



U.S. PRESIDENT'S MALARIA INITIATIVE



MOZAMBIQUE ENTOMOLOGICAL MONITORING

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
<i>Ace -1</i>	Acetylcholinesterase 1 gene
b/p/h	Bites per Person per Hour
b/p/n	Bites per Person per Night
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-linked Immunosorbent Assay
HLC	Human Landing Catch
IRS	Indoor Residual Spray
KD	Knock Down
<i>kdr</i>	knockdown resistance gene
LLIN	Long-lasting Insecticide-treated Bednet
m/t/n	Mosquitoes per Trap per Night
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PDH	Provincial Directorate of Health
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WHO	World Health Organization
WRBU	Walter Reed Biosystematics Unit

EXECUTIVE SUMMARY

Indoor residual spraying (IRS) and long-lasting insecticide-treated bednets (LLINs) remain the primary mosquito vector control interventions in many parts of world, including sub-Saharan Africa, where the disease continues to be a public health concern.

In Mozambique, Abt Associates (Abt) implemented the President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) project from July 1, 2017, through February 28, 2018. PMI AIRS has been followed by the PMI-VectorLink project, which began on March 1, 2018. Abt implements PMI VectorLink in close collaboration with Mozambique's National Malaria Control Program (NMCP), the Provincial Directorate of Health (PDH) in Zambézia Province, the District Services for Health, Women and Social Welfare (SDSMAS) at the district level, the Ministry of Agriculture and Food Security (MASA), and the Ministry of Land, Environment and Rural Development (MITADER) at the provincial and district levels.

During the 2017 spray campaign, AIRS Mozambique conducted IRS with pirimiphos-methyl (Actellic® 300CS) in seven target districts (Derre, Maganja da Costa, Milange, Mocuba, Molumbo, Mopeia, and Morrumbala). To guide proper targeting of IRS, monthly entomological monitoring was performed using CDC light traps, human landing catches (HLCs), pyrethrum spray catches (PSCs), and cone wall bioassays (used only in sprayed areas). Seasonal insecticide susceptibility tests were carried out in seven sprayed districts (Derre, Maganja da Costa, Milange, Mocuba, Molumbo, Mopeia, and Morrumbala).

In Mopeia district, a cluster-randomized trial investigating the impact and cost effectiveness of combining IRS with a non-pyrethroid, next generation IRS product and standard LLIN in an area with high malaria transmission is underway. Key methodological considerations related to the study are comparison of vector density, biting rate, and sporozoite rates between treatment and control arms. CDC light trap collections and HLCs were used for sampling mosquitoes in the study areas.

Mosquito collections using the methods described above demonstrated presence of highly diverse anopheline fauna, both the main vectors *Anopheles funestus* s.l. and *An. gambiae* s.l., and other potential vectors and non-vectors such as *An. coustani*, *An. ziemanni*, *An. tenebrosus*, *An. caliginosus*, *An. pretoriensis*, *An. maculipalpis*, *An. rufipes*, *An. squamosus*, *An. salbaii*, and *An. pharoensis*. Their role as malaria vectors remains to be investigated in the settings of surveyed districts. Our findings highlight high levels of heterogeneity and diversity in mosquito vector species composition and behavior in the monitored areas.

Following IRS, conducted October 17 to December 13, 2017, in general, *An. funestus* s.l. densities were suppressed in comparison with pre-IRS (July) densities. *An. gambiae* s.l. densities appear to have increased in January and February, potentially because of the rapid build-up of breeding habitats due to the high level of precipitation during that period.

Malaria vectors *An. gambiae* s.l. and *An. funestus* s.l. were collected both indoors and outdoors. In some districts the densities were higher outdoors and in others they were higher indoors. *An. funestus* s.l. tended to be found predominantly indoors, even after IRS, particularly in Mopeia District although biting there occurred both indoors and outdoors. Biting activity seemed to follow human sleeping patterns, with peak indoor biting activity occurring at 10:00-11:00 pm, 1:00-2:00 am, and 3:00-4:00 am, with high biting activity occurring mainly outdoors.

Both *An. gambiae* s.l. and *An. funestus* s.l. remained susceptible to pirimiphos-methyl, the insecticide used in the 2017 IRS campaign. Synergist tests with piperonyl butoxide (PBO) against *An. gambiae* s.l. indicated that the prevailing resistance mechanism involved was monooxygenases, since pre-exposure to 4% PBO-treated papers restored full susceptibility in resistant populations of *An. gambiae* s.l.

Cone wall bioassays performed for IRS quality assurance assays exhibited 99.5 percent mortality across different wall surface types, indicating that the spray operation achieved optimal quality spraying. The residual efficacy of the Actellic® 300CS used during the spray campaign appears to have remained effective for four to five months, a good portion of high malaria transmission season.

I. INTRODUCTION

Through support of the U. S. President's Malaria Initiative (PMI), the Africa Indoor Residual Spraying (AIRS) project has implemented five IRS spray rounds in Zambezia province of Mozambique. During the 2017 spray campaign, AIRS Mozambique conducted IRS in seven target districts (Derre, Maganja da Costa, Milange, Mocuba, Molumbo, Mopeia, and Morrumbala). PMI AIRS and the follow-on PMI VectorLink project also have carried out entomological monitoring activities in Zambezia, and they have supported the National Malaria Control Program's (NMCP's) entomological activities countrywide to enhance in-country capacity for entomological monitoring. Having data gathered by entomological monitoring activities to supplement epidemiological data is essential to guide proper targeting of indoor residual spraying (IRS); evaluate the susceptibility level of the local vectors to different insecticides and determine the underlying mechanisms; inform selection of insecticides; ensure the quality of spraying; monitor the impact of IRS on vector density, vector behavior, and composition; and determine parity rate and monitor the residual life of different insecticides on different types of wall surfaces. Parity rate determination was introduced at the end of February 2018. This entomological monitoring annual report covers the period from July 1, 2017 to June 30, 2018, of which July 1, 2017 to February 28, 2018, was under PMI AIRS and March 1 to June 30, 2018, was under PMI VectorLink.

Entomological monitoring was conducted in five IRS intervention districts: Maganja da Costa, Milange, Mocuba, Mopeia, and Morrumbala. Unsprayed Molevala district was used as a control district. No mosquito sampling was conducted during the month of April 2018 due to logistic issues associated with the transition from PMI-AIRS to PMI VectorLink.

In Mopeia, entomological monitoring data were collected using the Centers for Disease Control and Prevention (CDC) light trap and human landing catch (HLC) collection methods. In the other four IRS districts and in Molevala, the control district, entomological monitoring data were collected using the pyrethrum spray catch (PSC), CDC light trap, and HLC methodologies. For susceptibility tests, Prokopack collections were used in all districts to collect adult *An. funestus* s.l.; larval collections were conducted to collect *An. gambiae* s.l.

2. METHODOLOGY

2.1 BEHAVIOR AND DENSITY

2.1.1 PYRETHRUM SPRAY CATCH

In each district where PSC was conducted (intervention Maganja da Costa, Milange, Mocuba, Morrumbala, and control Molevala), two villages were selected and 10 houses per village were chosen, totalling 20 houses per district. PSC was conducted from 6:00 am to 8:00 am, once per month over four consecutive days in each district. The same houses were visited each month. Data were collected in five houses per day per district. The first collection was conducted three months prior to the IRS campaign and collection continued after the campaign. Baygon (commercial nomenclature) aerosol was used to knock down mosquitoes. It contains the pyrethroids deltamethrin 0.5 g/kg and imiprothrin 1.0 g/kg. In each house, one sleeping room was selected for spraying with Baygon. The room was closed for 10 minutes after spraying, and then knocked-down mosquitoes were collected using forceps into a labeled petri dish. The samples were identified morphologically and preserved in 1.5 ml Eppendorf tubes containing silica gel for further identification using the Polymerase Chain Reaction (PCR) technique, and a set of samples collected by this method during the mentioned period were sent for PCR at the Walter Reed Biosystematics Unit (WRBU) laboratory for further analysis.

2.1.2 HUMAN LANDING CATCHES

HLCs were conducted in the intervention districts of Maganja da Costa, Milange, Mocuba, Mopeia, and Morrumbala and the control district of Molevala. With the exception of Mopeia, two houses were sampled in a selected village on three consecutive nights to obtain six person-nights of collection per district per month (2 houses x 3 collection nights = 6 person-nights). In Mopeia, one house in each village in the intervention and control areas was selected for a total of eight houses (four in intervention and four in control districts). Collections were conducted on three consecutive nights to obtain 12 person-nights per area per month (4 houses x 3 collection nights = 12 person-nights). In all districts, two human volunteers were positioned, one inside the house and the other outside, to collect mosquitoes. Collections were conducted from 6:00 pm to 6:00 am. Over each hour of collection, collectors collected mosquitoes for 50 minutes and rested for 10 minutes, during which they exchanged positions and recorded humidity and temperature. During the time of collection, the collectors sat quietly on a small chair and exposed part of their legs (up to the knees); when they felt landing mosquitoes, they turned on a torch and collected the mosquitoes using a mouth aspirator. Collected mosquitoes were transferred into labeled paper cups assigned for each hourly collection. Collected mosquitoes were subsequently killed using cotton soaked in chloroform, identified, counted by species, location, and hour of collection, and preserved in 1.5 ml Eppendorf tubes with silica gel.

2.1.3 CDC LIGHT TRAP

CDC light traps were installed in four houses in four of the intervention districts (Maganja da Costa, Milange, Mocuba, and Morrumbala) and in the control district (Molevala). Additionally, monthly CDC light trap collections were conducted in Quelimane city to monitor trends following no IRS in Quelimane district in 2017. In Maganja da Costa, Milange, Mocuba, and Morrumbala, as well as in Molevala and Quelimane, data were collected for three consecutive nights, from 6:00 pm to 6:00 am, resulting in 12 trap nights per month for each district.

In Mopeia, ten villages were selected (five in intervention areas and five in control areas) as sentinel sites for CDC light trapping. Eight houses were selected in each village. Data were collected on three consecutive

nights, from 6:00 pm up to 6:00 am. This resulted in 240 traps nights per month equally, with an equal number in intervention and control areas.

The traps were set up inside the house in the bedroom beside the bed with humans sleeping under untreated bed nets, at the bed's footrest, about 1.5 m above the floor. After each night of collection, chloroform was used to kill the mosquitoes in the paper cups, and the mosquitoes were identified and preserved in 1.5 ml Eppendorf tubes for future species identification based on PCR. The same houses were used each month.

Data were collected from July 2017 up to June 2018.

A subset of samples from these collections in Mopeia was sent to WRBU for PCR analyses.

2.1.4 VECTOR SUSCEPTIBILITY TESTING

In September and October 2017, adult *An. funestus* s.l. mosquitoes were collected using Prokopacks and immediately used for susceptibility testing. Immature malaria vectors were collected from different larval habitats in Maganja da Costa, Mocuba, Mopeia, and Morrumbala districts from January to March 2018.

Field-collected larvae of *An. gambiae* s.l. were reared in the insectary to adult stage. Batches of 25 females, sugar-fed and aged from three to five days, were subsequently subjected to World Health Organization (WHO) tube tests following the standard WHO 2016 protocol. These females were exposed to pirimiphos-methyl 0.25%, alpha-cypermethrin 0.05%, permethrin 0.75%, DDT 4%, bendiocarb 0.1% and deltamethrin 0.05% on WHO impregnated filter papers for 60 minutes. Knockdown (KD) was scored at 60 minutes immediately after the exposure period, at which time all mosquitoes were gently transferred to holding tubes. Mortality was recorded at 24 hours after exposure. Where control mortality scored higher than 5 percent but below 20 percent, Abbott's correction was applied to test mortalities and those above 20 percent led to tests being discarded (Abbott 1925). Susceptibility levels of *An. gambiae* s.l. were evaluated based on WHO criteria (WHO 2016). WHO classifies 24-hour mortality rates higher than 98 percent as susceptible, between 90 percent and 97 percent as suggestive of resistance and requiring further investigation, and below 90 percent as resistant.

Intensity assays were conducted by exposing wild caught vector mosquitoes to insecticide dosages of 5× and 10× the diagnostic concentrations of permethrin and alpha-cypermethrin, according to the standard WHO bioassay method. All exposures were for one hour, and final mortality was scored after a 24-hour holding period during which a 10% sugar solution was made available to surviving mosquitoes.

The synergist assays were conducted using mosquitoes reared from field-collected larvae. Four bioassay exposures were done as follows: In the first group of replicates, the mosquitoes were exposed to the insecticide only (alpha-cypermethrin), the second group was exposed to 4% piperonyl butoxide (PBO) only, the third group to 4% PBO followed by insecticide, and the last group was exposed to the solvent (control). All replicates were exposed for 60 minutes and mortality was recorded 24 hours after exposure, according to the WHO (2016) protocol. This process was repeated three times based on the standard procedure.

For clothianidin susceptibility tests, freshly treated filter papers¹ were inserted into plastic cylinders and tested according to standard WHO susceptibility test protocols. The exposure time was 60 minutes. Afterward, mosquitoes were transferred into holding cylinders with filter paper treated only with distilled water and provided with lightly moistened cotton wool containing 10% sugar solution that was changed daily. Knockdown was recorded halfway through the test at 30 minutes and at the end of the test at 60 minutes. Mortality was recorded on days 1, 2, 3, 4, 5, and 6, and final mortality on day 7 after exposure. A negative control was tested at the same time and mortality recorded on days 1 through 7. The test was conducted with *An. gambiae* s.l. collected from several breeding sites in villages in two different districts (Mopeia and Morrumbala). For each village, four replicates of 25 mosquitoes were tested (total of 100 sugar-fed females) with clothianidin papers, and two replicates were used at the same time with the negative control papers (impregnated only

¹ Treated based on the AIRS protocol

with distilled water). In addition to the negative control described above, a positive control was done by similarly exposing a laboratory-reared susceptible *An. arabiensis* KGB strain.

All the above susceptibility tests were conducted to the extent possible under the recommended optimal conditions, at temperatures around 27°C ±2°C and 70–80 percent relative humidity. Similar to other collections, a portion of samples from these tests were sent to the Walter Reed Biosystematics Unit (WRBU) for PCR assays to identify sibling species and detect presence of knockdown (*kdr*) and acetylcholinesterase-1 (*Ace-1*) genes.

2.1.5 IRS QUALITY ASSAYS AND INSECTICIDE DECAY RATE MONITORING

Standard WHO cone bioassay tests were performed in Maganja da Costa, Milange, Mocuba, Morrumbala, and Mopeia (in the villages of Eduardo Mondlane, 24 de Julho, and Zero) districts, from October and November 2017 through July 2018 to evaluate spray quality and residual efficacy of the insecticide used in the 2017 spray campaign. Wall bioassays were conducted 24 hours after spraying and subsequently monitored monthly up to July 2018 until mortality dropped below 80 percent for two consecutive months. Quality assurance tests were carried out in October 2017.

In each district village, five houses were randomly selected in each village. The same houses were used each month. Cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m above the floor, arranged diagonally across a wall surface. Cones lined with self-adhesive tape were fixed on the sprayed walls for the assay. The control cone was affixed on a wall lined with a paperboard with adhesive in an unsprayed house or in the shade of a tree in the yard away from the sprayed house to avoid any potential airborne effect. Two- to five-day-old female mosquitoes were used for the tests. Susceptible *An. arabiensis* KGB strain mosquitoes were introduced into the plastic cones in batches of 10 and left exposed on the sprayed surface for 30 minutes at different heights. Numbers of mosquitoes knocked down at the 30th minute were recorded. At the end of the 30-minute exposure period, the mosquitoes were carefully collected and transferred to paper cups and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with net.

Tests for the airborne effect of pirimiphos-methyl (Actellic® 300CS) were conducted with mosquitoes placed inside a paper cup and hung 10 cm away from the sprayed wall surface at a height of 1.5 m above the floor. The mosquitoes were transferred into clean paper cups that were kept for a 24-hour holding period. Dead and live mosquitoes were counted after 24 hours, and the percentage mortality was calculated in the replicates for each house and recorded according to WHO protocol.

2.2 STATISTICAL TESTS

The average number of mosquitoes collected by the HLC method was calculated. To compare mean indoor and outdoor biting rates, Chi-square tests were used, and *P* values less than 0.05 were considered significant.

3. RESULTS

3.1 ANOPHELINE SPECIES COLLECTED BY THE DIFFERENT METHODS

During the reporting period, in Maganja da Costa, Milange, Mocuba, Molevala, Quelimane and Morrumbala, as well as Mopeia (intervention and control arms), a total of 14,511 anophelines belonging to 13 species/species complexes/groups were collected using the three collection methods (PSC, CDC, and HLC) and morphologically identified. Table 1 summarizes the number of mosquitoes collected, by district and species, from July 2017 to June 2018.

TABLE 1. NUMBER OF MOSQUITOES COLLECTED IN EACH DISTRICT BY ALL THREE COLLECTION METHODS

Species collected	Maganja da Costa	Mocuba	Morrumbala	Milange	Mopeia	Molevala	Quelimane	Total per species
<i>An. funestus</i> s.l.	1004	97	47	742	8146	1291	197	11524
<i>An. gambiae</i> s.l.	374	93	1057	144	619	391	24	2702
<i>An. coustani</i>	4	0	1	33	55	11	0	104
<i>An. zimmermanni</i>	0	0	0	1	12	0	0	13
<i>An. tenebrosus</i>	5	0	0	2	46	5	3	61
<i>An. deeringi</i>	0	0	0	1	0	0	0	1
<i>An. pretoriensis</i>	1	0	5	2	7	8	0	23
<i>An. natalensis</i>	0	0	8	0	4	0	0	12
<i>An. rufipes</i>	0	0	37	0	2	10	0	49
<i>An. squamosus</i>	0	0	0	0	16	0	0	16
<i>An. pharoensis</i>	2	0	0	0	2	0	0	4
<i>An. salbaii</i>	0	0	0	0	1	0	0	1
<i>An. maculipalpis</i>	0	0	0	0	1	0	0	1
Total	1390	190	1155	925	8911	1716	224	14511

3.1.1 PYRETHRUM SPRAY CATCH

PSC collections yielded 582 *Anopheles* mosquitoes (Table 2). By species they were 509 (87.5%) *An. funestus* s.l., 72 (12.4%) *An. gambiae* s.l., and 1 (0.17%) *An. tenebrosus*.

TABLE 2. NUMBER OF MOSQUITOES BY SPECIES COLLECTED USING PSC IN INTERVENTION AND CONTROL (MOLEVALA) DISTRICTS

	Milange	Mocuba	Maganja	Morrumbala	Molevala	Total
<i>An. funestus</i> s.l.	197	25	172	26	89	509
<i>An. gambiae</i> s.l.	9	9	18	8	28	72
<i>An. tenebrosus</i>	0	0	0	0	1	1
Total per district	206	34	190	34	118	582

The indoor resting density of *An. funestus* s.l. and *An. gambiae* s.l. was very low in both intervention and control sites. The mean vector density was estimated at less than three and one for *An. funestus* s.l. and *An.*

gambiae s.l. per room per day respectively. These low indoor resting densities were found at most of the collection sites before and after IRS intervention during the monitoring period (Figures 1A and 1B). Data for the month of April 2018 could not be collected due to unforeseen administrative challenges.

FIGURE 1 . INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN FIVE DISTRICTS BEFORE AND AFTER IRS INTERVENTION

FIGURE 1A. *AN. FUNESTUS* S.L.

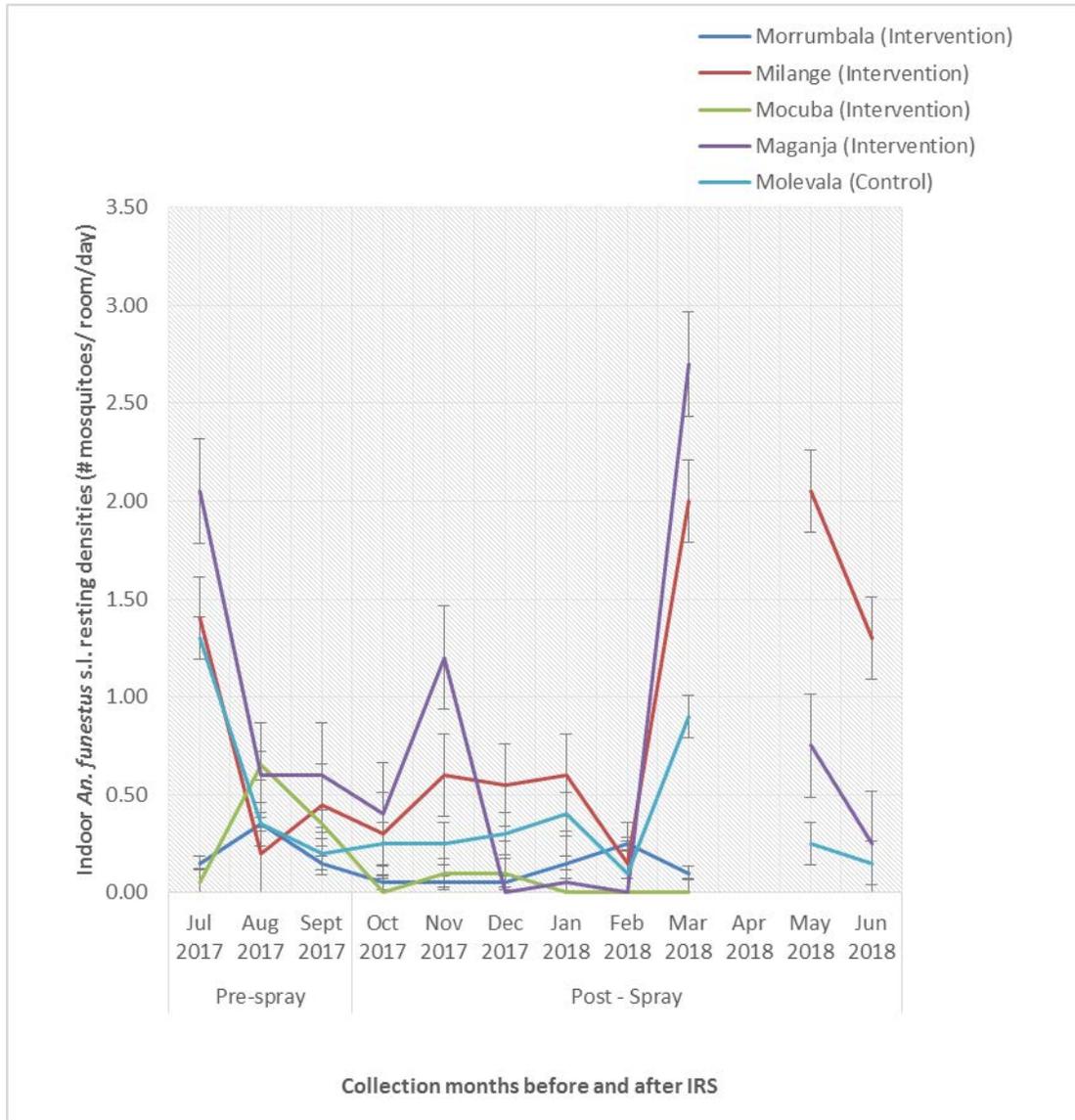
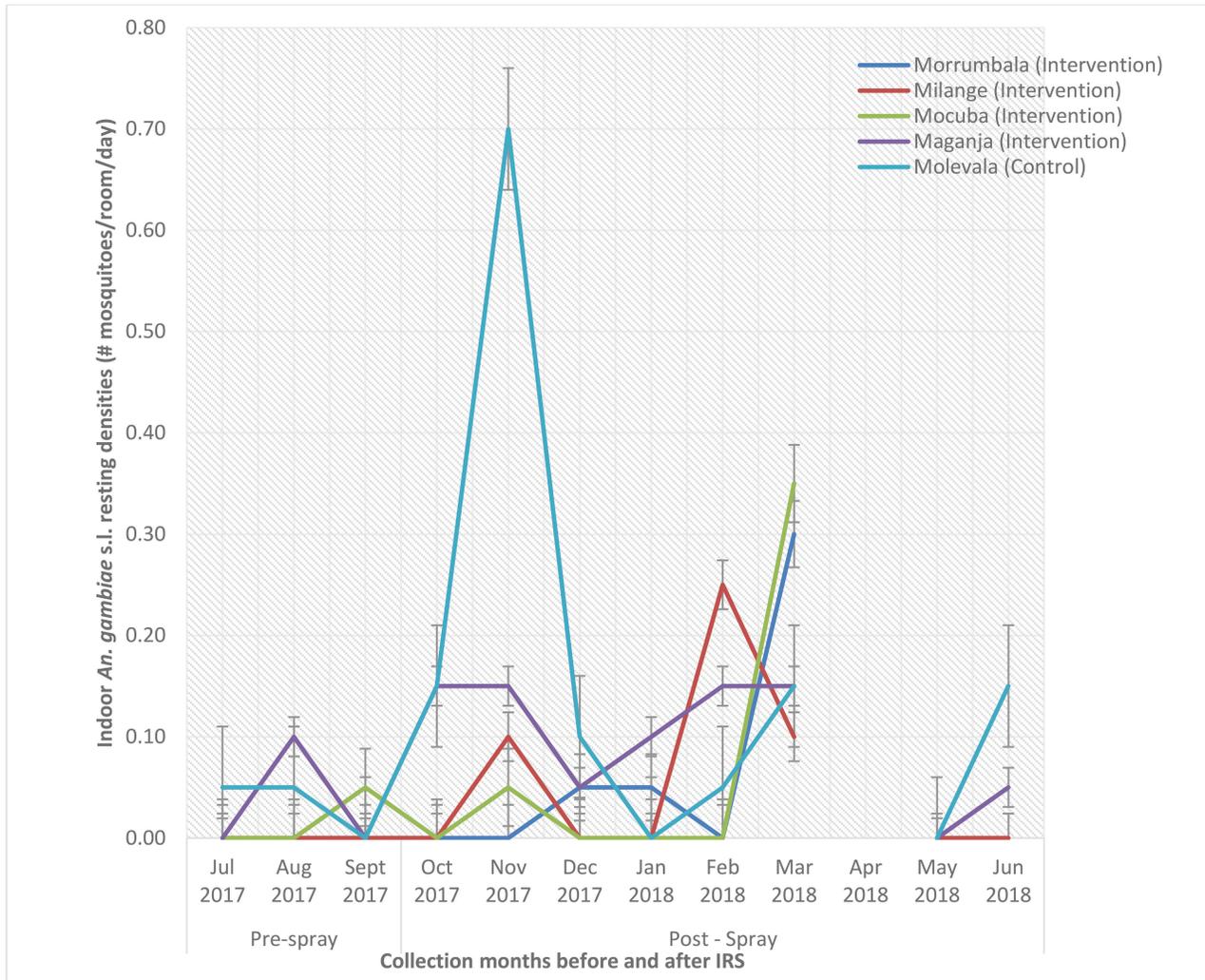


FIGURE 1B. *AN. GAMBIAE* S.L.



3.1.2 HUMAN LANDING CATCHES

A total of 3,266 *Anopheles* mosquitoes were collected using the HLC technique from July 2017 to June 2018. The species identified morphologically from this collection were: *An. funestus* s.l., (1680), *An. gambiae* s.l., (1356), *An. costani* (91), *An. tenebrosus* (38), *An. pretoriensis* (19), *An. squamosus* (15), *An. rufipes* (12), *An. ziemanni* (10), *An. natalensis* (7), *An. pharoensis* (4), *An. salbani* (1), *An. deemingi* (1), and *An. maculipalpis* (1). In Mocuba, no species other than *An. funestus* group and *An. gambiae* complex was collected. Maganja da Costa, Milange, Molevala and Mopeia districts appeared to have the highest anopheline species diversity.

All districts demonstrated a different pattern of *An. funestus* s.l. and *An. gambiae* s.l. human biting activity both indoors and outdoors and before and after IRS spraying, which began in October 2017 (Figure 2). In Milange, the mean *An. funestus* s.l. biting rates both indoors and outdoors dropped to 0.50 and 0.67 bites per person per night (b/p/n), respectively, after IRS began in October, but the two rates increased to 5.33 and 9.50 b/p/n, respectively, in December. The observed increase in outdoor biting rates was almost twice the increase for indoor biting. The *An. gambiae* s.l. indoor biting rates increased steadily in January and February, months characterized by rain and hot temperatures, and then fell back again from March to June 2018. The overall mean biting rate was found to be lower indoors (0.33 b/p/n) than outdoors (1.02 b/p/n). *An. funestus* s.l. activity indoors demonstrated a relatively higher biting rate from July to January. Most *An. gambiae* s.l. biting activity was observed to have increased in October, demonstrating that the species have different seasonality from that of *An. funestus* s.l. Figures 2A and 2B show that Molevala demonstrated the highest mean

An. funestus s.l. biting rates both indoors and outdoors over most of the monitoring period, except in November and December when Maganja da Costa and Milange had higher peaks for indoor and outdoor biting, respectively.

FIGURE 2. INDOOR AND OUTDOOR HUMAN BITING RATES FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN FOUR INTERVENTION DISTRICTS AND ONE CONTROL DISTRICT, BEFORE AND AFTER IRS INTERVENTION.

FIGURE 2A. *An. funestus* INDOOR

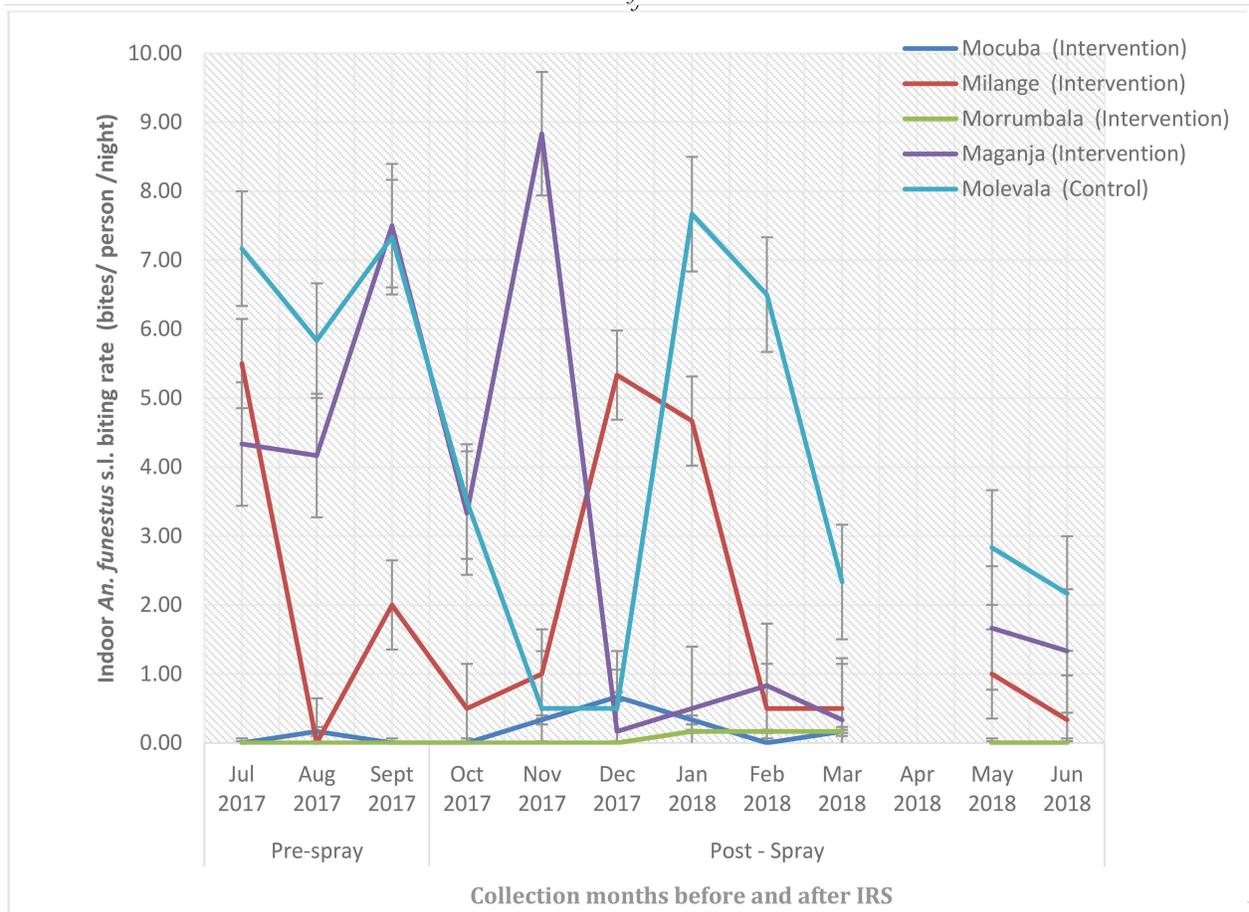


FIGURE 2B. *An. funestus* OUTDOOR

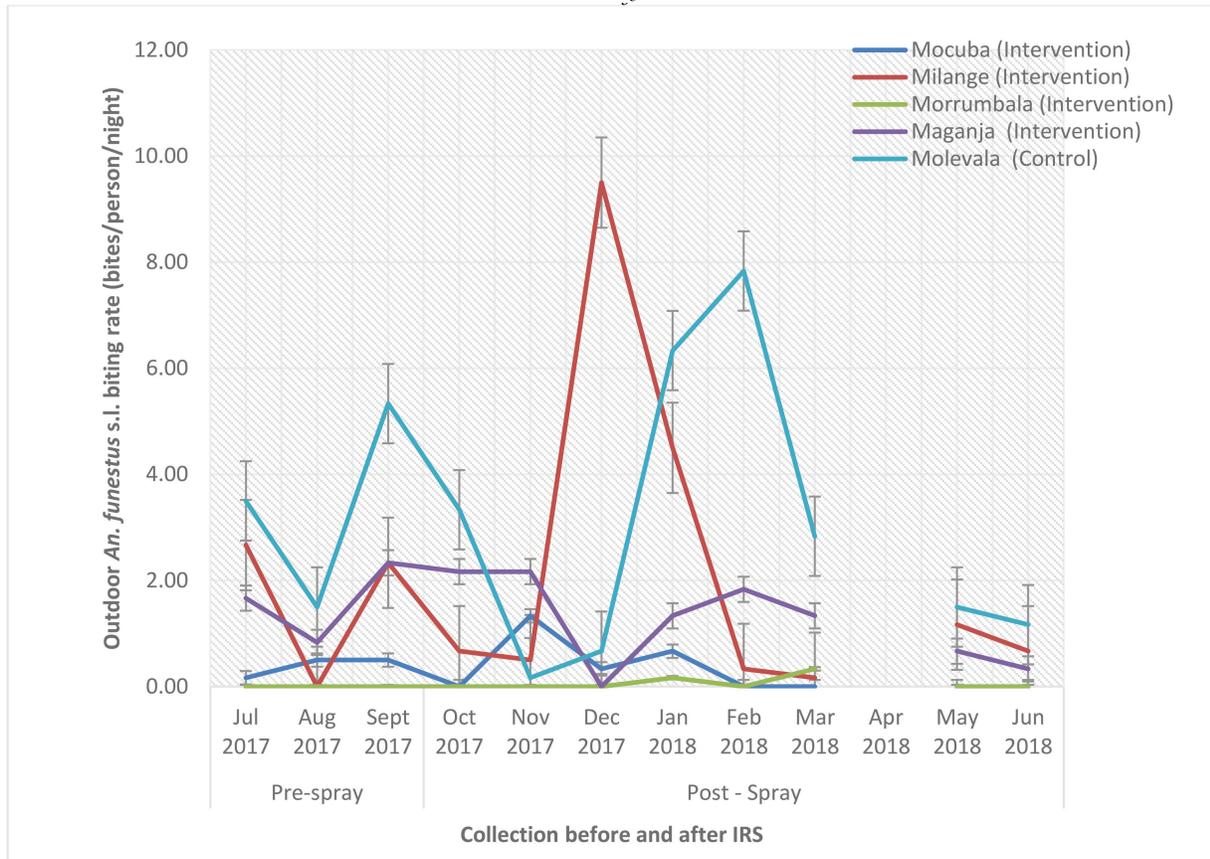


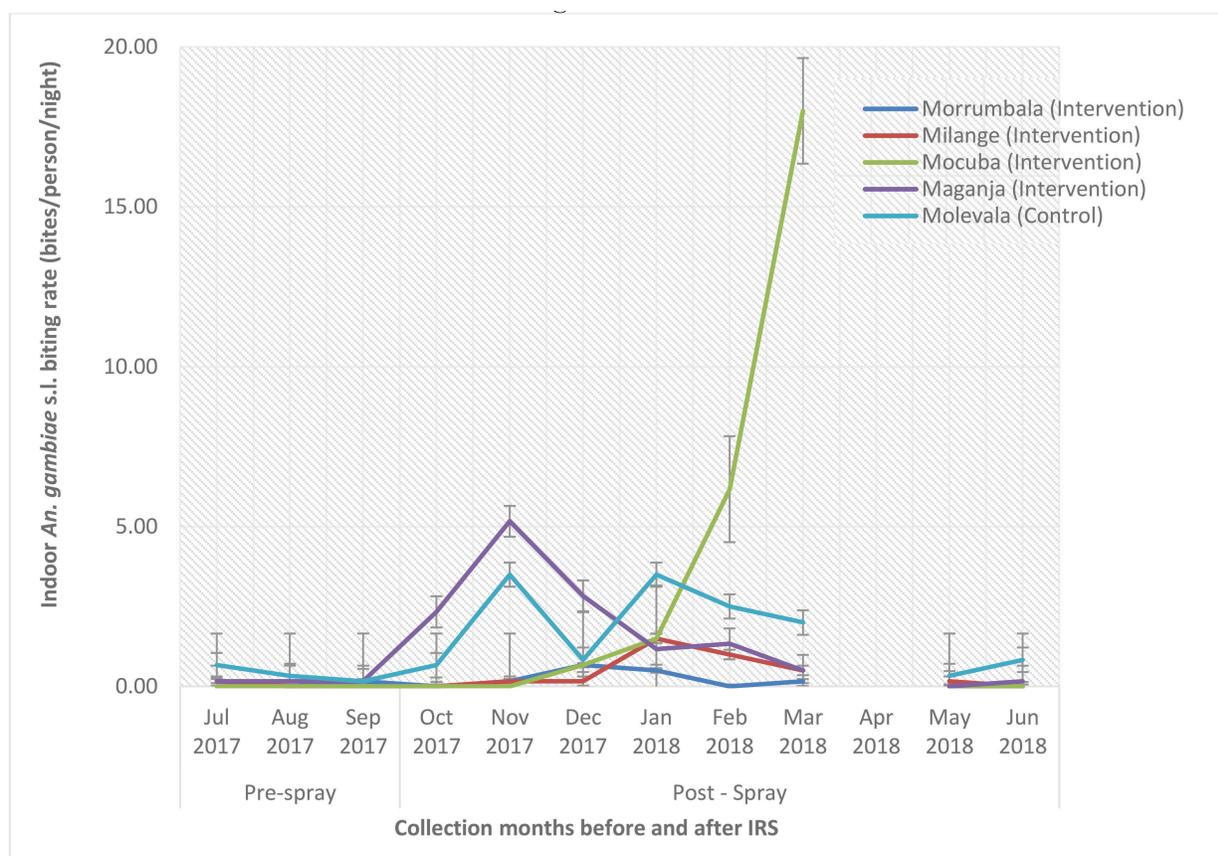
Table 3 shows the mean indoor and outdoor vector biting rates for *An. funestus* s.l. and *An. gambiae* s.l. after spraying. Molevala, the control district, had the highest pre-spray biting rates for *An. funestus* s.l. indoors and outdoors as well as for *An. gambiae* s.l. before spraying indoors. Following spraying, decreases and increases in biting rates were observed in both intervention and control districts. In Molevala, a drop in *An. funestus* s.l. biting rates was observed both indoors and outdoors, while an increase was observed for *An. gambiae* s.l. both indoors and outdoors. In the intervention districts, increases in *An. funestus* s.l. indoor biting rates were observed in Milange, Mocuba and Morrumbala, whereas a decrease was observed in Maganja da Costa. *An. funestus* s.l. outdoor biting rates were found to have increased after IRS in Milange and Morrumbala, while a decrease was observed in Maganja. Mocuba did not record any change after IRS. In contrast, *An. gambiae* s.l. biting rates both indoors and outdoors were found to have increased following IRS in all intervention districts of Maganja, Milange, Mocuba, and Morrumbala.

TABLE 3. INDOOR AND OUTDOOR MEAN BITING RATE FOR *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L., ESTIMATED USING HLC, EXPRESSED AS MEAN BITES PER PERSON PER NIGHT FROM ALL COLLECTION ROUNDS, BY DISTRICT, BEFORE AND AFTER SPRAYING

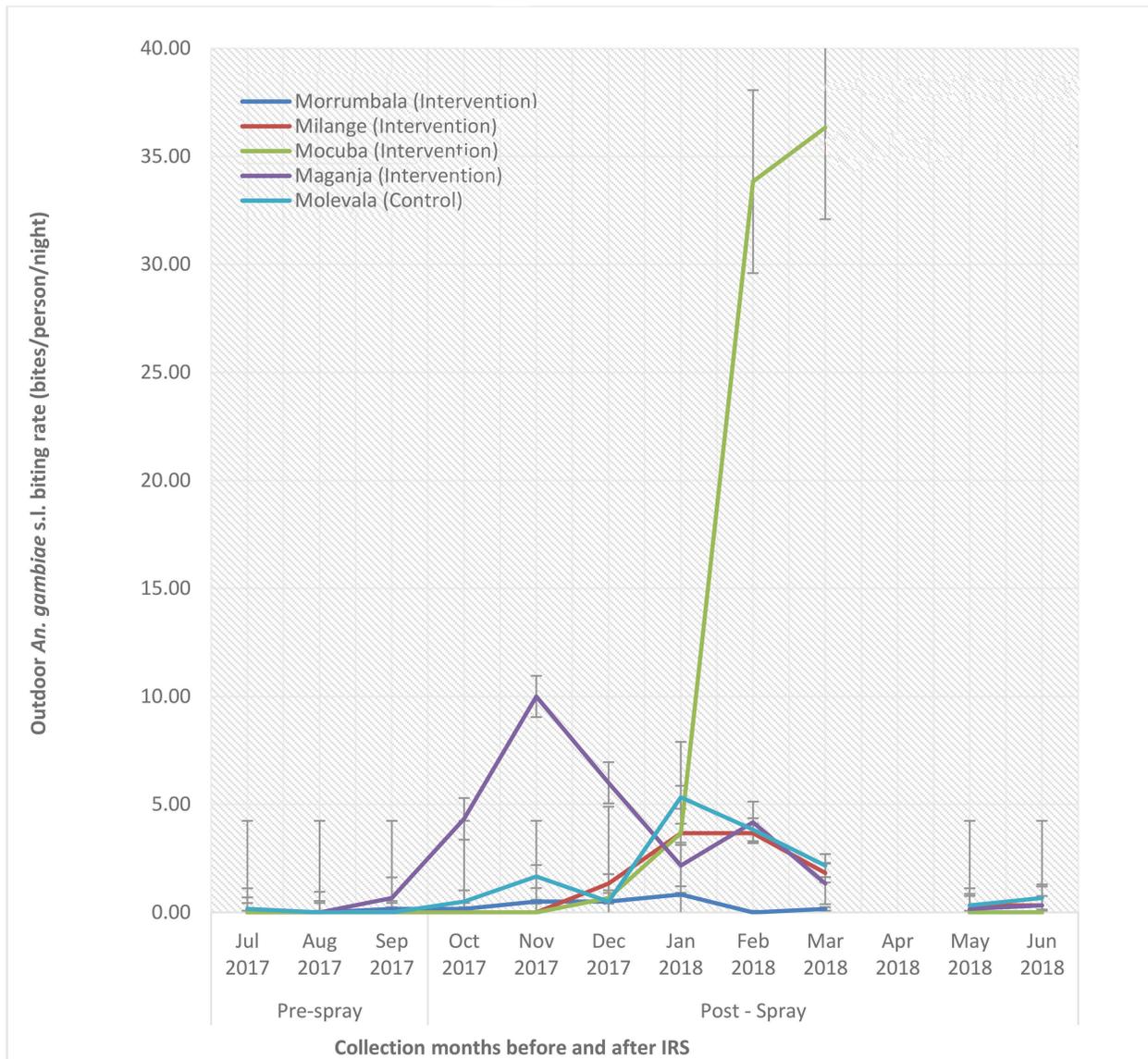
District	<i>Anopheles funestus</i> s.l. (b/p/n)				<i>Anopheles gambiae</i> s.l. (b/p/n)			
	Indoors		Outdoors		Indoors		Outdoors	
	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray
Maganja	5.33	2.125	1.61	1.23	0.28	3.56	0.28	3.56
Milange	2.50	4.27	1.67	2.19	0.17	0.438	0.00	1.40
Mocuba	0.06	0.25	0.39	0.39	0.11	0.250	0.06	0.36
Morrumbala	0.00	0.08	0.00	0.08	0.00	4.389	0.00	12.42
Molevala*	6.78	3.25	3.44	2.98	0.39	1.77	0.06	1.88

*Unsprayed control district. The pre- and post-spray estimates are based on the period when spraying was done in intervention districts: pre-spray was three months before spraying and post-spray was all the months after spraying up, to July 2018.

2C *An. gambiae* s.l. INDOOR



2D *An. gambiae* s.l. OUTDOOR



In Mopeia, the *An. funestus* s.l. biting rate in the intervention areas was low both indoors and outdoors throughout the collection period, scoring 0.42 b/p/n indoors and 0.30 b/p/n outdoors. In the Mopeia control areas, the mean indoor and outdoor biting rates were 2.34 and 1.80 b/p/n, respectively. The same was observed for *An. gambiae* s.l., where the mean biting rate was higher indoors and outdoors (0.30 and 0.80 b/p/n, respectively) in the control compared to intervention (0.10 and 0.28 b/p/n, respectively).

The low biting rates observed in the intervention areas could indicate the potential impact of spraying Actellic® 300CS against both predominant malaria vectors. In the control area, the biting rates for *An. funestus* s.l. and *An. gambiae* s.l. were high indoors and outdoors in July and January (Figures 3). The months with higher biting rates were July, May, and June, which could be partly due to the seasonal occurrence of the species (Annex Tables A-4 and A-5). The *An. gambiae* s.l. biting rates were basically zero indoors and outdoors in both intervention and control areas from July to December (Annex Tables A-6 and A-7). However, the biting rates increased slightly in January in both control (indoor and outdoor) and intervention (indoor and outdoor) areas following the rain and hot season (Figures 3C and 3D).

In Table 4, significant differences were observed between total numbers of *An. funestus* s.l. samples collected indoors and outdoors (with $p < 0.05$) in Mocuba, Maganja da Costa, Molevala (control), and Mopeia (control). More *An. funestus* s.l. mosquitoes were collected indoors than outdoors, an observation that was not expected in Maganja da Costa, since it had received IRS. In the intervention areas of Mopeia, no significant difference was detected between indoor and outdoor collections despite the low numbers of *An. funestus* s.l. collected in this area when compared with the district's control areas. Fewer *An. gambiae* s.l., were collected indoors than outdoors in all districts with the exception of Molevala (control). Significant differences between outdoor and indoor HLC collections were observed in Maganja da Costa, Milange, Mopeia (control) and Mopeia (intervention), and Morrumbala.

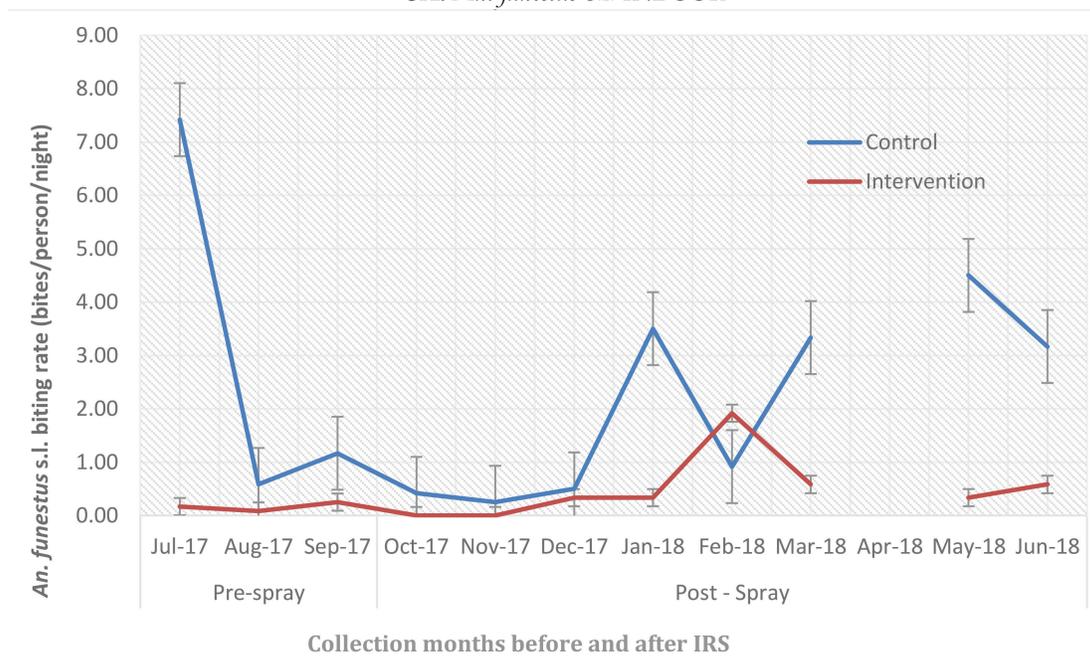
TABLE 4. COMPARISON OF TOTAL NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY HLC INDOORS AND OUTDOORS IN SIX DISTRICTS

District	<i>An. funestus</i> s.l.				<i>An. gambiae</i> s.l.			
	# Collected indoors	# Collected outdoors	X ²	p-value	# Collected indoors	# Collected outdoors	X ²	p-value
Maganja da Costa	198	88	42.31	<0.00001*	84	176	32.55	<0.00001*
Morrumbala	3	3	0.00	1	158	447	138.05	<0.00001*
Mocuba	10	21	3.90	0.048193*	10	14	0.67	0.414216
Milange	128	135	0.19	0.666004	22	67	22.75	<0.00001*
Molevala (Control)	278	205	11.03	0.000894*	92	91	0.01	0.941072
Mopeia (Control)	309	239	8.94	0.0027*	40	105	29.14	<0.00001*
Mopeia (Intervention)	55	39	2.72	0.098887	13	37	11.52	0.000688*

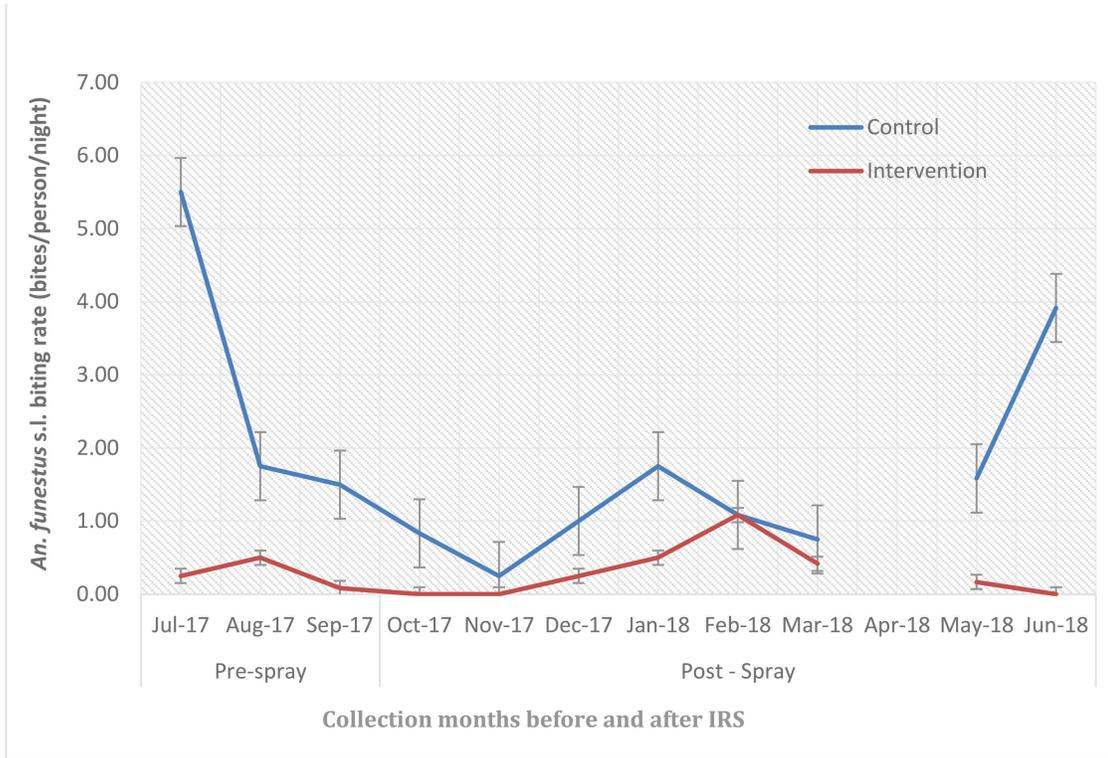
*p-value significant.

FIGURE 3. INDOOR AND OUTDOOR HUMAN BITING RATES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN MOPEIA INTERVENTION AND CONTROL AREAS

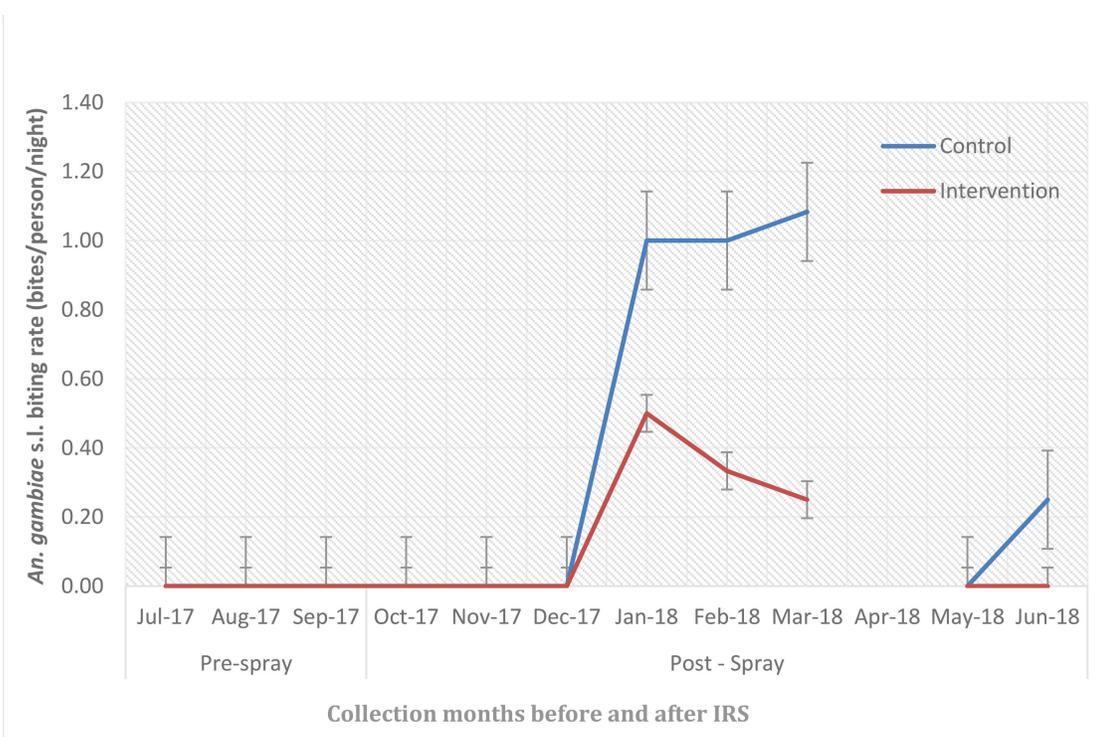
3A. *An. funestus* s.l. INDOOR



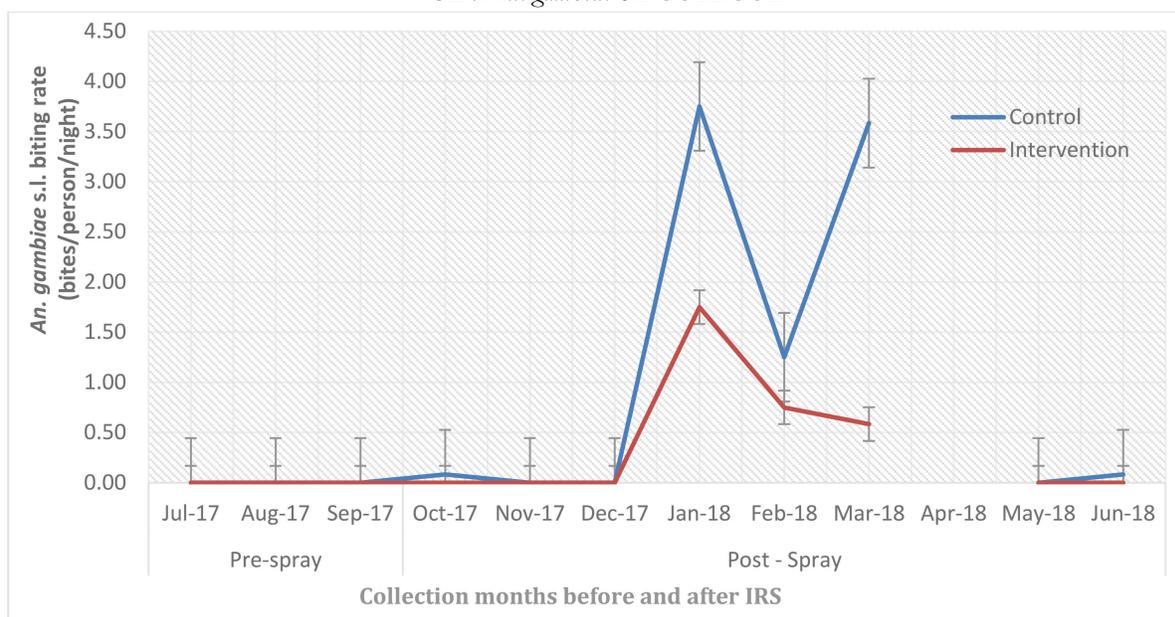
3B. *An. funestus* s.l. OUTDOOR



3C. AN. GAMBIAE S.L. INDOOR



3D. *An. gambiae* s.l. OUTDOOR



In Mopeia district, in both the intervention and control areas, the most abundant mosquito species collected were *An. funestus* s.l. (642) and *An. gambiae* s.l. (195). The remaining 114 mosquitoes were morphologically identified as *An. coustani* (49), *An. tenebrosus* (28), *An. squamosus* (15), *An. ziemanni* (9), *An. pretoriensis* (4), *An. natalensis* (4), *An. pharoensis* (2), *An. maculipalpis* (1), *An. rufipes* (1), and *An. salbaii* (1). Table 5 shows numbers of each mosquito species collected in control and intervention areas, indicating for each the total person-nights and subsequent biting rate expressed as b/p/n. *An. funestus* s.l. and *An. gambiae* s.l. were observed to contribute to 93 percent and 69 percent of the bites in the control and intervention areas, respectively. Other avid biters after the above included *An. coustani* and *An. tenebrosus*, in both sites.

TABLE 5. MOSQUITO SPECIES COLLECTED BY HLC AND THEIR MEAN BITING RATES IN MOPEIA CONTROL AND INTERVENTION AREAS, JULY 2017 TO JUNE 2018

Species collected	Intervention area			Control area		
	Total numbers collected	Total person nights	b/p/n	Total numbers collected	Total person nights	b/p/n
<i>An. funestus</i> s.l.	94	132	0.71	548	132	4.15
<i>An. gambiae</i> s.l.	50	132	0.38	145	132	1.09
<i>An. coustani</i>	29	132	0.22	20	132	0.15
<i>An. maculipalpis</i>	1	132	0.01	0	132	0
<i>An. natalensis</i>	3	132	0.02	1	132	0.01
<i>An. pharoensis</i>	1	132	0.01	1	132	0.01
<i>An. pretoriensis</i>	1	132	0.01	3	132	0.02
<i>An. squamosus</i>	7	132	0.05	8	132	0.06
<i>An. tenebrosus</i>	13	132	0.09	15	132	0.11
<i>An. ziemanni</i>	7	132	0.05	2	132	0.02
<i>An. rufipes</i>	0	132	0	1	132	0.01
<i>An. salbaii</i>	0	132	0	1	132	0.01
Total	206			745		

In the new IRS district of Maganja da Costa, the indoor biting activity of *An. funestus* s.l. was close to zero from 6:00 to 9:00 pm (Figure 4A). It started to increase after 10:00 pm, when the community went to bed, and peaked at 2:00 to 3:00 am, determined as 6 b/p/h. The indoor biting activity of *An. funestus* s.l. in Maganja da Costa followed almost the same pattern as in Molevala (control). However, in Molevala (control), peak indoor activity among *An. funestus* s.l. (6.33 b/p/h) was observed around 2:00 to 3:00 am. *An. funestus* s.l. indoor biting activity in Morrumbala and Mocuba was low, almost zero, while in Milange, biting started around 8:00 to 9:00 pm and extended through the night, with peaks observed from 1:00 to 2:00 am and 4:00 to 5:00 am (scored as 3 b/p/h). The outdoor biting of *An. funestus* s.l. in Molevala was highest of all districts. In general, the biting activity was observed from 6:00 pm to 6:00 am with two peaks, from 1:00 to 2:00 and 3:00 to 4:00 am, estimated as 4.17 and 4.33 b/p/h (Figure 4B). Similarly, in Milange and Maganja da Costa, the outdoor biting of *An. funestus* s.l. was observed from 6:00 pm to 6:00 am, with peak bites (4.17 and 2.50 b/p/h) in both sites and from 1:00 to 2:00 am (Figure 4B). In Morrumbala, the outdoor biting pattern was similar to the indoor, around zero, while in Mocuba; *An. funestus* s.l. tended to bite from 8:00 pm to 1:00 am. Likewise, biting in Mocuba was found to be consistently low (Figures 4A and B).

FIGURE 4. HOURLY BITING RATES OF *AN. FUNESTUS* S.L. IN MAGANJA DA COSTA, MORRUMBALA, MOCUBA, MILANGE, AND MOLEVALA AS DETERMINED THROUGH HLCS

FIGURE 4A. *An. funestus* s.l. INDOOR

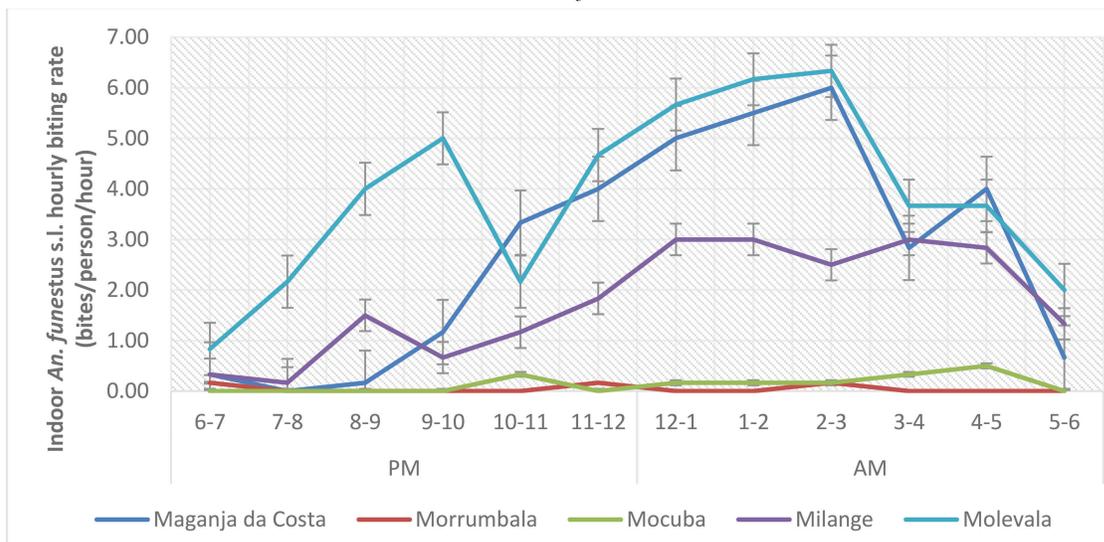
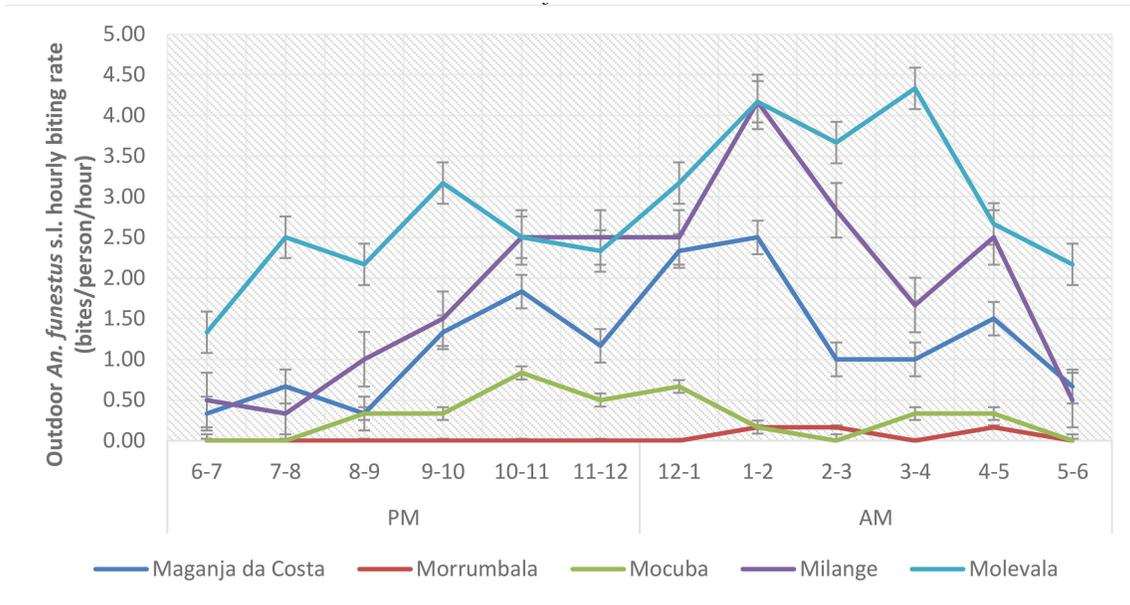


FIGURE 4B. *An. funestus* s.l. OUTDOOR



The indoor hourly biting activity for *An. gambiae* s.l. was highest in Morrumbala and Maganja da Costa (see Figures 4 C and D). The biting activity varied from <1 to 6.83 bites per person per hour (b/p/h), with peaks lasting from 10:00 pm to 11:00 pm, and from 2:00 am to 3:00 am, as well as between 4.00 and 5:00. In Molevala, the indoor biting activity was notable during the early and late morning hours, varying from 3.17 to 1.67 b/p/h from 1:00 am to 2:00 am and from 5:00 am to 6:00 am. In Mocuba and Milange, the indoor biting activity was low, less than 1.00 b/p/n in both districts (Figure 4C).

Results from *An. gambiae* s.l. outdoor biting shows that Morrumbala and Maganja da Costa had the highest biting activity, estimated at 12.17 and 7.50 b/p/h, from 12:00 am to 2:00 am and 12:00 am to 1:00 am, respectively (Figure 4D). Indoor and outdoor biting activity in the control district of Molevala was midway between the two highest sites (Morrumbala and Maganja da Costa) and two lowest (Mocuba and Milange), all of which are intervention districts (Figure 4D).

FIGURE 4C. *AN. GAMBIAE* S.L. INDOOR

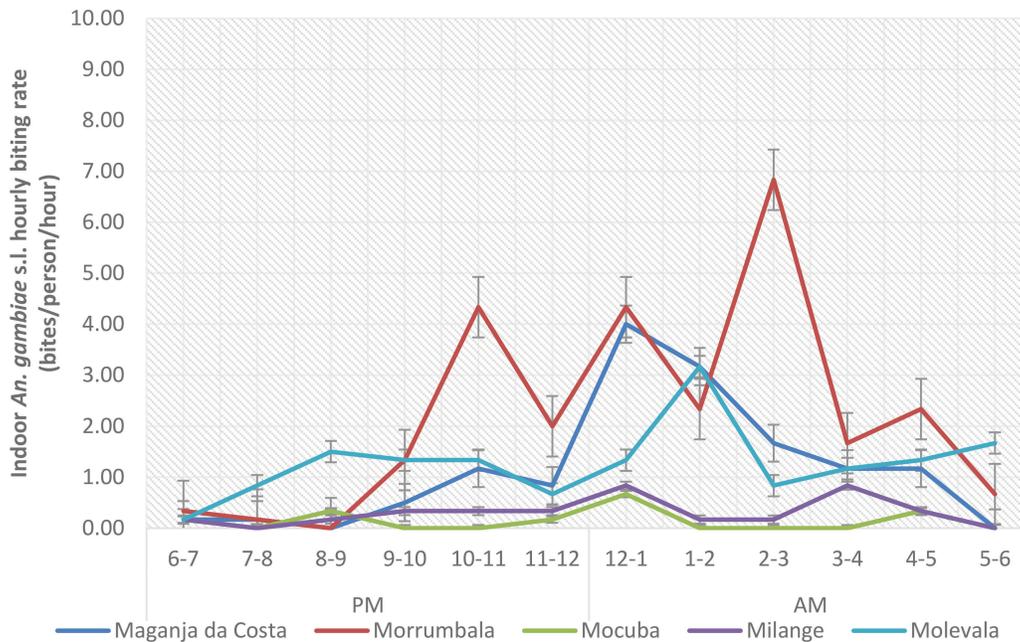
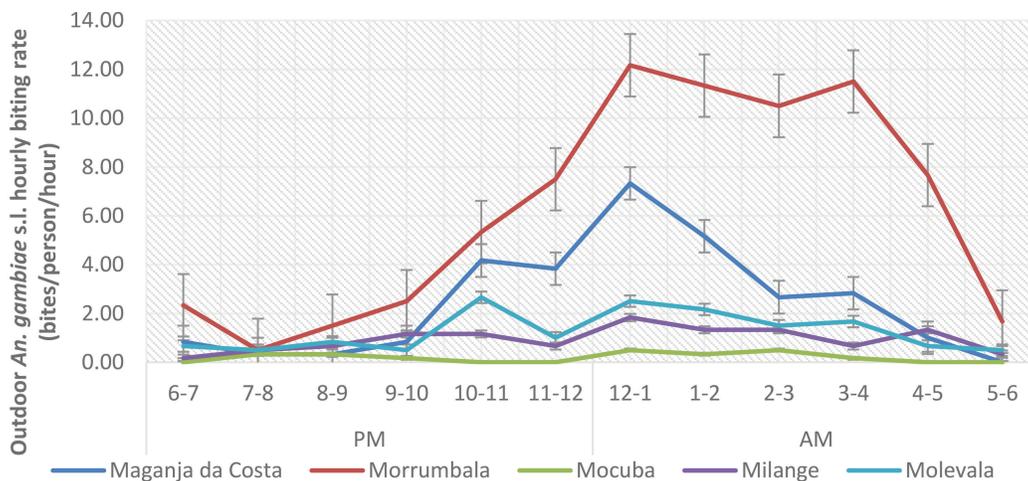


FIGURE 4D. *AN. GAMBIAE* S.L. OUTDOOR



An. gambiae s.l. biting activity in Mopeia district was found to be higher in the control area than in intervention areas, both indoors (0.5 versus 0.35 b/p/h) and outdoors (1.03 versus 0.75 b/p/h). Peak biting activity was observed outdoors from 10:00 to 12:00 pm in both control and intervention areas (Figures 5A and 5B).

Likewise, *An. funestus* s.l. biting activity was higher in the control areas than in the intervention areas, both indoors and outdoors. Results from indoor collections indicate that in the control area, the species could exhibit biting activity that was consistently above 1.5 b/p/h, increasing to a peak of 3.8 b/p/h, while in the intervention areas, it was consistently below 0.5 b/p/h without an obvious peak. Likewise, results from outdoor collections indicate that in the control area, biting activity was consistently above 1.5 b/p/h, peaking at 2.2 b/p/h, while in the intervention areas, it was consistently below 0.5 b/p/h without an obvious peak.

Peak biting time was observed in very early morning hours, between 1:00 am and 3:00 am, in the control areas (Figures 5C and 5D).

FIGURE 5. HOURLY BITING RATES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN MOPEIA DISTRICT, INTERVENTION AND CONTROL AS DETERMINED THROUGH HLCs

FIGURE 5A. INDOOR *AN. GAMBIAE* S.L. BITING RATE

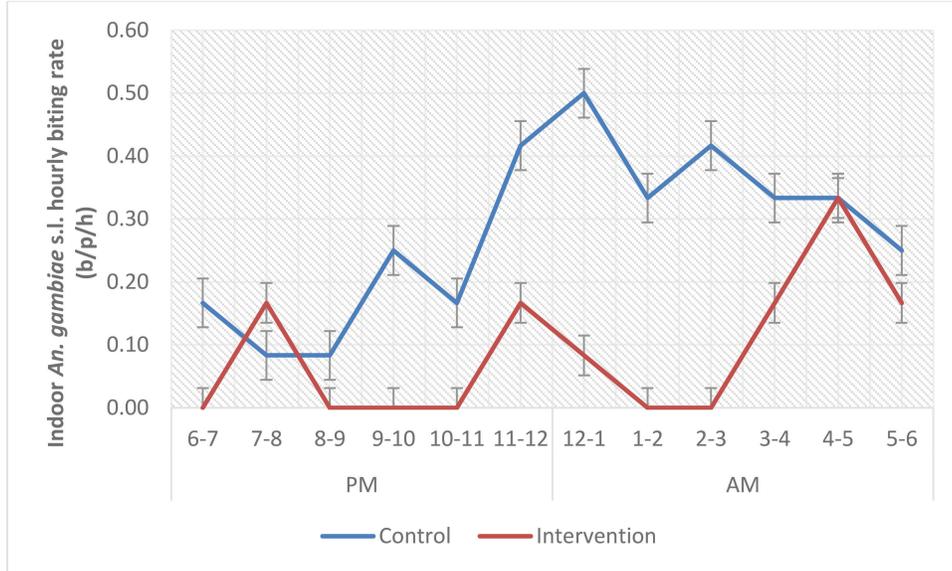


FIGURE 5B. OUTDOOR *AN. GAMBIAE* S.L. BITING RATE

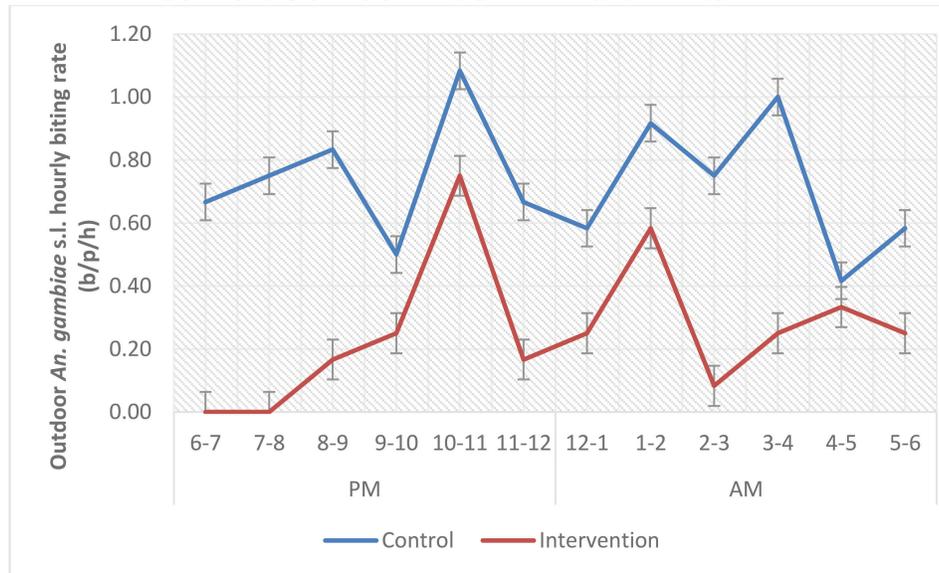


FIGURE 5C. INDOOR *AN. FUNESTUS* S.L. BITING RATE

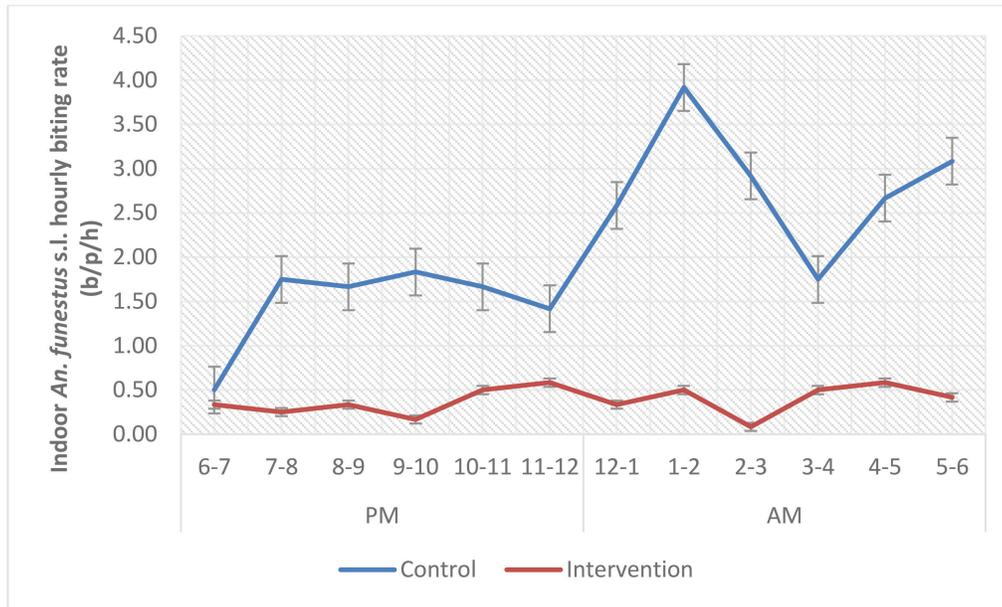
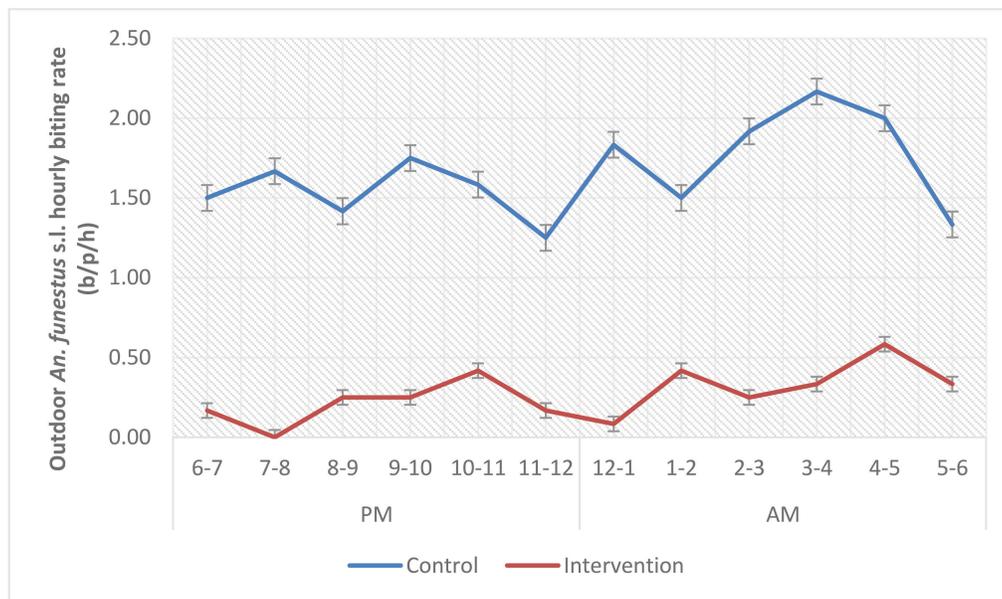


FIGURE 5D. OUTDOOR *AN. FUNESTUS* S.L. BITING RATE



3.1.3 CDC LIGHT TRAP (CDC-LT)

The CDC light trap collections yielded a total of 2,703 *Anopheles* mosquitoes from four intervention districts (excluding Mopeia) and the control district. Table 6 shows the monthly and total collections per district. Morphological identification of the mosquitoes revealed that 1,800 (66.59%) were *An. funestus* s.l., 850 (31.45%) were *An. gambiae* s.l., 36 (1.33%) were *An. rufipes*, 7 (0.26%) were *An. coustani*, 5 (0.18%) were *An. natalensis*, 4 (0.15%) were *An. tenebrosus*, and 1 (0.04) was *An. pretoriensis*. Molevala (control) had the highest percentage of all *An. funestus* s.l. collected, 39.94 percent, followed by Maganja da Costa at 30.33 percent.

TABLE 6. CDC LIGHT TRAP DATA FROM MONTHLY COLLECTION IN SIX DISTRICTS

Districts	Species	2017						2018						Total & average densities/month/night	
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr*	May	June		
Morrumbala	<i>An. funestus</i> s.l.	8	0	0	0	0	0	0	0	7		0	0	15	459
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58		0.00	0.00	0.11	
	<i>An. gambiae</i> s.l.	0	0	0	0	0	2	17	69	356		0	0	444	
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	0.00	0.00	0.00	0.00	0.00	0.17	1.42	5.75	29.67		0	0	3.36	
Milange	<i>An. funestus</i> s.l.	34	16	8	0	12	57	89	11	25		21	9	282	328
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	2.83	1.33	0.67	0.00	1.00	4.75	7.42	0.92	2.08		1.75	0.75	2.14	
	<i>An. gambiae</i> s.l.	0	0	0	0	0	4	9	16	17		0	0	46	
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	0.00	0.00	0.00	0.00	0.00	0.33	0.75	1.33	1.42		0.00	0.00	0.35	
Mocuba	<i>An. funestus</i> s.l.	0	17	16	0	0	8	0	0	0		0	0	41	101
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	0.00	1.42	1.33	0.00	0.00	0.67	0.00	0.00	0.00		0.00	0.00	0.311	
	<i>An. gambiae</i> s.l.	1	6	2	6	4	39	2	0	0		0	0	60	
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	0.08	0.50	0.17	0.50	0.33	3.25	0.17	0.00	0.00		0.00	0.00	0.45	
Maganja	<i>An. funestus</i> s.l.	61	37	117	158	155	4	1	0	10		0	3	546	642
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	5.08	3.08	9.75	13.17	12.92	0.33	0.08	0.00	0.83		0.00	0.25	4.14	
	<i>An. gambiae</i> s.l.	1	0	4	30	26	2	4	18	7		4	0	96	
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	0.08	0.00	0.33	2.50	2.17	0.17	0.33	1.50	0.58		0.33	0.00	0.73	
Molevala	<i>An. funestus</i> s.l.	22	23	12	12	4	29	145	224	130		107	11	719	899
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	1.83	1.92	1.00	1.00	0.33	2.42	12.08	18.67	10.83		8.92	0.92	5.45	
	<i>An. gambiae</i> s.l.	0	0	0	5	26	9	27	58	50		5	0	180	
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	0.00	0.00	0.00	0.42	2.17	0.75	2.25	4.83	4.17		0.42	0.00	1.36	
Quelimane	<i>An. funestus</i> s.l.	154	0	6	6	1	0	1	0	29		0	0	197	221
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	12.83	0.00	0.50	0.50	0.08	0.00	0.08	0.00	2.42		0.00	0.00	1.49	
	<i>An. gambiae</i> s.l.	5	0	0	0	1	2	5	0	11		0	0	24	
	Trap nights	12	12	12	12	12	12	12	12	12		12	12		
	Mean # Mosq/trap/night	0.42	0.00	0.00	0.00	0.08	0.17	0.42	0.00	0.92		0.00	0.00	0.18	
Total		286	99	165	217	229	156	300	396	642		137	23	2650	

In terms of mean collections, *An. funestus* s.l. was most abundant in Molevala (with mean collection of 5.45 mosquitoes per trap per night over the 11 collection months), followed by Maganja da Costa (with mean collection of 4.14 mosquitoes per trap per night over the 11 months). A sharp decrease in the *An. funestus* s.l. population density was observed in Maganja da Costa in December 2017, following introduction of IRS in October 2017.

In Milange, *An. funestus* s.l. densities decreased in October, the spray month, but they unexpectedly showed a slight increase in December 2017 and January 2018. The overall mean densities of mosquitoes per trap per night (m/t/n) across the collection cycle was 2.14. The highest densities observed in Morrumbala were 0.67 m/t/n in July and 0.58 m/t/n in March. For other months, the densities were zero. In Mocuba, the highest densities were reached in August (1.42) and September (1.33); the densities dropped to zero in October and November, then increased to 0.67 m/t/n in December. The overall mean densities for all collection rounds recorded in Morrumbala and Mocuba were low, 0.11 and 0.31 m/t/n, respectively.

Of note is the increase in *An. gambiae* s.l. densities across districts that are closely associated with the rainfall pattern (rainfall data not shown). Monthly densities of *An. gambiae* s.l. in Morrumbala, which were zero from July to November, increased slightly in December, and then rose sharply in March to peak at 29.67 m/t/n. Overall, Morrumbala recorded the highest mean density, estimated at 3.36 m/t/n. The CDC light traps in Milange collected no *An. gambiae* s.l. from July to November 2017. A few mosquitoes began to be seen in December, and their numbers rose steadily to 1.42 m/t/n in March.

An. gambiae s.l. densities in Maganja da Costa were observed to be around zero during the months before IRS (July to September). They increased after the spray campaign in October and subsequent months, with an overall mean density estimated as 0.73 m/t/n.

In Molevala (control), *An. funestus* s.l. was collected throughout the monitoring period. The mean density was estimated at 5.45 m/t/n. The highest densities were observed in the January to March period, as high as 12.08, 18.67, and 10.83 m/t/n in the three months, respectively. This aligns with the hot and rainy season, and high malaria transmission. Peak density for *An. gambiae* s.l., 4.83 m/t/n, was observed in February (Table 6 and Figures 6A and 6B)

In Quelimane district, where IRS was suspended in 2017, *An. funestus* s.l. was collected in low densities over most of the monitoring period. Peak density, 12.83 m/t/n, was recorded in July. The mean density was 1.49 m/t/n. *An. gambiae* s.l. densities were similarly very low over most of the collection period, with a mean density of 0.18 m/t/n.

These data demonstrate a significant difference between vector densities recorded in control and intervention areas ($p < 0.05$ was obtained with a X^2 of 142.56, 265.72, 636.80, 42.86, and 410.43 for Morrumbala, Milange, Mocuba, Maganja, and Quelimane districts, respectively).

FIGURE 6. INDOOR CDC LIGHT TRAP DENSITY PER TRAP PER NIGHT IN MORRUMBALA, MILANGE, MOCUBA, MAGANJA DA COSTA, QUELIMANE, AND MOLEVALA DISTRICTS

FIGURE 6A. *AN. FUNESTUS* S.L.

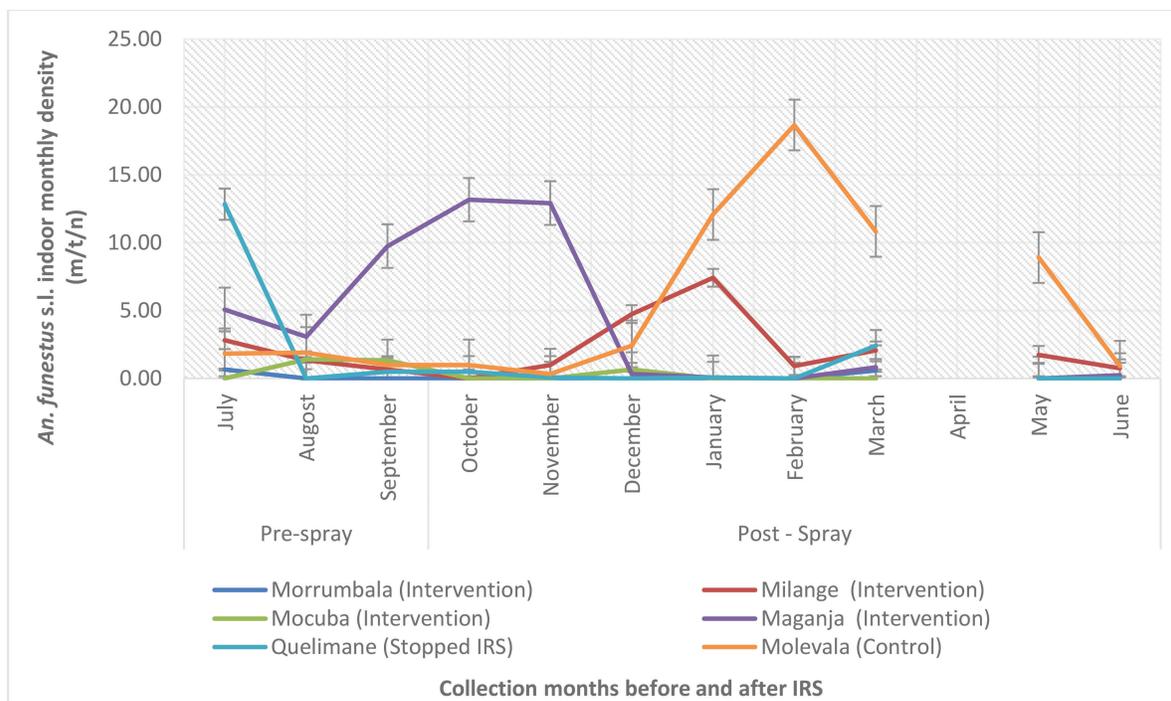
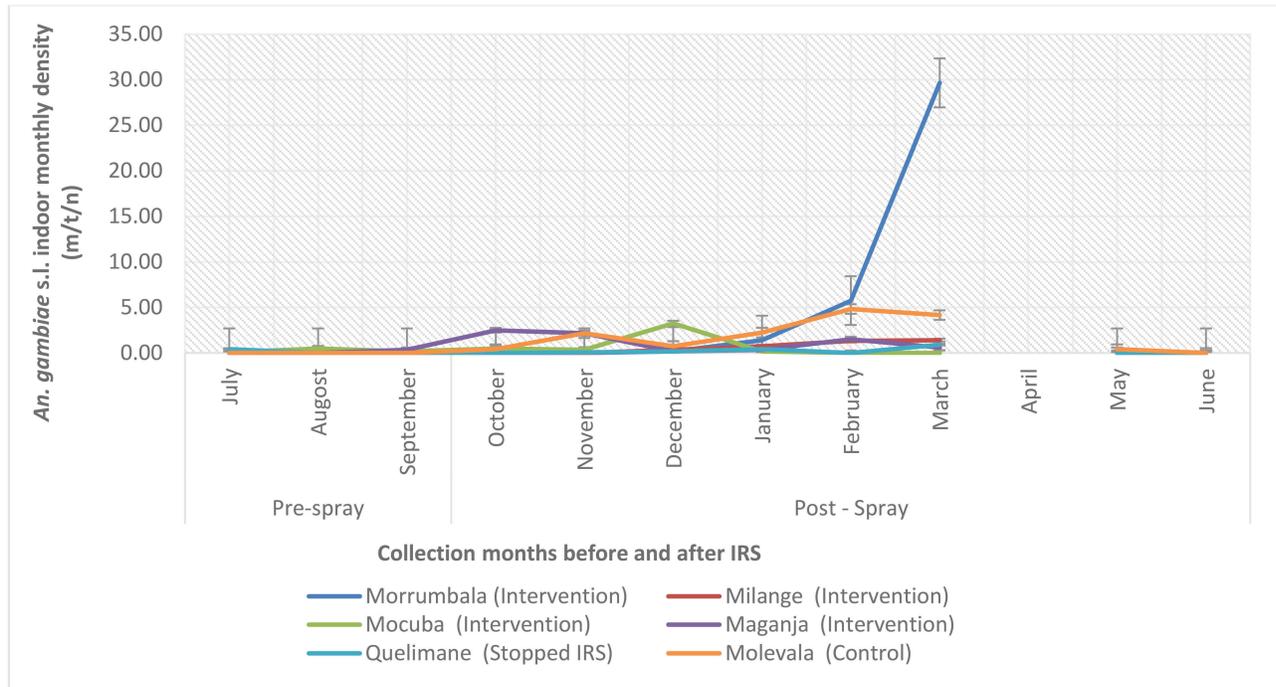


FIGURE 6B. *AN. GAMBIAE* S.L.



In Mopeia district, CDC light traps caught a total 7,960 *Anopheles* mosquitoes, 6,163 (77.42%) in control areas and 1,797 (22.58%) in intervention areas (Table 7). *An. funestus* s.l. was the most abundant mosquito species, with 7,504 (94.27%) collected, followed by *An. gambiae* s.l. with 424 (5.33%) mosquitoes collected. Other species caught in low numbers were *An. coustani*, 6 (0.08%), *An. rufipes* 1 (0.01%), *An. ziemanni* 3 (0.04%), *An. squamosus* 1 (0.01%), *An. pretoriensis* 3 (0.04%), and *An. tenebrosus* 18 (0.23%). Peak densities for *An. funestus* s.l. were observed in March and May 2018 in the control area. There were no notable peaks observed in the intervention area, except for a small increase recorded in October 2017 as IRS began. IRS seems to have suppressed further peaks. The *An. gambiae* s.l. population demonstrated consistently low densities in both intervention and control areas, except in January 2018, when its density increased slightly in the control area (Figure 7 A and B).

Table 7 also shows that the Mopeia intervention area yielded fewer anopheline mosquitoes than the control area did, for both *An. funestus* s.l. and *An. gambiae* s.l. The observed differences were found to be statistically significant for *An. funestus* s.l. ($X^2 = 2222, 69; p = 0$) and for *An. gambiae* s.l. ($X^2 = 174.49; p = 7.73 \times 10^{-40}$).

TABLE 7. ANOPHELINE SPECIES COLLECTED BY CDC LIGHT TRAP IN MOPEIA DISTRICT

District	Species	Total collection per month												Total	% per area	
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun			
Mopeia (Control)	<i>An. funestus</i> s.l.	567	659	394	125	196	428	533	444	835	0	903	710	5794	6,163	77.42
	<i>An. gambiae</i> s.l.	1	2	1	0	1	8	201	26	86	0	16	6	348		
	<i>An. coustani</i>	0	0	0	0	0	2	0	1	2	0	0	0	5		
	<i>An. pretoriensis</i>	0	0	0	0	0	3	0	0	0	0	0	0	3		
	<i>An. squamosus</i>	0	0	0	0	0	0	0	0	1	0	0	0	1		
	<i>An. tenebrosus</i>	0	0	0	0	0	0	0	5	4	0	0	2	11		
	<i>An. ziemanni</i>	0	0	0	0	0	0	0	0	0	0	1	0	1		
Mopeia (Intervention)	<i>An. funestus</i> s.l.	253	286	245	291	71	131	56	104	53	0	108	112	1710	1,797	22.58
	<i>An. gambiae</i> s.l.	0	2	0	3	1	2	36	4	22	0	2	4	76		
	<i>An. tenebrosus</i>	0	2	0	0	0	0	0	0	0	0	0	5	7		
	<i>An. coustani</i>	0	0	0	1	0	0	0	0	0	0	0	0	1		
	<i>An. rufipes</i>	0	0	0	0	0	1	0	0	0	0	0	0	1		
	<i>An. ziemanni</i>	0	2	0	0	0	0	0	0	0	0	0	0	2		
Total		821	953	640	420	269	575	826	584	1003	0	1030	839	7960		

FIGURE 7. INDOOR CDC LIGHT TRAP DENSITY PER TRAP PER NIGHT IN MOPEIA

FIGURE 7A. *AN. FUNESTUSS.L.*

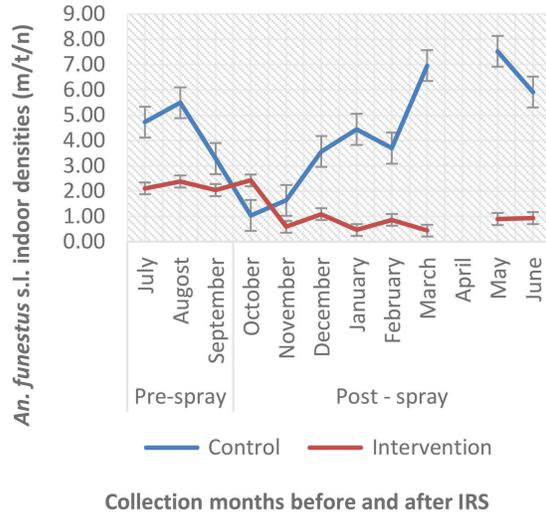
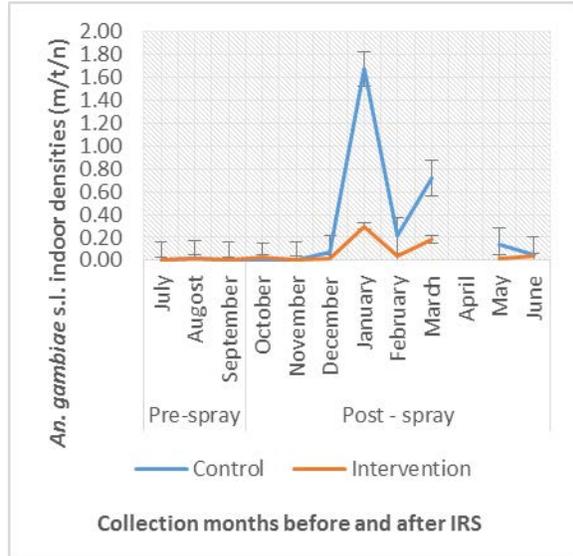


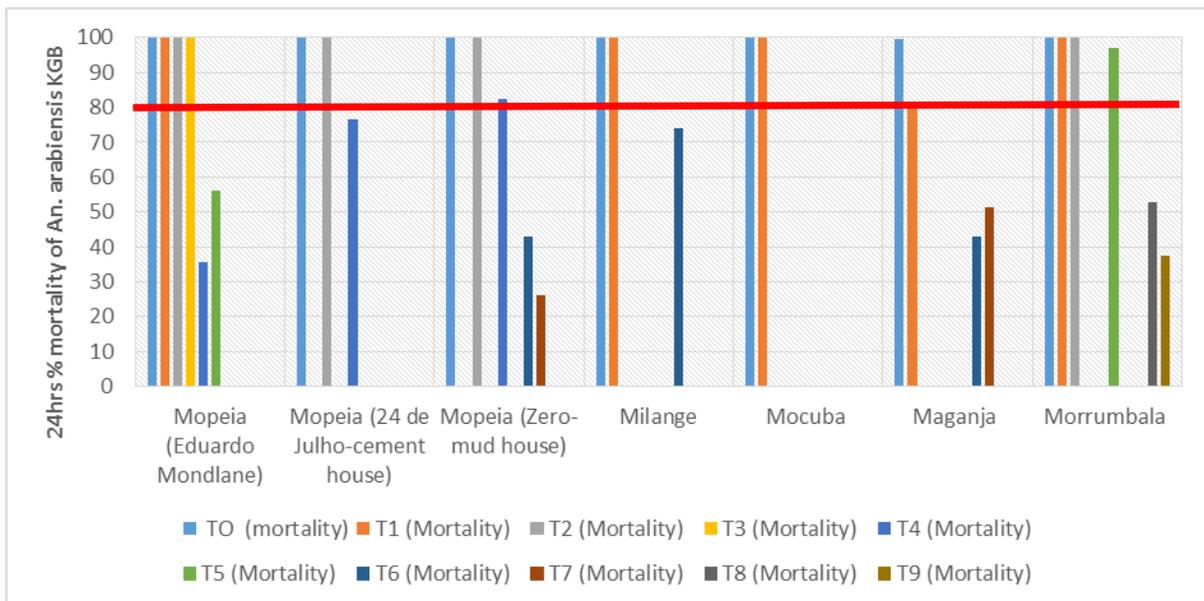
FIGURE 7B. *AN. GAMBIAES.S.L.*



3.2 CONE WALL BIOASSAY TESTS

During spray operations in October 2017, cone wall bioassays were conducted to measure the quality of the spray within the period from 24 hours to 14 days after spray. Thereafter, monthly assays were performed to monitor the insecticide decay rate on various sprayed wall surfaces. Figure 8 shows results of the quality assurance and decay rate monitoring of Actellic® 300CS in Maganja da Costa, Milange, Mocuba, and Morrumbala districts, as well as Mopeia (Eduardo Mondlane, 24 de Julho, and Zero).

FIGURE 8: SPRAY QUALITY ASSESSMENT AND RESIDUAL BIOEFFICACY OF ACTELIC® 300CS



Red line indicates the 80% mortality cutoff point

3.2.1 QUALITY OF SPRAYING

In all sites tested in all five intervention districts, 24-hour mortality was scored at 100 percent. The same mortality was observed at T₁, one month later, in all districts except in Maganja da Costa where T₁ mortality was 80 percent. (See separate, detailed report with data on spray quality, submitted previously.)

3.2.2 INSECTICIDE DECAY RATE

In 24 de Julho and Zero villages in Mopeia, T₀ was conducted in November. We were not able to conduct T₁ tests in these two villages in December, because the insectary did not produce enough mosquitoes for the tests. The most prevalent wall surface types that we assayed were cement and mud. Overall, the 24-hour mortality results of *An. arabiensis* KGB susceptible colony exposed on the sprayed surfaces were above 99.5 percent at T₀. In Eduardo Mondlane village, cone wall bioassays were conducted in all months following T₀. Mortality remained at 100 percent for T₂ and T₃, two and three months post IRS. However, the rates, dropped to 35.6 percent in February (T₄) and 56.3 percent in March (T₅), four and five months post IRS. In 24 de Julho and Zero villages, it was only possible to test up to T₄ and T₇.

Cone wall bioassay tests were not conducted for Mocuba after T₁. In Maganja da Costa, they were conducted up to T₇, in Milange up to T₆, and in Morrumbala up to T₉. Again, an acute shortage in mosquitoes from the insectary produced gaps in the monthly data series. From the available data, it appears that the longest the insecticide effectively persisted on sprayed surfaces was four months (in Mopeia) and five months (in Morrumbala).

3.2.3 THE AIRBORNE EFFECT

Tests for airborne effect of Actellic® 300CS were conducted alongside T₀ and T₁² wall bioassay tests in all five intervention districts (in Mopeia, in Eduardo Mondlane, 24 de Julho, and Zero villages). Results are in Table 8 and Annex Figure A-1. In Zero village (Mopeia), one more airborne effect bioassay test was conducted at T₄; the mortality was scored as 0 percent.

TABLE 8. PERCENT MORTALITY OF *AN. ARABIENSIS* KGB SUSCEPTIBLE STRAIN ON AIRBORNE EFFECT TEST

Districts (Villages)	% 24hr mortality of the airborne effect test		
	T ₀ Immediately after IRS	T ₁ 1 month post-IRS	T ₄ 4 months post IRS
Mopeia (Eduardo Mondlane)	15	18	
Mopeia (24 de Julho)	66.7	10	
Mopeia (Zero)	80	14	0
Milange	14	52	
Mocuba	60	30	
Maganja da Costa	62	48	
Morrumbala	16	10	

² In Zero village (Mopeia), T₁=T₂ because the wall bioassay started later in November, after submission of the quality assurance report.

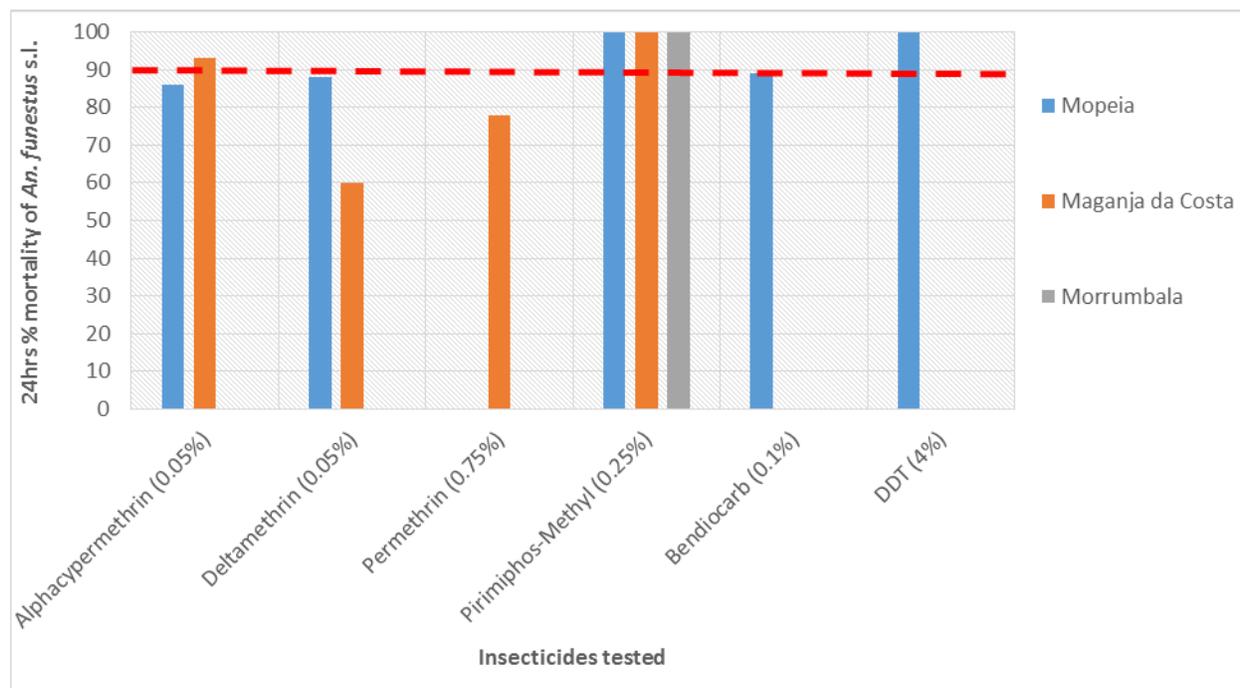
3.3 WHO SUSCEPTIBILITY TESTING

Susceptibility testing was conducted in Maganja da Costa, Milange, Mocuba, Molumbo, and Morrumbala, districts. Tests conducted in Derre and Mopeia could not cover all planned target insecticides due to a shortage of mosquitoes in the field.

The WHO 2016 standard susceptibility test method was used to test the main malaria vector collected, *An. gambiae* s.l. No *An. funestus* s.l. larvae were collected, but *An. funestus* s.l. adults were collected and immediately tested at Maganja da Costa, Mopeia, and Morrumbala.

An. funestus s.l. exposed to pirimiphos-methyl in Maganja da Costa, Mopeia, and Morrumbala were found to be fully susceptible to the product. Possible resistance was detected when testing against alpha-cypermethrin in Maganja da Costa and resistance was detected against alpha-cypermethrin, deltamethrin, and bendiocarb in Mopeia. Resistance to deltamethrin and permethrin was also detected in Maganja da Costa (Figure 9 and Annex Tables A-1 and A-1.1).

FIGURE 9. 24HR MORTALITY FROM THE WHO TUBE TESTS OF ADULT *AN. FUNESTUS* S.L. COLLECTED BY PROKOPACK COLLECTIONS, AUGUST-SEPTEMBER 2017

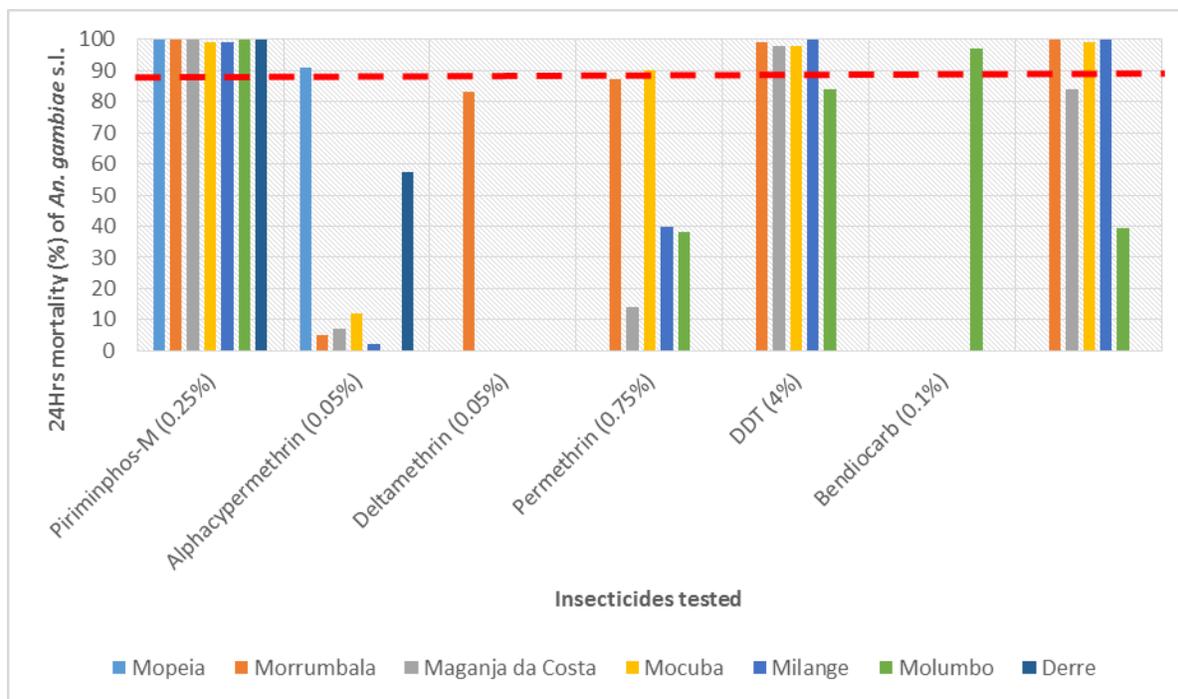


— Red line indicates mortality below 90% are resistant mosquitoes

In Mopeia, Morrumbala, Maganja da Costa, Mocuba, Milange, Molumbo, and Derre, *An. gambiae* s.l. tested against pirimiphos-methyl were found to be susceptible (Figure 10 and Annex Tables A-2 and A-2.1). *An. gambiae* s.l. exposed to DDT were susceptible in Morrumbala, Maganja da Costa, Mocuba, and Milange. Bendiocarb susceptibility was demonstrated by *An. gambiae* in Morrumbala, Mocuba, and Milange. Possible resistance to alpha-cypermethrin was noted in Mopeia, to permethrin in Mocuba, and to bendiocarb in Molumbo. A repeat test in Molumbo two months later gave a lower mortality, 39.36 percent, as opposed to the earlier 97.0 percent mortality. *An. gambiae* s.l. populations in Morrumbala, Maganja da Costa, Mocuba, Milange, and Derre were found to be resistant to alpha-cypermethrin. Resistance to permethrin was detected in the *An. gambiae* s.l. populations from Morrumbala, Maganja da Costa, Milange, and Molumbo. Resistance

to deltamethrin was detected in Morrumbala and to bendiocarb in Maganja da Costa. For the first time since 2012, we detected resistance in *An. gambiae* s.l. to DDT in Molumbo.

FIGURE 10. 24HR MORTALITY OF ADULT *AN. GAMBIAE* S.L. FROM LARVAL COLLECTIONS EXPOSED TO A RANGE OF INSECTICIDES AT RESPECTIVE DIAGNOSTIC CONCENTRATIONS (JANUARY-MARCH 2018)



Red line indicates mortality below 90% are resistant mosquitoes

3.3.1 DETERMINATION OF THE INTENSITY OF RESISTANCE AND SYNERGIST ASSAYS USING WHO TUBE TESTS

Bioassays for intensity of resistance were conducted where *An. gambiae* resistance was detected with the discriminating concentrations (24 hrs. mortality < 90%) of the respective insecticides.

Results of exposure to the 5× permethrin showed that the intensity of resistance was low in Molumbo (100% mortality) and moderate in Morrumbala (79% mortality), Mocuba (97% mortality), and Maganja da Costa (66% mortality) (Figure 11 and Annex Table A-2). Further exposure to 10× permethrin revealed the presence of high-intensity resistance in Milange (91% mortality).

Results of exposure to 5× and 10× alpha-cypermethrin revealed the presence high-intensity resistance in Morrumbala and Milange. Exposure to 10× alpha-cypermethrin confirmed presence of high-intensity resistance in both Morrumbala (74% mortality) and Milange (14% mortality).

FIGURE 11. 24HR MORTALITY OF ADULT *AN. GAMBIAE* S.L. FOLLOWING EXPOSURE TO MULTIPLES OF RESPECTIVE DIAGNOSTIC CONCENTRATIONS (JANUARY-MARCH 2018).

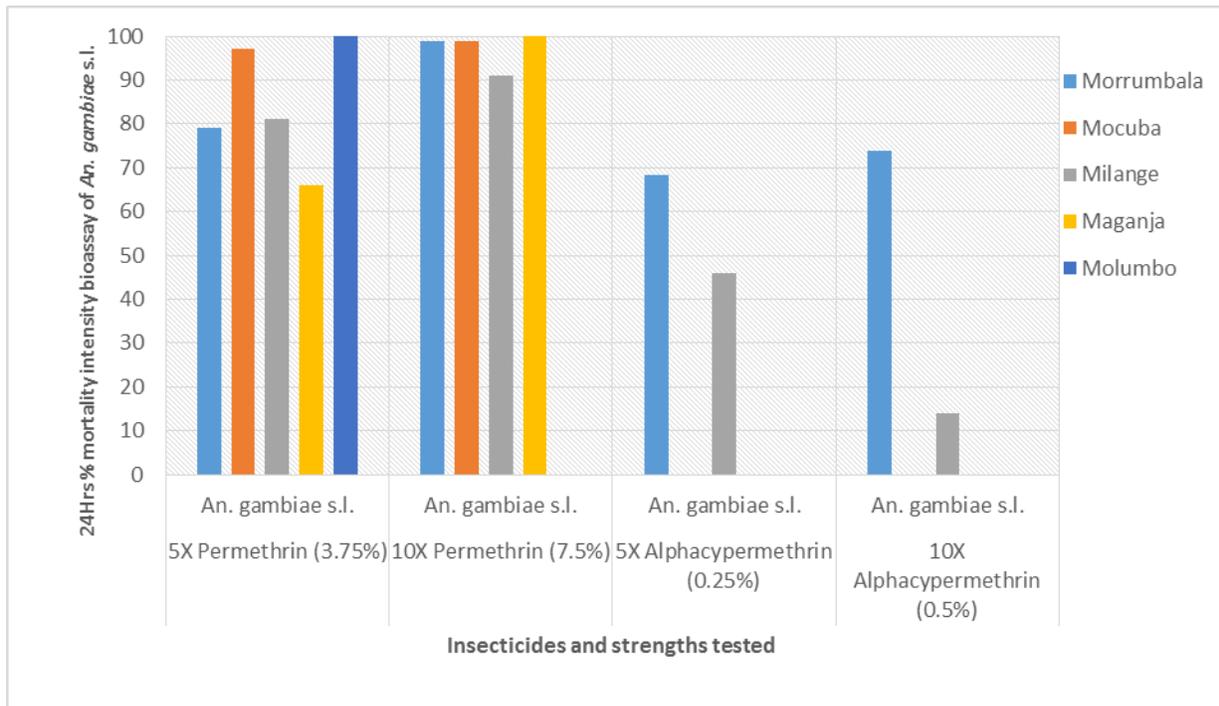
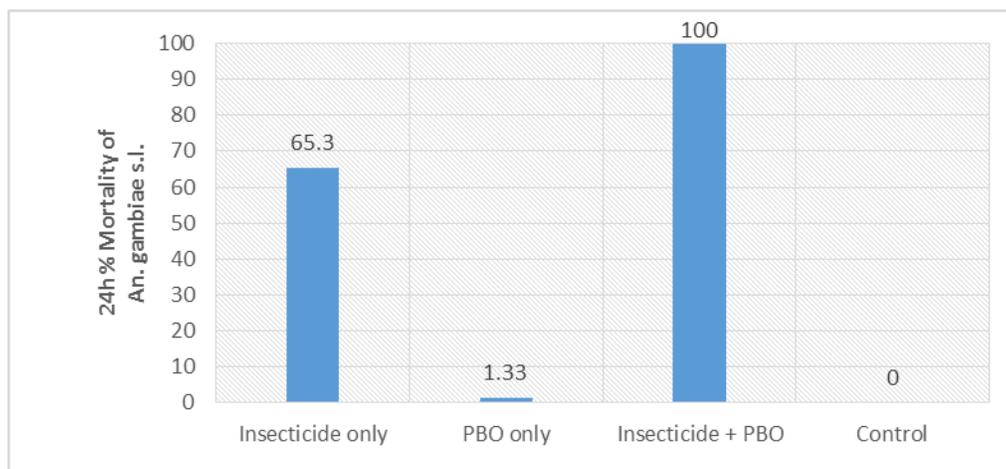


Figure 12 shows the results of synergist assays on *An. gambiae* s.l. from Morrumbala. The synergist PBO restored full susceptibility to alpha-cypermethrin, from 65.3 percent to 100 percent mortality among resistant populations of *An. gambiae* s.l. This observation suggests that monooxygenases are the only form of metabolic resistance prevailing in the area. The test was conducted in Morrumbala only due to shortage of field-collected mosquitoes in the rest of the districts with resistance to pyrethroids.

FIGURE 12: SYNERGIST ASSAY MORTALITY RESULTS IN *AN. GAMBIAE* S.L. FROM MORRUMBALA UPON EXPOSURE TO ALPHA-CYPERMETHRIN ONLY OR 4% PBO + ALPHA-CYPERMETHRIN

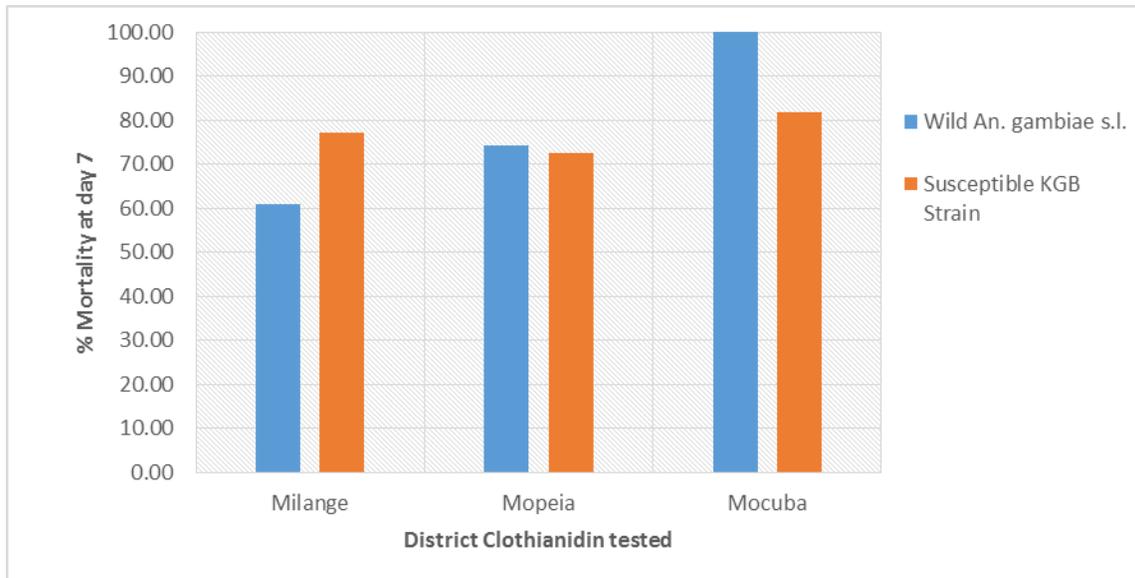


3.4 CLOTHIANIDIN SUSCEPTIBILITY TEST RESULTS

The clothianidin susceptibility test was done according to the PMI AIRS protocol with the standard operating procedure (SOP 001).

The tests were conducted on samples collected in Milange, Mopeia, and Mocuba. Figure 13 shows the results from tests on the wild *An. gambiae* s.l. and the laboratory standard *An. arabiensis* KGB strain. *An. gambiae* s.l. was susceptible to clothianidin in Mocuba after a seven-day holding period. However, tests from Milange and Mopeia showed some *An. gambiae* s.l. there survived. Some *An. arabiensis* KGB susceptible strain also survived. This might be due to potential variation in the process of impregnation of the papers. Variations were observed in mortality in the different replicates, possibly indicating variations of the insecticide doses. Temperature and humidity during clothianidin testing are presented in Annex Table A-9.

FIGURE 13. CLOTHIANIDIN WHO SUSCEPTIBILITY TEST RESULTS ON WILD *AN. GAMBIAE* S.L. AND SUSCEPTIBLE *AN. ARABIENSIS* KGB STRAIN (PERCENTAGE MORTALITY AT 7TH DAY POST-EXPOSURE)



4. DISCUSSION, LESSONS LEARNED, AND CHALLENGES

Results of PSCs show that *An. funestus* s.l. was the most abundant species collected while resting indoors in all surveyed districts. The districts of Milange and Maganja da Costa had the most abundant collections of *An. funestus* s.l. that were scored higher than those in the unsprayed control district of Molevala. In contrast, higher densities of *An. gambiae* s.l. were collected in Molevala than in all the intervention districts, where the species was found in very low numbers. This indicates a relatively quick response of *An. gambiae* s.l. to the IRS intervention.

HLCs demonstrated that the Mopeia control area had highest mean *An. funestus* s.l. biting rate both indoors and outdoors. *An. gambiae* s.l. also were found at higher densities in the control, albeit at much lower densities than *An. funestus* s.l. Furthermore, the HLC revealed significant differences between outdoor and indoor biting densities for both vector species except for *An. funestus* s.l. in Milange and *An. gambiae* s.l. in Molevala. While observed biting activity was consistently higher outdoors than indoors for *An. gambiae* s.l., there were mixed observations with *An. funestus* s.l., where indoor biting was higher than outdoor biting in the intervention sites in Maganja da Costa and Mopeia, as well as the control sites of Molevala and Mopeia. Regarding the overnight biting patterns observed, biting starts in early evening, between 6:00 pm and 8:00 pm at most sites, and generally peaks after midnight, towards morning hours.

Similar to PSC and HLC data, CDC light trap collections showed *An. funestus* s.l. (66%) as the most abundant species, followed by *An. gambiae* s.l. (31.5%), except in Morrumbala where the opposite was observed. The CDC light trap collections indoors demonstrated that the study area has a variety of anopheline species, specifically eight different species across the sampled sites.

The data from Mopeia operational research sites showed that application of IRS in addition to use of LLINs in the intervention sites reduced the vector population as compared with use of LLINs alone. Currently, the samples are being processed for molecular species identification and sporozoite infection in the vector populations. Detailed analysis will be reported as an addendum to this report when the results become available from the WRBU.

The WHO cone wall bioassay results obtained in five of seven sprayed districts showed that the spray quality of the 2017 spray campaign with Actellic® 300CS in all the districts was satisfactory at all monitored sites, with the exception of Maganja da Costa, where mortalities dropped to 81 percent just a month after spraying. The monitoring for insecticide decay rate was unfortunately marred by many gaps because some months had passed without any assays being conducted. Nevertheless, available evidence shows that the longest the insecticide stayed effective on sprayed surfaces was between four months (in Mopeia) and five months (in Morrumbala). This decay rate is slightly faster than what the product manufacturer estimates, as well as observations made elsewhere, which range from six to nine months.

When *An. funestus* s.l. collected by Prokopack in August and September 2017 were subjected to WHO susceptibility tests, results data indicated that the species is susceptible to pirimiphos-methyl in Mopeia, Maganja da Costa, and Morrumbala and to DDT in Mopeia. Resistance to alpha-cypermethrin, deltamethrin, and bendiocarb was noted in Mopeia. Resistance to deltamethrin and permethrin and possible resistance to alpha-cypermethrin was noted in Maganja da Costa. This observation is similar to the previous ones, as well as those observed elsewhere.

Vector susceptibility to pirimiphos-methyl was the same as in previous years. *An. gambiae* s.l. was found to be susceptible to pirimiphos-methyl in Derre, Maganja da Costa, Milange, Mocuba, Molumbo, Mopeia and

Morrumbala. Possible resistance to alpha-cypermethrin was detected in Mopeia, while resistance was detected in Derre, Maganja da Costa, Milange Mocuba, and Morrumbala. Vector resistance to deltamethrin was detected in Morrumbala, while possible resistance to permethrin was detected in Mocuba. In addition, resistance to permethrin was detected across Morrumbala, Maganja da Costa, Milange, and Molumbo. The observed widespread resistance to pyrethroids has become common in sub-Saharan Africa, particularly following extensive roll-out of LLINs that started about a decade ago, in order to achieve universal coverage. Continued lack of resistance to pirimiphos-methyl is good news, but decision makers should consider introducing insecticide rotation with alternative products well before resistance to pirimiphos-methyl sets in. The new compound clothianidin is expected to provide an alternative product to rotate with pirimiphos-methyl as part of good resistance management practice. It was unfortunate, however, that initial susceptibility tests with clothianidin produced doubtful results that urgently call for a repeat of the test.

In areas where resistance was detected on diagnostic doses of pyrethroids and where the mosquito samples were enough for the tests, intensity assays were conducted. Results of intensity assays with 5× and 10× concentrations for both permethrin and alpha-cypermethrin in *An. gambiae* s.l. from across the six districts showed variable results ranging from low intensity of resistance to moderate and high intensity of resistance. This has to be monitored regularly and in wider geographical ranges to determine areas where attention is needed to mitigate the impact of insecticide resistance on malaria vector control through deployment of next generation IRS and LLIN products.

Results of synergist assays conducted at Morrumbala suggest the presence of metabolic resistance possibly is due to monooxygenases. We propose to conduct similar tests more broadly in order to map the extent of this mechanism in Mozambique. Potentially, it is a good indicator that the newly recommended PBO net may be effective in managing the widely observed pyrethroid resistance through mass deployment.

Molecular assays are ongoing at WRBU in the United States. A separate addendum to this report will be prepared and submitted after the assays are completed.

5. REFERENCES

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6. ANNEX

TABLE A-1. PERCENTAGE MORTALITY *AN. FUNESTUS* S.L. USING WHO TUBE TESTS AFTER THE 24HR HOLDING PERIOD WITH DIAGNOSTIC CONCENTRATIONS (AUGUST-SEPTEMBER 2017)

Districts	Pyrethroids			Organophosphates	Carbamate	Organochlorine
	Alpha-cypermethrin (0.05%)	Deltamethrin (0.05%)	Permethrin (0.75%)	Pirimiphos-methyl (0.25%)	Bendiocarb (0.1%)	DDT (4%)
Mopeia	86% (100) R*	88% (100) R	NA	100% (100) S	89% (100) R	100% (100) S
Maganja da Costa	93% (100) PR	60% (100) R	78% (100) R	100% (100) S	NA	NA
Morrumbala	NA	NA	NA	100% (100) S	NA	NA

*No intensity testing with *An. funestus* was conducted due to insufficient mosquitoes.

**R (resistance)

** S (susceptible)

**PR (possible resistance)

**Numbers in parenthesis are total number of mosquitoes exposed.

TABLE A-1.1 PERCENTAGE MORTALITY *AN. FUNESTUS* S.L. USING WHO TUBE TESTS AFTER THE 24HR HOLDING PERIOD WITH DIAGNOSTIC CONCENTRATIONS (HISTORICAL DATA)

Districts	Years of susceptibility test	Pyrethroids				Organophosphates		Carbamate	Organochlorine
		Alpha-cypermethrin (0.05%)	Deltamethrin (0.05%)	Permethrin (0.75%)	Lambda-cyhalothrin (0.05%)	Pirimiphos-methyl (0.25%)	Fenitrothion 1%	Bendiocarb (0.1%)	DDT (4%)
Mopeia	2018	86% (100) R	88% (100) R	NA	NA	100% (100) S	NA	89% (100) R	100% (100) S
	2017	NA	NA	NA	NA	NA	NA	NA	NA
	2016	NA	NA	NA	NA	NA	NA	NA	NA
	2015	NA	NA	NA	NA	NA	NA	NA	NA
	2014	NA	NA	NA	NA	NA	NA	NA	NA
	2013	NA	NA	NA	NA	NA	NA	NA	NA
	2012	NA	NA	NA	NA	NA	NA	NA	NA
Maganja	2018	93% (100) PR	60% (100) R	78% (100) R	NA	100% (100) S	NA	NA	NA
	2017	NA	NA	NA	NA	NA	NA	NA	NA
	2016	NA	NA	NA	NA	NA	NA	NA	NA
	2015	NA	NA	NA	NA	NA	NA	NA	NA
	2014	NA	NA	NA	NA	NA	NA	NA	NA
	2013	NA	NA	NA	NA	NA	NA	NA	NA
	2012	NA	NA	NA	NA	NA	NA	NA	NA
Morrumbala	2018	NA	NA	NA	NA	100% (100) S	NA	NA	NA
	2017	NA	NA	NA	NA	NA	NA	NA	NA
	2016	NA	NA	NA	NA	NA	NA	NA	NA
	2015	NA	NA	NA	NA	NA	NA	NA	NA
	2014	NA	92.39% (100)PR	NA	100%(100) S	NA	100%(100) S	99%(100) S	100% (100)S
	2013	NA	NA	NA	NA	NA	NA	NA	NA
	2012	NA	NA	NA	NA	NA	NA	NA	NA

Districts	Years of susceptibility test	Pyrethroids				Organophosphates		Carbamate	Organochlorine
		Alpha-cypermethrin (0.05%)	Deltamethrin (0.05%)	Permethrin (0.75%)	Lambda-cyhalothrin (0.05%)	Pirimiphos-methyl (0.25%)	Fenitrothion 1%	Bendiocarb (0.1%)	DDT (4%)
Nicoadala	2018	NA	NA	NA	NA	NA	NA	NA	NA
	2017	NA	NA	NA	NA	NA	NA	NA	NA
	2016	NA	NA	NA	NA	NA	NA	NA	NA
	2015	NA	NA	NA	NA	NA	NA	NA	NA
	2014	NA	NA	NA	NA	NA	NA	NA	NA
	2013	NA	NA	NA	100% (100) S	NA	NA	NA	100%(100) S
	2012	NA	NA	NA		NA	NA	NA	NA
Derre	2018	NA	NA	NA	NA	NA	NA	NA	NA
	2017	NA	NA	NA	NA	NA	NA	NA	NA
	2016	NA	NA	NA	NA	NA	NA	NA	NA
	2015	NA	NA	NA	NA	NA	NA	NA	NA
	2014	NA	NA	NA	NA	NA	NA	NA	NA
	2013	NA	NA	NA	NA	NA	NA	NA	NA
	2012	NA	NA	NA	NA	NA	NA	NA	NA
Molumbo	2018	NA	NA	NA	NA	NA	NA	NA	NA
	2017	NA	NA	NA	NA	NA	NA	NA	NA
	2016	NA	NA	NA	NA	NA	NA	NA	NA
	2015	NA	NA	NA	NA	NA	NA	NA	NA
	2014	NA	NA	NA	NA	NA	NA	NA	NA
	2013	NA	NA	NA	NA	NA	NA	NA	NA
	2012	NA	NA	NA	NA	NA	NA	NA	NA
Milange	2018	NA	NA	NA	NA	NA	NA	NA	NA
	2017	NA	NA	NA	NA	NA	NA	NA	NA
	2016	NA	NA	NA	NA	NA	NA	NA	NA
	2015	NA	NA	NA	NA	NA	NA	NA	NA

Districts	Years of susceptibility test	Pyrethroids				Organophosphates		Carbamate	Organochlorine
		Alpha-cypermethrin (0.05%)	Deltamethrin (0.05%)	Permethrin (0.75%)	Lambda-cyhalothrin (0.05%)	Pirimiphos-methyl (0.25%)	Fenitrothion 1%	Bendiocarb (0.1%)	DDT (4%)
	2014	NA	100%(50) S	NA	NA	NA	NA	NA	NA
	2013	NA	92%(100) PR	NA	NA	NA	NA	NA	NA
	2012	NA	NA	NA	NA	NA	NA	NA	NA
	2018	NA	NA	NA	NA	NA	NA	NA	NA
	2017	NA	NA	NA	NA	NA	NA	NA	NA
	2016	NA	NA	NA	NA	NA	NA	NA	NA
	2015	NA	NA	NA	NA	NA	NA	NA	NA
	2014	NA	NA	NA	NA	NA	NA	NA	NA
	2013	NA	94%(100)PR	NA	93%(100)PR	NA	94%(100) PR	98%(100) S	100%(100) S

TABLE A-2. PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L., USING WHO TUBE TESTS AFTER 24HR HOLDING PERIOD WITH DIAGNOSTIC AND INTENSITY BIOASSAY CONCENTRATIONS (JANUARY-MARCH 2018)

Districts	Organophosphates	Pyrethroids							Organochlorine	Carbamate
	Pirimiphos-methyl (0.25%)	Alpha-cypermethrin (0.05%)	Alpha-cypermethrin (0.25%)	Alpha-cypermethrin (0.5%)	Deltamethrin (0.05%)	Permethrin (0.75%)	Permethrin (3.75%)	Permethrin (7.5%)	DDT (4%)	Bendiocarb (0.1%)
Mopeia	100% (100) S	91% (100) PR	NA	NA	NA	NA	NA	NA	NA	NA
Morrumbala	100% (100) S	5% (100) R	68.4% (100) R	74% (100) R	83% (100) R	87.21% (100) R	79% (100) R	99% (100) S	99% (100) S	100% (100) S
Maganja da Costa	100% (100) S	7% (100) R	NA	NA	NA	14%(100) R	66%(100) R	100% (100) S	98%(100) S	84%(100) R
Mocuba	99% (100) S	12%(100) R	NA	NA	NA	90% (100) PR	97%(100) PR	99%(100) S	98%(100) S	99%(100) S
Milange	99%(100) S	2%(100) R	46%(100) R	14%(100) ³ R	NA	40%(100) R	81%(100) R	91%(100) PR	100% (100) S	100% (100) S
Molumbo	100% (100) S	NA	NA	NA	NA	38%(100) R	100% (100) S	NA	84%(100) R	97%(100) PR
										39.36%(100) R
Derre	100% (100) S	57.44%(100) R	NA	NA	NA	NA	NA	NA	NA	NA

³ Alpha-cypermethrin intensity assay results in Milange are not what was expected; ideally, mortality at 10x intensity assay should be higher than at 5x.

TABLE A-2.1. PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L., USING WHO TUBE TESTS AFTER 24HR HOLDING PERIOD WITH DIAGNOSTIC BIOASSAY CONCENTRATIONS (HISTORICAL DATA)

Districts	Years of susceptibility test	Organophosphates		Pyrethroids				Organochlorine	Carbamate
		Pirimiphos-methyl (0.25%)	Fenitrothion (1%)	Alpha-cypermethrin (0.05%)	Deltamethrin (0.05%)	Permethrin (0.75%)	Lambda-cyhalothrin (0.05%)	DDT (4%)	Bendiocarb (0.1%)
Mopeia	2018	100% (100) S	NA	91% (100)R	NA	NA	NA	NA	NA
	2017	100 % (100) S	NA	100% (100) S	NA	NA	NA	NA	NA
Morumbala	2018	100% (100) S	NA	5% (100) R	83% (100) R	87.21% (100) R	NA	99% (100) S	100% (100) S
	2017	100 % (100) S	NA	97% (100) PR	NA	91% (100) PR	91 % (100)R	100 % (100) S	100 % (100) S
	2016	100% (100) S	NA	NA	34.44 (100)S	NA	33% (100) R	100% (100) S	100% (100) S
	2015	NA	100%(100) S	NA	90.67%(100)R	NA	68.75%(100)R	100%(100)S	100%(100)S
	2014	NA	NA	NA	NA	NA	NA	NA	NA
	2013	NA	100%(100) S	NA	97%(100) PR	NA	95%(100)R	98%(100)	100%(100) S
	2012	NA	NA	NA	100%(40)	NA	NA	NA	NA
Maganja da Costa	2018	100% (100) S	NA	7% (100) R	NA	14%(100) R	NA	98%(100) S	84%(100) R
	2017	100 % (100) S	NA	94 % (100) PR	NA	99 % (100) S	NA	98 % (100) S	99 % (100) S
Mocuba	2018	99% (100) S	NA	12%(100) R	NA	90% (100) PR	NA	98%(100) S	99%(100) S
	2017	99 % (100) S	NA	99% (100) S	NA	97% (100) PR	NA	100 % (100) S	97 % (100) Possible resistance
	2016	100% (100)S	NA	NA	52% (100)R	NA	40.21% (100)R	97.87 % (100) PR	98%(100)S
	2015	NA	100%(100)S	NA	74.33% (100) R	NA	92.33% (100)R	100%(100) S	98.9% (100) S
	2014	NA	100%(100) S	NA	100% (100)S	NA	100%(100) S	100% (100) S	100% (100) S
	2013	NA	NA	NA	NA	NA	NA	NA	NA
	2012	NA	NA	NA	100% (60) S	NA	90% (40)R	NA	NA

Districts	Years of susceptibility test	Organophosphates		Pyrethroids			Lambda-cyhalothrin (0.05%)	Organochlorine	Carbamate
		Pirimiphos-methyl (0.25%)	Fenitrothion (1%)	Alpha-cypermethrin (0.05%)	Deltamethrin (0.05%)	Permethrin (0.75%)		DDT (4%)	Bendiocarb (0.1%)
Milange	2018	99%(100) S	NA	2%(100) R	NA	40%(100) R	NA	100% (100) S	100% (100) S
	2017	99 % (100) S	NA	99% (100) S	NA	95% (100) PR	NA	99 % (100) S	100 % (100) S
	2016	100% (100) S	NA	NA	71% (100) R	NA	45% (100) R	100% (100) S	100% (100) S
	2015	NA	NA	NA	100%(100) S	NA	NA	100%(100)	NA
	2014	NA	NA	NA	NA	NA	NA	NA	NA
Molumbo	2018	100% (100) S	NA	NA	NA	38%(100) R	NA	84%(100) R	97%(100) PR
	2017	100 % (100) S	NA	97% (100) PR	NA	96 % (100) PR	NA	NA	NA
Derre	2018	100% (100) S	NA	57.44%(100) R	NA	NA	NA	NA	NA
	2017	100 % (100) S	NA	100% (100) S	NA	98 % (100) S	NA	100 % (100) S	NA
Nicoadala	2013	NA	NA	NA	100%(100)S	NA	NA	NA	100%(100) S
	2012	NA	NA	NA	100%(100)S	NA	NA	NA	NA

TABLE A-3. WHO INTERPRETATION OF SUSCEPTIBILITY TESTS FOR DETERMINING INTENSITY OF RESISTANCE

Susceptibility test with 5x intensity concentration		Susceptibility test with 10x intensity concentration	
≥ 98% mortality	Low intensity resistance	≥ 98% mortality	Moderate intensity resistance
< 98% mortality	Moderate to high intensity resistance	< 98% mortality	High intensity resistance

TABLE A-4. TOTAL HLC MONTHLY COLLECTION OF *AN. FUNESTUS* S.L., INDOOR IN THE INTERVENTION AND CONTROL

Collection months	Total collection for intervention and control districts				
	Mocuba	Milange	Morrumbala	Maganja	Molevala
	<i>An. funestus</i> s.l.	<i>An. funestus</i> s.l.	<i>An. funestus</i> s.l.	<i>An. funestus</i> s.l.	<i>An. funestus</i> s.l.
	Indoor (Intervention)	Indoor (Intervention)	Indoor (Intervention)	Indoor (Intervention)	Indoor (Control)
July	0	33	0	26	43
August	1	0	0	25	35
September	0	12	0	45	44
October	0	3	0	20	21
November	2	6	0	53	3
December	4	32	0	1	3
January	2	28	1	3	46
February	0	3	1	5	39
March	1	3	1	2	14
April	ND	ND	ND	ND	ND
May	---	6	---	10	17
June	---	2	---	8	13

TABLE A-5. TOTAL HLC MONTHLY COLLECTION OF *AN. FUNESTUS* S.L., OUTDOORS IN THE INTERVENTION AND CONTROL

Collection period and months		Total collection for intervention and control districts				
		Mocuba	Milange	Morrumbala	Maganja	Molevala
		<i>An. funestus</i> s.l.	<i>An. funestus</i> s.l.	<i>An. funestus</i> s.l.	<i>An. funestus</i> s.l.	<i>An. funestus</i> s.l.
		Outdoor (Intervention)	Outdoor (Intervention)	Outdoor (Intervention)	Outdoor (Intervention)	Outdoor (Control)
Pre-spray	July	1	16	0	10	21
	August	3	0	0	5	9
	September	3	14	0	14	32
Post - Spray	October	0	4	0	13	20
	November	8	3	0	13	1
	December	2	57	0	0	4
	January	4	27	1	8	38
	February	0	2	0	11	47
	March	0	1	2	8	17
	April	ND	ND	ND	ND	ND
	May	---	7	---	4	9
	June	---	4	---	2	7

TABLE A-6. TOTAL HLC MONTHLY COLLECTION OF *AN. GAMBIAE* S.L., INDOOR IN THE INTERVENTION AND CONTROL DISTRICTS

Collection period and months		Total collection for intervention and control districts				
		Mocuba	Milange	Morrumbala	Maganja	Molevala
		<i>An. gambiae</i> s.l.	<i>An. gambiae</i> s.l.	<i>An. gambiae</i> s.l.	<i>An. gambiae</i> s.l.	<i>An. gambiae</i> s.l.
		Indoor (Intervention)	Indoor (Intervention)	Indoor (Intervention)	Indoor (Intervention)	Indoor (Control)
Pre-spray	July	1	1	0	1	4
	August	0	0	0	1	2
	September	1	0	0	1	1
Post - Spray	October	0	0	0	14	4
	November	1	1	0	31	21
	December	4	1	4	17	5
	January	3	9	9	7	21
	February	0	6	37	8	15
	March	1	3	108	3	12
	April	ND	ND	ND	ND	0
	May	---	1	---	0	2
	June	---	0	---	1	5

TABLE A-7. TOTAL HLC MONTHLY COLLECTION OF *AN. GAMBIAE* S.L., OUTDOORS IN THE INTERVENTION AND CONTROL

Collection period and months		Total collection for intervention and control districts				
		Mocuba	Milange	Morrumbala	Maganja	Molevala
		<i>An. gambiae</i> s.l. Outdoor (Intervention)	<i>An. gambiae</i> s.l. Outdoor (Intervention)	<i>An. gambiae</i> s.l. Outdoor (Intervention)	<i>An. gambiae</i> s.l. Outdoor (Intervention)	<i>An. gambiae</i> s.l. Outdoor (Control)
Pre-spray	July	0	0	0	1	1
	August	0	0	0	0	0
	September	1	0	0	4	0
Post - Spray	October	1	0	0	26	3
	November	3	0	0	60	10
	December	3	8	4	36	3
	January	5	22	22	13	32
	February	0	22	203	25	23
	March	1	11	218	8	13
	April					
	May	---	2	---	1	2
	June	---	2	---	2	4

TABLE A-8. SPRAY DATE PER DISTRICT AND VILLAGES

District	Village	Spray date
Mocuba	Samora Machel	NA
	Muanaco	NA
Milange	12 de Outubro	11/12/2017
	3 de Fevereiro	13/12/2017
Morrumbala	Coqueiro	6/12/2017
	Franqueza	9/12/2017
Maganja da Costa	Motinho	25/11/2017
	Nante	19/10/17
Molevala	7 de Abril	NA
	25 de Junho	NA

TABLE A-9. TEMPERATURE AND HUMIDITY DURING CLOTHIANIDIN TEST

Days of test	Mopeia		Milange		Mocuba	
	Mean daily readings		Mean daily readings		Mean daily readings	
	Temp, °C	RH, %	Temp, °C	RH, %	Temp, °C	RH, %
30'	23.6	71.0	24.5	69	24.6	76
60'	22.9	79.0	24.5	69.0	24.6	76
24h	24.0	80.0	22.5	82.0	24.6	76
48h	24.1	84.0	25.9	78.0	24.6	76.0
72h	24.3	85.0	22.7	79.0	25.0	79.0
96h	22.0	78.0	23.4	84.0	25.3	81.0
120h	25.0	88.0	23.4	82.0	24.6	86.0
144h	25.0	86.0	24.6	83.0	24.6	76.0
168h	25.0	88.0	24.3	83.0	24.6	76.0

FIGURE A-1. THE AIRBORNE EFFECT OF SPRAYED INSECTICIDE ACTELIC® 300CS AT DIFFERENT SITES

