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PMI VECTORLINK MALAWI ANNUAL ENTOMOLOGICAL MONITORING REPORT

JULY 1, 2019 – JUNE 2020

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ACRONYMS

Ace-1	Acetylcholinesterase 1
b/p/n	bites/person/night
CDC	Centers for Disease Control and Prevention
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
F_0	Filial generation 0
F_1	Filial generation 1
HBR	Human Biting Rate
HLC	Human Landing Catch
ib/p/m	infective bites/person/month
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
kdr	*Knockdown Resistance*
LT	Light Trap
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
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

Malaria is the main cause of mortality and morbidity in Malawi, mainly affecting children under the age of 5 and pregnant women. Long-lasting insecticidal nets have been widely used as a major vector control intervention in Malawi, with indoor residual spraying (IRS) used as a complementary control measure in selected areas.

The U.S. President's Malaria Initiative (PMI) VectorLink Project in Malawi in collaboration with the Malaria Alert Centre conducted longitudinal monitoring from July 2019 to March 2020 in 15 sentinel sites in seven districts to assess malaria vector bionomics and susceptibility of the principal malaria vectors to insecticides in use for public health.

Vector Bionomics: Across all 15 sentinel sites, 29,356 male and female *Anopheles* mosquitoes were collected. Of these, 15,705 were collected using PSCs (53.5%), 12,409 using CDC-LTs (42.3%), and 1,242 (4.2%) using HLCs. *An. gambiae* s.l. was the predominant species, accounting for 62.8% (n=18,444) of all *Anopheles* mosquitoes collected. *An. funestus* s.l. (34.0%, n=9,994) was the second most common species, followed by *An. coustani* (3.1%, n=918). The highest numbers of *An. funestus* s.l. were collected in Chikwawa District using Centers for Disease Control and Prevention (CDC) light traps (LTs) (42 mosquitoes/trap/night), whereas the highest *An. gambiae* s.l. density was found in Karonga District, where the mosquitoes were collected by pyrethrum spray catches (PSCs) (336 mosquitoes/house/day). A total 2,324 *An. gambiae* s.l. were identified to the species-specific level by polymerase chain reaction (PCR); 99.1% were identified as *An. arabiensis* and 0.9% as *An. gambiae* s.s. A total of 711 *An. funestus* s.l. were also identified to the species- specific level by PCR; all were *An. funestus* s.s. *Anopheles funestus* s.s. and *An. arabiensis* are the major malaria vectors in all seven monitoring districts; *An. gambiae* s.s. has limited distribution.

Human Biting Rate (HBR) and Location: The overall indoor HBR for *An. funestus* s.l. was 1.2 bites/person/night (b/p/n) and outdoor HBR was 0.6 b/p/n in the five districts. The indoor HBR for *An. gambiae* s.l. was 1.7 b/p/n and outdoor was 2.2 b/p/n. The HBR for *An. coustani* were 0.7 b/p/n indoors and 1.9 b/p/n outdoors. *An. funestus* s.l. preferred biting indoors while *An. gambiae* s.l. and *An. coustani* largely fed outdoors. The highest biting activity of *An. funestus* s.l. was observed in Kasungu District in March; 10.1 b/p/n indoor and 5.8 b/p/n outdoor. The highest indoor biting activity of *An. gambiae* s.l. was observed in Nkhata Bay (5.5 b/p/n) and outdoor in Salima (5.2 b/p/n) both in March. The highest *An. coustani* indoor biting activity occurred in Nkhotakota (4.7 b/p/n) and outdoor in Kasungu (10.9 b/p/n) both in March. *An. funestus* s.l. and *An. gambiae* s.l. exhibited morning / day-time biting in Nkhata Bay, Kasungu, and Nkhotakota districts. The biting activity of both *An. gambiae* s.l. and *An. funestus* s.l. occurred from dawn to dusk with big or smaller peaks before and after midnight in all five districts.

Blood Meal Source: *An. gambiae* s.l. had fed predominantly on humans (97.4%) while a smaller proportion had fed on cattle (2.6%) in the seven districts. Similarly, 88.7% of *An. funestus* s.l. had fed on humans, while 11.3% had fed on cattle.

Resistance Mechanism No Acetylcholinesterase 1 (*Ace-1*) allele was detected in *An. gambiae* s.l. tested (n=161).

Parity Rate: The overall mean parity rates of *An. funestus* s.l. and *An. gambiae* s.l. were 81.9% and 69.7%, respectively, in the five districts of Nkhata Bay, Nkhotakota (IRS), Salima, Kasungu and Mangochi (IRS). In IRS districts, no clear trends of parous *An. gambiae* s.l. and *An. funestus* s.l. were observed before and after spraying.

In non-IRS districts, higher proportions of parous *An. gambiae* s.l.were collected during the dry season than the rainy seasons. No clear trend was observed on *An. funestus* s.l.

Infection Rate: A total of 5,290 female *Anopheles* mosquitoes were tested for *Plasmodium falciparum* (*Pf*) infection; 789 had been collected by human landing catches (HLCs), 1,718 by pyrethrum spray catches (PSCs), and 3, 283 by CDC-LTs. The overall *Pf* infection rate of *An. funestus* s.l. was 1.88% Fand of *An. gambiae* s.l. 1.19%. No *Pf* infection was detected in the *An. coustani* (n=520*)* that had been collected by HLCs or PSCs.

The estimated risk of malaria transmission for the 9 months (sum of monthly EIR for 9 months) was highest in Nkhotakota district, 165 infective bites/person/9 months (ib/p/9m) (*An. funestus* s.l. = 139 ib/p/9m) and *An. gambaie* s.l. = 26 ib/p/9m). However, the highest EIR in this district were recorded before spraying in July (17 ib/p/m), August (86 ib/p/m) and September (25 ib/p/m). The lowest EIR over the same period was recorded in Nkhata-Bay district 6.8 ib/p/9m (2.3 ib/p/m from*An. gambaie* s.l. and 5.3 ib/p/9m from *An. funestus* s.l.)).

Among the IRS districts, the estimated risk of malaria transmission over the 9 months period was higher in Nkhotakota (165 ib/p/9m) than in Mangochi district (21 ib/p/6m). In both IRS districts, *An. funestus* s.l. monthly EIRs were higher before spraying than after spraying. However, in these districts, higher monthly EIRs from *An. gambiae* s.l. were recorded after spraying than before spraying.

In non-IRS districts, there was variation in the monthly EIRs of *Anopheles* mosquitoes. Overall, the highest estimated risk of malaria transmission over the 9 months period was observed in standard net (SN) distribution districts compared to piperonyl butoxide (PBO) net distribution districts. Two of the three SN distribution districts, Chikwawa (94 ib/p/9m), Kasungu (83 ib/p/9m) recorded high EIRs while the EIR in Nkhata-bay was 3.7 ib/p/9m. Both PBO net distribution districts, Karonga (15 ib/p/9m) and Salima (6.8 ib/p/9m) recorded low EIRs. In Karonga (PBO), Nkhata-Bay (SN), Kasungu (SN) and Salima (PBO) higher monthly EIRs from *An. gambiae* s.l. were recorded during the rainy season than the dry season while in Chikwawa (SN), higher EIRs from *An. gambiae* s.l. were recorded in the dry season than rainy season. In SN districts of Kasungu and Chikwawa and Salima (PBO) district, high monthly EIRs from *An. funestus* s.l. were observed both in dry and rainy season while as in Karonga (PBO) district no sporozite infections were detected from this species in either dry or rainy season.

Residual Life of Sprayed Insecticide: Monthly wall cone bioassay results showed that Actellic 300CS had a relatively short residual life of 2–4 months on different wall surfaces in Nkhotakota and Mangochi districts, which is less than the World Health Organization's estimated residual life of 4–8 months. The residual life assessment was halted at month 5 due to restictions related to the COVID-19 pandemic.

Insecticide Resistance: Both *An. funestus* s.l. and *An. gambiae* s.l. are fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. The 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. funestus s.l. (exclusively *An. funestus* s.s.) is the major malaria vector followed by *An. gambiae* s.l. (predominantly *An. arabiensis*) in Malawi. Overall, a higher *Pf* infection rate was recorded in *An. funestus* s.l. (1.88%) than in *An. gambiae* s.l. (1.19%). No *Pf* infection was detected in *An. coustani*. The highest monthly EIR of *An. gambiae* s.l. was recored in Kasungu district in February (38 ib/p/m) during the rainy season. The highest *An. funestus* s.l. monthly EIR was recorded in Nkhotakota district (86 ib/p/m) in August before spraying and this was considerably reduced to 22 ib/p/m in January after spraying. In all other disticts, EIR was higher in January and February than August and September. The biting activity of both *An. gambiae* s.l. and *An. funestus* s.l. occurred from dawn to dusk exhibiting either low or higher peaks before and after midnight in all five districts. *An. funestus* s.l. primarily fed indoors while *An. gambiae* s.l. and *An. coustani* largely fed outdoors. *An. gambiae* s.l. and *An. funestus* s.l. predominantly fed on humans and a smaller proportion fed on cattle. *Kdr–e* resistance alleles have been detected in *An. gambiae* s.l. from Kasungu District. No previous reports indicated the existence of *kdr-e* resistance alleles in *Anopheles* mosquitoes in Malawi. The two important malaria vectors are highly resistant to pyrethroids but fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. Hence pirimiphos-methyl and clothianidin and chlofenapyr (once recommended by the World Health Organization for IRS) might be rotated in IRS programs in Malawi. The residual life of Actellic 300CS, which was used in 2019 -2020 season, is only 2–4

months on all the wall surfaces tested. Despite this, IRS had a considerable impact on *Anopheles* mosquito populations in Nkhotakota which remained low for five months before the assessment was halted due to restrictions related to the COVID-19 pandemic. SumiShield 50WG sprayed in one operational site in Nkhotakota District remained effective for 12 months. Overall, a higher estimated risk of malaria transmission over the 9 months period was observed in standard net (SN) distribution districts compared to piperonyl butoxide (PBO) net distribution districts.

I. INTRODUCTION

In September 2017, the U.S. Agency for International Development (USAID) through the U.S. President's Malaria Initiative (PMI) awarded Abt Associates the PMI VectorLink Project, a five-year malaria vector control contract. In October 2019, VectorLink Malawi carried out indoor residual spraying (IRS) in Nkhotakota District in the central region of Malawi. Actellic 300CS, a microencapsulated suspension formulation of pirimiphos-methyl, an organophosphate, and SumiShield 50WG, a clothianidin formulation, were used to spray structures in Nkhotakota District. In the same year, the Malawian government's National Malaria Control Program (NMCP), supported by World Vision International with funding from Global Fund conducted IRS in the southern region's Mangochi District. Only Actellic 300CS was sprayed in Mangochi. Two districts, Karonga and Salima, received piperonyl butoxide (PBO) nets during the mass campaign (2018/19 season) while Nkhata Bay, Kasungu, and Chikwawa received standard nets. No nets were distributed in the two districts where IRS was done (Nkhotakota and Mangochi).

PMI VectorLink Malawi, in collaboration with the Malaria Alert Centre of the College of Medicine at the University of Malawi, conducted entomological monitoring in three sites in Nkhotakota District and two sites in Mangochi District to assess the impact of IRS on entomological indices of malaria transmission. In addition, comprehensive longitudinal entomological monitoring was also conducted in 10 sentinel sites in five districts (Karonga, Nkhata Bay, 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, residual efficacy tests of Actellic 300CS and SumiShield 50WG, and the susceptibility status of malaria vectors to different insecticides across Malawi.

2. METHODOLOGY

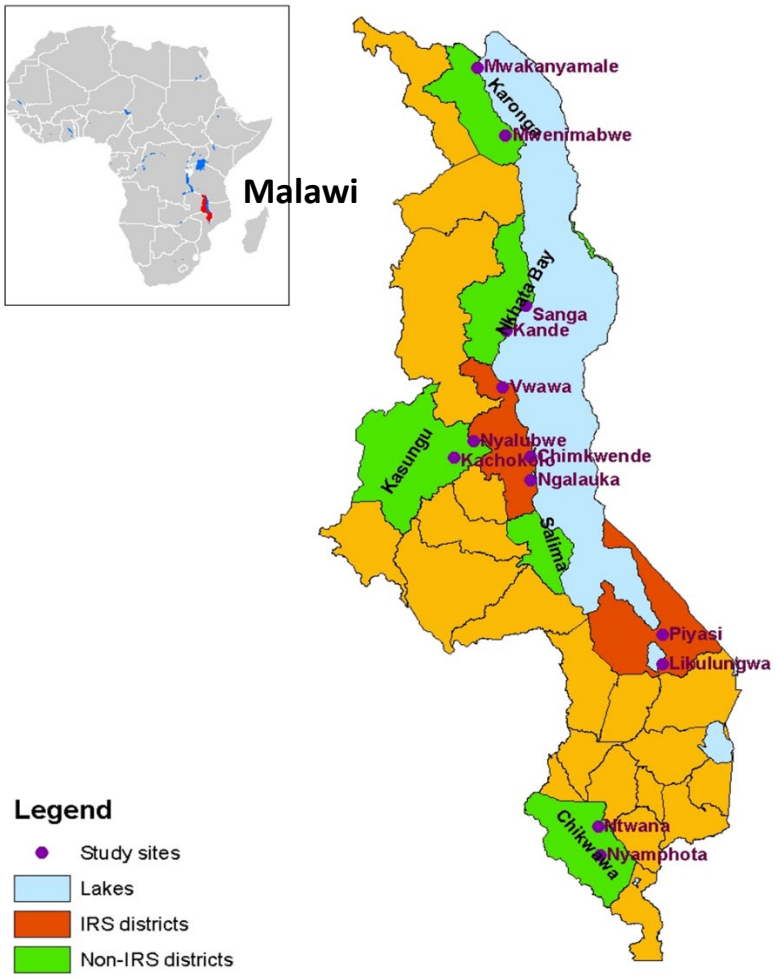
2.1 LONGITUDINAL MONITORING

The team sampled adult mosquitoes from July to December 2019 using pyrethrum spray catches (PSCs) and Centers for Disease Control and Prevention (CDC) miniature light traps (LTs) on a monthly basis in 15 sentinel sites in seven districts, located across Malawi (Figure 1). Sampling at two sites in Mangochi District did not begin until October because the district was incorporated into the monitoring program later. Four sites are located in the northern region of Malawi, seven in the central region, and four in the southern region (Table 1).

TABLE 1: FIFTEEN SENTINEL SITES WHERE ENTOMOLOGICAL MONITORING WAS CONDUCTED IN MALAWI

Region	District	Sentinel site	Latitude and longitude	Intervention
Northern	Karonga	Mwakanyamale	S 9° 47' 1.7"; E 33° 53' 34.36"	PBO nets
		Mwenimambwe	S 10° 20' 24.14"; E 34° 6' 41.62"	
	Nkhata Bay	Kande	S 11° 57' 3.3"; E 34° 7' 1.2"	Standard nets
		Sanga	S 11° 44' 18.58"; E 34° 16' 5.04"	
Central	Salima	Chilungo	S 14° 3' 44.41"; E 34° 31' 42.08"	PBO nets
		Cholokoto	S 13° 46' 20.77"; E 34° 35' 57.51"	
	Nkhotakota	Vwawa	S 12° 24' 54.4"; E 34° 5' 16.44"	IRS (Sites sprayed with Actellic and SumiShield)
		Chimkwende	S 12° 59' 3.49"; E 34° 18' 13.15"	
		Ngalauka	S 13° 10' 38.52"; E 34° 18' 12.84"	
	Kasungu	Kachokolo	S 12° 59' 35.09"; E 33° 43' 4.19"	Standard nets
		Nyalubwe	S 12° 51' 26.44"; E 33° 51' 57.79"	
Southern	Mangochi	Likulungwa	S 14° 41' 16.04"; E 35° 18' 54.53"	IRS (Sites sprayed with Actellic)
		Piyasi	S 14° 26' 56.39"; E 35° 19' 4.09"	
	Chikwawa	Nyamphota	S 16° 15' 31.71"; E 34° 50' 17"	Standard nets
		Ntwana	S 16° 1' 18.05"; E 34° 49' 7.16"	

FIGURE 1: MAP OF MALAWI SHOWING ENTOMOLOGICAL MONITORING SITES



The team also sampled adult mosquitoes using human landing catches (HLCs) on a quarterly basis in six sentinel sites: Sanga (Nkhata Bay), Vwawa and Chimkwende (Nkhotakota), Chilungo (Salima), Kachokolo (Kasungu), and Piyasi (Mangochi) (Table 2). All collected *Anopheles* mosquitoes were morphologically identified using the method described by Gillies and Coetzee (1987). Some of the unfed female *Anopheles* mosquitoes collected by HLC at the six sentinel sites were dissected to determine parity rates.

TABLE 2: LONGITUDINAL MONITORING OF ADULT MOSQUITOES IN EACH OF THE 15 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	4 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 seven 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. Mosquitoes were tested for susceptibility to pirimiphos-methyl 0.25%, deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05% using World Health Organization (WHO) susceptibility test kits or CDC bottle assays. The CDC bottle assay was used to determine intensity of resistance in the main malaria vectors by exposing adult mosquitoes to 1x, 2x, 5x, and 10x deltamethrin, permethrin, and alpha-cypermethrin. Tests for chlorfenapyr (pyrrole) and clothianidin (neonicotinoid) were performed using newly developed bottle or paper-based assay protocols. Efforts were made to perform all the tests in all the selected sentinel sites/districts. However, in the case of low numbers of mosquitoes, pirimiphos-methyl and clothianidin testing was a priority in IRS districts, both PMI and non-PMI, as well as planned and final sites. Deltamethrin, permethrin, and alpha-cypermethrin with and without a synergist, mainly PBO, was the top priority in non-IRS districts. Chlorfenapyr testing was also done in both IRS and non-IRS districts.

MOSQUITO COLLECTIONS

An. gambiae s.l. larvae were collected from their natural breeding habitats in all seven districts and reared to adults and subsequently tested. In addition, live blood-fed female *Anopheles* mosquitoes (F_0) resting inside people's homes were collected using Prokopack aspirators. The collected female mosquitoes were allowed to lay eggs, which were reared, and the resultant (F_1) progeny were tested for insecticide resistance. All mosquito-rearing activities were carried out in the insectary at the Malaria Alert Centre.

INSECTICIDES TESTED

Nine insecticides and their PBO combinations were tested: deltamethrin 0.05%, permethrin 0.75%, 4% PBO + deltamethrin 0.05%, 4% PBO + permethrin 0.75%, alpha-cypermethrin 0.05%, 4% PBO + alpha-cypermethrin 0.05%, chlorfenapyr 100µg/bottle, pirimiphos-methyl 0.25%, and clothianidin 13.2mg/paper. In Chikwawa, additional samples were exposed to deltamethrin 0.25% (5x) and permethrin 3.25% (5x). WHO tube assays and CDC bottle bioassays were used to assess the knockdown effect and mortality of the wild *Anopheles* populations.

WHO TUBE ASSAYS

Tests were performed according to standard WHO procedures (WHO 2016). F_1 progeny aged 2–5 days were used for susceptibility tests by exposing them to WHO-recommended diagnostic doses. For *An. gambiae* s.l. a mix of F1 progeny and reared for larvae were used. For *An. funestus* s.l., all tested were from F1 progeny.

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. or *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 introduced into the exposure tubes lined with insecticides or oil/water-impregnated control papers for an exposure period of one hour. Knockdown rates were scored at 10, 20, 30, 40, 50, and 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 seven days for slow-acting insecticides. 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. Then, each mosquito was placed in an individual tube, which was placed in a Ziploc bag with desiccants in it and clearly labeled with 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.

CDC Bottle Assays

A mix of F1 progeny and reared for larvae *An. gambiae* s.l. and ony F1 progeny of *An. funestus* s.l., were tested for susceptibility using the CDC bottle assay. The CDC bottle bioassay method (Brogdon and Chan 2010) with modifications was also used to test for the susceptibility of malaria vectors (*An. funestus* s.l. and *An. gambiae* s.l.) to chlorfenapyr (100µg/bottle) and to alpha-cypermethrin for samples collected from Kande, in Nkhata Bay District. Four Wheaton bottles (250mls) with caps were coated with 1ml of chlorfenapyr solution (100µg/bottle) or alpha-cypermethrin 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 the drawer covered with a napkin 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 at 60 minutes 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 five days post exposure. Mosquitoes exposed to alpha-cypermethrin were observed for 30 minutes, after which they were preserved in RNALater for further analysis at CDC.

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 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.

2.2.2 Mechanism of Insecticide Resistance

The Malaria Alert Centre worked on determining the mechanism of insecticide resistance of mosquitoes collected from sentinel sites. A sub-sample of the resistant and susceptible vectors from the WHO tube tests were identified and used for analysis by polymerase chain reaction (PCR) to determine resistance for knockdown resistance (*kdr*-) east and *kdr* – west) and Acetylcholinesterase 1 (*Ace-1*) following the procedure outlined in Malaria Research and Reference Reagent Resource Center (MR4, www.mr4.org) 2016 Manual. CDC bottle bioassays with synergists were also used to assess if detoxifying enzymes were contributing to the phenotypic resistance observed in Malawi. The synergist assay was used primarily to assess if oxidases have a role in the phenotypic resistance to pyrethroid insecticides. Mosquitoes were pre-exposed to PBO for one hour and tested against deltamethrin and permethrin.

2.3 Cone Bioassays

In 2019, PMI VectorLink Malawi, in collaboration with the NMCP and with support from PMI, and the NMCP, in collaboration with World Vision International with support from the Global Fund, and with technical assistance from PMI VectorLink, conducted IRS in Nkhotakota and Mangochi districts, respectively.

Mangochi was sprayed, with Actellic 300CS, in October 2019. Nkhotakota was sprayed in November; seven of its nine operations sites received Actellic and two received SumiShield 50WG. The Malaria Alert Centre, as a

PMI VectorLink Malawi partner, conducted wall bioassay tests in the two districts to assess the spray quality and the decay rate of the spayed insecticides. The residual life assessment was halted in March 2020 at T4 (4 months after spraying) due to COVID-19 restrictions in both districts. The activities resumed in July 2020 at T8 (8 months after spraying) in Mangochi and at T9 (9 months after spraying) in Nkhotakota and continued up to T10 (10 months after spraying). In two villages in each district, positive control structures were included due to the unusual residual life findings observed in Malawi in 2018.

Cone bioassay tests using a susceptible *An. gambiae* s.s. Kisumu strain were conducted less than one week after spray to assess spray quality. The fumigant effect of Actellic 300CS and SumiShield 50WG was also assessed in all the houses that were monitored.

In Nkhotakota District, the spray quality assessment was conducted in eight randomly selected villages (Table 3). Cone bioassay tests were performed within 24–48 hours after spray in all eight villages. In six villages (Thung'unda, Mtete 1, Bulumuti, Chimkwende, Ngalauka, and Chitsulo 1), six structures made of different wall surfaces (burned brick, cement plastered, and mud walls) were randomly selected at each site for the wall bioassay test. In the other two villages, Chiwawula and Muyande, 12 structures were randomly selected for the bioassay test. Six structures in each of the two villages were positive controls; they were sprayed under the close supervision of VectorLink Malawi staff. Cone bioassay tests were conducted in four villages, Chiwawula, Chimkwende, Ngalauka, and Muyande, to assess the residual life of the sprayed insecticides (Table 3).

In Mangochi District, the spray quality assessment was conducted in eight randomly selected sites (Table 3). Cone bioassay tests were performed from within 24 hours to one week after spray in all the sites. In seven villages (Likulungwa, Moto, Sawasawa, Mpembena, Makokola, Mwenda, and Chipoka), six structures made of the different wall surfaces (burned brick, cement plastered, and mud walls) were randomly selected for the wall bioassay tests. In the 8th village (Piyasi), 12 structures were randomly selected for the bioassay tests; the six were sprayed under close supervision of VectorLink Malawi staff to serve as positive controls. Cone bioassay tests were conducted in four villages; Piyasi, Makokola, Mwenda, and Chipoka, to assess the residual life of the sprayed insecticide (Table 3).

TABLE 3: VILLAGES IN NKHOTAKOTA AND MANGOCHI DISTRICTS WHERE SPRAY QUALITY AND RESIDUAL LIFE ASSESSMENTS WERE CONDUCTED

District	Sampled villages	Operations site	Latitude and longitude
Nkhotakota	Thung'unda,	Dwambazi	S 12°15' 43"; E 33° 57' 42"
	Mtete 1	Dwangwa	S 12°46' 23"; E 34° 14' 47"
	Chiwawula*	Ngala	S 12°26' 27"; E 34° 0' 54"
	Bulumuti 1	Bua	S 12°52' 18"; E 34° 12' 52"
	Chimkwende*	Boma	S 12°59' 2"; E 34° 18' 12"
	Ngalauka*	Mkaika	S 13°17' 46"; E 34° 30' 15"
	Chitsulo 1	Mwansambo	S 13°13' 21"; E 34° 10' 49"
	Muyande*	Benga	S 13°42' 82"; E 34° 27' 33"
Mangochi	Likulungwa 1	Malombe	S 14°40' 37"; E 35° 19' 26"
	Moto	Lungwena	S 14°17' 5"; E 35° 15' 41"
	Piyasi*	Nansenga	S 12°26' 27"; E 34° 0' 54"
	Sawasawa	Mnthiramanja	S 14°36' 4"; E 35° 7' 22"
	Mpembena	Nansenga	S 14°44' 15"; E 35° 10' 22"
	Makokola*	Nankumba	S 14°24' 16"; E 34° 19 17"
	Mwenda*	Monkey Bay	S 14°08' 12"; E 34° 50 45"
	Chipoka*	Namiyasi	S 14°19' 12"; E 35° 08 27"

* Residual life assessment village

2.4 LABORATORY ANALYSES

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 and subsequently calculate Entomological Inoculation Rates (EIRs). Blood mealconventional PCR as described in Malaria Research and Reference Reagent Resource Center (MR4, www.mr4.org) 2016 Manual was used to determine the source of blood in female blood fed *Anopheles* mosquitoes.

2.5 DATA ANALYSIS

The following parameters were calculated:

- Indoor resting density (IRD)=number of adult *Anopheles*/house/day.
- Human biting rate (HBR) per unit time=total number of vectors collected/number of collectors or traps per night or day/ number of nights of capture.
- Sporozoite rate (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 X SRs
- Monthly EIR=Nightly EIR X No. of days per month

3. RESULTS

3.1 LONGITUDINAL MONITORING

3.1.1 SPECIES COMPOSITION

A total of 29,356 *Anopheles* mosquitoes were collected from July 2019 to March 2020. Of these, 15,705 were collected using PSCs (53.5%), 12,409 using CDC-LTs (42.3%), and 1,242 (4.2%) using HLCs (Figures 2–4).

Overall, 62.8% (n=18,444) of the *Anopheles* collected were *An. gambiae* s.l.; 34.0% (n=9,994) *An. funestus* s.l., and 3.1% (n=918) *An. coustani*. However, species composition varied by sentinel site. The highest proportion of *An. gambiae* s.l. were collected from Karonga District from both PSC and CDC-LT collections (above 99%) (Figures 2 and 3). From HLCs, the highest proportion of this species was collected in Salima District (95 %) (Figure 4). The highest proportion of *An. funestus* s.l. collected from PSCs was observed in Nyalubwe site in Kasungu District and Vwawa site in Nkhotakota (90%) (Figure 2). From CDC-LTs, the highest proportion of *An. funestus* s.l. was observed again at Ntwana site in Kasungu (97 %) (Figure 3). The highest proportion of *An. funestus* s.l. collected from HLCs was observed at Kachokolo site in Kasungu (46%) (Figure 4). The highest proportion of *An. coustani* captured by HLCs was observed at Vwawa site in Nkhotakota (58 %). The highest proportion of *An. coustani* captured by CDC-LT was observed at Likulungwa site in Mangochi (15%) (Figure 3). No *An. coustani* were captured by PSC (Figure 2).

Furthermore, 14,824 *Culex* mosquitoes also were collected during the same period. Out of these, 36.9% (n=5,472) were collected by HLC, 36.2% (n=5,357) by CDC-LT, and 26.9% (n=3,995) by PSC.

FIGURE 2: *ANOPHELES* COMPOSITION, BY SENTINEL SITE ACROSS ALL SEVEN MONITORING DISTRICTS, FROM PSC COLLECTIONS

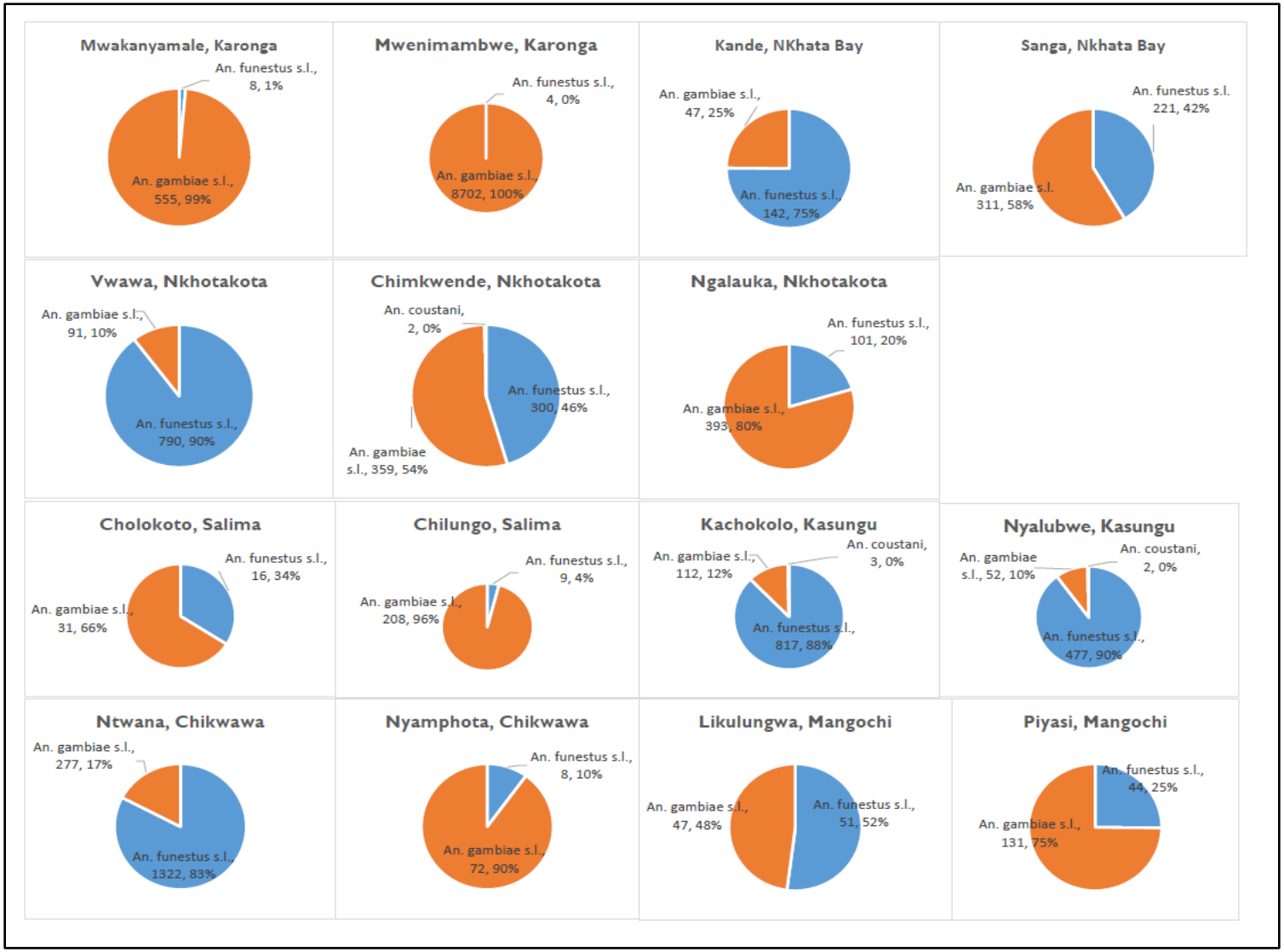


FIGURE 3: *ANOPHELES* COMPOSITION, BY SENTINEL SITE ACROSS ALL SEVEN MONITORING DISTRICTS, FROM CDC-LT COLLECTIONS

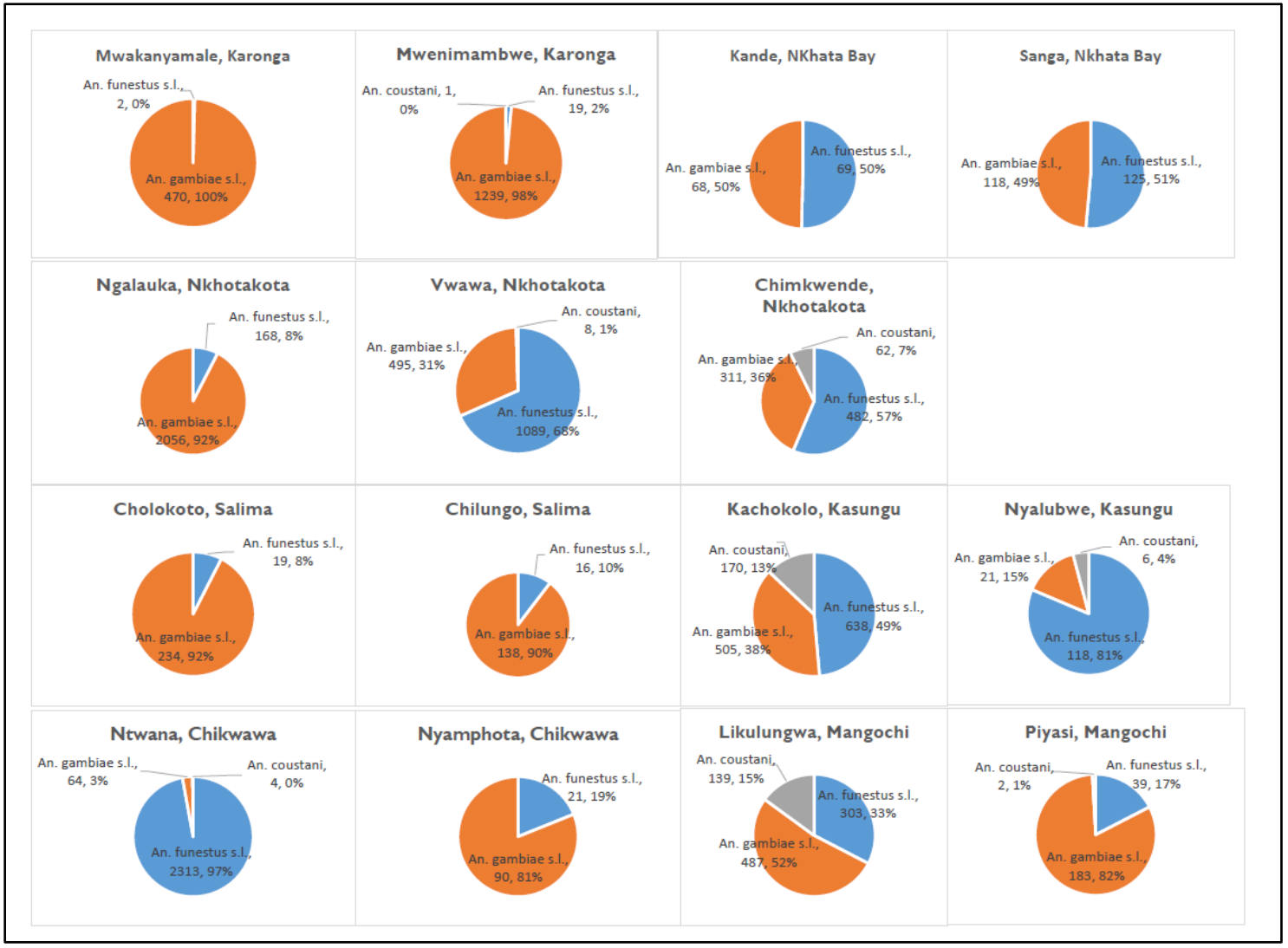
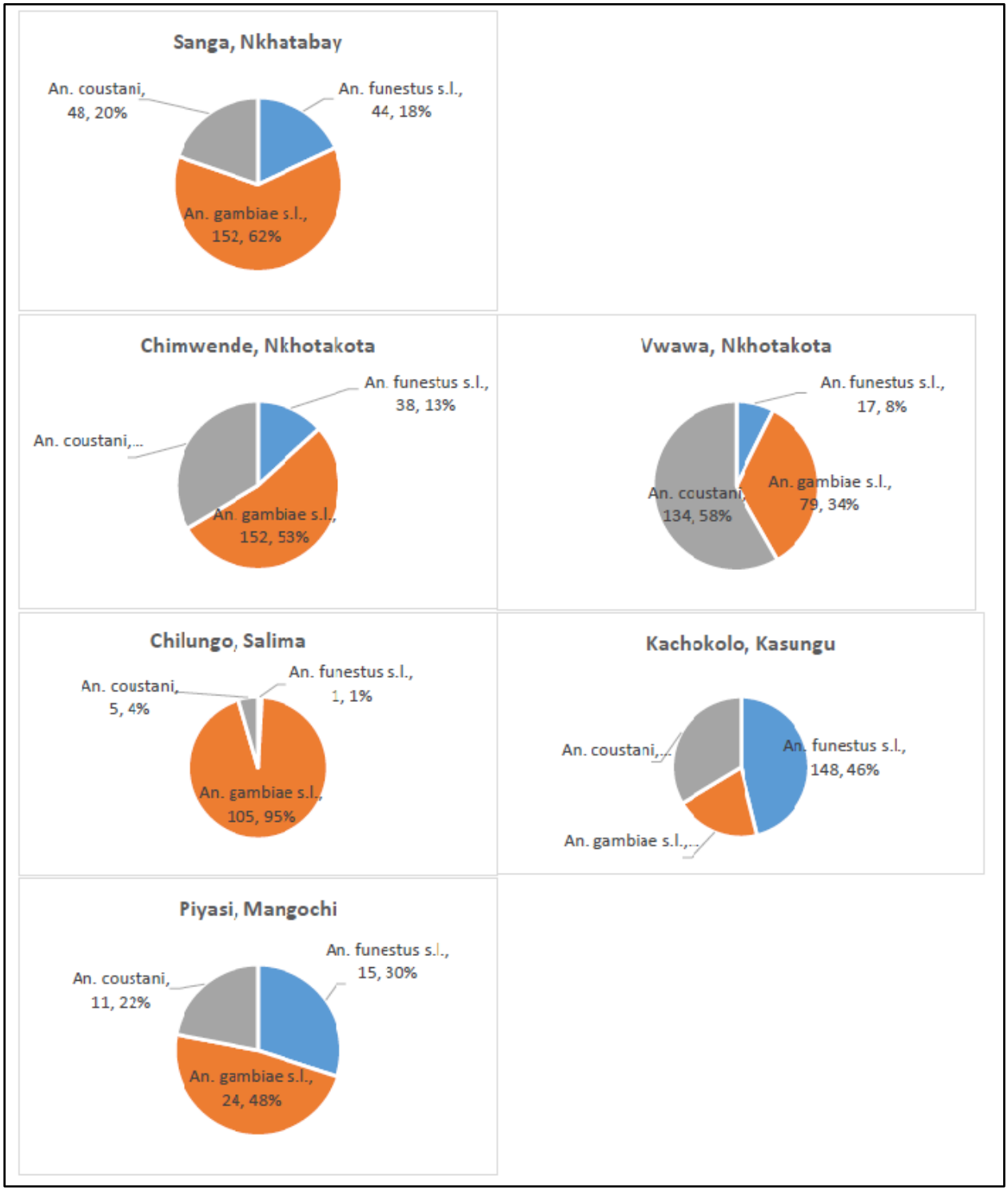


FIGURE 4: *ANOPHELES* COMPOSITION, BY SENTINEL SITE ACROSS ALL FIVE MONITORING DISTRICTS, FROM HLC COLLECTIONS



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

A) DENSITY OF AN. FUNESTUS S.L.

The overall indoor resting density (IRD) across all sentinel sites was 2.3 *An. funestus* s.l. per house per day. The highest mean IRD of *An. funestus* s.l. from PSC collections was observed in August 2019, with a mean catch of 4.8 *An. funestus* s.l. per house per day. The lowest was in November (<1.0 mosquito/house/day) (Figure 5).

Generally, the mean *An. funestus* s.l. IRD was highest in August 2019 at Vwawa site (Nkhotakota District) and Ntwana (Chikwawa). There was a slight peak of *An. funestus* s.l. density during the rainy season at Kachokolo (Kasungu) (15 mosquitoes/house/day) in March and Ntwana (Chikwawa) (15 mosquitoes/house/day) in January. The mean IRD of *An. funestus* s.l. remained very low (<2.0 mosquitoes/house/day) throughout the monitoring period in 10 sites: Mwenimambwe and Mwakanyamale (Karonga), Sanga and Kande (Nkhata Bay), Ngalauka (Nkhotakota), Cholokoto and Chilungo (Salima), Piyasi and Likulungwa (Mangochi), and Nyamphota (Chikwawa). The *An. funestus* s.l. population remained low (<1.0 mosquito/house/day) after spraying (November to March) in Nkhotakota District (Figure 6).

FIGURE 5: MEAN IRD ± SE OF AN. FUNESTUS S.L. COLLECTED BY PSCS IN THE SEVEN DISTRICTS, JULY 2019–MARCH 2020

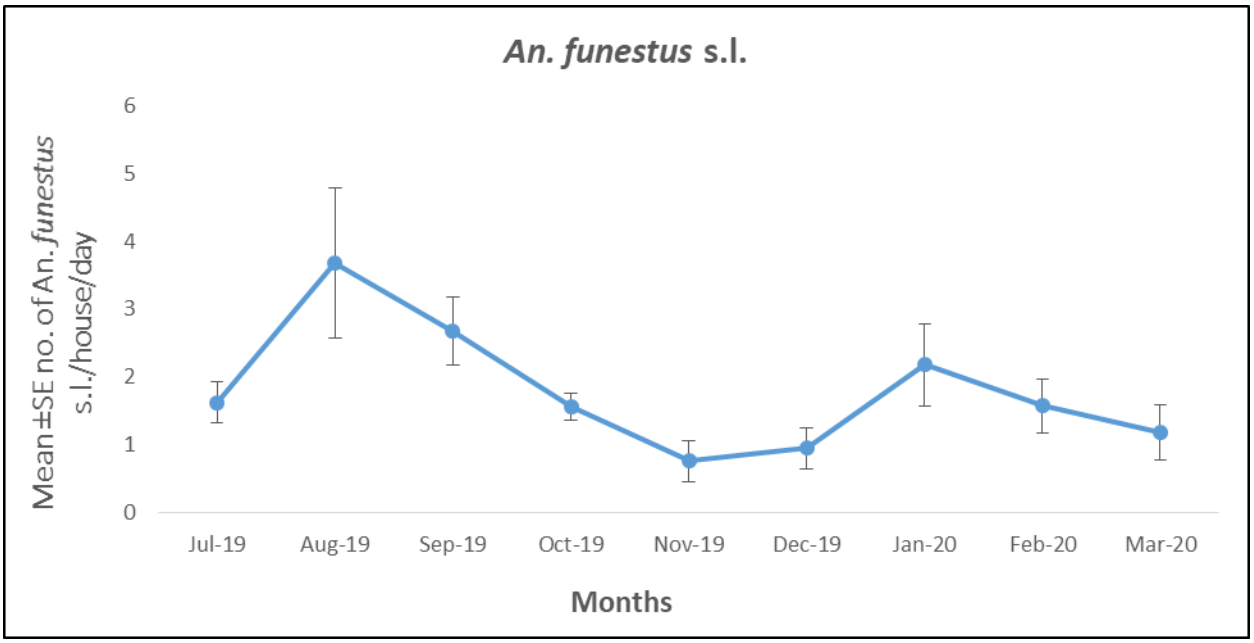
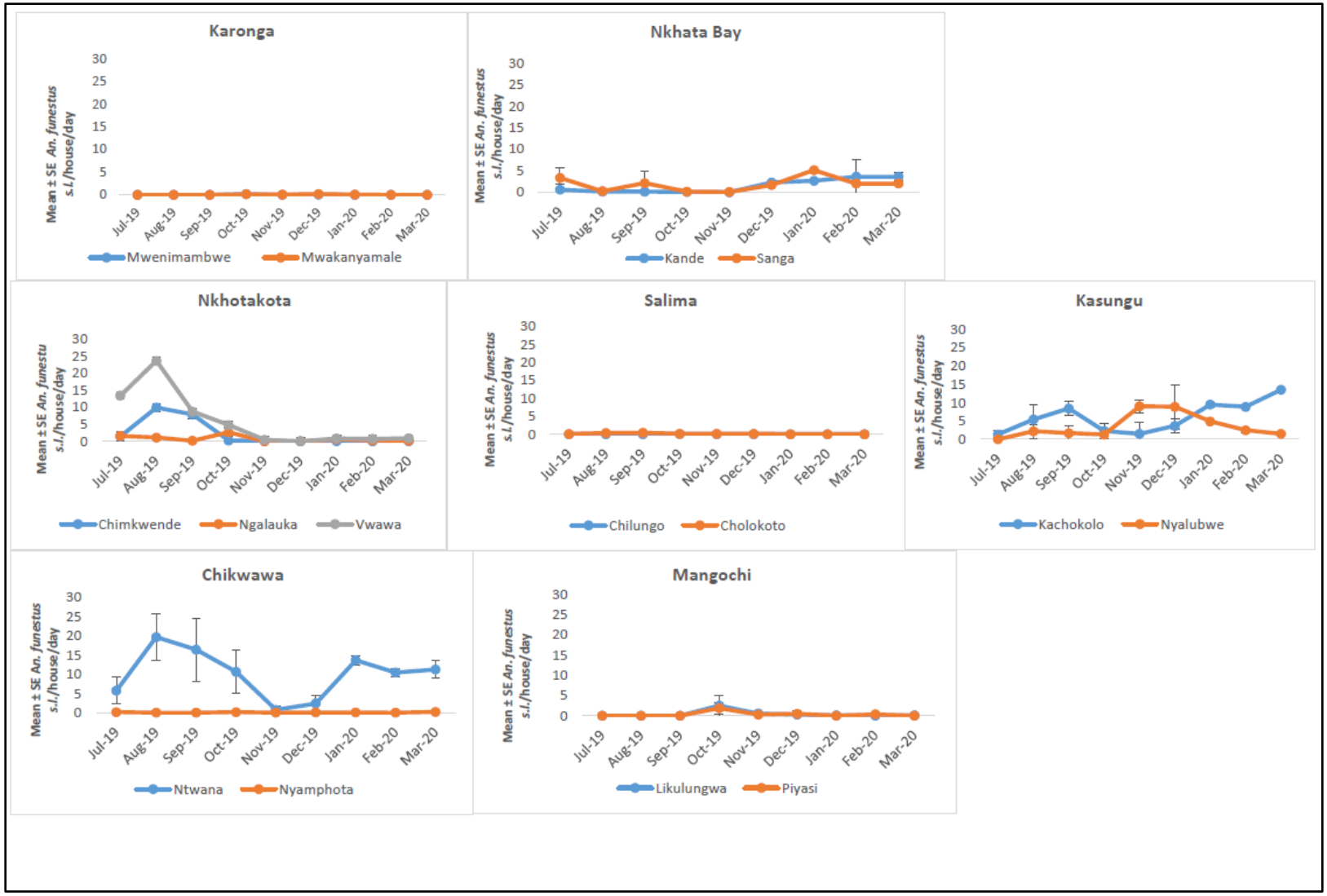


FIGURE 6: MEAN IRD ± SE OF *AN. FUNESTUS* S.L. COLLECTED BY PSCS IN THE SEVEN DISTRICTS, JULY 2019–MARCH 2020



B) DENSITY OF AN. GAMBIAE S.L.

The overall IRD of *An. gambiae* s.l. was 6.2 per house per day across all sentinel sites. The highest mean IRD of *An. gambiae* s.l. from PSC collections was observed in September 2019 with a mean 27.6 *An. gambiae* s.l. collected per house per day. The lowest IRD was in November (<1.0 mosquito/house/day) (Figure 7).

Mwenimambwe site in Karonga recorded the highest IRD in September with a mean *An. gambiae* s.l. IRD of 336.0 mosquitoes per house per day (Figure 8). Overall, IRD from PSCs remained low throughout the sampling period (<2.0 mosquitoes/house/day) at Kande (Nkhata Bay), Vwawa (Nkhotakota), Kachokolo and Nyalubwe (Kasungu), Cholokoto (Salima), Likulungwa and Piyasi (Mangochi), and Ntwana and Nyamphota (Chikwawa). There was a slight peak of *An. gambiae* s.l. density collected from PSCs at Chimkwende and Ngalauka (Nkhotakota) before spraying in August and September (10 mosquitoes/house/day) and during the rainy season at Sanga in Nkhata Bay (January) and Chilungo in Salima (December) (Figure 9).

FIGURE 7: MEAN IRD ± SE OF *AN. GAMBIAE* S.L. COLLECTED BY PSCS IN THE SEVEN DISTRICTS, JULY 2019–MARCH 2020

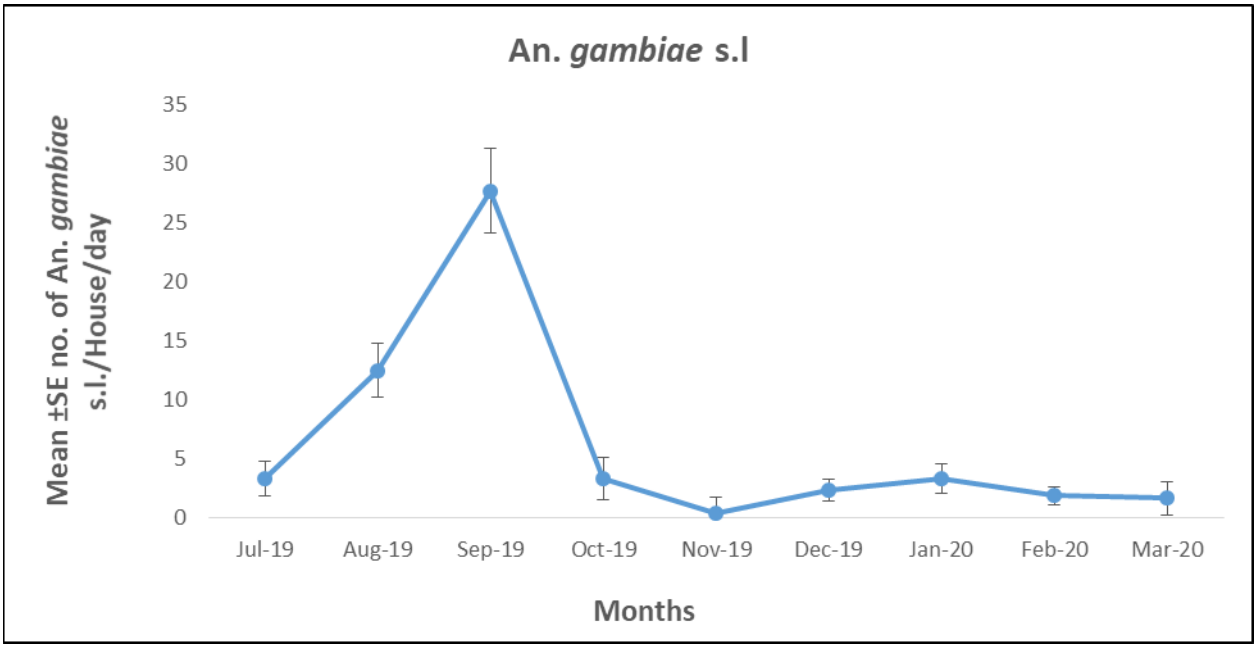


FIGURE 8: MEAN IRD ± SE OF *AN. GAMBIAE* S.L. COLLECTED BY PSCS IN KARONGA DISTRICT, JULY 2019–MARCH 2020

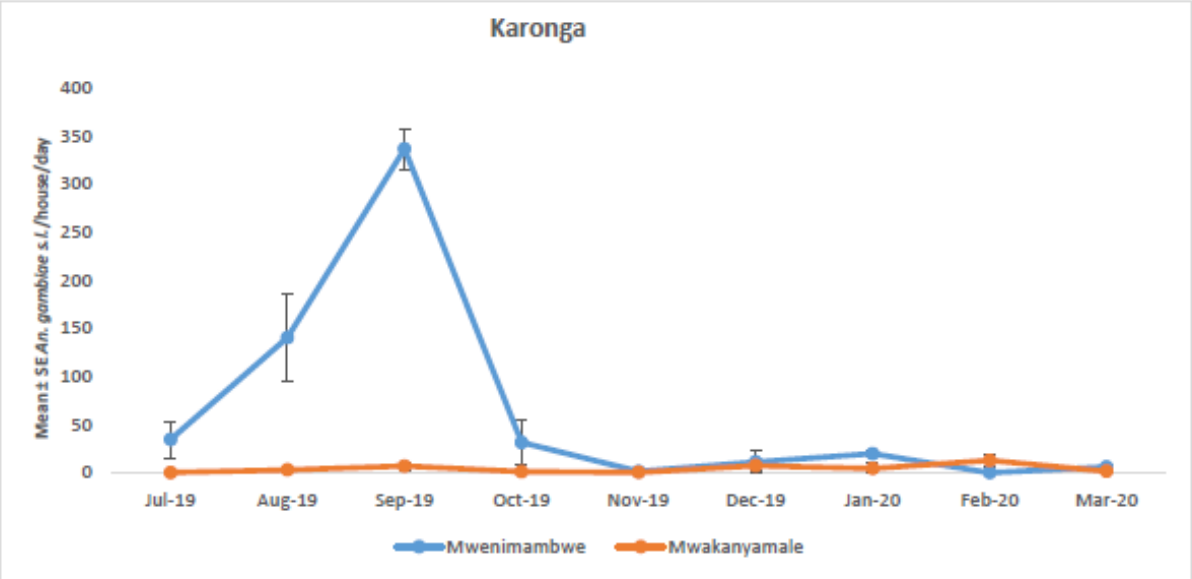
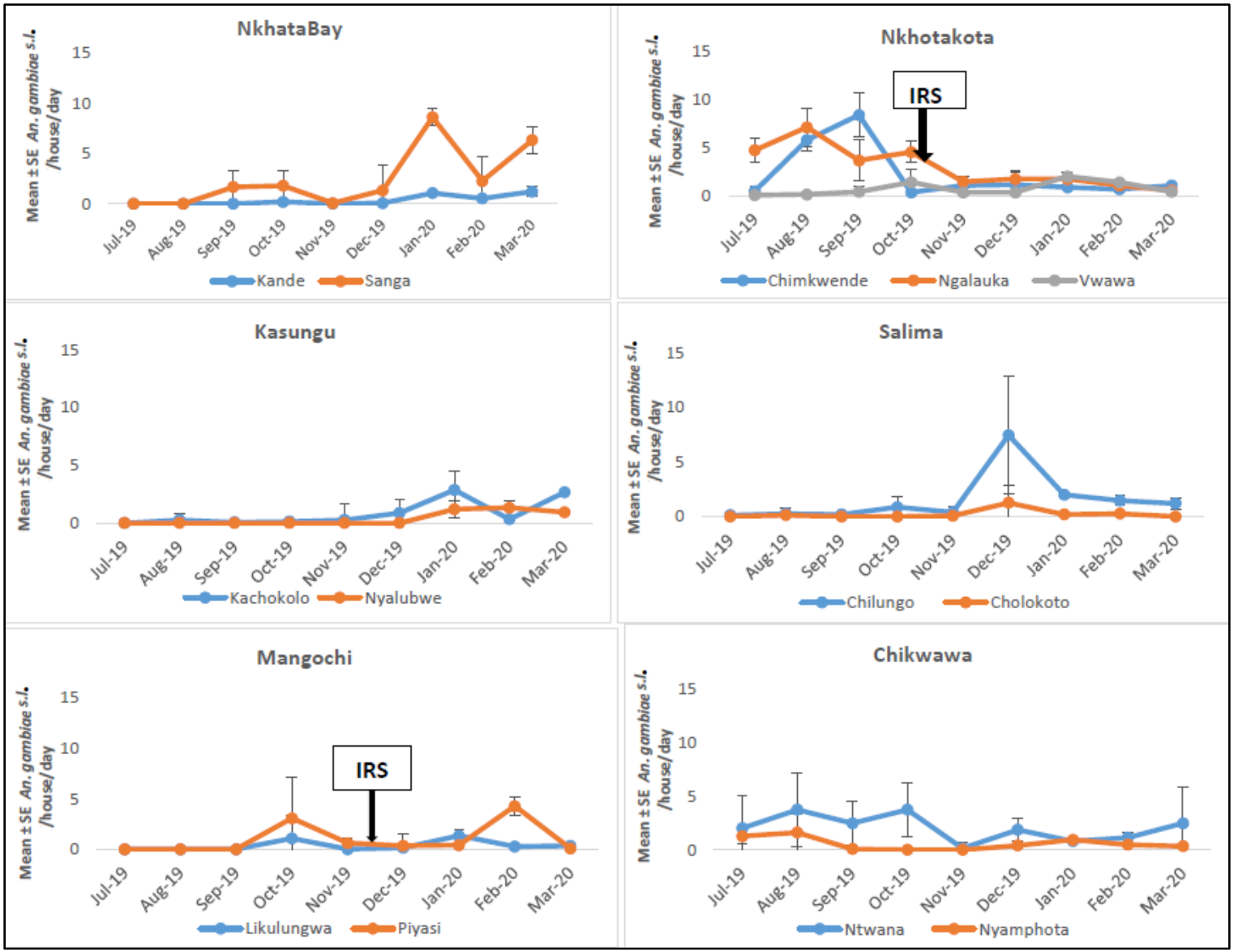


FIGURE 9: MEAN IRD ± SE OF *AN. GAMBIAE* S.L. COLLECTED BY PSCS IN SIX DISTRICTS, JULY 2019–MARCH 2020



C) Gonotrophic Status of An. funestus s.l. and An. gambiae s.l. Collected by PSC

Overall, a high proportion of the female *Anopheles* mosquitoes collected by PSC were blood fed. Out of 11,277 females collected by PSC, 2,596 (23%) were unfed, 6,169 (55%) were blood fed, 1,728 (15%) were half gravid, and 784 (7%) were gravid. The proportion fed was higher among *An. gambiae* s.l. than among *An. funestus* s.l. collected by PSC (Table 4). However, the proportion of gravid (39%) was higher among *An. funestus* s.l. than among *An. gambiae* s.l. (16%), indicating the more endophilic behavior of *An. funestus* s.l.

The total number of blood-fed mosquitoes in the IRS district of Nkhotakota was higher before spraying (August–September in Nkhotakota) than after spraying (November–March). The number of blood-fed mosquitoes also was higher before spraying in Mangochi. However, the number of fed *Anopheles* mosquitoes collected in Mangochi rose in January–March, during the rainy season. The same scenario was observed in Karonga District where more blood-fed *Anopheles* mosquitoes were collected in September than in October–March. In Nkhata Bay and Salima districts, more blood-fed *Anopheles* mosquitoes were collected during the rainy season (December–March), whereas in Kasungu and Chikwawa, high numbers of fed *Anopheles* mosquitoes were collected before (August–September) and during the rainy season (November–March) (Figure 10).

Table 4: Number and Proportion of Female *An. funestus* s.l. and *An. gambiae* s.l. and their Gonotrophic Status Collected by PSC in all 15 Sentinel Sites

Species	Gonotrophic Status				
	Unfed (N, %)	Fed (N, %)	Half gravid (N, %)	Gravid (N, %)	Total
An. funestus s.l.	388 (12%)	1649 (50%)	891 (27%)	397 (12%)	3,325
An. gambiae s.l.	2208 (28%)	4520 (57%)	837 (11%)	387 (5%)	7,952
Total	2596 (23%)	6169 (55%)	1728 (15%)	784 (7%)	11277

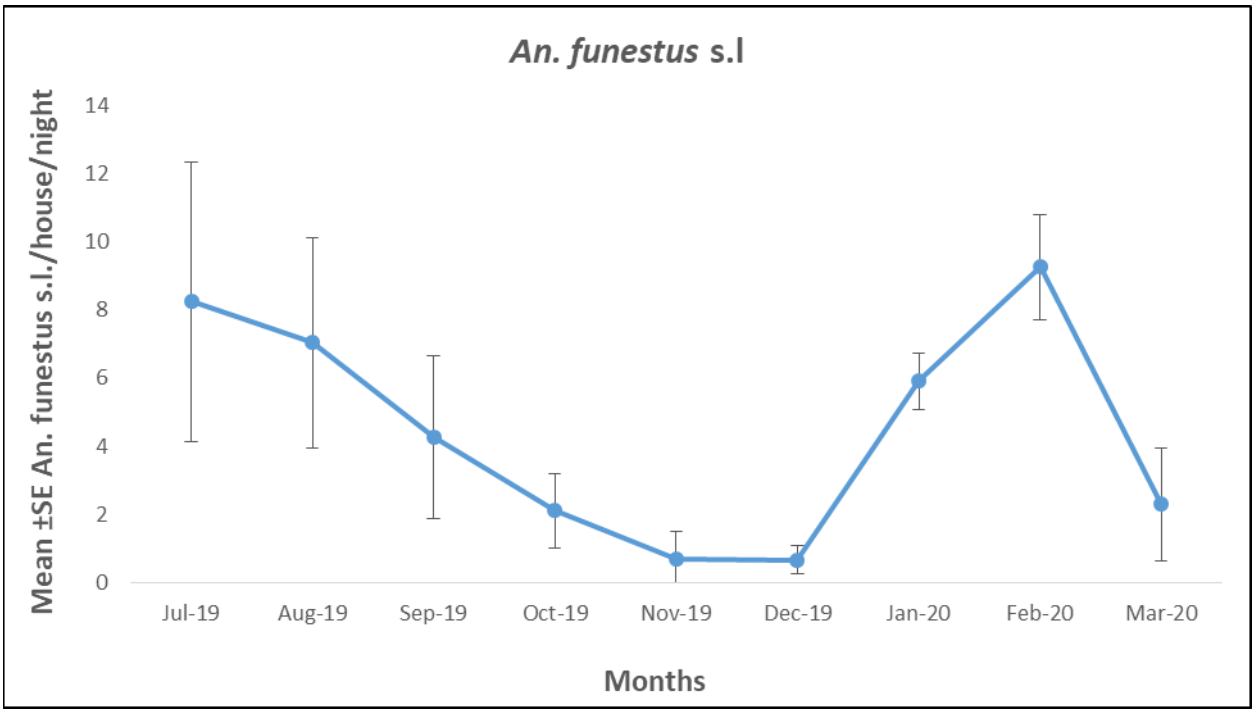
FIGURE 10: GONOTROPHIC STATUS OF FEMALE *ANOPHELES* MOSQUITOES COLLECTED BY PSC IN THE SEVEN MONITORING DISTRICTS

3.1.3 INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY CDC-LT

A) DENSITY OF AN. FUNESTUS S.L.

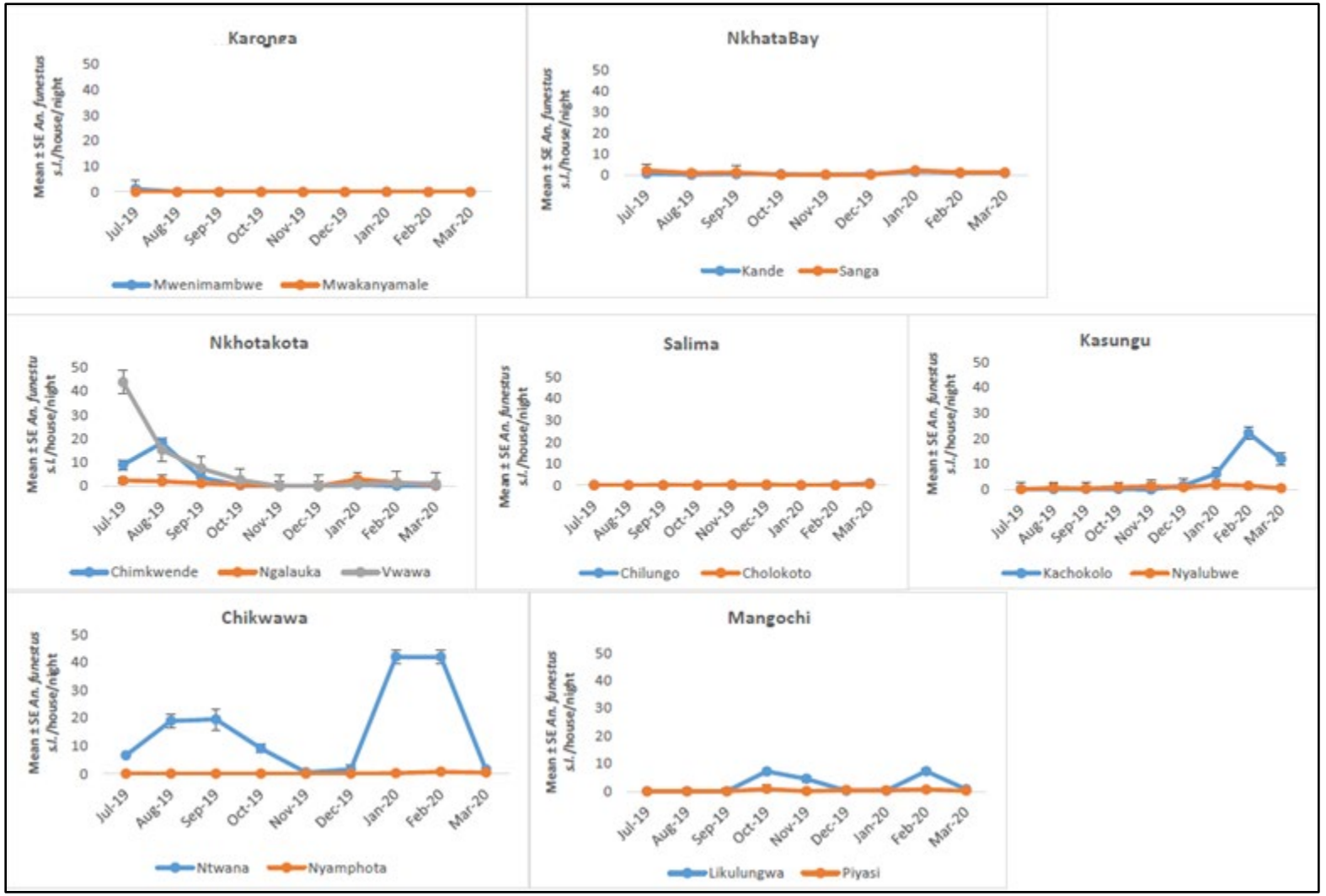
The mean number of *An. funestus* s.l. collected indoors by CDC-LT in all 15 sentinel sites from July 2019 to March 2020 is presented in Figure 11. The highest mean number of *An. funestus* s.l. was collected in February 2020 (9.0 mosquitoes/trap/night) and the lowest was recorded in November and December (<1.0 mosquito/trap/night). The mean density of *An. funestus* s.l. over the period was 2.2 mosquitoes/trap/night.

FIGURE 11: INDOOR DENSITY OF *AN. FUNESTUS* S.L., ALL MONITORING DISTRICTS, JULY 2019–MARCH 2020



The highest mean numbers of *An. funestus* s.l. were collected in Nkhotakota District; the number peaked at 43.7 mosquitoes/trap/night in July (Vwawa site), before spraying. The numbers steadily decreased from August to October 2019 and remained low after spraying (November 2019–March 2020). High *An. funestus* s.l. numbers were also recorded at Ntwana in Chikwawa (40.0 mosquitoes/trap/night) during the rainy season (December 2019–February 2020), then drastically decreased in March. A slight peak in *An. funestus* s.l. density was recorded at Kachokolo in Kasungu (10.0 mosquitoes/trap/night) in March, during the rainy season. Low *An. funestus* s.l. density was observed in the remaining sentinel sites throughout the sampling period (Figure 12).

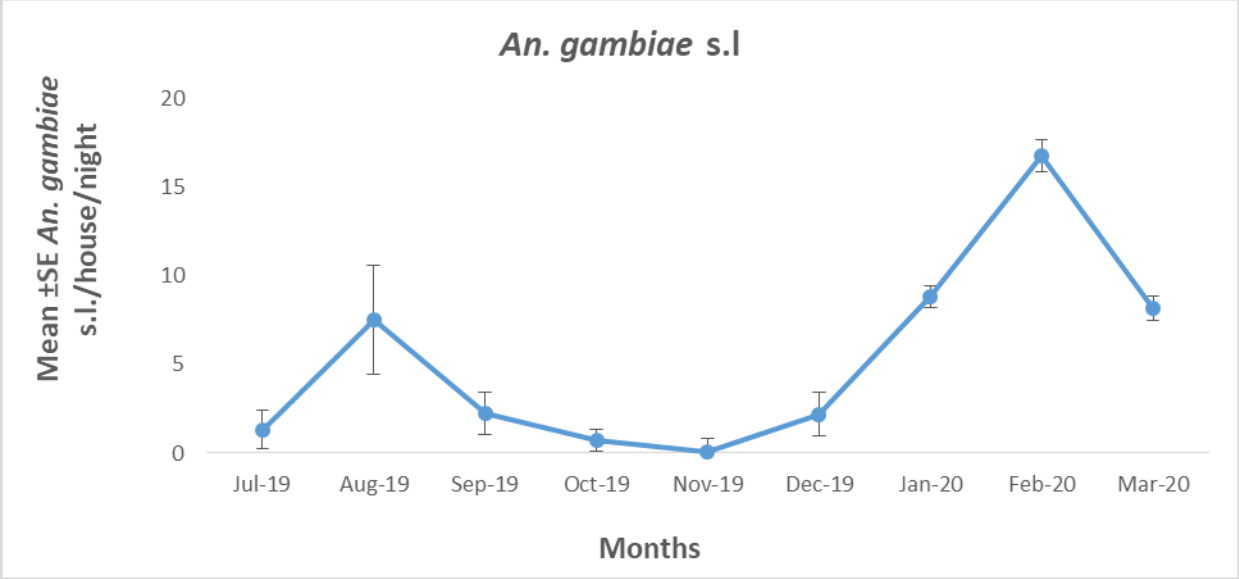
FIGURE 12:MEAN INDOOR DENSITY OF *AN. FUNESTUS* S.L. COLLECTED BY CDC-LT, BY SENTINEL SITE, JULY 2019–MARCH 2020



B) Density of An. gambiae s.l.

The overall numbers of *An. gambiae* s.l. collected indoors using CDC-LTs in the 15 sentinel sites from July 2019 to March 2020 is presented in Figure 13. The highest numbers of *An. gambiae* s.l. were captured in February (16.8 mosquitos per trap per night) and the lowest in November (<1.0 mosquito per trap per night). The overall mean density of *An. gambiae* s.l. was 5.3 mosquitoes per trap per night.

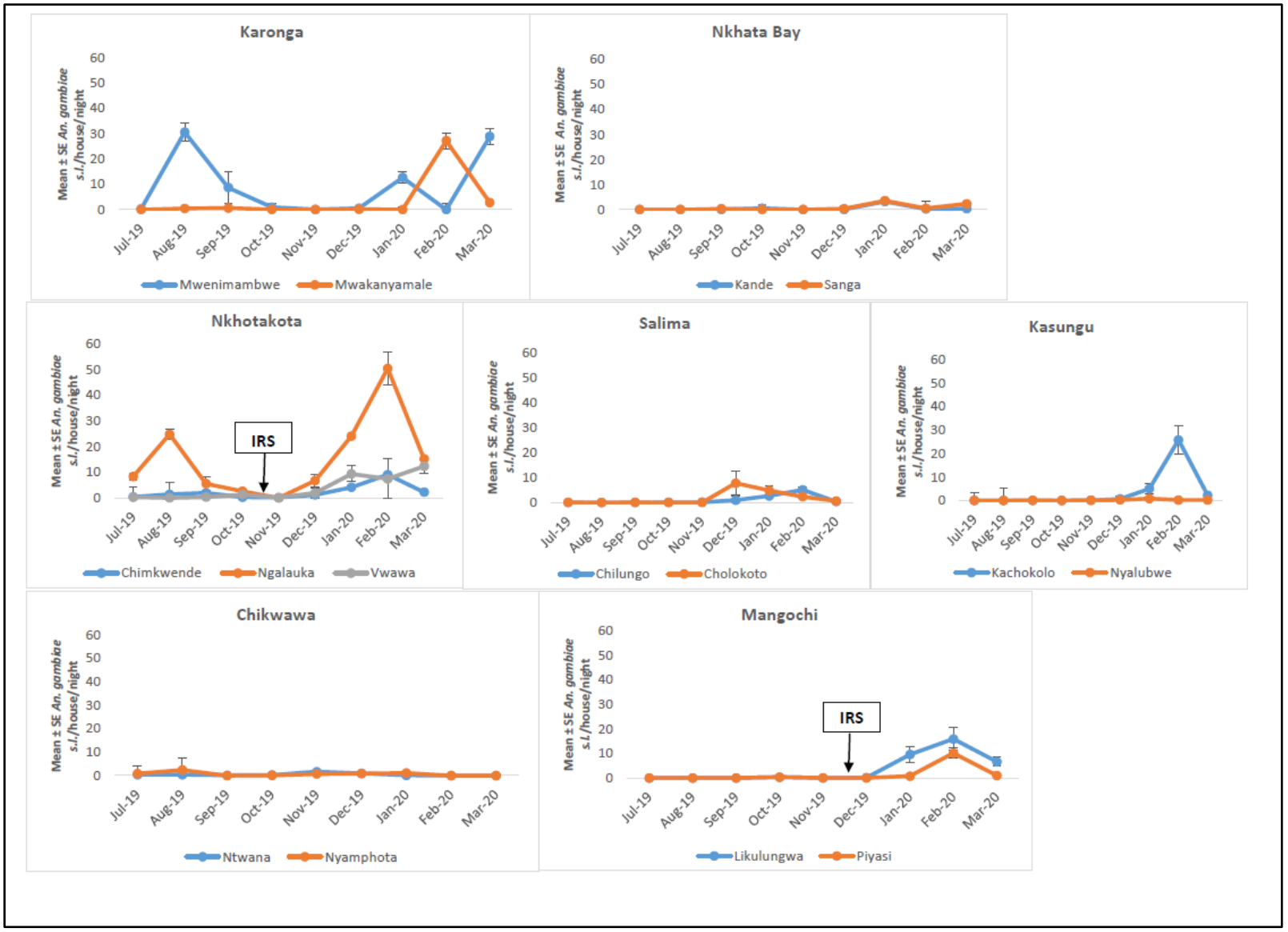
Figure 13: Indoor Density of *An. gambiae* s.l., all Monitoring Districts, July 2019–March 2020



The highest number of *An. gambiae* s.l. were captured at Ngalauka site in Nkhotakota District (50 mosquitoes/trap/night) in February, during the rainy season after spraying, followed by Mwenimambwe in Karonga (30 mosquitoes/trap/night) in August, before the rains, and March, during the rainy season, and Mwakanyamale, also in Karonga (30 mosquitoes/trap/night) in February, during the rainy season. A slight peak in *An. gambiae* s.l. numbers was observed at Kachokolo in Kasungu (25 mosquitoes/trap/night), and at Likulungwa and Piyasi in Mangochi (15 and 10 mosquitoes/trap/night, respectively) in February. *An. gambiae* s.l. numbers in the remaining 1sentinel sites were very low (<5 mosquitoes per trap per night) throughout the collection period (Figure 14).

It is important to note that there was a sharp rise in *An. gambiae* s.l. density during the rainy season, December–March, in the IRS districts of Nkhotakota and Mangochi.

FIGURE 14:MEAN INDOOR DENSITY OF *AN. GAMBIAE* S.L. COLLECTED BY CDC-LT, BY SENTINEL SITE, JULY 2019–MARCH 2020



C) Gonotrophic Status of An. funestus s.l. and An. gambiae s.l. Collected by CDC-LT

Overall, a high proportion of the female *Anopheles* mosquitoes collected were unfed. Out of the 11,251 females collected by CDC-LT, 10,172 (90%) were unfed, 860 (8%) were blood-fed, 78 (1%) were half gravid, and 141 (1%) were gravid. More unfed *An. gambiae* s.l. were collected using CDC-LTs compared with *An. funestus* s.l. (Table 5). The CDC-LTs captured more host-seeking (unfed) females than did the PSC collections, before they had the chance to bite people.

More unfed *Anopheles* mosquitoes were collected during the rainy season than before the rains in Salima, Mangochi, Nkhata Bay, Kasungu, and Karonga districts. The total number of unfed mosquitoes collected before and during the rainy season in Nkhotakota and Chikwawa was similar (Figure 15).

TABLE 5: NUMBER AND PROPORTION OF FEMALE *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. AND THEIR GONOTROPHIC STATUS SAMPLED BY CDC-LT IN ALL 15 SENTINEL SITES

Species	Gonotrophic Status				
	Unfed (N, %)	Fed (N, %)	Half-gravid (N, %)	Gravid (N, %)	Total
An. funestus s.l.	4,495(89%)	426(8%)	35(1%)	95(2%)	5,051
An. gambiae s.l.	5,677(92%)	434(7%)	43(1%)	46(1%)	6,200
Total	10172 (90%)	860 (8%)	78 (1%)	141(1%)	11251

FIGURE 15: GONOTROPHIC STATUS OF FEMALE *ANOPHELES* MOSQUITOES COLLECTED BY CDC-LTS IN THE SEVEN MONITORING DISTRICTS

3.2 INSECTICIDE RESISTANCE MONITORING

3.2.1 INSECTICIDE SUSCEPTIBILITY TESTS AND INTENSITY ASSAYS

The WHO tube assays and CDC bottle bioassay method with modifications were used for insecticide susceptibility tests for wild-caught *An. gambiae* s.l. and *An. funestus* s.l. mosquitoes from all seven districts. The results from the insecticide susceptibility tests indicate that both *An. gambiae* s.l. and *An. funestus* s.l. were resistant to pyrethroids (permethrin, deltamethrin, and alpha-cypermethrin). However, pre-exposure of *An. funestus* s.l. and *An. gambiae* s.l. to 4% PBO then to pyrethroids restored their susceptibility partially or fully.

Both *An. gambiae* s.l. and *An. funestus* s.l. were completely susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. **Mechanism of Insecticide Resistance**

A total of 161 *An. gambiae* s.l. mosquitoes collected using PSCs, CDC-LTs, and HLCs from entomological monitoring sentinel sites were tested for *Ace-1* by PCR. In general, no mutations for *Ace-1* were observed (Table 6).

TABLE 6: MECHANISM OF RESISTANCE

District	Site	Species	*Ace-1*		
			RR	**RS**	**SS**
Karonga	Mwenimambwe	*An. gambiae* s.l.	0	0	35
	Mwakanyamale	*An. gambiae* s.l.	0	0	28
Nkhata Bay	Kande	*An. gambiae* s.l.	-	-	-
	Sanga	*An. gambiae* s.l.	0	0	1
Nkhotakota	Vwawa	*An. gambiae* s.l.	-	-	-
	Chimkwende	*An. gambiae* s.l.	-	-	-
	Ngalauka	*An. gambiae* s.l.	0	0	24
Salima	Chilungo	*An. gambiae* s.l.	0	0	20
	Cholokoto	*An. gambiae* s.l.	0	0	14
	Nzembela	*An. gambiae* s.l.	0	0	38
Kasungu	Nyalubwe	*An. gambiae* s.l.	-	-	-
	Kachokolo	*An. gambiae* s.l.	0	0	0
Chikwawa	Nyamphota	*An. gambiae* s.l.	-	-	-
	Ntwana	*An. gambiae* s.l.	0	0	1
Number of genotypes			**0**	**0**	**161**
Number of alleles			**0**	**0**	**322**
Total number of alleles			**322**		
Frequency of the resistance R allele (RR+RS)/2[RR+RS+SS)]			**0%**		

3.3 LABORATORY ANALYSES

3.3.1 SPECIES IDENTIFICATION

A) AN. GAMBIAE S.L.

A total of 2,324 *An. gambiae* s.l. were randomly sampled and identified to the species-specific level by PCR (Table 7). The mosquito samples identified by PCR were collected using PSCs (n=1,570, 67.6%), CDC-LTs, (n=213, 9.1%), and HLCs (n=541, 23.3%). Out of the 2,324 *An. gambiae* s.l., 2,303 (99.1%) were identified as *An. arabiensis* and 21 (0.9%) as *An. gambiae* s.s. *An. arabiensis* occurred in all the seven districts, while *An. gambiae* s.s. occurred in three districts: Karonga (Mwenimambwe site), Nkhotakota (Vwawa and Ngalauka sites), and Salima (Cholokoto site). *An. arabiensis* was the predominant member of the *An. gambiae* complex in all the districts (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 Identified by PCR							
		PSCs		CDC-LTs		HLCs		Total	
		An. arabiensis N (%)	*An. gambiae* s.s. N (%)	*An. arabiensis* N (%)	*An. gambiae* s.s. N (%)	*An. arabiensis* N (%)	*An. gambiae* s.s. N (%)	*An. arabiensis* N (%)	*An. gambiae* s.s. N (%)
Karonga	Mwenimambwe	-665 (100)		-	2 (100)	*	*	665 (99.7)	2 (0.3)
	Mwakanyamale	-54 (100)		20 (100)	-	*	*	74 (100)	-
Nkhata Bay	Kande	7 (100)	-	6 (100)	-	*	*	13 (100)	-
	Sanga	18 (100)	-	2 (100)	-	128 (100)	-	148 (100)	-
Nkhotakota	Vwawa	7 (87.5)	1 (12.5)	45 (100)	-	75 (100)	-	127 (99.2)	1 (0.8)
	Chimkwende	237 (100)	-	-	-	141 (100)	-	376 (100)	-
	Ngalauka	412 (100)	-	34 (97.1)	1 (2.9)	*	*	446 (99.8)	1 (0.2)
Salima	Chilungo	98 (100)	-	1 (100)	-	99 (100)	-	198 (100)	-
	Cholokoto	12 (80)	3 (20)	47 (77)	14 (23)	*	*	59 (77.6)	17 (22.4)
Kasungu	Nyalubwe	-	-	-	-	*	*	-	-
	Kachokolo	26 (100	-	24 (100)	-	65 (100)	-	105 (100)	-
Chikwawa	Nyamphota	3 (100)	-	17 (100)	-	*	*	20 (100)	-
	Ntwana	39 (100)	-	-	-	*	*	39 (100)	-
Mangochi	Piyasi	-	-	-	-	33 (100)	-	33 (100)	-
Total		**1566 (99.7)**	**4 (0.3)**	**196 (92.0)**	**17 (8.0)**	**541 (100)**	**0 (0)**	**2303 (99.1)**	**21 (0.9)**

*Sentinel sites where HLCs are not conducted

B) AN. FUNESTUS S.L.

A total of 711 *An. funestus* s.l. were identified to species-specific level using PCR (Table 8) collected from PSCs (n=349, 49.0%), CDC-LTs (n=144, 20.3%), and HLCs (n=218, 30.7%). All 711 *An. funestus* s.l. were identified by PCR as *An. funestus* s.s. This species occurred in all districts except Karonga (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			
		PSCs	CDC-LTs	HLCs	Total
		An. funestus s.s. N (%)	*An. funestus* s.s. N (%)	*An. funestus* s.s. N (%)	*An. funestus* s.s. N (%)
Karonga	Mwenimambwe	-	-	*	-
	Mwakanyamale	-	-	*	-
Nkhata-Bay	Kande	46 (100)	2 (100)	*	48 (100)
	Sanga	45 (100)	60 (100)	19 (100)	124 (100)
Nkhotakota	Vwawa	29 (100)	19 (100)	13 (100)	61 (100)
	Chimkwende	13 (100)	1 (100)	33 (100)	47 (100)
	Ngalauka	78 (100)	19 (100)	*	87 (100)
Salima	Chilungo	1 (100)	1 (100)	1 (100)	3 (100)
	Cholokoto	-	1 (100)	*	1 (100)
Kasungu	Nyalubwe	38 (100)	10 (100)	*	48 (100)
	Kachokolo	62 (100)	12 (100)	139 (100)	213 (100)
Chikwawa	Nyamphota	11 (100)	-	*	11 (100)
	Ntwana	26 (100)	29 (100)	*	55 (100)
Mangochi	Piyasi	-	-	13 (100)	13 (100)
Total		349 (100)	144 (100)	218 (100)	711 (100)

* Sentinel sites where HLCs are not conducted

3.3.2 BLOOD MEAL ANALYSIS

A total of 77 blood-fed *An. funestus* s.l. mosquitoes from three districts (Nkhata Bay, Nkhotakota, and Chikwawa) were tested to identify the source of their blood meal. Human blood was the predominant source: 75 *An. funestus* s.l. (97.4%) were found to have fed on humans; only two (2.6%), from Nkhata Bay (Kande site), had fed on cattle (Table 9).

A total of 364 blood-fed *An. gambiae* s.l. mosquitoes from five districts (Karonga, Nkhata Bay, Nkhotakota, Salima, and Chikwawa) were tested to identify the source of their blood meal. Again, human blood was the predominant source: 323 (88.7%) had fed on humans; only 41 (11.3%), from Karonga, had fed on cattle (Table 9).

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.* Bloodmeal Source						*An. gambiae s.l.* Bloodmeal 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	-	-	-	-	-	-	154 (100)	-	-	-	-	154
	Mwakanyamale	-	-	-	-	-	-	4 (8.9)	41 (91.1)	-	-	-	45
Nkhata-Bay	Kande	29 (93.5)	2 (6.5)	-	-	-	31	4 (100)	-	-	-	-	4
	Sanga	10 (100)	-	-	-	-	10	7 (100)	-	-	-	-	7
Nkhotakota	Vwawa	2 (100)	-	-	-	-	2	34 (100)	-	-	-	-	34
	Chimkwende	-	-	-	-	-	0	24 (100)	-	-	-	-	24
	Ngalauka	3 (100)	-	-	-	-	3	71 (100)	-	-	-	-	71
Salima	Chilungo	-	-	-	-	-	0	19 (100)	-	-	-	-	19
	Cholokoto	-	-	-	-	-	0	2 (100)	-	-	-	-	2
Chikwawa	Nyamphota	-	-	-	-	-	0	2 (100)	-	-	-	-	2
	Ntwana	31 (100)	-	-	-	-	31	2 (100)	-	-	-	-	2
Total		**75 (97.4)**	**2 (2.6)**	**0 (0)**	**0 (0)**	**0 (0)**	**77**	**323 (88.7)**	**41 (11.3)**	**0 (0)**	**0 (0)**	**0 (0)**	**364**

3.3.3 INFECTION DETECTION

A) SRs FROM HLC COLLECTIONS (JULY 2019– MARCH 2020)

Tables 10 and 11 summarize the sporozoite infections of *An. gambiae* s.l. and *An. funestus* s.l. captured by HLCs respectively. The overall *An. gambiae* s.l. SR in the five districts where HLCs were conducted was 1.8% (n=570): 1.7% (n=238) indoors and 1.8% (n=332) outdoors.

In Nkhata Bay (standard net distribution), the highest *An. gambiae* s.l. SRs occurred in December, during the rainy season, at 5.9% indoors and 5.6% outdoors. No *An. gambiae* s.l. was found to be infected before the rains.

In Nkhotakota (IRS), the highest *An. gambiae* s.l. SR was observed outdoors in September (8.0%) and no *An. gambiae* s.l. was positive after spraying (November–March).

In Salima (PBO net distribution), the highest *An. gambiae* s.l. SRs were recorded in December both indoors (6.7%) and outdoors (6.5%).

In Kasungu (standard net distribution), no sporozoite-positive *An. gambiae* s.l. was found during the sampling period (July–March).

In Mangochi (IRS), no sporozoite-positive *An. gambiae* s.l. was recorded before spraying (July–October). However, infected *An. gambiae* s.l. were recorded after spraying, in March 2020: 5.6% indoors and 1.0% outdoors.

The overall *An. funestus* s.l. SR from the five districts was 5.0% (n=219), 6.8% (n=146) indoors and 1.4% (n=73) outdoors.

No *An. funestus* s.l. was found to be infected in Nkhata Bay during the sampling period (July–March) as the density of this species in this district was very low.

In Nkhotakota, the highest *An. funestus* s.l. SRs were recorded before spraying (in September): 17.1% indoors and 6.0% outdoors. No *An. funestus* s.l. was found to be infected after spraying (November–March) as the density of this species in this district was very low.

In Kasungu, the highest *An. funestus* s.l. SRs were recorded during the rainy season: 7.7% indoors (December) and 4.0% outdoors (March).

A total of 360 *An. coustani* (26.7% from indoor and 73.3% from outdoor collections) were tested for infection and no sporozoite-positive *An. coustani* was found in any of the five districts.

B) SRs FROM PSC COLLECTIONS (JULY 2019–MARCH 2020)

Table 12 summarizes the SR of *An. gambiae* s.l. and *An. funestus* s.l. collected from PSCs. A total of 1,124 *An. gambiae* s.l. were tested for *Pf* infection. The overall *An. gambiae* s.l. SR from all seven districts was 0.6%. The highest *An. gambiae* s.l. SR (3.1%) was recorded in Nkhata Bay District. No *An. gambiae* s.l. SRs were recorded in Kasungu, Salima, Mangochi, and Chikwawa districts.

A total of 594 *An. funestus* s.l. were tested for *Pf* infection. The overall *An. funestus* s.l. SR from the seven districts was 6.5%. High *An. funestus* s.l. SRs were recorded in Salima (15.4%) and Nkhotakota (13.1%). No sporozoite-positive *An. funestus* s.l. was recorded in Karonga, Mangochi, and Chikwawa districts. All 35 *An. coustani* collected from Nkhotakota tested negative for *Pf* infection.

C) SRs AND EIRs OF AN. GAMBIAE S.L. AND AN. FUNESTUS S.L. FROM CDC-LT COLLECTIONS (JULY 2019– MARCH 2020)

Table 13 summarizes the SRs and monthly EIRs of *An. gambiae* s.l. and *An. funestus* s.l. estimated from CDC-LTs collections over a 6-9 months period in the seven districts.

The estimated risk of malaria transmission for the 9 months (sum of monthly EIR for 9 months) was highest in Nkhotakota district, 165 infective bites/person/9 months (ib/p/9m) (*An. funestus* s.l. = 139 ib/p/9m) and *An. gambaie* s.l. = 26 ib/p/9m). However, the highest EIRs in this district were recorded before spraying in July (17 ib/p/m), August (86 ib/p/m) and September (25 ib/p/m). The second highest EIR was recorded in Chikwawa district (94 ib/p/9m; 8.40 ib/p/9m from *An. gambaie* s.l. and 85.38 ib/p/9m from*An. funestus* s.l.) followed by Kasungu district (83 ib/p/9m; *An. gambaie* s.l. = 49 ib/p/9m and *An. funestus* s.l. = 34 ib/p/9m), Mangochi district (21 ib/p/6m; *An. gambaie* s.l. = 11 ib/p/6m and *An. funestus* s.l. = 10 ib/p/6m), Karonga (13 ib/p/9m; *An. gambaie* s.l. = 13 ib/p/9m and *An. funestus* s.l. = no infective bites), Salima district (7.7 ib/p/9m; *An. gambaie* s.l. = 7.1 ib/p/9m and *An. funestus* s.l. = 0.6 ib/p/9m) and Nkhata-bay (6.8 ib/p/9m; *An. gambaie* s.l. = 2.3 ib/p/9m and *An. funestus* s.l. = 5.3 ib/p/9m).

In the IRS districts, the estimated risk of malaria transmission over the 9 months period was higher in Nkhotakota (165 ib/p/9m) than in Mangochi district (21 ib/p/9m). In Nkhotakota, monthly EIRs from *An. funestus* s.l. were higher before spray (86 ib/p/m recorded in August) than after spray (22 ib/p/m recorded in January). Similarly in Mangochi, *An. funestus* s.l. monthly EIRs were higher before spraying (14 ib/p/m in October) than after spraying (7.9 ib/p/m in February). However, in these districts, higher monthly EIRs from *An. gambiae* s.l. were recorded after spraying than before spraying; In Nkhotakota, 17 ib/p/m were recorded in January after spraying compared to 4.1 ib/p/m in September before spraying. Similarly, in Mangochi, 7.9 ib/p/m were recorded in February after spraying compared to 3.4 ib/p/m recorded in October before spraying.

In non-IRS districts, there was variation in the monthly EIRs of *Anopheles* mosquitoes. Overall, the highest estimated risk of malaria transmission over the 9 months period was observed in standard net (SN) distribution districts compared to piperonyl butoxide (PBO) net distribution districts. Two SN distribution districts, Chikwawa (94 ib/p/9m) and Kasungu (83 ib/p/9m) recorded high EIRs but the EIR in Nkhata-bay (6.78 ib/p/9m) was low. Both PBO net distribution districts, Karonga (13 ib/p/9m) and Salima (7.7 ib/p/9m) recorded low EIRs. In Karonga (PBO), Nkhata-Bay (SN), Kasungu (SN) and Salima (PBO), high monthly EIRs from *An. gambiae* s.l. were recorded during the rainy season than the dry season while in Chikwawa (SN) high EIRs from *An. gambaie* s.l. were recorded in the dry season than the rainy season. In SN districts of Kasungu and Chikwawa and Salima (PBO) district, high monthly EIRs from *An. funestus* s.l. were observed both in dry and rainy season while as in Karonga (PBO) district no sporozite infections were detected from this species in both dry and rainy season.

TABLE 10: RATE OF *PLASMODIUM FALCIPARUM* INFECTION IN *AN. GAMBIAE* S.L. BY LOCATION FROM HLC COLLECTIONS, JULY 2019–MARCH 2020

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	8	0	0.0	13	0	0.0	21	0	0.0
	December	17	1	5.9	18	1	5.6	35	2	5.7
	March	42	1	2.4	29	1	3.4	71	2	2.8
Nkhotakota	September	23	0	0.0	25	2	8.0	48	2	4.2
	December	16	0	0.0	44	0	0.0	60	0	0.0
	March	53	0	0.0	85	0	0.0	138	0	0.0
Salima	September	1	0	0.0	1	0	0.0	2	0	0.0
	December	15	1	6.7	31	2	6.5	46	3	6.5
	March	13	0	0.0	39	0	0.0	52	0	0.0
Kasungu	September	0	0	0.0	0	0	0.0	0	0	0.0
	December	4	0	0.0	6	0	0.0	10	0	0.0
	March	28	0	0.0	27	0	0.0	55	0	0.0
Mangochi	September	0	0	0.0	0	0	0.0	0	0	0.0
	December	0	0	0.0	0	0	0.0	0	0	0.0
	March	18	1	5.6	14	0	0.0	32	1	3.1
Total		**238**	**4**	**1.7**	**332**	**6**	**1.8**	**570**	**10**	**1.8**

TABLE 11: RATE OF *PLASMODIUM FALCIPARUM* INFECTION IN *AN. FUNESTUS* S.L. BY LOCATION FROM HLC COLLECTIONS BY DISTRICT, JULY 2019–MARCH 2020

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	7	0	0.0	10	0	0.0	17	0	0.0
	December	0	0	0.0	2	0	0.0	2	0	0.0
	March	0	0	0.0	0	0	0.0	0	0	0.0
Nkhotakota	September	35	6	17.1	6	0	0.0	41	6	14.6
	December	0	0	0.0	2	0	0.0	2	0	0.0
	March	3	0	0.0	1	0	0.0	4	0	0.0
Salima	September	0	0	0.0	0	0	0.0	0	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
Kasungu	September	0	0	0.0	0	0	0.0	0	0	0.0
	December	13	1	7.7	1	0	0.0	14	1	7.1
	March	82	3	3.7	43	1	2.3	125	4	3.2
Mangochi	September	0	0	0.0	0	0	0.0	0	0	0.0
	December	6	0	0.0	7	0	0.0	13	0	0.0
	March	0	0	0.0	0	0	0.0	0	0	0.0
Total		**146**	**10**	**6.8**	**73**	**1**	**1.4**	**219**	**11**	**5.0**

TABLE 12: SRS OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. FROM PSC COLLECTIONS BY DISTRICT

District	*An. gambiae* s.l.			*An. funestus* s.l.		
	Total No. tested	No. sporozoite positive	Sporozoite Rate (%)	Total No. tested	No. sporozoite positive	Sporozoite Rate (%)
Karonga	640	2	0.3	1	0	0.0
Nkhata Bay	66	2	3.1	99	2	2.0
Nkhotakota	314	3	1.0	45	6	13.3
Kasungu	8	0	0.0	311	31	9.7
Salima	50	0	0.0	13	2	15.4
Mangochi	3	0	0.0	29	0	0.0
Chikwawa	43	0	0.0	96	0	0.0
Total	1124	7	0.6	594	41	6.5

TABLE 13: SRS AND EIRS OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. FROM CDC-LT COLLECTIONS BY DISTRICT, JULY 2019–MARCH 2020

Species	Indicators	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total EIR/9 or 6 months
Karonga											
An. gambaie s.l.	Total No. tested	36	103	205	136	1	10	195	360	170	
	No. sporozoite positive	0	0	1	0	0	2	2	1	1	
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.20	0.01	0.00	0.01	
	HBR/night	0.4	23.1	6.95	0.75	0.05	0.5	9.55	20.4	23.75	
	Monthly EIR	0.0	0.0	1.0	0.0	0.0	3.0	3.0	1.7	4.2	13.02
An. funestus s.l.	Total No. tested	2	1	1	3	2	1	4	5	2	1
	No. sporozoite positive	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	
	HBR/night	0.9	0.3	0.5	0.15	0.1	0.05	0.2	0.05	0.05	
	Monthly EIR	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
	Total monthly EIR	0.0	0.0	1.0	0.0	0.0	3.0	3.0	1.7	4.2	13.02
Nkhata-Bay											
An. gambaie s.l.	Total No. tested	10	17	48	18	30	48	90	60	77	
	No. sporozoite positive	0	0	2	1	1	2	1	1	2	
	Sporozoite rate	0.00	0.00	0.04	0.06	0.03	0.04	0.01	0.02	0.03	
	HBR/night	0.67	1.13	0.35	0.4	2	0.35	5.1	0.6	2.05	
	Monthly EIR	0.0	0.0	0.4	0.4	0.4	0.4	1.7	0.3	1.6	5.33
An. funestus s.l.	Total No. tested	11	20	57	4	1	23	76	1	4	
	No. sporozoite positive	0	0	0	0	0	2	0	0	0	
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	
	HBR/night	1.95	0.7	1.15	0.25	0.2	0.85	2.8	0.25	1.55	
	Monthly EIR	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	2.25
	Total monthly EIR	0.0	0.0	0.4	0.4	0.4	2.7	1.7	0.3	1.6	7.78

Species	Indicators	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total EIR/9 or 6 months
Nkhotakota											
An. gambaie s.l.	Total No. tested	4	7	87	46	23	157	202	406	50	
	No. sporozoite positive	0	0	3	0	1	0	6	2	0	
	Sporozoite rate	0.00	0.00	0.03	0.00	0.04	0.00	0.03	0.00	0.00	
	HBR/night	4.5	13.1	3.9	2.07	0.13	4.93	18.77	33.43	14.87	
	Monthly EIR	0.0	0.0	4.1	0.0	0.2	0.0	16.9	5.0	0.0	26.22
An. funestus s.l.	Total No. tested	100	164	55	54	8	3	69	60	7	
	No. sporozoite positive	2	26	6	4	1	0	5	0	0	
	Sporozoite rate	0.02	0.16	0.11	0.07	0.13	0.00	0.07	0.00	0.00	
	HBR/night	27.53	17.8	6.2	1.8	1.8	0.03	2.2	1.5	1.0	
	Monthly EIR	16.7	85.8	20.5	4.1	6.8	0.0	4.8	0.0	0.0	138.72
	Total monthly EIR	16.7	85.8	24.5	4.1	7.0	0.0	21.8	5.0	0.0	164.94
Salima											
An. gambaie s.l.	Total No. tested	3	4	8	2	11	131	132	74	67	
	No. sporozoite positive	0	0	0	0	0	3	2	0	0	
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00	
	HBR/night	0.05	0.07	0.05	0.05	0.1	6.5	5.55	5.55	0.75	
	Monthly EIR	0.0	0.0	0.0	0.0	0.0	4.5	2.6	0.0	0.0	7.08
An. funestus s.l.	Total No. tested	2	5	1	4	2	1	4	1	22	
	No. sporozoite positive	0	2	0	0	0	0	0	0	0	
	Sporozoite rate	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	HBR/night	0.1	0.05	0.05	0.1	0.1	0.25	0.1	0.05	1.1	
	Monthly EIR	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.61
	Total monthly EIR	0.0	0.6	0.0	0.0	0.0	4.5	2.6	0.0	0.0	7.69
Kasungu											
An. gambaie s.l.	Total No. tested	1	2	1	3	7	21	14	32	70	
	No. sporozoite positive	0	0	0	0	0	2	1	2	1	
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.10	0.07	0.06	0.01	
	HBR/night	0.5	0.1	0.05	0.05	0.05	0.55	3.95	19.95	1.75	
	Monthly EIR	0.0	0.0	0.0	0.0	0.0	1.6	8.6	37.9	0.8	48.83
An. funestus s.l.	Total No. tested	19	7	52	47	129	150	22	57	214	
	No. sporozoite positive	0	2	1	0	20	19	0	1	6	
	Sporozoite rate	0.00	0.29	0.02	0.00	0.16	0.13	0.00	0.02	0.03	
	HBR/night	0.2	0.6	0.5	0.75	0.9	1.8	6	17.7	9.35	
	Monthly EIR	0.0	5.2	0.0	0.0	4.2	6.9	0.0	9.4	8.0	33.79
	Total monthly EIR	0.0	5.2	0.0	0.0	4.2	8.5	8.6	47.3	8.7	82.63
Mangochi											
An. gambaie s.l.	Total No. tested	**	**	**	9	3	2	12	75	91	
	No. sporozoite positive				2	0	0	0	1	0	
	Sporozoite rate				0.22	0.00	0.00	0.00	0.01	0.00	
	HBR/night	**	**	**	0.5	0.15	0.1	7.6	19.45	5.7	
	Monthly EIR				3.4	0.0	0.0	0.0	7.9	0.0	11.26
An. funestus s.l.	Total No. tested	**	**	**	91	74	17	1	24	3	
	No. sporozoite positive				5	0	0	0	0	0	
	Sporozoite rate				0.05	0.00	0.00	0.00	0.00	0.00	
	HBR/night	**	**	**	6.05	3.45	0.5	0.45	5.95	0.7	
	Monthly EIR				10.1	0.0	0.0	0.0	0.0	0.0	10.11
	Total monthly EIR				13.5	0.0	0.0	0.0	7.9	0.0	21.37

Species	Indicators	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total EIR/9 or 6 months
Chikwawa											
An. gambaie s.l.	Total No. tested	124	12	4	34	26	20	8	11	1	
	No. sporozoite positive	2	0	29	2	2	0	0	0	0	
	Sporozoite rate	0.02	0.00	0.00	0.06	0.08	0.00	0.00	0.00	0.00	
	HBR/night	6.2	2.1	0.1	1.3	1.3	1.35	1.75	1.35	0.95	
	Monthly EIR	3.0	0.0	0.0	2.3	3.0	0.0	0.0	0.0	0.0	8.40
An. funestus s.l.	Total No. tested	105	38	88	104	8	8	94	78	5	
	No. sporozoite positive	2	3	0	0	0	0	2	2	0	
	Sporozoite rate	0.02	0.08	0.00	0.00	0.00	0.0	0.02	0.03	0.00	
	HBR/night	5	14.25	14.65	4.75	0.35	1.2	31.2	36.05	1.95	
	Monthly EIR	2.9	34.2	0.0	0.0	0.0	0.0	20.2	28.1	0.0	85.38
	Total monthly EIR	5.9	34.2	0.0	2.3	3.0	0.0	20.2	28.1	0.0	93.78

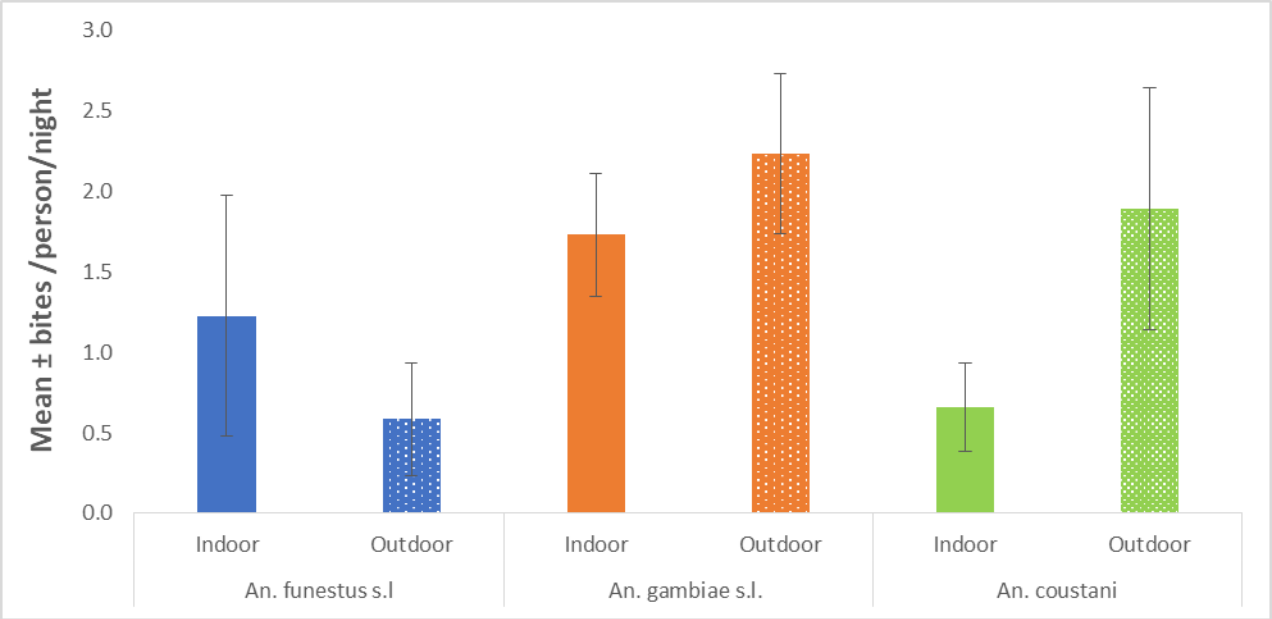
HBR/Person/Night = Number of female *Anophele gambiae* s.l. + An. *funestus* s.l. /trap/night

**No entomological activities were conducted; sites were established in October 2019.

3.4 Biting Rates of Malaria Vectors

HBR was measured in five districts: Nkhata Bay, Nkhotakota, Salima, Kasungu, and Mangochi. Overall, the HBRs for *An. funestus* s.l. were 1.2 bites per person per night (b/p/n) indoors and 0.6 b/p/n outdoors (Figure 16). The HBRs for *An. gambiae* s.l. were 1.7 b/p/n indoors and 2.2 b/p/n outdoors and for *An. coustani* 0.7 indoors and 1.9 b/p/n outdoors.

Figure 16: Average Bites of *Anopheles* Mosquitoes Per Person Per Night in Five Districts



The highest biting activity of *An. funestus* s.l. was recorded in Kasungu District in March, during the rainy season; the indoor HBR was 10.1 b/p/n and the outdoor 5.8 b/p/n (Table 14). The highest *An. gambiae* s.l. indoor biting activity was observed in Nkhata Bay (5.5 b/p/n) and the highest outdoor biting activity was in Nkhotakota (5.5 b/p/n) in March, during the rainy season. The highest biting activity of *An. coustani* was observed indoors in Nkhotakota (4.7 b/p/n) and outdoors in Kasungu (10.9 b/p/n), once again in March, during the rainy season (Table A-14 and Annex A).

TABLE 14: HBRS (B/P/N) OF *ANOPHELES* MOSQUITOES IN FIVE DISTRICTS, JULY 2019–MARCH 2020

District	Month	*An. funestus* s.l.		*An. gambiae* s.l.		*An. coustani*	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Nkhata Bay	September	0.8	0.4	1.4	2.3	1.4	0.9
	December	0.4	0.4	2.1	2.6	0.4	1.0
	March	0.0	0.0	5.5	3.8	0.6	1.6
Nkhotakota	September	2.4	0.4	1.3	1.8	0.1	0.2
	December	0.0	0.1	0.6	1.4	0.0	0.2
	March	0.3	0.1	3.4	5.5	4.7	9.2
Salima	September	0.0	0.0	0.1	0.0	0.0	0.0
	December	0.0	0.0	2.3	3.1	0.0	0.0
	March	0.0	0.1	1.9	5.3	0.1	0.5
Kasungu	September	0.8	0.0	0.1	0.0	0.3	0.5
	December	1.5	0.1	0.6	0.8	0.0	0.4
	March	10.1	5.8	3.0	3.4	1.5	10.9
Mangochi	December	0.8	0.8	0.0	0.0	0.0	0.5
	March	0.3	0.1	1.5	1.5	0.3	0.5
Overall		1.2	0.6	1.7	2.2	0.7	1.9

3.5 BITING PATTERN OF MALARIA VECTORS

Figure 17 shows the biting pattern of *An. funestus* s.l. and *An. gambiae* s.l. in the five districts of Nkhata Bay, Nkhotakota (two sites), Salima, Kasungu, and Mangochi collected quarterly from July 2019 to March 2020.

In Nkhata Bay District, the human biting activity of *An. gambiae* s.l. occurred from dawn to dusk both indoors and outdoors with three outdoor peaks: the major one occurred between 11:00 pm and midnight and the two smaller ones occurred at 1:00–2:00 am and 5:00–6:00 am. The indoor biting activity showed one major peak at 6:00–7: 00 am and three smaller peaks at 6:00–7:00 pm, 12:00–1:00 am, and 3:00–4:00 am. The biting activity of *An. funestus* s.l. in Nkhata Bay was very low but occurred throughout the night to early in the morning with a single smaller indoor biting peak at 1:00–2:00 am. The outdoor biting activity showed three smaller peaks, at 1:00–2:00 am, 5:00–6:00 am, and 8:00–9:00 am.

In Nkhotakota District, the biting activity of *An. gambiae* s.l. both indoors and outdoors occurred from dawn to dust. The biting was observed from 6: 00 pm throughout the night to 6:00 amThe biting activity of *An. funestus* s.l. in Nkhotakota occurred from early evening (6:00 pm) to late morning (11:00

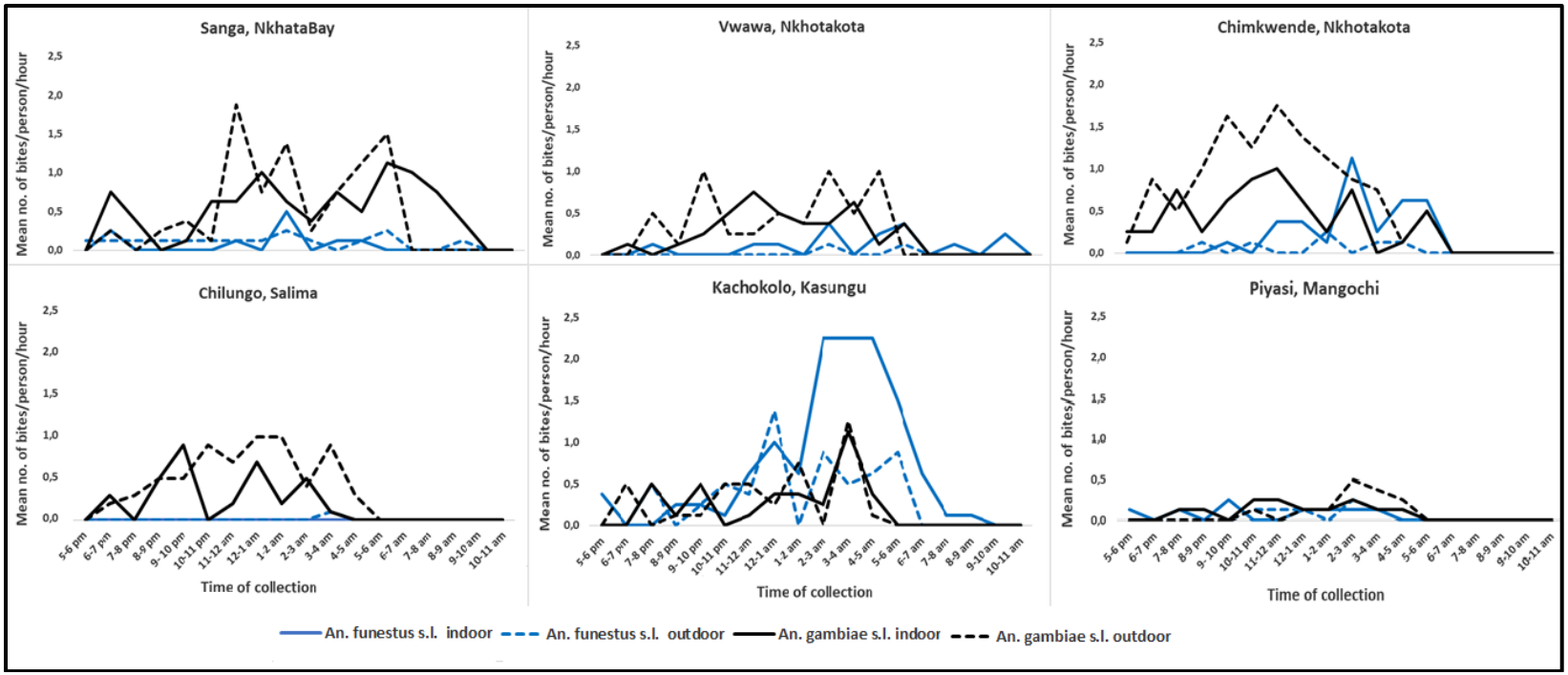
am). This species showed one major indoor biting peak, at 2:00–3:00 am, and four smaller peaks between 4:00 am and 11:00 am.

In Salima District, the biting activity of *An. gambiae* s.l. occurred from dawn to dusk. The outdoor biting activity of this species showed three distinct peaks: one before midnight, at 10:00–11:00 pm and two after midnight, at 12:00–2:00 am and 3:00–4:00 am. The indoor biting activity of this species also showed three peaks: one before midnight, at 9:00–10:00 pm, and the other two after midnight at 12:00–1:00 am and 2:00–3:00 am. The indoor and outdoor biting of *An. funestus* s.l. in this district was very low with no distinct peaks.

In Kasungu District, the biting activity of *An. gambiae* s.l. occurred from dawn to dusk with a one major indoor and outdoor biting peak occurring after midnight between 3:00 and 4:00 am. Two smaller outdoor peaks occurred at 7:00–8:00 pm and 9:00–10:00 pm while three indoor peaks occurred at 6:00–7:00 pm, 10:00–12:00 am and 1:00–2:00 am. The biting activity of *An. funestus* s.l. was higher than that of *An. gambiae* s.l. in this district and occurred from dawn to dusk. This species showed bimodal indoor biting peaks, a major one occurring after midnight at 2:00–5:00 am and a smaller one at 12:00–1:00 am. The outdoor biting activity of this species showed three peaks: a major one at 12:00–1:00 am and two smaller ones at 2:00–3:00 am and 5:00–6:00 am.

In Mangochi District, the biting activity of both *An. gambiae* s.l. and *An. funestus* s.l. was very low but occurred from early evening (5:00 pm) to early morning (6:00 am). *An. gambiae* s.l. showed a single smaller outdoor biting peak at 2:00–3:00 am.

FIGURE 17: AVERAGE HOURLY INDOOR AND OUTDOOR BITING RATES OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. IN SIX SENTINEL SITES, JULY 2019–MARCH 2020. DATA ARE AVERAGED ACROSS ALL COLLECTION PERIODS



3.6 PARITY RATES

The quarterly dissections of *An. funestus* s.l. and *An. gambiae* s.l. from the HLC sites are presented in Table 15. Total number of mosquitoes dissected depended on the number collected. All mosquitoes that were fresh/not brittle were dissected at each sentinel site. Overall, higher proportions of parous females were recorded among *An. funestus* s.l. (81.9%) than among *An. gambiae* s.l. (69.7%) in all five districts. The highest *An. funestus* s.l. parity rates were observed in Nkhata Bay (100%) and Mangochi (100%) despite the IRS intervention in the latter district. High parity rates for *An. gambiae* s.l. were observed in Mangochi (95%) and Kasungu (88%). The lowest *An. funestus* s.l. parity rate was recorded at Chimkwende site in Nkhotakota (59%) while the lowest *An. gambiae* s.l. parity rate was recorded at Chilungo site in Salima (57%).

Figure 18 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) dry vs rainy season in non-IRS districts based on HLC quarterly collection

In IRS district of Nkhotakota, the proportion of parous *An. funestus* s.l. was lower after spraying than befor spraying at Chimkwendebut at Vwawa site parity was slighty hight after spray. No clear trend was observed at Piyasi (Mangochi district) due to low numbers collected before spraying. Slightly higher proportion of parous *An. gambaie* s.l. were collected after spraying than before spraying at Chimkwende site in Nkhotakota but no An. gambiae s.l. was collected before spray at Vwawa (Nkhotakota) and Piyasi (Mangochi).

In non-IRS districts (Nkhata Bay and Kasungu), high proportions of parous *An. funestus* s.l. were collected both before and during the rainy season. For *An. gambiae* s.l. a higher proportion of parous were collected during the dry season than rainy season in all the three districts (Fig 18). However, these results should be interpreted cautiously as the numbers dissected are small. In addition, the dissections were done on the few unfed mosquitoes collected quarterly using HLC and these low numbers do not allow seasonal trend observation and comparison before and after spray over the whole sampling period. Moving forward, the team will use unfed mosquitoes from CDC-LT collections, which caught larger numbers of unfed females and are performed monthly. A preliminary trial in July 2020 showed that mosquitoes can be kept wet by placing a moist towel in the collection cups, making it possible to do ovary dissections in the morning.

TABLE 15: TOTAL NUMBER AND PROPORTION OF PAROUS FEMALE *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY HLC

District	Sentinel Site	*An. funestus* s.l.			*An. gambiae* s.l.		
		# Dissected	Parous	% Parity	# Dissected	Parous	% Parity
Nkhata Bay	Sanga	14	14	100	52	34	65
Nkhotakota	Vwawa	75	57	76	121	84	69
	Chimkwende	22	13	59	83	50	60
Salima	Chilungo	0	0	0	21	12	57
Mangochi	Piyasi	8	8	100	20	19	95
Kasungu	Kachokolo	72	67	93	32	28	88
Total		177	145	81.9	277	193	69.7

FIGURE 18: PROPORTION OF PAROUS FEMALE *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN IRS AND NON-IRS DISTRICTS

3.7 CONE BIOASSAYS

3.7.1 SPRAY QUALITY ASSESSMENT

In Nkhotakota, the assessment found that the spray quality in areas sprayed with Actellic 300CS was satisfactory: 100% mortality was recorded at all sites after 24 hours of observation (Figure 19). Similar results were obtained in areas sprayed with SumiShield 50WG; 100% mortality was recorded among mosquitoes from 16 structures on day 2 of observation. Only one structure recorded <100% mortality on day 5, but mortality was above 91% (Figure 20).

FIGURE 19: MEAN PERCENTAGE MORTALITY AT 24 HOURS AMONG KISUMU STRAIN OF *AN. GAMBIAE* S.S. EXPOSED TO SURFACES SPRAYED WITH ACTELLIC 300CS IN NKHOTAKOTA DISTRICT

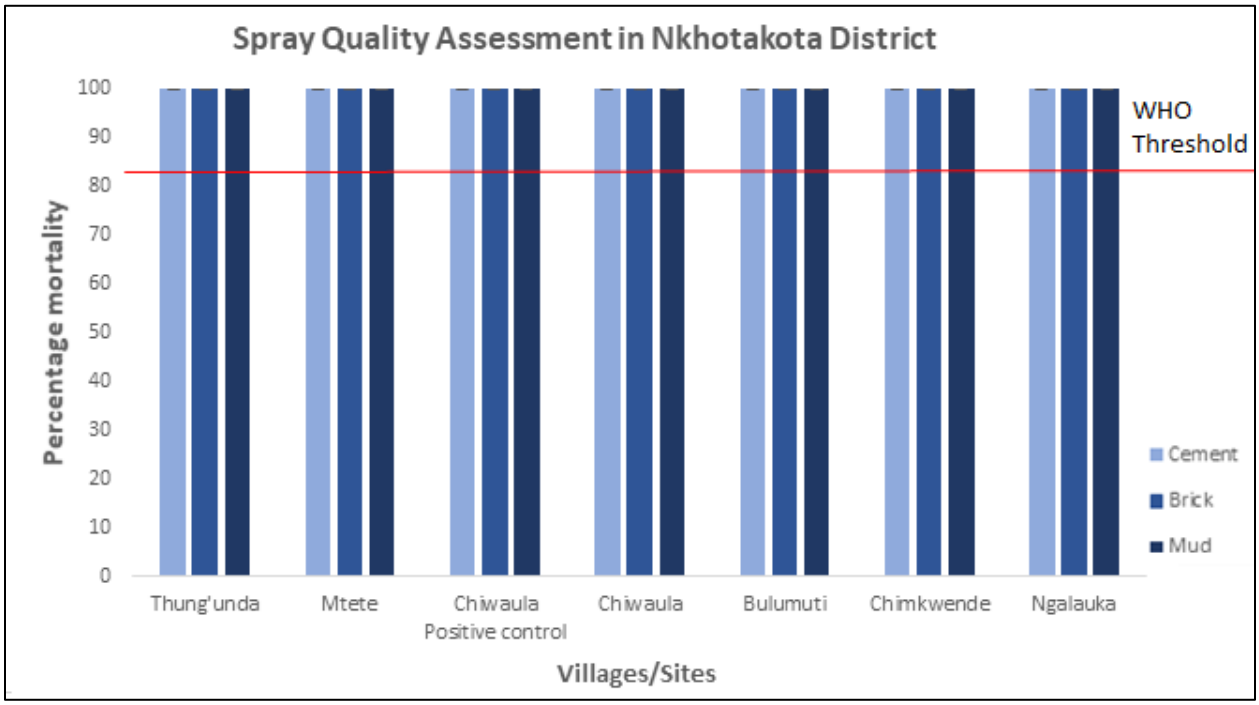
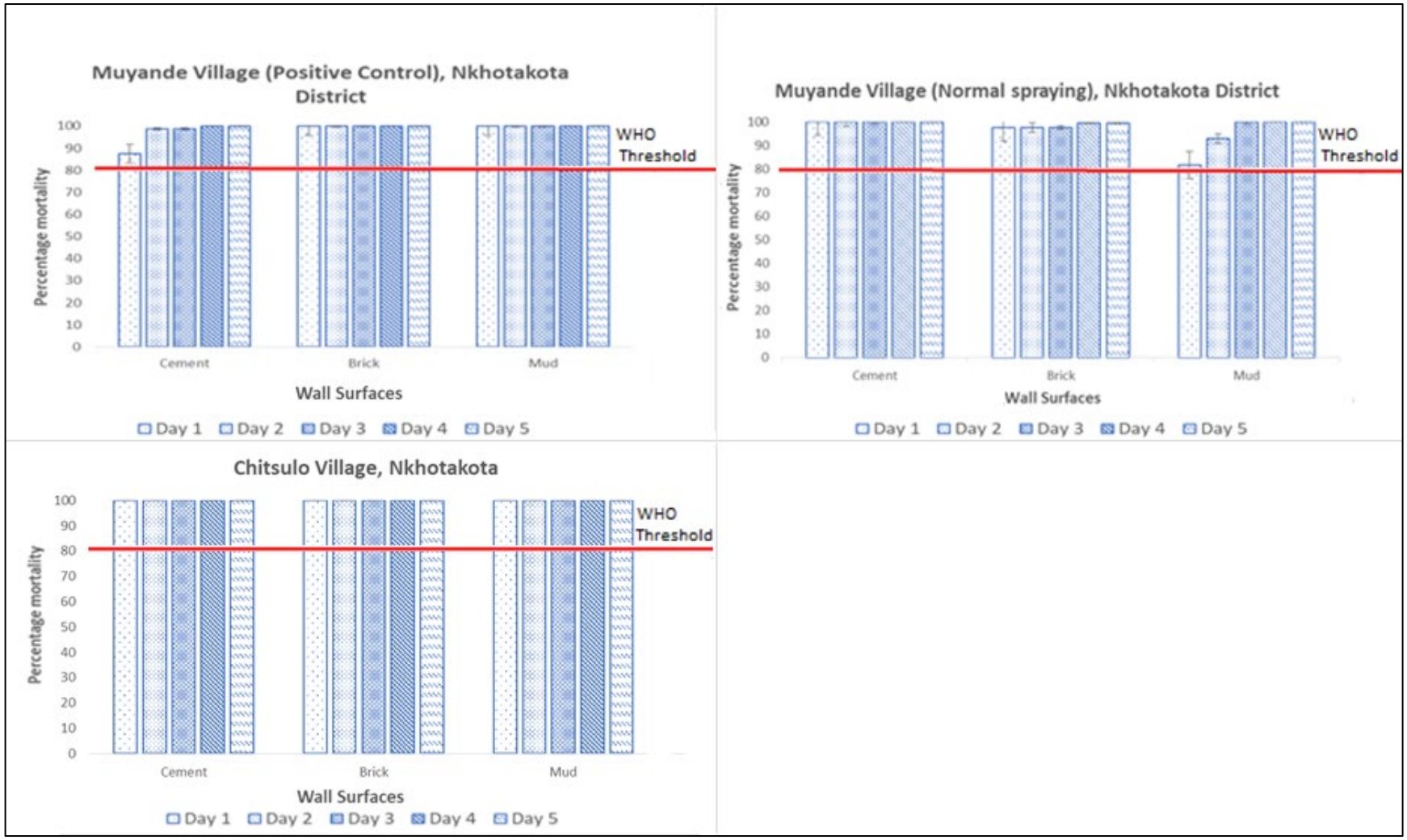


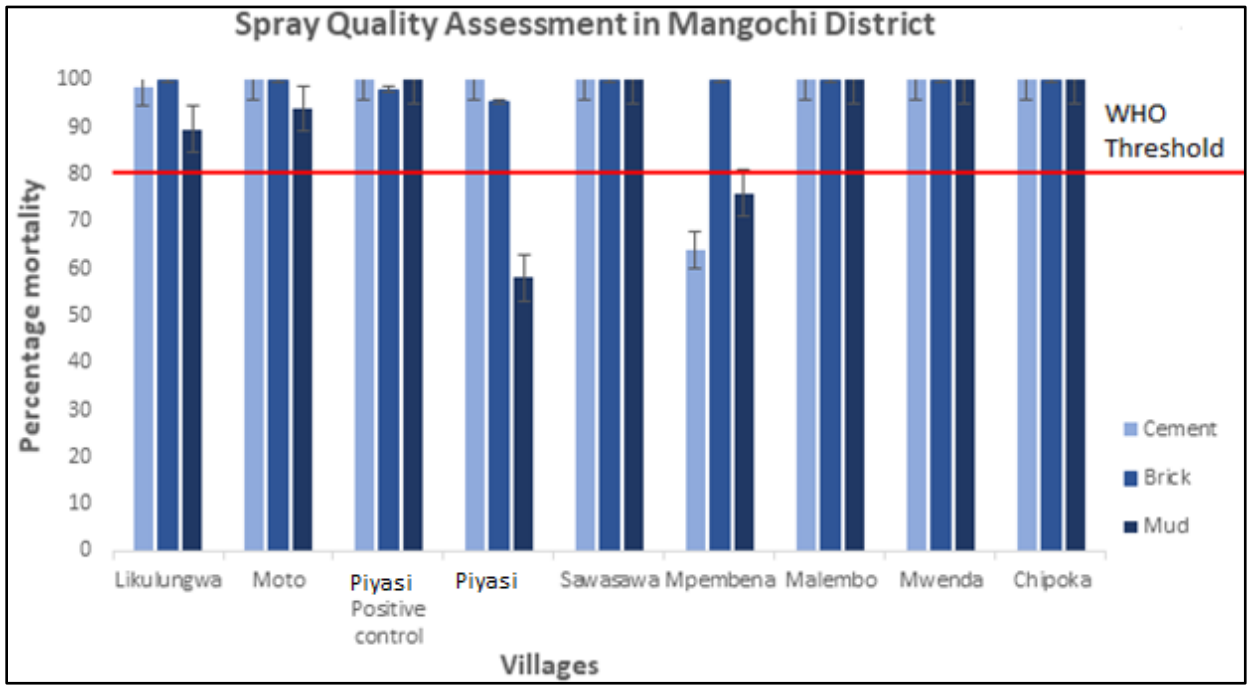
FIGURE 20: MEAN PERCENTAGE MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* S.S. EXPOSED TO POSITIVE CONTROL† SURFACES SPRAYED WITH SUMISHIELD IN NKHOTAKOTA DISTRICT, OBSERVED FOR FIVE DAYS POST-EXPOSURE



Note: To ensure quality of spraying is not associated with residual efficacy, 6 strucures were sprayed under the close supervision of VectorLink Malawi staff as a positive control.

In Mangochi, the spray quality assessment from areas sprayed with Actellic 300CS was satisfactory in all operations sites except Piyasi and Mpembena villages (Figure 21). A total of 54 structures were assessed for spray quality across the district; 18.5% of the structures recorded <100% mortality after 24 hours of observation. Most of the houses with less than 100% were from 2 sites (Piyasi and Mpembena) and spray oprators were retrained befor continuning with the spray campaign.

FIGURE 21: MEAN PERCENTAGE MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* S.S. AT 24 HOURS EXPOSED TO SURFACES SPRAYED WITH ACTELLIC 300CS IN MANGOCHI DISTRICT

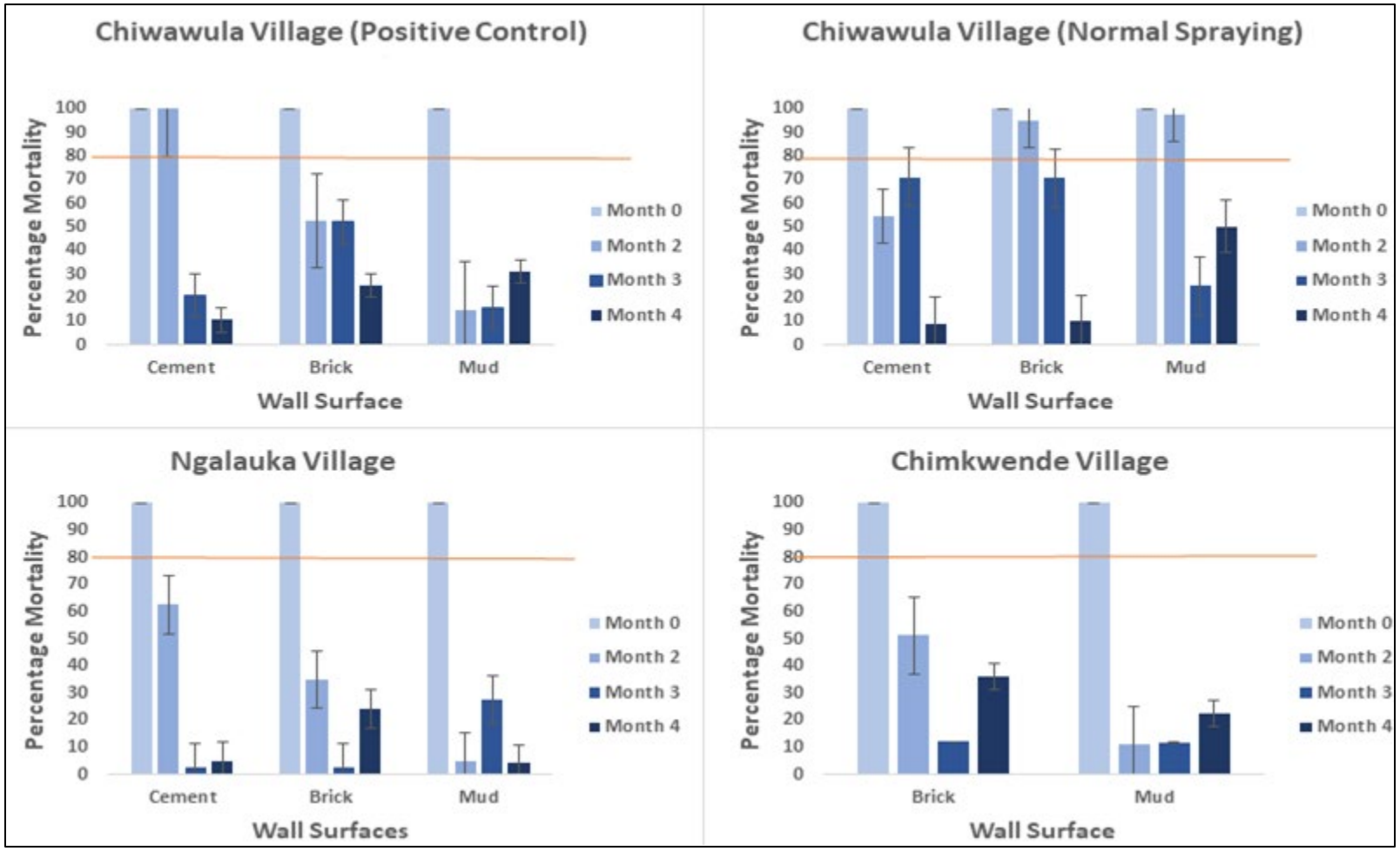


3.7.2 FUMIGATION EFFECT OF ACTELLIC 300CS AND SUMISHIELD 50WG

In Nkhotakota District, the fumigant effect of Actellic 300CS was very high; mortality was 100% on all wall surfaces after 24 hours of observation in all three villages (Figure 22). The fumigant effect of Actellic 300CS (>80% mosquito mortality) lasted two months after spraying in brick and mud structures in Chiwawula village, which received normal spraying (Figure 23). SumiShield 50WG also showed a fumigation effect; 100% mortality was recorded on day 5 in all the structures assessed 24 hours after spraying. Four months after spraying, SumiShield was still showing a fumigation effect in more than 80% of brick structures in the positive control (Figure 23) and for all surface types in the normal sprayed structures (Figure 24).

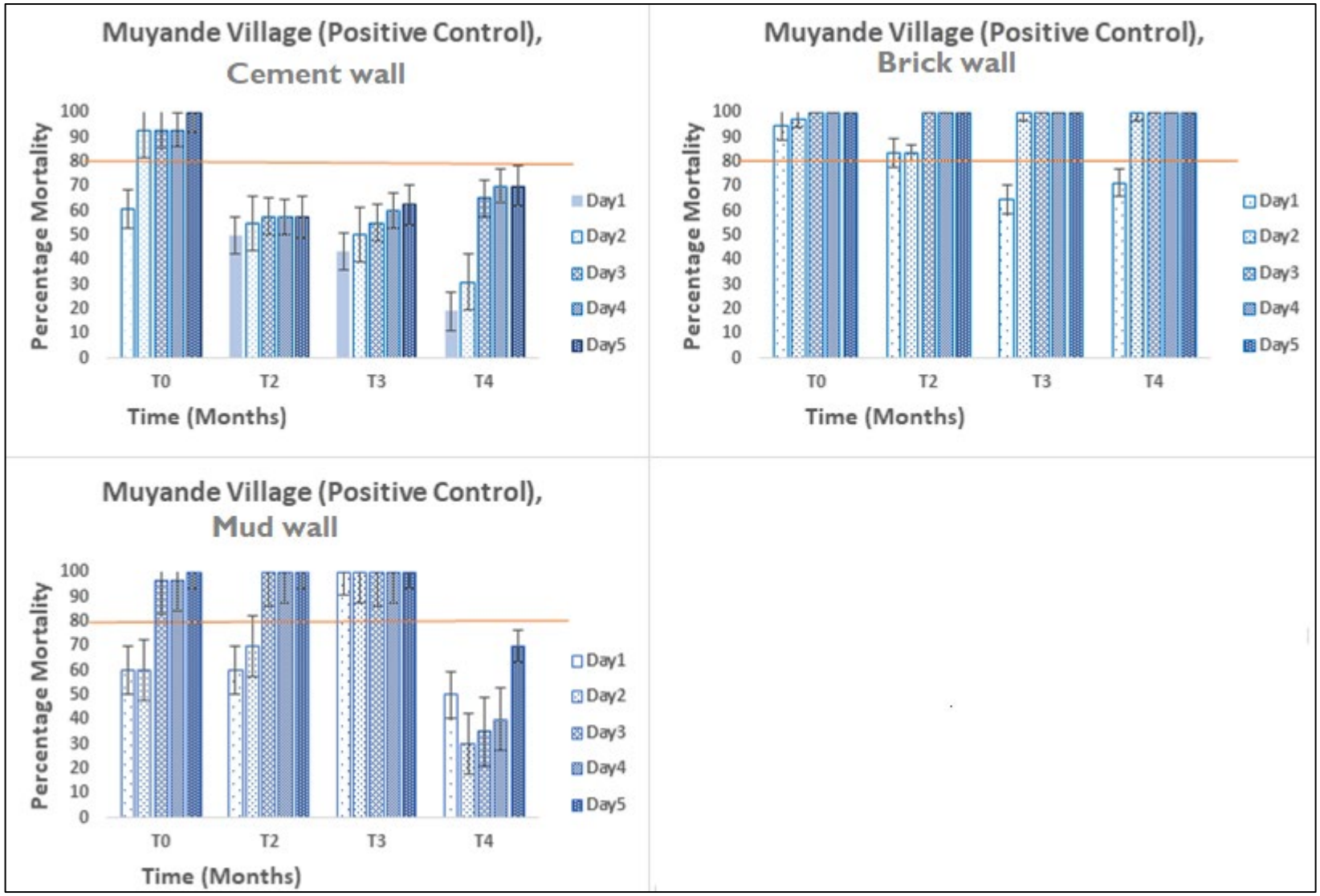
In Mangochi, the fumigant effect of Actellic 300CS was high, above 80% mosquito mortality on all wall surfaces after 24 hours of observation in all four villages except for the normal-spray structures at Piyasi village (Figure 25). The fumigant effect of Actellic 300CS (>80% mosquito mortality) lasted three months after spraying in mud structures at Makokola village.

FIGURE 22: FUMIGATION EFFECT OF ACTELLIC 300CS OBSERVED ON DIFFERENT WALL SURFACES IN NKHOTAKOTA DISTRICT



Note: To ensure quality of spraying is not associated with residual efficacy, 6 strucures were sprayed under the close supervision of VectorLink Malawi staff as a positive control.

FIGURE 23: FUMIGATION EFFECT OF SUMISHIELD OBSERVED IN MUYANDE VILLAGE (POSITIVE CONTROL) IN NKHOTAKOTA DISTRICT



Note: To ensure quality of spraying is not associated with residual efficacy, 6 strucures were sprayed under the close supervision of VectorLink Malawi staff as a positive control.

FIGURE 24: FUMIGATION EFFECT OF SUMISHIELD OBSERVED IN MUYANDE VILLAGE (NORMAL SPRAYING) IN NKHOTAKOTA DISTRICT.

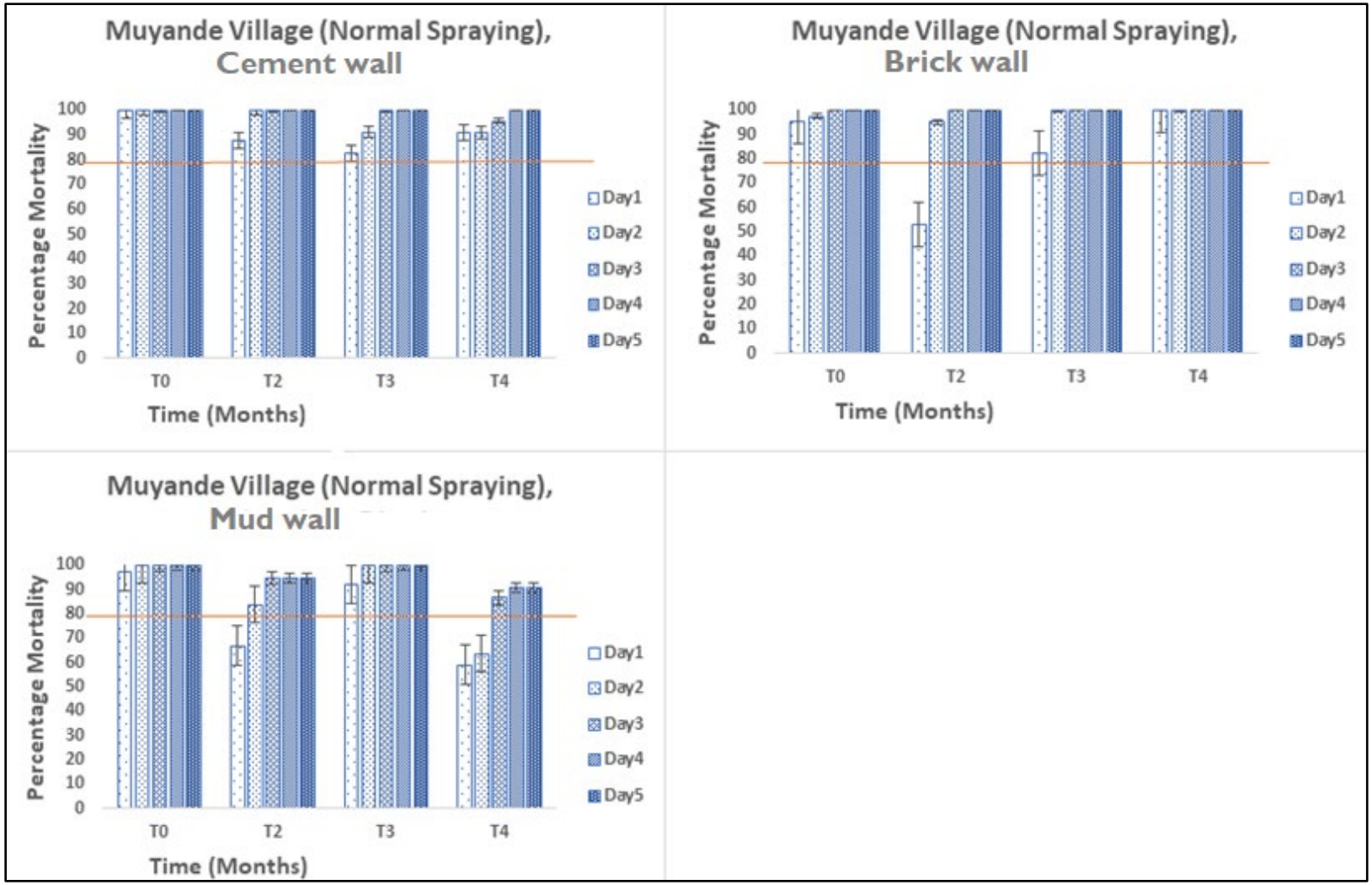
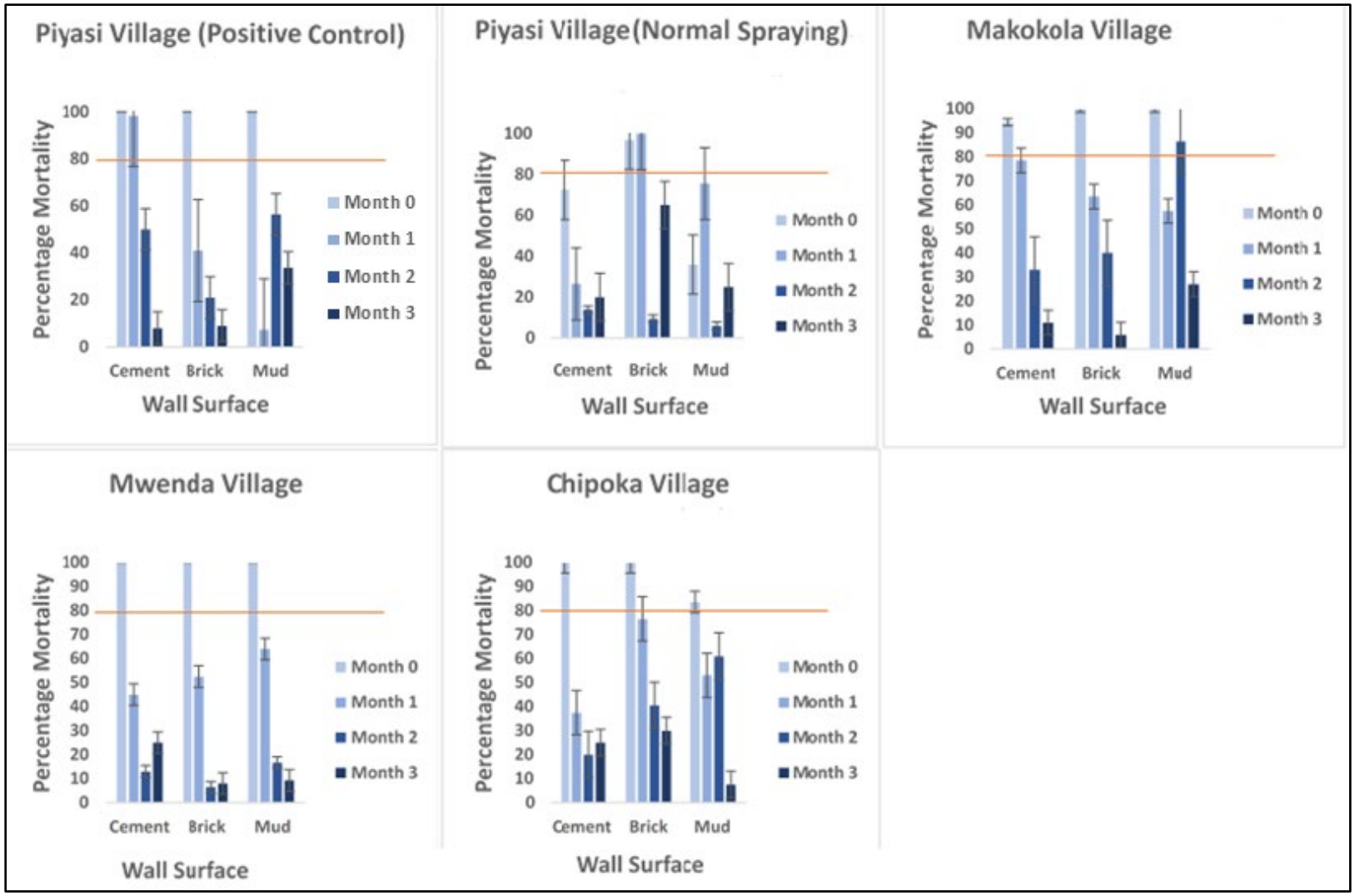


FIGURE 25: FUMIGATION EFFECT OF ACTELLIC 300CS OBSERVED IN DIFFERENT VILLAGES IN MANGOCHI DISTRICT



Note: To ensure quality of spraying is not associated with residual efficacy, 6 strucures were sprayed under the close supervision of VectorLink Malawi staff as a positive control.

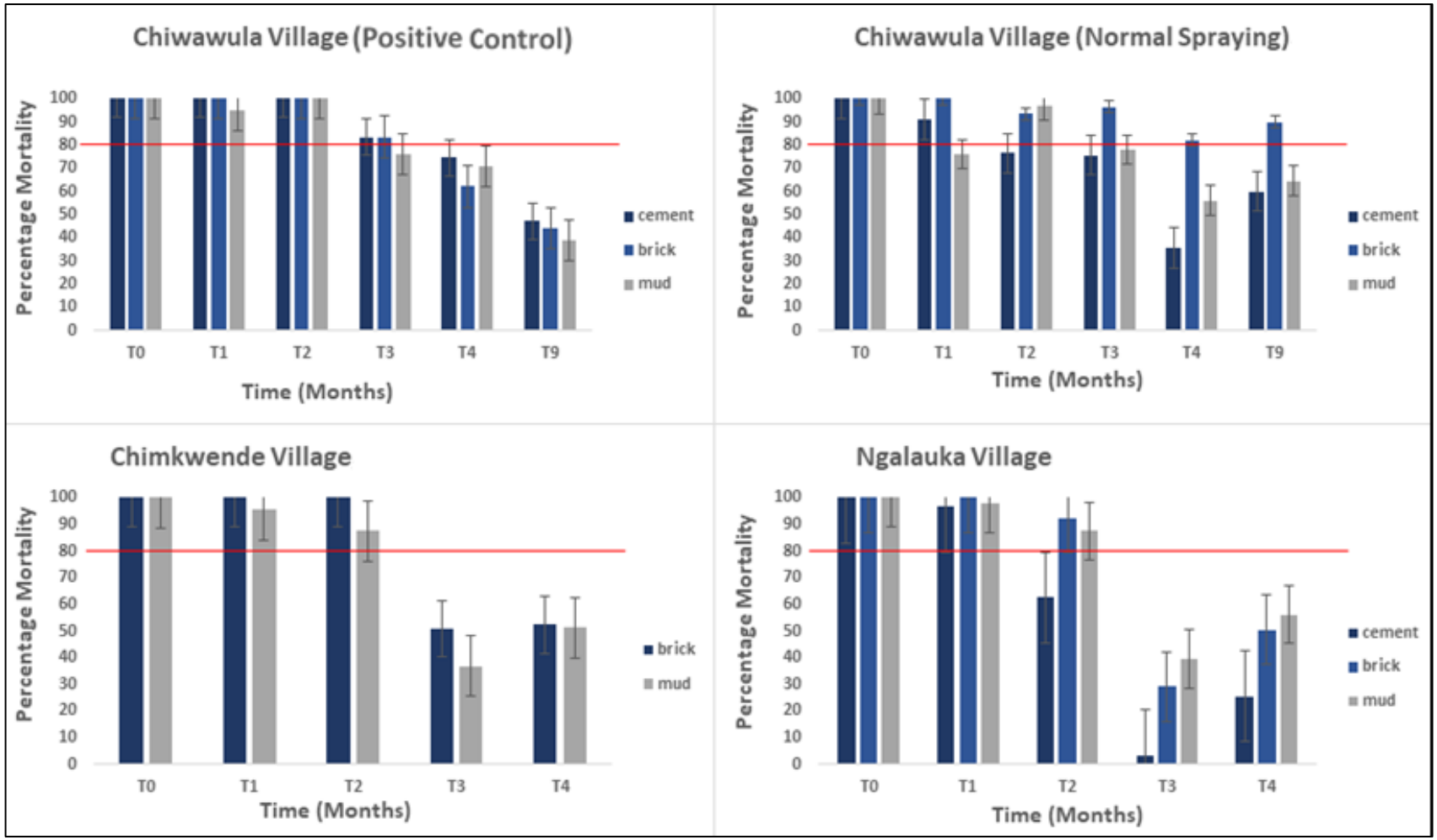
3.7.3 RESIDUAL LIFE OF ACTELLIC 300CS AND SUMISHIELD 50WG

In Nkhotakota, the residual efficacy of Actellic lasted 2–3 months after spraying (Figure 26). One hundred percent mosquito mortality was recorded in all the sites after 24 hours of observation. On average, less than 80% mosquito mortality was recorded between months 1 and 3 in all the villages except Chiwawula (normal spraying), where mosquito mortality was above 80%. At T4 and T9 (4 and 9 months after spraying, respectively), the average mortality rate at Chiwawula village was below 80%.

SumiShield was still effective at T10 (10 months after spraying) and the assessment will continue up to T12. The average mosquito mortality was above 80% threshold in both the positive control (where spraying was closely supervised by vectorlink staff) and the normal sprayed structures. One hundred percent mosquito mortality was observed in the positive control brick wall structures (Figure 27), and normal spraying cement and mud structures (Figure 28).

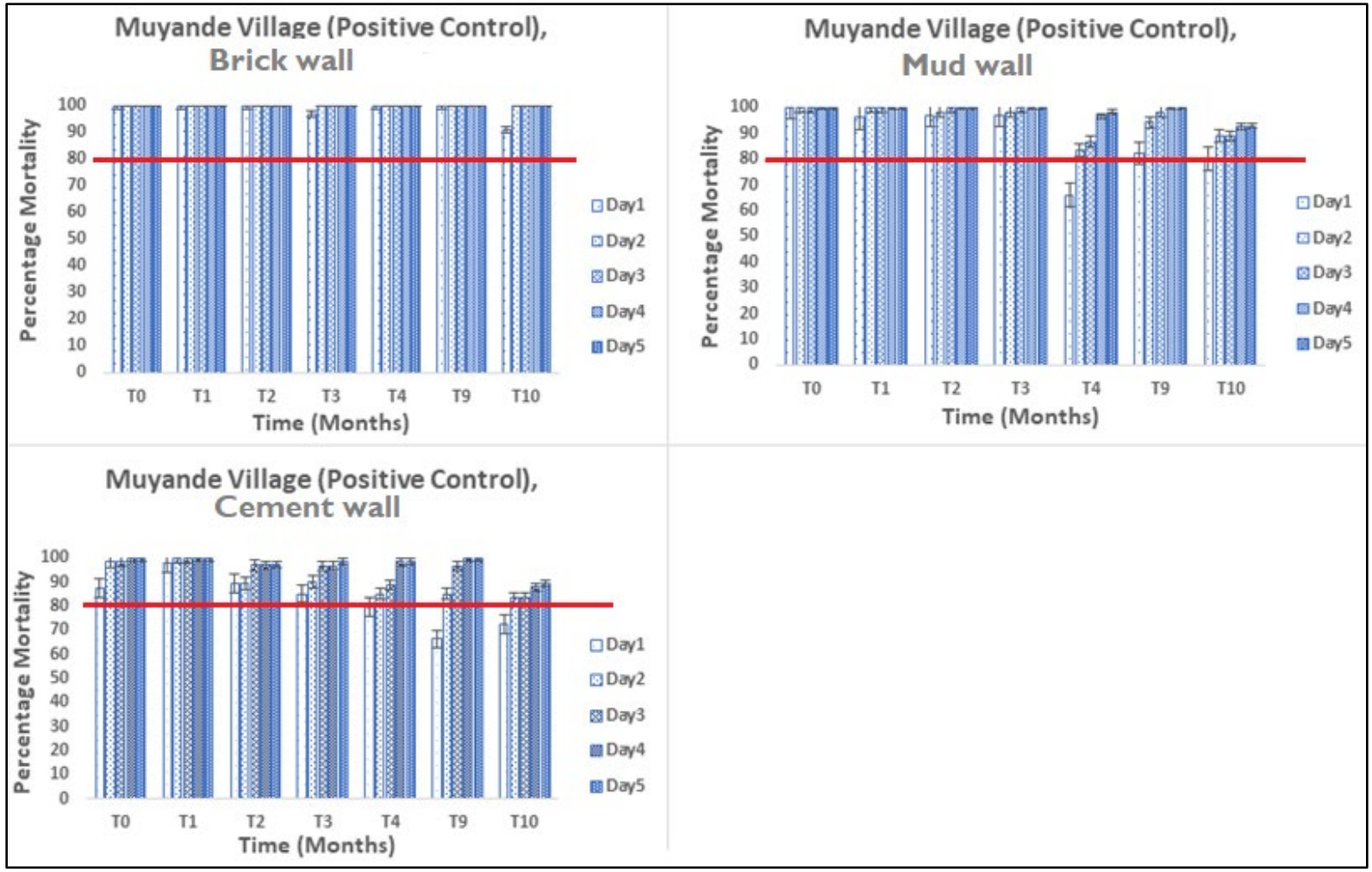
In Mangochi, the residual efficacy of Actellic lasted 2–4 months after spraying in (Figure 29). Average mosquito mortality in Mwenda and Piyasi (positive control – where spraying was closely supervised by Vectorlink staff) villages was less than 80% at T2; at Chipoka and Piyasi (normal spraying), the average mosquito mortality was also less than 80% at T2 but increased to more than 80% at T3 and dropped to less than 80% at T4(Figure 29). Average mortality at T0, T1 and T2 was higher for the control houses than the normal spraying houses.

FIGURE 26: MEAN PERCENTAGE MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* S.S. AT 24 HOURS EXPOSED TO SURFACES SPRAYED WITH ACTELLIC 300CS IN NKHOTAKOTA DISTRICT



Note: To ensure quality of spraying is not associated with residual efficacy, 6 strucures were sprayed under the close supervision of VectorLink Malawi staff as a positive control.

FIGURE 27: MEAN PERCENTAGE MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* S.S. EXPOSED TO POSITIVE CONTROL SURFACES SPRAYED WITH SUMISHIELD IN NKHOTAKOTA DISTRICT



Note: To ensure quality of spraying is not associated with residual efficacy, 6 strucures were sprayed under the close supervision of VectorLink Malawi staff as a positive control.

FIGURE 28: MEAN PERCENTAGE MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* S.S. EXPOSED TO NORMAL SPRAY SURFACES SPRAYED WITH SUMISHIELD IN NKHOTAKOTA DISTRICT

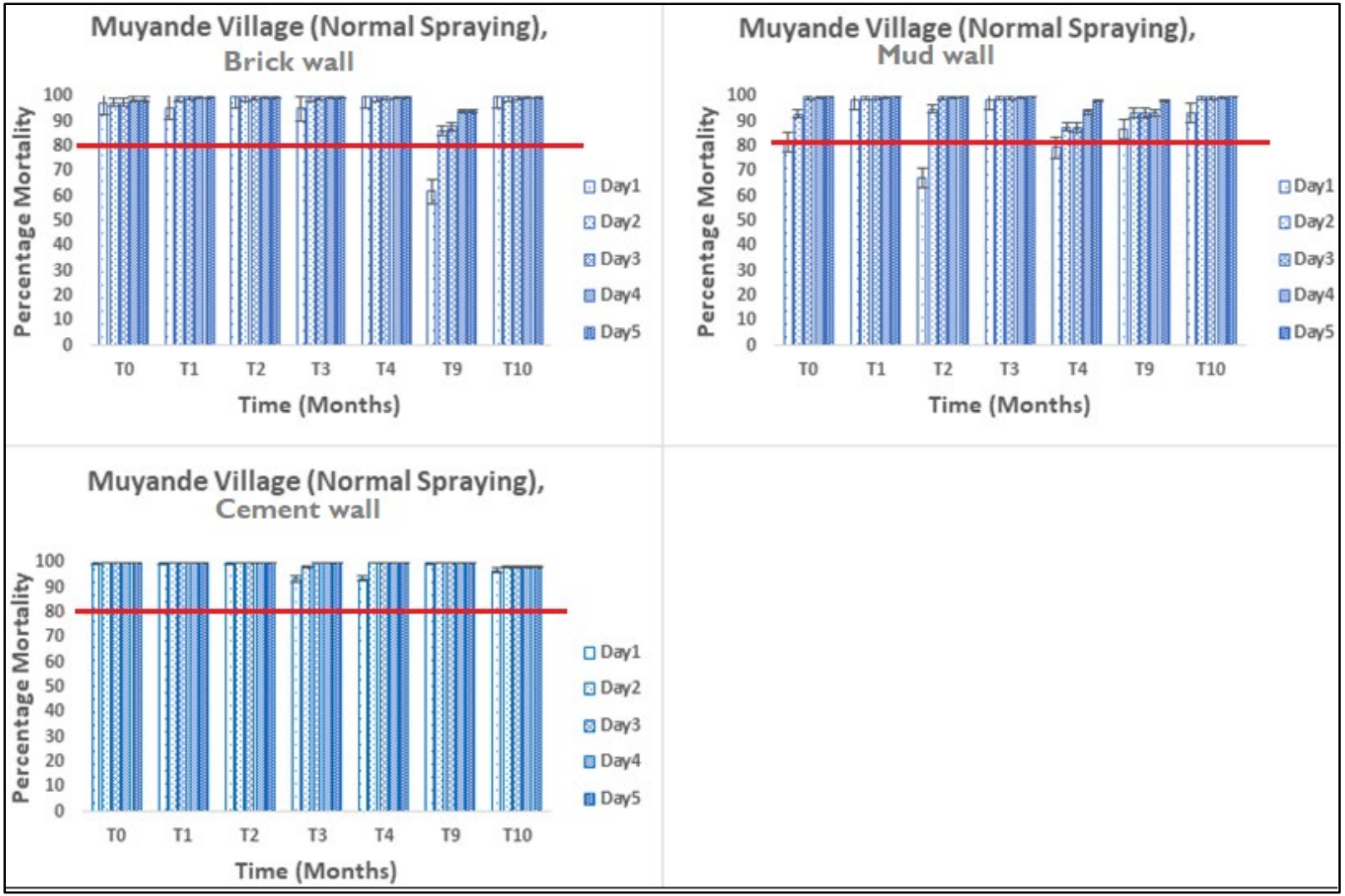
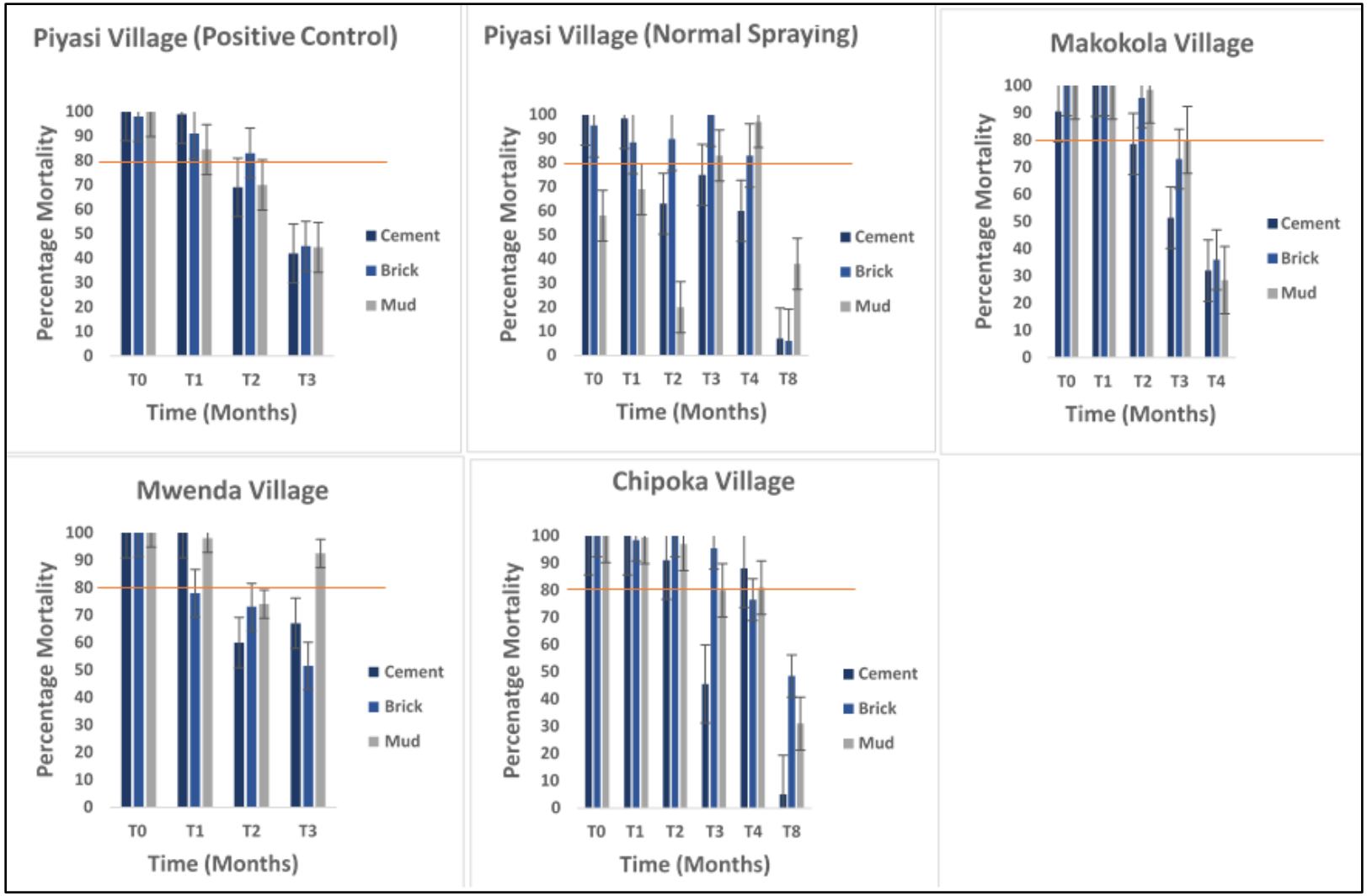


FIGURE 29: MEAN PERCENTAGE MORTALITY OF KISUMU STRAIN OF *AN. GAMBIAE* S.S. AT 24 HOURS EXPOSED TO SURFACES SPRAYED WITH ACTELLIC 300CS IN MANGOCHI DISTRICT



Note: To ensure quality of spraying is not associated with residual efficacy, 6 strucures were sprayed under the close supervision of VectorLink Malawi staff as a positive control.

3.8 INSECTICIDE RESISTANCE MONITORING

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

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

In Chikwawa District, *An. funestus* s.l. were exposed to different insecticides as shown in Figure 30a. *An. funestus* s.l. showed high resistance to deltamethrin 0.05% (13.8%, n=94), permethrin 0.75% (16.2%, n=111), and alpha-cypermethrin 0.05% (38.3%, n=115) (Annex C, Table C-1). Pre-exposing *An. funestus* s.l. to 4% PBO then to permethrin 0.75% restored its susceptibility (98.2%, n=111). However, pre-exposing *An. funestus* s.l. to 4% PBO then to deltamethrin 0.05% and alpha-cypermethrin 0.05% resulted in partial susceptibility restoration, 96.1% (n=102) and 97.3% (n=112), respectively. Exposure of *An. funestus* s.l. to chlorfenapyr 100µg/bottle resulted in 100% mortality (n=100) within 24 hours, while for clothianidin 13.2mg/paper (2%) tube test, mortality was >98% after 24hrs though it took four days to achieve 100% mortality (n=106) (Figure 30b). Exposure of *An. gambiae* s.l. collected from Mwamphanzi site in Chikwawa District to pirimiphos-methyl 0.25% resulted into 100% mortality (n=111) (Figure 30c). Exposure of *An. funestus* s.l. to higher doses of deltamethrin 0.25% (5x) and permethrin 3.75% (5x) resulted in 63.8% (n=105) and 60.4% (n=101) mortality, respectively (Figure 30a). This indicates that *An. funestus* s.l. mosquitoes are extremely resistant to both deltamethrin and permethrin. A further higher dose (10X) is required to test susceptibility status of *An. funestus* s.l. to these insecticides in Chikwawa.

FIGURE 30A: *AN. FUNESTUS* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN CHIKWAWA DISTRICT

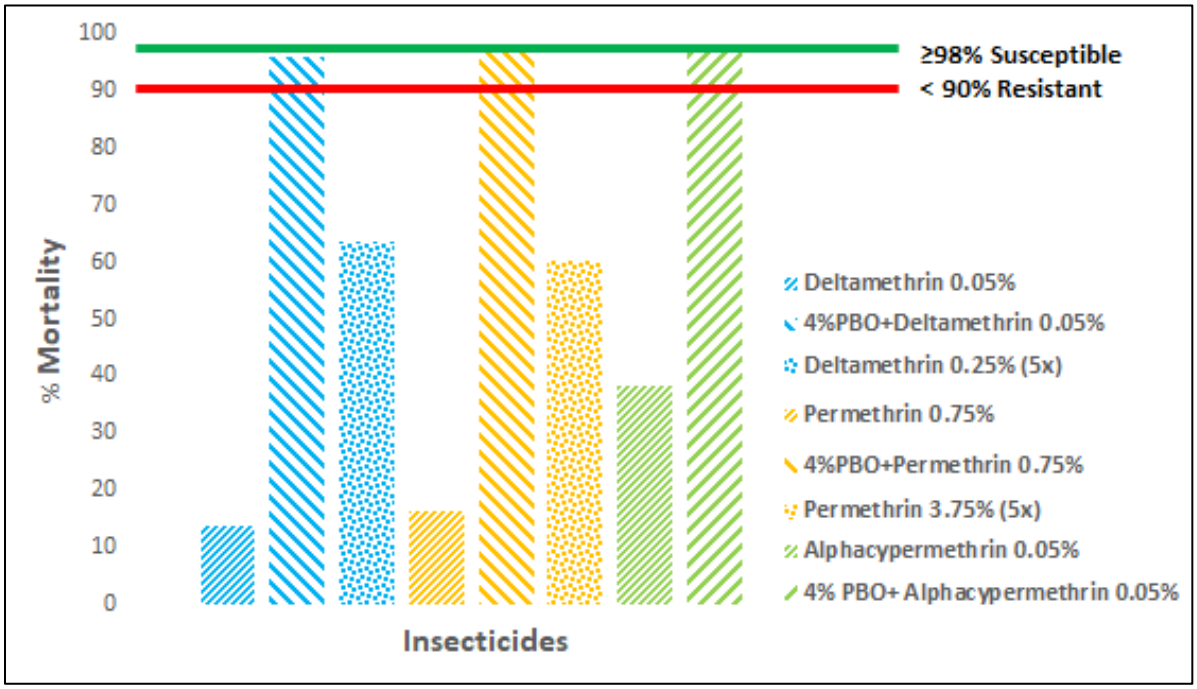


FIGURE 30B: *AN. FUNESTUS* S.L. RESPONSE TO CHLORFENAPYR 100µG/BOTTLE AND CLOTHIANIDIN 13.2MG/PAPER IN CHIKWAWA DISTRICT

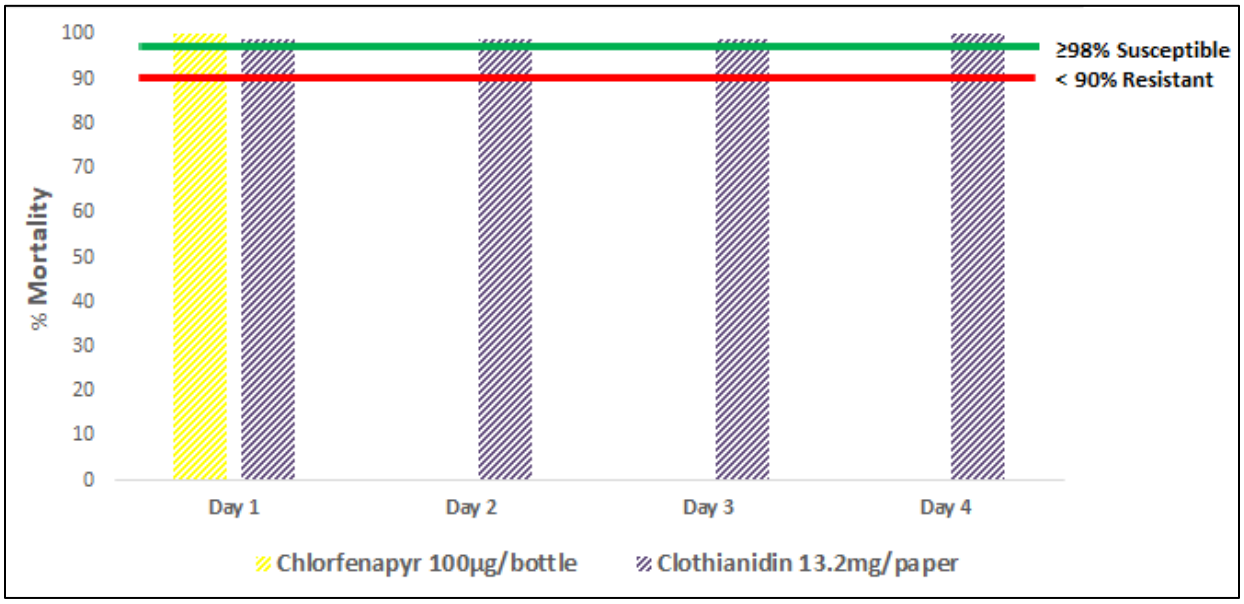
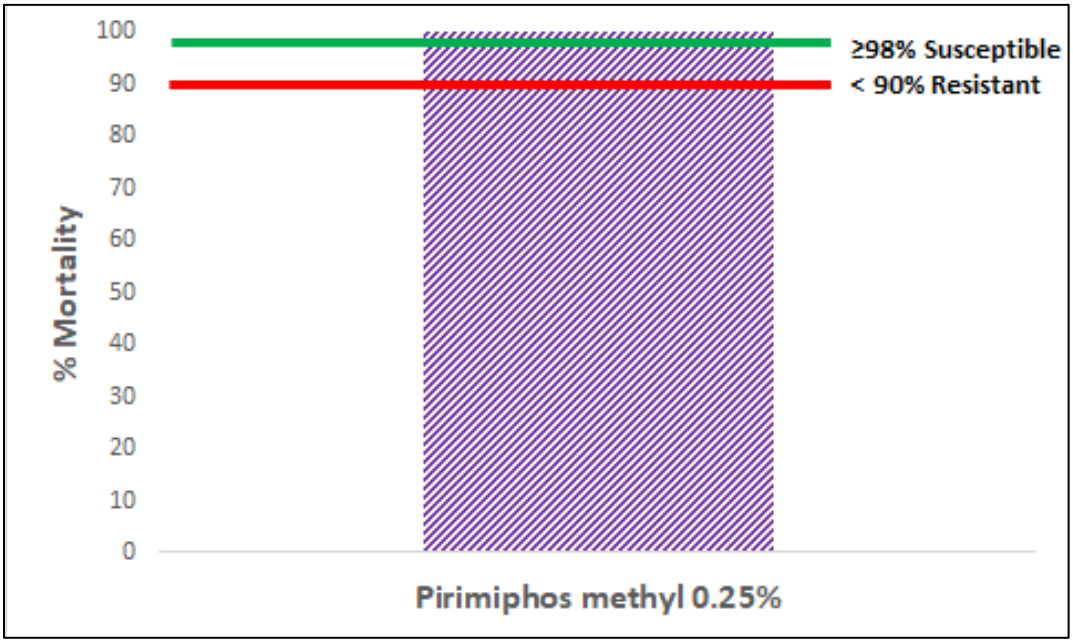


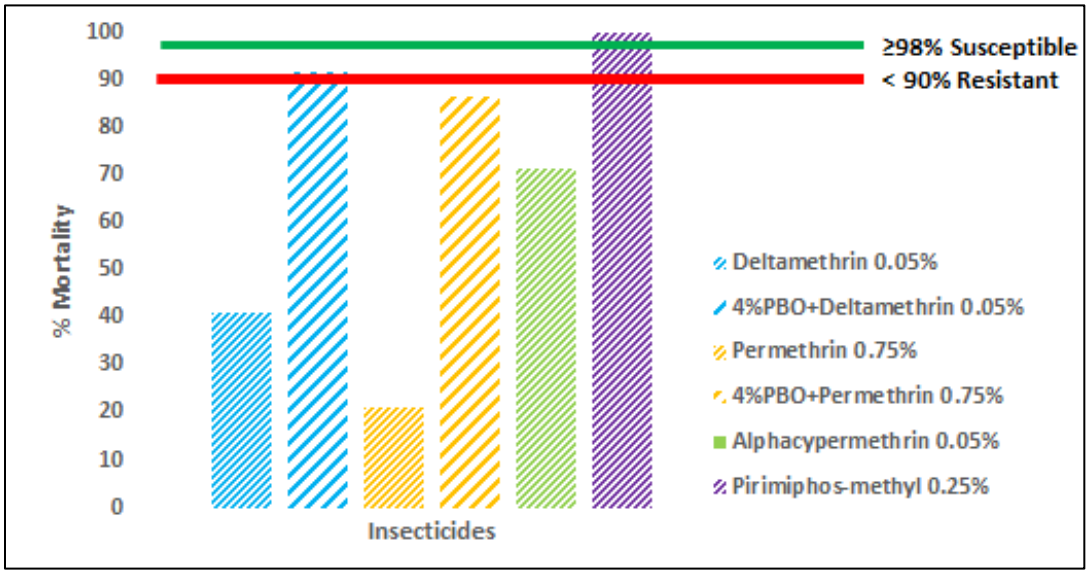
FIGURE 30C: *AN. GAMBIAE* S.L. RESPONSE TO PIRIMIPHOS-METHYL 0.25% IN CHIKWAWA DISTRICT



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

In Salima District, *An. gambiae* s.l. were resistant to deltamethrin 0.05% (40.8%, n=103), permethrin 0.75% (21%, n=100), and alpha-cypermethrin 0.05% (71.2%, n=73) (Figure 31 and Annex C, Table C-2). The mosquito samples pre-exposed to 4% PBO then to pyrethroids were not completely restored to susceptibility but the procedure greatly improved the efficacy of both deltamethrin 0.05% and permethrin 0.75%. PBO + deltamethrin 0.05% resulted in 91.6% mortality (n=95), which is 2.2 times that of deltamethrin alone. Likewise, PBO + permethrin 0.75% resulted in 86.5% mortality (n=104), which is 4.1 times that of permethrin alone. *An. gambiae* s.l. exposed to pirimiphos-methyl resulted into 100% mortality (n=57).

FIGURE 31: *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. were resistant to pyrethroids (Figure 32a and Annex C, Table C-3). Mortality rates for *An. funestus* s.l. exposed to deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05% were 2.8% (n=106), 4.8% (n=105), and 3.9% (n=102), respectively, indicating high resistance to pyrethroids in the district. However, when the same batch of *An. funestus* s.l. was pre-exposed to 4% PBO followed by deltamethrin 0.05% and permethrin 0.75%, full susceptibility was restored. Mortality was 100% for both 4% PBO + deltamethrin 0.05% (n=99) and 4% PBO + permethrin 0.75% (n=105), and for pirimiphos-methyl 0.25% (n=104). Similarly, *An. funestus* s.l. was susceptible with >=98% mortality to chlorfenapyr 100µg/bottle (n=104) and clothianidin 13.2mg/paper (n=110) within 24 hours(Figure 32b).

FIGURE 32A: *AN. FUNESTUS* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN KASUNGU DISTRICT

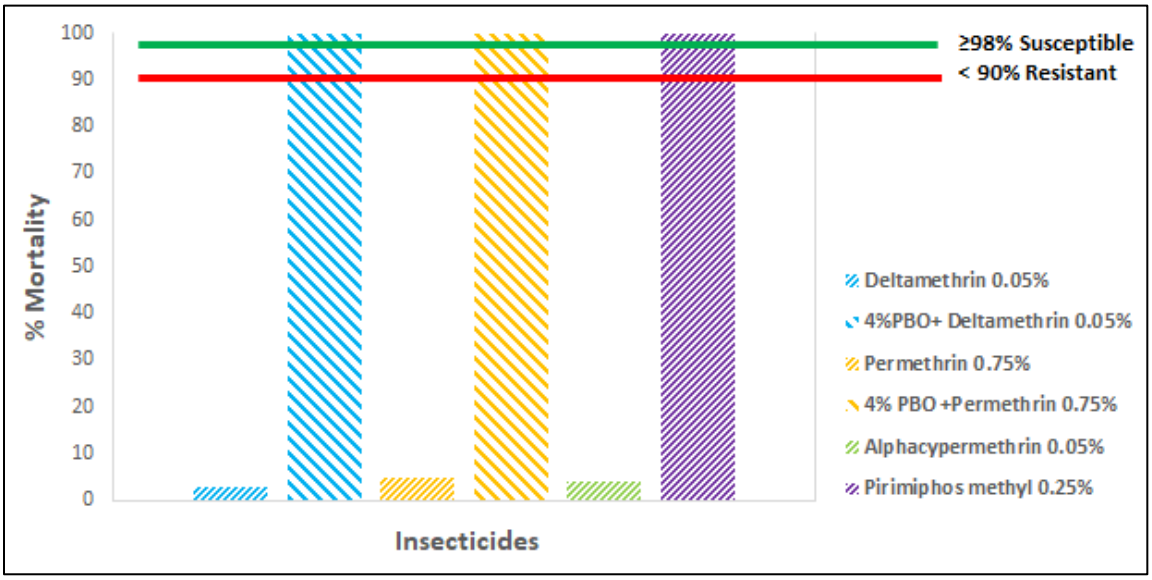
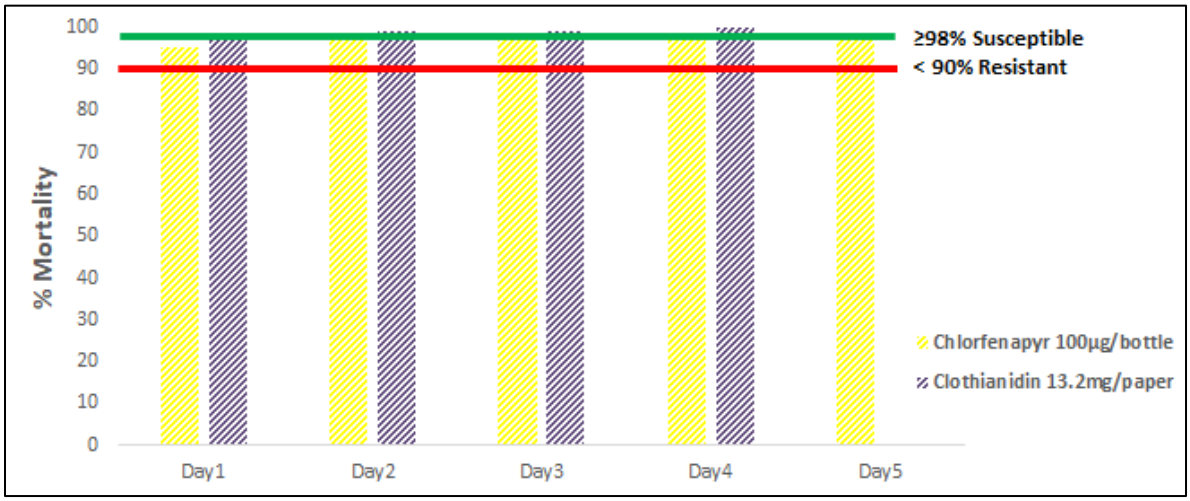


FIGURE 32B: *AN. FUNESTUS* S.L. RESPONSE TO CHLORFENAPYR 100µG/BOTTLE AND CLOTHIANIDIN 13.2MG/PAPER IN KASUNGU DISTRICT



3.8.4 *AN. GAMBIAE* S.L. AND *ANOPHELES FUNESTUS* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN MANGOCHI DISTRICT

In Mangochi District, *An. gambiae* s.l. were exposed to deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05%; the respective mortality rates were 83.7% (n=104), 37.4% (n=107), and 76.8% (n=99) (Figure 33a and Annex C, Table C-4). Pre-exposure of *An. gambiae* s.l. to 4% PBO then to pyrethroids resulted in improved efficacy. Pre-exposure to 4% PBO + deltamethrin 0.05% resulted in 100% mortality (n=102), to

4% PBO + permethrin 0.75% resulted in 93.2% mortality (n=103), and to 4% PBO + alpha-cypermethrin 0.05% resulted in 100% mortality (n=29). Exposure of *An. gambiae* s.l. to pirimiphos-methyl 0.25% resulted in mortality of 98.9% (n=90). *Anopheles gambiae* s.l. exposure to clothianidin 13.2mg/paper also resulted in 100% mortality (n=96) two days post exposure (Figure 33c).

Anopheles funestus s.l. exposure to chlorfenapyr 100µg/bottle resulted in 100% mortality (n=48) two days post exposure (Figure 33b).

FIGURE 33A: *AN. GAMBIAE* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN MANGOCHI DISTRICT

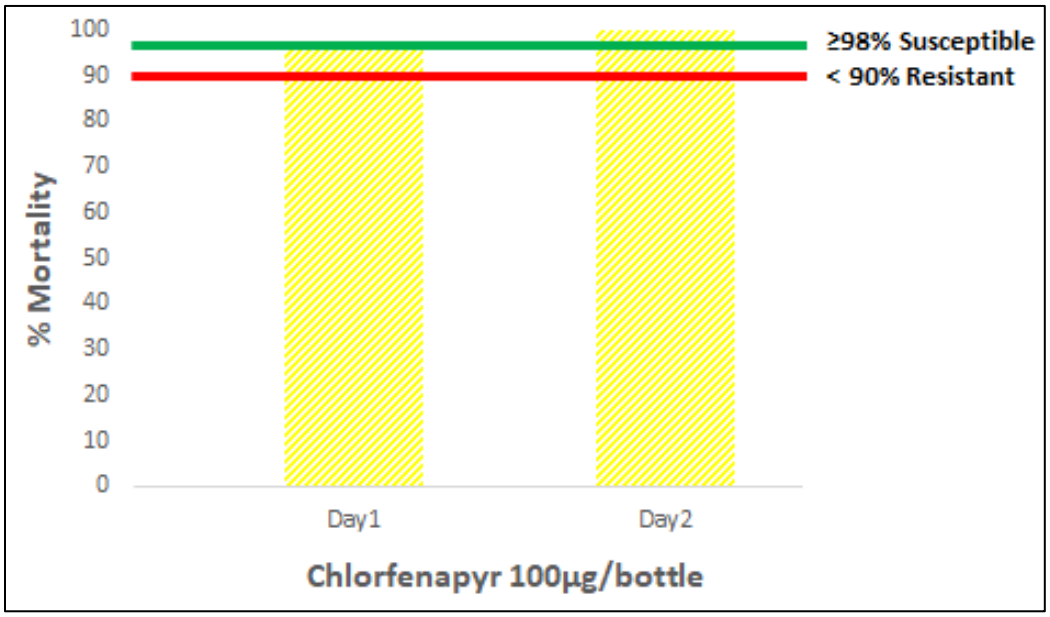


FIGURE 33B: *AN. FUNESTUS* S.L. RESPONSE TO CHLORFENAPYR 100µG/BOTTLE IN MANGOCHI DISTRICT

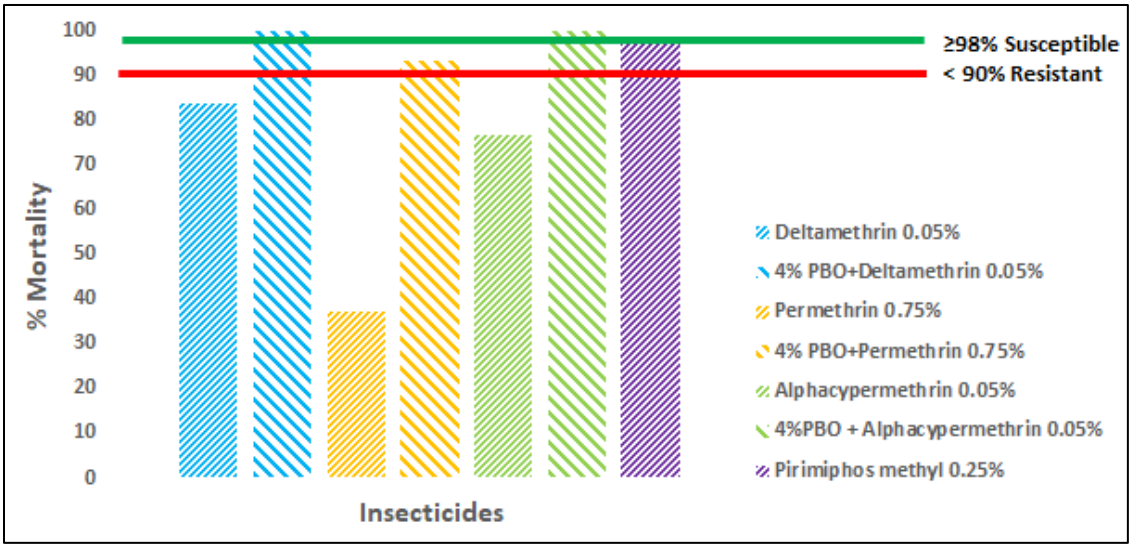
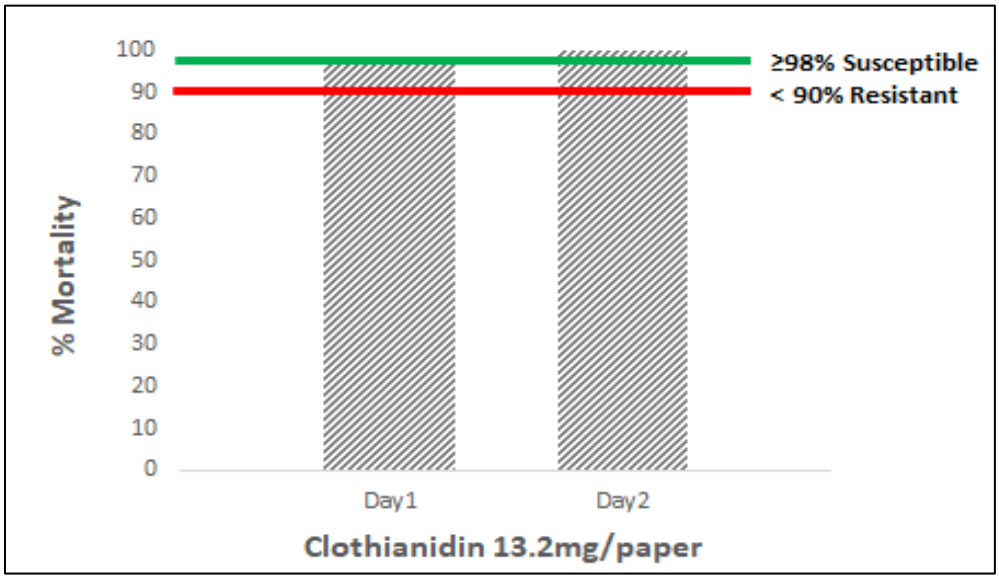


FIGURE 33C: *AN. GAMBIAE* S.L. RESPONSE TO CLOTHIANIDIN 13.2MG/PAPER IN MANGOCHI DISTRICT



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

In Nkhotakota District, *An. gambiae* s.l. exposure to pirimiphos-methyl 0.25% resulted in 100% mortality (n=107) within 24 hours, and their exposure to deltamethrin 0.05% resulted in 36.4% mortality (n=77) (Figure 34a and Annex C, Table C-5). *An. funestus* s.l. exposed to permethrin 0.75% produced a mortality rate of 37.5% (n=56) (Figure 34b), and to clothianidin 13.2mg/paper produced 100% mortality (n=106) within two days post exposure (Figure 34c).

FIGURE 34A: *AN. GAMBIAE* S.L. RESPONSE TO DELTAMETHRIN 0.05% AND PIRIMIPHOS-METHYL 0.25% IN CHIMKWENDE, NKHOTAKOTA DISTRICT

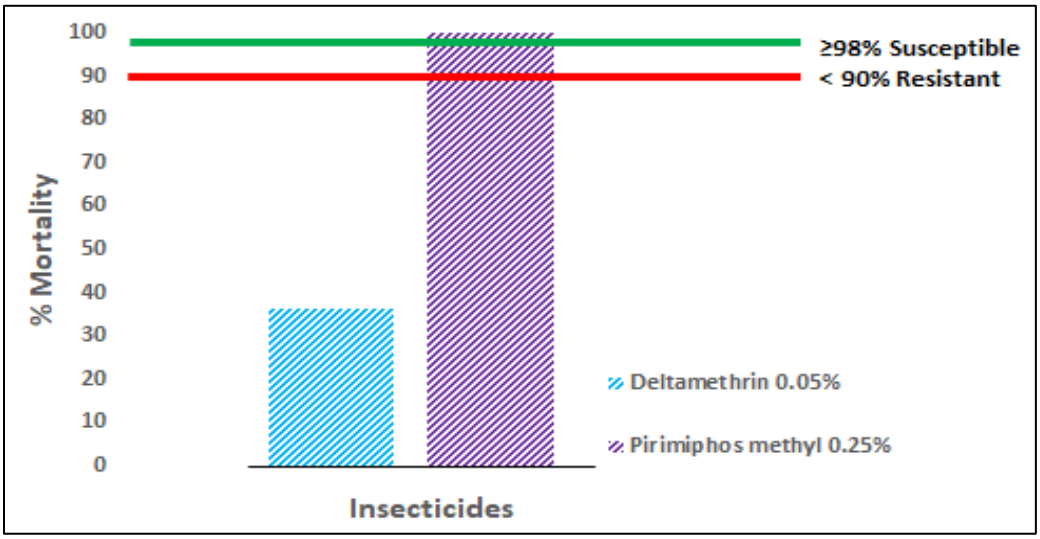


FIGURE 34B: *AN. FUNESTUS* S.L. RESPONSE TO PERMETHRIN 0.75% IN CHIMKWENDE, NKHOTAKOTA DISTRICT

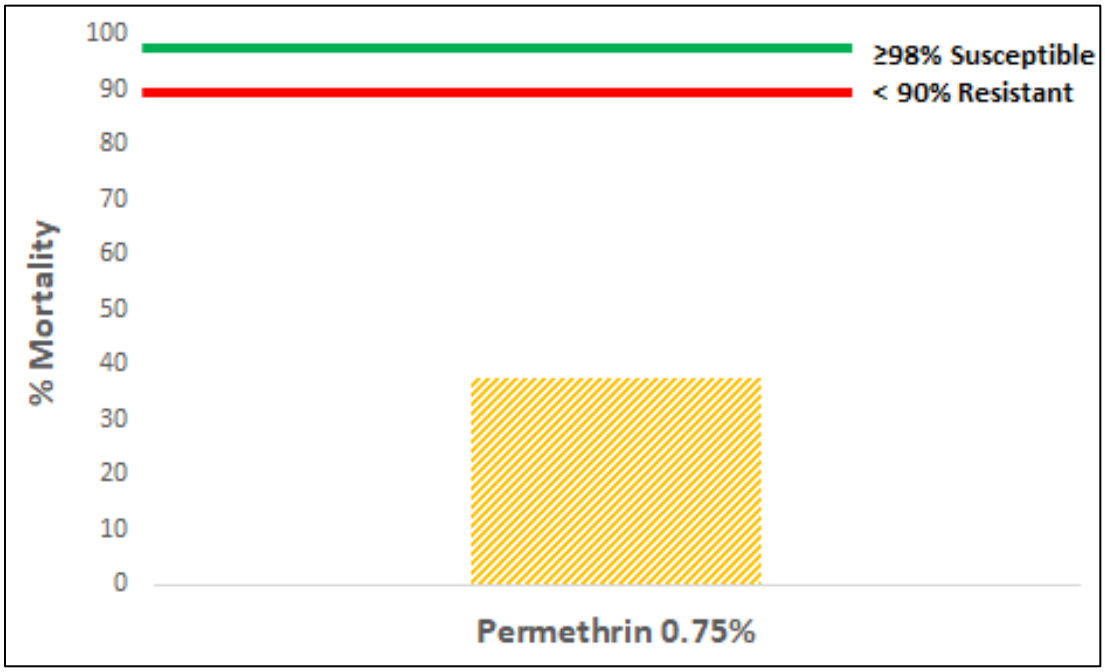
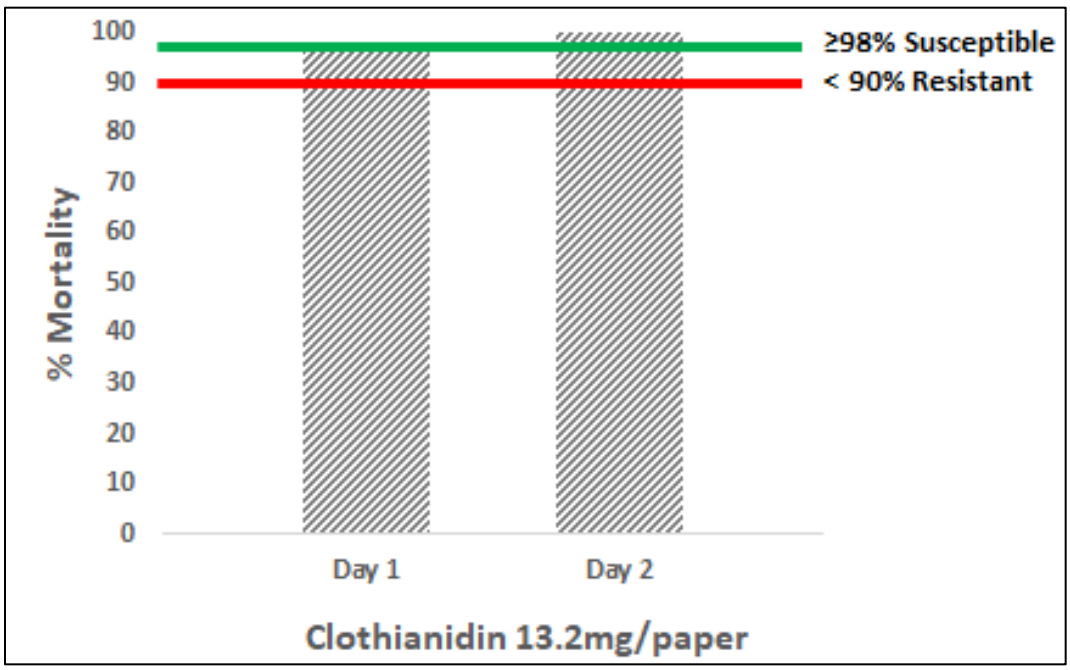


FIGURE 34C: *AN. FUNESTUS* S.L. EXPOSED TO CLOTHIANIDIN IN CHIMKWENDE, NKHOTAKOTA DISTRICT



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

In Nkhata Bay District, *An. funestus* s.l. were exposed (CDC bottle assay) to alpha-cypermethrin 5x and 10x doses (Figure 35a and Annex C, Table C-6). The knockdown effect of *An. funestus* s.l. to alpha-cypermethrin 5x was 76.7% (n=90), while to alpha-cypermethrin 10x it was 80.9% (n=89), indicating resistance even at the higher dose. In addition, *An. funestus* s.l. exposure to deltamethrin 0.05% and permethrin 0.75% resulted in mortality rates of 12.4% (n=105) and 4.3% (n=117), respectively (Figure 35b). Pre-exposure of *An. funestus* s.l. to 4% PBO improved the efficacy of pyrethroids: 4% PBO + deltamethrin 0.05% resulted in 97.6% mortality (n=124), 4% PBO + permethrin resulted in 98.2% mortality (n=109), and 4% PBO+ alpha-cypermethrin resulted in 87.4% mortality (n=103). *An. funestus* s.l. exposed to pirimiphos-methyl 0.25% resulted in 100% mortality (n=103). Exposure of *An. funestus* s.l. to chlorfenapyr 100µg/bottle resulted in 100% mortality (n=99) within 24 hours as did exposure to clothianidin 13.2mg/paper (n=90).

FIGURE 35A: *AN. FUNESTUS* S.L. RESPONSE TO ALPHA-CYPERMETHRIN 5X AND 10X IN KANDE, NKHATA BAY DISTRICT

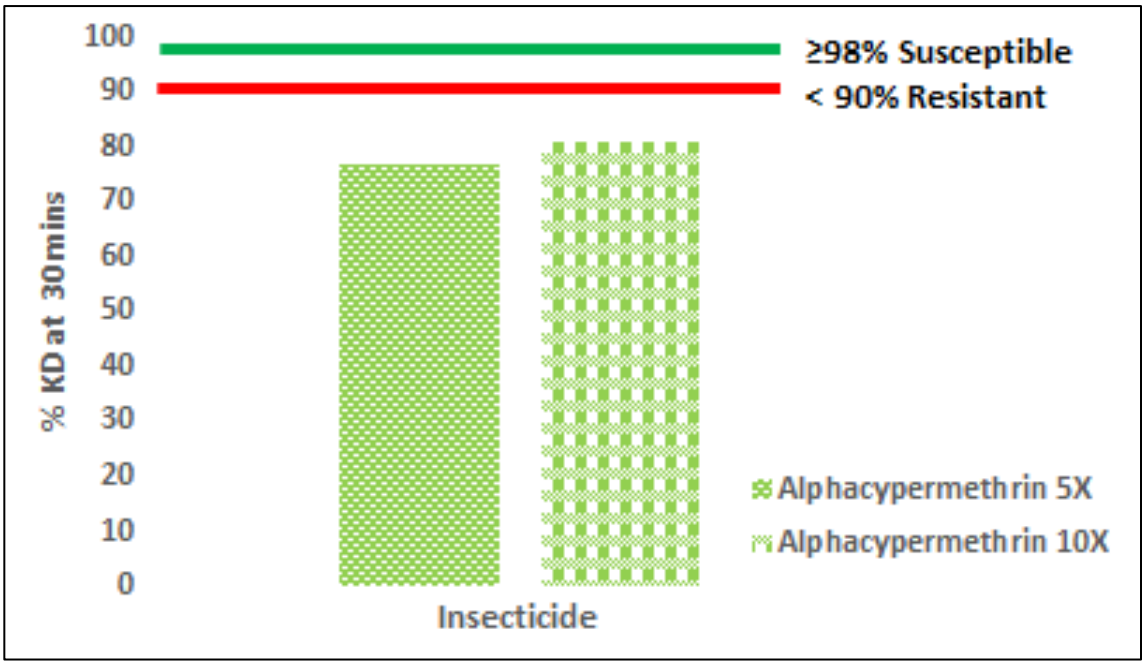
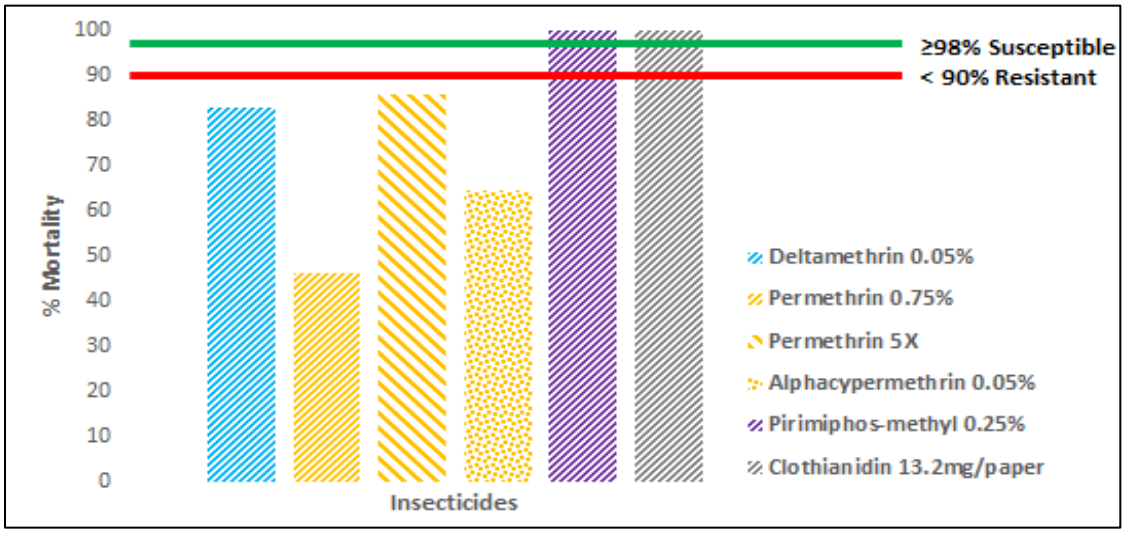


FIGURE 35B: *AN. FUNESTUS* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN KANDE, NKHATA BAY DISTRICT



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

In Karonga District, *An. gambiae* s.l. exposure to deltamethrin 0.05%, permethrin 0.75% and Alphacypermethrin 0.05% resulted in mortality rates of 83% (n=100), 46.3% (n=41) and 64.6%(n=99) respectively, indicating resistance to pyrethroid insecticides (Figure 36A and Annex C, Table C-7). *An. gambiae* s.l. were further subjected to a higher dose of permethrin 5X and resulted into 86% mortality (n=100) still indicating resistance to a higher dose. Exposing *An. gambiae* s.l. to pirimiphos-methyl 0.25%, clothianidin 13.2mg/paper and chlorfenapyr 100µg/bottle resulted to 100% mortality(n=100) within 24hrs, 100% mortality(n=101) within 24hrs and 100% mortality(n=102) within 3days, respectively (Figures 36A and 36B).

FIGURE 36: *AN. GAMBIAE* S.L. RESPONSE TO DIFFERENT INSECTICIDES AT MWENIMAMBWE, KARONGA DISTRICT

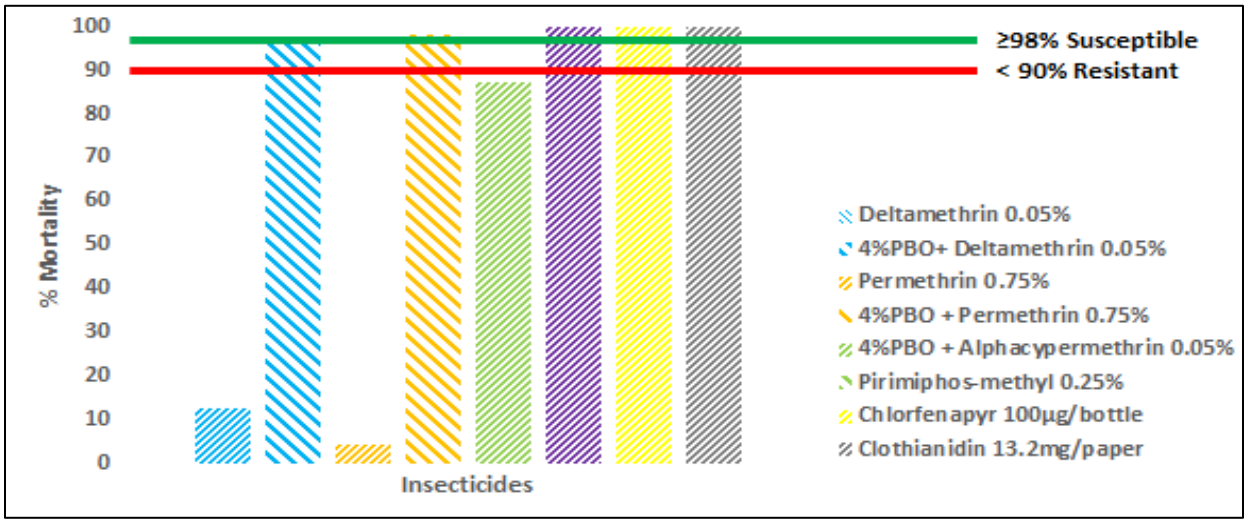
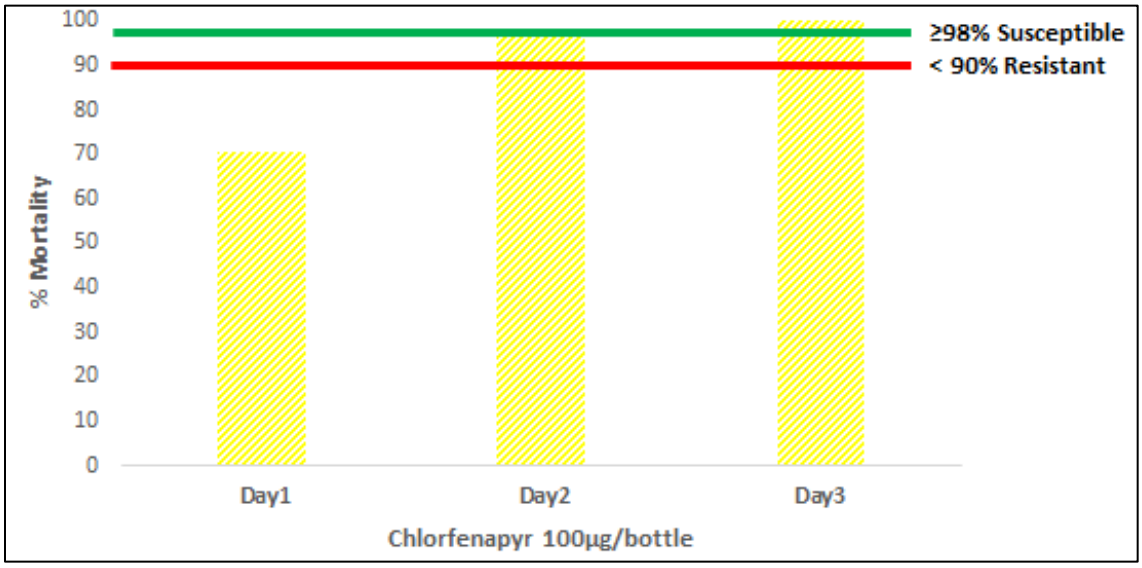


FIGURE 36B: *AN. GAMBIAE* S.L. EXPOSED TO CHLORFENAPYR AT MWENIMAMBWE, KARONGA DISTRICT



4. CONCLUSION AND RECOMMENDATIONS

- Overall, *An. gambiae* s.l. was the most abundant vector; it represented 63% of all the *Anopheles* mosquitoes collected in the seven entomological monitoring districts. The most important malaria vector *An. funestus* s.l., contributed 34.1% of all the *Anopheles* mosquitoes collected in the 7 disticts. However, *An. funestus* s.l. was also equally or more common in 4 districts.
- *An. funestus* s.l. (exclusively *An. funestus* s.s.) is the most important malaria vector in the seven districts, with an overall 1.88% sporozoite infection rate from the three collection methods. The highest EIR of this species over a 9 month period was recorded in Nkhotakota district (108.19 ib/p/9m). However, the highest EIRs of *An. funestus* s.l. in this district were recorded in July and August before spraying.
- *An. gambiae* s.l. (predominantly *An. arabiensis*) is the second most important malaria vector in the seven districts, with an overall 1.19% sporozoite infection rates from the three collection methods.The highest EIR of this species over a 9 month period was recorded in Kasungu district (53.07 ib/p/9m).In the IRS districts of Nkhotakota and Mangochi, *An. funestus* s.l. monthly EIRs were higher before spray than after spraying, while *An. gambiae* s.l. monthly EIRs were recorded higher after spraying.
- In non-IRS districts, high proportion of parous An. gambaie s.l. were collected during the dry season than the rainys season.
- Overall, the highest estimated risk of malaria transmission over the 9 months period was observed in standard net (SN) distribution districts compared to piperonyl butoxide (PBO) net distribution districts.
- *An. funestus* s.l. fed mainly indoors in the five districts where HLCs were conducted (Nkhata Bay, Nkhotakota, Kasungu, Salima, and Mangochi), whereas *An. gambiae* s.l. fed mainly outdoors.
- The biting activity of both *An. gambiae* s.l. and *An. funestus* s.l. occurred from dawn to dusk, with large and smaller peaks before and after midnight in all the five districts.
- Some morning / day-time indoor biting activity of *An. gambiae* s.l. was observed in Nkhata Bay sites and Chimkwende site in Nkhotakota, and the same activity was observed among *An. funestus* s.l. in Kasungu sites and Vwawa site in Nkhotakota. This calls for community education of household members in the three districts, to alert them to the daytime biting behavior of *Anopheles* mosquitoes.
- *An. gambiae* s.l. and *An. funestus* s.l. fed mainly on humans in the seven districts; a smaller proportion fed on cattle.
- IRS suppressed the *An. funestus* s.l. population in Nkhotakota and Mangochi districts during the rainy season (November 2019–March 2020). However, a sharp rise in the *An. gambiae* s.l. population was observed in these districts during the same period.
- The population of *Anopheles* mosquitoes varied greatly in districts where nets were distributed. In Salima District (PBO net distribution), the population of both *An. gambiae* s.l. and *An. funestus* s.l. remained low throughout the monitoring period while in Karonga (PBO net distribution), the

population of *An. gambiae* s.l. rose during the dry season and decreased during the rainy season. In Chikwawa and Kasungu (standard net distribution), the population of *An. funestus* s.l. rose during the rainy season.

- Spray quality was satisfactory in the IRS districts of Nkhotakota and Mangochi. The IRD of *Anopheles* mosquitoes remained low in these districts despite the onset of rainy season.
- The residual efficacy of Actellic 300CS ranged from 2 to 4 months in Nkhotakota and Mangochi districts.
- SumiShield 50WG was still effective (killing >80% of mosquitoes exposed to wall surfaces treated with the insecticide) at 10 months after spraying. Residual life of this insecticide will be monitored up to 12 months after spraying.
- Both *An. funestus* s.l. and *An. gambiae* s.l. were highly resistant to pyrethroids (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.05%) in all sentinel sites in the seven districts.
- Partial or full restoration of susceptibility was observed in *An. funestus* s.l. populations when pre-exposed to PBO, indicating the presence of the oxidase resistance mechanism. This means that long-lasting insecticidal nets that incorporate PBO would be effective against *Anopheles* vectors in Malawi.
- It is recommended that PBO nets be distributed in areas with moderate and high pyrethroid resistance in future mass distribution campaigns of nets in Malawi.
- Pirimiphos-methyl, clothianidin, and chlorfenapyr showed high efficacy against both *An. funestus* s.l. and *An. gambiae* s.l. across the country. The three insecticides should be considered in IRS rotation in Malawi, assuming an IRS formulation of chlorfenapyr becomes available. However, clothianidin should be priotised due to its longer residual
- No *Ace-1* allele mutation was detected in the *An. gambiae* s.l. tested.

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ANNEX A: HBRS OF *ANOPHELES* MOSQUITOES IN FIVE DISTRICTS: NKHATA BAY, NKHOTAKOTA, SALIMA, KASUNGU, AND MANGOCHI

TABLE A-1: HBRS OF *AN. FUNESTUS* S.L.

Sentinel Site	Month	Number Collected Indoor	Number Collected Outdoor	# of Collectors	# of Nights	# of Houses	Indoor HBR: (b/p/n)	Outdoor HBR: (b/p/n)	Bites/man/ night
Nkhata Bay	September	6	3	2	1	4	0.75	0.38	0.56
	December	3	3	2	1	4	0.38	0.38	0.38
	March	0	0	2	1	4	0.00	0.00	0.00
Average							0.38	0.25	0.31
Nkhotakota	September	38	6	2	1	8	2.38	0.38	1.38
	December	0	1	2	1	8	0.00	0.06	0.03
	March	5	1	2	1	8	0.31	0.06	0.19
Average							0.90	0.17	0.53
Salima	September	0	0	2	1	4	0.00	0.00	0.00
	December	0	0	2	1	4	0.00	0.00	0.00
	March	0	1	2	1	4	0.00	0.13	0.06
Average							0.00	0.04	0.02
Kasungu	September	6	0	2	1	4	0.75	0.00	0.38
	December	12	1	2	1	4	1.50	0.13	0.81
	March	81	46	2	1	4	10.13	5.75	7.94
Average							4.13	1.96	3.04
Mangochi	December	6	6	2	1	4	0.75	0.75	0.75
	March	2	1	2	1	4	0.25	0.13	0.19
Average							0.33	0.29	0.31

NB: Indoor/Outdoor HBR=Total collected/# of collectors/# of nights/# of houses; Nkhotakota District had two sites

TABLE A-2: HBRs OF *AN. GAMBIAE* S.L.

Sentinel Site	Month	Number Collected Indoor	Number Collected Outdoor	# of Collectors	# of Nights	# of Houses	Indoor HBR: Bites/man/ night	Outdoor HBR: Bites/man/ night	Bites/man/ night
Nkhata Bay	September	11	18	2	1	4	1.38	2.25	1.81
	December	17	21	2	1	4	2.13	2.63	2.38
	March	44	30	2	1	4	5.50	3.75	4.63
Average							3.00	2.88	2.94
Nkhotakota	September	20	28	2	1	8	1.25	1.75	1.50
	December	9	23	2	1	8	0.56	1.44	1.00
	March	54	88	2	1	8	3.38	5.50	4.44
Average							1.73	2.90	2.31
Salima	September	1	0	2	1	4	0.13	0.00	0.06
	December	18	25	2	1	4	2.25	3.13	2.69
	March	15	42	2	1	4	1.88	5.25	3.56
Average							1.42	2.79	2.10
Kasungu	September	1	0	2	1	4	0.13	0.00	0.06
	December	5	6	2	1	4	0.63	0.75	0.69
	March	24	27	2	1	4	3.00	3.38	3.19
Average							1.25	1.38	1.31
Mangochi	December	0	0	2	1	4	0.00	0.00	0.00
	March	12	12	2	1	4	1.50	1.50	1.50
Average							0.50	0.50	0.50

NB: Indoor/Outdoor HBR=Total collected/# of collectors/# of nights/# of houses; Nkhotakota District had two sites

TABLE A-3: HBRs OF *AN. COUSTANI*

Sentinel Site	Month	Number Collected Indoor	Number Collected Outdoor	# of Collectors	# of Nights	# of Houses	Indoor HBR: Bites/man/ night	Outdoor HBR: Bites/man/ night	Bites/man/ night
Nkhata Bay	September	11	7	2	1	4	1.38	0.88	1.13
	December	3	8	2	1	4	0.38	1.00	0.69
	March	5	13	2	1	4	0.63	1.63	1.13
Average							**0.79**	**1.17**	**0.98**
Nkhotakota	September	1	3	2	1	8	0.06	0.19	0.13
	December	0	3	2	1	8	0.00	0.19	0.09
	March	75	147	2	1	8	4.69	9.19	6.94
Average							**1.58**	**3.19**	**2.39**
Salima	September	0	0	2	1	4	0.00	0.00	0.00
	December	0	0	2	1	4	0.00	0.00	0.00
	March	1	4	2	1	4	0.13	0.50	0.31
Average							**0.04**	**0.17**	**0.10**
Kasungu	September	2	4	2	1	4	0.25	0.50	0.38
	December	0	3	2	1	4	0.00	0.38	0.19
	March	12	87	2	1	4	1.50	10.88	6.19
Average							**0.58**	**3.92**	**2.25**
Mangochi	December	0	4	2	1	4	0.00	0.50	0.25
	March	2	4	2	1	4	0.25	0.50	0.38
Average							**0.08**	**0.33**	**0.21**

NB: Indoor/Outdoor HBR=Total collected/# of collectors/# of nights/# of houses; Nkhotakota District had two sites

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

TABLE C-1: *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN CHAKANIRA AND MWAMPHANZI, CHIKWAWA DISTRICT

District	Site	Insecticide	Species	Source	No. Tested	No. Dead	% Mortality	Time at Final Mortality
Chikwawa	Chakanira	Deltamethrin 0.05%	*An. funestus* s.l.	F_1	94	13	13.8	24Hrs
		Permethrin 0.75%	*An. funestus* s.l.	F_1	111	18	16.2	24Hrs
		4% PBO + Deltamethrin 0.05%	*An. funestus* s.l.	F_1	102	98	96.1	24Hrs
		4% PBO + Permethrin 0.75%	*An. funestus* s.l.	F_1	111	109	98.2	24Hrs
		Alpha-cypermethrin 0.05%	*An. funestus* s.l.	F_1	115	44	38.3	24Hrs
		Chlorfenapyr 100µg/bottle	*An. funestus* s.l.	F_1	100	100	100	24Hrs
		Clothianidin 13.2mg/paper	*An. funestus* s.l.	F_1	106	106	100	4days
		4% PBO + Alpha-cypermethrin 0.05%	*An. funestus* s.l.	F_1	112	109	97.3	24Hrs
		Deltamethrin 0.25% (5x)	*An. funestus* s.l.	F_1	105	67	63.8	24Hrs
		Permethrin 3.75% (5x)	*An. funestus* s.l.	F_1	101	61	60.4	24Hrs
	Mwamphanzi	Pirimiphos-methyl 0.25%	*An. gambiae* s.l.	Larvae	111	111	100	24Hrs

Resistance Suspected resistance Susceptible

TABLE C-2: *AN. GAMBIAE* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN SALIMA DISTRICT

District	Site	Insecticide	Species	Source	No. Tested	No. Dead	% Mortality	Time at Final Mortality
Salima	Nzembela	Deltamethrin 0.05%	*An. gambiae* s.l.	Larvae	103	42	40.8	24Hrs
		Permethrin 0.75%	*An. gambiae* s.l.	Larvae	100	21	21	24Hrs
		4% PBO + Deltamethrin 0.05%	*An. gambiae* s.l.	Larvae	95	87	91.6	24Hrs
		4% PBO + Permethrin 0.75%	*An. gambiae* s.l.	Larvae	104	90	86.5	24Hrs
	Fifizi	Alpha-cypermethrin 0.05%	*An. gambiae* s.l.	Larvae	73	52	71.2	24Hrs
		Pirimiphos-methyl 0.25%	*An. gambiae* s.l.	Larvae	57	57	100	24Hrs

Resistance Suspected resistance Susceptible

TABLE C-3: *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 0.05%	*An. funestus s.l.*	F_1	106	3	2.8	24Hrs
		Permethrin 0.75%	*An. funestus s.l.*	F_1	105	5	4.8	24Hrs
		4%PBO + Deltamethrin 0.05%	*An. funestus s.l.*	F_1	99	99	100	24Hrs
		4%PBO + Permethrin 0.75%	*An. funestus s.l.*	F_1	105	105	100	24Hrs
		Alpha-cypermethrin 0.05%	*An. funestus s.l.*	F_1	102	4	3.9	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 13.2mg/paper	*An. funestus s.l.*	F_1	110	110	100	4Days

Resistance Suspected resistance Susceptible

TABLE C-4: *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN MANGOCHI DISTRICT

District	Site	Insecticide	Species	Source	No. Tested	No. Dead	% Mortality	Time at Final Mortality
Mangochi	Malindi	Pirimiphos-methyl 0.25%	*An. gambiae* s.l.	Larvae	90	89	98.9	24Hrs
	Malindi	Clothianidin 13.2mg/ml	*An. gambiae* s.l.	Larvae	96	96	100	2Days
	Kafulumila	Deltamethrin 0.05%	*An. gambiae* s.l.	Larvae	104	87	83.7	24Hrs
	Kafulumila	Permethrin 0.75%	*An. gambiae* s.l.	Larvae	107	40	37.4	24Hrs
	Kafulumila	4% PBO + Deltamethrin 0.05%	*An. gambiae* s.l.	Larvae	102	102	100	24Hrs
	Kafulumila	4% PBO + Permethrin 0.75%	*An. gambiae* s.l.	Larvae	103	96	93.2	24Hrs
	Malindi	Alpha-cypermethrin 0.05%	*An. gambiae* s.l.	Larvae	99	76	76.8	24Hrs
	Likulungwa	Chlorfenapyr 100µg/bottle	*An. funestus* s.l.	F_1	48	48	100	2Days
	Malindi	4% PBO + Alpha-cypermethrin 0.05%	*An. gambiae* s.l.	Larvae	29	29	100	24Hrs

Resistance Suspected resistance Susceptible

TABLE C-5: *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN CHIMKWENDE, NKHOTAKOTA DISTRICT

District	Site	Insecticide	Species	Source	No. Tested	No. Dead	% Mortality	Time at Final Mortality
Nkhotakota	Chimkwende	Pirimiphos-methyl 0.25%	*An. gambiae* s.l.	Larvae	107	107	100	24Hrs
		Deltamethrin 0.05%	*An. gambiae* s.l.	F_1	77	28	36.4	24Hrs
		Permethrin 0.75%	*An. funestus* s.l.	F_1	56	21	37.5	24Hrs
		Clothianidin 13.2mg/paper	*An. funestus* s.l.	F_1	106	106	100	2days

Resistance Suspected resistance Susceptible

TABLE C-6: *AN. FUNESTUS* S.L. RESPONSE TO ALPHA-CYPERMETHRIN IN KANDE, NKHATA BAY DISTRICT

District	Site	Insecticide	Species	Source	No. Tested	No. KD/Dead	% KD/ % Mortality	Time at Final Mortality
Nkhata- Bay	Kande	Alpha-cypermethrin 5X	*An. funestus* s.l.	F_1	90	69	76.7	30mins
		Alpha-cypermethrin 10X	*An. funestus* s.l.	F_1	89	72	80.9	30mins
		Chlorfenapyr 100µg/ml	*An. funestus* s.l.	F_1	99	99	100	24Hrs
		Clothianidin 13.2mg/ml	*An. funestus* s.l.	F_1	90	90	100	24Hrs
		Deltamethrin 0.05%	*An. funestus* s.l.	F_1	105	13	12.4	24Hrs
		Permethrin 0.75%	*An. funestus* s.l.	F_1	117	5	4.3	24Hrs
		Pirimiphos-methyl 0.25%	*An. funestus* s.l.	F_1	103	103	100	24Hrs
		4% PBO + Permethrin 0.75%	*An. funestus* s.l.	F_1	109	107	98.2	24Hrs
		4% PBO+ Deltamethrin 0.05%	*An. funestus* s.l.	F_1	124	121	97.6	24Hrs
		4% PBO + Alpha-cypermethrin 0.05%	*An. funestus* s.l.	F_1	103	90	87.4	24Hrs

Resistance Suspected resistance Susceptible

TABLE C-7: *AN. GAMBIAE* S.L. RESPONSE TO DELTAMETHRIN AND PERMETHRIN IN MWENIMAMBWE, KARONGA DISTRICT

District	Site	Insecticide	Species	Source	No. Tested	No. Dead	% Mortality	Time at final mortality
Karonga	Mwenimambwe	Deltamethrin 0.05%	*An. gambiae* s.l.	Larvae	100	83	83	24Hrs
		Permethrin 0.75%	*An. gambiae* s.l.	Larvae	41	19	46.3	24Hrs
		Permethrin 5X	*An. gambiae* s.l.	F_1	100	86	86	24Hrs
		Alpha-cypermethrin 0.05%	*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 13.2mg/ml	*An. gambiae* s.l.	F_1	101	101	100	24Hrs
		Chlorfenapyr 100µg/ml	*An. gambiae* s.l.	F_1	102	102	100	3days