USAID
FROM THE AMERICAN PEOPLE

U.S. PRESIDENT'S MALARIA INITIATIVE

U.S. PRESIDENT'S MALARIA INITIATIVE
vectorlink
INTELLIGENT › INNOVATIVE › INTEGRATED

THE PMI VECTORLINK PROJECT

UGANDA

ANNUAL ENTOMOLOGY REPORT

JANUARY 1 – DECEMBER 31, 2019

Recommended Citation: The PMI VectorLink Project. Uganda Annual Entomological Surveillance Report**,** January 1 – December 31, 2019. Rockville, MD. Abt Associates.

Contract No.: AID-OAA-17-00008

Task Order: AID-OAA-TO-17-00027

Submitted to: United States Agency for International Development/PMI

Submitted on: February 28, 2020

Approved:

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

Ace -1	Acetylcholinesterase 1 gene
AIRS	Africa Indoor Residual Spraying
b/p/h	Bites per Person per Hour
b/p/n	Bites per Person per Night
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
HBR	Human Biting Rate
HLC	Human Landing Catch
IDRC	Infectious Disease Research Collaboration
IRS	Indoor Residual Spray
kdr	Knockdown resistance gene
LLIN	Long-Lasting Insecticidal Net
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

Indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) remain the primary mosquito vector control interventions in many parts of world, including sub-Saharan Africa, where malaria continues to be a major public health concern.

During the 2019 spray campaign, the PMI VectorLink Uganda Project conducted IRS with pirimiphos-methyl (Actellic® 300CS) in 13 districts in eastern and northern Uganda (Alebtong, Amolatar, Budaka, Bugiri, Butaleja, Butebo, Kaberamaido, Kibuku, Namutumba, Otuke, Pallisa, Serere and Tororo) and with clothianidin (SumiShield 50WG) in the districts of Dokolo and Lira. IRS in Alebtong, Amolatar, Dokolo, Kaberamaido and Otuke was funded by the Department for International Development, United Kingdom (DFID-UK), while spraying in the remaining 10 districts was funded by USAID/PMI.

To guide vector control interventions, the project conducted monthly entomological monitoring using human landing catches (HLCs) and pyrethrum spray catches (PSCs) in six districts: Bugiri, Lira, Otuke and Tororo (current IRS districts), Apac (a former IRS district, sprayed in February–March 2017 by the Ministry of Health), and Soroti (a non-IRS control district); and cone wall bioassays (only in sprayed areas). Insecticide susceptibility tests were carried out on pirimiphos-methyl (organophosphate), bendiocarb (carbamate), three pyrethroids (alpha-cypermethrin, deltamethrin and permethrin), clothianidin (neonicotinoid) and chlorfenapyr (pyrrole), in three IRS districts (Bugiri, Lira and Tororo), one former IRS district (Apac), and four non-IRS districts (Arua, Kanungu, Moroto and Rakai), all in various parts of Uganda, to evaluate *Anopheles (An.) gambiae* s.l. susceptibility to those insecticides.

Our findings highlight high levels of heterogeneity and diversity in mosquito vector species composition and behavior in the monitored areas. However, the role of the various anopheline mosquitoes in malaria transmission apart from *An. gambiae* s.l. and *An. funestus* s.l. needs to be investigated in the study districts.

The two sampling methods (PSCs and HLCs) used in longitudinal mosquito collections yielded a total of 28,064 *Anopheles* mosquitoes from the six sentinel sites (Apac, Bugiri, Lira, Otuke, Soroti and Tororo districts). Morphological identification of the mosquitoes revealed that 20,645 (73.6 percent) were *An. funestus* s.l., 7,056 (25.1 percent) were *An. gambiae* s.l., and 363 (1.3 percent) were other anopheline mosquitoes such as *An. ardensis, An. coustani, An. maculipalpis, An. pharoensis, An. pretoriensis* and *An. ziemanni*. Soroti (control site) had the highest percentage of all *An. funestus* s.l. collected, 78.93 percent (n=16,295), followed by Apac at 13.97 percent (n=2,885), Lira at 3.69 percent (n=762), Otuke at 3.36 percent (n-693), and Bugiri and Tororo both at 0.02 percent (n=5) (Table 2).

Malaria vectors *An. gambiae* s.l. and *An. funestus* s.l. were collected both indoors and outdoors using human landing colllections. In some districts the densities were higher outdoors than indoors with the reverse being observed in other districts. *An. funestus* s.l. indoor biting collections were higher in Apac (61.5%, n=458/745), Lira (72.5%, n=150/207), Otuke (72.8%, 83/114) and Soroti (71.5%, n=5649/7899). For *An. gambiae* s.l. indoor biting collections were higher in Apac (50.1%, n=442/882), Lira (57.8%, n=466/806). In Otuke and Soroti outdoor collections were higher than indoors for *An. gambiae* s.l. Similarly for other anopheline mosquito species, outdoor collections were higher in all sentinel districts, varying from 60.0% in Soroti to 89.0% in Otuke.

An. gambiae s.l. remained susceptible to pirimiphos-methyl, the insecticide used in the 2019 IRS campaign. Synergist tests with piperonyl butoxide (PBO) against *An. gambiae* s.l. indicated that the main resistance mechanism involved was monoxygenases, since pre-exposure to 4 percent PBO-treated papers fully or partially restored susceptibility to pyrethroids in resistant populations of *An. gambiae* s.l. *An. gambiae* s.l. was generally susceptible to clothianidin and chlorfenapyr insecticides. However, *An. gambiae* s.l. was generally resistant to pyrethroids (alphacypermethrin, deltamethrin and permethrin).

Cone wall bioassays performed for IRS quality assurance assays exhibited 100 percent mortality across different wall surface types, indicating that the spray was not strongly underdosed. The residual efficacy of the Actellic® 300CS used during the spray campaign remained effective for six to eight months after the spray campaign, depending on the wall surface type sprayed, with plastered painted wall surfaces performing best and mud wall surfaces performing less so, while SumiShield 50WG remained effective for at least seven months after the spray campaign (testing continuing in Lira district).

Assays are yet to be performed for detection and identification of mutations on genetic resistance markers, knockdown resistance (*kdr*) and Acetylcholinesterase-1 (*Ace-1)* genes in *An. gambiae* s.l. due to delay in receipt of laboratory supplies by the Infectious Diseases Research Collaboration (IDRC).

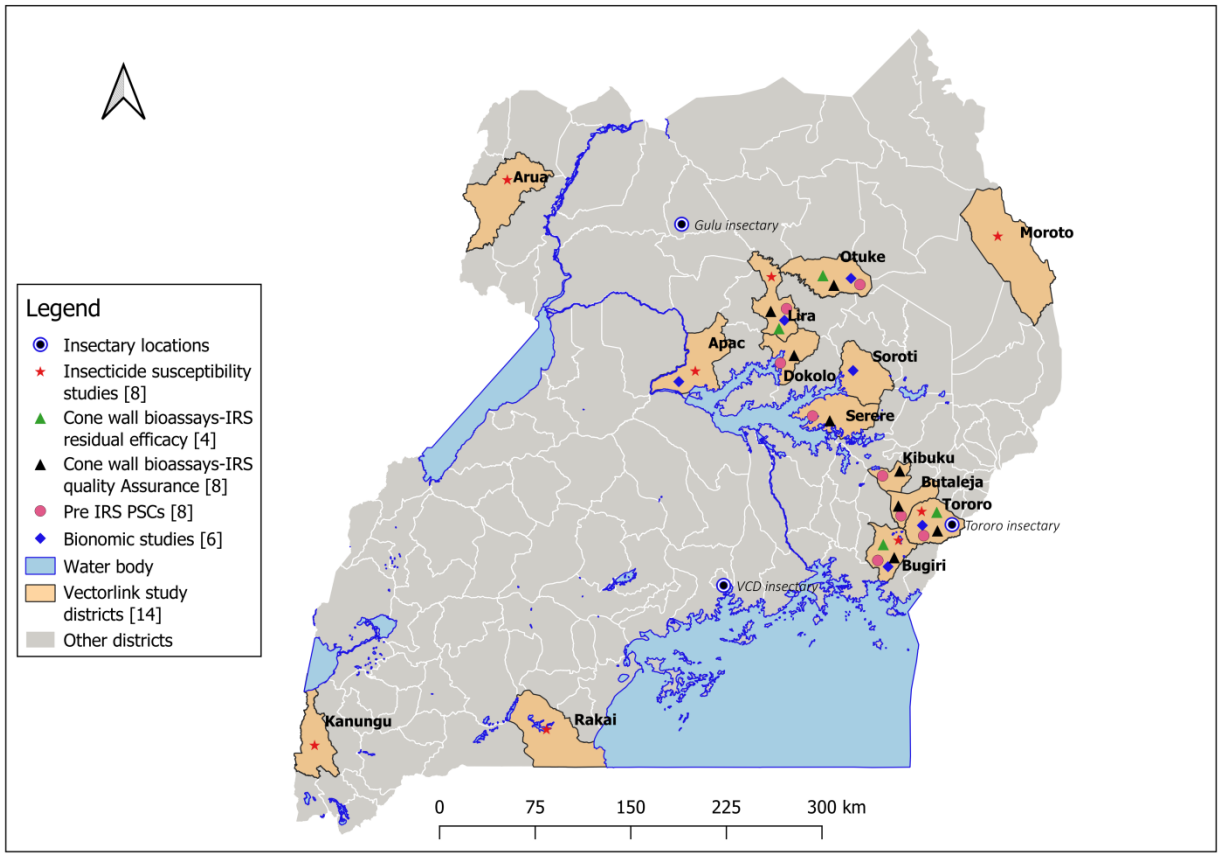
1. INTRODUCTION

The PMI VectorLink Project carried out entomological monitoring activities in six districts (Figure 1) and supported the National Malaria Control Division's entomological monitoring activities countrywide to enhance in-country capacity. Most of Uganda experiences a bi-modal rainfall pattern. March to May constitutes the first major rainfall season in Uganda (although rains were erratic in 2019 during this period), while September to November is the second rainfall season over most parts of the country. June to August is generally part of the dry season over most parts of south western, central, Lake Victoria basin and some parts of eastern region, but there is a continuation of rainfall season for much of the northern Uganda. December to February is a dry season over most parts of the country, except for some areas around Lake Victoria, Western and South-Western which sometimes receive isolated rainfall in December. For the eastern region IRS districts, the first rains are usually experienced between late February to mid-March and peaking around late April, with cessation of rains is expected around late May/mid-June except during El Ni ñ o years when there are above normal rains which usually extend beyond the usual rainfall season. For the northern region IRS districts, the first rains are usually experienced from mid-March with cessation of rains expected around late June/early July except during El Ni ñ o years. Seasonality of malaria transmission is associated with rainfall pattern with increases 2-4 weeks after the start of rains and decreasing during the dry seasons.

Entomological monitoring activities help to supplement epidemiological data essential in guiding proper targeting of IRS; evaluate the susceptibility level of the local vectors to different insecticides and determine the underlying mechanisms; inform selection of insecticides; evaluate the quality of spraying; monitor the impact of IRS on vector density, vector behavior, and composition; and determine parity rates and monitor the residual life of different insecticides on different types of wall surfaces. This entomological monitoring annual report covers the period from January 1 to December 31, 2019 under PMI VectorLink.

Longitudinal entomological monitoring was conducted in four IRS intervention districts: Bugiri, Lira, Otuke and Tororo; and Apac, a former IRS district and Soroti, a district which was never sprayed were used as control districts. In all these districts, entomological monitoring data was collected using pyrethrum spray catches (PSCs) and human landing catches (HLCs) indoors and outdoors. For susceptibility tests, trained village adult mosquito collectors used test tubes to collect adult *Anopheles funestus* s.l., while larval collections were used for *An. gambiae* s.l. collection.

Figure 1: PMI VectorLink Project Districts for Entomological Monitoring



2. METHODOLOGY

2.1 LONGITUDINAL MONITORING (BIONOMICS STUDIES)

VectorLink Uganda collected adult mosquitoes on a monthly basis from January through December 2019 using PSCs (SOP03/01) and HLCs (SOP02/01) in six sentinel sites: Bugiri, Lira, Otuke and Tororo (current IRS districts), Apac (a former IRS district, sprayed in February–March 2017 by the Ministry of Health), and Soroti (a non-IRS district used as the control district). Table 1 summarizes the longitudinal monitoring methods.

Table 1: Longitudinal monitoring adult mosquito collection methods

Collection Methods	Time	Frequency	Sample
PSCs	6:00 am to 10:00 am	Two days per site per month	Twenty houses per site (10 houses each day for two days) – same houses used every month
HLCs	6:00 pm to 7:00 am	Two consecutive nights per site per month	Two houses per site – same houses used every month

2.2 BEHAVIOR AND DENSITY

2.2.1 PYRETHRUM SPRAY CATCH

In each district where PSCs were conducted (Bugiri, Butaleja, Dokolo, Kibuku, Lira, Otuke, and Tororo), 20 houses in one village were selected. PSCs were conducted from 6:00 am to 10:00 am, once per month over two days in each district from January to December 2019. The same houses were visited each month. KillIt (commercial nomenclature) aerosol was used to knock down the mosquitoes. It contains the pyrethroids d-Tetramethrin 0.135% w/w, d-Allethrin 0.06% w/w and cypermethrin 0.46% w/w. One-roomed sleeping grass-thatched houses were selected for mosquito collection using PSCs. The room was closed for 10 minutes after spraying with KillIt, and then the knocked-down mosquitoes were collected using forceps into a labeled petri dish. The samples were identified morphologically[1] and preserved in 1.5 ml Eppendorf tubes with a hole pierced in it and kept in a plastic container containing silica gel for further identification using the Polymerase Chain Reaction (PCR) technique, and a set of samples collected by this method during the mentioned period were sent for PCR at Infectious Diseases Research Collaboration (IDRC) Molecular Laboratories for further analysis.

2.2.2 HUMAN LANDING CATCH

HLCs were conducted in Bugiri, Lira, Otuke, Tororo, Apac and Soroti. Two houses were sampled in each selected village on two consecutive nights to obtain four person-nights of collection per district per month (two houses x two collection nights = four person-nights indoors and 4 person-nights outdoors). In all districts, two human volunteers (trained adult mosquito collectors) were positioned, one inside the house and the other outside at least 5 meters from the house, to collect mosquitoes. Collections were conducted from 6:00 pm to 6:00 am using 12 volunteers working in shifts of 6 hours each. For each hour of collection, collectors collected mosquitoes for 55 minutes and rested for 5 minutes, during which they exchanged positions. During the time of collection, the collectors sat quietly on a small chair and exposed part of their legs (up to the knees) and arms up to the elbow; when they felt landing mosquitoes, they turned on a torch and collected the mosquitoes using

[1] Gillies MT and Coetzee M. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). South African Institute for Medical Research, 55: 33–81.

a mouth aspirator. Collected mosquitoes were transferred into labeled paper cups assigned for each hourly collection. Collected mosquitoes were subsequently killed using cotton soaked in diethyl ether, identified, counted by species, location, and hour of collection, and preserved in 1.5 ml Eppendorf tubes with a hole pierced in it and kept in a plastic container containing silica gel. A subset of samples from these collections were sent to IDRC Molecular Laboratories for PCR analyses. Data obtained from HLCs were used to directly determine human biting rate (HBR). HBR was calculated by dividing the number of mosquitoes of single species/ species complex (*An. gambiae* s.l. and/or *An. funestus* s.l.) collected by the number of persons per night.

2.3 VECTOR SUSCEPTIBILITY TESTING

Insecticide susceptibility studies were conducted using the World Health Organization (WHO) tube bioassays to determine insecticide susceptibility status and insecticide resistance intensity of major malaria vectors, *An. gambiae* s.l. and *An. funestus* s.l. to insecticides recommended by the WHO for use in public health. Synergist assays with PBO was conducted to assess the involvement of mixed function oxidases as a resistant mechanism. The five classes of insecticides tested included: neonicotinoids (clothianidin 50% WG) and pyrroles (chlorfenapyr 5%) organophosphates (pirimiphos-methyl 0.75%), pyrethroids (deltamethrin 0.05%, permethrin 0.75% and alphacypermethrin 0.05%,) and carbamates (bendiocarb 0.1%), % These studies were conducted in eight districts spread out throughout Uganda and thus somewhat representative of the different epidemiological settings in the country between June and July 2019.

Field-collected larvae of *An. gambiae* s.l. were reared to adult stage in the field insectaries established in the selected study sites in the districts. Batches of 20-25 females, sugar-fed and three to five-day old mosquitoes were subsequently subjected to WHO tube tests following the standard WHO 2016[2] protocol. Adult *An. funestus* s.l. mosquitoes collected in early mornings using test tubes were immediately used for susceptibility testing. These females were exposed to pirimiphos-methyl 0.25%, alpha-cypermethrin 0.05%, permethrin 0.75%, bendiocarb 0.1% and deltamethrin 0.05% on WHO impregnated filter papers for 60 minutes. Knockdown was scored at 60 minutes immediately after the exposure period, at which time all mosquitoes were gently transferred to holding tubes. Mortality was recorded at 24 hours after exposure. Where control mortality scored higher than 5 percent but below 20 percent, Abbott's correction was applied to test mortalities and those above 20 percent led to tests being discarded (Abbott [3]1925). Susceptibility levels of *An. gambiae* s.l. and *An. funestus* s.l. were evaluated based on WHO criteria (WHO 2016). WHO classifies 24-hour mortality rates higher than 98 percent as susceptible, between 90 percent and 97 percent as suggestive of resistance and requiring further investigation, and below 90 percent as resistant.

Intensity assays were conducted by exposing wild caught vector mosquitoes to insecticide dosages of 5× and 10× the diagnostic concentrations of alpha-cypermethrin, deltamethrin and permethrin, according to the standard WHO bioassay method (WHO 2016). All exposures were for one hour, and final mortality was scored after a 24-hour holding period during which a 10% sugar solution was made available to surviving mosquitoes.

The synergist assays were conducted using *An. gambiae* s.l. reared from field-collected larvae. Four bioassay exposures were done as follows: In the first group of replicates, the mosquitoes were exposed to the insecticide only (alpha-cypermethrin, delatmethrin or permethrin), the second group was exposed to 4% piperonyl butoxide (PBO) only, the third group to 4% PBO followed by an insecticide (alpha-cypermethrin, delatmethrin or permethrin), and the last group was exposed to the Acetone solvent (control). All replicates were exposed for 60 minutes and mortality was recorded 24 hours after exposure, according to the WHO (2016) protocol. This process was repeated three times based on the standard procedure.

Abbott's formula:

$$\text{Corrected mortality} = \frac{(\text{Test Mortality [\%]} - \text{Control Mortality [\%]})}{(100\% - \text{Control mortality [\%]}} \text{ x } 100$$

[2] World Health Organization (WHO). 2016. Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes, 2nd Edition. Geneva: WHO.

[3] Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267.

2.3 Clothianidin and Chlorfenapyr Susceptibility Test Results

For clothianidin susceptibility tests, freshly-treated filter papers (treated at 13.2 mg active ingredient per paper) were inserted into plastic cylinders and tested according to standard WHO susceptibility test protocols and according to the Africa Indoor Residual Spraying Project (AIRS)[4] standard operating procedure 001. The exposure time was 60 minutes. Afterward, mosquitoes were transferred into holding cylinders with filter paper treated only with distilled water and provided with lightly moistened cotton wool containing 10% sugar solution that was changed daily. Knock-down was recorded halfway through the test at 30 minutes and at the end of the test at 60 minutes. Mortality was recorded on days 1, 2, 3, 4, 5, and 6, and final mortality on day 7 after exposure. Mortality monitoring stopped whenever 100% test mortality was achieved before day 7. A negative control was tested at the same time and mortality recorded on days 1 through 7. The test was conducted with *An. gambiae* s.l. collected from several breeding sites in villages in four different districts (Apac, Bugiri, Lira and Tororo) between June and July 2019. For each district, four replicates of 25 mosquitoes were tested (total of 100 sugar-fed females) with clothianidin papers, and two replicates were used at the same time with the negative control papers (impregnated only with distilled water). In addition to the negative control described above, a positive control was done by similarly exposing a laboratory-reared susceptible *An. gambiae* s.s. Kisumu strain.

For chlorfenapyr, susceptibility tests were conducted according to standard WHO susceptibility test protocols using WHO insecticide-treated papers (5% concentration) procured from Malaysia. Final mortality was read at 3 days post-exposure.

The above susceptibility tests were conducted to the greatest extent possible under the recommended optimal conditions, at temperatures around 27°C +-2°C and 70–80 percent relative humidity. Similar to other collections, a portion of *An. gambiae* s.l. samples from these tests were sent to the IDRC molecular laboratories for PCR assays to identify sibling species and detect presence of knockdown (*kdr*) and acetylcolinesterase-1 (*Ace-1*) genes and to determine sporozoite rates.

2.4 IRS Quality Assays and Insecticide Decay Rate Monitoring

Standard WHO cone bioassay tests were performed in one site in each of the eight spray districts (Bugiri, Butaleja, Dokolo, Kibuku, Lira, Otuke, Serere, and Tororo) within two weeks of the start of spraying to assess the quality of spraying. Routine wall bioassays were subsequently monitored monthly in four districts of Bugiri, Lira, Otuke and Tororo up to December 2019 until mortality came below 80% for two consecutive months. Three houses of different wall types (plastered and painted, plain brick and mud-walled), were randomly selected in each study village. The same houses were used each month. Cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m above the floor. Cones lined with self-adhesive tape were fixed on the sprayed walls for the assay. The control cone was affixed on a wall lined with a paperboard with adhesive in an unsprayed house to avoid any potential airborne effect. Two- to five-day-old female mosquitoes were used for the tests. Susceptible *An. gambiae* s.s. Kisumu strain mosquitoes were introduced into the plastic cones in batches of 10 and left exposed on the sprayed surface for 30 minutes at different heights. Numbers of mosquitoes knocked down at the 30th minute were recorded. At the end of the 30-minute exposure period, the mosquitoes were carefully collected and transferred to paper cups and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with net with final mortality recorded after 24 hours holding period

[4] Clothianidin test protocol (03 08 2017)

post-exposure for pirimiphos–methyl CS. For SumiShield 50WG test mortality was monitored every 24 hours until 100% test mortality was achieved or day 5, whichever comes first.

Tests for the airborne effect of pirimiphos-methyl (Actellic® 300CS) and SumiShield 50WG were conducted with mosquitoes placed inside a mosquito net cage and hung 10 cm away from the sprayed wall surface at a height of 1.0 m above the floor with knock-down recorded at 30 mins and 60 mins after exposure. The mosquitoes were then transferred into clean paper cups that were kept for a 24-hour holding period for pirimiphos-methyl CS and the percentage mortality was calculated for each house and recorded according to WHO protocol. *An. gambiae* s.s. (Kisumu strain) was used for quality and decay rate, and fumigant effect assessment. For the SumiShield 50WG test mortality was monitored every 24 hours until 100% test mortality was achieved or day 5, whichever comes first

2.5 MOLECULAR ASSAYS

The molecular assays to be performed by IDRC molecular laboratory included the vector species identification, infection rate determination of malaria vectors (detection of *Plasmodium falciparum)* malaria parasites, blood meal analysis and detection of insecticide resistance genetic markers *kdr* and *Ace-1* using established protocols as stipulated in the Methods of *Anopheles* Research (MR4, 2014).

2.5.1 VECTOR SPECIES IDENTIFICATION

Following morphological identification of individual samples in the field, a selected proportion were amplified by PCR and directly sequenced using deoxyribonucleic acid (DNA) barcoding (mtDNA COI) and ITS2 (nDNA) primers and protocols for species confirmation. These initial screens provided verified positive controls for the PCR-based species diagnostic assays for downstream identifications. The MR4 *An. gambiae* s.l. assay for species diagnosis (Scott et al., 1993) was found to be highly reliable. *An. arabiensis* samples were identified using this assay.

2.5.2 DETECTION OF MALARIA PARASITES (DETERMINATION OF INFECTION RATES)

Detection and identification of malaria parasites in *An. gambiae* s.l. was restricted to *Plasmodium falciparum* ELISA assays by district and by month of collection. ELISAs following the method of Wirtz et al. (1987) were carried out on dried mosquito samples, kept dry (stored with desiccant) to prevent microbial growth. The sporozoite rates were calculated based on the results from the assays conducted.

2.5.3 DETECTION OF INSECTICIDE RESISTANCE MARKERS

To determine the prevailing resistance mechanisms, molecular assays are to be used to detect presence of knock down resistance (*kdr)* and Acetylcholinesterase (*Ace-1)* genes. This will be done in mosquito samples whose insecticide resistance phenotype has been determined using standard WHO susceptibility assays. DNA extraction will provide template to be used for determining the underlying genotype for the *kdr* mutation. Polymerase Chain Reaction (PCR) will be deployed as a diagnostic method for detection of *kdr* mutations following protocols described by Martinez-Torres et al. 1998 (*kdr* L1014F) and Ranson et al, 2000 (*kdr* L1014S). Mosquitoes will also be screened for insensitive Acetylcholinesterase (*Ace-1R*) by the PCR method of Weill et al, 2004.

3. RESULTS

3.1 *ANOPHELES* SPECIES COLLECTED BY DIFFERENT METHODS

3.1.1 LONGITUDINAL MONITORING

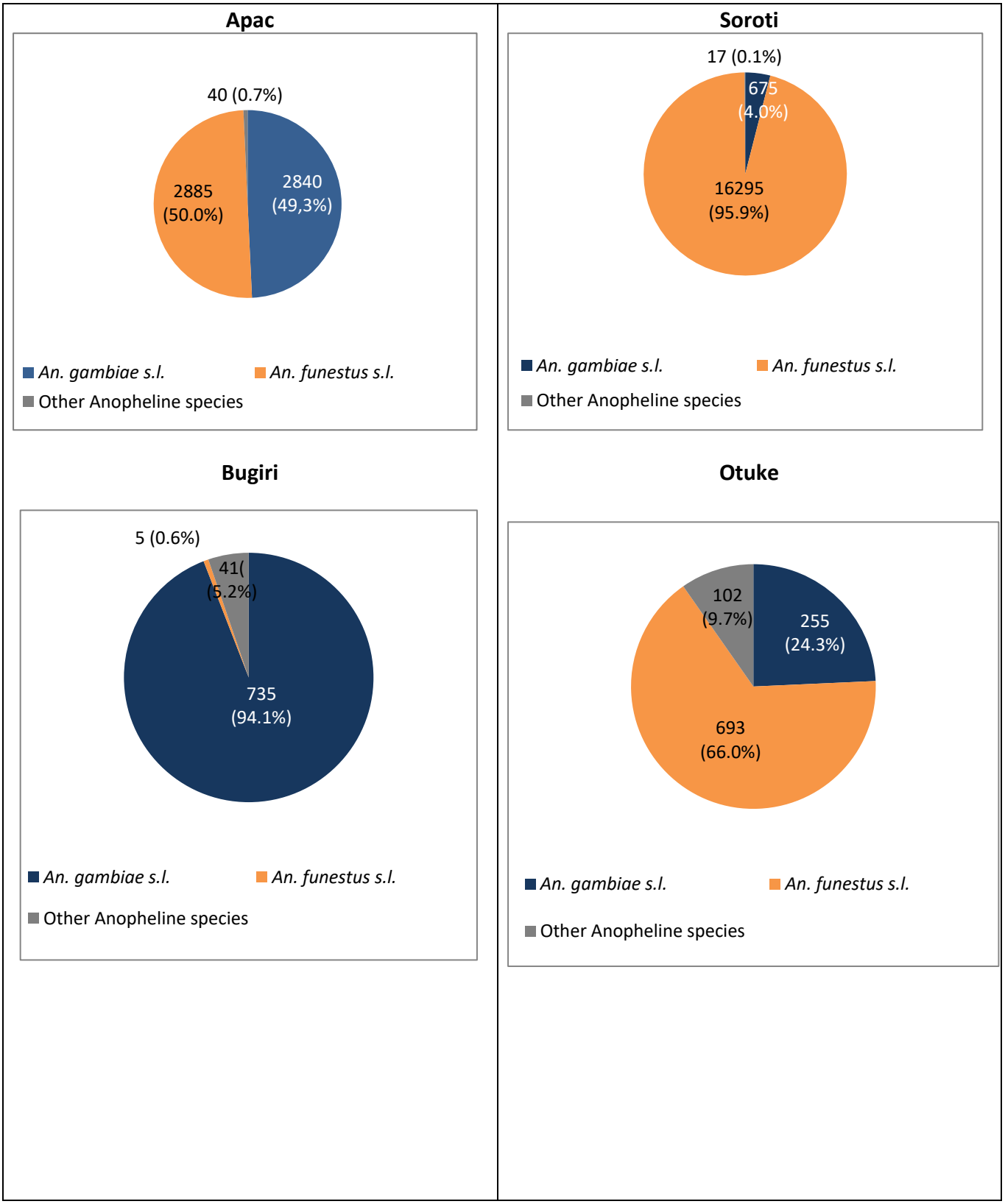
During the reporting period, in Bugiri, Lira, Otuke, and Tororo (current IRS districts), Apac (a former IRS district, sprayed in February–March 2017 by the Ministry of Health), and Soroti (a non-IRS district used as the control), a total of 28,065 female *Anopheles* mosquito species were collected using the two collection methods (PSCs and HLCs) and morphologically identified (Table 2).

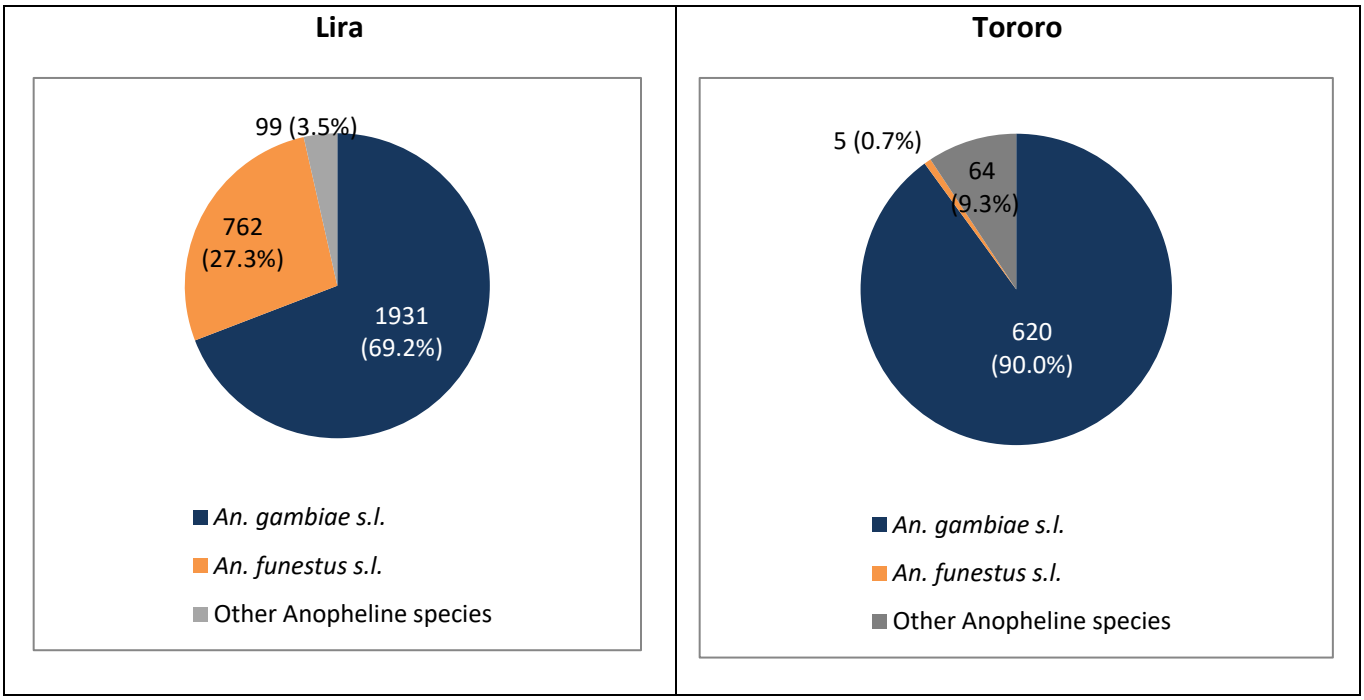
- A total of 7,056 *An. gambiae* s.l. were collected: 4,352 (61.7%) using PSCs and 2,704 (38.3%) using HLCs.
- A total of 20,645 *An. funestus* s.l. were collected: 11,676 (56.6%) using PSCs and 8,969 (43.4%) using HLCs.
- *An. funestus* s.l. was the most abundant (73.6%) *Anopheles* species collected, followed by *An. gambiae* s.l. (25.1%).
- Other *Anopheles* species including *An. ardensis, An. coustani, An. maculipalpis, An. pharoensis, An. pretoriensis* and *An. ziemanni* comprised the remaining 1.3% (Figure 2). However, vector distribution differed by study site. Overall *An. funestus* s.l. was the predominant vector in Soroti and Otuke districts while *An. gambiae* s.l. was dominant in Bugiri, Lira, and Tororo districts. In Apac the two vector species were found in fairly equal proportions (Table 2 and Figure 2).

Table 2: Number of female *Anopheles* mosquitoes collected in each district by PSC and HLC

Mosquito species	Apac	Bugiri	Lira	Otuke	Soroti	Tororo	Total	%
An. gambiae s.l.	2,840	735	1,931	255	675	620	7,056	25.1
An. funestus s.l.	2,885	5	762	693	16,295	5	20,645	73.6
Other *Anopheles* species	40	41	99	102	17	64	363	1.3
Total per district	5,765	781	2,792	1,050	16,987	689	28,064	100

Figure 2: *Anopheles* species composition by study site using PSCs and HLCs indoors and outdoors, January to December 2019





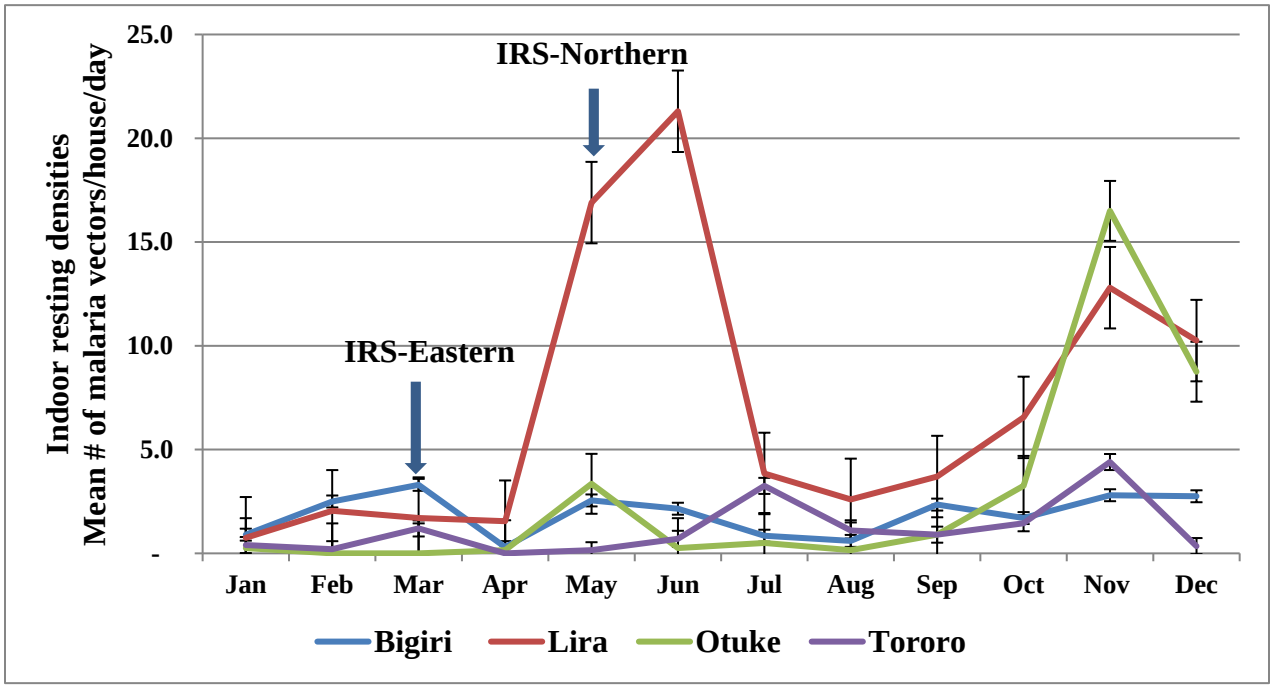
3.1.2 Pyrethrum Spray Catch

PSC collections yielded 16,035 *Anopheles* mosquitoes (Table 3 and Figures 3A and 3B). By species, there were 11,676 (72.82%) *An. funestus* s.l., 4,352 (27.14%) *An. gambiae* s.l. and 7 (0.04%) other *Anopheles* species. Most *Anopheles* vectors were caught in the unsprayed (control) sites of Apac and Soroiti. *An. gambiae* s.l. predominated in PSCs in the intervention areas (Bugiri, Lira and Tororo), while a greater proportion of *An. funestus* s.l. was caught in the control area (Soroti), former IRS district (Apac), and the current IRS district of Otuke.

Table 3: Number of mosquitoes by species collected using PSC in the study districts

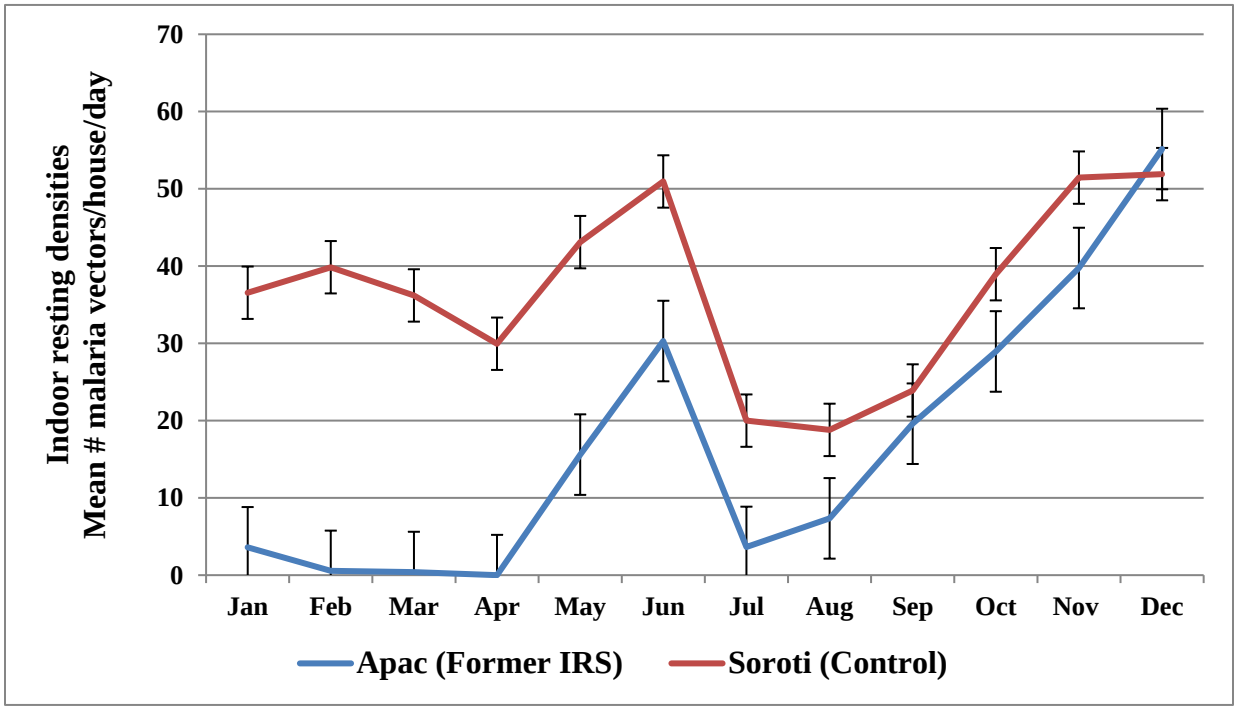
	Apac	Bugiri	Lira	Otuke	Soroti	Tororo	Total
An. gambiae s.l.	1,958	450	1,125	102	436	281	4,352
An. funestus s.l.	2,140	5	555	579	8,396	1	11,676
Other *Anopheles*	2	0	1	2	2	0	7
Total per district	**4,100**	**455**	**1,681**	**683**	**8,834**	**282**	**16,035**

Figure 3A: Combined Mean Indoor Resting Densities of *An. funestus* s.l. and *An. gambiae* s.l. in four intervention districts before and after IRS Intervention



Note: IRS was conducted in in Bugiri and Tororo in March and in Lira and Otuke in May 2019

Figure 3B: Combined Mean Indoor Resting Densities of *An. funestus* s.l. and *An. gambiae* s.l. in two control districts before and after IRS Intervention



3.1.3 HUMAN LANDING CATCHES

A total of 11,673 *Anopheles* mosquitoes were collected using HLCs from January to December 2019. The species identified morphologically from this collection included: 8,969 (74.56%) *An. funestus* s.l., 2,704 (22.48%) *An. gambiae* s.l., 356 (2.96%) other *Anopheles* species such as *An. ardensis, An. coustani, An. maculipalpis, An. pharoensis, An. pretoriensis* and *An. ziemanni. An. gambiae* s.l. was the most abundant mosquito species collected in the intervention areas (Bugiri, Lira, Otuke and Tororo) and Apac (former IRS district), while *An. funestus* s.l. was the most abundant mosquito species collected in the control area (Soroti) Table 4.

All districts demonstrated a different pattern of *An. funestus* s.l. and *An. gambiae* s.l. human biting activity both indoors and outdoors and before and after IRS spraying, which began in March 2019 for Bugiri and Tororo, and in May 2019 for Lira and Otuke. An overall a spike in *An. funestus* s.l. biting rates was observed between September and December 2019 in control and former IRS districts, while a similar increase was not observed in IRS districts. This is most likely due to the impact of IRS on the *An. funestus* s.l. population (Figure 4A-4F). Similarly, no spike in *An. gambiae* s.l. biting rates was observed in IRS districts following spraying, though the same trend was observed in control districts. High *An. gambiae* s.l. biting rates were recorded in the former IRS district (Figure 4G-4H).

Table 4: Human Landing Catches indoors and outdoors in six study districts in Uganda from January to December 2019

Anopheline species	Apac			Bugiri			Lira			Otuke			Soroti			Tororo			Total		Grand Total
	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	
An. gambiae s.l.	442	440	882	82	203	285	466	340	806	72	81	153	114	125	239	96	243	339	**1272**	**1432**	**2704**
An. funestus s.l.	458	287	745	0	0	0	150	57	207	83	31	114	5649	2250	7899	1	3	4	**6341**	**2628**	**8969**
Other *Anopheles* species	10	28	38	15	26	41	19	79	98	11	89	100	6	9	15	14	50	64	**75**	**281**	**356**
Total per district	**910**	**755**	**1665**	**97**	**229**	**326**	**635**	**476**	**1111**	**166**	**201**	**367**	**5769**	**2384**	**8153**	**111**	**296**	**407**	**7688**	**4341**	**12029**

Figure 4: Monthly indoor and outdoor human biting rates for *An. funestus* s.l. and *An. gambiae* s.l. in four intervention districts and two control districts, before and after IRS intervention, 2019. May 2019 data was used as baseline throughout the graphs, and June-December 2019 data was used as post-IRS data. January to April 2019 data from extended long dry season had a lot of zero outcomes that led to high variability so are excluded from the graphs

N.B.: May 2019 data is used as baseline, and June-December 2019 is post-IRS data. January to April 2019 data from extended long dry season had a lot of zero outcomes that led to high variability so excluded from the graphs..

Figure 4A: Combined *An. funestus* s.l. and *An. gambiae* s.l. mean bites/person/night indoor

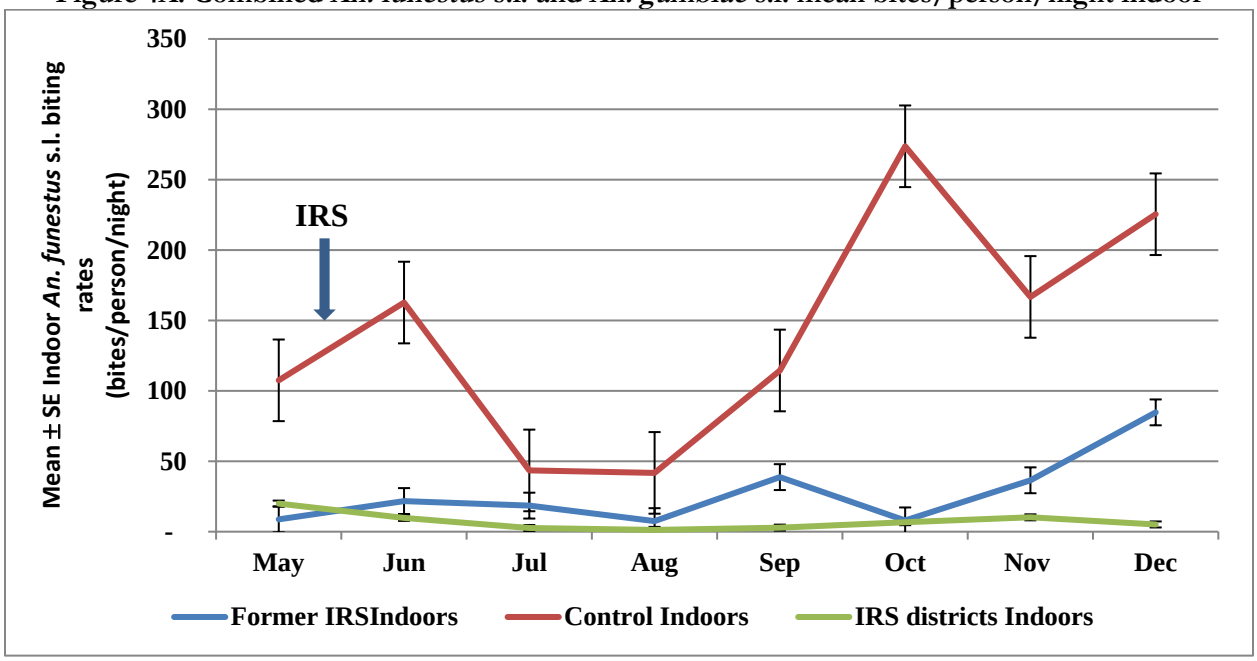


Figure 4B: Combined *An. funestus* s.l. and *An. gambiae* s.l. mean bites/person/night outdoor

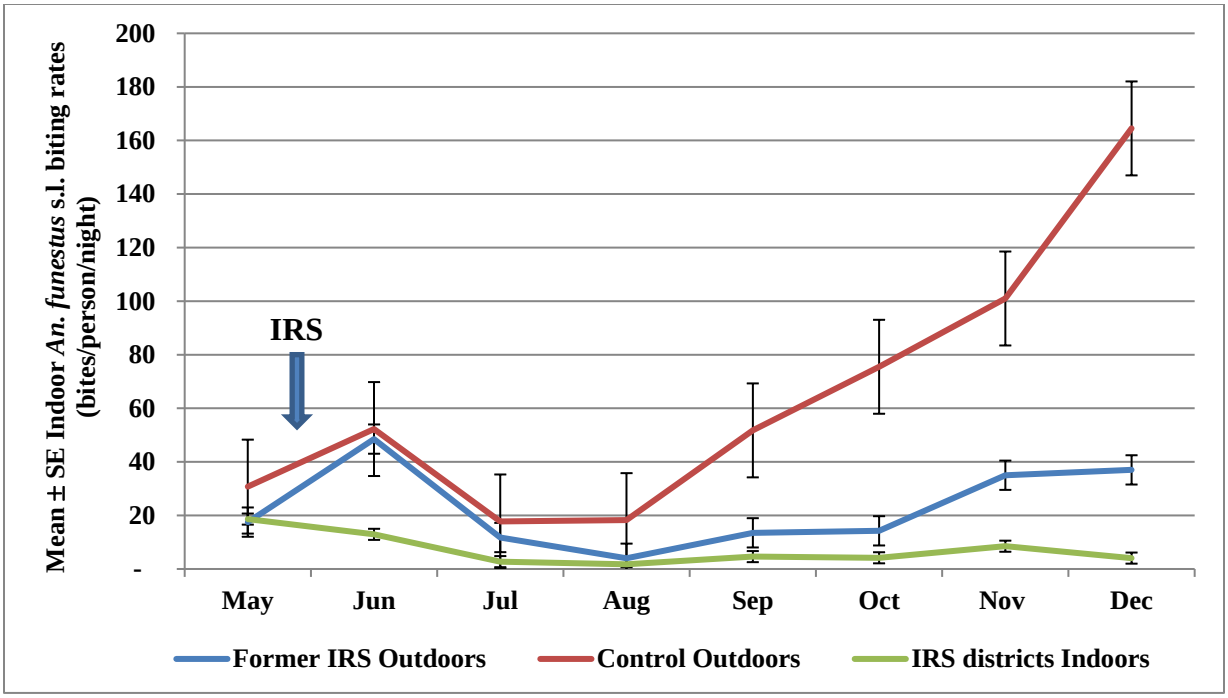


Figure 4C. *An. funestus* s.l. indoor in IRS districts

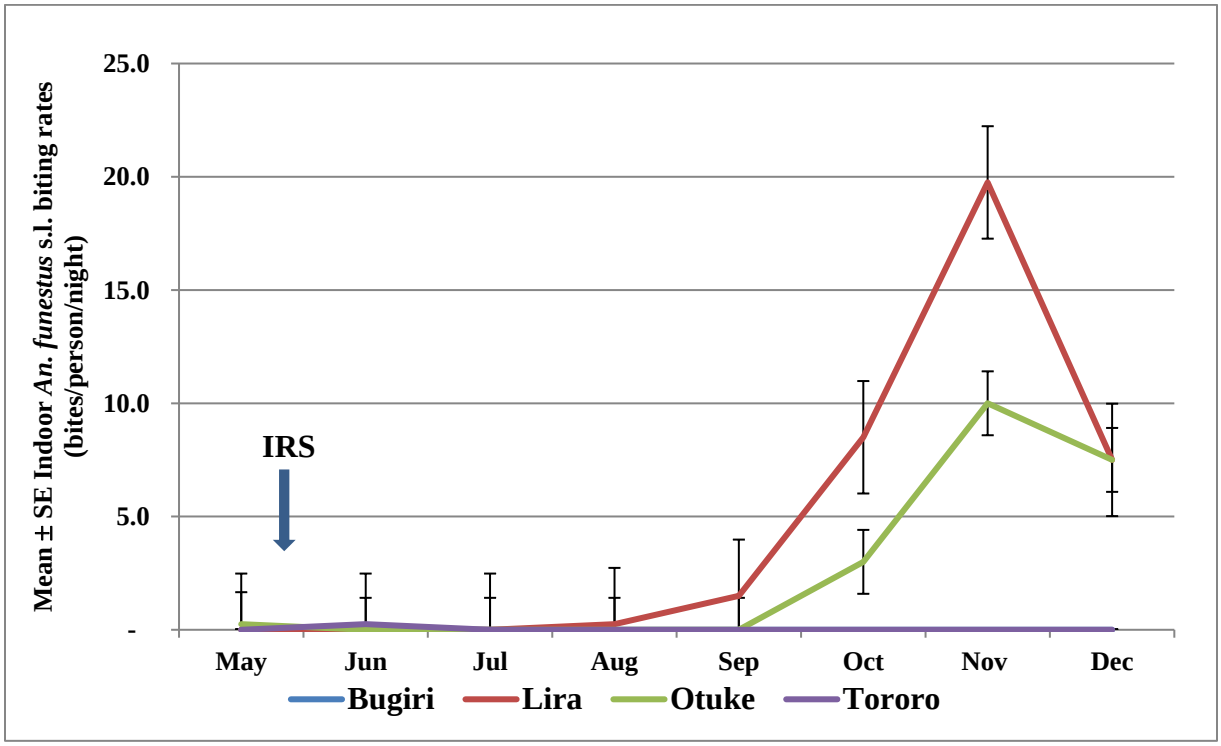


Figure 4D. *An. funestus* s.l. indoor in non-IRS districts

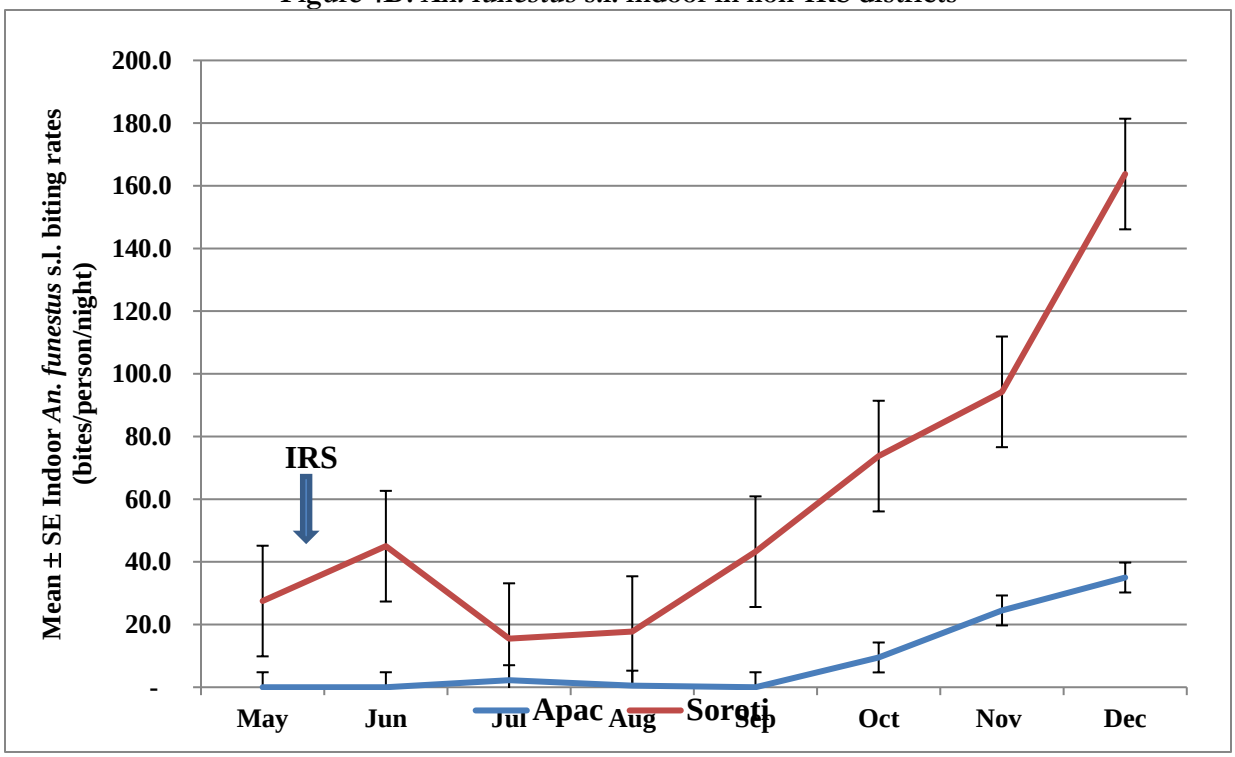


Figure 4E. *An. funestus* s.l. outdoor IRS districts

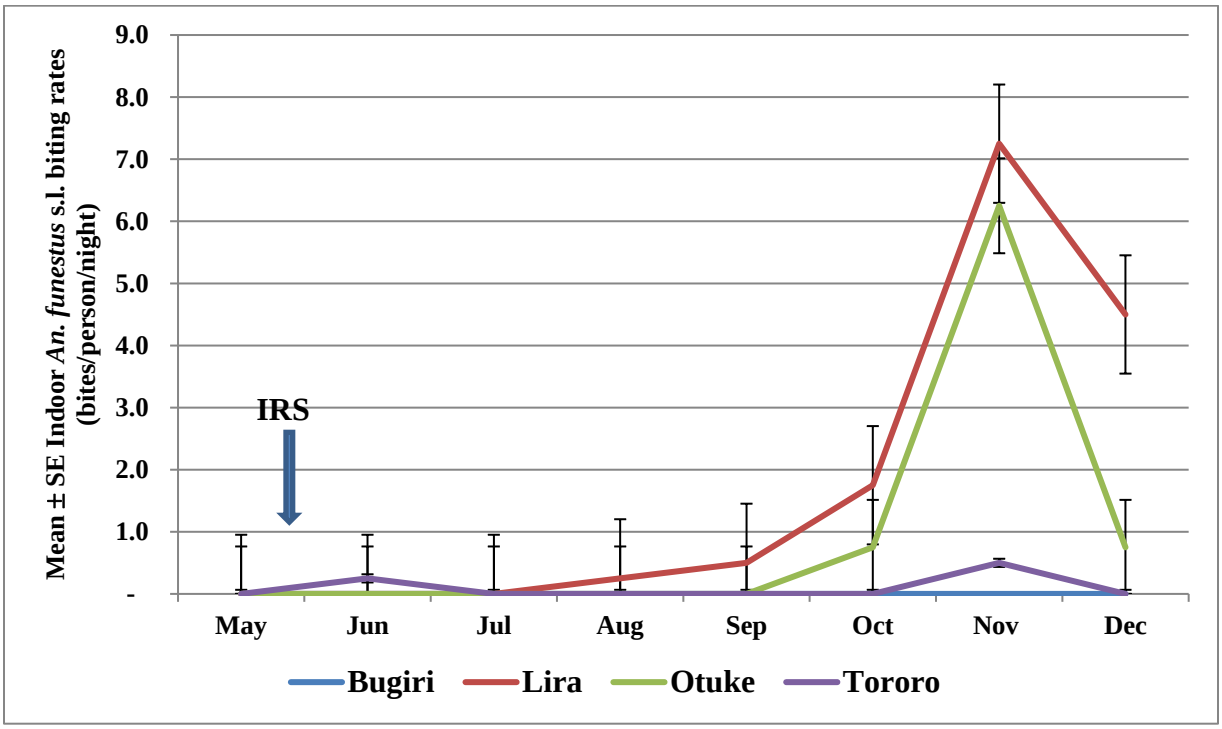


Figure 4F. *An. funestus* s.l. outdoor non-IRS districts

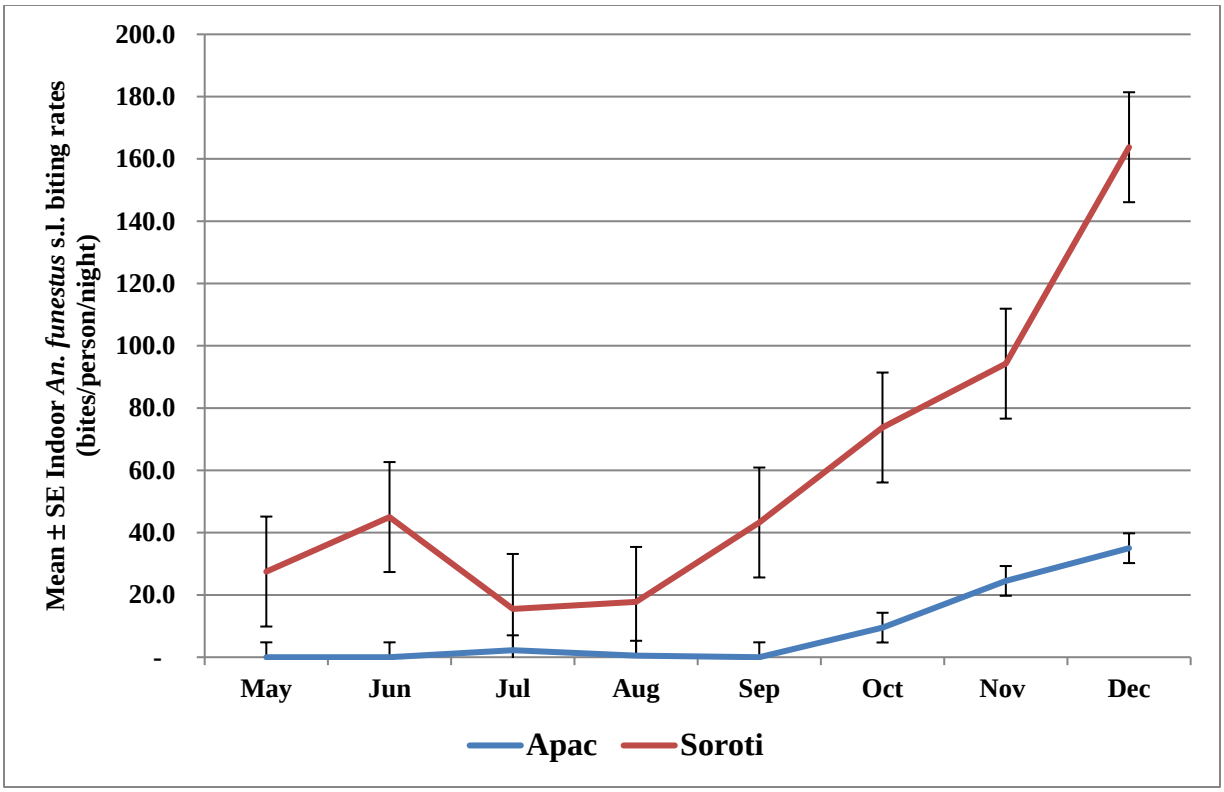


Figure 4G. *An. gambiae* s.l. outdoor

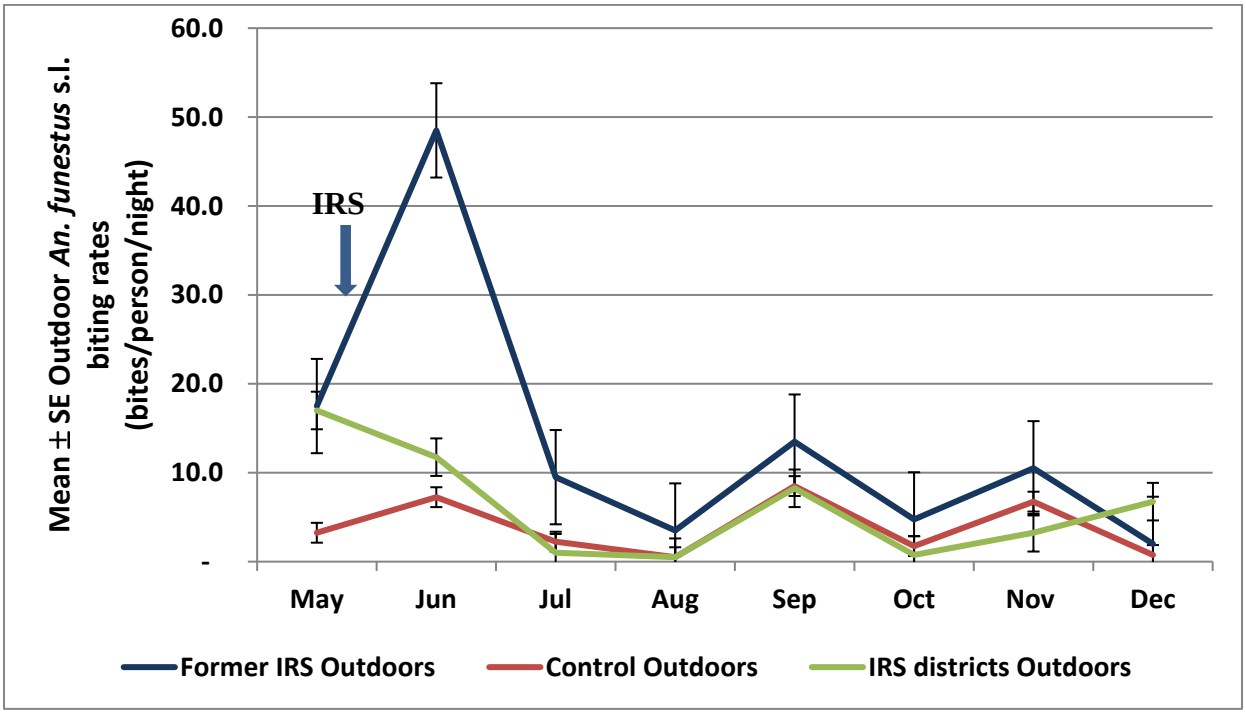
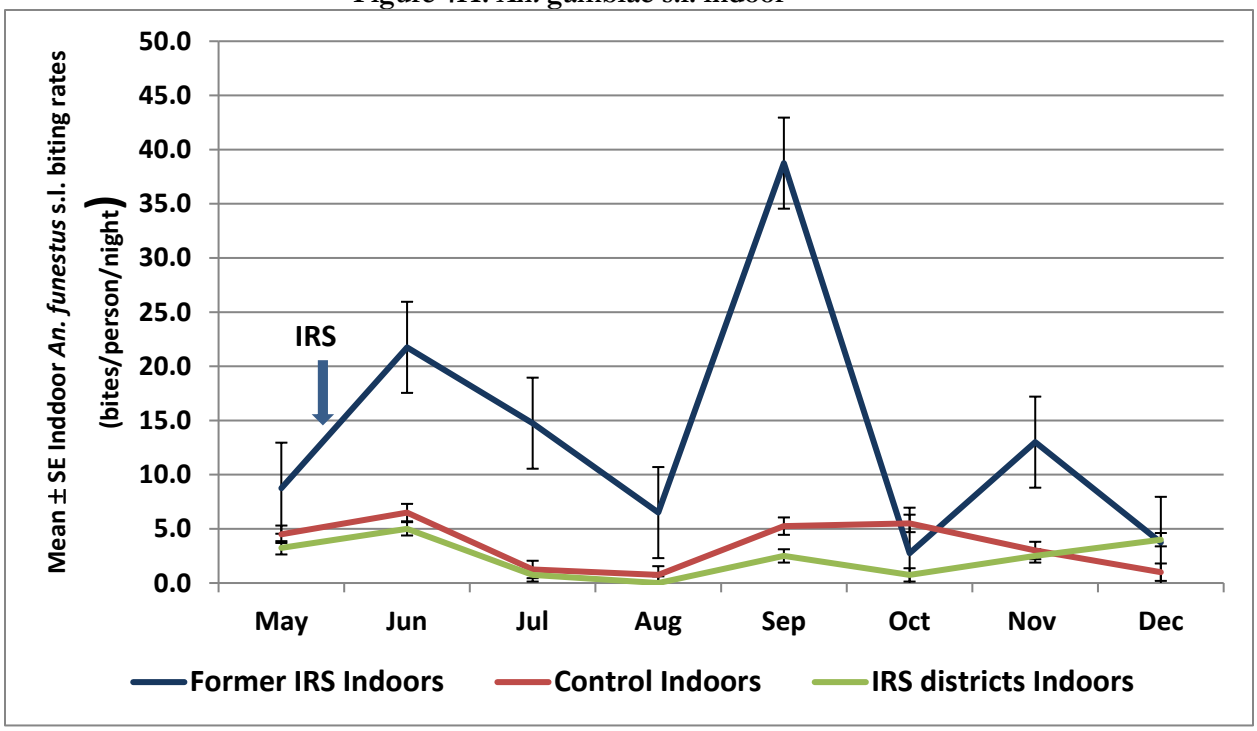


Figure 4H. *An.* gambiae s.l. indoor



.

Annex 1 Table A shows the mean indoor and outdoor vector biting rates for *An. gambiae* s.l. and *An. funestus* s.l. before and after spraying. Soroti, the control district, had the highest biting rates for *An. funestus* s.l. indoors and outdoors throughout the monitoring period. Following spraying, decreases in biting rates were observed in the intervention districts. There was a drop in both *An. gambiae* s.l. and *An. funestus* s.l. biting rates observed both indoors and outdoors in the intervention districts (Bugiri, Lira, Otuke and Tororo), while an increase was observed for both *An. gambiae* s.l. and *An. funestus* s.l., indoors and outdoors, in the former IRS district (Apac) and in the control district (Soroti). The low biting rates observed in the intervention areas could indicate the potential impact of spraying Actellic® 300CS and Sumishield 50WG against both predominant malaria vectors. However, *An. funestus* s.l. numbers were extremely low with the exception of Soroti where the sentinel site is located near a permanent swamp which is an ideal habitat for *An. funestus* s.l. breeeding. For *An. gambiae* s.l., the indoor numbers were quite similar and is related to the rainfall patterns in the country.

Table 5 shows the numbers of each mosquito species collected in intervention areas, former IRS and control areas, indicating for each the total person-nights and subsequent biting rate expressed as bites per person per night (b/p/n). *An. funestus* s.l. and *An. gambiae* s.l. were observed to contribute to 99.8 percent, 97.7 percent and 86.3 percent of the bites in the control, former IRS and intervention areas, respectively.

Table 5: Mosquito species collected by HLC and their mean biting rates in in intervention, former IRS and control areas, January to December 2019

Species Collected	Intervention Districts			Former IRS District			Control District		
	Total Numbers Collected	Total Person Nights	B/P/N	Total Numbers Collected	Total Person Nights	B/P/N	Total Numbers Collected	Total Person Nights	B/P/N
An. gambiae s.l.	1,583	192	8.2	882	48	18.4	239	48	5.0
An. funestus s.l.	325	192	1.7	745	48	15.5	7,899	48	164.6

Other *Anopheles* Species	303	192	1.6	38	48	0.8	15	48	0.3

In all the study districts, the indoor biting activity of *An. funestus* s.l. was close to zero from 6:00 to 10:00 pm except for Soroti where it appears to be active throughout the night. In Soroti increased biting activity occurs after 11.00 p.m. and peaks both indoors and outdoors between 4:00 a.m. and 7:00 a.m. (between 5-20 b/p/h) (Figure 5C). Biting activity of *An. funestus* s.l. is higher indoors than outdoors wherever it occurs (Figure 5A-5B). The biting activity of *An. gambiae* s.l. varies by district. Outdoor biting activity was higher in Bugiri and Tororo districts (Figure 5D) while outdoor biting activity was higher in Lira district (Figure -5E). No clear difference in feeding in location was observed in Otuke, Soroti and Apac (Figures 5E-5F). *An. funestus* s.l. biting activity was higher in the control area than in intervention areas, both indoors (1.22 versus 63.51b/p/h) and outdoors (0.47 versus 26.43 b/p/h). Generally, the hourly biting rates of *An. gambiae* s.l. was low in Bugiri, Otuke, Soroti and Tororo but high in Apac and Lira. The highest biting rates of *An. gambiae* s.l. recorded was 1.58 bites per person per hour in Apac (Figure 5F) unlike *An. funestus* s.l. which hits 20.21 bites per person per hour (Figure 5C) in the control site.

Figure 5: Hourly Biting Rates of *An. funestus* s.l. in Apac, Bugiri, Lira, Otuke, Soroti and Tororo as Determined through HLCs

Figure 5A. *An. funestus* s.l. indoor

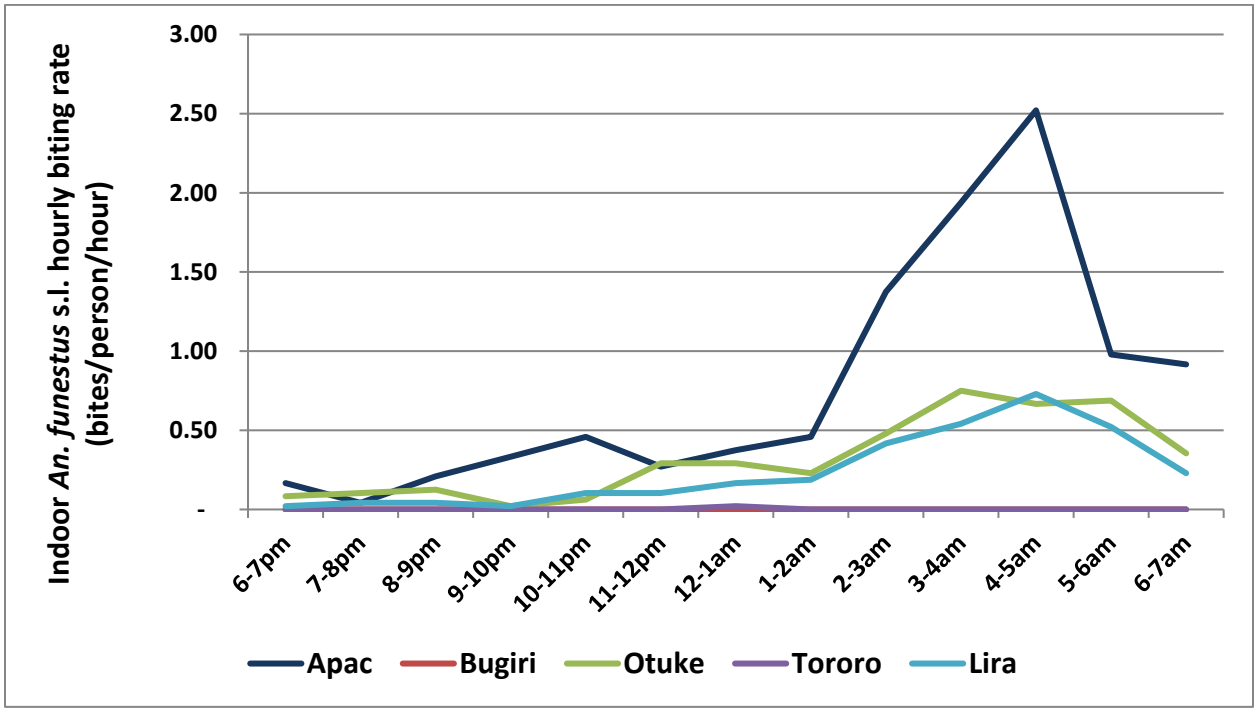


Figure 5B. *An. funestus* s.l. outdoor

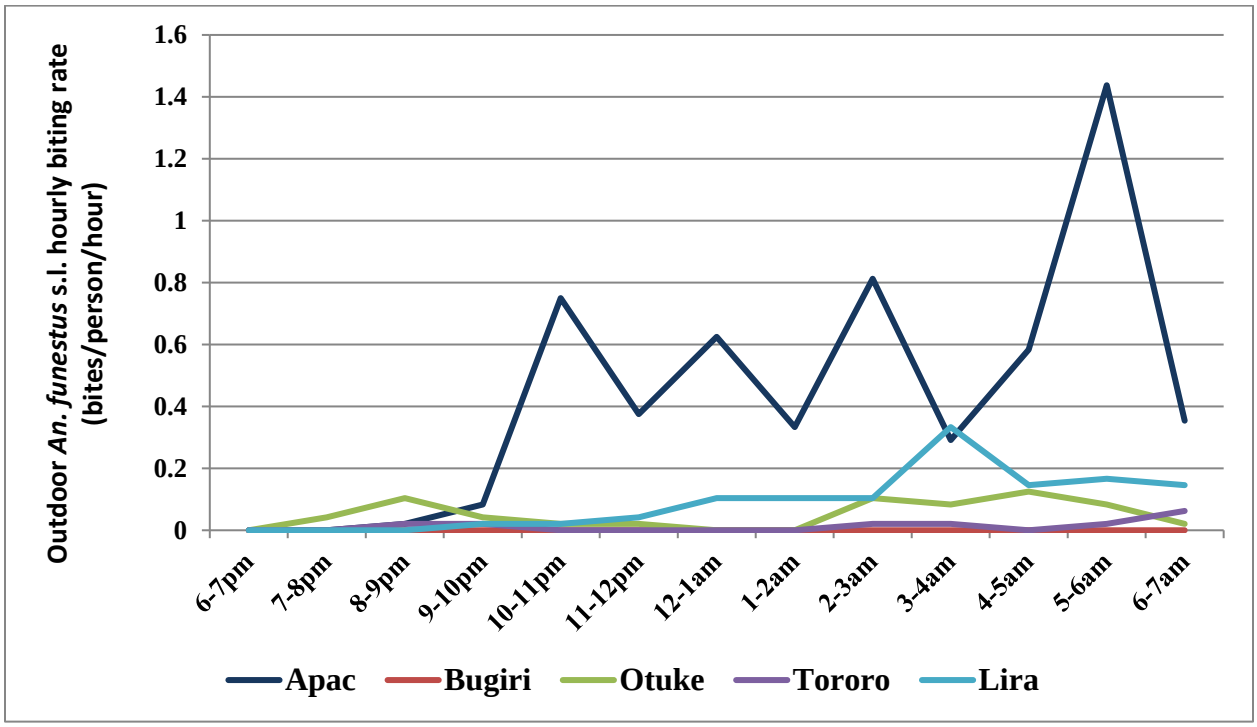


Figure 5C. *An. funestus* s.l. indoor and outdoor for Soroti

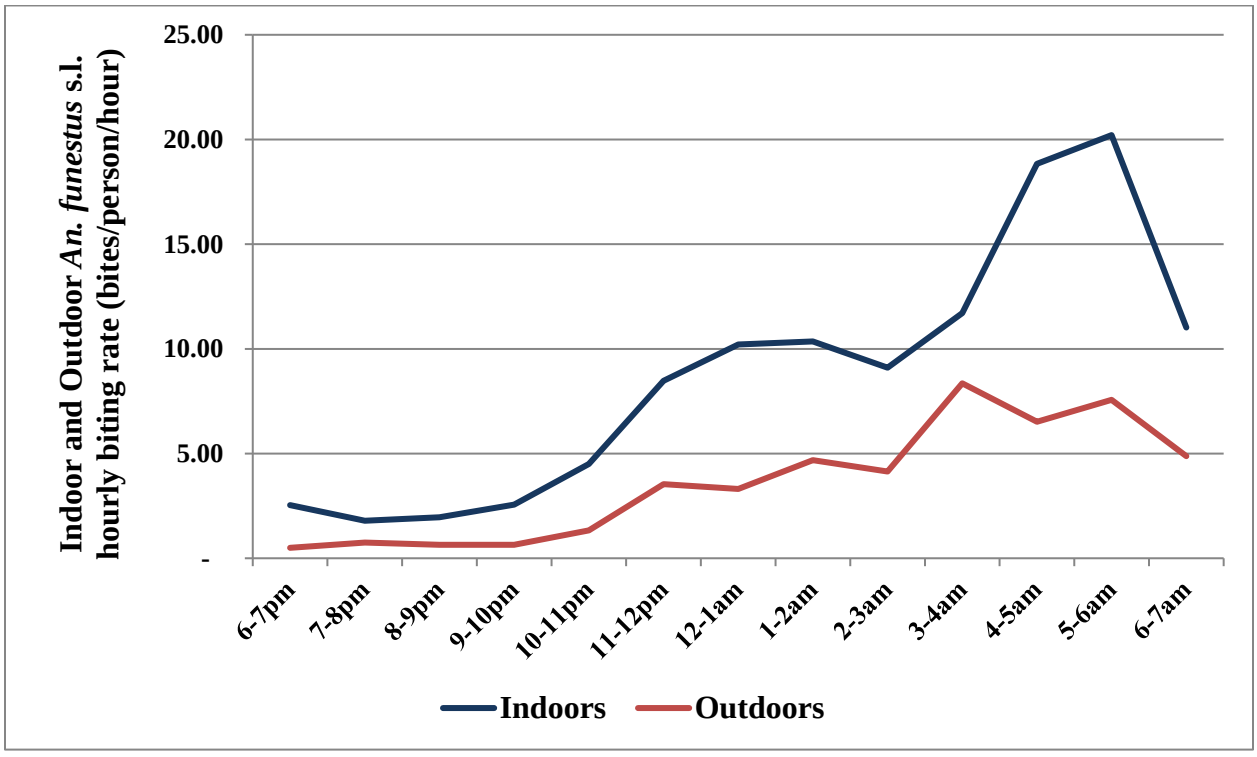


Figure 5D. *An. gambiae* s.l. indoors and outdoors in the IRS study districts of Bugiri, and Tororo

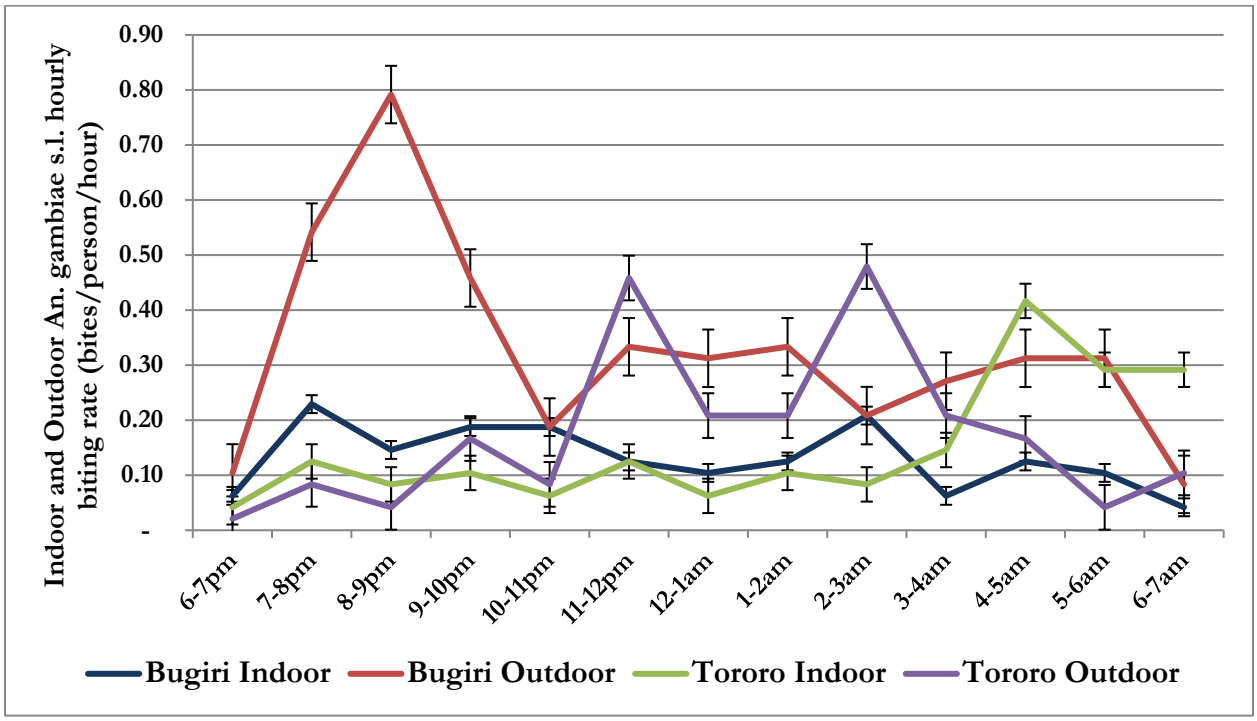


Figure 5E. *An. gambiae* s.l. indoors and outdoors in the districts of Lira and Otuke

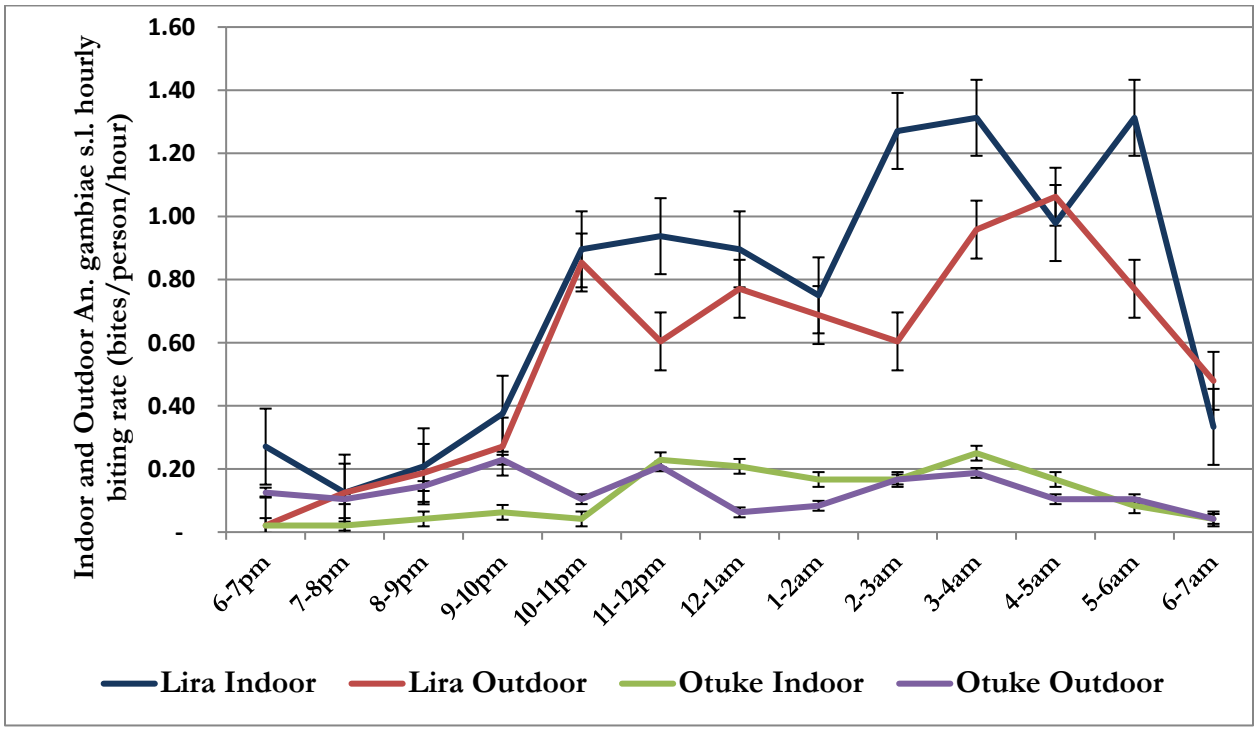
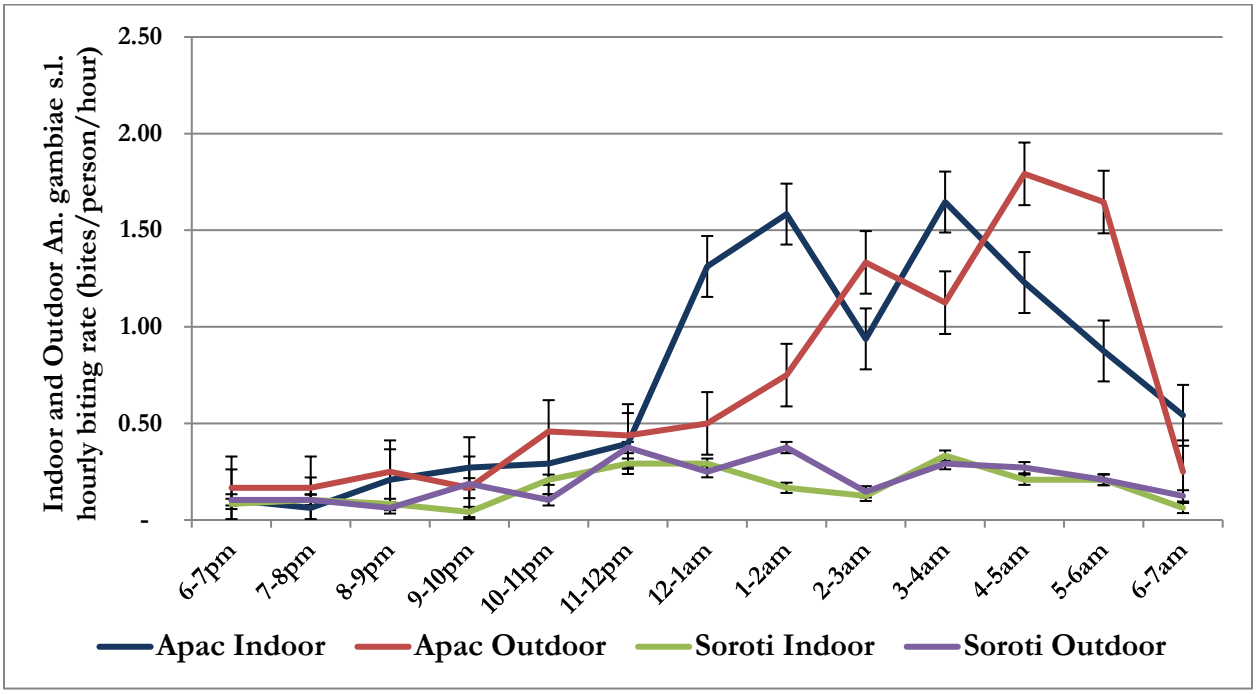


Figure 5F. *An. gambiae* s.l. Indoors and Outdoors in the in the former IRS study district of Apac and non-IRS district of Soroti



3.2 CONE WALL BIOASSAY TESTS

During the spray operations, WHO cone bioassays were conducted in one site per each of the eight spray districts (Bugiri, Butaleja, Dokolo, Kibuku, Lira, Otuke, Serere, and Tororo) within two weeks of the start of spraying to assess the quality of spraying. Thereafter, the residual efficacy and fumigant effect of Actellic 300CS was monitored in three districts (Bugiri, Otuke and Tororo) and that of SumiShield in Lira district on a monthly basis on three types of wall surfaces: plaster painted, plain brick, and mud and wattle, which constitute most wall surfaces in the IRS districts. The susceptible *An. gambiae* s.s. (Kisumu strain) was used for quality and decay rate assessment.

3.2.1 QUALITY OF IRS

The results for the spray quality bioassays (conducted within one week after spraying) showed adequate spray quality (100% mosquito mortality) in the eight sentinel spray districts.

3.2.2 INSECTICIDE DECAY RATE

Cone bioassay test results indicate that Actellic 300CS residual life ranged from six months in Otuke district to nine months in Bugiri district; while SumiShield 50WG remained effective with 100% test mortality rates on all three surfaces at seven months after spraying in Lira district. Actellic 300 CS lasted longer on painted surfaces compared to mud (Table 6). The majority of houses in Bugiri have plaster painted and plain brick wall surfaces while the majority of houses in Otuke have mud wall surfaces. Plastered and painted wall surfaces retain insecticides on the wall surface better than the plain brick and mud wall surfaces. Thus plastered and painted wall surfaces continue to kill mosquitoes for longer periods. There is a tendency for mud houses to absorb insecticides and insecticide availability is reduced on the surface. Also, due to the rough nature of mud and sometimes un-plastered plain brick houses, any contact by people on the walls tend to rub off the mud/soil and this removes insecticide thus reducing its efficacy on these walls. House modification to ensure wall are

plastered and painted, or at least plastered is likely to help retain insecticide on the surface for longer periods and thus allowing for longer residual efficacy of the insecticide.

Table 6: Wall Bio-Assay Results in Four Insecticide Decay Rate Monitoring Sites, December 2019

Time	% Mortality of *An. gambiae* s.s. (Kisumu strain)																Overall Mean
	Actellic 300 CS Bugiri (T0 = March 2019)				Clothianidin(SumiShield) Lira (T0 = May 2019)				Actellic 300 CS Otuke (T0 = May 2019)				Actellic 300CS Tororo (T0 = March 2019)				
	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	
T0	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T1	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T2	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	80 (24/30)	93.3	100 (30/30)	100 (30/30)	100 (30/30)	100	98.3
T3	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T4	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T5	93.3 (28/30)	100 (30/30)	100 (30/30)	97.7	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	97.7 (29/30)	86.7 (26/30)	94.8	70 (21/30)	23.40 (7/30)	70 (21/30)	54.5	86.7
T6	100 (30/30)	100 (30/30)	96.7 (29/30)	98.9	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	83.3 (25/30)	73.3 (22/30)	85.5	100 (30/30)	100 (30/30)	100 (30/30)	100	96.1
T7	100 (30/30)	96.7 (29/30)	86.7 (26/30)	94.5	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	76.7 (23/30)	60.0 (18/30)	78.9	100 (30/30)	93.3 (28/30)	86.7 (26/30)	90.0	90.8
T8	100 (30/30)	93.30 (28/30)	76.7 (23/30)	90.0	TBD	TBD	TBD		TBD	TBD	TBD		100 (30/30)	83.3 (25/30)	76.7 (23/30)	86.7	88.3
T9	93.3 (28/30)	86.7 (26/30)	73.3 (22/30)	84.4	TBD	TBD	TBD		TBD	TBD	TBD		90.0 (27/30)	76.7 (23/30)	63.3 (19/30)	76.7	80.6

Bugiri and Tororo, T0 =March 2019; Lira and Otuke, T0 = June 2019; TBD = To be Done

Key: T0 is the test done within 2 weeks after spraying an area; T1, T2, T3 etc represent the test results of studies conducted monthly after spraying an area i.e. 1, 2, 3 months post-spraying etc.

3.2.3 THE AIRBORNE EFFECT

Tests for airborne effect of Actellic 300CS were conducted alongside T1 to T9 in Bugiri and Tororo districts and T7 in Lira and Otuke districts (Table 7).

Table 7: Fumigant effect of Actellic 300CS spraying on the knockdown and mortality of *An. gambiae* s.s. by district in Bugiri (T9) and Tororo (T9) and Otuke (T7), and of SumiShield in Lira district (T7)

District	Spray date	Type of wall surface	Number of *An. gambiae* s.s. tested in sprayed houses	Number knocked down after 30 minutes (%)	Number knocked down after 60 minutes (%)	Number (%) dead after 24 hours
Bugiri (Actellic 300CS sprayed)	March 2019	Plaster Painted	10	0 (0%)	0 (0%)	9 (90%)
		Plain Brick	10	0 (0%)	0 (0%)	6 (60%)
		Mud	10	0 (0%)	0 (0%)	4 (40%)
Tororo (Actellic 300CS sprayed)	March 2019	Plaster Painted	10	0 (0%)	0 (0%)	8 (80%)
		Plain Brick	10	0 (0%)	0 (0%)	6 (60%)
		Mud	10	0 (0%)	0 (0%)	5 (50%)
Otuke (Actellic 300CS sprayed)	May 2019	Plaster Painted	10	0 (0%)	0 (0%)	8 (80%)
		Plain Brick	10	0 (0%)	0 (0%)	5 (50%)
		Mud and Wattle	10	0 (0%)	0 (0%)	5 (50%)
Total Actellic 300CS			**90**	**0 (0%)**	**0 (0.0%)**	**56 (62.2%)**
Lira (SumiShield 50WG sprayed)	May 2019	Plaster Painted	10	0 (0%)	0 (0%)	5 (50%)
		Plain Brick	10	0 (0%)	0 (0%)	8 (80%)
		Mud and Wattle	10	0 (0%)	1 (10%)	3 (30%)
Total SumiShield 50WG			**30**	**0 (0%)**	**1 (3.36.7%)**	**16 (53.3%)**

3.3 WHO Insecticide Susceptibility Testing

3.3.1 Determination of the Insecticide Susceptibility Status using WHO Tube Tests

Susceptibility testing was conducted in Apac, Arua, Bugiri, Kanungu, Lira, Moroto, Rakai and Tororo districts from June to July 2019 and January 2020 as tests conducted earlier could not cover all planned target insecticides due to a shortage of mosquitoes in the field in all the study districts.

The WHO 2016 standard susceptibility test method was used in 7/8 districts to test the main malaria vector *An. gambiae* s.l. CDC bottle assay was only used in Kanungu district to test pyrethroids.

An. gambiae s.l. was found to be susceptible (98-100% mortality) to pirimiphos-methyl in seven (Apac, Arua, Bugiri, Kanungu, Moroto, Rakai and Tororo) out of the eight districts while in Lira reduced susceptibility (97.6% mortality) was observed. The test result from Lira needs to be interpreted with caution as number of mosquitoes tested was few (n=41). Further study is needed prior to making any definitive conclusion about susceptibility status of *An. gambiae* s.l. population to pirimiphos-methyl in Lira (Figure 6A). *An. gambiae* s.l. was susceptible to bendiocarb in the five districts of Bugiri, Kanungu, Lira, Moroto and Tororo, with resistance observed in Apac, Arua and Rakai districts with 24 hour mortality varying between 81-87% (Annex 1 Table B and Figure 6A). *An. gambiae* s.l. was found resistant to alpha-cypermethrin, deltamethrin and permethrin where they were tested except in one site, Kanungu. Resistance of *An. gambiae* s.l. to deltamethrin observed in Apac, Arua, Bugiri, Lira, Moroto and Tororo with mortality varying between 25% and 89% with possible resistance observed in Kanungu. Resistance of *An. gambiae* s.l. to permethrin was recorded in Apac, Arua, Moroto and Tororo with mortality varying between 23% and 73%, with susceptibility observed in Kanungu where it was tested with CDC bottle bioassays. Resistance of *An. gambiae* s.l. to alpha-cypermethrin was observed in Apac, Arua, Moroto and Tororo with mortality varying between 23% and 73% with susceptibility observed in Kanungu (Figure 6B).

Figure 6: 24 hr Mortality of Adult *An. gambiae* s.l. From Larval Collections Exposed to a Range of Insecticides at Diagnostic Concentrations (June-July 2019 and January 2020)

Figure 6A. *An. gambiae* s.l. percent 24 hour holding mortality of female *Anopheles gambiae* s.l. after exposure to bendiocarb and pirimiphos-methyl in eight districts in Uganda, June – July 2019 and January 2020

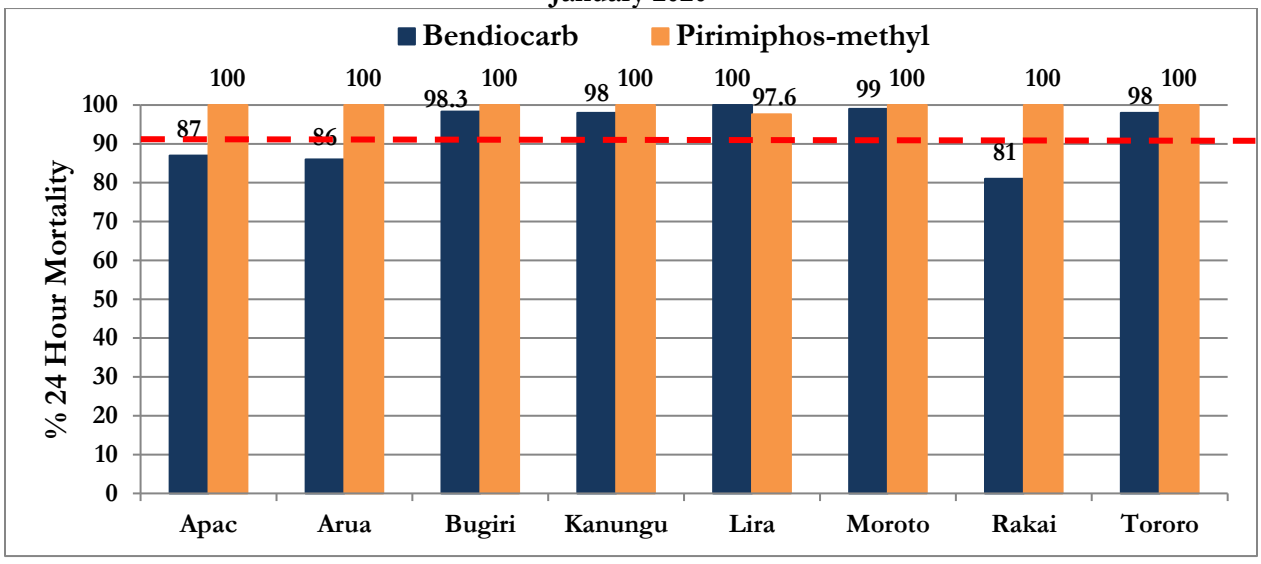
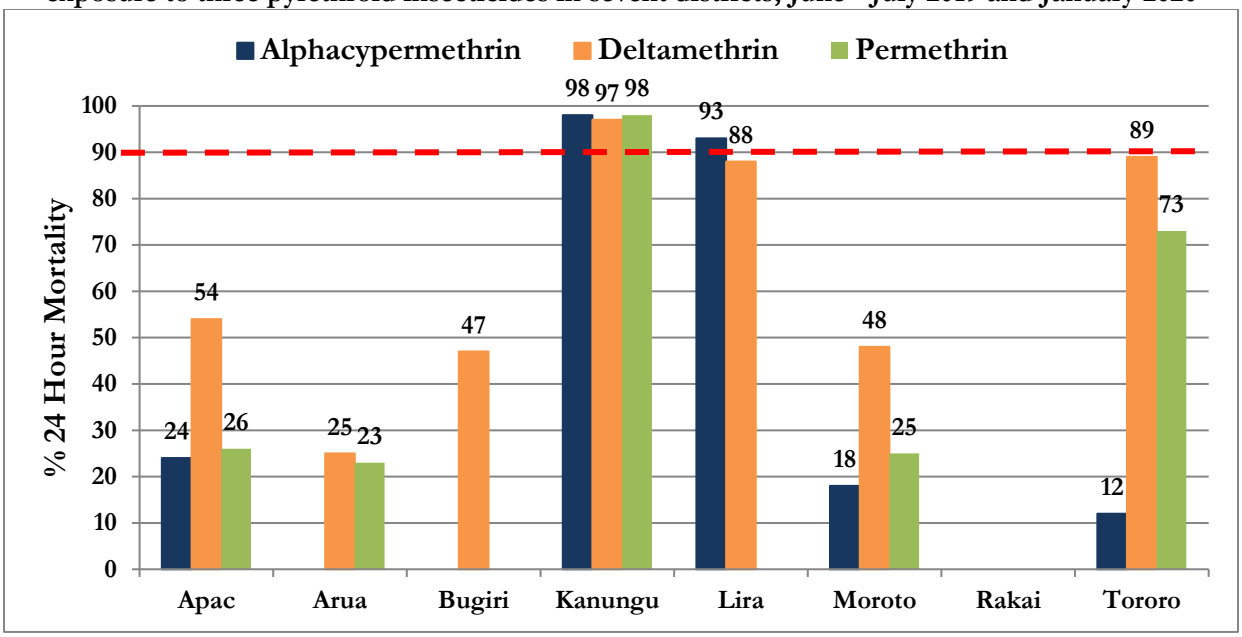


Figure 6B. *An. gambiae* s.l. Percent 24 hour holding mortality of female *Anopheles gambiae* s.l. after exposure to three pyrethroid insecticides in sevent districts, June - July 2019 and January 2020

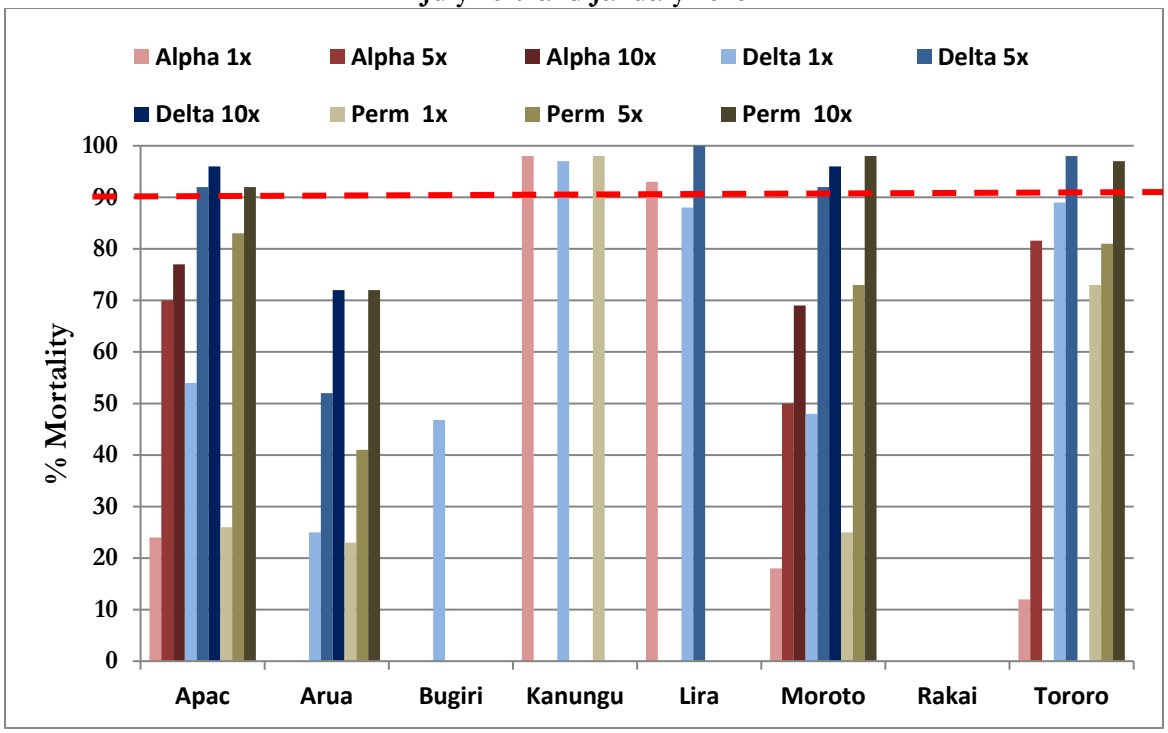


3.3.2 Determination of the Intensity of Resistance using WHO Tube Tests

Bioassays for intensity of resistance were conducted where *An. gambiae* s.l. resistance was detected with the discriminating concentrations (24 hr mortality <90%) of the respective insecticides using the WHO tube assay method in Apac, Arua, Moroto and Tororo districts.

Results of resistance intensity studies of *An. gambiae* s.l. using the WHO tube assay showed high resistance intensity of *An. gambiae* s.l. to alphacypermethrin in the districts of Apac, Moroto and Tororo, high resistance intensity to deltamethrin in Apac and Arua districts, while low resistance intensity observed to deltamethrin in Lira and Tororo. High resistance intensity was observed to permethrin in Apac and Arua and moderate resistance intensity in Apac and Moroto. (Annex 1 Table B. and Figure 7).

Figure 7: Percent mortality of *An. gambiae* s.l. after exposure to different concentrations of alphacypermethrin, deltamethrin and permethrin using WHO papers in four districts in Uganda, July 2019 and January 2020



Key: Alpha = alphacypermethrin; Delta = Deltamethrin; Perm = Permethrin

Red line indicates suceptibility cutoff (Results below 90% indicate resistance)

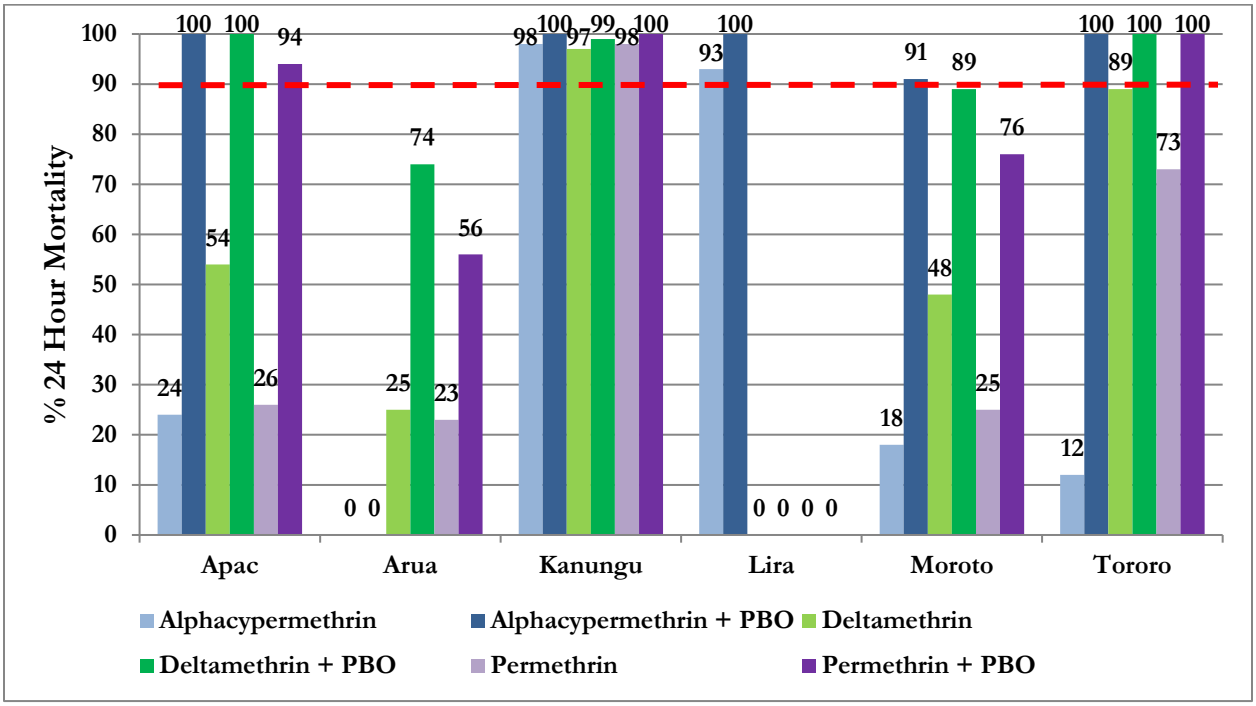
3.3.3 Determination of the Insecticide Resistance Mechanisms (Synergist Assays) using WHO Tube Tests

Synergist bioassays were conducted to assess the involvement of oxidase enzymes in *An. gambiae* s.l. resistance to pyrethroid insecticides using piperonyl butoxide (PBO).

- The synergist assay using PBO restored *An. gambiae* s.l. susceptibility to alpha-cypermethrin in Apac, Lira, and Tororo (100% mortality) and partially restored in Moroto (91% mortality)
- Pre-exposure to PBO restored susceptibility to deltamethrin in Apac, Lira and Tororo (99-100% mortality) and but did so only partially in Bugiri (74% mortality) and Moroto (89% mortality)
- Pre-exposure to PBO restored susceptibility to permethrin in Kanungu and Tororo (100% mortality) and partially restored in Apac (94% mortality), Bugiri (56% mortality) and Moroto (76% mortality) (Figure 8).

Full restoration of the efficacy of the insecticides in some districts to some insecticides suggest that monoxygenases are the only form of metabolic resistance prevailing in those sites. Partial restoration of the efficacy of the insecticides suggests that, though monoxygenases are the main form of metabolic resistance prevailing in the area, other forms of resistance mechanisms are also present and need to be investigated.

Figure 8: Synergist assay mortality results in *An. gambiae* s.l. from five out of eight study districts in Uganda upon exposure to alpha-cypermethrin, deltamethrin, and permethrin only or with 4% PBO pre-exposure June – July 2019



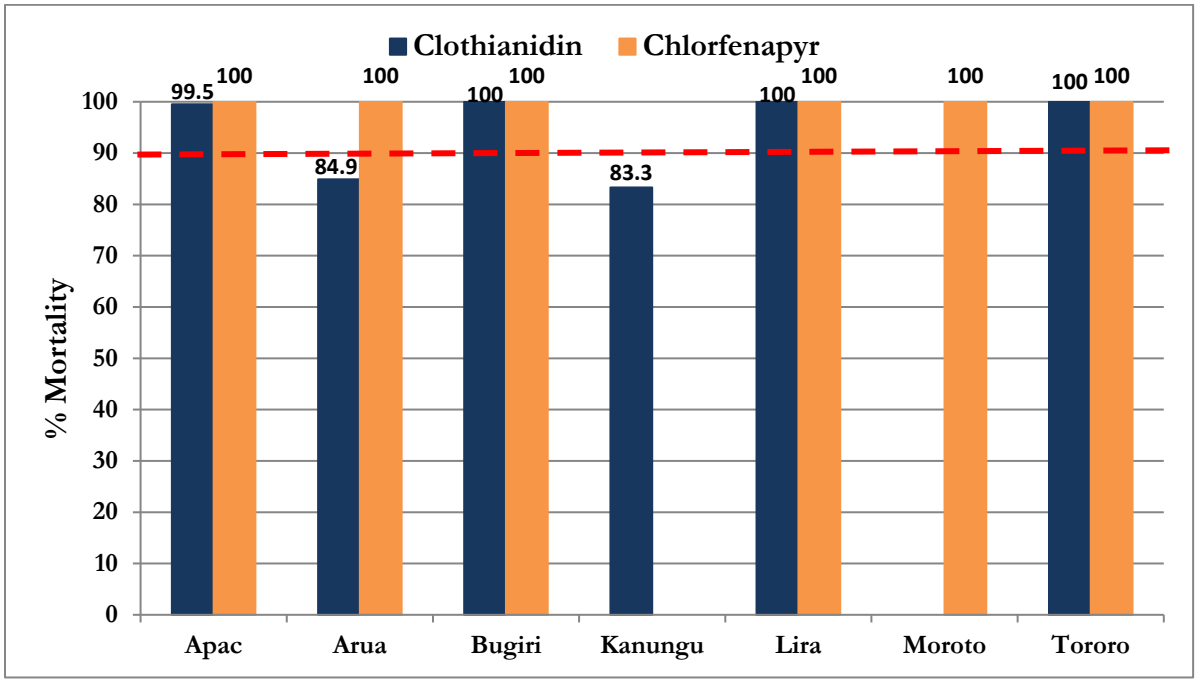
Key: Some tests not done in some districts due to inadequate mosquito samples

3.4 CLOTHIANIDIN AND CHLORFENAPYR SUSCEPTIBILITY TEST RESULTS

The clothianidin and chlorfenapyr susceptibility test were done according to the PMI AIRS protocol with the standard operating procedures SOP 001 and using WHO papers respectively.

The clothianidin tests were conducted on samples collected in Apac, Arua, Bugiri, Kanungu, Lira and Tororo districts. *An. gambiae* s.l. was found to be fully susceptible (99.5-100% mortality) to clothianidin in Apac, Bugiri, Lira, Tororo, resistant in Arua and Kanungu (though fewer than 100 mosquitoes were used for the test in these two districts) in Uganda after a seven-day holding period. (Figure 9). The chlorfenapyr tests were conducted on samples collected in Apac, Arua, Bugiri, Lira, Moroto and Tororo - districts (Figure 9). *An. gambiae* s.l. was found to be fully susceptible (100 percent mortality) to chlorfenapyr in all the six study districts where it was tested in Uganda after a three-day holding period. Both clothianidin and chlorfenapyr tests were conducted on the wild *An. gambiae* s.l. in parallel with the laboratory susceptible *An. gambiae* s.s. Kisumu strain which was also killed in all the tests except in Arua, Kanungu and Moroto where there was no nearby colony of susceptible *An. gambiae* s.s. to be used as reference.

Figure 9: Clothianidin and Chlorfenapyr Susceptibility Test Results on Wild *An. gambiae* s.l. (Percentage Mortality at 7th and 3rd Day respectively Post-Exposure)



3.5 PRE- AND POST-IRS PSCS IN THE CURRENT 4 IRS DISTRICTS, MARCH–JUNE 2018

The project collected a total of 266 female malaria vectors (264 *An. gambiae* s.l. and 2 *An. funestus* s.l.) during the pre-IRS PSCs in March and April 2019 compared to 59 female malaria mosquitoes (58 *An. gambiae* s.l. and 1 *An. funestus* s.l.) during the post-IRS PSCs in April and July 2019 in the current IRS districts of Bugiri, Lira, Otuke and Tororo (Table 9 and Figures 10 and 11). The data shows the indoor vector resting densities decreased following IRS, although the data was affected by the prolonged dry season during the pre-IRS PSCs and the intermittent heavy rains allowing for prolific vector breeding during the post-IRS PSCs from May 2019.

During pre-IRS PSCs in the four sentinel study districts of Bugiri, Tororo, Lira and Otuke, a total of 10 unfed female *An. gambiae* s.l. were dissected with 70% (n=7) samples being parous, indicating that most of the mosquitoes collected had laid eggs at least once. However, during post-IRS PSCs in the same four sentinel study districts, a total of 11 unfed female *An. gambiae* s.l. collected and dissected. Only 9.1% (n=1) *An. gambiae* s.l. dissected were parous, indicating that most of the mosquitoes collected were young and had not laid eggs therefore would not have transmitted malaria (Table 10)

Table 8: Comparison of female mosquito species caught during pre-IRS and post-IRS PSCs in Bugiri and Tororo districts, February and April 2019, and in Lira and Otuke in April and early July 2019

District	PSCs	Number of occupants	*An. gambiae* s.l.		*An. funestus* s.l.		Other *Anopheles*		*Culex* sp.		*Mansonia* sp.		Total female vectors	Vector density (Persons per vector)
			M	F	M	F	M	F	M	F	M	F		
Bugiri	Pre-IRS	50	11	110	0	0	0	0	2	1	0	0	110	2.20
	Post-IRS	69	1	2	0	0	0	0	0	2	0	2	2	0.03

Tororo	Pre-IRS	55	3	8	0	0	0	0	4	10	0	0	8	0.15
	Post-IRS	55	0	2	0	0	0	0	0	0	0	0	2	0.04
Lira	Pre-IRS	51	20	130	0	2	0	0	26	64	0	18	132	2.6
	Post-IRS	55	34	51	0	1	0	0	6	18	0	2	52	0.9
Otuke	Pre-IRS	67	3	16	0	0	0	0	4	26	0	6	16	0.2
	Post-IRS	50	2	3	0	0	0	0	0	2	0	0	3	0.1
Total	Pre-IRS	223	37	264	0	2	0	0	36	101	0	24	266	1.2
	Post-IRS	229	37	58	0	1	0	0	6	22	0	4	59	0.3

Figure 10: Mean number of vectors per house caught during pre- and post-PSCs in four IRS districts, 2019

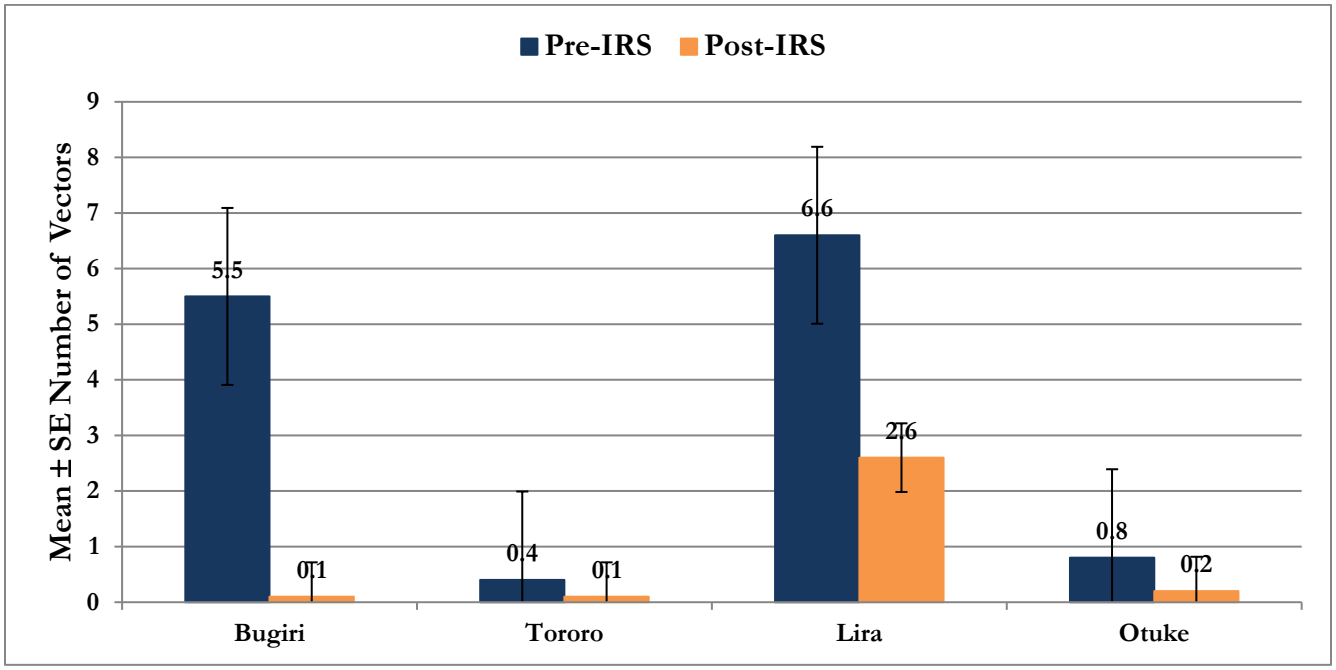


Figure 11: Comparison of female mosquito species caught during pre-IRS and post-IRS PSCs in the current four IRS districts, 2019

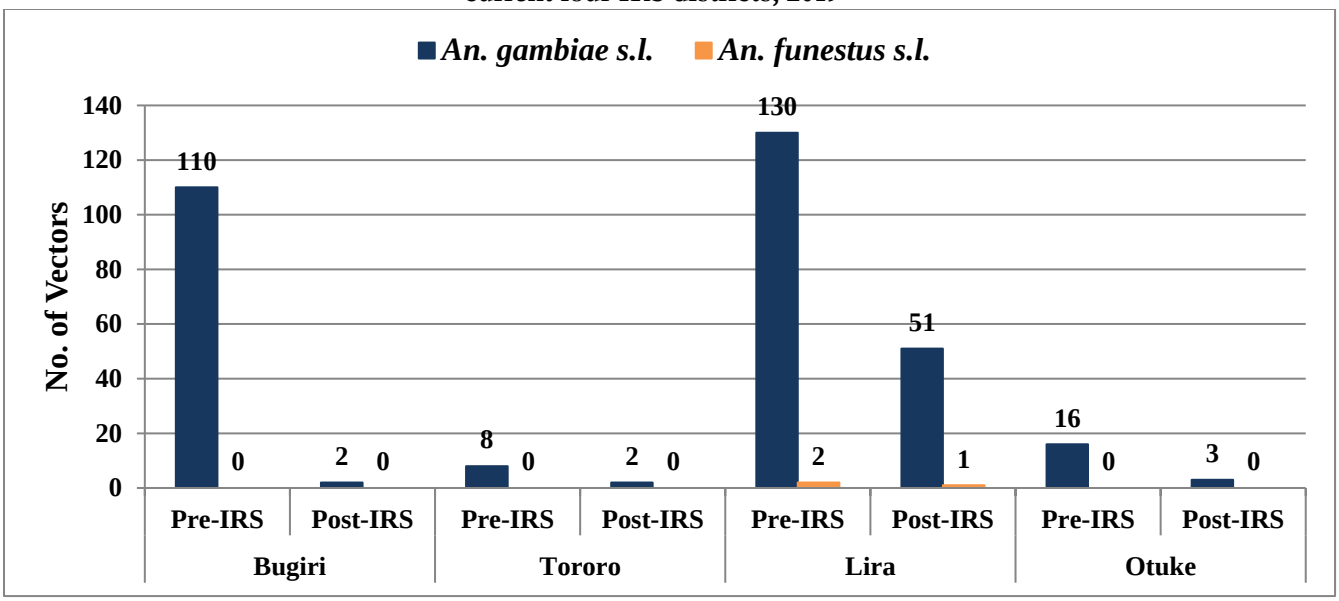


Table 9: Results of dissections of female malaria vectors for parity during one month pre-IRS and one month post-IRS PSCs in Bugiriri, Tororo, Lira and Otuke, district 2019

District	Study	Time	*An gambiae* s.l.				*An. funestus* s.l.			
			Total female dissected	Parity			Total female dissected	Parity		
				NP	P	%		NP	P	%
Bugiri	Pre-IRS	February	2	0	2	100	0	-	-	-
	Post-IRS	April	2	2	0	0	0	-	-	-
Tororo	Pre-IRS	February	0	-	-	-	0	-	-	-
	Post-IRS	April	2	2	0	0	0	-	-	-
Lira	Pre-IRS	April	8	3	5	62.5	0	-	-	-
	Post-IRS	July	6	5	1	16.7	0	-	-	-
Otuke	Pre-IRS	April	0	-	-	-	0	-	-	-
	Post-IRS	July	1	1	0	0	0	-	-	-
Total	**Pre-IRS**		**10**	**3**	**7**	**70.0**	**0**	**-**	**-**	**-**
	Post-IRS		**11**	**10**	**1**	**9.1**	**0**	**-**	**-**	**-**

Key: *NP = Nulliparous (never taken a blood meal); P = Parous (taken a blood meal); % = Percentage parous*

3.4 MOLECULAR ASSAY RESULTS

A total of 9,950 samples were given to the IDRC Molecular Laboratory for molecular analysis. 20 did not amplify during PCR speciation due to poor quality DNA.

3.4.1 IDENTIFICATION OF VECTOR SPECIES

Molecular assays performed on the samples identified morphologically as belonging to *An. gambiae* and *An. funestus* complexes. A total of 1,095 *An. gambiae* s.l. of morphologically identified mosquitoes were analyzed off which 397 (36.3%) were identified as *An. gambiae* s.s, 678 (61.9%) identified as *An. arabiensis*, while 20 (1.84%) could not be amplified due to poor quality DNA (Table 11).

An. gambiae s.s. was the dominant malaria vector of the *An. gambiae* complex in current IRS district of Lira (59.6%) and in the former IRS district of Apac (66.2%), while *An. arabiensis* was the dominant malaria vector in current IRS districts of Tororo (97.9%), Bugiri (84.1%) and Otuke (56.6%), and in the control district of Soroti

(78.2%). An. *funestsus* s.s. was the only malaria vector of the *An. funestus* group that was found in the study districts of Apac, Lira, Otuke, Soroti and Tororo where analysis was done (Table 11).

Table 10: Summary of PCR analysis of *An. gambiae* and *An. funestus* complexes from bionomics (longitudinal) studies conducted in various sentinel sites in various districts in Uganda, 2019 (Expecting additional data)

District	Sampling method and location	PCR Results of morphologically identified *An. gambiae* s.l.				PCR Results of morphologically identified *An. funestus* s.l.		
		Number analyzed	*An. gambiae* s.s.	*An. arabiensis*	Sample didn't amplify	Number analyzed	*An. funestus* s.s.	Didn't amplify
Apac (former IRS)	PSCs and HLCs (Atar village)	240	154 (66.2%)	81 (33.7%)	5 (2.1%)	25	24 (96.0%)	1 (4.0%)
Bugiri (current IRS)	PSCs and HLCs (Bubwoki village)	189	27 (14.3%)	159 (84.1%)	3 (1.6%)	0	0	0
Lira (current IRS)	PSCs and HLCs (Teokole village)	240	143 (59.6%)	93 (38.7%)	4 (1.7%)	25	24 (96.0%)	1 (4.0%)
Otuke (current IRS)	PSCs and HLCs (Oboloko village	76	31 (40.8%)	43 (56.6%	2 (2.6%)	21	20 (95.2%)	1 (4.8%)
Soroti (control)	PSCs and HLCs Awoja village)	206	41 (19.9%)	161 (78.2%)	4 (1.9%)	25	24 (96.0%)	1 (4.0%)
Tororo (current IRS)	PSCs and HLCs (Nangoke village)	144	1 (0.7%)	141 (97.9%)	2 (1.4%)	3	3 (100%)	0 (0%)
Total		**1095**	**397 (36.3%)**	**678 (61.9%)**	**20 (1.8%)**	**99**	**95 (96.0%)**	**4 (4.0%)**

3.4.2 DETECTION OF MALARIA PARASITES (DETERMINATION OF INFECTION RATES)

In the intervention area, *P. falciparum* sporozoites were detected in 0.63% (n= 10/1599) of the *An. gambiae* s.l. tested with 0.84% (n= 8/951) for mosquitoes collected using PSCs and 0.31% (n= 2/648) HLCs. Higher vector infection rates were detected in the control district, with a mean sporozoite rate estimated at 2.66% compared to the mean sporozoite rate of 0.63% in the intervention districts (Table 11). Table 12 shows sporozoite rates in An. funestus s.l. which was very few in in most district except for Soroti.

Table 11 *An. gambiae* s.l. *P. falciparum* sporozoites as detected by PCR assays of combined PSC and HLC collections (2019)

Month	Apac			Bugiri			Lira			Otuke			Soroti			Tororo			Non-IRS	IRS
	Number analyzed	No. positive	Sporozoite rate %	Number analyzed	No. positive	Sporozoite rate %	Number analyzed	No. positive	Sporozoite rate %	Number analyzed	No. positive	Sporozoite rate %	Number analyzed	No. positive	Sporozoite rate %	Number analyzed	No. positive	Sporozoite rate %	zoite rate %	zoite rate %
Jan	20	2	10.0	13	0	0	5	0	0	5	0	0	230	0	0	8	0	0	0.80	0
Feb	4	0	0	19	0	0	14	0	0	0	0	0	229	1	0.4	4	0	0	0.43	0

Mar	0	0	0	26	0	0	12	0	0	0	0	0	4	0	0	17	0	0	0	0
Apr	0	0	0	0	0	0	12	1	8.3	3	0	0	4	0	0	0	0	0	0	6.67
May	137	8	5.8	44	0	0	161	4	2.5	31	0	0	85	0	0	6	0	0	3.60	1.65
Jun	295	24	8.1	37	0	0	182	0	0	16	0	0	19	0	0	55	0	0	7.64	0
Jul	53	10	18.9	13	0	0	37	0	0	6	0	0	15	0	0	35	0	0	14.71	0
Aug	44	3	6.8	6	0	0	32	0	0	4	0	0	6	0	0	19	0	0	6.00	0
Sep	151	4	2.6	30	0	0	59	1	1.7	22	0	0	23	0	0	0	0	0	2.30	0.90
Oct	0	0	0	38	0	0	45	1	2.2	40	0	0	207	1	0.5	51	0	0	0.48	0.57
Nov	0	0	0	78	0	0	45	1	2.2	50	0	0	193	1	0.5	157	1	0.6	0.52	0.61
Dec	0	0	0	98	0	0	29	0	0	21	1	4.8	346	1	0.3	14	0	0	0.29	0.62
Total	**704**	**51**	7.2	**402**	**0**	**0**	**633**	**8**	**1.3**	**198**	**1**	0.5	**1361**	**4**	**0.3**	**366**	**1**	**0.3**	**2.66**	**0.63**

Table 12: Number of *An. funestus* s.l. vector species carrying *P. falciparum* sporozoites as detected by PCR assays of PSC and HLC collections (Expecting additional data)

District	Method of mosquito collection								
	PSC			HLC			TOTAL		
	Number analyzed	Sporozoite positive	Sporozoite rate	Number analyzed	Sporozoite positive	Sporozoite rate	Number analyzed	Sporozoite positive	Sporozoite rate
Apac	0	-	-	0	-	-	0	0	
Bugiri	3	0	0.00%	0	-	-	3	0	
Lira	0	-	-	0	-	-	0	-	-
Otuke	35	0	0.00%	10	1	10.00%	45	1	2.22%
Soroti	147	1	0.68%	138	0	0.00%	285	1	0.35%
Tororo	0	-	-	2	0	0.00%	2	0	0.00%
IRS	**38**	**0**	**0.00%**	**12**	**1**	**8.33%**	50	1	**2.00%**
Control	**147**	**1**	**0.68%**	**130**	**0**	**0.00%**	285	1	**0.35%**

3.4.3 DETECTION OF INSECTICIDE RESISTANCE MARKERS

These assays are yet to be done due to delay in receipt of laboratory supplies by IDRC.

4. Discussion, Lessons Learned, and Challenges

Results of PSCs during longitudinal studies show that *An. funestus* s.l. was the most abundant species collected while resting indoors in Soroti (control district) and in Otuke (current IRS district) while in the former IRS district of Apac, the two vector species were found in fairly equal proportions. In contrast, *An. gambiae* s.l. predominated the indoor catches in the IRS intervention districts of Bugiri, Lira and Tororo. This may indicate that the more endophillic and endophagic *An. funestus* s.l. responds faster to IRS than *An. gambiae* s.l. which may also include the more exophilic and exophagic *An. arabiensis* species, but could also relate to the sentinel site location with that of Soroti being near a permanent swamp, an ideal breeding habitat for *An. funestus* s.l..

HLCs demonstrated that the Soroti control area had highest mean *An. funestus* s.l. biting rate both indoors and outdoors. *An. gambiae* s.l. was found at higher densities in the control and former IRS district, albeit at much lower densities than *An. funestus* s.l. Furthermore, the HLCs revealed significant differences between outdoor and indoor biting densities for both vector species in some districts. Observed biting activity was consistently higher outdoors than indoors for *An. gambiae* s.l. in the IRS districts Bugiri, and Tororo, but higher indoors in Lira. *An. gambiae* s.l. biting rates were highest immediately preceding the start of spraying in the IRS districts of Lira and Otuke but not in Bugiri and Tororo where prolonged heavy rains increased anopheline mosquito breeding sites post-IRS. Biting activity of *An. funestus* s.l. was consistently higher indoors than outdoors especially in the control district of Soroti and the former IRS district of Apac and current IRS districts of Lira and Otuke (Annex 1). Regarding the overnight biting patterns observed, biting starts in early evening, between 6:00 pm and 8:00 pm at most sites, and generally peaks after midnight, towards morning hours of between 4:00 am and 6:00 am

The HLC collections indoors and outdoors demonstrated that the study areas have a variety of *Anopheles* species, specifically eight different species (*An. funestus* s.l., *An. gambiae* s.l., *An. ardensis, An. coustani, An. maculipalpis, An. pharoensis, An. pretoriensis* and *An. ziemanni*), with *An. funestus* s.l., *An. gambiae* s.l., which were the caught across the sampled sites, although specific species abundancy differed by sentinel site.

The WHO cone wall bioassay results obtained in 8 of 15 sprayed districts showed that the spray quality of the 2019 spray campaign was satisfactory at all monitored sites. The monitoring for insecticide decay rate showed that pirimiphos-methyl on average stayed effective on sprayed surfaces between six months (in Otuke) and nine months in Bugiri. It was slightly shorter on mud surfaces 8 months in Bugiri and Tororo and 9 months on brick and painted surfaces in the two districts.

An. gambiae s.l. was found susceptible to pirimiphos-methyl (98-100% mortality) in seven out of eight study districts with suspected resistance in Lira district, while *An. gambiae* s.l. was susceptible to bendiocarb in Bugiri, Kanungu, Mororto and Tororo but resistant in Apac, Arua and Rakai districts (percent mortality less than 90%). *An. gambiae* s.l. was found resistant to alpha-cypermethrin, deltamethrin and permethrin where they were tested except Kanungu district where possible resistance to deltamethrin and susceptibility to permethrin and alpha-cypermethrin was observed. 24 hour holding mortality varied from 12% for alphacypermethrin in Tororo district to 88 percent for deltamethrin in Lira based on WHO tube test results.

The observed widespread resistance to pyrethroids has become common in sub-Saharan Africa, particularly following extensive roll-out of LLINs that started about a decade ago, in order to achieve universal coverage. The impact of pyrethroid resistance on the efficacy of LLINs in malaria control needs to be assessed. It also points to the need to deploy next generation LLINs like the PBO-synergized LLINs and new WHO-prequalified dual active ingredient LLINs to combat insecticide resistance to pyrethroids and maximize the

effectiveness of LLINs in malaria control. Synergist assays using Piperonyl Butoxide (PBO) fully or partially restored *An. gambiae* s.l susceptibility to pyrethroids indicating that oxidases are the major resistance mechanisms in the study districts although other resistance mechanisms may also play a minor role in districts where PBO only partially restored susceptibility to pyrethroids.

Although resistance to pirimiphos-methyl is minimal in Uganda, there is a need to rotate it with other next generation insecticides with other modes of action against malaria vectors, since the country used pirimiphos-methyl for four consecutive years now. The susceptibility of *An. gambiae* s.l. to clothianidin observed in various study areas indicates that Uganda has other effective next generation insecticides that can be rotated with Actellic 300CS in IRS as an alternative to manage insecticide resistance in the country. Though resistance to clothianidin was detected in Arua and Kanungu, the number of mosquito samples used for the tests were below 95 recommended as ideal for coming to a definite conclusion, requiring further confirmatory testing. Although the current WHO recommendation is pro-active rotation, at least every 12 months, given financial constraints, in Uganda, the change of an insecticide will routinely be carried out every 3 years. In case resistance is detected before 3 years, a change for an effective class of insecticide is envisaged.

In areas where resistance was detected on diagnostic doses of pyrethroids and where the mosquito samples were enough for the tests, intensity assays were conducted. Results of intensity assays with 5× and 10× concentrations for alpha-cypermethrin, deltamethrin and permethrin on *An. gambiae* s.l. in six districts showed variable results ranging from low intensity of resistance to moderate and high intensity of resistance. This has to be monitored regularly and in wider geographical ranges to determine areas where attention is needed to mitigate the impact of insecticide resistance on malaria vector control through deployment of next generation IRS and LLIN products.

Results of synergist assays conducted in various districts suggest the presence of metabolic resistance mainly due to monoxygenases, although other resistance mechanisms appear to also play a minor role in some study districts. We therefore expect better malaria vector control with PBO synergized LLINs and the new WHO-pre-qualified next generation LLINs than pyrethroid-only LLINs which were deployed in some districts of Uganda during the last universal LLINs distribution coverage in 2017 to 2018. There is, however, need to conduct similar tests in various parts of Uganda in order to map the extent of this and other resistance mechanisms such as esterases and glutathione-s-transferases in the country.

Molecular assays are ongoing at IDRC Molecular Laboratories in Kampala, Uganda. The species identification performed at IDRC Molecular Laboratories using molecular assays (PCR) revealed *An. gambiae* s.s. and, *An. arabiensis* as the members of *An. gambiae* s.l.

Vector infection rate determination using PCR ELISA found sporozoite rates varying from 0%-1.8% (n= 59-395) in the current IRS districts in vectors collected using PSCs and 0% for vectors collected using HLCs, 0% in the control district for vectors collected using both PSCs and HLCs (n=331 & 294 respectively), and 5.5% and 10.4% for vectors collected using PSCs and HLCs, respectively, in the former IRS district (n=455 and 249, respectively). Overall the infection rates in IRS districts were very low for *An. gambiae* s.l. (Expecting additional data).

Assays for detection of mutations in resistance markers *kdr-E* and *Ace-1* are yet to be done at IDRC and a separate addendum to this report will be prepared and submitted after the assays are completed.

5. REFERENCES

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Brogdon EW and Chan A, 1998. Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay.

Centers for Disease Control and Prevention, 2012. Evaluating mosquitoes for insecticide resistance. Atlanta, GA. Centers for Disease Control and Prevention.

Gillies MT, Coetzee M. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region). *Publ South African Inst Med Res* [Internet] 55(55):1–143. Available from: http://ihi.eprints.org/1274/1/Gillies_%26_Coetzee_1984_Supplement_to_African_anophelines_1.pdf

World Health Organization (WHO). 2016. Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes, 2nd Edition. Geneva: WHO.

ANNEX 1

Table A: Mean indoor and outdoor vector biting rates per person per day for *An. funestus* s.l. and *An. gambiae* s.l. Indoors and Outdoors by district, January-December 2019

District	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec	
	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f
Apac																								
HLC indoors	0.5	0	0	0	0	0	0	0	8.8	0	21.8	0	14.8	3.8	6.5	1.0	38.8	0	2.8	5.25	13.0	23.5	3.8	81.0
HLC outdoors	0.3	0	0	0	0	0	0	0	17.5	0	48.5	0	9.5	2.3	3.5	0.5	13.5	0	4.8	9.50	10.5	24.50	2.0	35.0
Bugiri																								
HLC indoors	1.3	0	0	0	0.5	0	0	0	3.3	0	5.0	0	0.8	0	0	0	2.5	0	0.8	0	2.5	0	4.0	0
HLC outdoors	0.5	0	0.5	0	0.5	0	0	0	17.0	0	11.8	0	1.0	0	0.5	0	8.3	0	0.8	0	3.3	0	6.8	0
Lira																								
HLC indoors	0	0	0.3	0	0.3	0	0.3	0	68.8	0	18.8	0	5.3	0	4.0	0.3	6.5	1.5	9.3	8.50	2.3	19.8	1.0	7.5
HLC outdoors	0	0	0	0	0	0	0.3	0	52.5	0	11.0	0	2.8	0	3.0	0.3	4.5	0.5	6.5	1.75	2.0	7.3	2.5	4.5
Otuke																								
HLC indoors	0	0	0	0	0	0	0	0	7.0	0.3	4.5	0	0	0	0.5	0	1.0	-	2.8	3.00	2.0	10.0	0.3	7.5
HLC outdoors	0	0	0	0	0	0	0	0	4.8	0	3.5	0	2.0	-	0.5	0	2.3	0	4.3	0.75	2.8	6.3	0.3	0.8
Soroti																								
HLC indoors	-	92.8	0.3	57.0	0.5	72.8	0	79.0	4.5	103.0	6.5	156.3	1.3	42.3	0.8	41.0	5.3	109.3	5.5	268.3	3.0	163.8	1.0	224.5
HLC outdoors	-	11.5	-	14.0	0.3	27.3	0	29.0	3.3	27.5	7.3	45.0	2.3	15.5	0.5	17.8	8.5	43.3	1.8	73.75	6.8	94.3	0.8	163.8
Tororo																								
HLC indoors	0.8	-	0.3	-	-	-	-	-	0.5	-	10.8	0.3	4.3	-	0.3	-	-	-	2.8	-	4.3	-	0.3	-
HLC outdoors	-	-	-	-	7.0	-	0.3	-	0.3	-	25.5	0.3	5.3	-	2.8	-	3.0	-	2.8	-	12.5	0.5	1.5	-

Table B: Percent 24-hour holding mortality of *Anopheles gambiae* s.l. after exposure to seven public health insecticides, June 2018-January 2020 (results for adults reared from larvae)

Insecticide	Apac		Arua		Bugiri		Kanungu		Lira		Moroto		Rakai		Tororo	
	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality
Carbamate:																
Bendiocarb	100	87	93	86	115	98.3	100	98	100	100	103	99	80	81	101	98
Organophosphate:																
Pirimiphos-methyl	100	100	81	100	107	100	100	100	41	97.6	100	100	100	98	100	100
Pyrethroid:																
Alphacypermethrin 1x	100	24	-	-	-	-	100	98	100	93	107	18	-	-	109	12
Alphacypermethrin 5x	100	70	-	-	-	-	-	-	-	-	103	50	-	-	103	81.6
Alphacypermethrin 10x	100	77	-	-	-	-	-	-	-	-	102	69	-	-	-	-
Deltamethrin 1x	100	54	96	25	109	46.8	100	97	100	88	101	48	-	-	100	89
Deltamethrin 5x	100	92	84	52	-	-	-	-	100	100	104	92	-	-	109	98
Deltamethrin 10x	100	96	96	72	-	-	-	-	-	-	105	96	-	-	--	
Permethrin 1x	100	26	104	23	-	-	100	98	-	-	105	25	-	-	104	73
Permethrin 5x	100	83	72	41	-	-	-	-	-	-	102	73	-	-	114	81
Permethrin 10x	100	92	73	72	-	-	-	-	-	-	102	98	-	-	113	97
Clothianidin	200	99.5	86	84.9	231	100	30	83.3	200	100	-	-	-	-	217	100
Chlorfenapyr	100	100	100	100	69	100			102	100	80	100			99	100
KEY:	**Confirmed resistance**						**Probable resistance**						**Susceptible**			

* = Testing was not done due to inadequate mosquito samples.