U.S. PRESIDENT'S MALARIA INITIATIVE

PMI | AFRICA IRS (AIRS) PROJECT INDOOR RESIDUAL SPRAYING (IRS 2) TASK ORDER SIX

MOZAMBIQUE ENTOMOLOGICAL MONITORING ANNUAL REPORT JULY 2016 TO JUNE 2017

OCTOBER 2017

Recommended Citation: The PMI Africa Indoor Residual Spraying Project (PMI AIRS). August 2017. Mozambique: Entomological Monitoring Annual Report. Maputo, Mozambique: PMI AIRS, Abt Associates Inc.

Contract: GHN-I-00-09-00013-00

Task Order: AID-OAA-TO-14-00035

Submitted to: United States Agency for International Development/PMI

Abt Associates Inc. | 4550 Montgomery Avenue | Suite 800 North | Bethesda, Maryland 20814 | T. 301.347.5000 | F. 301.913.9061 | www.abtassociates.com

MOZAMBIQUE ENTOMOLOGICAL MONITORING ANNUAL REPORT

OCTOBER 2017

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-linked Immunosorbent Assay
HLC	Human Landing Catch
IDS	Health Demographic Inquiry
LLINs	Long Lasting Insecticide Treated Bednets
IRS	Indoor Residual Spray
KD	Knock Down
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WHO	World Health Organization
HBR	Human Biting Rate
WHOPES	World Health Organization Pesticide Evaluation Scheme
NICD	National Institute for Communicable Diseases
INS	National Institute of Health/Instituto Nacional de Saúde

Executive Summary

Indoor residual spraying (IRS) and 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) implements the President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) Project in close collaboration with Mozambique's National Malaria Control Program (NMCP), the Provincial Directorate of Health (PDH) in Zambezia 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 & Rural Development (MITADER) at the provincial and district levels.

During the 2016 spray campaign, AIRS Mozambique conducted IRS with pirimiphos-methyl in seven target districts (Derre, Milange, Mocuba, Molumbo, Quelimane, Mopeia, and Morrumbala). To guide proper targeting of IRS, monthly entomological monitoring was performed using CDC light traps, human landing catches, pyrethrum spray collections, and cone wall bioassays (used only in sprayed areas). Seasonal insecticide susceptibility tests were carried out in six sprayed districts (Quelimane, Mocuba, Morrumbala, Milange, Derre, Molumbo, and Mopeia) and one non-sprayed district (Maganja da Costa).

In Mopeia district a cluster-randomized trial on the impact and cost effectiveness of combining indoor residual spraying (IRS) with a non-pyrethroid, next generation IRS product and standard long-lasting insecticidal nets (LLIN) in an area with high malaria transmission is underway. Key methodological considerations related to the study are comparison of vector density, biting rate, sporozoite rate and EIR between the treatment and control arms. CDC light trap collections and human landing catches were used for sampling mosquitoes in the study areas.

From July 2016 to June 2017, 18,444 anopheline mosquitoes were collected, mainly *An. funestus* s.l. and *An. gambiae* s.l., which are regarded as the main vectors of malaria in Africa. Other species caught in small numbers included *An. coustani*, An. *ziemanni*, *An. tenebrosus*, *An. caliginosus*, *An. pretoriensis*, *An. maculipalpis*, *An. rufipes*, *An. squamosus*, *An. pharoensis*, and *An. rivulorum*, whose role as malaria vectors remain to be investigated in the settings of surveyed districts, although these species have been found with *Plasmodium falciparum* and *P. vivax* sporozoites in their salivary glands in other countries in Africa.

Following IRS, *An. funestus* s.l. and *An. gambiae* s.l. densities were suppressed. Two peaks of *An. gambiae* s.l. were observed in December and February to March, while peak indoor density of *An. funestus* s.l. occurred between April and June.

An. gambiae s.l. was collected mainly outdoors, whereas *An. funestus* s.l. was predominantly found indoors, even after IRS, except in Mopeia District where biting occurred both indoors and outdoors. Biting activity seems to follow human sleep patterns, with peak indoor biting activity between 23:00 and 04:00.

In Mopeia, *An. ziemanni* was observed to bite humans in intervention areas.

An. gambiae s.l. remains susceptible to pirimiphos-methyl, the insecticide used in the 2016 IRS campaign. Since adult *An. funestus* s.l. are the most abundant anopheline mosquitoes inside houses in all IRS target districts, it is critical that it be used in susceptibility testing. However, larval breeding sites of this species are nearly impossible to detect, and, therefore, susceptibility testing should be done with adults collected *An. funestus* s.l. mosquitoes.

The quality assurance results of Actellic 300CS were acceptable, with 100% mortality observed for susceptible *An. arabiensis* colony mosquitoes in all districts after spraying. Residual efficacy of the insecticide in most districts did not drop below the cutoff of 80% mortality until March (T5), except in Mopeia (Eduardo Mondlane and Cimento villages).

Our findings highlight heterogeneity in mosquito vector population composition and behavior in the monitoring areas.

1. INTRODUCTION

Through PMI support, Abt has implemented five rounds of IRS in Mozambique, specifically in Zambezia province. During the 2016 spray campaign, AIRS Mozambique conducted IRS in seven target districts (Derre, Milange, Mocuba, Molumbo, Quelimane, Mopeia, and Morrumbala). The PMI AIRS Mozambique program includes entomological monitoring activities in Zambezia and support to the NMCP's entomological activities countrywide to enhance capacity for entomological monitoring. Entomological activities are essential to (a) supplement epidemiological data to guide proper targeting of IRS; (b) evaluate the susceptibility level of the local vectors to different insecticides, and know the underlying mechanisms of resistance to inform selection of insecticides; (c) ensure the quality of spraying; (d) monitor the impact of IRS on vector density, vector behavior, and composition; and (e) monitor the residual life of different insecticides on different types of wall surfaces. This entomological monitoring final report covers the period July 1, 2016 – June 30, 2017.

Entomological monitoring was conducted in four IRS target districts, namely Mocuba, Milange, Morrumbala, and Mopeia, and in one control district, Maganja da Costa. Mopiea is the cost effectiveness study district, where entomological data collection is conducted in ten (10) villages, five from the intervention villages, and five in control villages. Data collection is based on two methods, indoor CDC light trap and human landing collection (HLC) for operational research in Mopeia. CDC light trap collection is conducted in all villages from both arms of the study, and HLC is conducted in eight villages, half from each of the study arms. Indoor CDC light traps are set up in eight houses per village, while human landing catches are conducted in one house per village.

In February 2017, the Zambezia insectary was destroyed by a fire at the provincial medical stores, resulting in a complete loss of the insectary, entomology laboratory and some entomological data. As the result of the fire, AIRS lost the entire susceptible mosquito colony, equipment, materials, data binders, digital data not backed up to the server, and data that was in the process of entry. In terms of samples, the loss included (i) PSC samples from July 2016 through January 2017 for Morrumbala, Milange, Mocuba, and Maganja da Costa districts; (ii) HLC samples from July 2016 through January 2017 for Morrumbala, Mocuba, Milange, and Maganja da Costa districts, and September 2016 to January 2017 for Mopeia; and (iii) CDC light trap samples from July 2016 through January 2017 for Morrumbala, Milange, Mocuba and Maganja da Costa, and for Mopeia from September 2016 through January 2017. In terms of digital data which had not been entered and/or not backed up, the loss included (i) PSC data from October 2016 through January 2017 for Morrumbala, July 2016 through October 2016, and December 2016 through January 2017 for Milange, and July 2016 through January 2017 for Mocuba and Maganja da Costa; (ii) HLC data from December 2016 through January 2017 from Morrumbala, Milange, Mocuba and Maganja da Costa, and for January and February 2017 for Mopeia district; and (iii) CDC light trap data for January 2017 for Morrumbala, Mocuba, and Milange and Maganja da Costa districts. For Mopeia District, January 2017 data were lost in the fire, and in February 2017 the collections were not conducted.

The sample and digital data loss had an impact on the content of this final report.

2. METHODOLOGY

2.1 BEHAVIOR AND DENSITY

Pyrethrum spray catch, human landing catches and CDC light trap collections were used for mosquito collections in IRS districts (Mocuba, Morrumbala and Milange) and a control district (Maganja da Costa) for routine entomological monitoring. CDC light trap collections and human landing catches were used for sampling mosquitoes in both the treatment and control arms of operational research sites in Mopeia. See Annex A for a summary of collection types, number of houses, and number of villages and sites.

2.1.1 PYRETHRUM SPRAY CATCH (PSC) COLLECTIONS

In each IRS district (Mocuba, Morrumbala, and Milange) and one control district (non-spraying area) (Maganja da Costa), indoor resting mosquitoes were collected by PSC in 20 selected houses per district from 6 AM to 8 AM. PSCs were conducted once per month over four consecutive days in each district. PSC data was collected in five houses per day per district and per site. The first collection was conducted three months before the spraying campaign and the second collection occurred during and after the spray campaign. The 20 houses were selected randomly at different distances to cover the area selected in each village for the monitoring. The aerosol used for PSC is Baygon (commercial nomenclature), which contains the pyrethroids Deltamethrin 0.5 g/kg and Imiprothrin 1,0g/kg. In each house, one sleeping room was selected for spraying with Baygon. The room was closed for 10 minutes after spraying, and knocked down mosquitoes were collected using forceps into a labeled petri dish. The samples were identified morphologically and preserved in 1.5 ml Eppendorf tube containing silica gel for further analyses.

2.1.2 HUMAN LANDING CATCHES (HLC)

HLC was conducted in Mocuba, Morrumbala, Milange, and Mopeia, and in Maganja da Costa, the control (non-spraying area) area. In all districts except Mopeia where operational research is ongoing, two houses were sampled in each selected village and the collection is performed on three (3) consecutive nights per month to obtain six (6) person-nights of collection/district/month (2 houses x 3 collection nights = 6 person-nights). In Mopeia, the cost – effectiveness study district, the data were collected in 8 houses, four in core areas of the intervention villages and other four in core areas of control villages. Collections were conducted for three nights to obtain 12 person-nights of collection in the core intervention and control villages/month (4 houses x 3 collection nights = 12 person-nights).

Across districts, two human volunteers were positioned with one inside of the house, and the other outside to collect mosquitoes. Collections were conducted from 6:00 PM to 6:00 AM for three consecutive nights per month. During each hour of collection, collectors collected mosquitoes for 50 minutes and rested for 10 minutes, during which time they exchanged positions and recorded humidity and temperature. During the time of collection, the collector sat quietly on a small chair and exposed part of his legs up to the knees, and when they felt landing mosquitoes, they turned on a torch and made collections with the help of 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 Collections

CDC light traps were installed in four houses in each of three standard IRS intervention districts, Mocuba, Morrumbala, and Milange, and in four houses in the control district, Maganja da Costa. In Mopeia district, for CDC light traps, 10 villages were selected (five in the intervention area and five in the control area). CDC light traps were set-up in 8 houses (1 trap per house) per village in the intervention areas, and 8 CDC light traps (1 trap per house) per village in the control area. The traps were set-up inside the houses in the bedroom about 1.5 m above the floor beside the foot of the bed where humans slept under untreated bed nets. After each night of collection, the mosquitoes were killed in paper cups with chloroform, identified, and preserved in 1.5 Eppendorf tube for future molecular species identification by PCR.

Data were collected monthly during three consecutive nights, from 6 pm to 6 am, from July 2016 to June 2017. In Mopeia, the data were collected from September 2016 and will continue until the end of the study in December 2018.

In Mocuba, Milange, Morrumbala, and Maganja da Costa, the CDC light trap data were collected monthly in four houses for three consecutive nights, resulting in 12 trap nights per month in each of the intervention and control district. In Mopeia the process is similar with increased numbers of houses. Data is collected in eight houses for three consecutive nights in five villages from the intervention area and in other five in the control area, resulting in 120 traps nights per month in the intervention areas and 120 trap nights in the control areas. In all intervention villages from Mopeia district, AIRS expected to collect CDC light trap data in 40 sprayed houses. But seven houses were found to be not-sprayed due to refusals and/ locked during spraying (four houses in 4 October village, two houses in 25 June village, and one house in 7 April village), as shown in Annex B. These houses were replaced with sprayed houses and data was collected for both the sprayed and non-sprayed houses. Collection of data in the non-sprayed houses of the intervention villages was discontinued between May and June 2017.

2.2 WHO Susceptibility Testing

An. gambiae s.l. was collected from different larval habitats in Mocuba, Morrumbala, Milange, Derre, Molumbo, Maganja, and Mopeia districts in September/October 2016 and January to March 2017.

In the insectary, the field collected larvae were reared to adult stage. Batches of 25 females, which were sugar-fed, aged from 3 – 5 days, were subsequently subjected to the WHO tube tests following the standard protocol (WHO, 2013). These females were exposed to pirimiphos-methyl 0.25%, alphacypermethrin 0.5%, permethrin 0.75%, DDT 4%, bendiocarb 0.1%, and lambdacyhalothrin 0.05% in WHO impregnated filter papers for 60 minutes, and the knockdown was checked at 10, 15, 20, 30, 40, 50, and 60 minutes. After this period, all mosquitoes were gently transferred to holding tubes and knockdown was again checked at 80 minutes, and mortality was recorded at 24 hours post-exposure. Susceptibility levels of *An. gambiae* s.l. were evaluated based on WHO criteria (WHO 2013). The WHO classifies 24 hour mortality rates from susceptibility tests higher than 98% as susceptible, between 90% and 97% as suggestive of resistance and requiring further investigation, and below 90% as resistant.

Every effort was made to collect *An. funestus* s.l. larvae in September and October. Unfortunately, the one potential breeding site for *An. funestus* s.l. was the Cucua River in Mopeia, where access was not possible due to the presence of crocodiles. All other breeding sites produced *An. gambiae* s.l., in Mopeia and other districts.

2.3 Cone Wall Bioassays

The standard WHO cone bioassay tests were performed in Mocuba, Milange, Morrumbala, Quelimane, and Mopeia (Eduardo Mondlane and Cimento) districts at 24 hours post-spraying, and subsequently monitored monthly until the 80% mortality *cutoff* point was observed or until June 2017, to evaluate spray quality and residual efficacy of the insecticide used in the 2016 spray campaign. The quality assurance tests were carried out in October 2016 (Morrumbala, Mocuba, Milange, Mopeia, and Quelimane) in houses sprayed with Actellic 300 CS.

In each district village, five houses were randomly selected. Cones were placed on sprayed surfaces at heights of 0.5 m, 1.0 m, and 1.5 m diagonally. There was another cone placed on the door and one control cone per house. At the same time, a test for the airborne effect of Actellic CS was conducted with mosquitoes placed inside a paper cup and placed 10cm away of the sprayed wall. WHO plastic cones lined with self-adhesive packing were fixed on the sprayed walls for the assay. The control cone was affixed on a wall with a paperboard with adhesive. Most house wall surfaces were made of mud, and some of them were rough and others were smooth. For these bioassays, 2 – 5 day-old susceptible *Anopheles arabiensis* (KGB) mosquitoes female were used from October 2016 up to February 2017 (T0 to T4). Due to the fire accident at the insectary at Quelimane all bioassay tests from March 2017 up to June 2017 (T5 to T8) were conducted using wild *An, gambiae* s.l collected from the field, except in Mopeia at T6 where tests were conducted on both wild *An. gambiae* s.l, and susceptible colony of *An. arabiensis*. A batch of 10 sugar fed females were introduced into the plastic cones and left exposed on the sprayed surfaces for 30 minutes at different heights (replicates) in the houses. Numbers of mosquitoes knocked down at 30 minutes were recorded. At the end of the exposure period, the mosquitoes were carefully collected and transferred to paper cups and provided with 10% sugar solution soaked in cotton wool placed on top of the paper cups covered with net. The mosquitoes in paper cups were kept for a 24-hour holding period. The 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.

2.4 Mosquito Identification

In the field, collected mosquitoes were sorted by genus, then *Anopheles* spp. females were morphologically identified to species using Gillies & Coetzee (1987) identification key.

2.5 Mosquito Rearing Conditions

An. arabiensis KGB susceptible colony mosquitoes used for cone wall bioassays were reared in an insectary with 25±2° C and 80±10% humidity and 12/12 h photoperiod. Larvae were fed on a mix of Tetramin®, Cerelac®, and Yeast twice a day. Pupae were collected on a daily basis and placed in a small bowl inside a 30x30x30 plastic cage. Adults were fed on 10% sucrose solution until they reached 3-5 days old in the original insectary.

2.5.1 Mosquito Rearing Conditions in 2017

The insectary and the entire susceptible colony was lost to a fire on February 6, 2017 that destroyed one of the buildings of the provincial medical stores where the insectary was located. Starting in March 2017, once a provisional insectary was established at the PMI AIRS project office facilities in Quelimane, KGB strain eggs were received from the National Institute of Health (INS) Mozambique and from the National Institute of Communicable Disease (NICD) from South Africa. Mosquitoes were transitioned to an animal feed system and reared to support bioassay and susceptibility tests. The transition into a start up insectary, as well as changes from human to animal feed, did not yield the necessary amount of mosquitoes in sufficient quantities, and, therefore, the decay rate testing continued with wild *An. gambiae* s.l. after T4.

2.6 STATISTICAL TESTS

The mean number of mosquitoes collected by method or by house 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 July 2016 to June 2017 in Maganja da Costa, Mocuba, Morrumbala, and Milange and September 2016 to June 2017 in Mopeia intervention and control arms), a total of 18,444 anophelines belonging to 12 species, species complexes, or groups were collected by three methods (HLC, CDC and PSC) and morphologically identified as:

16,299 *An. funestus* s.l.	86 *An. ziemanni*	24 *An. pretoriensis*	8 *An. squamosus*
1,757 *An. gambiae* s.l.	39 *An. tenebrosus*	18 *An. maculipalpis*	2 *An. pharoensis*
192 *An. coustani*	5 *An. caliginosus*	12 *An. rufipes*	2 *An. rivulorum*

3.1.1 PYRETHRUM SPRAY CATCHES

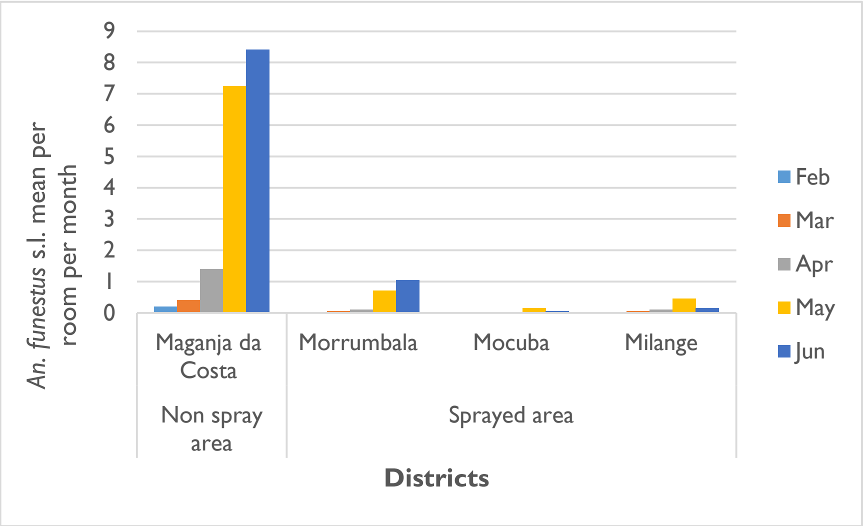
PSC data from August to December 2016 were lost in the insectary fire. Therefore, only data from February to June 2017 are presented.

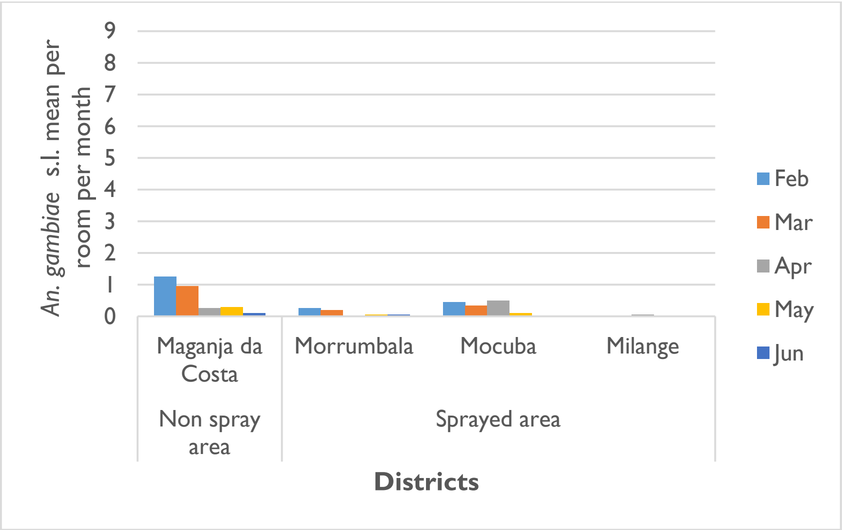
A total of 507 anophelines belonging to the *An. gambiae* complex (97; 19.1%) and *An. funestus* group (410; 80.9%) were collected in four districts, namely Maganja da Costa, Morrumbala, Mocuba, and Milange (see Table 1).

TABLE 1. MONTHLY COLLECTIONS BY PSC IN MORRUMBALA, MOCUBA, MILANGE, AND MAGANJA DA COSTA IN 2017

Months	Number of Houses per District	Non Spray Area		Spray Area						Total
		Maganja da Costa		Morrumbala		Mocuba		Milange		
		***An. funestus* s.l.**	***An. gambiae* s.l.**	***An. funestus* s.l.**	***An. gambiae* s.l.**	***An. funestus* s.l.**	***An. gambiae* s.l.**	***An. funestus* s.l.**	***An. gambiae* s.l.**	
Feb	20	4	25	0	5	0	9	0	0	**43**
Mar	20	8	19	1	4	0	7	1	0	**40**
Apr	20	28	5	2	0	0	10	2	1	**48**
May	20	145	6	14	1	3	2	9	0	**180**
Jun	20	168	2	21	1	1	0	3	0	**196**
Total		**353**	**57**	**38**	**11**	**4**	**28**	**15**	**1**	**507**

FIGURE 1. *ANOPHELES FUNESTUS* S.L. AND *ANOPHELES GAMBIAE* S.L. DENSITIES PER ROOM PER MONTH IN MAGANJA DA COSTA, MORRUMBALA, MOCUBA, AND MILANGE DISTRICTS BY PSC





As shown on Figure 1 above, only *An. funestus* s.l. and *An. gambiae* s.l. were found resting inside human dwellings in all districts surveyed.

In general, indoor resting density of *An. funestus* s.l. and *An. gambiae* s.l. seasonality has different trends. While *An. funestus* s.l. density gradually increases from February to June, the density of *An.gambiae* s.l. seems to decline in Morrumbala and Maganja da Costa. This may be related to a combination of factors, firstly due to the availability of suitable breeding sites for oviposition by the two species, and secondly, gradual reduction of residual activity of insecticide sprayed. The peak indoor resting density of *An. gambiae* s.l. was in February (rainy season), whereas the peak indoor resting period was in June (dry season) for *An. funestus* s.l.. In Mocuba and Milange, few *An. funestus* s.l. and *An. gambiae* s.l. were collected during this period to make a rational comparison, as shown in Figure 1.

Maganja da Costa district (the non-sprayed area) had the highest mosquito densities while Morrumbala, Mocuba and Milange (spray districts) districts had the lowest mosquito densities within the houses, Figure 1.

3.1.2 HUMAN LANDING CATCHES

From July 2016 to June 2017, in Mopeia, Maganja da Costa, Morrumbala, Milange, and Mocuba, a total of 3,211 anophelines were collected by human landing catches. Collected mosquitoes were morphologically identified as *An. funestus* s.l. (2254; 70.20%), *An. gambiae* s.l. (639; 19.90%), *An. rufipes* (6; 0.19%), *An. pretoriensis* (22; 0.69%), *An. rivulorum* (2; 0.06%), *An. pharoensis* (2; 0.06%), *An. maculipalpis* (17;0.53%), *An squamosus* (3; 0.09%) and *An. coustani* group members (*An. coustani* (163; 5.08%), *An. tenebrosus* (22; 0.69%), *An. ziemanni* (78; 2.43%), and *An. caliginosus* (3; 0.09%).

The highest collection of anophelines were from Mopeia (1592) and Maganja da Costa (941), while Mopeia and Milange had the highest diversity of species collected. In Mopeia district, AIRS collected *An. gambiae* s.l., *An. funestus* s.l., *An. coustani*, *An. ziemanni*, *An. tenebrosus*, *An. caliginosus*, *An. pretoriensis*, *An. rivulorum*, *An. pharoensis,* and *An. rufipes*. In Milange district, *An. gambiae* s.l., *An. funestus* s.l., *An. coustani*, *An. maculipalpis*, *An. pretoriensis,* and *An. rufipes* were caught. Mocuba was the district with lowest species diversity with only *An. gambiae* s.l. and *An. funestus* s.l. collected.

In Maganja da Costa, a higher number of *An. funestus* s.l. were collected indoors than outdoors ($p<0.05$), while in Mopeia and Milange, the number of mosquitos collected indoors and outdoors was similar (Table 2). Very few *An. funestus* s.l. were collected in Mocuba and Morrumbala, making any comparisons difficult. *An. gambiae* s.l. preferred biting mainly outdoors, except in Maganja da Costa and Mopeia, where no differences between indoor and outdoor collections were observed.

The indoor biting rate per person per night was near 0 (zero) between October at the start of the spray campaign and December in Maganja da Costa, Mocuba, Morrumbala, and Milange. In Maganja da Costa, the biting rate rapidly increased after the rainy season began, with the biting rate for *An. gambiae* s.l. reaching a maximum of 3.67 bites per person per night in March, while the maximum biting rate for *An. funestus* s.l. of 53 bites per person per night occurred in May, the end of rainy season.

TABLE 2. COMPARISON OF *ANOPHELES FUNESTUS* S.L. AND *ANOPHELES GAMBIAE* S.L. HLC INDOORS AND OUTDOORS IN SIX AREAS

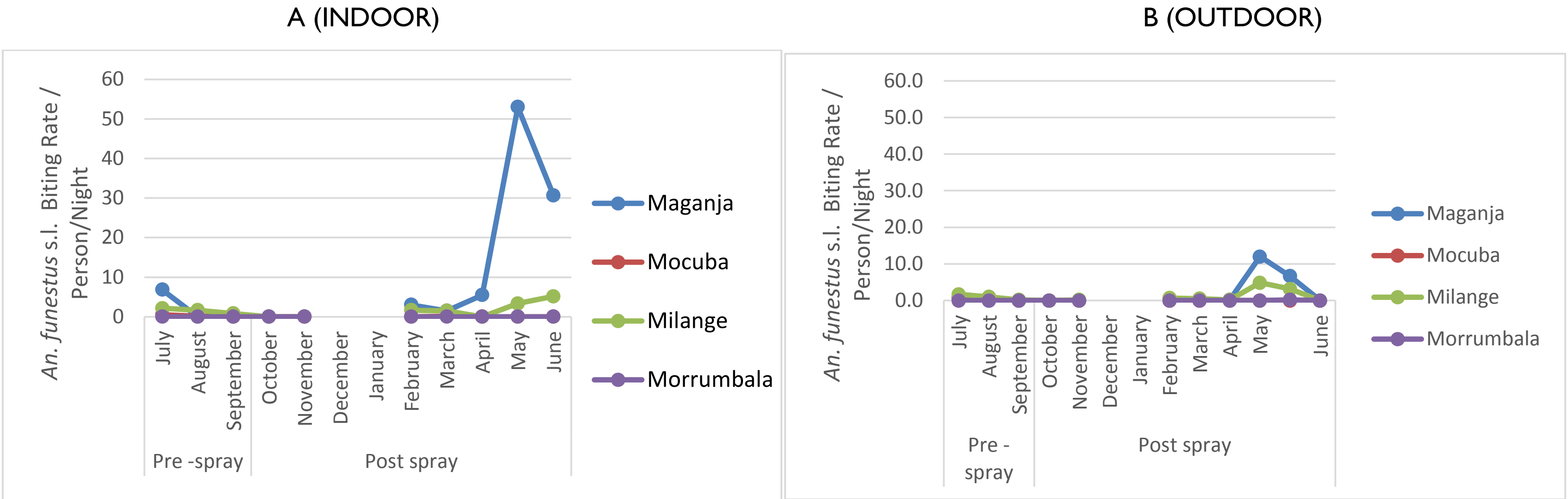
District	*An. funestus* s.l.				*An. gambiae* s.l.			
	Indoor	Outdoor	X^2	p-value	Indoor	Outdoor	X^2	p-value
Maganja da Costa	603	122	319	<0.00001*	77	71	0.24	0.62
Morrumbala	0	1	1	0.31	35	96	28.4	< 0.0001*
Mocuba	1	2	0.3	0.56	18	42	9.6	0.0019*
Milange	98	74	3.35	0.06	59	171	54.54	<0.0001*
Mopeia (Control)	565	631	3.64	0.056	25	19	0.82	0.36
Mopeia (Intervention)	82	77	0.1	0.75	14	15	0.03	0.85

*p-value significant.

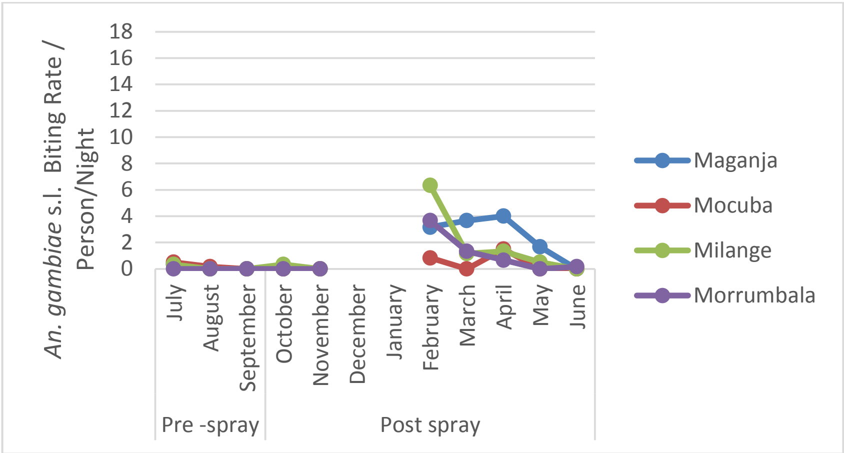
In Milange, in July, *An. funestus* s.l. indoor and outdoor biting rates declined from 2.2 and 1.7 bites per person per night, respectively to 0.0 bites per person per night in October, the first month of spraying (Figure 2A-B). For *An. gambiae* s.l., the indoor biting rate varied from 0.2 in July to 0.3 in October. This may be because October was the first month of spraying, and not all sampling areas were sprayed yet at the time of data collection.

In Mocuba, the *An. funestus* s.l. outdoor biting rate dropped from 0.2 in July to 0 bites per person per night in October, while no indoor biting mosquitoes were found for the same period. *An gambiae* s.l. biting was observed both indoors and outdoors with biting rates estimated as 0.5 in July and 0 after spraying in October (Figure 2C-D).

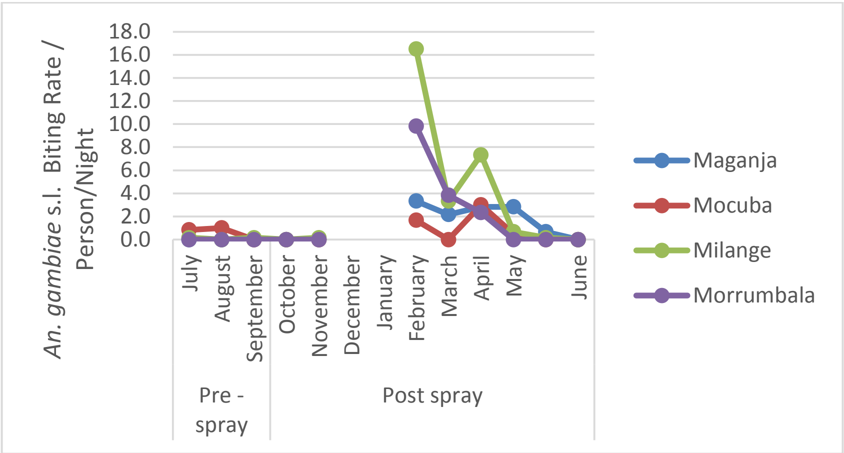
FIGURE 2. HLC INDOOR AND OUTDOOR HUMAN BITING RATES IN FOUR DISTRICTS SURVEYED BEFORE AND AFTER IRS INTERVENTION



C (INDOOR)

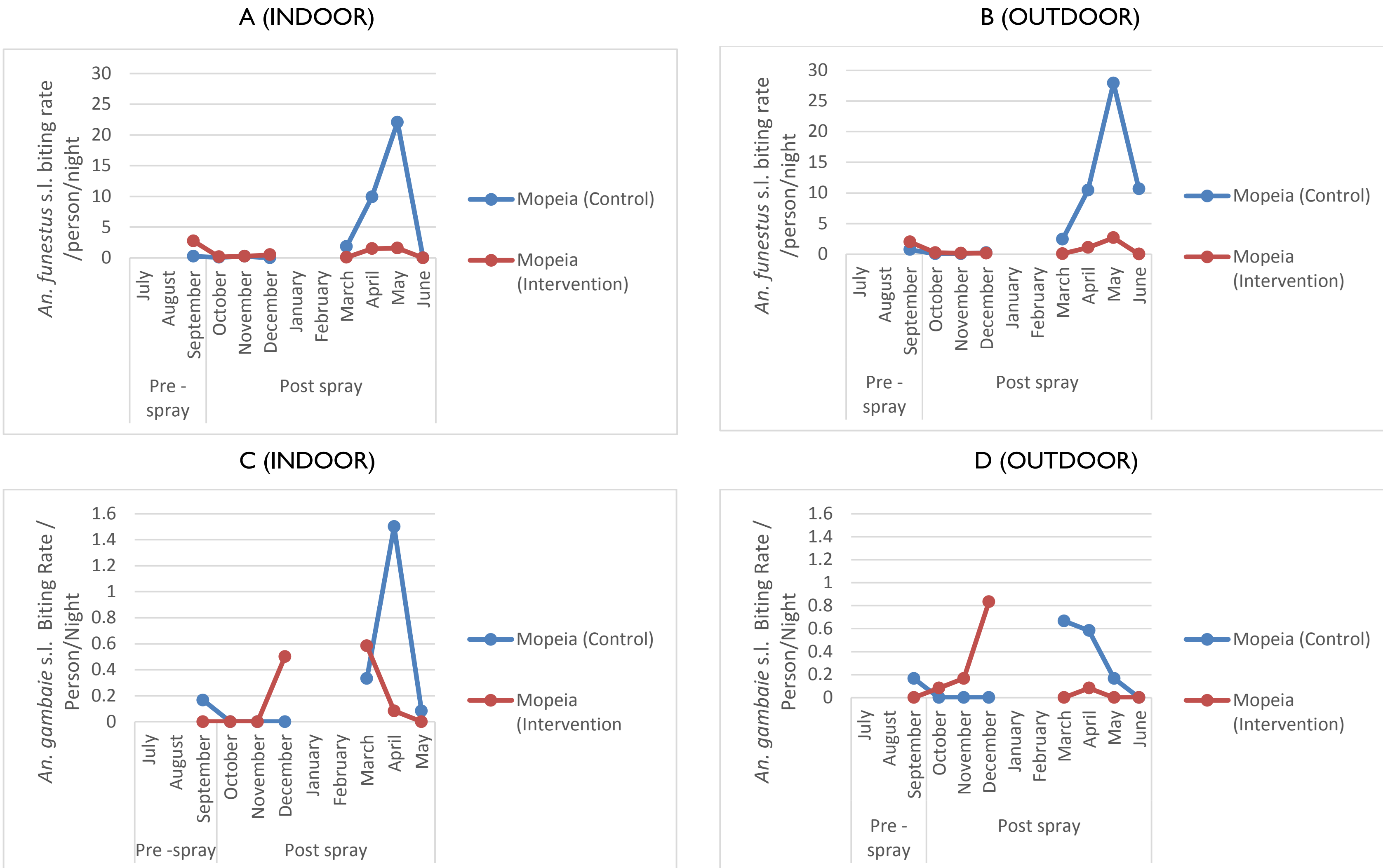


D (OUTDOOR)



In Figure 2, the term "post-spray" does not imply that Maganja da Costa, a control district, was sprayed, but rather defines the period of time prior and after the spray campaign in the other districts. Due to the fire that destroyed the Zambezia insectary in February 2017, no data from January were recorded for Maganja da Costa, Mocuba, Morrumbala, and Milange.

FIGURE 3. INDOOR AND OUTDOOR HUMAN BITING RATES OF *ANOPHELES FUNESTUS* S.L. AND *ANOPHELES GAMBIAE* S.L. IN MOPEIA INTERVENTION (IRS PLUS LLINS) AND CONTROL (LLINS ALONE) AREAS AS DETERMINED THROUGH THE HUMAN LANDING CATCHES



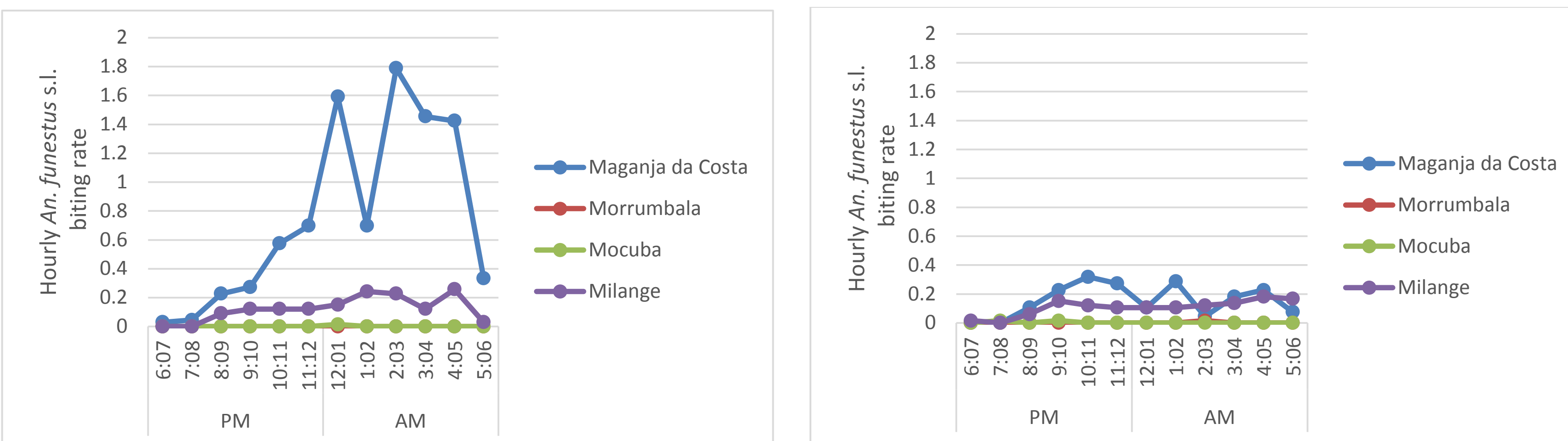
In Figure 3, pre- and post-spray refers to the months before and after spray. The term “post-spray” does not imply that control arm was sprayed, but defines the period of time prior and after the spray campaign in intervention arm. Both samples and data that had not been processed were lost in the insectary fire, and, therefore, no data is available for January and February.

In Figures 3 A and B, in both intervention and control areas before spraying in Mopiea, the biting rate of *An. funestus* s.l. was high, and it dropped immediately after spraying. Months after spray in the control area, biting rates remained high both indoors and outdoors. In the intervention area, biting rates were lower both indoors and outdoors, probably due to the spray effect.

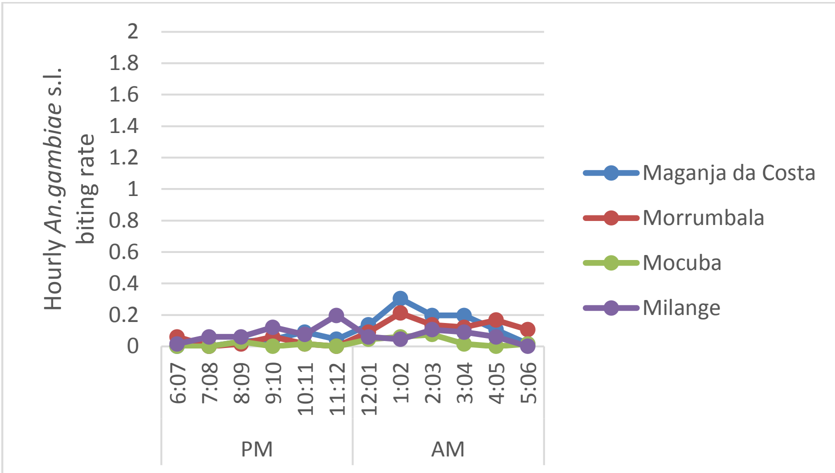
For *An. gambiae* s.l. the biting rate showed a lot of variation as shown in Figures 3 C and D. From March to April more mosquitoes were collected in the control areas both indoors and outdoors.

FIGURE 4. HOURLY BITING RATES OF *ANOPHELES FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN MAGANJA DA COSTA, MORRUMBALA, MOCUBA, AND MILANGE AS DETERMINED THROUGH THE HUMAN LANDING CATCHES

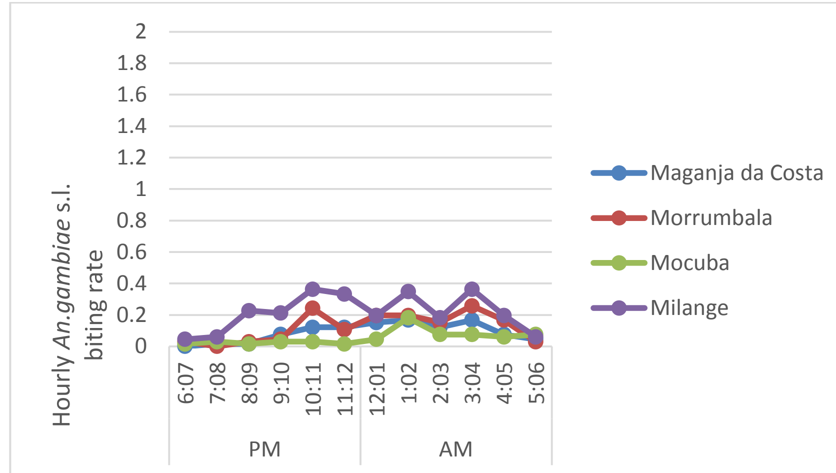
A (INDOOR) B (OUTDOOR)



C (INDOOR)



D (OUTDOOR)

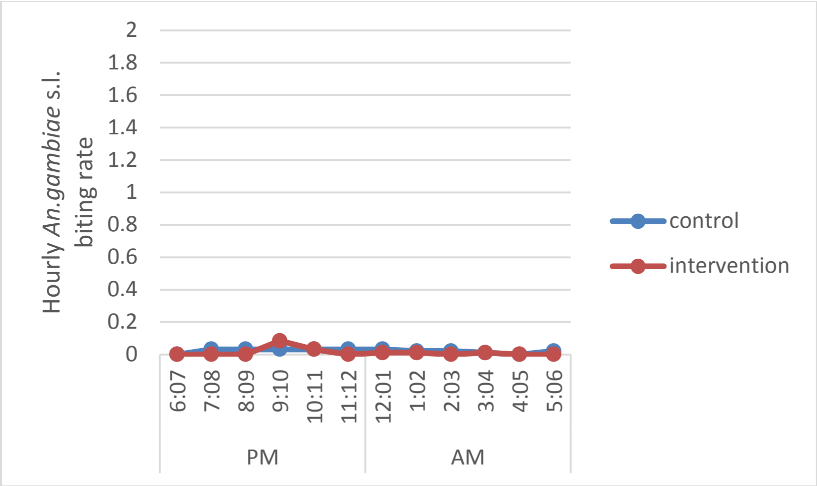


The peak indoor biting rate of *An. funestus* s.l. varied from 1.6 -1.8 from 12 to 1 AM and from 2 to 3 AM in Maganja da Costa while people are asleep. The outdoor peak biting rate was observed from 10 to 11 PM and 1 to 2 AM in Maganja da Costa. In Milange, the high indoor hourly biting peak was noted from 1 to 2 and 4 to 5 AM, while outdoors it appears constant from early evening into the night and in early morning (Figures 4 A and B).

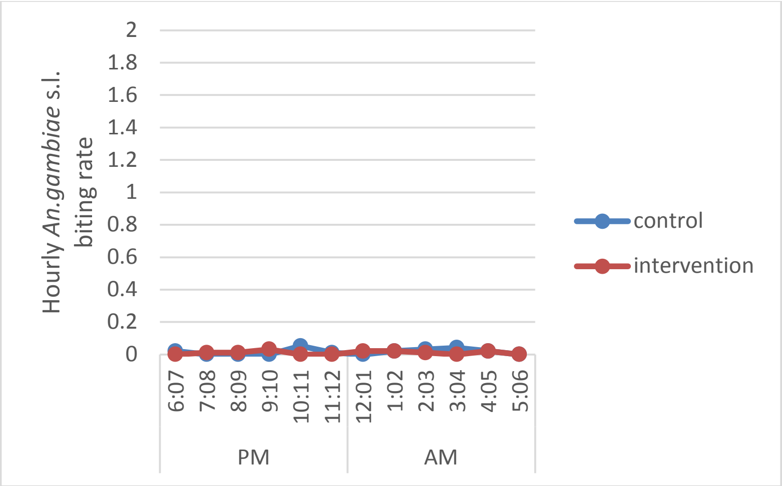
In general, the indoor hourly biting rate activity of *An. gambiae* s.l. occurred from 11 PM to 12 AM in Milange and 11 PM to 2 AM in Maganja da Costa and Morrumbala, while people are asleep (Figure 4C). Outdoor peak *An. gambiae* s.l. biting in Milange looks to vary from 10 PM to 12 AM, 1 to 2 AM and 3 to 4 AM, meaning that this population of mosquitoes are biting during regular sleeping time (Figure 4D).

FIGURE 5. HOURLY BITING RATES OF *ANOPHELES FUNESTUS* S.L. AND *ANOPHELES GAMBIAE* S.L. IN MOPEIA DISTRICT, INTERVENTION AND CONTROL AS DETERMINED THROUGH THE HUMAN LANDING CATCHES

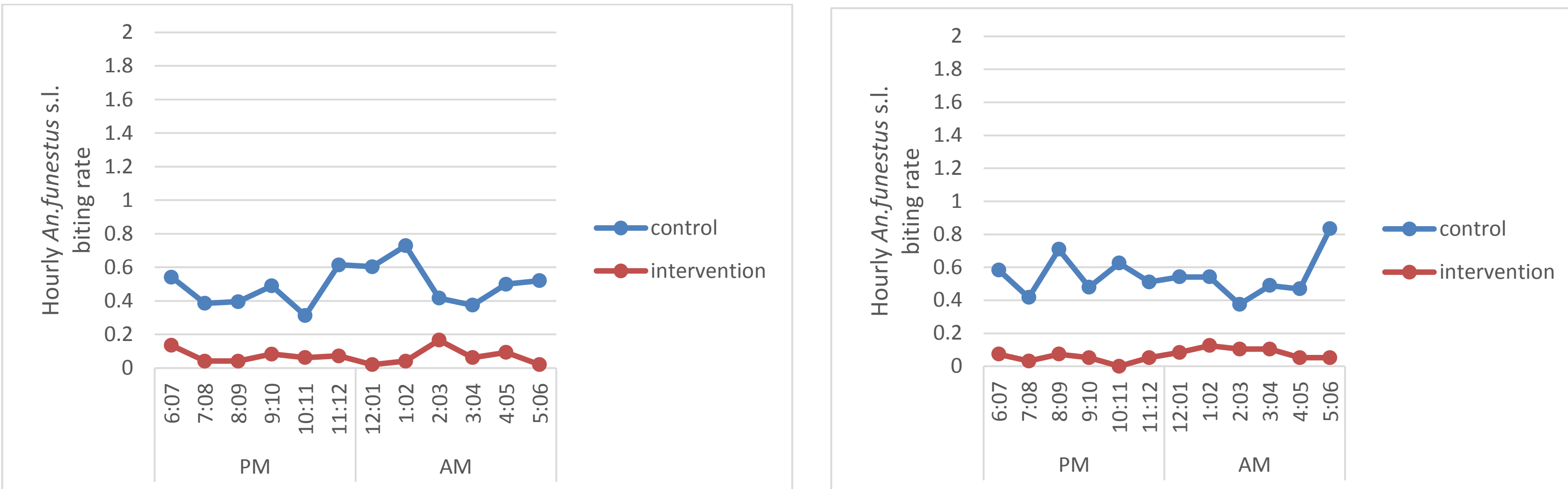
A (INDOOR)



B (OUTDOOR)



C (INDOOR) D (OUTDOOR)



In Mopeia, *An. gambiae* s.l., biting rates both indoors and outdoors are very low, and it is difficult to identify the peak biting hour for this species in both the control and intervention arms, as shown in Figures 5 A and B. For *An. funestus* s.l., the biting rate is generally low in intervention sites, but in the control areas, the peak biting hour indoors seems to be between 1 and 2 am. The peak biting hour outdoors was between 5am and 6 am (Figures 5C and D).

3.1.3 CDC Light Trap Collections

A total of 14,725 anophelines were caught in all sprayed and non-sprayed districts using CDC light traps, of which 13,635 (92.60%) were *An. funestus* s.l., 1,021 (6.93%) were *An. gambiae* s.l., 29 (0.2%) *An. coustani,* 17 (0.12%) *An. tenebrosus*, 8 (0.05%) *An. ziemanni,* 6 (0.04%) *An. rufipes*, 5 (0.03%) *An. squamosus*, 2 (0.01%) *An. caliginosus*, 2 (0.01%) *An. pretoriensis,* and 1 (0.01%) was *An. maculipalpis* (Table 3 and 4).

The majority of mosquitoes (97.1%) collected by CDC light trap were caught in Maganja da Costa and Mopeia. Comparing non-spray (Maganja da Costa) and spray districts (Mocuba, Milange and Morrumbala), 81.4% of all *An. funestus* s.l. and 31.7% of all *An. gambiae* s.l. were from the control district.

TABLE 3. CDC LIGHT TRAP DATA FROM MONTHLY COLLECTION

Districts	Species	2016-2017											Total Collected	
		Jul	Aug	Sep	Oct	Nov	Dec	Feb	Mar	Apr	May	Jun		
Maganja da Costa	*An. funestus* s.l.	24	26	47	0	6	26	48	1	7	160	198	543	717
	An. gambiae s.l.	0	4	5	12	6	60	37	12	17	18	3	174	
Morrumbala	*An. funestus* s.l.	18	0	0	0	0	0	1	0	0	8	15	42	203
	An. gambiae s.l.	0	0	0	0	0	0	41	66	39	5	3	154	
	An. tenebrosus	0	0	0	0	0	0	0	0	0	0	1	1	
	An. maculipalpis	0	0	0	0	0	0	0	1	0	0	0	1	
	An. rufipes	0	0	0	0	0	0	0	3	0	0	0	3	
	An. pretoriensis	0	0	0	0	0	0	2	0	0	0	0	2	
Milange	*An. funestus* s.l.	11	9	0	0	1	16	4	0	0	18	21	80	153
	An. gambiae s.l.	0	0	0	0	0	23	28	7	7	3	0	68	
	An. coustani	0	0	0	0	0	0	1	0	0	1	1	3	
	An. rufipes	0	0	0	0	0	1	0	0	0	1	0	2	
Mocuba	*An. funestus* s.l.	0	0	0	0	0	1	0	0	0	1	0	2	70
	An. gambiae s.l.	0	2	0	0	1	8	27	0	23	7	0	68	
Total		53	41	52	12	14	135	189	90	93	222	242		1143

Monthly collections by CDC light trap yielded less anopheline mosquitoes in sprayed districts, namely in Morrumbala, Mocuba, and Milange, than in Maganja da Costa, the control district, in all survey months ($p<0.05$; $X^2=74.09$).

TABLE 4. CDC LIGHT TRAP DATA FROM MONTHLY COLLECTION IN MOPEIA CONTROL AND INTERVENTION ARMS

Month/Mosquitoes Species	Control								Intervention							
	Sep	**Oct**	**Nov**	**Dec**	**Mar**	**Apr**	**May**	**Jun**	**Sep**	**Oct**	**Nov**	**Dec**	**Mar**	**Apr**	**May**	**Jun**
An. gambiae s.l.	3	1	1	21	77	182	18	4	2	2	12	55	70	89	19	1
An. funestus s.l.	24	112	32	106	1013	3915	2734	1150	342	134	25	109	88	1576	1148	456
An. tenebrosus	0	0	0	1	0	2	0	0	0	0	0	2	0	1	9	1
An. caliginosus	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
An. ziemanni	0	0	0	0	0	2	0	0	0	0	0	3	0	1	5	0
An. squamosus	0	0	0	0	0	0	1	0	0	0	0	0	0	2	2	0
An. rufipes	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
An. coustani	0	0	0	0	0	8	2	0	0	0	0	0	0	16	0	0
Total	**27**	**113**	**33**	**129**	**1090**	**4110**	**2755**	**1154**	**344**	**136**	**37**	**170**	**158**	**1685**	**1183**	**458**

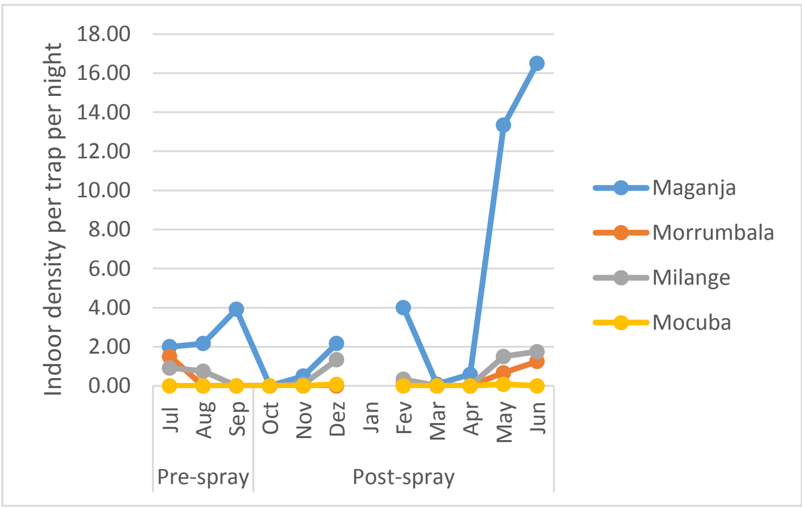
At the beginning of the cost study in September 2016, the densities of anopheline mosquitoes appeared to be higher in intervention areas (484 anophelines caught in two months) as compared to control areas (140) in Mopeia. After IRS spraying was conducted (October through November 2016), indoor density per trap dropped, mainly for *An. funestus* s.l., in sprayed and non-sprayed areas followed by a rapid increase of the anopheline population in non-sprayed areas.

In total, after spraying in Mopeia district, 69.3% of all Anophelinae were collected in the non-sprayed arm (control), whereas 30.7% were collected in the sprayed areas arm (intervention). *An. funestus* s.l. were the most predominant mosquitoes caught, representing 95.5% of mosquitoes collected, followed by *An. gambiae* s.l., (4.1%), and *An. coustani* group members (0.4%).

According to the CDC light trap collection data, *An. funestus* s.l. reached a peak of indoor density in April in Mopeia and June in Maganja da Costa (Figures 6 and 7). In Morrumbala and Milange, *An. funestus* s.l. peak density appears mainly between May and July, though the density is generally low in these areas. For Mocuba the density of *An. funestus* s.l. remained very low and similar with previous years. The peak density for the *An. gambiae* complex as determined through the CDC light trap collections was during the rainy seasons in Maganja da Costa, Mocuba, Morrumbala and Milange. However, in Mopeia the density is generally low for this species to determine the seasonality based on the CDC light trap collections as shown in Figure 7.

FIGURE 6. MONTHLY INDOOR CDC LIGHT TRAP DENSITY IN MAGANJA DA COSTA, MORRUMBALA, MILANGE, AND MOCUBA DISTRICTS

A (*Anopheles funestus* s.l.)



B (*Anopheles gambiae* s.l.)

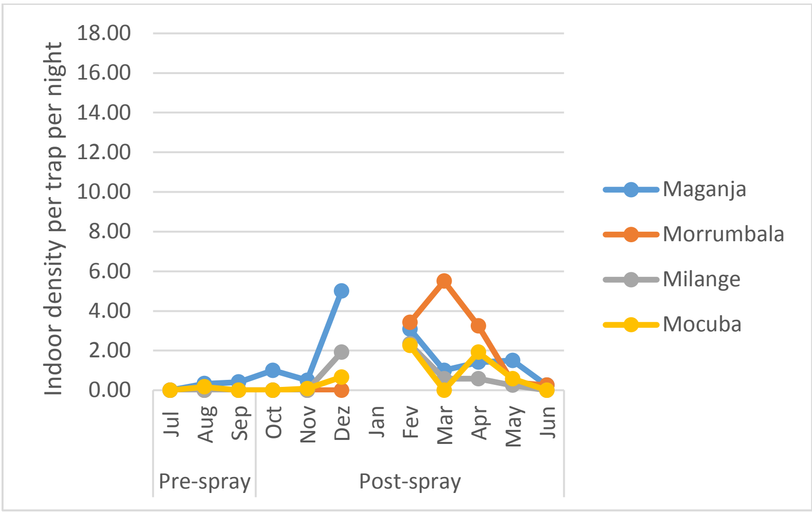
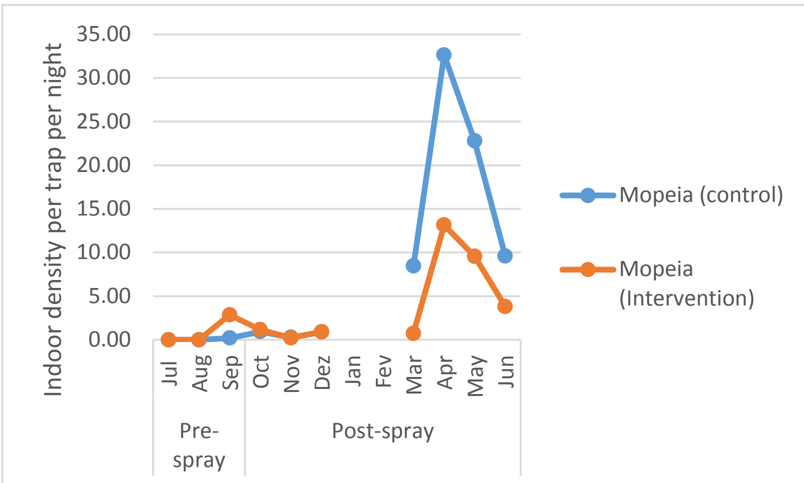
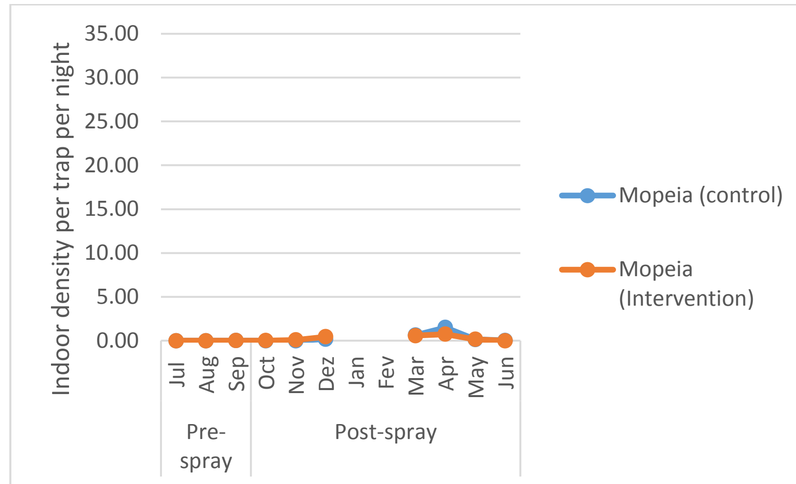


FIGURE 7. MONTHLY INDOOR CDC LIGHT TRAP DENSITY IN MOPEIA INTERVENTION AND CONTROL ARMS

A (*Anopheles funestus* s.l.)



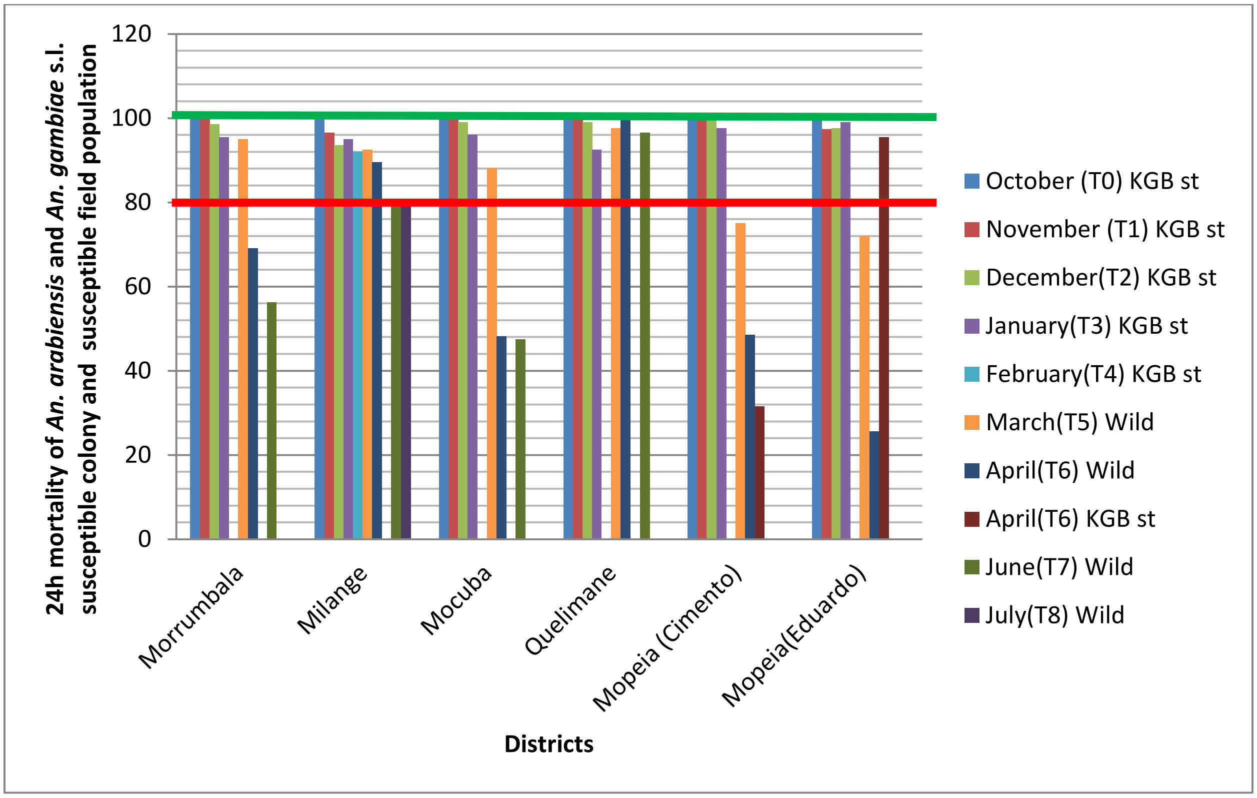
B (*Anopheles gambiae* s.l.)



3.2 CONE WALL BIOASSAY TESTS

Cone wall bioassays show that Actellic 300 CS remained effective in the districts of Quelimane (97.5%), Morrumbala (95%), Milange (92.5%), and Mocuba (88%) for at least five months following IRS. In the district of Mopeia, the residual life of the insecticide reached the cutoff point with mortality rates of 71.8% and 75% in Eduardo Mondlane and Cimento villages, respectively, at five months. Quelimane and Milange were the districts with the most prolonged residual activity, remaining above the cutoff point for at least seven months. The results obtained during months five to eight were based on *Anopheles gambiae* s.l. field collected larvae, since the *An. arabiensis* colony was destroyed by the fire at the Quelimane insectary (Figure 8).

FIGURE 8. RESIDUAL EFFICACY (ACTELLIC 300 CS)



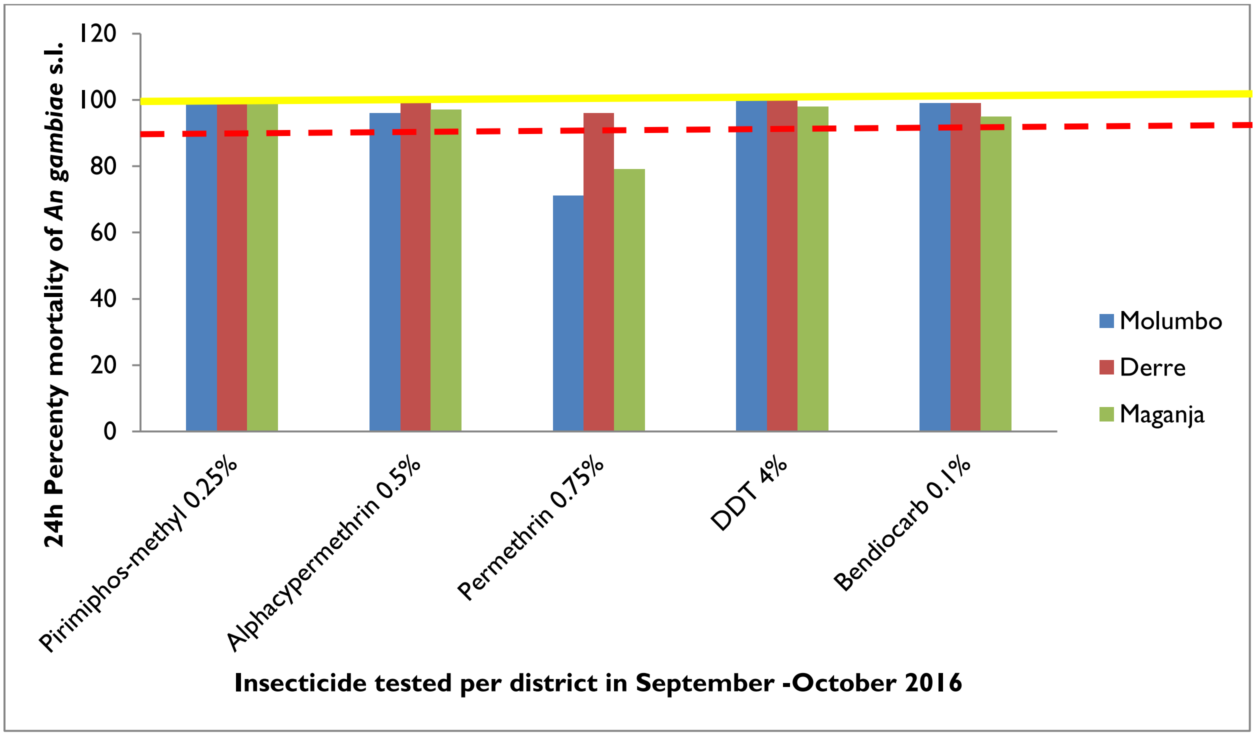
Green line shows 100% of insecticide activity against *An. arabiensis* (KGB strain) susceptible colony & *An. gambiae* s.l. (Wild), field population

Red line indicates the 80% efficacy cutoff point against *An. arabiensis* (KGB strain) susceptible colony & *An. gambiae* s.l. (Wild), field population

3.3 WHO SUSCEPTIBILITY TESTING

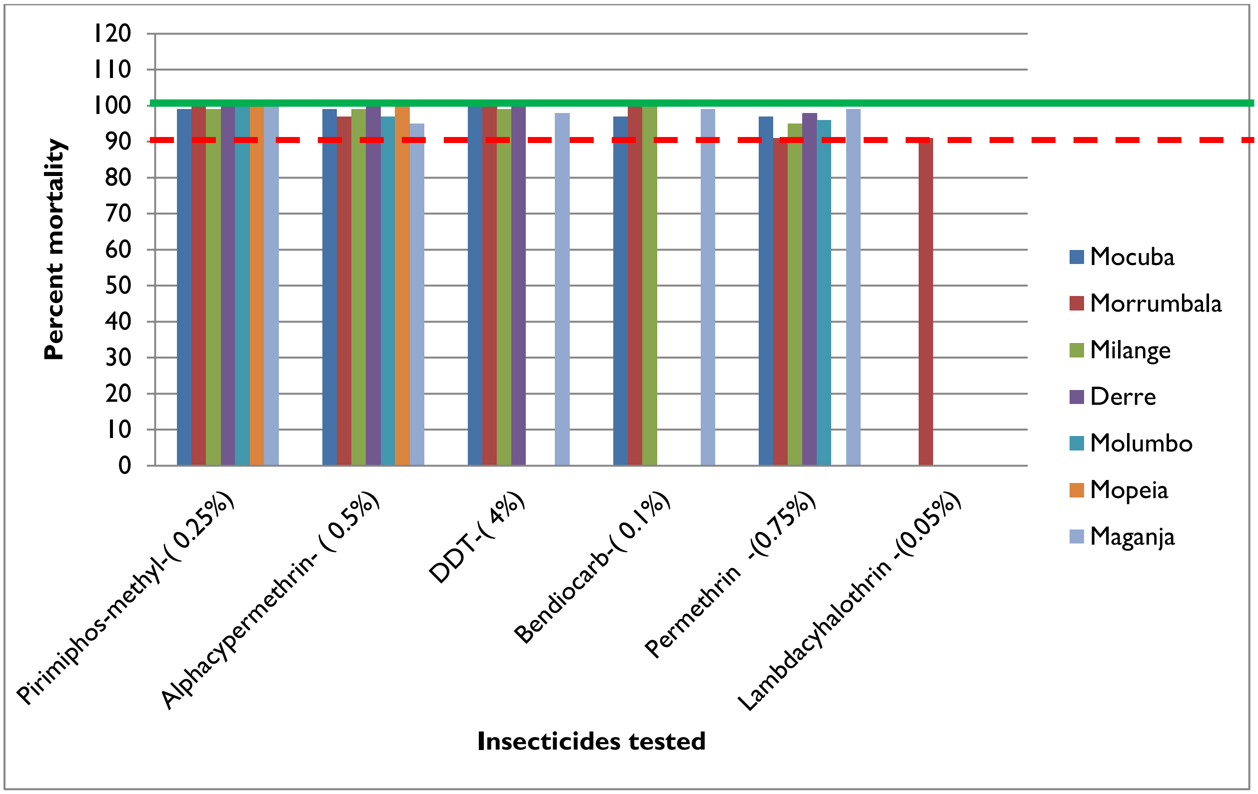
WHO susceptibility testing was conducted in September to early October 2016, prior to the start of the campaign, with the objective to find *An. funestus* population since most data of susceptibility tests was based on the *An. gambiae* population. Figure 9 shows results of *An. gambiae* s.l., collected in Molumbo, Derre and Maganja da Costa districts. Five insecticides were tested, pirimiphos-methyl (0.25%), Alphacypermethrin (0.5%), Permethrin (0.75%), DDT (4%) and Bendiocarb (0.1%). The results show that there is resistance to Permethrin (0.75%) in Molumbo and Maganja da Costa. No *An. funestus* were found for testing.

FIGURE 9. *AN. GAMBIAE* S.L. SUSCEPTIBILITY STATUS AGAINST INSECTICIDES RECOMMENDED BY WHOPES FOR PUBLIC HEALTH FROM SEPTEMBER TO OCTOBER 2016



From January to March 2017, another round of susceptibility testing was carried out on the *An. gambiae* s.l. population, which remains susceptible to pirimiphos-methyl, bendiocarb, DDT, and pyrethroids in all districts tested, with the exception of Morrumbala, where possible resistance to lambdacyhalothrin was detected for the second year in a row (Figure 10). However, in Morrumbala the mortality rate against lambdacyhalothrin rose from 33% in 2016 to 91% in 2017, suggesting that the pyrethroid has recovered some of its potency to kill *An. gambiae* s.l. in the district after two consecutive spray rounds using Actellic CS. In March 2017, the lowest percent mortality was observed to permethrin and lambdacyhalothrin (pyrethroids) in Morrumbala district with mortality rates of 91%. Before that, in September and October 2016, the lowest mortality rates were 71% and 79% to permethrin in Molumbo and Maganja da Costa districts respectively.

FIGURE 10. *AN. GAMBIAE* S.L. MORTALITY RATE IN WHO SUSCEPTIBILITY TESTS TO INSECTICIDES RECOMMENDED BY WHOPES FOR PUBLIC HEALTH FROM JANUARY TO MARCH 2017



Green line indicates 100% susceptibility

Red line indicates mortality below 90%; or mosquitoes' resistance

4. Discussion, Lessons Learned and Challenges

An. funestus s.l. remain the most abundant mosquitoes species collected from July 2016 to June 2017. Members of this group may play an important role as malaria vectors in the surveyed districts, as sporozoite infection rates of 2.15% in Maganja da Costa (a non-spraying area) and 1.61% in Milange (spray area) have previously been reported for *An. funestus* s.s., while an infection rate of 1.74% has been reported for *An. arabiensis* in Maganja da Costa (PMI AIRS 2016).

An. funestus s.l. and *An. gambiae* s.l. are the only Anophelinae collected by PSC in Morrumbala, Mocuba, Maganja da Costa, and Milange. Other species commonly caught by CDC light traps and HLC were not collected using PSC, most likely because they exited human dwellings after blood-feeding.

Apart from *An. gambiae* s.l. and *An. funestus* s.l., other *Anopheles* species collected by CDC light trap and HLC methods that have been considered as important vectors included *An. rivulorum* (Kawada et al. 2012), incriminated as a secondary vector in many parts of Africa, and *An. coustani*, *An. tenebrosus*, *An. ziemanni*, *An. pharoensis*, *An. pretoriensis*, *An. caliginosus*, *An. rufipes,* and *An. squamosus* (Afrane et al. 2016). Since this observation constitutes an alert that other species have the potential to transmit malaria in the settings of the districts surveyed, further tests for sporozoites using the ELISA method will be conducted on those species.

An. gambiae s.l. was collected mainly outdoors, whereas *An. funestus* s.l. was predominantly found indoors, even after IRS, except in Mopeia District where biting occurred both indoors and outdoors.

The quality assurance results of Actellic 300CS were acceptable, with 100% mortality observed for *An. arabiensis* susceptible colony mosquitoes in all districts. The efficacy of the insecticide remained high for 5 months post spray with the exception of Mopeia (Eduardo Mondlane and Cimento villages), where the residual efficacy dropped below the 80% efficacy cutoff point.

The observed increase in insecticide susceptibility to lambdacyhalothrin in Morrumbala from 33% in 2016 to 91% in 2017 may indicate the recovery of susceptibility to this pyrethroid in *An. gambiae* s.l. in the field after two consecutive spray rounds using Actellic CS.

Molecular analyses will be done at Wits laboratory in South Africa to identify sibling species belonging to *An. funestus* group and *An. gambiae* complex, and shall be submitted in a future report.

As mentioned in the Introduction chapter, the Zambezia insectary was destroyed by fire resulting in a complete loss of some entomological data, which was affected the depth of this report.

5. BIBLIOGRAPHY

The PMI Africa Indoor Residual Spraying Project (2016).Mozambique: Entomological Monitoring Annual Report. Maputo, Mozambique: PMI AIRS, Abt Associates Inc.

World Health Organization (2013). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, WHO ISBN 978 92 4 150515 4, (NLM Classification: WA 240). Geneva.

Gillies, MT, Coetzee, M. A (1987). Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region). Publication of the South Africa Institute of Medical Research.

Shidrawi GR (1972). The man biting activity of An. gambiae s.l. in DDT-sprayed and unsprayed villages of dry savanna in Nigeria. WHO/MAL/70.720.

Afrane A, M Bonizzoni, GYaw (2016). Secondary Malaria Vectors of Sub-Saharan Africa: Threat to Malaria Elimination on the Continent? https://cdn.intechopen.com/pdfs-wm/52512.pdf. Accessed 30 August 2017.

Kawada H, GO Dida, G Sonye, SM Njenga, C Mwandawiro, N Minakawa (2012) Reconsideration of *Anopheles rivulorum* as a vector of *Plasmodium falciparum* in western Kenya: some evidence from biting time, blood preference, sporozoite positive rate, and pyrethroid resistance. Parasites & Vectors, 5:230.

Annex A: Summary of Collection Type, Number of Houses, Village and Sites

Districts	Collection Type	# of houses per village	# of collection nights	# of villages	
				Control	Intervention
Mopeia	CDC Trap	8	3	5	5
	HLC	1	3	4	4
Mocuba	CDC Trap	4	3	NA	1
	HLC	2	3	NA	1
	PSC	20	NA	NA	2
Milange	CDC Trap	4	3	NA	1
	HLC	2	3	NA	1
	PSC	20	NA	NA	2
Morrumbala	CDC Trap	4	3	NA	1
	HLC	2	3	NA	1
	PSC	20	NA	NA	2
Maganja da Costa	CDC Trap	4	3	1	NA
	HLC	2	3	1	NA
	PSC	20	NA	2	NA

ANNEX B: *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED IN NON-SPRAYED HOUSES PER VILLAGE IN INTERVENTION AREA (SEPTEMBER 2016-MARCH 2017)

Village Name (Intervention Area)	No. of Sprayed Houses	Not Sprayed	*An. funestus* s.l.		%	*An. gambiae* s.l.		%
			Non Sprayed Houses	Total		Non-Sprayed Houses	Total Mosquitos	
Eduardo Mondlane	8	0	0	27	-	0	2	-
7 de Abril	7	1	38	328	11.6	5	58	8.6
Paz	8	0	0	25	-	0	9	-
4 de Outubro	4	4	22	22	100.0	1	1	100.0
25 de Junho	6	2	0	0	-	0	1	-