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Indoor Residual Spraying (IRS 2) Task Order Six

MOZAMBIQUE ENTOMOLOGICAL MONITORING ANNUAL REPORT

JULY 2015 TO JUNE 2016

NOVEMBER 2016

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The views expressed in this document do not necessarily reflect the views of the United States Agency for International Development or the United States Government.

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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
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

EXECUTIVE SUMMARY

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.

Through PMI support, Abt has implemented five spray rounds of IRS in Mozambique, specifically in Zambezia province. During the 2015 spray campaign, AIRS Mozambique conducted IRS in six target districts (Derre, Milange, Mocuba, Molumbo, Quelimane, and Morrumbala). The PMI AIRS Mozambique program also included 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 supplement epidemiological data to guide proper targeting of IRS; evaluate the susceptibility level of the local vectors to different insecticides and know 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 monitor the residual life of different insecticides on different types of wall surfaces. This entomological monitoring report covers the period July 1, 2015 – June 30, 2016.In Zambezia province, including PMI AIRS intervention and non-intervention sites (target districts), the *An. gambiae* complex, *An. funestus* group, *An. coustani*, *An. tenebrosus*, *An. dancalicus*, *An. caliginosus*, *An. pretoriensis*, *An. aruni*, *An. rufipes*, *An. daudai*, *An. salbai* and *An. vernus* were found. Biomolecular analysis conducted by Witwatersrand University, under a contract with Abt, showed that *An. arabiensis* and *An. funestus* s.s. are the most common malaria vectors.

Based on the results of the three rounds of entomological monitoring data collected using the human landing catch (HLC), pyrethrum spray collection (PSC), and indoor CDC Light trap methods, the indoor resting density of malaria vectors and the human biting rate (HBR), as well as the biting time were variable through the months, sites and collection rounds. In Maganja da Costa, a non-intervention area, the indoor resting density and biting rates of *An. funestus* have been high over the collection years with the peak indoor resting density in July (before the spray season) during the dry and cold season. The peak indoor resting density for *An. gambiae* s.l. was in November and March for the 2015/16 collection season. Since HLC is a gold standard method that indicates the direct contact between human and vector, samples from this collection method collected between July to November 2015 were analyzed for *Plasmodium falciparum* infection, and the results, in general, show a low infection rate.

To determine the susceptibility malaria vectors and inform selection of the appropriate insecticide to be used for the IRS, WHO susceptibility tests were conducted on mosquito samples collected at the intervention sites, and resistance was detected in mosquitoes exposed to pyrethroids in Milange, Morrumbala and Mocuba districts, and all mosquitoes tested with bendiocarb, pirimiphos-methyl and fenitrothion were fully susceptible. CDC intensity bottle assays were also used to measure intensity of resistance, and the results showed the intensity of pyrethroid resistance against *An. gambiae* s.l. was generally low. After the 2015 spray campaign, WHO cone wall bioassays were conducted to measure the quality of the spray and the residual life of the insecticides used for IRS. The results in Quelimane showed low quality of spraying and the area had to be re-sprayed. In the other sites, the results were generally acceptable. The data on the decay rate of insecticides showed differences in residual life of Actellic® 300 CS and deltamethrin. In Morrumbala and Mocuba the residual life of Actellic® 300 CS was five months and of deltamethrin in Milange and Quelimane (Maquival) six months. The residual life of Actellic® 300CS in Morrumbala was longer on mud surfaces than on cement.

1. INTRODUCTION

Through PMI support, AIRS Mozambique has implemented five spray rounds of IRS in Mozambique. During the 2015 spray campaign AIRS Mozambique in partnership with the Ministry of Health conducted IRS from October to December in six targeted districts, namely Derre, Milange, Mocuba, Molumbo, Quelimane and Morrumbala. In 2016, AIRS Mozambique in partnership with Ministry of health conducted IRS in seven target districts namely Derre, Milange, Mocuba, Quelimane, Morrumbala and Mopeia.

During this period AIRS Mozambique continued conducting entomological monitoring activities in five districts of centrally located Zambezia province, where the malaria burden is high – malaria prevalence there was 68% in the 2015 Immunization, Malaria and HIV/AIDS Indicator Survey. The entomological monitoring included monitoring of IRS impact on vector density, behavior, and composition; evaluating the susceptibility level of the local vectors to different insecticides; and understanding the potential mechanisms of resistance. Entomological monitoring is vital to determining vector susceptibility to different insecticides and the residual life of different insecticides on different types of walls under various environmental conditions. Results provide evidence to inform the selection of the insecticide(s) to be used for indoor residual spraying (IRS) and to make decisions about other operational criteria. As such, entomological monitoring is an essential part of properly targeting and planning IRS.

AIRS Mozambique continued routine entomological data collection, insecticide resistance testing, and determining the decay rate of insecticides sprayed for IRS in the project areas. Specific objectives of the entomological work were:

- Assess and determine *Anopheles* malaria vector species composition occurring in the intervention and control areas.
- Monitor malaria vector densities and behavior before and after IRS intervention.
- Assess malaria vector seasonality.
- Assess susceptibility of the main malaria vector to at least one insecticide of each class approved by the World Health Organization (WHO) Pesticide Evaluation Scheme (WHOPES).
- Determine the quality of spray operation and monitor insecticide decay rate.
- Maintain the susceptible *An. arabiensis* Durban colony in the Quelimane insectary for quality assurance and determine decay rate of insecticide sprayed.
- Provide financial support to the NMCP for resistance monitoring, PSCs, and cone bioassays to evaluate the quality of IRS operations and decay rate of insecticides sprayed for seven provinces, including Niassa, Tete, Manica, Sofala, Inhambane, Maputo and Gaza. Entomological monitoring activities in Cabo Delgado and Nampula provinces were covered through WHO in 2016.

In addition to reporting of the routine monthly collections to determine the species composition, behavior, abundance, and seasonality, this report summarizes other key seasonal activities in Zambezia province:

1. WHO insecticide susceptibility tests conducted in January and February with mosquito samples from Mocuba, Milange, and Morrumbala.
2. U.S. Centers for Disease Control and Prevention (CDC) bottle intensity assay conducted in January and February, also with mosquito samples from Mocuba, Milange, and Morrumbala.
3. Cone wall bioassay for quality assurance and decay rate of insecticides sprayed.

2. ACTIVITIES

2.1 MONITORING VECTOR BEHAVIOR AND DENSITY

AIRS Mozambique selected four districts (four sentinel sites) where it would collect entomological data on vector behavior and densities to monitor vector population dynamics. The project selected the three intervention (sprayed) districts of Mocuba, Milange, and Morrumbala, and one control (no spray), Maganja da Costa.

2.1.1 COLLECTION SCHEDULE

Entomological data collection took place in all four districts (intervention and control) throughout 2015/16. It started in July 2015, three months before spray (for a pre-spray baseline) and continued during the spray campaign (October 2015) and after spray for nine months. Final data were collected in June 2016 in all sites.

Two intervention districts (Mocuba and Morrumbala) were sprayed with Actellic® 300 CS; the third district (Milange) was sprayed with deltamethrin (Pali™ 250 WG) from October-December 2015.

2.1.2 COLLECTION METHODS

The methods used to collect entomological data in both intervention and control areas are outlined below.

2.1.2.1 HUMAN LANDING CATCHES

Human landing catches (HLCs) were conducted to collect blood-seeking mosquitoes. The HLCs were conducted in two households in each sentinel site for three consecutive nights per month. Four collectors worked in two two-person teams per house per night; each team served a six-hour shift, and so together they covered 12 hours of collection, from 6 p.m. to 6 a.m. One human collector was seated indoors and another seated outdoors. Outdoor mosquito collection was carried out about eight meters from the indoor sampling point in each house. Outdoor and indoor collectors switched places every hour.

Collectors adjusted their clothing so that their legs were exposed up to the knees. When they felt a mosquito landing, they quickly turned on their torch, collected the mosquito with the sucking tube, and transferred it to a paper cup. One cup was used for each hour of collection. Hourly temperature and humidity were recorded. Mosquitoes were identified, using taxonomic keys (Gilles and Coetzee, 1987).

2.1.2.2 PYRETHRUM SPRAY CATCH

Pyrethrum spray catch (PSC) was used to sample indoor resting mosquitoes in 10 houses in each of the intervention and control sites every month. Collections were carried out in the morning

between 5 a.m. and 7:00 a.m. Before the PSC was performed, all occupants were politely asked to move out of the house. The team recorded information from the head of household or an adult member about the number of people who had slept in the house the previous night. For the PSC, the floor was covered with white sheets. The eaves, windows, and other mosquito escape routes around the house were sprayed with Baygon (deltamethrin 0.5 g/kg and imiprothrin 1,0g/kg) (knockdown (KD) spray) as were the walls and roof space inside the house.

Ten minutes after spraying, collectors carefully removed the white sheets outside of the room, and all the mosquitoes that were knocked downed were collected and sorted by species. The abdominal status of all female anopheleses was determined, and individuals were identified as unfed, blood-fed, half-gravid, and gravid.

2.1.2.3 CDC Light Traps

CDC light traps were installed in four houses in the same area of the houses selected for HLC in each of the four sentinel sites. Also like the HLC collection, this collection was done for three consecutive nights every month. The CDC light-traps were suspended in a bedroom 1.5 meters from the floor and about 50 centimeters from a human sleeping under an untreated net. Traps were fitted with an incandescent bulb. The traps were set only indoors from 6 p.m. to 6 a.m. Mosquitoes were collected from the traps the next morning.

2.2 Vector Susceptibility Testing

Sample vector species were planned to be collected in the four entomological sentinel sites; however, only samples from the three intervention sentinel sites (in Mocuba, Morrumbala, and Milange) were tested for their susceptibility to insecticides. Testing in Maganja was not planned because of difficulty accessing the areas with potential breeding sites as some of the bridges were removed with flooding. Vector resistance to various insecticides approved by WHOPES for IRS use was monitored during this susceptibility testing. The following insecticides were tested using the WHO tube test 0.05% deltamethrin, 0.1% bendiocarb, 0.05 % lambdacyhalothrin, 4% DDT, 1% fenitrothion, and 0.25% pirimiphos-methyl.

The CDC bottle intensity assay was also used to assess the intensity of insecticide resistance in the areas. For 2016, the following insecticides were tested with the CDC bottle intensity assays: pirimiphos-methyl, deltamethrin, lambdacyhalothrin, and DDT. These insecticides were selected based on mosquito sample availability and historical use of the insecticides in the AIRS Mozambique intervention districts. Each insecticide was tested using the concentrations of 1x, 2x, 5x, and 10x.

2.2.1. Larval Collection

In early 2016, larval collections were conducted in three districts: in Mocuba on January 14-23, in Morrumbala on January 16-25, and in Milange on February 5-14. Purposefully oriented sampling was done to maximize collection of the aquatic stages of mosquitoes where it was possible to use an adapted metal dipper of approximately 200 ml. At each site, dips were made in places likely to harbor mosquito larvae, such as around tufts of submerged vegetation, edges of water bodies, and around floating debris. Larvae were classified either as *Anopheles* or culicines by the presence of the siphon, and only anopheles larvae were transported to the insectary located in

Quelimane in bottles of 1.5L with significant space on the top to allow larvae breathing. Each bottle was labeled with the date and site of the collection.

2.2.2 Larval Rearing

In the insectary, the larvae were kept in larval trays using water from the aquatic habitats from which the larvae were collected. The water was changed every other day to avoid scum formation. Each day, pupae from the same district were collected and put together in a small rounded tray and thereafter transferred into the mosquito cages. Adult female mosquitoes aged from 3 to 5 days were collected based on their morphological character and used for susceptibility tests.

2.2.3 WHO Tube Test Procedures

Emergent *Anopheles* mosquitoes were sorted according to species and given 10% sucrose solution. Twenty-five 3–5-day-old *Anopheles* mosquitoes were tested per replicate. According to the WHO protocol (WHO, 2013), only female mosquitoes were exposed to 0.05% deltamethrin, 0.1% bendiocarb, 0.05% lambdacyhalothrin, 4% DDT, 1% fenitrothion, and 0.25% pirimiphos-methyl impregnated papers and to oil impregnated papers in the control of each insecticide for one hour. KD was observed at 10, 15, 20, 30, 40, 50, and 60 minutes during the exposure period. KD was continuously observed up to the 80th minute during the holding period. The mosquitoes were kept in holding containers on 10% sugar solution for 24 hours prior to measuring mortality.

WHO criteria was used to interpret susceptibility test results:

- Susceptible = Mortality in the range 98 – 100%
- Possible Resistance = Mortality rate after 24h between 90 – 97%.
- Resistance = Mortality less than 90%

2.2.4 CDC Bottle Bioassays

The resistance intensity of deltamethrin, pirimiphos-methyl, DDT, and lambdacyhalothrin on *An. gambiae* s.l. populations from Morrumbala and Mocuba as well the resistance intensity of deltamethrin to *An. gambiae* s.l. from Milange was measured using the CDC bottle bioassay (Brogdon and Chan 2010). Four pre-measured vials of each insecticide, each containing different concentration of 1x, 2x, 5x, and 10x, were diluted in acetone.

A sufficient amount of acetone was added three times to each insecticide vial and washed off into a 50 ml graduated falcon tube. The falcon tube was topped up to the 50 ml mark. The prepared insecticide solutions were used to coat the 250 ml Wheaton bottle. The control bottle was prepared by adding 1 ml of acetone to a 250 ml Wheaton bottle and coating it as described by Brogdon and Chan (2010).

Four different test bottles for each insecticide were coated with 1 ml of different concentrations of the prepared insecticide solution to get each bottle with 1x, 2x, 5x, and 10x insecticide concentration. Between 6 and 25 mosquitoes were introduced into the four replicate bottles marked with their respective concentration. One control bottle coated with acetone was also run alongside the tests and the first KD observation was counted on time zero and at minute 15, 30, 45, 60, 75, 90, 105, and 120, and then transferred to the cage, then to carton cups. The final mortality count was done after 24 hours.

2.2.5 Sample Preservation

Mosquitos that survived the 24-hour post-exposure to WHO testing were killed with chloroform and separated from the dead mosquitoes and preserved separately on silica gel and RNA later (to perform enzymatic assays) for future morphological identification and polymerase chain reaction (PCR) analysis. At least 50% of all samples of each collection method, including all resistance testing, will be sent to a molecular laboratory to be processed for molecular species identification and detection of sporozoites using ELISA. For PCR on species ID and ELISA

sporozoite infection all samples collected from July to November 2015 using HLC were sent for molecular analyses; in parallel with these samples, all susceptibility test samples from 2015 were sent for species ID, kdr-west and east mutation detection, and differentiation between S and M (*An. coluzzii*) forms.

2.2.6 Cone Bioassay Test

In the 2015 spray campaign, the Ministry of Health, supported by AIRS Mozambique, sprayed in six of Zambezia province's 19 districts: Mocuba, Morrumbala, Milange, Derre, Quelimane, and Molumbo. The spray campaign started on October 19, 2015. Milange, Molumbo, and Quelimane were sprayed with deltamethrin (Pali™ 250 WG), Mocuba, Morrumbala, and Derre with Actellic® 300 CS.

Cone bioassay tests were used to evaluate quality of the spray operation. The objective was to assess the quality of the spray operations within the first two weeks of the spray campaign and the residual life of the insecticides sprayed. The bioassay tests were conducted in the villages of Pedreira (Mocuba), Coqueiro (Morrumbala), 12 de Outubro (Milange), and Madal (Quelimane). Tests were carried out using known susceptible mosquito colonies of *An. arabiensis* from Durban, South Africa, reared in the AIRS insectary located at the Provincial Health Directorate in Quelimane. Two to five-day-old sugar-fed females were exposed to the sprayed walls. Tests were performed in five houses randomly selected sprayed by different spray operators in each of the districts, 24 hours after spraying. Ten mosquitoes were introduced into each cone fixed on the wall, placed at top, middle, and bottom positions of the wall above the floor; a fourth cone was installed on the door. In each test site, one control was used per house and 10 mosquitoes were introduced in each control cone. Mosquitoes were exposed to the wall for 30 minutes, then transferred into paper cups and supplied with 10% sugar solution. Mosquito KD was recorded at 30 minutes on the end of exposure period, at minute 60, and after the 24-hour holding period. The residual life of the sprayed insecticides was also monitored monthly in the same four districts, following the same procedures, until the percentage mortality after the 24-hour holding period dropped below 80 percent.

3. RESULTS

3.1 ANOPHELINE SPECIES COLLECTED BY THE DIFFERENT COLLECTION METHODS

During the reporting period, a total of 3,320 adult female *Anopheles* mosquitoes were collected using PSC, HLC, and indoor CDC light traps (Table I). Detailed data are included in Annex 1. *An. gambiae* s.l. and *An. funestus* s.l. were common in all intervention and control sites. *An. funestus* s.l. is dominant at Milange and Maganja sites, whereas in Mocuba the dominant species was *An. gambiae* s.l. Both species were found at almost equal proportion in Morrumbala. The occurrence of other species varied from site to site. The species composition of collected mosquitoes by site is shown on Table 1, and Table 1A is showing the species composition per round by morphological identification done in the Quelimane insectary.

TABLE 1. SPECIES COMPOSITION OF MOSQUITO COLLECTED IN THE FOUR SENTINEL SITES (PERCENTAGES IN PARENTHESES)

Species	Milange	Morrumbala	Mocuba	Maganja
An. gambiae	232(24.5)	148(45.4)	123(90.4)	404(21.1)
An. funestus	683(72.2)	142(43.6)	12(8.8)	1490(78.0)
An. rivulorum	0(0.0)	0(0.0)	1(0.7)	0(0.0)
An. dancalicus	0(0.0)	31(9.5)	0(0.0)	0(0.0)
An. coustani	13(1.4)	2(0.6)	0(0.0)	9(0.5)
An. tenebrosus	0(0.0)	0(0.0)	0(0.0)	5(0.3)
An. caliginosus	1(0.1)	0(0.0)	0(0.0)	2(0.1)
An. pretoriensis	11(1.2)	1(0.3)	0(0.0)	0(0.0)
An. aruni	1(0.1)	0(0.0)	0(0.0)	1(0.1)
An. rufipes	4(0.4)	2(0.6)	0(0.0)	0(0.0)
An. daudi	1(0.1)	0(0.0)	0(0.0)	0(0.0)
An. vernus	1(0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Total	**947**	**326**	**136**	**1911**

TABLE 1A. HISTORICAL SUMMARY OF SPECIES COMPOSITION IN DIFERENT ROUND OF ENTOMOLOGICAL MONITORING (MORPHOLOGICAL IDENTIFICATION BY ABT TEAM)

	Years of Entomological monitoring			
Morphological species ID	**2012-2013**	**2013-2014**	**2014-2015**	**2015-2016**
An. gambiae	Yes	Yes	Yes	Yes
An. funestus	Yes	Yes	Yes	Yes
An. rivulorum	No	No	No	Yes
An. dancalicus	No	No	No	Yes
An. coustani	Yes	Yes	Yes	Yes
An. tenebrosus	No	No	No	Yes
An. caliginosus	No	No	No	Yes
An. pretoriensis	No	No	No	Yes
An. aruni	No	No	No	Yes
An. rufipes	No	No	No	Yes
An. daudi	No	No	No	Yes
An. salbai	No	No	No	Yes
An. vernus	No	No	Yes	NoYes

3.1.1 PYRETHRUM SPRAY COLLECTION

From July 2015 to June 2016, a total of 484 *Anopheles* mosquitoes were collected. The most common species were *An. gambiae* and *An. funestus,* representing, respectively, 18.4 percent and 81.2 percent of all mosquitoes collected. Other species collected included *An. rivulorum* and *An. dancalicus,* each representing 0.2 percent of the total collection.

Figure 1 (a and b) shows the PSC results, indicating variation of vector densities during the pre-spray and after the spray season. The figure presents the seasonality of malaria vectors in terms of indoor resting densities at both intervention and control sites. In Maganja (control site), the species peaked at different times, *An. funestus* before the spray during the dry and cold season, and *An. gambiae* s.l. in November and March (although they were present throughout the year). The indoor resting density of both species was generally low in all the intervention sites except in Morrumbala, where both species peaked during the pre-spray season, and decreased in number after the spraying.

In Figure 1 c, d, e, f, g and h, the indoor resting densities of malaria vector species in the previous years are presented, and there are variations in the density pattern over the years.

FIGURE 1: INDOOR RESTING DENSITY OF FEMALE *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. PER HOUSE PER DAY IN INTERVENTION AND CONTROL SITES DURING PRE- AND POST-SPRAY SEASONS AND HISTORICAL DENSITIES

Figure 1(a): *An. funestus* s.l.

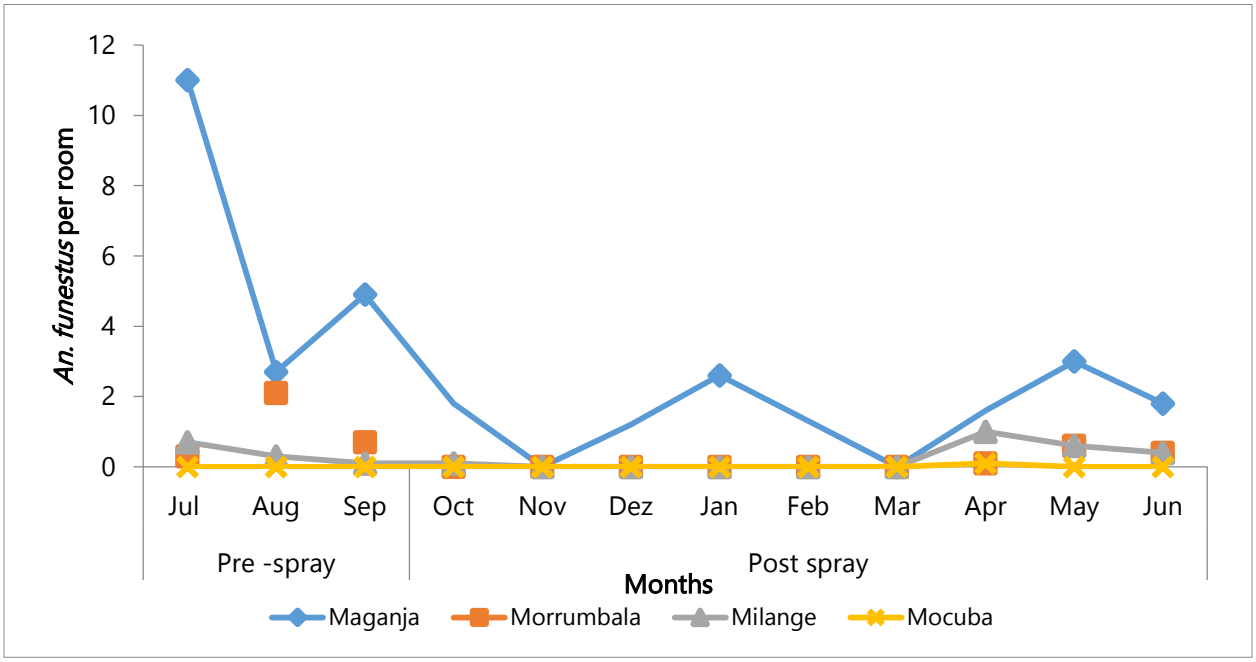


Figure 1(b): *An. gambiae* s.l.

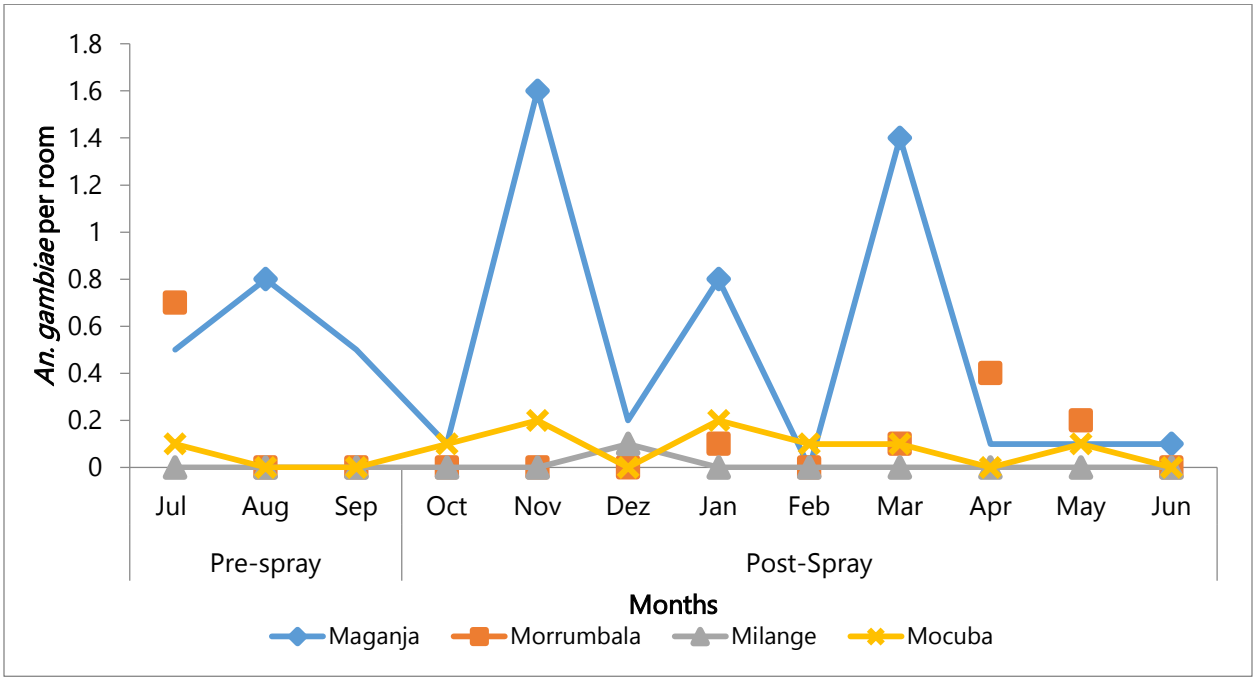


Figure 1(c): Historical Densities of *An. funestus* s.l. in Milange

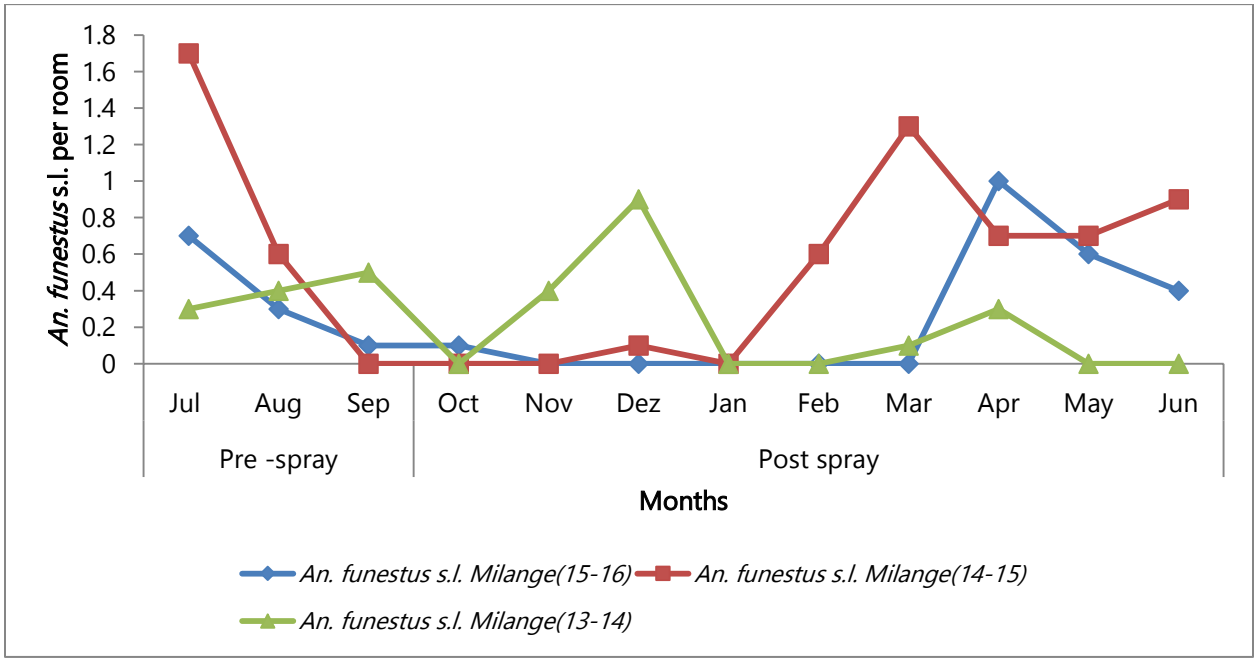


Figure 1(d): Historical Densities of *An. gambiae* s.l. in Milange

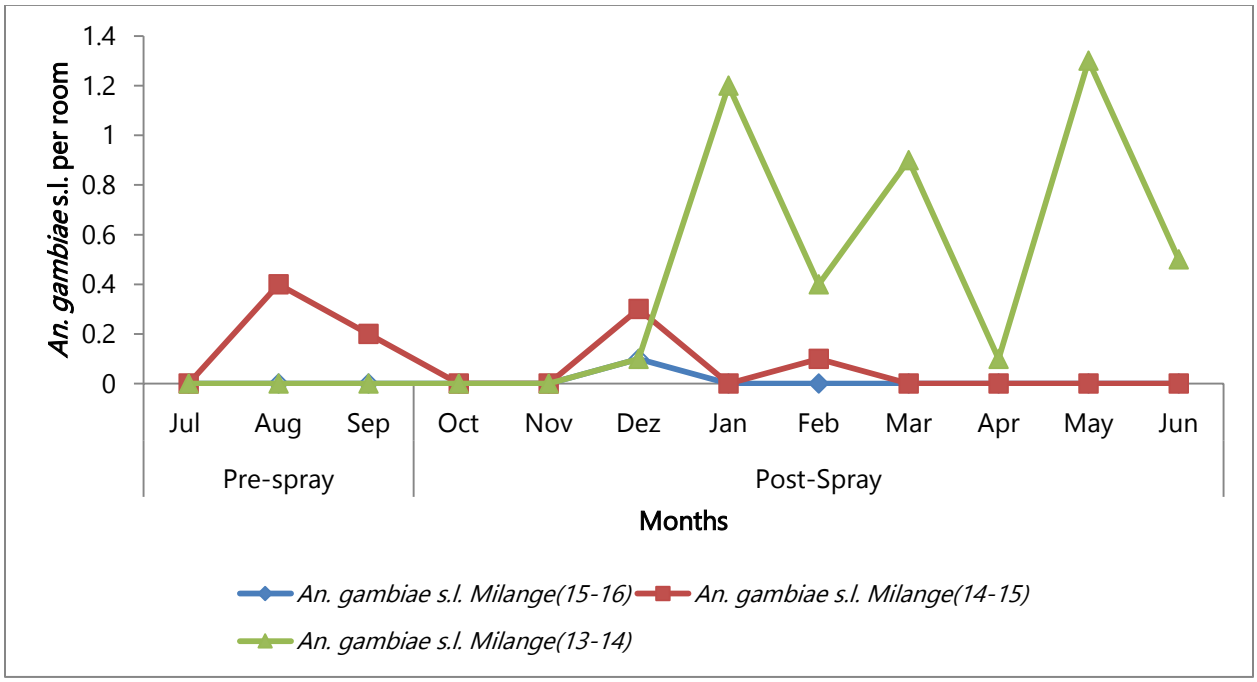


Figure 1(e): Historical Densities of *An. funestus* s.l. in Mocuba

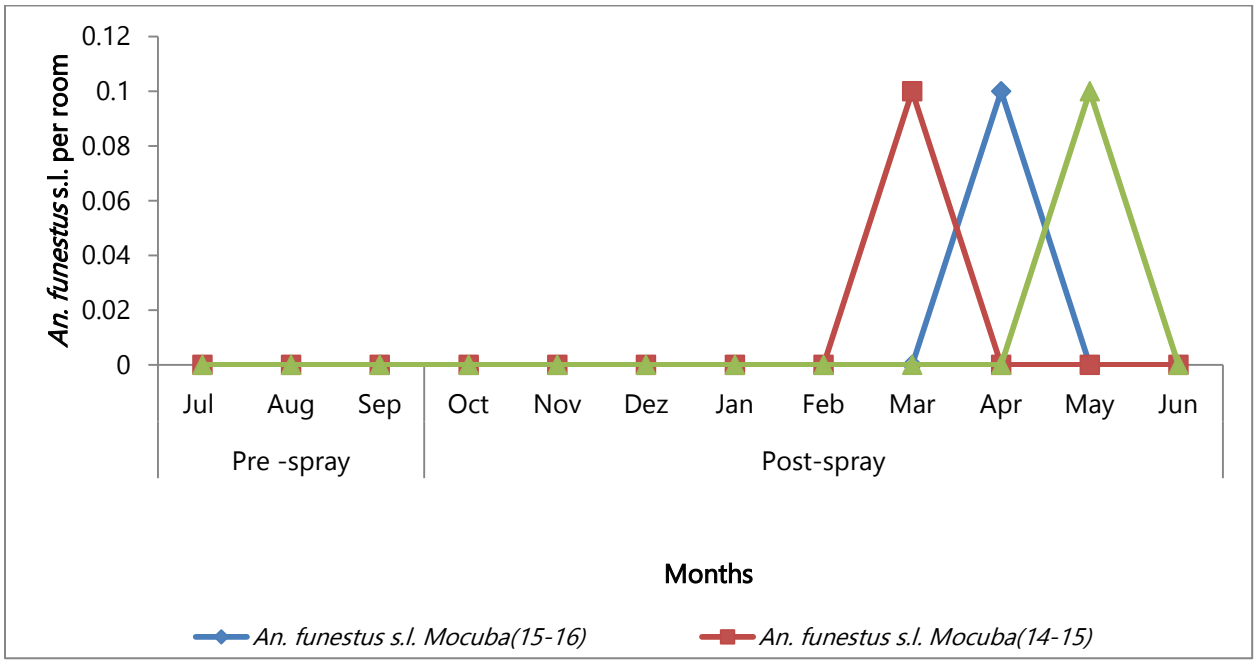


Figure 1(f): Historical Densities of *An. gambiae* s.l. in Mocuba

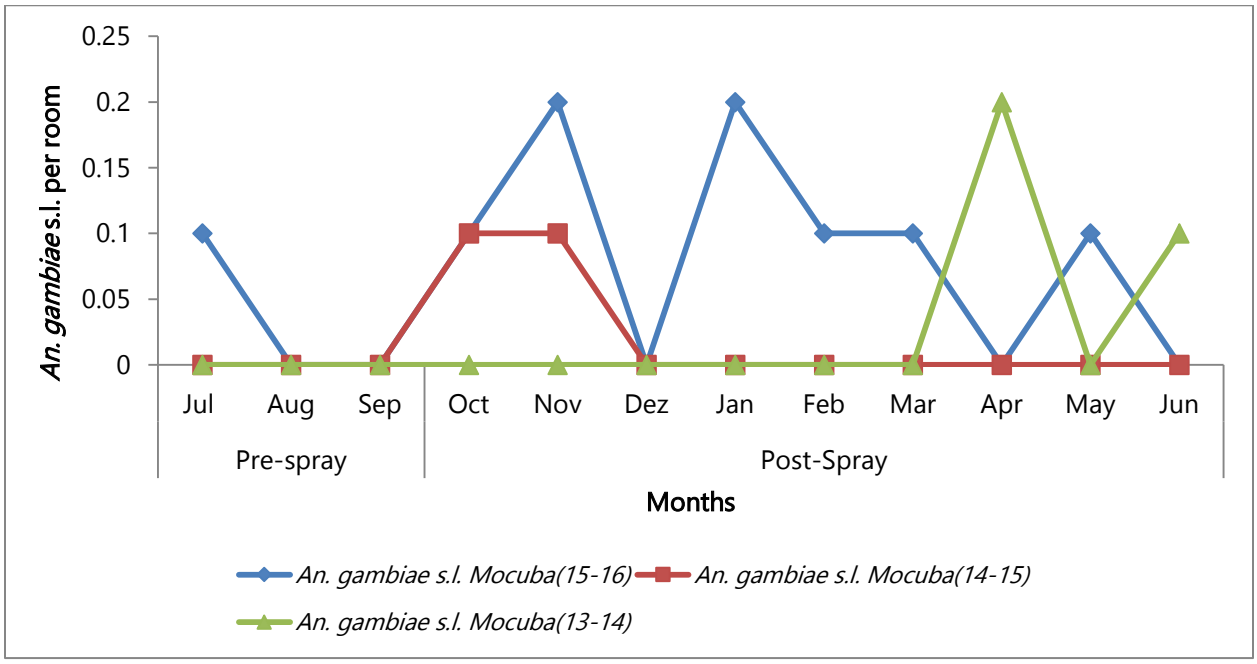


Figure 1(g): Historical Densities of *An. funestus* s.l. in Morrumbala

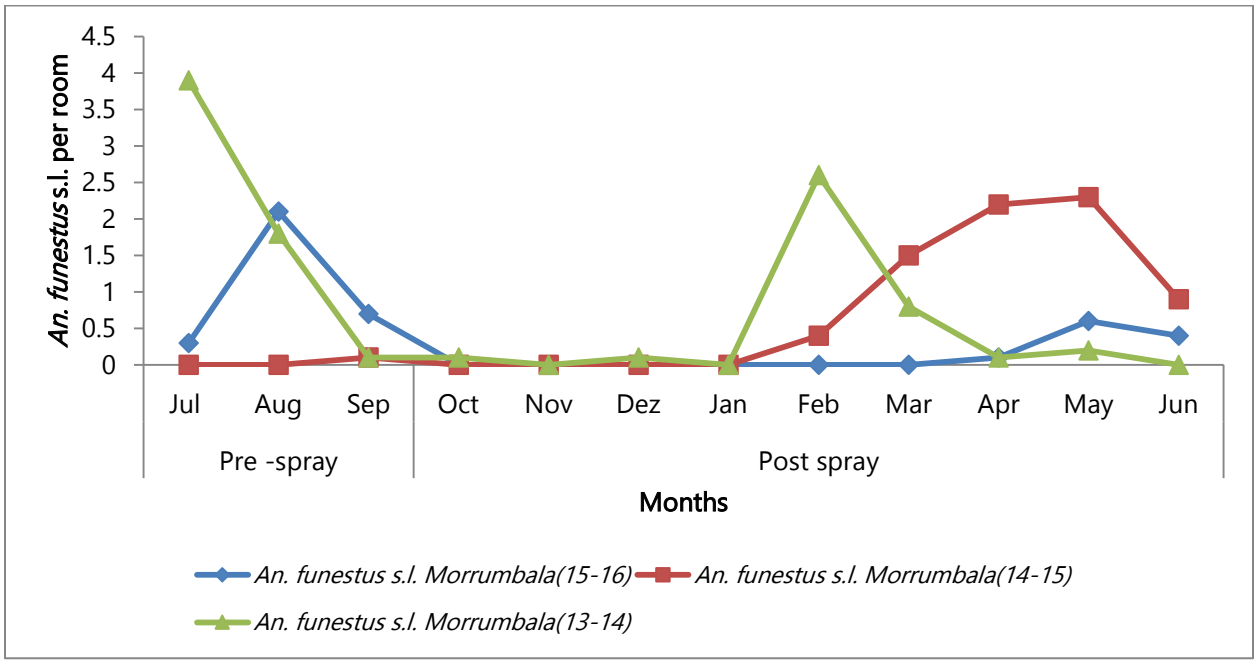


Figure 1(h): Historical Densities of *An. gambiae* s.l. in Morrumbala

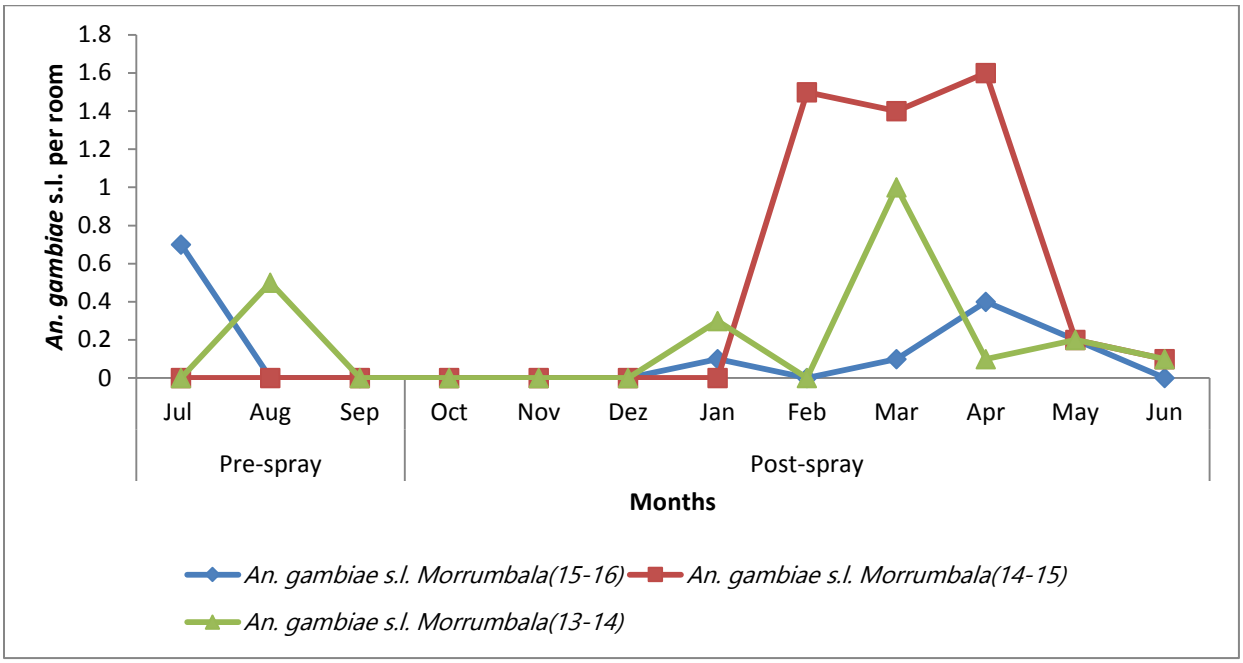


Figure 1(i): Historical Densities of *An. funestus* s.l. in Maganja

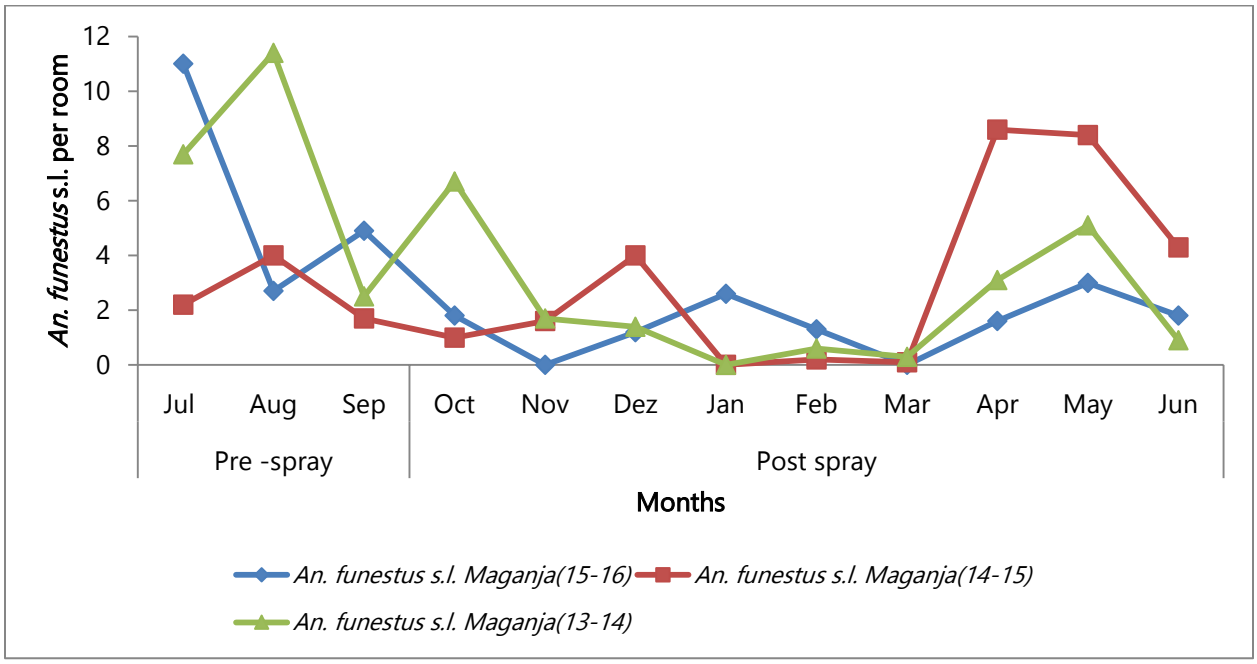
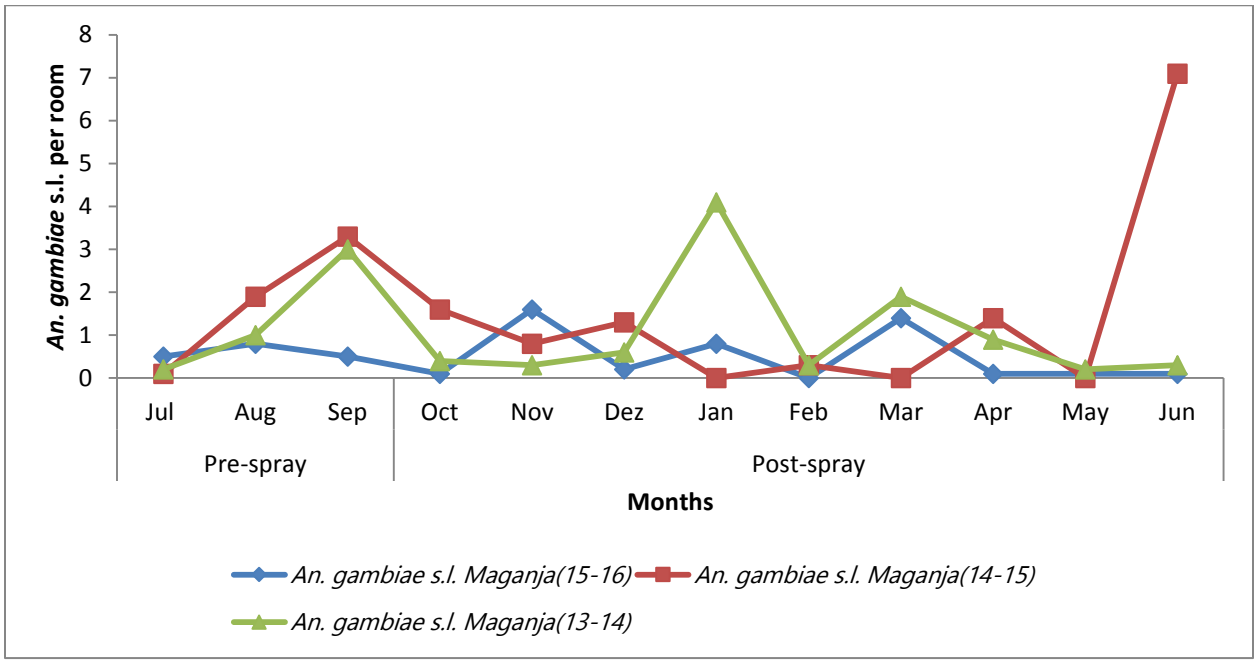


Figure 1(j): Historical Densities of *An. gambiae* s.l. in Maganja



3.1.2 HUMAN LANDING CATCHES

HLC collection was conducted once a month in each site in two houses for three consecutive nights for a total of three nights each month. During the monitoring period, a total of 1,787 *Anopheles* mosquitoes were collected while attempting to feed on human baits, as shown in Table 2.

TABLE 2. TOTAL NUMBER OF *ANOPHELES* SPP. COLLECTED PER SITE BY HLC

Districts										
	An.gambiae	*An.funestus*	*An.coustani*	*An. tenebrosus*	*An. caliginosus*	*An. dancalicus*	*An. pretoriensis*	*An.aruni*	*An.rufipes*	*An. daudi*
Milange	162	316	11	0	1	0	8	1	4	1
Mocuba	88	5	0	0	0	0	0	0	0	0
Morrumbala	39	2	0	0	0	3	1	0	2	0
Maganja	265	862	9	5	2	0	0	0	0	0
Total	554	1185	20	5	3	3	9	1	6	1

The proportions of indoor to outdoor collection for the main vectors were as follows: In Milange, *An. gambiae* s.l. was 63 (38.89%) vs 99 (61.11%) and *An. funestus* s.l. was 203 (97.13%) vs. 6 (2.87%), respectively. In Morrumbala, *An. gambiae* s.l. was 10 (25.64%) vs 29(74.36%) and *An. funestus* s.l. 1 (25%) vs 3 (75%). In Mocuba, *An. gambiae* s.l. was 18 (20.45%) vs 70 (79.55%) and *An. funestus* s.l. 3 (60%) vs 2 (40%). In the control, Maganja, *An. gambiae* s.l. was 117 (44.15%) vs 148 (55.85%) and *An. funestus* s.l. 654 (75.87%) vs 208(24.13%). These data indicate a tendency for outdoor feeding (exophagic behavior) for *An gambiae* s.l. in all the sites. In contrast, *An. funestus* s.l. tended to feed indoors (endophagic) in all sites except Morrumbala. Overall, the tendency to bite outdoors or indoors was variable with *An. gambiae* s.l.

Figure 2 shows hourly indoor and outdoor biting rates of *An. gambiae* s.l. and *An. funestus* s.l. Figure 2(a) shows that the peak indoor biting time of *An. gambiae* s.l. occurs from 1 to 2 a.m. in all districts (intervention and control). Its outdoor biting time peaks at two times, 10-11 p.m. and 12-2 a.m. in both intervention and control sites (Figure 2(b)). *Anopheles funestus* s.l. has low indoor biting rates in Mocuba and Morrumbala with no noticeable peak. In Milange, the biting activity is clearly notable, occurring from 12 -1 AM and 3-4 AM, while in Maganja da Costa (control) the peak biting time was from 12-1AM, 1-2 AM and 2-3 a.m. (Figure 2(c)). The peak outdoor biting time was high throughout the collection time in Maganja with peaks varying from 9-10 PM, 11PM-12AM, 12-1AM, 1-2AM and 4-5AM. In Mocuba and Morrumbala, the peak outdoor biting times was not notable, while in Milange, the outdoor biting peak happen from 10-11PM, 1-2 AM and 4-5 AM (Figure 2(d)).

3.1.3 Human Biting Rate, Monthly Profile and Historical Data

Figures 2(e) to 2(l) present historical indoor and outdoor human biting rate (HBR) data for comparison with monthly trends of malaria vectors over the time.

FIGURE 2: HOURLY AND MONTHLY INDOOR AND OUTDOOR BITING RATES OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. AND HISTORICAL AND MONTHLY INDOOR AND OUTDOOR HBR RATE

Figure 2(a): Indoor biting rate of *An. gambiae* s.l.

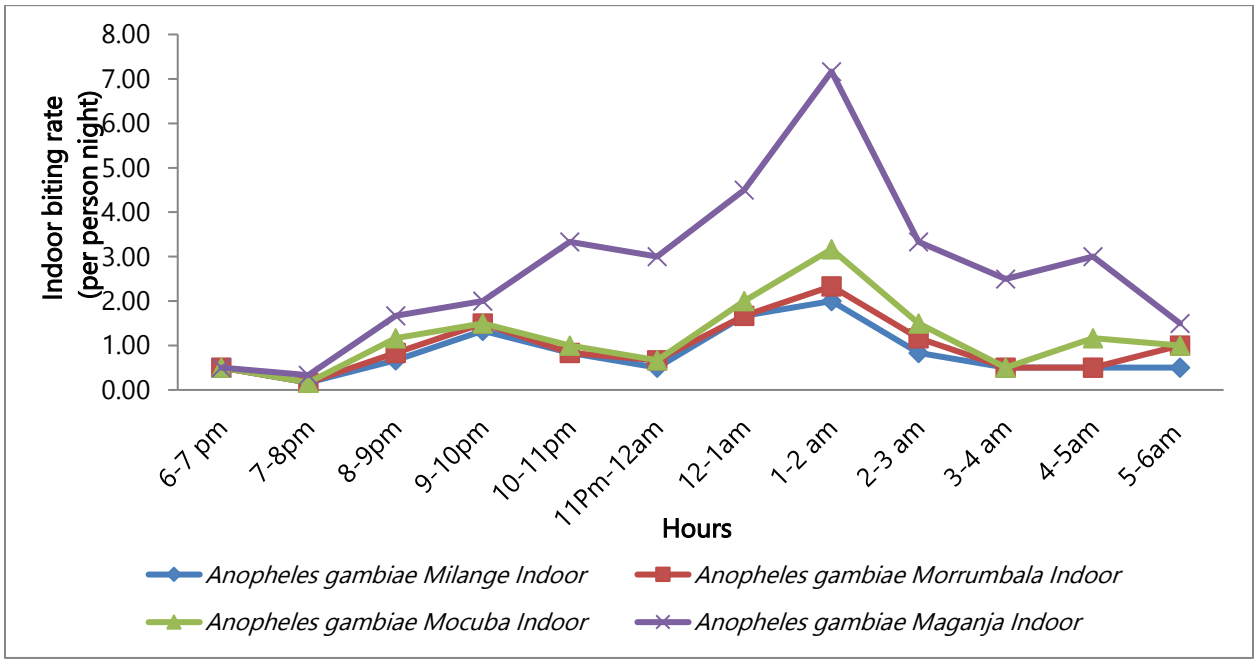


Figure 2(b): Outdoor biting rate of *An. gambiae* s.l

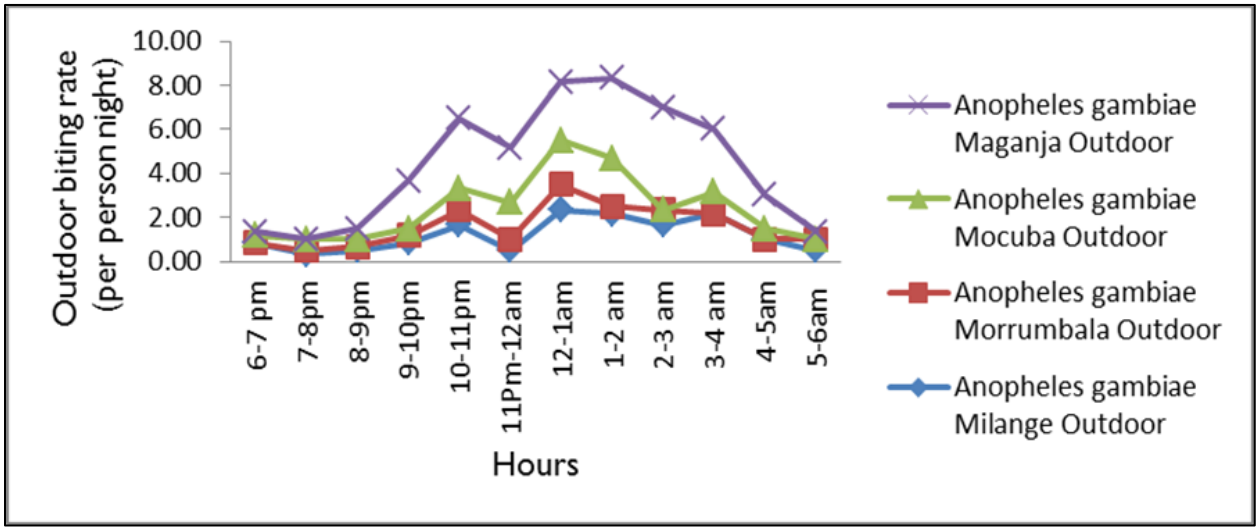


Figure 2(c): Indoor Biting Rate of *An. funestus* s.l.

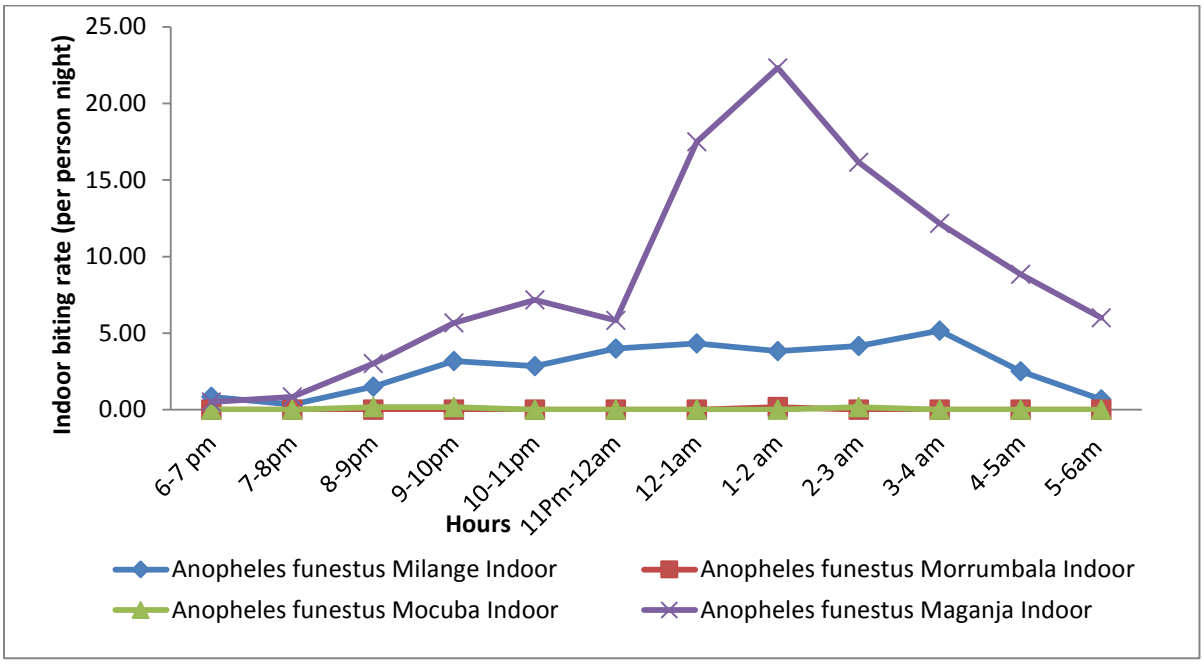


Figure 2(d): Outdoor Biting Rates of *An. funestus* s.l

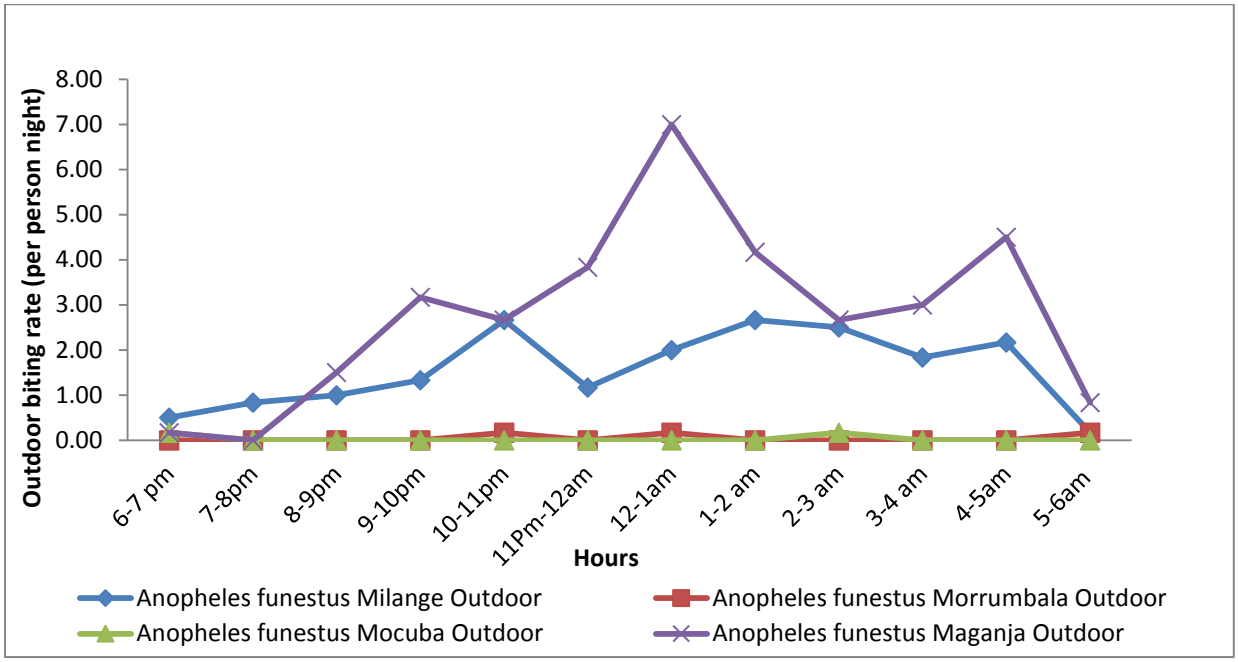


Figure 2(e): *An. funestus* s.l. Historical and Monthly Indoor and Outdoor HBR Rate in Milange

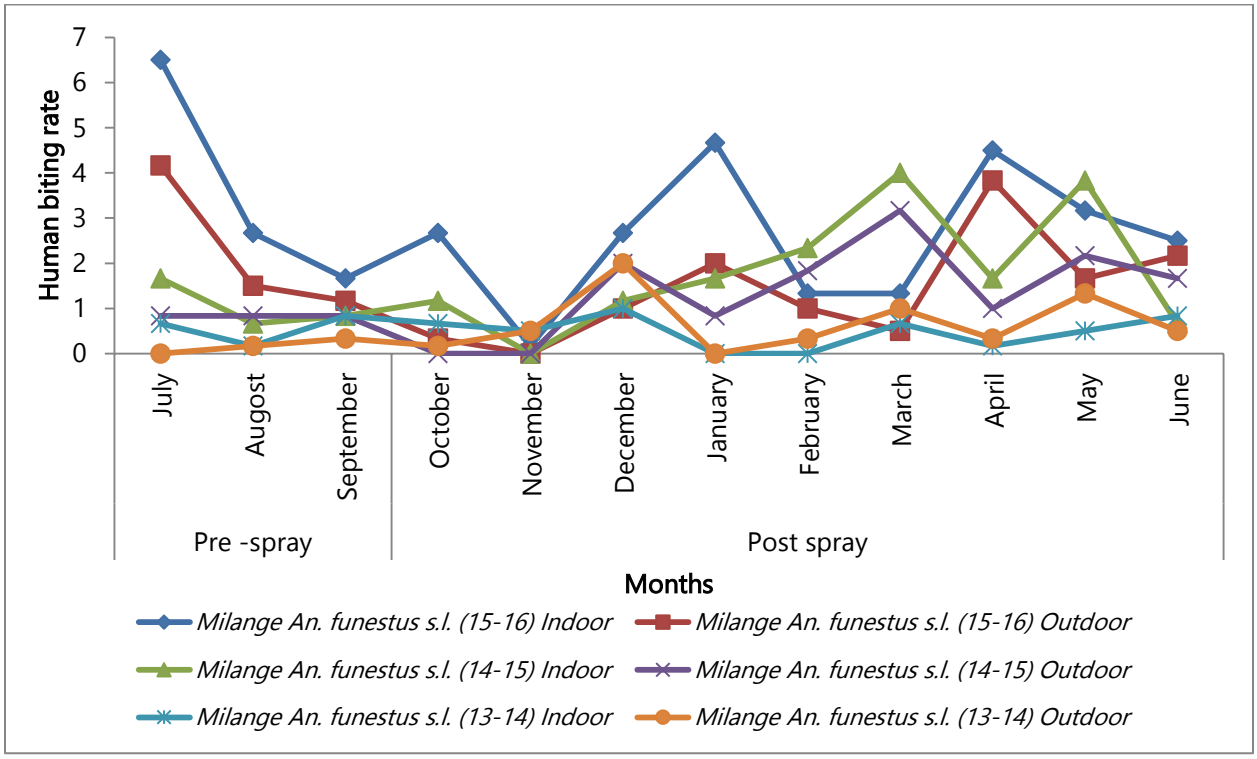


Figure 2(f): *An. gambiae* s.l. Historical and Monthly Indoor and Outdoor HBR Rate in Milange

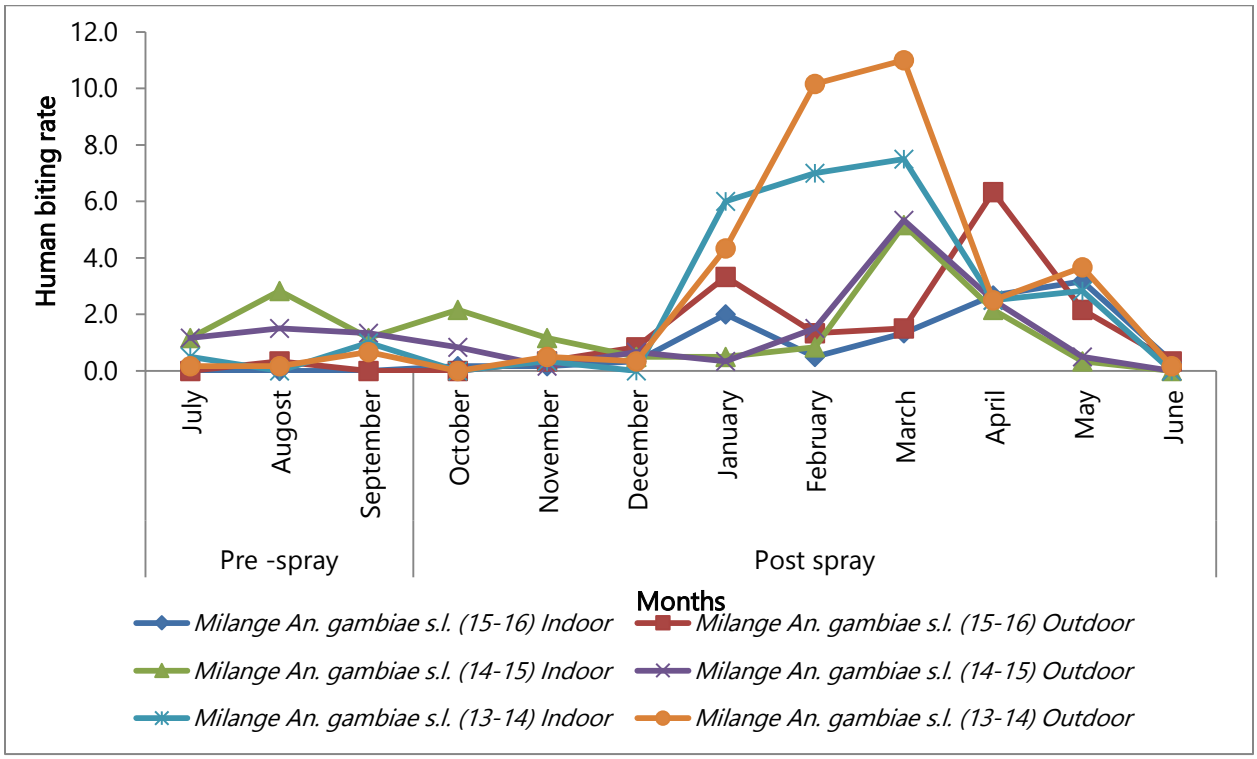


Figure 2(g): *An. funestus* s.l. Historical and Monthly Indoor and Outdoor HBR Rate in Morrumbala

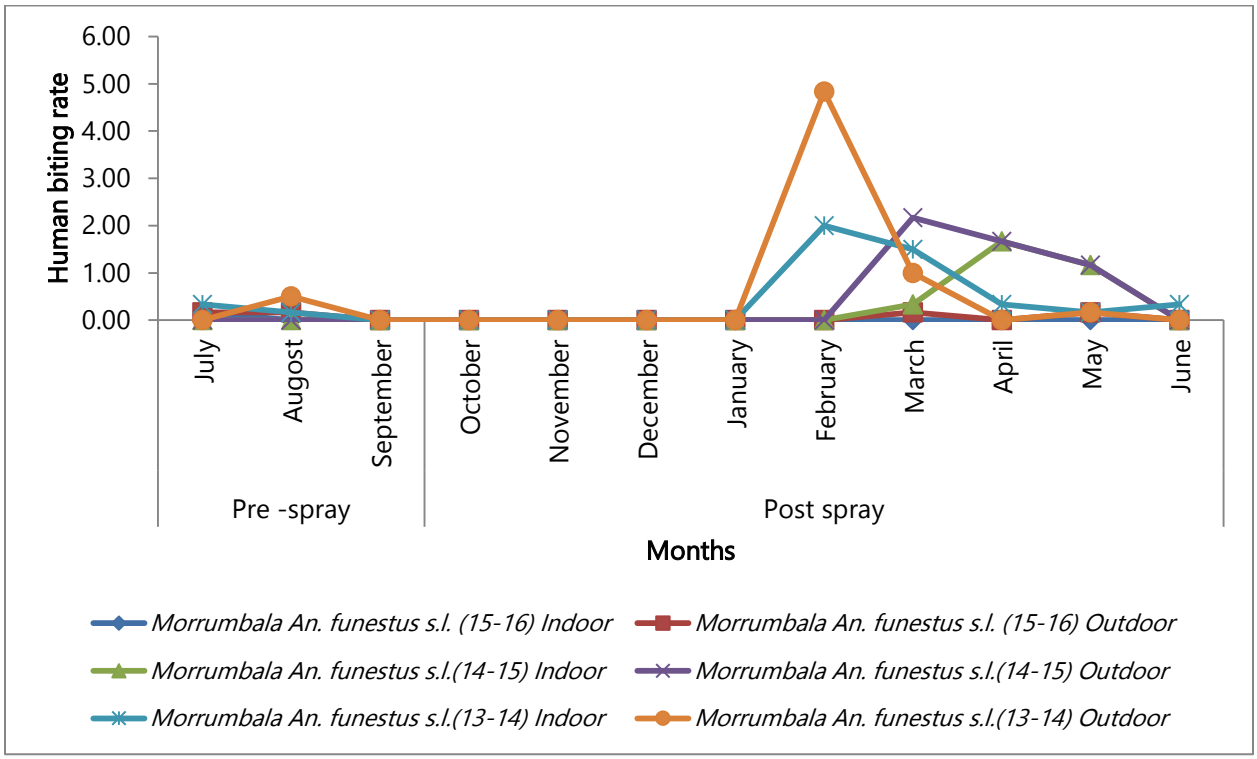


Figure 2(h): *An. gambiae* s.l. Historical and Monthly Indoor and Outdoor HBR Rate in Morrumbala

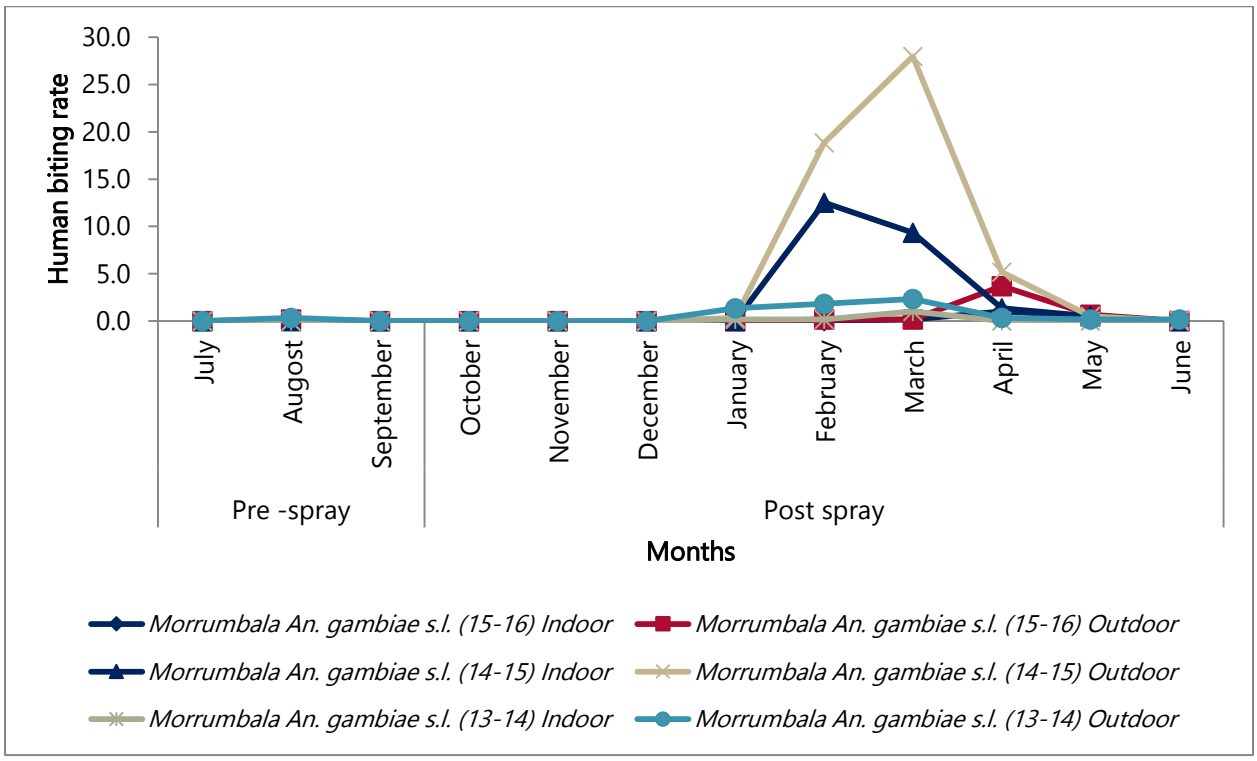


Figure 2(i): *An. funestus* s.l. Historical and Monthly Indoor and Outdoor HBR Rate in Mocuba

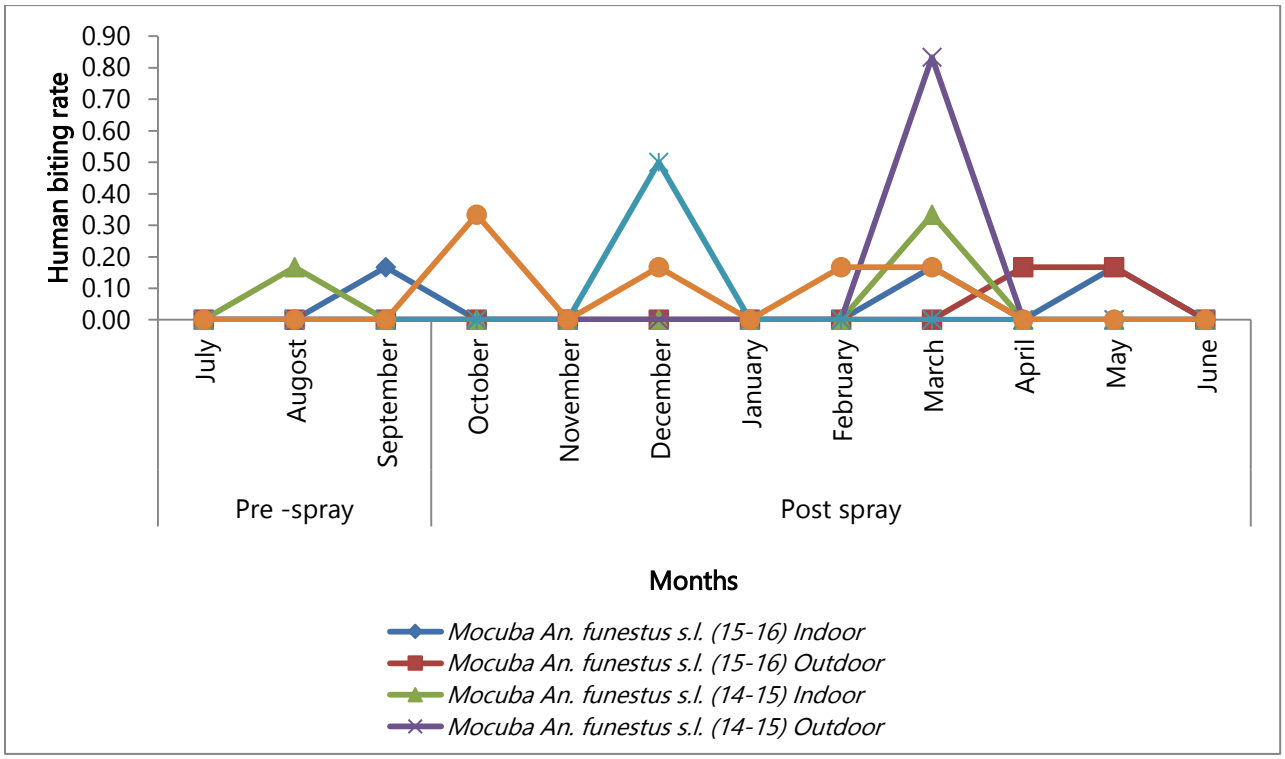


Figure 2(j): *An. gambiae* s.l. Historical and Monthly Indoor and Outdoor HBR Rate in Mocuba

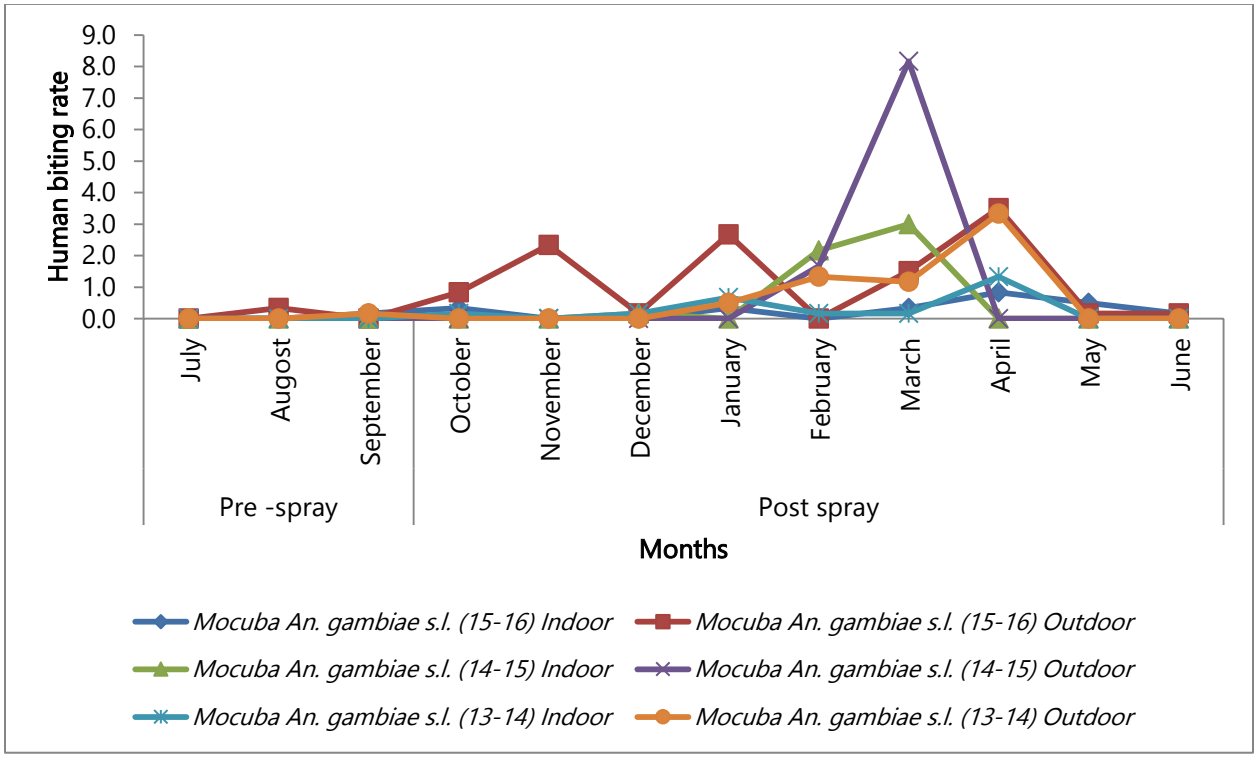


Figure 2(k): *An. funestus* s.l. Historical and Monthly Indoor and Outdoor HBR Rate in Maganja

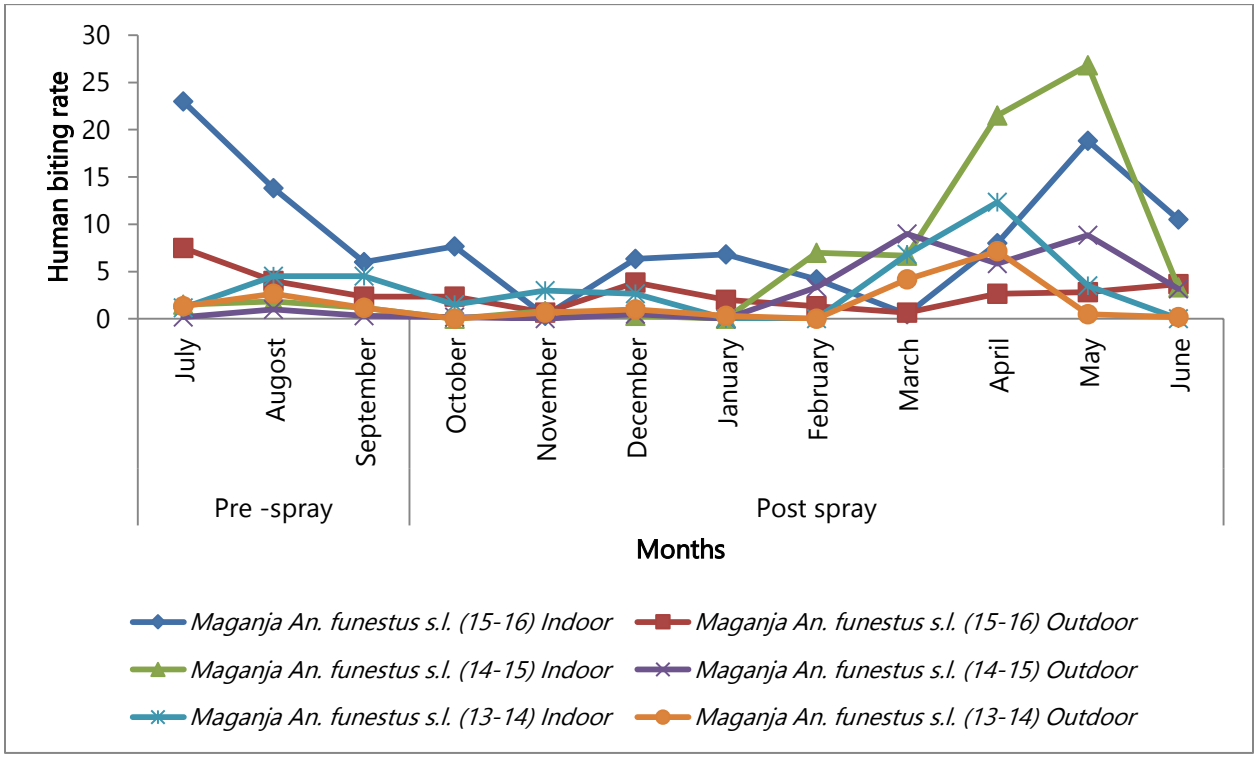
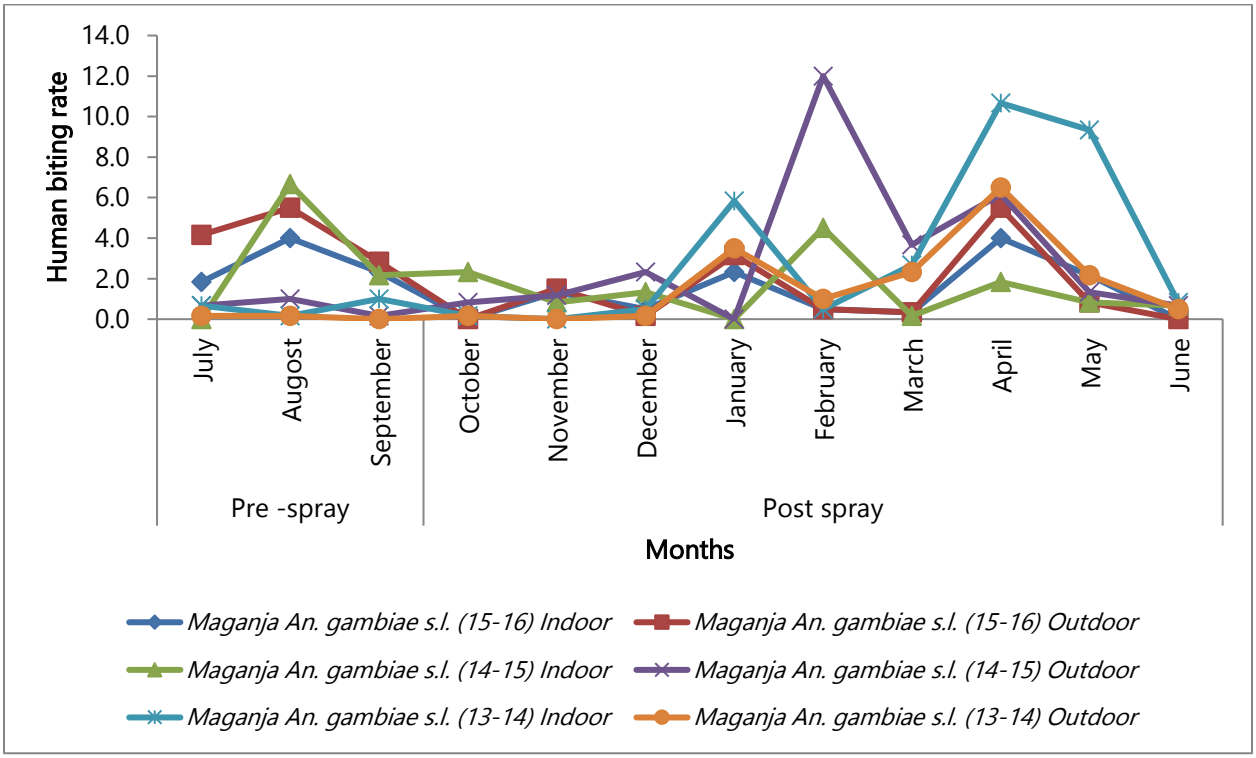


Figure 2(I): *An. gambiae* s.l. Historical and Monthly Indoor and Outdoor HBR Rate in Maganja



3.1.4 Biomolecular Analysis Report

AIRS Mozambique sent samples collected from July to November 2015 in the three intervention and the control districts to Witwatersrand University in South Africa for molecular analyses for species-specific identification of the *An. funestus* group and *An. gambiae* complex. In total, the program sent 734 samples identified as *An. funestus* s.l., *An. gambiae* s.l., *An. coustani*, *An. dancalicus*, *An. daudi,* and *An. rufipes*. Species for the complex was identified using PCR assays, the first based on Scott et al. (1993), which is not able to differentiate *An. gambiae* s.s. from *An. coluzzii* and the second one based on Fanello et al. (2002) to differentiate them from each other. To differentiate members of the *An. funestus* group, the multiplex PCR reaction was used according to Koekemoer et al. (2002). Of the total 734 samples sent, 534 were identified as *An. funestus* s.l. in the field and in the insectary, 493 as *An. funestus* s.s., *An. leesoni,* and *An. rivulorum*, at 92.3 percent accuracy. Of 184 identified as *An. gambiae* s.l. by the field team, the PCR assay identified 157 (*An. gambiae* s.s. and *An. arabiensis*) representing 85.3 percent accuracy on the morphological identification. Details are shown in Table 3.

Samples identified as *An. gambiae* s.s. on the PCR for species-specific identification were submitted to a second PCR to differentiate *An. gambiae* s.s. from *An. coluzzii* , previously known as S and M forms, as shown respectively in Table 3.1. Table 3.2 presents the distribution of malaria vectors per district and per month. *An. funestus* s.s. occurred mainly in July and August, and its abundance was reduced in the hot and rainy season, *An. arabiensis* was more common than *An. gambiae* s.s. during the same period.

About 1700 samples from susceptibility tests carried out in January and February 2015 were submitted for PCR species ID. Samples were mostly identified as *An. gambiae* s.l., and the results

of PCR show that most of them were members of the *An. gambiae* complex, mainly *An. arabiensis* and *An. gambiae* s.s. The samples identified as *An. gambiae* s.s., were submitted for a second PCR to differentiate the S and M (*An. coluzzii*) forms, Table 3.3. shows that most of mosquitoes were identified as *An. arabiensis* (n=886) and the remaining were *An. gambiae* s.s. (n=571). 93 samples were missing from the vials, 111 did not amplified, 38 registered as questionable M/S hybrids, and 3 samples were processed and preserved in RNAlater. Table 3.4 shows species breakdown by resistant or susceptible mosquitoes through PCR analysis. For some of the samples, it was not possible to distinguish as "S" or "R" since the samples were not individually labelled.

TABLE 3. SPECIES-SPECIFIC PCR IDENTIFICATION OF *AN. GAMBIAE* COMPLEX AND *AN. FUNESTUS* GROUP

Districts	Morpho ID						Wits ID								PCR ID				
	An.funestus	*An.gambiae*	*An.coustani*	*An.dancalicus*	*An. Daudi*	*An.rufipes*	*An.marshallii*	*An gambiae complex*	*An.rufipes*	*Unsure*	*An. tenebrosus*	*An. ziemanni*	*An.maculipalpis*	*Culine*	*An. demeilloni*	*An.funestus*	*An.leesoni*	*An. rivulorum*	*An.gambiae*	*An.arabiensis*
Maganja	410						1	5								353	4	14	12	21
		153						1	1	2						19		1	31	98
			7							1	2	4								
Morrumbala	2															2				
		1							1											
				3												2				1
Milange	122													1		120				1
		5														2			1	2
			3										3							
					1											1				
						2									1	1				
Mocuba		25																	7	18

TABLE 3.1. PCR IDENTIFICATION OF *AN. GAMBIAE* TO IDENTIFY *AN.GAMBIAE* S.S. AND *AN. COLUZZII*

An. gambiae s.s.	41	0	1	7	49
An. coluzzii	0	0	0	0	0

TABLE 3.2. BIOMOLECULAR ANALYSIS RESULTS OF SPECIES IDENTIFICATION PER MONTH AND DISTRICT

	Maganja					Milange			Morrumbala		Mocuba	
Months	*An. funestus* s.s.	*An. lessoni*	*An. rivulorum*	*An. gambiae*	*An. arabiensis*	*An. funestus* s.s.	*An. gambiae*	*An. arabiensis*	*An. funestus* s.s.	*An. arabiensis*	*An. gambiae*	*An. arabiensis*
July	158	2	8	8	31	64	0	0	3	1	0	0
August	101	1	3	7	49	23	0	1	1	0	0	2
September	47	1	2	9	22	16	0	0	0	0	1	1
October	45	0	2	7	2	18	0	1	0	0	2	5
November	21	0	0	10	8	3	1	1	0	0	4	10
Total	372	4	15	41	112	124	1	3	4	1	7	18

TABLE 3.3. BIOMOLECULAR ANALYSIS RESULTS OF MOSQUITOES FROM SUSCEPTIBILITY TESTS WITH SAMPLES COLLECTED IN 2015

District	*An.gambiae* s.s (S-form)	Hybrid ?	No. ID	*An.coluzzii*	*An. arabiensis*	Sample missing	RNAlater	Total
Mocuba	375	20	40	0	393	71	1	900
Milange	2	0	4	0	94	0	0	100
Morrumbala	194	18	67	0	399	22	2	702
Total	571	38	111	0	886	93	3	1702

TABLE 3.4 SPECIES BREAKDOWN BY RESISTANT OR SUSCEPTIBLE THROUGH PCR

Insecticide	Susceptible or Resistant	*An. gambiae* s.s. (S-form)	*An. arabiensis*
Deltamethrin	S	208	296
	R	6	7
Lambda-C	S	106	151
	R	14	9
Bendiocarb	S	34	50
	R	n/a	n/a
Fenitrothion	S	56	137
	R	0	0
DDT	S	85	106
	R	0	0

3.1.5 PLASMODIUM FALCIPARUM ELISA

For the *Plasmodium falciparum* ELISA, 707 samples from the total of 734 were submitted to the assay to detect sporozoites. Samples without head and thorax were not included and those not amplified on the PCR species-specific identification were also not included in the *Plasmodium falciparum* infection detection (Table 4).

TABLE 4. PLASMODIUM FALCIPARUM ELISA POSITIVE INFECTION PER DISTRICT AND % OF INFECTION

MAGANJA DA COSTA				
Species	**Sample size**	**ELISA pos**	**ELISA neg**	**%Infection**
An. arabiensis	115	2	113	1.74
An. funestus	372	8	364	2.15
An. gambiae	43	0	42	0.00
An. leesoni	4	0	4	0.00
An. rivulorum	15	0	15	0.00
MORRUMBALA				
Species	**Sample size**	**ELISA pos**	**ELISA neg**	**%Infection**
An. arabiensis	1	0	1	0.00
An. funestus	4	0	4	0.00
MILANGE				
Species	**Sample size**	**ELISA pos**	**ELISA neg**	**%Infection**
An. arabiensis	3	0	3	0.00
An. funestus	124	2	122	1.61
An. gambiae	1	0	1	0.00
MOCUBA				
Species	**Sample size**	**ELISA pos**	**ELISA neg**	**%Infection**
An. arabiensis	18	0	18	0.00
An. gambiae	7	0	7	0.00

3.1.6 INDOOR CDC LIGHT TRAP COLLECTION

The indoor CDC light traps collected 1,013 malaria vectors, including *An. funestus* s.l. and *An. gambiae* s.l., in four houses over three consecutive nights per month. The numbers collected per trap night are presented in Figure 3 for *An. funestus* s.l. and *An. gambiae* s.l. respectively. See also Annex A.

FIGURE 3: MONTHLY DENSITIES OF *AN.FUNESTUS* S.L. AND *AN.GAMBIAE* S.L. COLLECTED INDOORS BY CDC LIGHT TRAP DURING THE PRE AND POST SPRAY SEASONS

Figure 3(a). *An. funestus* s.l.

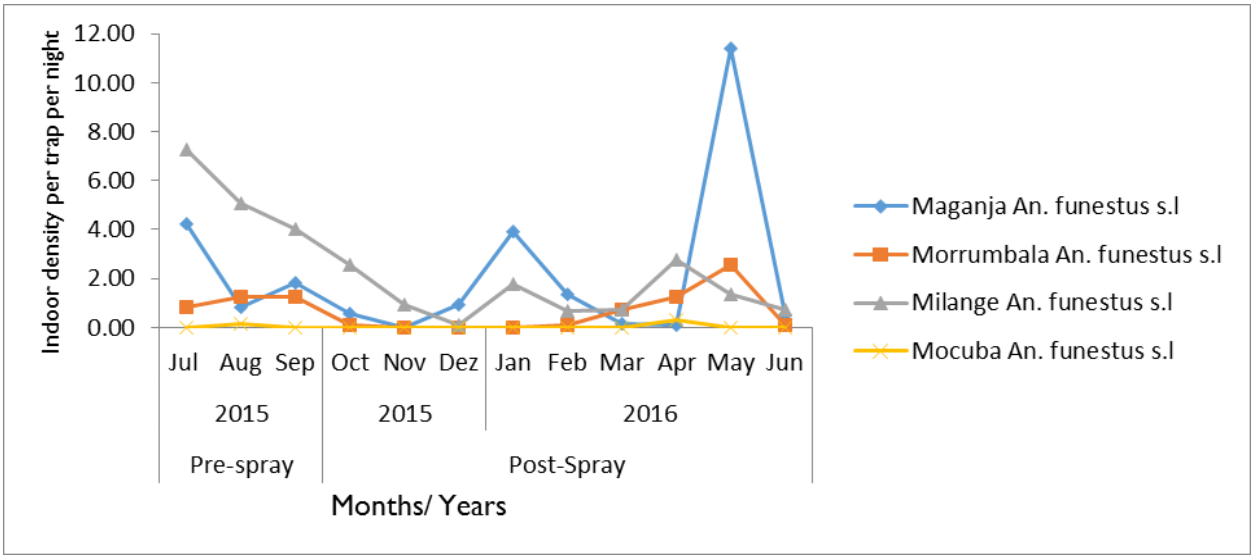
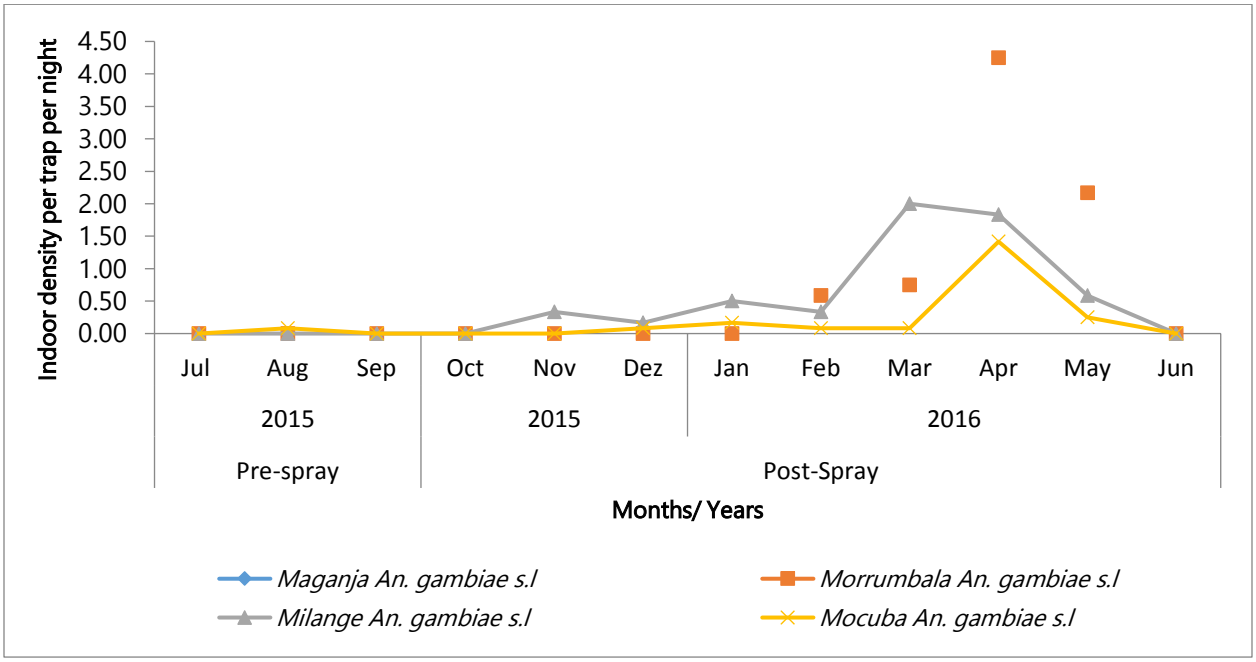


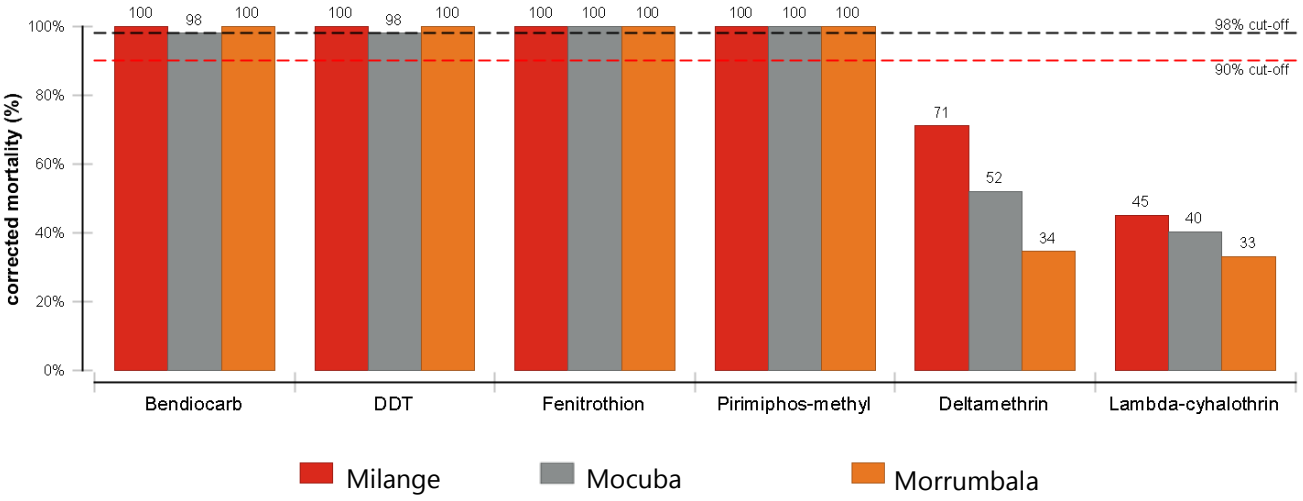
Figure 3(b): *An.gambiae* s.l.



3.2 WHO SUSCEPTIBILITY TESTING

In Morrumbala, Milange, and Mocuba, the *An. gambiae* s.l. tested showed clear signs of resistance to deltamethrin and lambdacyhalothrin, but susceptibility to the other insecticides tested (Table 5 and Figure 4 A and 4B).

FIGURE 4 (A). SUSCEPTIBILITY STATUS OF AN. GAMBIAE S.L. COLLECTED FROM IRS DISTRICTS INCLUDING ENTOMOLOGICAL SENTINEL SITES AREA, TESTED AGAINST SELECTED WHO RECOMMENDED INSECTICIDES FOR IRS (WHO TUBE TESTS)



Site	Carbamate	Organochlorine	Organophosphate		Pyrethroid	
	Bendiocarb	DDT	Fenitrothion	Pirimiphos methyl	Deltamethrin	Lambda cyhalothrin
Milange	100% (100)	100% (100)	100% (100)	100% (100)	71% (100)	45% (100)
Mocuba	98% (100)	98% (100)	100% (100)	100% (100)	52% (100)	40% (100)
Morrumbala	100% (100)	100% (100)	100% (100)	100% (100)	34% (100)	33% (100)

TABLE 5. PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. USING THE WHO INSECTICIDE RESISTANCE TESTS AFTER THE 24HRS HOLDING PERIOD

Resistant Potentially resistant Susceptible

Note: The top number in each cell is the corrected percentage mortality. The bottom number between parentheses is the number tested.

These maps show the corrected mortality using WHO tube assays to all active ingredients within each class. Multiple assays performed within a district have been aggregated. Green represents susceptible, Yellow potentially resistant, and Red are resistant.

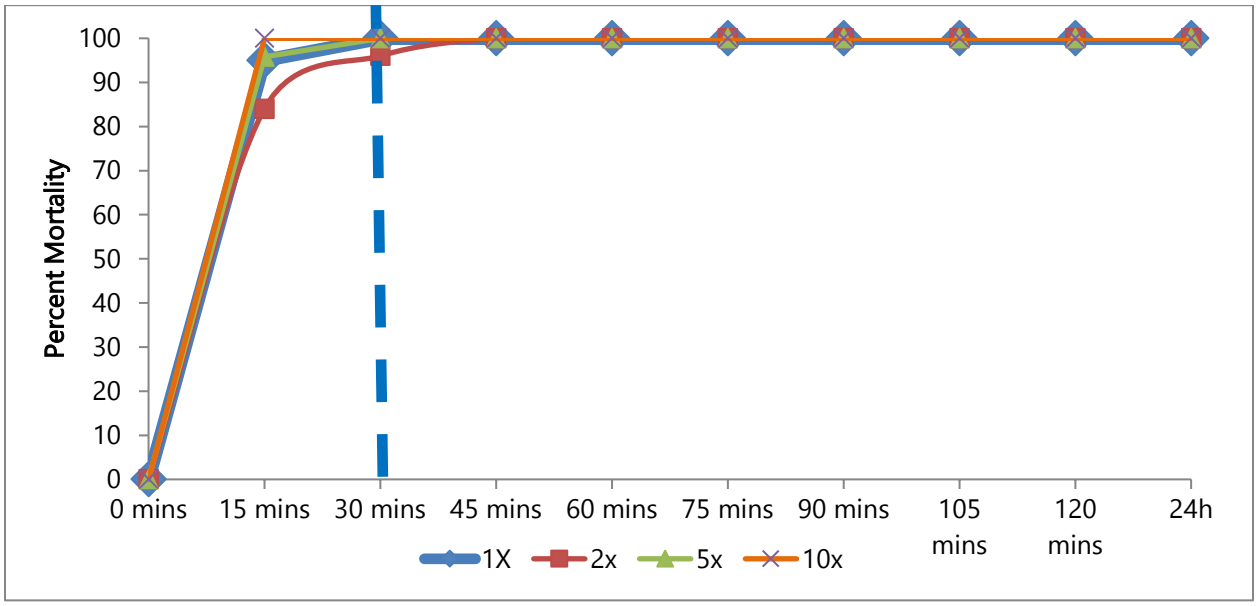
FIGURE 4 (B): MAPS SHOWING THE STATUS OF SUSCEPTIBILITY AND RESISTANCE OF *AN. GAMBIAE* S.L. FOR EACH INSECTICIDE TESTED PER SITE DISTRICT

Pyrethroids	DDT
	
Carbamates	**Organophosphates**
	

3.3 CDC Bottle Bioassay Testing

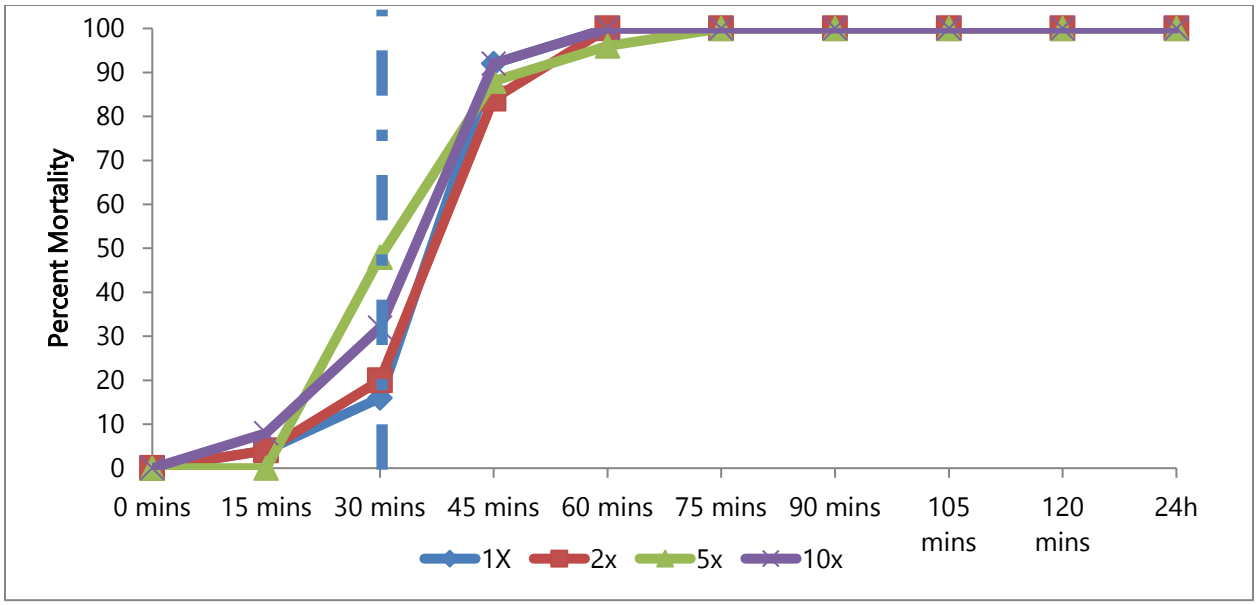
Figures 5-13 show the test results against *An. gambiae* s.l. from Morrumbala, Mocuba, and Milange districts using the CDC bottle bioassay method. Figures 5, 7, 8 and 9 show insecticide resistance intensity data for deltamethrin, pirimiphos-methyl, lambdacyhalothrin, and DDT, respectively for Morrumbala district. Figures 6, 10, 11 and 12 show the same data for Mocuba district and Figure 13 shows insecticide resistance intensity data for deltamethrin for Milange district.

FIGURE 5: MORRUMBALA DISTRICT INTENSITY OF RESISTANCE TO DELTAMETHRIN IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



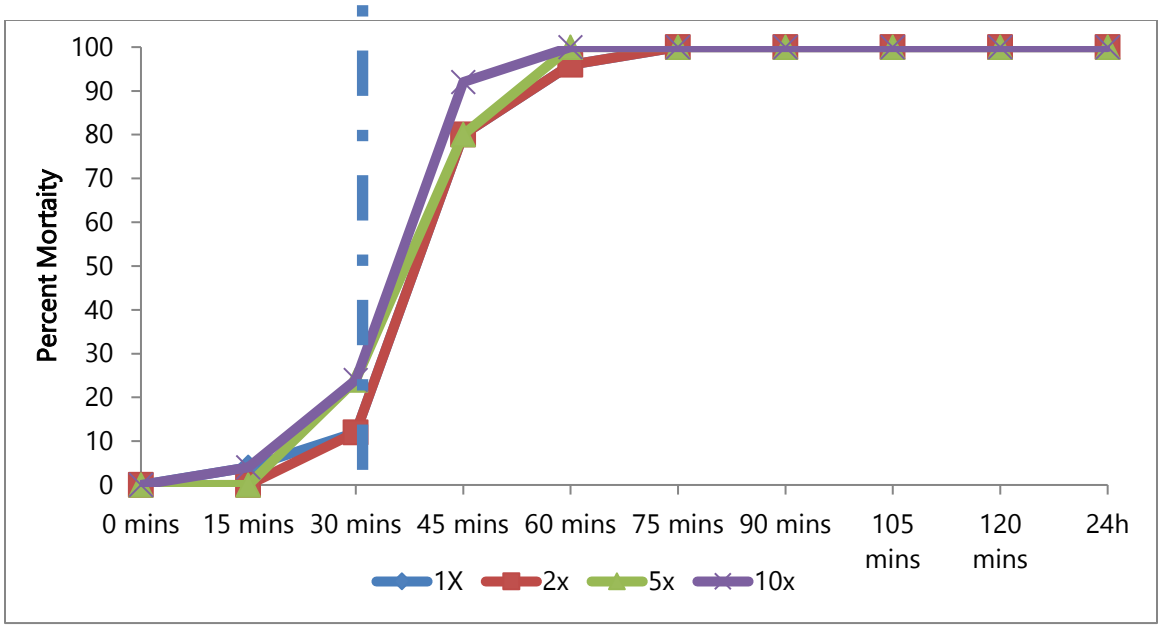
Note: Blue line shows the susceptibility threshold.

FIGURE 6: MOCUBA DISTRICT INTENSITY OF RESISTANCE TO PIRIMIPHOS-METHYL IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



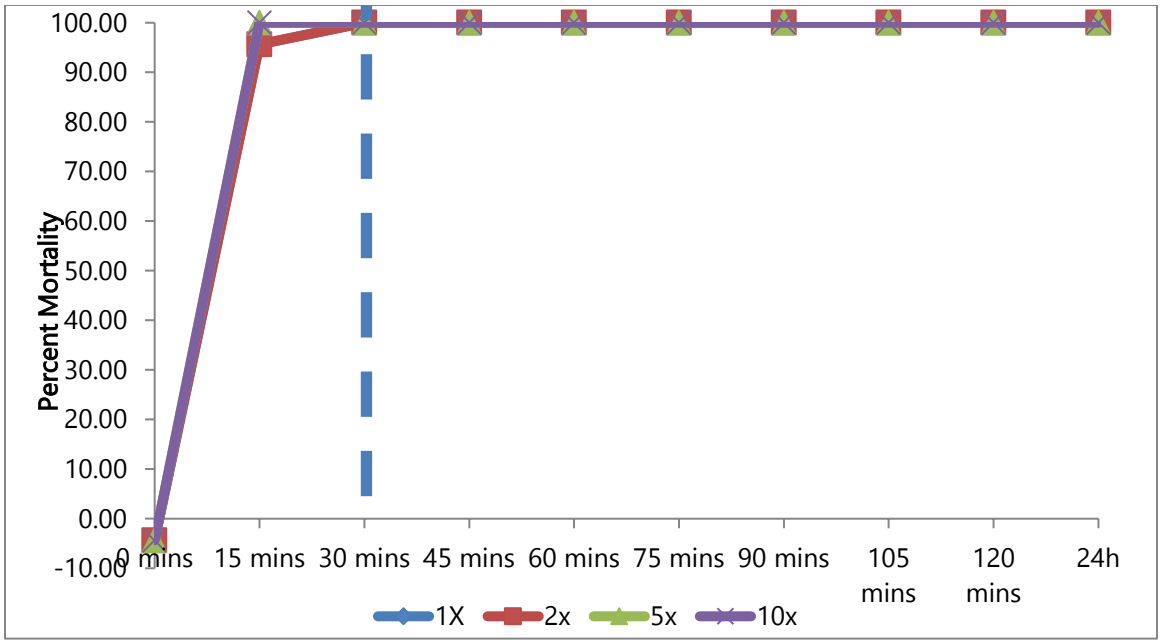
Note: Blue line shows the susceptibility threshold.

FIGURE 7: MORRUMBALA DISTRICT INTENSITY OF RESISTANCE TO PIRIMIPHOS-METHYL IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



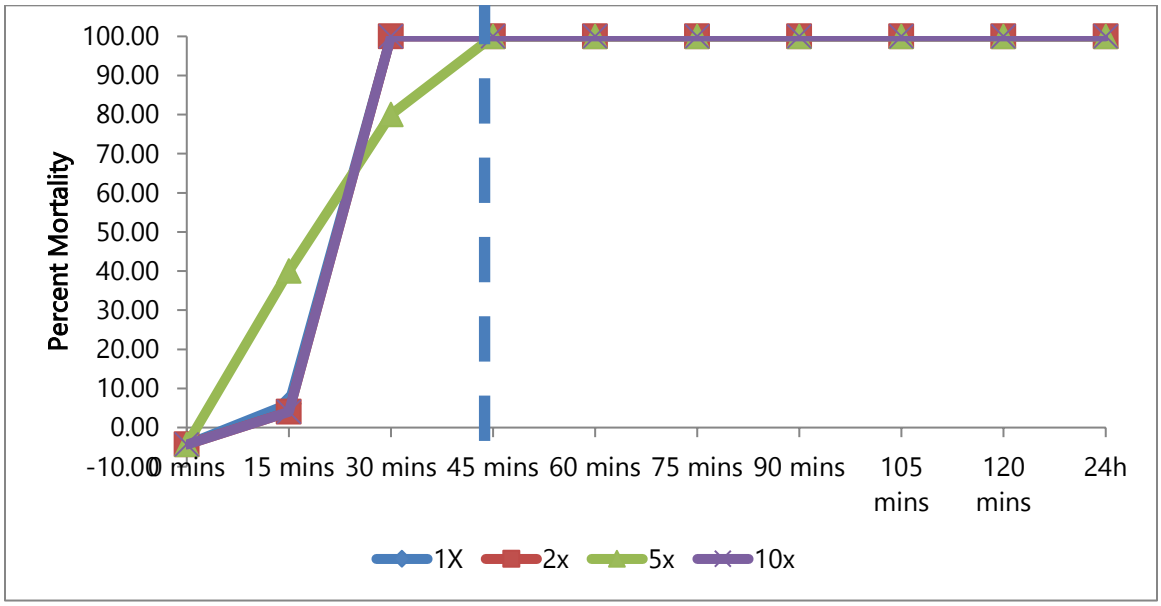
Note: Blue line shows the susceptibility threshold.

FIGURE 8: MORRUMBALA DISTRICT INTENSITY OF RESISTANCE TO LAMBDACYHALOTHRIN IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



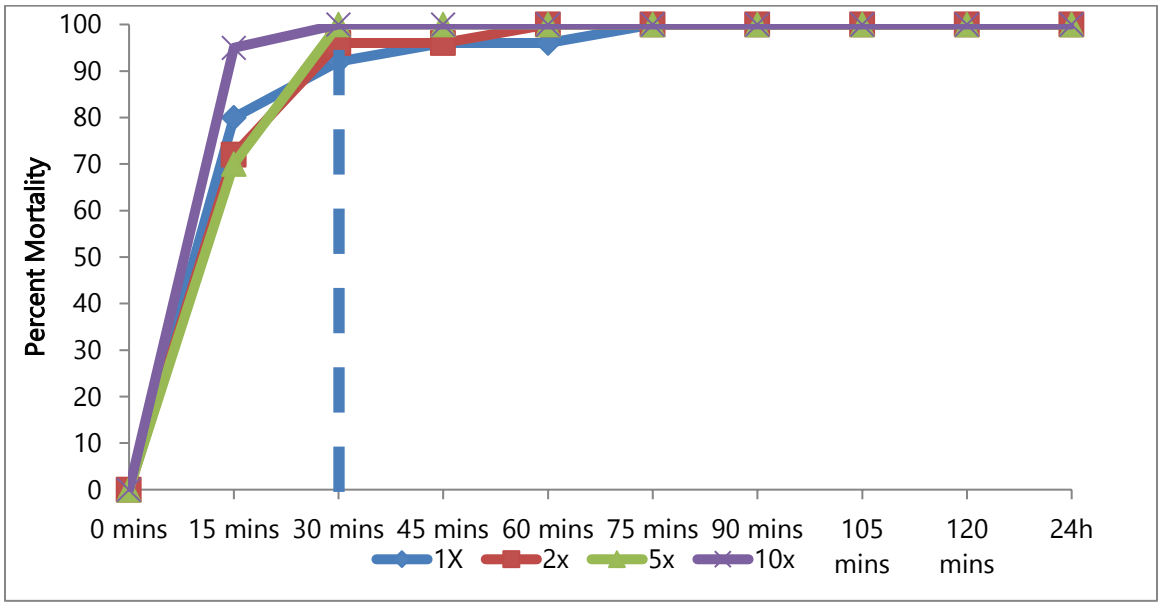
Note: Blue line shows the susceptibility threshold.

FIGURE 9: MORRUMBALA DISTRICT INTENSITY OF RESISTANCE TO DDT IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



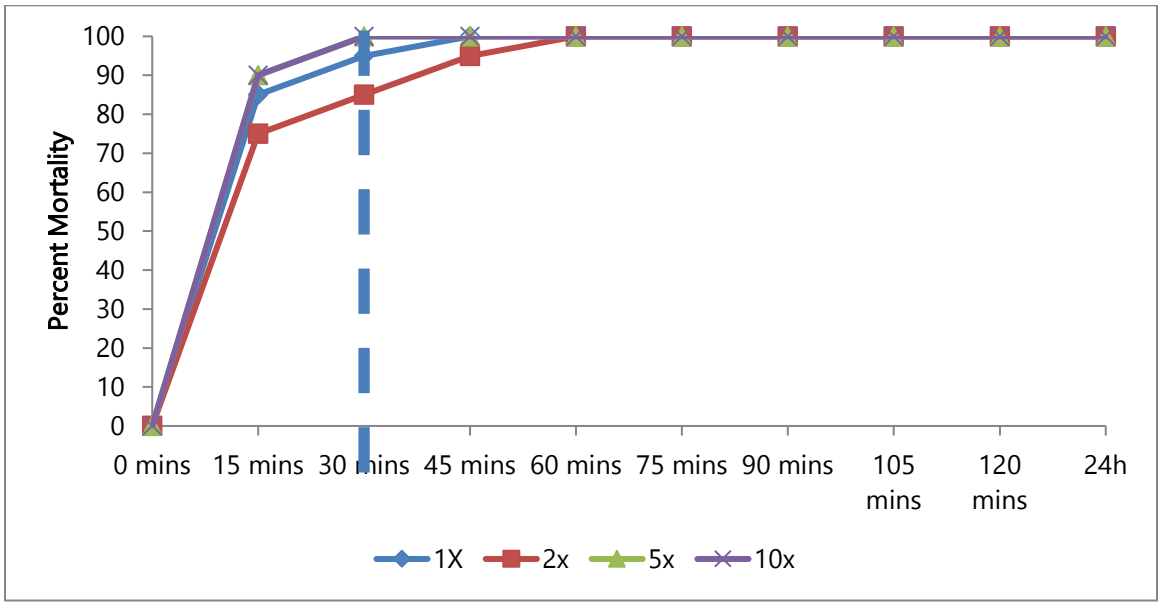
Note: Blue line shows the susceptibility threshold.

FIGURE 10: MOCUBA DISTRICT INTENSITY OF RESISTANCE TO DELTAMETHRIN IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



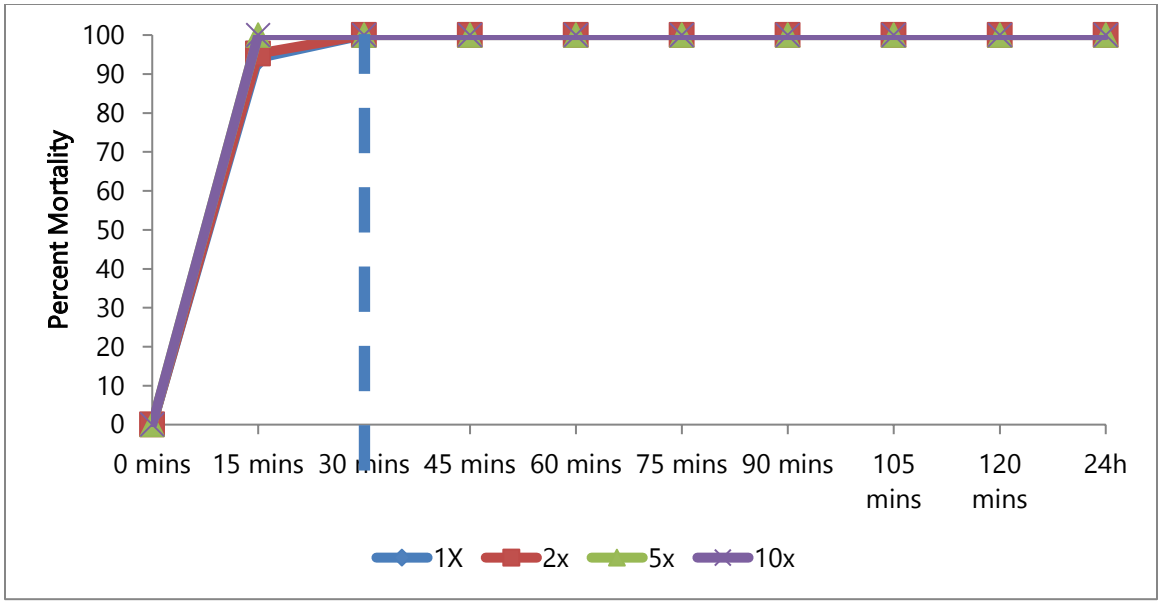
Note: Blue line shows the susceptibility threshold.

FIGURE 11: MOCUBA DISTRICT INTENSITY OF RESISTANCE TO DDT IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



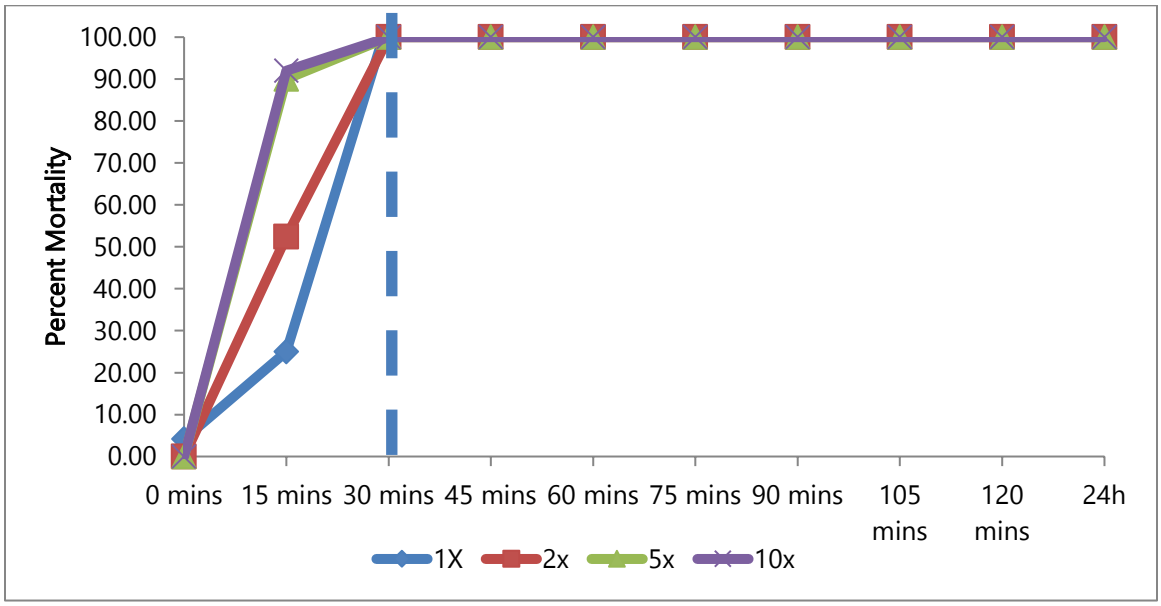
Note: Blue line shows the susceptibility threshold.

FIGURE 12: MOCUBA DISTRICT INTENSITY OF RESISTANCE TO LAMBDACYHALOTHRIN IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



Note: Blue line shows the susceptibility threshold.

FIGURE 13: MILANGE DISTRICT INTENSITY OF RESISTANCE TO DELTAMETHRIN IN *AN. GAMBIAE* S.L. USING CDC-RESISTANCE INTENSITY RAPID DIAGNOSTIC TEST



Note: Blue line shows the susceptibility threshold.

Based on WHO susceptibility test results, the resistance to pyrethroids (deltamethrin and lambdacyhalothrin) is consistent with data from previous years, 2014 and 2015 in Morrumbala and Mocuba. Resistance to deltamethrin and lambdacyhalothrin were detected this year in Milange. The percentage mortality rates observed in Mocuba were 52 percent and 40.22 percent; in Morrumbala 34.44 percent and 33.0 percent; and in Milange 71 percent and 45 percent, respectively for deltamethrin and lambdacyhalothrin insecticides. For other insecticide tested, the mortality rates were above 98 percent in all the districts.

With the CDC bottle bioassay, some mosquito survival was observed at diagnostic concentration and time for deltamethrin in Mocuba and Milange at 1x and 2x concentration and Morrumbala at 2x concentration. The *An. gambiae* s.l. mosquitoes from Mocuba and Morrumbala exposed to pirimiphos-methyl with the CDC-coated bottles indicated low mortality in all concentration ranges. In contrast to WHO methods, all samples exposed to pirimiphos-methyl and fenitrothion were fully susceptible. The results obtained from the CDC bottle assays might be related to the stability of pirimiphos-methyl preparations and would trust the WHO tube test results over the CDC bottle results for pirimiphos-methyl.

3.4 Cone Bioassay Tests

The results of the wall bioassay test conducted in the four selected districts are summarized in Table 6 and Figures 14 and 15.

1. Mortality rate of susceptible mosquitoes exposed to Actellic® 300 CS on sprayed walls was 100 percent in the 24-hour holding period for quality assurance test in Pedreira (Mocuba) and Coqueiro (Morrumbala) (Figure 14).
2. Mortality rate of susceptible mosquitoes exposed to deltamethrin (Pali™ 250 WG) on the quality assurance test was 100 percent in 12 de Outubro (Milange). However, the mean mortality was 78 percent in Madal (Quelimane) (Figure 14).
3. Control mortality was less than 5 percent in most houses. Where control mortality was between 5 and 20 percent, the Abbots formula was used to correct mortality. This was done on the quality assurance time as well as on the decay rate (residual efficacy times).
4. There was no difference between percentage mortality results after the 24-hour holding period within the Actellic® 300 CS sprayed districts. However in Quelimane, where a pyrethroid was sprayed, the 24-hour mortality was less than 80 percent.
5. The two structures that indicated the unacceptable mortality rate were identified and structures sprayed by the same spray operator were re-sprayed.
6. Team leaders and spray operators received morning refresher training with an emphasis on spray quality, insecticide mixing, structure door marking, followed by strengthened field supervision by members of AIRS Mozambique and the District Services for Health, Women and Social Welfare (SDSMAS).
7. Based on the results of the 24-hour mortality cone bioassay tests from three sites (Milange, Morrumbala, and Mocuba), the quality of spraying was acceptable.
8. The decay rate of the insecticide showed differences in residual life of Actellic® 300 CS and deltamethrin (Figure 15).
9. In Morrumbala and Mocuba the residual life of Actellic® 300 CS was five months and of deltamethrin in Milange and Quelimane (Maquival) six months (Figure 15).
10. In Morrumbala the decay rate of the Actellic® 300CS on the cement and mud surfaces last for 5 months post spray in general, but the cement surface last less than five months, (Figure 15A)

TABLE 6. CONE WALL BIOASSAY TEST RESULTS SUMMARY OF SPRAY QUALITY ASSURANCE

District	Site	# houses	# mosquitoes exposed	# mosquitoes killed after 24hrs	% exposure mortality (KD 30 min)	% 24h corrected mortality	% control mortality after 24 hrs
Morrumbala	Coqueiro	5	200	200	84.5	100	6/50
Mocuba	Pedreira	5	200	200	29	100	2/50

Milange	12 de Outubro	5	200	200	40	100	0/50
Quelimane	Madal	5	200	156	41.5	78	1/50

FIGURE 14: CONE WALL BIOASSAY, QUALITY ASSURANCE SUMMARY RESULTS

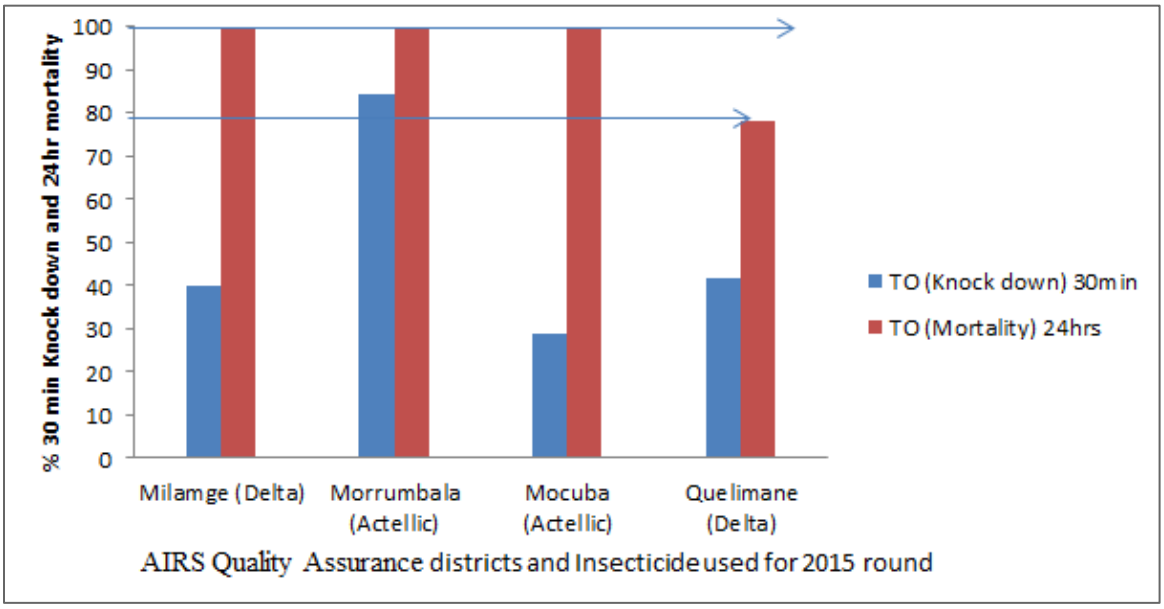


FIGURE 15: DECAY RATE (RESIDUAL ACTIVITIES) OF INSECTICIDES SPRAYED IN ALL INTERVENTION SITES

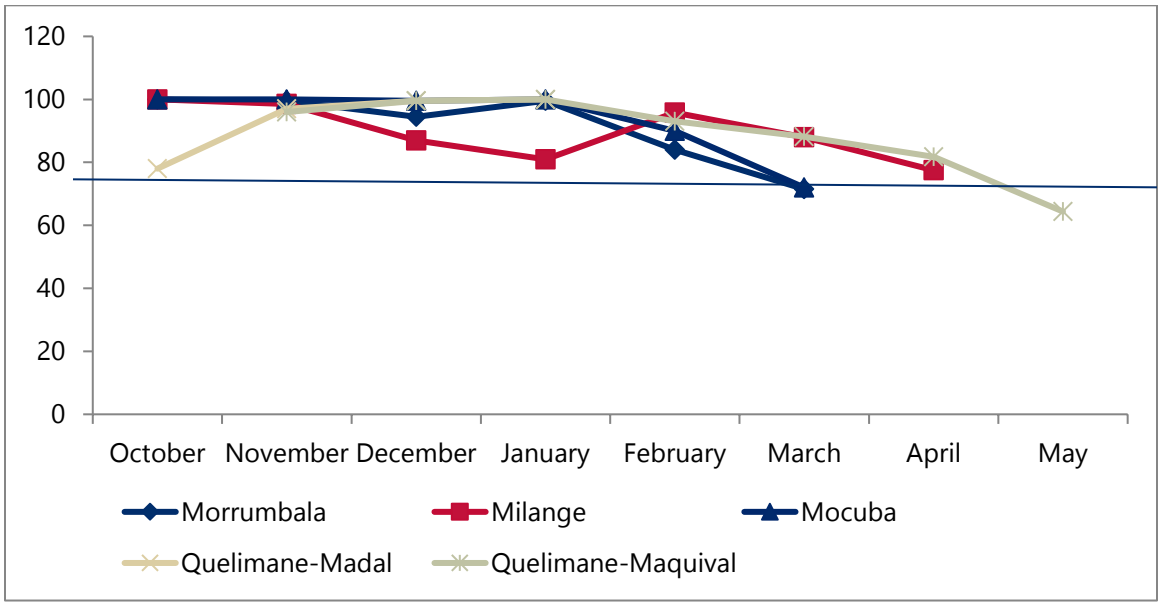
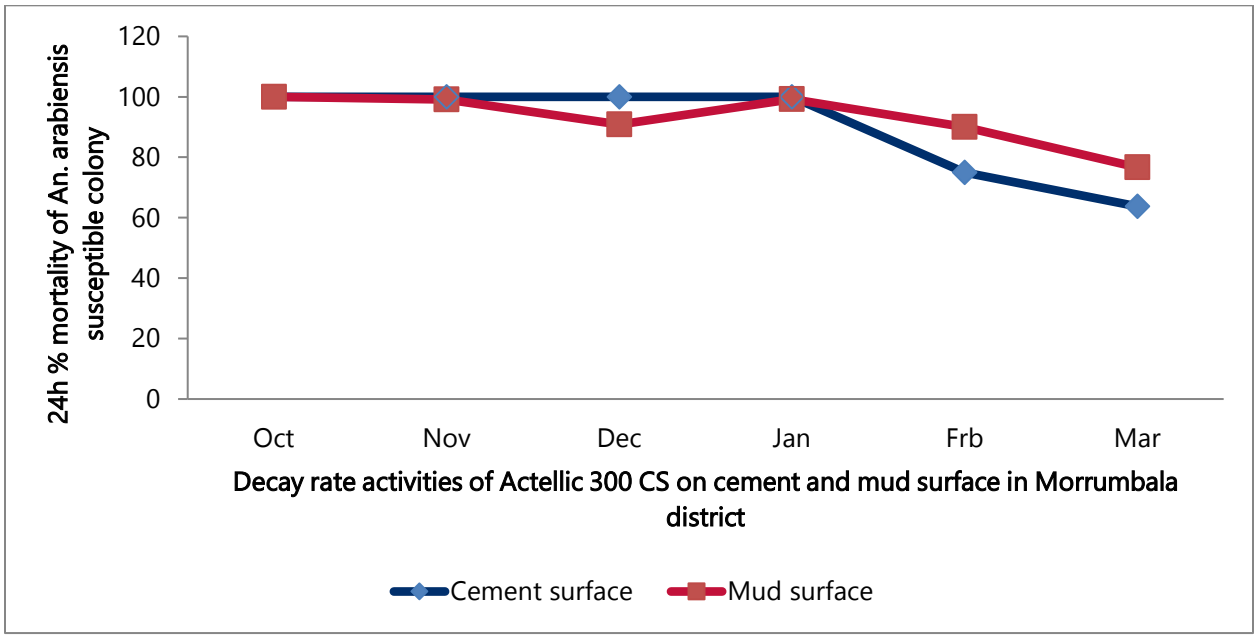


FIGURE 15(A). DECAY RATE (RESIDUAL ACTIVITIES) OF INSECTICIDES SPRAYED ON CEMENT AND MUD WALLS IN MORRUMBALA DISTRICT



4. Discussion, Lessons Learned, and Challenges

As Figure 2 showed, most bites by the malaria vectors occur after the human population is indoors, where there is a great chance they can be protected through the use of insecticide-treated bed nets and IRS. However, there is a small possibility of outdoor transmission by *An. gambiae* s.l. as they continue to bite outdoors to some extent during early morning hours. In rural areas of Mozambique, much of the population leaves their houses in early morning to begin farming activities.

Based on the PCR species-specific identification, the main species of the *An. gambiae* complex that occur in the study area are *An. gambiae* s.s. and *An. arabiensis;* in the *An. funestus* group, the main species is *An. funestus* s.s. From the samples identified as *An. gambiae* s.s., none are *An. coluzzii*. The results from sporozoite ELISA showed that the infection rates are generally low in the areas. No sporozoite was detected from the malaria vectors in two intervention areas (Mocuba and Morrumbala). The sporozoite rate observed in *An. funestus* from Milange, 1.61 percent, was lower than the 2.15 percent observed in Maganja (the control site). The sporozoite infection rate in *An. arabiensis* at the control site was 1.74 percent, but none observed in this vector in any intervention site.

Data from the CDC light trap collections showed higher indoor density in Morrumbala in April 2016 than in any other site, intervention or control. This might be associated with the decay rate of the insecticide sprayed there.

In general, the vectors remain susceptible to organophosphates tested with WHO tube tests. CDC bottle bioassay results for pirimiphos-methyl showed low mortality to this organophosphate insecticide in all concentrations (Figures 7 and 6) tested at Morrumbala and Mocuba. However, the results from the CDC bottle assays could be due to issues with the stability of the pirimiphos-methyl used for the tests.

AIRS Mozambique has also noted some of the tests for insecticide resistance intensity assays were done unnecessarily. In the future, the team will conduct insecticide resistance assays using the CDC bottle assays only when the vectors are resistant following the WHO tube tests.

In Morrumbala, Actellic® 300 CS looks to have a longer residual life on mud wall surfaces than on cement wall surfaces. In Milange, the residual life time of deltamethrin looks the same on both mud surfaces and wooden doors.

5. RECOMMENDATIONS

- We recommend sending more samples for PCR species identification and ELISA for *Plasmodium falciparum* infection during the next collection seasons to further understand the dynamics of malaria vectors during the period of collection and to continue observing any change in the species composition.
- We recommend performing the airborne effect test on the Actellic® 300 CS sprayed house when the mosquitoes are available and run the test in each house with the respective control until the mortality reaches less than 80 percent.
- On the susceptibility tests, we recommend to do further effort to collect *An. funestus* and test this population.

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ANNEX A: SUMMARY OF MOSQUITOES COLLECTED, DIFFERENT METHODS

TABLE A-1. TOTAL OF MOSQUITOES COLLECTED BY DIFFERENT METHODS ON THE INTERVENTION AND NON-INTERVENTION SITES

Species	CDC	PSC	HLC
An.gambiae	264	89	554
An.funestus	749	393	1185
An. rivolurum	0	1	0
An. dancalicus	27	1	3
An.coustani	4	0	20
An. tenebrosus	0	0	5
An. caliginosus	0	0	3
An. pretoriensis	3	0	9
An.aruni	1	0	1
An.rufipes	0	0	6
An. daudi	0	0	1
Total	1048	484	1787

TABLE A-2. PSC (*AN. GAMBIAE* S.L.) COLLECTION, MORRUMBALA INTERVENTION SITE, JULY 2015 TO JUNE 2016

Time		# of houses	*An.gambiae*	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	Fed per house
				UF^	F^	HG^	G^				
Pre-spray	Jul	10	7	0	4	3	0	3	42.86	0.7	0.40
	Aug	10	0	0	0	0	0	0	0.00	0	0.00
	Sep	10	0	0	0	0	0	0	0.00	0	0.00
After Spray	Oct	10	0	0	0	0	0	0	0.00	0	0.00
	Nov	10	0	0	0	0	0	0	0.00	0	0.00
	Dec	10	0	0	0	0	0	0	0.00	0	0.00
	Jan	10	1	0	0	1	0	1	100.00	0.1	0.00
	Feb	10	0	0	0	0	0	0	0.00	0	0.00
	Mar	10	1	0	1	0	0	0	0.00	0.1	0.10
	Apr	10	4	1	1	2	0	2	66.67	0.4	0.10
	May	10	2	0	1	1	0	1	50.00	0.2	0.10
	Jun	10	0	0	0	0	0	0	0.00	0	0.00
Total		120	15	1	7	7	0	7	259.52	1.5	0.06

UF^ – un-fed, F^-fed, HG^-half-gravid, G^ - gravid

TABLE A-3. PSC (*AN. FUNESTUS* S.L.) COLLECTION, MORRUMBALA INTERVENTION SITE, JULY 2015 TO JUNE 2016

Time		# of houses	*An. funestus*	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/	Female per house	Fed per house
				UF^	F^	HG^	G^				
Pre-spray	Jul	10	3	0	1	2	0	2	66.67	0.3	0.10
	Aug	10	21	1	19	1	0	1	5.00	2.1	1.90
	Sep	10	7	1	1	1	4	5	83.33	0.7	0.10
After Spray	Oct	10	0	0	0	0	0	0	0.00	0	0.00
	Nov	10	0	0	0	0	0	0	0.00	0	0.00
	Dec	10	0	0	0	0	0	0	0.00	0	0.00
	Jan	10	0	0	0	0	0	0	0.00	0	0.00
	Feb	10	0	0	0	0	0	0	0.00	0	0.00
	Mar	10	0	0	0	0	0	0	0.00	0	0.00
	Apr	10	1	1	0	0	0	0	0.00	0.1	0.00
	May	10	6	0	6	0	0	0	0.00	0.6	0.60
	Jun	10	4	0	1	1	2	3	75.00	0.4	0.10
Total		120	42	3	28	5	6	11	28.21	0.35	0.23

TABLE A-4. PSC. (*AN. FUNESTUS* S.L.) COLLECTION, MILANGE INTERVENTION SITE, JULY 2015 TO JUNE 2016

		houses		tages				Total (HG+G)	gravid (HG+G/ HG+G+F)	per house	Fed per house
				UF^	F^	HG^	G^				
Pre-spray	Jul	10	7	1	3	3	0	3	50.00	0.70	0.30
	Aug	10	3	0	2	0	1	1	33.33	0.30	0.20
	Sep	10	1	0	0	0	1	1	100.00	0.10	0.00
After Spray	Oct	10	1	1	0	0	0	0	0.00	0.10	0.00
	Nov	10	0	0	0	0	0	0	0.00	0.00	0.00
	Dec	10	0	0	0	0	0	0	0.00	0.00	0.00
	Jan	10	0	0	0	0	0	0	0.00	0.00	0.00
	Feb	10	0	0	0	0	0	0	0.00	0.00	0.00
	Mar	10	0	0	0	0	0	0	0.00	0.00	0.00
	Apr	10	10	0	2	8	0	8	80.00	1.00	0.20
	May	10	6	0	1	3	2	5	83.33	0.60	0.10
	Jun	10	4	0	3	1	0	1	25.00	0.40	0.30
Total		120	32	2	6	15	4	19	76.00	0.27	0.05

TABLE A-5. PSC (AN. GAMBIAE S.L.) COLLECTION, MOCUBA INTERVENTION SITE, JULY 2015 TO JUNE 2016

Time		# of houses	*An.gambiae*	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	Fed per house
				UF^	F^	HG^	G^				
Pre-spray	Jul	10	1	0	1	0	0	0	0.00	0.10	0.10
	Aug	10	0	0	0	0	0	0	0.00	0.00	0.00
	Sep	10	0	0	0	0	0	0	0.00	0.00	0.00
After Spray	Oct	10	1	0	1	0	0	0	0.00	0.10	0.10
	Nov	10	2	1	0	1	0	1	100.00	0.20	0.00
	Dec	10	0	0	0	0	0	0	0.00	0.00	0.00
	Jan	10	2	0	1	1	0	1	50.00	0.20	0.10
	Feb	10	1	0	0	0	1	1	100.00	0.10	0.00
	Mar	10	1	0	1	0	0	0	0.00	0.10	0.10
	Apr	10	0	0	0	0	0	0	0.00	0.00	0.00
	May	10	1	0	0	1	0	1	100.00	0.10	0.00
	Jun	10	0	0	0	0	0	0	0.00	0.00	0.00
Total		120	9	1	4	3	1	4	50.00	0.08	0.03

TABLE A-6. PSC (*AN. GAMBIAE* S.L.) COLLECTION, MAGANJA CONTROL SITE, JULY 2015 TO JUNE 2016

Time		# of houses	*An.gambiae*	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	Fed per house
				UF^	F^	HG^	G^				
Pre-spray	Jul	10	5	0	3	1	1	2	40.0	0.5	0.3
	Aug	10	8	4	2	0	2	2	50.0	0.8	0.2
	Sep	10	5	2	3	0	0	0	0.0	0.5	0.3
After Spray	Oct	10	1	0	1	0	0	0	0.0	0.1	0.1
	Nov	10	16	5	1	5	5	10	90.9	1.6	0.1
	Dec	10	2	0	0	2	0	2	100.0	0.2	0
	Jan	10	8	0	3	4	1	5	62.5	0.8	0.3
	Feb	10	0	0	0	0	0	0	0.0	0	0
	Mar	10	14	4	10	0	0	0	0.0	1.4	1
	Apr	10	1	0	0	1	0	1	100.0	0.1	0
	May	10	2	0	2	0	0	0	0.0	0.2	0.2
	Jun	10	1	0	0	1	0	1	100.0	0.1	0
Total		120	63	15	25	14	9	23	47.9	0.5	0.2

TABLE A-7. PSC (*AN. FUNESTUS* S.L.) COLLECTION, MAGANJA CONTROL SITE, JULY 2015 TO JUNE 2016

		houses	*funestus*	stages				Total (HG+G)	gravid (HG+G/ HG+G+F)	per house	Fed per house
				UF^	F^	HG^	G^				
Pre-spray	Jul	10	110	12	49	36	13	49	50.0	11	4.9
	Aug	10	27	10	3	6	8	14	82.4	2.7	0.3
	Sep	10	49	18	6	14	11	25	80.6	4.9	0.6
After Spray	Oct	10	18	5	10	3	0	3	23.1	1.8	1
	Nov	10	0	0	0	0	0	0	0	0	0
	Dec	10	12	0	6	4	2	6	50.0	1.2	0.6
	Jan	10	26	0	6	19	1	20	76.9	2.6	0.6
	Feb	10	13	1	2	8	2	10	83.3	1.3	0.2
	Mar	10	0	0	0	0	0	0	0	0	0
	Apr	10	16	0	0	12	4	16	100.0	1.6	0
	May	10	29	2	12	15	0	15	55.6	2.9	1.2
	Jun	10	18	2	4	5	7	12	75.0	1.8	0.4
Total		**120**	**318**	**50**	**98**	122	48	170	63.4	2.65	0.8

TABLE A-8. INDOOR CDC LIGHT TRAP DENSITIES OF *AN. FUNESTUS* S.L. AND *AN.GAMBIAE* S.L.

Years and Months of Collection													
Districts	Species	2015						2016					
		Jul	Aug	Sep	Oct	Nov	Dez	Jan	Feb	Mar	Apr	May	Jun
Maganja	*An. funestus s.l*	4.25	0.83	1.83	0.58	0.00	0.92	3.92	1.33	0.17	0.08	11.42	0.50
	An. gambiae s.l	0.17	0.08	0.25	0.00	0.00	0.00	0.92	0.08	0.25	1.58	3.00	0.00
Morrumbala	*An. funestus s.l*	0.83	1.25	1.25	0.08	0.00	0.00	0.00	0.08	0.75	1.25	2.58	0.08
	An. gambiae s.l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.75	4.25	2.17	0.00
Milange	*An. funestus s.l*	7.25	5.08	4.00	2.58	0.92	0.08	1.75	0.67	0.75	2.75	1.33	0.75
	An. gambiae s.l	0.00	0.00	0.00	0.00	0.33	0.17	0.50	0.33	2.00	1.83	0.58	0.00
Mocuba	*An. funestus s.l*	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
	An. gambiae s.l	0.00	0.08	0.00	0.00	0.00	0.08	0.17	0.08	0.08	1.42	0.25	0.00