



**Ministry of Community Development, Mother and Child Health**

# PMI | Africa IRS (AIRS) Project

## Indoor Residual Spraying (IRS Task Order 6)

# ZAMBIA 2015 ENTOMOLOGY REPORT

AUGUST 2015- FEBRUARY 2016

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# ACRONYMS

| | |
|---|---|
| AIRS | Africa Indoor Residual Spraying |
| CDC | Centers for Disease Control and Prevention |
| CSP | Circumsporozoite Antigens |
| DDMS | Disease Data Management System |
| DDT | Dichlorodiphenyltrichloroethane |
| EIR | Entomological Inoculation Rate |
| ELISA | Enzyme-linked Immunosorbent Assay |
| HLC | Human Landing Catch |
| I-RDT | Intensity Rapid Diagnostic Test |
| IRS | Indoor Residual Spraying |
| PSC | Pyrethrum Spray Catch |
| PCR | Polymerase Chain Reaction |
| PMI | President's Malaria Initiative |
| WHO | World Health Organization |
| WHOPES | World Health Organization Pesticides Evaluation Scheme |

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# EXECUTIVE SUMMARY

Zambia is implementing indoor residual spraying (IRS) for malaria control as part of an integrated vector management strategy. The President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) Project in Zambia conducted indoor residual spraying (IRS) in 39 districts in 2015 from September 28 – November 25, 2015. Entomological surveillance was carried out in six sprayed targeted sites and four control sites.

**Methods:**

Baseline data were collected in August 2015 using pyrethrum spray catch (PSC), Centers for Disease Control and Prevention (CDC) light trap and human landing catch (HLC). The impact of the IRS campaign on the malaria vectors was assessed from November 2015 to February 2016. The quality assurance of the IRS operations was assessed 24h after the spraying and the assessment of decay rate of insecticide sprayed was followed on a monthly basis. The level of the resistance of *Anopheles funestus* s.l. and *Anopheles gambiae* s.l. was assessed using the World Health Organization (WHO) tube tests. The resistance intensity assays was also performed in Mwense, Milenge, and Samfyia districts of Luapula Province using the CDC bottle assays.

**Results:**

**Vector density and behavior**: *Anopheles funestus* s.l. (12,758) is the most abundant malaria vector (64% of *Anopheles* species collected). The proportion of *An. gambiae* s.l. (2,111) was 11% of the total *Anopheles* collected. A total of 3,237 *An. tenebrosus* (16%), 692 *An. tchekedii* (3%), 732 *An. squamosus* (4%), 357 *An. coustani* (2%), 3 *An. rufipes* (0.02%), and 27 *An. ziemanni* (0.1%) were also collected.The mean average of *Anopheles funestus* s.l. was 6 per room per day in the intervention sites in August before IRS campaign and is similar to the density in the sprayed sites in November, one month after IRS (5 *An.funestus* s.l./day/room). The mean indoor resting density of *Anopheles funestus* s.l. dropped from six *Anopheles funestus* s.l. per room per day in August to two in the intervention sites in January three months after IRS. In contrary, in the control sites, the indoor resting density per room per day increased from three *Anopheles funestus* s.l. per room per day before IRS to 11 *Anopheles funestus* s.l. per room per day one month after the IRS campaign and was five *Anopheles funestus* s.l. per room per day in January three months after IRS campaign . five per room per day in January in the control sites..

The mean density of *Anopheles funestus* s.l. in the intervention sites (2.9 *Anopheles funestus* s.l. /trap/night) was four times lower than the density in the control sites (12.3/trap/night) in January using the CDC light trap collection method.

The HBR of *Anopheles funestus* s.l. (indoors) was reduced from three bites per person per night during the pre-spray period in August to 0.5 bites per person per night one month after spraying in November in the intervention sites. The drop of the HBR indoors in the intervention sites one month after the IRS campaign might be due to the IRS effect. However, the HBR increased from 0.5 per person per night in November to 4.6 bites per person per night in the

intervention sites in January three months after the spraying. In the control sites, the HBR indoors increased from 10.5 bites per person per night in August to 19 bites per person per night in November and 14.3 bites per person per night in January.

**Parity rate**: The parity rate is similar in both sprayed and control sites. There is no statistically significant difference between the parity in the sprayed and control sites. The parity is high at most of the sites.

**Quality assurance of the 2015 IRS campaign:** The WHO cone bioassay performed 24h and one month after spraying showed 100% mortality of the susceptible malaria vectors exposed to the mud and cement sprayed walls.

**Insecticide decay rate:** Pirimiphos-methyl was effective on both mud and cement in Kasama, Isoka, Katete, and Mwense in February four months after the spraying. The test mortality rate was less than the 80% WHO threshold on the mud and cement sprayed walls in Milenge and Serenje four months after spraying.The mortality rate was above 80% in Isoka and Mwense for both mud and cement sprayed walls and for cement walls in Kasama and Katete, five months after spraying. The residual life of pirimiphos-methyl at all sites was shorter than the expected residual life (six months).

**Susceptibility status:** *An. funestus* s.l. and *Anopheles gambiae* s.l. were susceptible to pirimiphos-methyl. The resistance intensity assay shows that *Anopheles funestus* s.l. was resistant to all the four pyrethroids tested at all selected sites in Milenge and Samfiya districts. No difference was observed between the sites in the intensity of deltamethrin resistance in the area except in Shitambulli. A difference was observed between the sites in the intensity of permethrin resistance.

# 1. INTRODUCTION

Malaria is endemic and the transmission is stable with a seasonal peak from November to April in Zambia. (IRS is one of the key malaria elimination strategies of the Zambian National Malaria Control Centre (NMCC)).

In 2014 PMI provided financial and technical support to the NMCC and district health offices for IRS and entomological surveillance activities through the Zambia Integrated Systems Strengthening Program and the PMI AIRS Project.

Entomological surveillance is a key component for IRS programming, providing information on the impact of IRS on vector density and the behavior of malaria vectors in IRS areas compared to non-IRS areas. Entomological activities also help to assess the quality of the IRS operations, the decay rates of the insecticide applied and the vectors susceptibility to insecticides recommended by World Health Organization Pesticides Evaluation Scheme (WHOPES) for use in malaria vector control. The susceptibility data collected are used to select the insecticide for the IRS campaign.

During the 2015 entomological surveillance, the entomological indicators assessed include:

- Malaria vector species composition
- Vector distribution and seasonality (Vector density)
- Vector behavior
- Vector susceptibility to insecticides
- Quality assurance of IRS and decay rate of insecticide applied
- Parity rates

Sporozoite rates and the Entomological Inoculate Rate (EIR) will be provided in the progress report as soon as the enzyme-linked immunosorbent assay (ELISA) circumsporozoite antigens (CSP) results are received from Macha Research Centre.

# 2. MATERIALS AND METHODS

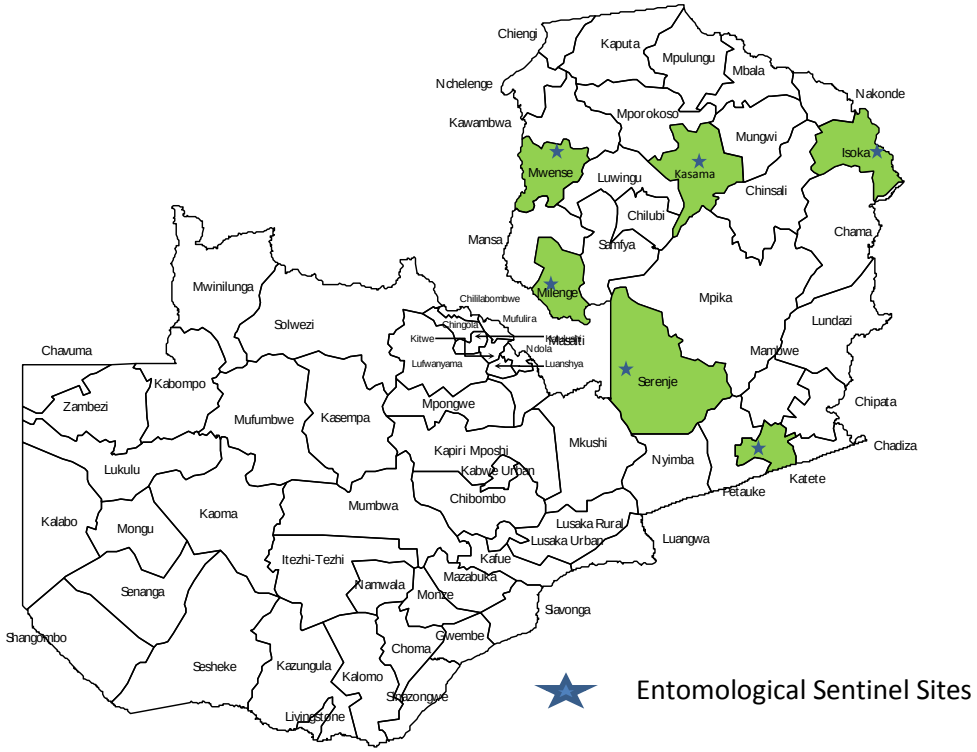
## 2.1 STUDY SITES

Entomological surveillance was performed in six sentinel districts to assess the 2015 IRS campaign that occurred from September 28 to November 25, 2015. In each district, one sprayed village and another unsprayed village as a control were selected (except in Kasama and Isoka districts where the control sites were sprayed during the 2014 IRS campaign). The sentinel sites covered all five provinces receiving IRS (Table I).

**Table 1: Entomological surveillance sentinel sites**

| Provinces | District | Sites | Spray Status | Percentage of Households Targeted for Spraying |
|---|---|---|---|---|
| Northern | Kasama | Kalonga | Sprayed | 60.5% |
| Eastern | Katete | Robert | Non-Sprayed (control) | 80% |
| | | Mbalani | Sprayed | |
| Muchinga | Isoka | Nsalamba | Sprayed | 87.1% |
| Central | Serenje | Chbobo | Sprayed | 86% |
| | | Chishi | Non-Sprayed (control) | |
| Luapula | Milenge | Lunga | Sprayed | 79% |
| | | Miyambo | Non-Sprayed (control) | |
| | Mwense | Shibesa | Sprayed | 97% |
| | | Chebele | Non-Sprayed (control) | |

**Figure** 1**: Geographical locations of entomological sentinel sites in Zambia**




## 2.2 MALARIA VECTOR DENSITY AND BEHAVIOR MONITORING

Adult mosquito collections were done using PSC, CDC light traps, and HLC.

### 2.2.1 Pyrethrum Spray Catch (PSC)

PSCs were used to sample indoor resting mosquitoes between 4:00 a.m. and 7:00 a.m. in 15 houses for three mornings in each sentinel site. Before the PSC was performed, all occupants were asked to vacate the house without disturbing the resting mosquitoes. The eaves, windows, and other escape routes around the house were sprayed with the pyrethrum mixture (0.025 percent pyrethrum emulsifiable concentrate with 0.1 percent piperonyl-butoxide in kerosene), using a small hand sprayer, followed by spraying of the walls and roof space inside the house. Ten minutes after spraying, all mosquitoes knocked down by the chemical were collected from the white sheets that were placed on the floor before spraying.

### 2.2.2 CDC light trap collections

CDC light traps were installed inside a total of four houses in each sentinel site for four consecutive nights. The CDC light-trap was suspended in a bedroom 1.5 meters above the floor and about 50 cm from a human sleeping under an insecticide-treated net on the foot side. Traps

were set from 18:00 until 06:00 to ensure that surveillance is conducted during the primary host-seeking periods.

### 2.2.3 Human Landing Catch (HLC)

The HLC was used to monitor mosquito feeding behavior in all 10 sentinel sites (six sprayed and four control sites). In each targeted village, the collection was done in a total of four houses for four consecutive nights every month. One collector was seated indoors and another seated outdoors from 6 p.m. to 6 a.m. A total of four volunteers per house per night collected from 6 p.m. to 6 a.m. The mosquitoes that tried to bite the volunteer were collected with the mouth aspirator and transferred to paper cups labelled with the hour of collection.

The following parameters were measured from the HLCs:

- Vector feeding behavior (biting time, location of biting)
- Parity
- Biting rate
- Sporozoite rate and EIR will be reported in the progress report as soon as we receive ELISA CSP results from Macha Research Centre.

Community health workers involved in the HLC were provided with chemoprophylaxis with deltaprim.

The mosquitoes collected by PSC, HLC, and CDC light trap were identified. Anophelines were sorted morphologically to species using the Gilles and Coetzee, 1987 identification key. The abdominal status of all female anophelines collected was categorized as unfed, blood-fed, half-gravid and gravid. The anopheline collected were preserved in Eppendorf tubes with silica gel. The preserved malaria vectors (*An. funestus* s.l. and *Anopheles gambiae* s.l.) will be used for polymerase chain reaction (PCR) analysis to identify the sibling species. The sample malaria vectors from the HLC will be analyzed by the ELISA method to look for CSPs and for blood meal determination.

## 2.3 SUSCEPTIBILITY TESTS

### 2.3.1 WHO susceptibility test

The CDC Backpack and Prokopack aspirators were used to capture adult indoor-resting mosquitoes. The malaria vectors collected were used in insecticide susceptibility bioassays. Backpack aspiration was performed in as many houses as possible between 04:00 and 07:00 to minimize the chance of mosquitoes leaving the house before the catch is performed. Captured mosquitoes were stored in mosquito cages and were provided access to cotton pads soaked with sugar water to keep them alive.

F1 generation female malaria main vectors aged 2-5 days reared from eggs of field-caught mosquitoes and malaria vectors reared from larvae collected from the field were used for the susceptibility test. The mosquitoes were exposed to diagnostic doses of various insecticides using insecticide-impregnated papers, as described by the WHO guideline. One insecticide from

each class was tested in some of the sentinel sites for *Anopheles funestus* s.l. and *Anopheles gambiae* s.l. A subsample of dead and surviving mosquitoes was preserved and sent to CDC laboratory for the mechanism of the resistance determination. All the resistance study data will be added to the DDMS database.

### 2.3.2 CDC Resistance Intensity Rapid Diagnostic Test (I-RDT)

AIRS Zambia performed the Rapid Resistance Intensity Diagnostic test (I-RDT) in four districts (Milenge, Samfya, Mwense, and Kawambwa) in Luapula Province to select the sites for an operational research project on resistance intensity.

Adult wild mosquitoes collected through backpack aspiration were used. Prior to the assay, a holding period of one day was observed after the mosquito collection to ensure that any damaged mosquitoes were not used for the test. CDC bottles treated with 1, 2, 5 and 10 times the diagnostic dose of each insecticide plus one control per insecticide were used. Five insecticides were tested withfour pyrethroids (deltamethrin, permethrin, lambda-cyhalothrin, alpha-cypermethrin) and PBO..  The mosquitoes were exposed for 30 minutes and the number of mosquitoes knocked down at 0, 15 and 30 minutes was recorded. The mortality was recorded at the diagnostic time (30 minutes). A subsample of dead and surviving mosquitoes was sent to CDC laboratory for the species identification using PCR.

## 2.4 QUALITY ASSURANCE OF IRS AND INSECTICIDE DECAY RATE

Cone bioassays were used to evaluate the quality of spraying and monitor the decay rate of the insecticide after spraying. The first wall bioassay was conducted 24 hours after spraying in 40 randomly selected houses. Six sprayed houses (three mud, three cement) and two unsprayed (control) houses (one mud and one cement) were used per sentinel site in Mwense, Milenge and Serenje districts. Four sprayed (two mud and two cement) and one control were selected per site in Isoka, Katete, and Kasama districts.

The Kisumu strain of *Anopheles gambiae* s.s. available in the insectary in October was not enough to cover all six sentinel sites for the T0 cone bioassay tests due to the ant invasion and destruction of some mosquitoes in the insectary in July-August. *Anopheles funestus* s.l. was collected using backpack aspirators from Milenge and Mwense districts. Previous surveys showed that *An. funestus* s.l. is most abundant in Milenge and Mwense districts. The WHO susceptibility test was done against pirimiphos-methyl CS (insecticide used for spraying) and showed 100% mortality. This known susceptible *Anopheles funestus* s.l. strain was used for the cone bioassay in Milenge, Mwense, and Serenje districts. The susceptible Kisumu strain (*Anopheles gambiae* s.s.) reared in the insectary at the NMCC was used for the cone bioassay tests in the remaining three districts namely Katete, Isoka, and Kasama. The two most commonly found surface types in the areas, cement and mud walls, were tested.

The cone bioassay tests were done according to the WHO test procedure. The cones were placed on the treated walls at three locations: 0.5m, 1m, and 1.5m above the ground. About 10 females of known susceptible malaria vectors were introduced per cone and exposed for 30 minutes. The number of mosquitoes knocked down after 30 minutes and 60 minutes and dead at the end of 24-hour holding period were recorded.

Subsequent tests were performed at T1 in November, T2 in December, T3 in January, T4 in February, T5 in March, and T6 in April to assess the decay rate of the insecticide applied.

# 3. RESULTS AND DISCUSSION

## 3.1 SPECIES COMPOSITION, VECTOR DENSITY, BEHAVIOR AND LONGEVITY

AIRS collected baseline data using indoor CDC light traps, PSC, and HLC in six sprayed sites and four control sites in August to assess the species composition, the vector density and the malaria vectors' behavior before the 2015 IRS campaign. Monthly collections were continued from November to January to assess the impact of the IRS campaign on malaria vectors.

### 3.1.1 Species composition

A total of 26,416 female mosquitoes were collected using PSC, HLC, and CDC light traps both from spray targeted and control sites, including 6,499 culicine (25%) and 19,917 anopheline (75%). *An. funestus* s.l. (12,758) was the most prevalent species (64%). A total of 2,111 *Anopheles gambiae* s.l. were caught (11%). The other anopheline species found were 3,237 *An. tenebrosus* (16%), 692 *An. tchekedii* (3%), 732 *An. squamosus* (4%), 357 *An. coustani* (2%), 3 *An. rufipes* (0.0006%) and 27 *An. ziemanni* (0.0014%). Tables A-H in the Annex show the number of each species collected per sites and per month.

**Figure 2: Species composition in the targeted sprayed sentinel sites (Aug 2015 - Jan 2016)**

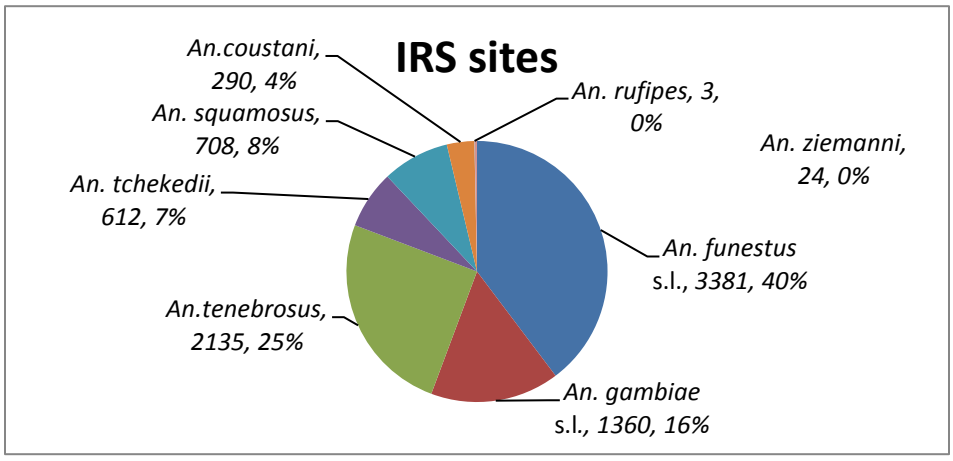
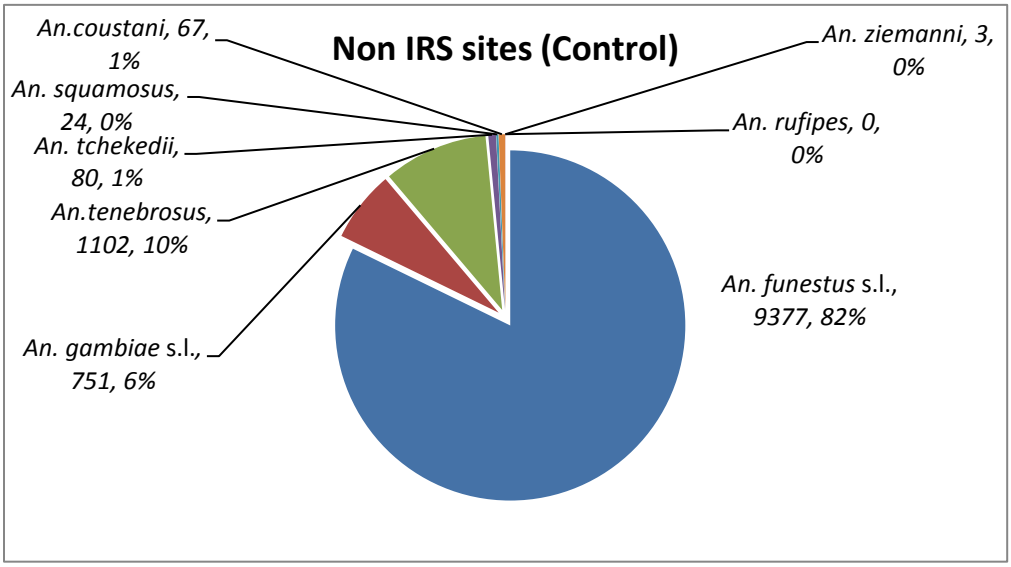


**Figure** 3 **: Species composition in the control sentinel sites (Aug 2015 - Jan 2016)**




## 3.2 VECTOR DENSITY, BEHAVIOR, AND LONGEVITY

### 3.2.1 Pyrethrum Spray Catch

As indicated in Tables 2, 3, 4, and 5, the average density of *Anopheles funestus* s.l. collected by PSC both from the intervention and control sites was low in August prior to the IRS campaign except in Milenge and Mwense. The highest density was recorded in Milenge where 28 *Anopheles funestus* s.l. were collected per room per day in Lunga (sprayed site) and nine *Anopheles funestus* s.l. per room per day in Miyambo (control site) in August. The average density in Lunga (7.9 *Anopheles funestus* s.l./room/day) was four times lower in January, three months after IRS campaign as compared to the density recorded in August. The average density of *An. funestus* s.l. per room per day in Shibesa dropped from 7.25 before IRS to 2.75 in January, three months after IRS campaign. Less than one *Anopheles funestus* s.l. was collected in Kalonga and Nsalamba during the study period. No *Anopheles funestus* s.l. was found in Katete District.

Overall, The mean average of *Anopheles funestus* s.l. was 6 per room per day in the intervention sites in August before IRS campaign and is similar to the density in the sprayed sites in November , one month after IRS (5 *An.funestus* s.l./day/room). The mean indoor resting density of *Anopheles funestus* s.l. dropped from six *Anopheles funestus* s.l. per room per day in August to two in January three months after IRS. In contrary, in the control sites, the indoor resting density per room per day increased from three *Anopheles funestus* s.l. per room per day before IRS in August to 11 *Anopheles funestus* s.l. per room per day one month after the IRS campaign and was five *Anopheles funestus* s.l. per room per day three months after spraying

The proportion of half and full gravid *Anopheles funestus* s.l. was similar and high in both sprayed and control sites (32% in sprayed sites and 31% in control sites), after the IRS campaign. More than half of the *Anopheles funestus* s.l. collected inside the houses (59.8% in the

intervention sites and 54% in the control) by PSC was found fed. The blood meal analysis will confirm if the vector-man contact is maintained even after IRS.

**Table 2 : Blood digestion stage and average density of *An. funestus* s.l. collected by PSC per room in Serenje District in August and from November 2015 to January 2016**

| Months | Number of rooms | *An. funestus s.l.* Collected | Physiological age | | | | Average density per room |
|---|---|---|---|---|---|---|---|
| | | | UF | F | HG | G | |
| **Chibobo (Sprayed site)** | | | | | | | |
| August (Baseline) | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| November | 15 | 40 | 4 | 6 | 15 | 15 | 2.7 |
| December | 15 | 27 | 2 | 19 | 1 | 5 | 1.8 |
| January | 15 | 34 | 3 | 24 | 5 | 2 | 2.3 |
| **Chishi (Control site)** | | | | | | | |
| August (Baseline) | 8 | 1 | 1 | 0 | 0 | 0 | 0.1 |
| November | 15 | 28 | 3 | 16 | 8 | 1 | 1.9 |
| December | 15 | 93 | 9 | 50 | 19 | 15 | 6.2 |
| January | 15 | 108 | 11 | 84 | 9 | 4 | 7.2 |

**Table 3 : Blood digestion stage and average density of *An. funestus* s.l. collected by PSC per room in Milenge District in August and from November 2015 to January 2016**

| Months | Number of rooms | *An. funestus s.l.* Collected | Physiological age | | | | Average density per room |
|---|---|---|---|---|---|---|---|
| | | | UF | F | HG | G | |
| **Lunga (Sprayed site)** | | | | | | | |
| August (Baseline) | 8 | 224 | 83 | 131 | 0 | 10 | 28 |
| November | 15 | 393 | 27 | 234 | 93 | 39 | 26.2 |
| December | 15 | 65 | 4 | 35 | 5 | 21 | 4.3 |
| January | 15 | 119 | 9 | 90 | 11 | 9 | 7.9 |
| **Miyambo (Control site)** | | | | | | | |
| August (Baseline) | 8 | 74 | 4 | 70 | 0 | 0 | 9.25 |
| November | 15 | 568 | 87 | 284 | 98 | 99 | 37.9 |
| December | 15 | 243 | 40 | 120 | 35 | 48 | 16.2 |
| January | 15 | 155 | 36 | 109 | 1 | 9 | 10.3 |

**Table 4 : Blood digestion stage and average density of *An. funestus* s.l. collected by PSC per room in Mwense District in August and from November 2015 to January 2016**

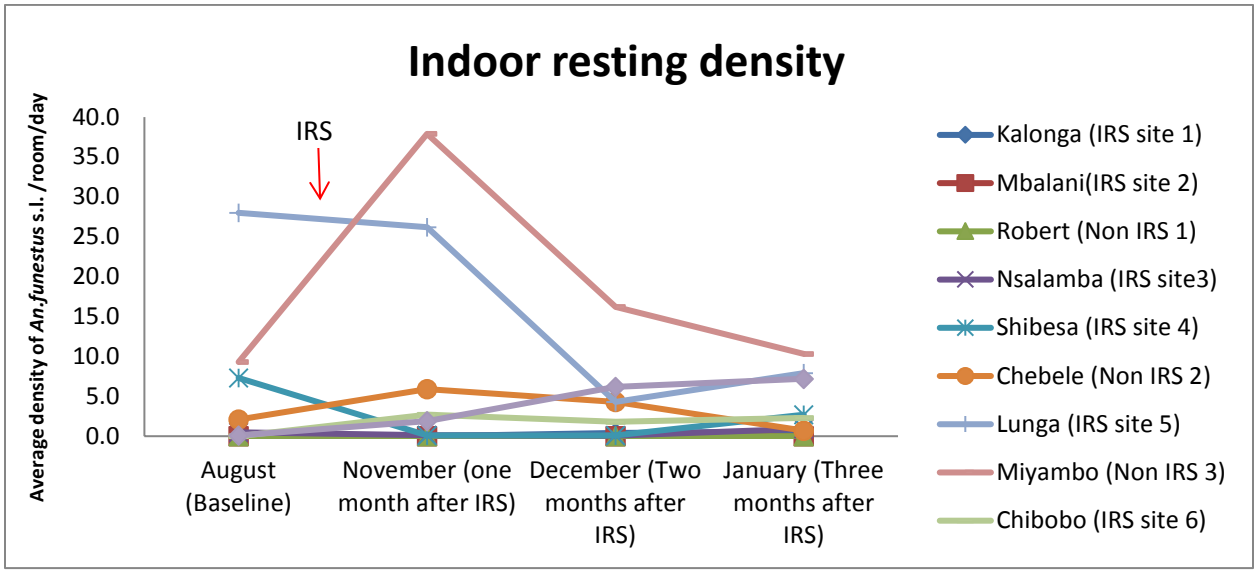
| Months | Number of rooms | *An. funestus s.l.* Collected | Physiological age | | | | Average density per room |
|---|---|---|---|---|---|---|---|
| | | | UF | F | HG | G | |
| **Shibesa (Sprayed site)** | | | | | | | |
| August (Baseline) | 8 | 58 | 5 | 53 | 0 | 0 | 7.25 |
| November | 15 | 1 | 0 | 1 | 0 | 0 | 0.1 |
| December | 15 | 2 | 0 | 2 | 0 | 0 | 0.1 |
| January | 15 | 41 | 3 | 29 | 2 | 7 | 2.7 |
| **Chebele (Control sites)** | | | | | | | |
| August (Baseline) | 8 | 17 | 4 | 13 | 0 | 0 | 2.1 |
| November | 15 | 89 | 7 | 36 | 30 | 16 | 5.9 |
| December | 15 | 64 | 9 | 27 | 15 | 13 | 4.3 |
| January | 15 | 11 | 3 | 8 | 0 | 0 | 0.7 |

**Table 5 : Blood digestion stage and average density of *An. funestus* s.l. collected by PSC per room in Kasama and Isoka districts in August and from November 2015 to January 2016**

| Months | Number of rooms | *An. funestus s.l.* Collected | Physiological age | | | | Average density per room |
|---|---|---|---|---|---|---|---|
| | | | UF | F | HG | G | |
| **Kalonga (Sprayed site) Kasama district** | | | | | | | |
| August (Baseline) | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| November | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| December | 15 | 6 | 3 | 3 | 0 | 0 | 0.4 |
| January | 15 | 9 | 0 | 2 | 4 | 3 | 0.6 |
| **Nsalamba (Sprayed site) Isoka district** | | | | | | | |
| August (Baseline) | 8 | 4 | 2 | 2 | 0 | 0 | 0.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| November | 15 | 2 | 2 | 0 | 0 | 0 | 0.13 |
| December | 15 | 1 | 1 | 0 | 0 | 0 | 0.07 |
| January | 15 | 14 | 3 | 6 | 1 | 4 | 0.93 |

**Figure 4: Indoor resting density of *Anopheles funestus* s.l. per room per night in August (baseline) and one, two and three months after IRS**




A total of 128 *Anopheles gambiae* s.l. were collected by PSC from the sprayed and control sites. A total of 79 *Anopheles gambiae* s.l. were collected in the sprayed sites and 49 in the control sites. *Anopheles gambiae* s.l. was not found in the rooms in Serenje and Katete districts. The average density of *Anopheles gambiae* s.l. was low, less than one *Anopheles gambiae* s.l. in Milenge and Kasama districts. The highest density was recorded in Mwense District where 3.4 *Anopheles gambiae* s.l. were found per room per day in the sprayed site and 2.6 in the control. Figures 5, 6, and 7 show the average density per room per day in Mwense, Milenge, Isoka, and Kasama districts.

**Figure 5: Average density of *Anopheles gambiae* s.l. per room per day in Mwense District (Aug 2015 - Jan 2016)**

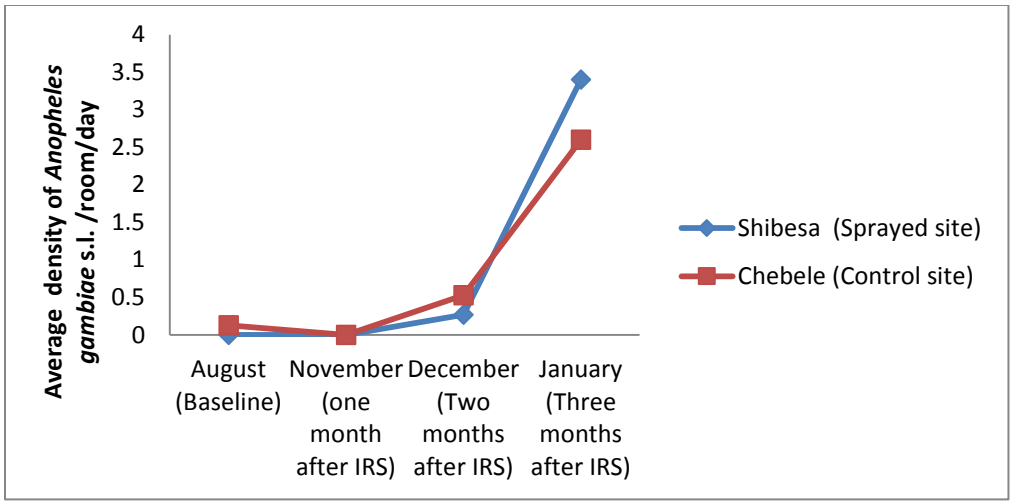



**Figure 6: Average density of *Anopheles gambiae* s.l. per room per day in Milenge District (Aug 2015 - Jan 2016)**

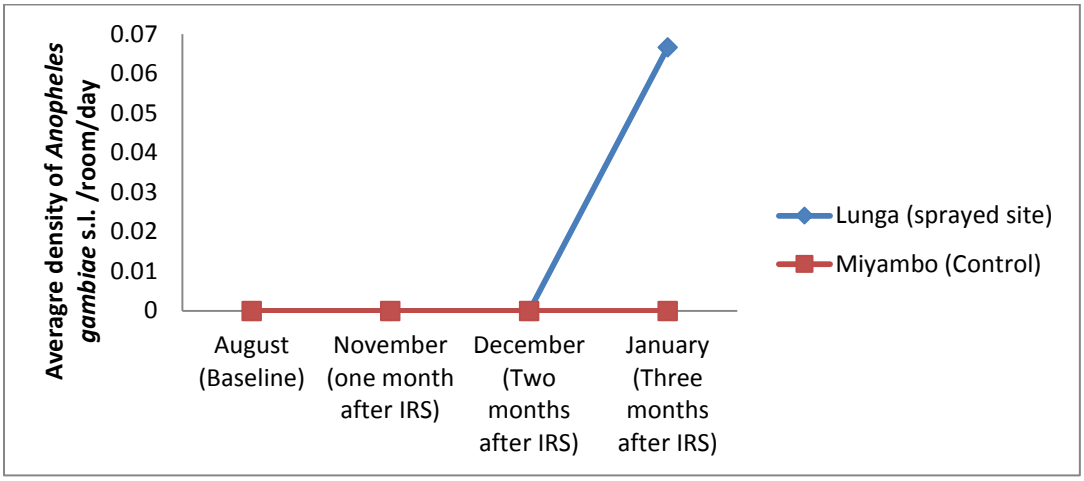
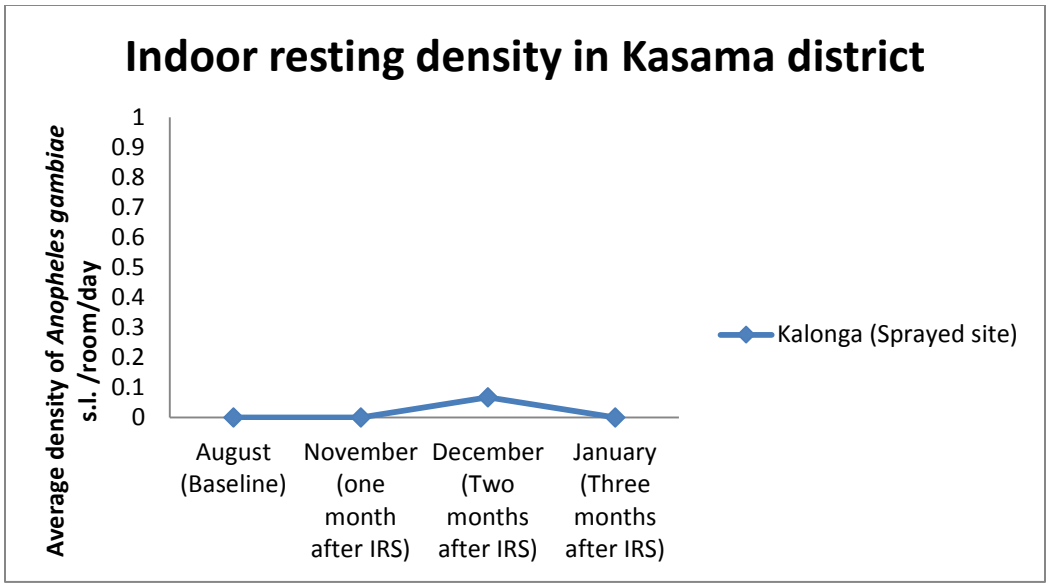
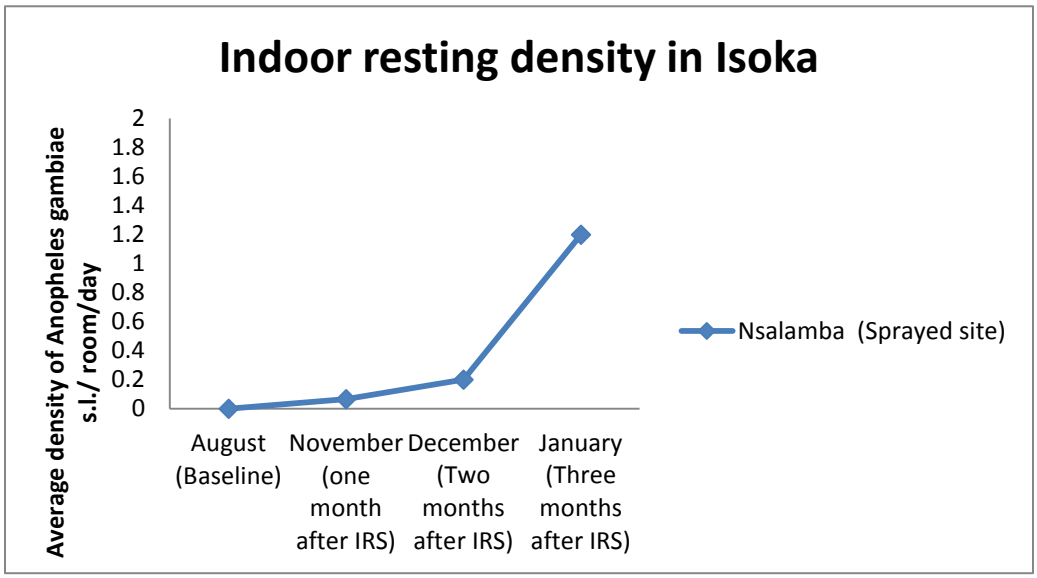



**Figure 7: Average density of *Anopheles gambiae* s.l. per room per day in Kasama and Isoka Districts (Aug 2015 - Jan 2016)**

**a) Kasama district**




**b) Isoka district**



### 3.2.2 CDC light trap collection

The average density of *An. funestus* s.l. per trap per night in the intervention sites dropped from five to 0.7 one month after IRS campaign. The density of *Anopheles funestus* s.l. per trap per night in the sprayed sites was 2.9 three months after the IRS campaign in January in the intervention sites. In contrary, the density of *Anopheles funestus* s.l. increased from seven in August to 12.2 per trap per night one month after IRS and was still high (12.3 per trap per night) three months in January in the control sites. The mean density of *Anopheles funestus* s.l. in the intervention site (2.9 *Anopheles funestus* s.l. /trap/night) was four times lower than the density in the control sites (12.3/trap/night) in January.

The average density of *An. funestus* s.l. in Miyambo (24 *Anopheles funestus* s.l./trap/night) in Milenge District was two times higher than the density recorded in Lunga, the intervention site (13.3 *Anopheles funestus* s.l /trap/night) in August before IRS. The density decreased from 13 *Anopheles funestus* s.l./ trap/night to 4.1 two months after spraying, but increased to 13 *Anopheles funestus* s.l. /trap/night in Lunga three months after IRS in January. However, the density recorded in Miyambo, the control site, was three times higher than the density of *Anopheles funestus* s.l. in Lunga in January 2016.

*Anopheles gambiae* s.l. was not collected in Katete District by CDC light trap during the study period. Less than one *Anopheles gambiae* s.l. was caught per trap per night in the sprayed sites except in January when the average density per trap per night was between zero in Chibobo in Serenje District and 14.4 in Nsalamba in Isoka District.

**Figure 8: Average density of *Anopheles funestus* s.l. per trap per night in Katete District (Aug 2015 - Jan 2016)**

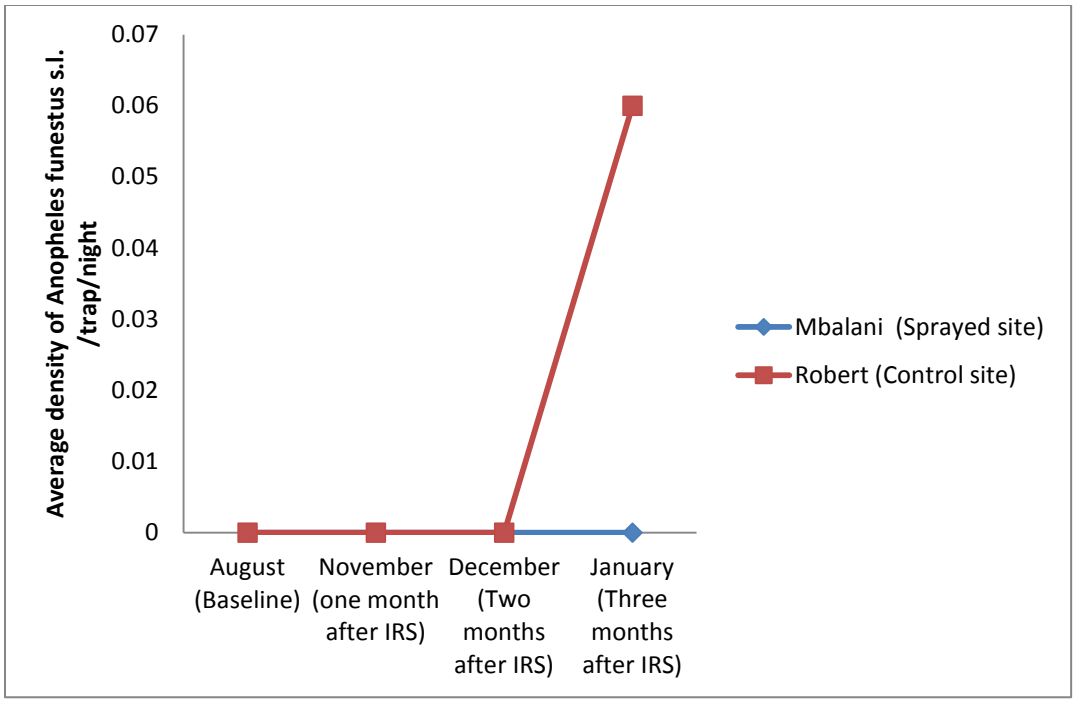



**Figure 9: Average density of *Anopheles funestus* s.l. per trap per night in Mwense District (Aug 2015 - Jan 2016)**

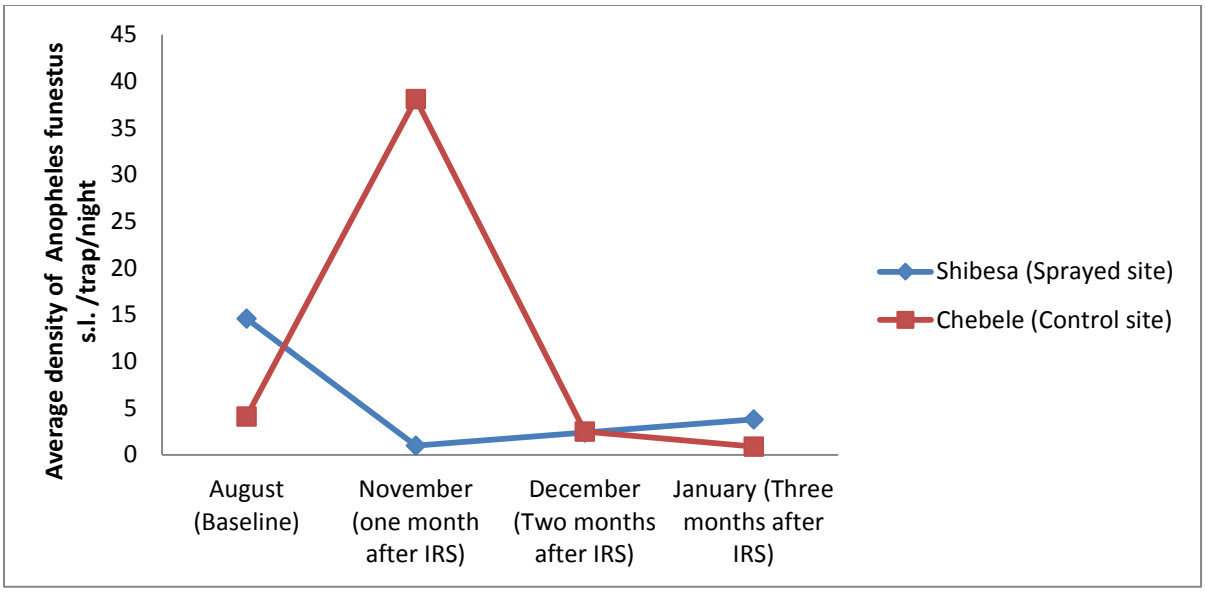



**Figure 10: Average density of *Anopheles gambiae* s.l. per trap per night in Mwense District (Aug 2015 - Jan 2016)**

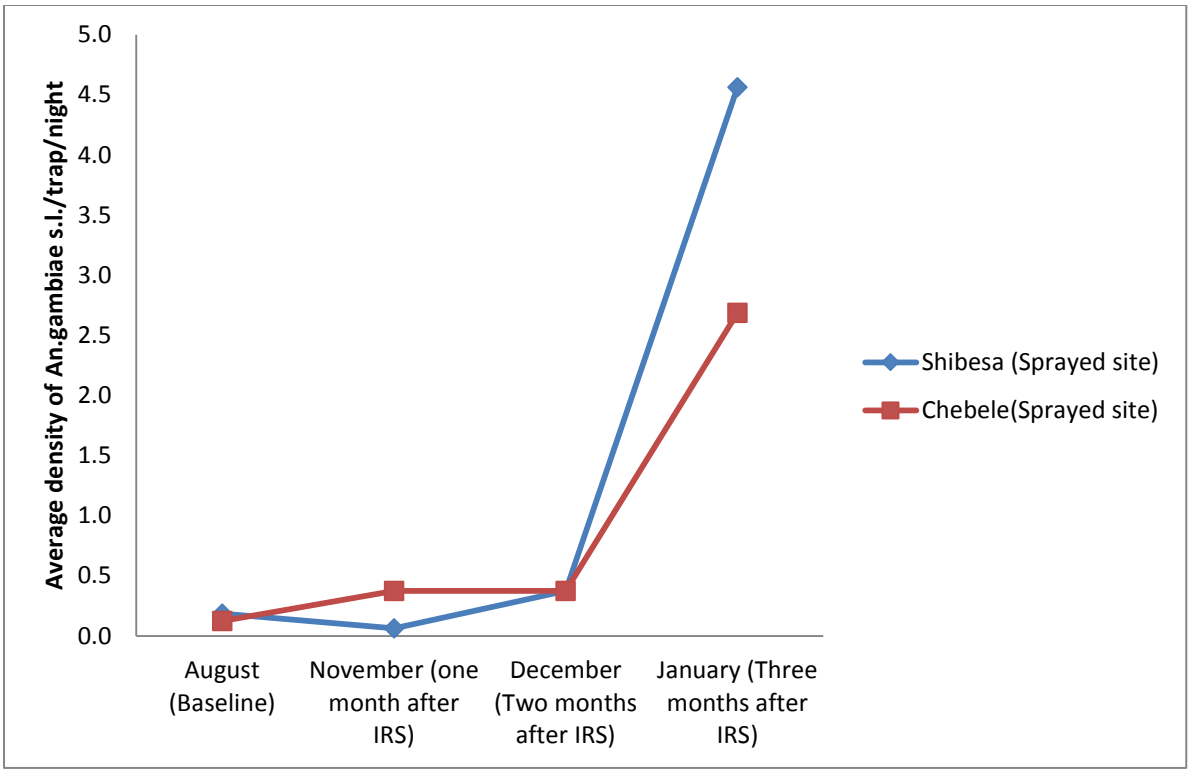



**Figure 11 : Average density of *Anopheles funestus* s.l. per trap per night in Milenge District (Aug 2015 - Jan 2016)**

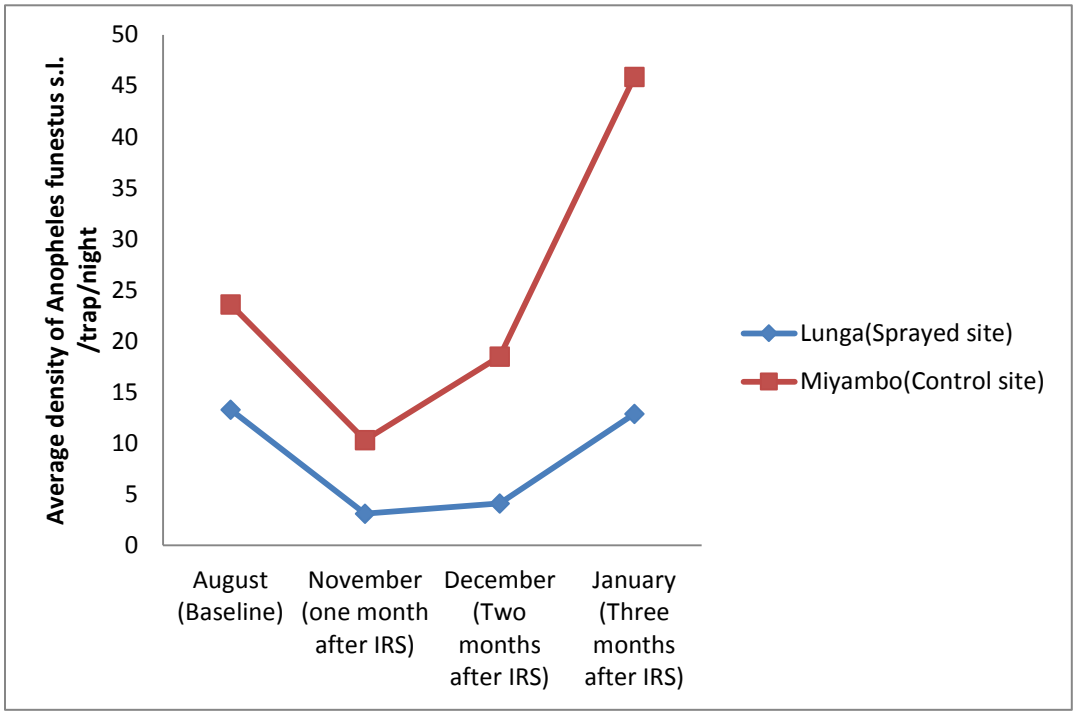


**Figure 12: Average density of *Anopheles gambiae* s.l. per trap per night in Milenge District (Aug 2015 - Jan 2016)**

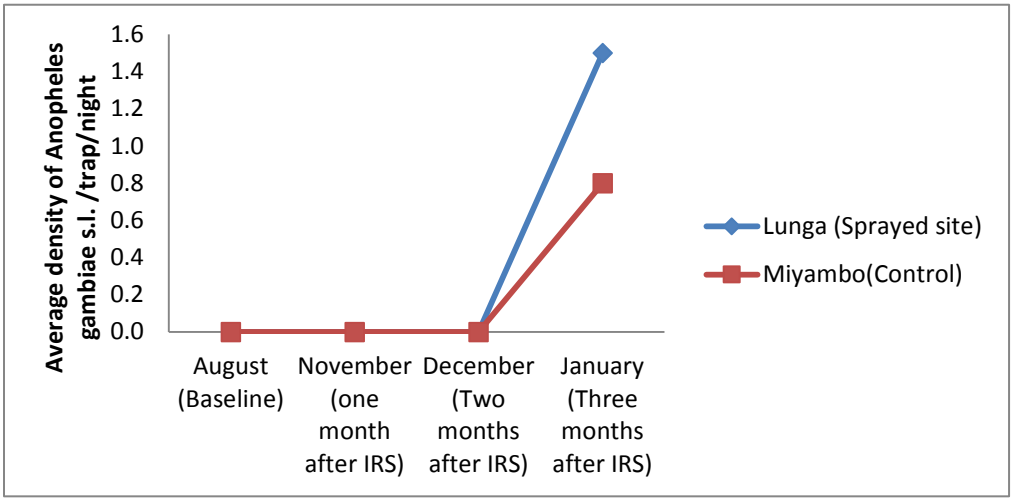



**Figure 13: Average density of *Anopheles funestus* s.l. per trap per night in Serenje District (Aug 2015 - Jan 2016)**

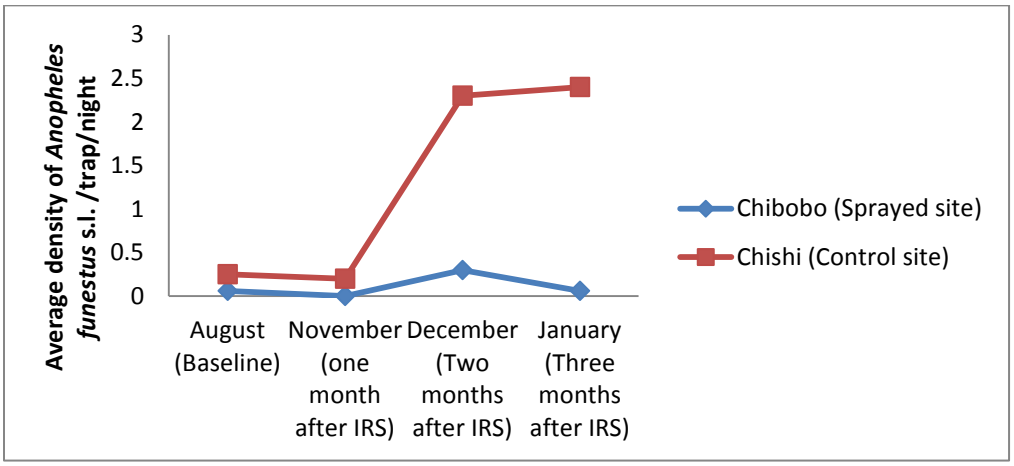
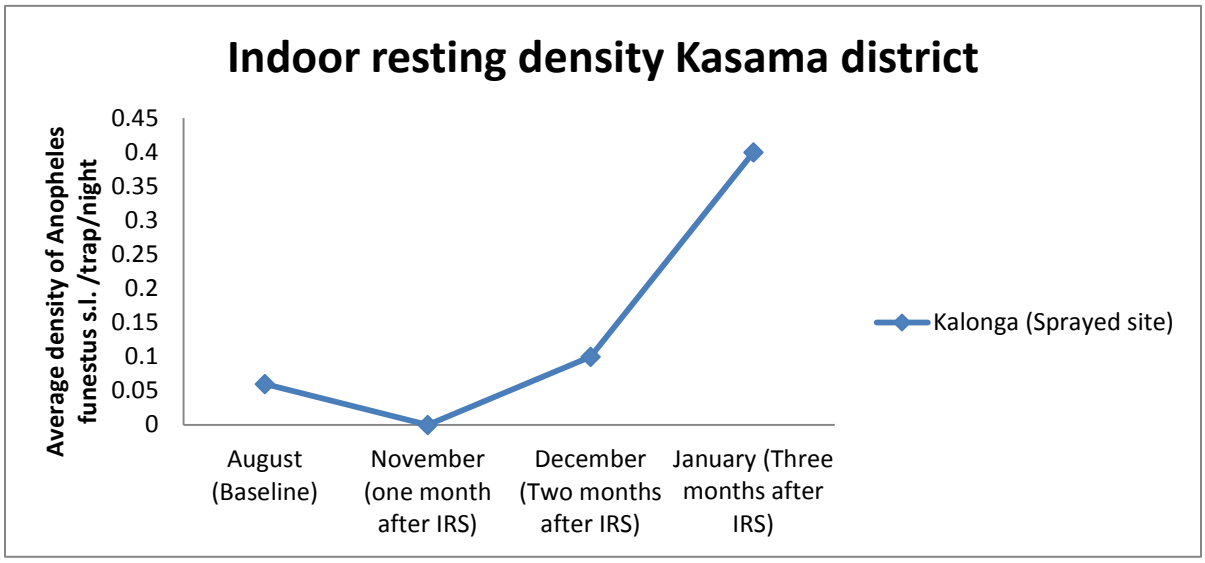


**Figure 14: Average density of *Anopheles funestus* s.l. per trap per night in Kasama and Isoka districts (Aug 2015 - Jan 2016)**

a) Kasama district




b) Isoka district

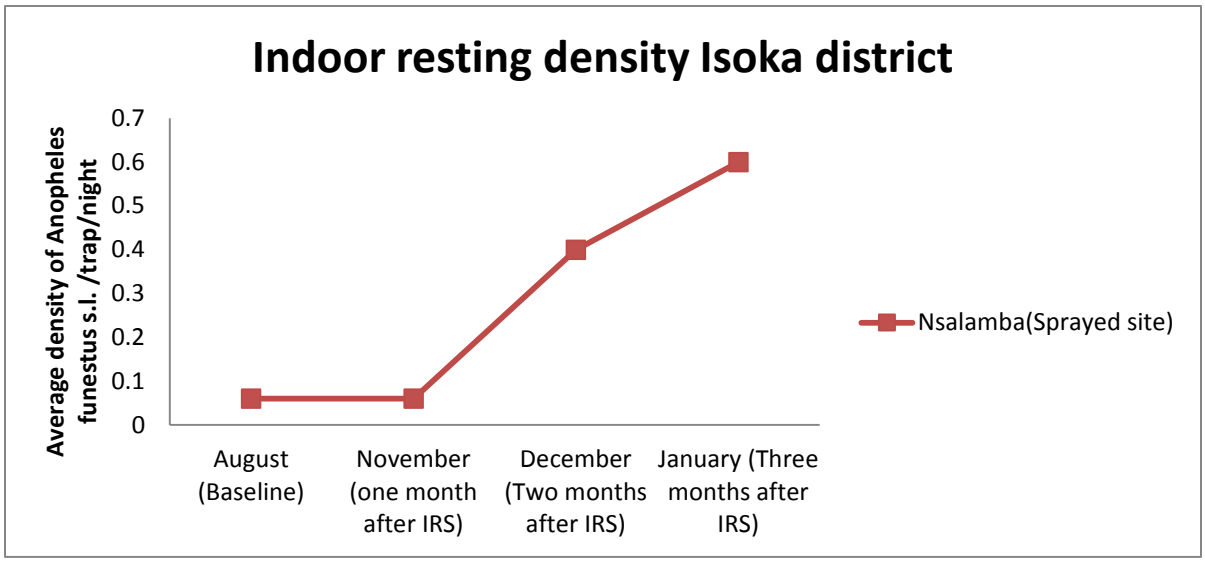
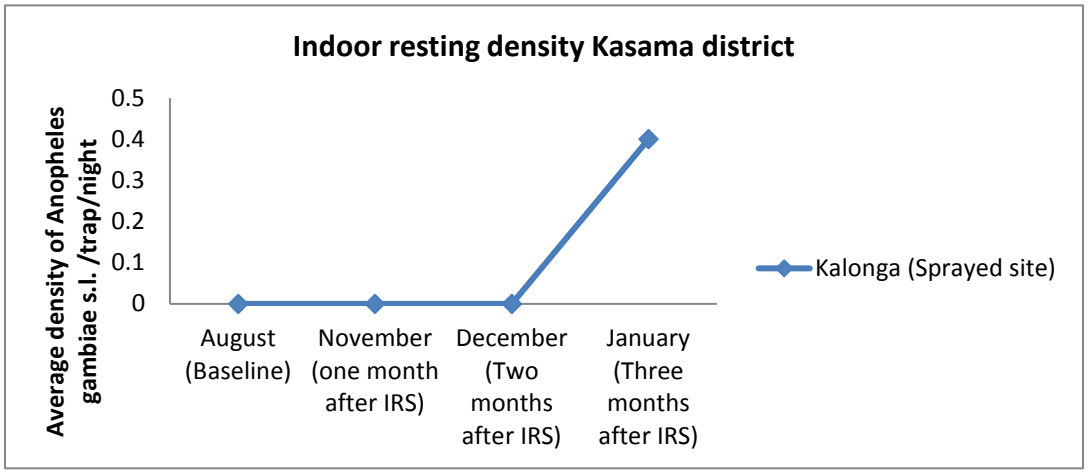
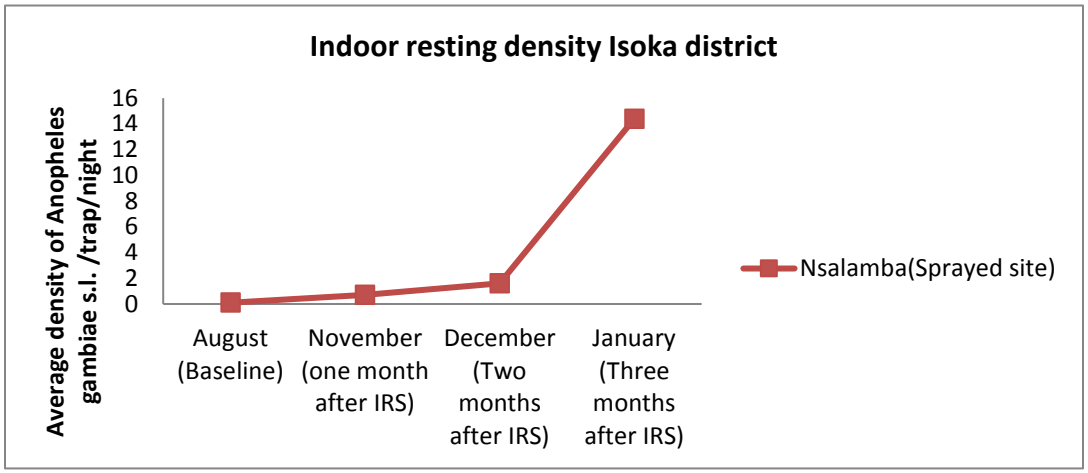


**Figure 15: Average density of *Anopheles gambiae* s.l. per trap per night in Kasama and Isoka Districts from August 2015 to January 2016**

a) Kasama district




b) Isoka district




### 3.2.3 HLC Collection

During the collection period, 13,025 female *Anopheles* were collected indoors and outdoors from the sprayed and control sites. A total of 6,965 *Anopheles funestus* s.l. and 1,532 *Anopheles gambiae* s.l. were collected. *An. tenebrous* (2,837*), An. tchekedii (616), An. squamosus (692), An. rufipes (3), An. coustani (353) and An. ziemanni* (27) were also collected biting indoors and outdoors. Figure 16 gives the composition of *Anopheles* species collected by HLC.

**Figure 16: *Anopheles* species composition of the indoor HLC (Aug 2015 to Jan 2016)**

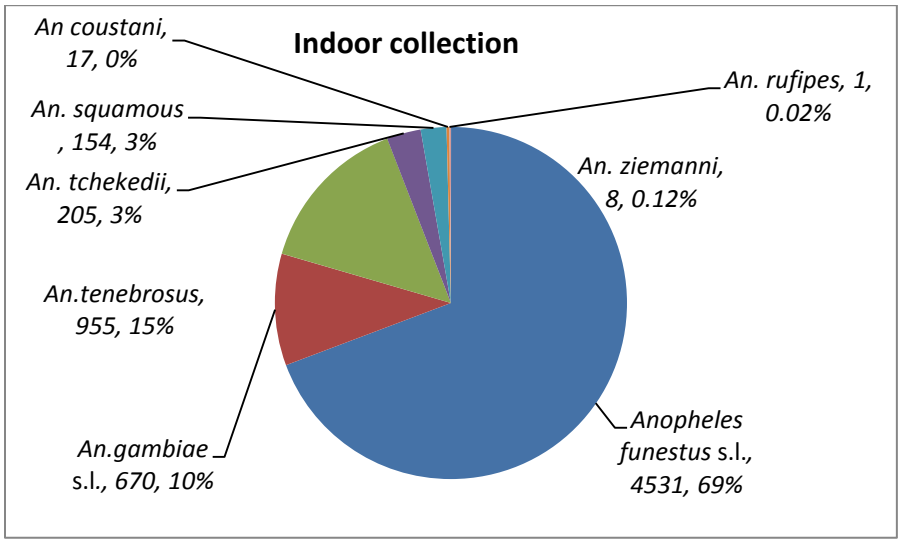
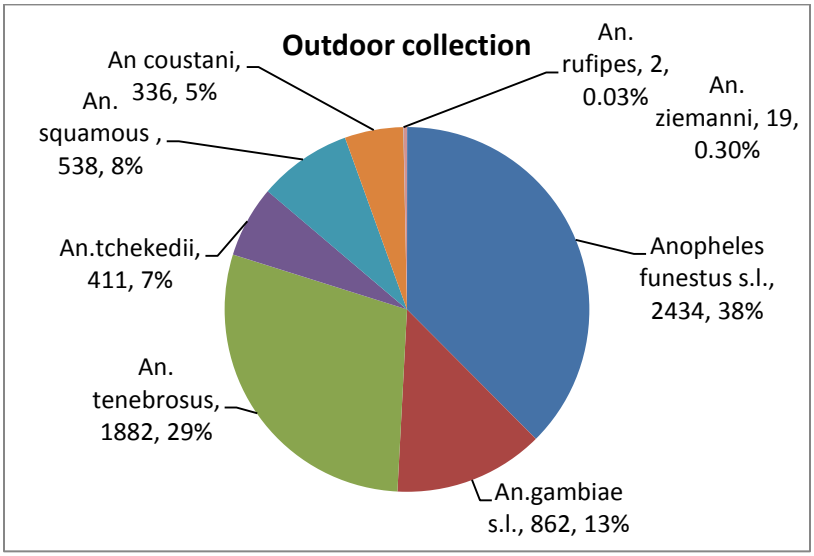



**Figure 17: *Anopheles* species composition of the outdoor HLC (Aug 2015 to Jan 2016)**




## Human biting rate of *Anopheles funestus* s.l.

The mean biting rate of *Anopheles funestus* s.l. was 10.5 per person per night inside the house and 4.3 per person per night outdoors in the control sites versus three per person per night inside and 1.3 per person per night outdoors in the spray targeted site in August.

The human biting rate of *Anopheles funestus* s.l. was reduced from three bites per person per night during the pre-spray period to 0.5 bites per person per night one month after spraying in the intervention sites. The drop of the human biting rate in the intervention sites one month after IRS campaign might be due to the IRS effect. However, the biting rate increased from 0.5 per person per night in November to 4.6 bites per person per night in the intervention sites in January three months after the spraying. In the control sites, the HBR indoors increased from 10.5 bites per person per night in August to 19 bites per person per night in November and 14.3 bites per person per night in January.

In Katete District during the pre-spray period and three months after spraying, no malaria vectors were collected from Mbalani, the sprayed site. In the control site the HBR indoors was low. Each person received only two *Anopheles funestus* s.l. bites per month inside house (0.0625 bites/night) in Robert (control site) in December and January versus no bites per month in the intervention site.

The HBR indoors in Shibesa (sprayed site) in Mwense District was 0.25 *Anopheles funestus* s.l. bites per person per night in November one month after spraying —240 times lower than the HBR in the control sites of Chebele (60 *Anopheles funestus* s.l. bites/person/night). However, the HBR inside houses increased from 0.25 to 3 bites per person per night in January and is similar to the HBR recorded indoors in the control site in January (2.75 bites/person/night).

The HBR indoors in Lunga (sprayed) in Milenge was 14 bites per person per night during the pre-spray period and dropped to 2.3 bites per person per night one month after IRS. The rate increased to 20 bites per person per night in January three months after spraying. The HBR indoors in Miyambo, the control site, increased from 16.25 bites/person/night in November to 52 bites/person/night.

Table 6 provides the HBR of *Anopheles funestus* s.l. from August to January in the control and sprayed sites. Tables 7, 8, 9, 10, and 11 show the HBR per district.

**Table** 6**: *Anopheles funestus* s.l. HBR in sprayed and control sites in August and from November 2015 to January 2016**

| | | August | November | December | January |
|---|---|---|---|---|---|
| **Total Intervention sites** | | | | | |
| Indoor | Total *Anopheles funestus* s.l. | 228 | 44 | 160 | 441 |
| | nb collectors | 20 | 24 | 24 | 24 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **2.9** | **0.5** | **1.7** | **4.6** |
| Outdoor | Total *Anopheles funestus* s.l. | 105 | 32 | 134 | 282 |
| | nb collectors | 20 | 24 | 24 | 24 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **1.3** | **0.3** | **1.4** | **3** |

| | | Total Control sites | | | |
|---|---|---|---|---|---|
| Indoor | Total *Anopheles funestus* s.l. | 503 | 1221 | 1021 | 913 |
| | nb collectors | 12 | 16 | 16 | 16 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **10.5** | **19.1** | **16** | **14.3** |
| Outdoor | Total *Anopheles funestus* s.l. | 205 | 602 | 545 | 529 |
| | nb collectors | 12 | 16 | 16 | 16 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **4.3** | **9.4** | **8.5** | **8.3** |

**Table 7: *Anopheles funestus* s.l. HBR in Katete District in August and from November 2015 to January 2016**

| | | August | November | December | January |
|---|---|---|---|---|---|
| **Mbalani (Intervention site)** | | | | | |
| **Indoor** | Total *Anopheles funestus* s.l. | 0 | 0 | 0 | 0 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0** | **0** |
| **Outdoor** | Total *Anopheles funestus* s.l. | 0 | 0 | 0 | 0 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0** | **0** |
| **Robert (Control)** | | | | | |
| **Indoor** | Total *Anopheles funestus* s.l. | 1 | 0 | 1 | 1 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0.0625** | **0** | **0.0625** | **0.0625** |
| **Outdoor** | Total *Anopheles funestus* s.l. | 0 | 0 | 1 | 0 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0.0625** | **0** |

**Table 8: *Anopheles funestus* s.l. HBR in Mwense District in August and from November 2015 to January 2016**

| | | August | November | December | January |
|---|---|---|---|---|---|
| **Shibesa (Intervention site)** | | | | | |
| Indoor | Total *Anopheles funestus* s.l. | | 4 | 18 | 49 |
| | nb collectors | | 4 | 4 | 4 |
| | nb nights | | 4 | 4 | 4 |
| | HBR/night | | **0.25** | **1.125** | **3.0625** |
| Outdoor | Total *Anopheles funestus* s.l. | | 1 | 31 | 40 |
| | nb collectors | | 4 | 4 | 4 |
| | nb nights | | 4 | 4 | 4 |
| | HBR/night | | **0.06** | **1.93** | **2.5** |
| **Chebele (Control)** | | | | | |
| Indoor | Total *Anopheles funestus* s.l. | | 960 | 179 | 44 |
| | nb collectors | | 4 | 4 | 4 |
| | nb nights | | 4 | 4 | 4 |
| | HBR/night | | **60** | **11.1875** | **2.75** |
| Outdoor | Total *Anopheles funestus* s.l. | | 510 | 100 | 36 |
| | nb collectors | | 4 | 4 | 4 |
| | nb nights | | 4 | 4 | 4 |
| | HBR/night | | **31.875** | **6.25** | **2.25** |

**Table 9 : *Anopheles funestus* s.l. HBR in Milenge District in August and from November 2015 to January 2016**

| | | August | November | December | January |
|---|---|---|---|---|---|
| **Lunga (Intervention site)** | | | | | |
| Indoor | Total *Anopheles funestus* s.l. | 220 | 36 | 126 | 327 |
| | nb collectors s | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **13.75** | **2.25** | **7.875** | **20.4375** |
| Outdoor | Total *Anopheles funestus* s.l. | 93 | 17 | 79 | 176 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **5.8125** | **1.0625** | **4.9375** | **11** |
| **Miyambo (Control)** | | | | | |
| Indoor | Total *Anopheles funestus* s.l. | 497 | 260 | 759 | 828 |

| | | | | | |
|---|---|---|---|---|---|
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **31.0625** | **16.25** | **47.4375** | **51.75** |
| Outdoor | Total *Anopheles funestus* s.l. | 202 | 91 | 397 | 483 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **12.625** | **5.6875** | **24.8125** | **30.1875** |

**Table 10: *Anopheles funestus* s.l. HBR in Mwense District in August and from November 2015 to January 2016**

| | | **August** | **November** | **December** | **January** |
|---|---|---|---|---|---|
| **Chibobo (Intervention site)** | | | | | |
| Indoor | Total *Anopheles funestus* s.l. | 0 | 3 | 6 | 12 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0.1875** | **0.375** | **0.75** |
| Outdoor | Total *Anopheles funestus* s.l. | 0 | 0 | 15 | 10 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0.9375** | **0.625** |
| **Chishi (control)** | | | | | |
| Indoor | Total *Anopheles funestus* s.l. | 5 | 1 | 82 | 40 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0.3125** | **0.0625** | **5.125** | **2.5** |
| Outdoor | Total *Anopheles funestus* s.l. | 3 | 1 | 47 | 10 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0.1875** | **0.0625** | **2.9375** | **0.625** |

**Table 11: *Anopheles funestus* s.l. biting rate in Kasama and Isoka districts in August and from November 2015 to January 2016**

| | **August** | **November** | **December** | **January** |
|---|---|---|---|---|
| **Kalonga (Intervention site)** | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Indoor | Total *Anopheles funestus* s.l. | 6 | 0 | 8 | 31 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0.375** | **0** | **0.5** | **1.9375** |
| Outdoor | Total *Anopheles funestus* s.l. | 1 | 1 | 2 | 16 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0.0625** | **0.0625** | **0.125** | **1** |
| **Nsalamba (Intervention site)** | | | | | |
| Indoor | Total *Anopheles funestus* s.l. | 2 | 1 | 2 | 22 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0.125** | **0.0625** | **0.125** | **1.375** |
| Outdoor | Total *Anopheles funestus* s.l. | 11 | 13 | 7 | 40 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0.6875** | **0.8125** | **0.4375** | **2.5** |

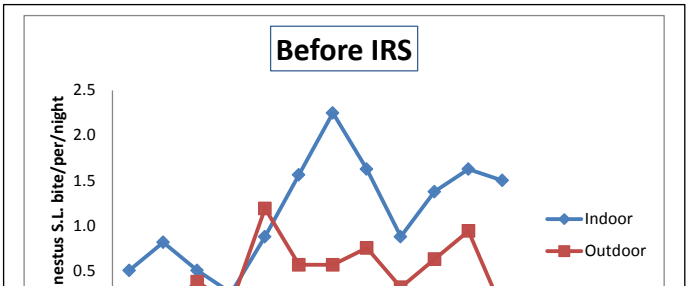
### ***Anopheles funestus* s.l. biting time**

*Anopheles funestus* s.l. started blood feeding indoors early in the evening in Lunga in Milenge in August. The peak of the biting rate was recorded between 0:00-1:00 a.m. After the IRS campaign, two peaks were observed, one between 1:00 -2:00 am and the second between 5:00-6:00 am.

*Anopheles funestus* s.l. started biting indoors between 7:00-8:00 pm. The biting peak was observed between 0:00 and 1:00 am after IRS in Shibesa in Mwense.

The peak biting of *Anopheles funestus* s.l. was recorded between 3:00 and 4:00 am in Chibobo in Serenje District, between 4:00-5:00 am in Kalonga in Kasama, and between 3:00 and 4:00 am in Nsalamba in Isoka District.

**Figure 18: *Anopheles funestus* s.l. feeding time in Lunga sprayed site in Milenge District, in August 2015 before IRS and from November to January 2015 after IRS**



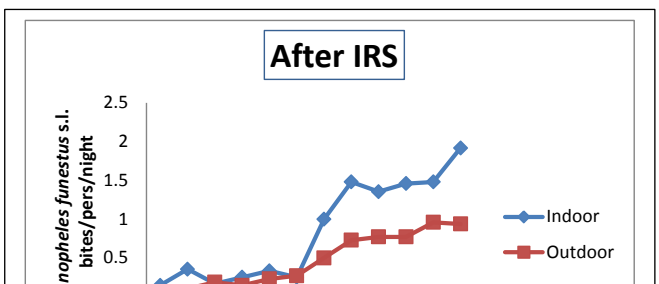



**Figure 19: *Anopheles funestus* s.l. feeding time in Shibesa sprayed site in Mwense District from November to January 2015 after IRS**

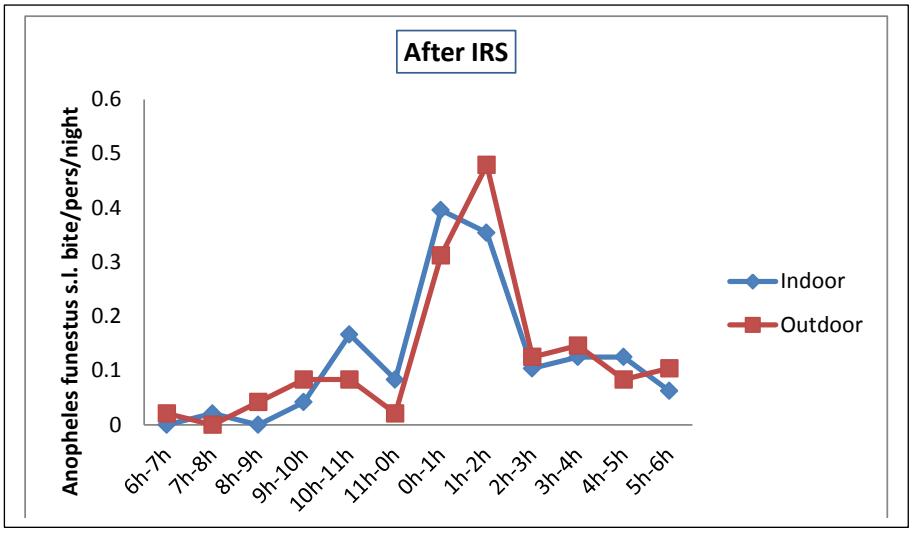
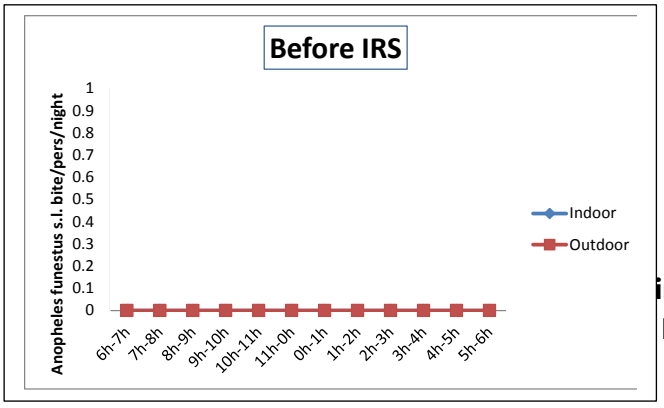
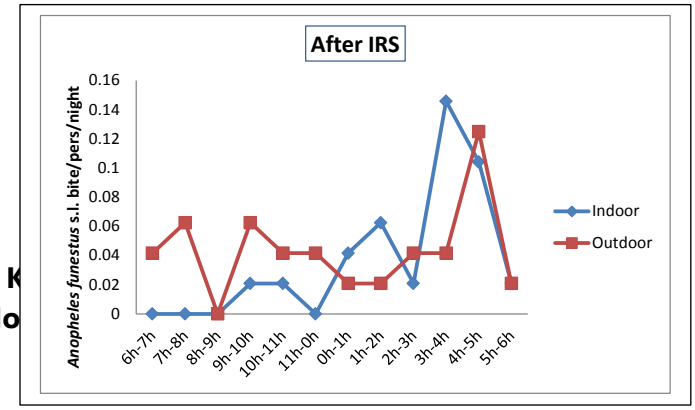



**Figure 20: *Anopheles funestus* s.l. feeding time in Chibobo sprayed site in Serenje district in August 2015 before IRS and from November to January 2015 after IRS**



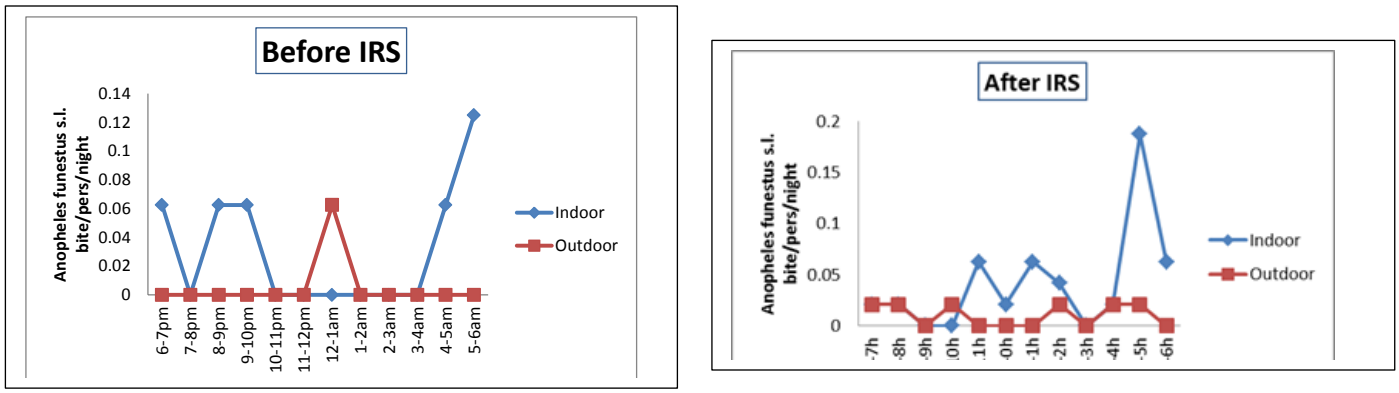
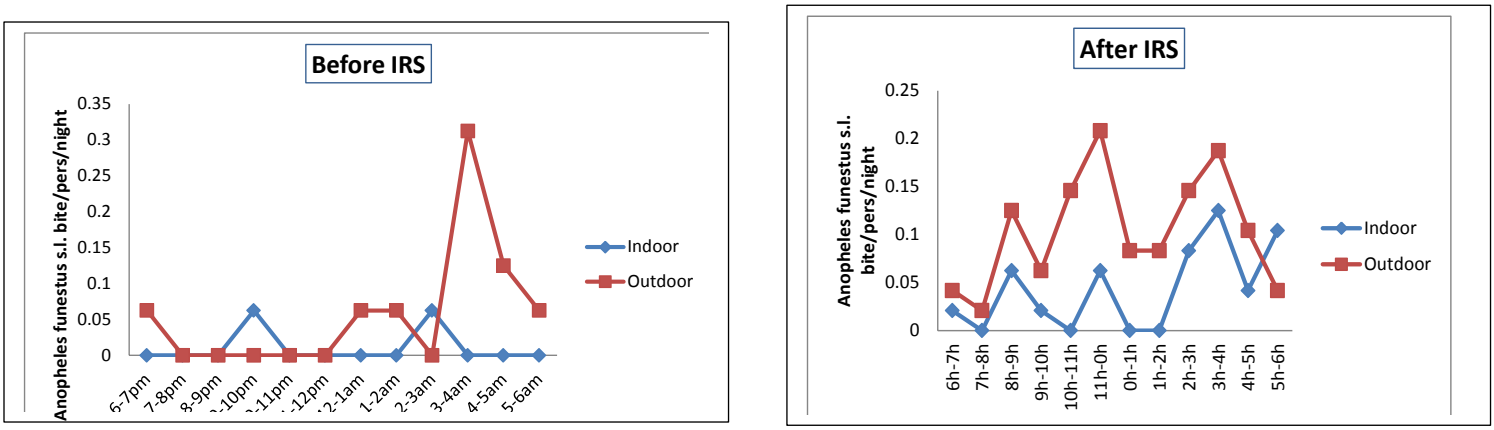



**Figure 22: *Anopheles funestus* s.l. feeding time in Nsalamba sprayed site in Isoka District in**

**August 2015 before and from November to January after IRS**




## 3.3 *ANOPHELES GAMBIAE* S.L. BITING RATE

*Anopheles gambiae* s.l. was not found biting in August one month before IRS except in Nsalamba in Isoka where it was found biting both indoors and outdoors. The biting rate in Shibesa was five *Anopheles gambiae* s.l. bites per person per night inside house in January three months after spraying. The highest biting rate of *Anopheles gambaie* s.l. (14 bites/person/night) inside was recorded in Nsalamba in Isoka District in January. *Anopheles gambiae* s.l. was not collected in Serenje during the study period. Tables 11, 12, 13, and 14 provide the HBR per district from August to January.

**Table 12: *Anopheles gambiae* s.l. biting rate in Milenge District in August and from November 2015 to January 2016**

| | | August | November | December | January |
|---|---|---|---|---|---|
| **Lunga (Intervention site)** | | | | | |
| **Indoor** | Total *Anopheles gambiae* s.l. | 0 | 0 | 0 | 11 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0** | **0.6875** |
| **Outdoor** | Total *Anopheles gambiae* s.l. | 0 | 0 | 1 | 8 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0.0625** | **0.5** |
| **Miyambo (Control)** | | | | | |
| **Indoor** | Total *Anopheles gambiae* s.l. | 0 | 0 | 2 | 7 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0.125** | **0.4375** |
| **Outdoor** | Total *Anopheles gambiae* s.l. | 0 | 0 | 0 | 11 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0** | **0.6875** |

**Table 13: *Anopheles gambiae* s.l. biting rate in Mwense District in August and from November 2015 to January 2016**

| | | August | November | December | January |
|---|---|---|---|---|---|
| **Shibesa (Intervention site)** | | | | | |
| Indoor | Total *Anopheles gambiae* s.l. | | 5 | 27 | 80 |
| | nb collectors | | 4 | 4 | 4 |
| | nb nights | | 4 | 4 | 4 |
| | HBR/night | | **0.3125** | **1.6875** | **5** |
| Outdoor | Total *Anopheles gambiae* s.l. | | 2 | 36 | 70 |
| | nb collectors | | 4 | 4 | 4 |
| | nb nights | | 4 | 4 | 4 |
| | HBR/night | | **0.125** | **2.25** | **4.375** |
| **Chebele (Control)** | | | | | |
| Indoor | Total *Anopheles gambiae* s.l. | | 70 | 52 | 151 |
| | nb collectors | | 4 | 4 | 4 |
| | nb nights | | 4 | 4 | 4 |
| | HBR/night | | **4.375** | **3.25** | **9.4375** |
| Outdoor | Total *Anopheles gambiae* s.l. | | 100 | 68 | 164 |
| | nb collectors | | 4 | 4 | 4 |
| | nb nights | | 4 | 4 | 4 |
| | HBR/night | | **6.25** | **4.25** | **10.25** |

**Table 14: *Anopheles gambiae* s.l. biting rate in Serenje District in August and from November 2015 to January 2016**

| | | August | November | December | January |
|---|---|---|---|---|---|
| **Chibobo (Intervention site)** | | | | | |
| Indoor | Total *Anopheles gambiae* s.l. | 0 | 0 | 0 | 0 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0** | **0** |

| | | | | | |
|---|---|---|---|---|---|
| Outdoor | Total *Anopheles gambiae* s.l. | 0 | 0 | 0 | 0 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0** | **0** |
| | **Chishi (Control)** | | | | |
| Indoor | Total *Anopheles gambiae* s.l. | 0 | 0 | 0 | 0 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0** | **0** |
| Outdoor | Total *Anopheles gambiae* s.l. | 0 | 0 | 0 | 0 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0** | **0** |

**Table 15: *Anopheles gambiae* s.l. biting rate in Kasama and Isoka districts in August and from November 2015 to January 2016**

| | | **August** | **November** | **December** | **January** |
|---|---|---|---|---|---|
| | **Kalonga (Intervention site)** | | | | |
| Indoor | Total *Anopheles gambiae* s.l. | 0 | 0 | 1 | 6 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0.0625** | **0.375** |
| Outdoor | Total *Anopheles gambiae* s.l. | 0 | 0 | 3 | 3 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0** | **0** | **0.1875** | **0.1875** |
| | **Nsalamba (Intervention site)** | | | | |
| Indoor | Total *Anopheles gambiae* s.l. | 5 | 15 | 8 | 222 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |
| | HBR/night | **0.3125** | **0.9375** | **0.5** | **13.875** |
| Outdoor | Total *Anopheles gambiae* s.l. | 2 | 18 | 44 | 332 |
| | nb collectors | 4 | 4 | 4 | 4 |
| | nb nights | 4 | 4 | 4 | 4 |

| | | | | |
|---|---|---|---|---|
| HBR/night | **0.125** | **1.125** | **2.75** | **20.75** |

## 3.4 *ANOPHELES GAMBIAE* S.L. BITING TIME

The peak biting period of the *Anopheles gambiae* s.l. was between 4:00 and 5:00 early in the morning inside in Lunga in Milenge after IRS. *Anopheles gambiae* s.l. started biting inside early in the evening between 7:00-8:00 pm and the peak was between 1:00 and 2:00 am in Shibesa in Mwense District. However, the biting rates are generally low and difficult to establish the trends for this species from the few collections of the human landing catches in most of the areas.

Figures 23, 24, 25, and 26 show the hourly biting rate of *Anopheles gambiae* s.l. per sprayed site after IRS.[1]

**Figure 23: *Anopheles gambiae* s.l. feeding time in Lunga (sprayed site) in Milenge**

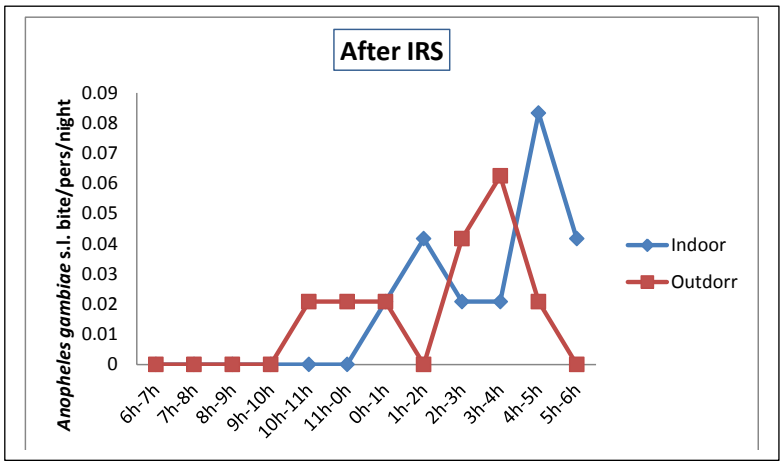



**Figure 24: *Anopheles gambiae* s.l. feeding time in Shibesa (sprayed site) in Mwense**

[1] *Anopheles gambiae* s.l. was not collected in Lunga in Milenge,in Kalonga, in Kasama and in Mbalani in Katete during the pre-spray period in August. Mosquitoe collection was also not performed in Shibesa in Mwense during the pre-spray period in August.

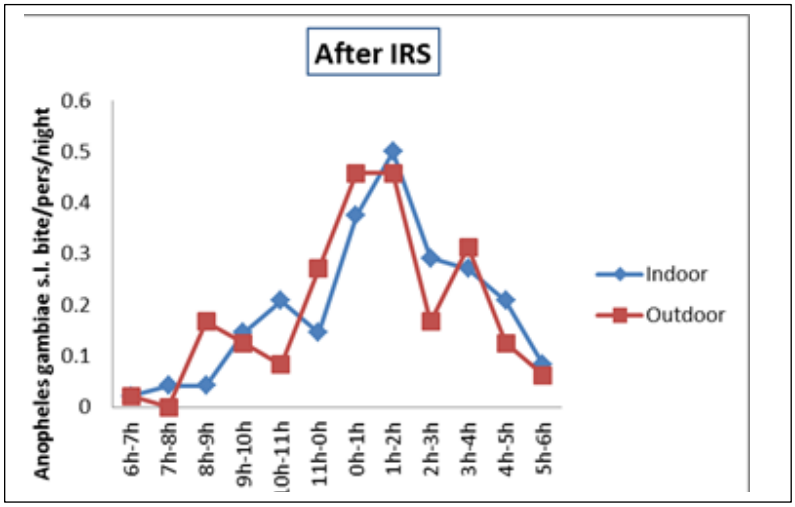



**Figure 25: *Anopheles gambiae* s.l. feeding time in Kalonga (sprayed site) in Kasama**

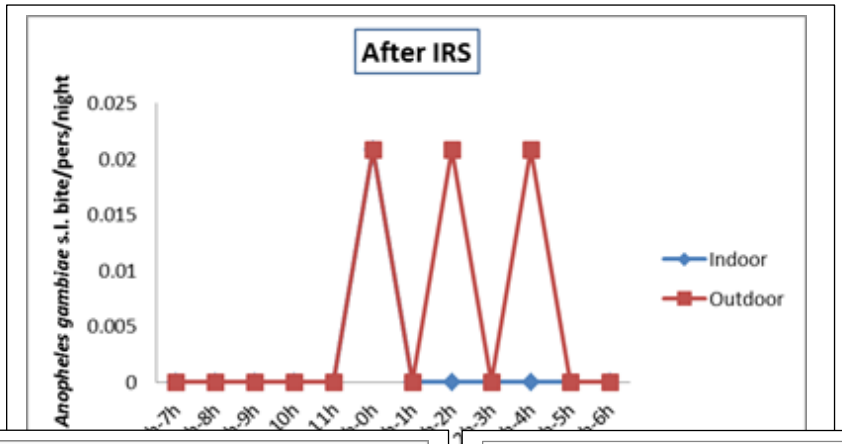
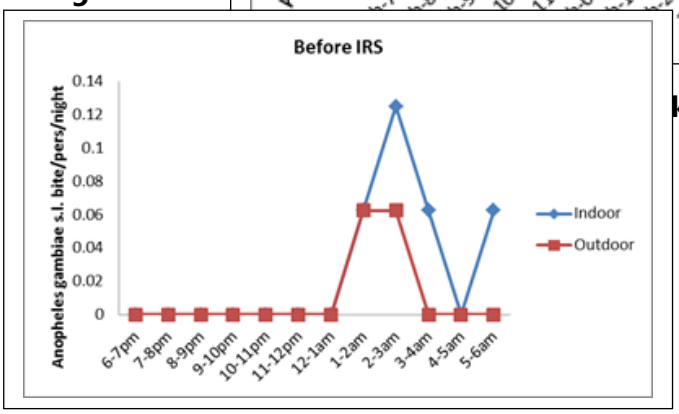
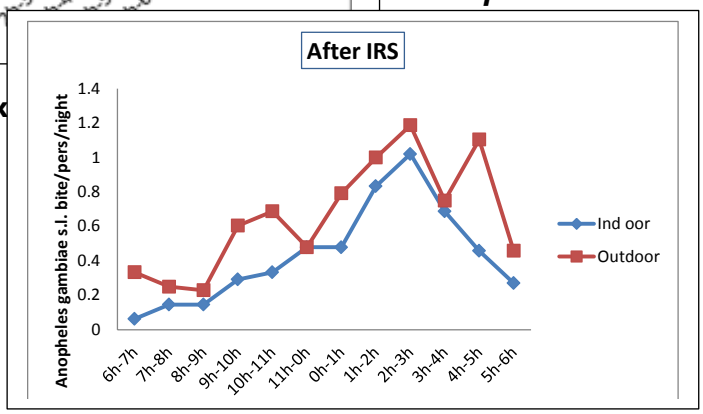



**Figure 26:** ***Anopheles***



## 3.5 PARITY RATE

The parity rate is similar both in sprayed and control sites. The overall parity rate for the IRS sites after IRS campaign was 45.5% (45/99) and 55.4% (243/439) for the control sites for *Anopheles funestus* s.l. and 54.3% (38/70) for IRS sites and 49.2% (31/63) for the control sites for *Anopheles gambiae* s.l. There is no statistically significant difference between the parity rate in the sprayed and control sites (p=0.07 for *Anopheles funestus* s.l. and p=0.55 for *Anopheles gambiae* s.l.), probably due to the low number of mosquitoes dissected for most of the sites.

**Table 16: *Anopheles funestus* s.l. parity rate in Milenge and Mwense districts**

| Time | Lunga | | | Miyambo | | | Schibesa | | | Chebele | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | # *An. funestus* s.l. dissected | Parous | % parous | # *An. funestus* s.l. dissected | Parous | % parous | # *An. funestus* s.l. dissected | Parous | % parous | # *An. funestus* s.l. dissected | Parous | % parous |
| **Pre-spray** | 4 | 2 | 50 | 51 | 41 | 80 | | | | | | |
| **November** | 5 | 3 | 60 | 28 | 14 | 50 | 2 | 0 | 0 | 11 | 7 | 64 |
| **December** | 62 | 30 | 48.4 | 113 | 72 | 64 | 4 | 2 | 50 | 73 | 28 | 38 |
| **January** | 2 | 2 | 100 | 196 | 109 | 56 | 3 | 1 | 33 | 0 | 0 | 0 |
| **Total** | **73** | **37** | 50.7 | **388** | **236** | **60.8** | **9** | **3** | **33** | **84** | **35** | 42 |

**Table 17: *Anopheles funestus* s.l. parity rate in Serenje and Katete districts**

| Time | Chibobo | | | Chishi | | | Mbalani | | | Robert | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | # *An. funestus* s.l. dissected | Parous | % parous | # *An. funestus* s.l. dissected | Parous | % parous | # *An. funestus* s.l. dissected | Parous | % parous | # *An. funestus* s.l. dissected | Parous | % parous |
| **Pre-spray** | 0 | 0 | 0 | 5 | 3 | 60 | 0 | 0 | 0 | 0 | 0 | |
| **November** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **December** | 3 | 2 | 66.7 | 18 | 13 | 72 | 0 | 0 | 0 | 0 | 0 | 0 |
| **January** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **3** | **2** | 66.7 | **23** | **16** | **69.6** | **0** | **0** | 0 | **0** | **0** | 0 |

**Table 18: *Anopheles funestus* s.l. parity rate in Kasama and Isoka districts**

| Time | Nsalamba | | | Kalonga | | |
|---|---|---|---|---|---|---|
| | # *An.* | Parous | % parous | # *An.* | Parous | % parous |

| | *funestus* s.l. dissected | | | *funestus* s.l. dissected | | |
|---|---|---|---|---|---|---|
| **Pre-spray** | 3 | 1 | 33 | 0 | 0 | 0 |
| **November** | 6 | 1 | 17 | 0 | 0 | 0 |
| **December** | 3 | 2 | 66.7 | 0 | 0 | 0 |
| **January** | 3 | 1 | 0 | 6 | 1 | 17 |
| **Total** | **15** | **5** | 33.3 | **6** | **1** | **16.7** |

## Table 19: *Anopheles gambiae* s.l. parity rate in Milenge and Mwense districts

| Time | Lunga | | | Miyambo | | | Schibesa | | | Chebele | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | # *An. gambiae* s.l. dissected | Parous | % parous | # *An. gambiae* s.l. dissected | Parous | % parous | # *An. gambiae* s.l. dissected | Parous | % parous | # *An. gambiae* s.l. dissected | Parous | % parous |
| **Pre-spray** | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | |
| **November** | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 33 | 1 | 0 | 0 |
| **December** | 0 | 0 | 0.0 | 0 | 0 | 0 | 7 | 2 | 29 | 42 | 22 | 52 |
| **January** | 2 | 2 | 100 | 4 | 4 | 100 | 17 | 9 | 53 | 16 | 5 | 31 |
| **Total** | **2** | **2** | 100.0 | **4** | **4** | **100.0** | **27** | **12** | **44** | **59** | **27** | 46 |

## Table 20: *Anopheles gambiae* s.l. parity rate in Serenje and Katete districts

| Time | Chibobo | | | Chishi | | | Mbalani | | | Robert | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | # *An. gambiae* s.l. dissected | Parous | % parous | # *An. gambiae* s.l. dissected | Parous | % parous | # *An. gambiae* s.l. dissected | Parous | % parous | # *An. gambiae* s.l. dissected | Parous | % parous |
| **Pre-spray** | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| **November** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **December** | 0 | 0 | 0.0 | 0 | 0 | 0 | | | | | | 0 |
| **January** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **0** | **0** | 0.0 | **5** | **0** | **0.0** | **0** | **0** | 0 | **0** | **0** | 0 |

## Table 21: *Anopheles gambiae* s.l. parity rate in Kasama and Isoka districts

| Time | Nsalamba | | | Kalonga | | |
|---|---|---|---|---|---|---|
| | # *An. gambiae* s.l. dissected | Parous | % parous | # *An. gambiae* s.l. dissected | Parous | % parous |
| **Pre-spray** | 11 | 3 | 27 | 0 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **November** | 4 | 1 | 25 | 0 | 0 | 0 |
| **December** | 23 | 18 | 78.3 | 2 | 2 | 0 |
| **January** | 11 | 3 | 0 | 1 | 0 | 0 |
| **Total** | **49** | **25** | 51.0 | **3** | **2** | **66.7** |

## 3.6 QUALITY ASSURANCE OF IRS AND INSECTICIDE DECAY RATE

The initial cone bioassay to determine the quality of spraying was carried out in six districts. The wall bioassay was conducted 24 hours after spraying in 40 randomly selected houses. The Kisumu strain of *Anopheles gambiae* s.s. available in the insectary in October was not enough to cover all six sentinel sites for the T0 cone bioassay tests due to the ant invasion and destruction of some mosquitoes in the insectary in July-August.

*Anopheles funestus* s.l. was collected using backpack aspirators from Milenge and Mwense districts. Previous surveys showed that *An. funestus* is most abundant in Milenge and Mwense districts. The WHO susceptibility test was done against pirimiphos-methyl CS (insecticide used for spraying) and showed 100% mortality. This known susceptible *Anopheles funestus* s.l. strain was used for the cone bioassay in Milenge, Mwense, and Serenje districts. Susceptible Kisumu strain (*Anopheles gambiae* s.s.) reared in the insectary at the NMCC was used for the cone bioassay tests in the remaining three districts, namely Katete, Isoka, and Kasama. The two most commonly found surface types in the areas, cement and mud walls, were tested.

The cone bioassay tests were done according to the WHO test procedure. During the tests, the number of mosquitoes knocked down after 30 minutes and dead after 24 hours were recorded. All the mosquitoes exposed were dead after the 24h holding period. The 100% mortality rate was noted at all sites at T0 and T1, respectively, 24 hours and one month after spraying, showing that the spraying was of good quality and pirimiphos-methyl CS was still effective one month after spray. Subsequent testing was done in December, January, February, March and April respectively, two, three, four, five and six months after the IRS campaign to determine the decay rate of insecticide applied on the walls. The mortality rate was 100% for both mud and cement sprayed walls except in Kasama District where the mortality rate in December was 96% for the mud and 98% for the cement sprayed walls.

In December 2015, two months after the IRS campaign, the insecticide applied continued to be effective and the WHO minimum threshold of effectiveness was met for both mud and cement walls. Less than 80% of susceptible mosquitoes exposed to the sprayed cement in Milenge (63%) and to the sprayed mud in Serenje (71%) were killed in January three months after the spraying. Pirimiphos-methyl was effective on both mud and cement in Kasama, Isoka, Katete, and Mwense in February four months after spraying. The test mortality rate was less than the 80% WHO threshold on the mud sprayed walls in Milenge and on the cement in Serenje. The residual life of pirimiphos-methyl in these two districts is short and needs to be investigated. Pirimiphos-methyl is still effective on both mud and cement sprayed walls five months after spraying in Mwense and Isoka districts. However the mortality rate was below 80% for mud sprayed walls in Kasama and Katete five months after spraying.

Figures 27 and 28 show the mortality rate at T0 (24 hours after spraying), at T1 (one month after in November), at T2 (two months after spraying in December), at T3 (three months after in January) at T4 (four months after spraying in February 2016), at T5 (five months after spraying in March 2016) and at T6 (Six months after spraying in April 2016).

**Figure 27: Mortality of Kisumu susceptible strain of *An. gambiae* s.s. after 30 mins exposure to pirimiphos-methyl CS and 24H holding period at T0, T1, T2, T3, T4, T5 and T6 in Kasama, Isoka, and Katete**

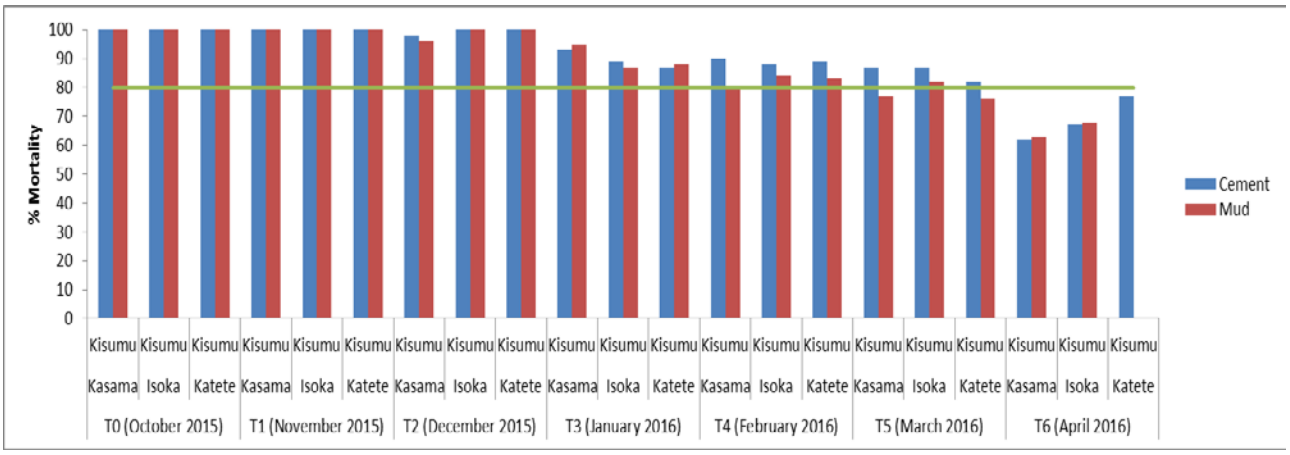
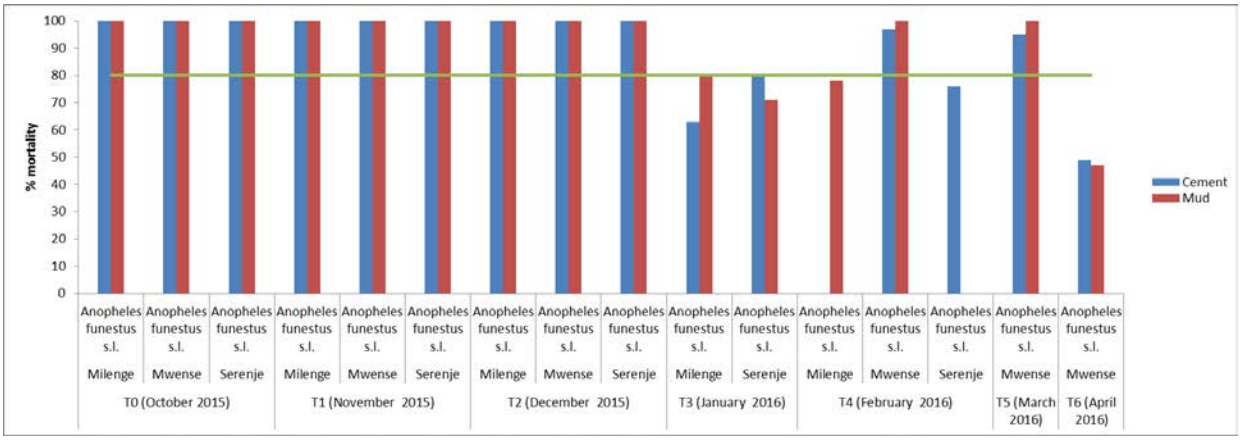



**Figure 28: Mortality of *Anopheles funestus* s.l. susceptible strain after 30 mins exposure to pirimiphos-methyl CS and 24H holding period at T0, T1, T2, T3 ,T4, T5 and T6**




## 3.7 MALARIA VECTOR SUSCEPTIBILITY TESTS

### WHO tube test

*An. funestus* s.l. and *Anopheles gambiae* s.l. were fully susceptible to pirimiphos-methyl. A 100% mortality rate was recorded at all sites in 2015 and 2016.*Anopheles funestus* s.l was resistant to deltamethrin at all sites but fully susceptible to Bendiocarb in Kalonga in Kasama and Chebele in Mwense districts. *Anopheles funestus* s.l. is resistant to Bendiocarb in Nandola in Kasama and in

Lunga and Miyambo in Milenge and  in Chibobo in Serenje districts.  *Anopheles funestus s.l.* is fully susceptible to DDT except in Mwemamusongo in Milenge.

*Anopheles gambiae* s.l. was fully susceptible to deltamethrin in Mbalani in Katete, in Chebele in Mwense, and in Lunga in Milenge and resistant in shibesa in Mwense and Robert in Katete. *Anopheles gambiae* s.l. is fully susceptible to Bendiocarb at all sites but resistant to DDT in chebele in Mwense and in Lunga in Milenge.

**Figure 29: Susceptibility status of *Anopheles funestus* s.l.**

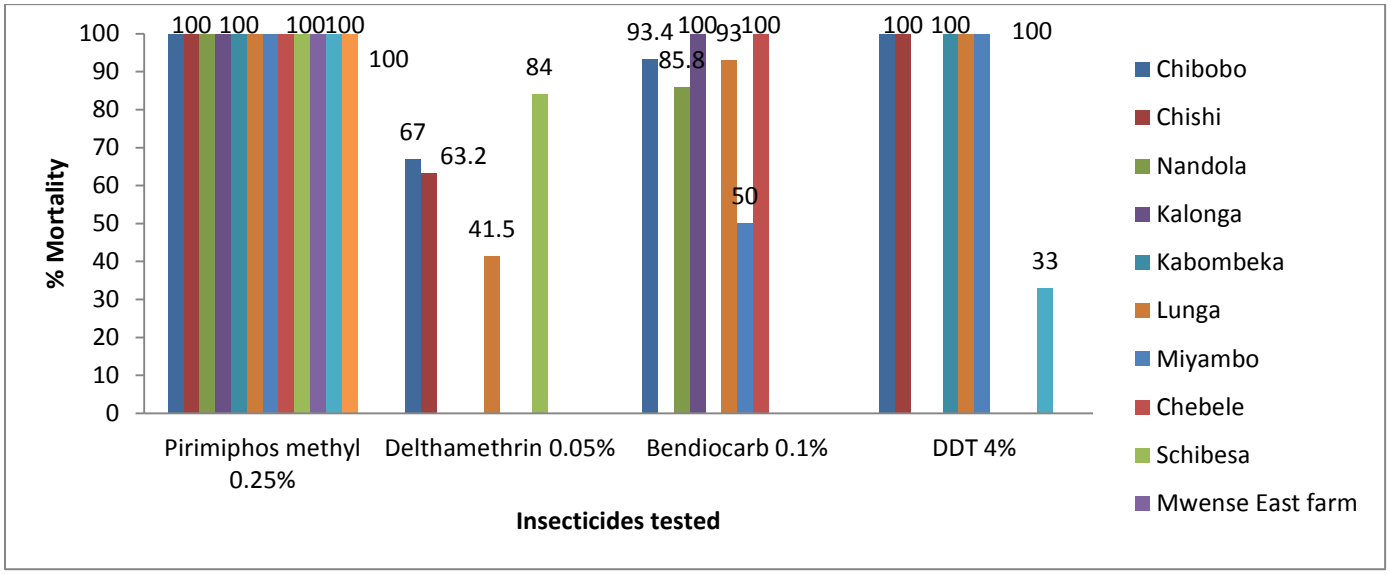
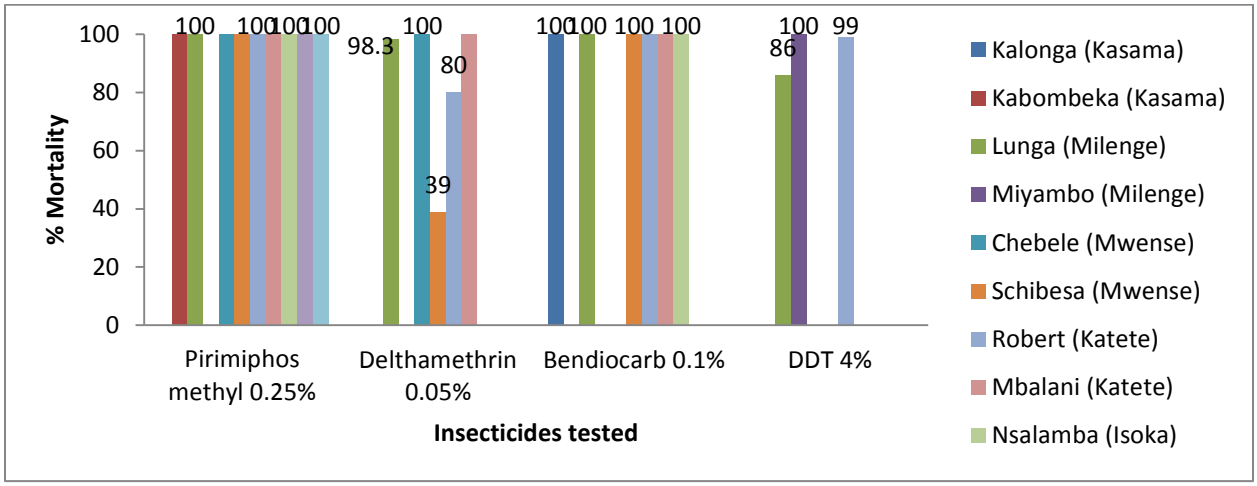



**Figure 30 : Susceptibility status of *Anopheles gambiae* s.l.**



## Insecticide resistance intensity assays

The Rapid Resistance Intensity Diagnostic test (I-RDT) was performed in September and November with the adult female *Anopheles funestus* s.s. collected from seven villages in Milenge, two villages in Samfiya, and three villages in Mwense District. Additional tests were run from March to May 2016.

High intensity of resistance to pyrethroid was observed except for permethrin. *Anopheles funestus* s.s. collected from Fumpa Kalusa and Yatema was fully susceptible to five and ten times the diagnostic dose of permethrin. *Anopheles funestus* s.s. was resistant to deltamethrin at all selected sites in Milenge and Samfyia districts. No difference was observed between the sites in the intensity of deltamethrin resistance except in Shitambulli where 44% of the *Anopheles funestus* s.s. survived to ten times the diagnostic dose of deltamethrin. . 21% of *Anopheles funestus* s.s. from Miyambo exposed to five times the diagnostic dose of deltamethrin survived and 18% survived to 10 times the diagnostic dose. A difference was observed between the sites in the intensity of resistance for permethrin, (100%) of the *Anopheles funestus* s.s. exposed to five and ten times the diagnostic dose of Permethrin died in Fumpa Kalusa and Yatema. For Pwele village, 26% of the mosquitoes exposed survived to 10 times the diagnostic dose of deltamethrin. In addition, 14% of *Anopheles funestus* s.s. from Fumpa Kulusa survived to 10 times the diagnostic dose of deltamethrin.

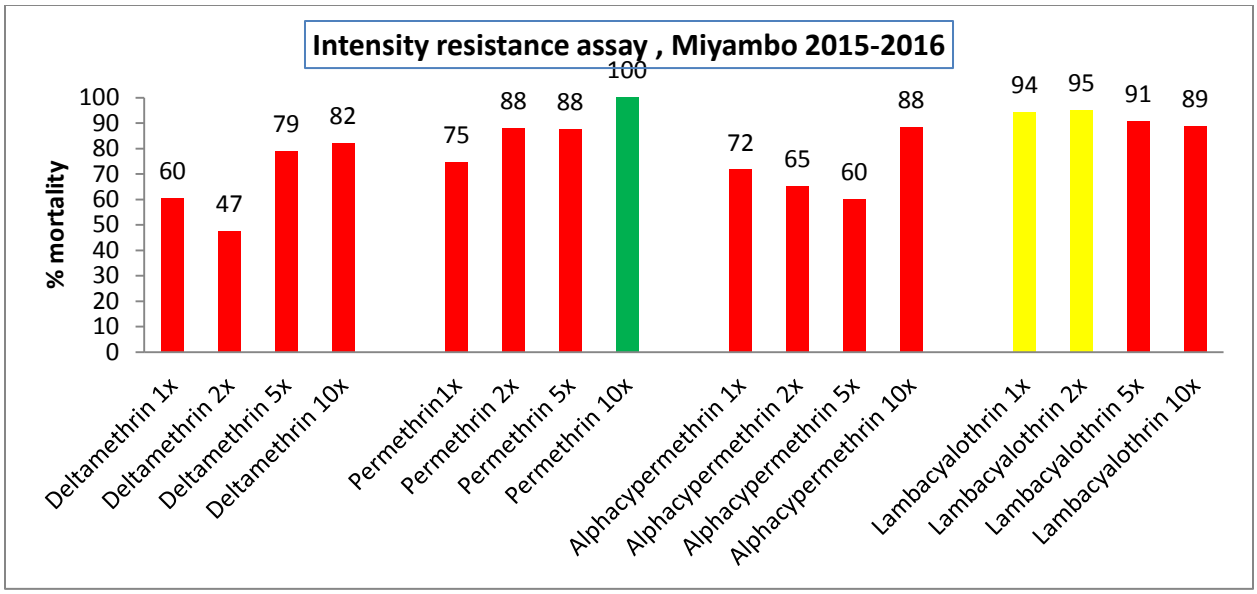
High intensity resistance is suspected in all the sites except in Shitambulli where the intensity of resistance seems moderate for deltamethrin.

Intensity resistance assay was performed in January for *Anopheles gambiae* s.l. but due to the heavy rain observed in January, few *Anopheles gambiae* were collected. Additional tests will be run during the next rainy season

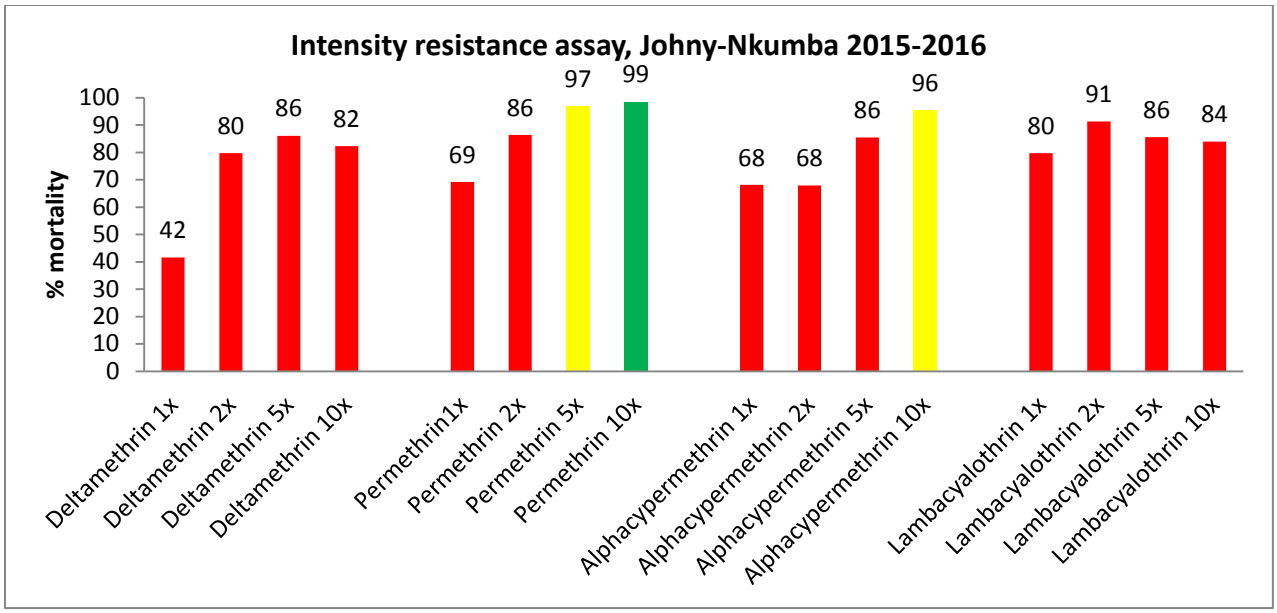
Figures 31 and 32 indicate the mortality rate per insecticide.

**Figure 31: Intensity resistance assay in Milenge district against *An. funestus* s.l.**

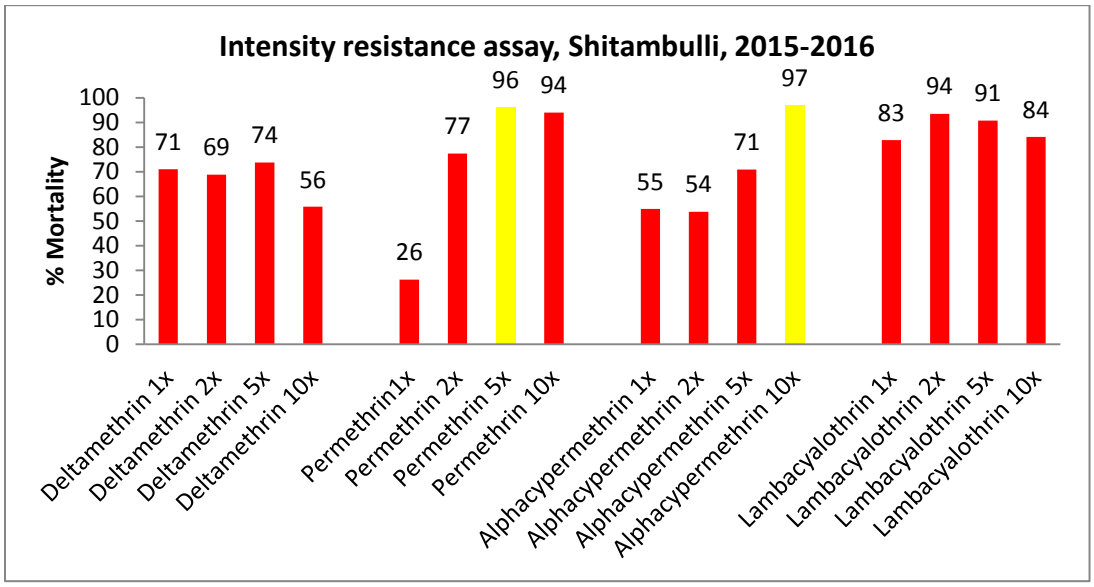
a)


Intensity resistance assay , Miyambo 2015-2016
% mortality
100
90
80
70
60
50
40
30
20
10
0
60
47
79
82
75
88
88
100
72
65
60
88
94
95
91
89
Deltamethrin 1x
Deltamethrin 2x
Deltamethrin 5x
Deltamethrin 10x
Permethrin1x
Permethrin 2x
Permethrin 5x
Permethrin 10x
Alphacypermethrin 1x
Alphacypermethrin 2x
Alphacypermethrin 5x
Alphacypermethrin 10x
Lambacyalothrin 1x
Lambacyalothrin 2x
Lambacyalothrin 5x
Lambacyalothrin 10x

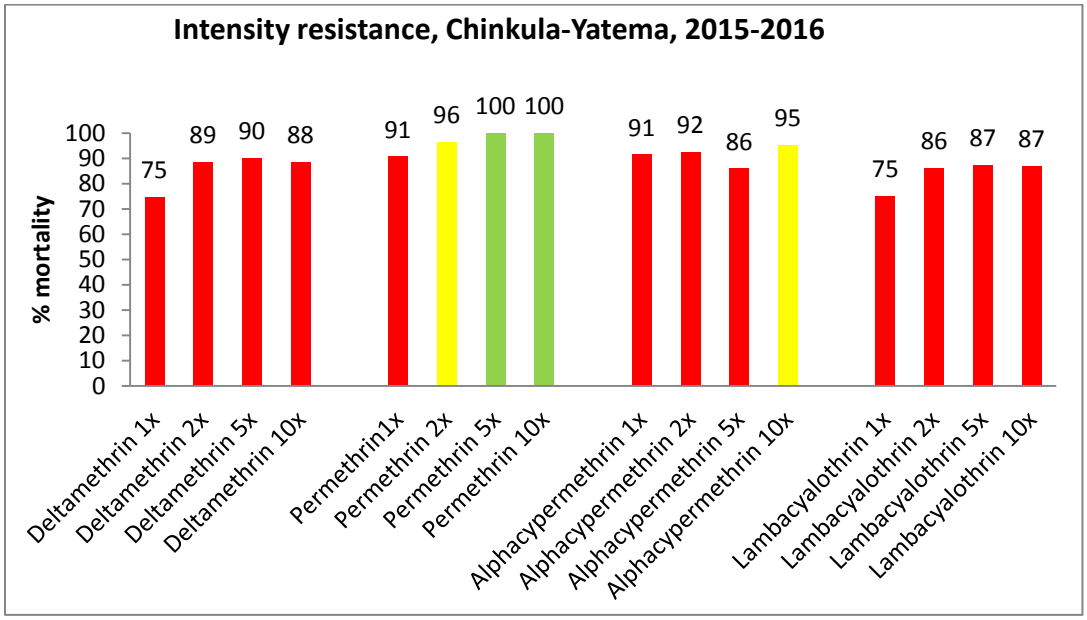
b)


Intensity resistance assay, Johny-Nkumba 2015-2016
% mortality
100
90
80
70
60
50
40
30
20
10
0
42
80
86
82
69
86
97
99
68
68
86
96
80
91
86
84
Deltamethrin 1x
Deltamethrin 2x
Deltamethrin 5x
Deltamethrin 10x
Permethrin1x
Permethrin 2x
Permethrin 5x
Permethrin 10x
Alphacypermethrin 1x
Alphacypermethrin 2x
Alphacypermethrin 5x
Alphacypermethrin 10x
Lambacyalothrin 1x
Lambacyalothrin 2x
Lambacyalothrin 5x
Lambacyalothrin 10x

c)


Intensity resistance assay, Shitambulli, 2015-2016
% Mortality
100
90
80
70
60
50
40
30
20
10
0
71
69
74
56
26
77
96
94
55
54
71
97
83
94
91
84
Deltamethrin 1x
Deltamethrin 2x
Deltamethrin 5x
Deltamethrin 10x
Permethrin1x
Permethrin 2x
Permethrin 5x
Permethrin 10x
Alphacypermethrin 1x
Alphacypermethrin 2x
Alphacypermethrin 5x
Alphacypermethrin 10x
Lambacyalothrin 1x
Lambacyalothrin 2x
Lambacyalothrin 5x
Lambacyalothrin 10x

d)


Intensity resistance, Chinkula-Yatema, 2015-2016
% mortality
100
90
80
70
60
50
40
30
20
10
0
75
89
90
88
91
96
100
100
91
92
86
95
75
86
87
87
Deltamethrin 1x
Deltamethrin 2x
Deltamethrin 5x
Deltamethrin 10x
Permethrin1x
Permethrin 2x
Permethrin 5x
Permethrin 10x
Alphacypermethrin 1x
Alphacypermethrin 2x
Alphacypermethrin 5x
Alphacypermethrin 10x
Lambacyalothrin 1x
Lambacyalothrin 2x
Lambacyalothrin 5x
Lambacyalothrin 10x

e)

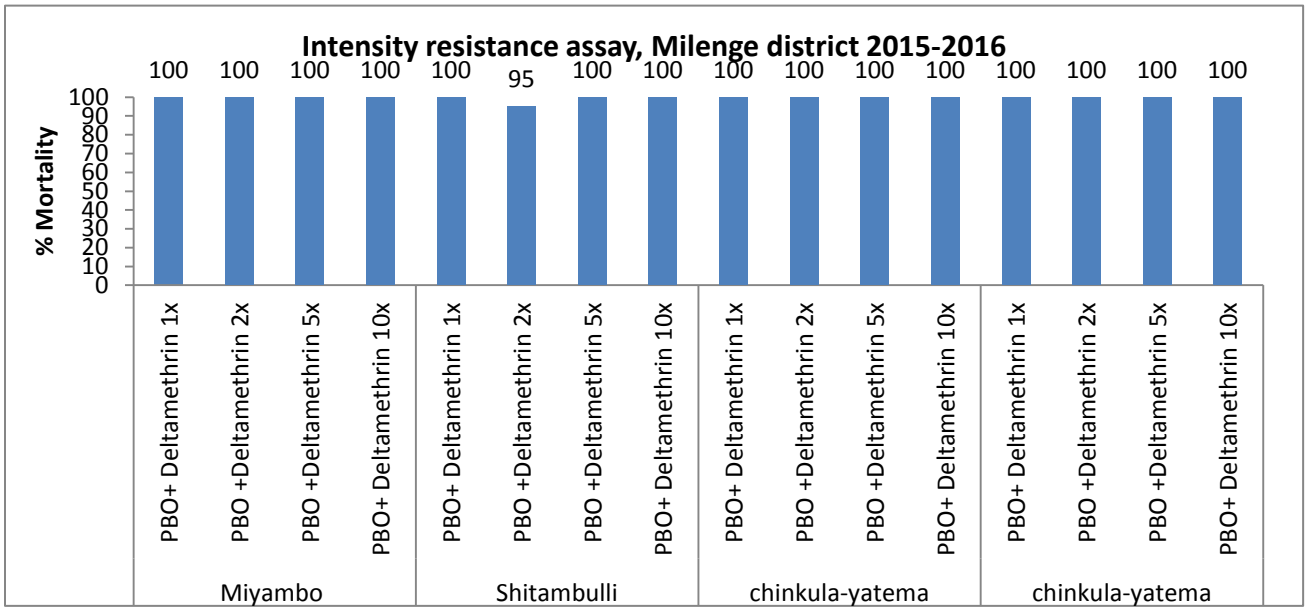
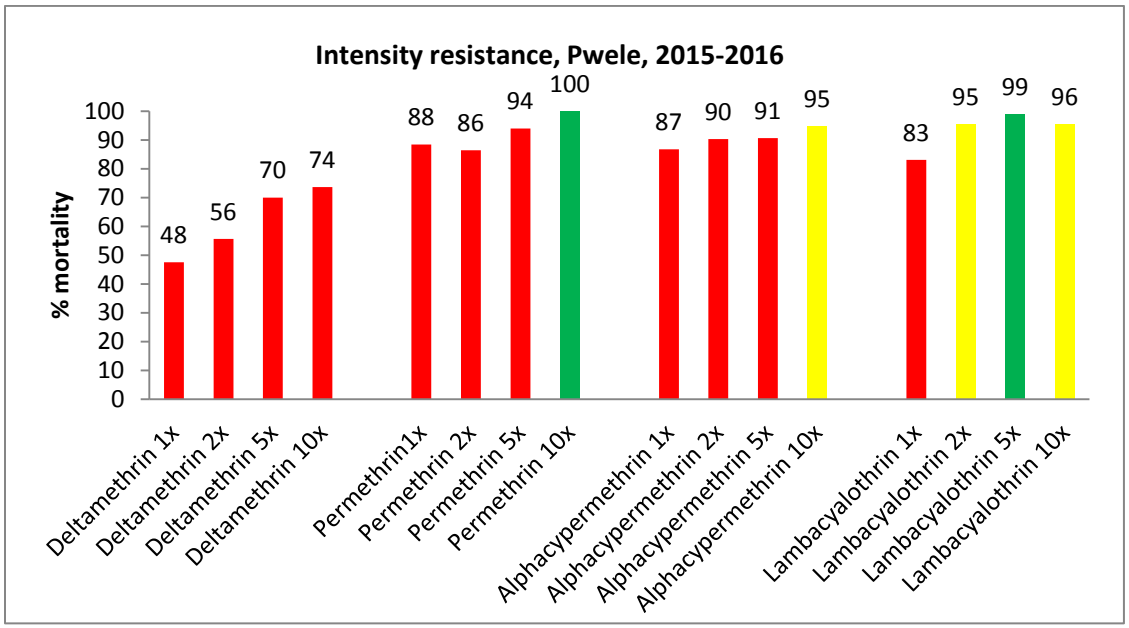
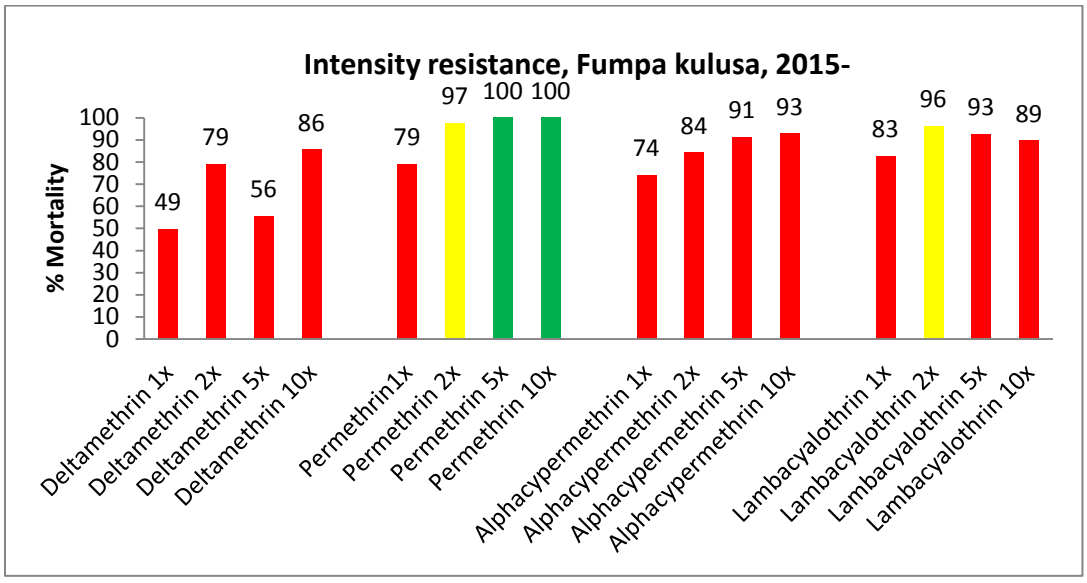

Intensity resistance assay, Milenge district 2015-2016
% Mortality
100
90
80
70
60
50
40
30
20
10
0
100
100
100
100
100
95
100
100
100
100
100
100
100
100
100
100
100
PBO+ Deltamethrin 1x
PBO +Deltamethrin 2x
PBO +Deltamethrin 5x
PBO+ Deltamethrin 10x
PBO+ Deltamethrin 1x
PBO +Deltamethrin 2x
PBO +Deltamethrin 5x
PBO+ Deltamethrin 10x
PBO+ Deltamethrin 1x
PBO +Deltamethrin 2x
PBO +Deltamethrin 5x
PBO+ Deltamethrin 10x
PBO+ Deltamethrin 1x
PBO +Deltamethrin 2x
PBO +Deltamethrin 5x
PBO+ Deltamethrin 10x
Miyambo
Shitambulli
chinkula-yatema
chinkula-yatema

**Figure 32: Intensity resistance assay in Samfyia District**

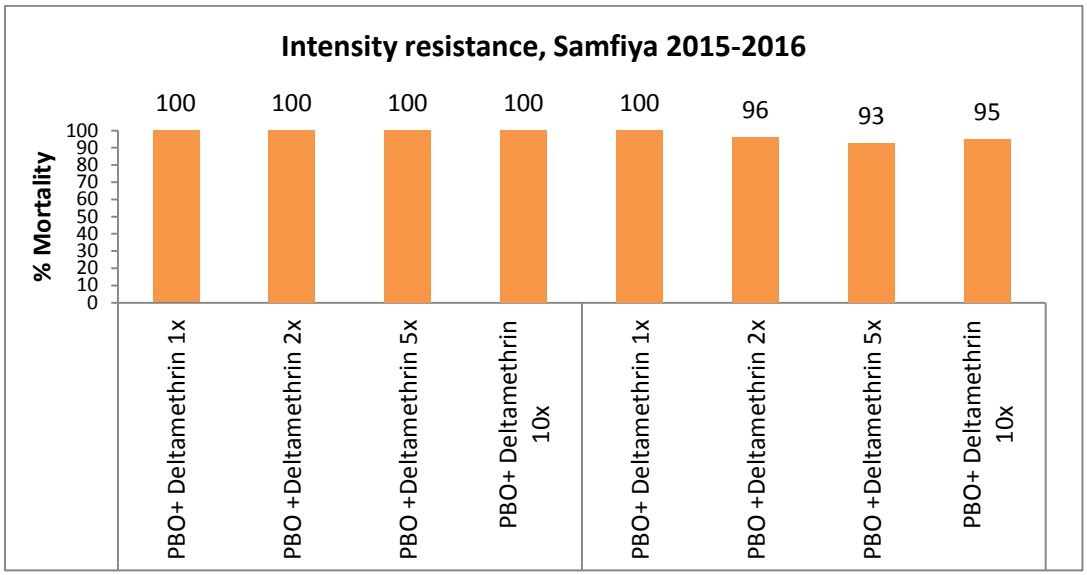
a)




b)



c)


Intensity resistance, Samfiya 2015-2016
% Mortality
100
90
80
70
60
50
40
30
20
10
0
100
100
100
100
100
96
93
95
PBO+ Deltamethrin 1x
PBO +Deltamethrin 2x
PBO +Deltamethrin 5x
PBO+ Deltamethrin 10x
PBO+ Deltamethrin 1x
PBO +Deltamethrin 2x
PBO +Deltamethrin 5x
PBO+ Deltamethrin 10x

# 4. Training on the Disease Data Management System (DDMS)

The AIRS Zambia team, including the technical manager, the insectary technician, one field entomologist, the M&E manager, the database manager, and the IT specialist were trained from December 7 -11, 2015. AIRS Zambia started entering the data in the database but unfortunately is facing technical issues accessing to the DDMS using the office internet. The concern was shared with the DDMS focal person and the AIRS Zambia IT specialist.

# 5. Country Capacity Building

AIRS Zambia has discussed and agreed with the National Malaria Control Program to establish new sentinel sites across the three epidemiological zones to create a representative sampling of geographical focus that will provide adequate entomological findings to inform planning for elimination interventions and strategies designed by the National Malaria Control Program and its partners.

Under ZISSP, 17 entomological kits were purchased, including microscope, backpack aspirators, larvae collections kits, and others entomology equipment. This equipment was sent to some districts but not used. AIRS Zambia assisted in retrieving these entomological surveillance field kits, which will be redistributed to the newly established sentinel sites, including the six PMI AIRS entomology sentinel districts.

Three Environmental Health Technicians were trained in 2015 by AIRS for entomology surveillance. Only one is involved in the entomology surveillance. Twelve technicians (two per sentinel site) were trained from March 2-4, 2016, on the mosquitoes sampling, the *Anopheles* species morphological identification using Gillies and M Coetzee, and 1987 identification key and ovary dissection. Theses trained staff will support the entomology surveillance at the district level.

# 6. CONCLUSION

The cone bioassay test conducted at T0, 24h and at T1, one month after spraying, showed 100% mortality of the mosquitoes exposed. The residual life of pirimiphos-methyl was less than six months at all sites. The vector density and the average HBR significantly decreased one and two months after spraying in the intervention sites compared to the control site and the baseline. An increase of the HBR was observed in January three months after the IRS campaign.

*An. gambiae* s.l. and *Anopheles funestus* s.l. are fully susceptible to pirimiphos-methyl. *Anopheles funestus* s.l. is resistant to deltamethrin at all sites; fully susceptible to Bendiocarb in Kalonga in Kasama and Chebele in Mwense. *Anopheles funestus* s.l. is resistant to Bendiocarb in Nandola in Kasama, in Lunga and Miyambo in Milenge, in Chibobo in Serenje. *Anopheles funestus* s.l. are fully susceptible to DDT except in Miyambo in Milenge. *Anopheles gambiae* s.l. was fully susceptible to deltamethrin in Mbalani in Katete, in Chebele in Mwense, and in Lunga in Milenge and resistant in Shibesa in Mwense and Robert in Katete. *Anopheles gambiae* s.l. is fully susceptible to Bendiocarb at all sites but resistant to DDT in chebele in Mwense and in Lunga in Milenge.

The resistance intensity assay shows that *Anopheles funestus* s.l. was resistant to all the four pyrethroids tested at all selected sites in Milenge and Samfiya districts. No difference was observed between the sites in the intensity of deltamethrin resistance in the area except in Shitambulli. However a difference was observed between the sites in the intensity of permethrin resistance.

The parity rate is similar in both sprayed and control sites. There is no statistically significant difference between the parity in the sprayed and control sites. The parity is high at most of the sites.

# 7. RECOMMENDATIONS

- Anopheles gambiae Kisumu strain should be used for the cone bioassay for the upcoming IRS campaign.
- In order to maximize our impact on entomological indicators, the focus for 2016 IRS season will be to continue to improve the quality of spraying by implementing DOS (directly observed spraying) by Team Leaders during the campaign.
- Adjust timing of spraying to start in November in the Eastern Province so it's in line with the peak of Anopheles funestus s.l. and the decay of pirimiphos methyl.

# ANNEX

**Table A:** Culicidae collected by PSC and CDC light trap in August before IRS campaign

| Districts | Villages | Status | CDC light trap | | | | | | Pyrethrum Spray Catch | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *An.funestus s.l.* | *An.gambiae s.l.* | *An. tenebrosus* | *An. tchekedi* | An. squamosus | *Culicinae* | *An. funestus s.l.* | *An .gambiae s.l.* | Culicinae |
| | | | n | n | n | n | n | n | n | n | n |
| Kasama | Kalonga | Sprayed | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Katete | Mbalani | Sprayed | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 27 |
| | Robert | Control | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Isoka | Nsalamba | Sprayed | 1 | 2 | | 1 | 0 | 16 | 4 | 0 | 10 |
| Mwense | Shibesa | Sprayed | 233 | 3 | 0 | 0 | 0 | 7 | 58 | 0 | 4 |
| | Chebele | Control | 66 | 2 | 0 | 0 | 0 | 11 | 17 | 1 | 6 |
| Milenge | Lunga | Sprayed | 213 | 0 | 161 | 46 | 0 | 1 | 224 | 0 | 0 |
| | Niyambo | Control | 378 | 0 | 18 | 8 | 1 | 6 | 74 | 0 | 0 |
| Serenje | chibobo | Sprayed | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Chichi | Control | 4 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **Total** | | | **897** | **7** | **184** | **55** | **1** | **54** | **378** | **1** | **50** |

**Table B:** Culicidae collected by Human Landing Catch in August 2015 before IRS campaign

| Districts | Villages | Status | HLC | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Indoor | | | | | | Outdoor | | | | | |
| | | | *An. funestus* s.l. | *An.gambiae* s.l. | *An. tenebrosus* | *An. tchekedii* | *An. squamosus* | *culicine* | *An. funestus* s.l. | *An.gambiae* s.l. | *An. tenebrosus* | *An. tchekedii* | *An. squamosus* | *Culicine* |
| | | | n | n | N | n | n | n | n | n | n | n | n | n |
| Kasama | Kalonga | Sprayed | 6 | 0 | 7 | 0 | 0 | 133 | 1 | 0 | 0 | 1 | 0 | 55 |
| Katete | Mbalani | Sprayed | 0 | 0 | 0 | 0 | 0 | 206 | 0 | 0 | 0 | 0 | 0 | 166 |
| | Robert | Control | 1 | 0 | 12 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 |
| Isoka | Nsalamba | Sprayed | 2 | 5 | 3 | 2 | 5 | 3 | 11 | 2 | 3 | 6 | 19 | 6 |
| Mwense | Shibesa | Sprayed | | | | | | | | | | | | |
| | Chebele | Control | | | | | | | | | | | | |
| Milenge | Lunga | Sprayed | 220 | 0 | 330 | 20 | 6 | 8 | 93 | 0 | 760 | 28 | 23 | 34 |
| | Niyambo | Control | 497 | 0 | 170 | 12 | 5 | 3 | 202 | 0 | 372 | 29 | 7 | 5 |
| Serenje | chibobo | Sprayed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Chichi | Control | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 |
| **Total** | | | **731** | **5** | **522** | **34** | **16** | **355** | **310** | **2** | **1136** | **64** | **50** | **266** |

**Table C:** Culicidae collected by PSC and CDC light trap in November 2015

| Districts | Villages | Status | CDC light trap | | | | | | Pyrethrum Spray Catch | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *An.funestus s.l.* | *An.gambiae s.l.* | An. tenebrosus | *An. tchekedi* | *An. squamosus* | *Culicinae* | *An. funestus* s.l. | *An .gambiae* s.l. | Culicinae |
| | | | n | N | n | n | n | n | n | n | n |
| Kasama | Kalonga | Sprayed | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 15 |
| Katete | Mbalani | Sprayed | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 8 |
| | Robert | Control | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 83 |
| Isoka | Nsalamba | Sprayed | 1 | 11 | 0 | 6 | 4 | 12 | 2 | 1 | 18 |
| Mwense | Shibesa | Sprayed | 16 | 1 | 0 | 0 | 0 | 3 | 1 | 0 | 0 |
| | Chebele | Control | 610 | 6 | 0 | 0 | 0 | 0 | 89 | 0 | 0 |
| Milenge | Lunga | Sprayed | 49 | 0 | 1 | 1 | 0 | 12 | 393 | 0 | 9 |
| | Niyambo | Control | 165 | 0 | 0 | 0 | 0 | 2 | 568 | 0 | 3 |
| Serenje | chibobo | Sprayed | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 |
| | Chichi | Control | 3 | 0 | 0 | 0 | 0 | 0 | 28 | 0 | 0 |
| **Total** | | | **844** | **18** | **1** | **7** | **4** | **69** | **1121** | **1** | **136** |

**Table D:** Culicidae collected by Human Landing Catch in November 2015

| Districts | Villages | Status | HLC | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Indoor | | | | | | | | Outdoor | | | | | | | |
| | | | *An. funestus s.l.* | *An.gambiae s.l.* | *An. tenebrosus* | *An. tchekedii* | *An. squamosus* | *An. Coustani* | *An. rufipes* | *Culicine* | *An. funestus s.l.* | *An.gambiae s.l.* | *An. tenebrosus* | *An. tchekedii* | *An. squamosus* | *An. coustani* | *An. rufipes* | *Culicine* |
| | | | n | n | n | N | n | n | n | n | | n | n | n | n | n | n | n |
| Kasama | Kalonga | Sprayed | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 8 | 1 | 0 | 9 | 1 | 2 | 0 | 0 | 30 |
| Katete | Mbalani | Sprayed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| | Robert | Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| Isoka | Nsalamba | Sprayed | 1 | 15 | 0 | 8 | 1 | 0 | 0 | 13 | 13 | 18 | 0 | 20 | 28 | 0 | 1 | 160 |
| Mwense | Shibesa | Sprayed | 4 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 22 |
| | Chebele | Control | 960 | 70 | 2 | 0 | 0 | 1 | 0 | 4 | 510 | 100 | 9 | 0 | 1 | 22 | 0 | 29 |
| Milenge | Lunga | Sprayed | 36 | 0 | 96 | 42 | 0 | 0 | 0 | 31 | 17 | 0 | 102 | 67 | 0 | 0 | 0 | 69 |
| | Niyambo | Control | 260 | 0 | 29 | 7 | 0 | 0 | 0 | 21 | 91 | 0 | 86 | 8 | 0 | 0 | 0 | 82 |
| Serenje | chibobo | Sprayed | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Chichi | Control | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| **Total** | | | **1265** | **90** | **127** | **58** | **2** | **1** | **1** | **117** | **634** | **120** | **206** | **96** | **31** | **22** | **2** | **412** |

**Table E**: Culicidae collected by PSC and CDC light trap in December 2015

| Districts | Villages | Status | CDC light trap | | | | | | Pyrethrum Spray Catch | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *An.funestus s.l.* | *An.gambiae s.l.* | *An. tenebrosus* | *An. tchekedi* | *An. squamosus* | *Culicinae* | *An. funestus* s.l. | *An .gambiae* s.l. | Culicinae |
| | | | n | N | n | | | N | n | n | n |
| Kasama | Kalonga | Sprayed | 2 | 0 | 0 | 0 | 0 | 12 | 6 | 1 | 44 |
| Katete | Mbalani | Sprayed | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 22 |
| | Robert | Control | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 50 |
| Isoka | Nsalamba | Sprayed | 7 | 25 | 0 | 2 | 4 | 46 | 1 | 3 | 0 |
| Mwense | Shibesa | Sprayed | 39 | 6 | 0 | 0 | 0 | 3 | 2 | 4 | 2 |
| | Chebele | Control | 40 | 6 | 0 | 0 | 0 | 16 | 64 | 8 | 2 |
| Milenge | Lunga | Sprayed | 65 | 0 | 8 | 0 | 0 | 9 | 65 | 0 | 0 |
| | Niyambo | Control | 296 | 0 | 0 | 0 | 0 | 3 | 243 | 0 | 0 |
| Serenje | chibobo | Sprayed | 5 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 3 |
| | Chichi | Control | 36 | 0 | 0 | 0 | 0 | 3 | 93 | 0 | 3 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total** | | | **490** | **37** | **8** | **2** | **4** | **121** | **501** | **16** | **126** |

**Table F:** Culicidae collected by Human Landing Catch in December 2015

| Districts | Villages | Status | HLC | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Indoor | | | | | | | | | Outdoor | | | | | | | |
| | | | An. funestus s.l. | An.gambiae s.l. | An. tenebrosus | An. tchekedii | An. squamosus | An. Coustani | An. rufipes | An. ziemanni | Culicine | An. funestus s.l. | An.gambiae s.l. | An. tenebrosus | An. tchekedii | An. squamosus | An. coustani | An. rufipes | An. Ziemanni | Culicine |
| Kasama | Kalonga | Sprayed | 8 | 1 | 42 | 3 | 11 | 0 | 0 | 0 | 13 | 2 | 3 | 68 | 6 | 8 | 0 | 0 | 0 | 19 |
| Katete | Mbalani | Sprayed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 284 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 118 |
| | Robert | Control | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 |
| Isoka | Nsalamba | Sprayed | 2 | 8 | 1 | 4 | 2 | 0 | 0 | 0 | 28 | 7 | 44 | 2 | 25 | 10 | 0 | 0 | 0 | 247 |
| Mwense | Shibesa | Sprayed | 18 | 27 | 0 | 0 | 1 | 0 | 0 | 0 | 6 | 31 | 36 | 0 | 0 | 1 | 0 | 0 | 0 | 348 |
| | Chebele | Control | 179 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 100 | 68 | 1 | 0 | 2 | 0 | 0 | 0 | 45 |
| Milenge | Lunga | Sprayed | 126 | 0 | 24 | 20 | 0 | 0 | 0 | 7 | 47 | 79 | 1 | 41 | 25 | 0 | 0 | 0 | 17 | 77 |
| | Niyambo | Control | 759 | 2 | 11 | 3 | 0 | 1 | 0 | 1 | 39 | 397 | 0 | 39 | 8 | 1 | 0 | 0 | 2 | 176 |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serenje | chibobo | Sprayed | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 10 |
| | Chichi | Control | 82 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 3 | 47 | 0 | 0 | 0 | 1 | 6 | 0 | 0 | 22 |
| **Total** | | | **1181** | **91** | **78** | **30** | **14** | **3** | **0** | **8** | **456** | **679** | **152** | **151** | **64** | **24** | **7** | **0** | **19** | **1075** |

**Table G**: Culicidae collected by PSC and CDC light trap in January 2016

| Districts | Villages | Status | CDC light trap | | | | | | | Pyrethrum Spray Catch | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *An.funestus* s.l. | *An.gambiae* s.l. | *An. tenebrosus* | *An. tchekedii* | *An. squamosus* | *An. coustani* | *Culicinae* | *An. funestus* s.l. | *An .gambiae* s.l. | *An. squamosus* | *An. tchekedii* | *An. coustani* | *An. tenebrosus* | *Culicinae* |
| | | | n | n | n | n | n | | n | n | n | | n | | | n |
| Kasama | Kalonga | Sprayed | 6 | 6 | 1 | 3 | 12 | 0 | 17 | 9 | 0 | n | 0 | 0 | 0 | 49 |
| Katete | Mbalani | Sprayed | 0 | 0 | 0 | 0 | 0 | 0 | 223 | 0 | 0 | 0 | 0 | 0 | 0 | 76 |
| | Robert | Control | 1 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 1 | 0 | 0 | 0 | 0 | 8 |
| Isoka | Nsalamba | Sprayed | 9 | 231 | 1 | 4 | 13 | 0 | 33 | 14 | 18 | 2 | 1 | 0 | 0 | 12 |
| Mwense | Shibesa | Sprayed | 60 | 73 | 0 | 0 | 0 | 2 | 76 | 41 | 51 | 0 | 0 | 0 | 0 | 4 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chebele | Control | 15 | 43 | 0 | 0 | 0 | 0 | 115 | 11 | 39 | 0 | 0 | 2 | 0 | 3 |
| Milenge | Lunga | Sprayed | 206 | 24 | 4 | 0 | 0 | 0 | 33 | 119 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Niyambo | Control | 735 | 12 | 182 | 4 | 2 | 0 | 143 | 155 | 0 | 2 | 0 | 0 | 19 | 33 |
| Serenje | chibobo | Sprayed | 1 | 0 | 0 | 0 | 0 | 0 | 26 | 34 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Chichi | Control | 38 | 0 | 0 | 0 | 0 | 0 | 17 | 108 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | | | **1071** | **389** | **188** | **11** | **27** | **2** | **702** | **491** | **110** | **4** | **1** | **2** | **19** | **186** |

**Table H:** Culicidae collected by Human Landing Catch in January 2016

| Districts | Villages | Status | HLC | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Indoor | | | | | | | | | Outdoor | | | | | | | |
| | | | *An. funestus* s.l. | *An.gambiae* s.l. | *An. tenebrosus* | *An. tchekedii* | *An. squamosus* | *An. Coustani* | *An. rufipes* | *An. ziemanni* | *Culicine* | *An. funestus* s.l. | *An.gambiae* s.l. | *An. tenebrosus* | *An. tchekedii* | *An. squamosus* | *An. coustani* | *An. rufipes* | *An. ziemanni* | *Culicine* |
| Kasama | Kalonga | Sprayed | 31 | 6 | 31 | 39 | 75 | 80 | 0 | 0 | 6 | 16 | 3 | 67 | 90 | 211 | 236 | 0 | 0 | 18 |
| Katete | Mbalani | Sprayed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 460 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 130 |
| | Robert | Contr | 1 | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ol | | | | | | | | | | | | | | | | | | |
| Isoka | Nsalamba | Sprayed | 22 | 222 | 15 | 31 | 47 | 3 | 0 | 0 | 106 | 40 | 332 | 42 | 83 | 219 | 31 | 0 | 0 | 470 |
| Mwense | Shibesa | Sprayed | 49 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 66 | 40 | 70 | 0 | 0 | 0 | 14 | 0 | 0 | 31 |
| | Chebele | Control | 44 | 151 | 0 | 0 | 0 | 9 | 0 | 0 | 95 | 36 | 164 | 0 | 0 | 0 | 21 | 0 | 0 | 30 |
| Milenge | Lunga | Sprayed | 327 | 11 | 131 | 12 | 0 | 0 | 0 | 0 | 217 | 176 | 8 | 185 | 14 | 2 | 0 | 0 | 0 | 317 |
| | Niyambo | Control | 828 | 7 | 50 | 1 | 0 | 0 | 0 | 0 | 159 | 483 | 11 | 95 | 0 | 1 | 0 | 0 | 0 | 196 |
| Serenje | chibobo | Sprayed | 12 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 3 | 10 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| | Chichi | Control | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 10 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 5 |
| **Total** | | | **1354** | **484** | **228** | **83** | **122** | **13** | **0** | **0** | **1165** | **811** | **588** | **389** | **187** | **433** | **307** | **0** | **0** | **1209** |

**Table I:** Number *of Anopheles gambiae* s.s. Kisumu strain exposed and dead in Kasama, Isoka and Katete

| District | Type of wall | October | | | November | | | December | | | January | | | February | | | Observa-tions |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number exposed | Number of dead at the end of the 24 hours | % of mortality observed at the end of the 24H | Number exposed | Number of dead at the end of the 24 hours | % of mortality observed at the end of the 24H | Number exposed | Number of dead at the end of the 24 hours | % of mortality observed at the end of the 24H | Number exposed | Number of dead at the end of the 24 hours | % of mortality observed at the end of the 24H | Number exposed | Number of dead at the end of the 24 hours | % of mortality observed at the end of the 24H of holding period | |

| | | | holding period | of holding period | | holding period | of holding period | | holding period | of holding period | | holding period | of holding period | | holding period | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mud | 60 | 60 | 100 | 60 | 60 | 100 | 60 | 58 | 97 | 61 | 58 | 95 | 60 | 48 | 80 | |
| Kasama | Cement | 60 | 60 | 100 | 60 | 60 | 100 | 60 | 59 | 98 | 61 | 57 | 93 | 60 | 54 | 90 | |
| | Mud | 60 | 60 | 100 | 60 | 60 | 100 | 60 | 60 | 100 | 60 | 52 | 87 | 60 | 51 | 84 | Mortality in the control was 7.5%. Abott formula was used |
| Isoka | Cement | 60 | 60 | 100 | 61 | 61 | 100 | 60 | 60 | 100 | 63 | 56 | 89 | 60 | 53 | 87 | |
| | Mud | 61 | 61 | 100 | 60 | 60 | 100 | 60 | 60 | 100 | 60 | 53 | 88 | 63 | 52 | 82.53968 | |
| Katete | Cement | 60 | 60 | 100 | 60 | 60 | 100 | 60 | 60 | 100 | 60 | 52 | 87 | 62 | 55 | 88.70968 | |

**Table J:** Number of *Anopheles funestus* s.l. susceptible strain exposed and dead per site and per month in Milenge, Mwense and Serenje

| | | October | | | November | | | December | | | January | | | February | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| District | Type of wall | Number exposed | Number of dead at the end of the 24 hours holding period | % of mortality observed at the end of the 24H of holding period | Number exposed | Number of dead at the end of the 24 hours holding period | % of mortality observed at the end of the 24H of holding period | Number exposed | Number of dead at the end of the 24 hours holding period | % of mortality observed at the end of the 24H of holding period | Number exposed | Number of dead at the end of the 24 hours holding period | % of mortality observed at the end of the 24H of holding period | Number exposed | Number of dead at the end of the 24 hours holding period | % of mortality observed at the end of the 24H of holding period |
| Milenge | Mud | 92 | 92 | 100 | 90 | 90 | 100 | 90 | 90 | 100 | 90 | 72 | 80 | 60 | 47 | 78 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cement | 91 | 91 | 100 | 90 | 90 | 100 | 91 | 91 | 100 | 90 | 57 | 63 | | | |
| | Mud | 90 | 90 | 100 | 95 | 95 | 100 | 90 | 90 | 100 | | | | 60 | 60 | 100 |
| Mwense | Cement | 90 | 90 | 100 | 93 | 93 | 100 | 90 | 90 | 100 | | | | 60 | 59 | 98 |
| | Mud | 95 | 95 | 100 | 90 | 90 | 100 | 94 | 94 | 100 | 90 | 64 | 71 | | | |
| Serenje | Cement | 93 | 93 | 100 | 90 | 90 | 100 | 97 | 97 | 100 | 90 | 72 | 80 | 62 | 47 | 76 |

**Table K:** Susceptibility status of *An. funestus* s.l.

| District | Pirimiphos-Methyl 0.25% | | | | Deltamethrin 0.05% | | Bendiocarb 0.1% | | DDT 4% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | February-August 2015 | | October 2015-March 2016 | | February-August 2015 | | February-August 2015 | | February-August 2015 | | October 2015-March 2016 | |
| | n | %M | n | %M | n | %M | n | %M | n | %M | n | % M |
| Kasama (Nandola) | 100 | 100 | | | | | 99 | 85.8 | | | | |
| Kasama (Kalonga) | 104 | 100 | | | | | 50 | 100 | | | | |
| Kabombeka (Kasama) | | | 24 | 100 | | | | | 5 | 100 | | |
| Milenge (Lunga) | 138 | 100 | 20 | 100 | 41 | 41.5 | 100 | 93 | 50 | 100 | | |
| Milenge (Miyambo) | 15 | 100 | | | | | 20 | 50 | | | 8 | 100 |
| Serenje (chibobo) | 65 | 100 | | | 64 | 67 | 60 | 90 | 144 | 100 | | |
| Serenje (Chishi) | 112 | 100 | | | 101 | 63.2 | | | 17 | 100 | | |
| Mwense (Chebele) | 33 | 100 | | | | | 31 | 100 | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mwense (Shibesa) | 31 | 100 | | | 74 | 84 | | | | | | |
| Mwense (East farm) | | | 50 | 100 | | | | | | | | |
| Milenge (Mwemamusongo) | | | 19 | 100 | | | | | | | | |
| Katete (Robert) | | | 40 | 100 | | | | | | | | |

**Table L:** Susceptibility status of *An. gambiae* s.l.

| District | Pirimiphos-Methyl 0.25% | | | | | | Deltamethrin 0.05% | | Bendiocarb 0.1% | | DDT 4% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | February-August 2015 | | October 2015-March 2016 | | April- June 2016 | | March- June 2016 | | March- June 2016 | | October 2015-March 2016 | | April- June 2016 | |
| | n | %M | n | %M | n | % M | n | % M | n | % M | n | % M | n | % M |
| Kasama (Kalonga) | | | | | | | | | 25 | 100 | 23 | 0 | | |
| Kabombeka (Kasama) | | | 39 | 100 | | | | | | | | | | |
| Milenge (Lunga) | | | | | 94 | 100 | 60 | 98.3 | 78 | 100 | | | 50 | 86 |
| Milenge (Miyambo) | | | | | | | | | | | | | 23 | 100 |
| Mwense (chebele) | 75 | 100 | | | | | 6 | 100 | | | 102 | 41 | | |
| Mwense (Shibesa) | | | 64 | 100 | | | 72 | 39 | 50 | 100 | | | | |
| Katete (Robert) | | | | | 146 | 100 | 122 | 80 | 136 | 100 | | | 136 | 99 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Katete (Mbalani) | | | | | 66 | 100 | 26 | 100 | 63 | 100 | | | | |
| Isoka (Nsalamba) | | | | | 73 | 100 | | | 29 | 100 | | | | |
| Isoka (Chilanga) | | | | | 23 | 100 | | | | | | | | |
| Isoka (Londamaka) | | | | | 18 | 100 | | | | | | | | |