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PMI VECTORLINK MALAWI ANNUAL ENTOMOLOGICAL MONITORING REPORT

JULY 1, 2018 – JUNE 30, 2019

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Prepared by: Abt Associates Inc.

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

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Acronyms

ANOVA	Analysis of Variance
b/p/n	bites/person/night
CDC-LT	Centers for Disease Control and Prevention Light Trap
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/yr	Infectious bites/person/year
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
LLIN	Long-Lasting Insecticidal Net
MAC	Malaria Alert Centre
NMCP	National Malaria Control Program
NKK	Nkhotakota
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
s.l.	Sensu lato
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, especially among children under the age of five and pregnant women. Long-lasting insecticidal nets have been widely used as a major vector control intervention in Malawi with indoor residual spraying (IRS) acting 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 2018 to June 2019 in 11 sentinel sites in five districts to assess malaria vector bionomics and susceptibility of principal malaria vectors across the country.

Vector Bionomics: Across all 11 sentinel sites, *Anopheles funestus* s.l. and *An. gambiae* s.l. were the dominant species accounting for 56.3% and 41.7%, respectively, of the 42,329 *Anopheles* mosquitoes collected. *An. coustani* (2.0%) was the least abundant species. The highest *An. funestus* s.l. density was collected in Nkhata-Bay from CDC-light traps (112.5 mosquitoes/trap/night) while as the highest *An. gambiae* s.l. density was collected in Karonga from pyrethrum spray catches (PSCs) (446.5 mosquitoes/house/day). A total of 2,109 *An. gambiae* s.l. were identified to the species level by polymerase chain reaction (PCR); 94.4% were identified as *An. arabiensis* and 5.6% *An. gambiae* s.s. A total of 1,614 *An. funestus* s.l. were also identified to the species level by PCR and all turned out to be *An. funestus* s.s. Hence, *An. funestus* s.s. and *An. arabiensis* are the major malaria vectors whereas *An. gambiae* s.s. has limited distribution. All the three species occur in the five districts monitored.

Human Biting Rate (HBR) and Location: The indoor HBR for *An. funestus* s.l. was 5.6 bites/person/night (b/p/n) and outdoor HBR was 2.8 b/p/n. The indoor HBR for *An. gambiae* s.l. was 1.5 b/p/n and outdoor was 9.1 b/p/n. The indoor HBR for *An. coustani* was 1.4 b/p/n and outdoor was 4.1 b/p/n. *An. funestus* s.l. preferred biting indoors while *An. gambiae* s.l. and *An. coustani* largely fed outdoors. Morning biting (6:00 am–11:00 am) by the three species was also observed. The proportion of morning biting of *An. funestus* s.l. was 24% (indoors) and 4.5% (outdoors); *An. gambiae* s.l., 2.8% (indoors) and 2.0% (outdoors); and *An. coustani*, 8.5% (indoors) and 1.1% (outdoors).

Parity Rates: The overall mean parity rates of *An. funestus* s.l. and *An. gambiae* s.l. were 63.8% and 58.1%, respectively, in the three districts of Nkhata-Bay, Nkhotakota (NKK), and Salima. In NKK (IRS) and Salima (non-IRS) districts, the proportion of parous female *An. funestus* s.l. and *An. gambiae* s.l. before spray (July–September) was lower than the period after spray (October–June). However, in Nkhata-Bay (non-IRS) district, the proportion of parous female *An. funestus* s.l. and *An. gambiae* s.l. was higher before spray than after spray.

Infection Rate and Entomological Inoculation Rate (EIR): The overall infection rate of *An. funestus* s.l. was 3.6% and of *An. gambiae* s.l. was 1.3%; no infection was detected in *An. coustani*. The annual EIR of *Anopheles* mosquitoes in Nkhata-Bay is estimated at 83.9 infectious bites/person/year (ib/p/yr); NKK, 52.1 ib/p/yr; and Salima, 37.3 ib/p/yr. In Nkhata-Bay, *An. funestus* s.l. accounted for all infectious bites. In NKK, *An. funestus* s.l. accounted for 92.4% (48.2 ib/p/yr) of the total EIR with the rest due to *An. gambiae* s.l. (3.9 ib/p/yr). In Salima, the annual EIR due to *An. gambiae* s.l. was 23.0 ib/p/yr while that of *An. funestus* s.l. was 14.3

ib/p/yr. In NKK the monthly EIR, 12.75 ib/p/month in September (just before the October IRS) was significantly higher than the EIR 0.09 ib/p/month in December (after the IRS (October); ($p<0.0001$). There was no difference between September and December EIR in Nkhata-Bay.

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 structures across NKK district, which is less than the World Health Organization's estimated residual life of 4–8 months. Despite the low residual life of Actellic 300CS observed through wall cone bioassays, IRS had a significant impact on mosquito density in NKK. Mosquito density remained low from November 2018 to June 2019, covering a period of 8 months after spraying.

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

Conclusions

An. funestus s.l. is the major malaria vector in Malawi. The estimated annual EIR for the 3 project districts ranged from 14.3 to 83.9 ib/p/yr, followed by *An. gambiae* s.l. with annual EIR estimated at 3.9 - 23.2 ib/p/yr. *An. funestus* s.l. mostly feeds indoors, while as *An. gambiae* s.l. mostly feeds outdoors. The two important malaria vectors are highly resistant to pyrethroids but fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. Hence pirimiphos-methyl and clothianidin can be rotated in IRS programs in Malawi. It is worth noting, however, that the residual life of Actellic 300CS, which was used in 2018, is only 2–4 months on different wall surfaces. Despite the low residual life, IRS had a significant impact on *Anopheles* mosquito populations in NKK which remained low for 8 months.

1. INTRODUCTION

In September 2017, the U.S. Agency for International Development (USAID) through the U.S. President's Malaria Initiative (PMI) awarded Abt Associates a five-year malaria vector control contract, the PMI VectorLink Project. In October 2018, VectorLink Malawi carried out indoor residual spraying (IRS) in Nkhotakota (NKK) District in the central region of Malawi. Actellic 300CS, a microencapsulated suspension formulation of pirimiphos-methyl, an organophosphate, was used to spray structures in NKK District. In the same year, Malawi government through NMCP distributed approximately 10.9 million nets (September-December). Out of these Nets, 2.0 million were nets with piperonyl butoxide (PBO) and were distributed in 10 districts; Mchinji, Ntchisi, Salima, Karonga, Likoma, Rumphi, Machinga, Mwanza, Neno, Nsanje.

PMI VectorLink Malawi in collaboration with the Malaria Alert Centre (MAC) of the College of Medicine, University of Malawi, conducted entomological monitoring in three sites in NKK District to assess the impact of IRS on entomological indices of malaria transmission. In addition, comprehensive longitudinal entomological monitoring was also conducted in eight sentinel sites in four districts (Karonga, Nkhata-Bay, Salima, and Chikwawa) across the country to assess vector bionomics (vector density, composition, distribution, and behavior), species identification, infection rates and insecticide resistance). Tests for resistance mechanism are pending and will be reported as addendum when completed.

This report summarizes the key findings of the longitudinal entomological monitoring, Actellic 300CS residual efficacy and susceptibility status of wild-caught malaria vectors to different insecticides across Malawi.

2. METHODOLOGY

2.1 LONGITUDINAL MONITORING

Adult mosquitoes were collected from 11 sentinel sites in five districts from July 2018 to June 2019 (Figure 1). Four sites are located in the Northern region of Malawi: Mwakanyamale and Mwenimambwe (Karonga, PBO District), and Sanga and Kande (Nkhata-Bay District). Five sites are in the Central region: Vwawa, Chimkwende, and Ngalauka (NKK, IRS District), and Cholokoto and Chilungo (Salima, PBO District). Two sites are in the Southern region: Ntwana and Nyamphota (Chikwawa District).

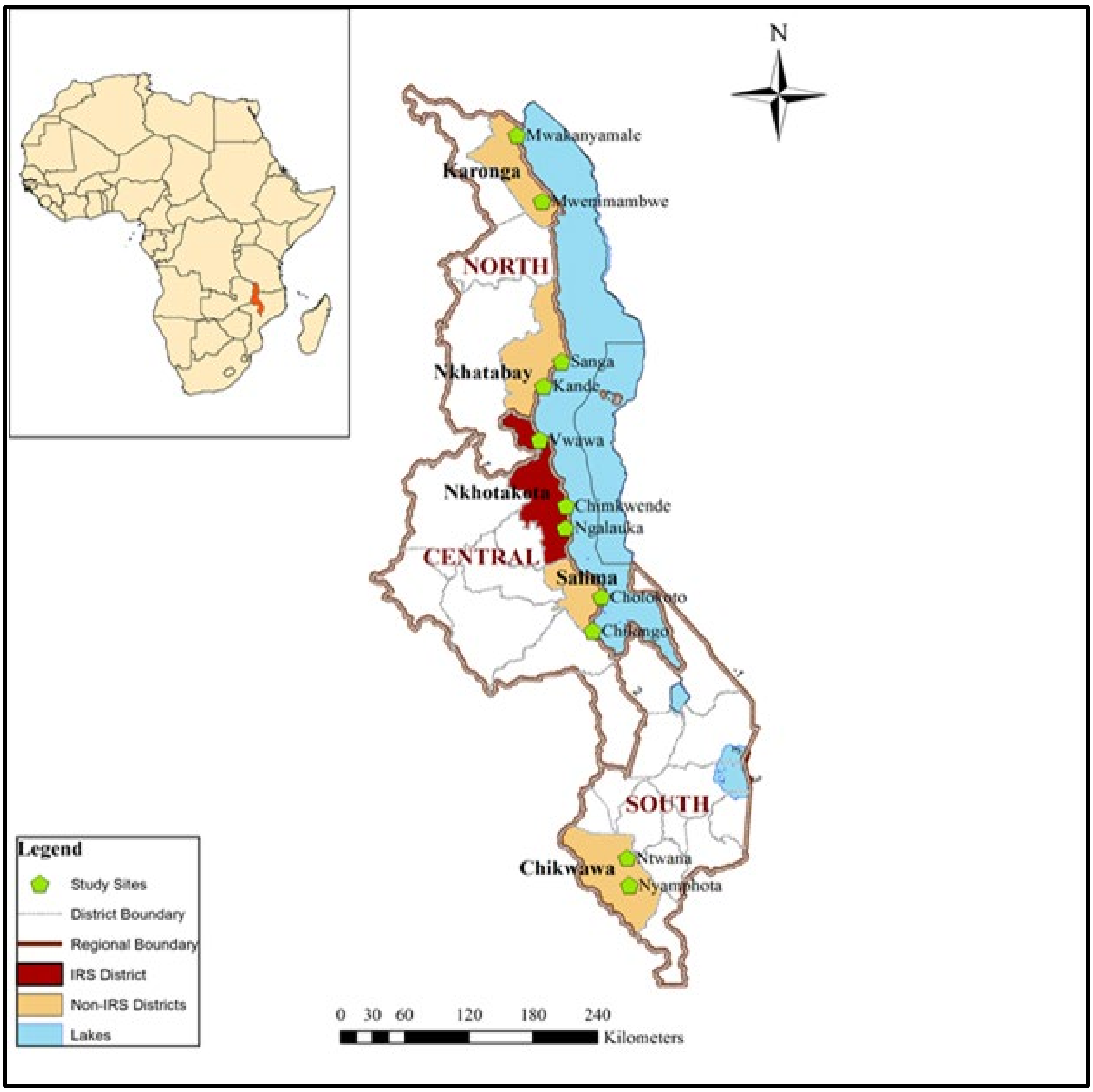


Figure 1: Entomological monitoring sites across Malawi, July 2018–June 2019

Three adult sampling methods were used for mosquito collection: pyrethrum spray catches (PSCs), conducted monthly; Centers for Disease Control and Prevention light traps (CDC-LTs), conducted monthly; and human landing catches (HLCs), conducted quarterly. Table 1 summarizes the three methods and sampling frequency.

Table 1: Longitudinal monitoring of adult mosquitoes in each of the 11 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
CDC-LTs	6:00 pm to 6:00 am	1 night per site per month	10 houses per site
HLCs	5:00 pm to 11:00 am	2 nights per house every three months	2 houses per site

PSCs were used to determine the indoor resting density (IRD) of malaria vectors and the seasonal variations in vector density and composition. Adult mosquitoes were collected monthly in a cohort of 15 randomly selected houses. The sampling was conducted in the same houses in each of the 11 sentinel sites, where at least one person had slept the previous night between 6:00 a.m. and 8:00 a.m. To do the PSC, a white cloth was spread in the selected house from wall to wall. Open spaces were blocked. The eaves, windows, other mosquito entry and escape routes, and space inside the house were sprayed using a commercial aerosol (Raid®, manufactured by SC Johnson & Son) to knock down mosquitoes resting inside the house. Ten minutes after spraying, all mosquitoes knocked down were collected from the white cloth.

CDC-LTs were installed inside a cohort of 10 separate randomly selected houses and sampling was conducted in the same houses for one night every month throughout the year (July 2018 to June 2019) at each sentinel site. CDC-LTs were suspended in a bedroom 1.5 meters above the floor and about 50 centimeters from a human sleeping under a bed net. Traps operated from 6:00 p.m. to 6:00 a.m. in the morning for one night in each of the 10 houses. During PSCs and CDC-LTs, other parameters such as number of people who slept in the house the previous night, number of animals, type of house, wall type, and roof type were also recorded.

HLCs were conducted on a quarterly basis in four sentinel sites (two in NKK, one in Nkhata-Bay, and one in Salima) to determine the biting rates, feeding locations, and peak biting time of various *Anopheles* vectors. HLCs were conducted from 5:00 p.m. to 11:00 a.m. Twelve volunteers in each of the four sentinel sites collected adult mosquitoes in two selected houses for two consecutive nights. Three pairs of collectors (two indoor and two outdoor) worked from 5:00 to 11:00 pm, 11:00 pm to 5:00 am, and 5:00 to 11:00 am, and they exchanged their positions between indoors and outdoors every hour to reduce possible differences in their attractiveness to mosquitoes. Collectors sat on chairs with their legs exposed up to the knee. Mosquitoes attempting to bite the collectors were caught using mouth aspirators and flashlights. Each hour, collected mosquitoes were transferred to individual labeled paper cups. Data on environmental parameters of each site, such as temperature and humidity, were recorded during the time of collection along with the use of vector control methods (long-lasting insecticidal nets (LLINs) and IRS) in the sampled houses.

All adult mosquitoes collected by each method were kept in moist petri dishes and later morphologically identified (Gillies and Coetzee 1987). All unfed mosquitoes from the HLCs identified as *An. gambiae* s.l. and *An. funestus* s.l. were dissected to assess the parity rate (Detinova 1962). Samples were preserved in properly labeled 1.5 ml Eppendorf tubes with silica gel for further analysis at the MAC laboratory.

2.2 Analysis and Molecular Evaluations

Molecular analyses were carried out on a sub-sample of specimens to:

1. Identify members of the *An. gambiae* s.l. and *An. funestus* s.l. to sibling species level.
2. Determine sporozoite rates and subsequently calculate EIRs.

Polymerase chain reaction (PCR) was used to identify *m*embers of *An. gambiae* complex and *An. funestus* group to the species-specific level as described by Benedict (2007). The heads and thoraxes of some of the *An. gambiae* s.l. and *An. funestus* s.l. were sorted and tested for the presence of circumsporozoite antigens of *Plasmodium falciparum* sporozoite using enzyme-linked immunosorbent assays (ELISA) described by Wirtz et al. (1987) to determine sporozoite rate.

2.3 Assessment of Spray Quality and Residual Efficacy of Actellic 300CS

Standard World Health Organization (WHO) cone bioassays (WHO 2006; 2013) were conducted using laboratory reared *An. gambiae* Kisumu strain to assess the spray quality and residual efficacy of Actellic 300CS. The airborne effect of pirimiphos-methyl was also assessed 24 hours and within 1week after spray.

2.3.1 Spray Quality Assessment

Cone bioassays were performed within one week of spray operations ending in four randomly selected villages in NKK District: Khufi 1, Chembakuka, Chimphewa, and Chikango (Figure 2).

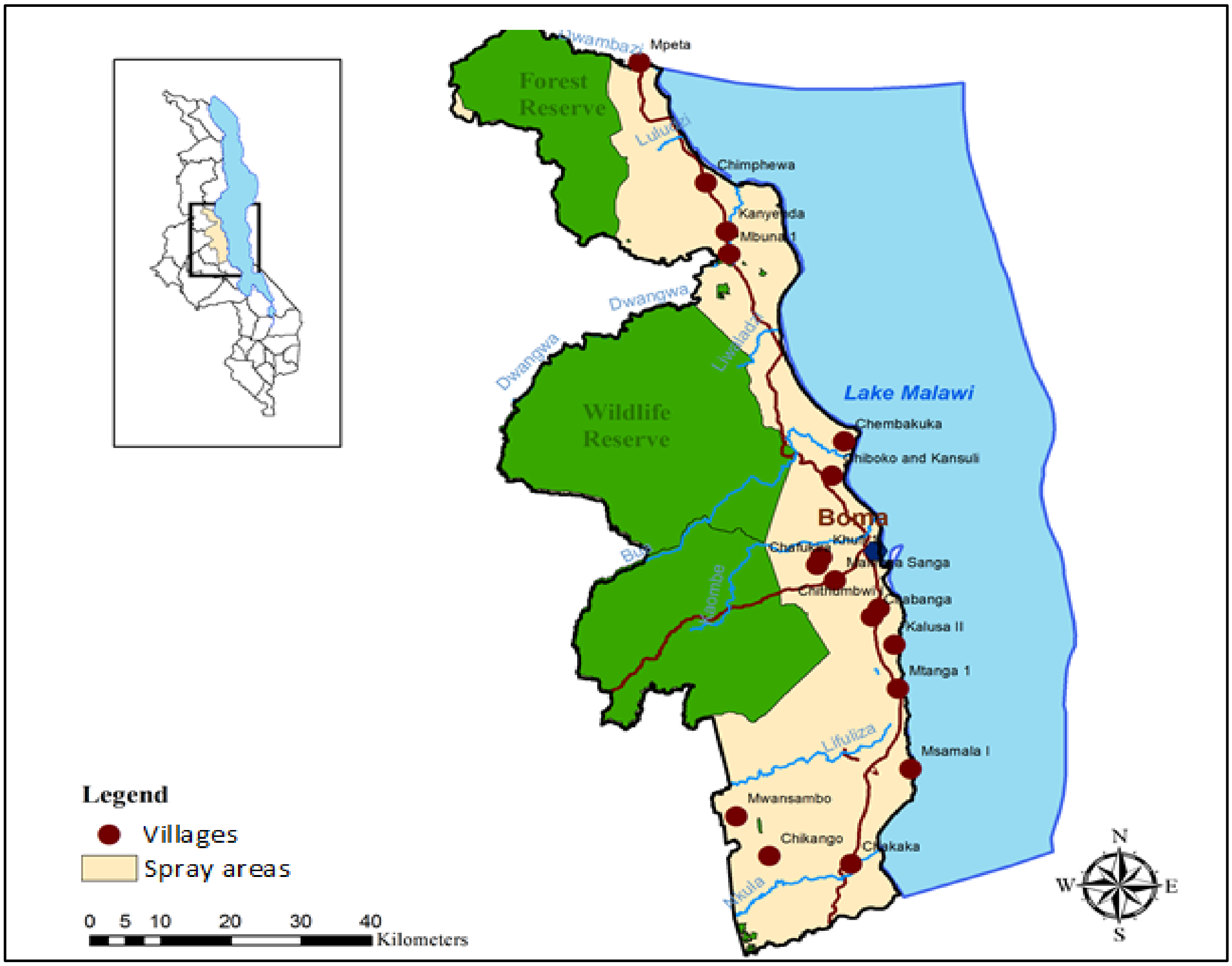


Figure 2: Villages in NKK District where spray quality and residual efficacy assessments of Actellic 300CS were conducted

The bioassays were conducted in six sleeping structures. All the structures had been sprayed with Actellic 300CS, but the wall surfaces were of different materials: two structures had mud walls, two had burnt brick, and two had plastered cement. Test cones were mounted at three heights (top, 1.5 m from floor; middle, 1.0 m; bottom, 0.5 m) on the sprayed walls. Two control cones were fixed on unsprayed surfaces, such as in kitchens and bathrooms, built outside the sleeping structures. Ten non-blood-fed females (2–5 days old) were introduced into each cone and were exposed to the walls for 30 minutes. After 30 minutes, the exposed mosquitoes were transferred to insecticide-free holding paper cups. Knockdown was observed and recorded at 30 and 60 minutes and mortality was recorded after 24 hours. Test mortality was corrected using Abbott's formula (Abbott 1925) when control mortality was between 5% and 20%.

Due to spray quality concerns from the NKK Boma operations site, spray teams in Boma were re-trained and an additional six houses were randomly selected to assess spray quality from each of the following villages: Chafukira, Kalusa II, and Mtanga 1; Malengasanga village acted as a control because it had been sprayed before the re-training of spray teams.

2.3.2 Fumigation Effect of Actellic 300CS

The airborne effect of pirimiphos-methyl was assessed in five villages: Khufi 1, Chembakuka, Chimphewa, Chikango, and Chinthumbwi 1. Twenty non-blood-fed female *An. gambiae* s.s. (Kisumu strain) were placed in a wire cage with netting, 10 cm away from the sprayed walls for 30 minutes. Knockdown was observed and recorded at 30 and 60 minutes, respectively, and mortality was recorded after 24 hours.

2.3.3 Residual Efficacy of Actellic 300cs

Residual efficacy monitoring was conducted in the two villages of Chembakuka and Chimphewa. Monthly visits were conducted in each village until mosquito mortality fell below the WHO threshold of 80%. Cone bioassays were conducted as described in Section 2.2.

Following a rapid decline in residual efficacy of Actellic 300CS, a one-off spray quality assessment was conducted in nine villages: Mwansambo, Chakaka, Msamala 1, Chiboko, Kansuli, Kanyenda, Mbuna 1, Chinthumbwi and Mpeta. A further spray quality assessment and residual efficacy of Actellic 300CS was conducted at Chinthumbwi 1 village (Figure 2). In Chinthumbwi 1, only six houses (two mud, two cement, and two burnt brick) were sprayed, under close supervision of PMI VectorLink Malawi's operations manager to rule out issues related to spray quality.

2.4 Insecticide Susceptibility Tests

Susceptibility tests were conducted at the peak of the rainy season between February and May 2019. Tests were planned for one site in each district where entomological monitoring was being conducted. However, due to low numbers of wild-caught mosquitoes in some districts, the tests were conducted in four districts: Karonga, Nkhata-Bay, Nkhotakota and Chikwawa. No tests were conducted in Salima district due to inadequate numbers of mosquitoes collected.

Live blood-fed female *Anopheles* mosquitoes (first generation, F_0) resting inside people's homes were collected from Ntwana, Sanga, Vwawa and Mwenimambwe sentinel sites in Chikwawa, Nkhata-Bay, Nkhotakota and Karonga districts, respectively, using Prokopack aspirators. The collected mosquitoes were allowed to lay eggs and reared, and the resultant (F_1) progeny were tested for insecticide resistance. All mosquito-rearing activities were carried out in the insectary at MAC.

2.4.1 WHO Tube Test

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-approved diagnostic doses. The following insecticides were tested: clothianidin (13.2mg/paper), pirimiphos-methyl 0.25%, permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.1%. Further tests, including pre-exposure to piperonyl butoxide (PBO) followed by a pyrethroid insecticide, were carried out: 4% PBO + permethrin 0.75%, 4% PBO + deltamethrin 0.05%, and 4% PBO + alpha-cypermethrin

0.10%. The same batch or family of mosquitoes was used for the PBO pre-exposure and pyrethroid-only tests.

Procedure: Four test replicates and one control were set up for each insecticide that was tested. 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 five replicates (four test and one control). Due to the low number of mosquitoes available, only a single control tube was run instead of two as per standard operating procedures. 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.

2.4.2 **CDC Bottle Assays**

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 permethrin 0.75% (samples collected from Vwawa, NKK district). Four Wheaton bottles (250mls) with caps were coated with 1ml of chlorfenapyr solution (100µg/bottle) or permethrin 0.75% by rolling and inverting the bottles. In addition, a control bottle was coated with 1ml of acetone. The coated bottles were then placed in the drawer covered with a napkin towel and left overnight to dry completely in the dark. Mosquitoes were exposed to chlorfenapyr 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. Mosquitoes exposed to permethrin 0.75% were observed for 30 minutes after which they were preserved in RNA later for further analysis.

2.4.3 **Interpretation of Results**

Susceptibility of *An. funestus* s.l. and *An. gambiae* s.l. was evaluated on the basis of the WHO criteria of test mortality (WHO 2016): 98–100% mortality indicates susceptibility. Mortality of less than 98% but more than or equal to 90% suggests the existence of resistance and the need for further investigation. 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.

2.4.4 Analysis of Data

The following parameters were calculated:

- Indoor resting density (IRD) = number of adult *Anopheles*/house/day collected using PSC. Student's T-test was used to compare IRD of *Anopheles* species.
- *Anopheles* mosquito numbers caught per trap per night (CDC-LT) and number of anopheles mosquitoes caught per house per day were compared using Student's T-test. All statistical analyses were conducted using Stata. P-value of less than 0.05 was considered to indicate statistical difference.
- Human biting rate (HBR) per unit time = total number of vectors collected/number of collectors per night/number of nights of capture.
- Sporozoite rate = the proportion of *Anopheles* found positive for the presence of circumsporozoite proteins (number positive for CSP/total number tested*100)
- EIRs were calculated by the formula:

  Quarterly EIR = Nightly HBRs X sporozoite rates X number of days in 3 months

  Annual EIR = Sum of quarterly EIRs

3. Results

3.1 Longitudinal Monitoring

3.1.1 Species Composition

A total of 42,329 *Anopheles* mosquitoes were collected from 11 sentinel sites in all five monitoring districts. Overall, PSCs contributed 55.9% (n = 23,679), CDC-LTs 39% (n = 16, 510), and HLCs 5.1 % (n = 2,140 to the total mosquito collections (Figure 3, and Annex A). Although the total number of *Anopheles* mosquitoes collected from PSCs was higher than from the other two collection methods, the mean difference per collection (PSCs = 12.0 mosquitoes/house/day and CDC-LTs = 12.5 mosquitoes/house/night was not statistically significant (p>0.05). The HBR from HLCs was 33.4 bites/person/night)

Overall, 56.3% of *Anopheles* mosquitoes collected were identified morphologically as *An. gambiae* s.l., 41.7% were *An. funestus* s.l., and 2.0% were *An. coustani* (Figure 4). However, species composition varied by sentinel site. *An. gambiae* s.l. was predominant in Mwenimambwe and Mwakanyamale sites (Karonga District), Chilungo site (Salima District), and Nyamphota site (Chikwawa District). *An. funestus* s.l. was predominant in Sanga and Kande sites (Nkhata-Bay District), Vwawa and Chimkwende sites (NKK District), and Cholokoto site (Salima District).

Further, 43,521 *Culex* mosquitoes were also collected during the same period. Of these, 8,399 were collected from HLCs (19.3%), 23,870 from CDC-LTs (54.8%), and 11,252 from PSCs (25.9%). *Mansonia* (n = 7,238) and *Aedes* (n = 47) mosquitoes were also collected using the three techniques.

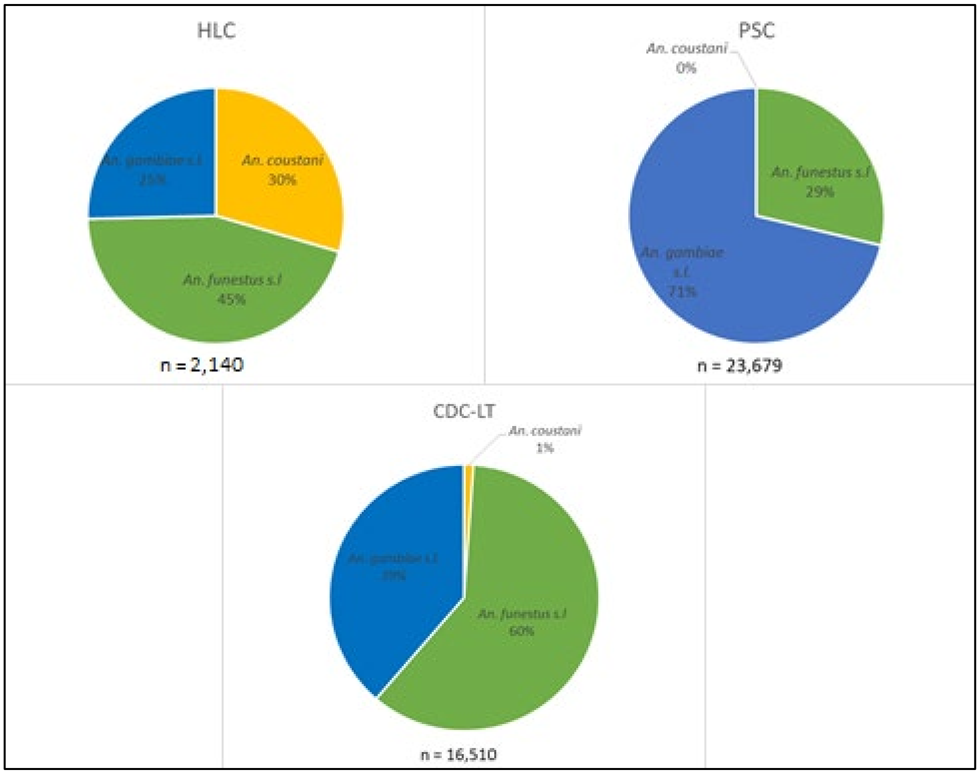


Figure 3: *Anopheles* mosquitoes collected by CDC-LT, PSCs, and HLCs, across all monitoring districts

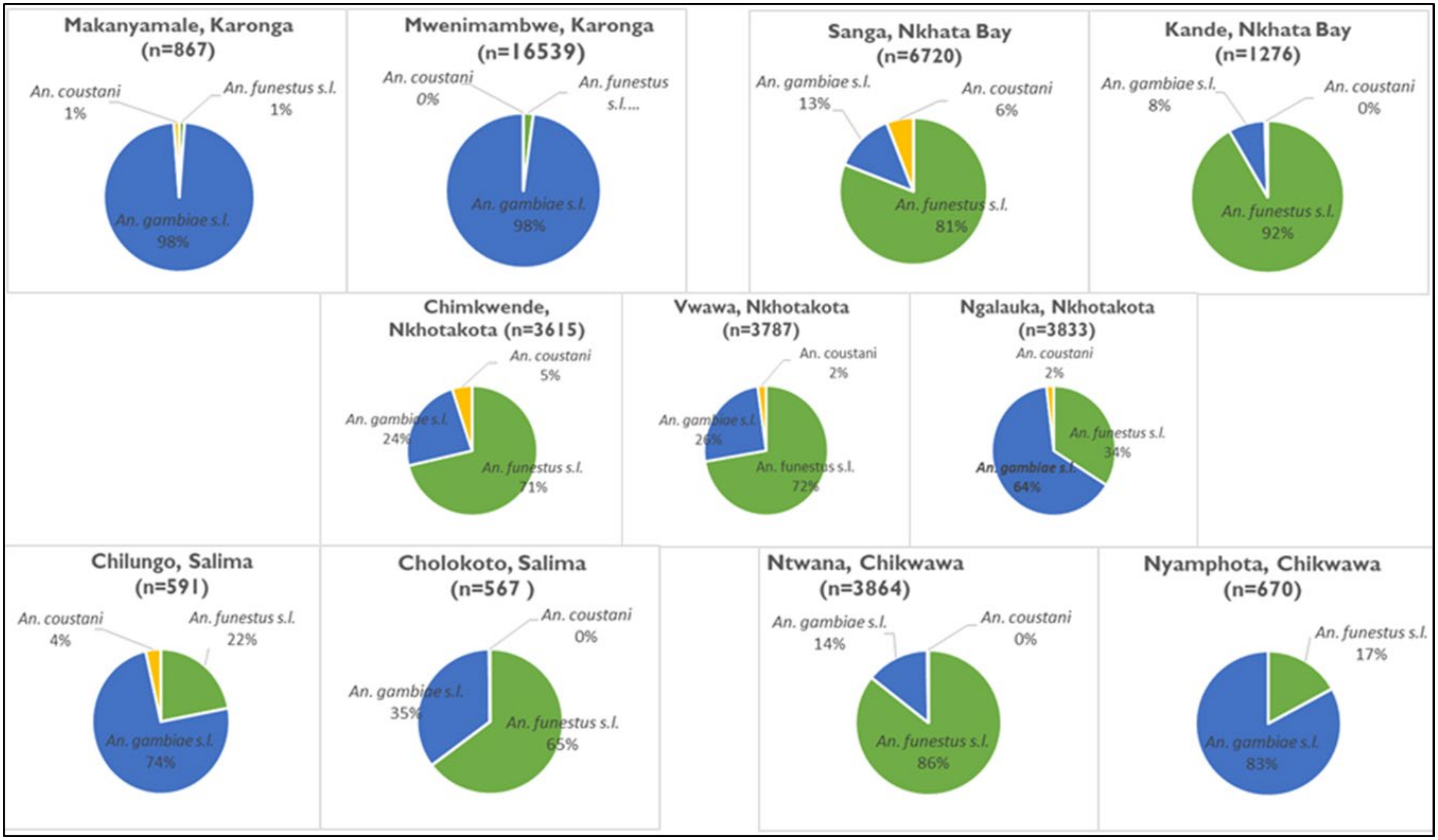


Figure 4: *Anopheles* composition, by sentinel site and collection method

3.1.2 INDOOR RESTING DENSITY (COLLECTED BY PSC)

a) *An. funestus* s.l. density

The IRD of *An. funestus* s.l. collected by PSC across all 11 sentinel sites from July 2018 to June 2019 is presented in Figure 5. The highest density of *An. funestus* s.l. was observed in September (8.1 mosquitoes/house/day) and the lowest in November and December (<1.0 mosquito/house/day). Overall, the IRD of *An. funestus* s.l. was 3.0 mosquitoes/house/day.

The highest mean IRD of *An. funestus* s.l. from PSC collections was observed in Chimkwende site (NKK District) in September 2018, with a mean catch of 38.0 *An. funestus* s.l./house/day. The mean IRD of *An. funestus* s.l. remained very low (<2.0 mosquitoes/house/day) throughout the year in six sites: Mwenimambwe and Mwakanyamale (Karonga), Cholokoto and Chilungo (Salima), Kande (Nkhata-Bay), and Nyamphota (Chikwawa) (Figure 6).

In the IRS district of NKK, the mean IRD of *An. funestus* s.l. was significantly higher before than after spraying ($p<0.05$).

Mean *An. funestus* s.l. IRD peaked between July and September 2018, and then dropped drastically in all sites except Ntwana (Chikwawa) and Sanga (Nkhata-Bay), where it rose during the peak rainy season, in January 2019.

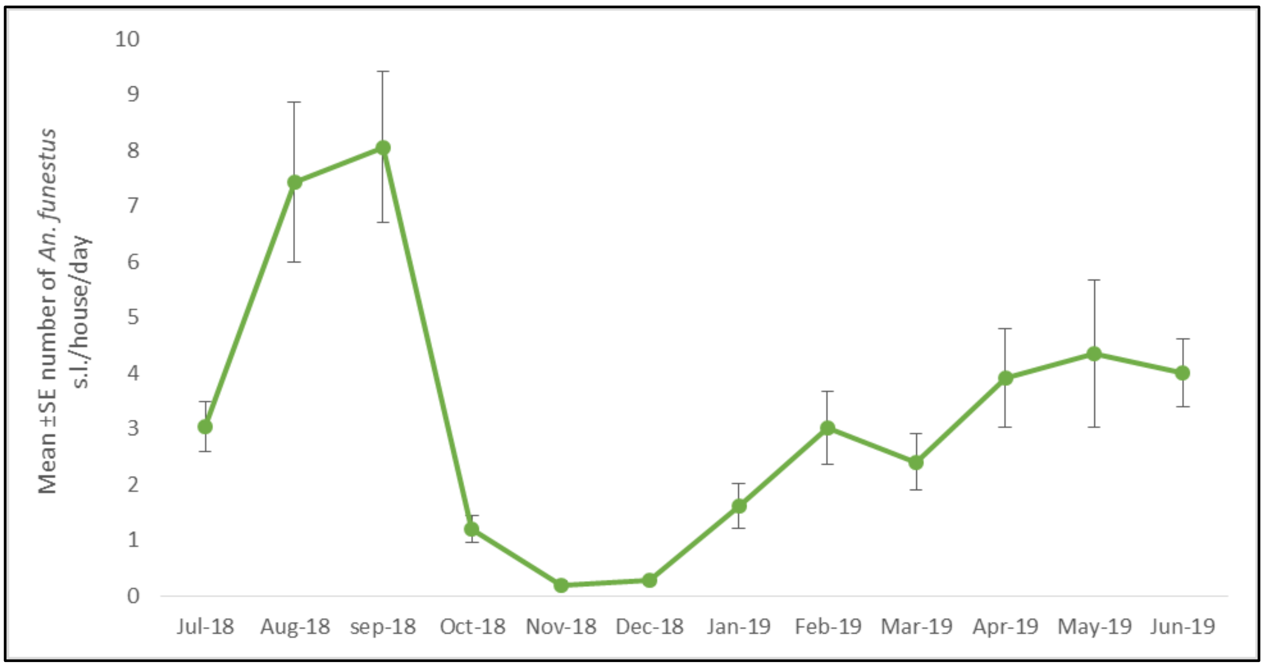


Figure 5: IRD of *An. funestus* s.l., all monitoring districts, July 2018–June 2019

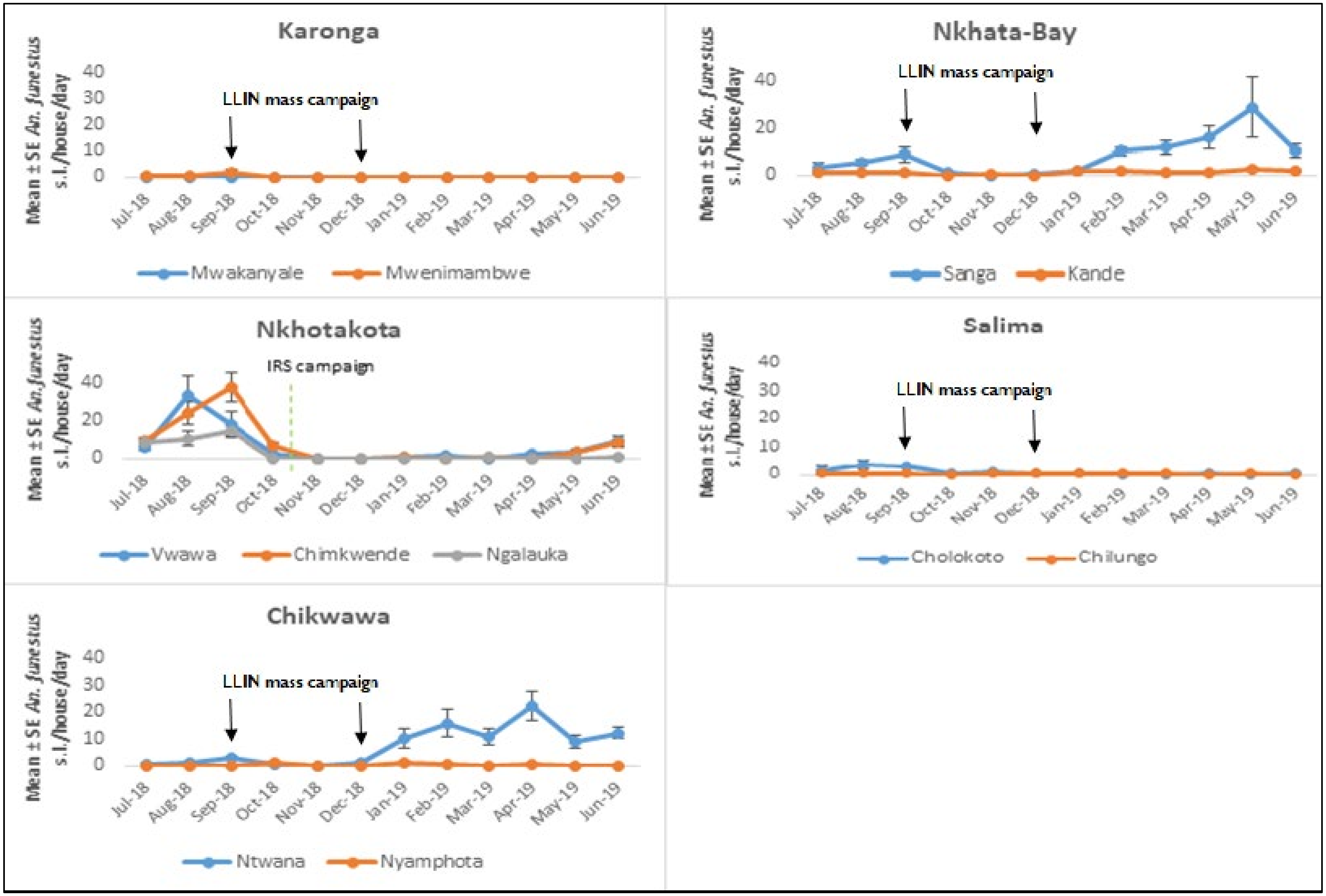


Figure 6: Average number of *An. funestus* s.l. collected by PSC, by sentinel site, July 2018–June 2019

b) *An. gambiae* s.l. density

The IRD of *An. gambiae* s.l. collected by PSC across all 11 sentinel sites from July 2018 to June 2019 is presented in Figure 7. The highest *An. gambiae* s.l. IRD was recorded in August (43.7 mosquitoes/house/night) and lowest in November and December (<1.0 mosquito/house/day). Overall, the mean IRD of *An. gambiae* s.l. was 9.0 mosquitoes/house/day in all sites.

With a mean catch of 446.5 *An. gambiae* s.l./house/day in August 2018, Mwenimambwe site in Karonga District by far recorded the highest mean *An. gambiae* s.l. IRD of all the sites. Overall, IRD of *An. gambiae* s.l. in Karonga was significantly higher than in the other districts ($p<0.05$). The lowest mean IRD of *An. gambiae* s.l. (<2.0 mosquitoes/house/day) was recorded at Kande site (Nkhata-bay District) and at Chilungo and Cholokoto sites (Salima) (Figures 8 and 9).

In NKK (IRS) District, the highest mean IRD of *An. gambiae* s.l. was obtained in September (9.5 mosquitoes/house/day), before spray, and in February (10.0 mosquitoes/house/day), after spray. The mean IRD for *An. gambiae s.l.* was significantly higher before than after spraying ($p<0.05$).

Again in Mwenimambwe site in Karonga, the mean IRD for *An. gambiae* s.l. was high in July (226.3 mosquitoes/house/day) and August (446.6 mosquitoes/house/day), then dropped to <1.0 mosquitoes/house/day in December. IRD rose slightly again in February to March, and then dropped again to <1.0 mosquito/house/day in June. In comparison, the mean IRD for *An. gambiae* s.l. in the remaining 10 sentinel sites was low throughout the year (<10.0 mosquitoes/house/day) (Figure 9).

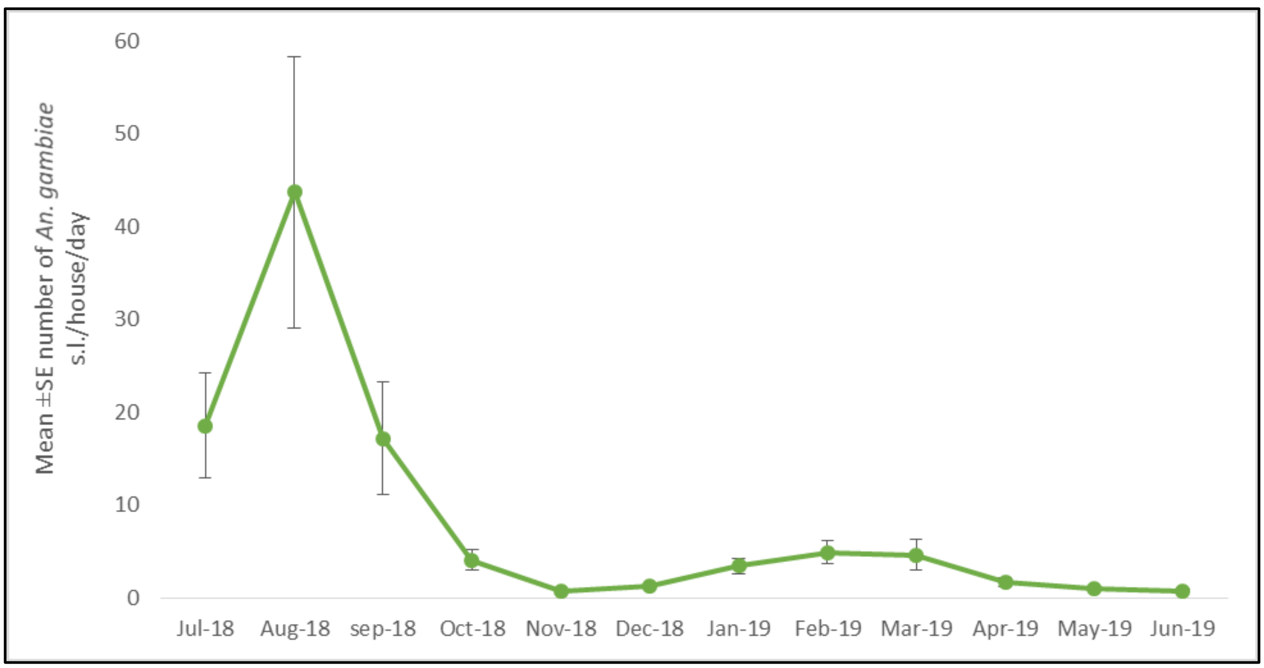


Figure 7: IRD of *An. gambiae* s.l., all monitoring districts, July 2018–June 2019

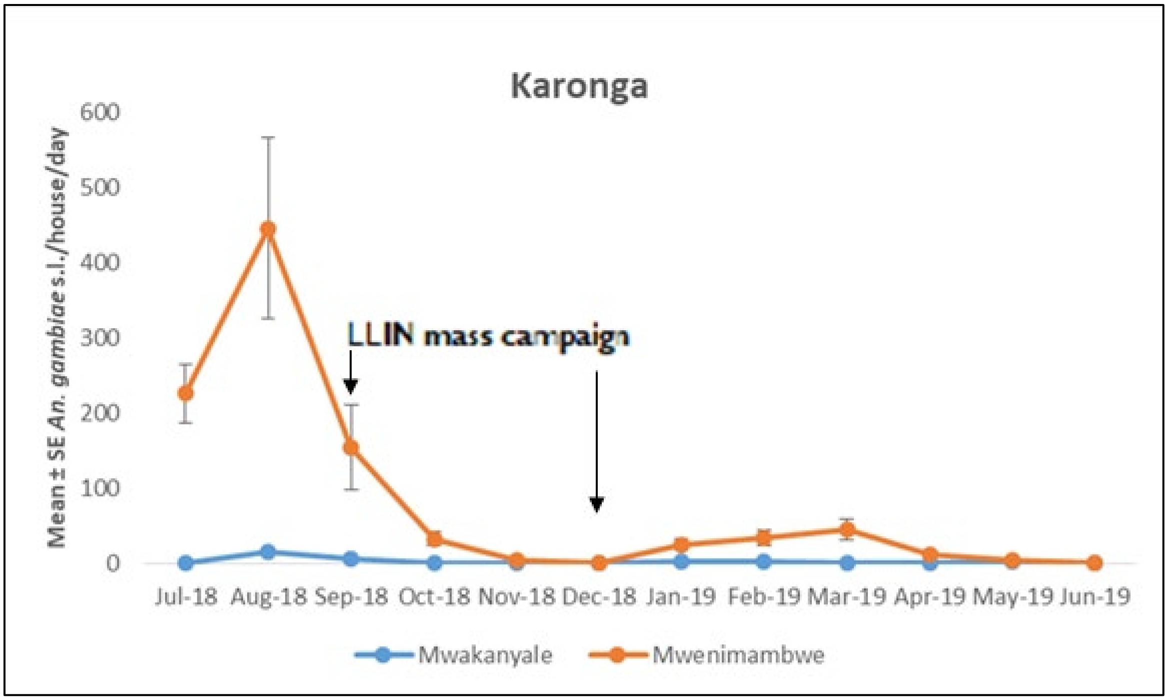


Figure 8: Average number of *An. gambiae* s.l. collected by PSC, by site in Karonga District, July 2018–June 2019

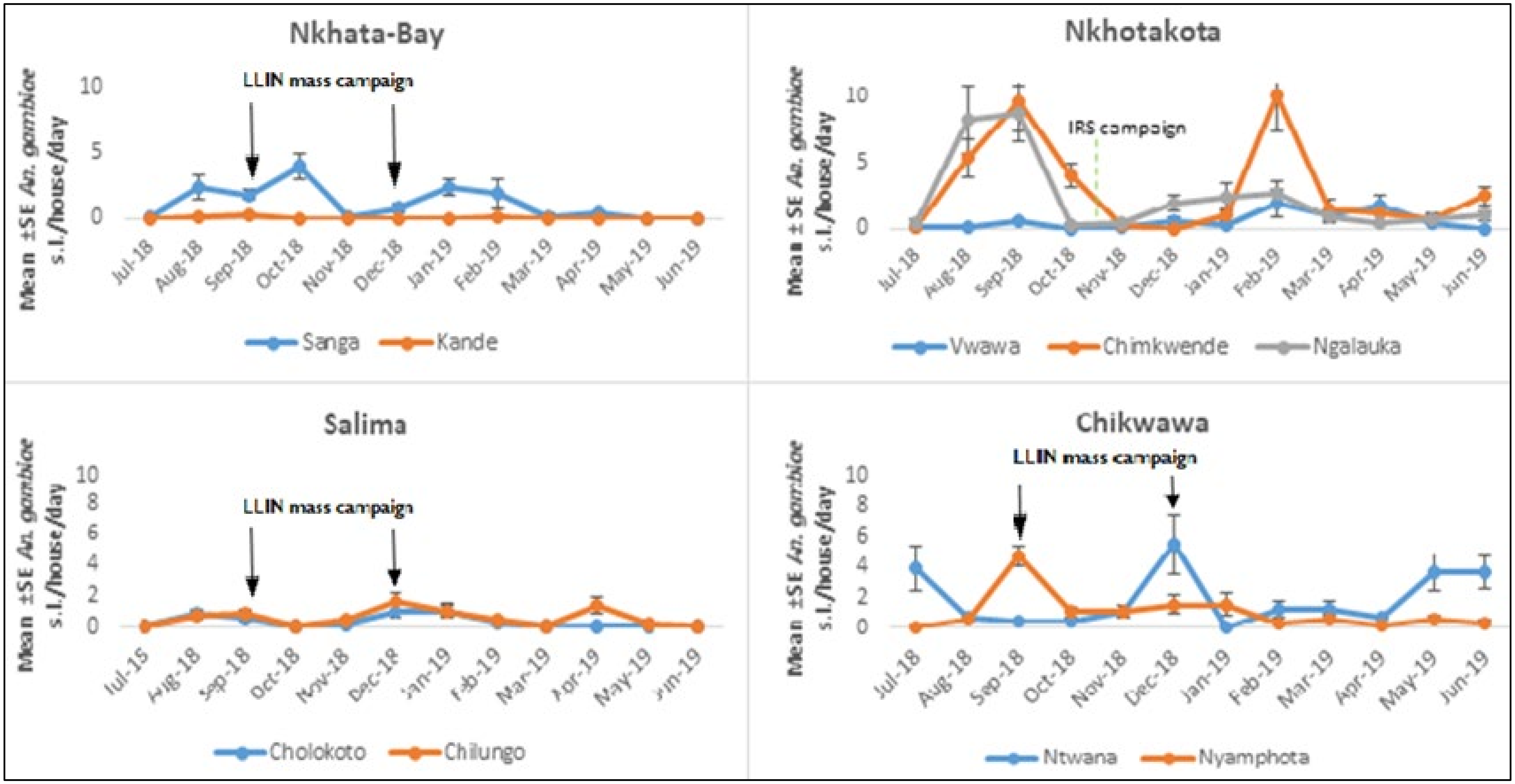


Figure 9: Average number of *An. gambiae* s.l. collected by PSC, by site in four monitoring districts, July 2018–June 2019

3.1.3 Indoor CDC-LT Collections

a) *An. funestus* density

The number of *An. funestus* s.l. collected by indoor CDC-LT in all 11 sites from July 2018 to June 2019 is presented in Figure 10. The peak density was recorded in March (13.3 mosquitoes/trap/night) and the lowest density was recorded in November (<1.0 mosquito/trap/night). The overall mean *An. funestus* s.l. density was 7.6 mosquitoes/trap/night.

The highest mean density of *An. funestus* s.l. was recorded in Sanga site (Nkhata-Bay) with 112.5 mosquitoes/trap/night in March 2019. The lowest densities (<10.0 mosquitoes/trap/ night) throughout the year were recorded in Cholokoto and Chilungo (Salima), Mwakanyamale and Mwenimambwe (Karonga), Kande (Nkhata-Bay), and Nyamphota (Chikwawa) (Figure 11).

In NKK (IRS) District, the highest densities of *An. funestus* s.l. were recorded before spraying (July to October). After spraying, *An. funestus* s.l. density drastically decreased for the rest of the study year (November–June) with the exception of Vwawa site where the IRD increased greatly in June (Figure 11).

In Sanga (Nkhata-Bay), *An. funestus* s.l. density was low prior to the rainy season (August to December), then increased between January and March (peak rainy season). It dropped in April to June, toward the end of the rainy season, and increase again in July (Figure 11).

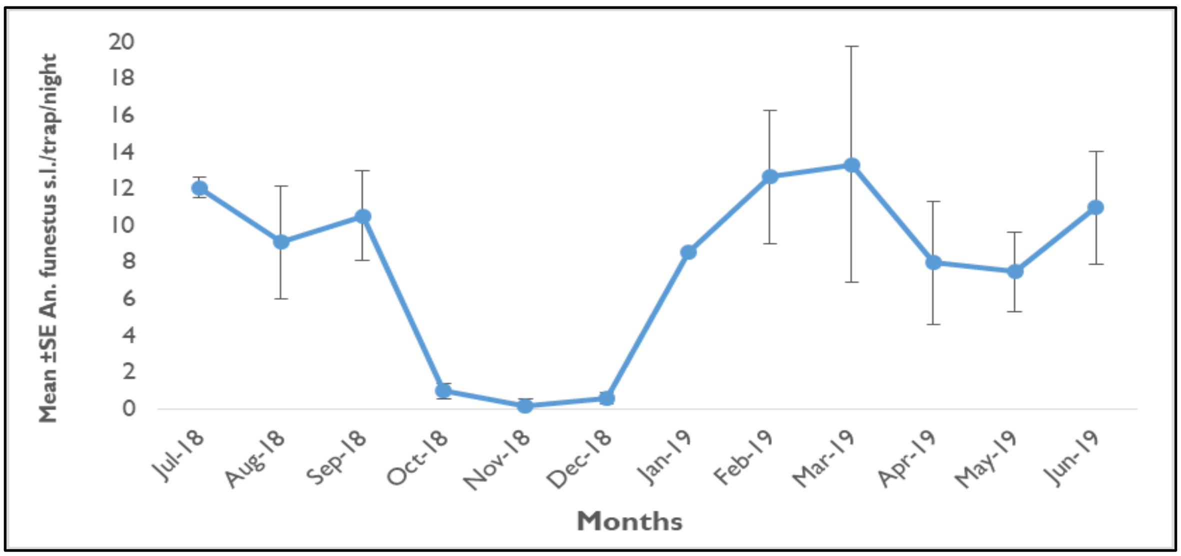


Figure 10: Indoor density of *An. funestus* s.l., all monitoring districts, July 2018–June 2019

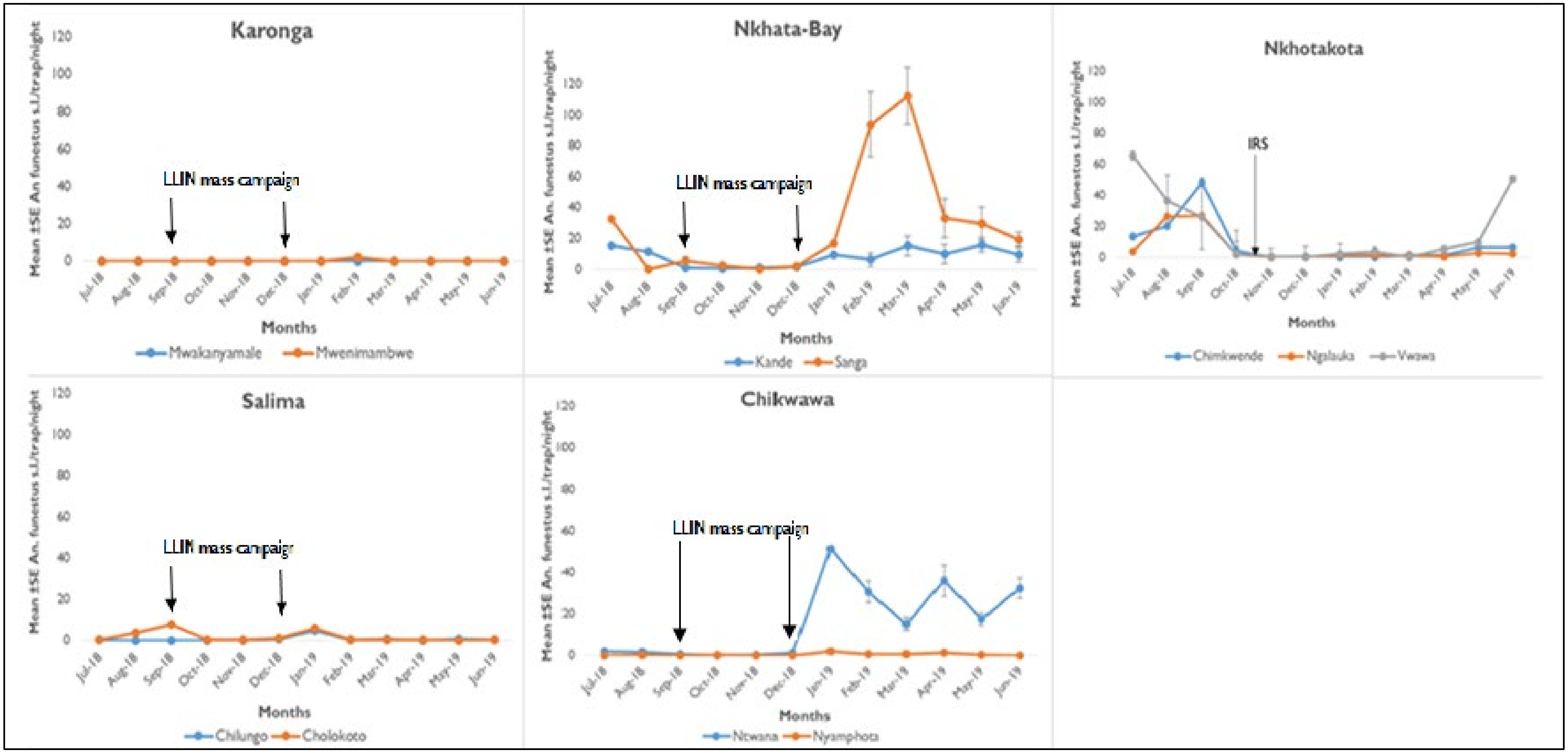


Figure 11: Mean indoor densities of *An. funestus* s.l. collected by CDC-LT, by sentinel site, July 2018–June 2019

b) *An. gambiae* s.l. density

The density of *An. gambiae* s.l. collected using CDC-LTs in the 11 sentinel sites is presented in Figure 12. Overall, the highest density of *An. gambiae* s.l. was observed in February (16.5 mosquitos per trap per night) and the lowest in November (<1.0 mosquito per trap per night). The mean density of *An. gambiae* s.l. was 4.7 mosquitoes per trap per night.

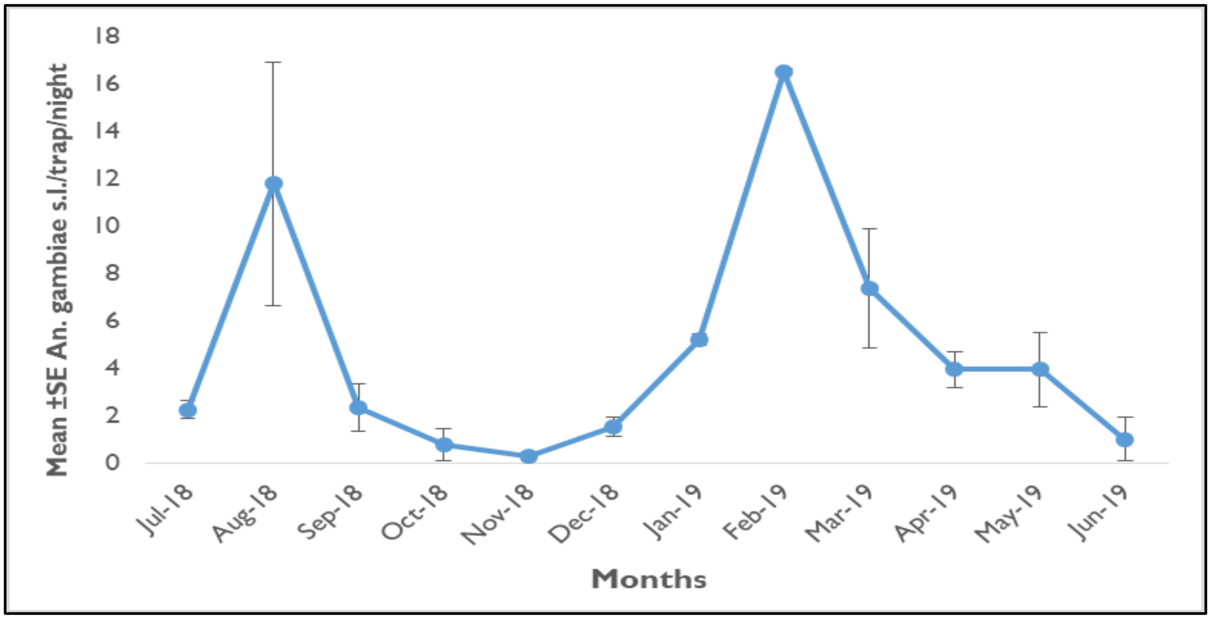


Figure 12: Indoor density of *An. gambiae* s.l., all monitoring districts, July 2018–June 2019

The highest *An. gambiae* s.l. density was obtained in NKK (IRS) District (Ngalauka site), 71.8 mosquitoes per trap per night) in February. The lowest densities (<10.0 mosquitoes per trap per night) throughout the year were recorded in Kande site (Nkhata-Bay), Cholokoto (Salima), and Ntwana and Nyamphota (Chikwawa) (Figure 13).

In NKK, *An. gambiae* s.l. densities at Vwawa and Chimkwende were relatively low throughout the year (<12.0 mosquitoes per trap per night) except in February, when the density reached 34.9 mosquitoes per trap per night at Vwawa. At Ngalauka site, *An. gambiae* s.l. density was low from September 2018 to January 2019, then started to rise and reached its peak in February, and then dropped between March and June (Figure 13).

In Karonga District, high *An. gambiae* s.l. density was recorded in August and dropped from September to January. The population started to rise again in February and March and then dropped from April to June (Figure 13).

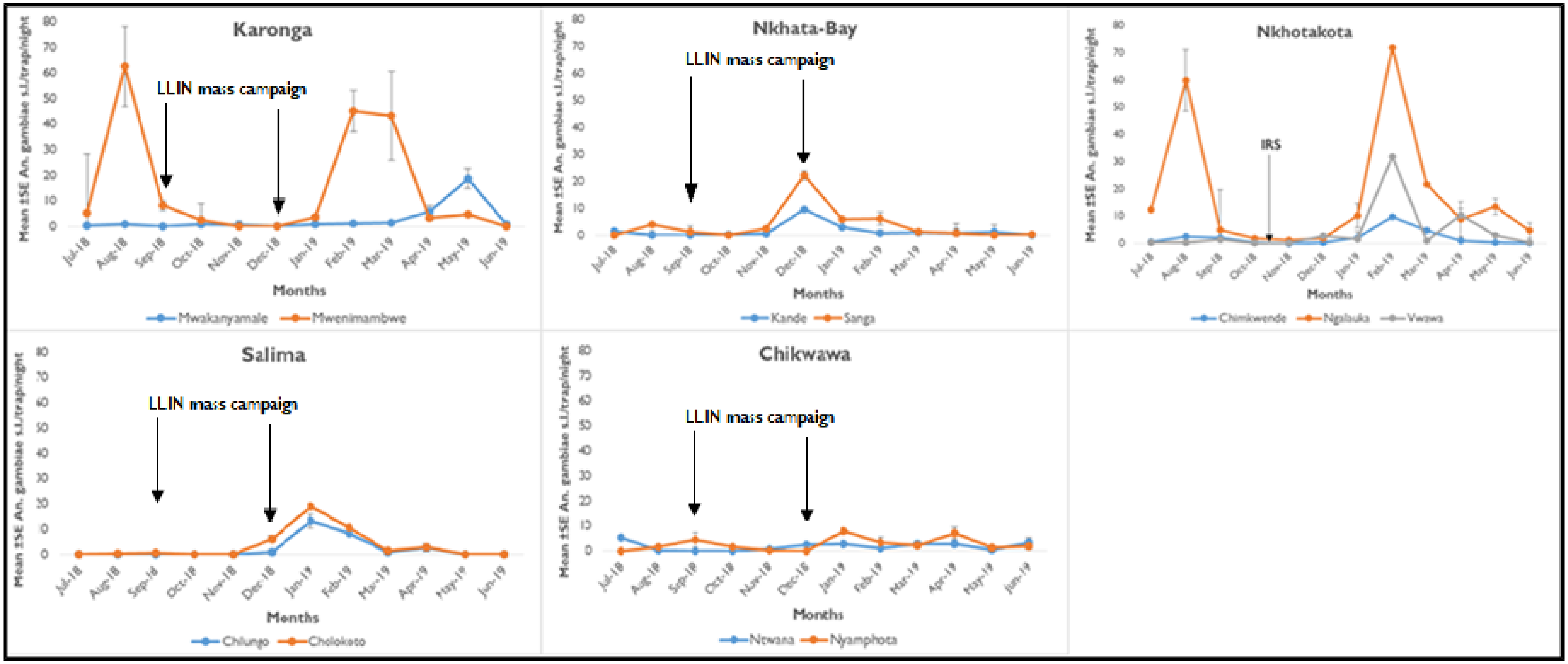


Figure 13: Mean indoor densities of *An. gambiae* s.l. collected by CDC-LT, by sentinel site, July 2018–June 2019

3.1.4 GONOTROPHIC STATUS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. (COLLECTED BY PSC)

Overall, the highest proportion of the female *Anopheles* mosquitoes collected were blood fed. Out of the 15,928 females collected by PSC, 3,016 (18.9%) were unfed; 7,094 (44.5%) were blood fed; 2,725 (17.1%) were gravid; and 3,046 (19.1%) were half gravid (Table 2). Gonotrophic status for 47 (0.3%) samples could not be determined because their abdomens were damaged. In addition, more female *An. gambiae* s.l. (unfed, fed, half gravid, or gravid) were collected than *An. funestus* s.l.

In NKK District, the proportion of half-gravid female *Anopheles* mosquitoes was higher before spray than after spray. However, the proportion of gravid female *Anopheles* mosquitoes remained constant before and after spray (Figure 14).

In Karonga District, the proportion of fed female *Anopheles* mosquitoes was higher before the rainy season (July–November) than during the rainy season (December–May). The opposite was observed in Salima District, where high numbers of fed female *Anopheles* were collected during the rainy season. In NKK, Nkhata-Bay, and Chikwawa districts, the proportions of fed female *Anopheles* mosquitoes collected before and during the rainy season were similar. A similar trend was observed for gravid female *Anopheles* mosquitoes in all five districts (Figure 15).

Table 2: Number and proportion of female *An. funestus* s.l. and *An. gambiae* s.l. and their gonotrophic status sampled by PSC in 11 sentinel sites

Species	**Gonotrophic status**					
	Unfed (N, %)	**Fed (N, %)**	**Half gravid (N, %)**	**Gravid (N, %)**	**Undetermined (N, %)**	**Total (N, %)**
An. funestus s.l.	673 (15.1)	1836 (41.2)	1136 (25.6)	784 (17.6)	21 (0.5)	4450 (27.9)
An. gambiae s.l.	2343 (20.4)	5258 (45.8)	1910 (16.6)	1941 (16.9)	26 (0.02)	11478 (72.1)
Total	**3016 (18.9)**	**7094 (44.5)**	**3046 (19.1)**	**2725 (17.1)**	**47 (0.3)**	**15928**

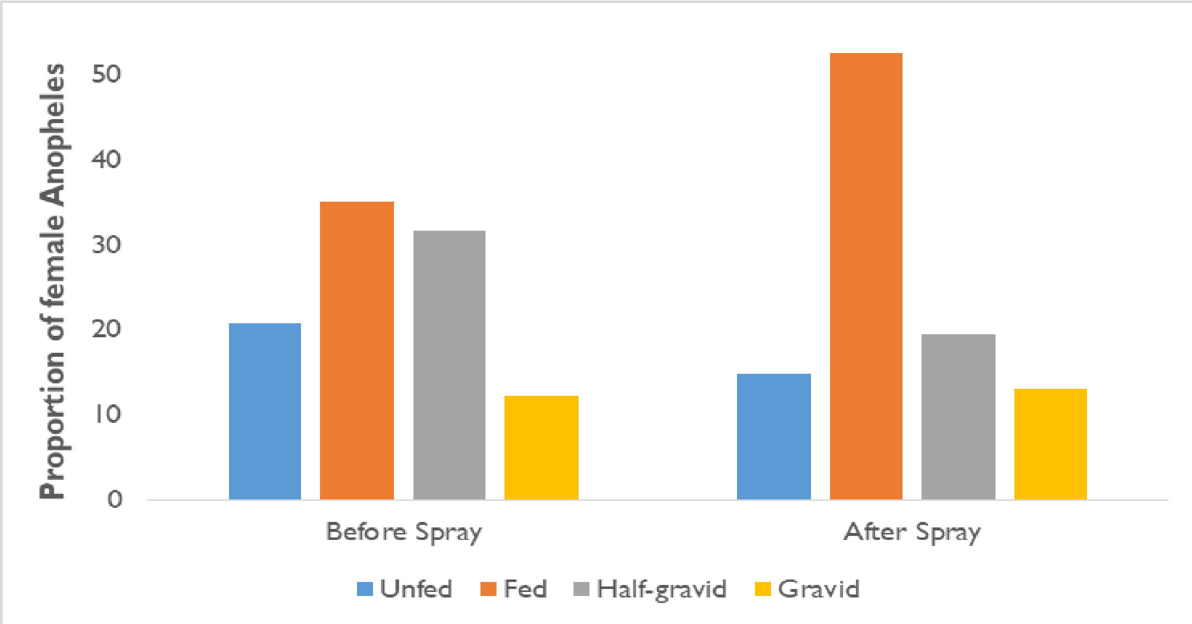


Figure 14: Gonotrophic status of female *An. funestus* s.l. and *An. gambiae* s.l. before and after spray in NKK District

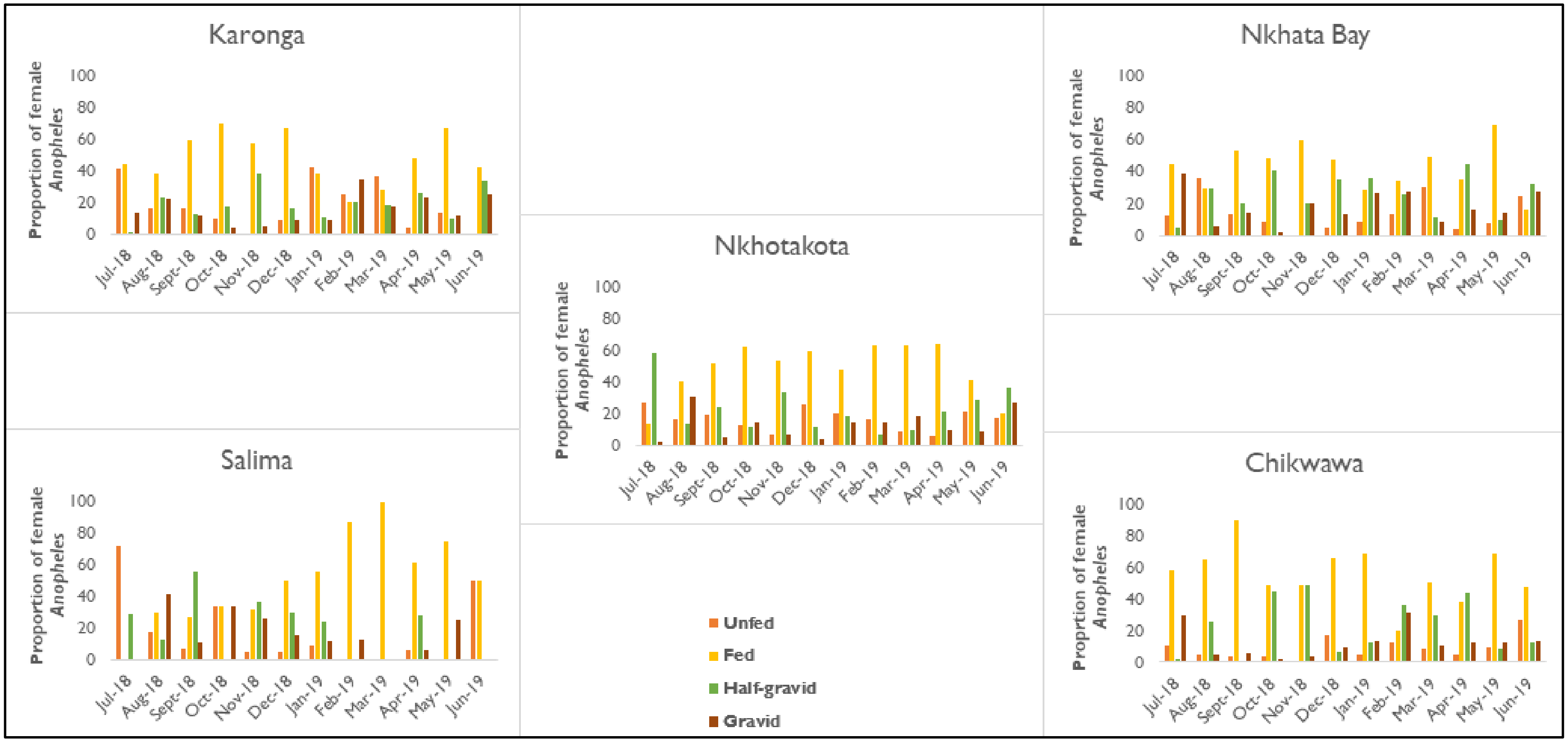


Figure 15: Gonotrophic status of *An. funestus* s.l. and *An. gambiae* s.l., by sentinel site, July 2018–June 2019

3.1.5 HUMAN BITING RATES (COLLECTED BY HLC)

(a) Biting rates of malaria vectors

The HBR was measured in three districts: Salima, NKK, and Nkhata-Bay. The overall HBR of *Anopheles* mosquitoes collected from the three districts is shown in Figure 16. The *An. funestus* s.l. HBR was 5.6 bites per person per night (b/p/n) indoors and 2.8 b/p/n outdoors. The HBRs for *An. gambiae* s.l. were 1.5 b/p/n indoors and 9.1 b/p/n outdoors, and for *An. coustani* were 1.4 indoors and 4.1 b/p/n outdoors.

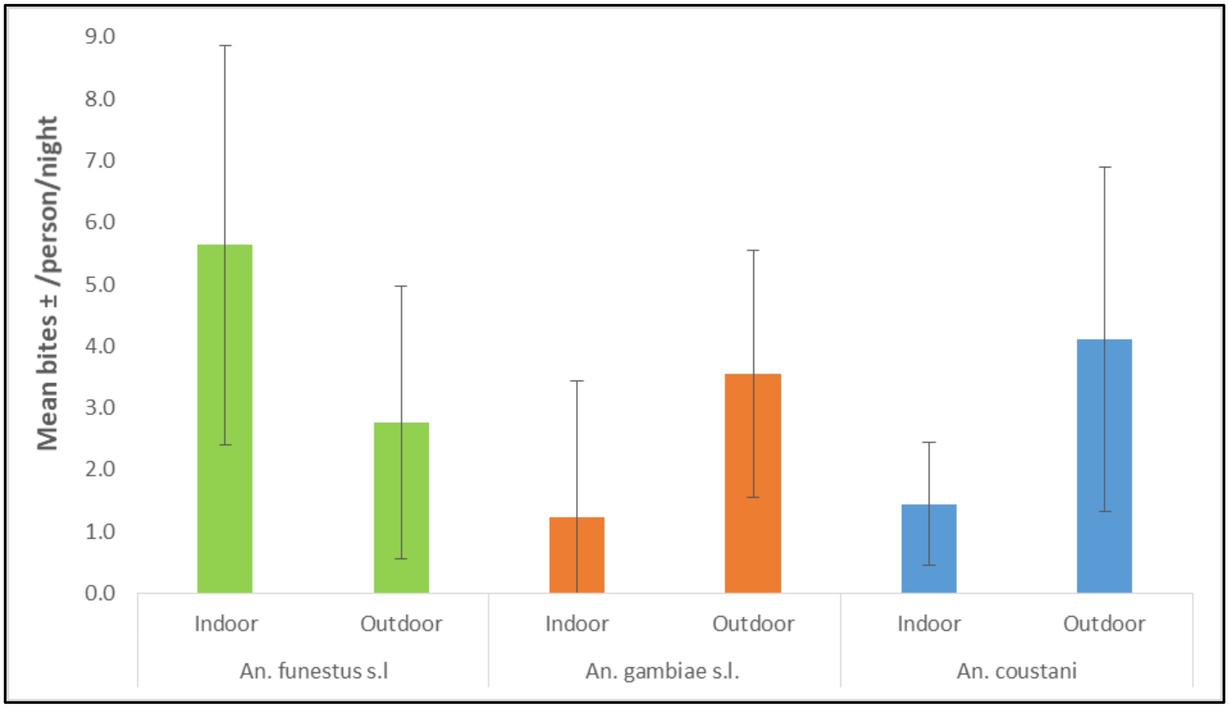


Figure 16: Average bites of *Anopheles* mosquitoes per person per night in three districts

Overall, the highest HBRs were recorded in Nkhata-Bay District. The highest biting activity of *An. funestus* s.l. was recorded there in March, the peak of the rainy season, with HBRs of 21.3 b/p/n indoors and 11.9 b/p/n outdoors. The highest *An. gambiae* s.l. biting activity was observed in September, before the rainy season, when the HBR was 5.8 b/p/n indoors and 27.7 b/p/n outdoors. The highest *An. coustani* indoor HBR, 6.5 b/p/n, was recorded in March and the highest outdoor HBR, 26.0 b/p/n, was recorded in September (Table 3).

Table 3: HBRs of *Anopheles* mosquitoes in three districts, July 2018 to June 2019

District	Month	*An. funestus* s.l.		*An. gambiae* s.l.		*An. coustani*	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Nkhata-Bay	September	8.00	6.88	5.75	27.63	3.00	26.00
	December	3.13	3.38	0.88	0.50	0.00	0.13
	March	21.25	11.88	0.63	2.13	6.50	6.25
	June	14.3	6.5	0.0	12.5	4.0	6.0

Nkhotakota	September	11.50	0.69	1.13	0.00	2.13	0.00
	December	0.06	0.06	1.00	1.75	0.00	0.19
	March	0.31	0.06	2.63	3.19	0.81	5.75
	June	6.75	0.69	0.13	0.69	0.56	2.88
Salima	September	0.13	0.75	0.00	0.00	0.38	2.00
	December	0.63	0.75	2.00	2.88	0.00	0.00
	March	1.25	1.50	0.50	3.75	0.00	0.13
	June	0.38	0.00	0.13	0.13	0.00	0.00
Overall		5.6	2.8	1.2	3.6	1.4	4.1

(b) Biting pattern of malaria vectors

Figure 17 shows the biting pattern of *An. funestus* s.l. and *An. gambiae* s.l., again in the three districts of Salima, NKK (two sites), and Nkhata-Bay. Almost all collections from Karonga were *An. gambiae* s.l.

In NKK District (both sites), the peak biting periods of *An. funestus* s.l. were 5:00–7:00 am indoors and 2:00–3:00 am outdoors. The outdoor biting activity was significantly less than the indoor biting activity ($p<0.05$). *An. gambiae* s.l. in this district fed both indoors and outdoors but its biting activity was significantly less than that of *An. funestus* s.l. The peak biting period of *An. gambiae* s.l. was 4:00–5:00 am indoors and 3:00–4:00 am outdoors.

In Chilungo site (Salima District), *An. gambiae* s.l. exhibited predominantly exophagic behavior and its peak biting period was 8:00–9:00 pm indoors, and 11:00 pm–12:00 am and 3:00–4:00 am outdoors. The biting activity of *An. funestus* s.l. in Salima was significantly less than that of *An. gambiae* s.l. ($p<0.05$). Its peak biting time was 11:00–12:00 am, indoors.

In Sanga site (Nkhata-Bay District), *An. gambiae* s.l. and *An. funestus* s.l. fed equally indoors and outdoors. The peak biting period of *An. gambiae s.l.* was 2:00–3:00 am indoors and 5:00–6:00 am outdoors, while the *An. funestus* s.l. peak biting period was 2:00–3:00 am and 4:00–5:00 am indoors and 4:00–5:00 am outdoors.

Morning/daytime biting was also observed and found to be mostly by *An. funestus* s.l., indoors, in NKK and Nkhata-Bay districts. The overall proportion of morning/daytime biting (6:00–11:00 am) was 23.1% indoors and 4.5% outdoors for *An. funestus*, 2.8% indoors and 2.0% outdoors *for An. gambiae* s.l., and 8.5% indoors and 1.1% outdoors for *An. coustani* (Annex B).

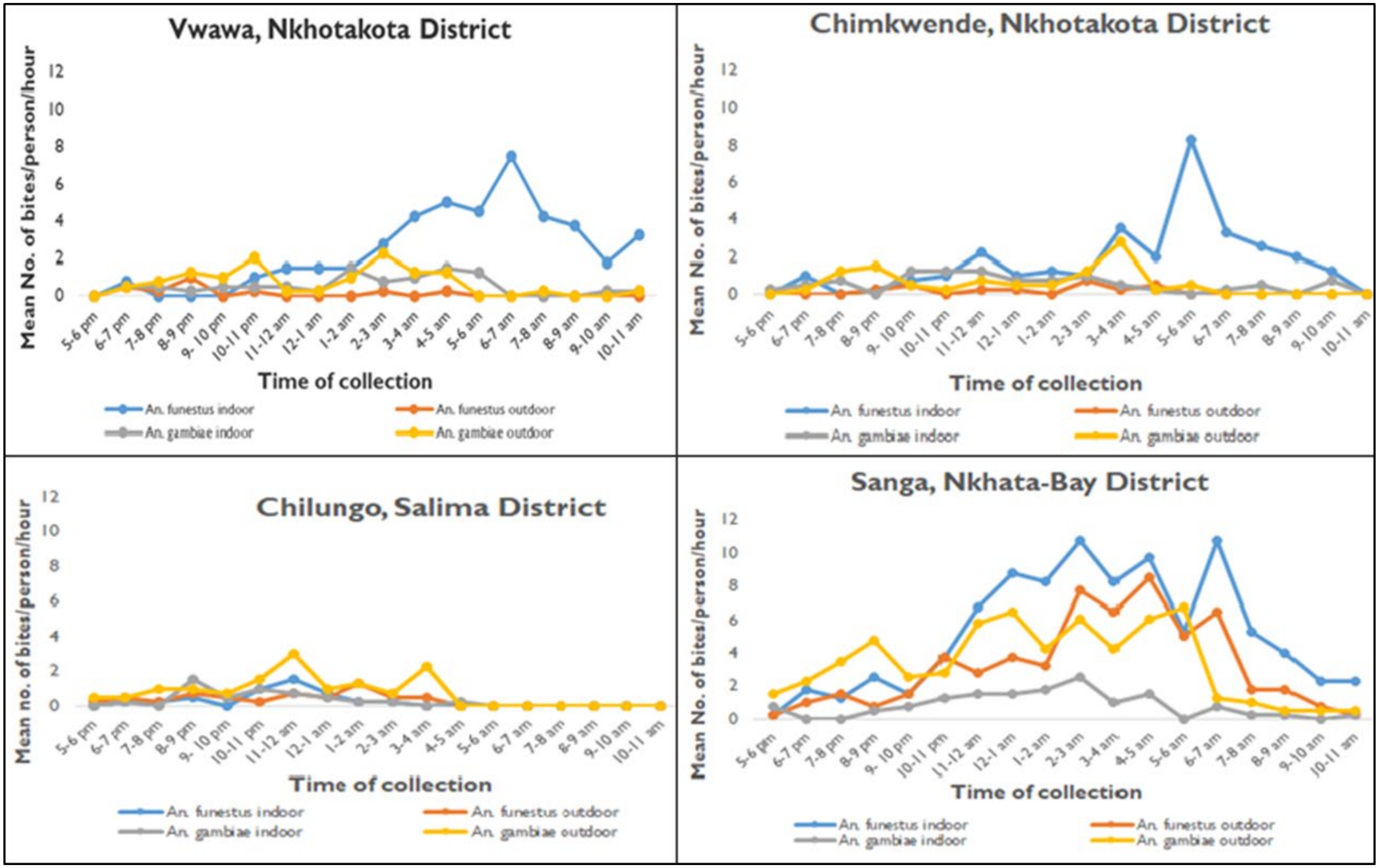


Figure 17: Average hourly indoor and outdoor biting rates of *An. gambiae* s.l. and *An. funestus* s.l. in four sentinel sites (July 2018–June 2019.

(c) Parity rates

The quarterly dissections of *An. funestus* s.l. and *An. gambiae* s.l. from the HLC sites are presented in Table 4. Overall, higher proportions of parous females were recorded among *An. funestus* s.l. (63.8%) than among *An. gambiae* s.l. (58.1%) in all four sites. Sanga site (Nkhata-Bay) had the highest proportion of parous mosquitoes.

Table 4: Total number and proportion of parous female *An. funestus* s.l. and *An. gambiae* s.l., by HLC site

District	Sentinel Site	*An. funestus* s.l.			*An. gambiae* s.l.		
		# Dissected	Parous	% Parity	# Dissected	Parous	% Parity
Nkhata-Bay	Sanga	373	279	74.8	101	67	66.3
Nkhotakota	Vwawa	103	53	51.5	69	27	39.1
	Chimkwende	91	33	36.3	51	30	58.8
Salima	Chilungo	24	12	50.0	46	31	67.4
Total		**591**	**377**	**63.8**	**267**	**155**	**58.1**

In NKK (IRS) District, the proportion of parous female *An. funestus* s.l. and *An. gambiae* s.l. before spray (July–September) was lower than after spray (October–June). The same trend was observed in non-IRS Salima District (Chilungo site). However, in non-IRS Nkhata-Bay (Sanga site), the proportion of female parous mosquitoes was higher in July-September (before the spray of NKK) than October-June (Figure 18).

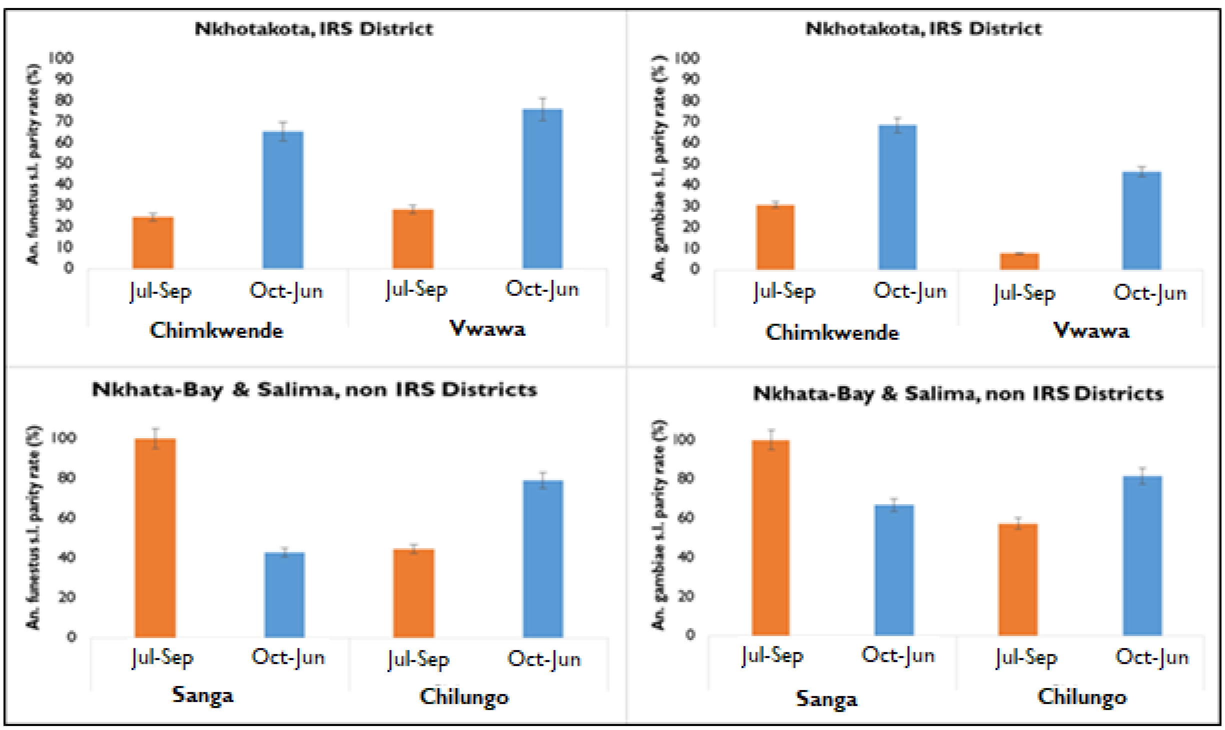


Figure 18: Proportion of parous female *An. funestus* s.l. from July - September and October - June, by HLC site.

3.2 Laboratory Analysis for Species Identification and Infection Detection

A total of 2,109 *An. gambiae* s.l. were identified to the species-specific level by PCR. These mosquito samples were collected using PSCs (n = 784, 37.2%), CDC-LTs (n = 945, 44.8%), HLCs (n =334, 15.8%), and susceptibility tests (n = 46, 2.2%) (Annex C). Out of the 2,109, 1,991 (94.4%) were identified as *An. arabiensis* and 118 (5.6%) as *An. gambiae* s.s. Both species are present in all the five districts, and *An. arabiensis* is predominant in each (Table 5).

Table 5: Number and proportion of *An. gambiae* s.l. identified by PCR, by sentinel site

District	Site	# Identified by PCR	*An. arabiensis* (N, %)	*An, gambiae* s.s. (N, %)
Karonga	Mwakanyamale	141	141 (100)	0 (0)
	Mwenimambwe	633	629 (99.4)	4 (0.6)
Nkhata-Bay	Kande	50	49 (98.0)	1(2.0)
	Sanga	242	238 (98.3)	4 (1.7)
Nkhotakota	Chimkwende	211	198 (93.8)	13 (6.2)
	Ngalauka	234	229 (97.9)	5 (2.1)
	Vwawa	143	137 (95.8)	6 (4.2)
Salima	Chilungo	142	111 (78.2)	31(21.8)
	Cholokoto	71	53 (74.6)	18 (25.4)
Chikwawa	Ntwana	143	111 (77.6)	32 (22.4)
	Nyamphota	99	95 (96.0)	4 (4.0)
Total		**2109**	**1991 (94.4)**	**118 (5.6)**

A total of 1,614 *An. funestus* s.l. were also identified to the species level using PCR; from PSCs (n = 626, 38.8%), CDC-LTs (n = 651, 40.1%), and HLCs (n = 337, 20.9%) (Annex C). Table 6 summarizes the number of *An. funestus* s.l. that were identified to species specific level using PCR. All 1,614 mosquito specimens were identified by PCR as *An. funestus* s.s. This species occurred across all five districts.

Sporozoite infections of *Anopheles* mosquitoes collected by HLC in the three districts are presented in Table 7. A total of 1,564 *Anopheles* mosquitoes (*An. gambiae* s.l. = 380, *An. funestus* s.l. = 915, *An. coustani* = 269) were screened for sporozoite infection by ELISA. The highest *An. funestus* s.l. sporozoite rate (10.0%) was observed in Salima District and the lowest (2.4%) was observed in Nkhata-Bay District. The highest and lowest *An. gambiae* s.l. sporozoite rates also were observed in Salima (5.3%) and Nkhata-Bay (0%). No sporozoite infections were found in *An. coustani* specimens. The overall sporozoite infection rates in all three districts were 1.3% for *An. gambiae* s.l. and 3.6% for *An. funestus* s.l.

Table 6: Number of *An. funestus* s.l. identified to species-specific level, by sentinel site

District	Site	# Identified by PCR	*An. funestus* s.s.
Karonga	Mwakanyamale	18	18
	Mwenimambwe	9	9
Nkhata-Bay	Kande	234	234
	Sanga	360	360
Nkhotakota	Chimkwende	419	419
	Ngalauka	70	70
	Vwawa	250	250
Salima	Chilungo	22	22
	Cholokoto	94	94
Chikwawa	Ntwana	110	110
	Nyamphota	28	28
Total		**1,614**	**1,614**

Table 7: Sporozoite infections in *Anopheles* mosquitoes sampled, by HLC district

District	Species	Number Examined by ELISA	Number positive for sporozoites	Sporozoite rate (%)
Nkhata-Bay	*An. gambiae* s.l.	131	0	0.0
	An. funestus s.l.	537	13	2.4
	An. coustani	150	0	0.0
	Total	**818**	**13**	**1.6**
Nkhotakota	*An. gambiae* s.l.	173	1	0.6
	An. funestus s.l.	338	16	4.7
	An. coustani	118	0	0.0
	Total	**629**	**17**	**2.7**
Salima	*An. gambiae* s.l.	76	4	5.3
	An. funestus s.l.	40	4	10.0
	An. coustani	1	0	0.0
	Total	**117**	**8**	**6.8**
All	*An. gambiae* s.l.	380	5	1.3
	An. funestus s.l.	915	33	3.6
	An. coustani	269	0	0.0
	Grand Total	**1,564**	**38**	**2.4**

The overall *An. gambiae* s.l. infectious bites per person per night in the three districts was estimated at 0.02 ib/p/n. However, the highest *An. gambiae* s.l. nightly EIR occurred in Salima between December and March ranging from 0.101-0.152 ib/p/n. The overall A*n. funestus* s.l. infectious bites per person per night was estimated at 0.14 ib/p/n and its highest nightly EIR occurred in Nkhata-Bay in June (0.43 ib/p/n) and NKK in September (0.425 ib/p/n) (Tables 8 and 9).

EIR was estimated using HBRs from HLC collections performed quarterly and sporozoite rates for quarters. Quarterly EIRs were estimated from nightly EIRs and quarterly EIRs were summed up to estimate the annual EIR. The highest *An. gambiae* s.l. annual EIR occurred in Salima; (23.2 ib/p/yr) and mostly occurred during the peak rainy season. In this district, the highest *An. gambiae* s.l. quarterly EIR was recorded between December-February [14 infective bites/per/quarter (ib/p/qr)]. In NKK, the highest *An. gambiae* s.l. quarterly EIR was recorded between March-May (3.9 ib/p/qr). No *An. gambiae* s.l. infective bites were recorded in Nkhata-Bay (Table 8)

Table 8: Entomological parameters of malaria transmission, *An. gambiae* s.l., by HLC district, July 2018–June 2019

District	Month	# Tested by ELISA	CS +ve*	Sporozoite Rate (%)	HBR	Nightly EIR	Estimated (Annual EIR=∑ Quarterly EIRs)
.Nkhata-Bay	September	88	0	0.0	16.7	0.000	0.0
	December	13	0	0.0	0.7	0.000	0.0
	March	23	0	0.0	1.4	0.000	0.0
	June	7	0	0.0	0.0	0.000	0.0
	Overall	**131**	**0**	**0.0**	**4.69**	**0.000**	**0.0**
Nkhotakota	September	103	0	0	0.56	0.000	0.0
	December	2	0	0.0	1.38	0.000	0.0
	March	68	1	1.5	2.91	0.043	3.9
	June	0	0	0.0	0.41	0.000	0.0
	Overall	**173**	**1**	**0.6**	**1.31**	**0.008**	**3.9**
Salima	September	7	0	0.0	0.00	0.000	0.0
	December	48	3	6.3	2.44	0.152	14.0
	March	21	1	4.8	2.13	0.101	9.2
	June	0	0	0.0	0.13	0.000	0.0
	Overall	**76**	**4**	**5.3**	**1.17**	**0.062**	**23.2**

∗ Circumsporozoite positive

The highest *An. funestus* s.l. annual EIR occurred in Nkhata-Bay (83.9 ib/p/yr). In this district, the highest *An. funestus* s.l. quarterly EIR was recorded between June - August (39.1 ib/p/qr). In NKK (IRS) District, highest *An. funestus* s.l. quarterly EIR was recorded between September -

November (39.1 ib/p/qr). In Salima, the highest *An. funestus* s.l. quarterly EIR was recorded between March - May (14.3 ib/p/qr) (Table 9).

Table 9: Entomological parameters of malaria transmission, *An. funestus* s.l., by HLC district, July 2018–June 2019

District	Months	# Tested by ELISA	CS +ve*	Sporozoite Rate (%)	HBR	Nightly EIR	Estimated (Annual EIR=∑ Quarterly EIRs)
Nkhata-Bay	September	71	2	2.8	7.44	0.210	19.3
	December	46	3	6.5	3.25	0.212	19.5
	March	251	1	0.4	16.56	0.066	6.0
	June	169	7	4.1	10.38	0.430	39.1
	Overall	**537**	**13**	**2.4**	**9.41**	**0.228**	**83.9**
Nkhotakota	September	129	9	7.0	6.09	0.425	39.1
	December	45	2	4.4	0.06	0.003	0.3
	March	82	3	3.7	0.19	0.007	0.6
	June	82	2	2.4	3.72	0.091	8.3
	Overall	**338**	**16**	**4.7**	**2.52**	**0.119**	**48.2**
Salima	September	0	0	0.0	0.44	0.000	0.0
	December	0	0	0.0	0.69	0.000	0.0
	March	35	4	11.4	1.38	0.157	14.3
	June	5	0	0.0	0.19	0.000	0.0
	Overall	**40**	**4**	**10.0**	**0.67**	**0.067**	**14.3**

* Circumsporozoite positive

Overall, the highest *Anopheles* mosquitoes annual EIR was recorded in Nkhata-Bay (83.9 ib/p/yr) followed by NKK (51.83 ib/p/yr) and then Salima (37.5 ib/p/yr) (Table 10).

Table 10: Entomological parameters of malaria transmission of *Anopheles* mosquitoes, by HLC district, July 2018–June 2019

District	Quarters	*An. gambiae* s.l.	*An. funestus* s.l.	Total

		Estimated Annual EIR=∑Quarterly EIRs)	Estimated Annual EIR=∑Quarterly EIRs)	Estimated Annual EIR=∑Quarterly EIRs
Nkhata-Bay	September	0	83.9	83.9
	December			
	March			
	June			
Nkhotakota	September	3.932	48.2	52.1
	December			
	March			
	June			
Salima	September	23.02	14.3	37.3
	December			
	March			
	June			

3.3 Spray Quality Assessment and Residual Efficacy of Actellic 300CS

There was a wide variation in the spray quality and residual efficacy of Actellic 300CS between villages across NKK district.

3.3.1 Spray Quality Assessment

Satisfactory results were obtained in Chembakuka, Chimphewa, and Chikango with a mean percentage mortality between 94% and 100% among the different wall surfaces after 24 hours of observation. However, unsatisfactory results were recorded at Khufi 1 village (an IRS operations site), where mortality was below WHO threshold of 80% after 24 hours of observation for cement plastered walls (46.8%) and burnt brick walls (62.7%) (Figure 19).

After re-training the spray teams from the NKK Boma operations site, the spray quality improved as evidenced in the following randomly selected villages: Kalusa II, Chafukira, and Mtanga I (Figure 20).

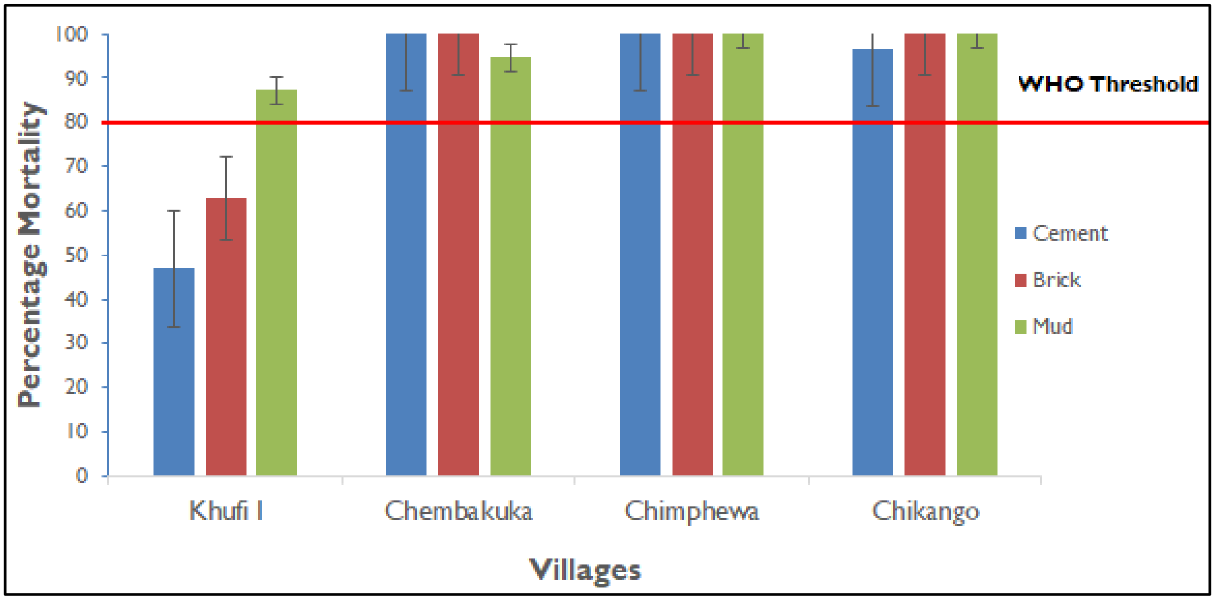


Figure 19: Mean percentage mortality after 24 hours of *An. gambiae* Kisumu in four villages after exposure to sprayed walls in NKK District, one week after spraying

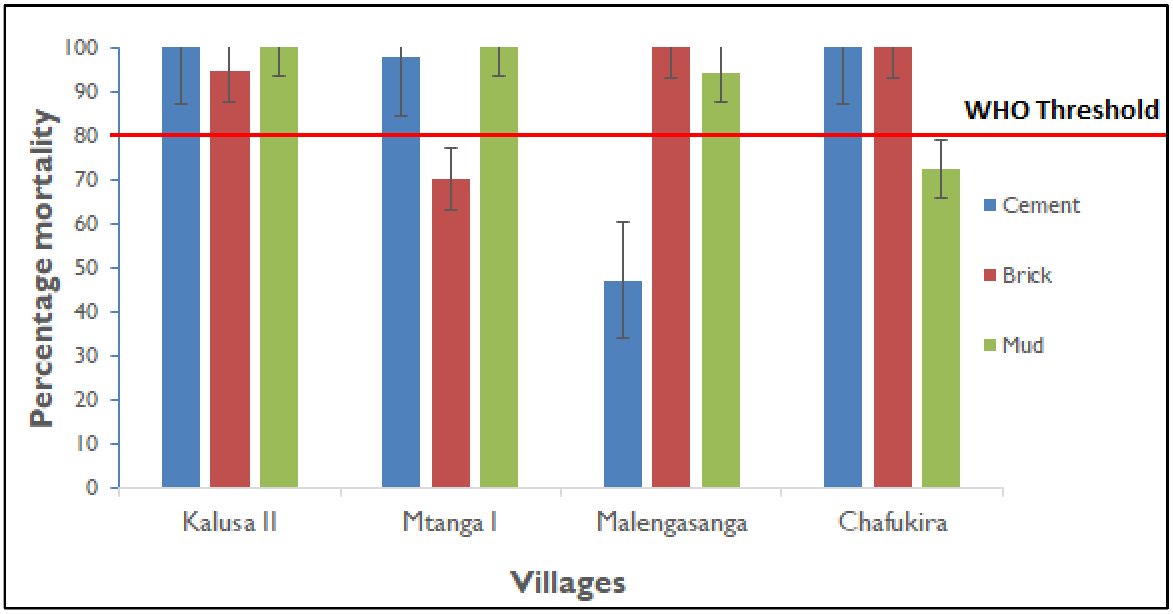


Figure 20: Mean percentage mortality after 24 hrs of *An. gambiae* Kisumu in four villages after exposure to sprayed walls in NKK Boma operations site after re-training the spray teams

3.3.2 Fumigation Effect of Actellic 300CS

The fumigation effect of Actellic 300CS was very low, less than 80% in all the houses with different wall surfaces except for mud wall surfaces in Chimphewa village (Figure 21).

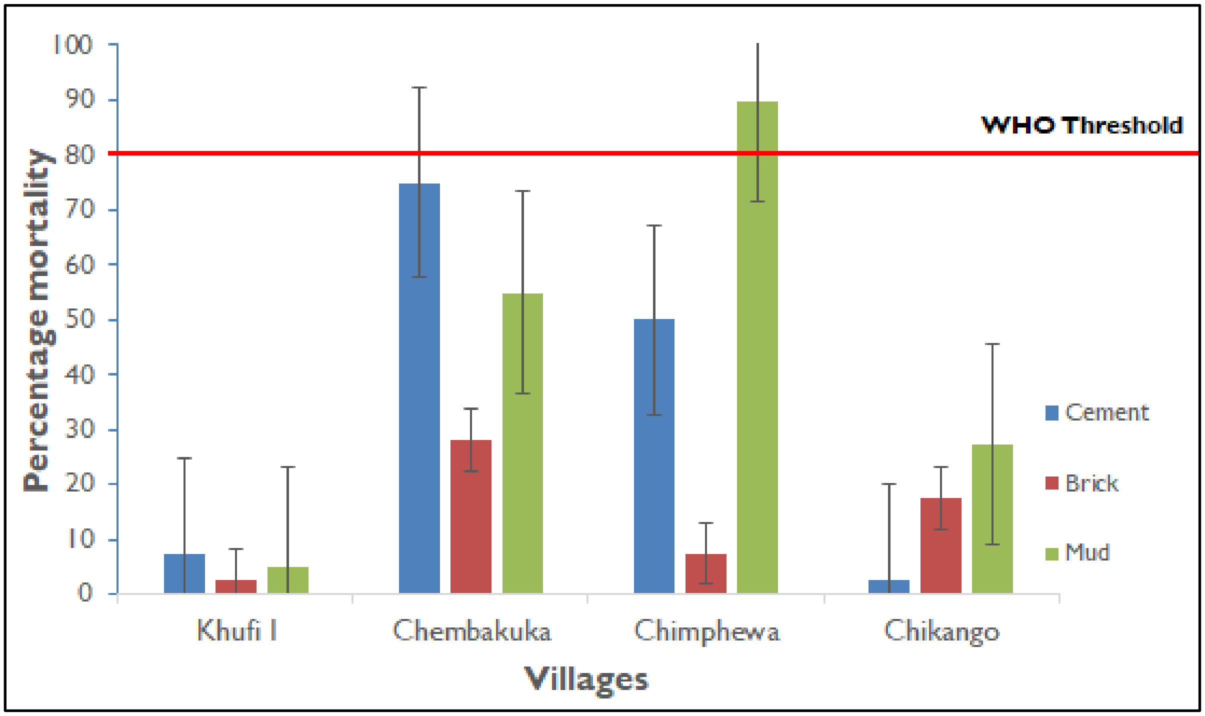


Figure 21: Mean percentage mortality after 24 hrs of *An. gambiae* Kisumu after exposure to airborne Actellic 300CS in four villages in NKK District, one week after spray

3.3.3 Residual Efficacy of Actellic 300CS

The residual efficacy of Actellic 300CS on different wall surfaces (mud, brick, and cement) ranged from two to four months across NKK district. (Annex C, Figures C1-C4).

3.4 Insecticide Susceptibility Results

3.4.1 *An. funestus* s.l. Susceptibility to Different Insecticides in Chikwawa District

Figure 22 summarizes the susceptibility status of *An. funestus* s.l. to pirimiphos-methyl 0.25%, and the pyrethroids alpha-cypermethrin 1.0%, permethrin 0.75%, and deltamethrin 0.05%. *An. funestus* s.l. was completely susceptible to pirimiphos-methyl (100% mortality) but resistant to all three pyrethroids. Pre-exposure of *An. funestus* s.l. to 4% PBO then pyrethroids resulted in complete restoration of susceptibility (100%) to alpha-cypermethrin and partial restoration to permethrin (94.6%) and deltamethrin (97.1%). This species was also susceptible to slow acting insecticides clothianidin 13.2mg/paper (100% mortality at day 4) and chlorfenapyr 100µg/bottle (100% mortality within 24 hours) (Figure 23) (Annex D, Tables D1 and D2).

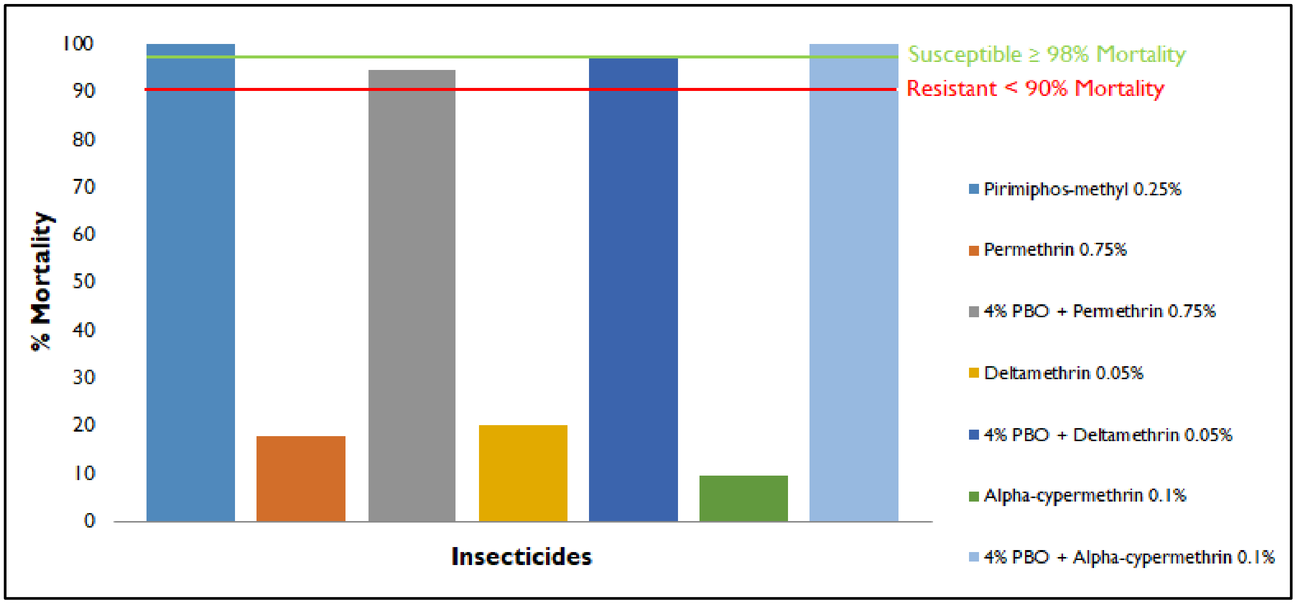


Figure 22: Susceptibility status of *An. funestus* s.l. to selected WHO-recommended insecticides, Chikwawa District

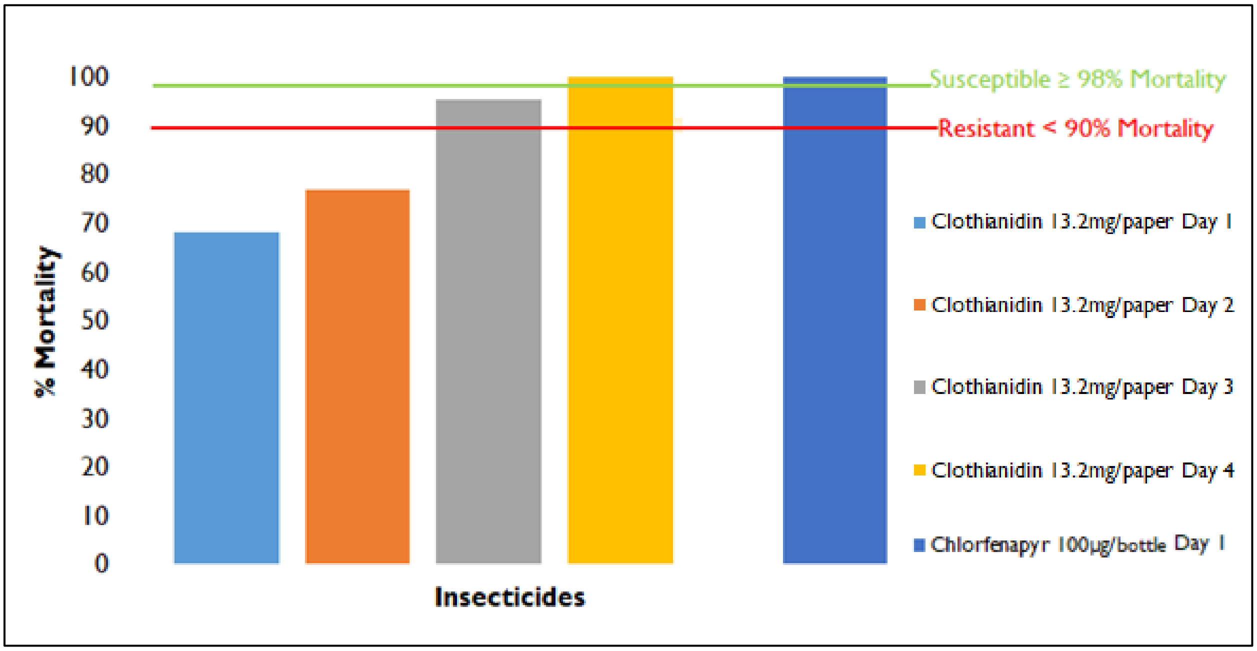


Figure 23: *An. funestus* s.l. response to clothianidin and chlorfenapyr, Chikwawa District

3.4.2 *AN. FUNESTUS* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN NKHOTAKOTA DISTRICT

Due to low numbers of *Anopheles* mosquitoes collected from NKK, only three insecticides [pirimiphos-methyl 0.25%, clothianidin 13.2mg/paper and permethrin 7.5% (10X)] were tested against *An. funestus* s.l. This species was fully susceptible to pirimiphos-methyl 0.25% and clothianidin 13.2mg/paper (100% mortality) (Figures 24, 25 and Annex D) but resistant to permethrin 7.5% (Figure 25; Annex D).

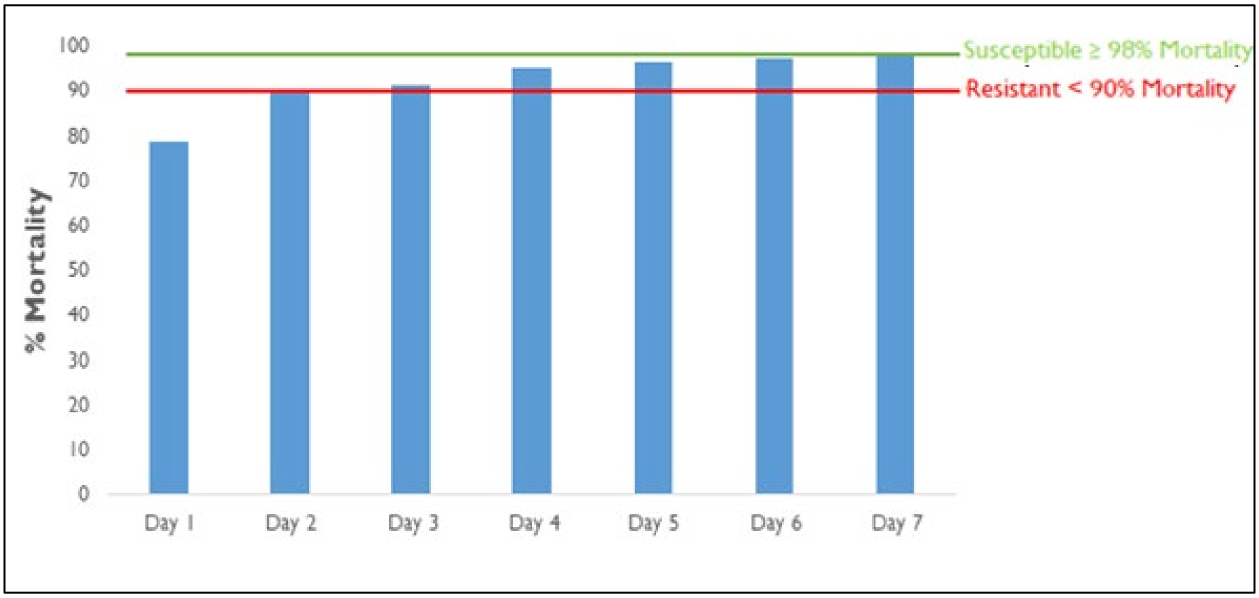
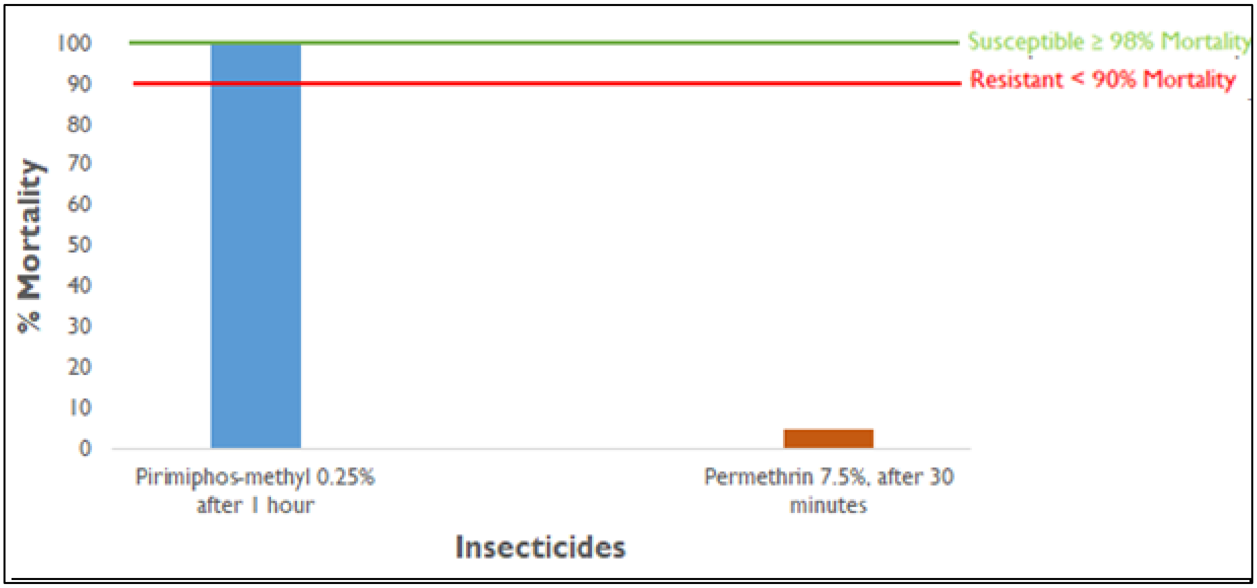


Figure 24: *An. funestus* s.l. response to clothianidin, Nkhotakota District



3.4.3 **FIGURE 25:** SUSCEPTIBILITY STATUS OF *AN. FUNESTUS* S.L. TO PIRIMIPHOS-METHYL 0.25% AND PERMETHRIN 7.5%, NKHOTAKOTA DISTRICT. ***ANOPHELES FUNESTUS* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN NKHATA-BAY DISTRICT**

An. funestus s.l. was completely susceptible to pirimiphos-methyl (100% mortality) but resistant to pyrethroids (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.1%). Pre-exposure to 4% PBO resulted in full restoration of susceptibility (100% mortality) for 4% PBO + permethrin and 4% PBO + deltamethrin but not to 4% PBO + alpha-cypermethrin which produced a 90.4% mortality rate (Figure 26). Exposing *An. funestus* s.l. to clothianidin 13.2mg/paper and chlorfenapyr100µg/bottle resulted in 100% mortality at days 4 and 1, respectively, (Figure 27) (Annex D, Tables D1 and D2).

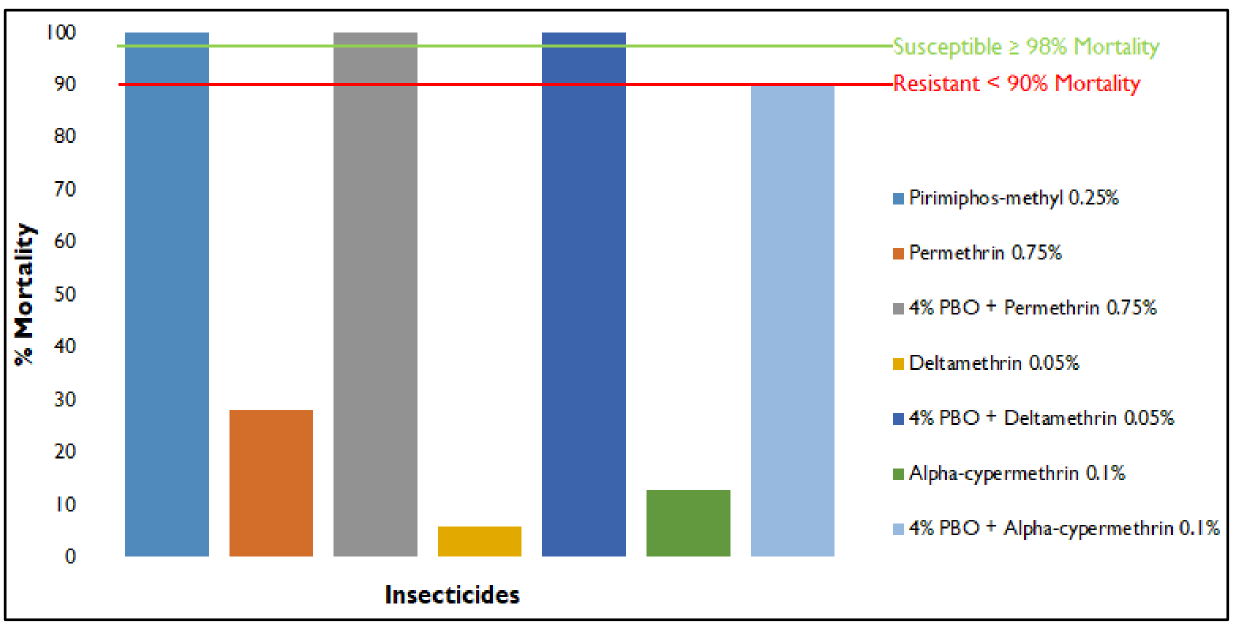


Figure 26: Susceptibility status of *An. funestus* s.l. to selected WHO-recommended insecticides, Nkhata-Bay District

Figure 27: *An. funestus* s.l. response to slow-acting insecticides, Nkhata-Bay District

3.4.4 *Anopheles funestus* s.l. Susceptibility to Different Insecticides in Karonga District

An. gambiae s.l. was susceptible to pirimiphos-methyl 0.25% (100% mortality) and alpha-cypermethrin 1.0% (99% mortality). However, this species was highly resistant to permethrin 0.75% (9.3% mortality) and moderately resistant to deltamethrin 0.05% (95.7% mortality). Pre-exposure to 4% PBO + permethrin 0.75% and 4% PBO + deltamethrin 0.05% restored full susceptibility (100%) (Figure 28) (Annex D, Tables D1 and D2). Exposing *An. gambiae* s.l. to clothianidin 13.2mg/paper resulted into 100% mortality on day 1, while the same species took two days to achieve 100% mortality when exposed to chlorfenapyr 100µg/bottle (Figure 29) (Annex D, Table D-2).

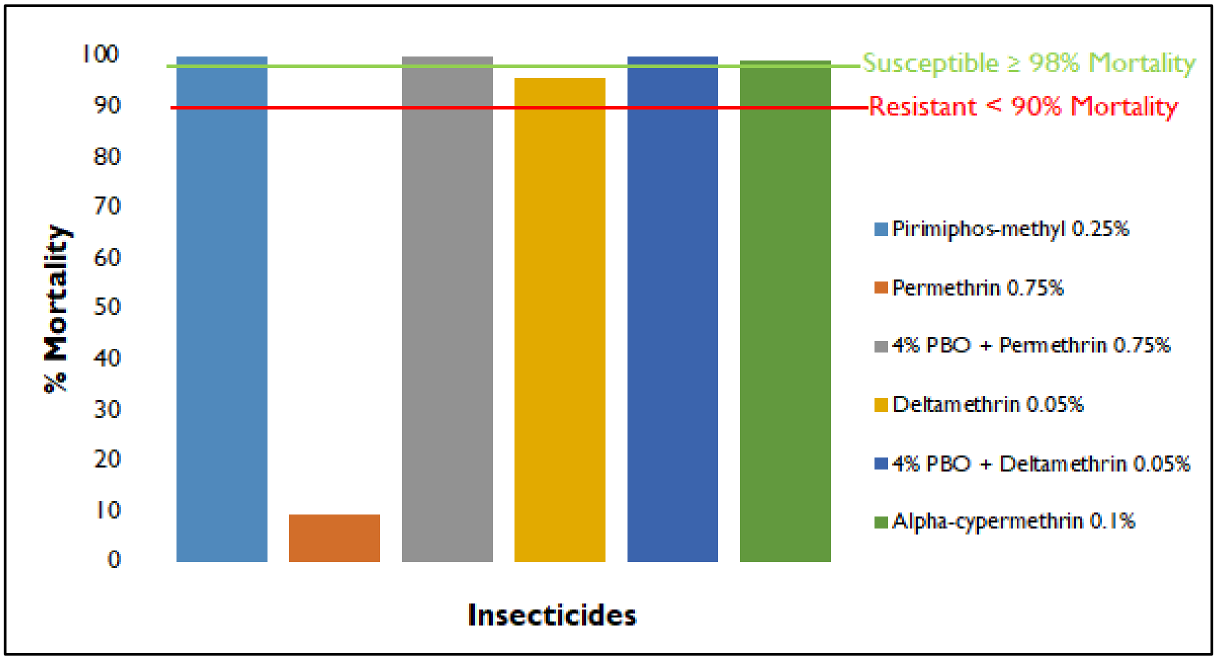


Figure 28: Susceptibility status of *An. gambiae* s.l. to selected WHO-recommended insecticides, Karonga District

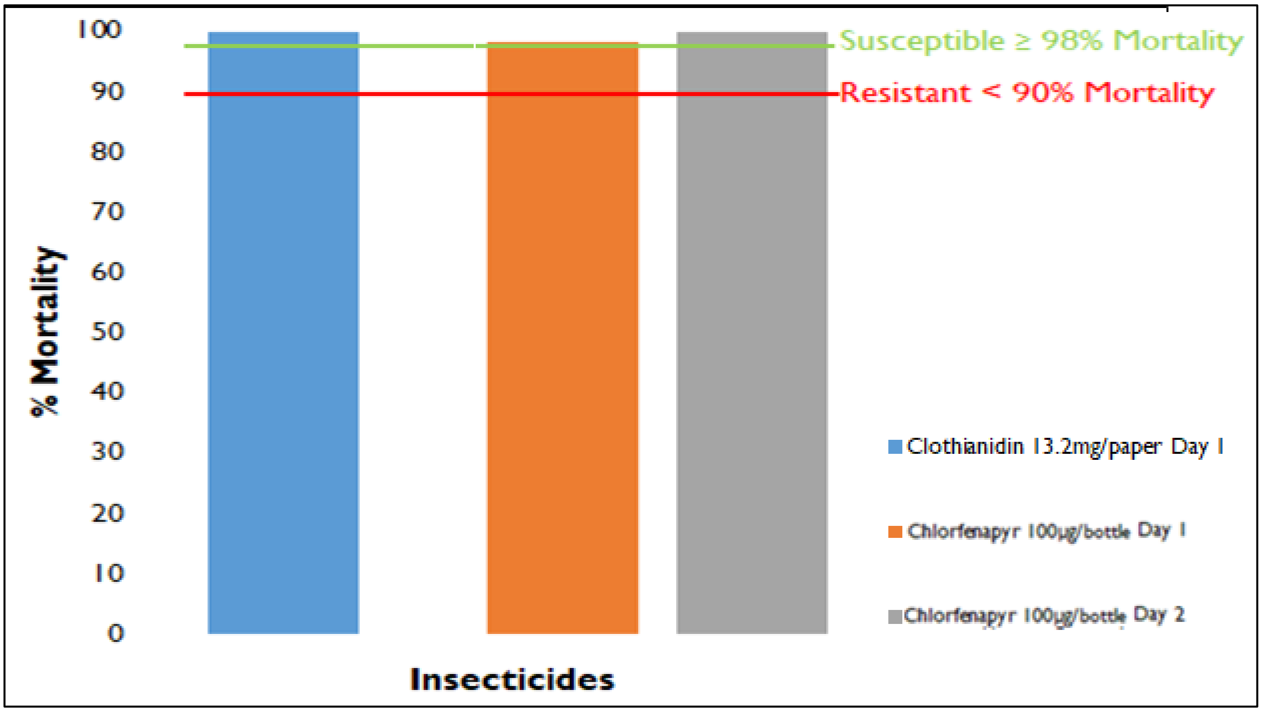


Figure 29: Susceptibility status of *An. gambiae* s.l. to clothianidin and chlorfenapyr, Karonga District

4. Discussion

Entomological monitoring results show that *An. gambiae* s.l. and *An. funestus* s.l. are the predominant species in Malawi as evidenced by their wide distribution across the 11 sentinel sites in the five monitored districts. PCR results show that *An. funestus* s.s. was the only member of the *An. funestus* group captured from the current collections. *An. funestus* s.l. is widely distributed in Malawi and is the major malaria vector based on the high sporozoite rates and EIRs estimated at 14.3 - 83.9 ib/p/yr. *An. funestus* s.l. density was very high in Nkhata-Bay, and relatively high in NKK (before rainy season) and in Chikwawa (during the rainy season). *An. gambiae* s.l. is the second most important malaria vector in Malawi with the highest annual EIR, estimated at 3.9 - 23.2 ib/p/yr. *An. gambiae* s.l. density was high in Karonga District, especially at Mwenimwambwe site, where there is persistent availability of water pools, an ideal habitat for mosquito breeding, because of rice cultivation at Wovwe irrigation scheme. The results also show that *An. gambiae* s.l. densities are rising relative to *An. funestus* s.l. in sites such as Ngalauka in NKK, Chilungo in Salima, and Nyamphota in Chikwawa. Among A*n. gambiae* s.l., PCR results show that *An. arabiensis* is the predominant species whereas *An. gambiae* s.s. density is low. Despite this low density of *An. gambiae* s.s., its distribution is now widespread across the five districts and accounted for over 20% of the *An. gambiae* collected in Salima and at one site in Chikwawa. Therefore, this species should be closely monitored, including insecticide susceptibility tests if possible, to assess its importance in malaria transmission in Malawi. Total annual EIRs were estimated at 83.9, 51.8 and 37.5 ib/p/yr in Nkhata-Bay, NKK, and Salima districts, respectively.

There was a variation in the biting pattern of *Anopheles* mosquitoes in the three districts where HLCs were conducted (Salima, NKK, and Nkhata-Bay). *An. funestus* s.l. preferred feeding indoors whereas *An. gambiae* s.l. and *An. coustani* predominantly fed outdoors. Morning/daytime biting also was observed among *Anopheles* species in these three districts. A high proportion of daytime biting was observed among *An. funestus* s.l.: between 6:00 am and 11:00 am with 24.0% of all females collected indoors and 4.5% of those collected outdoors obtained during these late morning hours.

Spray quality results showed that there was variation in spray quality across the operations sites in NKK District. The high mean percentage mortalities recorded in the three sites of Chembakuka, Chikango, and Chimphewa during the spray quality assessment suggested high quality of spray in the catchment areas surrounding these sites. Conversely, the low percentage mortality recorded from bioassays conducted at Khufi 1 site suggests that the spray quality was poor; hence, the NKK Boma catchment area might have suffered from inconsistent spray quality. The re-training of spray teams at the NKK operations site yielded positive results as evidenced by the high percentage mortalities recorded in the three sites of Kalusa 2, Mtanga 1, and Chafukira. Monthly follow-ups of wall cone bioassays in the two original villages selected (Chembakuka and Chimphewa) in NKK showed a significant variation and a rapid decline in the residual efficacy of Actellic 300 CS approximately four months after spray. This trend was also observed in the structures sprayed in 10 additional villages. Actellic 300CS also showed a fumigant effect one month after spray, but the effect was not long lasting. Due to low residual efficacy of

Actellic 300CS, PMI VectorLink Malawi investigated the possible causes: 1) sample size diversity, 2) spray quality, 3) sprayer discharge rate, which could affect the insecticide deposited, 4) insecticide quality, 4) quality of bioassays, 5) resistance status of Kisumu strain used in wall cone bioassay, and 6) the pH of water and soil samples. None of the investigations of the above causes explained the rapid decline in residual efficacy of Actellic. Syngenta is now doing bioefficacy tests to establish the cause.

Despite the low residual efficacy of Actellic 300CS in NKK, IRS played an important role as evidenced by low *Anopheles* mosquito density during after spray at all sites in that district (Chimkwende, Ngalauka, and Vwawa). *Anopheles* mosquito density was very high before spray, then drastically dropped after the spray, and this might have had an impact on malaria transmission. Of concern is the rise of *An. gambiae* s.l. (*An. arabiensis*) during the rainy season even as the population of *An. funestus* s.l. decreased.

The parity rates of both *An. funestus* s.l. and *An. gambiae* s.l. were lower before spray than after spray in NKK. This could be attributed to deficient numbers of young females coming from the breeding sites due to the effect of IRS. A similar trend was observed in Salima despite the distribution of PBO nets in this district. In Nkhata-Bay District, the reverse was observed: a higher number of parous female *An. funestus* s.l. and *An. gambiae* s.l. were observed before the spray than after spray. This could be related to the difference in seasonality of the mosquito population in the districts. In Nkhata-Bay, the mosquito population seems to peak after the spray months (Jan–Mar); this might add more young females to the population and thus less parity. LLIN distribution season could also affect parity.

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). This could be attributed to selection pressure maintained by the continuous distribution and mass campaign of pyrethroid-impregnated LLINs and by the commercial use of pyrethroids against mosquitoes (aerosols and coils) and in agriculture. However, pre-exposure of *An. gambiae* s.l. and *An. funestus* s.l. mosquitoes to PBO and then pyrethroids resulted in full or partial restoration of their susceptibility to pyrethroids. These results show that there was a variation in mosquito density in districts where PBO nets were distributed. In Salima, distribution of PBO nets had a significant impact in reducing the malaria burden throughout the year. However, no significant impact was observed in Karonga where mosquito population increased in the rainy season. This could be attributed to the species composition of *Anopheles* mosquitoes present in Karonga where *An. arabiensis* is the dominant species that has varying feeding behavior, feeds readily both on humans and animals, particularly, cattle and may be less likely to come into contact with insecticides on LLINs. Both *An. gambiae* s.l. and *An. funestus* s.l. were completely susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. These insecticides should be considered for rotation in IRS programs in Malawi.

5. Technical Support and Capacity Building

PMI VectorLink Malawi worked closely with National Malaria Control Programme (NMCP) during the implementation of the IRS program. During the quarterly Vector Control Working Group meetings that are carried out, PMI VectorLink Malawi provided technical support to the NMCP in selecting an insecticide/s for IRS for the 2019/2020 season based on the findings from the longitudinal insecticide resistance monitoring activities that were carried out in three districts in 2018/2019.

During the first year of the PMI VectorLink Malawi IRS project, an IRM Plan for 2019 to 2022 was developed. The plan will guide the NMCP in monitoring insecticides that will be used for vector control in Malawi to reduce the spread of insecticide resistance. The IRM plan will be a 'living" document and will be updated on a regular basis as new knowledge is gained.

6. CONCLUSIONS

- *An. funestus* s.l. is the primary malaria vector in Malawi with an estimated annual EIR ranging from 14.3 ib/p/yr to 83.9 ib/p/yr.
- *An. gambiae* s.l. is the second most important malaria vector in Malawi, with an estimated annual EIR of 23.2 ib/p/yr.
- Total annual EIRs were estimated at 83.9, 51.8, and 37.5 ib/p/yr in Nkhata-Bay, NKK, and Salima, respectively.
- *An. gambiae* s.s. density is currently low in Malawi but this species is widespread across the five monitored districts; it is important that this species be monitored to establish its role in malaria transmission in the country.
- *An. funestus* s.l. predominantly fed indoors while *An. gambiae* s.l. and *An. coustani* predominantly fed outdoors.
- Morning biting was observed among the *Anopheles* mosquitoes collected in the three districts where HLCs were conducted. A high proportion of daytime biting was recorded by *An. funestus* s.l., mainly occurring indoors.
- Despite inconsistent wall bioassay results, IRS reduced the indoor resting density of *Anopheles* mosquitoes, particularly *An. funestus* as shown by the decline in mosquito density during the rainy season when peak transmission normally occurs.
- *An. funestus* s.l. and *An. gambiae* s.l., the main malaria vectors in Malawi, are susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr. These three insecticides can be used in IRS programs in Malawi.
- Since no cases of resistance to pirimiphos-methyl were recorded in malaria vector species collected from the sentinel sites in Karonga, Nkhata-Bay, NKK and Chikwawa districts, members from the Vector Control Technical Working Group agreed that PMI/VectorLink Malawi and the NMCP should use pirimiphos-methyl in the 2019/2020 IRS campaign.
- Pre-exposure of *An. funestus* s.l. and *An. gambiae* s.l. to 4% PBO then pyrethroids resulted in complete restoration of their susceptibility. It is recommended that Malawi move to PBO LLINs for most of the country to effectively control these species.

Annex A: Anopheles Mosquitoes Collected, by Sentinel Site and Collection Method

District	Site	Collection Method	Species	Number of Collections	Number Collected	Average per collection effort
Karonga	Mwakanyamale	PSC	*An. funestus* s.l	12	8	0.7
			An. gambiae s.l.	12	509	42.4
			An. coustani	12	1	0.1
		CDC-Light Trap	*An. funestus* s.l	12	2	0.2
			An. gambiae s.l.	12	338	28.2
			An. coustani	12	9	0.8
	Mwenimambwe	PSC	*An. funestus* s.l	12	324	27.0
			An. gambiae s.l.	12	14380	1198.3
		CDC-Light Trap	*An. funestus* s.l	12	12	1.0
			An. gambiae s.l.	12	1821	151.8
			An. coustani	12	2	0.2
Nkhata-Bay	Sanga	PSC	*An. funestus* s.l	12	1528	127.3
			An. gambiae s.l.	12	212	17.7
		CDC-Light Trap	*An. funestus* s.l	12	3429	285.8
			An. gambiae s.l.	12	226	18.8
			An. coustani	12	8	0.7
		HLC	*An. funestus* s.l	4	602	150.5
			An. gambiae s.l.	4	300	75
			An. coustani	4	415	103.8
	Kande	PSC	*An. funestus* s.l	12	250	20.8
			An. gambiae s.l.	12	12	1.0
		CDC-Light Trap	*An. funestus* s.l	12	920	76.7
			An. gambiae s.l.	12	87	7.3
			An. coustani	12	7	0.6
Nkhotakota	Chimkwende	PSC	*An. funestus* s.l	12	1393	116.1
			An. gambiae s.l.	12	547	45.6
		CDC-Light Trap	*An. funestus* s.l	12	1048	87.3
			An. gambiae s.l.	12	231	19.3
			An. coustani	12	74	6.2
		HLC	*An. funestus* s.l	4	137	34.3
			An. gambiae s.l.	4	81	20.3
			An. coustani	4	104	26
	Vwawa	PSC	*An. funestus* s.l	12	1178	98.2
			An. gambiae s.l.	12	107	8.9
		CDC-Light Trap	*An. funestus* s.l	12	1453	121.1
			An. gambiae s.l.	12	677	56.4
			An. coustani	12	0	0
		HLC	*An. funestus* s.l	4	183	45.8
			An. gambiae s.l.	4	87	21.8
			An. coustani	4	93	23.3
	Ngalauka	PSC	*An. funestus* s.l	12	553	46.1

			An. gambiae s.l.	12	426	35.5
		CDC-Light Trap	*An. funestus* s.l	12	745	62.1
			An. gambiae s.l.	12	2036	169.7
			An. coustani	12	73	6.1
Salima	Cholokoto	PSC	*An. funestus* s.l	12	144	12.0
			An. gambiae s.l.	12	58	4.8
		CDC-Light Trap	*An. funestus* s.l	12	223	18.6
			An. gambiae s.l.	12	141	11.8
			An. coustani	12	1	0.1
		HLC	*An. funestus* s.l	4	20	5
			An. gambiae s.l.	4	43	10.8
			An. coustani	4	75	18.8
	Chilungo	PSC	*An. funestus* s.l	12	19	1.6
			An. gambiae s.l.	12	99	8.3
		CDC-Light Trap	*An. funestus* s.l	12	68	5.7
			An. gambiae s.l.	12	266	22.2
			An. coustani	12	1	0.1
Chikwawa	Ntwana	PSC	*An. funestus* s.l	12	1344	112.0
			An. gambiae s.l.	12	338	28.2
			An. coustani	12	5	0.4
		CDC-Light Trap	*An. funestus* s.l	12	1968	164.0
			An. gambiae s.l.	12	205	17.0
			An. coustani	12	4	0.3
	Nyamphota	PSC	*An. funestus* s.l	12	52	4.3
			An. gambiae s.l.	12	192	16.0
		CDC-Light Trap	*An. funestus* s.l	12	62	5.2
			An. gambiae s.l.	12	364	30.3

ANNEX B: PROPORTION OF DAYTIME/MORNING BITING OF *ANOPHELES* MOSQUITOES, ALL DISTRICTS

Table B-1: Total number of female *Anopheles* mosquitoes collected from all five districts collected between 5:00 pm and 11:00 am

District	Site	*An. funestus* s.l.		*An. gambiae* s.l.		*An. coustani*	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Nkhata-Bay	Sanga	373	227	36	244	130	307
Nkhotakota	Chimkwende	125	12	39	41	26	79
	Vwawa	173	10	55	49	14	62
Salima	Chilungo	19	24	21	54	3	17

Table B-2: Total number of female *Anopheles* mosquitoes collected from all five districts between 6:00 am and 11:00 am (morning biting)

District	Site	*An. funestus* s.l.		*An. gambiae* s.l.		*An. coustani*	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Nkhata-Bay	Sanga	89	41	1	15	5	13
Nkhotakota	Chimkwende	36	0	0	0	6	0
	Vwawa	69	0	2	1	1	0
Salima	Chilungo	0	0	1	0	0	0

Table B-3: Proportion of female *Anopheles* mosquitoes collected from all five districts between 6:00 am and 11:00 am (morning biting)

District	Site	*An. funestus* s.l.		*An. gambiae* s.l.		*An. coustani*	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Nkhata-Bay	Sanga	23.9	18.1	2.8	6.1	3.8	4.2
Nkhotakota	Chimkwende	28.8	0.0	0.0	0.0	23.1	0.0
	Vwawa	39.9	0.0	3.6	2.0	7.1	0.0
Salima	Chilungo	0.0	0.0	4.8	0.0	0.0	0.0
Overall		**23.1**	**4.5**	**2.8**	**2.0**	**8.5**	**1.1**

ANNEX C. RESIDUAL EFFICACY OF ACTELLIC 300CS ON DIFFERENT WALL STRUCTURES IN NKHOTAKOTA DISTRICT

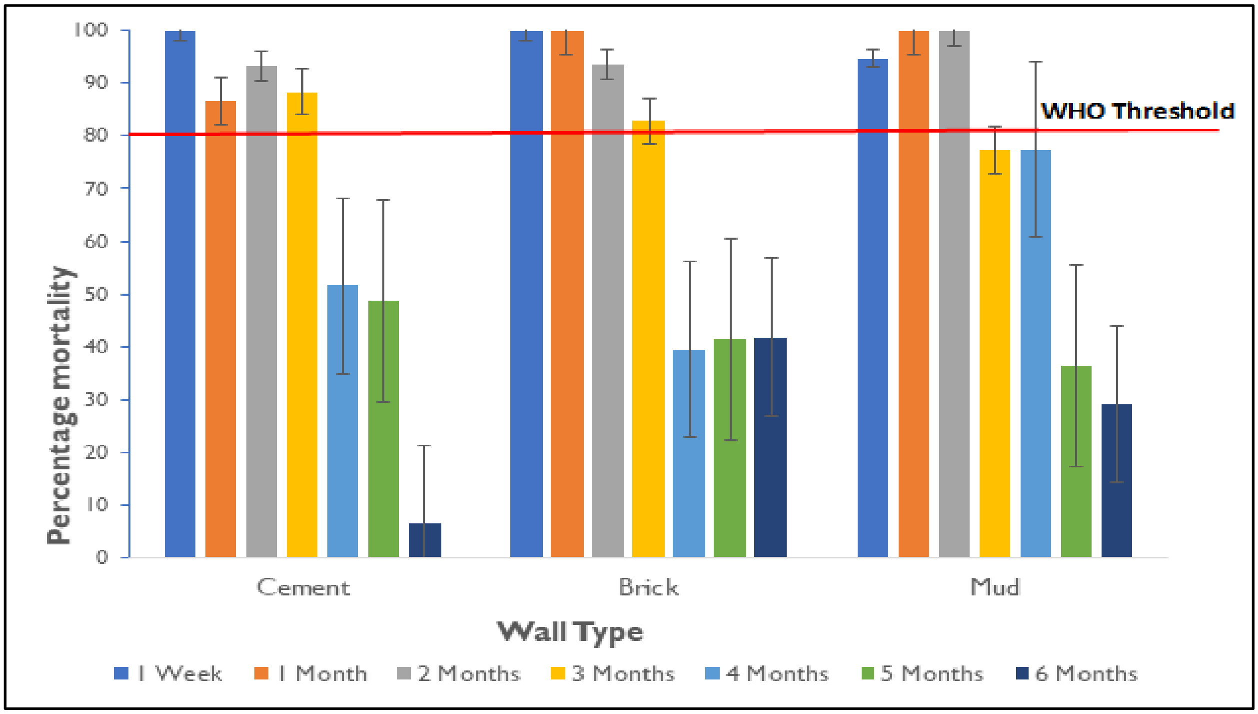


Figure C-1: Mean percentage mortality of *An. gambiae* Kisumu 24hrs after exposure to different wall surfaces at Chembakuka Village, 1–6 months after spray

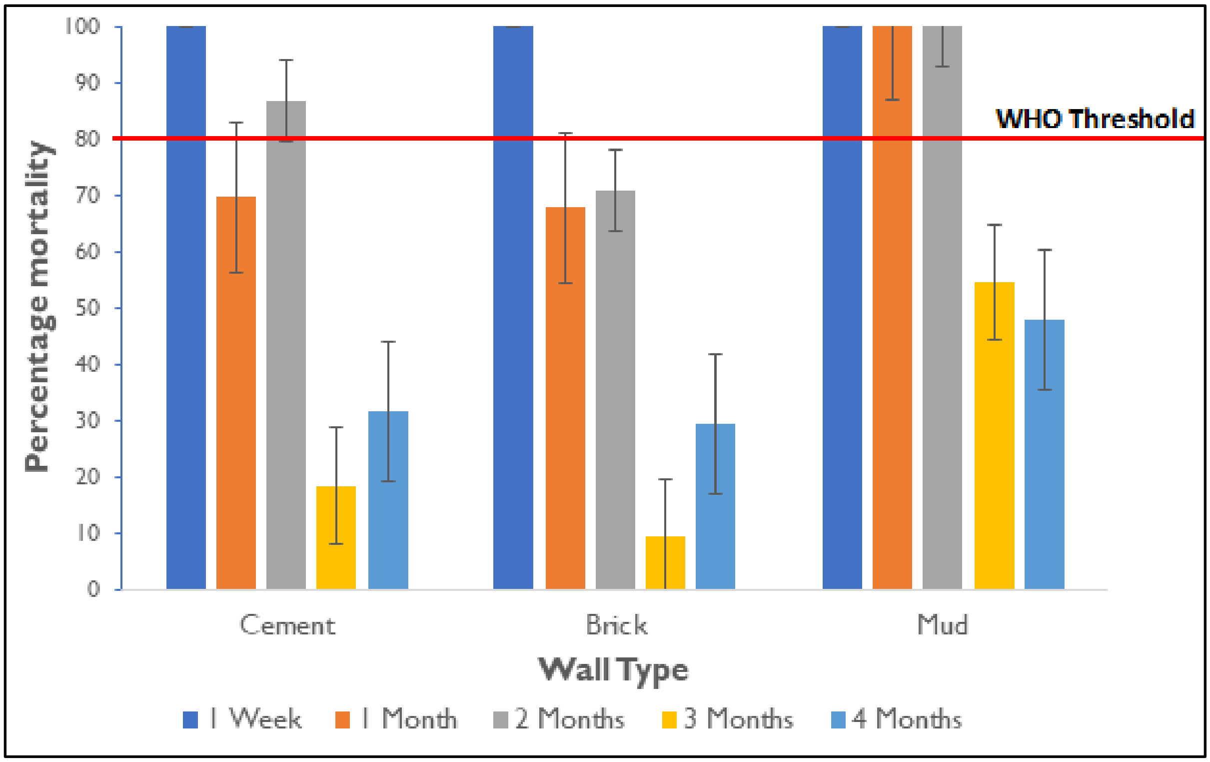


Figure C-2: Mean percentage mortality of *An. gambiae* Kisumu 24hrs after exposure to different wall surfaces at Chimphewa Village, 1–4 months after spray

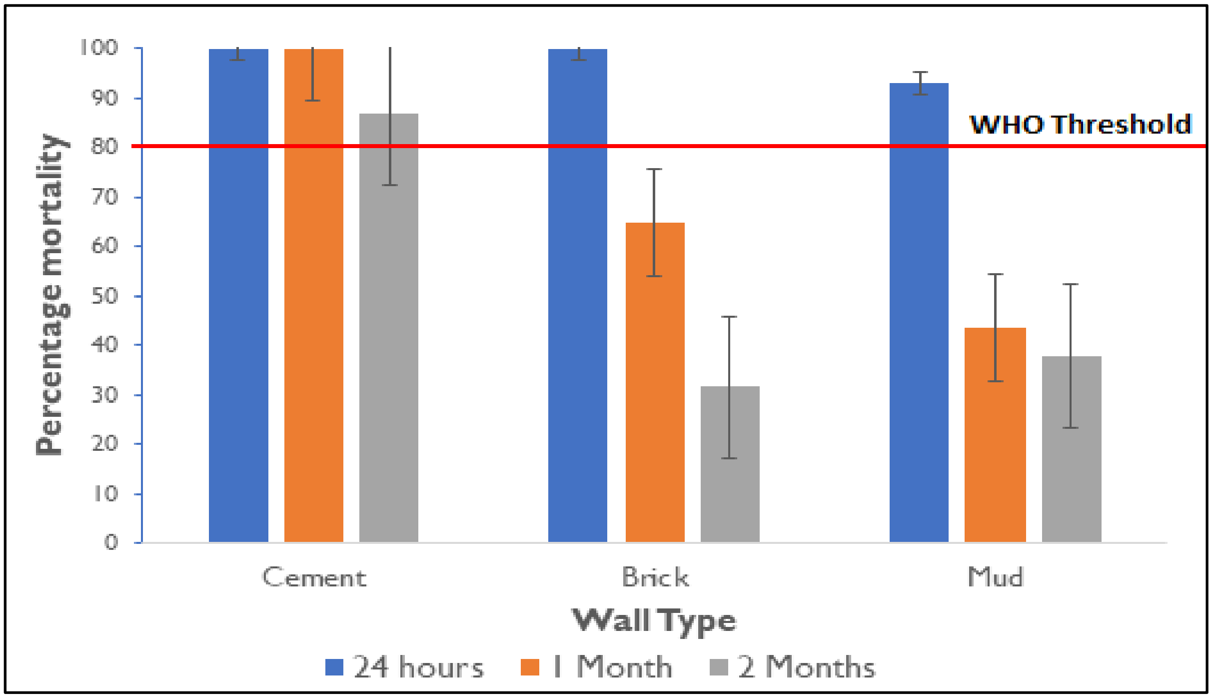


Figure C-3: Mean percentage mortality of *An. gambiae* Kisumu 24hrs after exposure to different wall surfaces at Chinthumbwi I Village, 1–2 months after spray

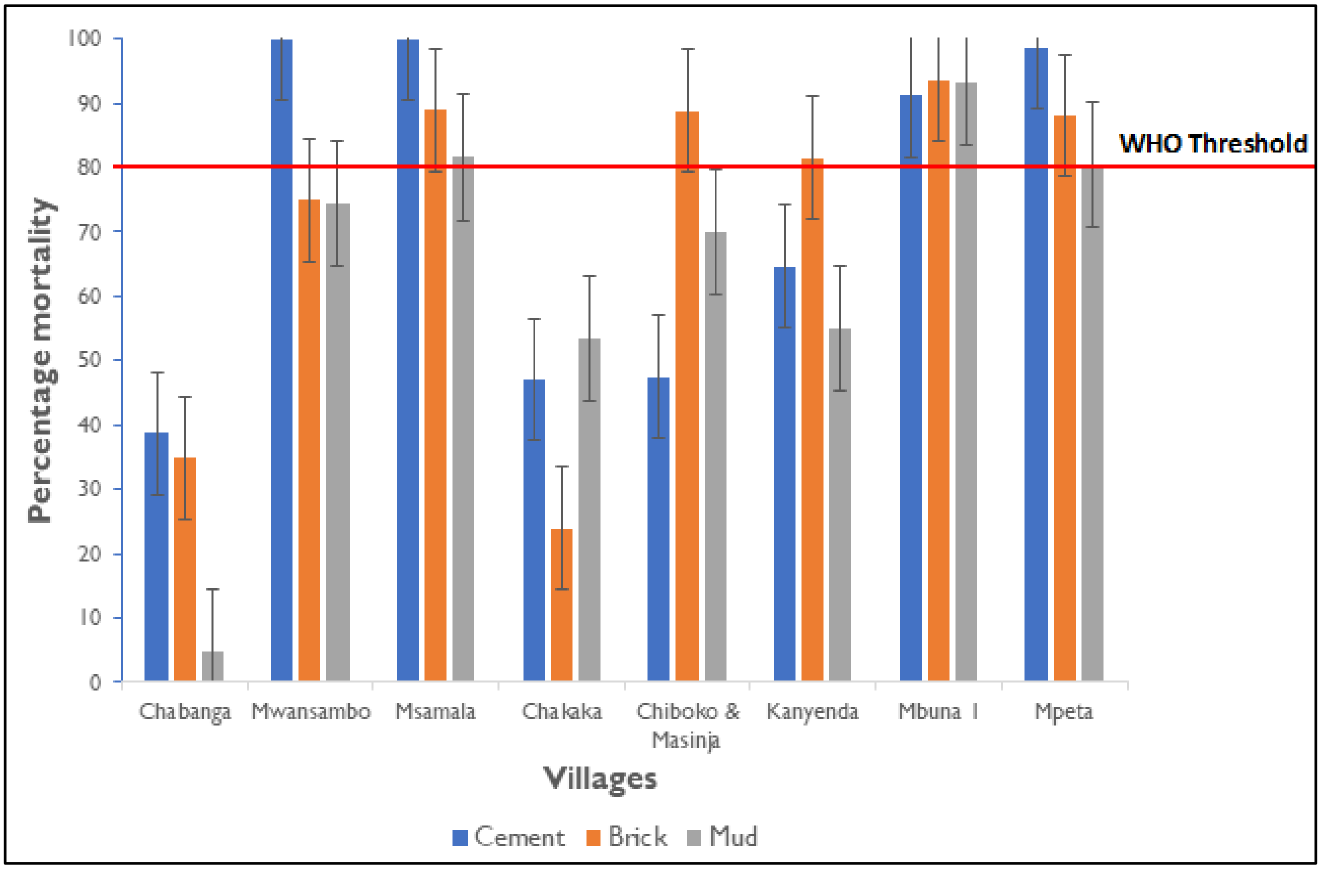


Figure C-4: Mean percentage mortality of *An. gambiae* Kisumu 24 hours after exposure to different wall surfaces in different villages, 4 months post-spray

ANNEX D. SUSCEPTIBILITY OF *ANOPHELES* MOSQUITOES TO DIFFERENT INSECTICIDES IN CHIKWAWA, NKHOTAKOTA, NKHATA-BAY, AND KARONGA DISTRICTS

Table D-1: Susceptibility status of *An. gambiae* s.l. and *An. funestus* s.l. to pyrethroids in Chikwawa, Nkhata-Bay, and Karonga districts

District	Deltamethrin 0.05%		4% PBO + Deltamethrin 0.05%		Permethrin 0.75%		4% PBO+ Permethrin 0.75%		Alpha-cypermethin 0.01%		4%PBO+Alpha-cypermethin 0.01%	
	# of mosquitoes	% mortality after 24hrs	# of mosquitoes	% mortality after 24hrs	# of mosquitoes	% mortality after 24hrs	# of mosquitoes	% mortality after 24hrs	# of mosquitoes	% mortality after 24hrs	# of mosquitoes	% mortality after 24hrs
Chikwawa	99	20.2	73	97.1	115	17.7	111	94.6	105	9.5	109	100
Nkhata-Bay	105	5.7	94	100	78	28.2	90	100	101	12.9	94	90.4
Karonga	93	95.7	103	100	97	9.3	105	100	109	99		

Table D-2: Susceptibility status of *An. gambiae* s.l. and *An. funestus* s.l. to **pirimiphos-methyl, clothianidin, and chlorfenapyr** in Chikwawa, Nkhata-Bay, and Karonga districts

District	Pirimiphos-methyl (0.25%)		Clothianidin (13.2mg/paper)		Chlorfenapyr (100µg/bottle)	
	# of mosquitoes	% Mortality after 24hrs	# of mosquitoes	% Mortality after 1-4 days	# of mosquitoes	% Mortality after 1 to 2days
Chikwawa	98	100	100	100	113	100
Nkhata-Bay	117	100	107	100	116	100
Karonga	102	100	97	100	111	100

Table D-3: Susceptibility status of *An. funestus* s.l. to **pirimiphos-methyl, clothianidin, and permethrin** in Nkhotakota district

Pirimiphos-methyl (0.25%)		**Clothianidin (13.2mg/paper)**		**Permethrin (7.5%)**	
# of mosquitoes	% Mortality after 24hrs	# of mosquitoes	% Mortality after 1-7 days	# of mosquitoes	% Mortality after 1 to 2days
97	100	109	98.1	88	4.5

ANNEX E. SPECIES IDENTIFICATION

Table E-1: Total number of *An. gambiae* s.l. identified to species level, by collection method

District	Site	PSC	CDC-LT	HLC	Susceptibility Test	Total
Karonga	Mwakanyamale	99	42	0	0	141
	Mwenimambwe	290	297	0	46	633
Nkhata-Bay	Kande	9	41	0	0	50
	Sanga	67	53	122		242
Nkhotakota	Chimkwende	54	93	64	0	211
	Ngalauka	60	174	0	0	234
	Vwawa	27	38	78		143
Salima	Chilungo	21	51	70	0	142
	Cholokoto	20	51	0	0	71
Chikwawa	Ntwana	87	56	0	0	143
	Nyamphota	50	49	0	0	99
Total		**784**	**945**	**334**	**46**	**2109**

Table E-2: Total number of *An. funestus* s.l. identified to species level, by collection method

District	Site	PSC	CDC-LT	HLC	Susceptibility Test	Total
Karonga	Mwakanyamale	16	11	0	0	27
	Mwenimambwe	0	0	0	0	0
Nkhata-Bay	Kande	84	150	0	0	234
	Sanga	79	164	117	0	360
Nkhotakota	Chimkwende	189	145	85	0	419
	Ngalauka	31	39	0	0	70
	Vwawa	72	54	124	0	250
Salima	Chilungo	7	4	11	0	22
	Cholokoto	55	39	0	0	94
Chikwawa	Ntwana	75	35	0	0	110
	Nyamphota	18	10	0	0	28
Total		**626**	**651**	**337**	**0**	**1614**

REFERENCES

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

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

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

Detinova, TS. 1962. *Age grouping methods in Diptera of medical importance, with special reference to some vectors of malaria*. World Health Organization Monographs series 47.

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

Wirtz, R. A., F. Zavala, Y., Charoenvit, G. H. Campbell, T. R. Burkot, I. Schneider, K. M. Esser, R. L. Beaudoin, and R. G. Andre. 1987. Comparative testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. *Bull. World Health Organization*. 65: 39–45.

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

World Health Organization. WHO. 2013. Malaria entomology and vector control, Guide for participants. Geneva: World Health Organisation.

World Health Organization. WHO. 2006. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. World Health Organization. http://apps.who.int/iris/bitstream/10665/69296/1/WHO_CDS_NTD_WHOPES_GCDPP_2006.3_eng.pdf