PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Six

AIRS ETHIOPIA ENTOMOLOGICAL MONITORING FINAL REPORT

MARCH 2015 –FEBRUARY 2016

APPROVED: APRIL 11, 2016

Recommended Citation: The PMI Africa Indoor Residual Spraying Project. January 2016. *Ethiopia Entomological Monitoring of 2015 IRS Activities*. Final Report. Addis Ababa, Ethiopia. The PMI AIRS Project, Abt Associates Inc.

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

Task Order: AID-OAA-TO-11-00035

Submitted to: United States Agency for International Development/PMI

Submitted: January 27, 2016

Re-Submitted: February 19, 2016

Re-Submitted: April 4, 2016

Approved: April 11, 2016

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	Centers for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
FMOH	Federal Ministry of Health
HLC	Human Landing Catch
IR	Insecticide resistance
IRS	Indoor Residual Spray
PSC	Pyrethrum Spray Catch
PMI	President Malaria Initiative
SOP	Spray Operator
WHO	World Health Organization

1. EXECUTIVE SUMMARY

Background

The 2015 entomological monitoring activities included year-round collection of data on vector density and species composition to help understand the abundance, seasonal patterns, biting behavior, parity of anopheline mosquitoes and assess the impact of IRS on entomological indicators. During the reporting period (March 2015 - February 2016), monthly pyrethrum spray catches (PSC), human landing catches (HLC), CDC light traps and window exit traps were carried out in two intervention (sprayed) sites and one control (not sprayed) site. The intervention sites were in Gobu Sayo and Seka Chekorsa Districts. One site from Ilugelan District, Ijaji Town, was selected as an unsprayed control site. HLC was used in two households in each sentinel site for two nights per month. PSC was used to sample indoor resting mosquitoes in 20 houses in each of the study sites every month. CDC light traps were installed in two houses adjacent to houses selected for HLC in each of the three sentinel sites, and window exit traps were installed in four selected houses in each site. In 2015, insecticide susceptibility using WHO tube test and CDC bottle bioassay were conducted in eight fixed PMI AIRS sentinel sites and five malarious districts, respectively, to determine the response of the main malaria vector to different insecticides used for IRS. Furthermore, wall bioassays were conducted to monitor the decay rate of pirimiphos-methyl (Actellic 300 CS) and bendiocarb 80 WP in four selected districts. The wall bioassay tests were conducted in 12 houses per site with a total of 48 houses sampled.

Results

Vector density and Seasonality: 7,427 female anopheline mosquitoes comprising six species were collected. The most abundant species were *An. gambiae* s.l. (32.6%), *An. coustani* (35.2%) and *An. pharoensis* (31.7%). Overall, the main vector of malaria in Ethiopia, *An. gambiae* s.l., started proliferation in the month of April and reached its peak at variable times between June and September, with densities dropping from October onwards. In the control site peak density was achieved in September. *An. gambiae* s.l. was most abundant during the peak rainy period (June – August) in all sites though peak density was achieved at variable times. *An. coustani* was the dominant anopheline species collected from August onwards. Indoor resting density and human biting rates as measured by PSC and human landing catches, respectively, dropped after IRS in both intervention sites but increased and peaked in September in the control site.

Resting habit: The resting habits of *An. gambiae* s.l. were variable by site. *An. gambiae* s.l. tended to exhibit endophilic tendencies in both intervention sites while it was more exophilic in the control site when we compared fed versus half gravid and gravid mosquitoes in PSC collections. The number of *An. gambiae* s.l. resting indoors reduced drastically after IRS in the intervention sites compared to the control site.

Feeding time and location: *An. gambiae* s.l. tended to feed more outdoors than indoors showing exophagic tendency in the two intervention sites (59.5% Gobu Sayo $p<0.001$; 61.8% with $p<0.001$ Seka Chekorsa) but tended to show endophagic tendencies in the control site (53.1%, $p>0.05$,Ijaji). The difference in feeding tendencies was statistically significant in the intervention site but not so in the control site. *An. gambiae* s.l. engaged in biting throughout the night but peak biting was variable between sites, with Gobu Sayo and Ijaji recording post-midnight biting activity (01.00 – 03.00 hours). In Seka Chokorsa a higher proportion of host-seeking *An. gambiae* s.l. was collected before midnight (19.00 – 23.00 hours).

Parity rate: Monthly parous rates for *An. gambiae* s.l. were variable between sites throughout the period of study with generally higher rates recorded between April and August in the intervention sites (Gobu Sayo: 76.8 – 100; Seka Chekorsa: 33.3 – 67). Parous rate greatly reduced in both intervention sites after IRS but remained the same in the control site. In the Ijaji control site parous rates remained high during the whole period of study (93.3 – 100).

Susceptibility test: The susceptibility of *An. gambiae* s.l. to 11 insecticides recommended for malaria vector control was tested using the WHO tube test in eight sites. The results showed that the vector was fully susceptible to pirimiphos-methyl, fenitrothion, and propoxur in all study sites. It was fully susceptible to bendiocarb in six sites. Suspected resistance was shown in one of eight sites and resistance to bendiocarb was noted in one of eight sites. *An. gambiae* s.l. was resistant to DDT and all the pyrethroids tested, including etofenprox in all sites. The vector was shown to be fully susceptible to malathion in two sites; possible resistance was detected in two sites and the vector was resistant to malathion in three sites. Susceptibility tests using CDC bottle bioassays were conducted in six sites with five different insecticides; namely, bendiocarb, propoxur, pirimiphos-methyl, deltamethrin, and permethrin. In three sites synergist (PBO) was used. *An. gambiae* s.l. was resistant to permethrin and deltamethrin in all sites. However, pre-treatment with PBO fully restored susceptibility at the diagnostic dosage to permethrin and deltamethrin in the Dugda sites indicating that an oxidase mechanism of resistance is probably involved. In Halaba site, pre-test PBO exposure restored susceptibility to deltamethrin with 100% knock down but not for permethrin. It is highly likely that permethrin resistance may be mediated by other metabolic enzymes and not only oxidase in addition to *kdr*.

Wall bioassay test: The test mortality of wild and susceptible mosquitoes was 100% for all wall surfaces conducted three to six days after spraying with pirimiphos-methyl. In bendiocarb sprayed houses the mortality of wild and susceptible mosquitoes was 100% for all dung plastered and painted houses within seven days of spraying. However, mortality of wild and susceptible *An. gambiae* s.l. mosquitoes ranged from 90-95% on mud wall surfaces. Average mortality of susceptible mosquitoes was 88.1% five months after spraying with pirimiphos-methyl and 58.3% four months after spraying with bendiocarb. At six and seven months after spraying with pirimiphos-methyl, the average mortality recorded was 68.1% and 57.8%, respectively.

Sporozoite Elisa: Sporozoite rates of 0.78% and 0.58%, respectively, were recorded for *P. vivax* and *P. falciparum* circumsporozoite protein (CSP) using the ELISA test for 506 *An. gambiae* s.l. samples tested from the three sites during the pre-spray period. *An. pharoensis* was positive for *P. vivax* (0.1%) and *P. falciparum* (0.1%) CSP during the pre-spray period. The sporozoite rates during the post-spray period were as follows: 0.04% and 0.2% for *P. vivax* and *P. falciparum* in *An. gambiae* s.l.; 0.08% for *P. facilciparum* in *An. coustani/ ziemanni* and 0.12% for *P. vivax* CSP in *An. pharoensis*. Overall, 84.2% (n= 16) of the specimens that tested positive for *P. vivax* and *P. falciparum* circumsporozoite protein were collected in Gobu Sayo. Furthermore, all the CSP positive *An. pharoensis* and *An. coustani/ ziemanni* were all collected from the same site. These results indicate the likely importance of both *An. pharoensis* and *An. coustani/ ziemanni* in malaria transmission in the country.

Molecular identification of *An. gambiae* s.l. and determination of kdr allelic frequency

All *An. gambiae* s.l. specimens analyzed using PCR showed *An. arabiensis* as the only species of the gambiae complex represented in the study sites. The West African *kdr* allele (L1014F) was common in populations of *An. gambiae* s.l. tested from the eight study sites. The *kdr* allele frequency in surviving mosquitoes following the bioassay tests ranged from 31% to 100% for DDT and 36% to 100% for deltamethrin. On the other hand, the *kdr* allele frequency in dead mosquitoes following bioassay test ranged from 13% to 88% for DDT and 13% to 75% for deltamethrin.

Conclusions

The present study characterizes the bionomics of *An. gambiae* s.l. and provides relevant information to be considered in planning and implementation of vector interventions. The longitudinal vector density monitoring studies conducted indicated that the main malaria vector *An. gambiae* s.l. started proliferation in April, reaching a peak in September based on results from the control site. Based on these results, conducting IRS in the month of May/June with long-lasting insecticides would most probably provide sufficient protection. In the use of insecticides with short residual life, implementation of IRS in early August would be recommended. Indoor resting densities as well as mean human biting rates considerably declined after IRS in both intervention sites, most likely due to the effect of insecticide sprayed. The main vector was found to be fully susceptible to pirimiphos-methyl, propoxur, and fenitrothion, indicating the potential use of these insecticides for IRS with continuous monitoring and application of measures to manage any likely emergence of resistance to these insecticides. These results provide a basis for improved targeting of IRS for enhanced impact on malaria transmission.

2. INTRODUCTION

In September 2014, Abt Associates was awarded the three-year Africa Indoor Residual Spraying (AIRS) Project, which is funded by the United States Agency for International Development (USAID) under the President's Malaria Initiative (PMI). This was a follow-on to the initial award in 2011 that saw the implementation of IRS and other activities in up to 17 countries in sub-Saharan Africa. The underlying objective of the current project is to limit exposure to malaria and reduce incidence and prevalence of malaria in up to 20 countries by implementing highly-effective indoor residual spraying campaigns.

Entomological activities are essential for proper targeting and planning of indoor residual spraying (IRS). They often include monitoring of IRS impact on vector density, behavior, and composition; evaluating the susceptibility level of the local vectors to different insecticides; and understanding the potential mechanisms of resistance. Entomological activities also are vital in determining the residual life of different insecticides on different types of wall surfaces under various environmental conditions. Entomological study results from susceptibility tests provide empirical evidence that inform selection of insecticides for IRS in addition to other operational criteria.

During 2015, AIRS Ethiopia continued routine entomological data collection and insecticide resistance (IR) testing in order to monitor the efficacy of IRS on malaria transmission in the project areas. Specific objectives of the 2015 entomological work were to:

- Determine the *Anopheles* species composition;
- Monitor year round vector density and behavior before and after spray operations;
- Assess susceptibility of the main vector to different insecticides;
- Assess quality of spray operations and decay rate of insecticides;
- Train Federal Ministry of Health (FMOH) staff on basic malaria entomology.

In addition to vector density and behavior studies, this report includes a brief summary of the following entomological monitoring activities performed by the Project in 2015:

- IR tests with the WHO tube test in eight sites (Annex D);
- IR tests using CDC bottle assays in six sites (Annex D);
- Cone bioassay for IRS quality check (Annex E);
- Cone bioassay for decay rate of pirimiphos-methyl and bendiocarb sprayed in project districts (Annex E);
- Training of district staff on basic malaria entomology (Annex F).

3. MONITORING VECTOR BEHAVIOR AND DENSITY

3.1 INTRODUCTION

In 2015, AIRS Ethiopia selected three sentinel sites to undertake a number of entomological studies, including vector population dynamics and behavior. The Project selected two intervention (sprayed) sites and one control (not sprayed) site to collect data on comprehensive entomological indicators that included vector behavior and density. The intervention sites were in Gobu Sayo District and Seka Chekorsa District. One site from Ilugelan District, Ijaji Town, was selected as an unsprayed control site. Gobu Sayo and Ijaji are located in Western Oromia 50 kilometers (km) from each other. Seka Chekorsa is in Southwest Oromia about 300 km from the two sites. The intervention sites were sprayed with bendiocarb in August 2015.

The AIRS entomology team led the data collection in Gobu Sayo and Ilugelan, and the Project contracted Jimma University to work in Seka Chekorsa sentinel site. Data collection started in March 2015 and continued for12 months until February 2016. This report covers the work that was performed from March 2015 to February 2016.

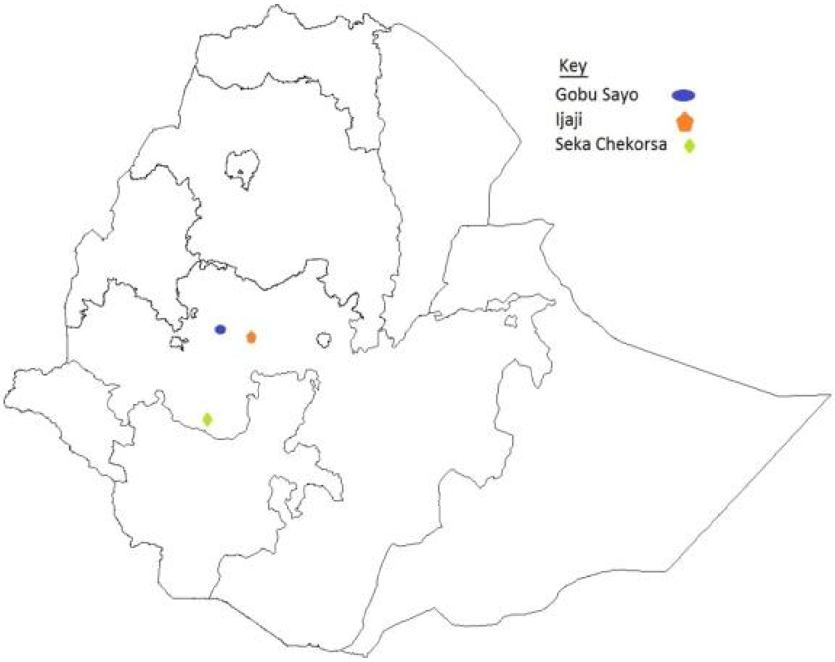
3.2 OBJECTIVES OF AIRS ETHIOPIA ENTOMOLOGICAL MONITORING:

- Identify the *Anopheles* mosquitoes present in the two intervention areas and one control area, indoor resting density, man biting rate(s), and biting cycles;
- Determine vector density, distribution, and seasonality in the intervention and control areas;
- Provide quality assurance of the IRS program through the World Health Organization (WHO) cone/wall bioassay;
- Determine the extent of endophagy (indoor feeding) and endophily (indoor resting);
- Determine parity as an entomological indicator to ascertain if the age composition of the mosquito population has been reduced to determine whether or not the IRS interventions are affecting the vectors and their ability to transmit malaria in the intervention areas.

4. COLLECTION METHODS

Ten rounds of entomological data collection were conducted in the three sentinel sites shown in Figure 1. The first collection was performed in March 2015 and continued at one-month intervals up to February 2016. Excel software was used to produce summary tables and graphics. Below are the descriptions of the methods and procedures used to collect the entomological data from the three sentinel sites.

FIGURE 1: 2014 SENTINEL SITES FOR MONITORING MOSQUITO DENSITY AND BEHAVIOR



4.1 HUMAN LANDING CATCHES

Human landing catches (HLC) were conducted in two houses in each sentinel site for two nights per month; thus data being collected for four nights per site per month. One mosquito collector was seated indoors and another seated outdoors from 6 p.m. to 8 a.m. to collect blood-seeking mosquitoes. Outdoor mosquito collection was carried out about eight meters from each of the two sampled houses. A team of two collectors was assigned a seven-hour shift. A total of four collectors per house per night covered 14 hours of collections from 6 p.m. to 8 a.m. The last shift had to collect for seven hours (1 a.m. - 8 a.m.). Outdoor and indoor collectors switched sites every hour. Collectors adjusted their clothing so that the legs were exposed up to the knees. When a mosquito was felt, collectors quickly turned on the torch, collected the mosquito with the sucking tube and transferred it to a paper cup. One cup was used for each hour of collection. Hourly temperature and humidity were recorded. At the end of the collection, mosquitoes were transported to the field lab and were identified using taxonomic keys (Gilles and Coetzee, 1987).

4.2 Pyrethrum Spray Catch

Pyrethrum Spray Catch (PSC) was used to sample indoor resting mosquitoes in 20 houses in each of the study sites every month. Collections were carried out in the morning between 6:00 a.m. and 7:30 a.m. Before the PSC was performed, all occupants were cordially asked to move out of the house. The team recorded information from the head of household or an adult member about the number of people who slept in the house the previous night and the number of treated nets present. The floor was then covered with white sheets and the eaves, windows, and other mosquito escape routes around the house were sprayed as were the walls and roof space inside the house with Baygon (knockdown spray). Ten minutes after spraying, collectors gathered all the mosquitoes that were knocked down from the sheets and sorted them by species. The abdominal status of all female anophelines was determined, and individual specimen recorded as unfed, blood-fed, half-gravid, and gravid females.

4.3 CDC Light Traps

Centers for Disease Control and Prevention (CDC) light traps were installed in two houses adjacent to the houses selected for HLC in each of the three sentinel sites, and collection was done for two nights every month. The CDC light-traps were suspended in a bedroom 1.5 meters high from the floor and about 50 centimeters from a human sleeping under a bed net. The light traps were fitted with an incandescent bulb. The traps were set from 6 p.m. to 6 a.m. Mosquitoes were collected from the traps the next morning and sorted at the field lab.

4.4 Window Exit Trap Collection

Window exit traps were installed in four selected houses that were well-sealed in each of the three sentinel sites and collection was done for four nights every month. The collection traps were mounted for four nights per month in each of the three sentinel sites. The traps were set from 6 p.m. to 6 a.m., and mosquitoes were collected from the traps the next morning. If the collected mosquitoes were alive, they were kept for 24 hours to monitor delayed mortality.

4.5 Identification of Malaria Vectors

Anopheles mosquitoes collected through HLC, PSC, CDC light traps, and window exit traps were preliminarily identified to the species level morphologically. All *Anopheles* specimens that were not dissected were labeled and stored individually in Eppendorf tubes on silica gel for further processing by Jimma University.

4.6 Determination of Parity

Unfed females belonging to *An. gambaie* s.l., presumably *An. arabiensis,* from HLC were dissected for ovary parity under a dissecting microscope to determine parity rate based on coiling of ovarian tracheoles (Detinova 1962). Mosquitoes were kept in wet petri dishes and dissected within 12 hours after the capture.

5. RESULTS

5.1 ANOPHELINE SPECIES DIVERSITY AND ABUNDANCE

During the 10 months of the study, a total of 7,427 adult female *Anopheles* mosquitoes were collected using PSC, HLC, CDC light traps, and window exit traps. Detailed data are included in Annex A. The species composition of collected mosquitoes follows:

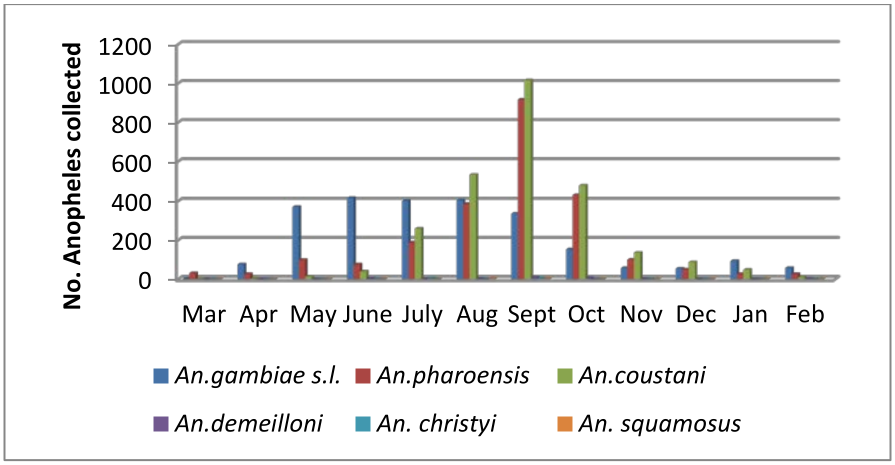
- 2,421 *An. gambiae* s.l.
- 2,353 *An. pharoensis*
- 2,616 *An. coustani/ziemanni*
- 23 *An. demeilloni*
- 8 *An. christyi*
- 6 A*n. squamosus.*

In addition to the *Anopheles*, 24,754 *Culex* mosquitoes were captured through the different collection techniques. *An. gambiae* s.l., *An. coustani, An. pharoensis, and An. christyi* were common in all the three sites. *An. demelloni* was collected from Gobu Sayo and Ijaji sites; *An. squamosus* from Seka Chekorsa site only.

5.2 TREND OF ANOPHELES OVER 12 MONTHS

As indicated in Figure 2 the proliferation of *Anopheles* particularly *An. gambiae* s.l. started in April, peaked in September 2015 and started to decline from October onwards. The peak density of *An. gambiae* s.l. was achieved at different times in the 3 sites: Seka Cherkosa (May); Gobu Sayo (July) and Ijaji (September). Based on these findings, it is possible to consider the start of IRS either in May/June if long-lasting insecticide is used or in early August if using insecticides with short residual life.

FIGURE 2: TREND OF *ANOPHELES* SPECIES OVER TIME FOR 3 SENTINEL SITES



NB: April to Aug: Pre-spray; Sept to Dec: Post IRS

5.3 PYRETHRUM SPRAY CATCH

Tables 1, 2 and 3 show the PSC results, which indicate that vector density was higher pre-spray (March – August) compared to subsequent months after spraying (September – December) in the intervention sites. In Gobu Sayo intervention site, mean indoor resting density of female *An. gambiae* s.l. pre-spray was 2.33 mosquitoes per house per day. To assess the impact IRS had on vector density, mean *An. gambiae* s.l. before IRS was compared with three months data post IRS assuming IRS with bendiocarb would be effective for up to three months in Ethiopia. Vector density declined from 2.33 mosquitos per house per day during pre-spray to 0.37 mosquitos per house per day post spray, which is a six fold decline (p=0.0408). The mean density dropped suddenly in September, one month after spraying, and remained low throughout the subsequent three months. The vector density reduction was more pronounced in the half gravid and gravid mosquitoes as compared to the freshly fed ones in the intervention sites (Figure 3). This indicates that the mosquitoes were either killed by the sprayed insecticides or repelled and left the house before they could reach the gravid stages. In the other intervention site, Seka Chekorsa, the mean indoor resting density of female *An. gambiae* s.l. per house per day was 1.8 during the pre-spray period. Following spraying, a sudden drop in the mean vector indoor resting density was observed with 0.07 female *An. gambiae* s.l. per house per day recorded after spray (Figure 4). Though the mean vector density declined from 1.8 *An. gambiae* s.l. per house per day pre spray to 0.07 after spray, the reduction was not statistically significant (p=0.062). This is most probably due to the small number of mosquitoes collected. In the control site of Ijaji, the mean indoor resting density per house per day increased from 1.03 pre-spray to 1.43 after spray (Figure 5). The increase, however, was not statistically significant (p=0.65).

The sudden drop in vector density noted one month after spraying in the two intervention sites compared to the increase in the control site might be attributed to the impact of IRS. However, starting two months after the spraying (October) a uniform decline in vector density was observed in all three sentinel sites irrespective of their treatment status. This uniform decline in indoor resting density appears to be linked more to the climatic factors rather than the intervention. In Ethiopia, the main rainy season usually ends in September hence impacting the number of breeding sites and mosquito densities in the subsequent months.

In indoor resting collections, such as PSC, the proportion of half-gravid and gravid mosquitoes is expected to be higher than fed mosquitoes if the vector's resting habit is endophilic. In Gobu Sayo, the proportion of gravid mosquitoes was higher during pre-spray (60-100%) but reduced to 10% one month after spraying and in subsequent months. In Seka Chekorsa, the proportion of gravid mosquitoes was higher during pre-spray and reduced to zero after spray. In the control site, the proportion of gravid mosquitoes was lower than fed mosquitoes throughout study period. Less gravid mosquitoes in the control villages indicate the preference of the vector to rest outdoors over indoors.

TABLE 1: PSC COLLECTIONS, GOBU SAYO INTERVENTION SITE, MARCH 2015-FEBRUARY 2016

Time	# of houses	# of Occupants	*# of LLINs	*An. gambiae* s.l. Collected	Abdominal/Blood Digestion stages	Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	# Fed per human host

		Human			UF^	F^	HG^	G^				
March	20	69	8	2	0	0	2	0	2	1	0.1	0
April	20	66	9	31	3	6	19	3	22	0.71	1.55	0.09
May	20	69	9	54	8	12	21	13	34	0.63	2.7	0.17
June	20	68	9	59	9	20	11	19	30	0.51	2.95	0.29
July	20	72	8	71	2	17	27	25	52	0.73	3.55	0.24
Aug	20	77	12	63	2	9	38	14	52	0.83	3.15	0.12
Sept	20	75	12	17	7	9	1	0	1	0.06	0.85	0.12
Oct	20	75	12	5	2	2	1	0	1	0.2	0.25	0.03
Nov	20	75	12	0	0	0	0	0	0	0	0	0
Dec	20	75	12	0	0	0	0	0	0	0	0	0
Jan	20	74	12	5	1	0	3	1	4	1	0.25	0.00
Feb	20	71	12	6	0	2	2	2	2	2	0.3	0.03

NB: April to Aug: Pre-spray; Sept to Dec: Post IRS
*LLINs - Long-Lasting Insecticidal Nets
^ UF – un-fed, F-fed, HG-half-gravid, G - gravid

TABLE 2: PSC COLLECTIONS, SEKA CHEKORSA INTERVENTION SITE, MARCH 2015-FEBRUARY 2016

Time	# of houses	# of Occupants	# of LLINs	*An. gambiae* s.l. Collected	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	# Fed per human host
		Human			UF^	F^	HG^	G^				
March	20	91	29	1	0	0	1	0	1	1.00	0.05	0
April	20	94	34	11	0	1	7	3	10	0.91	0.55	0.01
May	20	88	34	94	0	31	46	17	63	0.67	4.7	0.35
June	20	79	34	10	0	6	2	2	4	0.40	0.5	0.08
July	20	90	33	52	0	20	25	7	32	0.62	2.6	0.22
Aug	20	91	33	48	0	22	20	6	26	0.54	2.4	0.24
Sept	20	75	12	0	0	0	0	0	0	0	0	0
Oct	20	86	41	1	0	1	0	0	0	0	0.05	0.01
Nov	20	78	41	3	0	2	1	0	1	0.33	0.15	0.03
Dec	20	78	41	6	0	5	1	0	1	0.20	0.25	0.06

Jan	20	76	41	9	2	5	2	0	2	28.6	0.45	0.07
Feb	20	79	41	7	1	3	3	0	3	50.0	0.35	0.04

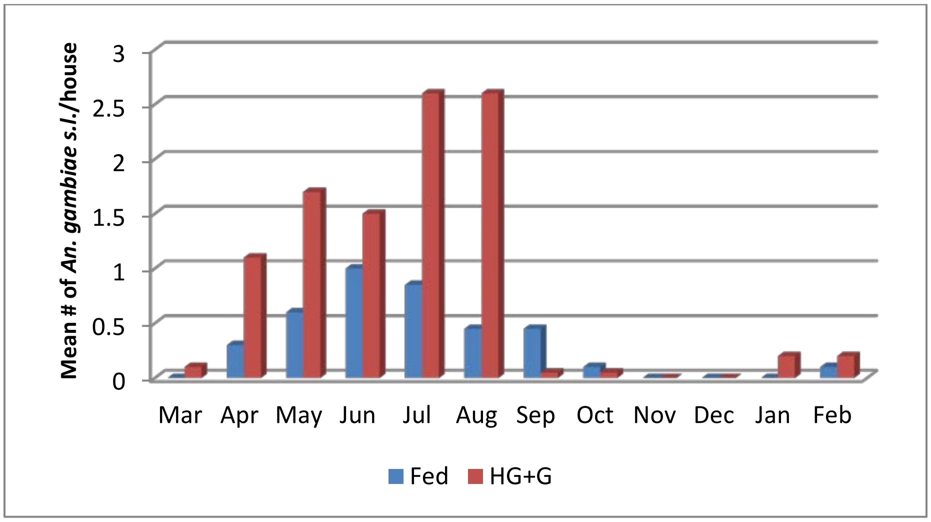
NB: April to Aug: Pre-spray; Sept to Dec: Post IRS. *LLINs - Long-Lasting Insecticidal Nets
^ UF – un-fed, F-fed, HG-half-gravid, G - gravid

TABLE 3: PSC COLLECTIONS, IJAJI CONTROL SITE, MARCH 2015-FEBRUARY 2016

Time	# of houses	# of Occupants	# of LLIN*s	*An. gambiae* s.l. Collected	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	# Fed per human host
					UF^	F^	HG^	G^				
March	20	79	10	0	0	0	0	0	0	0	0	0
April	20	81	9	6	0	2	1	3	4	0.67	0.3	0.02
May	20	81	10	27	1	16	10	0	10	0.37	1.4	0.20
June	20	81	9	27	2	15	10	1	11	0.41	1.4	0.19
July	20	81	9	24	2	17	3	2	5	0.21	1.2	0.21
Aug	20	80	18	40	3	25	7	5	12	0.30	2	0.31
Sept	20	79	15	74	6	47	17	4	21	0.28	3.7	0.59
Oct	20	77	14	9	0	5	2	2	4	0.44	0.5	0.06
Nov	20	75	12	3	1	1	0	1	1	0.33	0.2	0.01
Dec	20	83	11	2	0	2	0	0	0	0	0.1	0.02
Jan	20	75	12	0	0	0	0	0	0	0	0	0
Feb	20	75	12	0	0	0	0	0	0	0	0	0

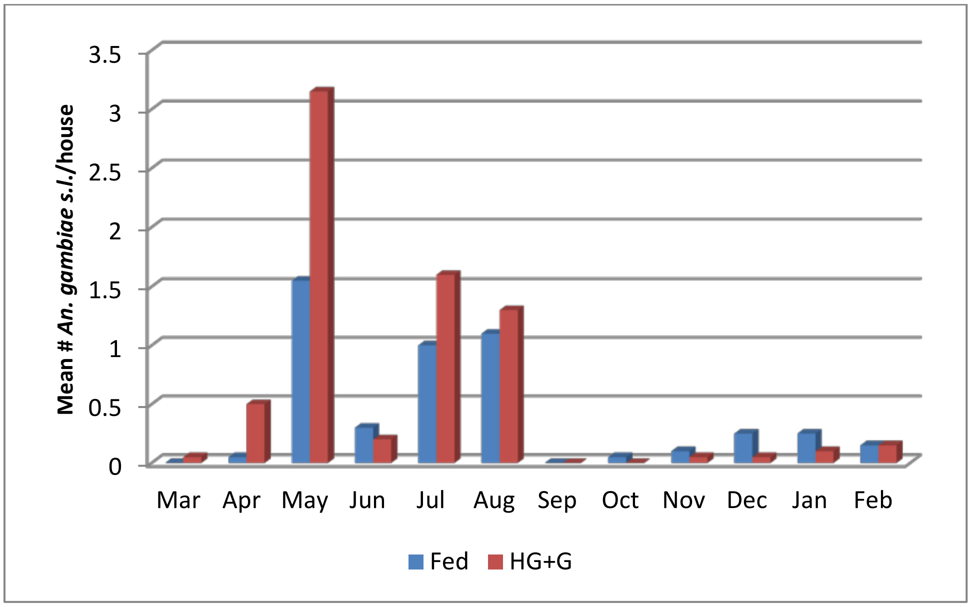
NB: April to Aug: Pre-spray; Sept to Dec: Post IRS. *LLINs - Long-Lasting Insecticidal Nets
^ UF – un-fed, F-fed, HG-half-gravid, G – gravid

FIGURE 3: PSC COLLECTIONS, GOBU SAYO INTERVENTION SITE, MARCH 2015-FEBRUARY 2016



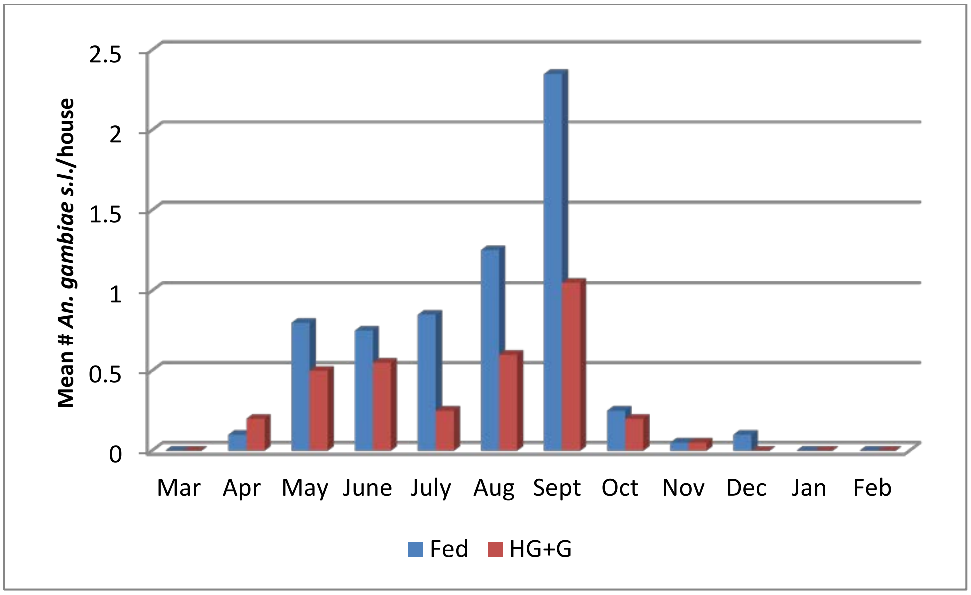
NB: April to Aug: Pre-spray; Sept to Dec: Post IRS

FIGURE 4: PSC COLLECTIONS, SEKA CHEKORSA INTERVENTION SITE, MARCH 2015-FEBRUARY 2016



NB: April to Aug: Pre-spray; Sept to Dec: Post IRS

FIGURE 5: PSC COLLECTIONS, IJAJI CONTROL SITE, MARCH 2015-FEBRUARY 2016



5.4 HUMAN LANDING CATCH

HLC collection was done once a month in each site in two houses for two consecutive nights for a total of four nights each month. During the study period, a total of 4,390 *Anopheles* mosquitoes were collected while attempting to feed on human baits. Of these, 1,287 were *An. gambiae* s.l., 1,423 *An. pharoensis,* 1,655 *An. coustani,* 16 *An. demeilloni* and 9 *An. christyi*. The proportion of indoor to outdoor collection for the main vector, *An. gambiae* s.l., in the intervention area was 368 (39%) vs. 576 (61%), respectively, indicating a tendency to outdoor feeding or exophagic habits. The difference in biting behaviour was significantly different ($p<0.001$) with the main vector exhibiting exophagic tendency. In the control site attempts to bite indoors were higher than outdoors for *An. gambiae* s.l. though it was not statistically significant ($p=0.108$)

An. pharoensis, An. coustani, An. demeilloni and *An. christyi* also preferred to bite outdoors over indoors even at higher rates than *An. gambiae* s.l. Details on HLC collections by sentinel site are provided in Tables 4, 5, and 6 and Figures 6, 7 and 8.

TABLE 4: HLC IN GOBU SAYO, INTERVENTION SITE, MARCH 2015-FEBRUARY 2016

Time	*An. gambiae* s.l.					*An. pharoensis*			*An. coustani*			*An. demeilloni*			An. christyi			Total Anophelines collected		
	In	MBR Indoor	Out	MBR Outdoor	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Grand total
March	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	0	1	1	2
April	6	1.5	6	1.5	12	0	4	4	0	2	2	0	0	0	0	0	0	6	12	18
May	34	8.5	37	9.25	71	1	5	6	0	0	0	0	0	0	0	0	0	35	42	77
June	78	19.5	64	16	142	2	9	11	3	9	12	0	0	0	0	0	0	83	82	165
July	43	10.75	69	17.25	112	17	83	100	4	19	23	0	0	0	0	0	0	64	171	235
Aug	36	9	80	20	116	33	112	145	8	101	109	0	2	2	0	0	0	77	293	370
Sept	24	6	69	17.25	93	170	360	530	70	457	527	1	7	8	0	2	2	264	888	1152
Oct	12	3	17	4.25	29	57	132	189	17	181	198	0	0	0	0	0	0	86	330	416
Nov	8	2	6	1.5	14	18	45	63	1	53	54	0	1	1	0	0	0	27	104	131
Dec	10	2.5	21	5.25	31	9	31	40	3	17	20	0	0	0	0	0	0	22	69	91
Jan	3	0.75	10	2.5	13	3	10	13	0	8	8	0	0	0	0	0	0	8	27	35
Feb	6	1.5	8	2	14	6	8	14	0	3	3	0	0	0	0	0	0	17	24	41

NB: April to Aug: Pre-spray; Sept to Nov: Post IRS

TABLE 5: HLC IN SEKA CHEKORSA, INTERVENTION SITE, MARCH 2015-FEBRUARY 2016

Time	*An. gambiae* s.l.					*An. pharoensis*			*An. coustani*			*An. demeilloni*			*An. christyi*			Total Anophelines collected		
	In	MBR Indoor	out	MBR Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Grand total
March	0	0	0	0	0	11	10	21	0	0	0	0	0	0	0	0	0	11	10	21
April	1	0.25	2	0.5	3	3	6	9	0	1	1	0	0	0	0	0	0	4	9	13
May	7	1.75	11	2.75	18	26	36	62	3	10	13	0	0	0	0	0	0	36	57	93
June	20	5	18	4.5	38	15	27	42	12	8	20	0	0	0	0	0	0	47	53	100

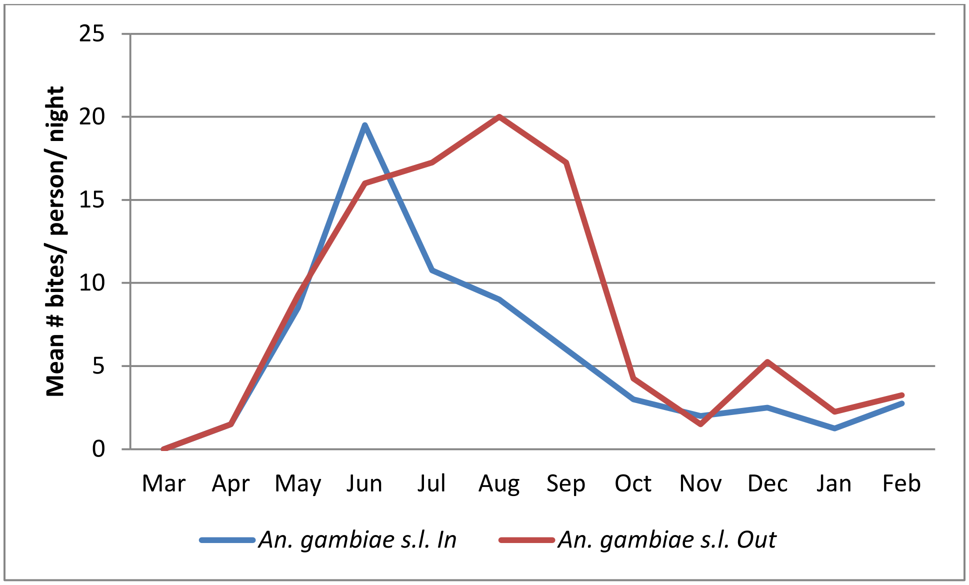
July	9	2.25	18	4.5	27	15	27	42	26	84	110	0	0	0	0	0	0	50	129	179
August	14	3.5	13	3.25	27	42	47	89	46	122	168	0	0	0	1	2	3	103	184	287
Sept	2	0.5	13	3.25	15	2	4	6	50	126	176	0	0	0	0	3	3	54	146	200
Oct	29	7.25	50	12.5	79	3	6	9	24	70	94	0	0	0	0	0	0	56	126	182
Nov	10	2.5	15	3.75	25	3	2	5	8	16	24	0	0	0	0	0	0	21	33	54
Dec	2	0.5	12	3	14	3	5	8	17	45	62	0	0	0	0	0	0	22	62	84
Jan	11	2.75	29	7.25	40	1	5	6	6	15	21	0	0	0	0	0	0	18	49	67
Feb	3	0.75	8	2	11	0	6	6	1	5	6	0	0	0	0	0	0	4	19	23

NB: April to Aug: Pre-spray; Sept to Nov: Post IRS

TABLE 6: HLC IN IJAJI, CONTROL SITE, MARCH 2015-FEBRUARY 2016

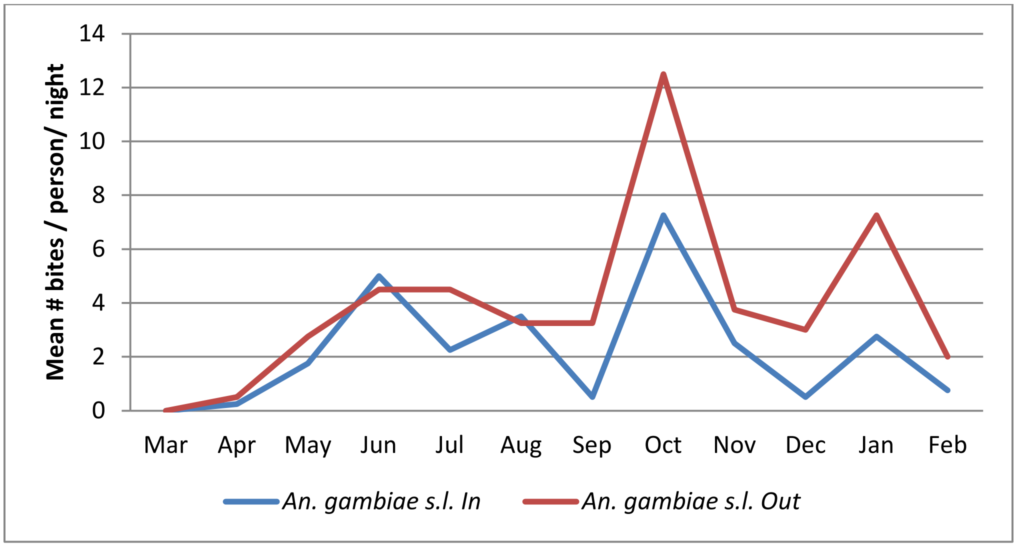
Time	*An. gambiae* s.l.					*An. pharoensis*			*An. coustani*			*An. demeilloni*			*An. christyi*			Total Anophelines collected		
	In	MBR In	Out	MBR Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Grand total
March	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
April	1	0.25	2	0.5	3	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3
May	32	8	18	4.5	50	0	0	0	0	0	0	0	0	0	0	0	0	32	18	50
June	15	3.75	16	4	31	0	0	0	0	0	0	0	3	3	1	0	1	16	19	35
July	39	9.75	29	7.25	68	0	0	0	0	0	0	0	0	0	0	0	0	39	29	68
August	27	6.75	17	4.25	44	0	0	0	0	0	0	0	0	0	0	0	0	27	17	44
Sept	52	13	66	16.5	118	0	0	0	0	1	1	0	1	1	0	0	0	52	68	120
Oct	10	2.5	10	2.5	20	0	1	1	1	2	3	1	0	1	0	0	0	12	13	25
Nov	5	1.25	3	0.75	8	0	0	0	0	0	0	0	0	0	0	0	0	5	3	8
Dec	1	0.25	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Jan	1	0.25	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Feb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

FIGURE 6: HLC IN GOBU SAYO, INTERVENTION SITE, MARCH 2015-FEBRUARY 2016



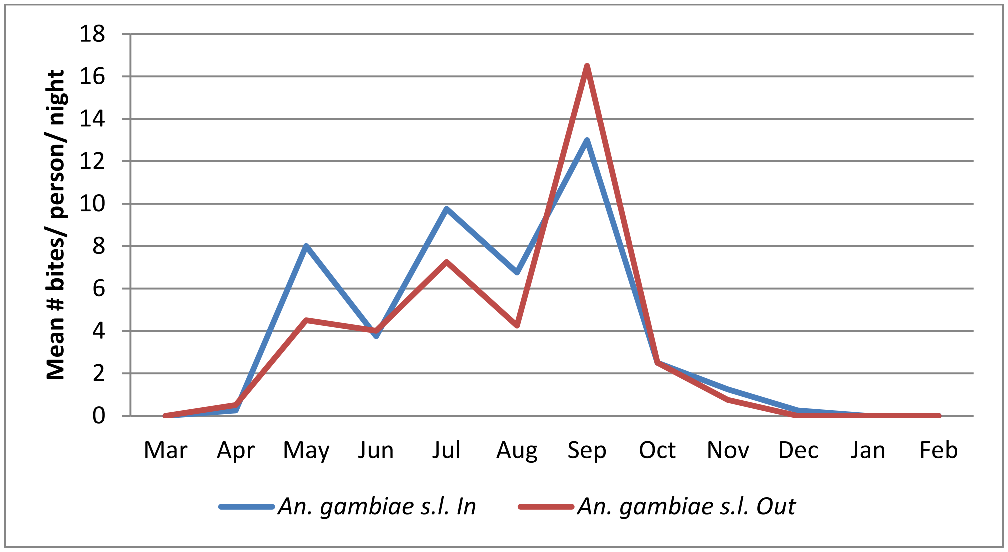
NB: April to Aug: Pre-spray; Sept to Dec: Post IRS

FIGURE 7: HLC IN SEKA CHEKORSA, INTERVENTION SITE, MARCH 2015-FEBRUARY 2016



NB: April to Aug: Pre-spray; Sept to Dec: Post IRS

FIGURE 8: HLC IN IJAJI, CONTROL SITE, MARCH 2015-FEBRUARY 2016



As indicated in Figures 9, 10, and 11, the biting time of the main malaria vector, *An. gambiae* s.l. is similar in Gobu Seyo intervention and Ijaji control sites (0.100 – 03.00 hours). *An. gambiae* s.l. in Seka Chekorsa sentinel sites attempted to bite at higher numbers in the first half of the night and decreased progressively throughout the night. In both intervention sites, there was a reduction in biting rate of the major vector following the spray operation. A larger proportion of *An. phareonsis* and *An. coustani* were caught seeking human hosts between 7:00pm and 10:00pm in the intervention sites (Annex- B and Annex- C). Since only one *An. pharoensis* and 4 *An. coustani* were found in Ijaji control site, it wasn't presented in the line graph.

In Gobu Sayo, the indoor mean biting rate decreased from 8.2 bites per person per night before IRS (March – August) to 3.67 bites per person per night after spray (September- November) though the decline was statistically not significant (p=0.3232). This could be attributed to the low number of mosquitoes collected during the monitoring period. In the same site, the mean outdoor biting rate decreased from 10.67 bites per person per night before IRS to 7.67 bites per person per night post IRS (p=0.63). However, in the control site, Ijaji, during the same period the mean indoor biting rates increased from 4.75 to 5.58 bites per person per night during the period coinciding with IRS in the intervention sites and the mean outdoor biting increased from 2.1 before IRS to 6.58 bites per person per night ; the difference was not statistically significant (P>0.05). The difference in the biting rate observed between the control and intervention sites (i.e. increase in the control and decrease in the intervention site post IRS) might be explained by the impact of IRS.

In Seka Chekorsa sentinel site, the mean indoor biting rates increased from 2.1 pre-spray (March – August) to 3.4 bites per person per night post-spray (September – November). Similarly, the mean outdoor biting rates also increased from 2.6 to 6.5 bites per person per night (p=0.123) over the period. In this district the biting rate dropped immediately after spraying but went up again two months later. The short residual life of bendiocarb might partially explain why mean biting rates went down immediately after spray but increased two months later.

In both intervention sites, Gobu Seyo and Seka Chekorsa, on average *An. gambiae* s.l. biting rates were higher outdoors compared to indoors. For example, in Gobu Seyo District the mean outdoor biting rate over the survey period was 8.06 bites per person per night outdoor and 5.42 indoor. In Seka Chekorsa the mean outdoor biting rate was 3.9, and the indoor biting rate was 2.3. However, in the control site, on average, *An. gambiae* s.l. was found to bite more indoors than outdoors, exhibiting endophagic tendencies. The mean biting rate was 3.73 and 3.79 bites per person per night outdoor and indoor, respectively. The difference in the outdoor and indoor biting rates was not statistically significant in the control site (P>0.05). Though bendiocarb is assumed to have little or no repellent effect, the difference in human biting rates noted between the intervention and control sites (i.e. outdoor biting in the intervention and indoor biting in the control), could be attributed to the impact of the sprayed insecticide (repellency or killing effect).

It seems apparent that the IRS intervention tended to suppress the vector biting as biting rates were greatly reduced after spray in both intervention sites when the vector density was expected to peak in September. The high number of mosquitoes collected pre-spray indicates the need to revisit the spraying time based on the residual life of insecticide used for IRS in the country.

FIGURE 9: BITING TREND OF *AN. GAMBIAE* S.L. GOBU SAYO, INTERVENTION SITE

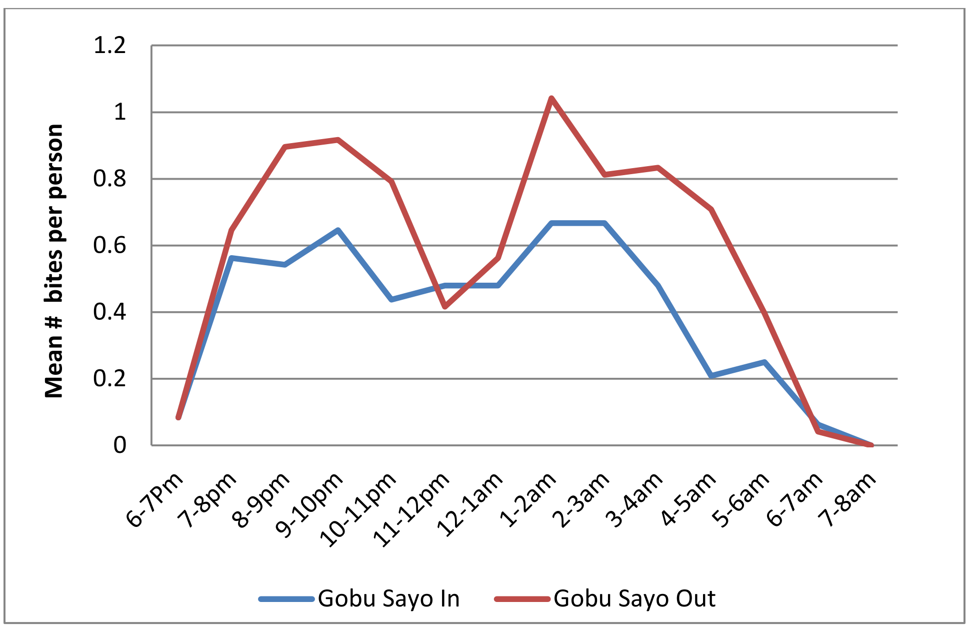


FIGURE 10: BITING TREND IN *AN. GAMBIAE* S.L. SEKA CHEKORSA, INTERVENTION SITE

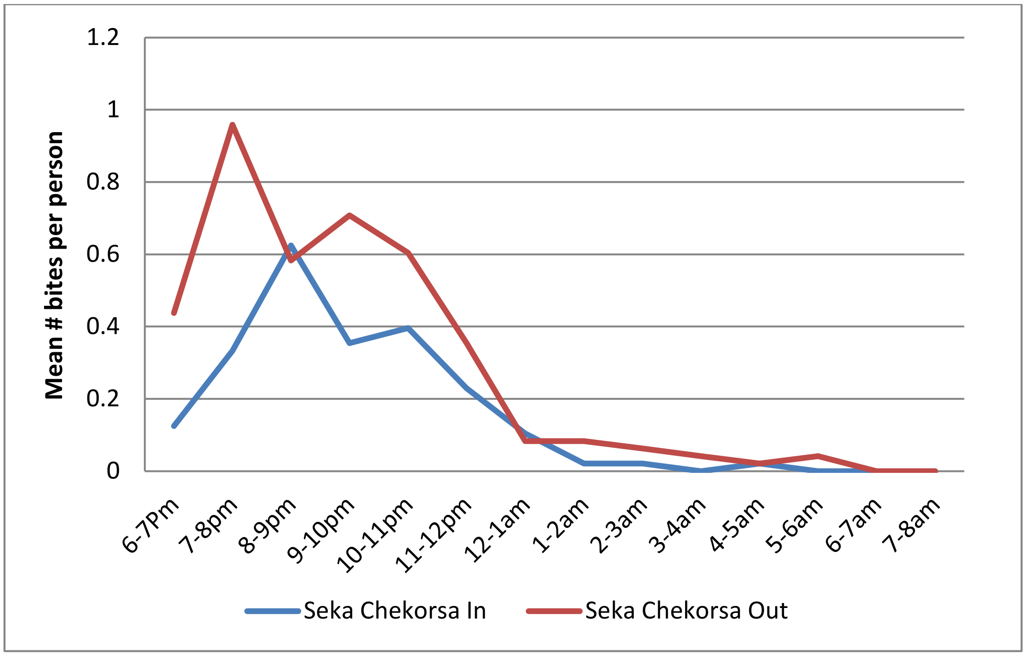
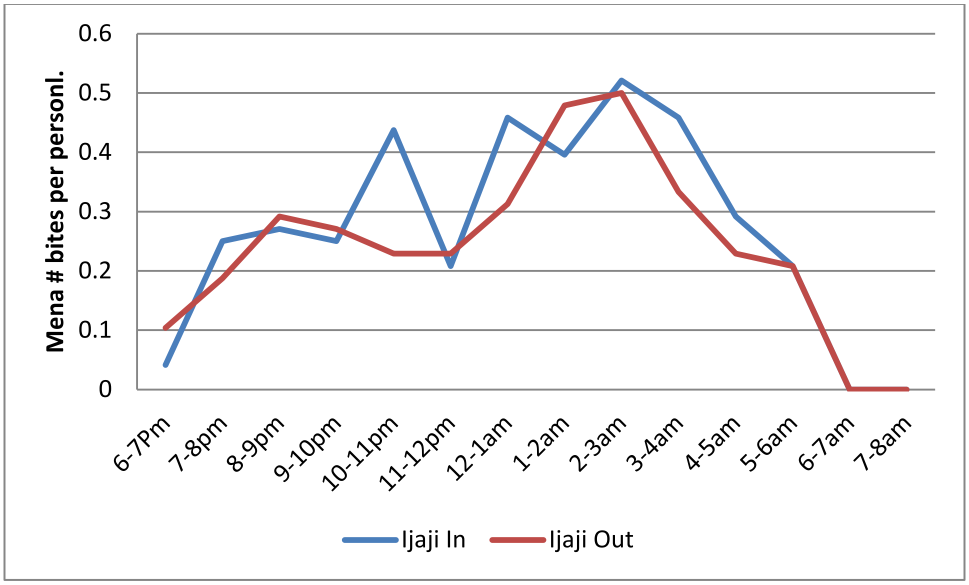


FIGURE 11: BITING TREND OF *AN. GAMBIAE* S.L. IJAJI, CONTROL SITE



5.5 CDC LIGHT TRAPS

An. gambiae s.l. comprised 13.3% (n= 306) of the total female anophelines collected from Gobu Sayo, Seka Chekorsa (interventions) and Ijaji (control) sites using CDC light trap indoors (Table 7 and 8).. There was a reduction in the number *An. gambiae* s.l. collected from intervention sites in the first two months after spraying (Figure 12). A reduction in the number of mosquitoes collected in the control site in September and subsequent months was observed. It is unclear why the number of mosquitoes collected from the control site using CDC light traps was low in September when the other sampling methods such PSC and HLC showed an increase.

TABLE 7: INDOOR CDC LIGHT TRAP COLLECTIONS, INTERVENTION SITES

Time	Gobu Sayo (Intervention)						Seka Chekorsa (Intervention)					
	An. gambiae s.l.	*An. pharoensis*	*An. coustani*	*An demeilloni*	*An. christyi*	Total	*An. gambiae* s.l.	*An. pharoensis*	*An. coustani*	*An. squamosus*	*An, christyi*	Total
March	0	3	0	0	0	3	0	5	0	0	0	5
April	8	8	0	0	0	16	0	5	0	0	0	5
May	23	4	0	0	0	27	16	27	0	0	0	43
June	41	11	6	0	0	58	19	8	0	0	0	27
July	26	38	118	0	1	183	6	3	0	0	0	9
Aug	25	116	247	0		388	8	14	13	0	0	35
Sept	7	360	295	0	0	662	1	1	11	0	0	13
Oct	3	228	200	0	0	431	0	0	0	0	0	0
Nov	3	31	57	0	0	91	23	0	3	0	0	**26**
Dec	0	1	3	0	0	4	2	0	0	0	0	**2**
Jan	2	3	4	0	0	9	23	0	0	0	0	23
Feb	8	7	1	0	0	16	0	0	0	0	0	0

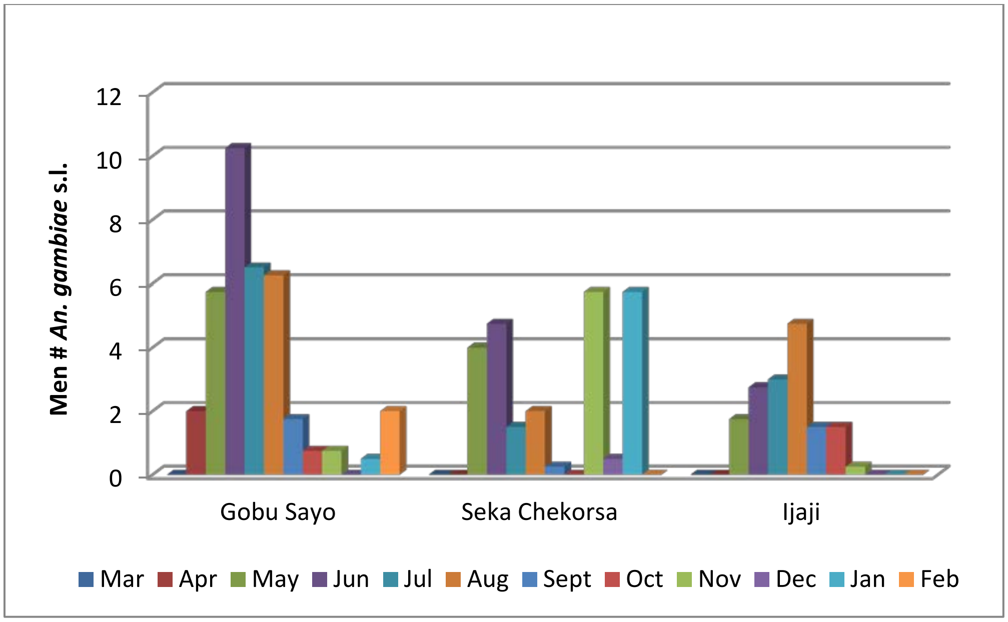
NB: April to Aug: Pre-spray; Sept to Dec: Post IRS

TABLE 8. INDOOR CDC LIGHT TRAP COLLECTIONS, CONTROL SITE

Time	Ijaji town						
	An. gambiae s.l.	*An. pharoensis*	*An. coustani*	*An. squamosus*	*An. demeilloni*	*An. chrysti*	Total
March	0	0	0	0	0	0	0
April	0	0	0	0	0	0	0
May	7	0	0	0	0	0	7
June	11	0	0	0	0	0	11
July	12	0	0	0	0	0	12

Aug	19	0	0	0	0	0	19
Sept	6	0	0	0	0	0	6
Oct	6	0	2	0	4	0	12
Nov	1	0	0	0	0	0	1
Dec	0	0	0	0	0	0	0
Jan	0	0	0	0	0	0	0
Feb	0	0	0	0	0	0	0

FIGURE 12: *AN. GAMBIAE* S.L. INDOOR CDC LIGHT TRAP COLLECTIONS IN THREE SITES



NB: Gobu Sayo and Seka Chekorsa: Intervention site; Ijaji: Control site

5.6 WINDOW EXIT TRAP COLLECTIONS

The objectives of window exit trap collection include:

- To determine mosquito species that bite indoors but try to move outdoors;
- To determine the effect of indoor residual spraying and insecticide-treated nets on the normal movement and feeding habits of mosquitoes;
- To determine the residual effects of insecticides as indicated by the numbers of dead mosquitoes collected and by the 24-hour mortality rate of mosquitoes found alive in the traps.

As indicated in Table 9, 10, 11 & Figure 13 generally few female *An. gambiae* s.l. were collected in exit traps hence no meaningful conclusion could be made on the effect of insecticide excito-repellency or residual life of insecticides.

TABLE 9: WINDOW EXIT TRAP COLLECTION, GOBU SEYO, INTERVENTION SITE

Time	Anopheles				Culex	*An. gambiae* s.l. collected	Dead at collection	Alive at collection	Dead after 24	Alive after 24 hrs	*Blood digestion stages ND			
	An. gambiae s.l.	*An. pharoensis*	*An. coustani*	Total							UF^	F^	HG^	G^
March	0	0	0	0	0	0	0	0	0	0				
April	8	8	0	16	66	2	1	1	0	1				
May	1	0	0	0	0	1	0	1	0	1				
June	30	0	0	0	5	30	0	30	0	30				
July	2	0	1	3	2	2	0	2	0	2				
August	5	7	0	0	3	5	0	5	0	5				
Sept	5	3	2	10	6	5	0	5	1	4				
Oct	1	0	0	1	0	1	0	1	0	1				
Nov	0	0	0	0	0	0	0	0	0	0				
Dec	0	0	0	0	9	0	0	0	0	0				
Jan	0	0	0	0	0	0	0	0	0	0				
Feb	0	0	0	0	0	0	0	0	0	0				

*ND: NOT DONE

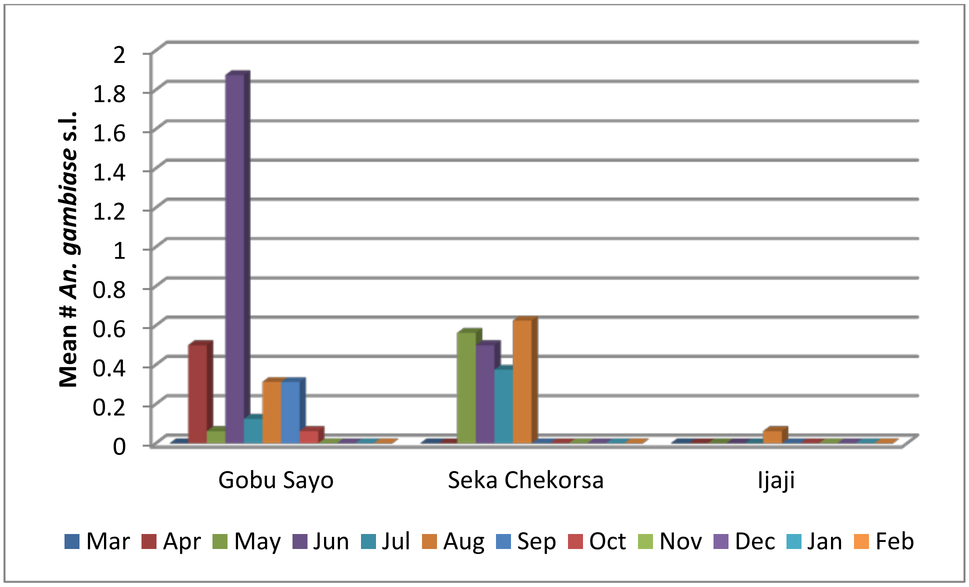
TABLE 10: WINDOW EXIT TRAP COLLECTION, SEKA CHEKORSA, INTERVENTION SITE

Time	Anopheles				Culex	*An. gambiae* s.l. collected	Dead at collection	Alive at collection	Dead after 24 hrs	Alive after 24 hrs	Blood digestion stages			
	An. gambiae s.l.	*An. pharoensis*	*An. coustani*	Total							UF^	F^	HG^	G^
March	0	0	0	0	0	0	0	0	0	0	0	0	0	0
April	0	2	0	2	0	0	0	0	0	0	0	0	0	0
May	9	0	0	9	0	9	0	9	0	9	2	1	3	3
June	8	0	0	8	0	8	0	8	0	8	0	3	1	4
July	6	0	0	6	28	6	0	6	0	6	2	1	0	3
August	10	0	0	10	0	10	0	10	0	10	0	4	5	1
Sept	0	0	0	0	6	0	0	0	0	0	0	0	0	0
Oct	0	0	0	0	4	0	0	0	0	0	0	0	0	0
Nov	0	0	0	0	11	0	0	0	0	0	0	0	0	0
Dec	0	0	0	0	5	0	0	0	0	0	0	0	0	0
Jan	0	0	0	0	66	0	0	0	0	0	0	0	0	0
Feb	0	0	0	0	37	1	0	1	0	1	0	1	0	0

TABLE 11: WINDOW EXIT TRAP COLLECTION, IJAJI, CONTROL SITE

Time	Anopheles				Culex	*An. gambiae* s.l. collected	Dead at collection	Alive at collection	Dead after 24 hrs	Alive after 24 hrs	Blood digestion stages			
	An. gambiae s.l.	*An. pharoensis*	*An. demoilonii*	Total							UF^	F^	HG^	G^
March	0	0	0	0	8	0	0	0	0	0	0	0	0	0
April	0	0	0	0	1	0	0	0	0	0	0	0	0	0
May	0	0	0	0	1	0	0	0	0	0	0	0	0	0
June	0	0	0	0	2	0	0	0	0	0	0	0	0	0
July	0	0	0	0	6	0	0	0	0	0	0	0	0	0
August	1	0	0	1	13	1	0	1	0	1	0	1	0	0
Sept	0	0	0	0	4	0	0	0	0	0	0	0	0	0
Oct	0	0	1	1	7	0	0	0	0	0	0	0	0	0
Nov	0	0	0	0	8	0	0	0	0	0	0	0	0	0
Dec	0	0	0	0	32	0	0	0	0	0	0	0	0	0
Jan	0	0	0	0	17	0	0	0	0	0	0	0	0	0
Feb	0	0	0	0	0	0	0	0	0	0	0	0	0	0

FIGURE 13: WINDOW EXIT TRAP COLLECTION IN THE STUDY SITES



NB: Gobu Sayo and Seka Chekorsa: Intervention site; Ijaji: Control site

5.7 DETERMINATION OF PARITY

The data for the study indicate that parous rates reduced drastically in both intervention sites 2–3 months after IRS but increased in subsequent months. However, the parity rate remained high in the control site (Table 12). Monthly parous rates for *An. gambiae* s.l. were variable between sites throughout the period of study with generally higher rates recorded between April and August in the intervention sites (Gobu Sayo: 76.8 – 100%; Seka Chekorsa: 33.3 – 67%). Parous rate greatly reduced in both intervention sites after IRS but remained the same in the control site. In Ijaji control site parous rates remained high during the whole period of study (93.3 – 100%).

TABLE 12: TRENDS IN PARITY RATE IN THREE SENTINEL SITES

Time	Gobu Sayo				Seka Chekorsa				Ijaji			
	An. gambiae s.l. Collected	# dissected	Parous	% parous	*An. gambiae* s.l. Collected	# dissected	Parous	% parous	*An. gambiae* s.l. Collected	# dissected	Parous	% parous
March	0	0	0	0	0	0	0	0	0	0	0	0
April	12	10	9	90	3	3	1	33.3	3	3	3	0
May	71	69	69	100	18	18	8	44	50	48	47	97.9
June	142	139	119	83.8	38	38	16	42	31	30	28	93.3
July	112	108	86	76.8	27	27	11	40.7	68	68	68	100

Aug	116	114	104	89.7	27	27	18	67	44	44	44	100
Sept	93	88	53	57.0	15	10	1	9	118	115	115	100
Oct	29	29	16	55.2	79	79	31	39	20	20	20	100
Nov	14	14	11	78.6	25	25	6	24	12	12	12	100
Dec	31	31	24	77.4	40	40	20	50	1	1	1	100
Jan	14	14	14	100	40	40	20	50.0	0	0	0	0
Feb	24	24	22	91.67	11	11	3	27.3	0	0	0	0

NB: April to Aug: Pre-spray; Sept to Dec: Post IRS

5.8 MOLECULAR AND ELISA TESTS

5.8.1 PRE-SPRAY SPOROZOITE ELISA TEST

Overall, 1,275 anopheline mosquitoes belonging to three species (*An. gambiae* s.l., *An. pharoensis,* and *An. coustani*) were tested before spray operations for *Plasmodium* circumsporozoite protein. Mosquitoes collected through HLC were used for this test. Of 509 *An. gambiae* s.l. samples tested from the three sites, four specimens were positive for *P. vivax* and three specimens for *P. falciparum* circumsporozoite protein, giving a sporozoite rate of 0.78 percent and 0.58 percent, respectively. Moreover, two *An. pharoensis* specimen were positive for *P. falciparum and P. vivax* (Table 13).

TABLE 13. SPOROZOITE RATES OF ANOPHELINE MOSQUITOES COLLECTED FROM SEKA CHEKORSA, GOBU SAYO AND IJAJI BEFORE SPRAY OPERATION

Site	Species	Number tested	Pv-210 positive	PV-247 positive	Pf positive
Seka Chekorsa	*An. gambiae* s.l.	120	0	0.83 (1)	0
	An. pharoensis	129	0	0	0
	An. coustani/ Ziemanni	269	0	0	0
Gobu Seyo	*An. gambiae* s.l.	290	0.34 (1)	(0.34 (1)	1.03 (3)
	An. pharoensis	191	0.52 (1)	0	0.52 (1)
	An. coustani/ Ziemanni	168	0	0	0
Ijaji	*An. gambiae* s.l.	99	1.01 (1)	0	0
	An. pharoensis	9	0	0	0
	An. coustani/ Ziemanni	0	0	0	0
Overall	*An. gambiae* s.l.	509	0.39 (2)	0.39 (2)	0.59 (3)
	An. pharoensis	329	0.30 (1)	0	0.30 (1)
	An. coustani/ Ziemanni	398	0	0	0

Values in parenthesis represent number of Anopheles positive for circumsporozoite protein

5.8.2 POST-SPRAY SPOROZOITE ELISA

A total of 2,465 anopheline mosquito specimens collected from three different sites after spray operations were tested for *Plasmodium* circumsporozoite proteins. About 46.5 percent of the analyzed specimens were *An. coustani,* 35.1 percent were *An. pharoensis* and the remaining 18.4 percent were *An. gambiae* s.l. Out of the analyzed specimens four were positive for *P. falciparum* and eight for *P. vivax* circumsporozoite proteins giving sporozoite rates of 0.162 percent and 0.324 percent, respectively (Table 14). The entomological inoculation rate (infective bites per person per night) for *An. gambiae* s.l. derived based on specimens that tested positive for *Plasmodium* circumsporozoite proteins (*P. falciparum* and *P. vivax*) is shown in Table 15.

TABLE 14. SPOROZOITE RATES OF ANOPHELINE MOSQUITOES COLLECTED FROM THREE SITES AFTER SPRAY OPERATION

Site	Species	Number tested	Pv-210	PV-247	Pf
Seka Chekorsa	*An. gambiae* s.l.	140	0	0.71 (1)	0
	An. pharoensis	55	0	0	0
	An. coustani/ Ziemanni	230	0	0	0
Gobu Seyo	*An. gambiae* s.l.	140	1.43 (2)	1.43 (2)	0.71 (1)
	An. pharoensis	810	0.37 (3)	0	0.12 (1)
	An. coustani/ Ziemanni	910	0	0	2
Ijaji	*An. gambiae* s.l.	170	0	0	0
	An. pharoensis	0	0	0	0
	An. coustani/ Ziemanni	10	0	0	0
Overall	*An. gambiae* s.l.	450	0.44 (2)	0.67 (3)	0.22 (1)
	An. pharoensis	865	0.35 (3)	0	0.12 (1)
	An. coustani/ Ziemanni	1150	0	0	0.73 (2)

Values in parenthesis represent number of Anopheles positive for circumsporozoite protein

TABLE 15. ENTOMOLOGICAL INOCULATION RATE FOR *AN. GAMBIAE* S.L

	Gobu Seyo			Seka Chekorsa			Ijaji		
	MBR	***SR***	***EIR+***	***MBR***	***SR***	***EIR***	***MBR***	***SR***	***EIR***
March	0	*	-	0	*	-	0	*	-
April	3	*	-	0.75	*	-	0.75	*	-
May	17.75	5.71	1.01	4.5	0	0	12.5	0	0
June	35.5	0	0	9.5	0	0	7.75	0	0
July	28	0	0	6.75	0	0	17	0	0

Aug	29	1.11	0.32	6.75	3.33	0.23	11	3.33	0.37
Sept	23.25	5.56	1.29	3.75	0	0	29.5	0	0
Oct	7.25	0	0	19.75	1.25	0.25	5	0	0
Nov	3.5	0	0	6.25	0	0	2	0	0
Dec	7.75	0	0	3.5	0	0	0.25	0	0
Jan	3.25	*	-	10	*	-	0	*	-
Feb	3.5	*	-	2.75	*	-	0	*	-

*Samples not tested for *Plasmodium* circumsporozoite protein

+EIR: Infective bites per person per night

5.8.3 MOLECULAR IDENTIFICATION OF *AN. GAMBIAE* S.L.

A total of 364 *An. gambiae* s.l. selected randomly from specimens collected from eight study sites (from five regions) were identified to species using species-specific PCR. The results of the molecular analysis showed that only *An. arabiensis* was the representative sibling species of the gambiae complex for the eight study sites (n = 345). Some 21 species could not be identified as specimen DNA could not be amplified (Table 16).

TABLE 16. IDENTIFICATION OF AN. GAMBIAE S.L. SAMPLES TO SPECIES FOLLOWING BIOASSAY TEST IN EIGHT SITES IN ETHIOPIA

Region	Village/ Site	# *An. gambiae* s.l assayed for species ID	# *An. gambiae* s.l. specimen not amplified	# *An. arabiensis*
Gambella	Gambella	70	3	67
Oromyia	Asendabo	70	4	66
SNNPR	Halaba	40	5	35
Amhara	Bahir Dar	40	2	38
Oromyia	Meki/Zeway	40	0	40
Tigray	Alamata	25	1	24
Oromyia	Chewaka	40	6	36
Afar	Amibara	39	0	39
Total		**364**	**21**	**345**

5.8.4 STATUS OF KDR RESISTANCE FOR DELTAMETHRIN AND DDT

AIRS Ethiopia provided support in supplies and logistics to Jimma University to conduct molecular analysis to determine the mechanism of resistance in *An. gambiae* s.l. In 2015, some 364 female anopheline mosquitoes collected from eight sites were analyzed for *kdr* mutations using the polymerase chain reaction (PCR). The *kdr* mutation was assessed in surviving and dead *An. gambiae* s.l. randomly selected following bioassay test. Of the specimens analyzed, only 275 (73.4%) specimens were amplified. The result of the analysis showed that the West African *kdr* allele (L1014F) was common in populations of *An. gambiae* s.l. tested from the eight study sites in five regions of the country. The *kdr* allele frequency in surviving mosquitoes following the bioassay tests ranged from 50% to 100% while the *kdr* allele frequency in dead mosquitoes following bioassay ranged from 13% to 88% (Table 17). The results therefore suggest that the West African *kdr* allele frequency is fixed in most study areas.

TABLE 17. RESULTS OF PCR ANALYSIS FOR KDR RESISTANCE MECHANISM

Village Site		**Survival status after exposure**	**# mosquitoes assayed**	**Homozygote mutation (RR)**	**Heterozygote (RS)**	**Homozygote Wild type (SS)**	***Kdr* allele frequency**	
							R	S
Gambella	DDT	Alive	30	17	10	3	0.74	0.26
		Dead	5	0	4	0	0.5	0.5
	Deltamethrin	Alive	30	18	3	3	0.81	0.19
		Dead	5	1	1	0	0.75	0.25
Asendabo	DDT	Alive	30	14	6	0	0.85	0.15
		Dead	4	0	1	3	0.13	0.89
	Deltamethrin	Alive	30	16	4	4	0.75	0.25
		Dead	6	2	2	1	0.6	0.4
Halaba	DDT	Alive	15	8	1	0	0.94	0.06
		Dead	5	1	0	2	0.3	0.7
	Deltamethrin	Alive	15	5	2	1	0.75	0.25
		Dead	5	2	1	1	0.63	0.37
Bahir Dar	DDT	Alive	15	3	4	1	0.63	0.37
		Dead	5	0	1	3	0.13	0.87
	Deltamethrin	Alive	15	3	6	3	0.5	0.5
		Dead	5	0	1	3	0.13	0.87
Meki/Zeway	DDT	Alive	15	3	4	3	0.5	0.5
		DDT dead	ND	ND	ND	ND	ND	ND
	Deltamethrin	Alive	15	3	4	3	0.5	0.5
		Dead	10	0	3	5	0.19	0.81
Alamata	DDT	Alive	9	9	0	0	1	0
		Dead	5	3	1	0	0.88	0.12
	Deltamethrin	Alive	8	6	0	0	1	0
	Lambda cyahalothrin	Dead	3	1	0	0	1	0
Chewaka	DDT	Alive	15	1	6	6	0.31	0.69
		Dead	5	1	1	3	0.3	0.7
	Deltamethrin	Alive	15	2	4	5	0.36	0.64
		Dead	5	1	1	0	0.75	0.25
Amibara	DDT	Alive	14	6	2	1	0.78	0.22
		Dead	5	1	2	2	0.4	0.6
	Deltamethrin	Alive	15	2	7	3	0.46	0.54
		Dead	5	1	3	1	0.5	0.5

6. DISCUSSION, LESSONS LEARNED, AND CHALLENGES

1. The longitudinal entomological study conducted in three sites to determine vector abundance, biting behavior, resting behavior and parity rates presents critical information to be considered in planning and implementing vector control interventions including IRS. Accurate targeting of IRS is critical in achieving desired results. The data so far indicate that it would be beneficial to commence IRS in May/early June to achieve effective control of the vectors before their population builds up in July and August during the peak rainy period. This is true while using an insecticide with long residual efficacy. While the implementation of IRS in August is practiced in Ethiopia using insecticides with short residual efficacy to provide protection during the period of peak malaria transmission, the efficiency of such application tends to be compromised due to the fact that transmission may already be on-going at high intensity.

2. The post spray assessment reduction in indoor resting density, human landing catches and parity rates indicated that IRS tended to achieve desired impact in controlling malaria vectors.

3. *Plasmodium falciparum and P. vivax* circumsporozoite antigens, detection in *An. pharoensis* and *An. coustani/ ziemanni* specimens tested, points to the importance of the two species in malaria transmission in the country. This therefore calls for further entomological monitoring in different parts of the country to better understand their role in malaria transmission against the changing climatic conditions.

4. The early evening biting (6:00 p.m. – 9:00 p.m.) of the malaria vectors in the three study sites is an indication that transmission may occur before people go to bed hence compromising the protection afforded by LLINs.

5. Since very few mosquitoes were collected in window exit traps, it is hard to make conclusions on either excito-repellency effect or residual life of insecticides.

Challenges:

- There was a delay in molecular analysis of mosquito samples and reporting by Jimma University. To address the delay and timeline, the AIRS Ethiopia signed an MOU with the Jimma University to resolve the challenges and also provided needed supplies.

7. RECOMMENDATIONS

- Based on these entomological findings in three sentinel sites, it would be important to expand similar activities to other areas of the country (sentinel sites) to provide a national profile of vector bionomics.

- Based on the temporal distribution of vectors it is recommended to implement IRS in May with insecticides with long residual efficacy.

- Since the density of *An. coustani* was found to be greater than the main vector, it is very important to focus on sporozoite ELISA tests as well as availability of the vector in permanent breeding sites during the dry season.

- The participation of the NMCP in entomological monitoring activities should be strengthened to ensure ownership and quality through supervision.

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ANNEX A: SUMMARY RESULTS OF MOSQUITO COLLECTIONS

TABLE A 1: ANOPHELINES SPECIES IN GOBU SAYO (INTERVENTION SITE)

Time	*An. gambiae s.l.*					*An. pharoensis*					*An. coustani*					*An. demilonii*					*An. christyi*		*Culicine*				
	PSC	CDC	Exit trap	HLC	Total	PSC	CDC	Exit trap	HLC	Total	PSC	CDC	Exit trap	HLC	Total	PSC	CDC	Exit trap	HLC	Total	HLC	Total	PSC	CDC	Exit trap	HLC	Total
Mar	2	0	0	0	2	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	57	47	0	5	109
April	31	8	2	12	53	0	8	0	4	12	0	0	0	2	2	0	0	0	0	0	0	0	114	88	3	198	403
May	54	23	1	71	149	0	4	0	6	10	0	0	0	0	0	0	0	0	0	0	0	0	28	32	0	111	171
June	59	41	30	142	272	0	11	0	11	22	0	6	0	12	18	0	0	0	0	0	0	0	16	179	5	338	538
July	71	26	2	112	211	5	38	0	100	143	5	118	1	23	147	0	0	0	0	0	0	0	39	1007	2	374	1422
Aug	63	25	5	116	209	3	116	7	145	271	4	241	0	109	354	0	0	0	2	2	0	0	8	1561	3	454	2026
Sept	17	7	5	93	122	14	360	3	530	907	2	295	2	527	826	0	0	0	8	8	2	2	18	2023	6	2008	4055
Oct	5	3	1	29	38	3	228	0	189	420	3	200	181	0	384	0	0	0	0	0	0	0	8	259	0	947	1214
Nov	0	3	0	14	17	0	31	0	63	94	0	57	0	54	111	0	0	0	1	1	0	0	5	478	0	0	483
Dec	0	0	0	31	31	0	1	0	40	41	0	3	0	20	23	0	0	0	0	0	0	0	8	924	9	197	1138
Jan	9	23	0	40	72	0	0	0	6	6	0	6	0	21	27	0	0	0	0	0	0	0	30	31	11	66	138
Feb	8	0	1	11	20	0	2	0	6	8	0	0	0	6	6	0	0	0	0	0	0	0	5	44	6	37	92

TABLE A 2: ANOPHELINES SPECIES IN SEKA CHOKORSA (INTERVENTION SITE)

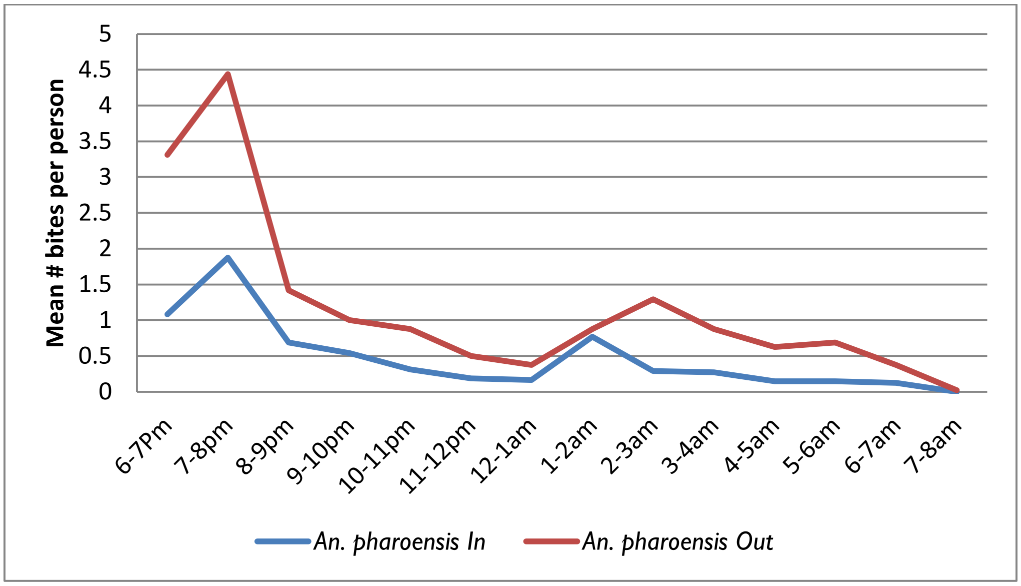
Time	*An. gambiae s.l.*					*An. pharoensis*					*An. coustani*					*An. squamosus*		An. christyi		*Culicine*				
	PSC	CDC	Exit trap	HLC	Total	PSC	CDC	Exit trap	HLC	Total	PSC	CDC	Exit trap	HLC	Total	HLC	Total	HLC	Total	PSC	CDC	Exit trap	HLC	Total
March	1	0	0	0	1	3	5	0	21	29	0	0	0	0	0	0	0	0	0	5	7	0	20	32
April	11	0	0	3	14	0	5	2	8	15	0	0	0	1	1	0	0	0	0	5	76	0	65	146
May	94	16	9	18	137	0	27	0	62	89	0	0	0	13	13	0	0	0	0	4	222	0	201	423
June	10	19	8	38	75	1	8	0	42	51	0	0	0	20	20	0	0	0	0	39	71	0	168	278
July	52	6	1	27	86	0	3	0	42	45	1	0	0	110	111	0	0	3	3	152	40	32	226	450
Aug	48	8	9	27	92	11	14	1	89	115	0	13	0	168	181	3	3	0	0	106	92	0	260	458
Sept	0	1	0	15	16	3	1	0	6	10	1	11	0	176	188	3	3	0	0	1	26	6	136	169
Oct	1	0	0	79	80	0	0	0	9	9	2	0	0	94	96	0	0	0	0	16	21	4	159	200
Nov	3	0	0	25	28	0	0	0	5	5	0	0	0	24	24	0	0	0	0	11	25	11	52	99
Dec	6	2	0	14	22	1	0	0	8	9	1	0	0	62	63	0	0	0	0	12	30	5	76	123
Jan	9	23	0	40	72	**0**	**0**	**0**	**6**	**6**	**0**	**6**	**0**	**21**	**27**	0	0	0	0	30	31	11	66	138
Feb	7	0	1	11	20	**0**	**2**	**0**	**6**	**8**	**0**	**0**	**0**	**6**	**6**	0	0	0	0	5	44	6	37	92

TABLE A 3: ANOPHELINES SPECIES IN IJAJI (CONTROL SITE)

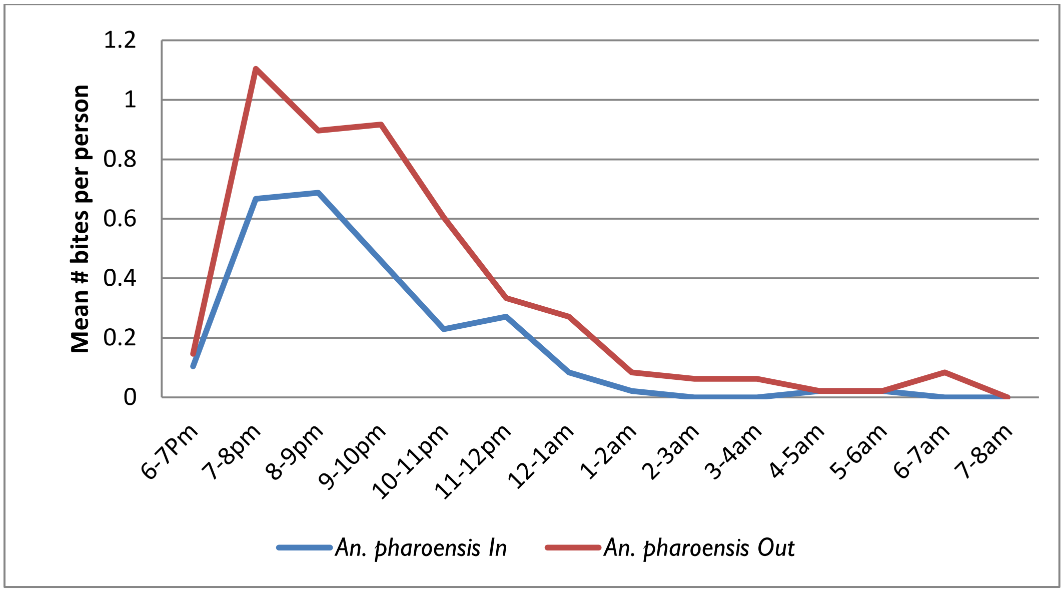
Time	*An. gambiae s.l.*					*An. pharoensis*			*An. coustani*				*An. demeilloni*					An. christyi		*Culicine*				
	PSC	CDC	Exit trap	HLC	Total	PSC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	Exit trap	HLC	Total	PSC	Total	PSC	CDC	Exit trap	HLC	Total
Mar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60	163	8	137	368
Apr	6	0	0	3	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	165	1	272	477
May	27	7	0	50	84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	27	1	39	845
June	27	11	0	31	69	0	0	0	0	0	0	0	0	0	0	3	3	0	1	161	97	2	178	445
July	24	12	0	68	104	0	0	0	0	0	0	0	1	0	0	0	1	0	0	400	112	6	408	928
Aug	40	19	1	44	104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	713	447	13	364	1373
Sept	74	6	0	118	198	0	0	0	0	0	1	1	0	0	0	1	1	2	2	2105	117	4	660	2894
Oct	9	6	0	20	35	1	1	2	1	2	3	6	0	4	1	1	6	0	0	391	289	7	1201	1888
Nov	3	1	0	8	12	1	0	1	0	0	0	0	0	0	0	0	0	0	0	283	240	8	578	4782
Dec	2	0	0	1	3	0	0	0	1	0	0	1	0	0	0	0	0	0	0	136	70	32	139	377
Jan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	63	17	82	192
Feb	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	37	15	0	18	71

ANNEX B: Biting Cycle of *An. pharoensis*

Biting cycle of *An. pharoensis* in Gobu Seyo intervention site

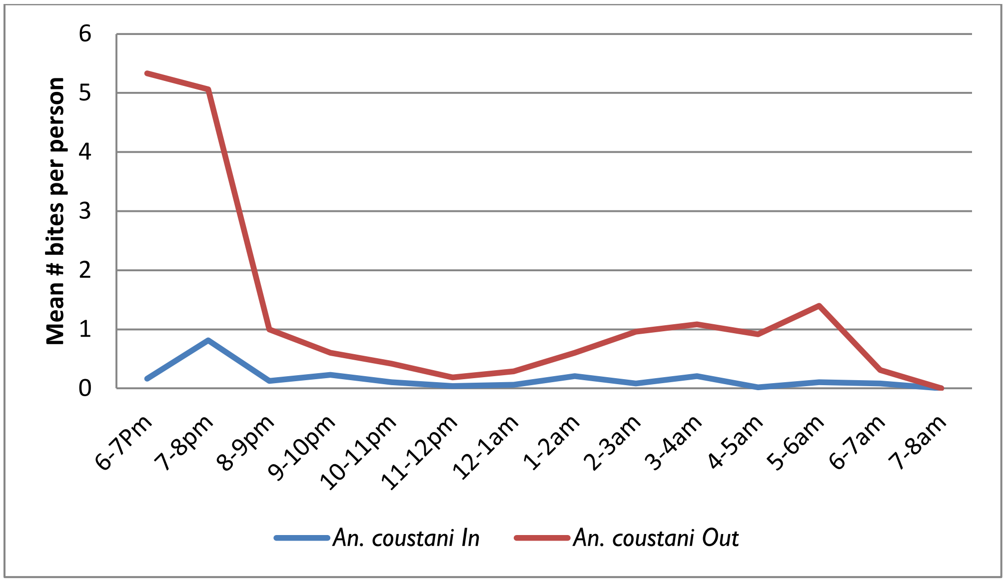


Biting cycle of *An. pharoensis* in Seka Chekorsa intervention site

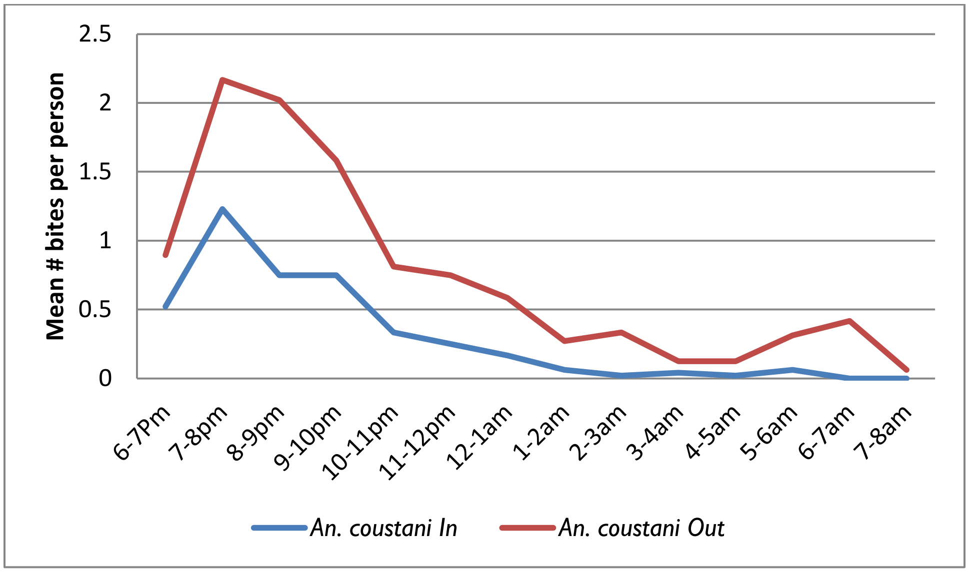


Annex C: Biting Cycle of *An. coustani*

Biting cycle of *An. coustani* in Gobu Seyo intervention site



Biting cycle of *An. coustani* in Seka Chokorsa intervention site



ANNEX D: 2015 VECTOR SUSCEPTIBILITY TESTING

INTRODUCTION

Entomological monitoring is one of the key activities that the PMI is supporting nationally in Ethiopia. When the PMI-funded IRS project, led by Research Triangle Institute, began its entomological monitoring activities in 2008, it detected a high level of vector resistance to dichlorodiphenyltrichloroethane (DDT). The insecticide had been used for IRS for five decades. As a result, in 2009, Ethiopia's government-funded national IRS program and the PMI IRS project switched to the use of a pyrethroid class of insecticides. However, 2010–2011 studies on IR by PMI/ Research Triangle Institute, the WHO, the FMOH, and Jimma, Mekelle, Dilla, and Addis Ababa universities showed that resistance to deltamethrin and other pyrethroids had spread to many parts of the country. This finding called on the national IRS program to reconsider the use of pyrethroids. In 2013 the national IRS program sprayed bendiocarb and propoxur and the PMI-funded IRS project used bendiocarb in its target districts. In 2014 PMI AIRS again sprayed bendiocarb. In 2015, PMI AIRS in collaboration with FMOH piloted Actellic 300 CS in eight project districts.

Nationwide entomological monitoring needs to be done to obtain a complete picture of IR in the country. PMI is supporting expanded entomological work to generate data on key entomological variables, in particular the vectors' resistance to different insecticides. These data will guide and help to refine vector control activities of the PMI/AIRS Ethiopia project and the national IRS program.

In 2012 and 2013 AIRS Ethiopia had five sites for longitudinal monitoring of IR. This number was increased to eight fixed sentinel sites in 2014 and 2015. The study in two sites was contracted to Mekelle University, two sites were contracted to Jimma University, and four sites were covered by the AIRS Ethiopia team.

METHODOLOGY

MOSQUITO COLLECTION AND REARING

The entomology team used mosquitoes reared from field-collected larvae or pupae. All efforts were made to collect larvae and pupae from various breeding sites so that the mosquitoes tested were fully representative of the vector population in the area. Mosquitoes were morphologically identified at an adult stage and only mosquitoes that appear to be belonging to the main vector, *An. gambiae* s.l., were selected for the resistance test. Identification was double checked with dead mosquitoes after the test. Non *An. gambiae* were excluded from the count.

WHO TUBE TEST FOR SUSCEPTIBILITY

The teams used standard WHO tube test methodology to test susceptibility of the main vector, *An. gambiae* s.l., for an array of insecticides (WHO, 2013). Three to four replicates of 25 non-blood fed, 2-3 day old mosquitoes were exposed to insecticide-impregnated papers for one hour. Similarly, control mosquitoes were exposed to oil-impregnated papers. The number of knocked down mosquitoes were recorded during the exposure time at intervals of 15, 30, 45, 60 minutes and for another hour after mosquitoes were transferred to holding tubes. A mosquito is considered knocked down if it lies on its side on the floor of the exposure tube and is consequently unable to fly (WHO, 2013). Mortality counts were taken after 24 hours of the holding period. Cotton wool soaked in 10 percent sugar solution was placed on top of the holding tube and optimum temperature and relative humidity was kept from a damp towel placed on top of holding boxes where tubes were kept.

CDC BOTTLE ASSAY TESTS

The CDC bottle assay (Brogdon et al. 2010) was also used during the peak mosquito population season to test the susceptibility of the main vector to different insecticides recommended for use in vector control. Mosquitoes reared from field-collected larvae or pupae were used for the tests. Efforts were made to collect larvae and pupae from various breeding sites so that the mosquitoes tested were fully representative of the vector population in the area.

Stock solution was prepared for each insecticide tested by diluting technical grade insecticide in 50 ml of acetone. Each bottle was internally coated with one ml of stock solution. The control bottle was coated with one ml of acetone. The bottles were covered with mats and kept overnight in a dark place to dry. The assay was run by introducing 15-25 mosquitoes to each bottle using an aspirator. As soon as the mosquitoes were transferred to bottles, a timer was set and knockdown recorded at 30 minutes diagnostic time. Mosquitoes that survived the diagnostic dosage and time were assumed to be resistant to the insecticide tested. The following insecticides were tested using CDC bottle bioassays: 12 ug/bottle for bendiocarb, propoxur, deltamethrin, 21.5ug/bottle for permethrin, 20ug/bottle for pirimiphos-methyl and 100 ug/bottle for DDT.

DATA ANALYSIS

Interpretation of the status of susceptibility or resistance was made based on the WHO 2013 classification criteria. If the 24-hour mortality rate is higher than 98 percent, the vector is fully susceptible to the insecticide; between 80 and 98 percent, the vector is classified as suspected resistant; and if mortality is below 80 percent, the vector is classified as resistant. When the control mortality was between 5 and 20 percent, the average observed mortality was corrected using Abbott's formula (Abbott, 1925). When the control mortality was above 20% the test result was discarded and the test was repeated.

RESULTS AND DISCUSSION

Table 1 below shows a summary of the test results for WHO tube tests in eight sites. The main malaria vector, *An. gambiae* s.l., was tested for susceptibility to 11 insecticides. The results showed that according to the WHO classification, the vector was fully susceptible to pirimiphos-methyl, fenitrothion and propoxur in all study sites. It was fully susceptible to bendiocarb in 6 sites, suspected resistant recorded in one site and the vector was resistant in one of the 8 sites. The vector was resistant to bendiocarb in Bahrdar (87%). *An. gambiae* s.l. is still highly resistant to DDT and all the pyrethroids tested, including etofenprox. The vector is fully susceptible to malathion in two sites, possible resistant and resistant population recoded in two and three other sites, respectively.

Table 2 shows resistance results to the insecticides tested in 2012, 2013, 2014, and 2015. The vector remains highly resistant to DDT and the pyrethroids. There is a slight sign of reversal of DDT resistance in Halaba, Alamata, Gambela, and Amibara. The vector's status in these eight sites is also 'resistant' or 'possibility resistant' to malathion but the level is much less than DDT and the pyrethroids. The vector is 100% susceptible to pirimiphos-methyl in all areas and at all times. The resistance level to bendiocarb has decreased from 14% in 2014 to 5% in 2015 in Omonada, which is also one of the project districts, and is consistently resistant in Bahrdar.

The CDC bottle assay test was planned to be done in selected districts from all regions of Ethiopia. The aim was to collect information on the status of vector resistance to selected insecticides using the CDC bottle assay and introduce the CDC bottle assay method as an alternative and addition to the WHO tube techniques. The test was completed only in six districts. The test was not done in 12 districts for two reasons: either the trained technician was not able to find enough mosquitoes for the test or the trained district focal person was transferred to another place.

Intensity studies were conducted in three sites by the AIRS team and the rest was done by Jimma University and trained district staffs in the other two sites. The addition of synergist (PBO) restored susceptibility to deltamethrin and permethrin. The vector is fully susceptible to propoxur and bendiocarb in all six sites. The CDC bottle produced higher mortality to pyrethroids compared to the WHO tube tests. The low mortality of the vector when exposed to pirimiphos-methyl CS using CDC bottle bioassay could be due to the instability of the stock solution rather than vector resistance to the insecticide. The vector was resistant to permethrin and deltamethrin at all sites. However, pre-treatment with PBO fully restored the susceptibility of the vector to the two insecticides in Dugda site, a clear indication of the involvement of oxidase metabolic enzyme. Similarly in Halaba site, pre-treatment of the vector with PBO fully restored susceptibility to deltamethrin but not for permethrin. It is highly likely that permethrin resistance was mediated by other metabolic enzymes and not only oxidase.

TABLE B 1: SUMMARY OF IR TESTS 2015

Insecticide	% mortality							
	Region: SNNPR	Region: Oromia	Region: Oromia	Region: Amhara	Region: Tigray	Region : Afar	Region: Gambella	Region: Oromia
	Dsitrict: Halaba	Dsitrict: Omonada/ Asendabo	District: Zwai Dugda	District: Bahrdar	Dsitrict: Alamata	District: Amibara	District: Lare	District: Chewaka
	Site: Habiba	Site: Asendabo/Osso Billi	Site: Shenen/Burka	Site: Zenzlima-Robit	Site: Hadish Kigni	Site: Were/Sedi	Site: Kurgeng	Site: Mender 1,2 and 3
DDT	25(25/100) (R)	4 (4/100) (R)	ND	10.8(10/93) (R)	40 (30/75) (R)	48.0 (48/100) (R)	24 (24/100) (R)	14.4(15/104)(R)
Lambda-cyhalothrin	21.4(22/103) (R)	9 (9/100) (R	ND	10.7(8/75) (R)	34.7 (26/75) (R)	34.9 (39/101) (R)	24 (24/100) (R)	53.7(51/95)(R)
Deltamethrin	43(46/107) (R)	32 (32/100) (R)	31.5(32/101)(R)	25.3(19/75) (R)	57.3 (43/75) (R)	49.2 (50/102) (R)	11 (11/100) (R)	48.5(48/99)(R)
Fenitrothion	100(104/104) (S)	100 (100/100) (S)	100 (98/98) (S)	100(75/75) (S)	100 (100/100) (S)	100 (99/99) (S)	100 (100/100) (S)	100(99/99) (S)
Malathion	96.2(101/105)(POR)	83 (83/100) (R)	ND	43(43/100) (R)	100 (100/100) (S)	100 (102/102) (S)	88 (88/100) (R)	95.7(88/92) (POR)
Pirimiphos-methyl	100(103/103) (S)	98 (98/100) (S)	100 (100/100) (S)	100(75/75)(S)	100 (100/100) (S)	100 (101/101) (S)	100 (100/100) (S)	100(83/83)(S)
Propoxur	100(102/102) (S)	100 (100/100) (S)	100 (100/100) (S)	99(99/100)(S)	100 (100/100) (S)	100 (102/102) (S)	100 (100/100) (S)	100(100/100) (S)
Bendiocarb	100(103/103) (S)	95 (95/100) (POR)	100 (100/100) (S)	87(87/100) (R)	100 (100/100) (S)	100 (100/100) (S)	100 (100/100) (S)	100(100/100)(S)
Permethrin	24.8(26/105) (R)	22(22/100) (R)	12.8(13/102)(R)	9(9/100) (R)	89 (89/100) (R)	60.9 (65/97) (R)	28(28/100) (R)	20(16/80) (R)
Etofenprox	42.7(44/103) (R)	50 (50/100) (R)	ND	9.3(7/75) (R)	53.2 (42/75) (R)	78.9(75/94) (R)	86 (86/100) (R)	9.9(9/91)(R)
Alpha-cypermethrin	16.3(17/105) (R)	4 (4/100) (R)	ND	17.3(13/75) (R)	80 (80/100) (R)	70.3(73/101) (R)	ND	17.4(16/92)(R)

Note: ND-Not done due to scarcity of mosquitoes. Test was done after spray in all sites.

TABLE B 2: COMPARISON OF IR STATUS OF AN. GAMBIAE S.L. IN 2012, 2013, 2014 AND 2015 IN 7 FIXED SAMPLING SITES

Insecticides	IR MONITORING SITES																							
	Omonada				Zwai				Chewaka				Bahrdar				Halaba		Alamata		Gambela		Amibara	
	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015	2012	2015	2014	2015	2014	2015	2014	2015
DDT	3.8	9	6.8	4	13	26	6.2	ND	3	22	6	14.4	6	16	9.3	10.8	0	25	25	40	12.5	24	18.8	48
Lambda cyhalothrin	25.7	15	39	9	ND	-	4.3	ND	-	44	11.2	53.7	-	-	24	10.7	-	21.4	58	34.7	14.7	24	46.2	34.9
Deltamethrin	12.8	26	42	32	27	36	10.7	32	12	51	45.5	48.5	44	20	25.3	25.3	1	43	44	45.4	18.1	11	45.4	49.2
Fenitrothion	99.1	97	100	100	99	-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Malathion	66.1	81	73	83	90	90	92.9	ND	58	71	93.7	95.7	26	33.3	89.3	43	48	96.2	89	100	95.5	88	100	100
Pirimiphos-methyl	100	100	100	98	ND	100	100	100	-	100	100	100	100	100	100	100	-	100	100	100	100	100	100	100
Propoxur	98	98	100	100	100	100	100	100	96	100	100	100	100	96	99	99	99	100	98	100	100	100	100	100
Bendiocarb	93	92	86.4	95	100	100	100	100	90	100	100	100	87	75	87	87	98	100	96	100	92	100	100	100
Permethrin	10.9	22	16	22	ND	-	2.9	13		-	31.3	20	-	-	66	9		24.8	10	89	28.4	28	19.1	60.9
Alpha cypermethrin	24.8	-	35	50		32	5	ND		-	32.2	17.4	50	42.7	61	9.3	ND	16.3	19	53.2	11.4	86	86.6(	70.3
Etofenprox	8.7	-	55	4		20	28.7	ND		-	24	9.9	23	54.7	22.6	17.3	ND	42.7	77	80	14.7	ND	72.5	78.9

TABLE B 3: SUMMARY OF CDC BOTTLE ASSAY RESULTS CONDUCTED IN 2015

Region	District	Type of insecticide	% Mortality after 30 minutes				% Mortality after 45 minutes			
			1X	2X	5X	10X	1X	2X	5X	10X
Oromia	Asendabo	DDT (Intensity)						50.9	39.6	51.8
		Permethrin(Intensity)	14.1	13	41.9	65.6				
		Deltamethrin(Intensity)	41.9	67	71.7	75.7				
		Bendiocarb(Intensity)	100	100	100	100				
		Propoxur (Intensity)	100	100	100	100				
	Abedogora	Permethrin(Intensity)	76.2	95	95.5	100				
		Deltamethrin(Intensity)	91.3	100	100	100				
		Bendiocarb(Intensity)	100	100	100	100				
		Propoxur (Intensity)	100	100	100	100				
		Pirimiphos methyl	4.2	7.7		95.8				
	Dugda	Permethrin (Intensity)	0*	0*	80	100				
		Deltamethrin(Intensity)	63.6	64	77.8	90				
		Bendiocarb(Intensity)	100	100	100	100				
		Propoxur (Intensity)	100	100	100	100				
		Pirimiphos-methyl	0*	0*	50	85.7				
		Deltamethrin + PBO	100	100	100	100				
		Permethrin + PBO	100	100	100	100				
	Chewaka	Permethrin(Intensity)	0	10	100	100				
		Deltamethrin(Intensity)	50	90	100	100				
		Bendiocarb(Intensity)	100	100	100	100				
		Propoxur (Intensity)	100	100	100	100				
		Deltamethrin + PBO	100							
		Permethrin + PBO	100							
SNNPR	Halaba	Permethrin(Intensity)	0	92	78.3	100				
		Deltamethrin(Intensity)	21.7	71	89.3	88.5				
		Bendiocarb(Intensity)	100	100	100	100				
		Propoxur (Intensity)	100	100	100	100				

		Pirimiphos-methyl	0	11	3.7	50					
		Deltamethrin + PBO	100	100	100	100					
		Permethrin + PBO	21.7	100	70.8	100					
	Kachabira	Permethrin(Intensity)	5	80	85	100					
		Deltamethrin (Intensity)	5	80	85	95					
		Bendiocarb (Intensity)	100	100	100	100					
		Propoxur (Intensity)	100	100	100	100					
		Pirimiphos-methyl	0	0	0	5					

******Pirimiphos-methyl: Likely instability of stock solution*

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ANNEX E: WALL BIOASSAY FOR SPRAY QUALITY ASSURANCE AND MONITORING DECAY RATE

1. WALL BIOASSAY FOR QUALITY CHECK

Objectives:

- To assess the quality of the 2015 spray operation implemented by AIRS Ethiopia;
- Collect baseline data for decay rate monitoring and assess the effect of different wall surfaces on the decay rate of pirimiphos-methyl and bendiocarb.

The AIRS Ethiopia team conducted cone bioassay tests for quality check and decay rate in four sites; two DB IRS and two CB IRS districts. One CB IRS district and one DB IRS district were selected for the bioassay test.

The AIRS Ethiopia team performed the tests in 12 houses per site purposefully selected to represent houses sprayed by different SOPs and wall types. A total of 48 houses were sampled from the four sites. This year's bioassay includes sampling of different types of walls. The tests were carried out using known susceptible mosquito colonies reared in the Adama Malaria Reference Training Center insectary and wild mosquitoes reared from larvae or pupae (2–3-day-old sugar-fed adult *An. gambiae* s.l.).

TABLE C 1: SUMMARY QUALITY ASSESSMENT RESULTS IN PIRIMIPHOS-METHYL SPRAYED STRUCTURES IN JULY 2015

Zone	District	Site	Time	Type of colony	Percent Mortality (N)			
					Dung	Mud	Painted	Paper
Elubabor	Chewaka	Mender 2 (CB)	T0	Wild	-	-	-	-
				Susceptible	100 (60)	100 (120)	100 (150)	100(30)
			T1	Wild	-	-	-	-
				Susceptible	100(60)	98.4(62)	100(62)	100 (62)
Jimma	Tiro-Afeta	Kejelo (DB)	T0	Wild	100 (60)	100 (90)	100 (30)	ND
				Susceptible	100 (50)	100 (60)	100 (60)	
			T1	Wild	100(60)	100(90)	100(30)	
				Susceptible	98.3(60)	100(60)	98.3(60)	

N= Number of mosquitoes tested in parenthesis

TABLE C 2: SUMMARY OF QUALITY ASSESSMENT RESULTS IN BENDIOCARB SPRAYED HOUSES IN AUGUST 2015

District	Site	Time	Type of colony	Percent Mortality (N)			
				Dung	Mud	Painted	Paper
Bako Tibe	Gudine Welkite (CB IRS)	T0	Wild	-	-	-	-
		T0	Susceptible	100 (120)	90 (120)	100 (120)	-
Shebe Sombo	Alo Sebeka (DB IRS)	T0	Wild	-	95.8 (120)	100 (60)	-
		T0	Susceptible	-	95 (120)	100 (60)	-

N= Number of mosquitoes tested in parenthesis

Summary of the results of the wall bioassay tests conducted three to six days after spraying with pirimiphos-methyl are shown in Table1. Mortality of wild and susceptible mosquitoes was 100% for all wall surfaces tested. There was no difference in mortality between wild and susceptible mosquitoes exposed to sprayed wall surfaces.

There was also no difference in mortality of mosquitoes between CB IRS and DB IRS model sites on all wall surfaces sprayed with pirimiphos-methyl. Mortality was 100% in both sites (CB and DB IRS districts) as well as in all types of wall surface types in both districts.

The cone bioassay test results conducted in bendiocarb sprayed houses are shown on Table 2. Mortality of wild and susceptible mosquitoes was 100% for all dung plastered and painted houses. Mortality of wild and susceptible *An. gambiae* s.l. mosquitoes ranged from 90 to 95% on mud wall surfaces.

There was also no difference between CB IRS and DB IRS model sites for mortality rate of mosquitoes exposed to mud walls sprayed with bendiocarb in these two districts. The fact that all houses with less than 100% mortality rate had walls plastered with mud raises the issue of low bio-efficacy of bendiocarb in mud surfaces.

TABLE C 3. SUMMARY RESULTS FOR DIFFERENT EXPOSURE TIMES ON BENDIOCARB SPRAYED MUD WALL SURFACES IN BAKO TIBE

Exposure time in minutes	Type of wall	#HH	Type of mosquito	Percent Mortality (N)			
				2 days after IRS (T0)	One month after IRS (T1)	Two month after IRS (T2)	Three months after IRS (T3)
30	Mud	2	Susceptible	95(60)	11.7(60)	10(60)	13.8(58)
60		2		100(60)	23.3(60)	11.6(60)	16.7(60)
120		2		100(60)	31.7(60)	18.3(60)	27.9(58)

N= Number of mosquitoes tested in parenthesis

2. Wall Bioassay for Monitoring Decay Rate of Actellic and Bendiocarb

As part of the 2015 entomological monitoring activities, wall bioassay tests were conducted to assess the quality and subsequently monitor the decay rate of pirimiphos-methyl and bendiocarb. The cone bioassay test was conducted in four districts: two community-based IRS and two district-based IRS sites: Tiro Afeta (DB IRS) and Chewaka (CB IRS) Districts were sprayed with pirimiphos-methyl, and Bako Tibe (CB IRS) and Shebe Sombo (DB IRS) Districts were sprayed with bendiocarb insecticides.

As indicated in Tables 4 and 5, residual life of pirimiphos-methyl and bendiocarb varied on different wall surfaces. In Chewaka, pirimiphos-methyl mortality rate was 92.4% and 98.1% after five months with susceptible mosquitoes on mud and dung wall surfaces, respectively. The results over the months were not consistent. In Tiro Afeta, pirimiphos-methyl performed well on all wall surfaces with average mortality rate of 82.5% after five months. The decay rate of bendiocarb was faster on mud wall surfaces compared to other surfaces. In Bako Tibe, average mortality rate was 7.6% and 39.9% in Shebe Sombo on mud wall surfaces after four months of IRS. The overall test results are shown in Tables 6–27.

TABLE C 4. RESULTS OF WALL BIOASSAY FOR DECAY RATE OF PIRIMIPHOS-METHYL

Time	Tiro Afeta (July 2015 – Feb 2016)							Chewaka (July 2015 – Feb 2016)				
	% mortality											
	Susceptible			Wild			Mean	Susceptible				Mean
	Mud	Dung	Painted	Mud	Dung	Painted		Mud	Dung	Painted	Paper	
T0	100	100	100	100	100	100	100	100	100	100	100	100
T1	100	100	100	96.7	98.3	100	99.2	98.4	100	100	100	99.6
T2	100	98.3	98.3	100	100	100	99.4	62.5	73.3	100	100	83.9
T3	81.7	77.3	89.2	ND			82.7	61	63.3	89.7	84.4	74.6
T4	89.3	76.6	87.5	ND			84.5	60.1	69.3	91.9	ND	73.8
T5	93.7	74.2	79.6	ND			82.5	92.4	98.1	90.5	ND	93.7
T6	83.9	78.9	76.0	ND			79.6	57.1	58.3	54.1	ND	56.6
T7	73.8	68.4	62.6	ND			68.3	45.8	36.2	59.8	ND	47.3

TABLE C 5. RESULTS OF WALL BIOASSAY FOR QUALITY CHECK AND DECAY RATE OF BENDIOCARB

Time	Shebe Sombo (Aug - Dec 2015)					Bako Tibe (Aug - Dec 2015)			
	% mortality								
	Susceptible		Wild		Mean	Susceptible			Mean
	Mud	Painted	Mud	Painted		Mud	Dung	painted	

T0	95	100	100	100	98.8	90	100	100	96.7
T1	82.5	100	61.7	83.3	81.9	53.3	93.3	92.2	79.6
T2	60.6	99.2	ND		79.9	61.7	66.7	99.2	75.9
T3	41.7	94.6	ND		68.1	5.9	83.5	87.6	59
T4	39.9	100	ND		70	7.6	52.6	79.3	46.5

ND= Not done due to scarcity of wild mosquitoes

TABLE C 6: DISTRICT: CHEWAKA; KEBELE: MENDER 2; SPRAYED ON JULY 23, 2015; TEST COMPLETED ON JULY 25, 2015 (T0)

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed % mortality**	**% Control mortality**	**Corrected mortality**
1	Dung	30	30	100	0	
2	Painted	30	30	100	0	
3	Mud	30	30	100	0	
4	Painted	30	30	100	0	
5	Mud	30	30	100	0	
6	Mud	30	30	100	0	
7	Dung	30	30	100	0	
8	Mud	30	30	100	0	
9	Painted	30	30	100	0	
10	Painted	30	30	100	0	
11	Painted	30	30	100	0	
12	Paper	30	30	100	0	
	Total	360	360	100	0	

TABLE C 7: DISTRICT: CHEWAKA; KEBELE: MENDER 2; SPRAYED ON JULY 23, 2015; TEST COMPLETED ON AUGUST 25, 2015 (T1)

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed Test mortality (%)**	**% Control mortality (%)**	**Corrected mortality**
1	Dung	30	30	100	0	
2	Painted	32	32	100	0	
3	Mud	31	31	100	0	
4	Painted	30	30	100	0	

5	Mud	31	30	96.8	0	
6	Dung	30	30	100	0	
7	Paper	30	30	100	0	
	TOTAL	214	213	99.5	0	

Mosquito type: Wild reared

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	29	29	100	0	
2	Painted	30	30	100	0	
3	Mud	30	29	96.7	0	
4	Mud	29	26	89.7	0	
5	Painted	28	28	100	0	
6	Painted	30	30	100	0	
7	Paper	30	30	100	0	
	Total	206	202	98.1	0	

TABLE C 8: DISTRICT: CHEWAKA; KEBELE: MENDER 2; SPRAYED ON JULY 23, 2015; TEST COMPLETED ON SEPT 20-22, 2015 (T2)

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed % mortality	% Control mortality	Corrected mortality
1	Dung	30	29	96.7	0	
2	Painted	30	30	100	0	
3	Mud	30	20	66.7	0	
4	Painted	30	30	100	0	
5	Mud	30	23	76.7	0	
6	Dung	30	15	50	0	
7	Paper	30	28	93.3	0	
8	Painted	29	29	100	0	
9	Mud	30	22	73.3	0	
10	Mud	30	10	33.3	0	
11	Painted	30	30	100	0	
	Total	329	266	80.9	0	

TABLE C 9: DISTRICT: CHEWAKA; KEBELE: MENDER 2; SPRAYED ON JULY 23, 2015; TEST COMPLETED ON OCT 24-26, 2015 (T3)

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed % mortality**	**% Control mortality**	**Corrected mortality**
1	Dung	30	21	70	0	
2	Painted	31	30	96.8	0	
3	Mud	31	18	58.1	1	53.4
4	Painted	30	29	96.7	0	
5	Mud	30	24	80	0	
6	Dung	30	17	56.7	0	
7	Paper	32	27	84.4	0	
8	Painted	29	26	89.7	0	
9	Mud	30	20	66.7	0	
10	Mud	30	13	43.3	0	
11	Painted	30	23	76.7	1	74.1
	Total	333	248	74.5	0	

TABLE C 10: DISTRICT: CHEWAKA; KEBELE: MENDER 2; SPRAYED ON JULY 23, 2015; TEST COMPLETED ON NOV 25, 2015 (T4)

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed % mortality**	**% Control mortality**	**Corrected mortality**
1	Dung	30	27	90	0	
2	Painted	35	35	100	0	
3	Mud	30	28	93.3	0	
4	Painted	32	30	93.8	0	
5	Mud	30	17	56.7	0	
6	Dung	32	16	50	0	
7	Paper		ND			
8	Painted	35	34	97.1		
9	Mud	32	15	46.9	0	
10	Mud	30	22	73.3	0	
11	Painted	34	26	76.5	0	
	Total	320	250	78.1	0	

TABLE C 11: DISTRICT: CHEWAKA; KEBELE: MENDER 2; SPRAYED ON JULY 23, 2015; TEST COMPLETED ON DEC 18, 2015 (T5)

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed % mortality**	**% Control mortality**	**Corrected mortality**
1	Dung	26	25	96.2	0	
2	Painted	29	29	100	0	
3	Mud	28	28	100	0	
4	Painted	27	27	100	0	
5	Mud	26	18	69.2	0	
6	Dung	27	27	100	0	
7	Paper	ND				
8	Painted	28	28	100	0	
9	Mud	26	26	100	0	
10	Mud	25	25	100	0	
11	Painted	28	21	75	0	
	Total	242	225	93	0	

TABLE C 12: DISTRICT: CHEWAKA; KEBELE: MENDER 2; SPRAYED ON JULY 23, 2015; TEST COMPLETED ON JAN 24, 2016 (T6)

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed % mortality**	**% Control mortality**	**Corrected mortality**
1	Dung	30	17	56.7	0	
2	Painted	29	21	72.4	0	
3	Mud	28	20	71.4	0	
4	Painted	30	30	100	0	
5	Mud	31	19	61.3	0	
6	Dung	30	18	60	0	
7	Paper	ND				
8	Painted	30	18	60	0	
9	Mud	29	12	41.4	0	
10	Mud	29	9	31.0	0	
11	Painted	31	15	48.4	0	

	Total	226	128	56.6	0	

TABLE C 13: DISTRICT: CHEWAKA; KEBELE: MENDER 2; SPRAYED ON JULY 23, 2015; TEST COMPLETED ON FEB 13, 2016 (T7)

Mosquito type: Susceptible						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed % mortality	% Control mortality	Corrected mortality
1	Dung	28	12	48.9	0	
2	Painted	30	16	53.3	0	
3	Mud	29	23	79.0	0	
4	Painted	29	26	89.7	0	
5	Mud	28	14	50.0	0	
6	Dung	30	9	30	0	
7	Paper	ND				
8	Painted	33	21	63.6	0	
9	Mud	31	16	51.6	0	
10	Mud	30	1	36.7	0	
11	Painted	30	10	33.3	0	
	Total	298	148	49.7	0	

TABLE C 14: DISTRICT: TIRO-AFETA; KEBELE: KEJELO; SPRAYED ON JULY 21, 2015; TEST CONDUCTED ON JULY 25, 2015 (T0)

Mosquito type: Susceptible						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	% Control mortality	Corrected mortality
1	Dung	30	30	100	10	
2	Painted	30	30	100	0	
3	Painted	30	30	100	0	
4	Mud	30	30	100	0	
5	Mud	30	30	100	10	
6	Dung	30	30	100	0	
	TOTAL	180	180	100	3.3	
Mosquito type: Wild reared						

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	30	30	100	0	
2	Mud	30	30	100	0	
3	Mud	30	30	100	0	
4	Mud	30	30	100	10	
5	Dung	30	30	100	0	
6	Painted	30	30	100	0	
	Test	180	180	100	1,6	

TABLE C 15: DISTRICT: TIRO-AFETA; KEBELE: KEJELO; SPRAYED ON JULY 21, 2015; TEST CONDUCTED ON AUGUST 23, 2015 (T1)

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	% Control mortality	Corrected mortality
1	Dung	30	30	100	0	
2	Painted	30	30	100	0	
3	Painted	30	30	100	0	
4	Mud	30	30	100	0	
5	Mud	30	30	100	0	
6	Dung	30	30	100	0	
	TOTAL	180	180	100	0	

Mosquito type: Wild reared

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	30	30	100	0	
2	Mud	30	30	100	0	
3	Mud	30	27	90	0	
4	Mud	30	30	100	0	
5	Dung	30	29	96.7	0	
6	Painted	30	30	100	0	
	Total	180	176	97.8	0	

TABLE C 16: DISTRICT: TIRO-AFETA; KEBELE: KEJELO; SPRAYED ON JULY 21, 2015; TEST CONDUCTED ON SEPT 17- 21, 2015 (T2)

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	% Control mortality	Corrected mortality
1	Dung	30	29	96.7	0	
2	Painted	30	30	100	0	
3	Painted	30	29	96.7	0	
4	Mud	30	30	100	0	
5	Mud	30	30	100	0	
6	Dung	30	30	100	0	
	TOTAL	180	178	98.9	0	

Mosquito type: Wild reared

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	30	30	100	0	
2	Mud	30	30	100	0	
3	Mud	30	30	100	0	
4	Mud	30	30	100	0	
5	Dung	30	30	100	0	
6	Painted	30	30	100	0	
	Total	180	180	100	0	

TABLE C 17: DISTRICT: TIRO-AFETA; KEBELE: KEJELO; SPRAYED ON JULY 21, 2015; TEST CONDUCTED ON OCT 17- 21, 2015 (T3)

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	% Control mortality	Corrected mortality
1	Dung	34	19	55.9	0	
2	Painted	33	29	87.9	0	
3	Painted	34	28	82.4	0	
4	Mud	31	29	93.5	0	
5	Mud	33	31	93.9	0	
6	Dung	31	19	61.3	0	
	TOTAL	196	155	79.1	0	

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	33	33	100	0	
2	Mud	40	17	100	0	
3	Mud	30	27	100	0	
4	Mud	30	30	100	0	
5	Dung	30	28	100	0	
6	Painted	35	34	100	0	
	Total	198	169	85.4	0	

TABLE C 18: DISTRICT: TIRO-AFETA; KEBELE: KEJELO; SPRAYED ON JULY 21, 2015; TEST CONDUCTED ON NOV 19, 2015 (T4)

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	Control mortality	Corrected mortality
1	Dung	32	23	71.9		
2	Painted	38	32	84.2		
3	Painted	34	27	79.4		
4	Mud	38	38	100		
5	Mud	31	31	100		
6	Dung	32	21	65.6		
	TOTAL	205	172	83.9		

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	30	25	83.3	0	
2	Mud	32	22	68.8	0	
3	Mud	36	29	80.6	0	
4	Mud	32	31	96.9	0	
5	Dung	34	29	85.3	0	
6	Painted	32	32	100	0	
	Total	196	168	85.7	0	

TABLE C 19: DISTRICT: TIRO-AFETA; KEBELE: KEJELO; SPRAYED ON JULY 21, 2015; TEST CONDUCTED ON DEC 17, 2015 (T5)

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	% Control mortality	Corrected mortality
1	Dung	30	23	76.7	0	
2	Painted	30	25	83.3	0	
3	Painted	34	24	70.6	0	
4	Mud	33	33	100	0	
5	Mud	32	32	100	0	
6	Dung	30	15	50	0	
	TOTAL	189	152	80.4	0	

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	30	24	80	0	
2	Mud	31	24	77.4	0	
3	Mud	30	28	93.3	0	
4	Mud	32	31	96.9	0	
5	Dung	30	27	90	0	
6	Painted	34	29	85.3	0	
	Total	376	315	83.8	0	

TABLE C 20: DISTRICT: TIRO-AFETA; KEBELE: KEJELO; SPRAYED ON JULY 21, 2015; TEST CONDUCTED ON JAN 7, 2016 (T6)

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	% Control mortality (%)	Corrected mortality
1	Dung	35	25	71.4	0	
2	Painted	33	25	75.7	0	
3	Painted	33	19	57.7	0	
4	Mud	30	25	83.7	8.3	
5	Mud	33	32	96.9	0	
6	Dung	31	26	83.8	0	
	TOTAL	195	152	77.9	1.4	

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	32	25	78.1	0	
2	Mud	30	20	66.6	0	
3	Mud	32	26	81.2	0	
4	Mud	30	27	90.0	0	
5	Dung	34	28	82.3	9.1	
6	Painted	34	32	94.1	0	
	Total	192	158	82.3	1.5	

TABLE C 21: DISTRICT: TIRO-AFETA; KEBELE: KEJELO; SPRAYED ON JULY 21, 2015; TEST CONDUCTED ON FEB 13, 2016 (T7)

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed Test mortality (%)**	**% Control mortality (%)**	**Corrected mortality**
1	Dung	28	10	35.7	0	
2	Painted	29	18	62.1	0	
3	Painted	32	13	40.6	0	
4	Mud	29	24	82.8	0	
5	Mud	31	25	80.6	0	
6	Dung	29	25	86.2	0	
	TotaL	178	115	64.6	0	

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed Test mortality (%)	%Control mortality	Corrected mortality
1	Dung	30	17	56.7	0	
2	Mud	29	16	55.2	0	
3	Mud	30	26	86.7	0	
4	Mud	30	19	63.3	0	
5	Dung	30	28	93.3	0	
6	Painted	30	26	86.7	0	
	Total	179	132	73.7	0	

TABLE C 22: DISTRICT: BAKO TIBE; KEBELE: GUDINE WELKITE SPRAYED ON AUGUST 14, 2015; TEST COMPLETED ON AUG 16-17, 2015

Mosquito type: Susceptible						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed % mortality	% Control mortality	Corrected mortality
1	Mud	30	30	100	0	
2	Mud	30	22	73.3	0	
3	Mud	30	28	93.3	0	
4	Mud	31	28	90.3	0	
5	Dung	30	30	100	0	
6	Painted	30	30	100	0	
7	Painted	30	30	100	0	
8	Painted	30	30	100	0	
9	Dung	30	30	100	0	
10	Dung	30	30	100	0	
11	Painted	30	30	100	0	
12	Dung	30	30	100	0	
	Total	361	348	96.4	0	

TABLE C 23: DISTRICT: BAKO TIBE; KEBELE: GUDINE WELKITE SPRAYED AUGUST 14, 2015; TEST COMPLETED ON SEPT 14-15, 2015

Mosquito type: Susceptible						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed % mortality	% Control mortality	Corrected mortality
1	Mud	30	27	90	10	89
2	Mud	30	14	46.7	10	41
3	Mud	30	11	36.7	10	30
4	Mud	31	12	40	10	33.3
5	Dung	30	30	100	10	
6	Painted	30	30	100	20	
7	Painted	30	30	100	0	
8	Painted	30	21	70	0	
9	Dung	30	28	93.3	0	
10	Dung	30	30	100	0	
11	Painted	30	30	100	20	
12	Dung	30	30	100	0	

	Total	361	293	81.2	7.5	

TABLE C 24: DISTRICT: BAKO TIBE; KEBELE: GUDINE WELKITE SPRAYED AUGUST 14, 2015; TEST COMPLETED ON OCT 16-18, 2015

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed % mortality**	**% Control mortality**	**Corrected mortality**
1	Mud	30	10	33.3	0	
2	Mud	30	5	16.7	0	
3	Mud	31	1	3.2	0	
4	Mud	30	4	13.3	0	
5	Dung	30	23	76.7	0	
6	Painted	30	29	96.7	0	
7	Painted	30	30	100	0	
8	Painted	30	30	100	0	
9	Dung	30	16	53.3	0	
10	Dung	30	30	100	0	
11	Painted	30	30	100	0	
12	Dung	30	11	36.7	0	
	Total	361	219	60.7	0	

TABLE C 25: DISTRICT: BAKO TIBE; KEBELE: GUDINE WELKITE SPRAYED ON AUGUST 14, 2015; TEST COMPLETED ON NOV 13-14, 2015

Mosquito type: Susceptible						
House #	**Surface Type**	**Total # of test mosquitoes**	**# of mosquitoes killed after 24hr**	**Observed % mortality**	**% Control mortality**	**Corrected mortality**
1	Mud	28	1	3.8	0	
2	Mud	29	1	3.5	0	
3	Mud	31	4	12.9	0	
4	Mud	30	1	3.3	0	
5	Dung	28	16	57.1	0	
6	Painted	29	21	72.4	0	
7	Painted	30	30	100	0	
8	Painted	29	22	75.9	0	
9	Dung	28	28	100	0	
10	Dung	29	29	100	0	

11	Painted	33	33	100	0	
12	Dung	28	14	50	0	

TABLE C 26: DISTRICT: BAKO TIBE; KEBELE: GUDINE WELKITE SPRAYED ON AUGUST 14, 2015; TEST COMPLETED ON DEC 12, 2015

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	Observed % mortality	% Control mortality	Corrected mortality
1	Mud	29	1	3.5	0	
2	Mud	29	5	17.2	0	
3	Mud	30	3	10	0	
4	Mud	30	0	0	0	
5	Dung	29	11	37.9	0	
6	Painted	29	22	75.9	0	
7	Painted	30	28	93.3	0	
8	Painted	28	10	35.7	0	
9	Dung	28	12	42.9	0	
10	Dung	30	29	96.7	0	
11	Painted	29	27	93.1	0	
12	Dung	28	1	3.8	0	

TABLE C 27: DISTRICT: SHEBE-SOMBO; KEBELE: ALO-SEBEKA; SPRAYED AUGUST 11, 2015; TEST COMPLETED AUG 15-17, 2015 TYPE OF INSECTICIDE: BENDIOCARB 80% WDP

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% mortality	% Control mortality	Corrected mortality
1	Painted	30	30	100	10	
2	Painted	30	30	100	10	
3	Mud	30	27	90	10	88.9
4	Mud	30	28	93.3	0	
5	Mud	30	30	100	0	
6	Mud	30	29	96.7	0	
	Total	180	176	97.8	5	

Mosquito type: Wild reared

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% observed mortality	% Control mortality	Corrected mortality
1	Mud	30	30	100	0	
2	Mud	30	28	93.3	0	
3	Mud	30	27	90	0	
4	Painted	30	30	100	0	
5	Mud	30	30	100	0	
6	Painted	30	30	100	0	
	Total	180	177	98.3	0	

TABLE C 28: DISTRICT: SHEBE-SOMBO; KEBELE: ALO-SEBEKA; SPRAYED AUGUST 11, 2015; TEST COMPLETED ON SEPT 19-20, 2015 TYPE OF INSECTICIDE: BENDIOCARB 80% WDP

Mosquito type: Susceptible						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% mortality	% Control mortality	Corrected mortality
1	Painted	30	30	100	10	
2	Painted	30	30	100	0	
3	Mud	30	25	83.3	10	81.5
4	Mud	30	27	90	10	88.9
5	Mud	30	27	90	0	
6	Mud	30	20	66.7	10	63.0
		180	159	88.3	5	
Mosquito type: Wild reared						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% observed mortality	% Control mortality	Corrected mortality
1	Mud	30	24	80	0	
2	Mud	30	17	56.7	20	45.9
3	Mud	30	3	10	0	
4	Painted	30	20	66.7	0	
5	Mud	30	30	100	0	
6	Painted	30	30	100	0	
	Total	180	124	68.9	3.3	

TABLE C 29: DISTRICT: SHEBE-SOMBO; KEBELE: ALO-SEBEKA; SPRAYED AUGUST 11, 2015; TEST COMPLETED ON OCT 20-22, 2015 TYPE OF INSECTICIDE: BENDIOCARB 80% WDP

Mosquito type: Susceptible						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% mortality	% Control mortality	Corrected mortality
1	Painted	32	32	100	10	
2	Painted	34	34	100	0	
3	Mud	34	5	14.7	0	
4	Mud	32	3	9.4	10	
5	Mud	33	28	84.8	0	
6	Mud	32	21	65.6	10	
	Total	197	123	62.4	5	

Mosquito type: Wild						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% observed mortality	% Control mortality	Corrected mortality
1	Mud	32	29	90.6	0	
2	Mud	30	18	60	10	58.8
3	Mud	35	22	62.9	0	
4	Painted	30	29	96.7	10	96.3
5	Mud	31	31	100	10	
6	Painted	32	32	100	0	
	Total	190	161	84.7	5	

TABLE C 30: DISTRICT: SHEBE-SOMBO; KEBELE: ALO-SEBEKA; SPRAYED AUGUST 11, 2015; TEST COMPLETED ON NOV 19-22, 2015 TYPE OF INSECTICIDE: BENDIOCARB 80% WDP

Mosquito type: Susceptible						
House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% mortality	% Control mortality	Corrected mortality
1	Painted	30	30	100	0	
2	Painted	30	24	80	10	
3	Mud	30	27	90	0	
4	Mud	30	24	80	0	
5	Mud	30	23	76.7	0	
6	Mud	30	17	56.7	0	
	Total	**180**	**145**	**80.6**	**1.7**	
Mosquito type: Susceptible						

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% observed mortality	% Control mortality	Corrected mortality
1	Mud	30	3	10	0	
2	Mud	31	4	12.9	10	58.8
3	Mud	32	4	12.5	0	
4	Painted	31	3	9.7	0	
5	Mud	32	10	31.3	0	
6	Painted	33	32	96.9	0	96.3
	Total	189	56	29.6	1.7	

TABLE C 31: DISTRICT: SHEBE-SOMBO; KEBELE: ALO-SEBEKA; SPRAYED AUGUST 11, 2015; TEST COMPLETED ON DEC 19-22, 2015 TYPE OF INSECTICIDE: BENDIOCARB 80% WDP

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% mortality	% Control mortality	Corrected mortality
1	Painted	30	30	100	0	
2	Painted	30	24	80	10	
3	Mud	30	27	90	0	
4	Mud	30	24	80	0	
5	Mud	30	23	76.7	0	
6	Mud	30	17	56.7	0	
	Total	180	145	80.6	1.7	

Mosquito type: Susceptible

House #	Surface Type	Total # of test mosquitoes	# of mosquitoes killed after 24hr	% observed mortality	% Control mortality	Corrected mortality
1	Mud	30	3	10	0	
2	Mud	31	4	12.9	10	58.8
3	Mud	32	4	12.5	0	
4	Painted	31	3	9.7	0	
5	Mud	32	10	31.3	0	
6	Painted	33	32	96.9	0	96.3
	Total	189	56	29.6	1.7	

REFERENCES

Abbott W.S.1925. A method of computing the effectiveness of insecticide. *J. Econ. Ent.* 18(2), pp. 265-267.

ANNEX F: NATIONAL TRAINING ON BASIC MALARIA ENTOMOLOGY

USAID/PMI in collaboration with the FMOH conducted national-level basic malaria entomology training in Tokkuma Hotel, Adama town from March 23-26, 2015, for 34 health professionals from ten regions and FMOH (Table 28).

TABLE D 1. REGIONAL HEALTH STAFF TRAINED ON BASIC MALARIA ENTOMOLOGY

Region	Number	
	Invited	Attended
Oromia	15	8
Amahara	10	7
SNNPR	8	5
Tigray	4	4
Somali	3	3
Afar	2	0
Benshangul-Gumuz	2	2
Gambella	2	2
Harari	1	1
Diredawa	1	1
FMOH	2	1
Total	50	34