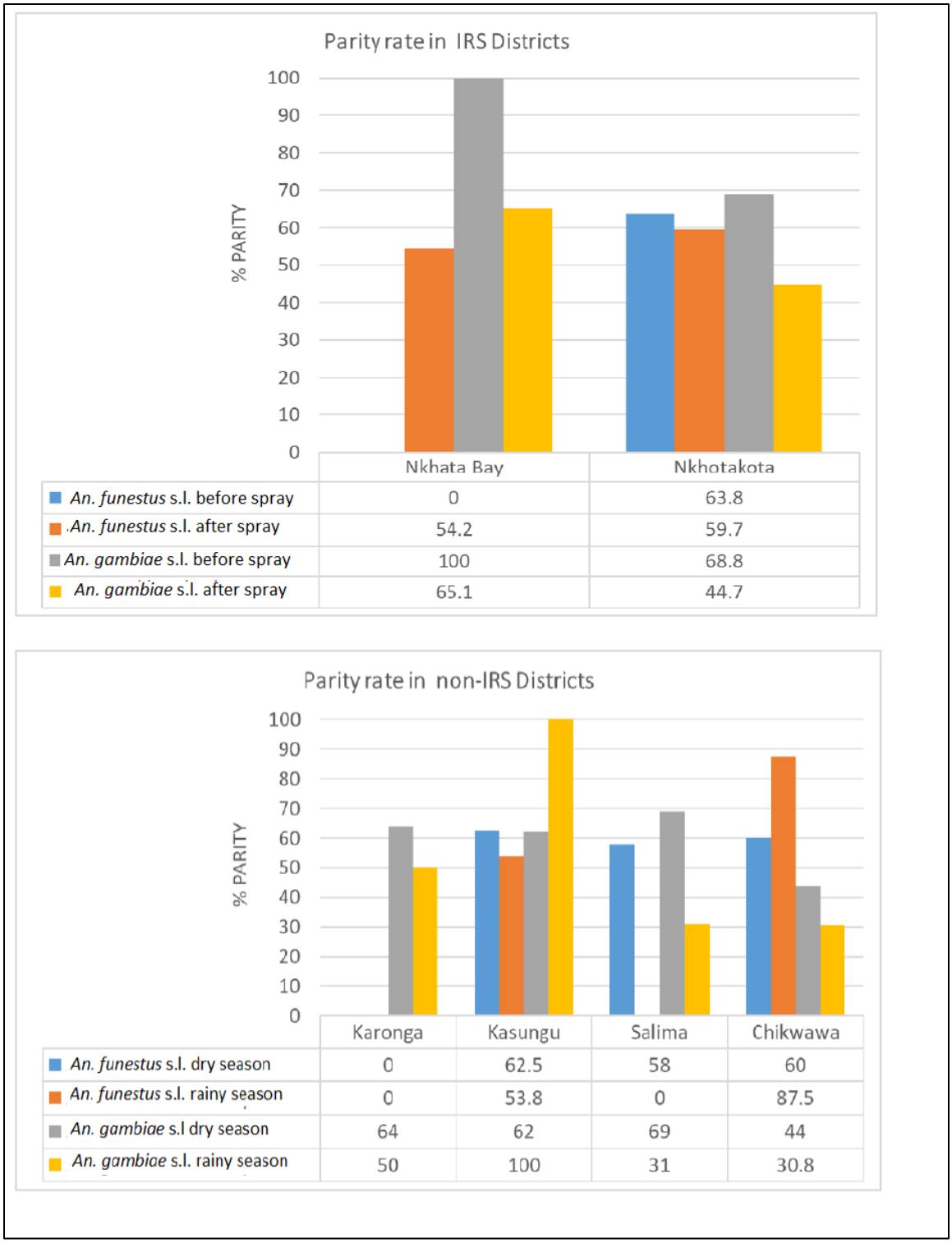
## Table 14: Total Number and Proportion of Parous Female *An. funestus* s.l. Collected by CDC-LTs Across All Six Monitoring Districts

| District | Status | Jul-20 | Aug-20 | Sep-20 | Oct-20 | Nov-20 | Dec-20 | Jan-21 | Feb-21 | Mar-21 | Apr-21 | May-21 | Jun-21 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Karonga** | Total No. collected | 0 | 97 | 1 | 2 | 0 | 0 | 18 | 14 | 0 | 0 | 0 | 1 | 133 |
| | Total No. dissected | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total No. parous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Parity rate (%) | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** |
| **Nkhata Bay** | Total No. collected | 178 | 39 | 51 | 0 | 1 | 0 | 8 | 0 | 26 | 47 | 50 | 176 | 576 |
| | Total No. dissected | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 22 | 12 | 19 | 69 |
| | Total No. parous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 16 | 6 | 5 | 38 |
| | Parity rate (%) | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **68.8** | **72.7** | **50.0** | **26.3** | **55.1** |
| **Kasungu** | Total No. collected | 28 | 16 | 13 | 4 | 1 | | 19 | 132 | 182 | 84 | 4 | 39 | 522 |
| | Total No. dissected | 0 | 12 | 0 | 0 | 0 | 0 | 3 | 12 | 37 | 7 | 3 | 12 | 86 |
| | Total No. parous | 0 | 7 | 0 | 0 | 0 | 0 | 2 | 10 | 26 | 2 | 1 | 4 | 52 |
| | Parity rate (%) | **0.0** | **58.3** | **0.0** | **0.0** | **0.0** | **0.0** | **66.7** | **83.3** | **70.3** | **28.6** | **33.3** | **33.3** | **60.5** |
| **Nkhotakota** | Total No. collected | 232 | 506 | 45 | 150 | 101 | 12 | 50 | 101 | 176 | 121 | 1463 | 350 | 3307 |
| | Total No. dissected | 0 | 36 | 12 | 5 | 4 | 1 | 0 | 5 | 27 | 30 | 114 | 79 | 313 |
| | Total No. parous | 0 | 21 | 8 | 2 | 3 | 1 | 0 | 2 | 12 | 15 | 67 | 48 | 179 |
| | Parity rate (%) | **0.0** | **58.3** | **66.7** | **40.0** | **75.0** | **100.0** | **0.0** | **40.0** | **44.4** | **50.0** | **58.8** | **60.8** | **57.2** |
| **Salima** | Total No. collected | 21 | 8 | 4 | 0 | 1 | 0 | 5 | 122 | 49 | 15 | 10 | 16 | 251 |
| | Total No. dissected | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 34 | 19 | 4 | 3 | 5 | 67 |
| | Total No. parous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 11 | 2 | 3 | 4 | 38 |
| | Parity rate (%) | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **52.9** | **57.9** | **50.0** | **100.0** | **80.0** | **56.7** |
| **Chikwawa** | Total No. collected | 141 | 266 | 10 | 37 | 5 | 1 | 129 | 77 | 67 | 34 | 74 | 0 | 841 |
| | Total No. dissected | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 11 | 12 | 10 | 0 | 47 |
| | Total No. parous | 0 | 7 | 0 | 0 | 0 | 0 | 8 | 0 | 5 | 6 | 9 | 0 | 35 |
| | Parity rate (%) | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **57.1** | **0.0** | **45.5** | **50.0** | **90.0** | **0.0** | **74.5** |
| **Overall** | Total No. collected | 600 | 932 | 124 | 193 | 109 | 13 | 229 | 446 | 500 | 301 | 1601 | 582 | 5630 |
| | Total No. dissected | 0 | 50 | 12 | 5 | 4 | 1 | 17 | 51 | 110 | 75 | 142 | 115 | 582 |
| | Total No. parous | 0 | 35 | 8 | 2 | 3 | 1 | 10 | 30 | 65 | 41 | 86 | 61 | 342 |
| | Parity rate (%) | **0.0** | **70.0** | **66.7** | **40.0** | **75.0** | **100.0** | **58.8** | **58.8** | **59.1** | **54.7** | **60.6** | **53.0** | **58.8** |

## Table 15: Total Number and Proportion of Parous Female *An. gambiae* s.l. Collected by CDC-LT Across All Six Monitoring Districts

| District | Status | Jul-20 | Aug-20 | Sep-20 | Oct-20 | Nov-20 | Dec-20 | Jan-21 | Feb-21 | Mar-21 | Apr-21 | May-21 | Jun-21 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Karonga** | Total No. collected | 8 | 339 | 390 | 11 | 0 | 1 | 89 | 137 | 356 | 87 | 24 | 7 | 1449 |
| | Total No. dissected | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 16 | 37 | 7 | 0 | 0 | 61 |
| | Total No. parous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 25 | 2 | 0 | 0 | 39 |
| | Parity rate (%) | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **75.0** | **67.6** | **28.6** | **0.0** | **0.0** | **63.9** |
| **Nkhata Bay** | Total No. collected | 2 | 16 | 3 | 0 | 1 | 1 | 12 | 55 | 102 | 30 | 6 | 1 | 229 |
| | Total No. dissected | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 35 | 10 | 2 | 0 | 68 |
| | Total No. parous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 25 | 6 | 0 | 0 | 44 |
| | Parity rate (%) | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **61.9** | **71.4** | **60.0** | **0.0** | **0.0** | **64.7** |
| **Kasungu** | Total No. collected | 1 | 0 | 15 | 9 | 0 | 0 | 44 | 179 | 177 | 39 | 104 | 3 | 571 |
| | Total No. dissected | 0 | 0 | 3 | 0 | 0 | 0 | 7 | 11 | 55 | 16 | 37 | 0 | 129 |
| | Total No. parous | 0 | 0 | 3 | 0 | 0 | 0 | 5 | 10 | 36 | 7 | 0 | 0 | 61 |
| | Parity rate (%) | **0.0** | **0.0** | **100.0** | **0.0** | **0.0** | **0.0** | **71.4** | **90.9** | **65.5** | **43.8** | **0.0** | **0.0** | **47.3** |
| **Nkhotakota** | Total No. collected | 12 | 194 | 7 | 30 | 17 | 56 | 126 | 334 | 260 | 134 | 76 | 304 | 1550 |
| | Total No. dissected | 0 | 2 | 4 | 4 | 4 | 9 | 0 | 32 | 36 | 21 | 11 | 15 | 138 |
| | Total No. parous | 0 | 2 | 1 | 4 | 1 | 3 | 0 | 22 | 14 | 11 | 17 | 6 | 81 |
| | Parity rate (%) | **0.0** | **100.0** | **25.0** | **100.0** | **25.0** | **33.3** | **0.0** | **68.8** | **38.9** | **52.4** | **154.5** | **40.0** | **58.7** |
| **Salima** | Total No. collected | 1 | 3 | 12 | 0 | 1 | 0 | 19 | 132 | 30 | 11 | 35 | 0 | 244 |
| | Total No. dissected | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 31 | 8 | 6 | 0 | 0 | 49 |
| | Total No. parous | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 24 | 6 | 2 | 7 | 0 | 40 |
| | Parity rate (%) | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **50.0** | **77.4** | **75.0** | **33.3** | **0.0** | **0.0** | **81.6** |
| **Chikwawa** | Total No. collected | 24 | 60 | 0 | 4 | 5 | 36 | 65 | 49 | 26 | 26 | 1 | 2 | 298 |
| | Total No. dissected | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 7 | 12 | 0 | 0 | 32 |
| | Total No. parous | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 4 | 4 | 0 | 0 | 14 |
| | Parity rate (%) | **0.0** | **0.0** | **0.0** | **0.0** | **0.0** | **46.2** | **0.0** | **0.0** | **57.1** | **33.3** | **0.0** | **0.0** | **43.8** |
| **Overall** | Total No. collected | 48 | 612 | 427 | 54 | 24 | 94 | 355 | 886 | 951 | 327 | 246 | 317 | 4341 |
| | Total No. dissected | 0 | 4 | 7 | 4 | 4 | 22 | 10 | 111 | 178 | 72 | 50 | 15 | 477 |
| | Total No. parous | 0 | 2 | 4 | 4 | 1 | 9 | 6 | 69 | 85 | 30 | 24 | 6 | 240 |
| | Parity rate (%) | **0.0** | **50.0** | **57.1** | **100.0** | **25.0** | **40.9** | **60.0** | **62.2** | **47.8** | **41.7** | **48.0** | **40.0** | **50.3** |

## Figure 21: Proportion of Parous Female *An. funestus* s.l. and *An. gambiae* s.l. in IRS and Non-IRS Districts




*NB: 1) Before spray = July 2020 to September 2020 and "After spray" = November 2020–June 2021.*
*2) Dry season = July 2020 to October 2020 and "Rainy season" = November 2020–May 2020.*

## 3.7 CONE BIOASSAYS

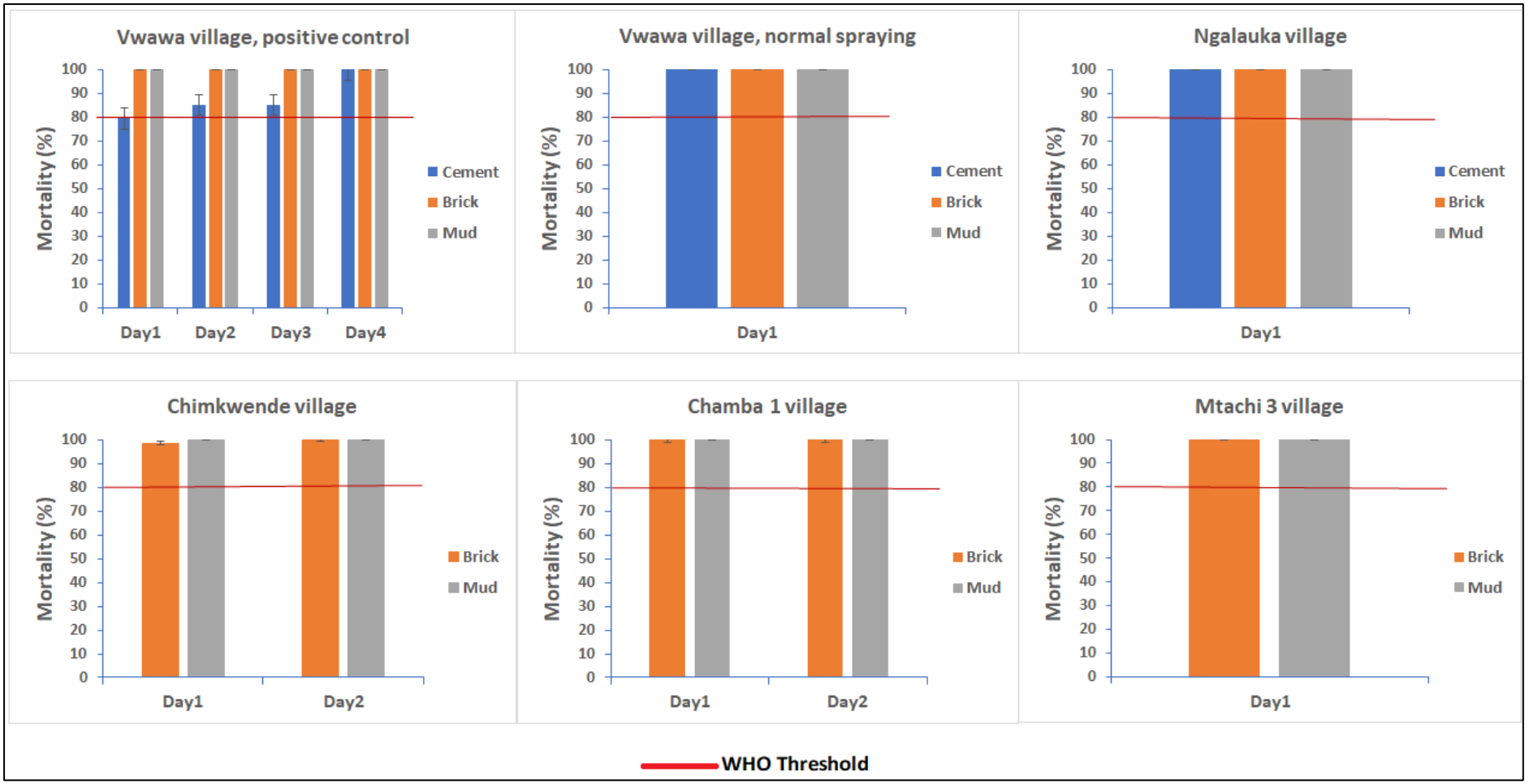
### 3.7.1 SPRAY QUALITY ASSESSMENT

The spray quality assessment from catchment areas sprayed with either SumiShield 50WG and Actellic 300CS in Nkhotakota District was satisfactory in all operational sites. At Vwawa site (Positive Control, SumiShield site), 100% mosquito mortality was recorded after 24 hours of observation in all structures except in one cement house, where 100% mortality was recorded after 4 days of observation (Figure 22). From the other SumiShield 50WG-sprayed sites, 100% mosquito mortality was observed after 1–2 days of observation from all 24 structures tested (Figure 22). At Kalungama (Actellic 300CS sprayed village), 100% mosquito mortality was observed after 24 hours in all six structures tested (Table 16).

Table 16: Spray Quality Assessment Results for Actellic 300CS in Nkhotakota District

| Village | Wall Surface Type | No. of Mosquitoes Exposed | No. Dead After 24 Hrs. | Mortality 24 Hrs. (%) |
|---|---|---|---|---|
| Kalungama | Cement plastered | 28 | 28 | 100 |
| | Cement plastered | 31 | 31 | 100 |
| | Burnt bricks | 30 | 30 | 100 |
| | Burnt bricks | 30 | 30 | 100 |
| | Mud | 30 | 30 | 100 |
| | Mud | 31 | 31 | 100 |
| | Average | 30 | 30 | 100 |

Figure 22: Spray Quality Assessment of SumiShield 50WG in Nkhotakota District




*NB: Positive control: Spray operators were closely supervised by PMI Vectorlink staff.*

### 3.7.2 FUMIGANT EFFECT OF ACTELLIC 300 CS AND SUMISHIELD 50WG

Both SumiShield 50WG and Actellic 300CS showed fumigant effect in Nkhotakota District. In the positive control structures at Vwawa (SumiShield), 100% mortality was observed after 24 hours in all six structures except one structure (cement house) where 100% mosquito mortality was observed after 4 days (Figure 23). At Chamba 1 and Mtachi 3, 100% mosquito mortality was observed after 24 hours and 48 hours, respectively. Similarly, at Vwawa (normal spraying – spray operators conducted spraying without close supervision) and Ngalauka, 100% mosquito mortality was observed after 24 hours to 48 hours (2 days) (Figures 23 and 24) while in Chimkwende it was observed after 72 hours (3 days) (Figure 24). SumiShield exhibited a fumigant effect 8 months after spraying in Nkhotakota District (Figures 23 and 24).

The fumigant effect of Actellic 300CS was only observed during spray quality assessment at Kalungama village where 100% mosquito mortality was observed after 24 hours in all six structures tested. One month after spraying, mosquito mortality dropped to below 80%. In Nkhata Bay, the fumigation effect of Actellic 300CS was very low when the tests were conducted two months after spraying; mosquito mortality fell below 80% (Figure 25).

Figure 23: Fumigation Effect of SumiShield 50WG at Vwawa Village in Nkhotakota District

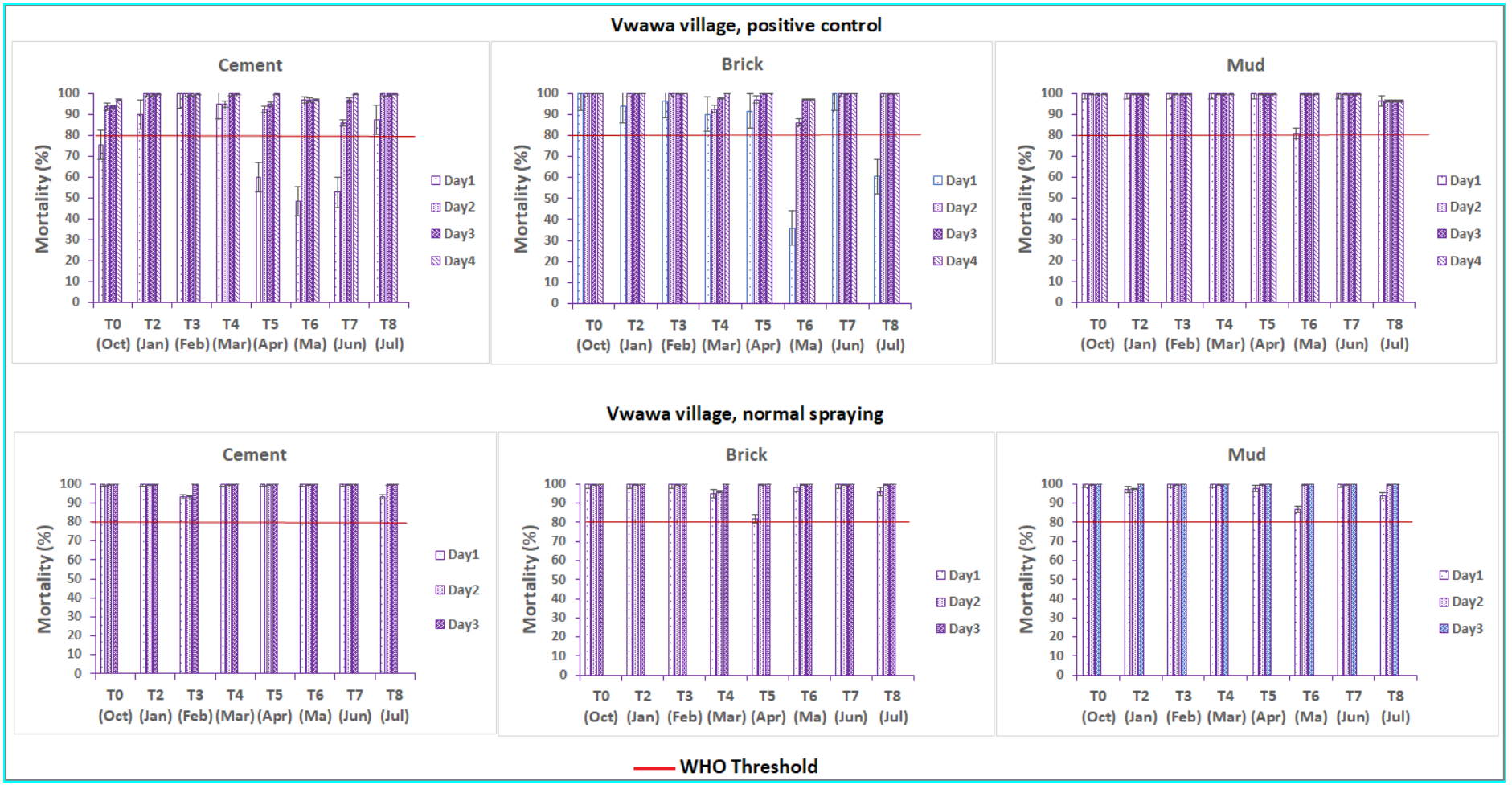


Figure 24: Fumigation Effect of SumiShield at Ngalauka and Chimkwende Villages in Nkhotakota District

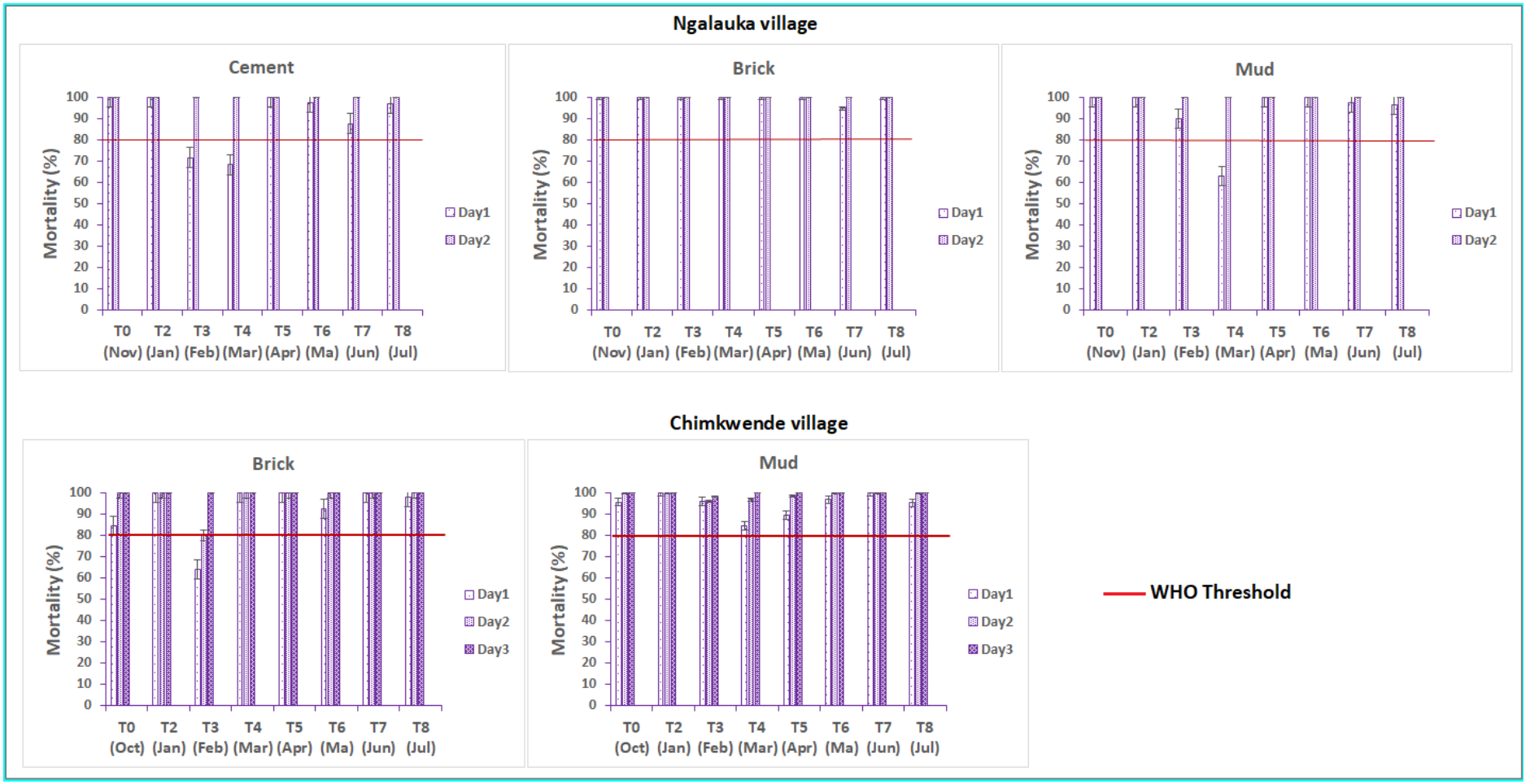
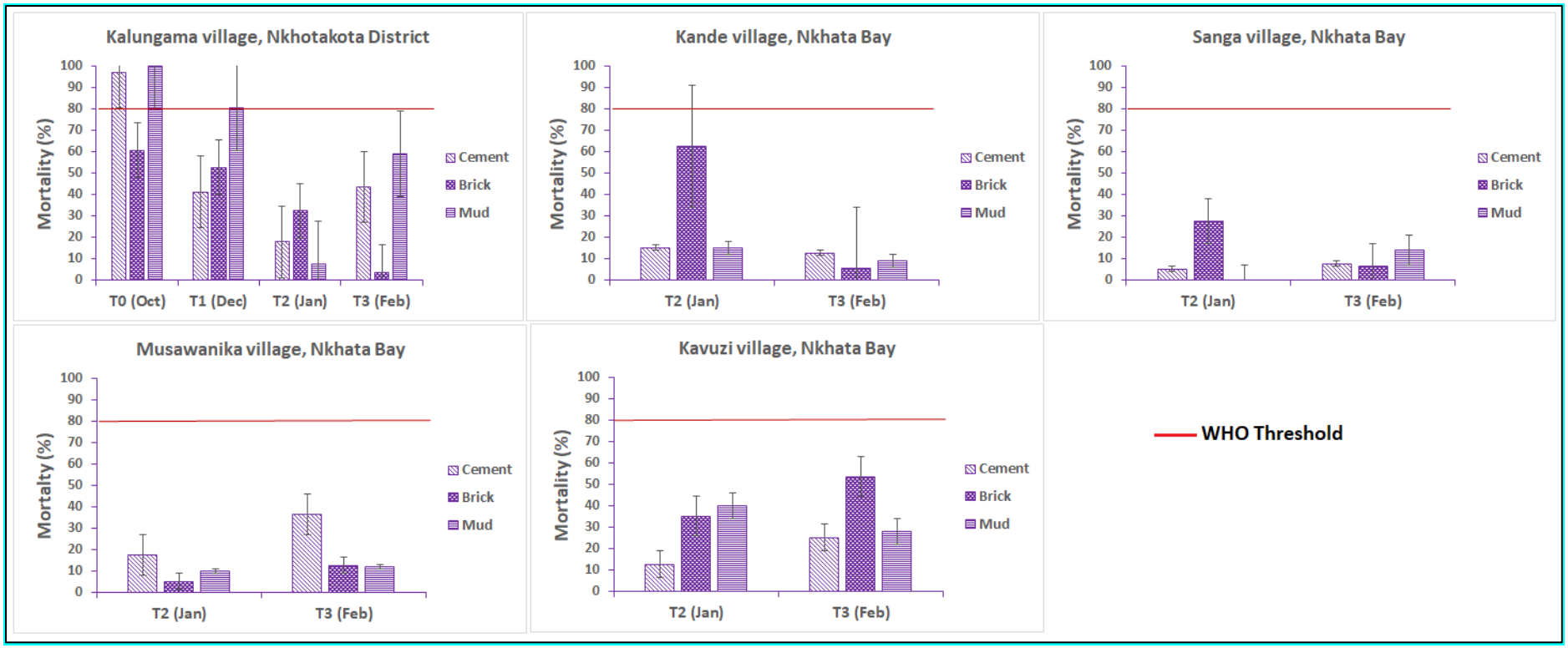


Figure 25: Fumigation Effect of Actellic 300CS in Nkhotakota and Nkhata Bay Districts



### 3.7.3 RESIDUAL LIFE OF ACTELLIC 300CS AND SUMISHIELD 50WG

Quality assessment (T0) and T1 tests were not performed in Nkhata Bay due to shortage of mosquitoes from the insectary. Residual efficacy assessment of Actellic 300CS in this district commenced two months after spraying (T2) in four villages: Kande, Sanga, Kavuzi, and Musawanika. In Nkhotakota District, monthly residual life assessment of insecticides sprayed also commenced at T2 in four villages: Vwawa, Chimkwende, Ngalauka, and Kalungama. The same six houses selected during the quality check were assessed in Nkhotakota District; in Nkhata Bay District, six houses assessed were randomly selected at T2.

Results of monthly follow-up of the residual lifespan of Actellic 300CS are shown in Figure 26. In Nkhotakota, the residual efficacy of Actellic lasted 2–3 months after spraying, when mosquito mortality fell below the 80% WHO threshold. Monitoring continued for the cement houses and the residual life lasted five months. In Nkhata Bay District, no residual effect of Actellic 300CS was observed when assessment was conducted two months after spraying. Average mosquito mortality fell below 80% in all four villages.

SumiShield 50WG is still highly effective (100% mortality) both in the control and routine spray houses 8 months after spray in Nkhotakota District and monitoring is continuing (Figures 27 and 28).

Figure 26: Residual Efficacy of Actellic 300CS in Nkhotakota and Nkhata Bay Districts

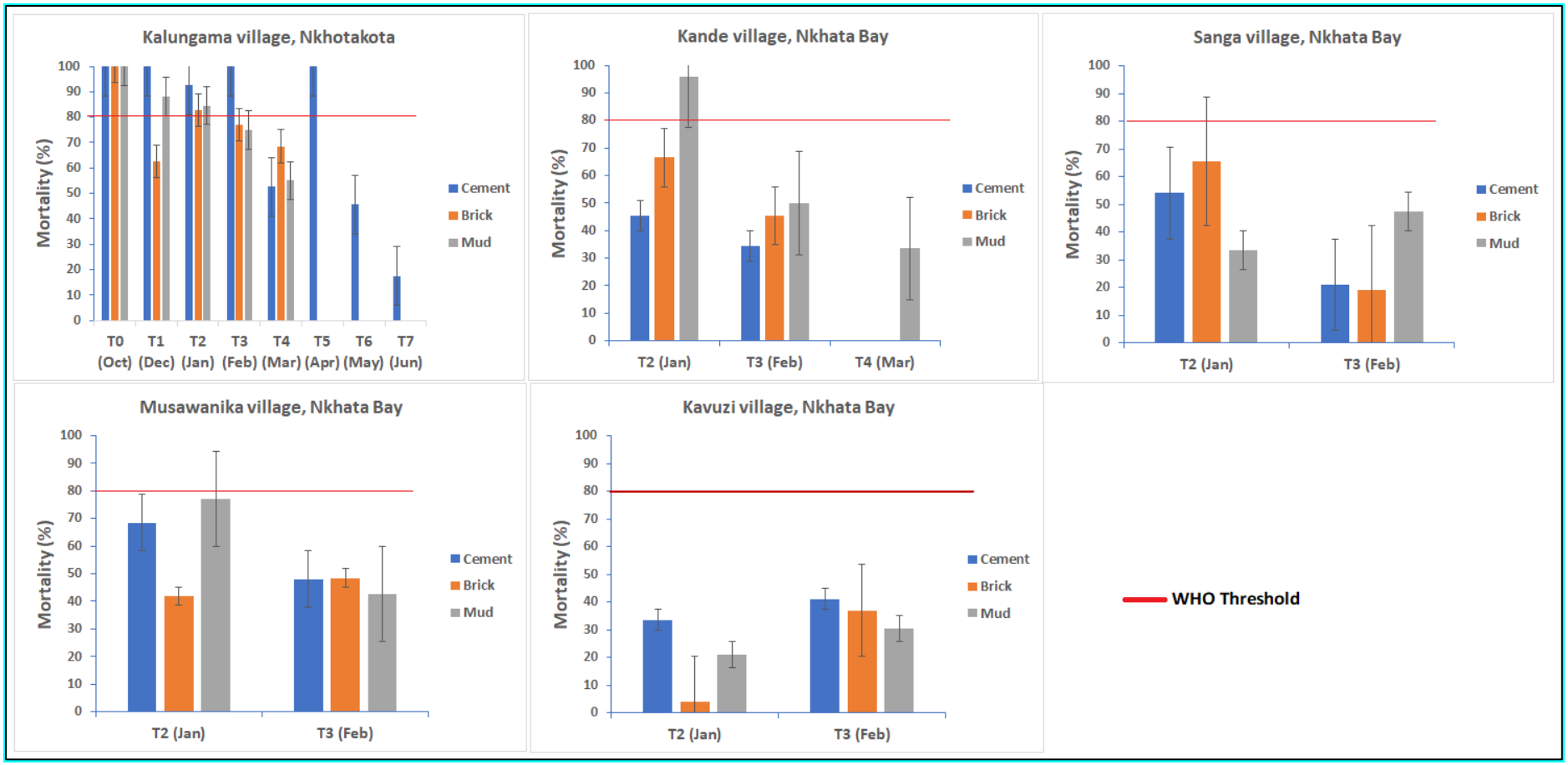


Figure 27: Residual Efficacy of SumiShield Monitored at Vwawa in Nkhotakota District

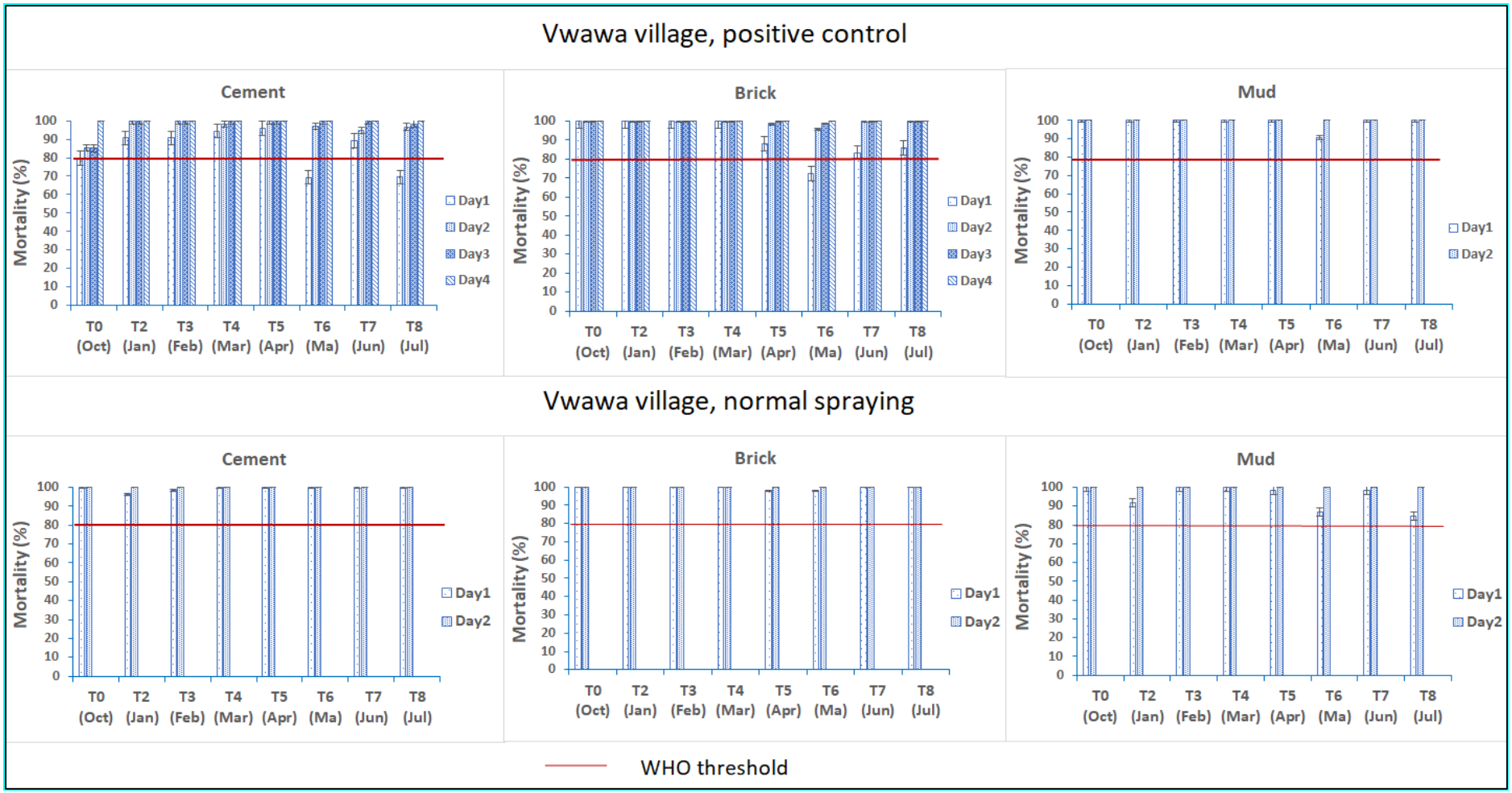
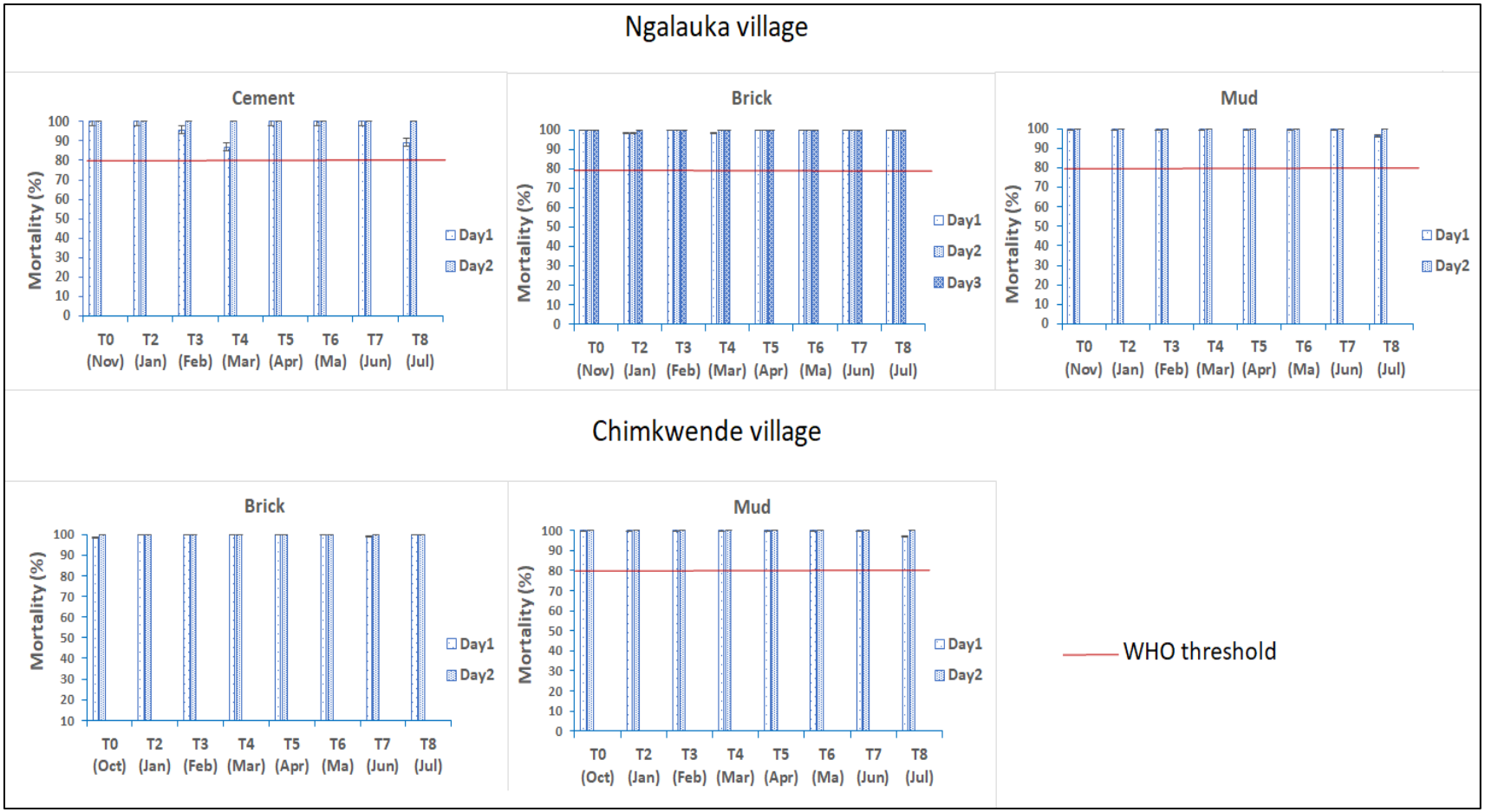


Figure 28: Residual Efficacy of SumiShield Monitored at Ngalauka and Chimkwende in Nkhotakota District



## 3.8 INSECTICIDE RESISTANCE MONITORING

This section discusses *An. funestus* and *An. gambiae* susceptibility to various insecticides in the six districts. Detailed results are in Annex D.

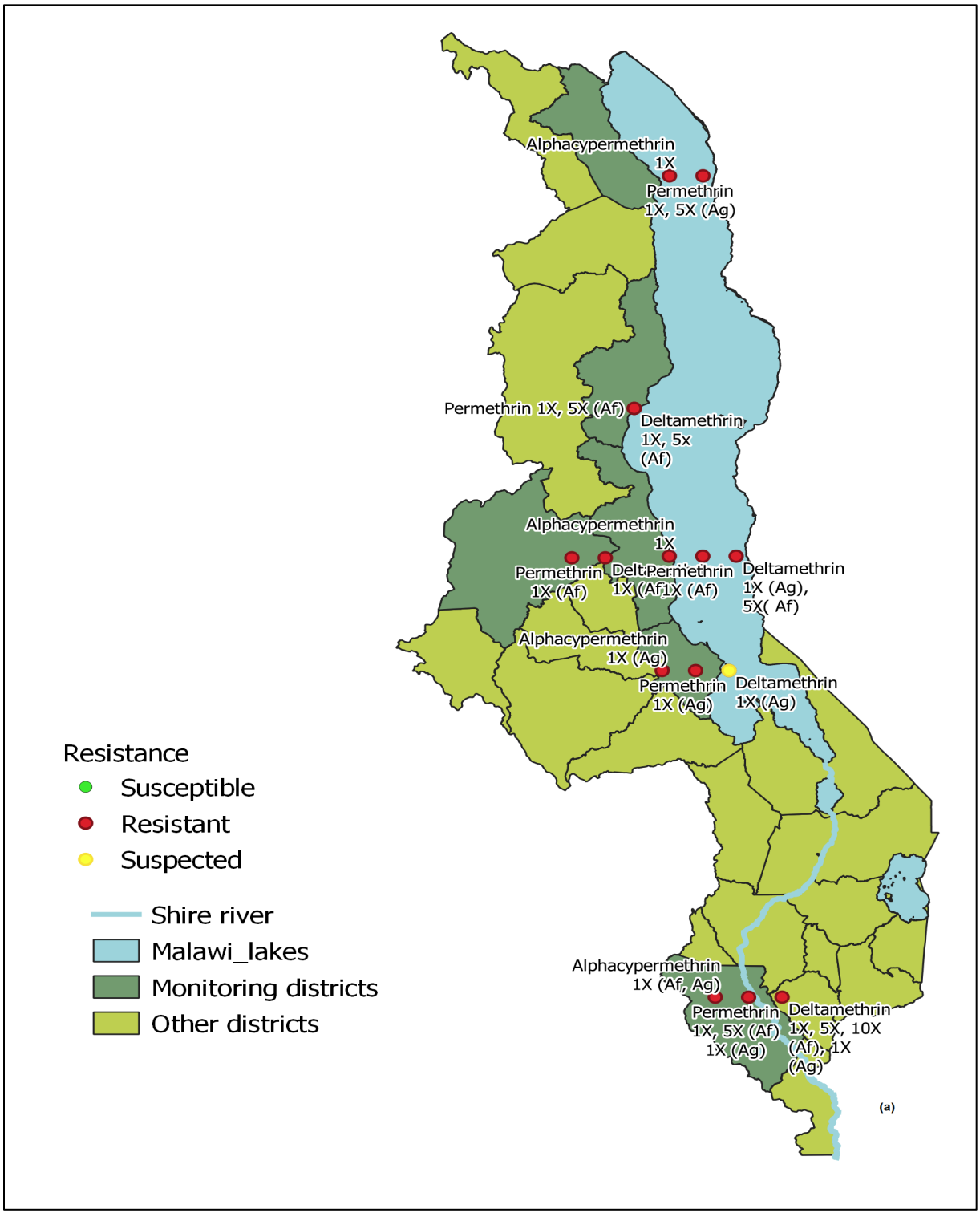
*An. gambiae* s.l. and *An. funestus* s.l. were tested against different diagnostic doses of different insecticides as indicated in Table 17.

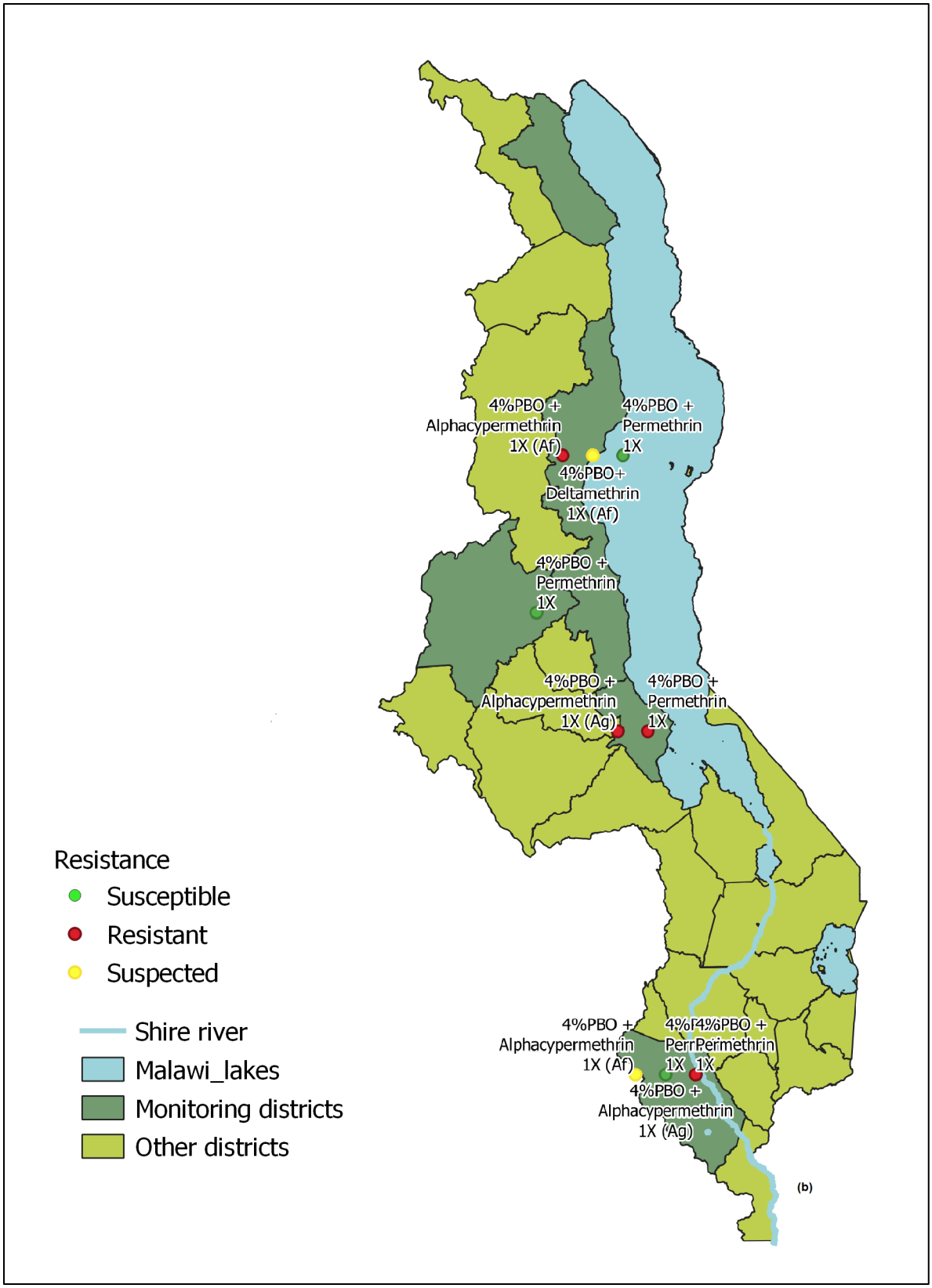
Table 17: *An. funestus* s.l. and *An. gambiae* s.l. Exposed to Different Doses of Insecticides

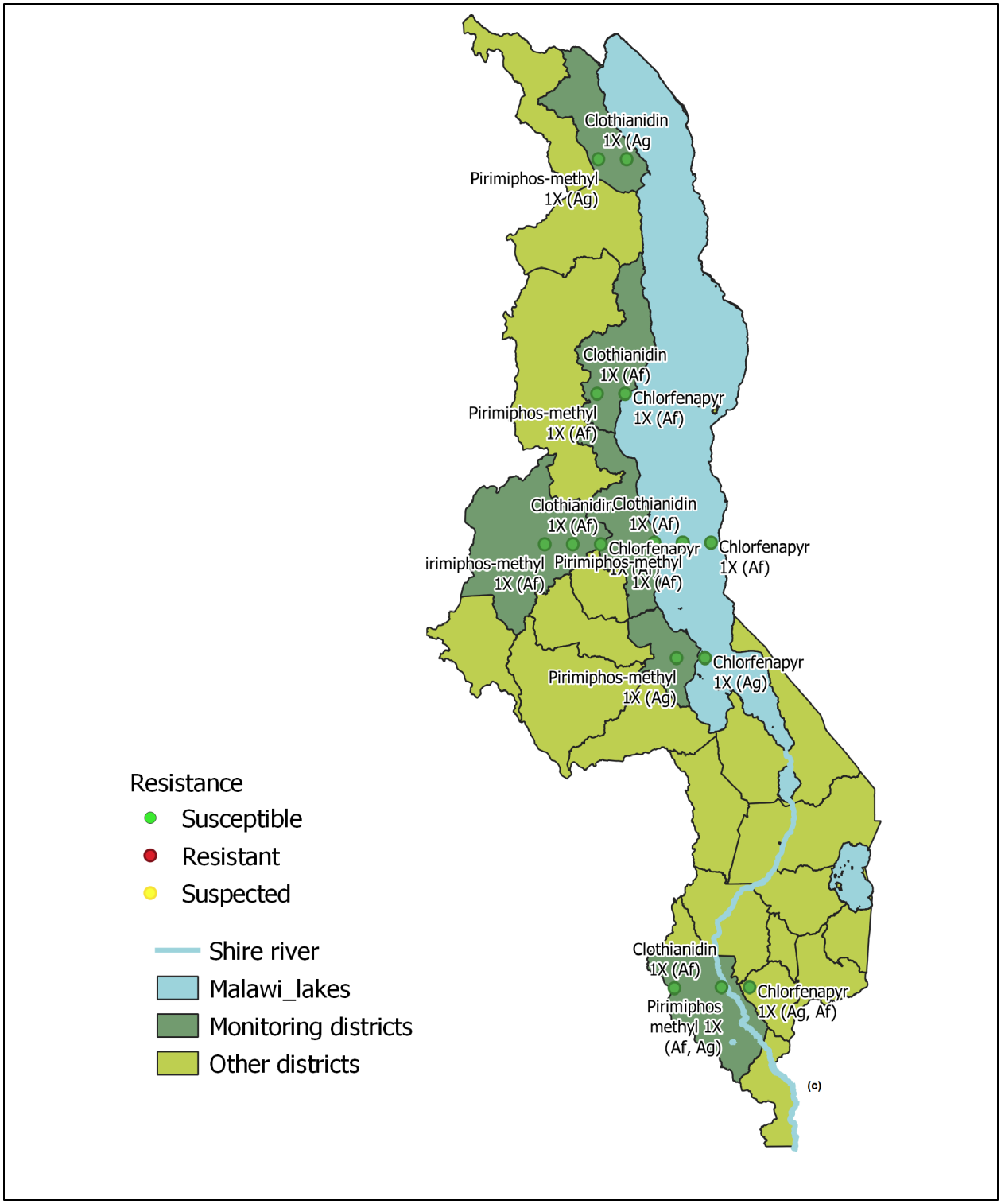
| Insecticide | Diagnostic dose | Intensity | Test Method |
|---|---|---|---|
| Deltamethrin | 0.05% | 1x | WHO Tube Assay |
| Deltamethrin | 0.25% | 5x | WHO Tube Assay |
| Deltamethrin | 62.5µg/bottle | 5x | CDC Bottle Assay |
| Deltamethrin | 0.5% | 10x | WHO Tube Assay |
| Permethrin | 0.75% | 1x | WHO Tube Assay |
| Permethrin | 3.75% | 5x | WHO Tube Assay |
| Permethrin | 107.5µg/bottle | 5x | CDC Bottle Assay |
| Alpha-cypermethrin | 0.05% | 1x | WHO Tube Assay |
| Pirimiphos-methyl | 0.25% | 1x | WHO Tube Assay |
| Chlorfenapyr | 100µg/bottle | 1x | CDC Bottle Assay |
| Clothianidin | 2% | 1x | WHO Tube Assay |

The overall insecticide resistance profile in the six entomological monitoring districts for the period July 2020–June 2021 is presented in Figure 29. (The entomological monitoring districts are presented above in Figure 1, in Section 2.1).

Figure 29: Resistance to Pyrethroids Alone (a); After Pre-Exposure to PBO (b); Organophosphate, Chlorfenapyr, and Clothianidin (c)




4%PBO +
Alphacypermethrin
1X (Af)
4%PBO +
Permethrin
1X
4%PBO+
Deltamethrin
1X (Af)
4%PBO +
Permethrin
1X
4%PBO +
Alphacypermethrin
1X (Ag)
4%PBO +
Permethrin
1X
4%PBO +
Alphacypermethrin
1X (Af)
4%PBO +
PerrPermethrin
1X
1X
4%PBO +
Alphacypermethrin
1X (Ag)
(b)
Resistance
Susceptible
Resistant
Suspected
Shire river
Malawi_lakes
Monitoring districts
Other districts



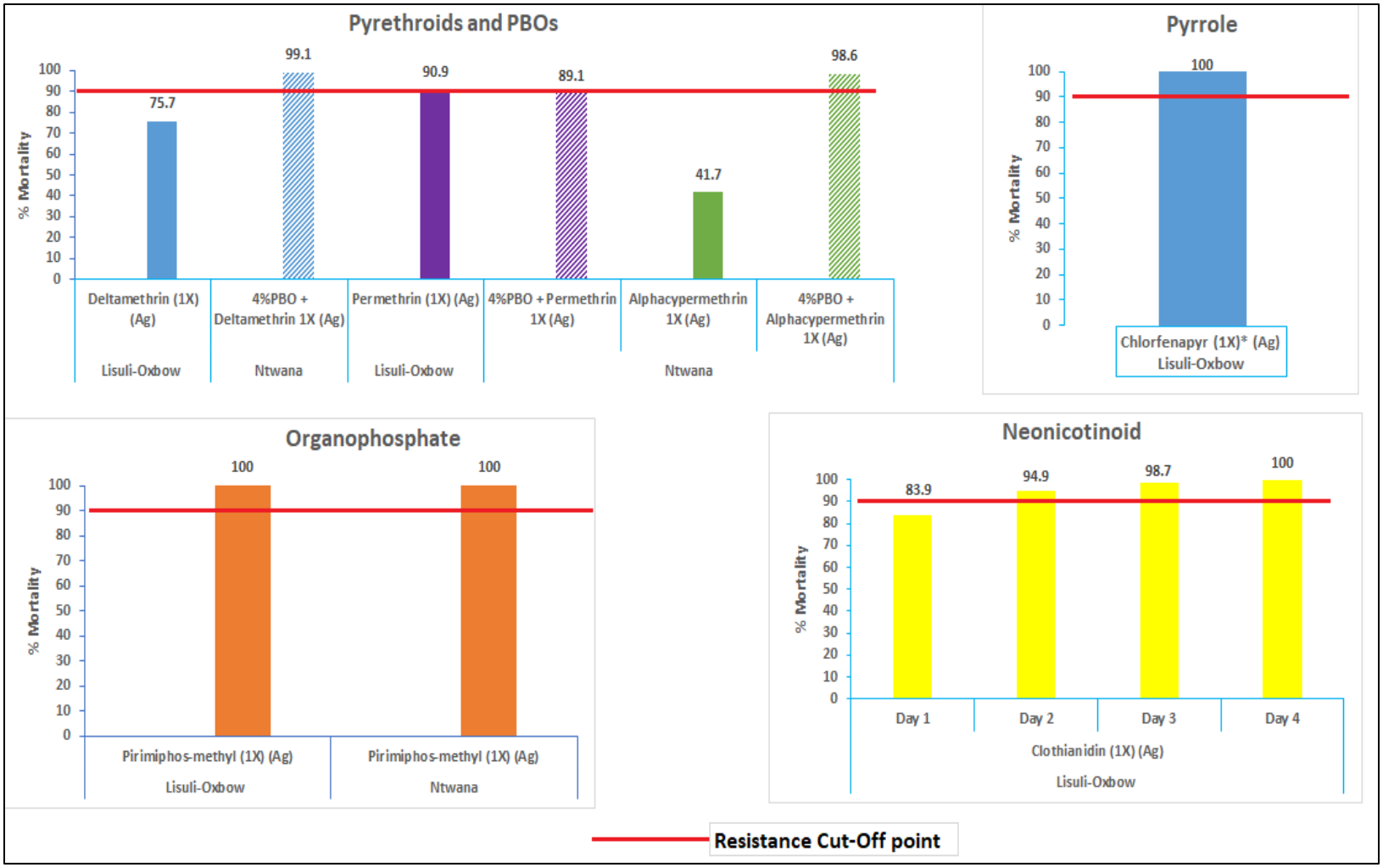

Note: *Af* = *An. funestus* and *Ag* = *An. gambiae*
(A) pyrethroid resistance frequency, (B) pyrethroid resistance frequency following exposure to 4% PBO, (C) insecticide resistance to pirimiphos-methyl (organophosphate), chlorfenapyr(pyrrole), and clothianidin (neonicotinoid)

### 3.8.1 *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN CHIKWAWA

In Chikwawa, *An. gambiae* s.l. were resistant to deltamethrin 1x, permethrin 1x, and alpha-cypermethrin 1x. Pre-exposure of these species to 4% PBO, then to deltamethrin and alphacypermethrin reverted to its susceptibility as defined by WHO, while pre-exposure to 4% PBO then to permethrin did not increase its efficacy and still remained below the threshold point. *An. arabiensis* and *An. gambiae* s.s. were susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin (Figure 30, and Annex D, Table D1).

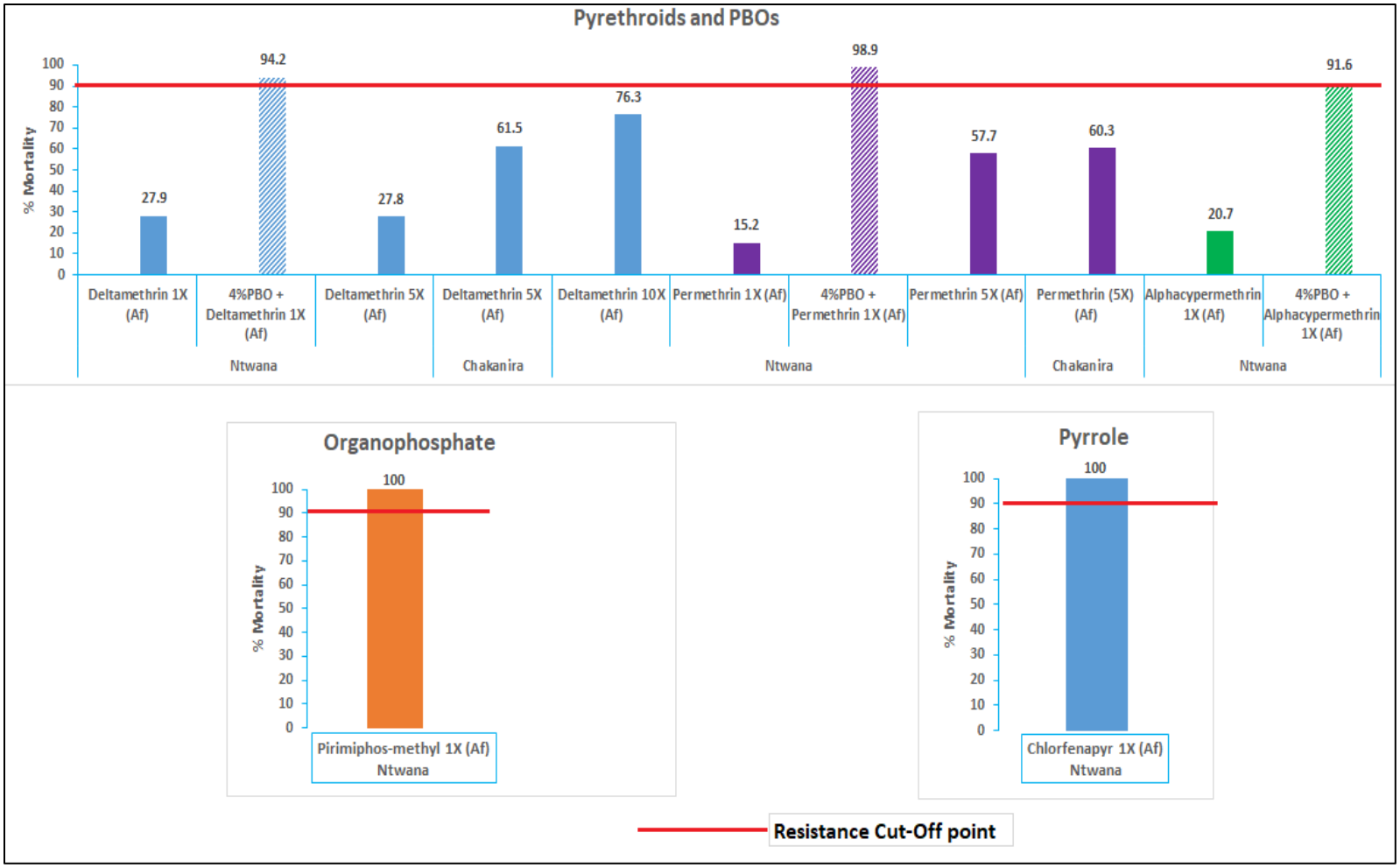
Similarly, *An. funestus* s.l. was resistant to deltamethrin 1x, deltamethrin 5x, deltamethrin 10x, permethrin 1x, permethrin 5x, and alpha-cypermethrin 1x. Pre-exposure to 4% PBO of *An. funestus* s.l. to 4% PBO then permethrin 1x reverted to its susceptibility as defined by WHO, while pre-exposure to 4% PBO then to deltamethrin 1x and alphacypermethrin 1x increased susceptibility but still below the threshold point. *An. funestus* s.l. was susceptible to pirimiphos-methyl and chlorfenapyr (Figure 31, and Annex D1).

Figure 30: *An. gambiae* s.l. *(Ag)* Response to Different Insecticides in Chikwawa District




** CDC bottle assay test*

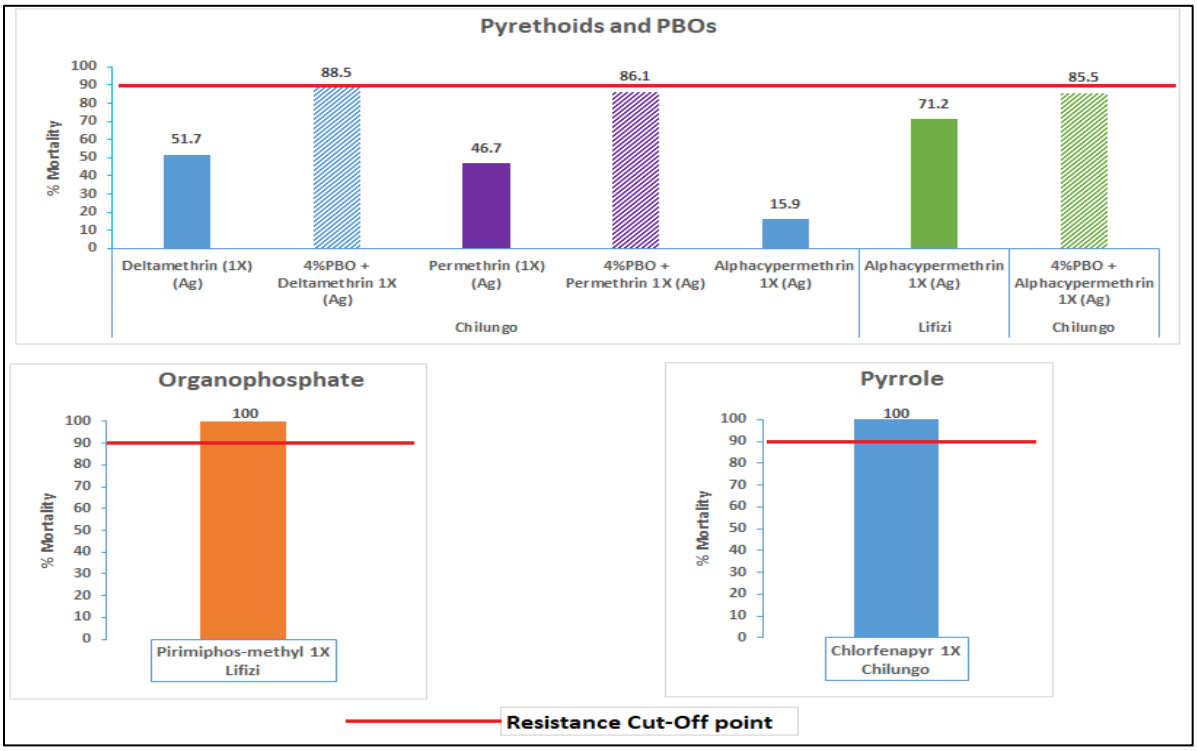
Figure 31: *An. funestus* s.l. (*Af*) Response to Different Insecticides and Concentrations in Chikwawa District



### 3.8.2 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN SALIMA DISTRICT

In Salima District, *An. gambiae* s.l. was resistant to alpha-cypermethrin 1x, deltamethrin 1x, and permethrin 1x. Pre-exposure of *An. gambiae* s.l. to 4% PBO then to the pyrethroids improved the efficacy of the pyrethroids but remained below threshold point. *An. gambiae* s.l. were susceptible to pirimiphos-methyl and chlorfenapyr; 100% mosquito mortality occurred within 24 hours for both insecticides (Figure 32, and Annex D2).

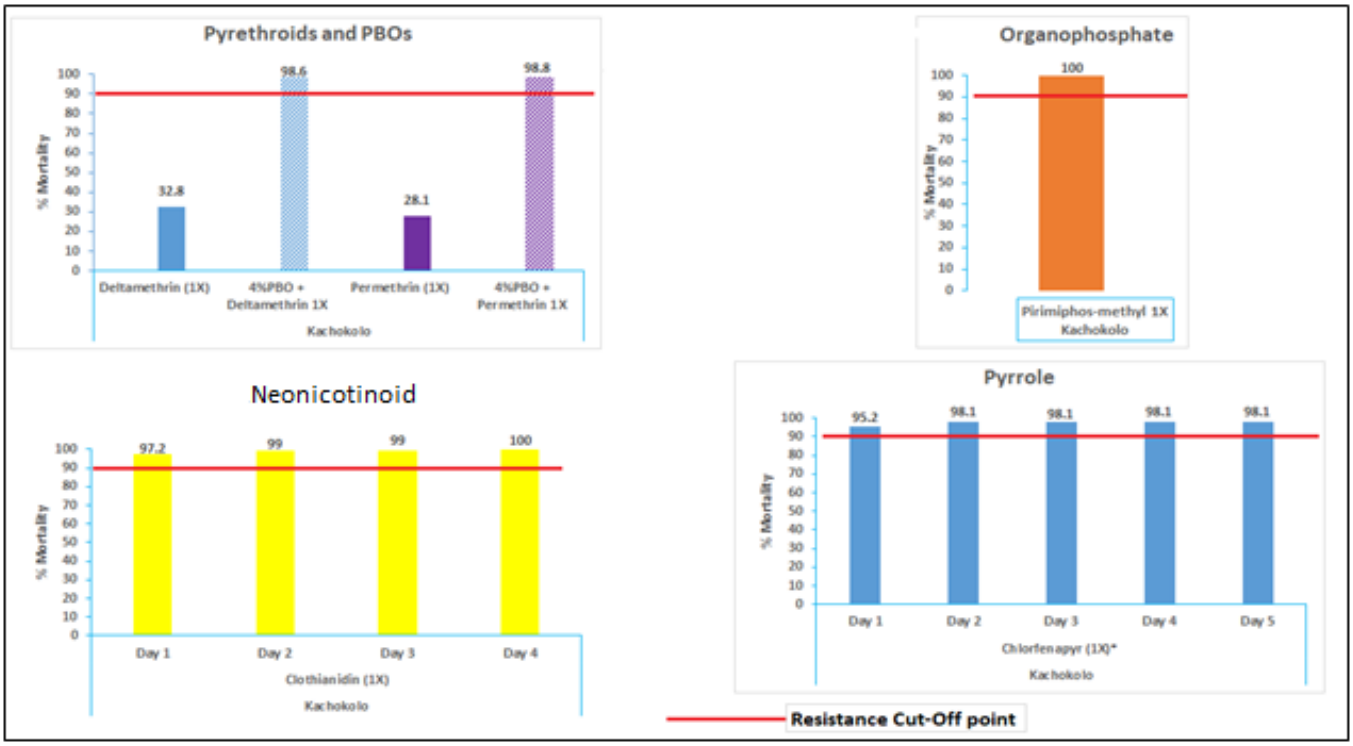
Figure 32: *An. gambiae* s.l. Response to Different Insecticides in Salima District



### 3.8.3 *AN. FUNESTUS* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN KASUNGU DISTRICT

In Kasungu District, *An. funestus* s.l. was resistant to deltamethrin 1x and permethrin 1x. Pre-exposure of *An. funestus* s.l. to 4% PBO then to pyrethroids reverted to its susceptibility as defined by WHO. *An. funestus* s.l. were susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr; 100% mosquito mortality occurred within 24 hours for pirimiphos-methyl and 96 hours (4 days) for clothianidin, and 98.1% mosquito mortality occurred after 96 hours for chlorfenapyr (Figure 33) (Annex D3).

Figure 33: *An. funestus* s.l. Response to Different Insecticides in Kachokolo, Kasungu District

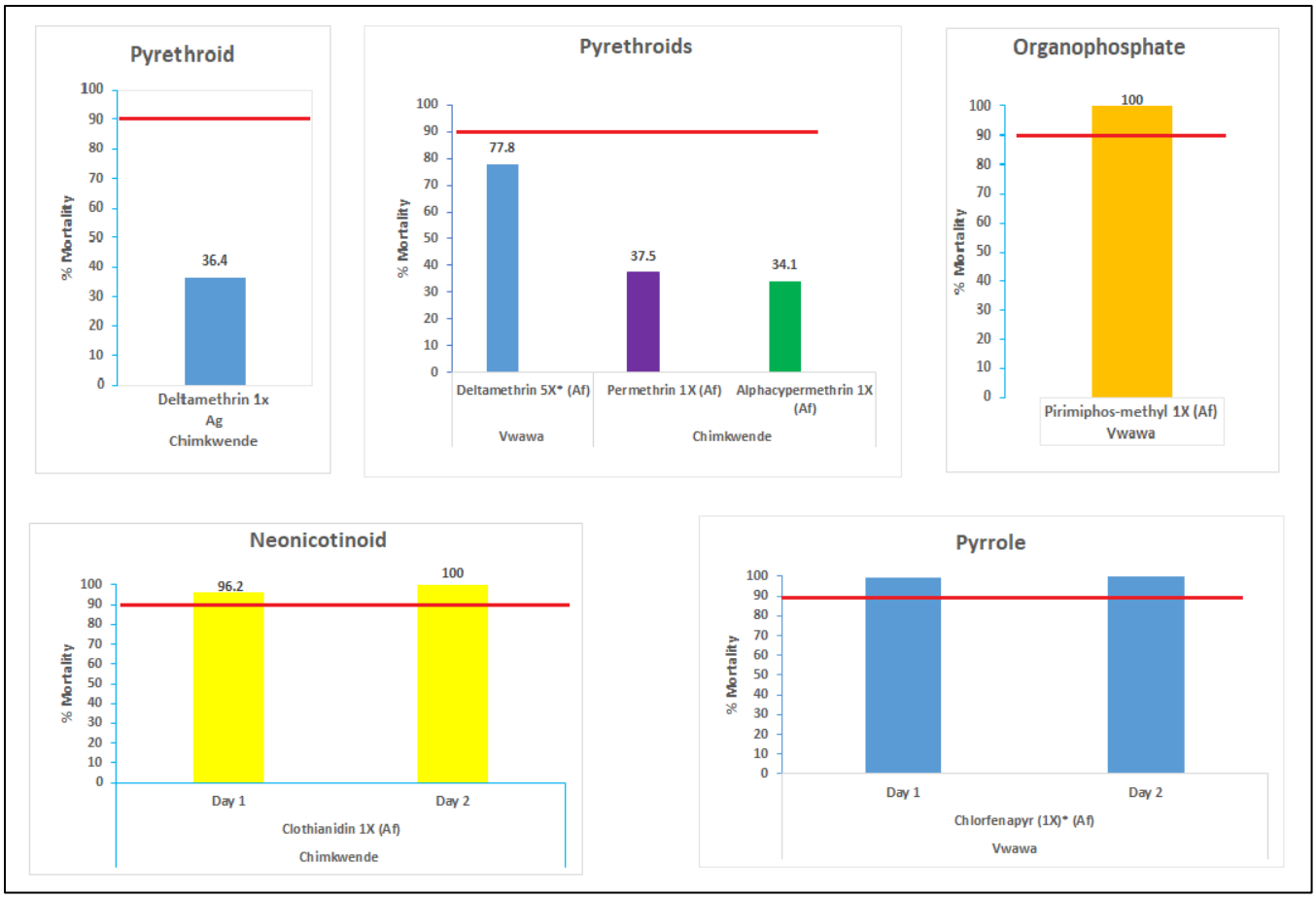



* CDC bottle assay test

### 3.8.4 *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN NKHOTAKOTA DISTRICT

In Nkhotakota District, *An. gambiae* s.l. exposed to deltamethrin 1x were resistant. Likewise, *An. funestus* s.l. was resistant to permethrin 1x, alpha-cypermethrin 1x, and deltamethrin 5x, but susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr. One hundred percent mosquito mortality occurred within 24 hours for pirimiphos-methyl and 48 hours (2 days) for clothianidin and chlorfenapyr (Figure 34, and AnnexD4).

Figure 34: *An. gambiae* s.l. (*Ag*) and *An. funestus* s.l. (*Af*) Response to Different Insecticides in Nkhotakota District

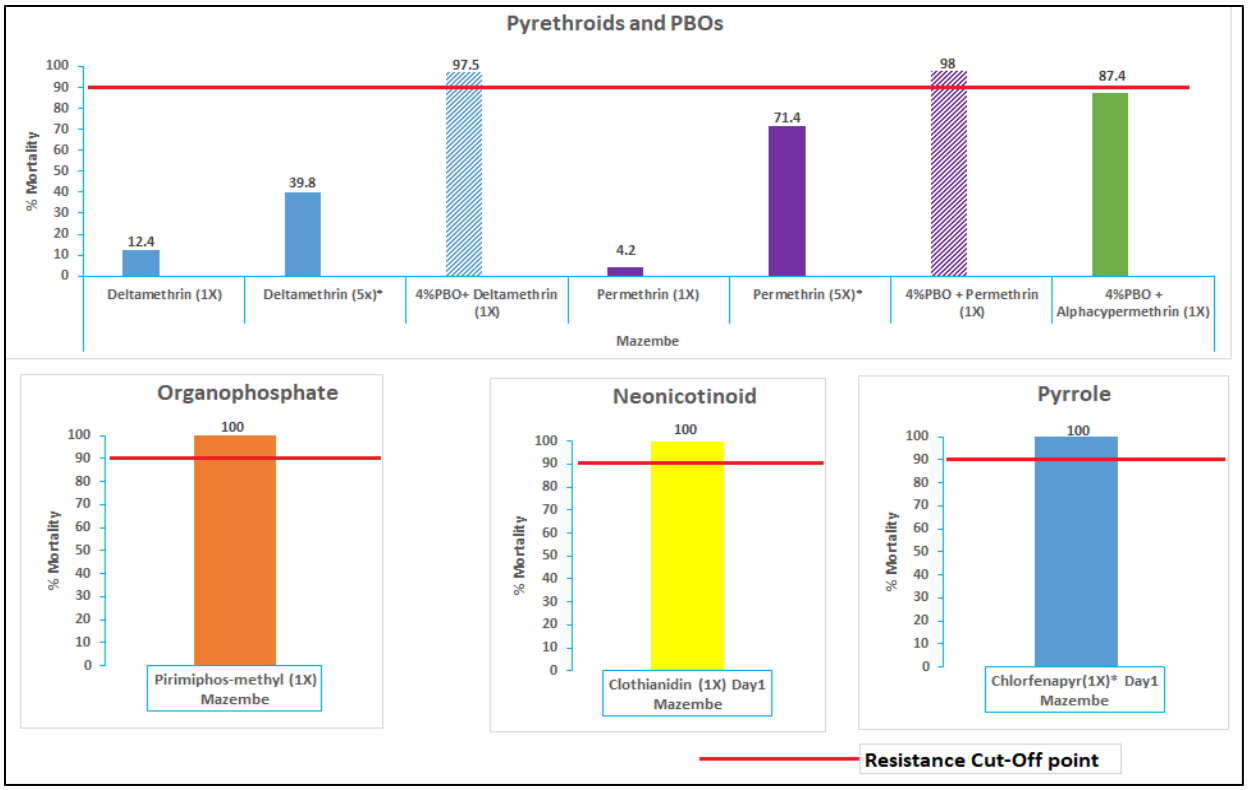



* CDC bottle assay test

## 3.8.5 *AN. FUNESTUS* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN NKHATA BAY DISTRICT

In Nkhata Bay District, *An. funestus* s.l. was resistant to deltamethrin 1x, deltamethrin 5x, permethrin 1x, and permethrin 5x. Pre-exposure of *An. funestus* s.l. to 4% PBO then permethrin 1x reverted to its susceptibility as defined by WHO, while pre-exposure to 4% PBO then to deltamethrin 1x and alphacypermethrin 1x increased susceptibility but still below the threshold point. *An. funestus* s.l. was susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr; 100% mosquito mortality occurred with 24 hours for all the three insecticides (Figure 35, and Annex D5).

Figure 35: *An. funestus* s.l. Response to Different Insecticides in Nkhata Bay District

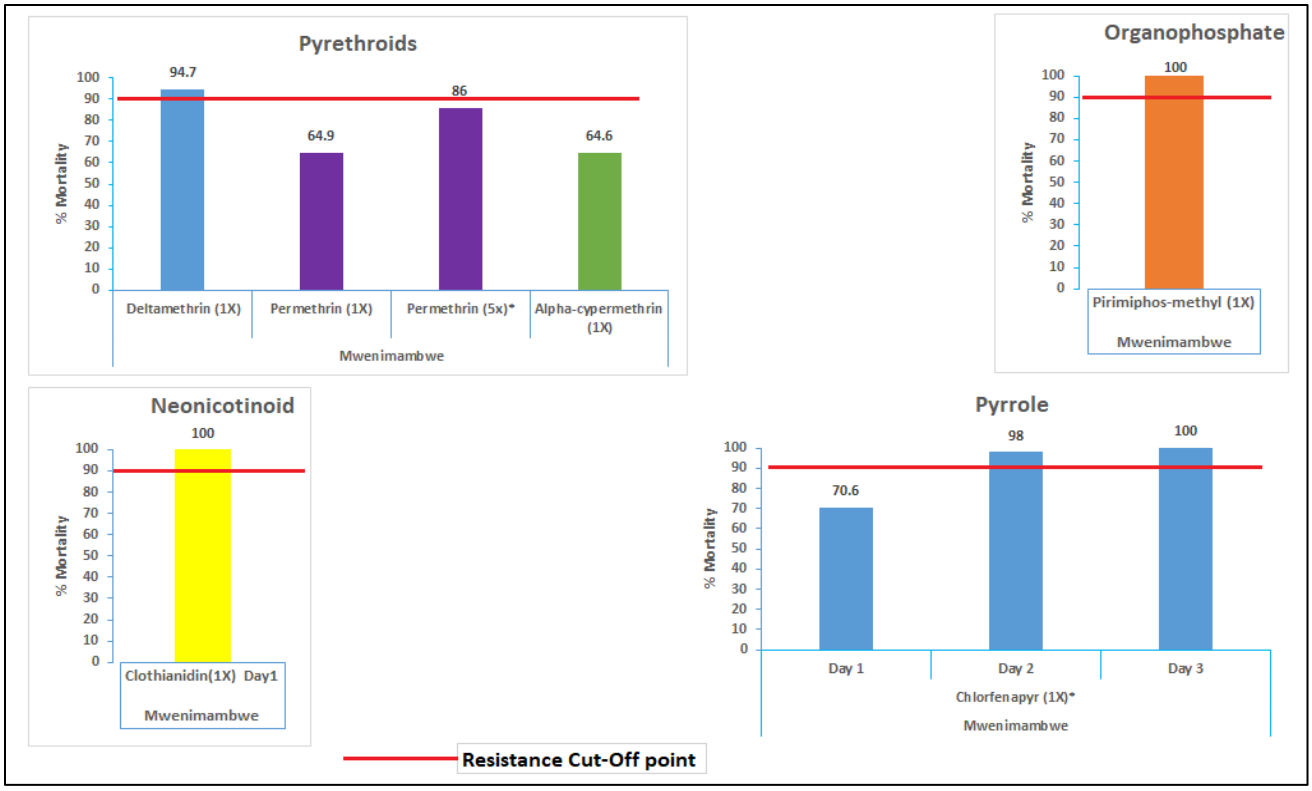



* CDC bottle assay test

### 3.8.6 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN KARONGA DISTRICT

In Karonga District, *An. gambiae* s.l. was resistant to permethrin (1X) and alpha-cypermethrin (1X) and possible resistance recorded for deltamethrin (1X). Mortality was also only 86% for permethrin 5X. The vector was susceptible to pirimiphos-methyl 1X, clothianidin 1X, and chlorfenapyr 1X. One hundred percent mosquito mortality occurred within 24 hours for pirimiphos-methyl and clothianidin, and after 72 hours for chlorfenapyr (Figure 36, and Annex D6).

Figure 36: *An. gambiae* s.l. Response to Different Insecticides in Mwenimambwe, Karonga District




* CDC bottle assay test

## 3.8.7 DETECTION OF *ACE-1*

A total of 310 female *An. gambiae* s.l. [(70% (n=217) *An. arabiensis* and 30% (n=93) *An. gambiae* s.s.] were analyzed for *Ace-1*. The processed samples were collected from six sentinel sites in Chikwawa, Salima, Kasungu, Nkhotakota, Nkhata Bay, and Karonga districts using PSCs, CDC-LTs, and live mosquito collections. (See Table 3 above in Section 2.2.3.) Out of the processed samples, 14.5% (n=45) were collected from Chikwawa, 19% (n=59) from Salima, 51% (n=16.5) from Kasungu, 22.9% (n=71) from Nkhotakota, 7.4% (n=23) from Nkhata Bay, and 19.7% (n=61) from Karonga. All the samples analyzed were homozygous susceptible and no G119S mutation was detected (Table 18).

Table 18: *Ace-1* and *kdr* Results

| District | Species | *Ace - 1* | | | *kdr-e* | | | f(R) for kdr-e |
|---|---|---|---|---|---|---|---|---|
| | | RR | RS | SS | RR | RS | SS | |
| Karonga | *An. arabiensis* | 0 | 0 | 57 | 0 | 0 | 44 | |
| | *An. gambiae* s.s. | 0 | 0 | 4 | 0 | 0 | 4 | |
| Nkhata Bay | *An. arabiensis* | 0 | 0 | 23 | 0 | 0 | 23 | |
| | *An.gambiae* s.s | 0 | 0 | 0 | 0 | 0 | 0 | |
| Nkhota kota | *An. arabiensis* | 0 | 0 | 53 | 0 | 0 | 20 | |
| | *An.gambiae* s.s. | 0 | 0 | 18 | 0 | 0 | 23 | |
| Salima | *An. arabiensis* | 0 | 0 | 9 | 1 | 0 | 3 | **(0.25)** |
| | *An. gambiae* s.s. | 0 | 0 | 50 | 26 | 0 | 27 | **(0.49)** |

| District | Species | *Ace - 1* | | | *kdr-e* | | | f(R) for kdr-e |
|---|---|---|---|---|---|---|---|---|
| | | RR | RS | SS | RR | RS | SS | |
| Kasungu | *An. arabiensis* | 0 | 0 | 32 | 0 | 0 | 22 | |
| | *An. gambiae* s.s | 0 | 0 | 19 | 5 | 6 | 7 | **(0.61)** |
| Chikwawa | *An. arabiensis* | 0 | 0 | 43 | 0 | 0 | 28 | |
| | *An. gambiae* s.s | 0 | 0 | 2 | 0 | 0 | 2 | |
| Number of genotypes | | 0 | 0 | 310 | 32 | 6 | 203 | |
| Number of alleles | | 0 | 0 | 620 | 64 | 12 | 406 | |
| Total number of alleles | | 620 | | | 482 | | | |
| Frequency of the resistance R allele [2RR+RS)/2[RR+RS+SS)] | | **0 %** | | | **14.5%** | | | |

### 3.8.8 DETECTION OF L1014S (*KDR-EAST*)

*Kdr-east* analysis was conducted on samples collected from all six sentinel sites using PSCs, CDC-LTs, and live mosquito collections. A total of 241 female *An. gambiae* s.l. samples (58.5% (n= 141) *An. arabiensis* and 41.5% (n=100) *An. gambiae* s.s.) were analyzed. Out of the processed samples, 12.4% (n=30) were from Chikwawa, 23.7% (n=57) from Salima, 16.6% (n=40) from Kasungu, 17.8% (n=43) from Nkhotakota, 9.5% (n=23) from Nkhata Bay, and 16.6% (n=48) from Karonga.

The L1014S mutation was detected in Salima (in both *An. arabiensis* and *An. gambiae* s.s.) and Kasungu (*An. gambiae* s.s.) districts only. No mutation was detected in the rest of the districts. The overall frequency for both species in Salima and Kasungu was 14.5% (Table 18).

# 4. CONCLUSION AND RECOMMENDATIONS

- Overall, *An. gambiae* s.l. was the most abundant vector, representing 55.6% of all the *Anopheles* mosquitoes collected in the six entomological monitoring districts.
- *An. gambiae* s.l. was the predominant vector in Karonga District, Salima (Chilungo site) and Chikwawa (Nyamphota site). Low numbers of *An. gambiae* s.l. were recorded in Nkhata Bay District.
- *An. funestus* s.l. was dominant in Nkhotakota District (Vwawa and Chimkwende sites), Salima (Cholokoto site) and Kasungu District. Low numbers of *An. funestus* s.l. were recorded in Karonga District.
- CDC-light traps, PSCs, and HLCs were all effective in *Anopheles* mosquito collection.
- All mosquitoes that were identified to species-specific level from the *An. funestus* group in all the six districts turned out to be *An. funestus* s.s.
- Two species from *An. gambiae* complex were identified from the six districts: *An. arabiensis* and *An. gambiae* s.s. *An. arabiensis* was the predominant, accounting for 86% of all mosquiotoes that were identified to species-specific level by PCR.
- *An. gambiae* s.l. predominantly fed on cows while *An. funestus* s.l. fed mainly on humans in all the six districts.
- *An. funestus* s.l. is the most important malaria vector in five of the 6 districts, except in Karonga, where *An. gambiae* s.l. was responsible for all observed transmission. The overall sporozoite rate was also higher for *An, funestus* s.l. (3,5%) than for *An. gambiae* s.l. (1.2%). The overall EIR rate (30.1 ib/p/yr) due to *An. funestus* s.l. was also higher than that of *An. gambiae* s.l. (14.0 ib/p/yr). .
- *An. gambiae* s.l. (predominantly *An. arabiensis*) is the second most important malaria vector in the six districts, with an overall SR of 1.2%. The highest annual EIR of this species was recorded in Chikwawa District (27.25 ib/p/yr).
- The biting activity of *An. funestus* s.l. and *An. gambiae* s.l. occurred from dawn to dusk in all the four districts where quarterly HLC was conducted. Morning/daytime biting after 5 am was also observed when people were awake.
- The proportion of parous *An. funestus* s.l. . was higher before IRS than after spraying in both Nkhata Bay and Nkhotakota districts. A similar trend was observed in Nkhotakota for *An. gambiae* s.l. but no clear trend was obsrved in Nkhata Bay due to low number collected during the dry season.
- In non-IRS districts (Karonga, Salima, and Chikwawa), higher proportions of parous *An. gambiae* s.l. were collected during the dry season than during the rainy season; the reverse was true in Kasungu District.
- In Kasungu District, high parity rates of *An. funestus* s.l. were observed in the dry season compared to the rainy season. Conversely, in Chikwawa District, higher rates in *An. funestus* s.l. were observed during the rainy season than during the dry season.
- Spray quality was satisfactory in both IRS districts of Nkhotakota and Nkhata Bay.
- The residual efficacy of Actellic 300CS, 2 to 3 months in Nkhotakota and Nkhata Bay districts was much shorter than the residual efficacy of SumiShield 50WG which was still effective 8 months

after spraying in Nkhotakota District. However, entomological indicators of malaria transmission were not substantially different between the sites sprayed with Actellic and SumiShield. Overall, the mean HBR for *An. funestus* s.l. in Nkhata Bay (Actellic) was 8.8 b/p/n compared to 11.7 b/p/n in Nkhotakota (SumiShield). The HBR of An. gambiae s.l. was 10.1 and 0.05 b/p/n for Nkhotakota and Nkhata Bay districts, respectively. The annual EIR (71 ib/p/yr) was higher in Nkhotakota than the EIR in Nkhata Bay (47.3 ib/p/yr) though much of the EIR for NKK was due to *An. gambiae* s.l. which was less affected by IRS. Though the numbers dissected were few, there was no significant difference in parity between the Actellic and SumiShield sprayed sites. The parity rate for Nkhotakota for *An. funestus* s.l. (179/313; 57.2%) was not statistically different from the parity rate in Nkhata Bay (18/69; 55.1%); (p=0.75). The difference in parity for *An. gambiae* s.l. with 58.7% (81/138) for Nkhotakota and 64.7% (44/68) for Nkhata Bay was not also statistically significant (p=0.41). Preliminary analysis of HMIS case data (unpublished and data not shown in this report) also showed that IRS had an impact in reducing malaria cases but IRS with Actellic and SumiShield had similar effect in malaria case reduction. Therefore, for reasons that are not clear, IRS with Actellic and SumiShield seem to have similar impact on entomological and epidemiological indicators of malara, despite the reported shorter residual efficay of Actellic compared to SumiShield.

- 
- *An. funestus* s.s., and *An. gambiae* s.l. were susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. Clothianidin for IRS and ITNs with the active ingredient chlorfenapyr can be used as part the malaria vector control interventions in Malawi.
- *An. funestus* s.l. and *An. gambiae* s.l. were highly resistant to the three pyrethroids tested: deltamethrin, permethrin, and alpha-cypermethrin
- Pre-exposure of *An. funestus* s.l., and *An. gambiae* s.l. to 4% PBO followed by pyrethroids greatly improved the efficacy of pyrethroids. Therefore, ITNs with PBO can be recommended for distribution in Malawi.
- The absence of the *Ace-1* resistant allele is consistent with the absence of phenotypic resistance to pirimiphos-methyl (organophosphate) in *An. gambiae* s.l. in Malawi.
- There appears to be *kdr-east* resistance in *An. gambiae* s.l. populations in Malawi. However, these results need to be verified further to ascertain their validity. Furthermore, historical samples of this species need to be analyzed.
- Given its impact on entomological and epidemiological indicators of malaria and the absence of phenotypic or genotypic resistance to organophosphates, pirimiphos-methyl is recommended for IRS as part of the rotation strategy for insecticide resistance management.
- However, further investigation is also recommended to understand the causes for the short residual efficacy of Actellic as determined by cone bioassays and why these do not appear to be predictive of the entomological and epidemiological effect of IRS with Actellic..
- Due to the long residual life of SumiShield, it is recommended that IRS with this insecticide be conducted in September when *An. funestus* s.l population is at its lowest to avoid a rise in mosquito population observed in October before spraying.

# 5. REFERENCES


Abbott, WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265–267.

Benedict, M. 2007. *Methods in Anopheles research.* Atlanta, USA: Malaria Research and Reference.

Brogdon, W, and Chan A. 2010. *Guidelines for Evaluating Insecticide Resistance in Vectors using the CDC Bottle Bioassay/ Methods in Anopheles Research*. Second edition. CDC technical report. Atlanta, GA, USA: CDC.

Dandalo, L, Yihdego, Y, and Mzilahowa, T. 2019. PMI VectorLink Malawi Susceptibility Tests Preliminary Report (April 2019). Rockville, MD, USA: Abt Associates.

Gillies, MT, and Coetzee M. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). *South African Institute for Medical Research* 55: 33–81.

Malaria Research and Reference Reagent Resource (MR4) Center Manual. 2106. Methods in Anopheles Research.

Monroe, A, Msaky, D, Kiware, S, Tarimo, BB, Moore, S, Haji, K, Koenker, H, Harvey, S, Finda, M, Ngowo, H, Mihayo, K, Greer, G, Ali, A, and Okumu, F. 2020. Patterns of human exposure to malaria vectors in Zanzibar and implications for malaria elimination efforts. *Malaria Journal*, *19*(1), 1–14. https://doi.org/10.1186/s12936-020-03266-w

Wirtz, RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, and Andre RG. 1987. Comparative testing of Plasmodium falciparum sporozoite monoclonal antibodies for ELISA development. *Bull. World Health Organization* 65: 39–45.

World Health Organization. 2016. *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*.

World Health Organization. 2019. *Guidelines for malaria vector control.*

# ANNEX A: SPOROZOITE RATE IN *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. FROM PSC AND CDC-LT COLLECTIONS

Annex A1: SRs in *An. gambiae* s.l. by Location from HLC Collections, July 2020–June 2021

| District | Month | *An. gambiae* s.l. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Indoor | | | Outdoor | | | Total | | |
| | | Total tested | Total +ve | Sporozoite rate (%) | Total tested | Total +ve | Sporozoite rate (%) | Total tested | Total +ve | Sporozoite rate (%) |
| Nkhata Bay | September | 4 | 0 | 0.0 | 4 | 0 | 0.0 | 8 | 0 | 0.0 |
| | December | 1 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 0 | 0.0 |
| | March | 25 | 0 | 0.0 | 38 | 0 | 0.0 | 63 | 0 | 0.0 |
| | June | - | - | - | - | - | - | - | - | - |
| Nkhotakota | September | 45 | 0 | 0.0 | 46 | 0 | 0.0 | 91 | 0 | 0.0 |
| | December | 1 | 0 | 0.0 | 9 | 0 | 0.0 | 10 | 0 | 0.0 |
| | March | 128 | 1 | 0.8 | 229 | 3 | 1.3 | 357 | 4 | 1.1 |
| | June | - | - | - | -- | - | - | - | - | - |
| Salima | September | 6 | 0 | 0.0 | 15 | 0 | 0.0 | 21 | 0 | 0.0 |
| | December | 2 | 0 | 0.0 | 1 | 0 | 0.0 | 3 | 0 | 0.0 |
| | March | 15 | 3 | 20.0 | 1 | 0 | 0.0 | 16 | 3 | 18.8 |
| | June | - | - | - | - | - | - | - | - | - |
| Kasungu | September | 6 | 0.0 | 0.0 | 1 | 0 | 0.0 | 7 | 0 | 0.0 |
| | December | 3 | 0.0 | 0.0 | 31 | 0 | 0.0 | 34 | 0 | 0.0 |
| | March | 12 | 0.0 | 0.0 | 40 | 0 | 0.0 | 42 | 0 | 0.0 |
| | June | 1 | 0.0 | 0.0 | 0 | 0 | 0.0 | 1 | 0 | 0.0 |
| Total | | **249** | **4** | **1.6** | **415** | **3** | **0.7** | **654** | **7** | **1.1** |

## Annex A2: SRs in *An. funestus* s.l. by Location from HLC Collections by District, July 2020–June 2021

| District | Month | *An. funestus* s.l. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Indoor | | | Outdoor | | | Total | | |
| | | Total tested | Total +ve | Sporozoite rate (%) | Total tested | Total +ve | Sporozoite rate (%) | Total tested | Total +ve | Sporozoite rate (%) |
| Nkhata Bay | September | 3 | 0 | 0.0 | 1 | 0 | 0.0 | 4 | 0 | 0.0 |
| | December | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| | March | 0 | 0 | 0.0 | 1 | 0 | 0.0 | 1 | 0 | 0.0 |
| Nkhotakota | September | 82 | 3 | 3.7 | 12 | 0 | 0.0 | 94 | 3 | 3.2 |
| | December | 1 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 0 | 0.0 |
| | March | 33 | 0 | 0.0 | 7 | 0 | 0.0 | 40 | 0 | 0.0 |
| Salima | September | 1 | 0 | 0.0 | 5 | 0 | 0.0 | 6 | 0 | 0.0 |
| | December | 1 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 0 | 0.0 |
| | March | 15 | 1 | 6.3 | 20 | 2 | 10.0 | 35 | 3 | 8.6 |
| Kasungu | September | 0 | 0 | 0.0 | 1 | 0 | 0.0 | 1 | 0 | 0.0 |
| | December | 11 | 0 | 0.0 | 4 | 0 | 0.0 | 15 | 0 | 0.0 |
| | March | 2 | 1 | 50.0 | 7 | 0 | 0.0 | 9 | 1 | 11.1 |
| Total | | **149** | **5** | **3.4** | **58** | **2** | **3.4** | **207** | **7** | **3.4** |

## Annex A3: SRs of *An. gambiae* s.l. from PSC Collections by District

| District | Indicator | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Karonga | Total No. tested | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Nkhata Bay | Total No. tested | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Nkhotakota | Total No. tested | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Salima | Total No. tested | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Kasungu | Total No. tested | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Chikwawa | Total No. tested | 0 | 0 | 6 | 27 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Total | Total No. tested | 0 | 7 | 6 | 31 | 7 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 52 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |

## Annex A4: SRs of *An. funestus* s.l. from PSC Collections by District

| District | Indicator | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Karonga | Total No. tested | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Nkhata Bay | Total No. tested | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Nkhotakota | Total No. tested | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Salima | Total No. tested | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Kasungu | Total No. tested | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 6 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **20** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **16.7** |
| Chikwawa | Total No. tested | 0 | 0 | 19 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26 |
| | No. sporozoites (+ve) | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | Sporozoite rate (%) | **0** | **0** | **5.3** | **33.3** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **11.5** |
| Total | Total No. tested | 0 | 3 | 19 | 6 | 6 | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 40 |
| | No. sporozoites (+ve) | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | Sporozoite rate (%) | **0** | **0** | **5.3** | **33.3** | **16.7** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **10** |

## Annex A5: SRs of *An. gambiae* s.l. from CDC-LT Collections by District

| District | Indicator | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Karonga | Total No. tested | 1 | 104 | 194 | 11 | 0 | 1 | 42 | 60 | 231 | 77 | 19 | 2 | 742 |
| | No. sporozoites (+ve) | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 8 |
| | Sporozoite rate (%) | **0** | **1.9** | **1** | **0** | **0** | **0** | **0** | **1.7** | **0.9** | **1.3** | **0** | **0** | **1.1** |
| Nkhata Bay | Total No. tested | 1 | 11 | 3 | 0 | 1 | 1 | 11 | 35 | 64 | 20 | 3 | 1 | 151 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **33.3** | **0** | **0.7** |
| Nkhotakota | Total No. tested | 1 | 72 | 50 | 43 | 21 | 40 | 120 | 140 | 86 | 56 | 1 | 0 | 630 |
| | No. sporozoites (+ve) | 0 | 0 | 2 | 5 | 0 | 1 | 2 | 1 | 0 | 1 | 0 | 0 | 12 |
| | Sporozoite rate (%) | **0** | **0** | **4** | **11.6** | **0** | **2.5** | **1.7** | **0.7** | **0** | **1.8** | **0** | **0** | **1.9** |
| Salima | Total No. tested | 1 | 1 | 12 | 0 | 1 | 0 | 19 | 126 | 22 | 8 | 30 | 0 | 220 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 4 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **2.4** | **4.5** | **0** | **0** | **0** | **1.9** |
| Kasungu | Total No. tested | 0 | 0 | 9 | 9 | 1 | 0 | 38 | 143 | 91 | 37 | 23 | 1 | 352 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **8.1** | **0** | **0** | **0.9** |
| Chikwawa | Total No. tested | 1 | 13 | 5 | 4 | 5 | 31 | 37 | 35 | 21 | 18 | 0 | 0 | 170 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **5.6** | **0** | **0** | **0.5** |
| | Total No. tested | 5 | 201 | 273 | 67 | 29 | 73 | 267 | 539 | 515 | 216 | 76 | 4 | 2265 |
| | No. sporozoites (+ve) | 0 | 2 | 4 | 5 | 0 | 1 | 2 | 5 | 3 | 6 | 1 | 0 | 29 |
| | Sporozoite rate (%) | **0** | **1.0** | **1.5** | **7.5** | **0.0** | **1.4** | **0.7** | **0.9** | **0.6** | **2.8** | **1.3** | **0.0** | **1.3** |

## Annex A6: SRs of *An. funestus* s.l. from CDC-LT Collections by District

| District | Indicator | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Karonga | Total No. tested | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 5 |
| | No. sporozoites (+ve) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sporozoite rate (%) | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Nkhata Bay | Total No. tested | 135 | 28 | 46 | 0 | 1 | 6 | 5 | 0 | 9 | 23 | 32 | 48 | 333 |
| | No. sporozoites (+ve) | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 16 |
| | Sporozoite rate (%) | **5.9** | **10.7** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **4.3** | **3.1** | **6.3** | **4.8** |
| Nkhotakota | Total No. tested | 65 | 118 | 143 | 79 | 33 | 10 | 30 | 33 | 40 | 72 | 2 | 0 | 625 |
| | No. sporozoites (+ve) | 2 | 2 | 5 | 6 | 6 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 26 |
| | Sporozoite rate (%) | **3.1** | **1.7** | **3.5** | **7.6** | **18.2** | **10** | **0** | **3** | **2.5** | **2.8** | **0** | **0** | **4.2** |
| Salima | Total No. tested | 5 | 0 | 62 | 0 | 1 | 0 | 5 | 110 | 37 | 14 | 9 | 10 | 253 |
| | No. sporozoites (+ve) | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 5 | 2 | 0 | 0 | 0 | 9 |
| | Sporozoite rate (%) | **0** | **0** | **1.6** | **0** | **0** | **0** | **20** | **4.5** | **5.4** | **0** | **0** | **0** | **3.6** |
| Kasungu | Total No. tested | 13 | 16 | 11 | 4 | 1 | 0 | 18 | 111 | 123 | 59 | 3 | 26 | 385 |
| | No. sporozoites (+ve) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 0 | 0 | 6 |
| | Sporozoite rate (%) | **7.7** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **3.3** | **1.7** | **0** | **0** | **1.6** |
| Chikwawa | Total No. tested | 0 | 34 | 36 | 37 | 5 | 1 | 94 | 47 | 43 | 19 | 51 | 0 | 367 |
| | No. sporozoites (+ve) | 0 | 3 | 2 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 9 |
| | Sporozoite rate (%) | **0** | **8.8** | **5.6** | **2.7** | **0** | **0** | **1.1** | **0** | **2.3** | **5.3** | **0** | **0** | **2.5** |
| Total | Total No. tested | 218 | 196 | 300 | 122 | 41 | 17 | 152 | 302 | 252 | 187 | 97 | 84 | 1968 |
| | No. sporozoites (+ve) | 11 | 8 | 8 | 7 | 6 | 1 | 2 | 6 | 8 | 5 | 1 | 3 | 66 |
| | Sporozoite rate (%) | **5.0** | **4.1** | **2.7** | **5.7** | **14.6** | **5.9** | **1.3** | **2.0** | **3.2** | **2.7** | **1.0** | **3.6** | **3.4** |

# ANNEX B: TABLE B1: SRS AND ANNUAL EIRS OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. ESTIMATED FROM CDC-LT COLLECTIONS IN THE SIX DISTRICTS

| Species | Indicators | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mwakanyamale, Karonga** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 3 | 4 | 4 | 0 | 1 | 10 | 1 | 73 | 75 | 0 | 2 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 2 | 3 | 4 | 0 | 0 | 1 | 54 | 37 | 91 | 85 | 1 | 3 | |
| | HBR/night | 0.2 | 0.3 | 0.4 | 0 | 0 | 0.1 | 5.4 | 3.7 | 9.1 | 8.5 | 0.1 | 0.3 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.11 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.4 | 0 | 0 | 3.40 |
| *An. funestus* s.l. | Total No. tested | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Total collected by CDC-LTs | 0 | 0 | 0 | 2 | 0 | 0 | 18 | 7 | 0 | 0 | 0 | 1 | |
| | HBR/night | 0 | 0 | 0 | 0.2 | 0 | 0 | 1.8 | 0.7 | 0 | 0 | 0 | 0.1 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| | Total Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.4 | 0 | 0 | 3.40 |
| **Mwenimambwe, Karonga** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 103 | 190 | 11 | 0 | 0 | 32 | 59 | 158 | 2 | 19 | 0 | |
| | No. sporozoite positive | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 6 | 336 | 386 | 11 | 0 | 0 | 35 | 100 | 265 | 2 | 23 | 4 | |
| | HBR/night | 0.6 | 33.6 | 38.6 | 1.1 | 0 | 0 | 3.5 | 10 | 26.5 | 0.2 | 2.3 | 0.4 | |
| | Nightly EIR | 0.0 | 0.33 | 0.41 | 0.00 | 0.00 | 0.00 | 0.00 | 0.17 | 0.34 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 10.11 | 12.2 | 0 | 0 | 0 | 0 | 4.746 | 10.399 | 0 | 0 | 0 | 37.45 |

| Species | Indicators | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *An. funestus* s.l. | Total No. tested | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 0 | 97 | 1 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | |
| | HBR/night | 0 | 9.7 | 0.1 | 0 | 0 | 0 | 0 | 0.7 | 0 | 0 | 0 | 0 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| | Total Monthly EIR | 0 | 10.11 | 12.2 | 0 | 0 | 0 | 0 | 4.746 | 10.399 | 0 | 0 | 0 | 37.45 |
| **Kande, Nkhata Bay** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 22 | 3 | 0 | 1 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 27 | 3 | 0 | 1 | |
| | HBR/night | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4 | 2.7 | 0.3 | 0 | 0.1 | |
| | Nightly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| *An. funestus* s.l. | Total No. tested | 24 | 5 | 0 | 0 | 1 | | 3 | 0 | 2 | 2 | 9 | 0 | |
| | No. sporozoite positive | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.04 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 27 | 6 | 6 | | 1 | 0 | 6 | 0 | 2 | 2 | 12 | 2 | |
| | HBR/night | 2.7 | 0.6 | 0.6 | 0 | 0.1 | 0 | 0.6 | 0 | 0.2 | 0.2 | 1.2 | 0.2 | |
| | Nightly EIR | 0.11 | 0.24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 3.49 | 7.44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10.93 |
| | Total Monthly EIR | 3.49 | 7.44 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 10.93 |
| **Sanga, Nkhata Bay** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 16 | 11 | 0 | 1 | 2 | 11 | 32 | 105 | 17 | 3 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.33 | 0.00 | |
| | Total collected by CDC-LTs | 1 | 16 | 3 | 0 | 1 | 1 | 12 | 51 | 75 | 27 | 6 | 0 | |
| | HBR/night | 0.1 | 1.6 | 0.3 | 0 | 0.1 | 0.1 | 1.2 | 5.1 | 7.5 | 2.7 | 0.6 | 0 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.2 | 0 | 6.20 |
| *An. funestus* s.l. | Total No. tested | 111 | 26 | 50 | 0 | 0 | 6 | 2 | 0 | 8 | 21 | 23 | 48 | |
| | No. sporozoite positive | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | |
| | Sporozoite rate | 0.06 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.04 | 0.06 | |
| | Total collected by CDC-LTs | 151 | 33 | 45 | 0 | 0 | 0 | 2 | | 24 | 45 | 38 | 174 | |
| | HBR/night | 15.1 | 3.3 | 4.5 | 0 | 0 | 0 | 0.2 | 0 | 2.4 | 4.5 | 3.8 | 17.4 | |
| | Nightly EIR | 0.95 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.21 | 0.17 | 1.09 | |

| Species | Indicators | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Monthly EIR | 29.52 | 3.935 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.429 | 5.12 | 32.63 | 77.63 |
| | Total Monthly EIR | 29.52 | 3.93 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.43 | 11.32 | 32.63 | 83.83 |
| **Chimkwende, Nkhotakota** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 24 | 24 | 12 | 1 | 10 | 67 | 18 | 39 | 13 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 2 | 3 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.08 | 0.25 | 0.00 | 0.10 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 5 | 34 | 0 | 13 | 1 | 18 | 52 | 101 | 84 | 24 | 8 | 261 | |
| | HBR/night | 0.5 | 3.4 | 0 | 1.3 | 0.1 | 1.8 | 5.2 | 10.1 | 8.4 | 2.4 | 0.8 | 26.1 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.33 | 0.00 | 0.18 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 10.08 | 0 | 5.58 | 4.81 | 0 | 0 | 0 | 0 | 0 | 20.47 |
| *An. funestus* s.l. | Total No. tested | 1 | 21 | 19 | 32 | 15 | 8 | 14 | 8 | 12 | 40 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 1 | 4 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.05 | 0.13 | 0.13 | 0.00 | 0.00 | 0.00 | 0.08 | 0.03 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 9 | 60 | | 43 | 16 | 9 | 28 | 29 | 16 | 66 | 392 | 6 | |
| | HBR/night | 0.9 | 6 | 0 | 4.3 | 1.6 | 0.9 | 2.8 | 2.9 | 1.6 | 6.6 | 39.2 | 0.6 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.54 | 0.21 | 0.00 | 0.00 | 0.00 | 0.13 | 0.17 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 16.66 | 6.4 | 0 | 0 | 0 | 4.1333 | 4.95 | 0 | 0 | 32.15 |
| | Total Monthly EIR | 0.00 | 0.00 | 0.00 | 26.74 | 6.40 | 5.58 | 4.81 | 0.00 | 4.13 | 4.95 | 0.00 | 0.00 | 52.61 |
| **Ngalauka, Nkhotakota** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 42 | 86 | 14 | 1 | 27 | 0 | 57 | 310 | 9 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 6 | 120 | | 10 | 2 | 26 | 14 | 137 | 87 | 11 | 13 | 13 | |
| | HBR/night | 0.6 | 12 | 0 | 1 | 0.2 | 2.6 | 1.4 | 13.7 | 8.7 | 1.1 | 1.3 | 1.3 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.24 | 0.08 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.73 | 2.61 | 0 | 0 | 0 | 9.34 |
| *An. funestus* s.l. | Total No. tested | 1 | 57 | 76 | 17 | 0 | 0 | 0 | 25 | 42 | 32 | 0 | 0 | |
| | No. sporozoite positive | 0 | 2 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.04 | 0.04 | 0.06 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.03 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 117 | 252 | 0 | 25 | 1 | 0 | 5 | 49 | 141 | 55 | 729 | 214 | |
| | HBR/night | 11.7 | 25.2 | 0 | 2.5 | 0.1 | 0 | 0.5 | 4.9 | 14.1 | 5.5 | 72.9 | 21.4 | |
| | Nightly EIR | 0.00 | 0.88 | 0.00 | 0.15 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.17 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 27.41 | 0 | 4.559 | 0 | 0 | 0 | 5.488 | 0 | 5.156 | 0 | 0 | 42.61 |
| | Total Monthly EIR | 0.00 | 27.41 | 0.00 | 4.56 | 0.00 | 0.00 | 0.00 | 12.22 | 2.61 | 5.16 | 0.00 | 0.00 | 51.95 |

| Species | Indicators | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Vwawa, Nkhotakota** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 42 | 86 | 14 | 1 | 27 | 0 | 57 | 310 | 9 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.11 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 1 | 40 | 7 | 7 | 14 | 12 | 60 | 91 | 89 | 99 | 55 | 30 | |
| | HBR/night | 0.1 | 4 | 0.7 | 0.7 | 1.4 | 1.2 | 6 | 9.1 | 8.9 | 9.9 | 5.5 | 3 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 1.10 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 3.1 | 0 | 0 | 0 | 0 | 0.89 | 33 | 0 | 0 | 36.99 |
| *An. funestus* s.l. | No. sporozoite positive | 62 | 40 | 142 | 30 | 18 | 3 | 16 | 16 | 13 | 0 | 2 | 0 | |
| | No. sporozoites | 1 | 0 | 4 | 1 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.02 | 0.00 | 0.03 | 0.03 | 0.22 | 0.33 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 106 | 194 | 45 | 82 | 84 | 3 | 17 | 22 | 19 | | 342 | 131 | |
| | HBR/night | 10.6 | 19.4 | 4.5 | 8.2 | 8.4 | 0.3 | 1.7 | 2.2 | 1.9 | 0 | 34.2 | 13.1 | |
| | Nightly EIR | 0.17 | 0.00 | 0.13 | 0.27 | 1.87 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 5.3 | 0 | 3.8 | 8.473 | 56 | 3.1 | 0 | 0 | 0 | 0 | 0 | 0 | 76.68 |
| | Total Monthly EIR | 5.30 | 0.00 | 3.80 | 11.57 | 56.00 | 3.10 | 0.00 | 0.00 | 0.89 | 33.00 | 0.00 | 0.00 | 113.67 |
| **Chilungo, Salima** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 1 | 29 | 0 | 0 | 0 | 21 | 78 | 20 | 5 | 20 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.20 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 1 | 2 | 10 | 0 | 0 | 0 | 19 | 82 | 6 | 7 | 24 | 0 | |
| | HBR/night | 0.1 | 0.2 | 1 | 0 | 0 | 0 | 1.9 | 8.2 | 0.6 | 0.7 | 2.4 | 0 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.28 | 0.12 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 91.84 | 3.72 | 0 | 0 | 0 | 95.56 |
| *An. funestus* s.l. | Total No. tested | 2 | 0 | 59 | 0 | 0 | 0 | 6 | 21 | 61 | 11 | 8 | 0 | |
| | No. sporozoite positive | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | 5 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 2 | 2 | 3 | 0 | 0 | 0 | 5 | 25 | 30 | 11 | 9 | 0 | |
| | HBR/night | 0.2 | 0.2 | 0.3 | 0 | 0 | 0 | 0.5 | 2.5 | 3 | 1.1 | 0.9 | 0 | |
| | Nightly EIR | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0.15 | 0 | 0 | 0 | 2.58 | 0 | 0 | 0 | 0 | 0 | 2.74 |
| | Total Monthly EIR | 0.00 | 0.00 | 0.15 | 0.00 | 0.00 | 0.00 | 2.58 | 91.84 | 3.72 | 0.00 | 0.00 | 0.00 | 98.30 |

| Species | Indicators | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cholokoto, Salima** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 0 | 4 | 0 | 1 | 0 | 1 | 48 | 18 | 3 | 10 | 20 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 50 | 24 | 4 | 11 | 0 | |
| | HBR/night | 0 | 0.1 | 0.2 | 0 | 0.1 | 0 | 0 | 5 | 2.4 | 0.4 | 1.1 | 0 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.92 | 0 | 0 | 0 | 0 | 2.92 |
| *An. funestus* s.l. | Total No. tested | 3 | 0 | 9 | 0 | 1 | 0 | 0 | 89 | 12 | 3 | 1 | 10 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 19 | 0 | 1 | 0 | 1 | 0 | 0 | 97 | 19 | 4 | 1 | 16 | |
| | HBR/night | 1.9 | 0 | 0.1 | 0 | 0.1 | 0 | 0 | 9.7 | 1.9 | 0.4 | 0.1 | 1.6 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.33 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9.16 | 0 | 0 | 0 | 0 | 9.16 |
| | Total Monthly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 12.07 | 0.00 | 0.00 | 0.00 | 0.00 | 12.07 |
| **Kachokolo, Kasungu** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 0 | 1 | 1 | 1 | 37 | 23 | 145 | 133 | 24 | 0 | 1 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 0 | 0 | 0 | 1 | 0 | 0 | 28 | 175 | 162 | 26 | 82 | 0 | |
| | HBR/night | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 2.8 | 17.5 | 16.2 | 2.6 | 8.2 | 0.0 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.11 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | 0 | 3.25 | 0 | 0 | 3.25 |
| *An. funestus* s.l. | Total No. tested | 4 | 12 | 12 | 0 | 5 | 15 | 7 | 111 | 129 | 55 | 0 | 20 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 4 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.03 | 0.02 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 4 | 12 | 13 | 0 | 1 | 0 | 8 | 127 | 174 | 80 | 0 | 30 | |
| | HBR/night | 0.4 | 1.2 | 1.3 | 0 | 0.1 | 0 | 0.8 | 12.7 | 17.4 | 8 | 0 | 3 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.54 | 0.15 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0.6 | 0 | 0 | 0.00 | 16.726 | 4.364 | 0 | 0 | 21.69 |
| | Total Monthly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.60 | 0.00 | 0.00 | 0.00 | 16.73 | 7.61 | 0.00 | 0.00 | 24.94 |

| Species | Indicators | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nyalubwe, Kasungu** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 0 | 9 | 8 | 0 | 0 | 15 | 1 | 10 | 14 | 23 | 1 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 0 | 0 | 0 | 1 | 0 | 0 | 28 | 175 | 162 | 26 | 82 | 0 | |
| | HBR/night | 0 | 0 | 0 | 0.1 | 0 | 0 | 2.8 | 17.5 | 16.2 | 2.6 | 8.2 | 0 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.37 | 0.00 | 0.00 | |
| | Monthly EIR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | 0 | 11.14 | 0 | 0 | 11.14 |
| *An. funestus* s.l. | Total No. tested | 9 | 4 | 0 | 4 | 1 | 0 | 11 | 0 | 4 | 4 | 3 | 6 | |
| | No. sporozoite positive | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.25 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 24 | 4 | 0 | 4 | 0 | 0 | 11 | 5 | 8 | 4 | 4 | 9 | |
| | HBR/night | 2.4 | 0.4 | 0 | 0.4 | 0 | 0 | 1.1 | 0.5 | 0.8 | 0.4 | 0.4 | 0.9 | |
| | Nightly EIR | 0.27 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 8.27 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | 6.2 | 0 | 0 | 0 | 14.47 |
| | Total Monthly EIR | 8.27 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.20 | 11.14 | 0.00 | 0.00 | 25.61 |
| **Nyampota, Chikwawa** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 0 | 12 | 0 | 27 | 9 | 9 | 4 | 8 | 8 | 0 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 13 | 12 | 0 | 0 | 2 | 11 | 4 | 13 | 11 | 0 | 0 | 2 | |
| | HBR/night | 1.3 | 1.2 | 0 | 0 | 0.2 | 1.1 | 0.4 | 1.3 | 1.1 | 0 | 0 | 0.2 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | 0 | 0 | 0 | 0 | 0.00 |
| *An. funestus* s.l. | Total No. tested | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 5 | 2 | 0 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 10 | 3 | 0 | 0 | 0 | |
| | HBR/night | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.2 | 1 | 0.3 | 0 | 0 | 0 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | Monthly EIR | 0.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | 0 | 0 | 0 | 0 | 0.00 |
| | Total Monthly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| Species | Indicators | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Total Annual EIR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ntwana, Chikwawa** | | | | | | | | | | | | | | |
| *An. gambiae* s.l. | Total No. tested | 1 | 1 | 11 | 4 | 3 | 22 | 33 | 27 | 13 | 18 | 0 | 0 | |
| | No. sporozoite positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 11 | 48 | 0 | 4 | 3 | 25 | 61 | 36 | 15 | 26 | 1 | 0 | |
| | HBR/night | 1.1 | 4.8 | 0 | 0.4 | 0.3 | 2.5 | 6.1 | 3.6 | 1.5 | 2.6 | 0.1 | 0 | |
| | Nightly EIR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 | 0.00 | |
| | Monthly EIR | 0.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | 0 | 4.333 | 0 | 0 | 4.33 |
| *An. funestus* s.l. | Total No. tested | 0 | 34 | 55 | 43 | 5 | 1 | 92 | 42 | 41 | 19 | 51 | 0 | |
| | No. sporozoite positive | 0 | 3 | 3 | 3 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | |
| | Sporozoite rate | 0.00 | 0.09 | 0.05 | 0.07 | 0.00 | 0.00 | 0.01 | 0.00 | 0.02 | 0.05 | 0.00 | 0.00 | |
| | Total collected by CDC-LTs | 140 | 266 | 10 | 37 | 5 | 1 | 127 | 67 | 64 | 34 | 74 | 0 | |
| | HBR/night | 14 | 26.6 | 1 | 3.7 | 0.5 | 0.1 | 12.7 | 6.7 | 6.4 | 3.4 | 7.4 | 0 | |
| | Nightly EIR | 0.00 | 2.35 | 0.05 | 0.26 | 0.00 | 0.00 | 0.14 | 0.00 | 0.16 | 0.18 | 0.00 | 0.00 | |
| | Monthly EIR | 0.00 | 72.76 | 1.64 | 8.002 | 0 | 0 | 4.28 | 0.00 | 4.839 | 5.368 | 0 | 0 | 96.88 |
| | Total Monthly EIR | 0.00 | 72.76 | 1.64 | 8.00 | 0.00 | 0.00 | 4.28 | 0.00 | 4.84 | 9.70 | 0.00 | 0.00 | 101.22 |

# ANNEX C: HBRS AND PARITY OF *ANOPHELES* MOSQUITOES

Annex C1: HBRs of *An. funestus* s.l. Collected by HLC, July 2020–June 2021

| District | Month | Indoor | Outdoor | # of Collectors | # of Nights | # of Houses | INDOOR HBR (b/p/n) | OUTDOOR HBR (b/p/n) | Bites/Person/ Night |
|---|---|---|---|---|---|---|---|---|---|
| **Kasungu** | September | 1 | 0 | 1 | 1 | 6 | 0.17 | 0.00 | 0.08 |
| | December | 11 | 4 | 1 | 1 | 6 | 1.83 | 0.67 | 1.25 |
| | March | 12 | 17 | 1 | 1 | 6 | 2.00 | 2.83 | 2.42 |
| | June | 58 | 8 | 1 | 1 | 6 | 9.67 | 1.33 | 5.50 |
| **Average** | | | | | | | **3.42** | **1.21** | **2.31** |
| **Nkhata Bay** | September | 0 | 1 | 1 | 1 | 6 | 0.00 | 0.17 | 0.08 |
| | December | 0 | 0 | 1 | 1 | 6 | 0.00 | 0.00 | 0.00 |
| | March | 1 | 2 | 1 | 1 | 6 | 0.17 | 0.33 | 0.25 |
| | June | 29 | 26 | 1 | 1 | 6 | 4.83 | 4.33 | 4.58 |
| **Average** | | | | | | | **1.25** | **1.21** | **1.23** |
| **Nkhotakota** | September | 19 | 6 | 1 | 1 | 12 | 1.58 | 0.50 | 1.04 |
| | December | 1 | 0 | 1 | 1 | 12 | 0.08 | 0.00 | 0.04 |
| | March | 30 | 8 | 1 | 1 | 12 | 2.50 | 0.67 | 1.58 |
| | June | 180 | 93 | 1 | 1 | 12 | 15.00 | 7.75 | 11.38 |
| **Average** | | | | | | | **4.79** | **2.23** | **3.51** |
| **Salima** | September | 1 | 5 | 1 | 1 | 6 | 0.17 | 0.83 | 0.50 |
| | December | 0 | 0 | 1 | 1 | 6 | 0.00 | 0.00 | 0.00 |
| | March | 26 | 32 | 1 | 1 | 6 | 4.33 | 5.33 | 4.83 |
| | June | 98 | 16 | 1 | 1 | 6 | 16.33 | 2.67 | 9.50 |
| **Average** | | | | | | | **5.21** | **2.21** | **3.71** |
| **Overall** | | | | | | | **3.67** | **1.71** | |

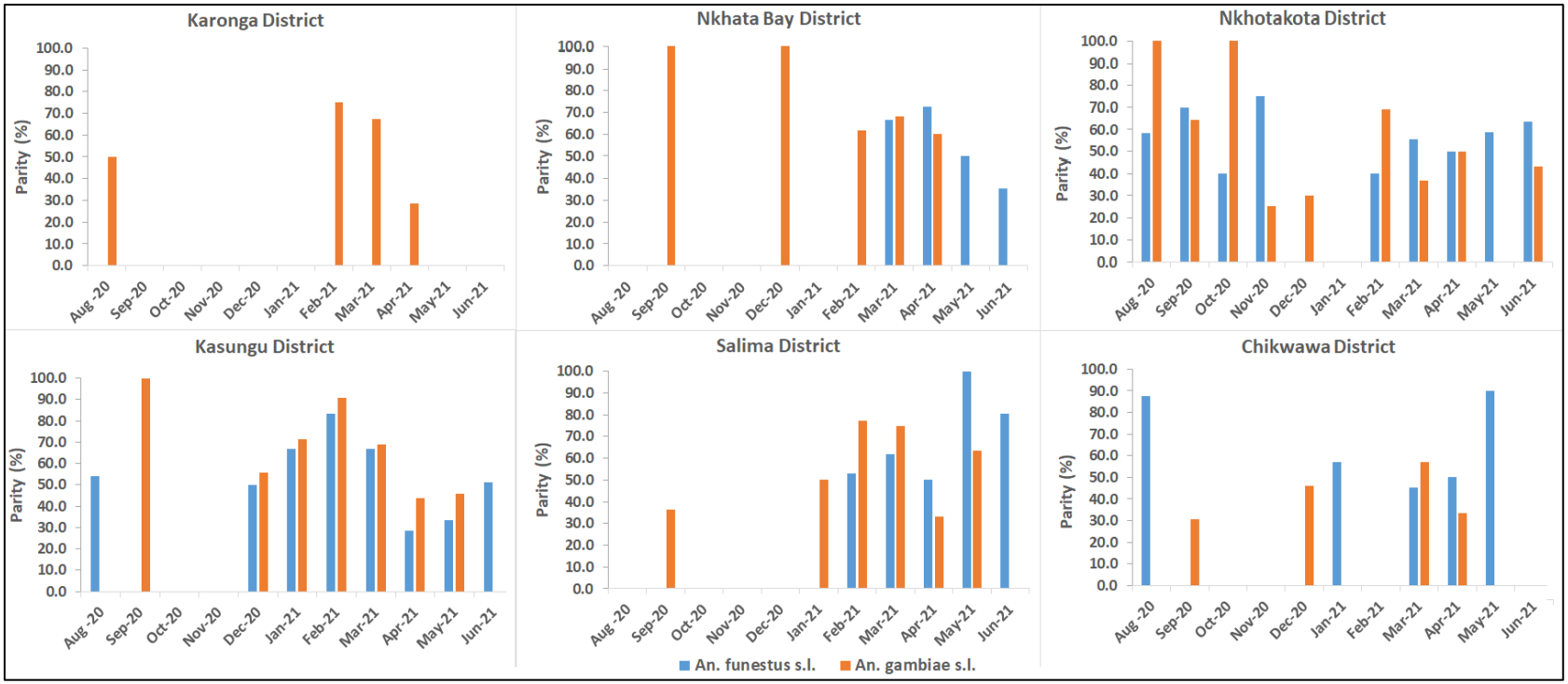
*Note: Nkhotakota had two sites*

## Annex C2: HBRs of *An. gambiae* s.l. Collected by HLC, July 2020–July 2021

| Site | Month | Indoor | Outdoor | # of Collectors | # of Nights | # of Houses | INDOOR HBR (b/p/n) | OUTDOOR HBR (b/p/n) | Bites/Person/ Night |
|---|---|---|---|---|---|---|---|---|---|
| **Kasungu** | September | 0 | 0 | 1 | 1 | 6 | 0.00 | 0.00 | 0.00 |
| | December | 7 | 30 | 1 | 1 | 6 | 1.17 | 5.00 | 3.08 |
| | March | 70 | 105 | 1 | 1 | 6 | 11.67 | 17.50 | 14.58 |
| | June | 2 | 0 | 1 | 1 | 6 | 0.33 | 0.00 | 0.17 |
| **Average** | | | | | | | **3.29** | **5.63** | **4.46** |
| **Nkhata Bay** | September | 4 | 3 | 1 | 1 | 6 | 0.67 | 0.50 | 0.58 |
| | December | 1 | 0 | 1 | 1 | 6 | 0.17 | 0.00 | 0.08 |
| | March | 46 | 61 | 1 | 1 | 6 | 7.67 | 10.17 | 8.92 |
| | June | | | 1 | 1 | 6 | 0.00 | 0.00 | 0.00 |
| **Average** | | | | | | | **2.13** | **2.67** | **2.40** |
| **Nkhotakota** | September | 23 | 37 | 1 | 1 | 12 | 1.92 | 3.08 | 2.50 |
| | December | 4 | 9 | 1 | 1 | 12 | 0.33 | 0.75 | 0.54 |
| | March | 140 | 193 | 1 | 1 | 12 | 11.67 | 16.08 | 13.88 |
| | June | 1 | 18 | 1 | 1 | 12 | 0.08 | 1.50 | 0.79 |
| **Average** | | | | | | | **3.50** | **5.35** | **4.43** |
| **Salima** | September | 8 | 16 | 1 | 1 | 6 | 1.33 | 2.67 | 2.00 |
| | December | 0 | 0 | 1 | 1 | 6 | 0.00 | 0.00 | 0.00 |
| | March | 27 | 15 | 1 | 1 | 6 | 4.50 | 2.50 | 3.50 |
| | June | 0 | 0 | 1 | 1 | 6 | 0.00 | 0.00 | 0.00 |
| **Average** | | | | | | | **1.46** | **1.29** | **1.38** |
| **Overall** | | | | | | | **2.59** | **3.73** | |

*Note: Nkhotakota had two sites*

## Annex C3: Proportion of Parous Female *An. funestus* s.l. and *An. gambiae* s.l. from the Six Districts



# ANNEX D: *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. RESPONSE TO DIFFERENT INSECTICIDES

Annex D1: *An. funestus* s.l. and *An. gambiae s.l.* Response to Different Insecticides in Lisuli-Oxbow, Chakanira and Ntwana, Chikwawa District

| District | Site | Insecticide | Species | Source | No Tested | No Dead | %Mortality | Time at Final Mortality |
|---|---|---|---|---|---|---|---|---|
| Chikwawa | Lisuli-Oxbow | Deltamethrin 1x | *An. gambiae* s.l. | Larvae | 107 | 84 | 75.7 | 24Hrs |
| | | Permethrin 1x | *An. gambiae* s.l. | Larvae | 114 | 105 | 90.9 | 24Hrs |
| | | Pirimiphos methyl (0.25%) | *An. gambiae* s.l. | Larvae | 113 | 113 | 100 | 24Hrs |
| | | Chlorfenapyr (100µg/bottle) | *An. gambiae* s.l. | Larvae | 102 | 102 | 100 | 24Hrs |
| | | Clothianidin (2%) | *An. gambiae* s.l. | Larvae | 93 | 78 | 100 | 4Days |
| | Chakanira | Deltamethrin 5x | *An. funestus* s.l. | F1 | 105 | 67 | 61.5 | 24Hrs |
| | | Permethrin 5x | *An. funestus* s.l. | F1 | 101 | 61 | 60.3 | 24Hrs |
| | Ntwana | Deltamethrin 1x | *An. funestus* s.l. | F1 | 113 | 40 | 27.9 | 24Hrs |
| | | 4%PBO + Deltamethrin 1x | *An. funestus* s.l. | F1 | 102 | 97 | 94.2 | 24Hrs |
| | | Deltamethrin 5x | *An. funestus* s.l. | F1 | 115 | 32 | 27.8 | 24Hrs |
| | | Deltamethrin 10x | *An. funestus* s.l. | F1 | 80 | 61 | 76.3 | 24Hrs |
| | | Permethrin 1x | *An. funestus* s.l. | F1 | 101 | 20 | 15.2 | 24Hrs |
| | | 4%PBO + Permethrin 1x | *An. funestus* s.l. | F1 | 110 | 109 | 98.9 | 24Hrs |
| | | Permethrin 5x | *An. funestus* s.l. | F1 | 103 | 59 | 57.3 | 24Hrs |
| | | Alpha-cypermethrin 1x | *An. funestus* s.l. | F1 | 124 | 31 | 20.7 | 24Hrs |
| | | 4%PBO+ Alpha-cypermethrin 1x | *An. funestus* s.l. | F1 | 129 | 119 | 91.6 | 24Hrs |
| | | Pirimiphos methyl 1x | *An. funestus* s.l. | F1 | 102 | 102 | 100 | 24Hrs |
| | | Chlorfenapyr (100µg/bottle) | *An. funestus* s.l. | F1 | 100 | 100 | 100 | 24Hrs |
| | | Alpha-cypermethrin 1x | *An. gambiae* s.l. | F1 | 108 | 45 | 41.7 | 24Hrs |
| | | 4%PBO + Deltamethrin 1x | *An. gambiae* s.l. | F1 | 107 | 108 | 99.1 | 24Hrs |
| | | 4%PBO + Permethrin 1x | *An. gambiae* s.l. | F1 | 101 | 90 | 89.1 | 24Hrs |
| | | 4%PBO + Alpha-cypermethrin 1x | *An. gambiae* s.l. | F1 | 74 | 73 | 98.6 | 24Hrs |
| | | Pirimiphos-methyl (0.25%) | *An. gambiae* s.l. | F1 | 75 | 75 | 100 | 24Hrs |

**Key:** Resistant  Suspected resistance  Susceptible

## Annex D2: *An. gambiae* s.l. Response to Different Insecticides in Lifizi, Salima District

| District | Site | Insecticide | Species | Source | No Tested | No Dead | %Mortality | Time at Final Mortality |
|---|---|---|---|---|---|---|---|---|
| Salima | Lifizi | Alpha-cypermethrin 1x | *An. gambiae* s.l. | Larvae | 73 | 52 | 71.2 | 24hrrs |
| | | Pirimiphos-methyl (0.25%) | *An. gambiae* s.l. | Larvae | 57 | 57 | 100 | 24Hrs |
| | Chilungo | Deltamethrin 1x | *An. gambiae* s.l. | Larvae | 102 | 62 | 51.7 | 24Hrs |
| | | 4%PBO + Deltamethrin 1x | *An. gambiae* s.l. | Larvae | 96 | 85 | 88.5 | 24Hrs |
| | | Permethrin 1x | *An. gambiae* s.l. | Larvae | 97 | 55 | 46.7 | 24Hrs |
| | | 4%PBO + Permethrin 1x | *An. gambiae* s.l. | Larvae | 112 | 98 | 86.1 | 24Hrs |
| | | Alpha-cypermethrin 1x | *An. gambiae* s.l. | Larvae | 113 | 18 | 15.9 | 24Hrs |
| | | 4%PBO+ Alpha-cypermethrin 1x | *An. gambiae* s.l. | Larvae | 50 | 44 | 85.5 | 24Hrs |
| | | Chlorfenapyr (100µg/bottle) | *An. gambiae* s.l. | Larvae | 103 | 103 | 100 | 24Hrs |

**Key:** Resistant Suspected resistance Susceptible

## Annex D3: *An. funestus* s.l. Response to Different Insecticides in Kachokolo, Kasungu District

| District | Site | Insecticide | Species | Source | No Tested | No Dead | %Mortality | Time at Final Mortality |
|---|---|---|---|---|---|---|---|---|
| Kasungu | Kachokolo | Deltamethrin 1x | *An. funestus* s.l. | F1 | 93 | 37 | 32.8 | 24Hrs |
| | | 4%PBO + Deltamethrin 1x | *An. funestus* s.l. | F1 | 83 | 82 | 98.6 | 24Hrs |
| | | Permethrin 1x | *An. funestus* s.l. | F1 | 69 | 25 | 28.1 | 24Hrs |
| | | 4%PBO + Permethrin 1x | *An. funestus* s.l. | F1 | 104 | 103 | 98.8 | 24Hrs |
| | | Pirimiphos-methyl (0.25%) | *An. funestus* s.l. | $F_1$ | 104 | 104 | 100 | 24Hrs |
| | | Chlorfenapyr (100µg/bottle) | *An. funestus* s.l. | $F_1$ | 104 | 102 | 98.0 | 5Days |
| | | Clothianidin (2%) | *An. funestus* s.l. | $F_1$ | 110 | 110 | 100 | 4Days |

**Key:** Resistant Suspected resistance Susceptible

## Annex D4: *An. funestus* s.l. and *An. gambiae* s.l. Response to Different Insecticides in Chimkwende and Vwawa, Nkhotakota District

| District | Site | Insecticide | Species | Source | No. Tested | No. Dead/ No. KD | %Mortality/ %KD | Time at Final Mortality/KD |
|---|---|---|---|---|---|---|---|---|
| Nkhotakota | Chimkwende | Deltamethrin 1x | *An. gambiae* s.l. | F1 | 77 | 28 | 36.4 | 24Hrs |
| | | Permethrin 1x | *An. funestus* s.l. | F1 | 56 | 21 | 37.5 | 24Hrs |
| | | Alpha-cypermethrin 1x | *An. funestus* s.l. | Larvae | 44 | 15 | 34.1 | 24Hrs |
| | | Clothianidin (2%) | *An. funestus* s.l. | F1 | 106 | 106 | 100 | 2days |
| | Vwawa | Deltamethrin 5x | *An. funestus* s.l. | F1 | 45 | 35 | 77.8 | 30Mins |
| | | Pirimiphos-methyl (0.25%) | *An. funestus* s.l. | F1 | 110 | 110 | 100 | 24Hrs |
| | | Chlorfenapyr (100µg/bottle) | *An. funestus* s.l. | F1 | 118 | 118 | 100 | 2Days |

**Key:** Resistant Suspected resistance Susceptible

## AnnexD5: *An. funestus* s.l. Response to Different Insecticides in Kande, Nkhata Bay District

| District | Site | Insecticide | Species | Source | No. Tested | No. KD/Dead | %KD/ %Mortality | Time at Final KD/Mortality |
|---|---|---|---|---|---|---|---|---|
| Nkhata Bay | Mazembe | Deltamethrin 1x | *An. funestus* s.l. | F1 | 105 | 13 | 12.4 | 24Hrs |
| | | 4%PBO+ Deltamethrin 1x | *An. funestus* s.l. | F1 | 124 | 121 | 97.5 | 24Hrs |
| | | Permethrin 1x | *An. funestus* s.l. | F1 | 119 | 5 | 4.2 | 24Hrs |
| | | 4%PBO + Permethrin 1x | *An. funestus* s.l. | F1 | 109 | 107 | 98.0 | 24Hrs |
| | | 4%PBO + Alpha-cypermethrin 1x | *An. funestus* s.l. | F1 | 103 | 90 | 87.4 | 24Hrs |
| | | Pirimiphos-methyl (0.25%) | *An. funestus* s.l. | F1 | 103 | 103 | 100 | 24Hrs |
| | | Clothianidin (2%) | *An. funestus* s.l. | F1 | 90 | 90 | 100 | 24Hrs |
| | | Chlorfenapyr (100µg/bottle) | *An. funestus* s.l. | F1 | 99 | 99 | 100 | 24Hrs |
| | | Deltamethrin 5x | *An. funestus* s.l. | F1 | 128 | 51 | 39.8 | 30Mins |
| | | Permethrin 5x | *An. funestus* s.l. | F1 | 98 | 70 | 71.4 | 30Mins |

**Key:** Resistant Suspected resistance Susceptible

## Annex D6: *An. gambiae* s.l. Response to Different Insecticides in Mwenimambwe, Karonga District

| District | Site | Insecticide | Species | Source | No. Tested | No. Dead | %Mortality | Time at Final Mortality |
|---|---|---|---|---|---|---|---|---|
| Karonga | Mwenimambwe | Deltamethrin 1x | *An. gambiae* s.l. | Larvae | 57 | 54 | 94.7 | 24Hrs |
| | | Permethrin 1x | *An. gambiae* s.l. | Larvae | 37 | 24 | 64.9 | 24Hrs |
| | | Permethrin 5x | *An. gambiae* s.l. | $F_1$ | 100 | 86 | 86 | 24Hrs |
| | | Alpha-cypermethrin 1x | *An. gambiae* s.l. | $F_1$ | 99 | 64 | 64.6 | 24Hrs |
| | | Pirimiphos-methyl (0.25%) | *An. gambiae* s.l. | $F_1$ | 100 | 100 | 100 | 24Hrs |
| | | Clothianidin (2%) | *An. gambiae* s.l. | $F_1$ | 101 | 101 | 100 | 24Hrs |
| | | Chlorfenapyr (100µg/bottle) | *An. gambiae* s.l. | F1 | 102 | 102 | 100 | 3 days |

**Key:** Resistant Suspected resistance Susceptible