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THE PMI VECTORLINK PROJECT

UGANDA

ANNUAL ENTOMOLOGY REPORT

JANUARY 1 – DECEMBER 31, 2022

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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
COVID-19	Coronavirus disease of 2019
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
ITN	Insecticide treated Net
Kdr	Knockdown resistance gene
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
VCD	Vector Control Division
WET	Window Exit Traps
WHO	World Health Organization

EXECUTIVE SUMMARY

Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) remain the primary mosquito vector control interventions in many parts of world, including Uganda, where malaria continues to be a major public health concern. Between January 1 – December 31, 2022, the President's Malaria Initiative (PMI) VectorLink project implemented robust entomological monitoring activities in support of vector control implementation in Uganda.

In 2022, the PMI VectorLink Uganda Project conducted a single-phase IRS campaign in 10 districts (Budaka, Bugiri, Butaleja, Butebo, Kibuku, Lira, Namutumba, Pallisa, Serere, and Tororo) from March 1–31, 2022. The project used two insecticides, SumiShield 50WG in Bugiri and Tororo, and Fludora Fusion 56.25 WP-SB, both being a clothianidin product, in the other eight districts. All IRS implemented in these districts was funded by PMI. Cone wall bioassays for quality assurance of the IRS were conducted in six districts (Bugiri, Butaleja, Kibuku, Lira, Serere and Tororo), and for monthly residual efficacy in four districts (Bugiri, Lira, Serere and Tororo).

To assess the quality and impact of vector control interventions, the project conducted monthly entomological surveillance using human landing catches (HLCs) indoors and outdoors, and pyrethrum spray catches (PSCs) plus window exit traps (WETs) in six districts: Bugiri, Lira, Serere and Tororo (IRS districts), Apac and Soroti (non-IRS districts). In addition, insecticide susceptibility tests were carried out to assess the susceptibility of *An. gambiae* s.l. and *An. funestus* s.l. to insecticides used in public health vector control: pyrethroids (alpha-cypermethrin, deltamethrin and permethrin), carbamates (bendiocarb), organophosphates (pirimiphos-methyl), pyrroles (chlorfenapyr) and neonicotinoids (clothianidin), in 11 geographically dispersed districts in different epidemiological settings in the country: three IRS districts (Bugiri, Lira and Tororo) and eight non-IRS districts (Hoima, Gulu, Kamwenge, Katakwi, Kitgum, Nakaseke, Soroti and Wakiso). These activities were conducted with the participation of district Vector Control Officers (VCOs) based in the various entomological monitoring districts, thus strengthening their capacity in entomological monitoring and surveillance activities.

The three longitudinal sampling methods utilized (PSCs, WETs and HLCs) yielded a total of 37,393 *Anopheles* mosquitoes from the six sentinel sites combined of which 27,506 (73.6%) were *An. funestus* s.l., 9,798 (26.2%) were *An. gambiae* s.l., and 89 (0.2%) were other *Anopheles* mosquitoes, including *An. coustani* and *An. ziemanni*. However, vector species composition differed considerably between study sites: *An. gambiae* s.l. was dominant in Bugiri and Tororo (and has been since at least 2017/18) while *An. funestus* s.l. was the predominant vector in Apac, Lira, Serere and Soroti. *An. funestus* has been predominant in Soroti and Apac since at least 2017, and in Lira since 2020. This was the first year for monitoring species composition in Serere. The non-IRS sites Soroti and Apac yielded the highest total numbers of *An. funestus* s.l. collected (Soroti: n=16,962; Apac: n=3,447).

In three districts the human biting rates were higher outdoors than indoors for *An. funestus* s.l., *An. gambiae* s.l. or both. This was true in Apac for both *An. funestus* s.l. (64.4%, n=215/334) and *An. gambiae* s.l. (72.3%, n=86/119), and for *An. gambiae* s.l. in Bugiri (59.9%, n=97/162) and in Serere (52.2%, n=282/540). In all other sites indoor biting rates were higher than outdoor. For *An. gambiae* s.l. the percentage biting indoors ranged from 55.9-56.6% and for *An. funestus* s.l. from 56.6-66.7%. The higher indoor than outdoor biting rates suggest that both IRS and ITNs remain, or would be, effective tools for malaria control in Tororo, Lira, and Soroti. In Lira and Soroti the endophagic index for the predominant vector has remained above 0.5 for all years monitored under VectorLink. Elsewhere, particularly before IRS is implemented, a spot check survey of biting and resting behavior is recommended. An estimation of exposure that factors in human behavior is also needed to better interpret the ratio of indoor-to-outdoor biting..

No spray quality concerns were revealed by cone wall bioassays performed for IRS quality assurance: 100% mortality was reported within 24 hours post-exposure across different wall surface types sprayed with either

Fludora Fusion or SumiShield. The residual efficacy of Fludora Fusion was between four to seven months in Kibuku, Lira and Serere districts, depending on the wall surface type, with painted cement wall surfaces performing best and mud wall surfaces performing worst. The residual efficacy of SumiShield was seven months in Bugiri and Tororo districts, on all the wall surface types sprayed. The relatively short residual efficacy of Fludora Fusion, particularly on mud, is concerning as this may not provide sufficient protection during peak transmission. This performance was worse than the previous year and this has negative implications in the control of malaria in parts of Uganda which experience two rainy seasons and perennial malaria transmission.

Insecticide resistance testing was conducted using 2-5day old adults reared from field larval collections of *An. gambiae* s.l., while for *An. funestus* s.l. resistance tests, adults were obtained from early morning prokopack collections. Susceptibility to priority IRS insecticides remained widespread: *An. gambiae* s.l. remained susceptible to pirimiphos-methyl (98-100% mortality at 24 hours post-exposure) in all 10 districts where this insecticide was tested (for one district, Katakwi, tests were done using *An. funestus* s.l.). *An. gambiae* s.l. was susceptible to bendiocarb in all four districts where the tests were conducted (Gulu, Kitgum, Lira and Tororo) with a 24-hour holding mortality of 99-100% *An. gambiae* s.l. was susceptible to clothianidin and chlorfenapyr insecticides in all districts where they were tested. However, *An. gambiae* s.l. was resistant to all pyrethroids tested (alpha-cypermethrin, deltamethrin and permethrin with the exception of one instance of probable resistance detected for deltamethrin in Kitgum (95% mortality). Synergist tests with piperonyl butoxide (PBO) against *An. gambiae* s.l. indicated that the main resistance mechanism involved was mixed function oxidases, since pre-exposure to PBO-treated papers fully restored (in Nakaseke district) or partially restored (in Hoima, Katakwi and Lira districts) susceptibility to pyrethroids in pyrethroid-resistant populations of *An. gambiae* s.l. *An. funestus* s.l. remained susceptible to pirimiphos-methyl, clothianidin, chlorfenapyr and bendiocarb but resistant to alpha-cypermethrin, deltamethrin and permethrinin Katakwi and Soroti districts. These results clearly indicate that there is no resistance in the primary malaria vectors to either active ingredient (pirimiphos-methyl and clothianidin) of IRS products recently sprayed in Uganda. The IRS deployment decision between Actellic 300CS, Fludora Fusion and SumiShield can therefore be taken on the basis of residual efficacy and rotation strategy.. The results also indicate that PBO nets are likely to perform better than pyrethroid-only nets for malaria control in Uganda, but a decision between PBO nets and dual active ingredient nets for replacing pyrethroid-only nets should be taken on a district-by-district basis and based on up-to-date insecticide resistance testing for each district.

Molecular assays for identification of species within species complexes, sporozoite infection rate determination, detection and identification of mutations on genetic resistance markers (knockdown resistance (*kdr*) and Acetylcholinesterase-1 (*Ace-1)* genes) in *An. gambiae* s.l. were conducted. *Anopheles gambiae* s.s. was the predominant vector identified in the districts of Apac, Lira, Tororo, and Soroti, while *An. arabiensis* was more common in Serere and Bugiri districts although in Bugiri the vector was caught in almost equal portions with *An. gambiae* s.s. All the 240 *An. funestus* s.l. samples analysed from six bionomics study districts were *An. funestus* s.s. The PCR results from mosquitoes assayed in the insecticide resistance tests show that *An. arabiensis* was represented in greater numbers 60.4% (436/722) than *An. gambiae* s.s. 39.6% (286/722).

Of the 829 samples from insecticide resistance studies to pyrethroids analysed for *L101F* and *L1014S* knockdown (*kdr*) markers of insecticide resistance to pyrethroids, the majority (41.62% [345/829]) were wildtype. Overall, the *L1014S* mutation was more prevalent compared to the *L101F* mutation, with Kamwenge district having the highest *L1014S* allelic frequency mutation (60.4%) and Kitgum the lowest (8.0%). On the other hand, the *L1014F* mutation allelic frequency was highest in Kitgum (16.2%). Gulu and Katakwi districts had almost equal allelic frequency proportions of the *L1014F* and *L1014S* mutations. No Ace-1R mutation was found in 256 mosquitoes tested (190 alive and 66 dead) from eight districts.

Sporozoite ELISA analysis of 7,726 (3,682 *An. gambiae* s.I. and 4,044 *An. funestus* s.l.) mosquito samples showed the sporozoite rate of *An. gambiae* s.l. ranged from 1.1% to 1.6% from PSC collections and from 1.4% to 6.2% in HLC collections, while the sporozoite rate of *An. funestus* s.l. varied between 0.4% and 2.8% in PSCs and 0.0% and 9.1% in HLCs. Sporozoite-positive *An. funestus* samples were found throughout the year with a peak in June; for *An. gambiae* the sporozoite positivity rates were generally high during the months of April, through August with a peak in May. Results indicate that in the non-IRS districts (Apac and Soroti) the sporozoite rate in *An. gambiae* s.l. varied between 1.3% and 2.5% and in *An. funestus* s.l. varied between 1.5.% and 6.5%; while

in the IRS districts (Bugiri, Lira, Serere and Tororo) the sporozoite rate in *An. gambiae* s.l. varied between 0.4% and 1.8% and in *An. funestus* s.l. varied between 0.0.% and 9.1%.(refer to Annex 9) The sporozoite positivity rates of malaria vectors caught using HLCs were generally higher than those caught using PSCs for both species, (higher for *An. gambiae* s.l. in Apac, Bugiri, Lira, Serere and Soroti except for Tororo where PSC catches were higher, and higher for *An. funestus* s.l. in Apac, Bugiri, Serere, Soroti and Tororo except for Lira where PSC catches were higher) and higher for *An. funestus* s.l. compared to *An. gambiae* s.l. Compared to previous years, sporozoite positivity rates of malaria vectors caught using HLCs were generally higher than those caught using PSCs in 2020 but generally higher for *An. gambiae* s.l. mosquitoes caught using PSCs in 2019 and 2021. However, there were differences in the various districts. *An. funestus* s.l. is more endophilic and endophagic than *An. gambiae* s.l. and this may account for the higher sporozoite rate in *An. funestus* s.l. if this species is also more anthropophilic. The higher infection rates in *An. funestsus* s.l. than in *An. gambiae* s.l. in most study districts indicates that both IRS and LLINs are effective tools for controlling the more anthropophilic and endophagic *An. funestus* s.l. as it both will protect people sleeping in sprayed houses or under LLINs. Assessment of the human blood index for these two species is needed to verify if this is true. The human blood index measures the source of blood found in fed mosquitoes which indicates the biting preferences of mosquitoes (humans or various animals).

1. INTRODUCTION

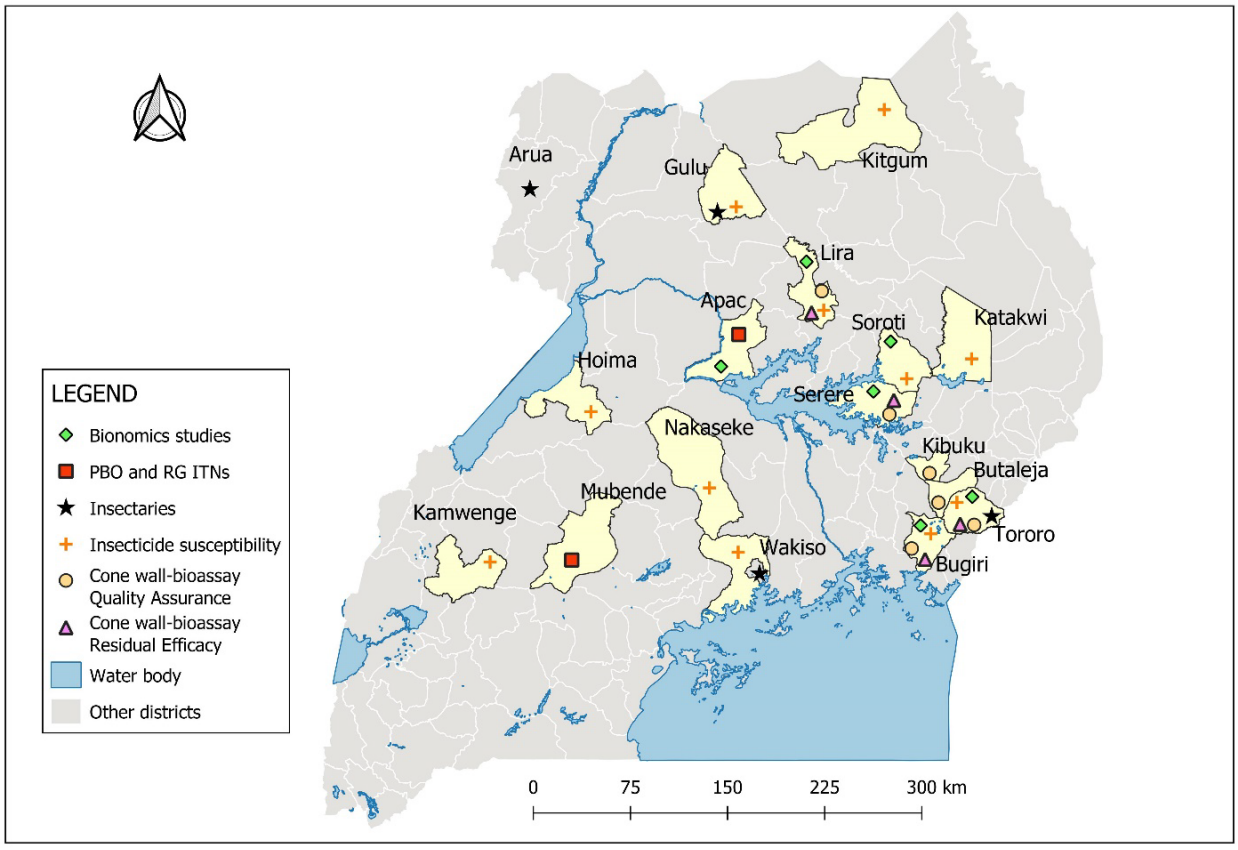
During the reporting period, the PMI VectorLink Project carried out entomological monitoring activities in 11 districts (Figure 1) in partnership with district Vector Control Officers (VCOs), strengthening in-country capacity to conduct similar entomological surveillance activities in the future. The entomological data generated by the project informs the selection of insecticides for vector control in Uganda and adds to the evidence base for evaluation of vector control impact and targeting. These data are collected through susceptibility tests of local vector species to different insecticides and determination of any underlying resistance mechanisms, cone bioassays to evaluate the quality and residual efficacy of spraying on different wall types, and monthly longitudinal monitoring of the vector density, behavior, and composition in districts that received vector control interventions in 2022 and those that did not.

Longitudinal entomological monitoring was conducted in four indoor residual spraying (IRS) intervention districts: Bugiri, Lira, Serere and Tororo; and in Apac and Soroti where IRS was not implemented in 2022. Apac previously received both piperonyl butoxide (PBO) nets (PermaNet 3.0) and Royal Guard ITNs in 2020, while the other longitudinal monitoring districts received pyrethroid-only insecticide treated nets (ITNs) (PermaNet2.0 and Yorkool LN). Pyrethrum spray catches (PSCs) to measure indoor resting densities and human landing catches (HLCs) indoors and outdoors to measure human biting rates were conducted every month of the reporting period. Window exit traps were added to supplement PSCs and measure house exiting behavior from September to December 2022. For insecticide resistance testing conducted between July and November 2022, larval collections of *An. gambiae* s.l. provided adults of known age, while for *An. funestus* s.l. resistance tests adult collections provided specimens of unknown age. Insecticide susceptibility monitoring was conducted in 11 districts representative of the different epidemiological settings in the country. This was done to determine the susceptibility status of the major malaria vectors in different settings in the country which helps to guide the Ministry of Health in the deployment of different LLINs in the country during mass LLIN distribution campaigns and selection of insecticides for IRS when implemented. IRS quality assurance studies were conducted within one week of IRS and this was followed by residual insecticide efficacy monitoring monthly post-IRS in the same structures until the mortality fell below 80% for two consecutive months.

Malaria transmission seasonality in Uganda is largely influenced by the tropical climate (mainly temperature and rainfall), with malaria vectors and malaria transmission increasing within 2-4 weeks after the peak of the rains. Most of Uganda experiences a bi-modal rainfall pattern. March to May constitutes the first major rainfall season, whilst most of the country experiences a second rainy season between September through November. June to August is generally dry in the south-western, central, Lake Victoria basin regions, and some parts of the eastern region, but there is a continuation of rainfall for much of northern Uganda during these months. 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 13 non-PMI IRS districts (Adjumani Arua, Koboko, Mad-Okollo, Maracha, Moyo, Obongi, Terogo and Yumbe in North-western (West Nile) region and Amolatar, Dokolo, Kaberamaido and Kalaki in mid-north region), implemented by Ministry of Health and supported by Global Fund, the onset of the rains is expected around early to late March. The peak of the seasonal rain is expected around late April to early May, followed by moderate relaxation around mid-June. For the eastern region nine PMI IRS districts (Bugiri, Budaka, Butaleja, Butebo, Kibuku, Numutumba, Pallisa, Serere and Tororo), the first rains are usually experienced between late February to mid-March and peaking around late April, with cessation of rains around late May/mid-June, except during El Nino years when there are above normal rains usually extending beyond the typical rainfall season. For the northern region PMI IRS district (Lira), the first rains are usually experienced from mid-March with cessation of rains expected around late June/early July except during El Nino years. However, in recent years, rainfall patterns have become more unpredictable, unusually heavy and extended rains flushing out breeding sites have made it difficult to plan when to conduct susceptibility studies. The districts of Bugiri, Budaka, Butaleja, Butebo,

Kibuku, Namutumba, Pallisa and Tororo all grow rice with extensive fields except for Serere and Lira, which are done at a much smaller scale.

FIGURE 1: PMI VECTORLINK PROJECT DISTRICTS FOR ENTOMOLOGICAL MONITORING IN 2022



2. METHODOLOGY

2.1 LONGITUDINAL MONITORING (VECTOR BIONOMICS)

PMI VectorLink Uganda collected adult mosquitoes monthly from January through December 2022 using PSCs (in accordance with PMI VectorLink Standard Operating Procedure (SOP) 03/01[1]), HLCs (PMI VectorLink SOP 02/01) and window exit traps (WETs) in six sentinel sites: Bugiri, Lira, Serere and Tororo (current IRS districts), and Apac and Soroti (non-IRS/control districts). 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 village (10 houses each day for two days) – same houses used every month. (Villages: **Apac:** Atar, Aporotuku & Amwonyocao-B; **Soroti**: Awoja, Madera Central cell & Agora; **Bugiri**: Bubwoki, Nangalama & Mugera; **Lira**: Te-Okole, Araki & Alikport; **Serere**: Angolei, Ogobai & Adepi; **Tororo**: Nagoke, Rugot & Kajarau North)
WETs	6:00 pm to 7:00 am	One night per site per month	One window exit trap in two houses per village in three villages per district where PSCs were conducted – 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 (PSC)

In each district where PSCs were conducted according to the standard PMI VectorLink SOP 03/01[2] protocol, 20 grass-thatch roof houses with a single sleeping room in each of three villages were selected. PSCs were conducted from 6:00 am to 10:00 am, two days per month over in each district from January to December 2022. The same houses were visited each month. A pyrethroid based aerosol (Polygard KillIt insecticide containing d-Tetramethrin 0.135% w/w, d-Allethrin 0.06% w/w and cypermethrin 0.46% w/w) was used to knock down indoor resting mosquitoes. The room was closed for 10 minutes after spraying with KillIt aerosol. The mosquitoes that were knocked down were put in a petri dish and taken to a field insectary for morphological identification and dissection for parity. Female *An. gambiae* s.l. and *An. funestus* s.l. were preserved, in 1.5mL microfuge tubes inside a plastic container containing silica gel, for further species identification using the Polymerase Chain Reaction (PCR) at Infectious Diseases Research Collaboration (IDRC) and Vector Control Divisions (VCD) molecular laboratories. Mosquito samples taken to IDRC

[1] Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/

[2] PMI VectorLink SOP03/01 Pyrethrum Spray Catch

molecular laboratory include *Anopheles gambiae* s.l. and *An. funestus* s.l. from longitudinal monitoring (PSCs and HLCs) and from insecticide susceptibility monitoring for confirmation of morphological identification and PCR analysis for speciation. *Anopheles gambiae* s.l. from insecticide susceptibility monitoring are taken for identification of *kdr* mutations in *An. gambiae* s.l. (homozygous or heterozygous/L1014S or L1014F) and of Acetylcholinesterase resistance (Ace-1R). Mosquito samples taken to VCD molecular laboratory include *Anopheles gambiae* s.l. and *An. funestus* s.l. from longitudinal monitoring (PSCs and HLCs) for sporozoite rate determination using ELISA to monitor the possible changes in the infection rates in *An. gambiae* s.l. and *An. funestus* s.l.

2.2.2 Window Exit Traps (WET)

In each bionomics sentinel district two houses where PSCs were conducted according to the standard PMI VectorLink SOP 03/01[3] protocol in each of three sentinel villages per district were selected for window exit traps (WETs). A trap was installed covering one window per house one day before the PSCs were conducted and removed after each PSC and kept in a safe place to be reused the following month WETs were included in the trappings that took place from September through December 2022. The addition of WETs was intended determine the density and species of mosquitoes that exited houses before or after feeding, to determine if early exiting without (prolonged) contact with IRS on the walls could partially explain why malaria transmission continued in districts after IRS. All the trapped mosquitoes in each WET were aspirated into a paper cup just before conducting the PSCs in the target houses. Samples were preserved and identified as described in section 2.2.1.

2.2.3 Human Landing Catch (HLC)

HLCs were conducted according to the standard PMI VectorLink SOP 02/01[4] protocol in all six sentinel districts. Two houses were sampled in one selected village per sentinel district on two consecutive nights to obtain eight 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 trained adult mosquito collectors collected mosquitoes, one inside the house and the other outside at least 5 meters from the house. HLCs were conducted from 6:00 pm to 7:00 am using 12 volunteers for each house, working in shifts of 4 hours each. For every hour, collectors caught mosquitoes for 55 minutes and rested for 5 minutes, during which they exchanged positions. During the time of collection, the collectors exposed part of their legs (up to the knees) and arms up to the elbow; when they felt mosquitoes landing, they turned on a torch and collected the mosquitoes using a mouth aspirator. Mosquitoes were transferred into labeled paper cups assigned for each collection hour. Mosquitoes were subsequently killed using cotton wool soaked in diethyl ether, morphologically identified, counted by species, location, and hour of collection, and preserved in 1.5 mL microfuge tubes and kept in a plastic container with silica gel. A subset of samples from these collections were sent to IDRC molecular laboratories for PCR analyses for species identification. Data obtained from HLCs were used to determine indoor and outdoor human biting rate (HBR). Mean hourly HBR was calculated by dividing the number of mosquitoes of single species or species complex (*An. gambiae* s.l./*An. funestus* s.l.) collected by the number of persons per hour.

2.3 Vector Susceptibility Testing

Insecticide susceptibility studies were conducted using the WHO tube bioassays according to the standard PMI VectorLink SOP 06/01[5] protocol and Centers for Disease Control and Prevention (CDC) bottle bioassays according to the standard PMI VectorLink SOP 04/01[6] protocol to determine *An. gambiae* s.l and *An. funestus* s.l. susceptibility or resistance to insecticides recommended by the WHO for use in public health. The five

[3] PMI VectorLink SOP03/01 Pyrethrum Spray Catch
[4] PMI VectorLink SOP02/01 Indoor and Outdoor Human Landing Catch (HLC)
[5] PMI VectorLink SOP06/01. Insecticide Susceptibility Test, Intensity and Synergist Assay Using WHO Test Kits
[6] PMI VectorLink SOP04/01. Susceptibility Testing, Resistance Intensity and Synergist Assays Using the WHO Bottle Bioassay

classes of insecticides tested included (in order of priority testing): neonicotinoids (clothianidin), organophosphates (pirimiphos-methyl 0.25%), pyrethroids (deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05%), pyrroles (chlorfenapyr) and carbamates (bendiocarb 0.1%). The pyrethroids, bendiocarb, and pirimiphos-methyl were tested using the WHO tube bioassays. These studies were conducted between July and November 2022 in 11 districts: Bugiri, Hoima, Gulu, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti, Tororo and Wakiso districts, representative of the different epidemiological settings in the country. Diagnostic doses of the insecticides were used following the PMI VectorLink SOP 06/01[7] based on the standard WHO bioassay method (WHO 2016). When pyrethroid resistance was detected, resistance intensity was determined using 5x and 10x diagnostic doses. Synergist assays with a pre-exposure to 4% piperonyl butoxide (PBO) were conducted in tube bioassays to assess the contribution of mixed function oxidases to phenotypic resistance. Synergist assays using PBO were run in parallel to controls using the insecticide only, PBO only, and acetone only. All exposures using the WHO tube tests 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.

Clothianidin and chlorfenapyr were tested in CDC bottle bioassays. For both insecticides, 20 to 25 mosquitoes were aspirated into each of four 250mL glass Wheaton bottle bottles coated with solutions prepared from technical grade insecticide. After the exposure time of 1 hour, the mosquitoes were released in a clean cage and aspirated back to paper cups and fed with 10% sugar solution on wet cotton balls to assess delayed mortality. For clothianidin, mortality was scored 24 hours after exposure. For chlorfenapyr, mortality was scored every 24 hours up to 72 hours after exposure.

Technical grade clothianidin was dissolved in a mixture of Mero (81% Rapeseed oil methyl ester) and acetone. Clothianidin tends to crystallize if used with acetone alone and the uptake of active ingredient by the mosquito becomes low. Therefore, the Mero adjuvant manufactured by Bayer Crop Science was used to prevent crystallization. Bottles were coated with 4µg technical grade clothianidin dissolved in a mixture of 800ppm Mero® (81% Rapeseed oil methyl ester) and acetone. Two control bottles were coated using a solution of the acetone/Mero mixture. Clothianidin stock and working solutions were made fresh or stored at 4°C in the fridge in amber bottles to protect against UV light and used within 24 hours. The solution was left for 1h at room temperature and vigorously shaken for 10 seconds before pipetting into bottles. Testing of the susceptibility of *An. gambiae* s.l. to chlorfenapyr followed the PMI VectorLink SOP 04/01. The bottles were coated with chlorfenapyr provided by CDC Atlanta at a concentration of 100µg/mL. Two bottles impregnated with acetone only were set up similarly and served as negative controls. All bottles were treated a day before conducting the tests.

All WHO tube and CDC bottle susceptibility tests were performed using adults reared from field-collected larvae of *An. gambiae* s.l. at field insectaries established in the selected study sites in the districts, following the PMI VectorLink SOP 14/01[8] protocol. Adult *An. funestus* s.l. mosquitoes were collected using prokopack aspirators in Katakwi and Soroti districts for resistance assays in those districts. All susceptibility tests were conducted to the greatest extent possible under the recommended optimal conditions, at temperatures of 27±2°C and 75%±10% relative humidity during exposure and post-exposure holding periods. Maximum and minimum temperature and humidity were recorded at the end of the testing and holding periods and recorded on the results form. When control mortality was higher than 5% but equal to or less than 10%, Abbott's correction was applied to test mortalities (Abbott 1925). Control-adjusted mortality rates equal to or higher than 98% were classified as susceptible, between 90% and 97% as suggestive of resistance and requiring further investigation, and below 90% as resistant. A subset of *An. gambiae* s.l. and *An. funestus* s.l. samples from the susceptibility tests were sent to the IDRC molecular laboratories for PCR assays to identify sibling species and detect the presence of knockdown (*kdr*) and acetylcolinesterase-1 (*Ace-1*) genes and to determine sporozoite rates.

[7] PMI VectorLink SOP06/01. Insecticide Susceptibility Test, Intensity and Synergist Assay Using WHO Test Kits
[8] PMI VectorLink SOP14/01. Standard Operating Procedures for Rearing of Anopheles gambiae s.l. Mosquitoes

2.4 IRS Quality Assays and Insecticide Residual Efficacy Monitoring

Standard WHO cone wall bioassay tests were performed according the standard PMI VectorLink SOP 09/01 protocol in one sentinel village in six of ten IRS districts (Bugiri, Butaleja, Kibuku, Lira, Serere, and Tororo) within one week of the start of spraying to assess the spray quality. These bioassays continued monthly to measure residual efficacy in four districts (Bugiri, Lira, Serere and Tororo) until mortality dropped below 80% for two consecutive months for any given wall surface type. Two houses of different wall types (painted cement, plain brick and mud-walled) were randomly selected in each village. The same houses were used each month. Cones were fixed, using self-adhesive tape, at heights of 0.5 m, 1.0 m, and 1.5 m above the floor. The control cone was affixed on a wall lined with a paperboard with adhesive in an unsprayed house. Two- to five-day-old female susceptible *An. gambiae* s.s. Kisumu strain mosquitoes were introduced into the plastic cones in batches of 10 and exposed for 30 minutes. The number of mosquitoes knocked down at 30 minutes was recorded, after which the mosquitoes were carefully collected, and transferred to paper cups and provided with 10% sugar solution. Final mortality was recorded every 24 hours up to 120 hours, or until 100% test mortality was achieved.

2.5 Molecular assays

Molecular assays performed by IDRC molecular laboratory included vector species identification and detection of insecticide resistance genetic markers *kdr* and *Ace-1* using established protocols as stipulated in the methods of *Anopheles* Research (MR4, 2014). Sporozoite Enzyme-linked Immunosorbent Assay (ELISA) for sporozoite rate determination of malaria vectors (detection of *Plasmodium falciparum* malaria parasites) was conducted by Vector Control Division (VCD) molecular laboratory following the protocol by Wirtz, R.A., et al. (1989).

2.5.1 Vector Species Identification

Following morphological identification of individual specimens in the field, a selected proportion of samples collected from the longitudinal monitoring districts and susceptibility monitoring districts (Bugiri, Gulu, Hoima, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti, Tororo and Wakiso) based on the total mosquitoes collected from each study district were subjected to PCR assays (Musapa, M., et al., 2013) to identify sibling species using amplified and sequenced mosquito 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 identification using Scott et al., 1993. *An. arabiensis, An. gambiae s.s.* and other sibling species samples were identified using this assay. *An. gambiae* s.s. identified from this assay are to be further analyzed and separated into *An. gambiae* s.s. and *An. coluzzii* at the VCD molecular laboratory using the rapid high throughput SYBR green assay for identifying the malaria vectors *Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles gambiae* s.s. (Giles | PLOS ONE) (Chabi J., et al., 2019).

Detection of the members of the *An. funestus* complex was conducted in accordance with the Koekemoer et al. (2002) protocol at the VCD molecular laboratory. The amplicons were analyzed using a 2.5% gel stained with EtBr for detection of fragments of 587bp *An. vaneedeni*, 505bp *An. funestus*,411bp, *An. rivulorum*, 313bp *An. rivulorum*-like, 252bp *An. parensis* and 146bp *An. leesoni.*

2.5.2 Detection of malaria parasites (determination of infection rates)

Detection and identification of malaria parasites in *An. gambiae* s.l. and *An. funestus* s.l. were restricted to *Plasmodium falciparum* ELISA assays by district and by month of collection. ELISA following the method of Wirtz et al. (1987) was carried out on dried mosquito samples (preserved with desiccant).

2.5.3 Detection of insecticide resistance markers

To determine genetic resistance mechanisms underlying phenotypic resistance, molecular assays were used to detect the presence of knock down resistance (*kdr)* and Acetylcholinesterase (*Ace-1)* genes. This was done in mosquito samples whose insecticide resistance phenotype had been determined using standard WHO susceptibility assays. Mosquitoes that survived the phenotypic assay were prioritized for testing. PCR detection

of *kdr* mutations followed protocols described by Martinez-Torres et al. 1998 (*kdr* L1014F) and Ranson et al, 2000 (*kdr* L1014S) and detection of *Ace-1* mutations followed the 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, Serere and Tororo (current IRS districts), Apac and Soroti (non-IRS districts) a total of 37,393 female *Anopheles* mosquito species were collected using the three collection methods (PSCs, WET and HLCs) and morphologically identified (Table 2).

A total of 9,798 *An. gambiae* s.l. were collected: 7,408 (62.2%) using PSCs, 2,174 (22.2%) using HLCs and 216 (2.2%) using WETs. A total of 27,506 *An. funestus* s.l. were collected: 17,121 (62.3%) were collected using PSCs, 9,746 (35.4%) using HLCs and 639 (2.3%) using WETs. Other *Anopheles* species including *An. coustani* and *An. ziemanni* comprised the remaining 0.2% (n=89) of *Anopeheles* species collected. Vector composition differed by study site. *An. funestus* s.l. was the predominant vector in Apac, Lira Serere and Soroti districts while *An. gambiae* s.l. was dominant in Bugiri and Tororo districts, which has not changed since 2017/18, although increased numbers of *An. funestus* s.l. were collected in these districts in 2022 compared to previous years (Table 2 and Figures 2-5).

TABLE 2: NUMBER OF FEMALE *ANOPHELES* MOSQUITOES COLLECTED IN EACH DISTRICT BY PSC, WET AND HLC COMBINED, JANUARY THROUGH DECEMBER 2022

Mosquito Species	Non-IRS Districts		IRS Districts				All Districts	
	Apac	Soroti	Lira	Serere	Tororo	Bugiri	Total	%
An. funestus s.l.	3,447	16,962	2,892	3,238	431	536	27,506	73.6
An. gambiae s.l.	732	4,032	1,314	1,656	1,072	992	9,798	26.2
Other *Anopheles* species	1	12	19	21	0	36	89	0.2
Total per district	**4,180**	**21,006**	**4,225**	**4,915**	**1,503**	**1,564**	**37,393**	**100**

FIGURE 2: ANOPHELES SPECIES COMPOSITION BY STUDY SITE USING PSCS, JANUARY THROUGH DECEMBER 2022

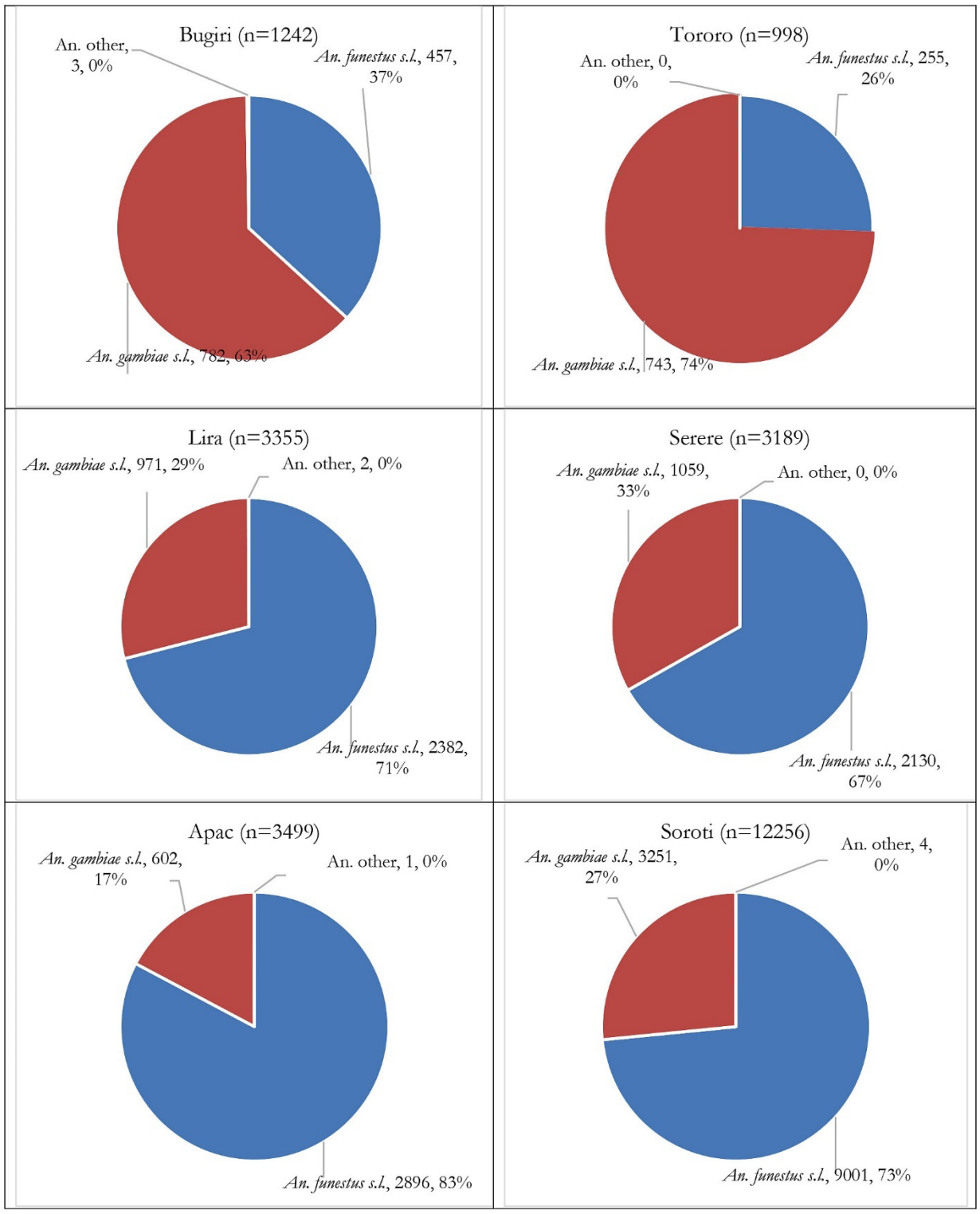


FIGURE 3: ANOPHELES SPECIES COMPOSITION BY STUDY DISTRICT USING HLCS INDOORS, JANUARY THROUGH DECEMBER 2022

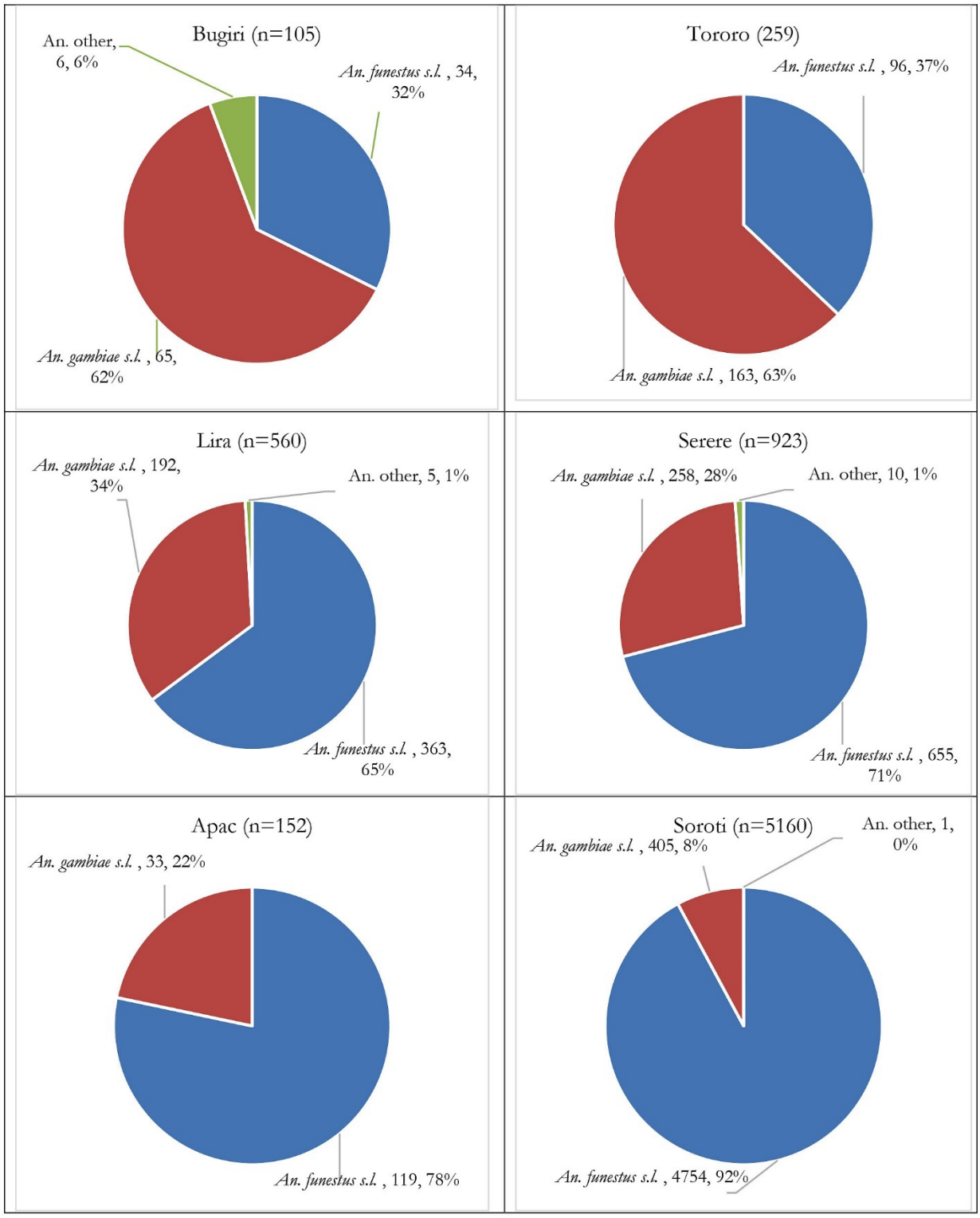


FIGURE 4: ANOPHELES SPECIES COMPOSITION BY STUDY DISTRICT USING HLCS OUTDOORS, JANUARY THROUGH DECEMBER 2022

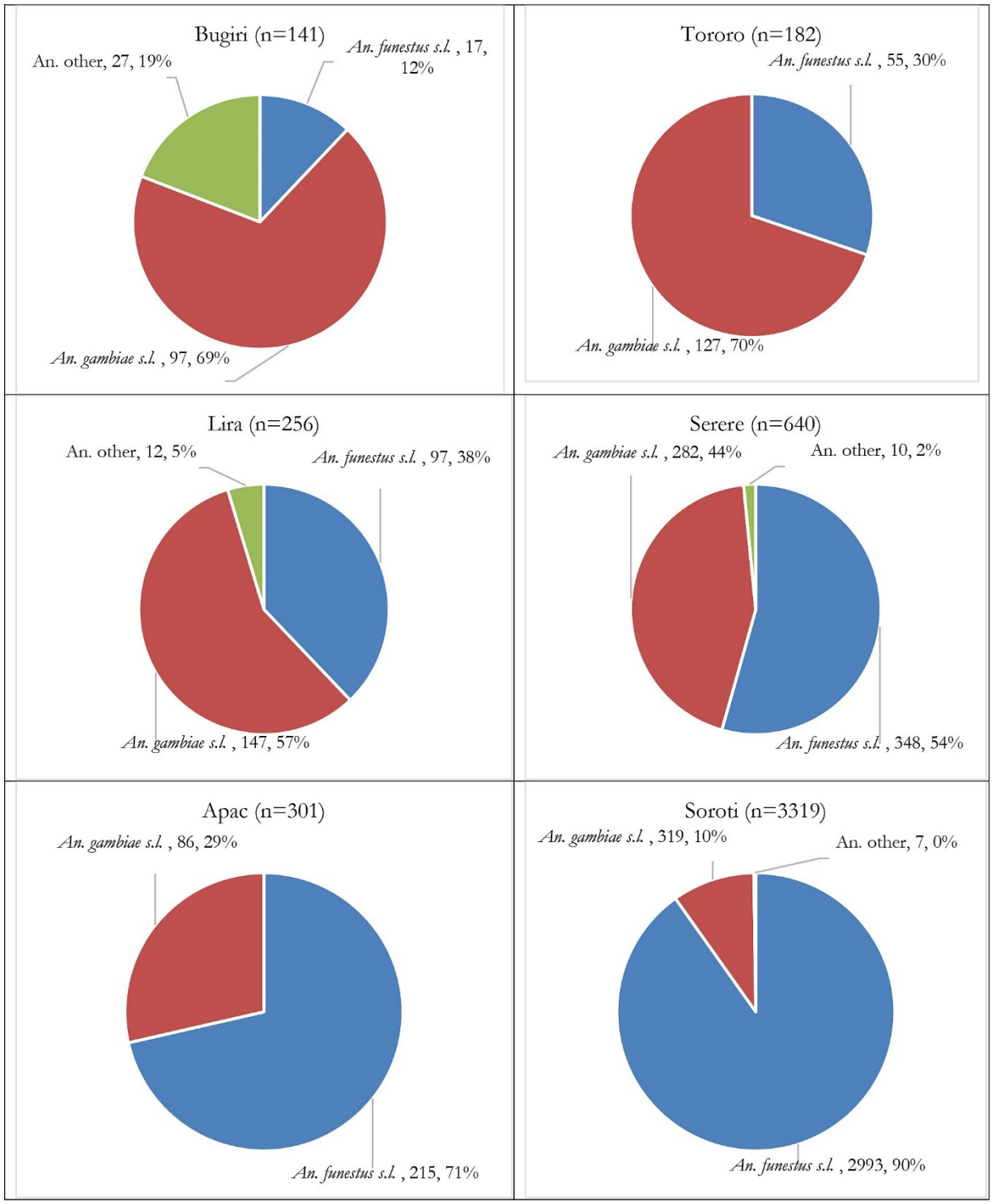
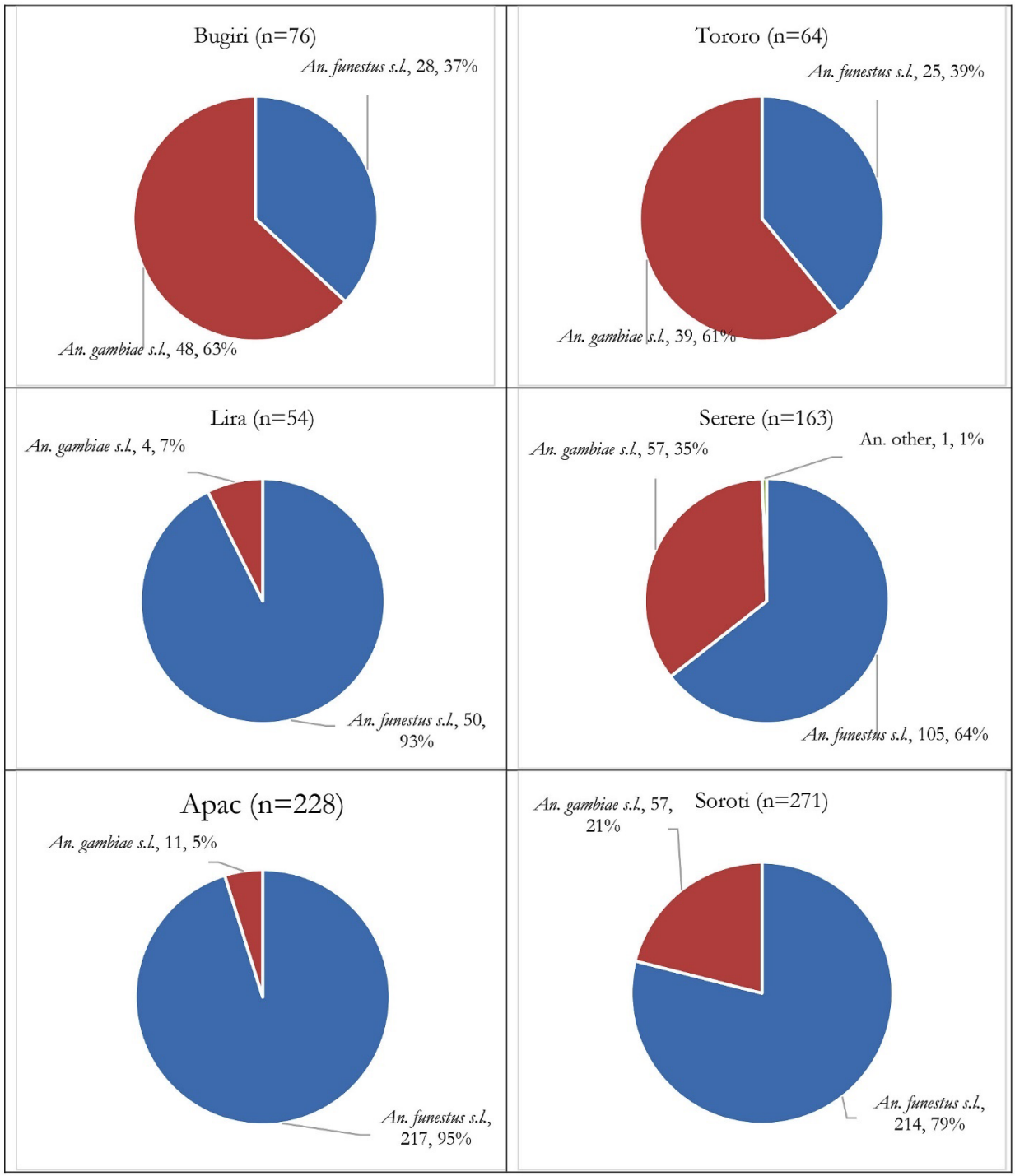


FIGURE 5: ANOPHELES SPECIES COMPOSITION BY STUDY DISTRICT USING WINDOW EXIT TRAPS, SEPTEMBER THROUGH DECEMBER 2022



3.1.2 Pyrethrum Spray Catch

PSC collections yielded 24,539*Anopheles* mosquitoes (Table 3 and Figure 3). By species, there were 17,121 (69.77%) *An. funestus* s.l., 7,408 (30.19%) *An. gambiae* s.l. and 10 (0.04%) other *Anopheles* species. Most *Anopheles* vectors caught by PSC were from unsprayed sites of Apac and Soroti (Table 3 and Figure 6). *An. gambiae* s.l. predominated in PSCs in the IRS districts of Bugiri and Tororo but not in Lira or Serere. *An. funestus* s.l. was predominant in the non-IRS districts of Apac and Soroti. The predominance of *An. gambiae* s.l. in the IRS districts of Bugiri and Tororo could have been a result of prolonged IRS campaigns in Tororo and Bugiri which had greater impact on more endophilic *An. funestus* s.l. compared to *An. gambiae* s.l.

Of the IRS districts, increasing *An. gambiae* s.l. densities were observed in Bugiri and Tororo before IRS was sprayed, but not in Lira or Serere. This implies the timing of IRS was appropriate in the latter two districts but slightly late in Bugiri and Tororo. No immediate impact of IRS on indoor resting densities was observed except for *An. gambiae* s.s. in Bugiri and Tororo, though in Tororo this impact lasted only one month (figure 6). In both Bugiri and Tororo *An. funestus* s.s. indoor resting densities were low before and after IRS; in contrast = *An. funestus* s.l. increased in the second half of the year in Apac and Soroti (non-IRS districts). In Lira and Serere indoor resting densities were higher than those observed in Bugiri and Tororo and the impact of IRS is less apparent. In Serere *An. funestus* s.l. densities were low after IRS until November-December, when they returned to pre-IRS levels. In Lira, *An. funestus* s.l. densities were low before and after IRS until October, when densities reached a peak equivalent to that seen in Apac where there was no IRS. In all districts, IRS and non-IRS alike, except in Bugiri, a peak in *An. gambiae* s.l. densities were seen between April and June. These observations relate to relative changes in densities at each site; when combining all sites on the same graph (figures 7 and 8) it can be seen that the highest absolute numbers caught for both species were recorded in Soroti, the site that did not receive either IRS, dual-active nets or PBO nets.

Figures 9 and 10 show the proportion of the abdominal stages of *An. gambiae* s.l. and *An. funestus* s.l. collected from PSCs in IRS and non-IRS districts. There are no obvious differences between IRS and non-IRS districts for either mosquito species in the proportion of half gravid + fully gravid compared to unfed and blood-fed. This is different from 2021 when for *An. gambiae* s.s. the proportion of half gravid + fully gravid was much smaller for IRS than non-IRS districts.

Table 3: Number of mosquitoes by species collected using PSC in the study districts, January through December 2022

Mosquito Species	Non-IRS Districts		IRS Districts				All Districts	
	Apac	Soroti	Lira	Serere	Tororo	Bugiri	Total	%
An. funestus s.l.	2,896	9,001	2,382	2,130	255	457	17,121	69.77
An. gambiae s.l.	602	3,251	971	1,059	743	782	7,408	30.19
Other *Anopheles* species	1	4	2	0	0	3	10	0.04
Total per district	**3,499**	**12,256**	**3,355**	**3,189**	**998**	**1,242**	**24,539**	**100**

3.1.3 Window Exit Trap (WET) Catch

Proportion of mosquitoes by species exiting houses collected using WETs in the study districts, September through December 2022 is shown in Table 4 below. WET collections yielded 856 *Anopheles* mosquitoes by species, there were 639 (74.6%) *An. funestus* s.l., 216 (25.2%) *An. gambiae* s.l. and 1 (0.12%) other *Anopheles* species. Most *Anopheles* vectors were caught in the unsprayed (control) sites of Apac and Soroti (Table 4). *An. gambiae* s.l. predominated in WETs in the intervention areas (Bugiri and Tororo) correlating with the indoor resting density data and reflecting that this the major mosquito species complex at those sites, while a greater proportion of *An. funestus* s.l. was caught in the control areas (Apac and Soroti) and the current IRS districts of Lira and Serere.

In the IRS districts of Serere and Bugiri, higher numbers of mosquitoes were caught exiting than remaining in the houses to be caught by PSC. However, in Lira (both species) and *An. funestus* in Tororo, this was not the case. It's not certain that this exiting behavior can be attributed to the IRS, partly because the WETs were not deployed until 6 months after IRS was sprayed, and it might more likely reflect the relative composition of *An. gambiae* s.s. and *An. arabiensis* as the latter is typically more exophilic. *An. arabiensis* is more prevalent in Bugiri and Serere, whilst *An. gambiae* s.s. is more prevalent in Lira and Tororo (Table 7). The percentage exiting is also not consistent in the controls – in Soroti the majority of *An. funestus* s.l. remained indoors but that was not seen in Apac, and more *An. gambiae* s.l. were caught in exit traps than by PSC in both control districts. This suggests something other than the IRS is affecting exiting behavior. Furthermore, it cannot be concluded from these data that the mosquitoes exiting in the IRS districts are surviving long enough to take a subsequent bloodmeal, especially as clothianidin is known to have a delayed mortality effect. There was no obvious difference in mosquito exit rates between SumiShield-sprayed districts (Lira and Serere) and the Fludora Fusion-sprayed districts (Bugiri and Tororo), as there were no consistent exiting behavior patterns for either insecticide as shown in Table 4. The abdominal status of mosquitoes caught exiting houses showed that for all districts except Apac, the unfed plus freshly blood fed *An. gambiae* s.l. and *An. funestus* s.l. account for more than 50% of the vectors that exit out of the houses (75-100% for *An. gambiae* s.l. and 54-92% for *An. funestus* s.l.) (Table 5).

TABLE 4: PERCENTAGE OF MOSQUITOES BY SPECIES THAT STAYED IN THE HOUSES COLLECTED BY PSCS AND THOSE THAT EXITED HOUSES AND WERE COLLECTED USING WETS BY DISTRICT, SEPTEMBER THROUGH DECEMBER 2022

Mosquito Species	Non-IRS Districts				IRS Districts								All Districts	
	Apac		Soroti		Lira		Serere		Tororo		Bugiri			
	PSC	WET	PSC	WET	PSC	WET	PSC	WET	PSC	WET	PSC	WET	PSC	WET
An. funestus s.l.	48 (202)	52 (217)	68 (447)	32 (214)	66 (99)	34 (50)	42 (76)	58 (105)	46 (21)	54 (25)	36 (16)	64 (28)	57 (861)	43 (639)
An. gambiae s.l.	42 (8)	58 (11)	49 (55)	51 (57)	82 (18)	18 (4)	37 (33)	63 (57)	65 (63)	38 (39)	36 (27)	64 (48)	49 (206)	51 (216)
Other *Anopheles* species	0	0	0	100 (1)	0	0	0	100 (1)	0	0	0	0	50 (1)	50 (1)

N.B.: Numbers in parentheses represent total number (N) of mosquitoes caught.

TABLE 5: PERCENTAGE BY ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. EXITING HOUSES AND CAUGHT IN WETS SEPTEMBER TO DECEMBER 2022

	District	*An. gambiae* s.l.				*An. funestus* s.l.			
		Unfed	Fed	Half gravid	Gravid	Unfed	Fed	Half gravid	Gravid
IRS Districts	Bugiri	35 (72.9%)	10 (20.8%)	3 (6.3%)	0 (0.0%)	11 (39.3%)	10 (35.7%)	1 (3.6%)	6 (21.4%)
	Lira	2 (50.0%)	1 (25.0%)	1 (25.0%)	0 (0.0%)	18 (36.0%)	9 (18.0%)	19 (38.0%)	4 (8.0%)
	Serere	30 (52.6%)	27 (47.4%)	0 (0.0%)	0 (0.0%)	81 (77.1%)	15 (14.3%)	3 (2.9%)	6 (5.7%)
	Tororo	15 (38.5%)	17 (43.6%)	7 (17.9%)	0 (0.0%)	19 (76.0%	4 (16.0%)	2 (8.0%)	0 (0.0%)
Non-IRS Districts	Apac	0 (0.0%)	4 (36.4%)	7 (63.6%)	0 (0.0%)	23 (10.6%)	58 (26.7%)	89 (41.0%)	47 (21.7%)
	Soroti	49 (86.0%)	5 (8.8%)	1 (1.8%)	2 (3.5%)	66.8	17.3	2.3	13.6

FIGURE 6: INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. FROM PSCS BY MONTH BY DISTRICT, JANUARY THROUGH DECEMBER 2022

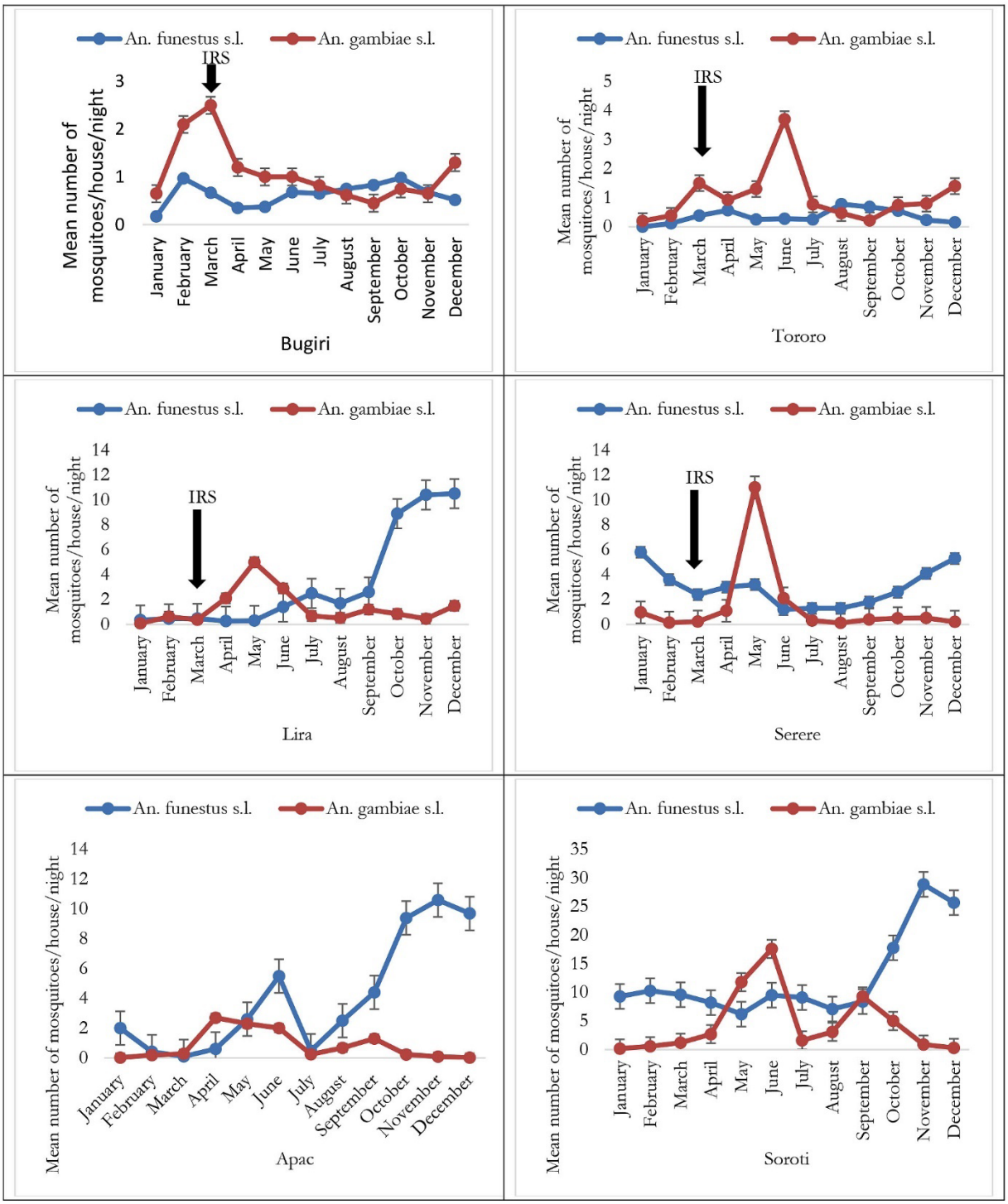


FIGURE 7: INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. FROM PSCS BY MONTH, JANUARY THROUGH DECEMBER 2022

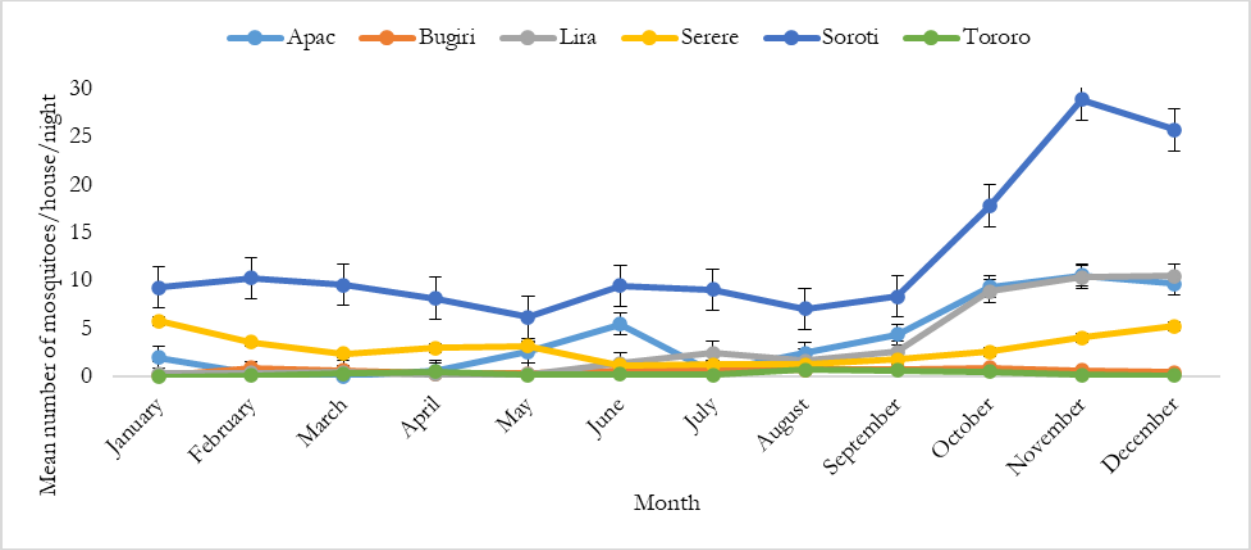


FIGURE 8: INDOOR RESTING DENSITIES OF *AN. GAMBIAE* S.L. FROM PSCS BY MONTH, JANUARY THROUGH DECEMBER 2022

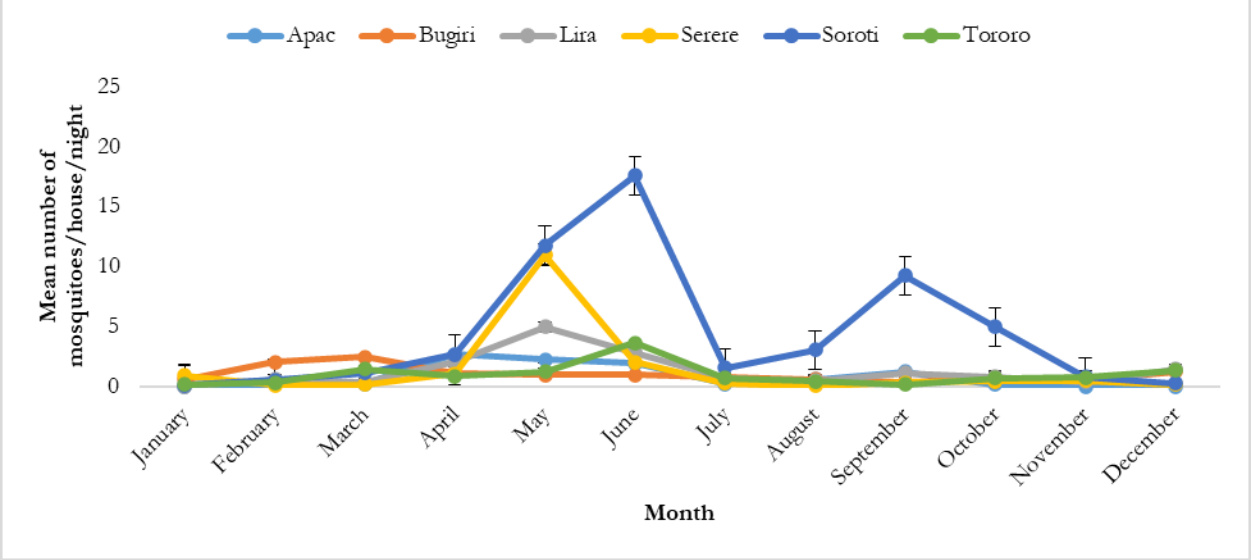


FIGURE 9: PROPORTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE COLLECTED BY PSC FROM A: IRS DISTRICTS; B: NON-IRS DISTRICTS

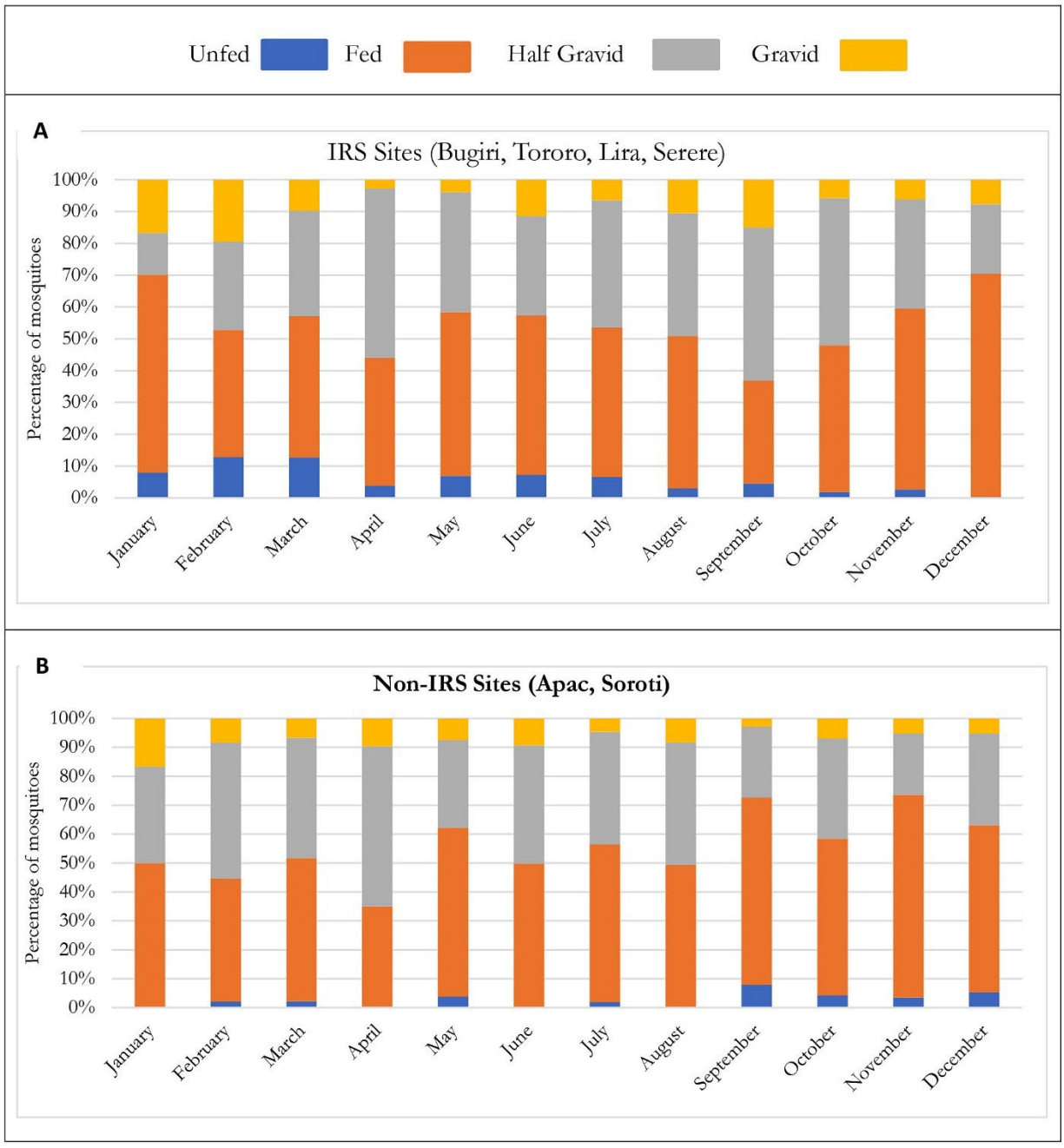
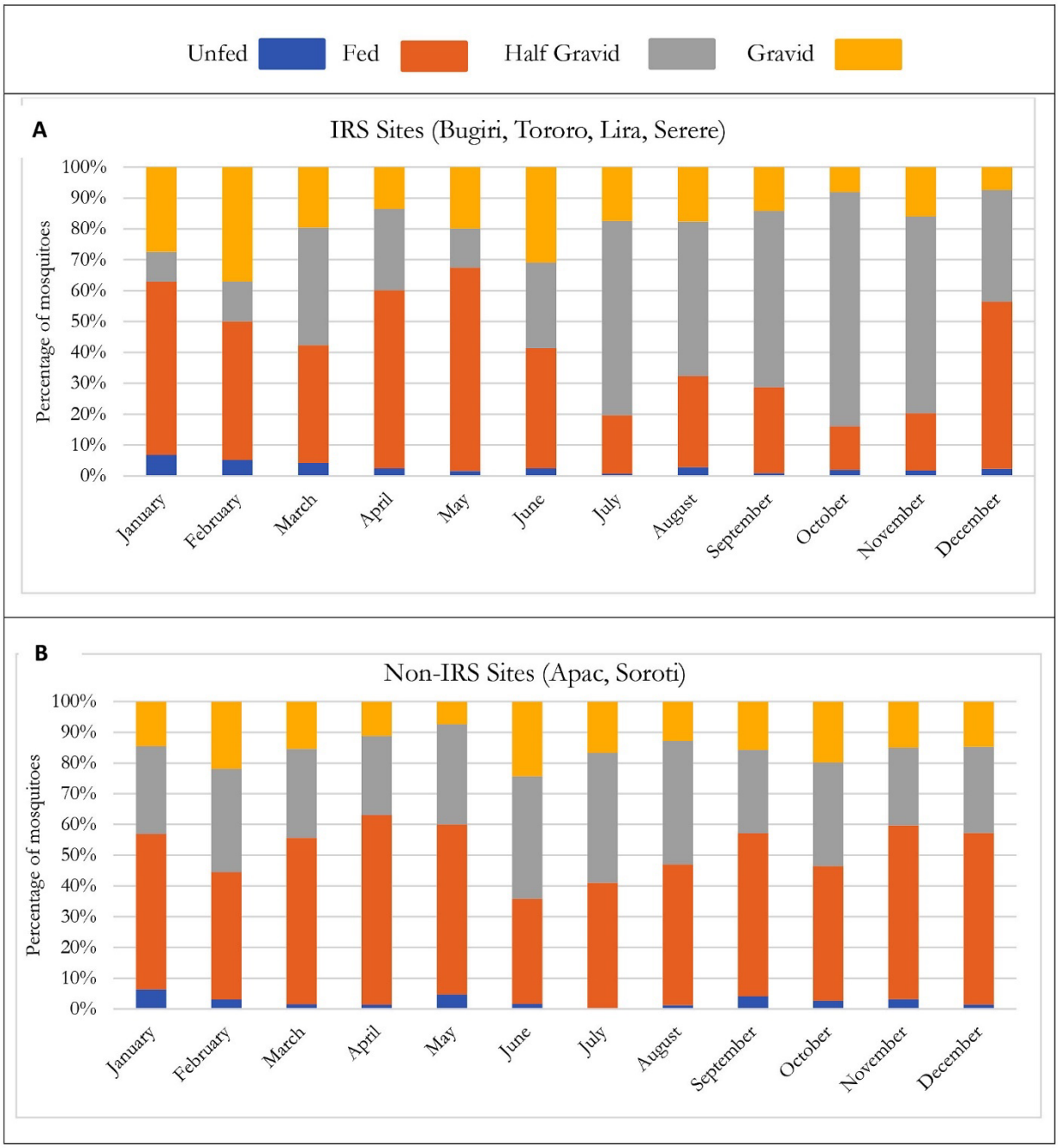


FIGURE 10: PROPORTION OF *AN. FUNESTUS* S.L. MOSQUITOES BY ABDOMINAL STAGE COLLECTED BY PSC FROM A: IRS DISTRICTS; B: NON-IRS DISTRICTS



3.1.4 HUMAN LANDING CATCHES

A total of 11,998 *Anopheles* mosquitoes were collected using HLCs from January to December 2022. The species identified morphologically from this collection included 9,746 (81.2%) *An. funestus* s.l., 2,174 (18.1%) *An. gambiae* s.l., 78 (0.7%) other *Anopheles* species, including *An. coustani* and *An. ziemanni*. *An. gambiae* s.l. was the most abundant mosquito species collected both indoors and outdoors in Bugiri and Tororo, while *An. funestus* s.l. was the most abundant mosquito species collected both indoors and outdoors in the districts of Apac and

Soroti (control districts) and the IRS districts of Lira and Serere (Table 6). Both IRS and non-IRS 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 (March). An overall a spike in *An. funestus* s.l. biting rates was observed from April to June 2022 in the non-IRS districts, with the highest mean number of bites per person per night exceeding 210 in Soroti in December. These increases are probably related to increases in the rainfall during the two main rainfall seasons. A second peak in April in Soroti was not seen in IRS districts, where HBR indoors and outdoors remained low until 5-6 months after spraying when there was an increase of *An. funestus* s.l. vectors especially in Lira and Serere. High HBR indoors was observed in Serere before IRS (Figure 11B).

More concerningly, the peak in *An. gambiae s.l.* HBR indoors in Soroti in June is replicated in the IRS districts of Lira and Serere in May, and this is similar for outdoor biting too, though high HBR is not observed indoor or outdoor in the IRS districts of Tororo and Bugiri. (Figures 11-14)

TABLE 6: HUMAN LANDING CATCHES INDOORS AND OUTDOORS IN SIX STUDY DISTRICTS IN UGANDA, JANUARY THROUGH DECEMBER 2022

Anopheles species	IRS Districts												Non-IRS Districts					
	Bugiri			Lira			Tororo			Serere			Apac			Soroti		
	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total
An. gambiae s.l.	65	97	162	192	147	339	163	127	290	258	282	540	33	86	119	405	319	724
An. funestus s.l.	34	17	51	363	97	460	96	55	151	655	348	1003	119	215	334	4754	2993	7747
Other *Anopheles* species	6	27	33	5	12	17	0	0	0	10	10	20	0	0	0	1	7	8
Total per district	105	241	346	560	256	816	259	182	441	923	640	1563	152	301	453	5160	3319	8479

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, 2022.

FIGURE 11: *AN. FUNESTUS* S.L. MEAN BITES/PERSON/MONTH INDOOR IN: A: ALL DISTRICTS; B: IRS DISTRICTS; C: NON-IRS DISTRICTS

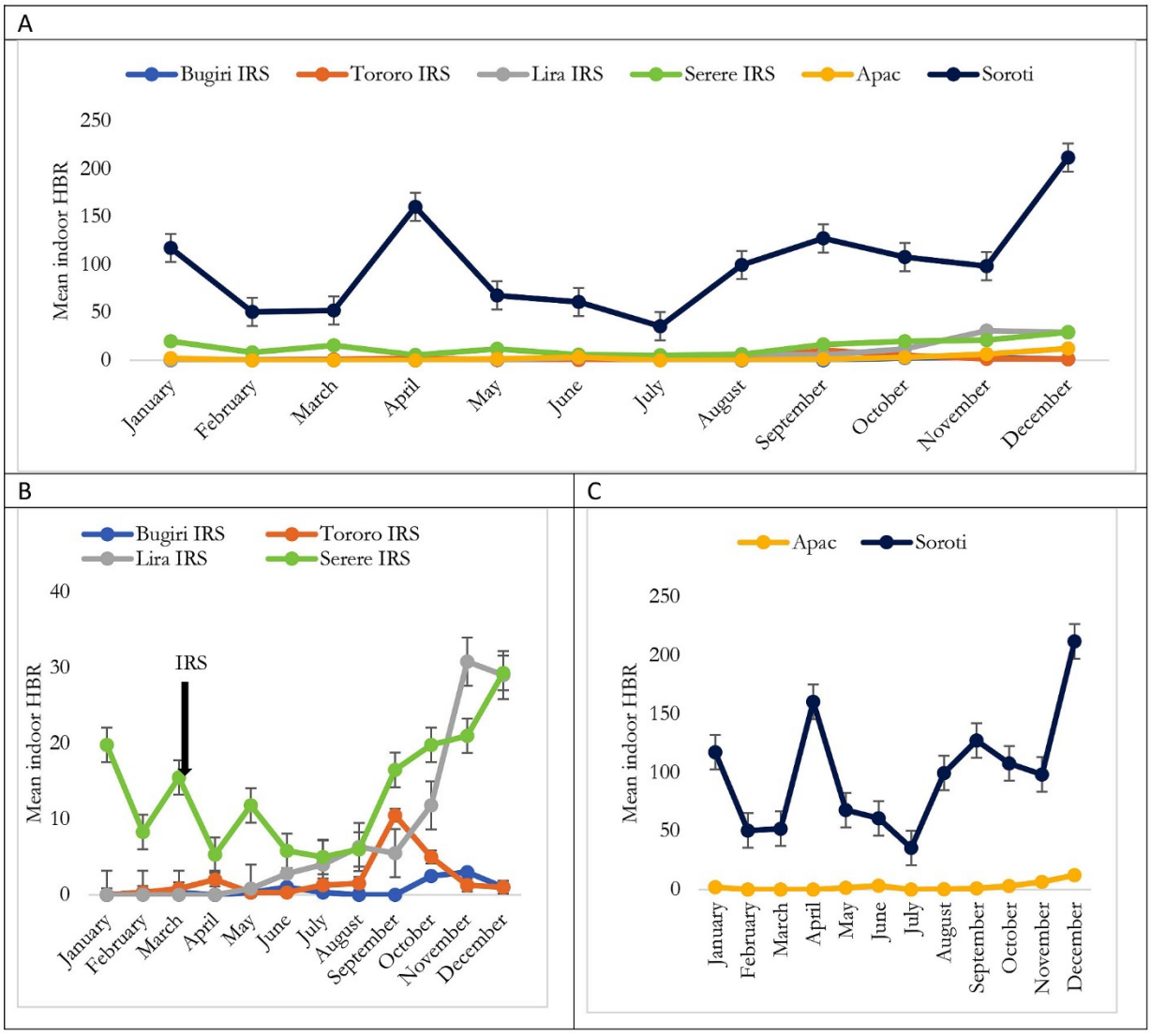


FIGURE 12: *AN. FUNESTUS* S.L. MEAN BITES/PERSON/MONTH OUTDOOR IN: A: ALL DISTRICTS; B: IRS DISTRICTS; C: NON-IRS DISTRICTS

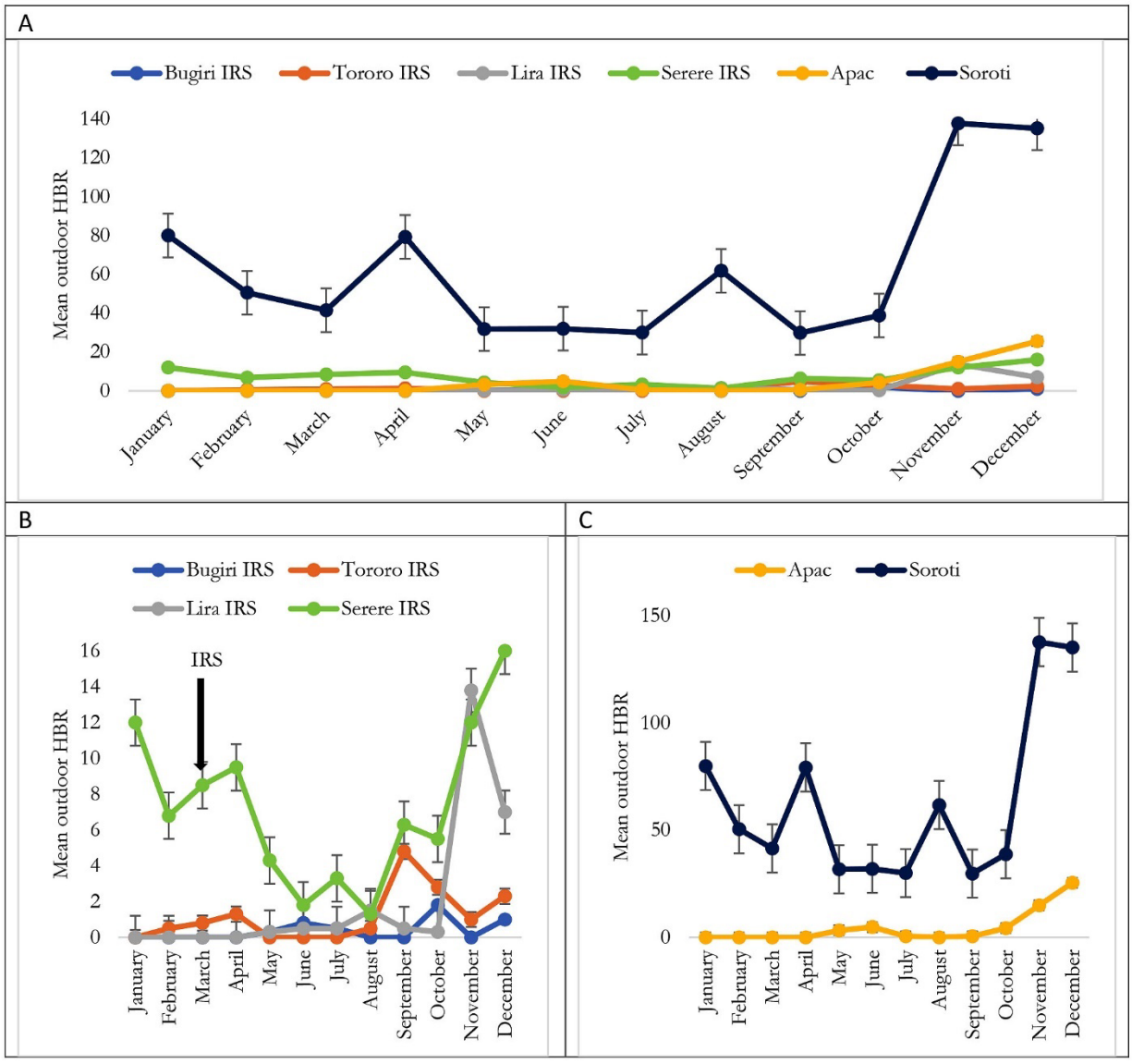


FIGURE 13: *AN. GAMBIAE* S.L. MEAN BITES/PERSON/MONTH INDOOR IN A: ALL DISTRICTS; B: IRS DISTRICTS; C: NON-IRS DISTRICTS.

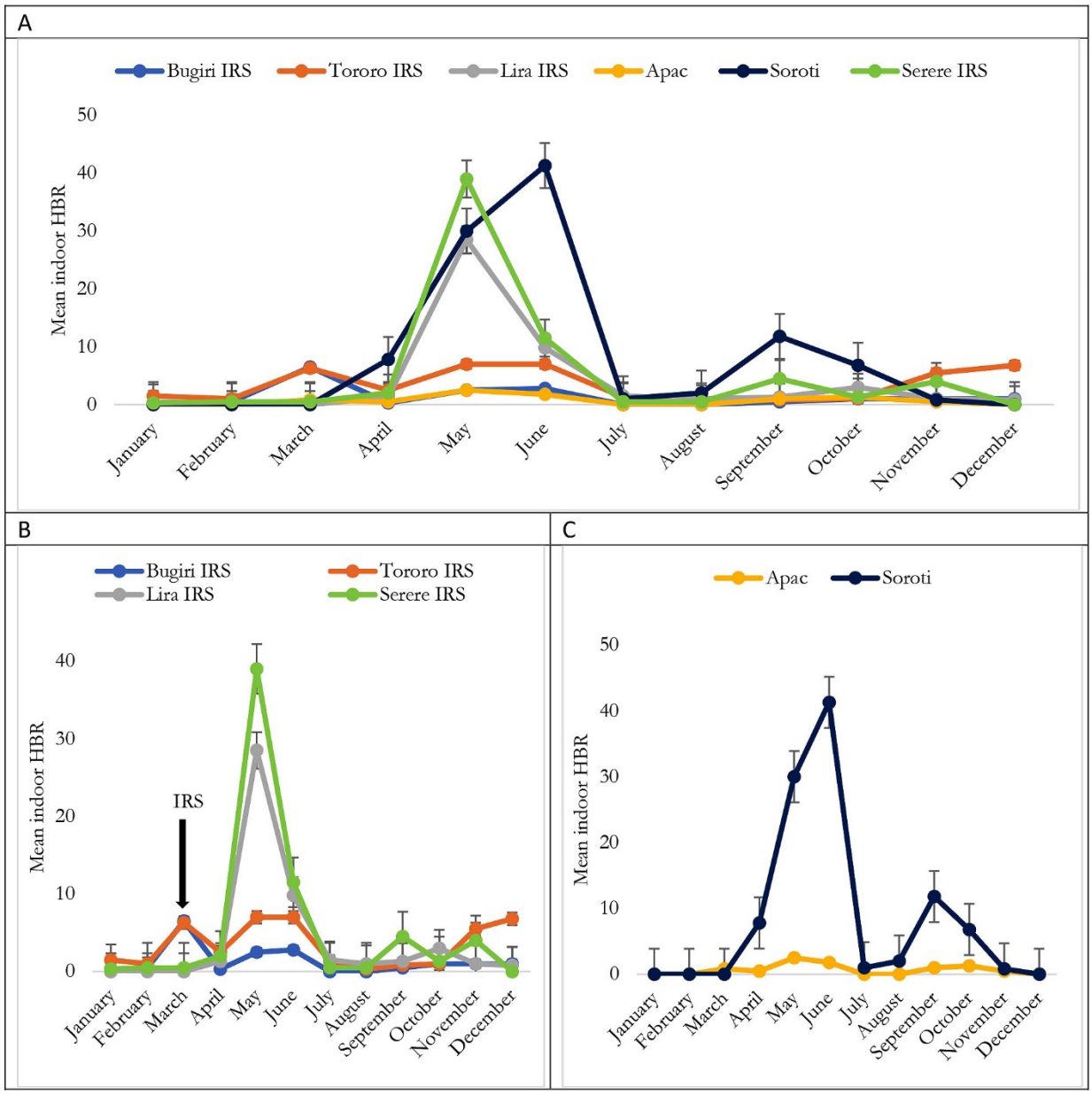
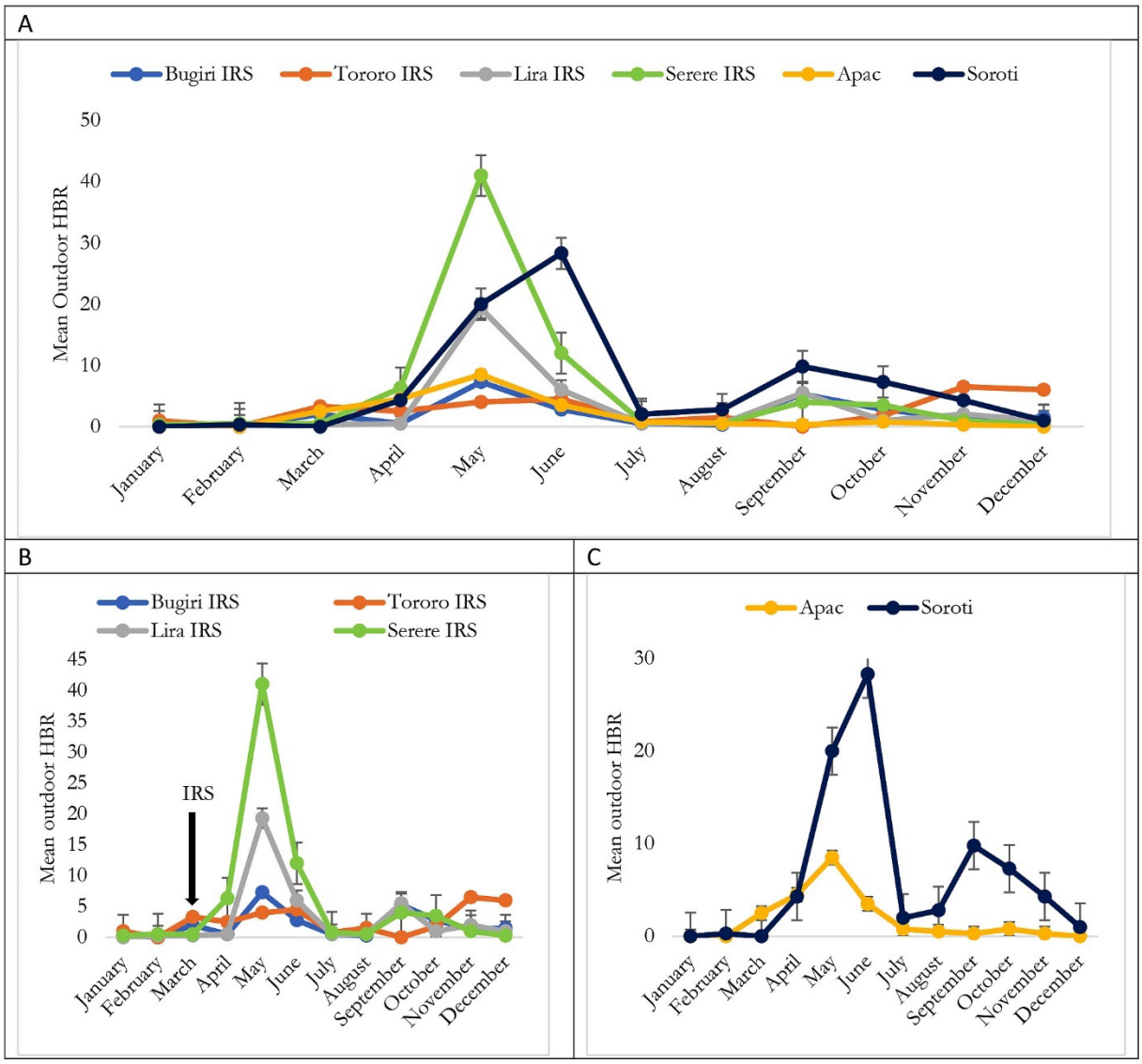


FIGURE 14: *AN. GAMBIAE* S.L. MEAN BITES/PERSON/MONTH OUTDOOR IN A: ALL DISTRICTS; B: IRS DISTRICTS; C: NON-IRS DISTRICTS



Annexes 1 and 2 show the hourly biting rates of *An. gambiae* s.l. and *An. funestus* s.l. in IRS intervention areas and control areas respectively. Figure 15 shows the mean number of *An. gambiae* s.l. and *An. funestus* s.l. per person per hour indoor and outdoor respectively.

There is little indication of early biting indoors or outdoors in either the IRS or non-IRS districts for either species. For *An. gambiae* s.l., in IRS districts of Bugiri, Lira, Serere and Tororo, the indoor biting peaked at between 2a.m.-3a.m. in Tororo and 3a.m.-4 a.m. Lira and Serere, while outdoor biting peaks varied greatly from between 1a.m.-2a.m. in Tororo and 5a.m.-6a.m. in Bugiri. For *An. gambiae* s.l., in non-IRS districts of Apac and Soroti, the indoor biting peaked at between midnight–1a.m. in Soroti and 1a.m.-2a.m. in Apac, while outdoor biting peaks varied greatly from between midnight-1a.m. in Soroti and 1-2a.m. in Apac (Annex 1 and Figure 15).

For *An. funestus* s.l., in IRS districts of Bugiri, Lira, Serere and Tororo, the indoor biting peaked at between 12 midnight-1a.m. in Tororo, 2-a.m.-3a.m. in Bugiri, 3a.m.-4.00a.m. in Serere and Lira (Annex 2 and Figure 16).

For *An. funestus* s.l., in non-IRS districts of Apac and Soroti, the indoor biting peaked at between 4a.m.–5a.m. in Soroti and 5a.m.-6a.m. in Apac, while outdoor biting peaks varied greatly from between 3a.m.-4a.m. in Soroti and 4a.m.-5a.m. in Apac (Annex 1 and Figure 16).

FIGURE 15: MEAN NUMBER OF *AN. GAMBIAE* S.L. COLLECTED PER PERSON PER HOUR BY HLC: A: ALL DISTRICTS INDOORS; B: IRS DISTRICTS INDOORS; C: NON-IRS DISTRICTS INDOORS; D: ALL DISTRICTS OUTDOORS; E: IRS DISTRICTS OUTDOORS; F: NON-IRS DISTRICTS OUTDOORS

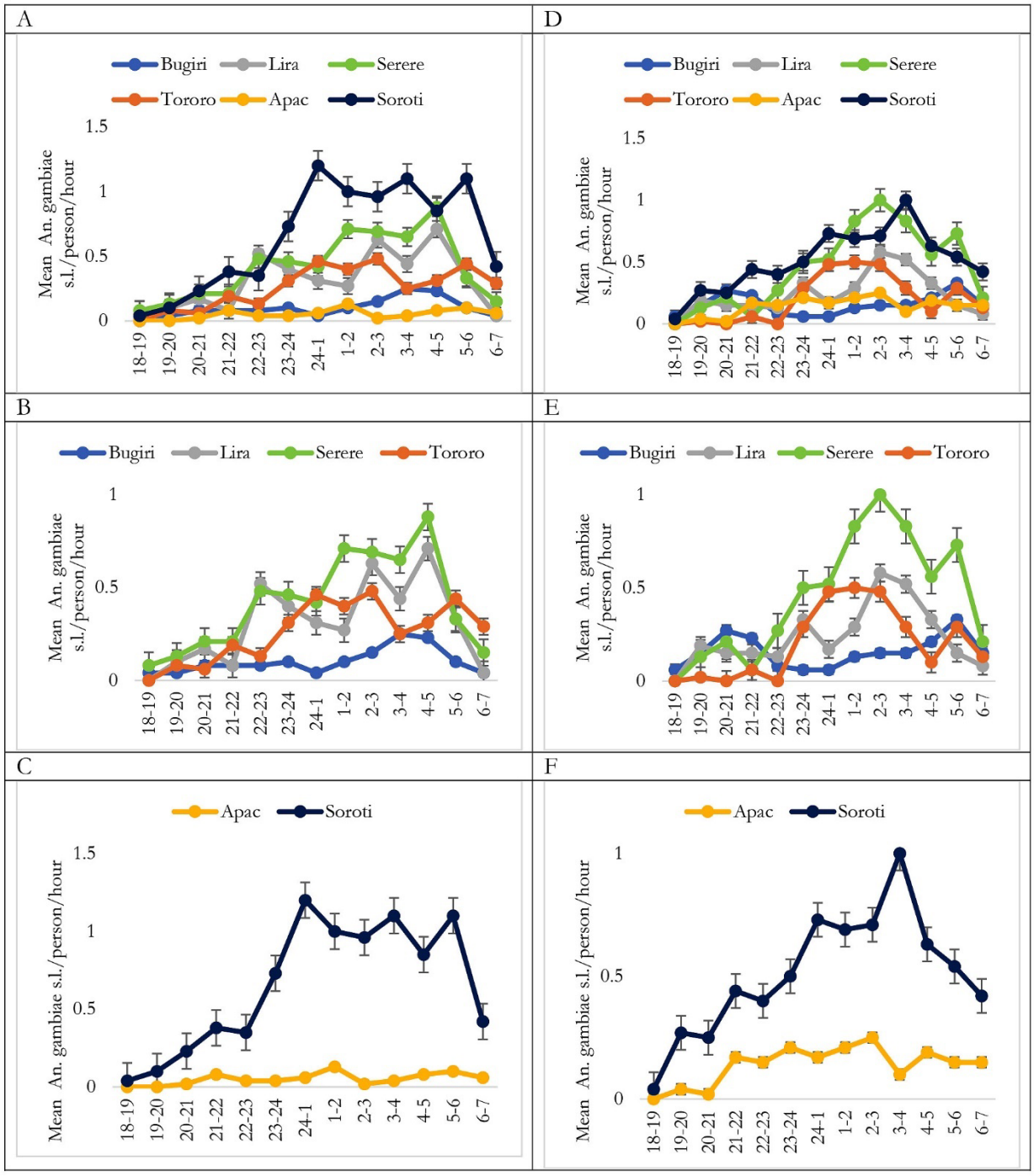
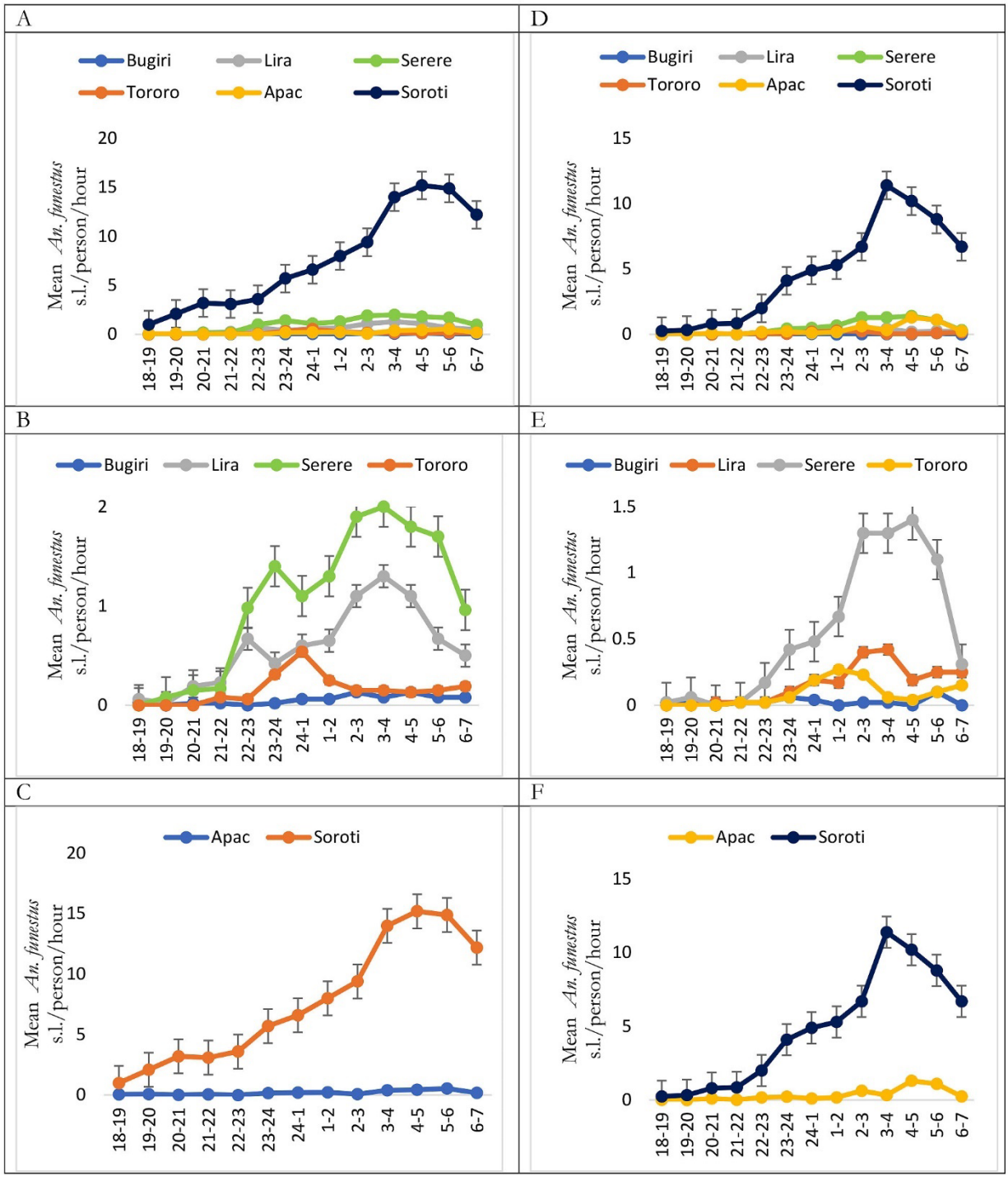


FIGURE 16: MEAN NUMBER OF *AN. FUNESTUS* S.L. COLLECTED PER PERSON PER HOUR BY HLC. A: ALL DISTRICTS INDOORS; B: IRS DISTRICTS INDOORS; C: NON-IRS DISTRICTS INDOORS; D: ALL DISTRICTS OUTDOORS; E: IRS DISTRICTS OUTDOORS; F: NON-IRS DISTRICTS OUTDOORS, UGANDA, 2022



3.2 Cone Wall Bioassay Tests

During the spray operations, WHO quality assurance cone bioassays were conducted in one site for each of six spray districts (Bugiri, Butaleja, Kibuku, Lira, Serere, and Tororo) within one week of the start of spraying to assess the quality of spraying. Thereafter, the residual efficacy of Fludora Fusion was monitored in three districts (Kibuku, Lira and Serere) while the residual efficacy of SumiShield was monitored in Bugiri and Tororo monthly on three types of wall surfaces: cement painted, plain brick, and mud, which constitute most wall surfaces in the IRS districts. Susceptible *An. gambiae* s.s. (Kisumu strain) was used for spray quality and residual efficacy assessment.

3.2.1 Quality of IRS

The results for the spray quality bioassays showed adequate spray quality (100% mosquito mortality) in the six sentinel spray districts.

3.2.2 Insecticide Residual Efficacy

Cone bioassay test results indicate that SumiShield residual efficacy was seven months in both Bugiri and Tororo districts on all wall types (Annex 3 and Figure 17). For Fludora Fusion residual efficacy was four to five months in Kibuku, five months in Lira and six to seven months in Serere districts (Annex 4 and Figure 18). Fludora Fusion insecticides lasted longest on painted surfaces and least on mud wall surfaces while SumiShield lasted seven months on all wall types. 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.

Figure 17: Results of the 2022 residual insecticide efficacy monitoring of SumiShield in Bugiri and Tororo districts, Mortality Day 5, March – December 2022

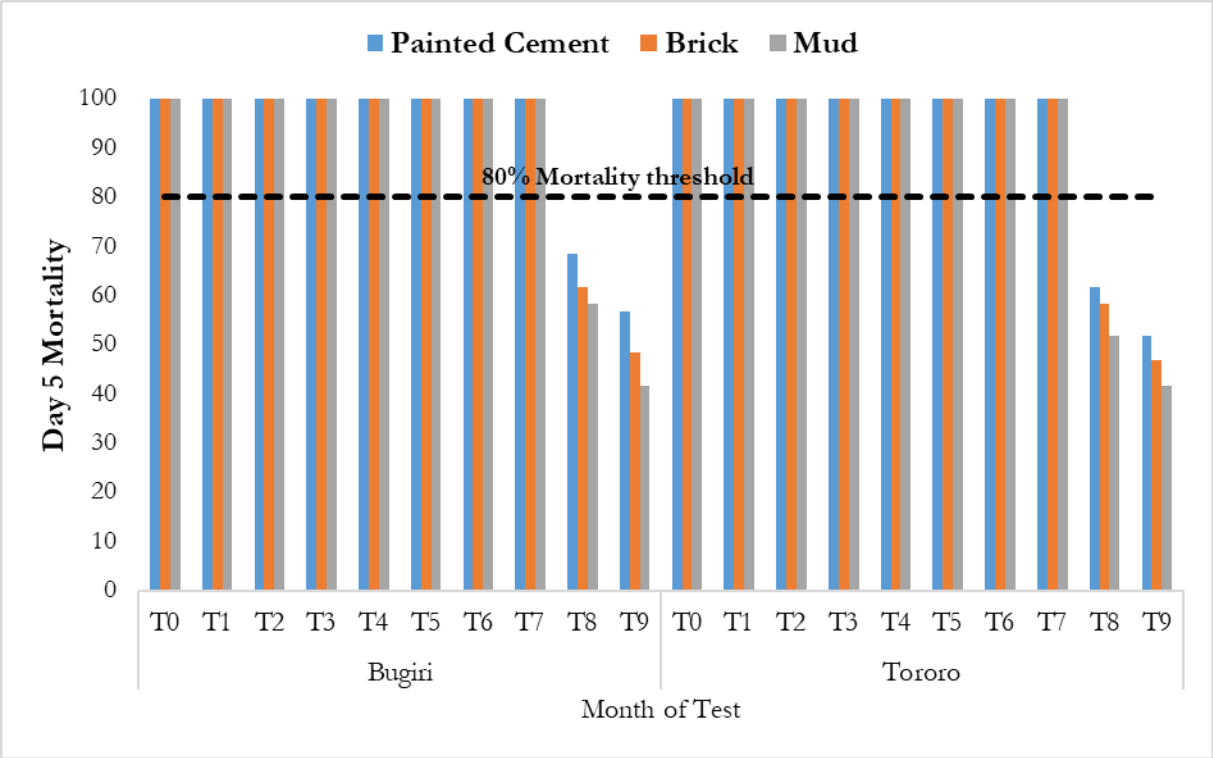
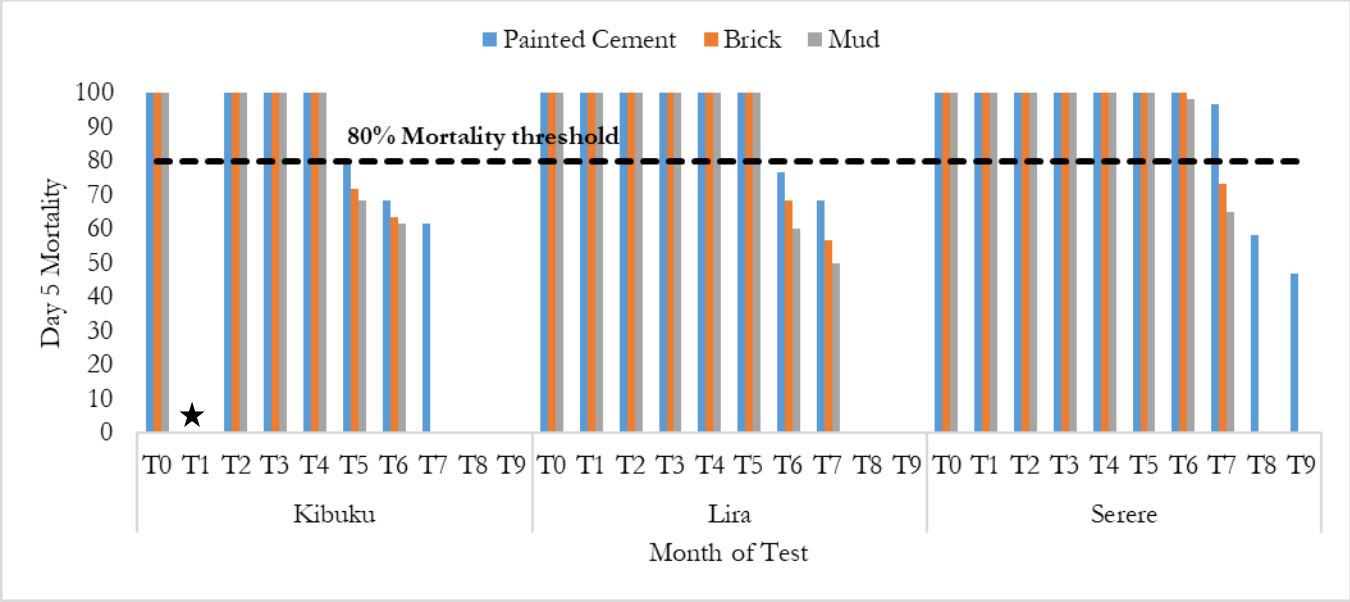


FIGURE 18: RESULTS OF THE 2022 RESIDUAL INSECTICIDE EFFICACY MONITORING OF FLUDORA FUSION IN KIBUKU, LIRA AND SERERE DISTRICTS, MORTALITY DAY 5, MARCH – DECEMBER 2022



Key: * - No wall bioassays were conducted during the month in Kibuku. It was originally planned for IRS QA wall bioassays not for residual efficacy monitoring, but PMI/USAID Uganda office requested the PMI VL to conduct residual efficacy studies in the district from T2 onwards.

The team that went to conduct residual efficacy tests in Serere at T8 did so only for painted cement wall surfacesas there were insufficient mosquito numbers to repeat test the plain brick and mud wall surfaces that were below the threshold the previous month. There is therefore only one month of mortality <80% of those surfaces.

3.3 WHO INSECTICIDE SUSCEPTIBILITY TESTING

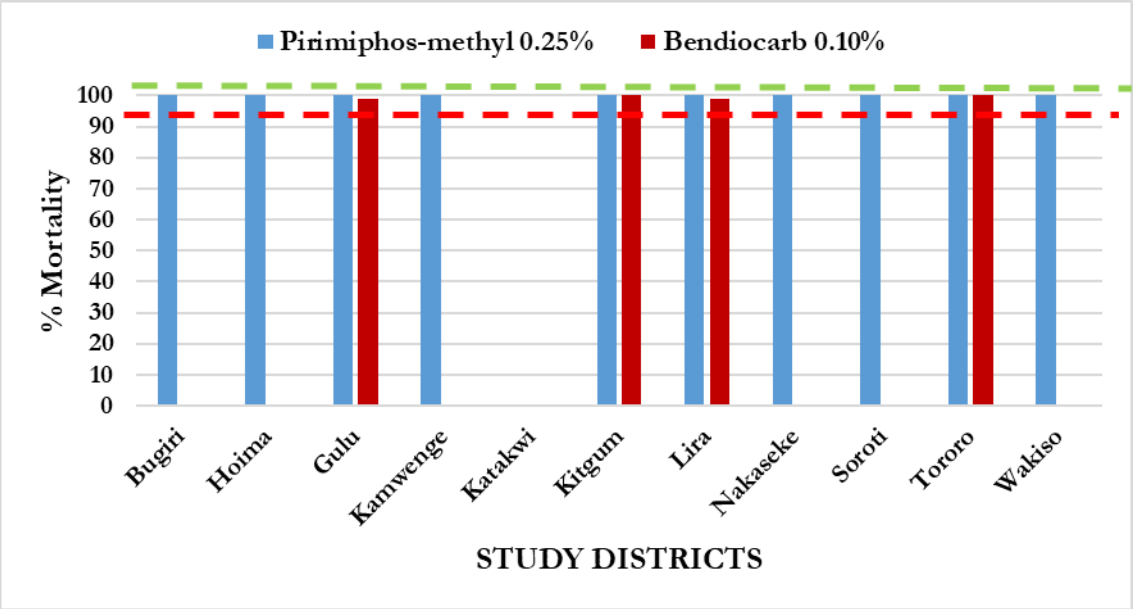
3.3.1 DETERMINATION OF THE INSECTICIDE SUSCEPTIBILITY STATUS USING WHO TUBE TESTS

Susceptibility testing was conducted in 11 districts of Bugiri, Gulu, Hoima, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti, Tororo and Wakiso from July-November 2022. Control mortality was always at an acceptable level, no Abbott's adjustments were made and no tests were invalidated. The unpredictable rainfall pattern in the country over the recent years has made it difficult to plan when it is most appropriate to conduct susceptibility studies and resulted in the extended period of performing the insecticide susceptibility monitoring activities.

The susceptibility status of *An. gambiae* s.l. in tube tests to the different insecticides is shown in Figures 19 and 20 and Annex 5. *An. gambiae* s.l. was susceptible to pirimiphos-methyl in all 11 districts surveyed. *An. gambiae* s.l. was susceptible to bendiocarb in four districts (Gulu, Kitgum, Lira and Tororo) out of 11 districts where the test was completed (Figure 19). Bendiocarb tests were not performed in the other seven districts due to inadequate number of vectors collected and prioritization of testing other insecticides over bendiocarb. Three pyrethroids were tested in Katakwi and Lira; elsewhere deltamethrin was prioritized. *An. gambiae* s.l. was resistant to alpha-cypermethrin and permethrin in Katakwi (48% & 39% mortality) and Lira (52% and 28% mortality). *An. gambiae* s.l. was confirmed resistant to deltamethrin in seven districts (Gulu, Hoima, Kamwenge, Katakwi, Lira, Nakaseke and Tororo) where tests were completed, with mortality varying between 23.0% in Nakaseke and 83.0% in Tororo, and with probable resistance in Kitgum.

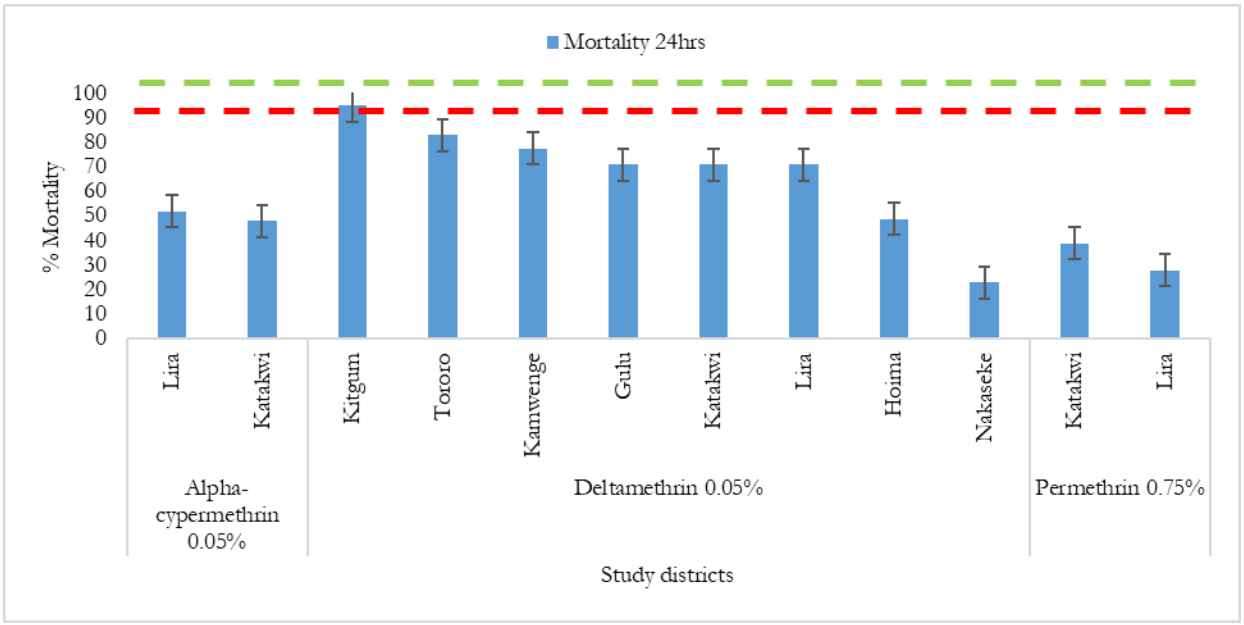
An. funestus s.l. was susceptible to pirimiphos-methyl, clothianidin, chlorfenapyr and bendiocarb in Soroti and in Katakwi, but resistant to deltamethrin (33% mortality), permethrin (23% mortality) and alpha-cypermethrin (28% mortality) - (Figure 21 and Annex 6).

FIGURE 19: PERCENTAGE 24HOUR MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO PIRIMIPHOS-METHYL AND BENDIOCARB IN 9 DISTRICTS JULY-NOVEMBER 2022 (LARVAL COLLECTIONS)



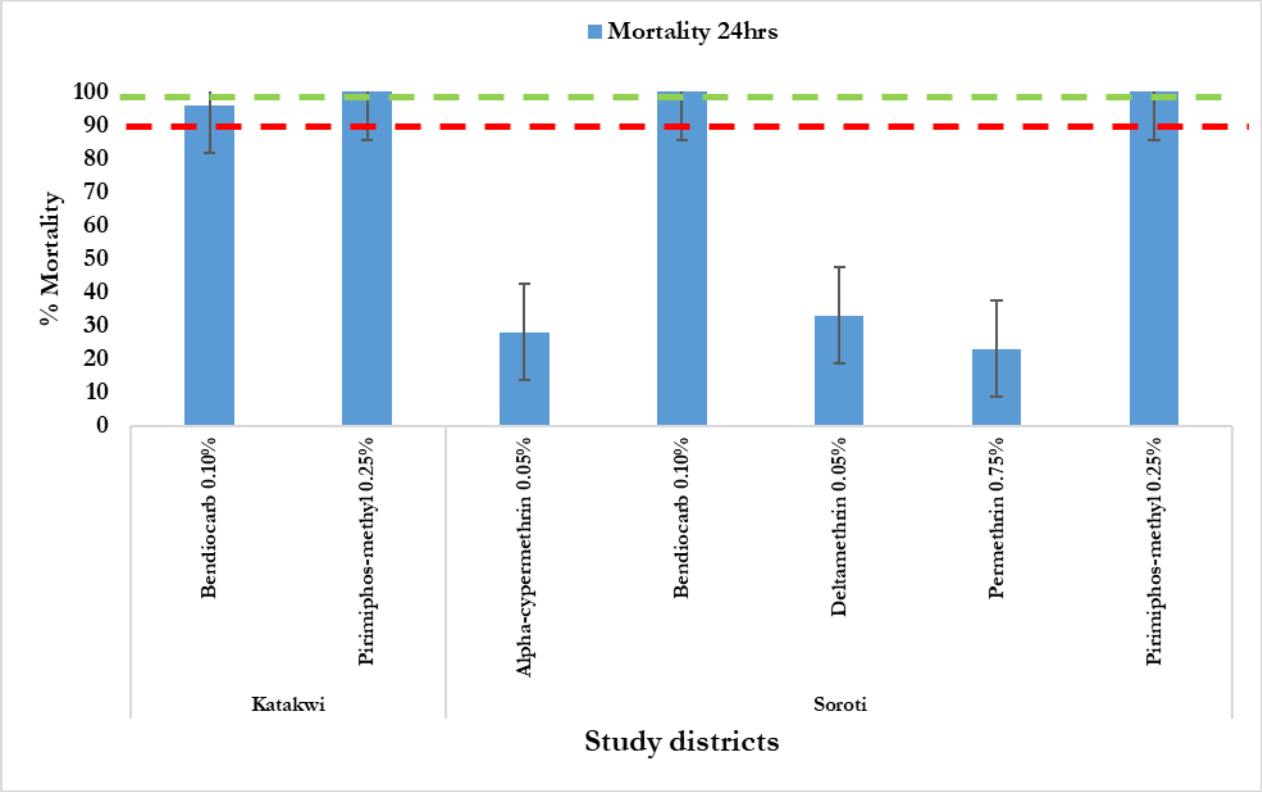
Key: Line indicates resistance threshold Line indicates susceptibility threshold

FIGURE 20: PERCENTAGE 24 HOUR MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO THREE PYRETHROID INSECTICIDES AT 1X DIAGNOSTIC CONCENTRATION IN NINE DISTRICTS, JULY – NOVEMBER 2022 (LARVAL COLLECTIONS)



Key: Line indicates resistance threshold Line indicates susceptibility threshold

FIGURE 21.: PERCENTAGE 24-HOUR MORTALITY OF ADULT *AN. FUNESTUS* S.L. FROM EARLY MORNING COLLECTIONS EXPOSED TO A RANGE OF INSECTICIDES AT 1X DIAGNOSTIC CONCENTRATIONS IN TWO DISTRICTS, JULY–NOVEMBER 2022



Key: Line indicates resistance threshold Line indicates susceptibility threshold

3.3.2 DETERMINATION OF THE INTENSITY OF RESISTANCE USING WHO TUBE TESTS

In Lira, 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 The results showed at least moderate deltamethrin and permethrin resistance intensity in Lira (92% and 94.0% mortality respectively after 24h exposure at 5× concentration) - (Figure 22 and Annex 5). The resistance intensity could be high, but tests were not done at 10x the discriminating concentration due to inadequate mosquito samples. No insecticide intensity tests were performed in the other five study districts due to inadequate number of vectors collected.

Resistance intensity studies of *An. funestus* s.l. using the WHO tube assay were conducted in Soroti district only. The results showed high resistance intensity to deltamethrin (94% mortality at x10 diagnostic dose) and alpha-cypermethrin (71% mortality at x10 diagnostic dose), and moderate resistance intensity to permethrin (99% mortality at x10 diagnostic dose) – (Figure 23 and Annex 6).

FIGURE 22: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN AND PERMETHRIN IN LIRA AND HOIMA DISTRICTS, JULY-NOVEMBER 2022

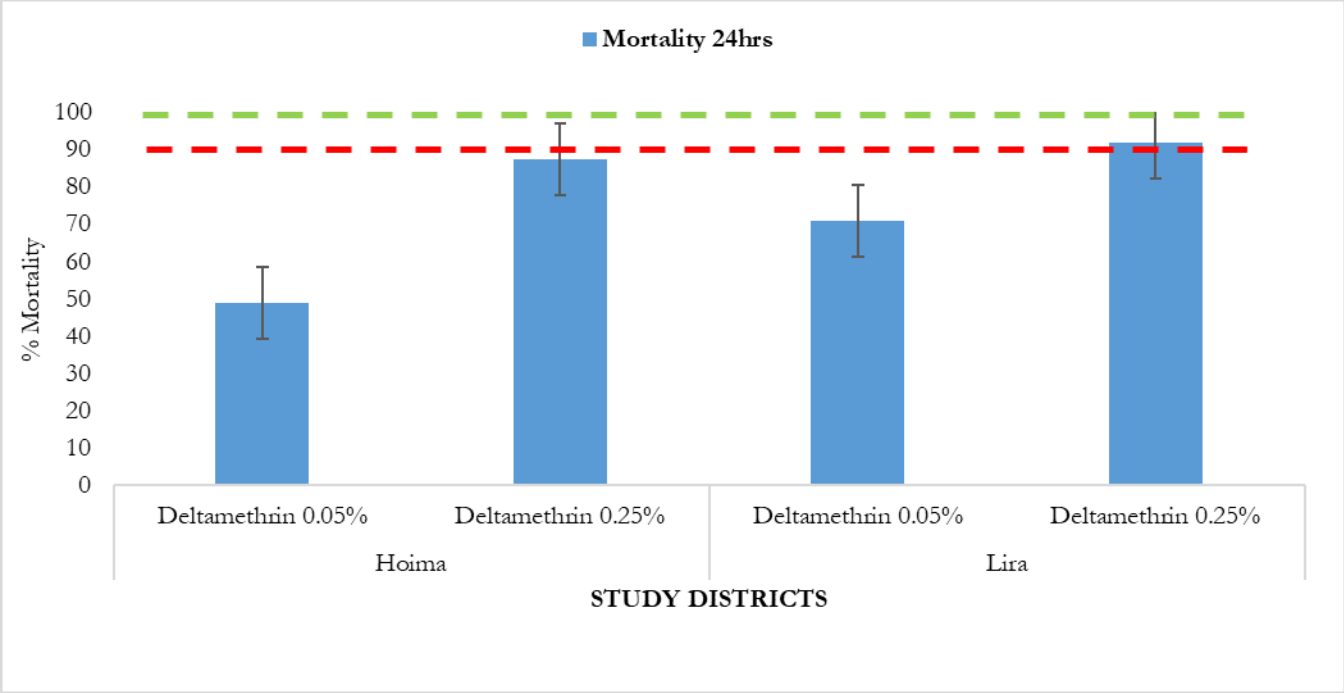
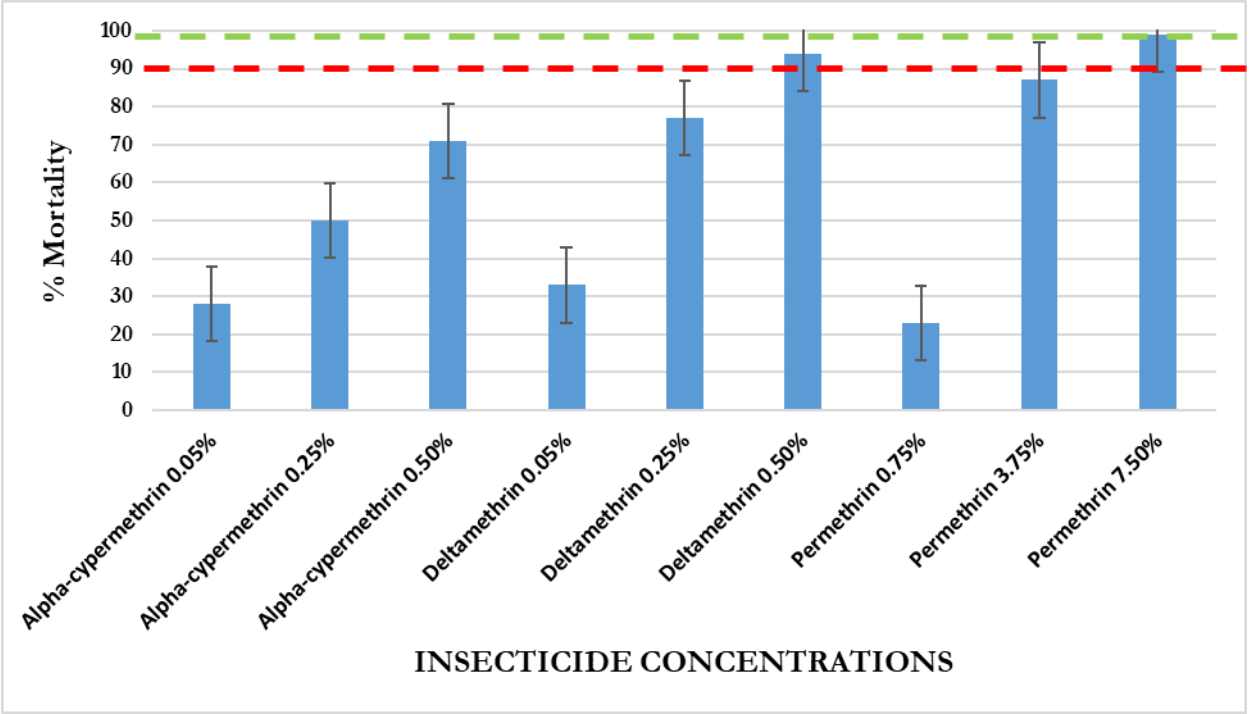


FIGURE 23: PERCENTAGE MORTALITY OF *AN. FUNESTUS* S.L. AFTER EXPOSURE TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN AND PERMETHRIN IN SOROTI DISTRICT, JULY 2022



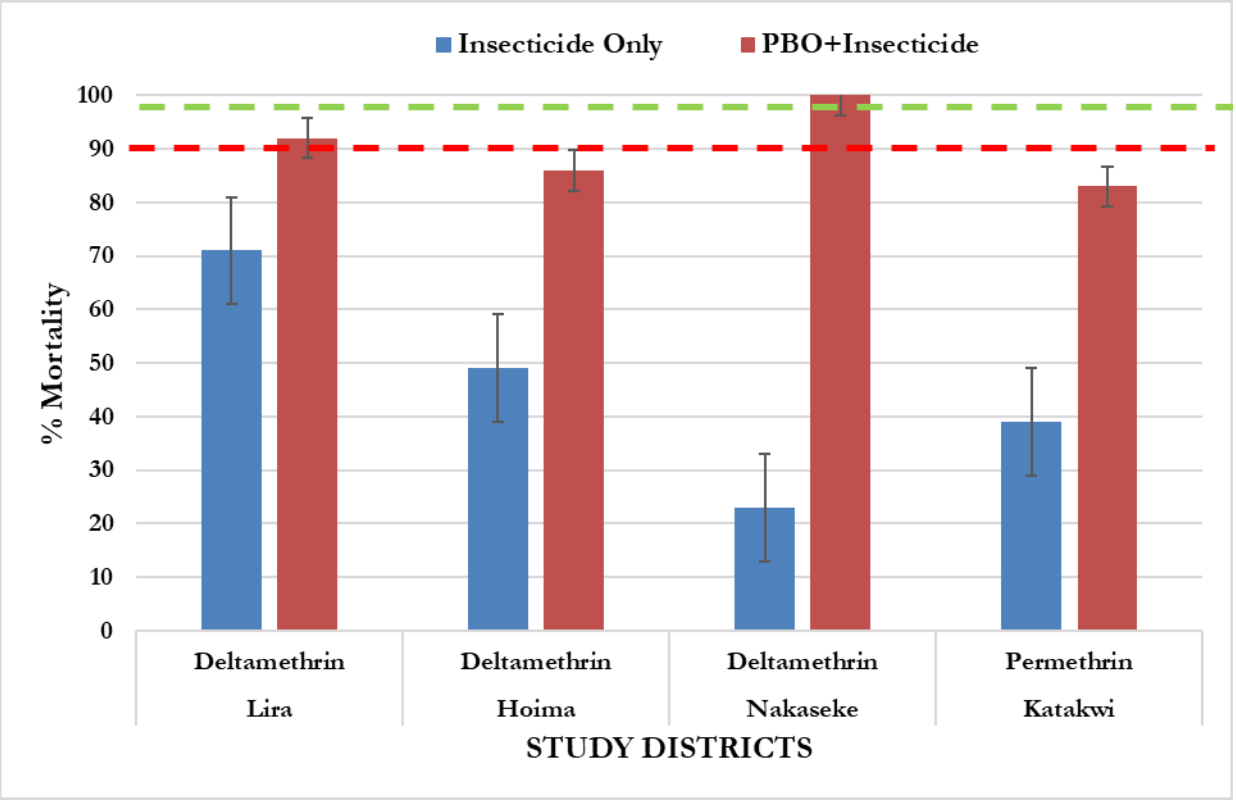
Key: Line indicates resistance threshold Line indicates susceptibility threshold

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). Synergist assays of *An. gambiae* s.l. using the WHO tube assay were conducted in only four districts: Hoima, Katakwi, Lira and Nakaseke due to inadequate number of vectors collected in other districts.

Pre-exposure to the synergist piperonyl butoxide (PBO) fully restored *An. gambiae* s.l. susceptibility to deltamethrin in Nakaseke but partially in Hoima and Lira districts from 59% to 92.0% (Figure 24 and Annex 1). No synergist assays tests were conducted in the other five study districts due to inadequate number of vectors collected.

Figure 24: Percent 24-hour mortality of *An. gambiae* s.l. after PBO pre-exposure in four districts in Uganda, 2022



Key: Line indicates resistance threshold Line indicates susceptibility threshold

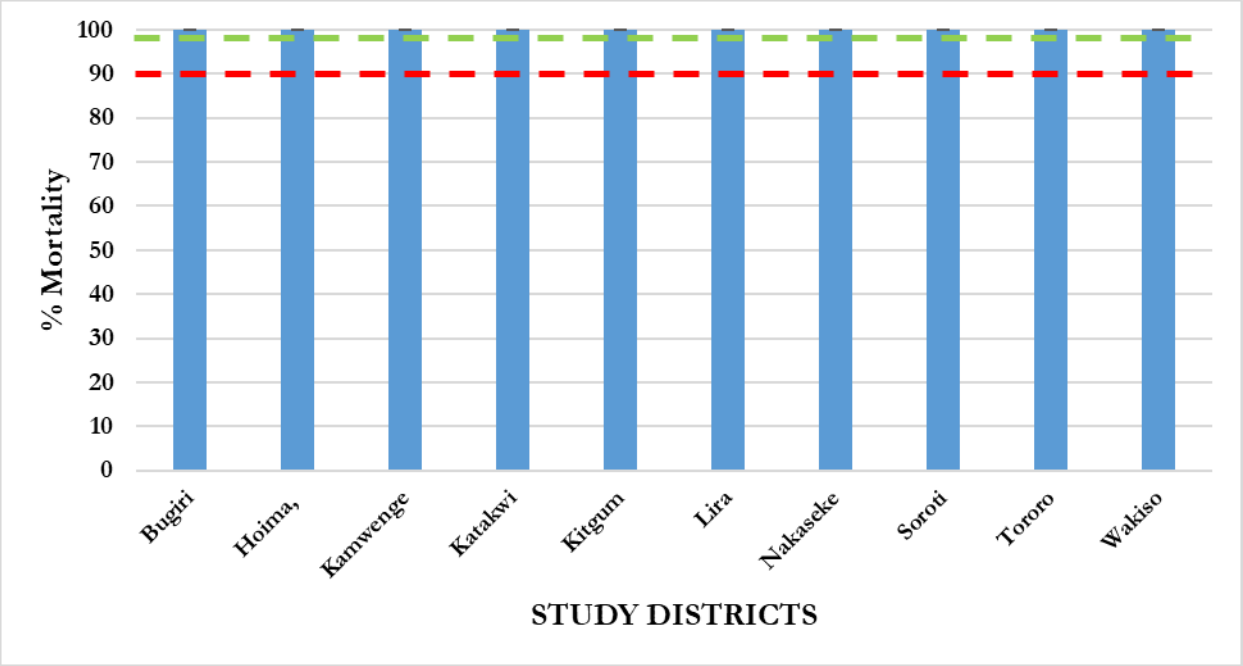
3.3.4 Clothianidin Susceptibility Test Results

The clothianidin susceptibility test were done according to the study protocol provided by PMI VL [8], whereby 250ml glass Wheaton bottles were coated with technical grade clothianidin dissolved in a mixture of Mero® (81% Rapeseed oil methyl ester) and acetone at the diagnostic dose of clothianidin of 4µg AI/bottle. The CDC susceptibility bioassays using the coated clothianidin bottles was conducted the day after according to the standard VectorLink SOP 04/01.

The clothianidin tests were conducted on samples collected in all 11 districts surveyed. *An. gambiae* s.l. was also susceptible to clothianidin in all eight districts surveyed. One hundred percent (100%) mortality was recorded within 24 hours post exposure (Figure 25 and Annex 5). *An. gambiae* s.l. was susceptible to clothianidin in all 11

districts surveyed with ninety-nine to one hundred percent (99 %-100%) mortality recorded at 24 hours post-exposure (Figure 25).

FIGURE 25.: PERCENT 24-HOUR MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO CLOTHIANIDIN AT A CONCENTRATION OF 4 µG/BOTTLE + 800PPM MERO, JULY-NOVEMBER 2022 (RESULTS FOR ADULTS REARED)



Key: Line indicates resistance threshold Line indicates susceptibility threshold

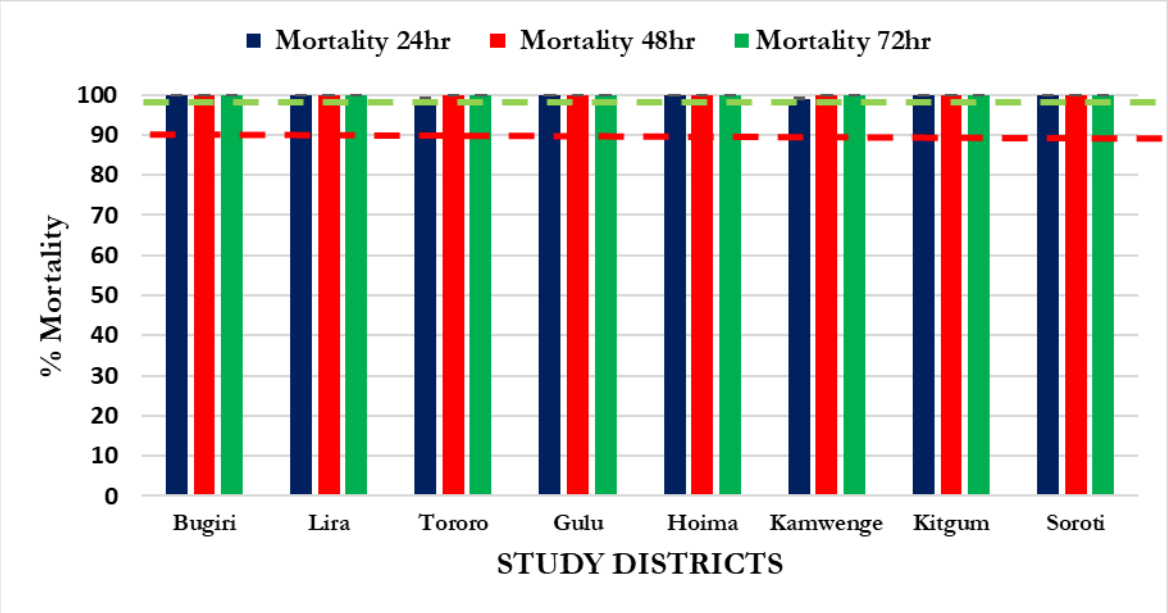
3.3.5 CHLORFENAPYR SUSCEPTIBILITY TEST RESULTS:

Chlorfenapyr tests were conducted on samples collected in 8 out of 11 study districts surveyed (Figure 26).

All the *An. gambiae* s.l. mosquitoes exposed to chlorfenapyr at a concentration of 100µg/bottle achieved 99-100 percent mortality within 1-day post-exposure in all the six districts (Figure 26 and Annex 7).

Chlorfenapyr tests were conducted on the wild *An. gambiae* s.l. in parallel with the laboratory susceptible *An. gambiae* s.s. Kisumu strain in the districts of Bugiri, Gulu, Kitgum and Tororo where access to susceptible *An. gambiae* s.s. strains was possible, which were all killed in all the tests. Parallel test with the laboratory susceptible *An. gambiae* s.s. Kisumu strain were not conducted in the other study districts where, in most cases, there was no nearby colony of susceptible *An. gambiae* s.s. to be used as reference. *An. gambiae* s.l. was susceptible to chlorfenapyr in nine out of 11 study districts where studies were done, with 99-100% mortality was recorded in day one post-exposure in all locations (Annex 7 and Figure 26).

FIGURE 26: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO CHLORFENAPYR AT A CONCENTRATION OF 100MG/BOTTLE IN 6 DISTRICTS IN UGANDA, JULY – NOVEMBER 2022



Key: Line indicates resistance threshold Line indicates susceptibility threshold

3.4 MOLECULAR ASSAY RESULTS

Molecular assay analysis for the species identification of the *An. gambiae* and *An. funestus* complexes, infection rates and characterization of insecticide resistance markers, knockdown resistance (*kdr*) and Acetylcholinesterase-1 (*Ace-1)* genes were conducted at Vector Control Division (MoH) and IDRC Molecular Laboratories. Molecular assays were performed on a proportion of samples collected from January to December 2022 in the longitudinal study districts of Bugiri, Lira, Serere and Tororo (IRS intervention districts) and Apac and Soroti (non-IRS intervention districts) and susceptibility study districts of Gulu, Hoima, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti and Tororo. No tests were conducted with pyrethroids in Bugii and Wakiso districts due to inadequate vector samples collected.

3.4.1 IDENTIFICATION OF VECTOR SPECIES

Of the *Anopheles gambiae* s.l. complex only *An. arabiensis* and *An. gambiae* s.s were detected, and of the *An. funestus* s.l. species group only *An. funestus* s.s. was detected. Further separation of *An. gambiae* s.s. into *An. gambiae* s.s. and *An. coluzzii* was completed in April 2023. A total of 1,114 of the 2022 entomological monitoring mosquito samples identified as *An. gambiae* s.s. by IDRC Molecular Laboratory were analyzed further: 150 from Tororo district, 257 from Soroti district, 49 from Gulu district 24 from Katakwi district, 129 from Serere district, 92 from Apach district, 41 from Nakaseke District, 30 from Kitgum district, 196 from Lira district 96 from Bugiri district and 50 from Hoima district. Of these, 1109 were confirmed as *An. gambiae* s.s. while 05 didn't amplify. No *An. coluzzii* was found in these samples.

3.4.1.1. PCR ANALYSIS RESULTS OF MOSQUITO SAMPLES FROM LONGITUDINAL DATA COLLECTION

A summary of the PCR molecular analysis for the speciation of the *An. gambiae* s.l. and *An. funestus* group from longitudinal surveillance is given in Table 6 and Figure 27.

Anopheles gambiae s.s. was the predominant vector identified in the districts of Apac, Lira, Tororo and Soroti, while *An. arabiensis* was more common in Serere and Bugiri, although in Bugiri the vector was in almost equal portions with *An. gambiae* s.s. *(*Table 2A and Figure 1). All 240 *An. funestus* s.l, samples analyzed from six bionomics study districts were *An. funestus* s.s.

Disaggregated by method, PSC collected higher numbers of *An. gambiae* s.s. in Lira, Soroti and Tororo; while almost equal numbers of *An. gambiae* s.s. and *An. arabiensis* were collected in Apac and Bugiri, but more *An. arabiensis* than *An. gambiae* s.s. were collected in Serere (Table 7).

Human Landing Collections (HLCs) indoor caught more *An. gambiae* s.s. [65.41% 87/133) compared to *An. arabiensis* [34.59% (46/133)] in all districts except Bugiri (Table 8), while HLCs outdoor recorded higher proportions of *An. gambiae* s.s. than *An. arabiensis* in Bugiri, Soroti and Tororo, but recorded higher proportions of *An. arabiensis* compared to *An. gambiae* s.s. in Lira, Serere and Apac (Table 9). Window exit traps (WET) method collected higher proportions of *An. gambiae* s.s. overall in Apac, Serere and Tororo, but collected a higher proportion of *An. arabiensis* in Bugiri district.

An. gambiae s.s. was consistently collected in higher proportions than *An. arabiensis* in most months in the districts of Lira, Soroti and Tororo, while in the districts of Apac, Bugiri and Serere, there were months where *An. gambiae* s.s. was collected in higher proportions than *An. arabiensis*, while in others, *An. arabiensis* was predominant.

TABLE 7: SPECIES COMPOSITION OF THE *ANOPHELES GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. MOSQUITOES COLLECTED USING PSCS IN 6 BIONOMICS STUDY DISTRICTS IN 2022

District	*An. gambiae* s.l.					*An. funestus* s.l.				
	Number Amplified	*An. gambiae* s.s.		*An. arabiensis*		Number Amplified	*An. funestus* s.s.		Other *An. funestus species*	
		No.	%	No.	%		No.	%	No.	%
Apac	122	64	52.46	58	47.54	37	37	100	0	0
Bugiri	183	85	46.45	98	53.55	30	30	100	0	0
Lira	203	148	72.91	55	27.09	25	25	100	0	0
Serere	119	41	34.45	78	65.55	15	15	100	0	0
Soroti	153	138	90.2	15	9.8	15	15	100	0	0
Tororo	160	92	57.5	68	42.5	13	13	100	0	0
Total	**940**	**568**	**60.43**	**372**	**39.57**	**135**	**135**	**100**	**0**	**0**

FIGURE 27: *AN. GAMBIAE* S.L. SPECIES COMPOSITION BY PCR ACROSS BIONOMICS STUDY DISTRICTS JAN-DEC 2022

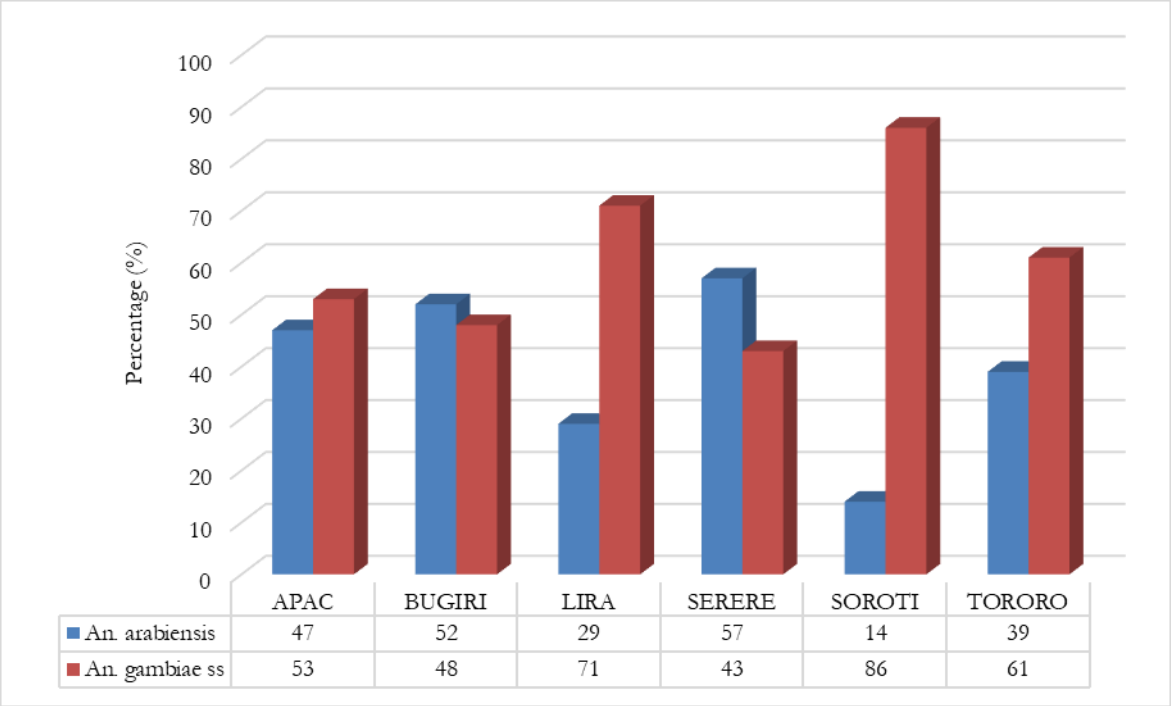


TABLE 8: SPECIES COMPOSITION OF THE *ANOPHELES GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. MOSQUITOES COLLECTED USING HLCS INDOOR IN 6 BIONOMICS STUDY DISTRICTS IN 2022

District	*An. gambiae* s.l.					*An. funestus* s.l.				
	Number Amplified	*An. gambiae* s.s.		*An. arabiensis*		Number Amplified	*An. funestus* s.s.		Other An. *funestus* species	
		No.	*%*	*No.*	*%*		*No.*	*%*	*No.*	*%*
Apac	14	9	64.29	5	35.71	7	7	100	0	0
Bugiri	23	10	43.48	13	56.52	4	4	100	0	0
Lira	18	14	77.78	4	22.22	9	9	100	0	0
Serere	44	28	63.64	16	36.36	18	18	100	0	0
Soroti	11	9	81.82	2	18.18	20	20	100	0	0
Tororo	23	17	73.91	6	26.09	3	3	100	0	0
Total	**133**	**87**	**65.41**	**46**	**34.59**	**61**	**61**	**100**	**0**	**0**

TABLE 9: SPECIES COMPOSITION OF THE *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. MOSQUITOES COLLECTED USING HLCS OUTDOOR IN 6 BIONOMICS STUDY DISTRICTS IN 2022

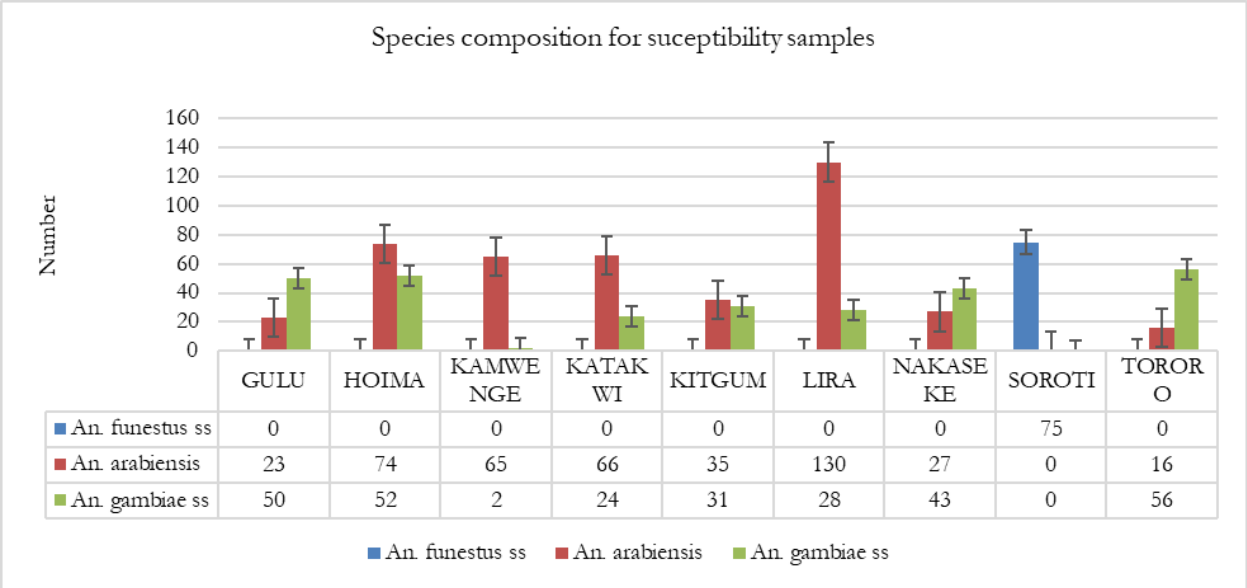
District	*An. gambiae* s.l.					*An. funestus* s.l.				
	Number Amplified	*An. gambiae* s.s.		*An. arabiensis*		Number Amplified	*An. funestus* s.s.		Other *An. funestus* species	
		No.	%	No.	%		No.	%	No.	%
Apac	29	13	44.83	16	55.17	6	6	100	0	0
Bugiri	30	18	60.00	12	40.00	2	2	100	0	0
Lira	15	5	33.33	10	66.67	7	7	100	0	0
Serere	29	12	41.38	17	58.62	10	10	100	0	0
Soroti	25	16	64.00	9	36.00	13	13	100	0	0
Tororo	19	16	84.21	3	15.79	5	5	100	0	0
Total	**147**	**80**	**54.42**	**67**	**45.58**	**43**	**43**	**100**	**0**	**0**

3.4.1.2 PCR ANALYSIS RESULTS OF MOSQUITO SAMPLES FROM INSECTICIDE RESISTANCE TESTS

Molecular analysis of the *An. gambiae* s.l. found that *An. gambiae* s.s. was mostly used in susceptibility tests in Gulu, Nakaseke and Tororo, whilst *An. arabiensis* was mostly used in tests in the IRS districts of Bugiri and Lira and in the non-IRS district of Soroti, Hoima, Kamwenge, Katakwi, and Kitgum (Figure 28).

A total of 722 *An. gambiae* s.l. susceptibility samples that amplified and were analysed for species ID from the 9 districts of Gulu, Hoima, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti and Tororo out of the 11 districts. *An. arabiensis* was the more predominant species accounting for 60.4% (436/722) and *An. gambiae* s.s. accounting for 39.6% (286/722) of the total sample analysed. The 75 *An. funestus* s.l. samples analysed from Soroti district were all *An. funestus* s.s.

FIGURE 28: SPECIES COMPOSITION OF MALARIA VECTORS COLLECTED DURING SUSCEPTIBILITY STUDIES IN 9 DISTRICTS

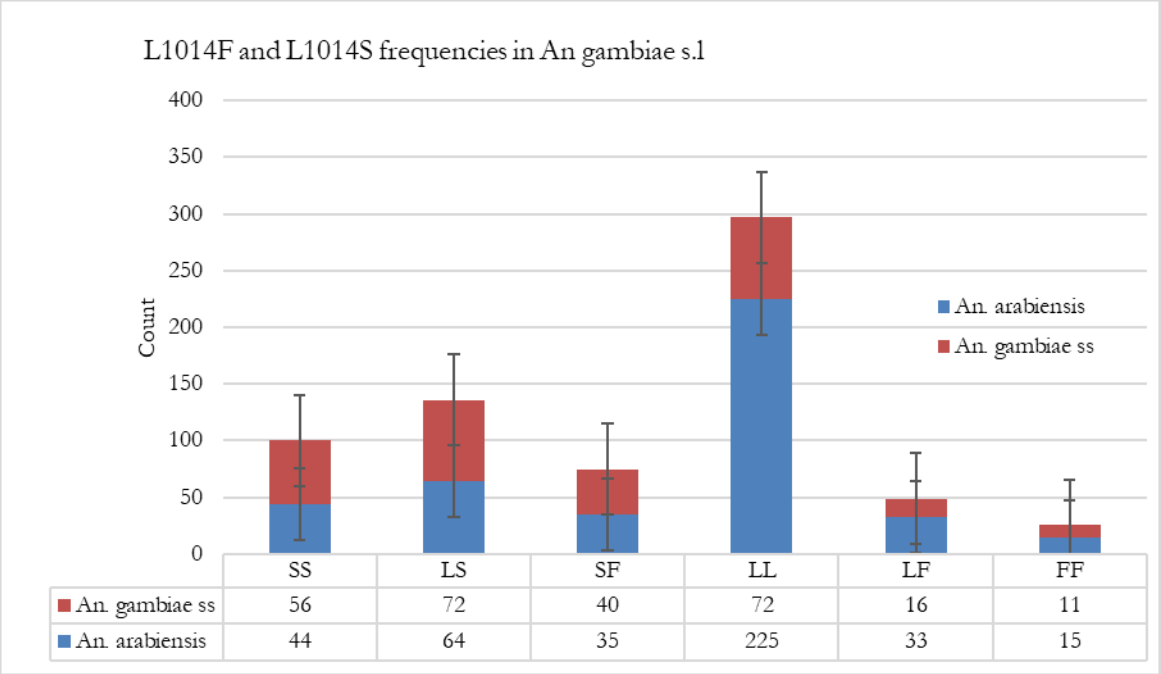


3.4.1.3. KDR- L1014 F AND L1014S MUTATION ALLELIC FREQUENCY

A total of 829 samples were analysed for *L1014F* and *L1014S* knockdown (*kdr*) markers of insecticide resistance to pyrethroids. Of the analysed samples, the majority (41.62% [345/829]) were wildtype. Overall, the *L1014S* mutation was more prevalent compared to the *L1014F* mutation in the districts, with Kamwenge district having the highest *L1014S* allelic frequency mutation at 60.4% while Kitgum had the lowest allelic frequency for the mutation (8.0%). On the other hand, the *L1014F* mutation allelic frequency was highest in Kitgum district at 16.2% frequency while Gulu and Katakwi districts had almost equal allelic frequency proportions of the *L1014F* and *L1014S* mutations (Tables 10 and 11). Of the 696 mosquito samples analysed for the *kdr L1014F* and *L1014S* mutations, 450 mosquitoes were alive while 246 samples were dead; the allelic frequency for the *kdr* (both *L1014 F* and *L1014S)* mutation were almost similar in the alive (46.4%) and dead (41.5%) mosquitoes.

A proportion (25.94% [215/829]) of the samples were homozygous mutant for either the *L1014F* or *L1014S* mutation, with the *L1014S* mutation being more prevalent at 19.06% (158/829). 17.61% (146/829) of the samples were homozygous mutant; 10.37% (86/829) were double mutants (having both *L1014S* and *L1014F* alleles). Analysis of mutation disaggregated by species showed that 14.2% (59/416) of the *An. arabiensis* mosquitoes carried the homozygous form of either of the *L1014F* or *L1014S* SNP mutation while 25.1 (67/267) of the *An. gambiae* s.s. mosquitoes have the homozygous form of either the *L1014F* or *L1014S* mutations. Notably, the wildtype was very common in the *An. arabiensis* mosquitoes (54.1%; 225/416) as compared to *An. gambiae* s.s. (27%;72/267) (Figure 29).

FIGURE 29: L1014F AND L1014S MUTATION DISTRIBUTION ACROSS THE DIFFERENT SPECIES.



KEY: AR=An. arabiensis; GA= An. gambiae s.s; SS= homozygous resistant-east; LS= heterozygous resistant-east; LL= homozygous susceptible wild FF= homozygous resistant west; LF= heterozygous resistant west; FS=homozygous east/west mutation (double mutant)

TABLE 10: OVERALL *KDR* L1014S/F MUTATION ALLELE FREQUENCY

District	SS	LS	SF	LL	LF	FF	NA	Total
GULU	12	12	29	15	4	6	6	84
HOIMA	21	20	9	85	6	12	6	159
KAMWENGE	18	25	26	3	1	3	1	77
KATAKWI	3	11	12	54	13	3	4	100
KITGUM	1	5	2	48	11	4	6	77
LIRA	8	37	5	98	17	2	4	171
NAKASEKE	38	4	1	30	4	1	2	80
TORORO	14	44	2	12	1	0	8	81
Total (%)	115 (13.87)	158 (19.06)	86 (10.37)	345 (41.62)	57 (6.88)	31 (3.74)	3 (4.46)	829

Key: SS= homozygous resistant-east; LS= heterozygous resistant-east; LL= homozygous susceptible wild FF= homozygous resistant west; LF= heterozygous resistant west; FS=homozygous east/west mutation (double mutant); NA= No amplification

TABLE 11: *KDR* L1014F AND L101S ALLELIC FREQUENCY DISTRIBUTION IN THE ALIVE MOSQUITOES

District	Total	L1014S					L1014F				
		SS	LS	SF	LL	Allelicfreq S %	LF	FF	SF	LL	Allelic freq F%
Gulu	69	11	12	26	12	**49.2**	3	5	26	12	**42.4**
Hoima	57	7	18	5	19	**37.8**	5	3	5	19	**25.0**
Kamwenge	16	2	6	5	1	**53.6**	0	2	5	1	**56.3**
Katakwi	96	3	11	12	54	**18.1**	13	3	12	54	**18.9**
Kitgum	5	0	0	1	2	**16.7**	2	0	1	2	**30.0**
Lira	106	6	36	2	43	**28.7**	17	2	2	43	**18.0**
Nakaseke	28	12	0	0	13	**48.0**	2	1	0	13	**12.5**
Tororo	73	14	44	2	12	**51.4**	1	0	2	12	**10.0**
Total	**450**	**55**	**127**	**53**	**156**		**43**	**16**	**53**	**156**	

Key: SS= homozygous resistant-east; LS= heterozygous resistant-east; LL= homozygous susceptible wild FF= homozygous resistant west; LF heterozygous resistant west; FS=homozygous east/west mutation (double mutant)

3.4.1.4 DETECTION OF RESISTANCE MARKERS ACE-1R

Assays for detection of *Ace-1* genes were performed by the IDRC Molecular laboratory. Analysis of the Ace-1R mutation showed that all 256 mosquitos (190 alive and 66 dead) from eight study districts were wild type for this gene, and no Ace-1R mutations were observed (Table 11).

TABLE 12: MOSQUITO SAMPLES EXAMINED FOR ACETYLCHOLINESTERASE MUTATION (ACE-1R)

District	No. tested	Phenotype	AA	Aa	Aa
Gulu	33	Alive	33	0	0
		Dead	0	0	0
Hoima	41	Alive	41	0	0
		Dead	0	0	0
Kamwenge	30	Alive	17	0	0
		Dead	13	0	0
Katakwi	30	Alive	30	0	0
		Dead	0	0	0
Lira	34	Alive	34	0	0
		Dead	0	0	0
Tororo	35	Alive	35	0	0
		Dead	0	0	0
Kitgum	23	Alive	0	0	0
		Dead	23	0	0
Nakaseke	30	Alive	0	0	0
		Dead	30	0	0
Total	**256**	**Alive**	**190**	**0**	**0**
		Dead	**66**	**0**	**0**

Key: AA= homozygous susceptible Ace-1; Aa= Heterozygous resistant Ace-1; aa=Homozygous resistant Ace-1R

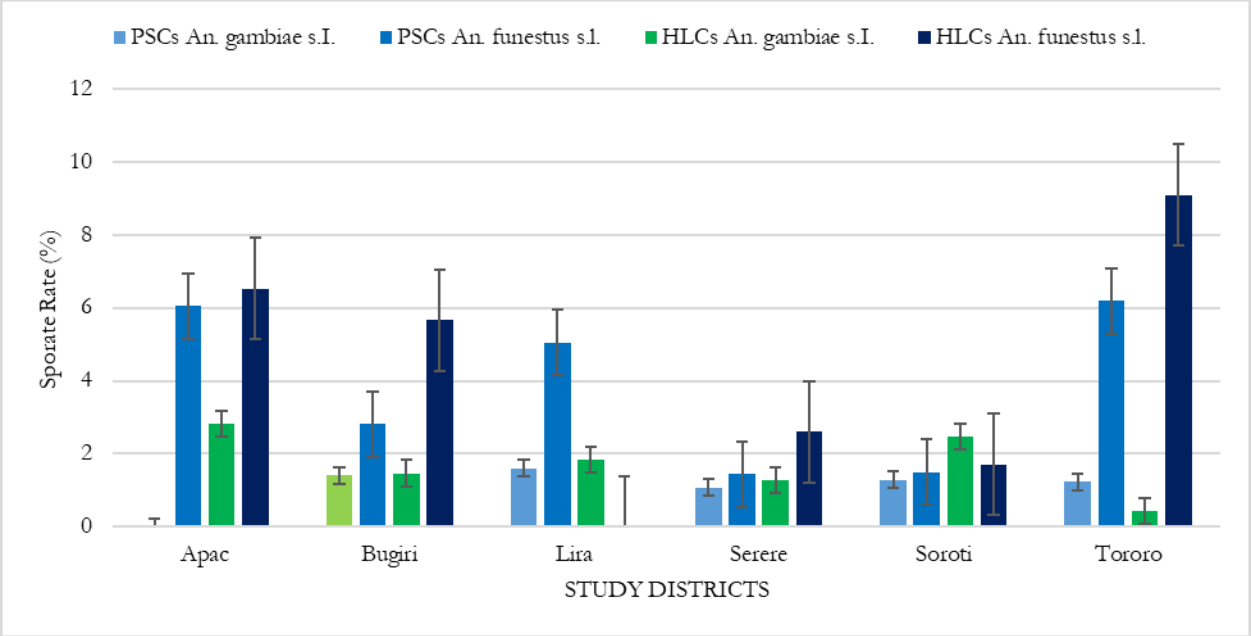
3.4.2 Detection and Identification of Malaria Parasites *Plasmodium Falciparum*

Sporozoite ELISA analysis of 7,726 (3,682 *An. gambiae* s.l. and 4,044 *An. funestus* s.l.) mosquito samples show more parasite-positive mosquitoes were found in the *An. funestus* group. The sporozoite positivity of *An. funestus* complex was distributed throughout the year with peak in June 2022, while that of *An. gambiae* complex was not distributed throughout the year but with peak in May 2022 (Tables 13, Figure 30 and Annexes 8 and 9). The sporozoite positivity rates were generally high during the months of April, May, June, July, and August 2022. Results indicate that Apac, Bugiri, Lira and Tororo had relatively high sporozoite rates while Serere and Soroti had relatively lower sporozoite rates. The sporozoite positivity rates of malaria vectors caught using HLCs were generally higher than those caught using PSCs for both species complexes. Comparing HLC indoor and outdoor sporozoite rates, mosquitoes caught biting indoors generally had higher sporozoite rates for both species complexes, though the sample size being compared are low. In IRS districts, for *An. gambiae* s.l., the mosquitoes caught indoors had higher rates in Lira than outdoors but higher outdoor than indoor in Serere, and for *An.* funestus s.l. the mosquitoes caught indoors had higher rates than outdoors in Bugiri, Serere and Tororo but similar inLira.

Table 13: Summary of sporozoite rates of *An. gambiae* s.l. and *An. funestus* s.l. in the bionomics study districts, January to December 2022

District	PSCs						HLCs Indoor						HLCs Outdoor					
							HLCs Indoor			HLCs Outdoor			HLCs Indoor			HLCs Outdoor		
	An. gambiae s.I.			*An. funestus* s.l.			*An. gambiae* s.I.			*An. gambiae* s.I.			*An. funestus* s.l.			*An. funestus* s.l.		
	No. tested	No. Pf +ve	% Pf +ve	No. tested	No. Pf +ve	% Pf +ve	No. tested	No. Pf +ve	% Pf +ve	No. tested	No. Pf +ve	% Pf +ve	No. tested	No. Pf +ve	% Pf +ve	No. tested	No. Pf +ve	% Pf +ve
Apac	428	0	0	562	34	6	132	1	0.8	41	00	0.0	85	03	3.5	43	02	4.7
Bugiri	570	8	1.4	355	10	2.8	32	0	0.0	25	00	0.0	24	03	12.5	40	01	2.5
Lira	436	7	1.6	496	25	5	31	2	6.5	87	03	3.5	09	00	0.0	00	00	0.0
Serere	371	4	1.1	554	8	1.4	121	1	0.8	106	02	1.9	114	04	3.5	86	01	1.2
Soroti	390	5	1.3	798	12	1.5	96	1	1.0	72	02	2.8	491	11	2.2	221	03	1.4
Tororo	486	6	1.2	113	7	6.2	87	0	0.0	123	00	0.0	34	03	8.8	77	02	2.6

FIGURE 30: SPOROZOITE RATES OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. IN THE BIONOMICS STUDY DISTRICTS, JANUARY TO DECEMBER 2022



4. Discussion, Lessons Learned, and Challenges

4.1 Discussion

Results of PSCs during longitudinal studies showed a general increase in malaria vectors (*An. gambiae* s.l. and *An. funestus* s.l.) caught in 2022 compared to 2021 except for a slight reduction in the number of *An. gambiae* s.l. caught in Soroti which reduced from 9,075 in 2021 to 9,001 in 2022. *An. funestus* s.l. was the most abundant species collected while resting indoors in Apac, and Soroti (control districts) and in Lira and Serere (current IRS districts). However, there was a surprising great increase in the number of *An. funestus* s.l. mosquitoes caught in 2022 in Bugiri and Tororo districts compared to the previous years where very few of the species were caught in these districts. For example, the number of *An. funestus* s.l. caught in Bugiri increased from 47 in 2021 to 457 in 2022 and in Tororo from 0 in 2021 to 255 in 2022. In contrast, *An. gambiae* s.l. predominated the indoor catches in the IRS intervention districts of Bugiri and Tororo. The increased numbers of *An. funestus* s.l., a highly anthropophilic, endophilic and endophagic malaria vector, more than *An. gambiae* s.l., caught in Bugiri, Tororo and Lira in 2022 may partially explain increases in the malaria cases that were observed in these three IRS districts. The combined (IRS + non-IRS) relative composition is always skewed to An. funestus s.l. in all years because Soroti has disproportionately higher numbers compared to all other sites. When considering the relative composition for just Bugiri, Lira and Tororo (sites with continuous ento surveillance and annual IRS) IRS sites over time, funestus is becoming relatively more predominant (23% in 2019-58% in 2020-41% in 2021-53% in 2022).

Indoor resting densities of *An. funestus* s.l. increased more in the second half of the year especially in Apac and Soroti (non-IRS districts) and in Lira and Serere (IRS districts), while higher numbers of *An. gambiae* s.l. were recorded in the first half of the year but reduced in the second half probably associated with the impact of IRS. Analysis of the abdominal stages of *An. gambiae* s.l. and *An. funestus* s.l. clearly showed that the proportion of half-gravd and fully gravid *An. gambiae* s.l. mosquitoes caught in non-IRS districts was significantly higher than in IRS districts indicating that IRS was effectively killing mosquitoes resting on the sprayed walls.

Results of WETs during longitudinal studies showed that a proportion of both malaria vectors (*An. gambiae* s.l. and *An. funestus* s.l.) exited the houses either before feeding or after feeding. This may be mechanism that the malaria vectors have developed to avoid resting on insecticide treated wall surfaces and ITNs. This may also probably explain the increased malaria transmission after IRS and in areas with ITNs.

HLCs demonstrated that the Soroti control area had the highest mean *An. funestus* s.l. biting rate both indoors and outdoors, like what was reported for 2021. *An. gambiae* s.l. was found at higher densities in the control Soroti district at much higher densities than in Apac, another control district. 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 Serere and the non-IRS districts of Apac, but higher indoors in Lira and Tororo and the control district of Soroti. The increased biting of *An. gambiae* s.l. biting rates observed in all IRS districts within the first and second months after IRS, especially in Lira and Serere districts, is difficult explain although the number of vectors subsequently reduced in the following months. This may be due to the delayed mortally attributed to Fludora Fusion. An increase of biting rates of *An. gambiae* s.l. was observed in Lira, Bugiri and Tororo in 2021 which was mirrored in 2022. In 2021, the biting rates after IRS in Tororo increased which was partly due to the prolonged heavy rains – the heaviest in the district in the last five years – which could have significantly

increased the number of anopheline mosquito breeding sites in the months after IRS. *An. funestus* s.l. consistently observed, biting indoor in all study districts except in Apac district where outdoor biting was higher. Regarding the overnight biting patterns of *An. funestus* s.l. observed, biting starts in early evening, between 6:00 pm and 8:00 pm especially in Soroti and Apac, 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 four different species (*An. funestus* s.l., *An. gambiae* s.l., *An. coustani 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. Other species like *An. ardensis, An. pretoriensis and An. squamosuss* that were caught in 2020 were not caught in 2022.

Our findings highlight high levels of heterogeneity and diversity in mosquito vector species composition and behavior in the monitored areas. The early biting of both *An. funestus* s.l., and *An. gambiae* s.l., outdoors before people enter the sprayed houses or under LLINs may contribute to malaria transmission despite the presence of both IRS and LLINs. However, the role of the various anopheline mosquitoes like *An. coustani An. ziemanni* which have been caught in various study districts in malaria transmission apart from *An. gambiae* s.l. and *An. funestus* s.l. needs to be investigated in the study districts.

The WHO cone wall bioassay results obtained in 6 of 8 sprayed districts showed that the spray quality of the 2022 spray campaign was satisfactory at all monitored sites for both SumiShield and Fludora Fusion. The monitoring for insecticide decay rate showed that Fludora fusion on average stayed effective on sprayed surfaces between four and seven months in Kibuku, Lira and Serere, while that of SumiShield remained effective for seven months in Tororo and Bugiri on all the wall types sprayed.

An. gambiae s.l. was found to be susceptible to pirimiphos-methyl (98-100% mortality) in the 10 out of 11 districts where the test was completed, while *An. gambiae* s.l. was susceptible to bendiocarb in four districts (Gulu, Kitgum, Lira and Tororo) out of 11 districts where the test was completed *An. gambiae* s.l. was found susceptible to clothianidin and chlorfenapyr (99-100% mortality) in all the 11 study districts. *An. gambiae* s.l. was resistant to alpha-cypermethrin, deltamethrin and permethrin in all the study districts where they were tested with mortality varying between 23.0% in Nakaseke and 83.0% in Tororo for deltamethrin based on WHO tube test results. *An. funestus* s.l. was susceptible to pirimiphos-methyl, clothianidin, chlorfenapyr and bendiocarb in Soroti and in Katakwi but resistant to deltamethrin (33% mortality), permethrin (23% mortality) and alpha-cypermethrin (28% mortality.

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

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 insecticide treated nets (ITNs) and the new WHO-pre-qualified next generation ITNs such as Interceptor® G2 (Alpha-cypermethrin and chlorfenapyr coated on polyester) and Royal Guard® *(*Alpha-cypermethrin and pyriproxyfen incorporated into polyethylene, all panels), some of which are being tested in Uganda, than with pyrethroid-only ITNs which were deployed in most districts of Uganda during the last universal ITNs distribution coverage from July 2020 to March 2021. Indeed, there was a great reduction observed in the number of malaria vectors caught by PSC and HLCs in Apac in 2021 and 2022 following the PBO synergized LLINs and Royal Guard® LLINs

distribution in 2020. There is, however, need to conduct similar tests in various parts of Uganda to map the extent of this and other resistance mechanisms such as esterases and glutathione-s-transferases in the country.

In conclusion, the vector bionomics indicates that IRS and ITNs continue to be the most appropriate interventions for control of malaria vectors in all these districts as the majority of malaria vectors bite from 10.00 p.m. up to 6.00 a.m. However, some malaria vectors start biting as early as 7.00 p.m. and yet most people in bionomics study sites retire to bed at around 10.00 p.m., meaning people can still be infected before they are protected by IRS and ITNs. The slightly high numbers of vectors exiting houses before or after they have fed may be an insecticide exposure avoidance mechanism that malaria vectors have developed to avoid being exposed to ITNs and sprayed wall surfaces which may partially explain increased malaria cases despite the presence of IRS and or ITNs. There is a need to monitor the rainfall pattern as it affects the abundance of malaria vectors and therefore, malaria transmission levels in the country during the different seasons. Increased rainfall results in increased vector breeding habitats and therefore malaria transmission. The full susceptibility of *An. gambiae* s.l. to pirimiphos-methyl, clothianidin and chlorfenapyr (98-100% mortality) indicates that both pirimiphos-methyl and clothianidin can be used in rotation to assist in the management of insecticide resistance in IRS programs.

The species identification performed at the IDRC Molecular laboratory using molecular assays, *Anopheles gambiae* s.s. was found to be the dominant malaria vector of the *An. gambiae* s.l. complex followed by *An. arabiensis*, more so in the IRS districts of Lira and Tororo and the non-IRS districts of Apac and Soroti, while *An. funestus* was the only species of *An. funestus* group recorded from six longitudinal surveillance districts of Apac, Bugiri, Lira , Serere, Soroti, and Tororo, which is consistent with findings during the previous years as well. Molecular analysis of the *An. gambiae* s.l. found that *An. arabiensis* was also the most identified member of the *An. gambiae* complex used in susceptibility tests in the non-IRS districts of Hoima, Kamwenge, Katakwi and Kitgum and the IRS district of Lira, while *An. gambiae* s.s. was the most identified member of the *An. gambiae* complex used in tests in the IRS district of Tororo and in the non-IRS district of Gulu and Nakaseke. Of note, *An. gambiae* s.s. of the *An. gambiae* complex has been recorded in increasing proportions in the districts of Bugiri and Tororo where *An. arabiensis* predominated in these districts a few years back. In Bugiri, for example, the proportion of *An. gambiae* s.s. amongst the *An. gambiae* complex species analyzed was as follows: 14.5%, 13.1%, 50.8% and 47.5% in 2019, 2020, 2021 and 2022 respectively, while in Tororo, it was 0.7% 1.3%, 33.7% and 61.4% in 2019, 2020, 2021 and 2022 respectively. Increase in the proportions of *An. funestus* s.s. and *An. gambiae* s.s. collected in Bugiri and Tororo districts, coupled with the relatively high sporozoite rates in these districts, may have contributed to the increased malaria prevalence and upsurges reported in these two districts in the last two years. *An. funestus* s.s. and *An. gambiae* s.s. are more efficient vectors than *An. arabiensis* which used to predominate collections in the two districts.

Of the samples analyzed for *L101F* and *L1014S* knockdown (*kdr*) markers of insecticide resistance to pyrethroids the majority were wildtype. Overall, the *L1014S* mutation was more prevalent compared to the *L101F* mutation in the districts, which is in line with earlier findings where the *L101F* and *L1014S* knockdown (*kdr*) markers were found to predominate in East Africa and West Africa respectively. Analysis of the Ace-1R mutation showed that all mosquitos analyzed in the eight study districts were wild type for this gene with no Ace-1R mutations were observed. This is consistent with findings during the previous years.

4.2 CHALLENGES

There was delayed molecular speciation of *Anopheles gambiae* s.s. into *An. gambiae* s.s. and *An. colluzzii* due to delayed receipt of the necessary *An. coluzzii* primers by VCD Molecular Laboratories in Kampala, Uganda which has not enabled the incorporation of these results in this report. The procurement of relevant missing equipment and addition of staff to the VCD Lab in the new project would help to have all entomological samples run at VCD.

4.3 LESSONS LEARNED

The involvement of unemployed VCOs in entomological monitoring activities strengthens their capacity to conduct similar studies and increases the manpower available to the Ministry of Health for deployment in future

similar studies in various areas of the country. However, some of the VCOs still need capacity strengthening to be able to excel professionally.

Due to the unpredictable rainfall pattern in the country over the recent years it is difficult to plan when it is most appropriate to conduct susceptibility studies. Though the studies were staggered in 2022 to cater for the rainfall patterns in the different study districts, the fast drying of breeding sites affected the number tests conducted in various study districts. Involving and facilitating district based VCOs and/or community mosquito collectors to collect and rear mosquitoes in advance of study teams reaching the districts would enable studies to be conducted in a shorter period of time, improving results and reducing the study costs.

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ANNEX 1: MEAN HOURLY BITING RATES OF *AN. GAMBIAE* S.L. IN APAC, BUGIRI, LIRA, SERERE, SOROTI AND TORORO DETERMINED THROUGH HLCS, JANUARY THROUGH DECEMBER 2022

District	Collection location	Time of Collection												
		6–7 p.m.	7-8 p.m.	8-9 p.m.	9-10 p.m.	10-11 p.m.	11-12 p.m.	12-1 a.m.	1-2 a.m.	2-3 a.m.	3-4 a.m.	4-5 a.m.	5-6 a.m.	6-7 a.m.
Bugiri (IRS)	Indoor	0.04	0.04	0.08	0.08	0.08	0.1	0.04	0.1	0.15	0.25	0.23	0.1	0.04
	Outdoor	0.06	0.15	0.27	0.23	0.08	0.06	0.06	0.13	0.15	0.15	0.21	0.33	0.15
Lira (IRS)	Outdoor	0	0.1	0.17	0.08	0.52	0.4	0.31	0.27	0.63	0.44	0.71	0.33	0.04
	Indoor	0	0.19	0.15	0.15	0.13	0.33	0.17	0.29	0.58	0.52	0.33	0.15	0.08
Serere (IRS)	Indoor	0.08	0.13	0.21	0.21	0.48	0.46	0.42	0.71	0.69	0.65	0.88	0.33	0.15
	Outdoor	0	0.13	0.21	0.06	0.27	0.5	0.52	0.83	1	0.83	0.56	0.73	0.21
Tororo (IRS)	Indoor	0	0.08	0.06	0.19	0.13	0.31	0.46	0.4	0.48	0.25	0.31	0.44	0.29
	Outdoor	0	0.02	0	0.06	0	0.29	0.48	0.5	0.48	0.29	0.1	0.29	0.13
Apac (non-IRS)	Indoor	0	0	0.02	0.08	0.04	0.04	0.06	0.13	0.02	0.04	0.08	0.1	0.06
	Outdoor	0	0.04	0.02	0.17	0.15	0.21	0.17	0.21	0.25	0.1	0.19	0.15	0.15
Soroti (non-IRS)	Indoor	0.04	0.1	0.23	0.38	0.35	0.73	1.2	1	0.96	1.1	0.85	1.1	0.42
	Outdoor	0.04	0.27	0.25	0.44	0.4	0.5	0.73	0.69	0.71	1	0.63	0.54	0.42

ANNEX 2: MEAN HOURLY BITING RATES OF *AN. FUNESTUS* S.L. IN APAC, BUGIRI, LIRA, SERERE, SOROTI AND TORORO DETERMINED THROUGH HLCS, JANUARY THROUGH DECEMBER 2022

District	Collection location	Time of Collection												
		6–7 p.m.	7-8 p.m.	8-9 p.m.	9-10 p.m.	10-11 p.m.	11-12 p.m.	12-1 a.m.	1-2 a.m.	2-3 a.m.	3-4 a.m.	4-5 a.m.	5-6 a.m.	6-7 a.m.
Bugiri (IRS)	Indoor	0.02	0	0.02	0.02	0	0.02	0.06	0.06	0.13	0.08	0.13	0.08	0.08
	Outdoor	0.02	0.04	0	0.02	0.02	0.06	0.04	0	0.02	0.02	0	0.1	0
Lira (IRS)	Indoor	0	0	0.02	0.02	0.02	0.1	0.19	0.17	0.4	0.42	0.19	0.25	0.25
	Outdoor	0.06	0.02	0.19	0.23	0.67	0.42	0.6	0.65	1.1	1.3	1.1	0.67	0.5
Serere (IRS)	Indoor	0	0.08	0.15	0.17	0.98	1.4	1.1	1.3	1.9	2	1.8	1.7	0.96
	Outdoor	0.02	0.06	0	0.02	0.17	0.42	0.48	0.67	1.3	1.3	1.4	1.1	0.31
Tororo (IRS)	Indoor	0	0	0	0.08	0.06	0.31	0.54	0.25	0.15	0.15	0.13	0.15	0.19
	Outdoor	0	0	0	0.02	0.02	0.06	0.19	0.27	0.23	0.06	0.04	0.1	0.15
Apac (non-IRS)	Indoor	0.06	0.08	0.02	0.06	0	0.17	0.21	0.23	0.08	0.4	0.44	0.54	0.19
	Outdoor	0.02	0	0.1	0.02	0.17	0.23	0.1	0.17	0.63	0.33	1.3	1.1	0.25
Soroti (non-IRS)	Indoor	1	2.1	3.2	3.1	3.6	5.7	6.6	8	9.4	14	15.2	14.9	12.2
	Outdoor	0.25	0.33	0.81	0.85	2	4.1	4.9	5.3	6.7	11.4	10.2	8.8	6.7

ANNEX 3: WALL BIO-ASSAY RESULTS IN BUGIRI, LIRA, SERERE AND TORORO SUMISHIELD INSECTICIDE DECAY RATE MONITORING SITES, DECEMBER 2022

Time	% Mortality of *An. gambiae* s.s. (Kisumu strain)								Overall Mean
	SumiShield Bugiri (T0 = March 2022)				SumiShield Tororo (T0 = March 2022)				
	Painted Cement	Plain Brick	Mud	Mean	Painted Cement	Plain Brick	Mud	Mean	
T0	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T1	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T2	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T3	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T4	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T5	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T6	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T7	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T8	68.3 (41/60)	61.7 (37/60)	58.3 (35/60)	62.8	61.7 (37/60)	58.3 (35/60)	51.7 (31/60)	57.2	60.0
T9	56.7 (34/60)	48.3 (29/60)	41.7 (25/60)	48.9	51.6 (31/60)	46.7 (28/60)	41.7 (25/60)	46.7	47.8

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.

ANNEX 4: WALL BIO-ASSAY RESULTS IN KIBUKU LIRA AND SERERE AND TORORO FLUDORA FUSION INSECTICIDE DECAY RATE MONITORING SITES, DECEMBER 2022

Time	% Mortality of *An. gambiae* s.s. (Kisumu strain)												Overall Mean
	Fludora Fusion Kibuku (T0 = March 2022)				Fludora Fusion Lira (T0 = March 2022)				Fludora Fusion Serere (T0 = April 2021)				
	Painted Cement	Plain Brick	Mud	Mean	Painted Cement	Plain Brick	Mud	Mean	Painted Cement	Plain Brick	Mud	Mean	
T0	100 (60/60)	100(60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T1					100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T2	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T3	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T4	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	100
T5	80 (48/40)	71.7 (43/60)	68.3 (41/60)	71.1	100 (60/60)	100 (60/60)	100 (60/60)	100	100 (60/60)	100 (60/60)	100 (60/60)	100	91.1
T6	68.3 (41/60)	63.3 (38/60)	61.7 (37/60)	64.4	76.7 (46/60)	68.3 (41/60)	60 (36/60)	68.3	100 (60/60)	100 (60/60)	98.3 (59/60)	99.4	77.4
T7	61.7 (37/60)	55 (33/60)	51.7 (31/60)	56.1	68.3 (41/60)	56.7 (34/60)	50 (30/60)	58.3	96.7 (58/60)	73.3 (44/60)	65 (39/60)	78.3	64.3
T8	ND	ND	ND	ND	ND	ND	ND	ND	58.3 (35/60)	ND	ND	56.3	58.3
T9	ND	ND	ND	ND	ND	ND	ND	ND	46.7 (28/60)	ND	ND	46.7	46.7

Key: T0 is the test done within 1 week 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. ND = Not Donew

ANNEX 5: PERCENT 24-HOUR HOLDING MORTALITY OF *AN. GAMBIAE S.L.* AFTER EXPOSURE TO INSECTICIDES, JULY-NOVEMBER 2022 (RESULTS FOR ADULTS REARED FROM LARVAE)

Insecticide	Bugiri # Tested (Mortality)	Hoima # Tested (Mortality)	Gulu # Tested (Mortality)	Kamwenge # Tested (Mortality)	Katakwi # Tested (Mortality)	Kitgum # Tested (Mortality)	Lira # Tested (Mortality)	Nakaseke # Tested (Mortality)	Soroti # Tested (Mortality)	Tororo # Tested (Mortality)	Wakiso # Tested (Mortality)
Organophosphate:											
Pirimiphos-methyl 0.25%	100 (100%)	100 (100%)	100 (100%)	100 (98%)	-	100 (100%)	100 (100%)	100 (100%)	100 (100%)	100 (100%)	100 (100%)
Carbamate:											
Bendiocarb 0.10%	-	-	100 (99.0%)	-	-	100 (100%)	100 (99.0%)	-		100 (100%)	
Pyrethroids:											
Deltamethrin 0.05%	-	100 (49%)	100 (71.0%)	76 (77.6%)	100 (71.0%)	100 (95.0%)	100 (71.0%)	100 (23%)	-	100 (83.0%)	-
PBO + Deltamethrin 0.05%	-	100 (86%)	-	-	-	-	100 (92.0%)	100 (100%)	-	-	-
Deltamethrin 0.25% (x5)	-	40 (87.5%)	-	-	-	-	100 (92.0%)	-	-	-	-
Deltamethrin 0.5% (x10)	-	-	-	-	-	-	-	-	-	-	-
Permethrin 0.75% (x1)	-	-	-	-	100 (39.0%)	-	100 (28.0%)	-	-	-	-
PBO + Permethrin 0.75%	-	-	-	-	100 (83.0%)	-		-	-	-	-
Permethrin 3.75% (x5)	-	-	-	-	-	-	100 (94.0%)	-	-	-	-
Permethrin 7.5% (x10)	-	-	-	-	-	-		-	-	-	-
Alpha-cypermethrin 0.05%	-	-	-	-	100 (48.0%)	-	100 (52.0%)	-	-	-	-
PBO + Alpha-cypermethrin 0.05%	-	-	-	-	-	-	-	-	-	-	-
Alpha-cypermethrin 0.25% (x5)	-	-	-	-	-	-	-	-	-	-	-
Alpha-cypermethrin 0.5% (x10)	-	-	-	-	-	-	-	-	-	-	-
Neocotinoid:											
Clothianidin 4 µg/bottle + 800ppm MERO	100 (100%)	100 (100%)	100 (99%)	100 (100%)	100 (99%)	100 (100%)	100 (99%)	100 (100%)	-	100 (100%)	100 (100%)
KEY:	Confirmed resistance					Probable resistance				Susceptible	

ANNEX 6: PERCENT 24-HOUR HOLDING MORTALITY OF *AN. FUNESTUS* S.L. AFTER EXPOSURE TO VARIOUS INSECTICIDES, IN SOROTI DISTRICT, EASTERN UGANDA, OCTOBER 2022 (RESULTS FOR EARLY MORNING PROKOPACK ASPIRATOR COLLECTED ADULTS)

District	Insecticide	Pirimiphos-methyl 0.25%	Bendiocarb 0.1%	Deltamethrin 0.05% (x1)	Deltamethrin 0.25% (x5)	Deltamethrin 0.5% (x10)	Clothianidin 4 µg/bottle + 800ppm MERO	Permethrin 0.75% (x1)	Permethrin 375% (x5)	Permethrin 7.5% (x10)	Alpha-cypermethrin 0.05% (x1)	Alpha-cypermethrin 0.25% (x5)	Alpha-cypermethrin 0.5% (x10)
Soroti	# Tested (Mortality)	100 (100%)	100 (100%)	100 (33%)	100 (77%)	100 (94%)	100 (100%)	100 (23%)	100 (87%)	100 (99%)	100 (28%)	100 (50%)	100 (71%)
Katakwi	# Tested (Mortality)	100 (100%)	100 (96.0%)	-	-	-	-	-	-	-	-	-	-

Key:	Confirmed resistance	Probable resistance	Susceptible

ANNEX 7. PERCENT MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO CHLORFENAPYR AT A CONCENTRATION OF 100 µG/BOTTLE, JULY 02-NOVEMBER 13, 2022 (RESULTS FOR ADULTS REARED FROM LARVAE)

District	No. of mosquitoes exposed	No. of control mosquitoes	Chlorfenapyr at 100µg/bottle Mortality at days post-exposure		
			Day 1	Day 2	Day 3
Bugiri	100	20	100	100	100
Hoima	100	20	100	100	100
Gulu	100	20	100	100	100
Kamwenge	100	20	99	100	100
Katakwi	100	20	100	100	100
Kitgum	100	20	100	100	100
Lira	100	20	100	100	100
Nakaseke	ND	ND	ND	ND	ND
Soroti	100	20	100	100	100
Tororo	100	20	99	100	100
Wakiso	ND	ND	ND	ND	ND

N.D. = Not done.

ANNEX 8: SPOROZOITE RATES OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED USING PSCS IN THE BIONOMICS STUDY DISTRICTS, JANUARY TO DECEMBER 2022

Month	Apac				Bugiri				Serere				Soroti				Tororo				Lira			
	An. gambiae s.l.		*An. funestus* s.l.		*An. gambiae* s.l.		*An. funestus* s.l.		*An. gambiae* s.l.		*An. funestus* s.l.		*An. gambiae* s.l.		*An. funestus* s.l.		*An. gambiae* s.l.		*An. funestus* s.l.		*An. gambiae* s.I.		*An. funestus* s.I.	
	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos
Jan	0	0	112	2	17	0	3	0	13	0	24	0	1	0	60	1	0	0	0	0	0	0	15	0
Feb	0	0	19	1	129	4	28	2	2	0	26	0	18	0	86	1	5	1	1	0	23	2	20	0
Mar	06	0	09	0	134	1	31	0	0	0	20	0	40	0	86	0	64	2	6	0	13	0	12	1
Apr	143	0	31`	1	50	0	14	1	49	0	25	1	46	1	48	1	40	0	6	0	100	1	10	1
May	123	0	153	7	49	0	17	0	135	3	25	0	47	0	68	1	67	1	6	1	61	2	13	0
Jun	100	0	210	2	45	0	36	3	114	1	24	3	47	1	105	3	207	0	6	0	100	0	78	13
Jul	00	0	23	0	29	0	25	0	4	0	57	1	46	2	96	3	31	0	10	0	16	0	10	0
Aug	21	0	124	10	17	1	39	1	0	0	74	1	41	0	57	0	10	1	10	1	15	0	100	2
Sept	35	0	101	4	16	0	44	0	11	0	52	0	45	0	0	0	0	0	38	0	57	0	50	3
Oct	0	0	100	4	28	1	54	2	17	0	27	0	30	0	92	1	12	0	20	5	33	2	33	0
Nov	0	0	0	0	27	0	50	1	26	0	100	0	29	1	100	1	50	1	10	0	9	0	55	2
Dec	0	0	100	3	29	1	14	0	0	0	100	1	0	0	0	0	0	0	0	0	9	0	100	03
Total	**428**	**0**	**562**	**34**	**570**	**8**	**355**	**10**	**371**	**4**	**554**	**8**	**390**	**5**	**798**	**12**	**486**	**6**	**113**	**7**	**436**	**7**	**496**	**25**

ANNEX 9: SPOROZOITE RATES OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED USING HLCS IN THE BIONOMICS STUDY DISTRICTS, JANUARY TO DECEMBER 2022

Month	Apac				Bugiri				Serere				Soroti				Tororo				Lira			
	An. gambiae s.l.		*An. funestus* s.l.		*An. gambiae* s.l.		*An. funestus* s.l.		*An. gambiae* s.l.		*An. funestus* s.l.		*An. gambiae* s.l.		*An. funestus* s.l.		*An. gambiae* s.l.		*An. funestus* s.l.		*An. gambiae* s.I.		*An. funestus* s.I.	
	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos	No tested	No pos
Jan	0	0	0	0	0	0	0	0	0	0	8	0	0	0	83	0	0	0	0	0	0	0	0	0
Feb	0	0	0	0	0	0	0	0	0	0	8	0	0	0	56	0	0	0	0	0	0	0	0	0
Mar	03	0	0	0	22	1	0	0	0	0	13	0	0	0	63	0	30	0	2	1	0	0	0	0
Apr	13	0	0	0	0	0	0	0	23	1	8	0	17	0	96	1	14	0	8	0	0	0	0	0
May	37	1	6	1	30	0	0	0	81	1	9	0	14	0	73	1	36	1	0	1	182	2	0	0
Jun	18	1	18	0	16	0	0	0	84	1	24	2	23	0	37	0	43	0	0	0	58	3	0	0
Jul	0	0	0	0	0	0	0	0	0	0	29	1	4	0	46	2	4	0	2	0	2	0	0	0
Aug	0	0	0	0	0	0	0	0	0	0	26	1	9	1	86	4	3	0	4	1	00	0	0	0
Sept	0	0	0	0	0	0	0	0	15	0	55	1	6	0	79	2	0	0	0	0	23	0	0	0
Oct	0	0	22	2	0	0	27	1	7	0	12	0	20	2	40	0	3	0	26	2	6	0	0	0
Nov	0	0	0	0	0	0	26	2	25	0	0	0	28	0	0	0	50	0	13	1	0	0	50	0
Dec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	3	52	0	11	0	0	0	0	0
Total	**71**	**02**	**46**	**03**	**68**	**01**	**53**	**03**	**235**	**03**	**192**	**5**	**121**	**03**	**759**	**13**	**235**	**01**	**66**	**06**	**271**	**5**	**50**	**0**