PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Four

AIRS MOZAMBIQUE
ZAMBÉZIA PROVINCE
ENTOMOLOGICAL MONITORING

FINAL REPORT

AUGUST 2015

Recommended Citation: PMI|Africa IRS (AIRS) Project. August 2015. AIRS Mozambique Entomological Monitoring, Final Report. Bethesda, MD. PMI|Africa IRS (AIRS) Project Indoor Residual Spraying (IRS 2) Task Order Four, Abt Associates Inc.

Contract GHN-I-00-09-00013-00; **Task Order:** AID-OAA-TO-11-00039

And Contract No.: Contract: GHN-I-00-09-00013-00; **Task Order**: AID-OAA-TO-14-00035

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CONTENTS

ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	Center for Disease Control and Prevention
HLC	Human Landing Catches
INS	National Institute of Health
IRS	Indoor Residual Spraying
KDR	Knockdown Resistance
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme
QA	Quality assurance

1. RATIONALE AND OBJECTIVES

Malaria, especially that caused by *Plasmodium falciparum*, remains one of the primary causes of morbidity and mortality among impoverished communities across endemic regions of Mozambique. Overall, malaria is responsible for about 44% of all outpatient consultations; 57% of admissions to health facilities, especially pediatric services; and about 30% of total deaths in all country [3,4]. *Plasmodium falciparum* is the most common parasite and is responsible for more than 90% of the malaria cases [4, 5, 6]. The most important malaria vectors are members of the *Anopheles gambiae* complex and *Anopheles funestus* group, whose distribution varies across different eco-epidemiological settings in the country [1,2].

The principal malaria intervention strategies in Mozambique include case management with prompt use of artemisinin-based combination therapies, vector control, intermittent preventive treatment for pregnant women and health promotion. Indoor Residual Spraying (IRS) remains the cornerstone strategy for malaria vector control.

In August 2011, Abt Associates was awarded a three-year Africa-wide Indoor Residual Spraying (AIRS) project, IRS2 Task Order 4, which was funded by the United States Agency for International Development (USAID) under the United States President's Malaria Initiative (PMI). The objective of the project was to contribute to PMI's Global Health Initiative's goal to halve the burden of malaria in 70 percent of at-risk populations in sub-Saharan Africa. Abt works closely with ministries of health (MOHs), and national malaria control programs (NMCPs), district health offices, local non-governmental organizations, and community and business leaders to ensure that government, the private sector, and communities are able to sustain and lead future indoor residual spraying (IRS) and malaria control programs. In September 2014, Abt Associates was awarded another three-year Task Order #6 (The PMI AIRS Project) to support the implementation of IRS in 15 African countries including the continuation of support to Mozambique (Zambézia Province). The PMI AIRS Mozambique program also includes entomological monitoring activities in the province of Zambézia.

In accordance to PMI's technical guidelines for entomological monitoring, insecticide resistance testing must be conducted annually to inform insecticide selection for annual spray campaign. The National Malaria Control Program of Mozambique (NMCP) is scaling up IRS as one of the key interventions for malaria control in 8 provinces, across 21 districts in 2015. PMI, through the AIRS project, provides technical and financial support to the NMCP for the implementation of IRS, enhanced surveillance and entomological monitoring activities in 6 districts in the Zambezia province. Entomological monitoring is implemented in four districts of intervention and one control district. The Centers for Disease Control and Prevention (CDC) light trap was a method introduced later than other methods (pyrethrum spray collection and human landing catches).

The PMI AIRS Mozambique team in collaboration with NMCP and the Provincial Directorate of Health (DPS) was responsible for entomological data collection and insecticide resistance testing in order to monitor the efficacy of the IRS on malaria transmission in the project selected areas. Overall, Abt was responsible for the following activities:

- Develop and implement a detailed entomological work plan.
- Determine vector densities and species composition that exist in the selected intervention area / control area through Pyrethrum Spray Collections (PSC).

- Monitor susceptibility status of local *Anopheles* species to World Health Organization Pesticide Evaluation Scheme (WHOPES) approved insecticides using WHO Tube test.
- Quality Assurance of the IRS operations using WHO Cone wall bioassays.
- Monitoring vector biting time and location through Human Landing Catches (HLC).

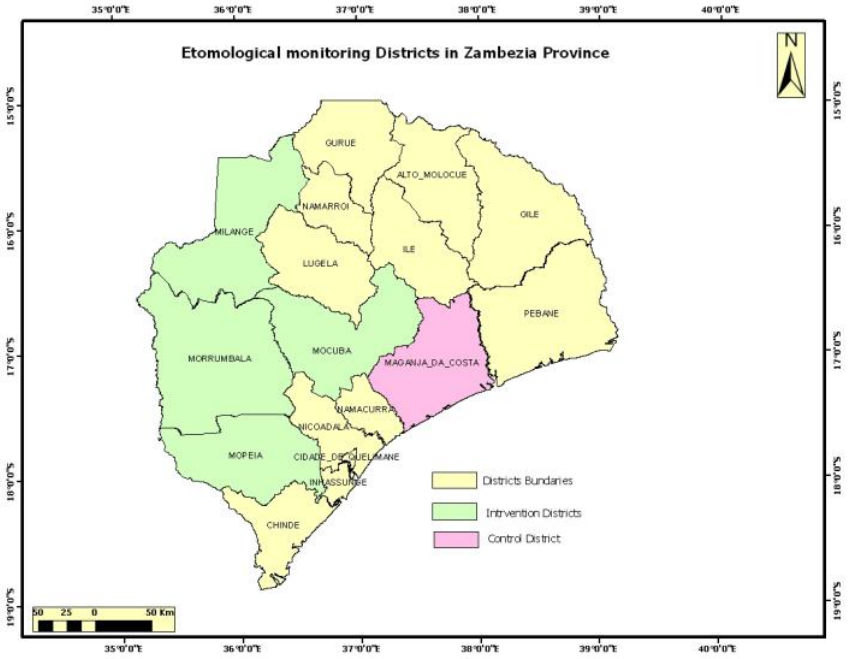
2. Data Collection Methods and Sites

2.1 Study Sites

In Mozambique, the National Malaria Control Program (NMCP) used DDT for IRS before changing the policy in1993, when pyrethroids were introduced in the country. Before 2005 the NMCP had carried out its vector control program with IRS in a few areas in central Mozambique (Zambézia Province). From 2007 up to now, with PMI support, the districts of Milange, Morrumbala and Mocuba have been sprayed with pyrethroids and DDT (supplied by NMCP Mozambique via the Global Fund). Since 2009 pyrethroids have been the only class of insecticides used for IRS [1], and from 2010 to date, pyrethroids have been the insecticide of choice for the PMI funded IRS campaigns in Zambezia. However, with the development of insecticide resistance to pyrethroids, the PMI AIRS project will start using organophosphate insecticide in 2015; Actelic CS 300 will be used in the districts of Derre, Morrumbala and Mocuba, with the remaining districts receiving pyrethroid IRS before shifting all districts to organophosphates in 2016.

Surveys were conducted in the districts of Mocuba, Morrumbala, Milange and Mopeia (intervention areas), and Maganja da Costa (control area). Mopeia was later introduced in 2014 as an intervention site where cone wall bioassay tests and CDC light trap collections were carried out as shown in Figure 1 below. The landscape of Zambézia province is characterized as savannah, in general the rainy season usually occurs from October to April, and the cold and dry season occurs from mid-May to September. Malaria transmission in the province is perennial with peak occurring from December to April, with *Anopheles gambiae* s.l. and *An. funestus* s.l. identified as the major malaria vectors in the areas. These monitoring sites were selected based on the human population on the site, malaria prevalence, easy accessibility throughout the year and availability of potential breeding sites of malaria vectors.

Figure 1. Zambézia Province entomological monitoring districts of intervention and control



2.2 ENTOMOLOGICAL SURVEY METHODOLOGIES

Table 1: Summary of indoor residual spraying and entolomological survey methodologies in each district in Zambézia, Mozambique (July 2014-June 2015)

District	Type	Jul. 2014	Aug. 2014	Sept. 2014	Oct. 2014 Spray start	Nov. 2014	Dec. 2014	Jan. 2015	Feb. 2015	Mar. 2015	Apr. 2015	May.2015	Jun.2015
Mocuba	Interv,	PSC, HLC	PSC, HLC	PSC, HLC	PSC, HLC, CDC, QA	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate, resistance test	PSC, HLC, CDC, Decae rate, resistance test	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate
Morrumbala	Interv,	PSC, HLC	PSC, HLC	PSC, HLC	PSC, HLC, CDC, QA	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate, resistance test	PSC, HLC, CDC, Decae rate, resistance test	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate
Milange	Interv,	PSC, HLC	PSC, HLC	PSC, HLC	PSC, HLC, CDC, QA	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate, resistance test	PSC, HLC, CDC, Decae rate, resistance test	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate	PSC, HLC, CDC, Decae rate
Mopeia	Interv,				QA, CDC	Decae rate, CDC	Decae rate, CDC	Decae rate, CDC	Decae rate, CDC	Decae rate, CDC	Decae rate, CDC	Decae rate, CDC	Decae rate, CDC
Maganja	Control	PSC, HLC	PSC, HLC	PSC, HLC	PSC, HLC, CDC	PSC, HLC, CDC	PSC, HLC, CDC	PSC, HLC, CDC	PSC, HLC, CDC	PSC, HLC, CDC	PSC, HLC, CDC	PSC, HLC, CDC	PSC, HLC, CDC

2.2.1 Impact of IRS on Mosquito Density, Species Composition and biting behavior

Pre-spray collections were conducted from July through September 2014, before the spray campaign which begun on October 20, 2014, in order to assess vector density, composition, biting time and location. After the spray campaign, mosquitoes were sampled on a monthly basis through June 2015 in four intervention and one control district using PSC, HLC and CDC light traps.

2.2.2 Pyrethrum Spray Catch (PSC)

In each selected district, one village was selected in which indoor resting mosquitoes were collected by PSC in 10 selected houses in each of the three intervention and one control areas from 6 am to 9 am. The PSC was conducted once per month for 12 consecutive months and the collection was carried out for two consecutive days in each study site. PSC data was collected in five houses per day per district and per site (there was one site per district). The first collection was carried out three months before the spraying campaign and aimed to obtain baseline data on important entomological indicators (species composition, indoor resting vector density, and behaviour) before the intervention. The other nine data collection points occurred after the IRS campaign. The 10 houses were selected randomly at different distances; the selection of houses was done to cover the area selected in each village for the study. The aerosol used for PSC was Baygon (commercial nomenclature) and it contains Pyrethroids (these include Deltamethrin 0.5 g/kg and Imiprothrin 1,0g/kg). In each house, one sleeping room was selected to be sprayed with Baygon. The room was closed for 10 minutes, after which knocked down mosquitoes were collected using forceps into a labeled petri dish. The samples were identified morphologically and preserved in 1.5 µl Eppendorf tube containing silica gel awaiting further identification using Polymerase Chain Reaction (PCR) technique.

2.2.3 Human Landing Catch (HLC)

In three of the selected intervention districts (Mocuba, Morrumbala and Milange), and in the control area, two houses were sampled from a selected village and two human volunteers were positioned one inside a 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 were collecting mosquitoes during 50 minutes and rested for 10 minutes during which they exchanged positions, 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. A total of four cups were used for each hour of collection, two inside and another two outside, collected mosquitoes were subsequently killed using cotton soaked in chloroform covered with petri dish, identified, counted by species, location, hour of collection, and deposited into 1.5 Eppendorf tubes in silica gel.

2.2.4 CDC Light Traps

The CDC Light trap was installed in four houses in each of four intervention districts and the control district. The traps were installed inside the houses in the room beside the bed with humans sleeping under untreated bed net; the trap was installed near the foot end of the person and 1.5 m above the ground. Those data were collected during three consecutive nights, from 6 pm up to 6 am, from

October 2014 to June 2015. After each night of collection the mosquitoes were transferred to paper cups and killed with chloroform, identified and preserved in1.5 Eppendorf tube for future specie identification based on PCR.

2.3 Quality Assurance and Insecticide Residual Efficacy Tests

The standard WHO cone bioassay tests were performed in Mocuba, Milange, Morrumbala and Mopeia districts, from October 2014 through June 2015 to evaluate spray quality and residual efficacy of the insecticide used in the 2014 spray campaign. Wall bioassay for quality assurance evaluation was conducted 24 hours after spraying and subsequently monitored monthly from October and December 2014 up to the month where each of them reached mortality below 80% in treated houses (Table 8). The quality assurance tests were carried out at two different times, one in October 2014 (Morrumbala, Mocuba, Mopeia and Milange) with deltamethrin from Bayer and another in December 2014 with deltamethrin from Tagros (in Mopeia). For quality assurance with deltamethrin from Bayer, 80 cone bioassay tests were conducted on 800 *An. arabiensis* female mosquitoes in 20 houses. For the cone bioassay tests with deltamethrin from Tagros, 10 houses were selected in Mopeia and 40 tests were conducted with 400 *An. arabiensis*.

In each district five to ten houses were randomly selected and, following householder acceptance, cones were placed at selected resting surface heights of 0.5 m, 1.0 m and 1.5 m diagonally and one control cone was also used per house. For each cone a batch of 10 sugar-fed female susceptible *Anopheles arabiensis* mosquitoes were used on the sprayed surface as well as on the control; the control was fixed on a paperboard fixed with adhesive. Most surfaces of the wall on the houses were made of mud, and some of them were rough and others were smooth, no more than one house was of cement. For these bioassays 2 – 5 day-old females were used. WHO plastic cones lined with the self-adhesive packing were fixed on the sprayed walls for the assay. A batch of 10 mosquitoes were introduced into the plastic cones and left exposed on the sprayed surface for 30 minutes at different heights (replicates) in the houses. Numbers of mosquitos knocked down at the 30th minute 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 24 hours holding period. The dead and live mosquitoes were counted after 24 hours of holding period, and the percentage mortalities were calculated in the replicates for each house and recorded according to WHO protocol.

2.3.1 Insecticide Resistance Monitoring

An. gambiae s.l. was collected from different larval habitats in Mocuba (Ceta and Aeroporto I & II villages), Morrumbala (Franqueza village) and Milange (12 de Outubro and Nhamzombe villages) districts in January and February 2015. In 2015 the collection was moderate as some breeding sites were not accessible because they were highly affected by the excessive rain and flooding, At least one insecticide per class was tested in Mocuba and Morrumbala with the exception of Milange district where only deltamethrin was tested and it was not possible to complete all replicates.

In the insectary, the field collected larvae that were reared to adult stage and batches of 25 females who were sugar-fed and aged from 3 – 5 days, were subsequently subjected to the WHO tube tests following the standard protocol (WHO, 2013). These females were exposed to deltamethrin and

lambdacyhalothrin 0.05%, bendiocarb 0.1 %, DDT 4% and fenithrotion 1% in WHO impregnated filter papers for 60 minutes, the knockdown was checked on 10, 15, 20, 30, 40, 50 and 60 minutes, after this period all mosquitoes were gently transferred to holding tube and knockdown was again checked on 80 minutes on holding period and mortality was recorded 24 hours later. 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 susceptibility: less than 98% suggestive of existence of resistance and future investigation is needed, between 90% to 97% presence of resistance gene in vector population must be confirmed and below 90% as resistant.

After the tests were conducted, the mosquitoes were preserved in 1.5 µl Eppendorf tubes containing silica gel, and on RNALater® solution conserved at environmental temperature and on fridge at -20°C, awaiting for further identification using PCR technique as well as for the allelic frequency of knockdown resistance (KDR) and to measure of RNA levels associated with up-regulated enzyme mechanisms if possible.

2.4 RESULTS

2.4.1 PYRETHRUM SPRAY COLLECTIONS

A total of 740 *Anopheles* mosquitoes were collected in four districts over 96 days - two days in each district per month for twelve months -results as shown in Table2 below. The major malaria vector in Morrumbala, Milange and Maganja was *An. funestus* s.l. and in Mocuba the abundance of both vector species was very low. In general the most abundant malaria vector was *An. funestus* s.l. representing 67.84% and *An. gambiae* s.l. 32.16% of all collection. The peak of mosquito density of malaria vectors was observed in April and June in the control area Maganja da costa 86 (11.62%) and 71 (9.59%) for *An. funestus* s.l. and *An. gambiae* s.l., respectively. For Morrumbala the peak density was observed in April and May 16(2.16%) and 23(3.11%) for *An. gambiae* s.l. and *An. funestus* s.l. (Table2). The entomological monitoring was conducted year round to fully assess the seasonal abundance and species composition of the malaria vectors and potential impact of IRS interventions.

The total number of mosquitoes collected per site, month and species as well as the percentage contribution of each species out of the total malaria vectors collected is shown in Table 2. Morrumbala (intervention) was the district where more *An. funestus* s.l. and *An. gambiae* s.l. vectors were collected than the other two intervention districts (Mocuba and Milange). The distribution of malaria vectors per month and respective densities are shown in Table 3. Figures 2 a and b illustrate the trend of malaria vector density in both intervention and control areas over the monitoring period, including the base line (Pre-spray), per intervention versus control area.

Table 2: Total Number of Mosquitoes Collected per Month and Species using PSC

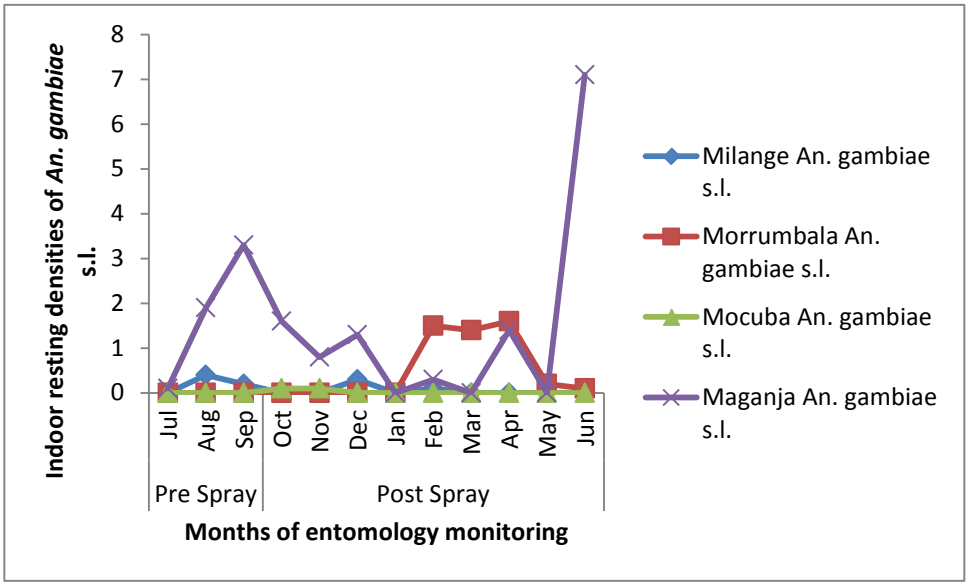
Districts	Species	Period of Entomological Monitoring													% collected
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total	
Mocuba (Int.)	*An. gambiae* s.l.	0	0	0	1	1	0	0	0	0	0	0	0	2	0.27
	An. funestus s.l.	0	0	0	0	0	0	0	0	1	0	0	0	1	0.14
Milange (Int.)	*An. gambiae* s.l.	0	4	2	0	0	3	0	1	0	0	0	0	10	1.35
	An. funestus s.l.	17	6	0	0	0	1	0	6	13	7	7	9	66	8.92
Morrumbala(Int.)	*An. gambiae* s.l.	0	0	0	0	0	0	0	15	14	16	2	1	48	6.49
	An. funestus s.l.	0	0	1	0	0	0	0	4	15	22	23	9	74	10.00
Maganja(Cont.)	*An. gambiae* s.l.	1	19	33	16	8	13	0	3	0	14	0	71	178	24.05
	An. funestus s.l.	22	40	17	10	16	40	0	2	1	86	84	43	361	48.78
Total		40	69	53	27	25	57	0	31	44	145	116	133	740	

Table 3. Malaria Vector Species Collected by PSC in the Intervention area and Control areas by Month and Respective Densities (indoor resting mosquitoes per room per day)

Maganja	*An. funestus s.l*	22 (2.2)	40 (4.0)	17 (1.7)	10 (1.0)	16 (1.6)	40 (4.0)	--[1]	2 (2.0)	1 (0.1)	86 (8.6)	84 (8.4)	43 (4.3)
	An. gambiae s.l	1 (0.1)	19 (1.9)	33 (3.3)	16 (1.6)	8 (0.8)	13 (1.3)	--[1]	3 (0.3)	0 (0.0)	14 (1.4)	0 (0.0)	71 (7.1)
Morrumbala	*An. funestus s.l*	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (0.4)	15 (1.5)	22 (2.2)	23 (2.3)	9 (0.9)
	An. gambiae s.l	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	15 (1.5)	14 (1.4)	16 (1.6)	2 (0.2)	1 (0.1)
Milange	*An. funestus s.l*	17 (1.7)	6 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	6 (0.6)	13 (1.3)	7 (0.7)	7 (0.7)	9 (0.9)
	An. gambiae s.l	0 (0.0)	4 (0.4)	2 (0.2)	0 (0.0)	0 (0.0)	3 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mocuba	*An. funestus s.l*	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	--[1]	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
	An. gambiae s.l	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.1)	0 (0.0)	--[1]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

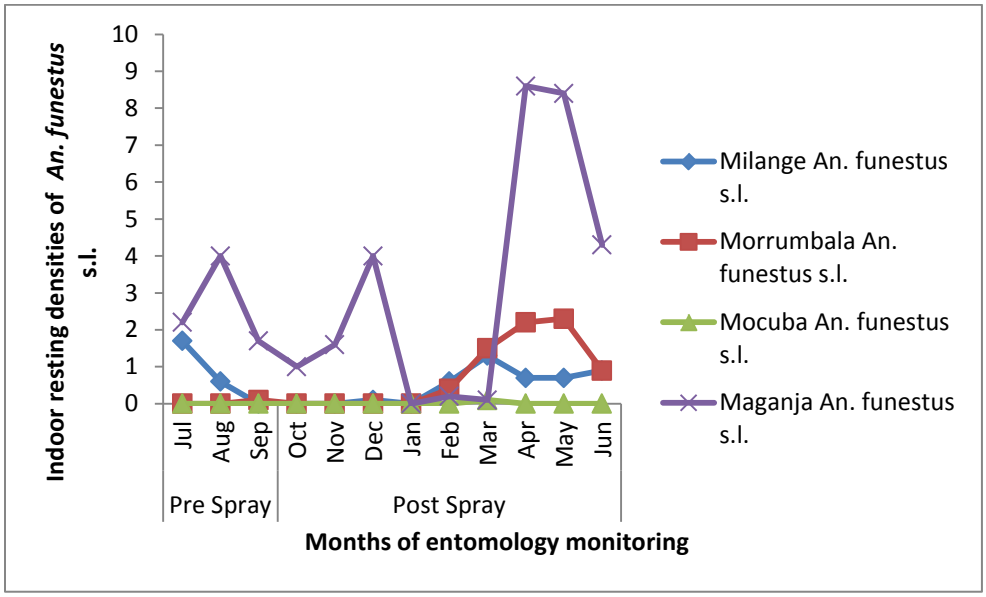
Figures 2 (a & b). Trend of Densities of Female *An. gambiae* s.l. and *An. funestus* s.l. per house per day in Mocuba, Morrumbala and Milange Intervention and Maganja Control Sites over time on the Pre and Post Spray Seasons

Figure 2a) Trend Densities of An. *gambiae* s.l. indoor resting (*intervention versus control*)



[1] No collections were conducted in January

Figure 2b). Trend Densities of An. funestus* s.l. indoor resting (intervention verus control*



At the baseline period (pre-spray), as well as during the post-spray period, the trend of the malaria vector in Mocuba (intervention) was the same, with low malaria vector collected. In Maganja (control), during both periods, the malaria vector presence was high when compared with Mocuba (Figure 2a and b). In Morrumbala, Figure 2a and b from all pre spray period up to three months after spray the densities of malaria vector was low, and from February to May 2015 there was a trend of increase for *An. funestus* s.l. as well *An. gambiae* s.l., but in May *An. gambiae* s.l. began decrease while in Maganja (Control) the densities of the vectors remain high, with inconsistent trend. In Milange (IRS-district) figure 2a and b, the densities of *An. funestus* s.l. and *An. gambiae* s.l., was relatively lower than the control one during the pre-spray season; after spray (October to June) the *An. funestus* densities seem to be low like the *An. gambiae*. After January, the *An. funestus* population increased but remained lower when compared with the control. Generally low densities were observed in the intervention areas during pre-spray as well after spray seasons, and this tendency maybe associated with the cumulative effect of the IRS, particularly in Mocuba.

2.5 HUMAN LANDING COLLECTION

A total of 1937 malaria vectors were collected using HLC method over 144 nights with three consecutive nights of collection per month per district from July 2014 to June 2015 (Table 4).

In most intervention districts most of the malaria vectors (*An. gambiae* s.l. and *An. funestus* s.l.) were collected outdoors; 422 mosquitoes were collected indoors and 587 outdoors (Table 4). In the control area *An. funestus* s.l. was collected more indoors than outdoors (Table 4). The proportion of *An. gambiae* s.l. mosquitoes collected outdoors was significantly higher than indoors for Mocuba, Morrumbala and Maganja sites (p values, 0.0046, <0.0001, and 0.0030, respectively). However, for *An. funestus*, the indoor

biting was significantly higher than outdoor for Milange and Maganja (p = 0.0069 and < 0.0001, respectively). No significant differences were observed for biting indoor and outdoor in the other sites.

In general, there is a tendency for exophagic behavior for both malaria vectors in intervention districts (Mocuba and Morrumbala) when compared to the control district; however in Milange both vectors look to be endophagic. Assuming that all inhabitants of the communities are indoors during the peak biting hours, IRS should protect the population from the vector as there does not appear to be any significant early evening or late morning biting.

Table 4. Distribution by genus, species and place of collection of adult mosquitoes collected with HLC in 4 Districts

	Anopheles spp. Collected					
	An. gambiae s.l.			*An. funestus* s.l.		
Districts	Indoor	Outdoor	p	Indoor	Outdoor	p
Mocuba	32 (35.16%)	59 (64.84%)	0.0046	3 (37.50%)	5 (62.50%)	0.4795
Morrumbala	142 (30.94%)	317 (69.06%)	< 0.0001	19 (38.00%)	31 (62.00%)	0.0897
Milange	108 (53.20%)	95 (46.80%)	0.3615	118 (59.60%)	80 (40.40%)	0.0069
Maganja	128 (41.56%)	180 (58.44%)	0.003	426 (68.71%)	194 (31.29%)	< 0.0001
Total	410 (38.64%)	651 (61.36%)	< 0.0001	566 (64.61%)	310 (35.39%)	< 0.0001

The number of malaria vectors collected (per hour) in intervention and control villages are shown in Tables 5 and 6.

Table 5. Data showing biting location and time for *An. gambiae* s.l. in intervention (Mocuba, Morrumbala and Milange) and control (Maganja) in Zambézia province over all collection rounds (pre and post spray)

Biting Time		*Anopheles gambiae* s.l.							
		Intervention (Districts)						Control (District)	
		Mocuba		Morrumbala		Milange		Maganja	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
All round	6-7 pm	0	4	0	8	3	1	0	2
	7-8pm	2	5	14	7	3	6	3	0
	8-9pm	2	1	6	6	5	4	3	2
	9-10pm	0	2	3	34	8	10	9	16
	10-11pm	2	4	12	12	9	3	17	22
	11Pm-12am	4	2	15	31	17	19	9	26
	12-1am	0	8	17	43	18	8	21	42
	1-2 am	5	10	23	60	8	4	20	21

	2-3 am	4	5	22	34	9	14	9	31
	3-4 am	8	4	13	46	11	10	18	7
	4-5am	4	3	12	19	10	9	17	8
	5-6am	1	11	5	17	7	7	2	3
	Total	32	59	142	317	108	95	128	180
Proportion of endophagy/exophagy		0.35	0.65	0.31	0.71	0.53	0.47	0.42	0.58

Table 6. Data showing biting location and time for *An. funestus* s.l. in intervention (Mocuba, Morrumbala and Milange and Control (Maganja) in Zambézia province over all round (Pre and Post spray)

Biting Time		*Anopheles funestus* s.l.							
		Intervention (Districts)						Control(District)	
		Mocuba		Morrumbala		Milange		Maganja	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
All round	6-7 pm	1	0	0	0	4	0	1	1
	7-8pm	0	0	0	0	6	2	4	2
	8-9pm	0	2	0	1	10	4	5	10
	9-10pm	0	0	1	0	6	8	16	16
	10-11pm	0	1	1	3	10	6	22	20
	11Pm-12am	1	0	3	1	9	15	30	19
	12-1am	0	0	2	9	7	9	45	34
	1-2 am	0	0	4	1	12	6	96	24
	2-3 am	0	2	6	3	11	8	77	25
	3-4 am	1	0	0	4	17	8	57	20
	4-5am	0	0	1	9	12	3	63	17
	5-6am	0	0	1	0	14	11	10	6
Total		3	5	19	31	118	80	426	194
Proportion of endophagy/exophagy		0.38	0.63	0.38	0.62	0.60	0.40	0.93	0.31

The peak biting periods outdoors for *An. gambiae* s.l. in Mocuba (Figure 3a) during post spray were from 1 to 2 and 5 to 6 am, while for the control (Maganja) Figure 3d, the peak was observed from 12 to 1 am. Indoors, peak biting was observed between 3 and 4 am in Mocuba and between 1 and 2 am in Maganja. For *An. funestus* s.l. it is difficult to define the biting peaks in Mocuba (Figure 3a) due to the low biting rate both indoors and outdoors. In the control sentinel site the biting peak period indoors for *An. funestus* s.l. was from 1 am to 2 am, while it was between 12and 1am outdoors. In Mocuba the biting rates were lower for both *An. gambiae* and *An. funestus* as compared with the control district, as was the same for the PSC method.

Overall, the peak biting time for *An. gambiae* s.l. in Morrumbala occurs at different times between 9 pm to 4 am with the highest biting activity occurring from 1 to 2 am outdoors (figure 3b). It was difficult to know the peak biting hours of *An. funestus* for Morrumbala due to very low biting rates both indoors and outdoors. Compared to the control district, more *An. gambiae* s.l. were caught in Morrumbala both indoors and outdoors, but significantly fewer *An. funestus* were captured both indoors and outdoors.

In Milange (figure 3c), for *An. gambiae* the peak biting time was 11 pm to 12 am and 12 to 1 am for outdoor and indoor biting, respectively. For *An. funestus* the peak biting time indoors was between 3 and 4 am and outdoors was between 11pm and 12am. For both vectors fewer mosquitoes were caught both indoors and outdoors in Milange compared to Maganja.

Figures 3 (a – d). Peak biting times of *An. gambiae* s.l. and *Anopheles funestus* s.l. in Mocuba, Morrumbala and Milange intervention and Maganja control site

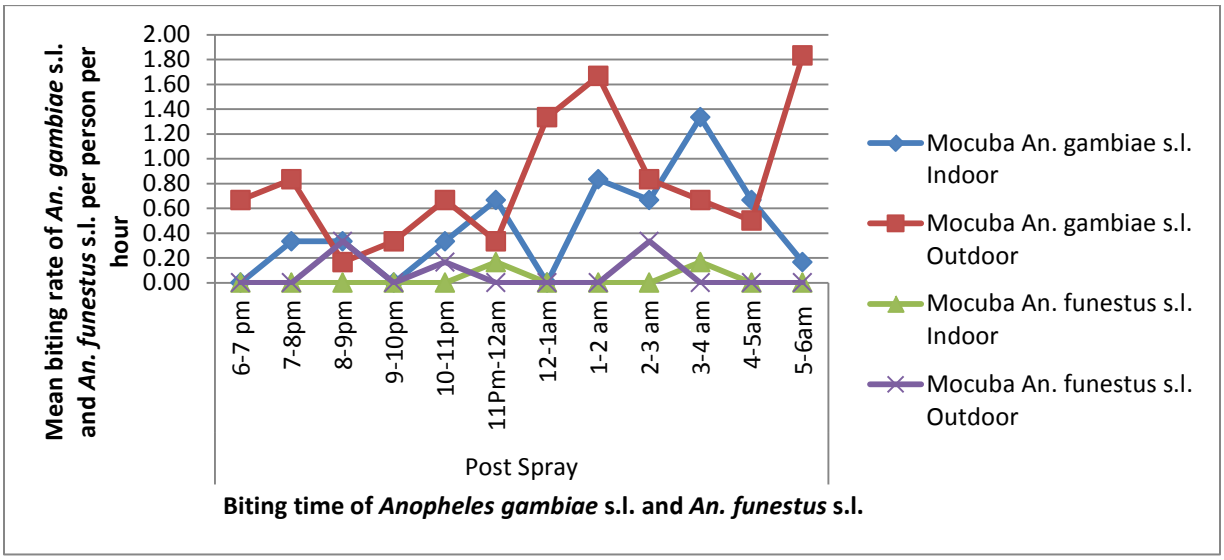


Figure 3a). Peak biting time of An. gambiae* s.l. and *An. funestus* s.l. *in Mocuba

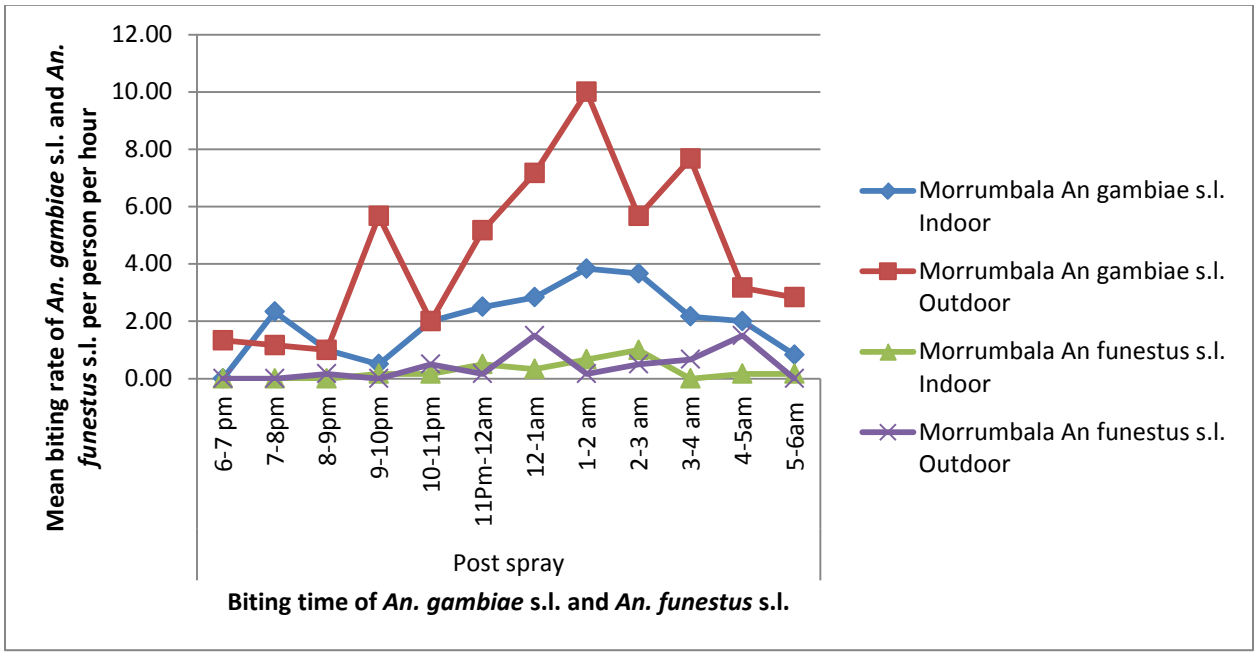


Figure 3b). Peak biting time of Anopheles gambiae* s.l. and *Anopheles funestus* s.l. *in Morrumbala

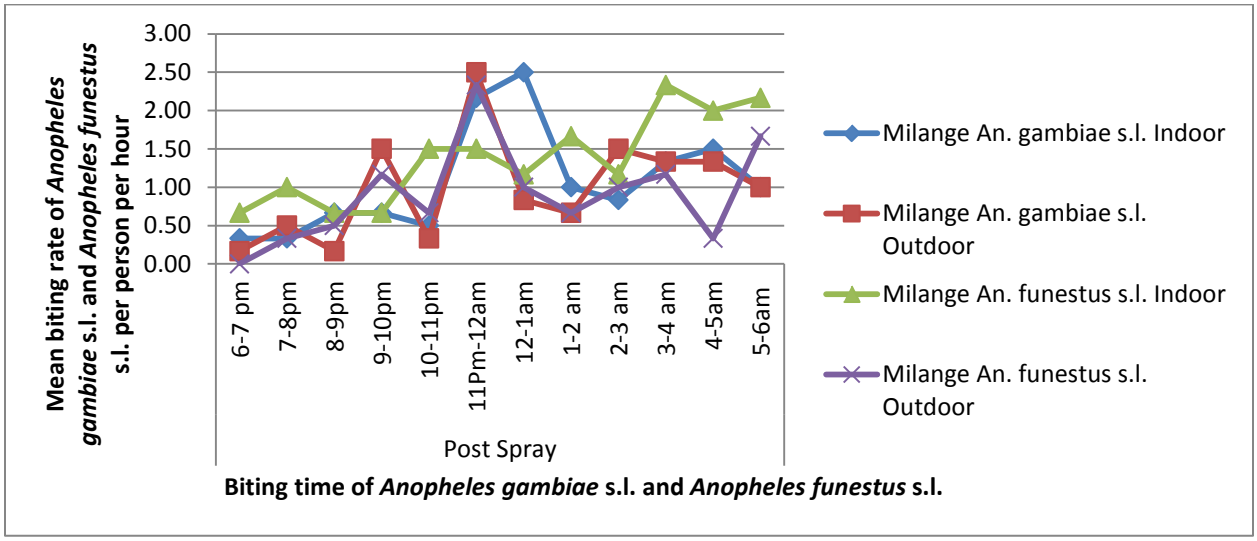


Figure 3c). Peak biting time of Anopheles gambiae* s.l. and *Anopheles funestus* s.l. *in Milange

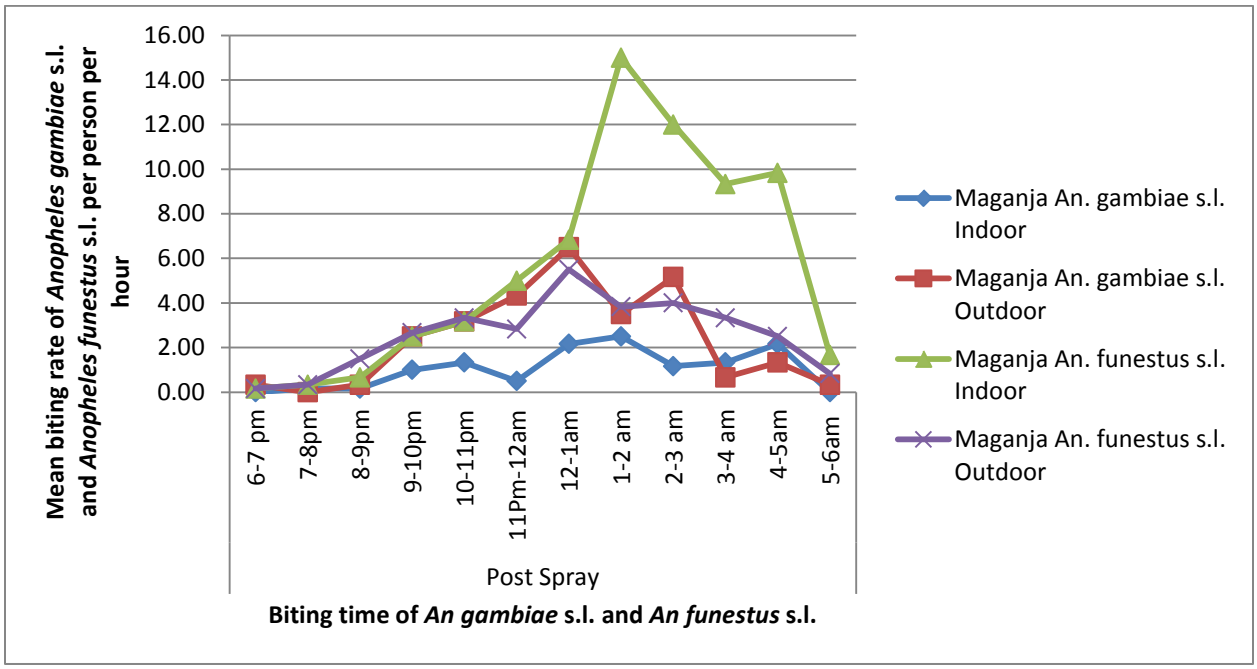


Figure 3d). Peak biting time of Anopheles gambiae* s.l. and *Anopheles funestus* s.l. *in Maganja

2.5. CDC LIGHT TRAP COLLECTION

This collection method was the last one introduced in October 2014, but it had interruptions in all districts in November due to battery charger issues and in January due to the heavy rain and flooding in Mocuba, Mopeia and Maganja. A total of 3206 *Anopheles* mosquitos were collected using the CDC light trap, of which 2105 were identified as *An. funestus*, 1091 as *An. gambiae* s.l., 4 *An. coustani,* 1 *An. vernus*, 2 *An. salbaii* and 3 *An. dancalicus* (Table 7). When compared with other collection methods, the CDC light traps collected more mosquitoes than the pyrethrum spray catches and human landing catches; Table 7a showe the monthly distribution of main malaria vector collected per district and respective densities per night trap of collection.

Figures 4a and b present the occurrence and trend distributions of both malaria vectors (*An. gambiae* s.l. and *An. funestus*) collected per trap per night in each intervention district (Mocuba, Morrumbala, Mopeia and Milange) and the control district (Maganja). Milange showed high densities of *Anopheles funestus* s.l. from February to June, while in Morrumbala the highest density was observed in the months of May to June with the peak in June, holding the possibility of this vector playing a role in malaria transmission in the cold months. The occurrence of *An. funestus* in cold months was also observed in Mopeia and Maganja. In Mocuba the densities of this vector were low (figure 4a).

Anopheles gambiae s.l. densities in figure 4b show that the species occurs mostly in the months of February to April in the summer, with high temperature, humidity and rain fall. After April this species abundance is reduced, and this pattern tends to be similar in all intervention and control districts.

Table 7. Number of *Anopheles* mosquitoes collected by CDC Light Trap

Milange	1116	524	1	3	2	1
Morrumbala	643	413	1	0	0	0
Mopeia	51	61	0	0	0	0
Mocuba	8	24	0	0	0	0
Maganja	287	69	2	0	0	0

Table 7a. Monthly distribution of *Anopheles funestus* and *gambiae* densities per trap per night

Months of collection	Districts of Entomological Monitoring									
	Intervention Areas (Four Districts)								Control site	
	Mocuba		Milange		Mopeia		Morrumbala		Maganja	
	An. funestus	*An. gambiae*	*An. funestus*	*An. gambiae*	*An. funestus*	*An. gambiae*	*An. funestus*	*An. gambiae*	*An. funestus*	*An. gambiae*
October	0 (0)	0 (0)	9 (0.75)	16 (1.33)	0 (0)	3 (0.25)	0 (0)	0 (0)	7 (0.58)	2 (0.17)
November	--	--	--	--	--	--	--	--	--	--
December	0 (0)	0 (0)	27 (2.25)	17 (1.41)	0 (0)	10 (0.83)	0 (0)	0 (0)	10 (0.83)	3 (0.25)
January	--	--	154 (12.83)	20 (1.67)	--	--	0 (0)	3 (0.25)	--	--
February	0 (0)	8 (0.66)	313 (26.08)	191 (15.92)	17 (1.42)	21 (1.75)	12 (1)	212 (17.67)	18 (1.5)	32 (2.67)
March	6 (0.5)	12 (1)	248 (20.66)	118 (9.83)	5(0.42)	9 (0.75)	50 (4.17)	176 (14.67)	17 (1.42)	9 (0.75)
April	0 (0)	4 (0.33)	63 (5.25)	143 (11.92)	13 (1.08)	8 (0.67)	224 (18.67)	21(1.75)	106 (8.83)	23 (1.92)
May	1 (0.08)	0 (0)	152 (12.66)	12 (1)	2 (1.17)	0 (0)	48 (4)	1 (0.09)	117 (9.75)	0 (0)
June	1 (0.08)	0 (0)	150 (12.5)	7 (0.58)	14 (1.17)	10 (0.83)	309 (25.75)	0 (0)	12 (1)	0 (0)

Figure 4a. Monthly *Anopheles funestus* s.l. distributions per night per trap of collection (CDC Light Trap)

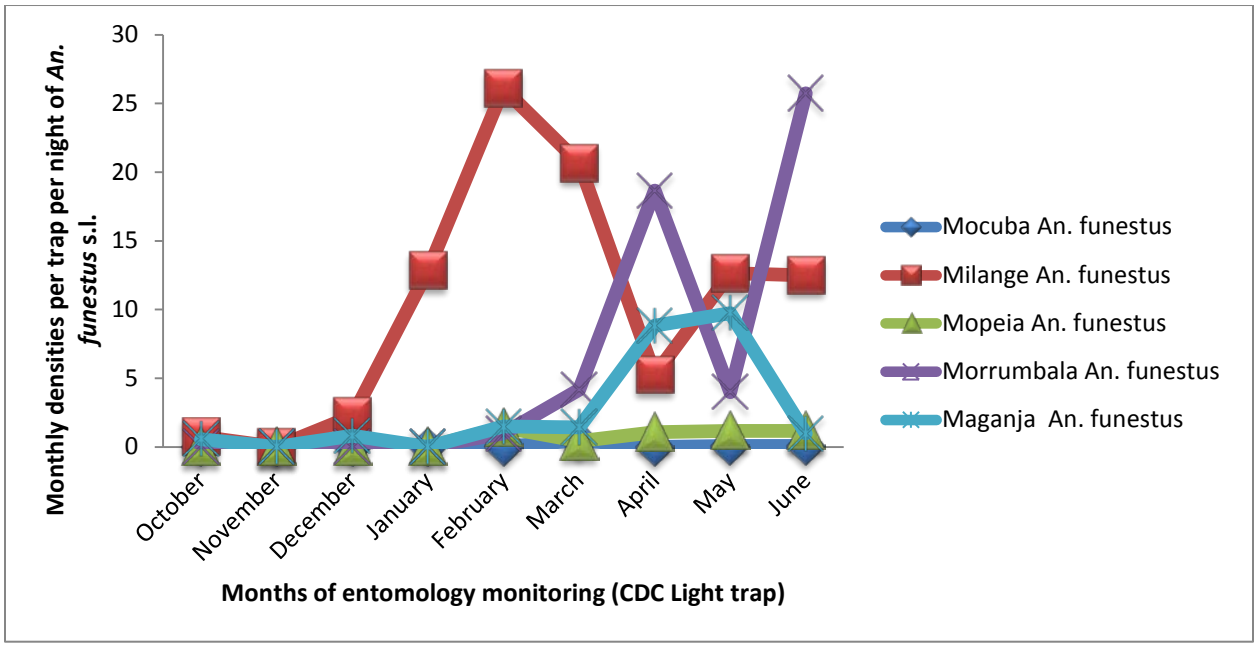
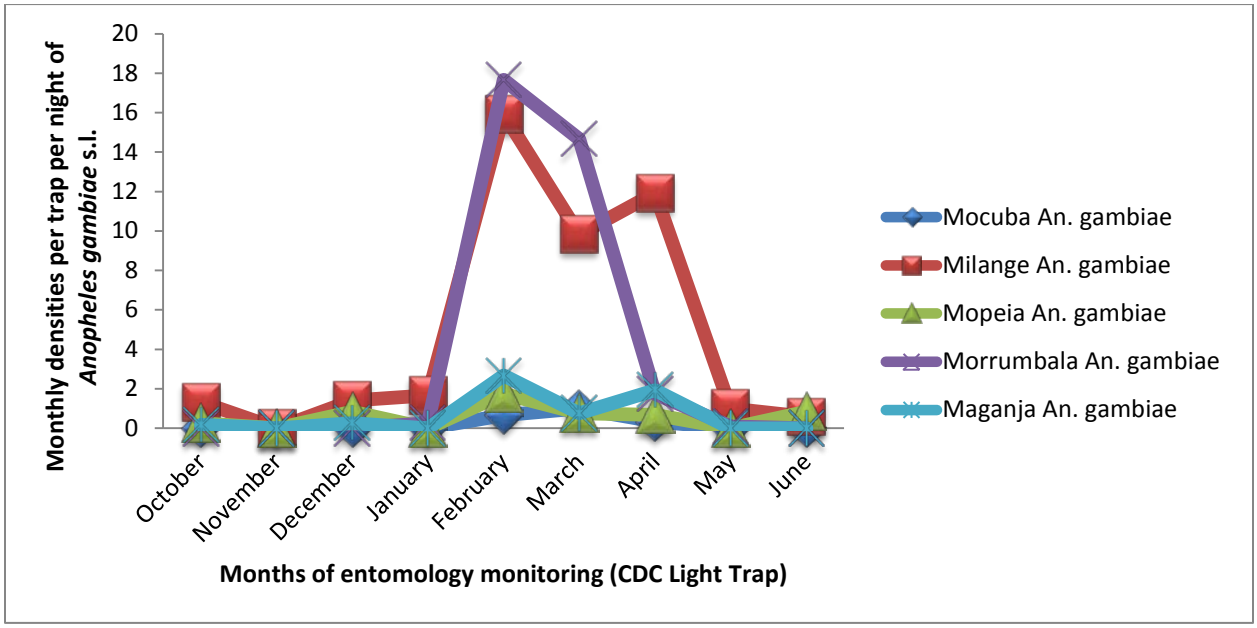


Figure 4b. Monthly *Anopheles gambiae* s.l. distributions per night per trap of collection (CDC Light trap)



2.6 Quality Assurance and Insecticide Residual Efficacy Tests using WHO Cone Wall Bioassay

The control mortality in the carton paper was zero for Morrumbala, Mopeia, Mopeia T and Milange, and only a total of three for all the control tests in Mocuba. The mortality of mosquitoes after the 24 hours holding period was 100% in all districts for both insecticides for the quality assurance tests (Table 9). The residual efficacy was no longer than three months in Mopeia with deltamethrin from Tagros, while in areas sprayed with deltamethrin from Bayer, generally the cutoff point of less than 80% was reached approximately six to seven months after spraying. This residual life shows to be covering the high transmission season (Table 10 and Figure 5).

Table 8. WHO cone bioassay calendar and design

Time	24 hr. post spray (October)	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
No of tests	80	80	120	100	120	120	120	60	60
No of mosquitos tested	800	800	1200	1000	1200	1200	1200	600	600
No of houses	20	20	30	25	30	30	30	15	15

Table 9. Wall Bioassay Test Results for Spray Quality in the Spray districts 24h after spray

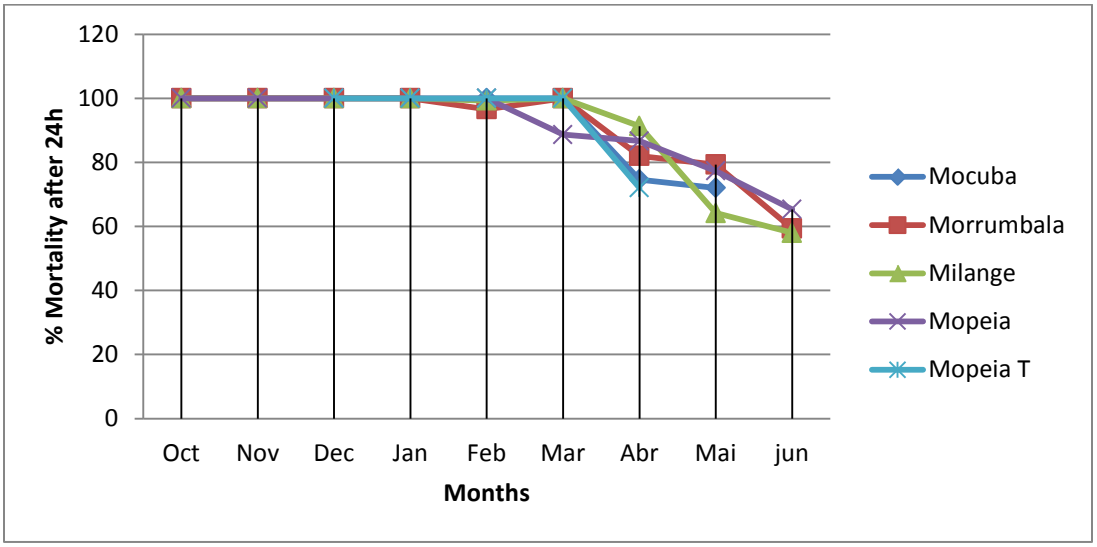
District	Site	# houses	# mosquito exposed	# mosquito killed after 24hrs	% test mortality rates post 24hrs holding period	% control mortality after 24hrs
Mocuba	Samora Machel	5	150	150	100	3/50
Morrumbala	Coqueiro	5	150	150	100	0/50
Milange	12 de Outubro	5	150	150	100	0/50
Mopeia	24 de julho	5	150	150	100	0/50
Mopeia T*	Força da Mudança	10	300	300	100	0/100

* Tagros

Table 10. Wall Bioassay Decay Rate by month and sites

Months	24 h Mortality %				
	Mocuba	Morrumbala	Milange	Mopeia	Mopeia T
Oct	100	100	100	100	---
Nov	100	100	100	100	---
Dec	100	100	100	100	100
Jan	---	100	100	100	100
Feb	100	96.6	99.3	100	100
Mar	100	100	90.67	88.67	100
Apr	74.6	82	91.33	86.67	72
May	72	79.33	64.17	77.33	---
June	---	59.33	58	65.33	---

Figure 5. Quality Assurance of the Spray and Decay Rates Measurements



2.7 INSECTICIDE RESISTANCE MONITORING

Overall, the number of mosquitoes used for the WHO susceptibility tests was within the recommended numbers (WHO, 2013) in two districts Mocuba and Morrumbala. However, in Milange it was below the recommended numbers due to the difficulty to access potential breeding sites in the area. Even in Mocuba and Morrumbala where the required numbers were achieved, overall there was site to site variation in terms of larval abundance. Tests with the WHO impregnated paper in Mocuba and Morrumbala showed that *An, gambiae* s.l., is resistant against Deltamethrin and Lambdacyhalothrin, in Mocuba and Morrumbala respectively. In both districts after first result using 100 female against Deltamethrin 0.05% on the test, two other repetitions using the same number of mosquitos were done

and the general 24h mortality indicated resistance. The mosquitoes were susceptible for all other insecticide tested (bendiocarb, DDT and fenithrothion) Table 11. Possible resistance was noted for lambdacyhalothrin and deltamethrin in Mocuba and Morrumbala, respectively. No resistance to deltamethrin was indicated for the tests on mosquitoes collected from Milange.

Table 11. Insecticide resistance test results of *An. gambiae* s.l. February 2015

Mocuba	*An. gambiae* s.l.	Deltamethrin	12	300	74.33
	An. gambiae s.l.	Lambdacyhalothrin	12	300	92.33
	An. gambiae s.l.	Bendiocarb	4	100	98.9
	An. gambiae s.l.	DDT	4	100	100
	An. gambiae s.l.	Fenitrothion	4	100	100
Morrumbala	*An. gambiae* s.l.	Deltamethrin	12	300	90.67
	An. gambiae s.l.	Lambdacyhalothrin	4	100	68.75
	An. gambiae s.l.	Bendiocarb	4	100	100
	An. gambiae s.l.	DDT	4	100	100
	An. gambiae s.l.	Fenitrothion	4	100	100
Milange	*An. gambiae* s.l.	Deltamethrin	4	100	100

2.8. Molecular Identification with PCR (sample from susceptibility test, PSC, CDC Light Trap and HLC)

At the moment, none of the samples collected during the reporting period from susceptibility test, CDC Light Trap, PSC and HLC, were processed by this method, or sent to laboratory outside of the country. There is however plans to send samples abroad (South Africa or CDC Atlanta) for molecular species identification and insecticide resistance alleles.

3. Conclusion

In October and December 2014, the 24h mortality result for quality assurance was 100% for all four districts and both insecticides used. The residual effect of the insecticide remained active for about three months (Tagros Deltamethrin) and six to seven months (Bayer deltamethrin) and began to lose the residual activity in subsequent months. With the PSC collection method, very few mosquitoes where collected on the site located in Mocuba during the post spray period, which may potentially be due to the cumulative residual effect of the insecticide on the wall or might naturally have lower vector

abundance. In Morrumbala from February to June there were malaria vectors of both species resting indoors (PSC collection), which could be due to flood and rain occurred in this period and associated with the seasonality of mosquito abundance in the area. This was the rainy and hot season, during which there is greater abundance of *An. gambiae* s.l. in the area, however *An. funestus* s.l. was also found in larger numbers. For Milange there was a relatively high number of *An. funestus* s.l. collected as compared to *An. gambiae* s.l. For the same period of collections by the PSC method in the control village (Maganga) a relatively higher number of malaria vectors were collected than in each intervention site. HLC will remain the gold standard to measure human vector contact, with our data in most of the intervention areas *An. gambiae* s.l. tends to feed mainly outdoor. The more recently introduced CDC Light trap showed to be a very productive method to monitor mosquitoes abundance in the area, and should be considered as an important monitoring tool for malaria vectors seasonal abundance.

4. RECOMMENDATIONS

AIRS Mozambique recommends to continue conducting HLC, as well as PSC data collection, in the intervention and control areas. The recently introduced method (CDC light trap) is equally recommended, it looks to be productive on mosquitoes collection in the areas, which is more or less independent of human bias, although HLC is the gold standard method to measure the human-vector contact directly.

For susceptibility testing AIRS Mozambique recommends spending more time for larvae collection in Milange to increase the number of mosquitoes tested using the standard WHO protocol and to achieve the target of testing at least one insecticide from each of the four classes of insecticides.

In order to improve the quality of the information in the report we recommend sending the mosquitoes samples every three month after collection for PCR laboratory to guarantee the inclusion of data on molecular analysis in each report.

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