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THE PMI VECTORLINK PROJECT ZAMBIA

ENTOMOLOGY ANNUAL REPORT

AUGUST 2018 - JUNE 2019

Recommended Citation: The President's Malaria Initiative (PMI)/VectorLink Project. *Zambia 2018-2019 Entomology Annual Report.* Rockville, MD. The PMI VectorLink Project, Abt Associates.

Contract: AID-OAA-I-17-00008

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

Submitted to: United States Agency for International Development/PMI

Submitted on: October 1, 2019

Approved on: December 16, 2019

Abt Associates Inc. | 6130 Executive Blvd | Rockville, Maryland 20814
| T. 301.347.5000 | F. 301.913.9061
abtassociates.com

THE PMI VECTORLINK PROJECT ZAMBIA ENTOMOLOGY ANNUAL REPORT

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	U.S. Centers for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
GRZ	Government of the Republic of Zambia
HBR	Human Biting Rate
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
ITN	Insecticide-treated net
NMEP	National Malaria Elimination Program
PCR	Polymerase Chain Reaction
PSC	Pyrethrum Spray Catch
PMI	President's Malaria Initiative
WHO	World Health Organization

EXECUTIVE SUMMARY

The main malaria vector control interventions implemented in Zambia are indoor residual spraying (IRS) and insecticide-treated nets (ITNs). The U.S. President's Malaria Initiative (PMI) VectorLink Project, funded by the U.S. Agency for International Development (USAID) and implemented by Abt Associates, supports the implementation of IRS in Zambia. The PMI VectorLink Project conducted its 2018 IRS campaign in Zambia from October 15 to December 15, 2018, using Actellic 300CS in 26 high-burden districts in Luapula, Northern, and Muchinga Provinces, and SumiShield 50WG (active ingredient: clothianidin) in the three pre-elimination districts of Eastern Province (Katete, Sinda, and Chadiza).

Entomological monitoring associated with the 2018 IRS campaign was conducted in a total of 21 sites—14 sites for vector surveillance and insecticide resistance monitoring and seven sites for monitoring insecticide residual efficacy. Vector surveillance to assess the impact of IRS was conducted from August 2018 to June 2019 in 14 sentinel sites—five IRS and five control sites across the four provinces where IRS was supported by PMI VectorLink Project, plus an additional two sites in Central Province and two sites in Eastern Province—one IRS site sprayed by the Government of Zambia (GRZ) and one control site in each. Mosquitoes were collected using pyrethrum spray catches (PSCs) and human landing catches (HLCs). Baseline data were collected in August and October 2018 and post-intervention data collections started in December 2018, one month after the campaign. Spray quality was assessed 24 hours after IRS followed by monthly assessments of the insecticide decay rate on walls at seven sprayed sites, in seven districts supported by PMI VectorLink project (including the five districts where vector surveillance was conducted). Insecticide susceptibility tests were conducted between January and April 2019 using the World Health Organization (WHO) tube tests in the 14 surveillance sites.

Data from August 2018 to June 2019 indicated that *Anopheles funestus* s.l. was the most abundant (27,844, or 47.4%) *Anopheles* species collected. A total of 3,942 *An. gambiae* s.l. were also caught (6.7%). The density of An. funestus s.l. decreased from 2.1 vectors per house one month before IRS to 1.2 vectors per house one month after IRS. During the same period, the density of An. funestus s.l. increased at the control sites from 1.5 vectors to 3.9 vectors per house. The density of *An. gambiae* s.l. increased at both IRS and control sites after IRS implementation, though the increase was less pronounced at the IRS sites. The average human biting rates of *An. funestus* s.l. at IRS sites reduced immediately after IRS but returned to pre-IRS levels within five months of IRS.

There was 100 percent mortality of susceptible *An. gambiae* s.s. mosquitoes exposed to walls sprayed with pirimiphos-methyl or clothianidin insecticides at the time of the 2018 IRS campaign (T0) in all seven districts (Kawambwa, Mporokoso, Kasama, Isoka, Milenge, Mwense, and Katete). The observed mortality implies good spray quality during the campaign. Based on longitudinal data of the effectiveness of the two insecticides deployed in the 2018 IRS campaign on sprayed surfaces, the effective duration of pirimiphos-methyl (Actellic 300CS) was four months, while clothianidin (SumiShield) persisted for seven months (using the standard criteria of at least 80% mortality for two consecutive months).

An. funestus s.l. and *An. gambiae* s.l. were susceptible to clothianidin, pirimiphos-methyl, bendiocarb, and chlorfenapyr in all areas where the insecticides were tested. *An. funestus* s.l. were fully susceptible to dichlorodiphenyltrichloroethane (DDT) at all sites, while probable resistance to DDT was observed for *An. gambiae* s.l. at one site (Chebele, Mwense district in Luapula Province). In general, IRS implementation reduced the indoor resting density, human biting rate, and longevity of the major malaria vector at the sprayed sites. However, the duration of this effect only lasted 3-5 months, likely due to the short duration of the insecticide. The NMEP plans to deploy longer-lasting insecticides across the country during the 2019 IRS campaign. Continuous entomological monitoring will help assess whether a longer-lasting insecticide will provide the much-needed, sustained reductions in vector populations after IRS.

1. INTRODUCTION

Malaria is endemic to Zambia and transmission is stable, with a seasonal peak associated with the rainy season from November to May and peak parasite prevalence occurring towards the end of the transmission season in April and May. Malaria in Zambia is transmitted by the *Anopheles gambiae* and the *An. funestus* group of mosquitoes; the main vector species are *An. gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. Indoor residual spraying (IRS) is one of the primary vector control interventions of the Zambian National Malaria Elimination Program (NMEP).

The U.S. President's Malaria Initiative (PMI) has provided financial and technical support to the NMEP and district health offices for IRS and entomological surveillance activities since 2008 through the Africa Indoor Residual Spraying (AIRS) Project and now the PMI VectorLink Project, which began in 2018. VectorLink Zambia supports the NMEP through routine entomological surveillance and generates data on key entomological indicators including malaria vector species composition, density, feeding behavior, feeding habits, and parity rate in seven districts. In addition, VectorLink conducts insecticide susceptibility tests, assesses the quality of spray during the IRS campaign, and monitors the duration of efficacy of the insecticide on the walls after IRS. These data guide the NMEP and other stakeholders on vector control decision making, including insecticide selection, IRS programming, and insecticide resistance management.

Entomological surveillance is a key component of IRS programming, providing information on the impact of IRS on malaria vector density and behavior in geographic areas where IRS has occurred compared to non-IRS areas. This report covers the period August 2018 to June 2019 and is linked to the 2018 IRS campaign. Table 1 below outlines the entomological indicators covered in this report. Indicators are delineated as basic or advanced per PMI's 2018 Technical Guidance.

Table 1: Basic and Advanced Entomological Indicators by Collection Method and Frequency of Collection

Indicator	Collection Method(s)	Frequency	Parameters measured
Basic Indicators			
Vector species composition	PSC, HLC	Once every two months	Percentage of mosquito species captured
Vector abundance	PSC, HLC	Once every two months	Indoor resting density and human biting rate
Seasonality	PSC, HLC	Once every two months	Indoor resting density and human biting rate
Insecticide susceptibility	Larval and Adult collections	Once per year	Percentage mortality at 24 hrs. or five days (for clothianidin)
Spray quality assurance	Insectary colony mosquitoes	Once per year, within 48 hours of spray	Percentage mortality at 24 hrs.; or at five days (for clothianidin)
Residual efficacy monitoring	Insectary colony mosquitoes	Monthly[1]	Percentage mortality at 24 hrs.; or at five days (for clothianidin)
Vector feeding time	HLC	Once every two months	Hourly human biting rates
Vector feeding location	HLC	Once every two months	Indoor and outdoor biting rates
Advanced Indicators			
Sporozoite rate*	HLC	Once every two months	Percent positive for sporozoites
Entomological Inoculation Rate*	HLC	Once every two months	Product of biting rate and sporozoite rate
Parity rate	HLC	Once every two months	Percentage parous

HLC=Human Landing Catch, PSC=Pyrethrum Spray Catch; [1]Collected monthly after spray campaign until mortality below 80% for two consecutive months, *These indicators will be reported on in an addendum to this report.

2. MATERIALS AND METHODS

2.1 STUDY SITES

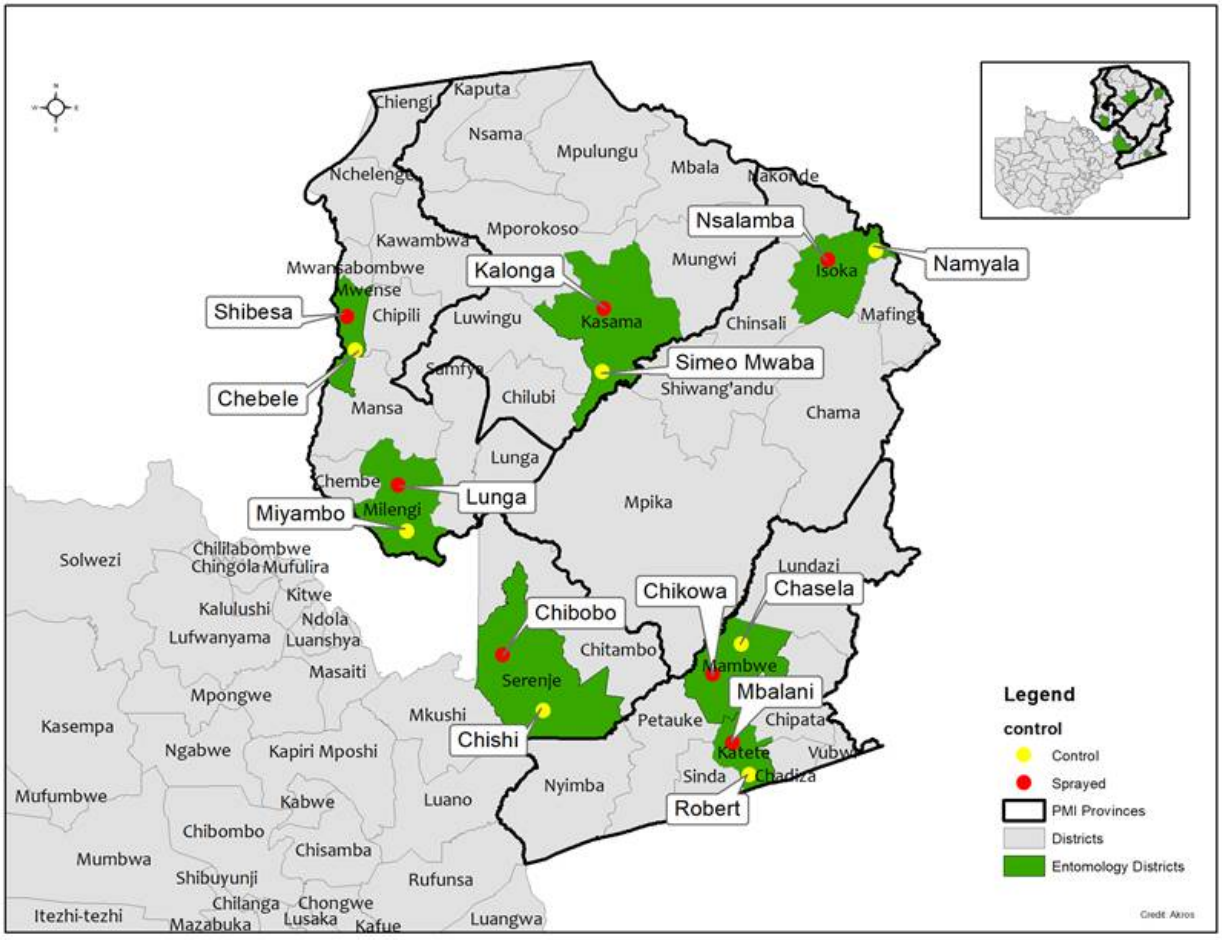
From August 2018 to June 2019, VectorLink Zambia conducted entomological surveillance and insecticide resistance monitoring activities in 14 sentinel sites—10 sites within districts that received IRS in the 2018 PMI VectorLink IRS campaign, and four sites in two districts that were sprayed by the Government of the Republic of Zambia (GRZ) (Figure 1). Each district consisted of two matched sentinel sites—one sprayed (sites sprayed with either Actellic 300CS or SumiShield in the 2018 IRS campaign) and one unsprayed (control site with no IRS) as shown in Table 2—to enable direct comparisons. A site is a cluster of households and is typically a single village within a catchment area of the district. The control (unsprayed) sites were selected as the nearest available unsprayed cluster to the corresponding sprayed cluster. The clusters selected as control sites were usually not targeted for IRS due to factors such as hard to reach areas and sparsely distributed houses. Control sites were at least two kilometers from any sprayed structures. IRS quality assurance and the monitoring of insecticide decay rates on walls after IRS was monitored at seven sprayed sites as shown in Table 2.

Table 2: Entomological Monitoring Sites

Province	District	Health Facility Catchment Area	Sentinel Site (Village)	Spray Status	Distance to Nearest Sprayed Community (km)	Percent of Households Targeted for IRS by PMI/VL in 2018
Muchinga	Isoka	Kapililonga	Nsalamba	Sprayed with Actellic 300CS	-	100%
		Nsasamwenje	Namyala	Non-Sprayed (control)	30	0%
Northern	Kasama	ZNS	Kalonga	Sprayed with Actellic 300CS	-	100%
		ZNS	Simeo Mwaba	Non-Sprayed (control)	5	0%
Eastern	Katete	Kamphambe	Mbalani	Sprayed with SumiShield	-	100%
		Mwandafisi	Robert	Non-Sprayed (control)	10	0%
	Mambwe	Chikowa	Chikowa	Sprayed with Actellic 300CS	-	100% (by GRZ)
		Chikowa	Chasela	Non-Sprayed (control)	6	0%
Luapula	Milenge	East Seven	Lunga	Sprayed with Actellic 300CS	-	100%
		Sokontwe	Miyambo	Non-Sprayed (control)	7	0%
	Mwense	Kashiba	Shibesa	Sprayed with Actellic 300CS	-	100%
		Mwense Stage 2	Chebele	Non-Sprayed (control)	2	0%
Central	Serenje	Chibobo	Chibobo	Sprayed with Actellic 300CS	-	100% (by GRZ)
		Chibobo	Chishi	Non-Sprayed (control)	5	0%

IRS Quality Assurance and Insecticide Residual Efficacy Monitoring Sites				
Province	**District**	**Location of site (Village name)**	**Spray Status**	**Percent of Households Targeted for IRS by PMI/VL in 2018**
Luapula	Kawambwa	Chama	Sprayed with Actellic 300CS	100%
Luapula	Milenge	Kapalala	Sprayed with Actellic 300CS	100%
Luapula	Mwense	Lukwesa	Sprayed with Actellic 300CS	100%
Northern	Mporokoso	Chalabesa	Sprayed with Actellic 300CS	100%
Northern	Kasama	Kalonga	Sprayed with Actellic 300CS	100%
Eastern	Katete	Undi	Sprayed with SumiShield	100%
Muchinga	Isoka	Katyetye	Sprayed with Actellic 300CS	100%

Figure 1: Geographical Locations of PMI Entomological Sentinel Sites in Zambia



2.2 Longitudinal Monitoring of Malaria Vector Density and Behavior

Vector surveillance was conducted at two sites (one sprayed and one unsprayed) in each of the seven sentinel districts. Adult mosquitoes were collected at the vector surveillance sites once every two months from August 2018 to June 2019 using pyrethrum spray catches (PSCs) and human landing catches (HLCs) (see Table 1). Collections at the two sentinel sites in Mambwe (the new sentinel district in Eastern Province) commenced in November after identification of the catchment areas. VectorLink conducted IRS in five of the intervention sites (Kalonga, Mbalani, Nsalamba, Lunga, and Shibesa) in November 2018. The NMEP sprayed Chikowa in Mambwe district and Chibobo in Serenje district in December 2018. Actellic 300CS was sprayed at all sites except Mbalani in Katete district which was sprayed using SumiShield 50WG. Baseline collections were carried out in August and October in all sentinel districts except for Mambwe where baseline collections were done in November.

Table 3: Adult Mosquito Collection Methods for Vector Surveillance

Method	Time	Frequency	Sample
PSC	4:00 a.m. to 6:00 a.m.	Once every two months	15 houses per site; five houses per day
HLC	6:00 p.m. to 8:00 a.m.	Once every two months	Four houses, four consecutive nights, indoor and outdoor

2.2.1 Pyrethrum Spray Catches

PSCs were used to sample indoor-resting mosquitoes between 4:00 a.m. and 6:00 a.m. in 15 houses at each of the 14 sentinel sites in each collection month (five distinct houses per day over three consecutive days). Collections were done in the same 15 houses throughout the data collection period, except in a few cases where the house owner was absent and the nearest available house was recruited for that day. Before the PSCs were performed, all occupants were asked to vacate the house without disturbing the resting mosquitoes. A pyrethroid-based insecticide product, Raid (SC Johnson & Son S.A. Ltd), in pressurized 300ml spray cans was used to knock down the mosquitoes. Raid contains the pyrethroids tetramethrin 0.2% w/w, prallethrin 0.04% w/w, imiprothrin 0.034% w/w; and the synergist piperonyl-butoxide 1.15% w/w. The eaves, windows, and other openings in the house were sprayed followed by the inside of the walls and roof of the house. Ten minutes after spraying, all mosquitoes knocked down by the insecticide were collected using white sheets placed on the floor before spraying.

The species composition and indoor resting density were determined from the PSCs at each sentinel site.

2.2.2 Human Landing Catches

HLCs were used to monitor mosquito feeding behavior across all the 14 sentinel sites. At each site, mosquitoes were collected indoors and outdoors at four houses for four consecutive nights every other month to yield 16 person-nights indoors and 16 person-nights outdoors per site per month. The same houses were used each time throughout the surveillance period. Community-based volunteers trained on the HLC technique served as the collectors and worked in pairs—one collector was seated indoors and another seated outdoors (within 5 meters of the front of the house) from 6:00 p.m. to 1:00 am. The pair was replaced by another pair of volunteers from 1:00 to 8:00 a.m., meaning four volunteers per house per night participated in collections from 6:00 p.m. to 8:00 a.m.

During collection, each collector sat on a small chair and exposed their legs from the ankle to the knee (collectors wore long sleeved shirts to cover their arms). When a mosquito landed on their legs, they used a flashlight to locate the mosquito and a mouth aspirator to collect it and carefully transfer it into paper cups labelled with the hour of collection, site name, and house ID. For each hour of collection, the volunteers

collected mosquitoes for 50 minutes and took a 10 minute break. During breaks, the collectors swapped positions—that is, the outdoor collector moved indoors and the indoor collector relocated outdoors. All community based volunteers involved in the HLCs were provided chemoprophylaxis with deltaprim.

The following parameters were measured from the HLCs:

- Vector feeding behavior (time and location of biting)
- Human biting rate (HBR)
- Parity rate
- Sporozoite rate
- Entomological inoculation rate (EIR)

2.2.3 Laboratory Analysis

Mosquitoes collected by HLCs were killed using cotton wool soaked in formalin to enable pre-laboratory handling. *Anopheles* mosquitoes collected by PSC and HLC were identified morphologically by species using the Gilles and Coetzee 1987 identification key[1], and counted according to house number (in case of PSC samples) and by house number, location, and hour of collection (for HLC samples). The abdominal status of all female *Anopheles* collected by PSC were categorized as either unfed, blood-fed, or gravid. The *Anopheles* collected were preserved in 1.5ml Eppendorf tubes with silica gel desiccant. A hole was pierced in the cap of the tube and the tubes were kept in transparent Ziploc bags also containing silica gel. A sub-sample of preserved *An. funestus* s.l. and *An. gambiae* s.l. were preserved for further processing using polymerase chain reaction (PCR) to identify the sibling species, enzyme-linked immunosorbent assay (ELISA) method to detect circumsporozoite proteins of *Plasmodium falciparum* sporozoites, and blood meal PCR to identify the source of the blood meal (blood-fed samples only).

Laboratory processing of selected subsamples will be completed by the end of November, following a delay in receiving the reagents and supplies that have recently arrived in-country. A separate report will be submitted with the results from the laboratory analysis as an addendum to this report.

2.2.4 Data Presentation

Data obtained from PSC were used to determine the indoor resting density (the average number of mosquitoes per house per night), while data from HLCs were used to estimate the HBR (mean number of mosquitoes collected per person per night). Indoor resting densities are presented with standard errors to compare variations between IRS and non-IRS sites. Biting rates are presented as averages of hourly collections from each of the bimonthly HLC efforts.

2.3 Quality Assurance of IRS and Insecticide Decay Rate

To assess the decay rate of the insecticide on walls after IRS, VectorLink conducted cone bioassays in 42 randomly selected houses located across the seven districts where IRS was implemented (Kawambwa, Milenge, Mwense, Mporokoso, Kasama, Katete, Isoka). Cone bioassays were conducted 24 to 48 hours after spraying and within two weeks of the spray campaign (T0) to gauge quality of spray. The bioassays were conducted in Kawambwa and Mporokoso in October 2018 and in Kasama, Isoka, Milenge, Mwense, and Katete in November 2018. The bioassays were repeated monthly until mosquito mortality dropped below 80% for two consecutive months to assess the duration of efficacy of the insecticide on the sprayed walls against susceptible vectors. Six sprayed houses—three mud and three cement—plus two unsprayed, control houses (one mud and one cement) were used at each of the seven sprayed sites.

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

The cone bioassay tests were done according to the WHO test procedure. The cones were placed on the treated walls at three locations: 0.5m, 1m, and 1.5m above the ground. Ten female *An. gambiae* s.s. Kisumu strain mosquitoes were introduced per cone and exposed for 30 minutes. A fourth replicate of 10 mosquitoes was placed in a paper cup 1 meter above the floor and about 0.1 meter from the sprayed wall to assess the fumigant effect of the insecticide. After 30 minutes of exposure, the mosquitoes were transferred to insecticide-free paper cups supplied with 10% sugar solution. The cups were then placed in plastic buckets covered with a damp napkin to create favorable humidity for the mosquitoes and held for 24 hours (for mosquitoes exposed to Actellic 300CS) and up to 5-7 days (for mosquitoes exposed to SumiShield). The number of mosquitoes knocked down after 30 minutes and 60 minutes and the number dead after every 24-hour holding period were recorded. When the mortality of the control was between 5-20%, corrected mortality was determined using Abbot's formula.

Table 4: Quality Assurance and Insecticide Residual Efficacy Activities

Activity	Frequency	Sample
Quality assurance of IRS	Once in the first two weeks of campaign, and within 24-48 hrs. of spraying	Eight houses per site (sprayed: three mud and three cement; Unsprayed: one mud and one cement)
Monitoring of Insecticide Decay rate on walls	Monthly, until exposed mosquito mortality falls below 80% for two consecutive months	Eight houses per (sprayed: three mud and three cement; unsprayed: one mud and one cement)

2.4 Insecticide Resistance Monitoring

2.4.1 WHO Susceptibility Tests

U.S. Centers for Disease Control and Prevention (CDC) backpack and Prokopack aspirators were used to capture indoor-resting adult, female mosquitoes and dippers were used to collect larvae from larval habitats for insecticide susceptibility testing. Wild unfed female *An. funestus* s.l. aged 2-5 days and *An. gambiae* s.l. (reared from larvae collected in the field) were used for the susceptibility tests. The mosquitoes were exposed to diagnostic doses of various insecticides using insecticide-impregnated papers, as described by WHO guidelines. Susceptibility of *An. funestus* s.l. and *An. gambiae* s.l. to clothianidin 2.0% (a neonicotinoid), DDT 4.0% (an organochlorine), pirimiphos methyl 0.25% (an organophosphate), and deltamethrin 0.05% (a pyrethroid), were tested in select sentinel sites. Given the susceptibility of the mosquitos have shown to DDT, the NMEP has expressed interest in deploying DDT in the country as part of the Elimination 8 strategy in southern Africa. In fact, in the 2019 IRS campaign, the government will spray DDT in Northern Province (all districts except Chilubi which will use Fludora Fusion) and Luapula Province (except Nchelenge district, which will be sprayed with support from PMI VectorLink with Fludora Fusion). The exposure time was 60 minutes, after which mosquitoes were transferred into the holding tubes and provided with 10% sugar solution. For the clothianidin tests, mortality was recorded after 24 hours, and again after two, three, four, five, six, and seven days while, for the other insecticides, mortality was recorded after 24 hours only. Mortality for clothianidin-exposed mosquitoes is recorded over a longer period due to the slow-acting nature of the insecticide on mosquitoes. The sugar solution was changed daily during the holding periods. Susceptibility tests were done from January to April 2019.

Clothianidin papers used in the susceptibility tests were locally impregnated following procedures developed by PMI VectorLink project. In this procedure, Whatman® No. 1 filter papers measuring 12cm by 15cm were treated with the diagnostic dose of clothianidin (2% w/v) which is 13.2 mg active ingredient per paper, equivalent to 734 mg ai/m^2. Firstly, 26.4 mg of SumiShield 50WG (containing 50% clothianidin as active ingredient) was suspended in 2 milliliters of distilled water and the resulting suspension (containing 13.2mg ai) was shaken well before pipetting it onto the filter paper. After drying overnight, the filter papers were stored

in aluminum foil at 4°C in the fridge. Papers were freshly prepared for each test. Control papers were prepared by pipetting 2 ml of distilled water on the Whatman® No. 1 filter paper.

2.4.2 CDC Bottle Assays

CDC bottle assays were used to assess the susceptibility status of *An. funestus* s.l. to different concentrations of chlorfenapyr in Lunga and Miyambo sites in Milenge district. The standard CDC bottle assays procedures were followed with some modifications. Two doses of chlorfenapyr (12.5 µg and 100 µg) were tested. The exposure time was 60 minutes and mortality was recorded at 1 hour, 24 hours, 48 hours, and 72 hours.

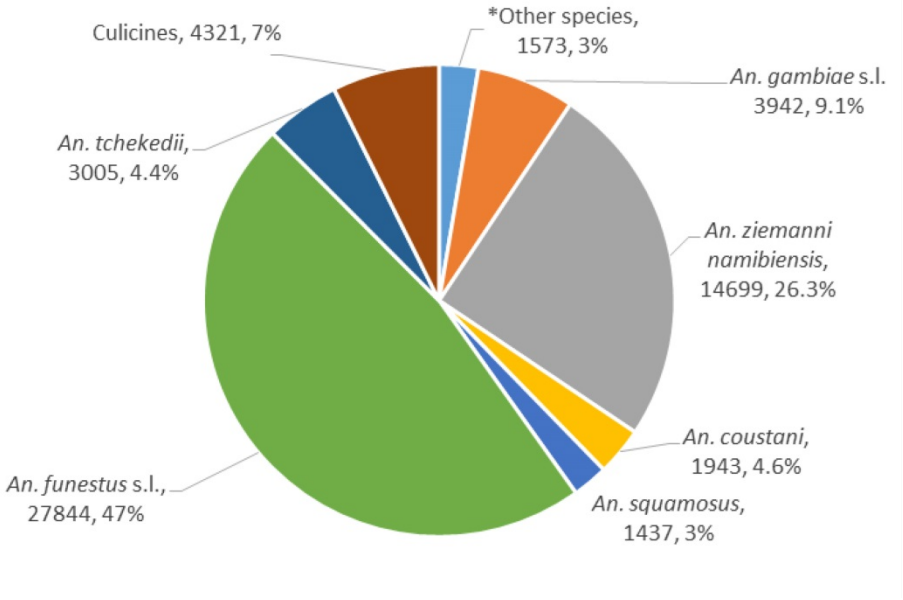
3. Results and Discussion

From August 2018 to June 2019, mosquitoes were collected bimonthly in the sentinel districts to assess vector species composition, density, and behavior. Baseline data were collected in August and October 2018 and IRS was implemented in November 2018.

3.1 Species Composition

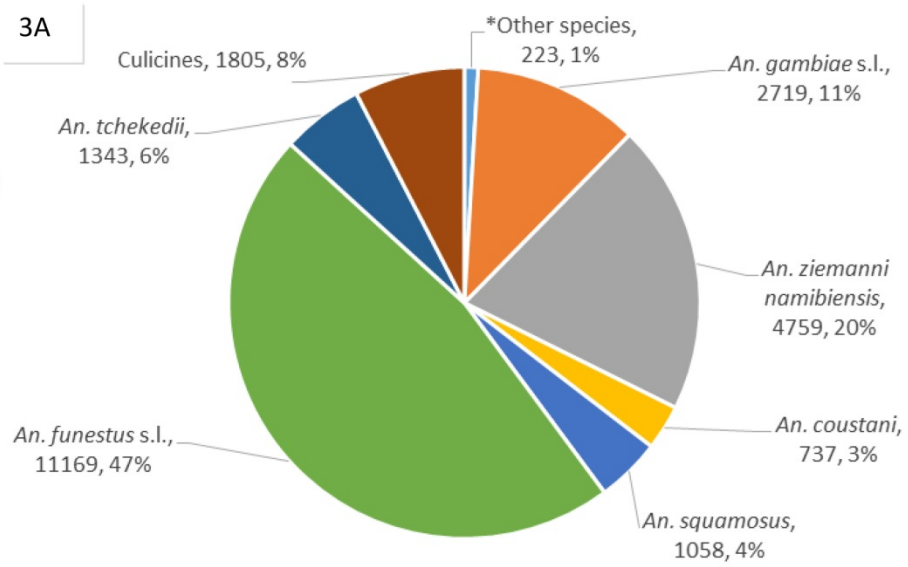
A total of 58,764 female mosquitoes were collected using PSCs and HLCs from the spray and control sites. The most abundant anopheline species collected was *An. funestus* s.l. 27,844 (47.4%) followed by *An. ziemanni namibiensis* 14,699 (25.9%), *An. gambiae* s.l. 3,942 (6.7%), and *An. tchekedii* 3,005 (5.1%). The other *Anopheles* species (*An. squamosus, An. argenteolobatus, An. maculipalpis, An. rufipes, An. tenebrosus, An. gibbinsi,* and *An. pretoriensis)* together accounted for less than 9% of the total mosquitoes collected. Figures 2, 3 and 4 below show the number and percentage of each species collected in all sites, sprayed sites, and control sites, respectively.

Figure 2: Species Composition of Samples from All Sites (Aug 2018-Jun 2019)

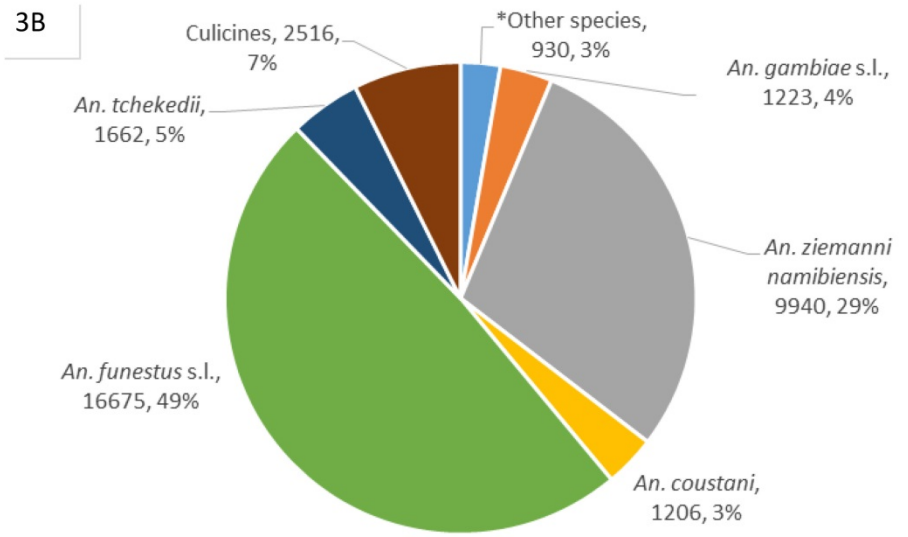


*Other species include: *An. argentiolobatus* (1061, 1.8%), *An. maculipalpis* (362, 0.6%), *An. rufipes* (90, 0.2%), *An. tenebrosus* (39, 0.1%), *An. gibinnsi* (19, 0.03%), and *An. pretoriensis* (2, 0.003%).

Figure 3: Species Composition in Sprayed (3A) and Unsprayed (3B) Sentinel Sites (Aug 2018-Jun 2019)



*Other species include: *An. maculipalpis* (188, 0.8%), *An. tenebrosus* (12, 0.05%), *An. gibinnsi* (12, 0.05%), *An. rufipes* (9, 0.04%), and *An. pretoriensis* (2, 0.008%).



*Other species include: *An. squamosus* (379, 1.1%), *An. argentiolobatus* (262, 0.8%), *An. maculipalpis* (174, 0.5%), *An. rufipes* (81, 0.2%), *An. tenebrosus* (27, 0.08%), and *An. gibinnsi* (7, 0.02%).

All main *Anopheles* species were collected from both sprayed and unsprayed sites. More *An. gambiae* s.l. were collected in sprayed sites compared to unsprayed sites; more *An. funestus* s.l. were collected from unsprayed sites than sprayed sites.

Out of the 31,786 primary vectors collected, *An. funestus* s.l. accounted for 87.6% (27,844) while *An. gambiae* s.l. accounted for 12.4% (3,942). Ninety-one percent of the vectors (28,993) were collected from HLCs (both indoors and outdoors) and 9% (2,793) were collected from PSCs. The collection effort during the entire surveillance period was 2,624 (62%) for HLCs and 1,230 (32%) for PSCs. Though the number collected by

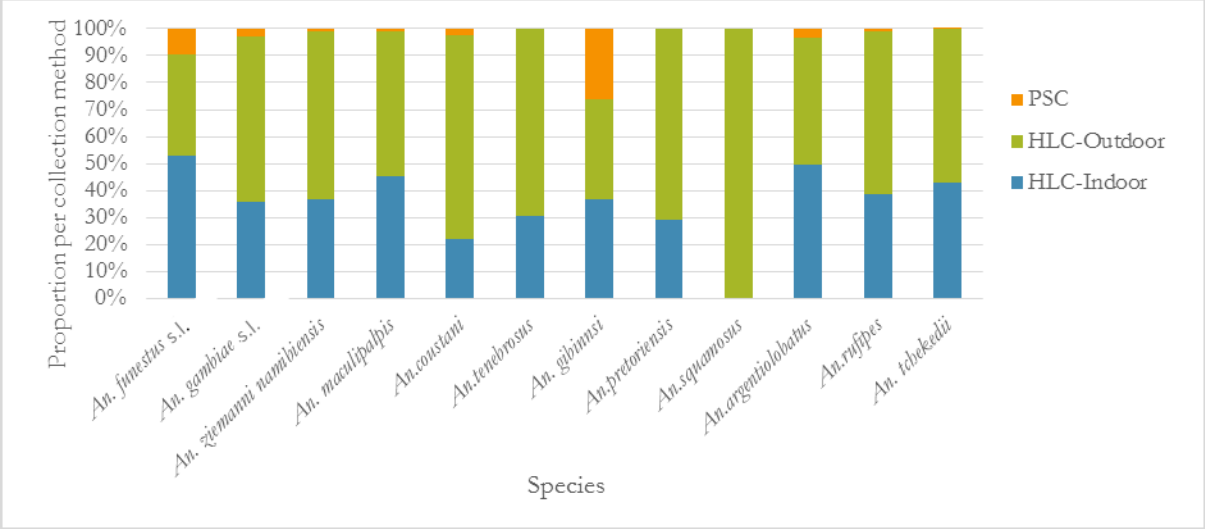
HLCs was twice that of PSC, the total vectors collected by HLC were more than seven times that collected by PSC. Among those collected by PSCs, *An. funestus* s.l. accounted for 96% while *An. gambiae* s.l. accounted for 4%. Among those collected by HLCs, 87% were *An. funestus* s.l. and 13% were *An. gambiae* s.l.

An. funestus s.l. was the predominant malaria vector in all districts except Mambwe, where *An. gambiae* s.l. was the most common species collected (Figure 4). In Isoka district, 37% were *An. gambiae* s.l. and 63% were *An. funestus* s.l. The total number of mosquitoes collected by site and collection method are presented in Annex A and the number of *An. funestus* and *An. gambiae* s.l. collected are shown in Annex B.

Figure 4: Relative Proportions of *An. funestus* s.l. and *An. gambiae* s.l. by District (Aug 2018-Feb 2019)

Figure 5 shows the proportion of each *Anopheles* species collected by each collection method. The most *An. funestus* s.l. were collected from HLC indoors, while HLC outdoors yielded the majority of the other species collected.

Figure 5: Proportion of *Anopheles* Species Collected by PSCs and HLCs (Indoor and Outdoor) across Sites (August 2018-June 2019)



3.2 Vector Density and Behavior

3.2.1 Indoor Resting Density of *An. funestus* s.l. and *An. gambiae* s.l., Collected by PSC

Given that the districts were not all sprayed at the same time—for instance, Mambwe was sprayed in December while the other districts were sprayed in November—graphs in the following section present data according to the time relative to the month of IRS (e.g., T-1 is one month before spraying, T+1 is one month after spraying) instead of calendar months, enabling comparison between districts and summing across districts.

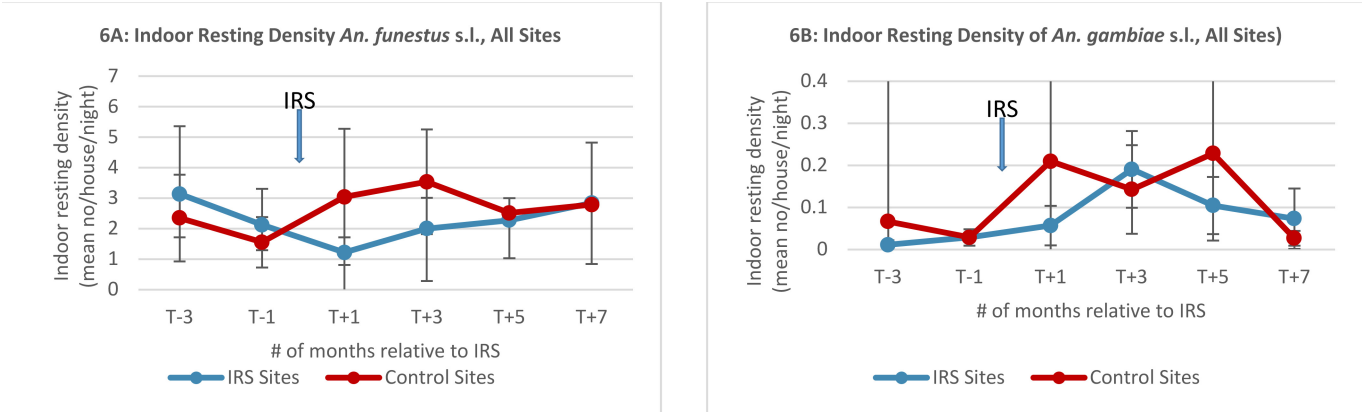
At the IRS sites, the indoor resting density of *An. funestus* s.l. reduced immediately after IRS (from 2.1 to 1.2 vectors per house) and increased (from 1.5 to 3.9 vectors per house) at the unsprayed sites (Fig 6A). The indoor resting density of *An. gambiae* s.l. increased after IRS at both the IRS and unsprayed sites (Fig 6A) although the increase was less pronounced at the IRS sites (from 0.03 to 0.06 vectors per house) compared to the unsprayed sites (from 0.03 to 0.21 vectors per house).

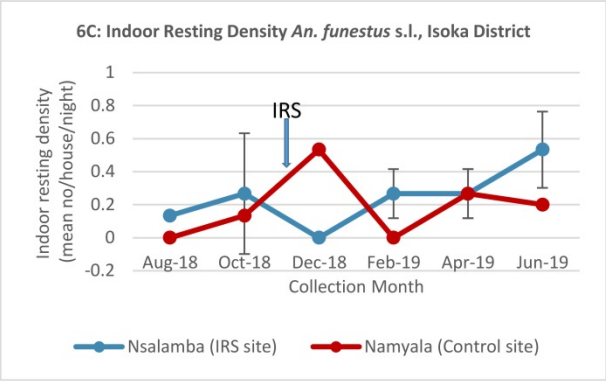
Figure 6 shows the mean densities of *An. funestus* s.l. and *An. gambiae* s.l. per house per night in sprayed and control sites between August 2018 and June 2019 with 95% confidence intervals. Indoor densities of *An. funestus* s.l. decreased after IRS at four of the seven IRS sites (Nsalamba-6C, Kalonga-6E, Chikowa-6I, and Lunga-6K) and for *An. gambiae* s.l. at one site (Nsalamba-6D). At all other sites, vector densities either increased (Chikowa-6J and Shibesa-6N) or were unaffected because no *An. funestus* s.l. or *An. gambiae* s.l. vectors were collected (Kalonga-6F, Mbalani-6H, Lunga-6L, and Chibobo-6P).

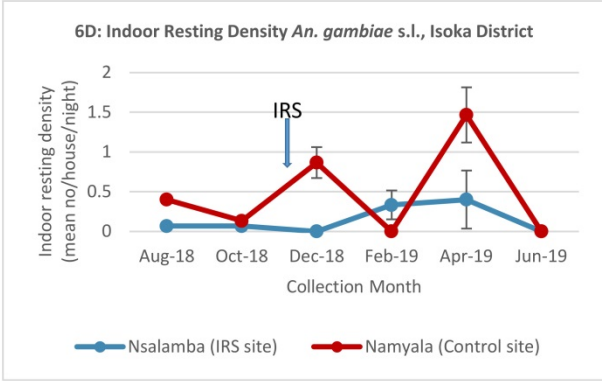
In Kasama and Mwense, we observed higher monthly density of *An. funestus* s.l. at the sprayed site compared to the control site throughout the reporting period, including the baseline (Figure 6E and 6M), while in Katete (Fig. 6G), Milenge (Fig. 6K), and Serenje (Fig. 6L), lower densities of *An. funestus* s.l. were observed at the sprayed site compared to the control sites in most months, with modest longitudinal impact of IRS at the sprayed site in Milenge. The fewest indoor resting mosquitoes were recorded at the unsprayed site in Mambwe in Eastern Province, with an average of 0.13 *An. funestus* s.l. per house per night throughout the surveillance period, while the most were collected at the unsprayed site in Milenge district in Luapula Province with a mean of 8.68 *An. funestus* s.l. per house per night.

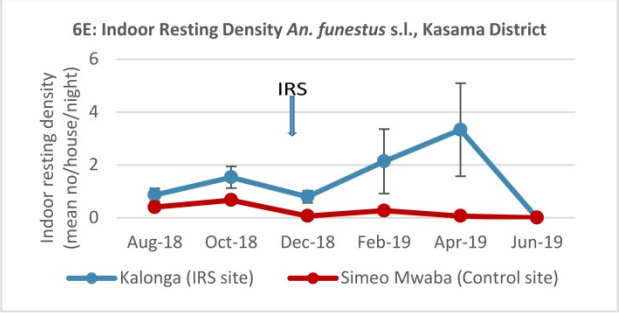
A total of 112 *An. gambiae* s.l. were collected indoors across all sites (41 from the sprayed sites, 71 from the control sites. Given the densities in both sprayed and control sites were low, it may be difficult to draw conclusions on the impact of IRS on the indoor resting density of this species.

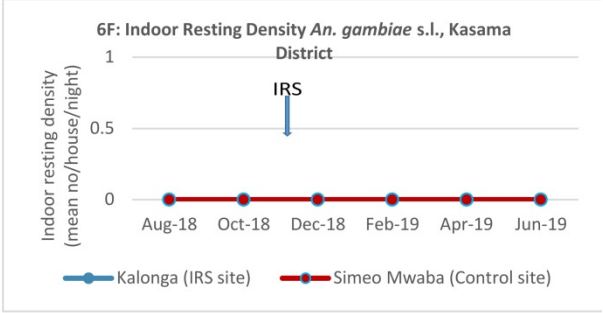
Figure 6: Indoor Resting Density of *An. funestus* s.l. and *An. gambiae* s.l. across Sites (August 2018-June 2019)

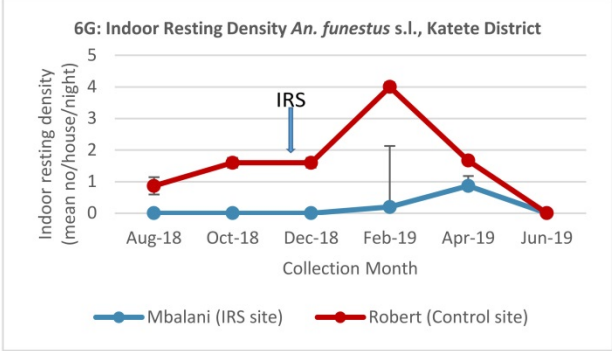


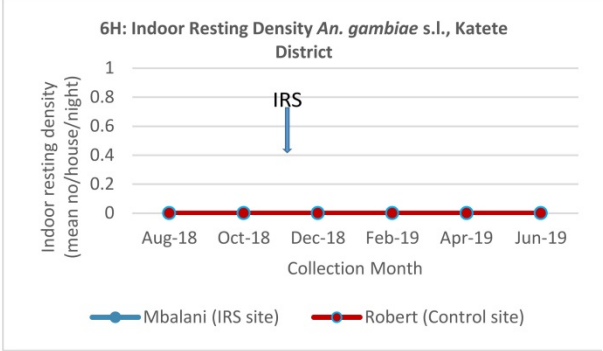

6C: Indoor Resting Density An. funestus s.l., Isoka District
Indoor resting density (mean no/house/night)
1
0.8
0.6
0.4
0.2
0
-0.2
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Nsalamba (IRS site)
Namyala (Control site)

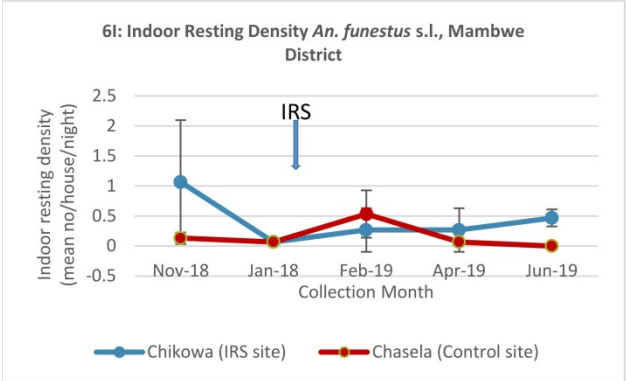

6D: Indoor Resting Density An. gambiae s.l., Isoka District
Indoor resting density (mean no/house/night)
2
1.5
1
0.5
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Nsalamba (IRS site)
Namyala (Control site)

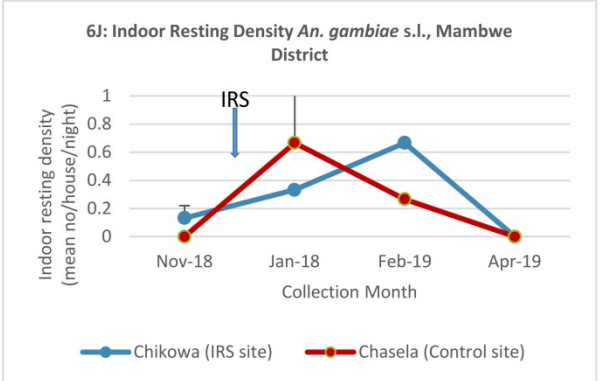

6E: Indoor Resting Density An. funestus s.l., Kasama District
Indoor resting density (mean no/house/night)
6
4
2
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Kalonga (IRS site)
Simeo Mwaba (Control site)

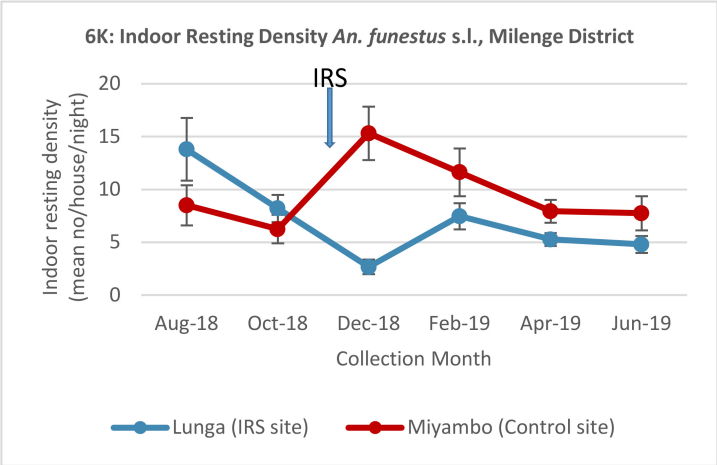

6F: Indoor Resting Density An. gambiae s.l., Kasama District
Indoor resting density (mean no/house/night)
1
0.5
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Kalonga (IRS site)
Simeo Mwaba (Control site)

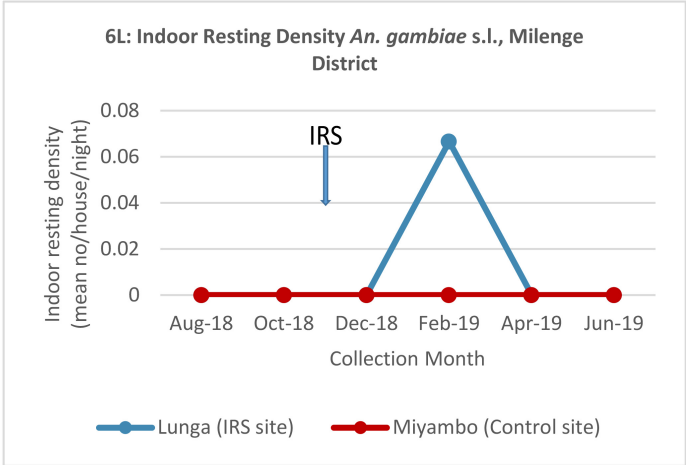

6G: Indoor Resting Density An. funestus s.l., Katete District
Indoor resting density (mean no/house/night)
5
4
3
2
1
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Mbalani (IRS site)
Robert (Control site)

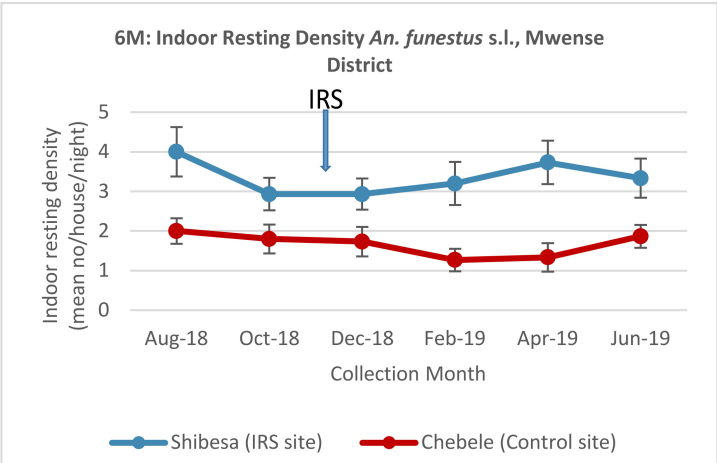

6H: Indoor Resting Density An. gambiae s.l., Katete District
Indoor resting density (mean no/house/night)
1
0.8
0.6
0.4
0.2
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Mbalani (IRS site)
Robert (Control site)

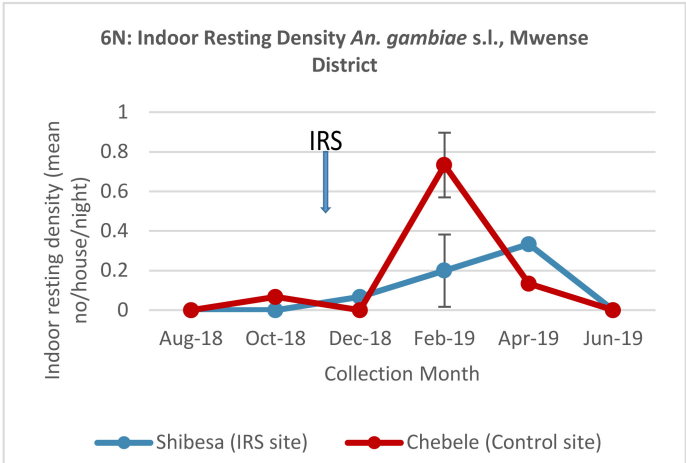

6I: Indoor Resting Density An. funestus s.l., Mambwe District
Indoor resting density (mean no/house/night)
2.5
2
1.5
1
0.5
0
-0.5
IRS
Nov-18
Jan-18
Feb-19
Apr-19
Jun-19
Collection Month
Chikowa (IRS site)
Chasela (Control site)

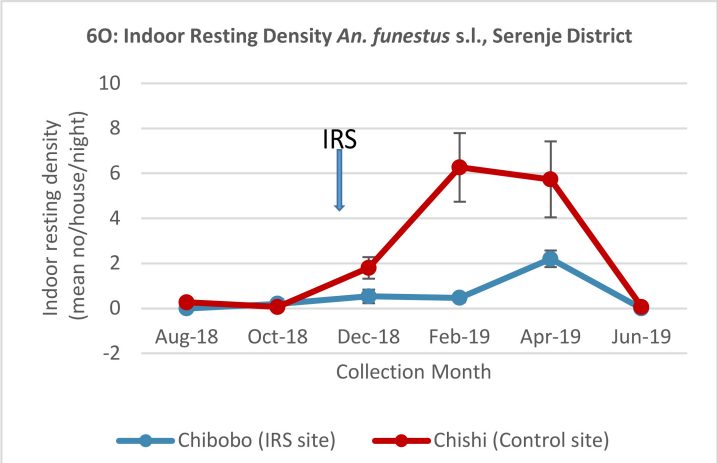

6J: Indoor Resting Density An. gambiae s.l., Mambwe District
Indoor resting density (mean no/house/night)
1
0.8
0.6
0.4
0.2
0
IRS
Nov-18
Jan-18
Feb-19
Apr-19
Collection Month
Chikowa (IRS site)
Chasela (Control site)

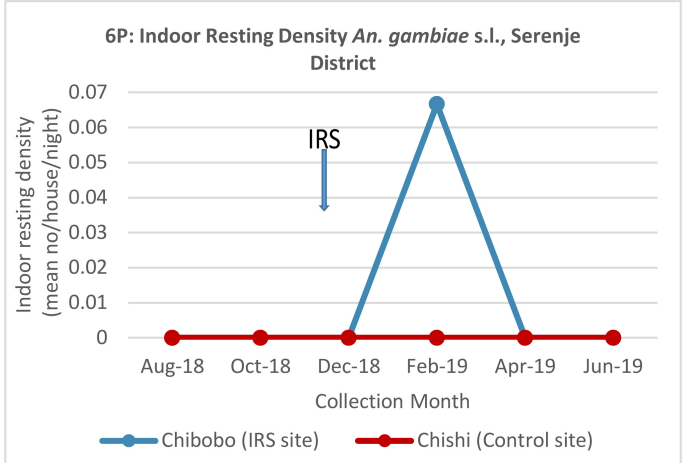








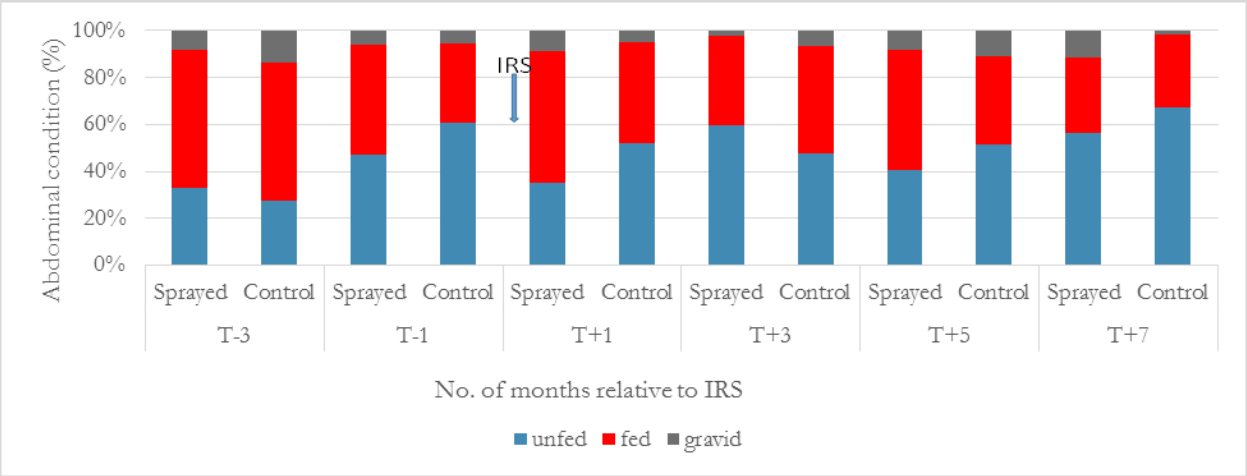




3.2.2 Abdominal Condition of An. funestus s.l. Collected by PSCs

Figure 7 shows the abdominal status of *An. funestus* s.l. collected indoors by PSCs from sprayed and control sites before and after IRS. Overall, the proportion of fed or gravid *An. funestus* s.l. collected indoors was 55.5% in the sprayed sites and 49.7% in the control sites. The proportion of fed or gravid *An. funestus* s.l. collected indoors was 53.1% one month before IRS and 64.4% one month after IRS at the sprayed sites compared to 39.2% and 47.8% at the control sites.

Figure 7: Abdominal Condition of *An. funestus* s.l. Collected by PSCs in Sprayed and Control Sites (August 2018-June 2019)

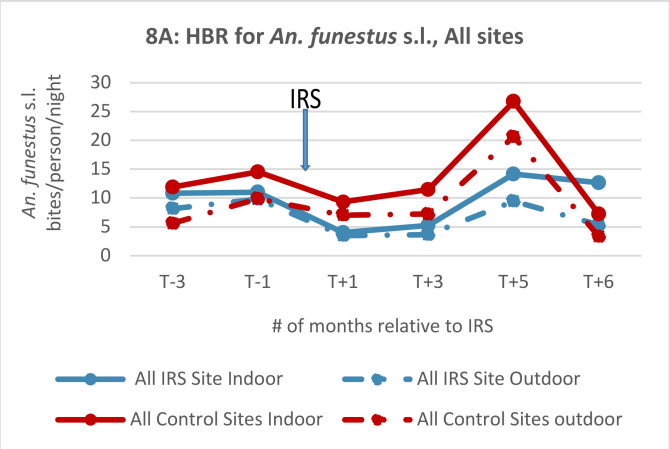


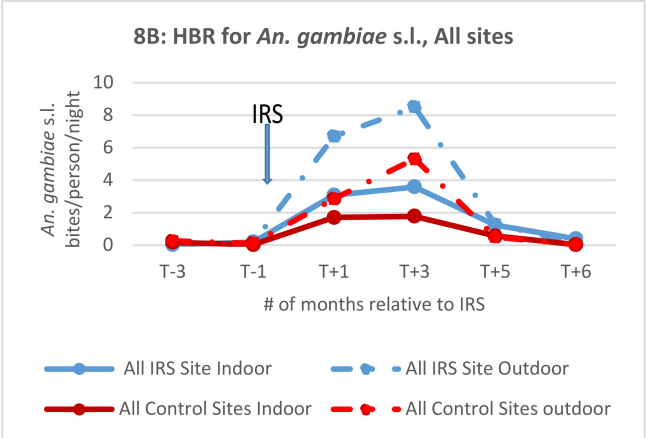
3.2.3 Biting Rate of *An. funestus* s.l. and *An. gambiae* s.l. Collected By HLC

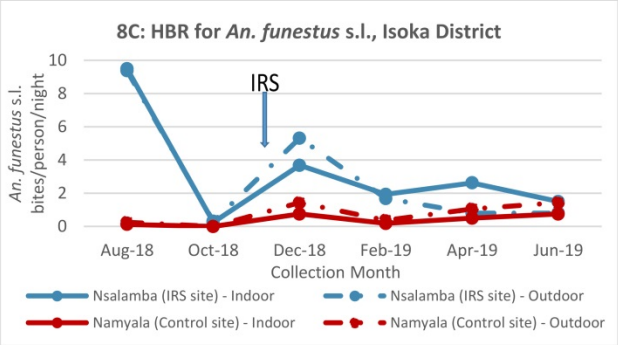
The HBR of *An. funestus* s.l. and *An. gambiae* s.l. indoors and outdoors in the sprayed and control sites are presented in Figure 8. One month after IRS, the average HBR for *An. funestus* s.l. across sites was lower than before IRS; indoors, the average bites per person per night before IRS was 11.0 compared to 3.9 bites after IRS, and outdoors, the average bites per person per night was 9.8 before IRS and 3.4 afterwards (Fig 8A). Reductions in human biting were also observed at the control sites, though the amount of reduction was lower at the control sites (14.5 bites to 9.3 bites indoors, and 9.8 to 7.0 bites outdoors). The overall biting rates for *An. gambiae* s.l. increased after IRS from 0.19 to 3.09 bites per person per night indoors and 0.17 to 6.7 outdoors (Fig 8B). This trend is likely due to the seasonal abundance of this species during the rainy season. At the site level, *An. funestus* s.l. human biting rates reduced after IRS at three of the seven IRS sites (Kalonga -8E, Lunga -8K and Shibesa -8M) and for *An. gambiae* s.l. at one site (Kalonga -8F). At all other sites, vector numbers either increased or were unaffected because no vectors were collected. There was more biting indoors than outdoors at 11 of the 14 sentinel sites for *An. funestus* s.l. and at six out of the 14 sites for *An. gambiae* s.l.

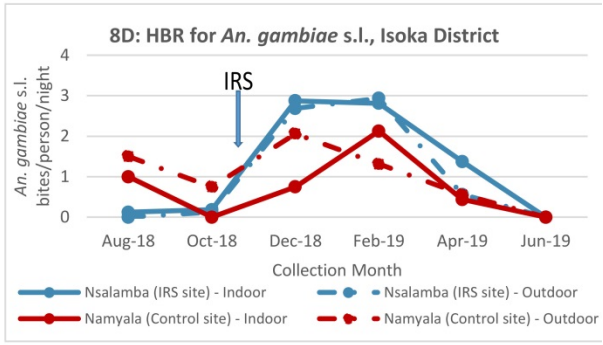
Figure 8: Human Biting Rate of *An. funestus* s.l. and *An. gambiae* s.l. across Sites (August 2018-June 2019)

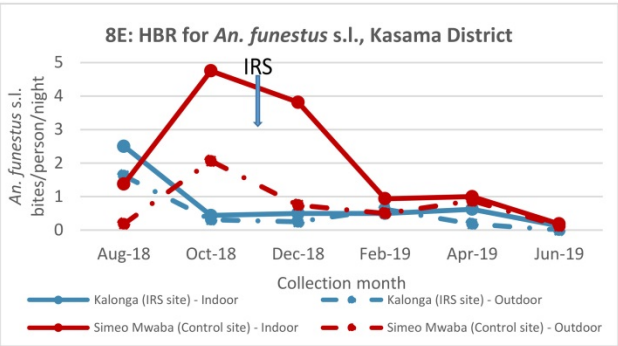
[Arrow indicates when IRS was implemented.]

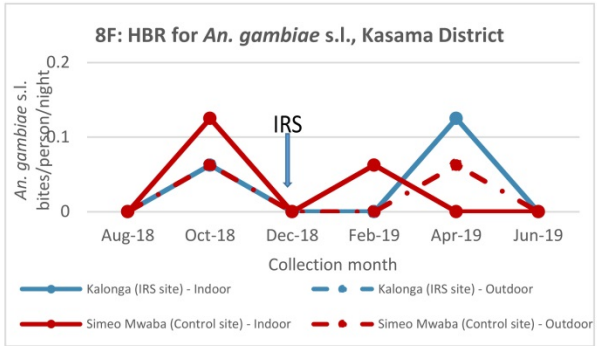


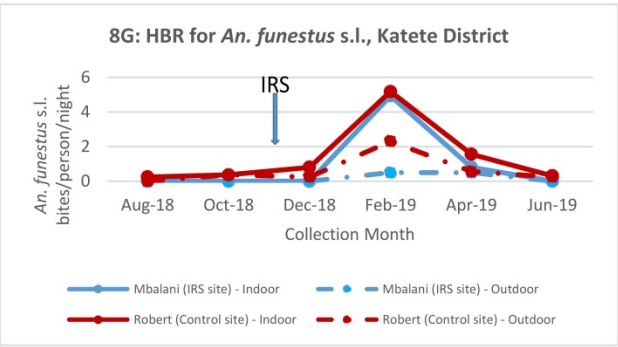


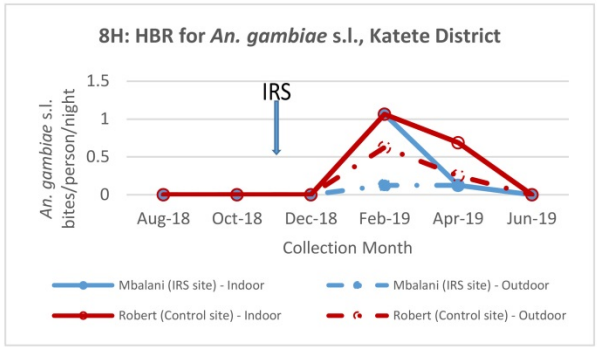

8C: HBR for An. funestus s.l., Isoka District
An. funestus s.l. bites/person/night
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Nsalamba (IRS site) - Indoor
Nsalamba (IRS site) - Outdoor
Namyala (Control site) - Indoor
Namyala (Control site) - Outdoor

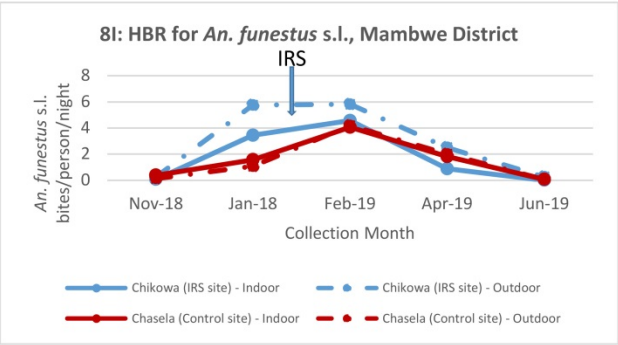

8D: HBR for An. gambiae s.l., Isoka District
An. gambiae s.l. bites/person/night
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Nsalamba (IRS site) - Indoor
Nsalamba (IRS site) - Outdoor
Namyala (Control site) - Indoor
Namyala (Control site) - Outdoor

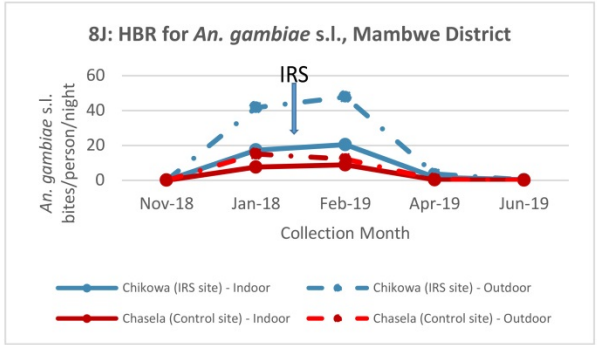

8E: HBR for An. funestus s.l., Kasama District
An. funestus s.l. bites/person/night
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection month
Kalonga (IRS site) - Indoor
Kalonga (IRS site) - Outdoor
Simeo Mwaba (Control site) - Indoor
Simeo Mwaba (Control site) - Outdoor

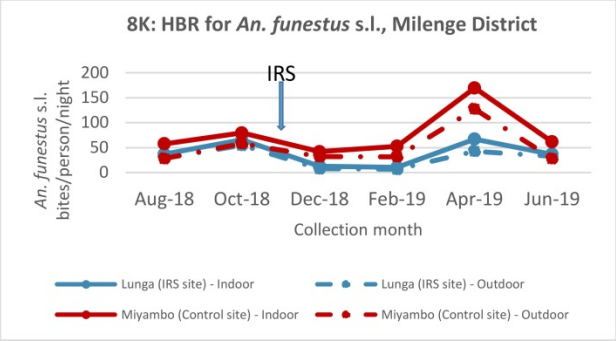

8F: HBR for An. gambiae s.l., Kasama District
An. gambiae s.l. bites/person/night
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection month
Kalonga (IRS site) - Indoor
Kalonga (IRS site) - Outdoor
Simeo Mwaba (Control site) - Indoor
Simeo Mwaba (Control site) - Outdoor

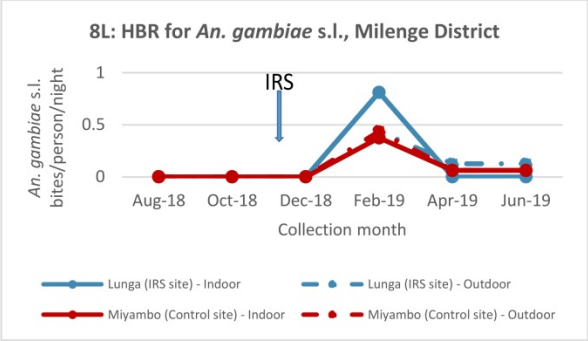

8G: HBR for An. funestus s.l., Katete District
An. funestus s.l. bites/person/night
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Mbalani (IRS site) - Indoor
Mbalani (IRS site) - Outdoor
Robert (Control site) - Indoor
Robert (Control site) - Outdoor

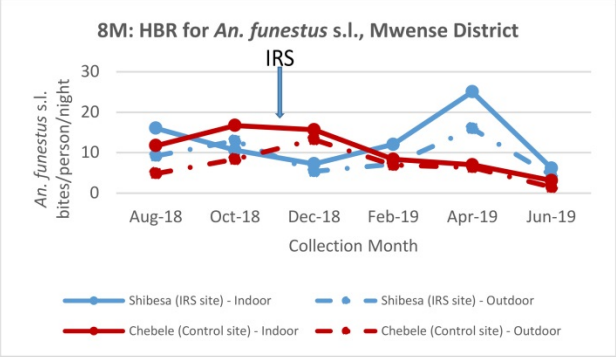

8H: HBR for An. gambiae s.l., Katete District
An. gambiae s.l. bites/person/night
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Mbalani (IRS site) - Indoor
Mbalani (IRS site) - Outdoor
Robert (Control site) - Indoor
Robert (Control site) - Outdoor

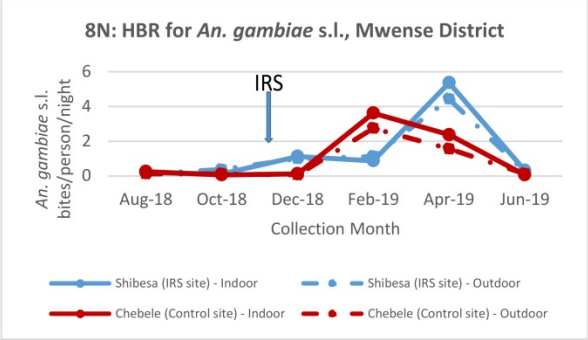

8I: HBR for An. funestus s.l., Mambwe District
An. funestus s.l. bites/person/night
IRS
Nov-18
Jan-18
Feb-19
Apr-19
Jun-19
Collection Month
Chikowa (IRS site) - Indoor
Chikowa (IRS site) - Outdoor
Chasela (Control site) - Indoor
Chasela (Control site) - Outdoor

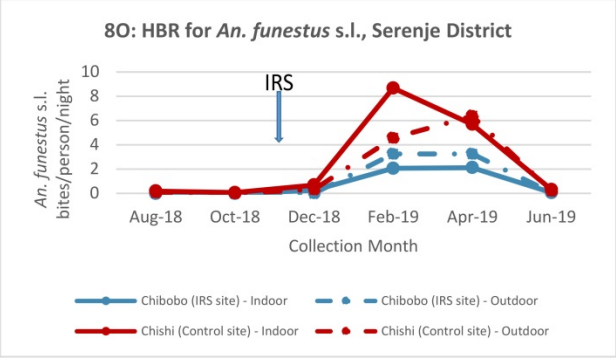

8J: HBR for An. gambiae s.l., Mambwe District
An. gambiae s.l. bites/person/night
IRS
Nov-18
Jan-18
Feb-19
Apr-19
Jun-19
Collection Month
Chikowa (IRS site) - Indoor
Chikowa (IRS site) - Outdoor
Chasela (Control site) - Indoor
Chasela (Control site) - Outdoor


8K: HBR for An. funestus s.l., Milenge District
An. funestus s.l. bites/person/night
200
150
100
50
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection month
Lunga (IRS site) - Indoor
Lunga (IRS site) - Outdoor
Miyambo (Control site) - Indoor
Miyambo (Control site) - Outdoor


8L: HBR for An. gambiae s.l., Milenge District
An. gambiae s.l. bites/person/night
1
0.5
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection month
Lunga (IRS site) - Indoor
Lunga (IRS site) - Outdoor
Miyambo (Control site) - Indoor
Miyambo (Control site) - Outdoor


8M: HBR for An. funestus s.l., Mwense District
An. funestus s.l. bites/person/night
30
20
10
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Shibesa (IRS site) - Indoor
Shibesa (IRS site) - Outdoor
Chebele (Control site) - Indoor
Chebele (Control site) - Outdoor


8N: HBR for An. gambiae s.l., Mwense District
An. gambiae s.l. bites/person/night
6
4
2
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Shibesa (IRS site) - Indoor
Shibesa (IRS site) - Outdoor
Chebele (Control site) - Indoor
Chebele (Control site) - Outdoor


8O: HBR for An. funestus s.l., Serenje District
An. funestus s.l. bites/person/night
10
8
6
4
2
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Chibobo (IRS site) - Indoor
Chibobo (IRS site) - Outdoor
Chishi (Control site) - Indoor
Chishi (Control site) - Outdoor

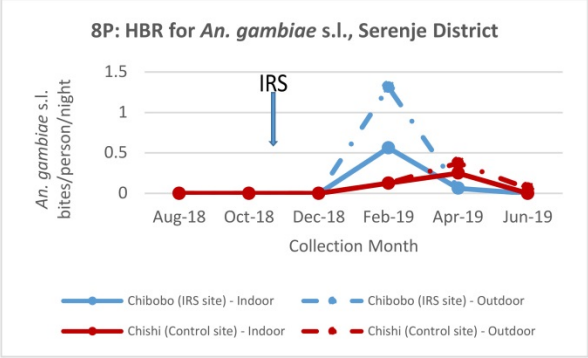
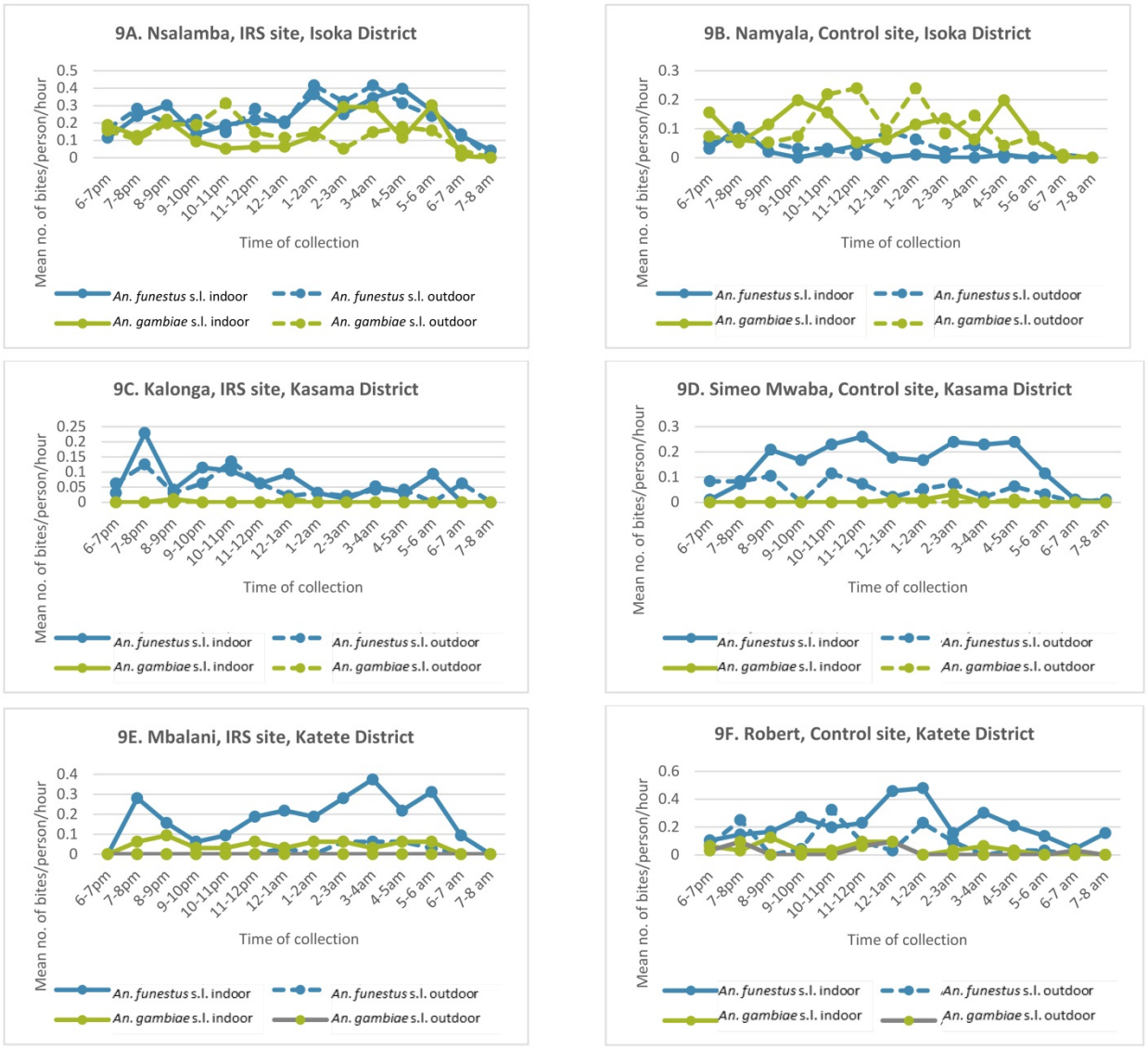
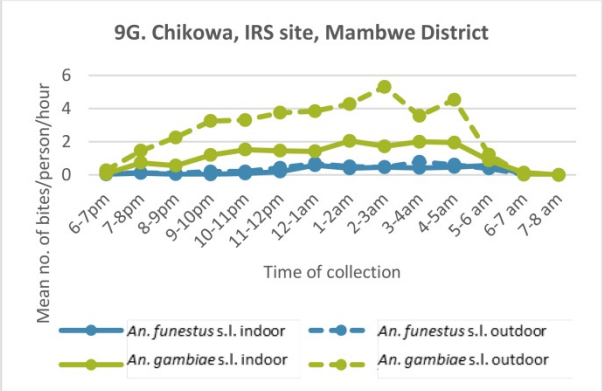
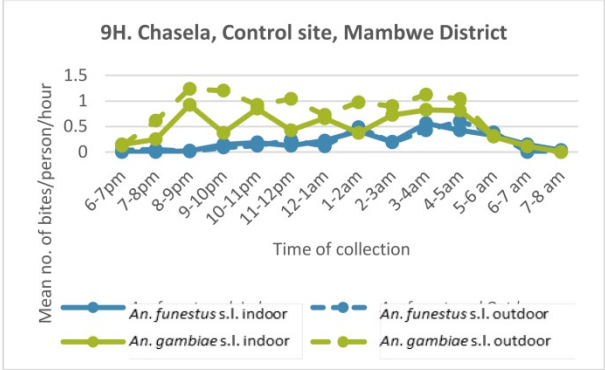
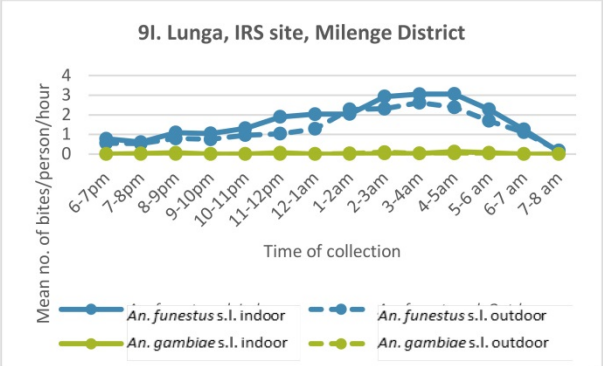

8P: HBR for An. gambiae s.l., Serenje District
An. gambiae s.l. bites/person/night
1.5
1
0.5
0
IRS
Aug-18
Oct-18
Dec-18
Feb-19
Apr-19
Jun-19
Collection Month
Chibobo (IRS site) - Indoor
Chibobo (IRS site) - Outdoor
Chishi (Control site) - Indoor
Chishi (Control site) - Outdoor

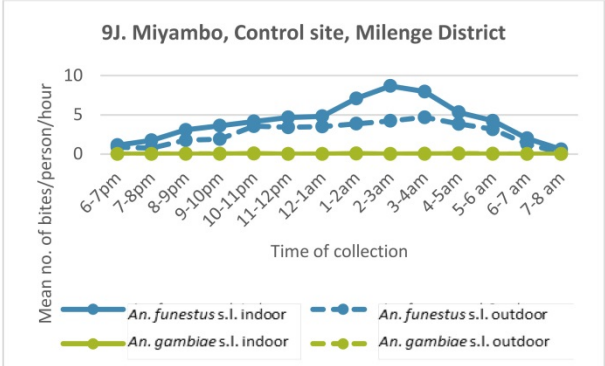
Figure 9: Hourly Biting Rates of *An. funestus* s.l. and *An. gambiae* s.l. by Site (August 2018-June 2019)

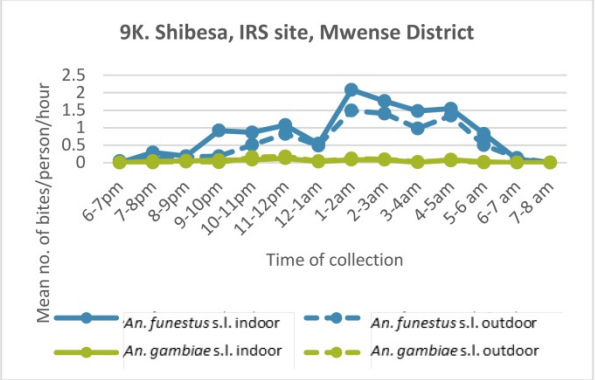


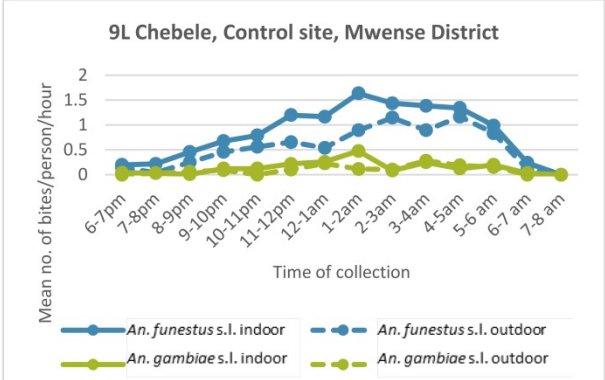

9G. Chikowa, IRS site, Mambwe District
Mean no. of bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

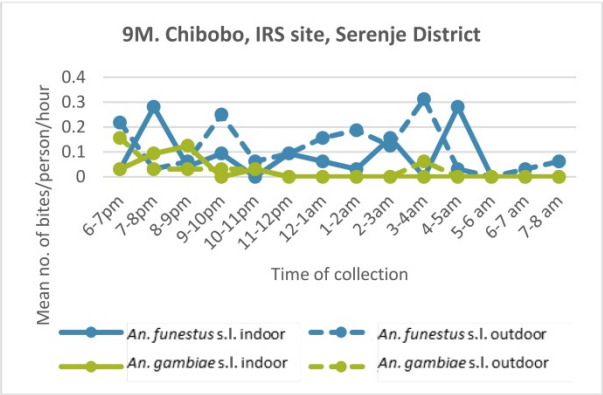

9H. Chasela, Control site, Mambwe District
Mean no. of bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

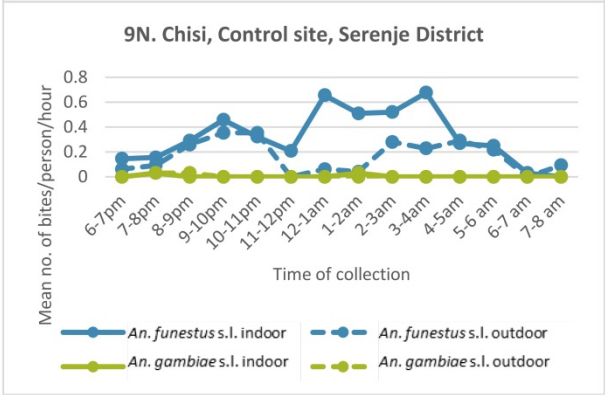

9I. Lunga, IRS site, Milenge District
Mean no. of bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9J. Miyambo, Control site, Milenge District
Mean no. of bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9K. Shibesa, IRS site, Mwense District
Mean no. of bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9L Chebele, Control site, Mwense District
Mean no. of bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

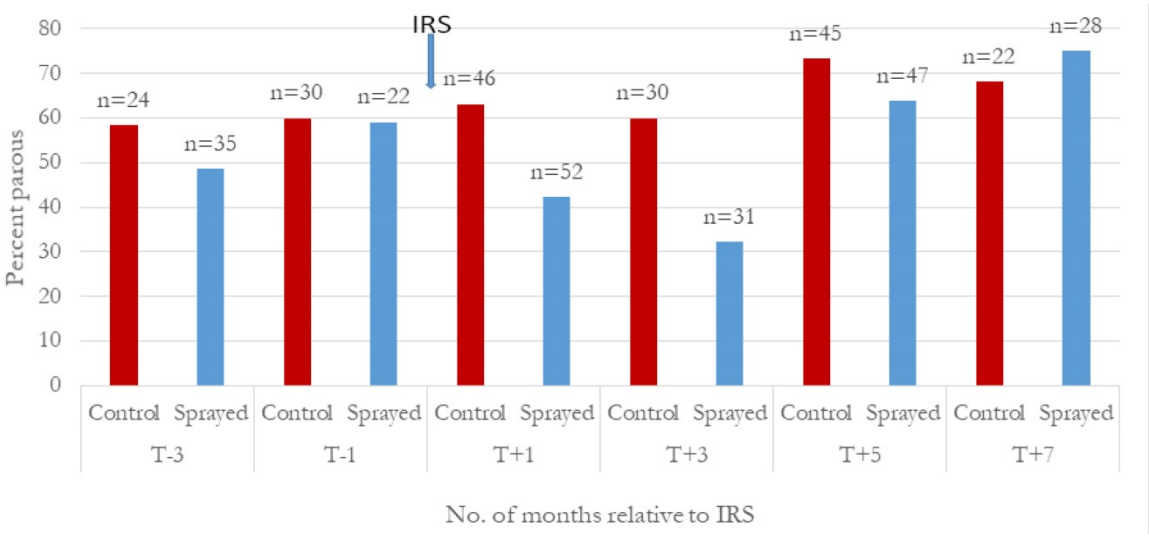

9M. Chibobo, IRS site, Serenje District
Mean no. of bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9N. Chisi, Control site, Serenje District
Mean no. of bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

3.3 PARITY RATE

A total of 412 unfed female *An. funestus* s.l. and *An. gambiae* s.l. collected by HLC were examined for parity status. Parity at the IRS sites reduced from 59.1% before IRS to 42.3% after IRS, while parity rate remained almost constant in the control sites—63.0% before IRS and 60.0% after IRS (Fig. 10). Overall, parity rate at all sites during the entire surveillance period was 52.6% at the sprayed sites and 64.5% at the control sites. IRS thus reduced parity rates; fewer parous females were caught at the IRS sites after IRS compared to the control sites.

Figure 10: Parity Rate of *An. funestus* in Intervention and Control Sites (August 2018-June 2019)



3.4 QUALITY ASSURANCE OF IRS AND INSECTICIDE DECAY RATE

All mosquitoes exposed to the sprayed walls at T0 (during the quality of spray determination) were dead after the 24-hour holding period in the districts sprayed with Actellic 300CS districts (Figure 11) and after 48-hour holding period in the district sprayed with SumiShield (Figure 12).. Mortality of all control tests was below the 5% threshold, thus there was no correction of exposed mortality required. The data indicates a good quality of spray during the 2018 campaign.

As shown in Figure 11, the residual efficacy of Actellic 300CS represented by the monthly mosquito mortality after bioassays indicated five months of efficacy on both cement and mud walls in Mporokoso and Kawambwa, four months in Mwense, Kasama, Isoka, and two months on mud walls and three months on cement walls in Milenge. The average residual efficacy of Actellic 300CS was four months.

In Katete, the only site where SumiShield residual efficacy was monitored, mortality initially dropped below the 80% cut-off at T6. At T7, we observed mortalities above 80% which required additional testing since mortality must fall below 80% for two consecutive months before residual efficacy is confirmed (PMI VectorLink SOP #9). Mortality dropped below 80% at both T8 and T9 and residual efficacy of SumiShield was determined to be seven months on both mud and cement walls.

Figure 11: Mortality of *An. gambiae* s.l. Kisumu Strain on Surfaces Sprayed with Actellic 300CS (pirimiphos-methyl) (Oct 2018-May 2019)

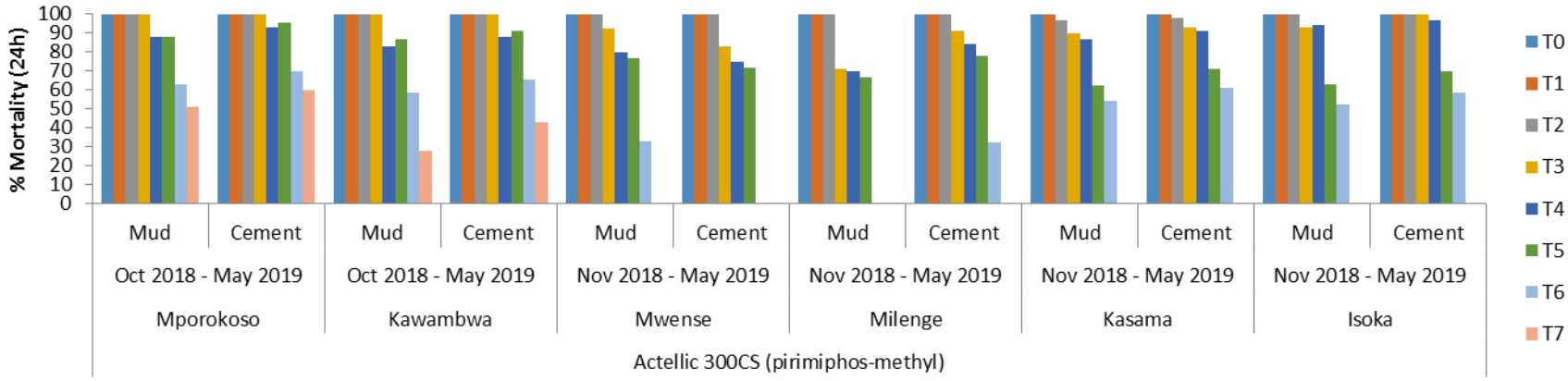
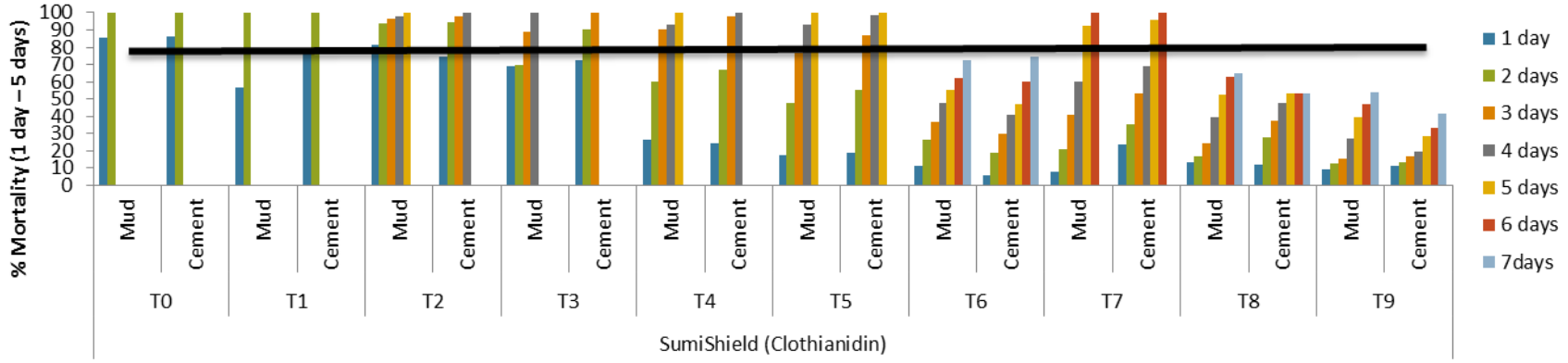


Figure 12: Mortality of *An. gambiae* s.l. Kisumu Strain to Surfaces Sprayed with SumiShield (clothianidin) in Katete District (Nov 2018-Aug 2019)



Note: In Figures 11 and 12, the black line indicates the 80% minimum mortality threshold for insecticide efficacy; the rate of insecticide decay is measured according to when the mosquito mortality falls below 80% for two consecutive occurrences.

3.5 INSECTICIDE SUSCEPTIBILITY TESTS

Both *An. funestus* s.l. and *An. gambiae* s.l. were fully susceptible to clothianidin, pirimiphos methyl, bendiocarb, and chlorfenapyr at all sites tested. While *An. funestus* s.l. was fully susceptible to DDT at all the sites tested, a probable resistance was observed among *An. gambiae* s.l. in Chebele in Mwense district, Luapula Province. Mortality in all control tests (non-insecticide-treated papers) was below the 5% threshold, thus no correction of exposed mortality was needed. Figures 13 and 14 show susceptibility data for *An. funestus* s.l. and *An. gambiae* s.l. for all insecticides and monitoring sites. Exposed mosquito mortality of 98% (shown by the top dotted line) or above indicates susceptibility, while mortality below 90% (shown by the bottom line) indicates confirmed resistance. Mortality between the two is indicative of probable resistance. A table of the insecticide susceptibility test results is provided in Annex C. Note that for some insecticides, susceptibility tests were not conducted at all the sites due to the low numbers of test mosquitoes collected from those sites.

Figure 13: Insecticide Susceptibility Status of *An. funestus* s.l. (Jan-Apr 2019)

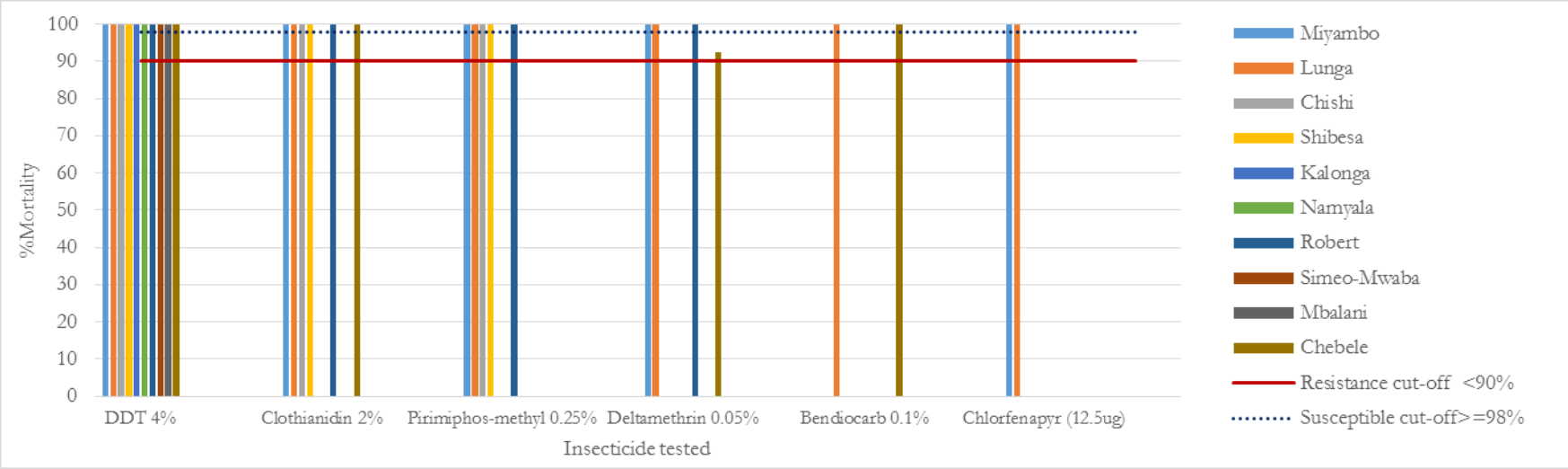
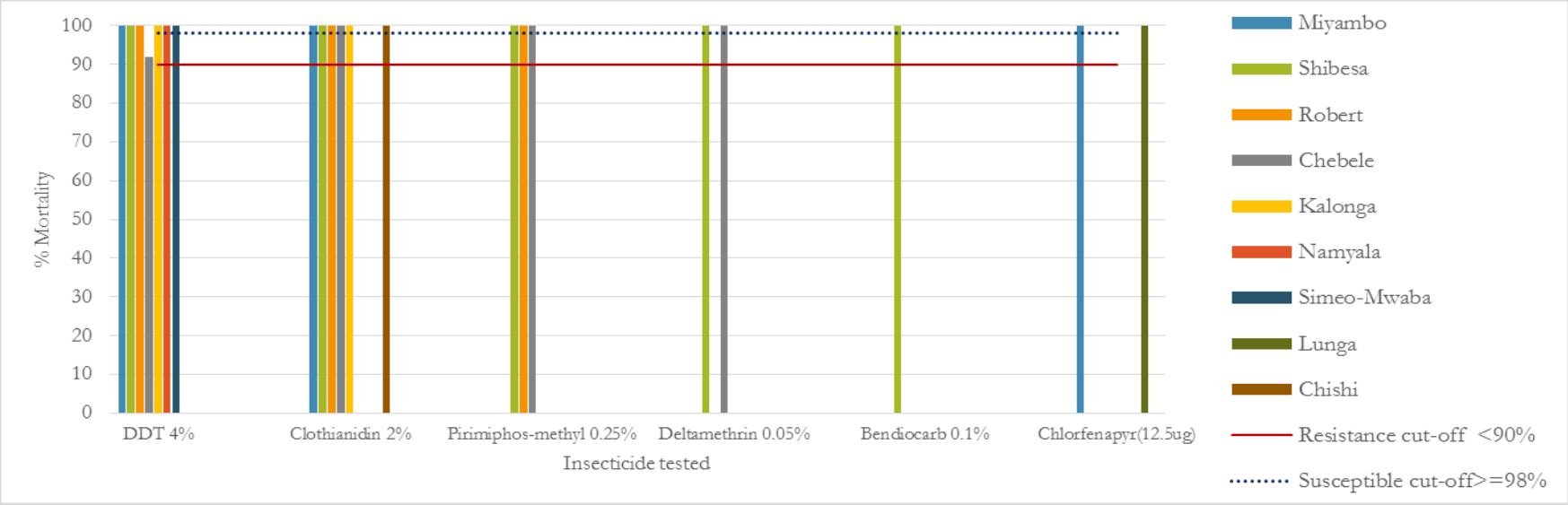


Figure 14: Insecticide Susceptibility Status of *An. gambiae* s.l. (Jan-Apr 2019)



4. Discussion and Conclusion

4.1 Species Composition and Vector Density

Entomological monitoring results from August 2018 to June 2019 indicate that *An. funestus* s.l. remains the predominant *Anopheles* species and predominant malaria vector at all surveillance sites. The diversity of *Anopheles* vector species observed during this surveillance period was similar to previous years with a significant presence of *An. ziemanni namibiensis* s.l. and *An. tchekedii*. The abundance of some of these species around human dwellings warrants further investigation into their role, if any, in local malaria transmission. *Anopheles* species were collected by both PSCs and HLCs (indoors and outdoors) at the surveillance sites. Of the two main malaria vectors in the region, *An. funestus* s.l. remains dominant over *An. gambiae* s.l. with an overall proportion of 87.6%. In this reporting period, we found 46% of *Anopheles* from the sprayed sites were *An. funestus* s.l. compared to 27% in the 2017-2018 reporting period and 67% in 2016-2017).

Indoor densities of *An. funestus* s.l. vectors were reduced immediately after IRS at most of the surveillance sites but this reduction was not sustained as densities returned to pre-spray levels within three months of IRS. A clear impact of IRS on indoor resting density was observed in Milenge where pre-IRS densities were higher at the IRS sites compared to the control sites and, immediately after IRS, the density in the IRS sites was lower compared to the control sites. Higher indoor densities were seen at the IRS sites in Kasama and Mwense districts compared to the control sites. This finding was similar to what was reported in 2017-2018. Differences in the landscape and ecological characteristics between the IRS and control sites are suspected to explain the observed trends. Most notably, the IRS sites are located closer to disproportionately more potential vector habitats than the control sites. Vector numbers were highest in Milenge district in Luapula Province compared to the other districts and lowest in Isoka in Muchinga Province. Similar trends in vector numbers have been reported in this province and attributed to the formation of marshes and other water bodies from the Luapula River in many parts of the province which creates permanent *An. funestus* s.l. breeding sites. *An. gambiae* s.l. indoor densities increased after IRS at most sites, which may be due to coincidental rains that followed the IRS campaign.

4.2 Vector Biting Behavior

Human biting rates of *An. funestus* s.l. reduced at the IRS and control sites immediately after IRS though the reductions at the IRS sites were greater than those at the control sites. Human biting rates of *An. gambiae* s.l. at the intervention sites did not decrease after IRS. Vector human biting rates were higher throughout the surveillance period in Kalonga (the sprayed site in Kasama) compared to Simeo Mwaba (control site), and in Shibesa (sprayed site in Mwense) compared to Chebele (control site). In Kasama, biting did not decrease immediately after IRS at the IRS site; this was also the case at the control site. Early evening biting was observed in Kalonga (Kasama district) and in Mbalani (Katete district) while late-night biting was the predominant behavior at the rest of the sites.

4.3 Impact of IRS

Vector parity was lower at all IRS sites compared to control sites from 1-3 months after IRS implementation. A decrease in parity rates implies a reduction in the average longevity of the vectors which reduces the ability of the vector to transmit malaria.

4.4 Duration of Efficacy of Actellic 300CS and SumiShield

Actellic 300CS was effective on both mud and cement walls but decayed faster on mud surfaces compared to cement. The average duration of efficacy across all sentinel sites was four months. The short residual life on

mud surfaces and the average duration of efficacy is consistent with previous reports from the same areas. Though both local vectors were fully susceptible to pirimiphos-methyl at all sites, the relatively short residual life on sprayed surfaces might explain for increase in mosquito abundance after IRS. Zambia's sustained deployment of this chemical prompted a shift to longer-lasting insecticides in the 2019 IRS campaign.

SumiShield, a clothianidin-based insecticide, was monitored at one site and yielded seven months of efficacy. More sites will be monitored during the 2019 IRS campaign (when clothianidin products will be used widely) and the length of residual efficacy will be confirmed.

4.5 Insecticide Susceptibility

The predominant vector, *An. funestus* s.l., was fully susceptible to DDT and clothianidin which are currently used for IRS in Zambia. *An. gambiae* s.l. was susceptible to clothianidin but not DDT (we observed a probable resistance at one site out of seven). Both vectors were fully susceptible to chlorfenapyr, a compound earmarked for future deployment in vector control. Some of the target insecticides were not fully covered during the tests due to low numbers of live mosquitoes collected at some of the sites.

4.6 Conclusions

- *An. funestus* s.l. was the predominant primary malaria vector in six of the seven sentinel districts where entomological surveillance was conducted; *An. gambiae* s.l. was the predominant vector in Mambwe.
- The indoor resting density of *An. funestus* s.l. decreased after IRS in most of the sprayed sites compared to the control sites.
- IRS did not reduce *An. gambiae* s.l. human biting rates, presumably due to proliferation of the vector during the rainy season that coincides with the post-intervention period. Also, *Anopheles arabiensis*, a member of this complex is known to exhibit exophagic behavior and may not be subject to the full effect of the sprayed walls.
- The majority of human biting occurred late at night, when people were likely asleep; thus ITNs are also an appropriate vector control intervention. This supports the new vector control strategy of rolling out ITNs in areas where IRS will not been implemented.
- *An. funestus* s.l., the predominant vector species, was highly endophilic at most of the sites, thus IRS remains an appropriate malaria intervention for this part of Zambia.
- There was a positive impact of IRS on *An. funestus* s.l. parity rates, with fewer parous vectors biting people after IRS compared to before IRS.
- The quality of IRS by VectorLink during the 2018 campaign was very high as shown by the high rate of mortality of mosquitoes exposed to the walls immediately after spray.
- The residual life of pirimiphos-methyl on sprayed walls based on monthly bioassays was about four months, while the residual efficacy for SumiShield was seven months based on 80% mortality for two consecutive months.
- The short effective duration of Actellic 300CS in Zambia (four months) is unlikely to protect against *An. funestus* s.l. and *An. gambiae* s.l. during the entire transmission season regardless when it is sprayed.
- *An. funestus* s.l. and *An. gambiae* s.l. were fully susceptible to clothianidin and chlorfenapyr at all sites.
- *An. funestus* s.l. was susceptible to DDT at all sites. Probable resistance by *An. gambiae* s.l. was seen at one site (Chebele, Mwense district, Luapula Province).

5. RECOMMENDATIONS

5.1 FOR NMEP & VECTORLINK ZAMBIA

- Shift insecticide(s) used for IRS from pirimiphos-methyl to longer-duration insecticides, such as Fludora Fusion or SumiShield, and possibly DDT in non-PMI supported areas, to cover the long malaria transmission season.
- Due to resistance of local vectors to pyrethroid insecticides, consider introducing next-generation ITNs in select areas, especially where ITNs are the major vector control intervention.
- Given limited or short-lasting impact of IRS in some sites, continue and expand the mass net distribution campaigns and strengthen continuous net distribution strategy at health

5.2 FOR VECTORLINK ZAMBIA

- Conduct post-IRS coverage surveys to confirm IRS coverage levels at vector surveillance sites. This is important to be certain that surveillance sites obtained high spray coverage that provides community effect.
- Conduct additional susceptibility assays for insecticides not fully represented in terms of sites and number of samples tested.
- Explore the possibility of implementing vector monitoring strategies that are informed by malaria cases in districts with reduced vector populations. In such districts, the recommendation is to move away from the current fixed monitoring sites to the investigation of malaria hot spots determined by health facility malaria cases data collated at the District Health Office.
- Expand and maintain the collaboration with NMEP laboratory in Lusaka (which is receiving support from the Malaria Control and Elimination Partnership in Africa project, or MACEPA) to supplement molecular analyses of mosquito samples to solve the recurrent issue of delays in obtaining vital data from mosquito samples collected in the field.

ANNEX A: CULICIDAE COLLECTED IN SPRAYED AND CONTROL SITES (AUGUST 2018-JUNE 2019)

District	Village	Status	HLC Indoor												
			An. funestus s.l.	*An. gambiae* s.l.	*An. ziemanni namibiensis*	*An. maculipalpis*	*An. coustani*	*An. tenebrosus*	*An. gibbinsi*	*An. rufipes*	*An. pretoriensis*	*An. squamosus*	*An. argentiolobatus*	*An. tchekedii*	*Culicines*
Isoka	Nsalamba	Sprayed	313	118	398	88	12	0	5	2	0	387	299	0	215
	Namyala	Control	43	69	125	44	4	0	0	2	0	28	53	0	113
Kasama	Kalonga	Sprayed	75	3	85	0	40	0	0	0	0	88	13	0	41
	Simeo Mwaba	Control	193	3	94	0	129	1	0	0	0	52	14	0	1
Katete	Mbalani	Sprayed	94	19	0	3	8	0	0	1	0	9	0	0	96
	Robert	Control	136	28	4	27	34	1	2	4	0	7	0	0	57
Mambwe	Chikowa	Sprayed	143	634	0	0	36	0	0	0	0	2	0	0	160
	Chasela	Control	126	265	0	2	122	4	0	10	0	10	0	0	602
Mwense	Shibesa	Sprayed	1236	127	2	0	18	0	0	0	0	0	0	0	73
	Chebele	Control	1003	113	0	0	9	0	0	2	0	1	0	1	123
Milenge	Lunga	Sprayed	3671	14	1453	0	1	0	0	1	0	71	8	572	34
	Miyambo	Control	7412	8	3249	0	0	0	0	0	0	56	21	715	49
Serenje	Chibobo	Sprayed	72	10	0	0	2	1	0	0	0	0	0	0	5
	Chishi	Control	250	6	0	0	10	5	0	4	0	0	0	0	20
Total			14767	1417	5410	164	425	12	7	26	0	711	408	1288	1589

District	Village	Status	HLC Outdoor												
			An. funestus s.l.	*An. gambiae* s.l.	*An. ziemanni namibiensis*	*An. maculipalpis*	*An. coustani*	*An. tenebrosus*	*An. gibbinsi*	*An. rufipes*	*An. pretoriensis*	*An. squamosus*	*An. argentiolobatus*	*An. tchekedii*	*Culicines*
Isoka	Nsalamba	Sprayed	293	101	588	92	15	0	5	1	2	313	451	0	279
	Namyala	Control	64	99	248	59	17	0	1	0	0	44	114	0	181
Kasama	Kalonga	Sprayed	48	3	89	0	40	0	0	0	0	40	7	0	44
	Simeo Mwaba	Control	72	2	60	0	271	0	0	0	0	51	12	0	5
Katete	Mbalani	Sprayed	16	4	0	1	2	0	0	2	0	5	0	0	45
	Robert	Control	60	14	8	29	15	0	0	0	0	3	0	0	26
Mambwe	Chikowa	Sprayed	233	1496	1	0	480	0	0	1	0	30	0	0	304
	Chasela	Control	119	447	0	11	543	6	0	41	0	24	0	0	786
Mwense	Shibesa	Sprayed	882	118	0	1	56	0	0	0	0	1	0	0	338
	Chebele	Control	659	79	0	0	14	0	0	2	0	2	0	0	292
Milenge	Lunga	Sprayed	2769	9	2030	0	2	0	0	0	0	70	11	768	37
	Miyambo	Control	4892	10	6121	0	0	0	0	3	0	97	48	946	50
Serenje	Chibobo	Sprayed	105	22	0	0	5	11	0	1	0	0	0	0	17
	Chishi	Control	184	9	0	1	11	10	1	13	0	0	0	0	23
Total			10396	2413	9145	194	1471	27	7	64	2	680	643	1714	2427

ANNEX B: *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. BY SITE AND COLLECTION METHOD (AUGUST 2018-JUNE 2019)

District	Site	Status	No. of Collection Months	*An. funestus* s.l.			*An. gambiae* s.l.			GRAND TOTAL
				Number collected by PSC	Number collected by HLC	Total Collected	Number collected by PSC	Number collected by HLC	Total Collected	
Isoka	Namyala	Sprayed	6	18	107	125	42	168	210	335
	Nsalamba	Control	6	22	606	628	13	219	232	860
Kasama	Kalonga	Sprayed	6	130	123	253	0	6	6	259
	Simeo Mwaba	Control	6	22	265	287	0	5	5	292
Katete	Mbalani	Sprayed	6	16	110	126	0	23	23	149
	Robert	Control	6	146	196	342	0	42	42	384
Mambwe	Chikowa	Sprayed	5	32	376	408	17	2,130	2,147	2,555
	Chasela	Control	5	12	245	257	15	712	727	984
Milenge	Lunga	Sprayed	6	642	6440	7082	1	23	24	7106
	Miyambo	Control	6	912	12,304	13,216	0	18	18	13,234
Mwense	Shibesa	Sprayed	6	304	2118	2422	9	245	254	2676
	Chebele	Control	6	151	1662	1813	14	192	206	2019
Serenje	Chibobo	Sprayed	6	73	177	250	1	32	33	283
	Chishi	Control	6	201	434	635	0	15	15	650
TOTAL			82	2,681	25,163	27,844	112	3,830	3,942	31,786

ANNEX C: INSECTICIDE SUSCEPTIBILITY TEST RESULTS

***An. gambiae* s.l. Insecticide Susceptibility Test Results (Jan-Feb 2019)**

Chemical	District/Sentinel Site	Intervention Status	# Exposed	# Dead after 24 hours	% Mortality after 24 hours
DDT (4%)	Mwense, Chebele	Control	41	41	100
	Mwense, Shibesa	Sprayed	47	47	100
	Serenje, Chishi	Control	52	52	100
	Milenge, Miyambo	Control	201	201	100
	Milenge, Lunga	Sprayed	100	100	100
	Katete, Robert	Control	20	20	100
	Katete, Mbalani	Sprayed	7	7	100
	Isoka, Namyala	Control	23	23	100
	Kasama, Kalonga	Sprayed	40	40	100
	Kasama, Simeo-Mwaba	Control	10	10	100
Clothianidin (2%)	Mwense, Chebele	Sprayed	33	33	100
	Mwense, Shibesa	Sprayed	24	24	100
	Serenje, Chishi	Control	15	15	100
	Milenge, Miyambo	Control	192	192	100
	Milenge, Lunga	Sprayed	98	98	100
	Katete, Robert	Control	50	50	100
Pirimiphos-methyl (0.25%)	Mwense, Shibesa	Sprayed	20	20	100
	Serenje, Chishi	Control	70	70	100
	Milenge, Miyambo	Control	133	133	100
	Milenge, Lunga	Sprayed	15	15	100
	Katete, Robert	Control	50	50	100
Deltamethrin (0.05%)	Mwense, Chebele	Control	27	25	92.6
	Milenge, Lunga	Sprayed	24	24	100
	Milenge, Miyambo	Control	75	75	100
	Katete, Robert	Control	19	19	100
Bendiocarb (0.1%)	Mwense, Chebele	Control	22	22	100
	Milenge, Lunga	Sprayed	20	20	100
	Mwense, Shibesa	Sprayed	10	10	100
Chlorfenapyr (12.2 µg)	Milenge, Lunga	Sprayed	70	70	100
	Milenge, Miyambo	Control	100	100	100

An. gambiae s.l. Insecticide Susceptibility Test Results (Jan-Feb 2019)

Chemical	District/ Sentinel Site	Intervention status	# Exposed	# Dead after 24 hrs	% Mortality after 24 hrs
DDT (4%)	Milenge, Miyambo	Control	100	100	100
	Mwense, Chebele	Control	63	58	92.1
	Kasama, Kalonga	Sprayed	21	21	100
	Isoka, Namyala	Control	28	28	100
	Katete, Robert	Control	40	40	100
	Mwense, Shibesa	Sprayed	45	45	100
	Kasama, Simeo-Mwaba	Control	4	4	100
Clothianidin (2%)	Mwense, Chebele	Control	22	22	100
	Serenje, Chishi	Control	2	2	100
	Kasama, Kalonga	Sprayed	17	17	100
	Milenge, Miyambo	Control	9	9	100
	Katete, Robert	Control	5	5	100
	Mwense, Shibesa	Sprayed	2	2	100
	Serenje, Chishi	Control	2	2	100
Pirimiphos-methyl (0.25%)	Mwense, Chebele	Control	25	25	100
	Katete, Robert	Control	7	7	100
	Mwense, Shibesa	Sprayed	47	47	100
Deltamethrin (0.05%)	Mwense, Chebele	Control	23	23	100
	Katete, Robert	Control	2	1	50
	Mwense, Shibesa	Sprayed	18	18	100
Bendiocarb (0.1%)	Mwense, Shibesa	Sprayed	2	2	100