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THE PMI VECTORLINK PROJECT

UGANDA

ANNUAL ENTOMOLOGY REPORT

JANUARY 1 – DECEMBER 31, 2020

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ACRONYMS AND ABBREVIATIONS

Ace-1	Acetylcholinesterase 1 gene
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 Spraying
ITNs	Insecticide Treated Nets
kdr	Knockdown resistance gene
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SOP	Standard Operating Procedure
UMRESP	The Uganda Malaria Reduction and Elimination Strategic Plan, 2014-2020
WHO	World Health Organization

EXECUTIVE SUMMARY

Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) remain the primary mosquito vector control interventions in many endemic countries, including sub-Saharan African countries where malaria continues to be a major public health concern.

During the 2020 spray campaign, the President's Malaria Initiative (PMI) VectorLink Uganda Project conducted IRS in two phases, with Phase I conducted from March 2–March 28, 2020 and Phase II from May 25–June 20, 2020. The Phase II campaign which was initially planned to start on April 20, 2020 was delayed due to the lockdown caused by the Coronavirus pandemic of 2019 (COVID-19). The project used three insecticides for the 2020 spray campaign: Fludora Fusion WP-SB and the remaining balances of Actellic® 300CS and SumiShield 50WG, which were left over from the 2019 campaign. In Phase I, the project conducted IRS with Actellic® 300CS during the first week in seven districts in eastern Uganda (Budaka, Bugiri, Butebo, Kibuku, Namutumba, Pallisa and Tororo) and with Fludora Fusion for the rest of the spraying period in these districts with the exception of Butaleja, where Fludora Fusion was sprayed throughout the entirety of the spray period. During Phase II the project conducted IRS with the left over SumiShield 50WG during the first week in eight districts in eastern and northern Uganda (Alebtong, Amolatar, Dokolo, Kaberamaido, Kalaki, Lira, Otuke and Serere) and with Fludora Fusion for the rest of the spraying period. IRS in Alebtong, Amolatar, Dokolo, Kaberamaido, Kalaki and Otuke was funded by the Department for International Development, United Kingdom (DFID-UK), while spraying in the remaining 10 districts (Budaka, Bugiri, Butaleja, Butebo, Kibuku, Lira, Namutumba, Pallisa, Serere and Tororo) was funded by United States Agency for International Development (USAID)/PMI.

To guide vector control interventions and assess impact, the project conducted monthly entomological monitoring using human landing catches (HLCs) and pyrethrum spray catches (PSCs) in six districts: Bugiri, Lira, Otuke and Tororo (current IRS districts), Apac and Soroti (non-IRS control districts); and wall cone bioassays (only in eight current IRS districts for IRS quality assurance studies for only the first month and subsequently in four of these IRS districts for residual efficacy studies). Insecticide susceptibility tests were carried out on pirimiphos-methyl (organophosphate), bendiocarb (carbamate), three pyrethroids (alpha-cypermethrin, deltamethrin and permethrin), clothianidin (neonicotinoid) and chlorfenapyr (pyrrole), in three IRS districts (Bugiri, Lira and Tororo) and eight non-IRS districts (Hoima, Gulu, Kamwenge, Katakwi, Kitgum, Nakaseke, Soroti and Wakiso), all located in various parts of Uganda, to evaluate *Anopheles (An.) gambiae* s.l. susceptibility status to those insecticides and resistance intensity to pyrethroids.

Our findings highlight high levels of heterogeneity and diversity in mosquito vector species composition and behavior in the longitudinal monitoring areas.

A total of 36,805 *Anopheles* mosquitoes were collected from the six sentinel sites (Apac, Bugiri, Lira, Otuke, Soroti and Tororo districts) using both PSCs and HLCs. Morphological identification of the mosquitoes revealed that 32,536 (88.4 percent) were *An. funestus* s.l., 3,891 (10.6 percent) were *An. gambiae* s.l., and 378 (1.0 percent) were other *Anopheles* mosquito species such as *An. ardensis, An. coustani, An. pretoriensis, An. squamosus* and *An. ziemanni*. However, vector distribution differed by study site. *An. gambiae* s.l. was dominant in Bugiri and Tororo districts while *An. funestus* s.l. was the predominant vector in Apac, Lira Otuke and Soroti districts. Soroti (control site) had the highest percentage of all *An. funestus* s.l. collected, 52.32 percent (n=17,002), followed by Apac at 31.62 percent (n=10,288), Otuke at 8.21 percent (n-2,672), Lira at 7.70 percent (n=2,505), Bugiri at 0.10 percent (n=31), and Tororo at 0.06 percent (n=18).

Malaria vectors *An. gambiae* s.l. and *An. funestus* s.l. were collected both indoors and outdoors using HLCs. *An. funestus* s.l. densities were higher indoors than outdoors at all sites while *An. gambiae* s.l. bites almost equally indoors and outdoors in all sites except in Bugiri, Lira and Apac where higher densities were recorded indoors than outdoors (Lira and Apac) or inversely (Bugiri).

An. funestus s.l. indoor resting density collected using PSCs was higher in Apac (53.7%, n=382/712), Lira (83.6%, n=556/665), Otuke (72.0%, 255/354) and Soroti (64.5%, n=5537/8579). For *An. gambiae* s.l. higher indoor density was recorded in Apac (55.3%, n=183/331), Lira (70.8%, n=196/277). Similarly, the other anopheline mosquito species bite more outdoor than indoor in all sentinel districts, varying from 33.3% in Soroti to 100% in Apac and Tororo.

The results of the susceptibility tests showed that *An. gambiae* s.l. remained susceptible to pirimiphos-methyl, the insecticide used during the 2019 and partly in the 2020 IRS campaigns. Synergist tests with 4% piperonyl butoxide (PBO) and pyrethroids against *An. gambiae* s.l. restored ful or partial susceptibility to pyrethroids in resistant populations of *An. gambiae* s.l. indicating that monoxygenase enzymes could be involved in the insecticide resistance of the vectors. Also, *An. gambiae* s.l. was susceptible to clothianidin and chlorfenapyr insecticides. However, *An. gambiae* s.l. was resistant to pyrethroids (alpha-cypermethrin, deltamethrin and permethrin) at all sites surveyed. For *An. funestus* s.l., resistance was observed to alpha-cypermethrin in Soroti (5% mortality) and Katakwi (83.2% mortality), to deltamethrin in Soroti (20% mortality) and Katakwi (88% mortality) and to permethrin in Katakwi (72.1% mortality).

Wall cone bioassays performed for IRS quality assurance exhibited 100% mortality across different wall surface types (mud, plain brick and plaster painted walls), with all the three insecticides sprayed (Actellic® 300CS, SumiShield 50WG and Fludora Fusion) indicating that the spray was correctly applied. The residual efficacy of both Actellic® 300CS and Fludora Fusion used during the spray campaign remained effective for six to eight months after the spray campaign in Bugiri and Tororo district, depending on the wall surface type sprayed. Plastered painted wall surfaces performed better than mud wall surfaces in terms of length of effectiveness of the insecticides sprayed. SumiShield 50WG remained effective for at least six months after the spray campaign (testing continuing in Lira and Otuke districts).

Molecular laboratory analyses are yet to be performed for vector mosquito identification, infection rate determination, detection and identification of insecticide resistance markers (knockdown resistance (*kdr*) and Acetylcholinesterase-1 (*Ace-1*)) genes in *An. gambiae* s.l. However, Infectious Diseases Research Collaboration (IDRC) has started with extraction of Deoxyribonucleic Acid (DNA) for mosquito molecular analysis and an addendum will be submitted after all analysis is completed by April 2021.

1. INTRODUCTION

The PMI VectorLink Project carried out entomological monitoring activities in six districts (Figure 1) and supported the National Malaria Control Division's entomological monitoring activities countrywide to enhance in-country capacity. Uganda has varying rainfall patterns, with a large part of the country experiencing a bi-modal rainfall pattern. March to May constitutes the first major rainfall season in Uganda, while September to November represents the second rainfall season in the majority of the country. June to August is generally the dry season in south western, central, Lake Victoria basin and some parts of the eastern region in the country. However, there is a continuation of rainfall for much of northern Uganda from June to August. December to February is the dry season in most of the country, except for some areas around Lake Victoria, western and south-western which sometimes receive isolated rainfall in December. For the eastern region IRS districts, the first rains are usually experienced between late February to mid-March which peak around late April, with cessation of rains occurring around late May/mid-June except during El Nino years when there were abnormal rains, which typically extend beyond the usual rainfall season. For the northern region IRS districts, the first rains are usually experienced from mid-March with cessation of rains occurring around late June/early July except during El Nino years. Seasonality of malaria transmission is associated with rainfall patterns with increases 2-4 weeks after the start of rains and decreasing during the dry seasons.

In Uganda, malaria remains a leading cause of morbidity and mortality. The entire population of about 41 million is at risk of malaria and the disease accounts for 30-50% of outpatient consultations, 22% of admissions, 11 % of deaths, and costs a family on average nine US dollars or 3% of annual household income per malaria episode (The Uganda Malaria Reduction and Elimination Strategic Plan 2021-2025). Globally Uganda is the third highest contributor of malaria cases and the seventh highest contributor of malaria deaths in 2018-2019 according to the World Malaria Report (WMR) 2020. Uganda contributed the second largest reduction in malaria cases of 1.5 million cases between 2017 and 2018. Since 2019, however, there has been a rise in malaria cases. Confirmed Malaria Cases in Uganda were increasingly recorded between 2018, and 2020 from 7,396,793 in 2018; to 12,423,079; and 13,317,355 in 2019 and 2020 respectively (Uganda DHIS2 Extracted on 10th February 2021). In 2020, the Coronavirus (COVID-19) pandemic had furthered contributed to the increase in malaria cases due to reduced supplies of anti-malarial drugs in some districts and also the postponement of IRS in eight districts in northern Uganda.

Over the last decade, significant gains have been made: parasite prevalence dropped by 80% from 45% in 2009 to 9% in 2019; mortality reduced from a high of 20 per 100,000 population in 2016 to 9 in 2019 and incidence of total malaria cases declined from 460 per 1000 population in 2013 to 281 in 2019. However, these achievements still fall short of the targets set in the previous strategic plan – The Uganda Malaria Reduction Strategic Plan (UMRSP) 2014-2020 targets were to reduce annual malaria deaths from the 2013 levels (30 per 100,000) to less than 1 death per 100,000 population; reduce malaria morbidity from 150 to 30 confirmed cases per 1000 population and reduce malaria parasite prevalence from 19% to less than 7%. The malaria transmission in Uganda has become unstable with increased occurrences of outbreaks and epidemics in most parts of the country.

Entomological monitoring activities help to supplement epidemiological data essential in guiding proper targeting of IRS; evaluate the susceptibility level of the local vectors to different insecticides and determine the underlying mechanisms; inform selection of insecticides; evaluate the quality of spraying; monitor the impact of IRS on vector density, vector behavior, species composition, and determine parity rates; and monitor the residual life of different insecticides on different types of wall surfaces. This PMI VectorLink Uganda entomological monitoring annual report covers the period from January 1 to December 31, 2020.

2. METHODOLOGY

2.1 MONITORING SITES

Longitudinal entomological monitoring was conducted in four IRS intervention districts: Bugiri, Lira, Otuke and Tororo; and two control sites: Apac and Soroti (non-IRS districts) from January - March 2020 and August-December 2020, representing a total of eight collection months. Collections could not be conducted between April and July due to the COVID-19 pandemic and following PMI guidance. In all the districts, entomological monitoring data was collected using pyrethrum spray catches (PSCs) and human landing catches (HLCs) indoors and outdoors. Insecticide susceptibility tests were conducted in 11 sites including four of the longitudinal monitoring sites (Bugiri, Lira, Soroti and Tororo) (Table 1 and Figure 1).

During the spray operations, IRS quality checks were conducted using wall cone bioassays in one site per each of the eight spray districts (Bugiri, Butaleja, Dokolo, Kibuku, Lira, Otuke, Serere, and Tororo) within a week of the start of spraying to assess the quality of spraying. Thereafter, the residual efficacy and fumigant effect of Actellic 300CS and Fludora Fusion was monitored in Bugiri and Tororo districts and that of SumiShield 50WG and Fludora Fusion in Lira and Otuke districts.

Due to the COVID-19 pandemic, entomological monitoring activities were conducted taking precautions to reduce transmission of COVID-19 based on the COVID-19 mitigation plans as per PMI VectorLink Operating Requirements to mitigate the risk of exposure in field staff of August 17, 2020[1]

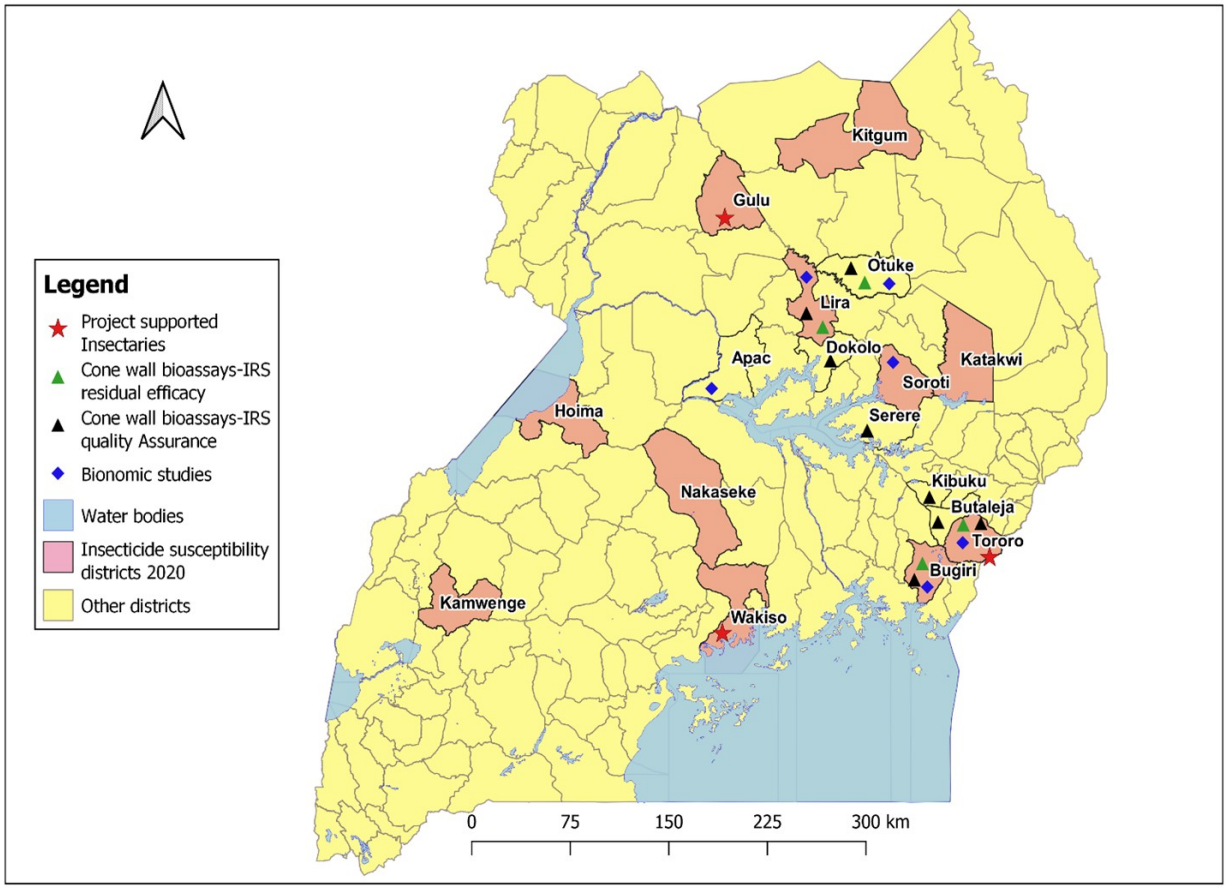
TABLE 1: ENTOMOLOGICAL MONITORING SITES

Region	District	Sentinel Site	Spray Status	Entomological monitoring activities
Eastern	Bugiri	Bubwoki (HLC&PSC), Nangalama (PSC) and Mugera (PSC)	Sprayed with both Fludora Fusion and Actellic	HLC; PSC, Cone bioassay, Susceptibility test
Northern	Lira	Te-Okole (HLC&PSC), Araki (PSC) Alikpot (PSC)	Sprayed with both Fludora Fusion and SumiShield	HLC; PSC, Cone bioassay, Susceptibility test
Northern	Otuke	Oboloko (HLC&PSC), Barodugu (PSC) and Baraliro (PSC)	Sprayed with both Fludora Fusion and SumiShield	HLC; PSC, Cone bioassay
Eastern	Tororo	Nagoke (HLC&PSC), Rugot (PSC) and Kajarau North (PSC)	Sprayed with Fludora Fusion and Actellic	HLC; PSC, Cone bioassay, Susceptibility test
Northern	Apac	Atar (HLC&PSC), Aporotuku (PSC) and Amwonyocao-B (PSC)	Non-Sprayed, IRS control	HLC; PSC
Eastern	Soroti	Awoja (HLC&PSC), Madera Central cell (PSC) and Agora (PSC)	Non-Sprayed, IRS control	HLC; PSC, Susceptibility test
Western	Hoima		Non-Sprayed	Susceptibility test
Northern	Gulu		Non-Sprayed	Susceptibility test
Western	Kamwenge		Non-Sprayed	Susceptibility test

[1] The PMI VectorLink Project. PMI VectorLink Operating Requirements to Reduce Transmission of COVID-19. August 17, 2020. Abt Associates Inc.

Region	District	Sentinel Site	Spray Status	Entomological monitoring activities
Eastern	Katakwi		Non-Sprayed	Susceptibility test
Northern	Kitgum		Non-Sprayed	Susceptibility test
Central	Nakaseke		Non-Sprayed	Susceptibility test
Central	Wakiso		Non-Sprayed	Susceptibility test

FIGURE 1: PMI VECTORLINK PROJECT DISTRICTS FOR ENTOMOLOGICAL MONITORING



2.2 LONGITUDINAL MONITORING OF MALARIA VECTOR DENSITY AND BEHAVIOR

VectorLink Uganda collected adult mosquitoes on a monthly basis from January to March and then from July through December 2020 using PSCs and HLCs in six sentinel sites: Bugiri, Lira, Otuke and Tororo (current IRS districts), Apac and Soroti (non-IRS districts used as the control districts). All entomological data collections were conducted following PMI standard operating procedures[2]. Table 2 summarizes the longitudinal monitoring methods and frequency.

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

TABLE 2: LONGITUDINAL MONITORING ADULT MOSQUITO COLLECTION METHODS

Collection Methods	Time	Frequency	Collection period	Sample
PSCs	6:00 am to 10:00 am	Two days per site per month	January-March 2020 and July-December 2020	Twenty houses per site (10 houses each day for two days) – same houses used every month
HLCs	6:00 pm to 7:00 am	Two consecutive nights per site per month	January-March 2020 and August-December 2020	Two houses per site – same houses used every month

2.2.1 PYRETHRUM SPRAY CATCH

In each district where PSCs were conducted (Apac, Bugiri, Lira, Otuke Soroti and Tororo), 20 houses in each of three villages were selected. PSCs were conducted from 6:00 am to 10:00 am, once per month over two days in each district from January to December 2020, except during the months of April to June when the country experienced a lockdown down due to the COVID-19 pandemic. The same houses were visited each month. A commercial aerosol made of the pyrethroids d-Tetramethrin 0.135% w/w, d-Allethrin 0.06% w/w and cypermethrin 0.46% w/w was used to knock down the mosquitoes. One-roomed sleeping grass-thatched houses were selected for mosquito collection using PSCs. The room was closed for 10 minutes after spraying with the aerosol, and then the knocked-down mosquitoes on the white sheets on the floor were collected using forceps into a labeled petri dish. The samples were identified morphologically[3] and preserved in 1.5 ml Eppendorf tubes with a hole pierced in it and kept in a plastic container containing silica gel, of which a sub-set of collected samples were sent to Infectious Diseases Research Collaboration (IDRC) Molecular Laboratories for further species identification by Polymerase Chain Reaction (PCR) and infection rate determination using Enzyme-linked Immunosorbent Assay (ELISA).

2.2.2 HUMAN LANDING CATCH

HLCs were conducted in Bugiri, Lira, Otuke, Tororo, Apac and Soroti. Two houses were sampled in each selected village during two consecutive nights to obtain four person-nights of collection per district per month (two houses x two collection nights = four person-nights indoors and 4 person-nights outdoors). In all districts, two human volunteers (trained adult mosquito collectors) were positioned, one inside the house and the other outside at least 5 meters from the house, to collect mosquitoes. Collections were conducted from 6:00 pm to 7:00 am using 12 volunteers working in shifts of 6 and 7 hours each. For each hour of collection, both indoor and outdoor collectors exchanged positions. During the time of collection, the collectors sat quietly on a small chair and exposed part of their legs (up to the knees); when they felt mosquitoes landing, they turned on their torches and collected the mosquitoes using a mouth aspirator. Collected mosquitoes were transferred into labeled paper cups assigned for each hourly collection. Collected mosquitoes were subsequently killed using cotton soaked in diethyl ether, identified, counted by species, location, and hour of collection, and preserved in 1.5 ml Eppendorf tubes with a hole pierced in it and kept in a plastic container containing silica gel. A subset of samples from these collections were sent to IDRC Molecular Laboratories for PCR analyses.

2.3 SUSCEPTIBILITY STATUS FOR INSECTARY REARED *AN. GAMBIAE* S.S. (KISUMU STRAIN)

During the reporting period, the project conducted susceptibility testing of (2-5 day old) *An. gambiae* s.s. (Kisumu) reared at Vector Control Division Kampala, Tororo and Gulu University insectaries (Figure 1). The tests were done for the following insecticides: deltamethrin 0.05%, alpha-cypermethrin 0.05%, permethrin 0.75%, bendiocarb 0.1% and pirimiphos-methyl 0.25% using the World Health Organization (WHO) tube assay protocol as described above.

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

2.4 IRS Quality Assays and Insecticide Decay Rate Monitoring

Standard WHO cone bioassay tests were performed in one site in each of the eight spray districts (Bugiri, Butaleja, Dokolo, Kibuku, Lira, Otuke, Serere, and Tororo) within one week of the start of spraying to assess the quality of spraying. Routine wall bioassays were subsequently monitored monthly in four districts of Bugiri, Lira, Otuke and Tororo until mortality dropped below 80% for two consecutive months (Table 3). Three houses of different wall types (plastered and painted, plain brick and mud-walled) were randomly selected in each study village. The same houses were used each month. Cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m above the floor. Cones lined with self-adhesive tape were fixed on the sprayed walls for the assay. The control cone was affixed on a wall lined with a paperboard with adhesive in an unsprayed house to avoid any potential airborne effect. Two- to five-day-old female mosquitoes were used for the tests. Susceptible *An. gambiae* s.s. Kisumu strain mosquitoes were introduced into the plastic cones in batches of 10 and left exposed on the sprayed surface for 30 minutes at different heights. When available, larvae of *An. gambiae* s.l. was collected in each of the sprayed district and reared to adults. The wild mosquitoes were exposed to the wall following the same protocol. Numbers of mosquitoes knocked down at the 30th minute were recorded. At the end of the 30-minute exposure period, the mosquitoes were carefully collected and transferred to paper cups and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with net with final mortality recorded after 24 hours holding period post-exposure for Actellic 300CS. For SumiShield 50WG and Fludora Fusion, test mortality was monitored every 24 hours until 100% test mortality was achieved or up to day 5, whichever came first.

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

TABLE 3: QUALITY ASSURANCE AND INSECTICIDE RESIDUAL EFFICACY ACTIVITIES

Activity	Frequency	Sample
Quality assurance of IRS	Once within one week of spraying during the first two weeks of campaign at eight sites	Three houses per site (sprayed mud, sprayed plastered paint and sprayed plain bricks) and one unsprayed control
Monitoring of Insecticide Decay rate on walls	Monthly, until exposed mosquito mortality falls below 80% for two consecutive months at five sites	Three houses per site (sprayed mud, sprayed plastered paint and sprayed plain bricks) and one unsprayed control

2.5 Insecticide Resistance Monitoring

2.5.1 Vector Susceptibility Testing

Insecticide susceptibility studies were conducted using the WHO tube bioassays to determine insecticide susceptibility status and insecticide resistance intensity of major malaria vectors, *An. gambiae* s.l. and *An. funestus* s.l. to insecticides recommended by the WHO for use in public health. When pyrethroid resistance was confirmed, resistance intensity was determined using 5x and 10x diagnostic doses of some of the insecticides. Synergist assays with piperonyl butoxide (PBO) was conducted to assess the involvement of mixed function oxidases as a resistant mechanism. All the tested mosquitoes were then preserved in Eppendorf tubes and stored in plastic containers with silica gel for further molecular analysis. The five classes of insecticides tested included: neonicotinoids (clothianidin 50% WG), pyrroles (chlorfenapyr 5%), organophosphates (pirimiphos-

methyl 0.75%), pyrethroids (deltamethrin 0.05%, permethrin 0.75% and alpha-cypermethrin 0.05%,) and carbamates (bendiocarb 0.1%). These studies were conducted in 11 districts: Bugiri, Hoima, Gulu, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti, Tororo and Wakiso between September and November 2020.

Field-collected larvae of *An. gambiae* s.l. were reared to adult in the field temporal insectaries established in identified and rented non-sprayed houses in the selected study sites in the districts. Batches of 20-25 female, sugar-fed and three to five-day old mosquitoes were subsequently subjected to WHO tube tests following the standard PMI VectorLink SOP 06/01. Adult *An. funestus* s.l. mosquitoes collected in early mornings using mouth aspirators were morphologically identified and immediately used for susceptibility testing. These females were exposed to pirimiphos-methyl 0.25%, alpha-cypermethrin 0.05%, permethrin 0.75%, bendiocarb 0.1% and deltamethrin 0.05% on WHO impregnated filter papers for 60 minutes. Knockdown was scored at 60 minutes immediately after the exposure period, at which time all mosquitoes were gently transferred to holding tubes. Mortality was recorded at 24 hours after exposure. Where control mortality scored higher than 5% but below 20%, Abbott's correction was applied to test mortalities and those above 20% led to tests being discarded (Abbott [4]1925).

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

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

Abbott's formula:

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

2.5.2 CLOTHIANIDIN AND CHLORFENAPYR SUSCEPTIBILITY TEST RESULTS

For clothianidin susceptibility tests, freshly treated filter papers (treated at 13.2 mg active ingredient per paper) were used for WHO susceptibility. The test was conducted with *An. gambiae* s.l. collected from several breeding sites in villages in the different districts between September and November 2020. For each district, four replicates of 25 mosquitoes were tested (total of 100 sugar-fed females) with clothianidin papers, and two replicates were used at the same time with the negative control papers (impregnated only with distilled water). The exposure time was 60 minutes. Afterward, mosquitoes were transferred into holding cylinders with filter paper treated only with distilled water and provided with lightly moistened cotton wool containing 10% sugar solution that was changed daily. Knock-down was recorded halfway through the test at 30 minutes and at the end of the test at 60 minutes. Mortality was recorded up to 7 days post exposure. Mortality monitoring stopped whenever 100% test mortality was achieved before day 7. A negative control was tested at the same time and mortality recorded on days 1 through 7. In addition to the negative control described above, a positive control was done by similarly exposing a laboratory-reared susceptible *An. gambiae* s.s. Kisumu strain to confirm the quality of the treated papers.

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

For chlorfenapyr, Centers for Disease Control and Prevention (CDC) bottles were treated at a selected dose of 100 µg/bottle. The mosquitoes were exposed for one hour and the mortality was recorded up to three days. All tests, paper impregnation, and coating of bottles were conducted following PMI VectorLink SOPs.

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

2.6 MOLECULAR ASSAYS

The molecular assays will be performed by the IDRC Molecular Laboratory included the vector species identification, infection rate determination of malaria vectors (detection of *Plasmodium falciparum)* malaria parasites and detection of insecticide resistance genetic markers *kdr* and *Ace-1* using established and published protocols.

2.6.1 VECTOR SPECIES IDENTIFICATION

Following morphological identification of individual samples in the field, a selected proportion of *An. gambiae* s.l. were further identified using PCR methods. The DNA of each mosquito was extracted from the leg using chelex protocol and amplified by PCR (Musapa, M., et al., 2013) and directly sequenced using DNA barcoding (mtDNA COI) and ITS2 (nDNA) primers and protocols for species confirmation. Amplified samples were run on a 1.5% agarose gel stained with EtBr. Amplicon fragments of 153bp (QD) or 415bp (QDA) for*An. quadriannulatus*, 464bp/466bp for *An. melas/merus*, 390bp for *An. gambiae* and 315bp for *An. arabiensis* were identified using standard PCR methods of diagnosis (Scott et al., 1993) and MR4 control reference. The Short INterspersed Element (SINE) PCR protocol described by Santolamazza *et al*, was used to differentiate *An. gambiae* and *An. coluzzii* (Santolamazza et al, 2008)

2.6.2 DETECTION OF MALARIA PARASITES (DETERMINATION OF INFECTION RATES)

Detection of *Plasmodium falciparum* infection status in *An. gambiae* s.l. was performed with Enzyme-linked Immunosorbent Assay (ELISA) assays following the method described by Wirtz et al. (1987). The calculation of sporozoite rates is based on the number of mosquitoes positive for sporozoites divided by the number of mosquitoes tested using ELISA method.

2.6.3 DETECTION OF INSECTICIDE RESISTANCE MARKERS

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

2.7 DATA PRESENTATION AND INTERPRETATION

Data obtained from PSCs were used to determine the indoor resting density (the average number of mosquitoes per house per night) over collection months and compared between IRS sprayed sites and non-IRS control sites., while data from HLCs were used to estimate the human biting rate (mean number of mosquitoes collected per person per night) and the peak biting time (highest mean number of bites per person per hour). Biting times are presented as averages of hourly human bites of both main vectors (*An. funestus* s.l. and *An. gambiae* s.l.) from each of the monthly HLC collections. For each collection method, the proportion of malaria vectors collected was estimated by sites as the percentage of each vector out of the total *Anopheles* species collected during the eight-month collection periods.

The results of the insecticide susceptibility tests were described following the WHO discrimination criteria[5] with corrected mortality after 24 h for pyrethroids, carbamate and organophosphates; 72 h for chlorfenapyr and 7 days for clothianidin < 90% as confirmed resistance, between 90 and < 98% as possible resistance, and ≥ 98% as susceptible. For the intensity assays of the pyrethroids, corrected mortality of: 98–100% at 5× the diagnostic dose indicates low resistance intensity, less than 98% at 5× diagnostic dose implies testing the 10× diagnostic dose and 98–100% at 10× the diagnostic dose confirms a moderate resistance intensity and less than 98% at 10× the diagnostic dose indicates high resistance intensity. For synergist assays with PBO, significant increase of mortality after pre-exposure to PBO indicates that enzymes such as P450s could be involved in the resistance of the tested vectors.

[5] World Health Organization; 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd edition, Geneva: (http://www.who.int/malaria/publications/atoz/9789241511575/, accessed March 2017).

3. RESULTS

Laboratory analysis for measuring advanced entomological indicators on the 2020 mosquito samples commenced in February due to delayed contractual process of IDRC and reagent procurement. The data are not presented in the current report and will be submit later in a separate addendum.

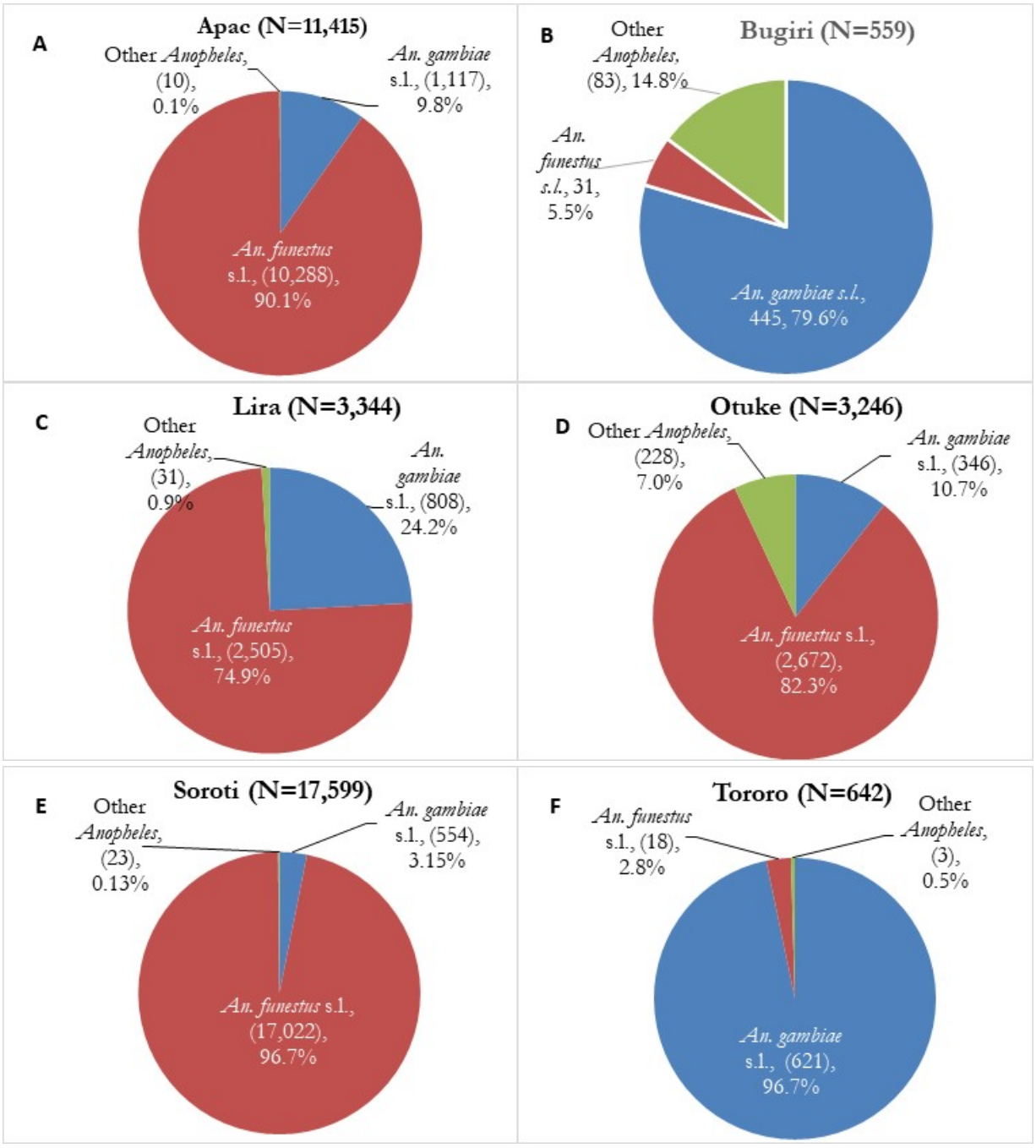
3.1 *ANOPHELES* SPECIES COLLECTED BY DIFFERENT METHODS

3.1.1 LONGITUDINAL MONITORING

During the reporting period (January 2020 to March and from August to December 2020), in Bugiri, Lira, Otuke, and Tororo (current IRS districts), Apac and Soroti (non-IRS districts used as the control), a total of 36,805 female *Anopheles* mosquito species were collected using the two collection methods (PSCs and HLCs) and morphologically identified (Annex 1-Table1 and Figure 2).

- A total of 3,891 *An. gambiae* s.l. were collected: 2,797 (71.9%) using PSCs and 1,094 (28.1 %) using HLCs.
- A total of 32,536 *An. funestus* s.l. were collected: 22,221 (68.3%) using PSCs and 10,315 (31.7%) using HLCs.
- *An. funestus* s.l. was the most abundant (88.4%, n=32,536) *Anopheles* species collected, followed by *An. gambiae* s.l. (10.6%, n=3,891).
- Other *Anopheles* species including *An. ardensis, An. coustani, An. pretoriensis, An. squamosus* and *An. ziemanni* comprised the remaining 1.0% (n=378) (Figure 2). However, vector distribution differed by study site. Overall *An. funestus* s.l. was the predominant vector in Apac, Lira Otuke and Soroti districts while *An. gambiae* s.l. was dominant in Bugiri and Tororo districts (Figure 2).

FIGURE 2: SPECIES COMPOSITION OF THE TOTAL *ANOPHELES* COLLECTED BY SITE USING PSCS AND HLCS FROM JANUARY TO DECEMBER 2020 (*THE NUMBERS IN BRACKET IN THE PIE CHARTS REPRESENT THE NUMBER OF EACH SPECIES COLLECTED*)

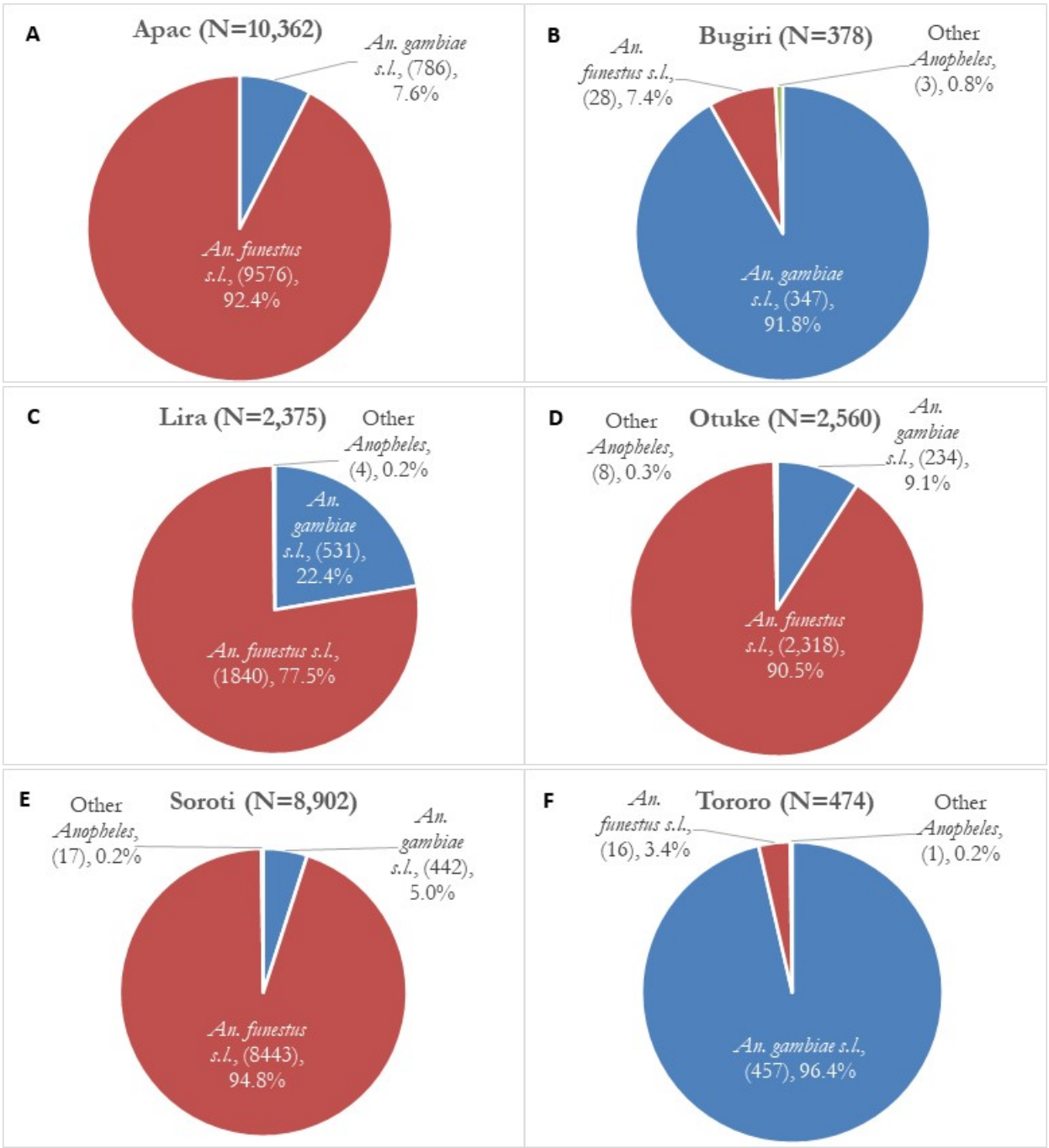


3.1.2 PYRETHRUM SPRAY CATCH

PSC collections yielded 25,051*Anopheles* mosquitoes (Annex1-Table 2 and Figures 3A-F). In order from the most to the least abundant species, there were 22,221 (88.7%) *An. funestus* s.l., 2,797 (11.2%) *An. gambiae* s.l. and 33 (0.1%) other *Anopheles* species. Most *Anopheles* vectors were caught in the unsprayed (control) sites of Apac and Soroiti. *An. gambiae* s.l. predominated in PSCs in the intervention areas of Bugiri and Tororo (Figures 3B

& F), while a greater proportion of *An. funestus* s.l. was caught in the control areas (Apac and Soroti (Figures 3A & E) and the current IRS districts of Lira and Otuke (Figures 3C & D).

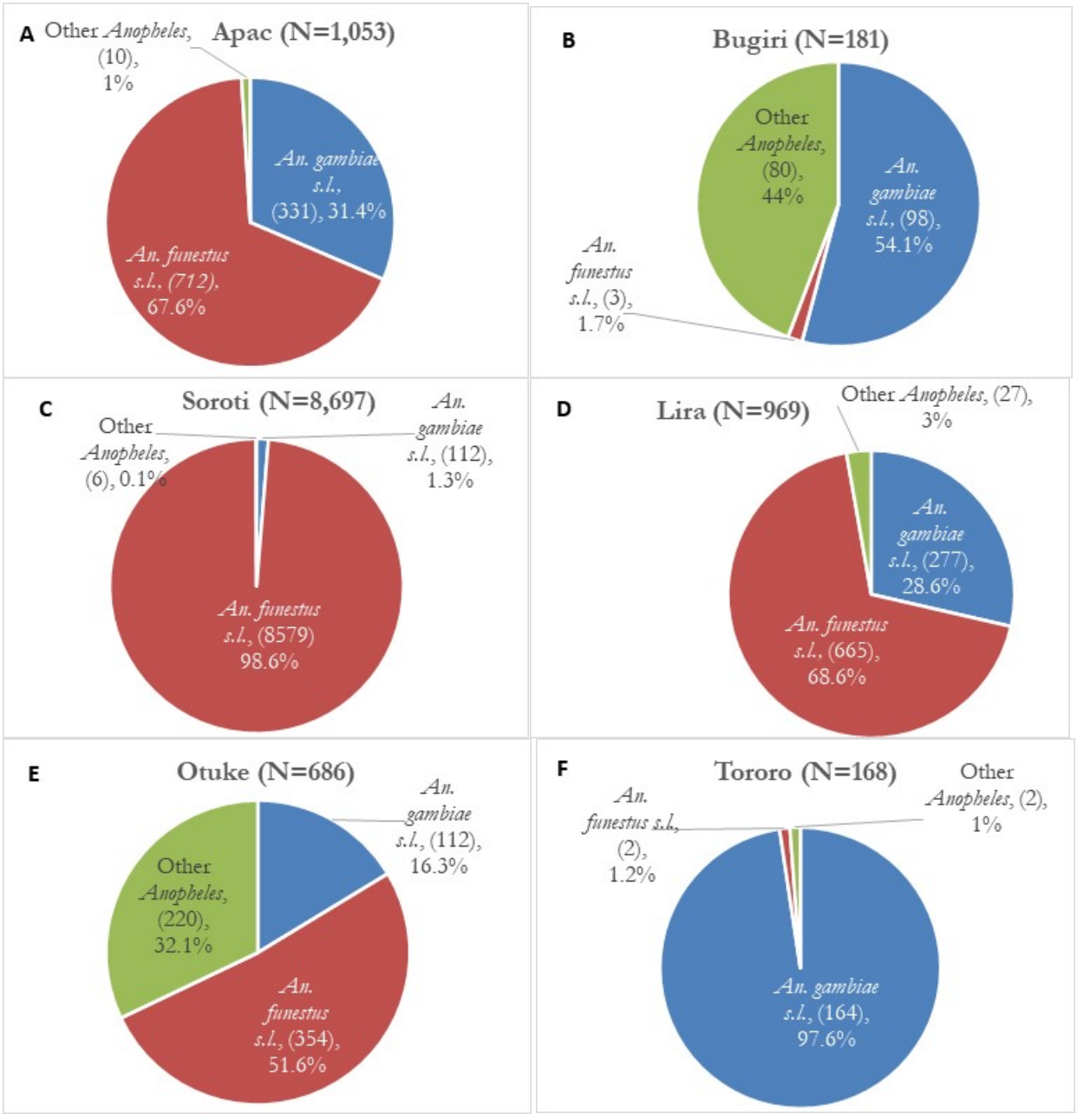
FIGURE 3: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES COLLECTED BY SITE USING PSCS FROM JANUARY 2020 -MARCH AND AUGUST-DECEMBER 2020 (*THE NUMBERS IN BRACKET REPRESENT THE NUMBER OF EACH SPECIES COLLECTED*)



3.1.3 HUMAN LANDING CATCHES

A total of 11,754 *Anopheles* mosquitoes were collected using HLCs from January to March and from August to December 2020 (Annex 1-Table 3 and Figures 4A-F) for a total of eight collection months. The species identified morphologically from this collection included: 10,314 (87.75%) *An. funestus* s.l., 1,098 (9.34%) *An. gambiae* s.l., 342 (2.91%) other *Anopheles* species such as *An. ardensis, An. coustani, An. pretoriensis, An. squamosus* and *An. ziemanni*. Most other *Anopheles* species collected was recorded in Bugiri. *An. gambiae* s.l. was the most abundant mosquito species collected in the intervention areas (Bugiri and Tororo), while *An. funestus* s.l. was the most abundant mosquito species collected in the districts of Lira and Otuke (IRS districts) and Apac and Soroti (control districts).

FIGURE 4: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES COLLECTED USING HLCS PER SITE FROM JANUARY 2020 - MARCH AND AUGUST - DECEMBER 2020 (*THE NUMBERS IN BRACKET REPRESENT THE NUMBER OF EACH SPECIES COLLECTED*)



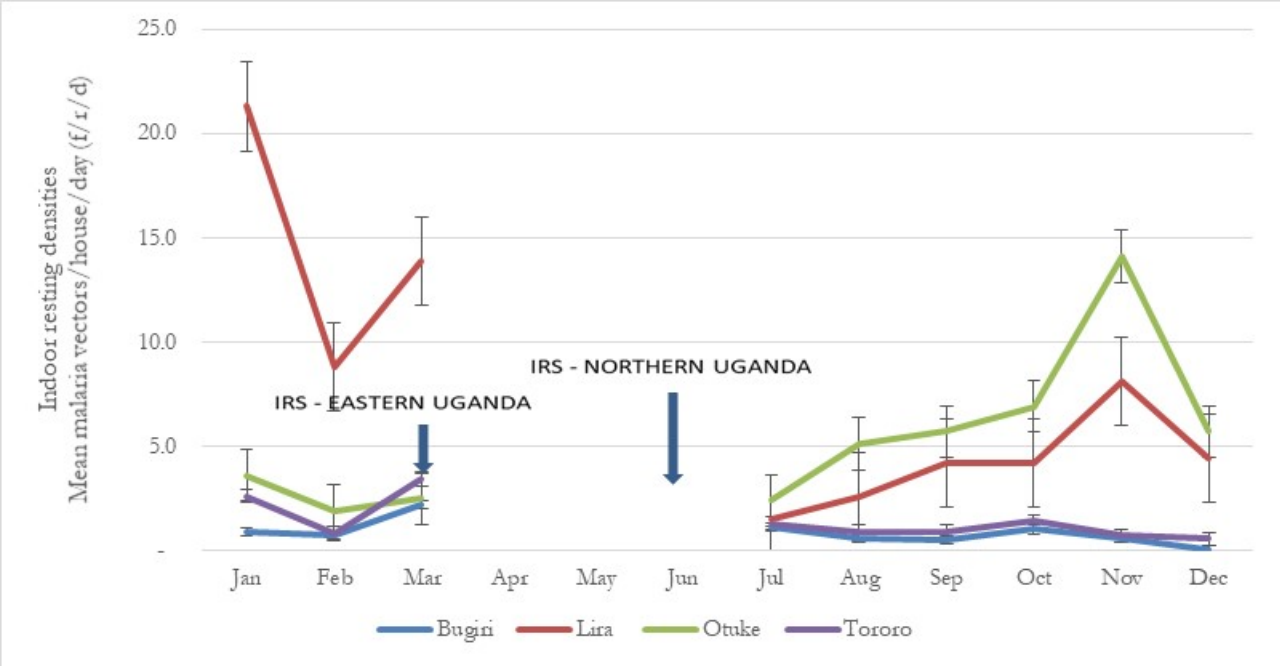
3.2 VECTOR DENSITY AND BEHAVIOR

3.2.1 INDOOR RESTING DENSITY OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY PSC

Overall, the indoor density of both *An. gambiae* s.l. and *An. funestus* s.l. decreased considerably after the spray campaigns. The impact of the different phases of IRS on the density of the vectors could not be clearly appreciated due to the suspension of monitoring activities between March and July 2020, coinciding with the first quarter post-spray for Phase I (Bugiri and Tororo) and pre-spray quarter for Phase II (Otuke and Lira). However, the overall indoor resting density of the vectors before IRS was between 21.3 females/room per day (f/r/d) in January to 14 f/r/d in March in Lira, while less than 4 f/r/d was recorded in all the other three sites between the same period. After spraying, the indoor resting density was less than 1.5 f/r/d in Bugiri and Tororo and less than 8 f/r/d in Lira from July to December 2020. A slight increase in the number of vectors collected was observed in Otuke, but this is an assumption as there was not data between April and June for comparison which could have helped to determine whether there was an increase or a reduction (Figure 5).

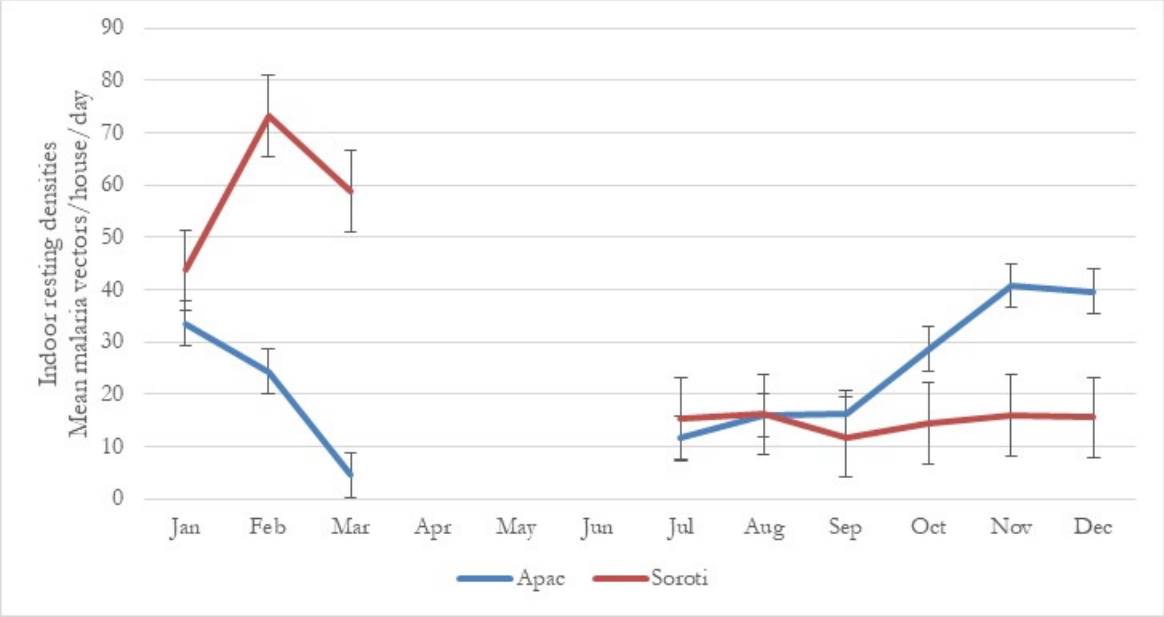
In the control sites of Apac and Soroti, higher indoor resting density was observed with a mean peak of 59 f/r/d in February in Soroti and 34 f/r/d in Apac in January. The densities also decreased from July to December 2020 in Soroti with a mean of 15 f/r/d. In contrast, the mean indoor resting density in Apac increased progressively from 11.6 f/r/d in July to about 40 f/r/d in November and December 2020 (Figure 6)

FIGURE 5: COMBINED MEAN INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN THE FOUR INTERVENTION DISTRICTS BEFORE AND AFTER IRS INTERVENTION



Note: IRS was conducted in in Bugiri and Tororo in March and in Lira and Otuke in May-June 2020
Error bars represent the mean standard deviation bars.

FIGURE 6: COMBINED MEAN INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN THE TWO IRS CONTROL DISTRICTS

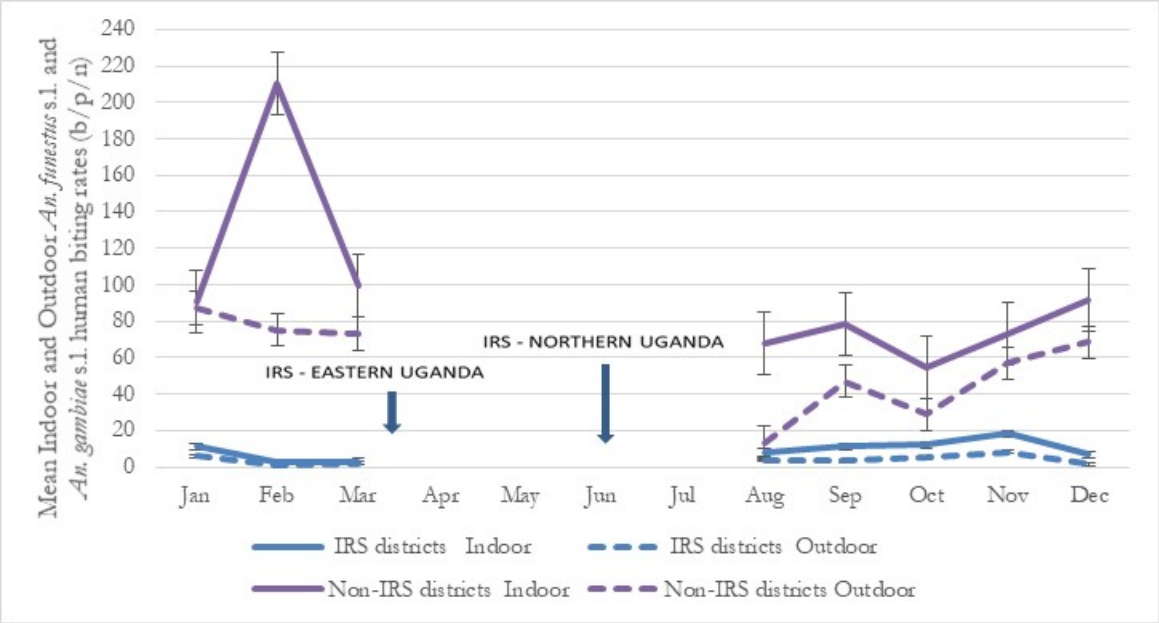


Error bars represent the mean standard deviation bars.

3.2.2 HUMAN BITING RATE OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY HLC

The human biting rates (HBRs) indoors and outdoors of both *An. funestus* s.l. and *An. gambiae* s.l. in the IRS and control sites are presented in Figure 7. There were overall higher number of *An. funestus* s.l. (10,314) than *An. gambiae* s.l. (1,098) at both IRS and non-IRS sites. All districts demonstrated different *An. funestus* s.l. and *An. gambiae* s.l. densities, both indoors and outdoors (Annex 1, Table 4), before and after IRS spraying, which began in March 2020 for Bugiri and Tororo, and in late May 2020 for Lira and Otuke. When combined, a slight impact of IRS on HBR was observed after the spraying when compared with the control sites (Figure 7). But this needs to be considered cautiously due to the lack of data from April to July 2020.

FIGURE 7: MONTHLY INDOOR AND OUTDOOR HUMAN BITING RATES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN THE FOUR IRS DISTRICTS AND THE TWO CONTROL DISTRICTS, BEFORE AND AFTER SPRAYING



Error bars represent the mean standard deviation bars.

Table 4 illustrates the numbers of each *Anopheles* species collected in IRS intervention areas and control areas, indicating for each the total person-nights and subsequent biting rate expressed as bites per person per night (b/p/n). *An. funestus* s.l. and *An. gambiae* s.l. were observed to contribute to 99.8% and 84.5% of the anopheline bites in the control and IRS areas, respectively.

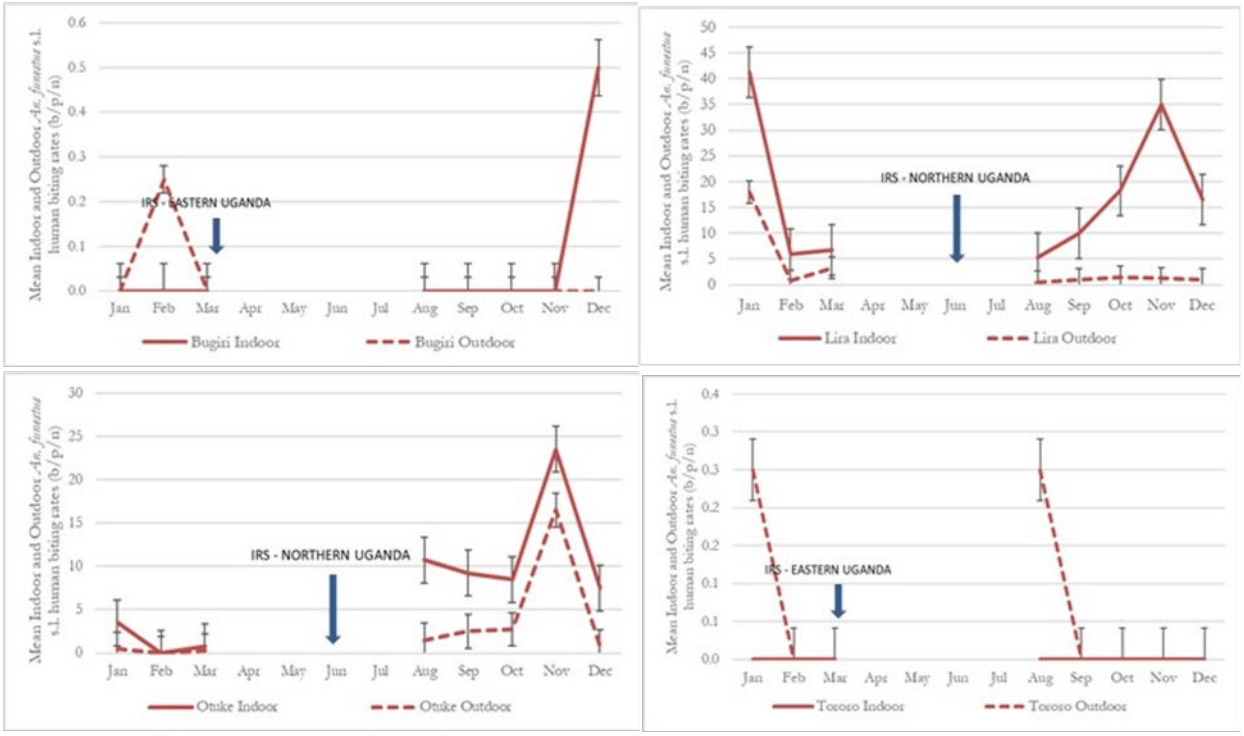
TABLE 4: MOSQUITO SPECIES COLLECTED BY HLC AND THEIR MEAN BITING RATES IN INTERVENTION, FORMER IRS AND CONTROL AREAS, JANUARY TO DECEMBER 2020

Species Collected	IRS Districts			Control non-IRS District		
	Total Numbers Collected	Total Person Nights	B/P/N	Total Numbers Collected	Total Person Nights	B/P/N
An. gambiae s.l.	651	128	5.1	443	64	6.9
An. funestus s.l.	1024	128	8.0	9291	64	145.2
Other *Anopheles* Species	308	128	2.4	16	64	0.3

HUMAN BITING RATE OF AN. FUNESTUS S.L. BY SITES

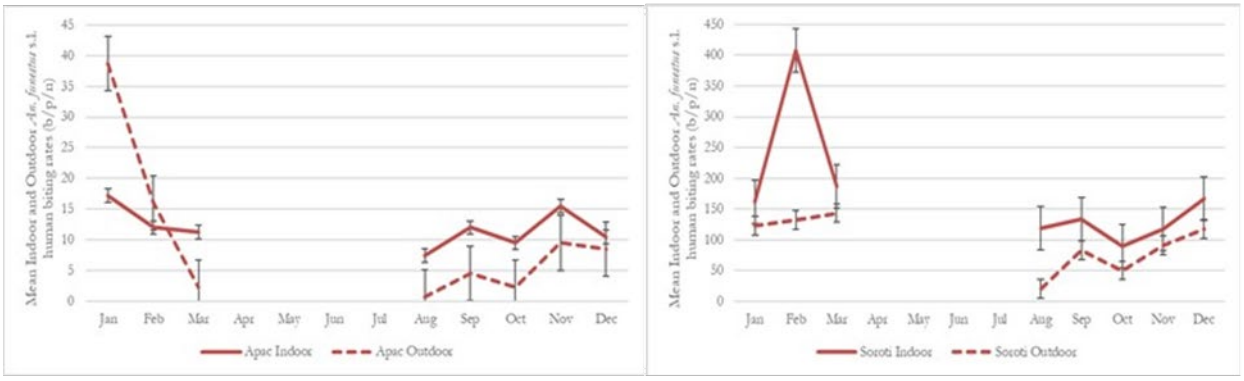
The HBR of *An. funestus* s.l. is presented by collection site in Figure 8 below. Overall, the *An. funestus* s.l. vector bites more indoors than outdoors, and the IRS northern sites of Lira and Otuke yielded higher HBRs (12.67 b/p/n) than the eastern sites of Bugiri and Tororo (0.03 b/p/n). Lira recorded the highest indoor HBR in February 2020 (41.3 b/p/n) and November 2020 (35 b/p/n) and Otuke with 23.5 b/p/n indoor in November 2020 and 15 b/p/n outdoor. The eastern IRS sites recorded very low HBR with less than 1 b/p/n all year round. As described above, the trends observed before and after IRS in all sites could not be compared due to the suspension of activities. However, when compared to the control sites, Soroti and Apac show high HBRs, and particularly Soroti with trends of more than 400 b/p/n in February 2020 and over 100 b/p/n from July to December 2020 (Figure 9).

FIGURE 8: *AN. FUNESTUS* S.L. MEAN INDOOR AND OUTDOOR HUMAN BITING RATES BY IRS DISTRICTS



Error bars represent the mean standard deviation bars

FIGURE 9: *AN. FUNESTUS* S.L. MEAN INDOOR AND OUTDOOR HUMAN BITING RATES BY NON-IRS DISTRICTS



Error bars represent the mean standard deviation bars.

HUMAN BITING RATE OF AN. GAMBIAE S.L. BY SITES

Contrary to *An. funestus* s.l., the HBRs of *An. gambiae* s.l. were slightly similar at all IRS sites with trends between 0 and 8 b/p/n except in Lira where a peak of about 16 b/p/n was recorded in September 2020. The HBRs of the IRS sites (2.77 b/p/n) were also like those of the control sites (3.34 b/p/n). Furthermore, the densities recorded at the IRS sites after spraying were higher than before the IRS intervention, both indoors and outdoors (Figure 10). The trend was different for Soroti but that could be explained by the fact that *An. funestus* s.l. was predominantly collected in Soroti throughout the year (Figure 11).

FIGURE 10: *AN. GAMBIAE* S.L. MEAN INDOOR AND OUTDOOR HUMAN BITING RATES BY IRS DISTRICTS

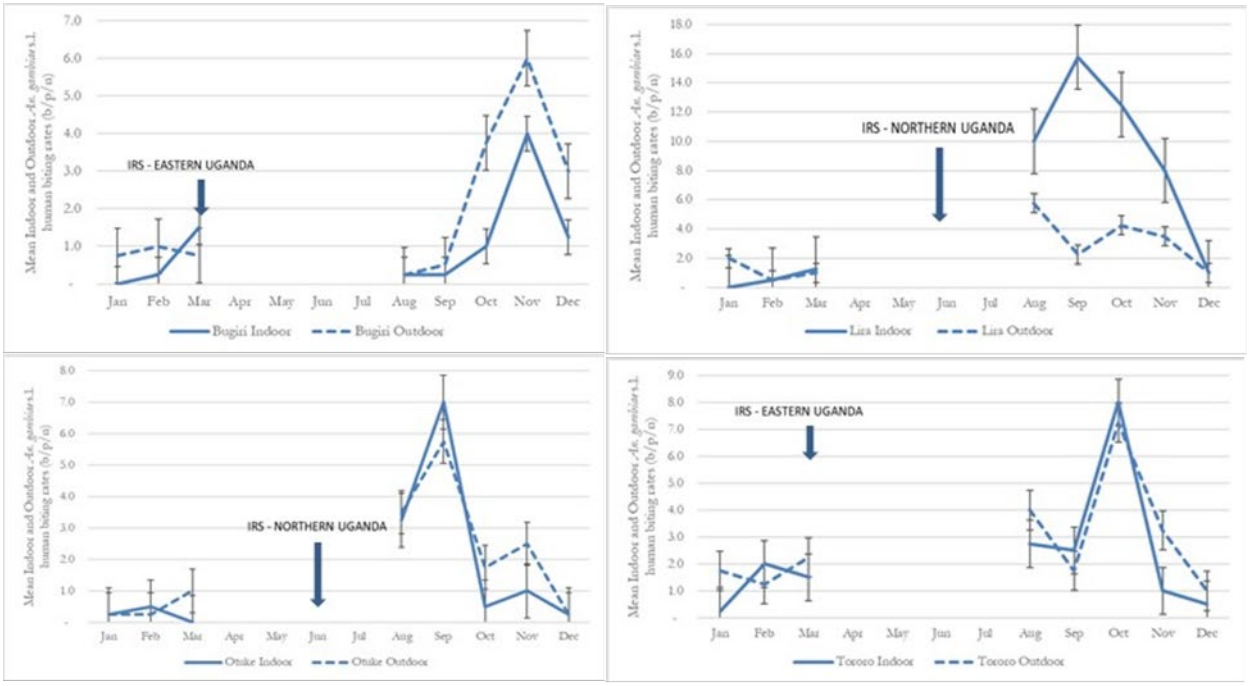
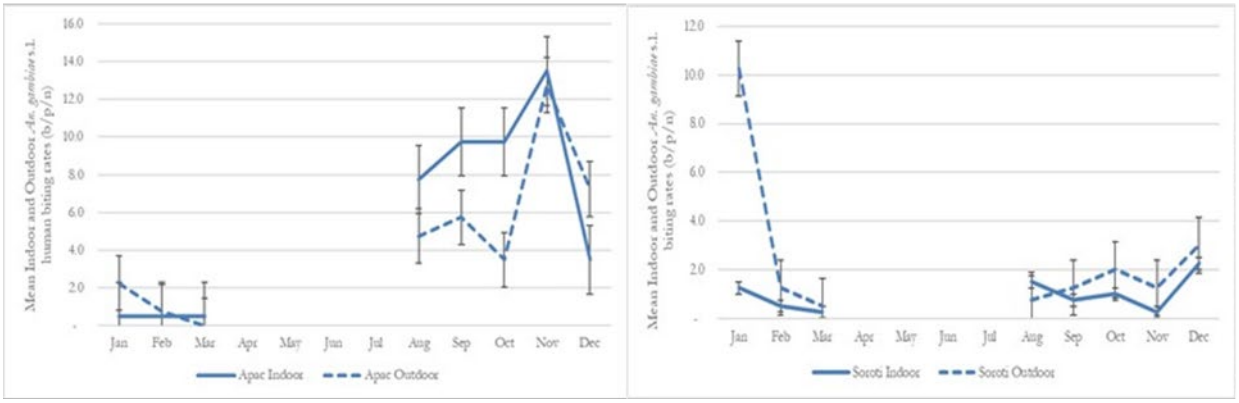


FIGURE 11: *AN. GAMBIAE* S.L. MEAN INDOOR AND OUTDOOR HUMAN BITING RATES BY NON-IRS DISTRICTS



Error bars represent the mean standard deviation bars.

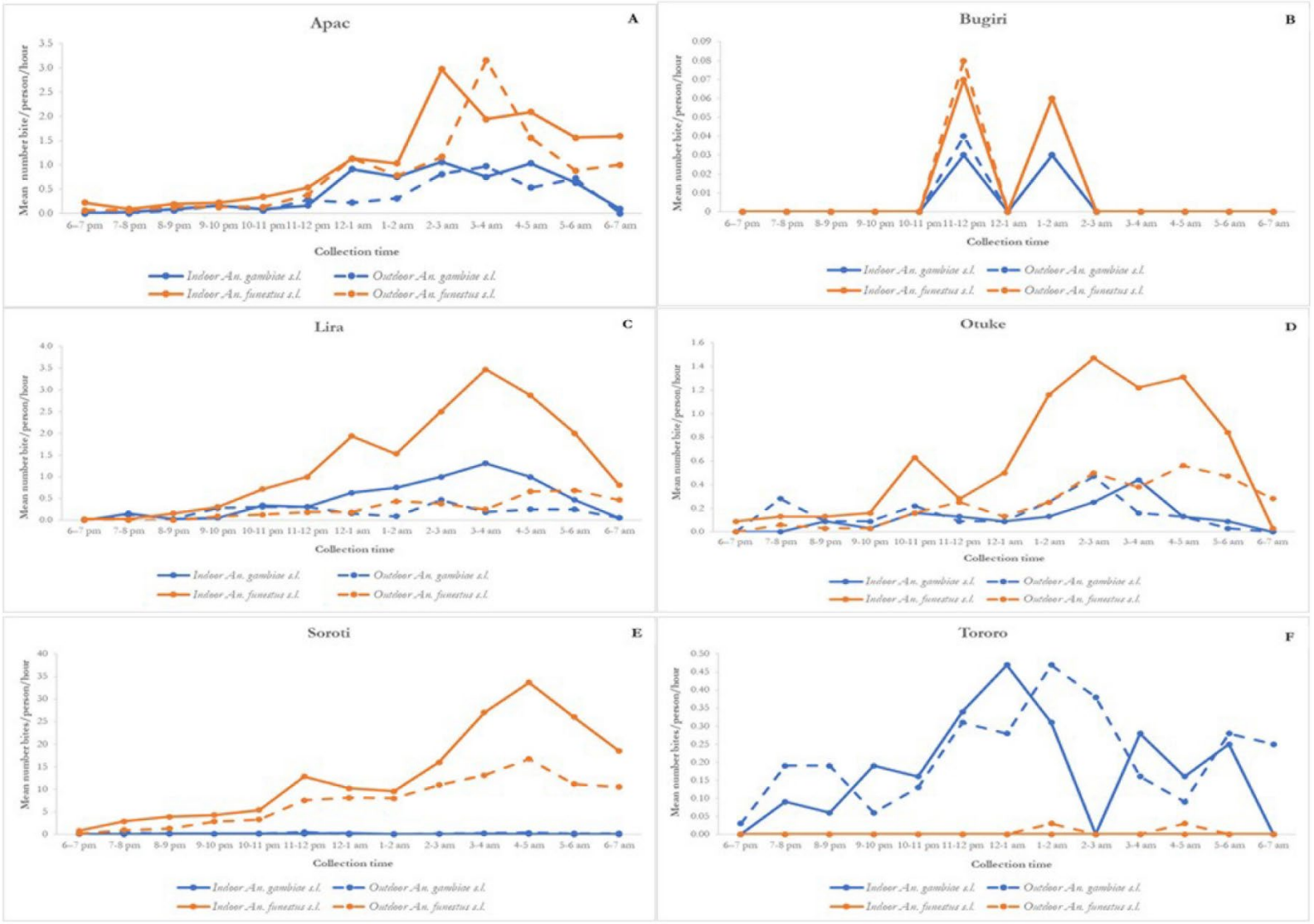
3.2.3 BITING TIME AND LOCATION OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L.

The biting time of both vectors *An. gambiae* s.l. and *An. funestus* s.l. was estimated at each collection site using HLCs. Night biting behavior of both *An. funestus* s.l. and *An. gambiae* s.l. indicates that vectors bite throughout the night indoors and outdoors at all districts surveyed, with few numbers of vectors starting to feed as early as 7:00 p.m. Also, it was observed that both vectors continue to bite until 7:00 am at the end of the collection time and particularly indoor for *An. funestus* s.l. and outdoor for *An. gambiae* s.l. (Figure 12). The numbers of vectors feeding increased from around 10:00-11:00 pm Overall, the mean hourly biting rate of *An. funestus* s.l. was higher in all the sites except in Tororo, both indoors and outdoors. The peak biting occurred between 2:00 am and 4:00 am in Apac, Lira, Otuke and Soroti while the peak was recorded between 11:00 pm and 2:00 am

in Bugiri. Soroti recorded the highest hourly peak HBR with 33.7 bites/person/hour (b/p/h) indoors and 16.7 b/p/h outdoors.

The overall peak biting time of *An. gambiae* s.l. occurred between 12:00 pm and 4:00 am In Apac, *An. gambiae* s.l. bite more between 12:00 pm and 4:00 am and in Lira and Otuke between 2:00 am and 4:00 am both indoors and outdoors. In Tororo, the indoor and outdoor peak hourly biting was recorded between 11:00 pm and 2:00 am with another slight peak observed after 5:00 am both indoor and outdoor.

FIGURE 12: INDOOR AND OUTDOOR BITING TIME OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED USING HLCS PER SITE FROM JANUARY 2020 - MARCH AND AUGUST - DECEMBER 2020



3.3 CONE WALL BIOASSAY TESTS

3.3.1 QUALITY OF IRS

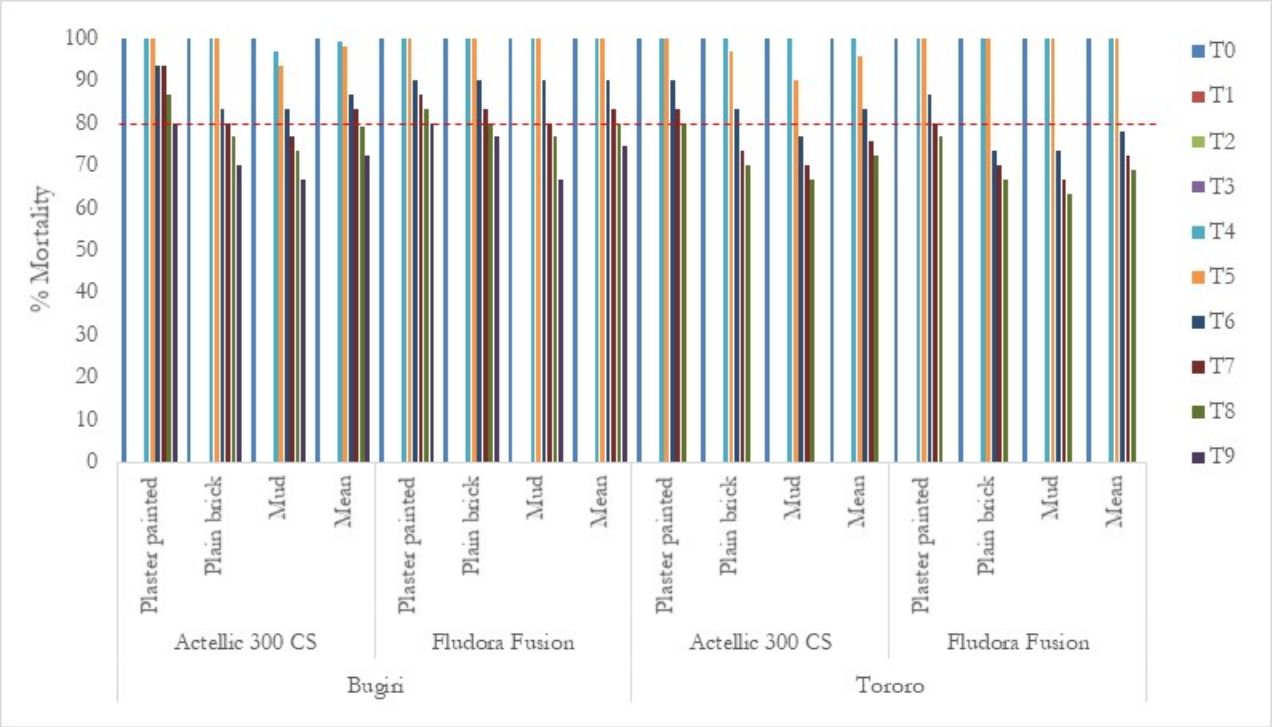
During the spray operations, WHO cone bioassays were conducted in one site per each of the eight spray districts (Bugiri, Butaleja, Dokolo, Kibuku, Lira, Otuke, Serere, and Tororo) using the susceptible *An. gambiae* s.s. (Kisumu strain). The results of the spray quality bioassays (conducted within one week after spraying) showed adequate spray quality (100% mosquito mortality) in the eight sentinel spray districts.

3.3.2 INSECTICIDE DECAY RATE

Cone bioassay test results indicate that Actellic 300CS and Fludora Fusion residual life ranged from six months in Tororo district to seven months in Bugiri district, with residual effectiveness on plastered painted wall surfaces extending up to eight months. However, SumiShield 50WG remained effective up to six months on all three surfaces after spraying in Lira and Otuke districts. All the three insecticides lasted longer on painted surfaces and least on mud wall surfaces (Annex 2- Table 1 and Figure 13 below). The months 1-3 could not be completed in Bugiri and Tororo due to the suspension of the activities as a result of the COVID-19 pandemic.

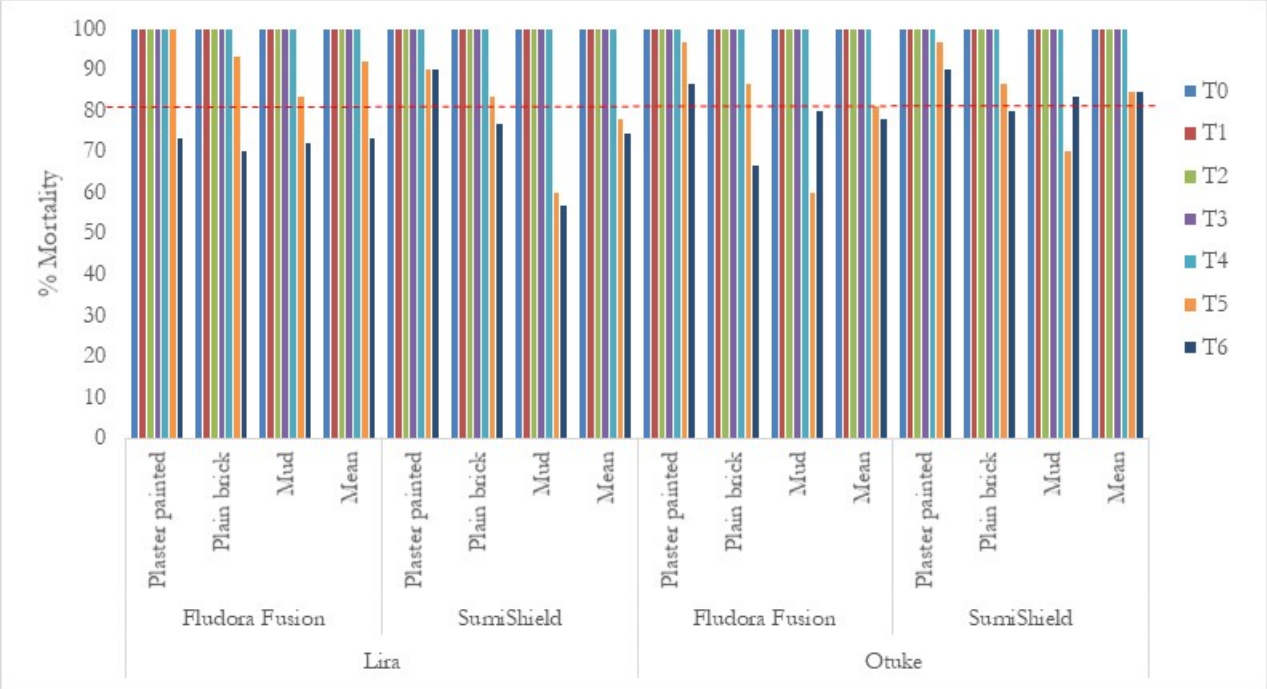
Similar results were recorded for Lira and Otuke six months after spraying. The bioassays are still ongoing (Annex 2-Table 2 and Figure 14 below).

FIGURE 13: WALL BIOASSAY RESULTS IN BUGIRI AND TORORO INSECTICIDE DECAY RATE MONITORING SITES, DECEMBER 2020



-------- The red line represents the 80% efficacy threshold.

FIGURE 14: WALL BIOASSAY RESULTS IN LIRA AND OTUKE INSECTICIDE DECAY RATE MONITORING SITES, DECEMBER 2020

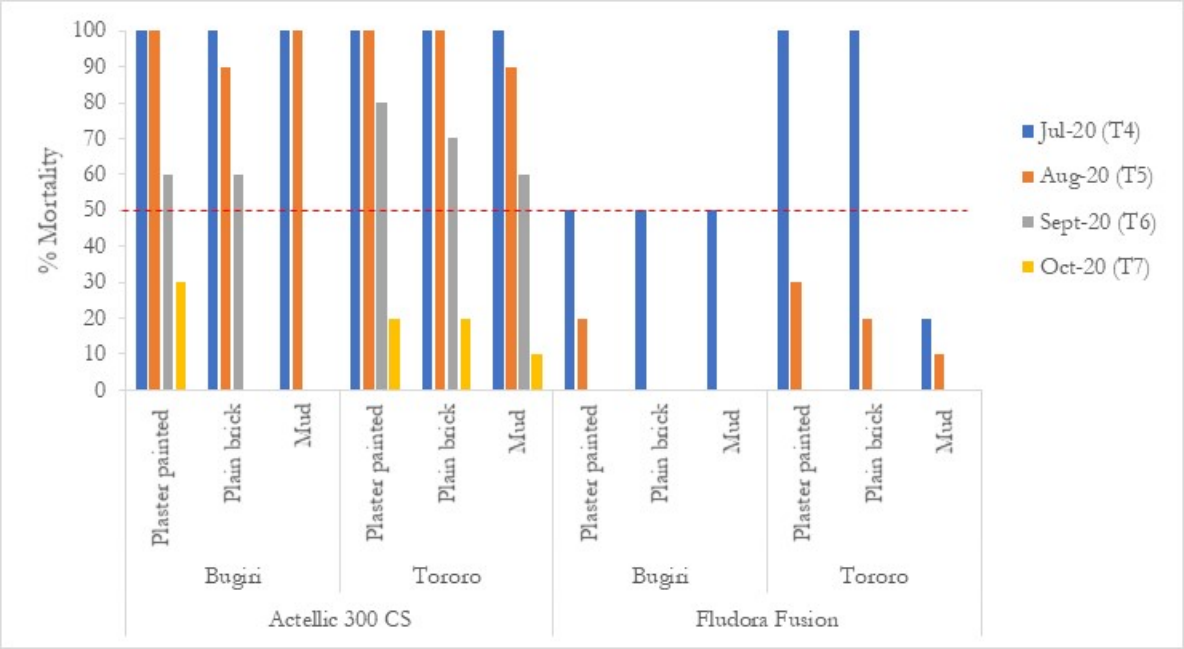


The red line represents the 80% efficacy threshold.

3.3.3 THE AIRBORNE EFFECT

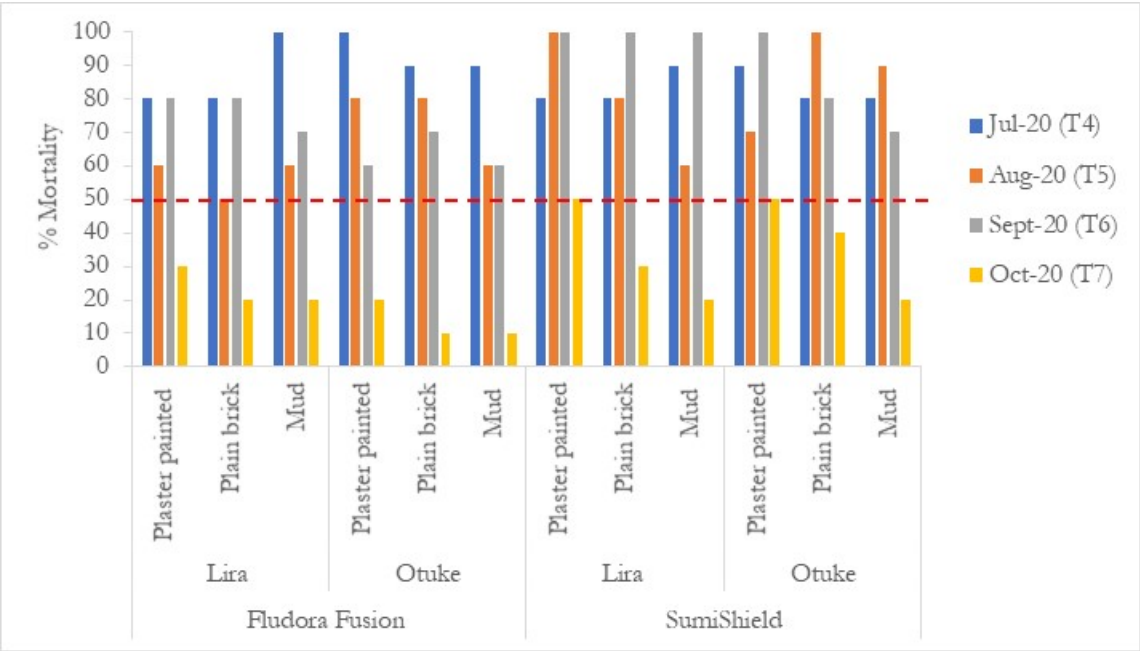
Tests on the airborne effect of Actellic 300CS and Fludora Fusion were conducted alongside from the fourth month (T4) post-IRS to eight months post-IRS (T8) in Bugiri and Tororo districts (Annex 2-Table 3 and Figure 15 below) and the tests for airborne effect of SumiShield and Fludora Fusion from one month up to six months (T6) post-IRS in Lira and Otuke districts (Annex-table 4 and Figure 16 below). Initial follow up could not be done due to the suspension of entomological monitoring activities per COVID-19 pandemic guidance. However, the fumigant effect of Actellic 300 CS was observed until September 2020 on the three tested surfaces (plaster painted, plain brick and mud surfaces) representing seven months post spraying in Bugiri and Tororo, while Fludora Fusion airborne effect lasted till July (four months) on all tested surfaces. In Lira and Otuke, both Fludora Fusion and SumiShield airborne effect were observed up to four months (T4) in October 2020 (Figure 16).

FIGURE 15: FUMIGANT EFFECT OF ACTELLIC 300CS AND FLUDORA FUSION SPRAYING ON MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE* S.S. IN BUGIRI AND TORORO DISTRICTS, EASTERN UGANDA, AT 4-7 MONTHS POST-IRS



--------- The red line represents the 50% efficacy threshold.

FIGURE 16: FUMIGANT EFFECT OF SUMISHIELD AND FLUDORA FUSION SPRAYING ON MORTALITY OF *AN. GAMBIAE* S.S. IN LIRA AND OTUKE DISTRICTS, NORTHERN UGANDA, AT 1-4 MONTHS POST-IRS



--------- The red line represents the 50% efficacy threshold.

3.4 WHO INSECTICIDE SUSCEPTIBILITY TESTING

3.4.1 DETERMINATION OF THE INSECTICIDE SUSCEPTIBILITY STATUS USING WHO TUBE TESTS

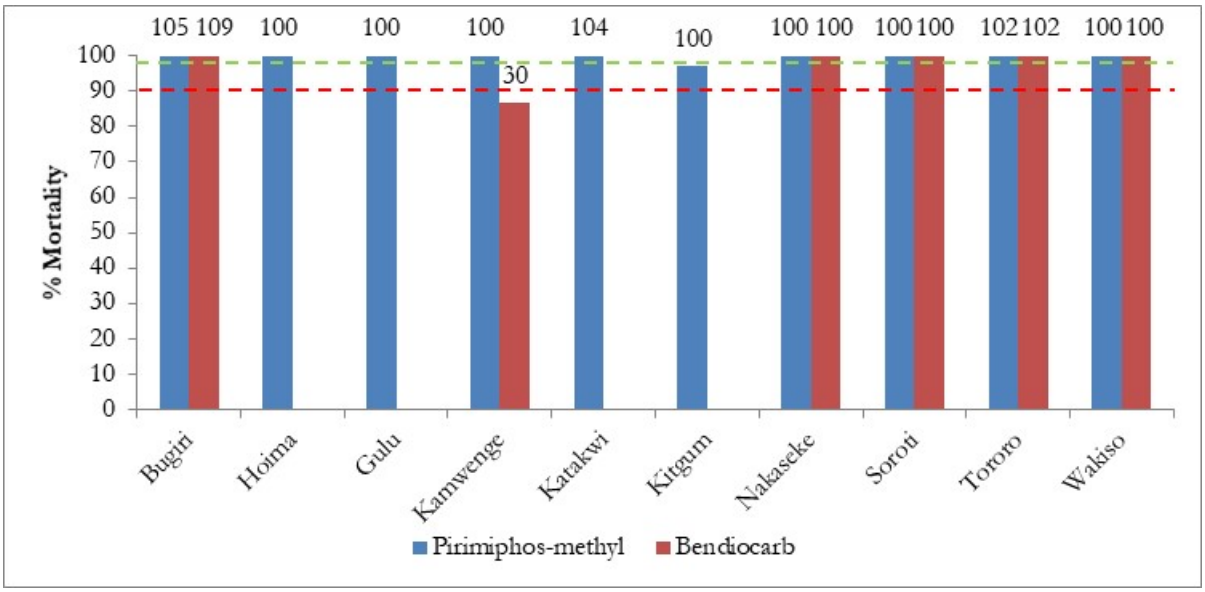
Susceptibility testing was conducted in 11 districts of Bugiri, Hoima, Gulu, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti, Tororo and Wakiso from September-October 2020.

An. gambiae s.l. was found to be susceptible (98-100% mortality) to pirimiphos-methyl in nine out of the 10 districts (Bugiri, Hoima, Gulu, Kamwenge, Katakwi, Nakaseke, Soroti, Tororo and Wakiso) where the test was completed. Only one district (Kitgum) recorded probable resistance (97.0% mortality). Further investigation is needed for confirmation of the observed data prior to making any definitive conclusion about susceptibility status of *An. gambiae* s.l. population to pirimiphos-methyl in Kitgum (Figure 17). *An. gambiae* s.l. was susceptible to bendiocarb in five districts (Bugiri, Nakaseke, Soroti, Tororo and Wakiso) out of six districts where the test was completed. Resistance was observed in Kamwenge district (Annex 3-Table 1 and Figure 17 below).

An. gambiae s.l. was resistant to alpha-cypermethrin in all 11 study districts (Bugiri, Hoima, Gulu, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti, Tororo and Wakiso) with mortality varying between 6% in Gulu and 82% in Lira. *An. gambiae* s.l. was resistant to deltamethrin in all the nine study districts where the test was completed (Bugiri, Hoima, Gulu, Kamwenge, Katakwi, Lira, Nakaseke, Tororo and Wakiso) with mortality varying between 4% in Gulu and 89% in Nakaseke. *An. gambiae* s.l. was resistant to permethrin in all the seven study districts where the test was completed (Bugiri, Gulu, Kamwenge, Katakwi, Soroti, Tororo and Wakiso), with mortality varying between 0% in Gulu and 72.8% in Katakwi (Figure 18).

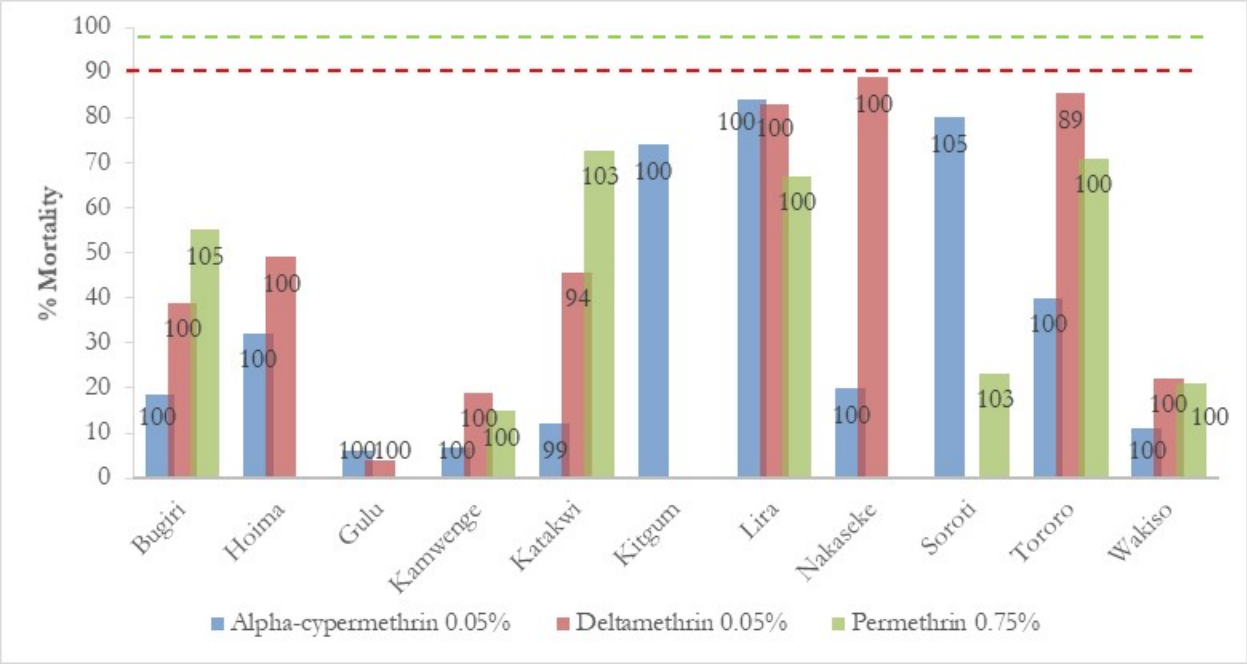
For *An. funestus* s.l., resistance was observed to alpha-cypermethrin in both Soroti (5% mortality) and Katakwi (83.2% mortality), to deltamethrin in both Soroti (20% mortality) and Katakwi (88% mortality) and to permethrin in Katakwi (72.1% mortality) (Annex 3, Table1 and Figure 19 below).

FIGURE 17: PERCENT 24 HOUR HOLDING MORTALITY OF FEMALE *AN. GAMBIAE* S.L. AFTER EXPOSURE TO BENDIOCARB IN 6 DISTRICTS AND PIRIMIPHOS-METHYL IN 10 DISTRICTS IN UGANDA, SEPTEMBER – OCTOBER 2020



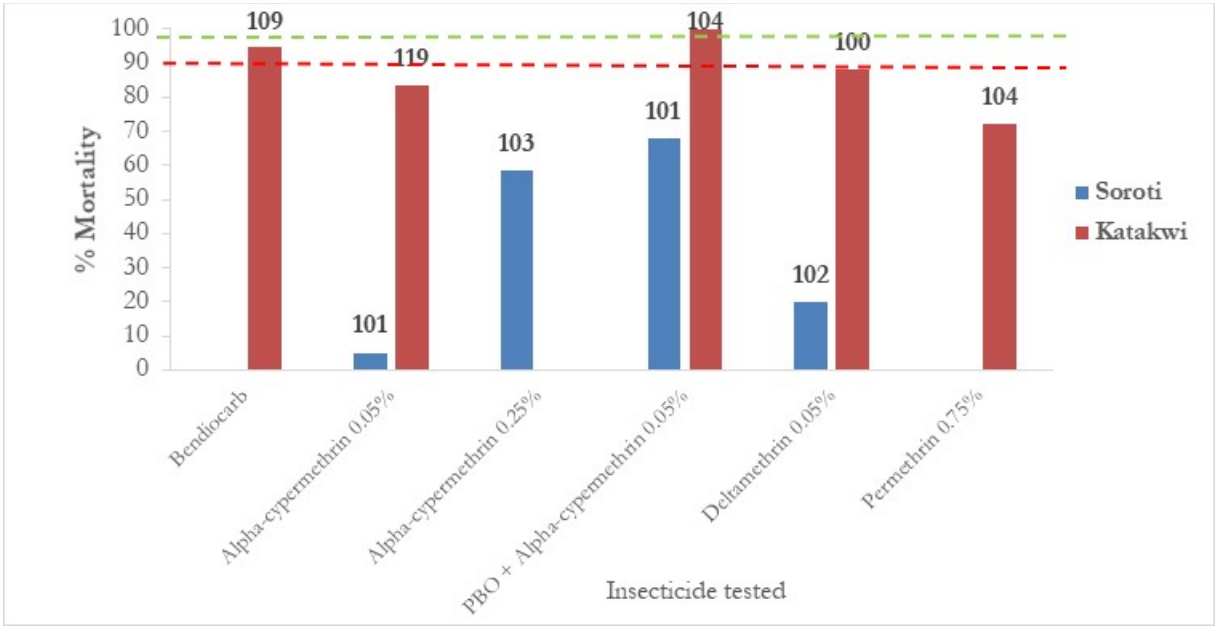
Line indicates resistance threshold Line indicates susceptibility threshold ; Numbers on the top of bars represent the number of mosquitoes tested per district and per insecticide

FIGURE 18: PERCENT 24 HOUR HOLDING MORTALITY OF FEMALE *AN. GAMBIAE* S.L. AFTER EXPOSURE TO THREE PYRETHROID INSECTICIDES IN 11 DISTRICTS IN UGANDA, SEPTEMBER – OCTOBER 2020



Line indicates resistance threshold Line indicates susceptibility threshold, Numbers on the top of bars represent the number of mosquitoes tested per district and per insecticide

FIGURE 19: PERCENT 24 HOUR HOLDING MORTALITY OF FEMALE *AN. FUNESTUS* S.L. AFTER EXPOSURE TO BENDIOCARB, THREE PYRETHROID INSECTICIDES AND PBO + ALPHA-CYPERMETHRIN IN TWO DISTRICTS IN EASTERN UGANDA, SEPTEMBER – OCTOBER 2020



Line indicates resistance threshold Line indicates susceptibility threshold, Numbers on the top of bars represent the number of mosquitoes tested per district and per insecticide

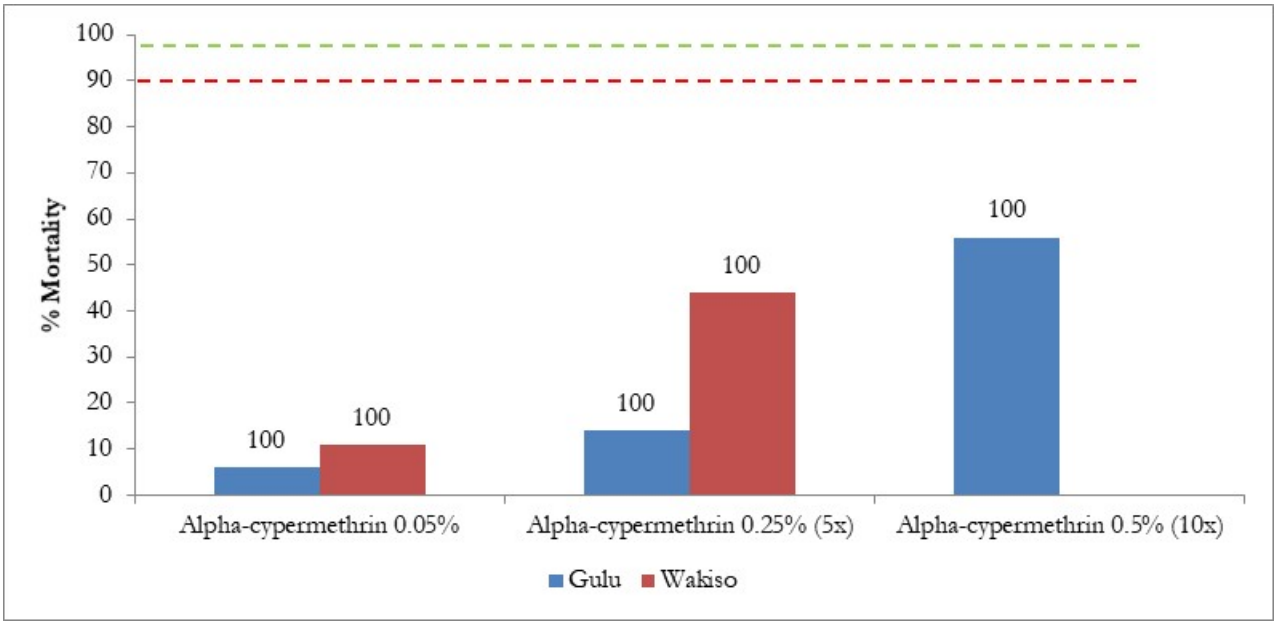
3.4.2 Determination of the Intensity of Resistance using WHO Tube Tests

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

Results of resistance intensity studies of *An. gambiae* s.l. using the WHO tube assay showed high resistance intensity of *An. gambiae* s.l. to alphacypermethrin in Gulu (56% mortality after 24h at 10× concentration), while Wakiso recorded 44% mortality after 24h at 5× diagnostic dose of alpha-cypermethrin (Annex 3-Table 1 and Figure 20 below).

Resistance intensity studies of *An. funestus* s.l. using the WHO tube assay showed 58.3% mortality after 24h at 5× alpha-cypermethrin diagnostic dose in Soroti (Figure 19).

Figure 20: Percent Mortality of *An. gambiae* s.l. after Exposure To Different Concentrations Of Alpha-cypermethrin in Two Districts in Uganda, September-October 2020



Line indicates resistance threshold Line indicates susceptibility threshold, Numbers on the top of bars represent the number of mosquitoes tested per district and per insecticide

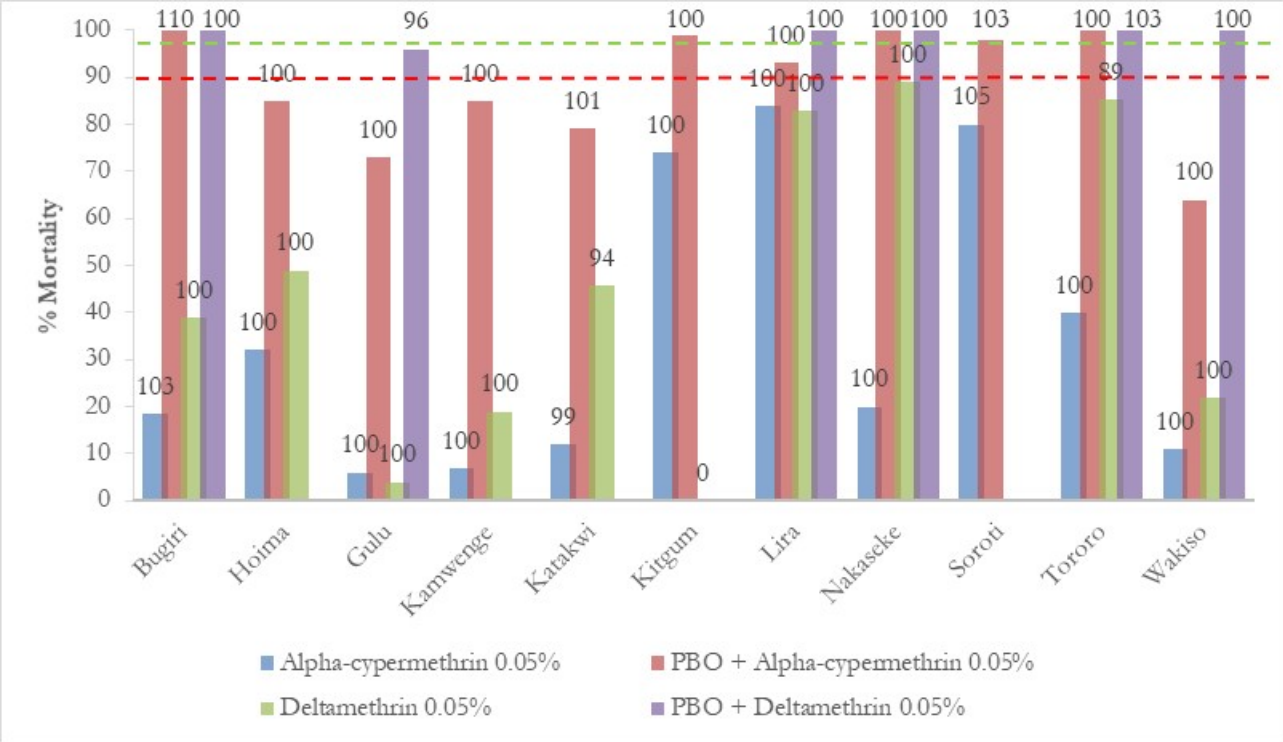
3.4.3 Synergist Assays of Pyrethroids using WHO Tube Tests

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

The synergist assay using PBO fully restored *An. gambiae* s.l. susceptibility (98-100%) to alpha-cypermethrin in 6/11 sites (Bugiri, Kitgum, Lira, Nakaseke, Soroti and Tororo) and partially restored susceptibility in 5/11 sites (Hoima, Gulu, Kamwenge, Katakwi and Wakiso) with mortality varying between 64% mortality in Wakiso and 85% in Hoima and Kamwenge. PBO fully restored *An. gambiae* s.l. susceptibility to deltamethrin in 5/6 sites (Bugiri, Lira, Nakaseke, Soroti, Tororo) and partially restored susceptibility in Gulu (96% mortality). PBO partially restored *An. gambiae* s.l. susceptibility to permethrin in Wakiso (85% mortality) (Annex 3, Table 1 and Figure 18). The synergist assay using PBO fully restored *An. funestus* s.l. susceptibility to alpha-cypermethrin in Katakwi (100% mortality) and partially in Soroti (68% mortality).

Full restoration or significant increase of mortality after pre-exposure to PBO in some districts indicates that monooxygenase enzyme such as P450s might be involved in the resistance of the vectors.

FIGURE 21: SYNERGIST ASSAY MORTALITY RESULTS IN *AN. GAMBIAE* S.L. FROM FIVE OUT OF EIGHT STUDY DISTRICTS IN UGANDA UPON EXPOSURE TO ALPHA-CYPERMETHRIN AND DELTAMETHRIN ONLY OR WITH 4% PBO PRE-EXPOSURE SEPTEMBER – OCTOBER 2020



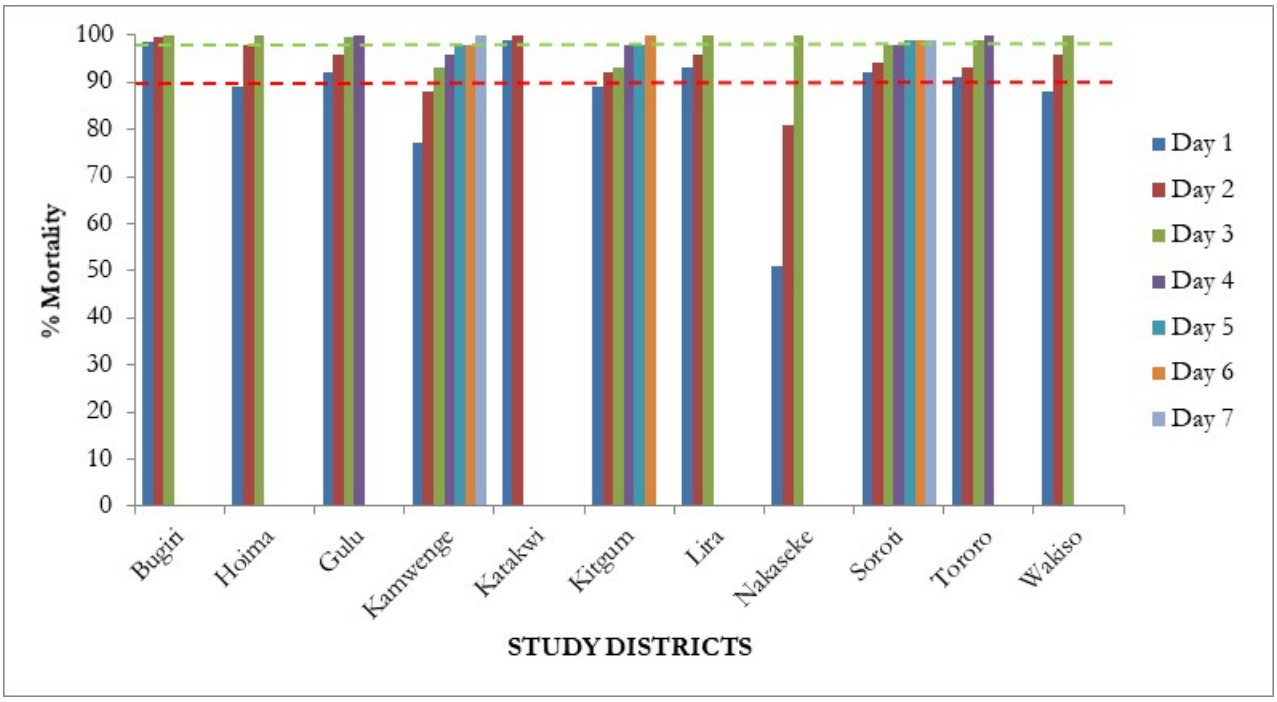
Line indicates resistance threshold Line indicates susceptibility threshold , Numbers on the top of bars represent the number of mosquitoes tested per district and per insecticide

3.4.4 CLOTHIANIDIN AND CHLORFENAPYR SUSCEPTIBILITY TEST RESULTS

The clothianidin tests were conducted on samples collected in all 11 districts surveyed. *An. gambiae* s.l. was susceptible to clothianidin in all 11 districts surveyed. One hundred percent (100%) mortality was recorded within 2-3 days in Bugiri, Hoima, Lira, Katakwi, Nakaseke, and Wakiso while the other sites recorded susceptibility between 4 to 7 days post exposure. However, the test was repeated in Bugiri after recording a possible resistance (94.2%) during the initial testing in order to confirm the possible resistance status of the *An. gambiae* s.l. population to clothianidin which resulted on 100% mortality and confirming susceptibility to the insecticide (Annex 3, Table 2 and Figure 19).

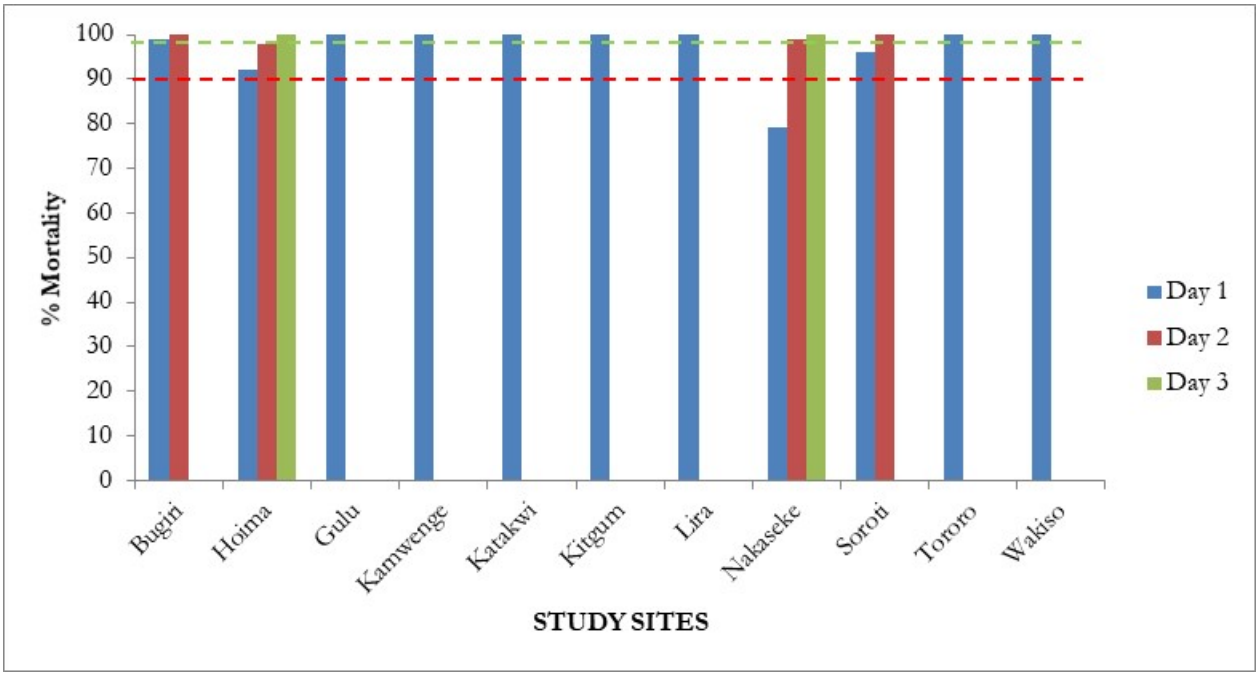
Chlorfenapyr tests were conducted on samples collected in all 11 districts surveyed (Figure 20). *An. gambiae* s.l. was susceptible to chlorfenapyr at all 11 sites at the dose of 100 µg/bottle after a three-day holding period. Both clothianidin and chlorfenapyr tests were conducted on the wild *An. gambiae* s.l. in parallel with the laboratory susceptible *An. gambiae* s.s. Kisumu strain in the districts of Bugiri, Gulu, Katakwi, Lira, Soroti, Tororo where access to susceptible *An. gambiae* s.s. strains was possible. Hundred percent mortality of *An. gambiae* s.s. Kisumu was also recorded in all the tests and sites. Hoima, Kamwenge, Kitgum, Nakaseke and Wakiso where there was no nearby colony of susceptible *An. gambiae* s.s. was not tested, but the wild collected *An. gambiae* s.l. showed 100% mortality confirming therefore the good coating of the bottle.

FIGURE 22: CLOTHIANIDIN DELAYED MORTALITY DURING SUSCEPTIBILITY STUDIES, SEPTEMBER TO OCTOBER 2020 (RESULTS FOR ADULTS REARED FROM LARVAE)



Line indicates resistance threshold Line indicates susceptibility threshold

FIGURE 23: PERCENT MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO CHLORFENAPYR AT A CONCENTRATION OF 100MG/BOTTLE, SEPTEMBER-OCTOBER 2020 (RESULTS FOR ADULTS REARED FROM LARVAE)



Line indicates resistance threshold Line indicates susceptibility threshold

3.4.5 SUSCEPTIBILITY STATUS FOR INSECTARY REARED *AN. GAMBIAE* S.S. (KISUMU STRAIN)

The study results of the susceptibility studies conducted on 2–5-day old *An. gambiae* s.s (Kisumu) reared at Vector Control Division Kampala, Tororo and Gulu University showed that the *An. gambiae* s.s (Kisumu Strain) reared were still susceptible (100% mortality for various insecticides tested) and therefore suitable for use in wall bioassays and as controls in susceptibility studies (Table 5).

TABLE 5: RESULTS OF INSECTICIDE SUSCEPTIBILITY STATUS OF INSECTARY REARED *AN. GAMBIAE* S.S. (KISUMU STRAIN) IN FEBRUARY 2020

Insecticide	Vector Control Division insectary Kampala	Gulu University insectary	Tororo Hospital insectary
	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)
Alpha-cypermethrin 0.05%	100 (100.0%)	100 (100.0%)	101 (100.0%)
Permethrin 0.75%	100 (100.0%)	100 (100.0%)	121 (100.0%)
Deltamethrin 0.05%	100 (100.0%)	100 (100.0%)	109 (100.0%)
Bendiocarb 0.10%	100 (100.0%)	100 (100.0%)	100 (100.0%)
Pirimiphos methyl 0.25%	100 (100.0%)	100 (100.0%)	101 (100.0%)

4. Discussion, Lessons Learned, and Challenges

Entomological longitudinal monitoring conducted in six sites that included four IRS sites and two control sites during eight months from January to December showed *An. funestus* s.l. and *An. gambiae* s.l. as the main malaria vectors in Uganda. The two methods used for data collection were PSCs and HLCs, conducted monthly except from April to June where collection was suspended due to the COVID-19 pandemic.

Results of PSCs showed that *An. funestus* s.l. was the most abundant species collected while resting indoors in Apac and Soroti (control districts) and in Lira and Otuke (current IRS district) while very few numbers were collected in Bugiri and Tororo (IRS districts). In contrast, *An. gambiae* s.l. predominated the indoor catches in the IRS intervention districts of Bugiri and Tororo. These trends were similar to what was observed during previous years which may indicate that the more endophillic and endophagic populations of *An. funestus* s.l. may be more impacted by IRS than *An. gambiae* s.l. as the *An. funestus* s.l. indoor resting densities were lower than those of *An. gambiae* s.l. in IRS districts. Furthermore, the difference species proportions recorded between districts could also relate to the mosquito collection sentinel site's geographical location with that of Apac and Soroti being near a permanent swamp, an ideal breeding habitat for *An. funestus* s.l.

A variety of *Anopheles* species was caught in the sites using HLC collections indoors and outdoors, including six different species (*An. funestus* s.l., *An. gambiae* s.l., *An. coustani, An. pretoriensis, An. squamosus,* and *An. ziemanni*). *An. funestus* s.l., and *An. gambiae* s.l. were caught at all sites, although specific species abundance differed by sentinel site. HLCs also demonstrated that Soroti yielded the highest mean *An. funestus* s.l. biting rate both indoors and outdoors. *An. gambiae* s.l. was found at higher densities in the control Apac district, albeit at much lower densities than *An. funestus* s.l. Furthermore, the HLCs revealed differences in biting behavior between outdoor and indoor for both vector species in each district with either higher indoor biting than outdoors in some districts and inversely in the others throughout the collection period.

The HBR was consistently higher outdoors than indoors for *An. gambiae* s.l. in only Bugiri among the IRS districts. *An. gambiae* s.l. of Otuke and Tororo showed similar biting behavior with approximate equal mean indoor and outdoor overall biting rates. Only Lira recorded higher indoor density than the other sites. The different behavior observed in each site could not be fairly compared and adjusted with the IRS campaigns as no collection was conducted during the April to June period representing a crucial time for data comparison. However, in the control district of Apac and Soroti, *An. gambiae* s.l. bite more indoors and outdoors respectively. Additionally, *An. funestus* s.l. and *An. gambiae* s.l. were consistently biting more indoors than outdoors throughout the night in all sites with biting peaks occurring between 11 p.m. and 4 a.m. in majority. The biting behavior of the vectors is an important part of the effectiveness of all vector control intervention to overcome the vectors, particularly for IRS and insecticide treated net (ITN) impact. Both vector control strategies are more effective when the vectors bite mostly indoors. The laboratory analyses determining the infection and entomological inoculation rates will help understand the impact of both interventions in IRS sites enabling good comparison between control and IRS sites on the reduction or not of malaria transmission within each district.

The WHO cone wall bioassay results obtained in 8 of 16 sprayed districts showed that the spray quality of the 2020 spray campaign was satisfactory at all monitored sites. The monthly insecticide decay test showed that Actellic 300CS and Fludora Fusion on average stayed effective on sprayed surfaces between six and eight months in Tororo and Bugiri depending on the wall surface type sprayed. Both insecticides had slightly shorter residual efficacy on mud surfaces; six months in Bugiri and Tororo and nine months on painted wall surfaces in the two districts. SumiShield 50WG was found to be effective for at least six months after the spray campaign

on the various wall surface sprayed in Lira and Otuke districts. The duration of the insecticide on the plastered or plastered painted walls indicates that more the houses are well constructed with good materials, more the insecticide could last to protect the populations. This showed that 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 and community protection against malaria vectors. Additionally, the airborne effect of all the three insecticides sprayed was observed at an average of four months post-IRS on all the three surfaces tested. This is slightly surprising compared to other reports showing that the Fludora Fusion and SumiShield have very low airborne effect with less than one month fumigant effect in some countries. The airborne effect of both insecticides will need to be check closely in the future for confirmation of the trends observed in Uganda in terms of the length of the airborne effect of the insecticides after spraying.

Insecticide resistance monitored across the country showed that *An. gambiae* s.l. was susceptible to pirimiphos-methyl (98-100% mortality) in nine out of 10 study districts with suspected resistance in Kitgum district, while *An. gambiae* s.l. was susceptible to bendiocarb in five districts (Bugiri, Nakaseke, Soroti, Tororo and Wakiso) out of six sites where the test was completed, with resistance observed in Kamwenge district. *An. gambiae* s.l. was resistant to alpha-cypermethrin in all the 11 study districts (Bugiri, Hoima, Gulu, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti, Tororo and Wakiso) with mortality varying between 6% in Gulu and 82% in Lira based on WHO tube test results.

An. funestus s.l., was resistant to both alpha-cypermethrin and deltamethrin in both Soroti and Katakwi and to permethrin in Katakwi, similarly to what was observed for *An. gambiae* s.l. against the three pyrethroids tested in all districts. This indicates that both *An. gambiae* s.l and *An. funestus* s.l., are resistant to pyrethroids (alpha-cypermethrin, deltamethrin and permethrin) which are used for net impregnation and this should be a cause for concern for the deployment of pyrethroid-only ITNs for malaria control in Uganda. Synergist assays using PBO fully or partially restored *An. gambiae* s.l. susceptibility to pyrethroids indicating that oxidases might be involved in the insecticide resistance of the vectors, although other resistance mechanisms like *Kdr* (east and west) may also play a major role in districts where PBO only partially restored susceptibility to pyrethroids.

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, in order to achieve universal coverage and also excessive use of the same insecticide and chemicals in agriculture. It also points out the need to roll -out extensively next generation ITNs like the PBO ITNs and the new WHO-prequalified dual active ingredient ITNs such as Interceptor® G2 (alpha-cypermethrin and chlorfenapyr coated on polyester) and Royal Guard® *(*alpha-cypermethrin and pyriproxyfen incorporated into polyethylene) to control pyrethroid resistant vectors and maximize the effectiveness of ITNs in malaria control. Interestingly, the NMCP has already embarked on the distribution of these new ITNs since the last ITN mass distribution campaign 2017-2020, and there is the need to ensure that there is widespread coverage across the country. There is also a need to conduct a follow up monitoring of the new ITNs distributed in various parts of Uganda in order to appreciate the gains achieved for further consideration.

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

Although resistance to pirimiphos-methyl is still not widespread in Uganda (possible resistance observed in only one site (Kitgum) among those tested), but having been used for four consecutive years now, the use of both SumiShield and Fludora Fusion in IRS in Uganda is a welcome development in the management of insecticide resistance in the country. This is especially true as malaria vectors in Uganda were susceptible to clothianidin. These insecticides can be used in rotation to maintain their efficacy against the local malaria vectors. Although the current WHO recommendation is proactive rotation, at least every 12 months, given

financial constraints, in Uganda, the change of an insecticide will routinely be carried out every three years. In case resistance is detected before three years, a change for an effective class of insecticide is envisaged.

Molecular assays are ongoing at IDRC Molecular Laboratories in Kampala, Uganda involving vector mosquito speciation, infection rate determination, detection and identification of mutations on genetic resistance markers, knockdown resistance (*kdr*) and *Ace-1* genes in *An. gambiae* s.l. The laboratory analysis is still a challenge in Uganda and change in activity planning needs to be undertaken. The proposed number of samples to analyze can be shared with IDRC either monthly or quarterly to enabling less workload. This implies that the contract with IDRC needs to be reviewed and extended from three-month contract to a full year contract as it won't have any budget implication.

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ANNEX A: LONGITUDINAL MONITORING

TABLE 1 A1: NUMBER OF FEMALE *ANOPHELES* MOSQUITOES COLLECTED IN EACH DISTRICT BY PSC AND HLC

Mosquito species	Apac	Bugiri	Lira	Otuke	Soroti	Tororo	Total	%
An. gambiae s.l.	1,117	445	808	346	554	621	3,891	10.6
An. funestus s.l.	10,288	31	2,505	2,672	17,022	18	32,536	88.4
Other *Anopheles* species	10	83	31	228	23	3	378	1.0
Total per district	**11,415**	**559**	**3,344**	**3,246**	**17,599**	**642**	**36,805**	**100**

TABLE 2 A1: NUMBER OF MOSQUITOES BY SPECIES COLLECTED USING PSC IN THE STUDY DISTRICTS

	Apac	Bugiri	Lira	Otuke	Soroti	Tororo	Total	%
An. gambiae s.l.	786	347	531	234	442	457	2797	11.2
An. funestus s.l.	9576	28	1840	2318	8443	16	22221	88.7
Other *Anopheles*	0	3	4	8	17	1	33	0.1
Total per district	**10,362**	**378**	**2,375**	**2560**	**8,902**	**474**	**25,051**	**100**

TABLE 3 A1: NUMBER OF MOSQUITOES BY SPECIES COLLECTED USING HLC IN THE STUDY DISTRICTS

	Apac	Bugiri	Lira	Otuke	Soroti	Tororo	Total	%
An. gambiae s.l.	331	98	277	112	112	164	1,094	9.3
An. funestus s.l.	712	3	665	354	8579	2	10,315	87.8
Other *Anopheles*	10	80	27	220	6	2	345	2.9
Total per district	**1,053**	**181**	**969**	**686**	**8,697**	**168**	11,754	100

TABLE 4 A1: HUMAN LANDING CATCHES INDOORS AND OUTDOORS IN SIX STUDY DISTRICTS IN UGANDA FROM JANUARY TO DECEMBER 2020

Anopheles species	Apac			Bugiri			Lira			Otuke			Soroti			Tororo			Total		Grand Total
	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	
An. gambiae s.l.	183	148	331	34	57	91	196	81	277		72	123	31	81	112	74	90	164	569	529	1,098
An. funestus s.l.	382	330	712	2	3	5	556	109	665	255	96	351	5,537	3,042	8,579	0	2	2	6,732	3,582	10,314
Other *Anopheles* species	0	10	10	25	50	75	10	17	27	38	184	222	4	2	6	0	2	2	77	265	342
Total per district	**565**	**488**	**1,053**	**61**	**110**	**171**	**762**	**207**	**969**	**344**	**352**	**696**	**,5572**	**3,125**	**8,697**	**74**	**94**	**168**	**7,378**	**4,376**	**11,754**

TABLE 5 A1: HOURLY BITING RATES OF *AN. GAMBIAE* S.L. IN APAC, BUGIRI, LIRA, OTUKE, SOROTI AND TORORO DETERMINED THROUGH HLCS, JANUARY TO DECEMBER 2020

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.
Apac	Indoor	0.0	0.03	0.09	0.16	0.09	0.16	0.91	0.75	1.06	0.75	1.03	0.63	0.09
	Outdoor	0.03	0.0	0.06	0.16	0.06	0.28	0.22	0.31	0.81	0.97	0.53	0.72	`0.44
Bugiri`	Indoor	0.06	0.0	0.03	0.06	0.16	0.22	0.13	0.09	0.0	0.06	0.06	0.09	0.09
	Outdoor	0.22	0.06	0.13	0.19	0.16	0.16	0.16	0.09	0.19	0.25	0.13	0.16	0.13
Lira	Indoor	0.0	0.16	0.03	0.06	0.34	0.31	0.63	0.75	1.00	1.31	1.00	0.47	0.06
	Outdoor	0.0	0.13	0.03	0.28	0.31	0.31	0.16	0.09	0.47	0.19	0.25	0.25	0.06
Otuke	Indoor	0.0	0.0	0.09	0.03	0.16	0.13	0.09	0.13	0.25	0.44	0.13	0.09	0.0
	Outdoor	0.0	0.28	0.09	0.09	0.22	0.09	0.09	0.25	0.47	0.16	0.13	0.03	0.0
Soroti	Indoor	0.06	0.03	0.09	0.13	0.13	0.09	0.22	0.06	0.06	0.16	0.06	0.0	0.03
	Outdoor	0.09	0.34	0.16	0.13	0.16	0.53	0.03	0.03	0.13	0.22	0.34	0.19	0.19
Tororo	Indoor	0.0	0.09	0.06	0.19	0.16	0.34	0.47	0.31	0.0	0.28	0.16	0.25	0.0
	Outdoor	0.03	0.19	0.19	0.06	0.13	0.31	0.28	0.47	0.38	0.16	0.09	0.28	0.25

TABLE 6 A1: HOURLY BITING RATES OF *AN. FUNESTUS* S.L. IN APAC, BUGIRI, LIRA, OTUKE, SOROTI AND TORORO DETERMINED THROUGH HLCS, JANUARY TO DECEMBER 2020

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.
Apac	Indoor	0.22	0.09	0.19	0.22	0.34	0.53	1.13	1.03	2.97	1.94	2.09	1.56	1.59
	Outdoor	0.06	0.06	0.13	0.13	0.13	0.38	1.13	0.78	1.16	3.16	1.56	0.88	1.00
Bugiri`	Indoor	0.0	0.0	0.0	0.0	0.0	0.03	0.0	0.03	0.0	0.0	0.0	0.0	0.0
	Outdoor	0.0	0.0	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lira	Indoor	0.03	0.03	0.16	0.31	0.72	1.00	1.94	1.53	2.50	3.47	2.88	2.00	0.81
	Outdoor	0.0	0.0	0.0	0.09	0.13	0.19	0.19	0.44	0.38	0.25	0.66	0.69	0.47
Otuke	Indoor	0.09	0.13	0.13	0.16	0.63	0.28	0.50	1.16	1.47	1.22	1.31	0.84	0.03
	Outdoor	0.0	0.06	0.03	0.03	0.16	0.25	0.13	0.25	0.50	0.38	0.56	0.47	0.28
Soroti	Indoor	0.78	2.91	3.91	4.28	5.41	12.81	10.22	9.53	15.97	27.03	33.72	26.06	18.50
	Outdoor	0.19	0.94	1.28	2.81	3.28	7.53	8.09	8.00	10.94	13.09	16.69	11.16	10.50
Tororo	Indoor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Outdoor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.03	0.0	0.0	0.03	0.0	0.0

TABLE 7 A1: MEAN INDOOR AND OUTDOOR VECTOR BITING RATES PER PERSON PER DAY FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. INDOORS AND OUTDOORS BY DISTRICT, JANUARY-DECEMBER 2020

District	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec	
	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f
Apac																								
HLC indoors	0.5	17.3	0.5	12.0	0.8	27.0	ND	ND	ND	ND	ND	ND	ND	ND	7.8	7.5	9.8	12.0	9.8	9.5	13.5	15.5	3.5	10.5
HLC outdoors	2.3	38.8	0.5	16.0	0.0	4.0	ND	ND	ND	ND	ND	ND	ND	ND	4.8	0.8	5.8	4.5	3.5	2.3	12.8	9.5	7.3	8.5
Bugiri																								
HLC indoors	0.0	0.0	0.3	0.0	1.5	0.0	ND	ND	ND	ND	ND	ND	ND	ND	0.3	0.0	0.3	0.0	1.0	0.0	4.0	0.0	1.3	0.5
HLC outdoors	0.8	0.0	1.0	0.3	0.8	0.0	ND	ND	ND	ND	ND	ND	ND	ND	0.3	0.0	0.5	0.0	3.8	0.0	6.0	0.0	3.0	0.0
Lira																								
HLC indoors	0.0	41.3	0.5	6.0	1.3	6.8	ND	ND	ND	ND	ND	ND	ND	ND	10.0	5.3	15.8	10.0	12.5	18.3	8.0	35.0	1.0	16.5
HLC outdoors	2.0	18.0	0.5	0.8	1.0	3.3	ND	ND	ND	ND	ND	ND	ND	ND	5.8	1.0	2.3	1.0	4.3	1.5	3.5	1.3	1.0	1.0
Otuke																								
HLC indoors	0.3	3.3	0.3	0.0	0.0	0.8	ND	ND	ND	ND	ND	ND	ND	ND	3.3	10.8	7.0	9.3	0.5	8.5	1.0	23.5	0.0	7.5
HLC outdoors	0.3	0.5	0.3	0.0	1.0	0.3	ND	ND	ND	ND	ND	ND	ND	ND	3.5	1.5	5.8	2.5	1.8	2.8	2.5	16.5	0.3	0.8
Soroti																								
HLC indoors	1.5	154.3	0.8	408.0	0.3	184.5	ND	ND	ND	ND	ND	ND	ND	ND	1.5	119.3	0.8	134.0	1.0	89.8	1.0	112.5	2.3	166.8
HLC outdoors	10.3	123.0	0.8	131.8	1.0	143.5	ND	ND	ND	ND	ND	ND	ND	ND	0.8	20.3	1.3	82.8	2.0	50.3	1.3	90.8	3.0	113.8
Tororo																								
HLC indoors	0.3	0.0	2.0	0.0	1.5	0.0	ND	ND	ND	ND	ND	ND	ND	ND	2.8	0.0	2.5	0.0	8.0	0.0	1.0	0.0	0.5	0.0
HLC outdoors	1.8	0.3	1.3	0.0	2.3	0.0	ND	ND	ND	ND	ND	ND	ND	ND	4.0	0.3	1.8	0.0	7.3	0.0	3.3	0.0	1.0	0.0

Key: A.g. = Anopheles gambiae s.l.; **A.f.** = Anopheles funestus s.l.; **ND** = Not done (due to COVID-19 lockdown)

ANNEX B: WALL CONE BIOASSAYS

TABLE 1 A2: WALL BIO-ASSAY RESULTS IN BUGIRI AND TORORO INSECTICIDE DECAY RATE MONITORING SITES, DECEMBER 2020

Time	% Mortality of *An. gambiae* s.s. (Kisumu strain)																Overall Mean
	Actellic 300 CS Bugiri (T0 = March 2020)				Fludora Fusion Bugiri (T0 = March 2020)				Actellic 300 CS Tororo (T0 = March 2020)				Fludora Fusion Tororo (T0 = March 2020)				
	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	
T0	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T4	100 (30/30)	100 (30/30)	96.7 (29/30)	98.9	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	99.7
T5	100 (30/30)	100 (30/30)	93.3 (28/30)	97.8	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	96.7 (29/30)	90.0 (27/30)	95.6	100 (30/30)	100 (30/30)	100 (30/30)	100	98.3
T6	93.3 (28/30)	83.3 (25/30)	83.3 (25/30)	86.6	90 (27/30)	90 (27/30)	90 (27/30)	90	90 (27/30)	83.3 (25/30)	76.7 (23/30)	83.3	86.7 (26/30)	73.3 (22/30)	73.3 (22/30)	77.8	84.4
T7	93.3 (28/30)	80.0 (24/30)	76.7 (23/30)	83.3	86.7 (26/30)	83.3 (25/30)	80.0 (24/30)	83.3	83.3 (25/30	73.3 (22/30)	70.0 (21/30)	75.5	80.0 (24/30)	70.0 (21/30)	66.7 (20/30)	72.2	78.6
T8	86.7 (26/30)	76.7 (23/30)	73.3 (22/30)	78.9	83.3 (25/30)	80.0 (24/30)	76.7 (23/30)	80.0	80.0 (24/30)	70.0 (21/30)	66.7 (20/30)	72.2	76.7 (23/30)	66.7 (20/30)	63.3 (19/30)	68.9	75.0

Key: Bugiri and Tororo, **T0 =** March 2020, 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.

TABLE 2 A2: WALL BIO-ASSAY RESULTS IN LIRA AND OTUKE INSECTICIDE DECAY RATE MONITORING SITES, DECEMBER 2020

Time	% Mortality of *An. gambiae* s.s. (Kisumu strain)																Overall Mean
	Fludora Fusion Lira (T0 = June 2020)				Clothianidin (SumiShield) Lira (T0 = June 2020)				Fludora Fusion Otuke (T0 = June 2020)				Clothianidin (SumiShield) Otuke (T0 = June 2020)				
	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	
T0	100 (30/30)	100 (60/60)	100 (60/60)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (60/60)	100 (60/60)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T1	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T2	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T3	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T4	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T5	100 (30/30)	93.3 (30/30)	83.3 (30/30	92.2	90.0 (30/30)	83.3 (30/30	60.0 (30/30)	77.8	96.7 (30/30)	86.7 (30/30)	60.0 (30/30)	81.1	96.7 (30/30)	86.7 (30/30)	70.0 (30/30)	84.5	83.9
T6	73.3 (22/30)	70.0 (21/30)	73.3 (22/30	72.2	90.0 (27/30)	76.7 (23/30	56.7 (17/30)	74.5	86.7 (26/30)	66.7 (20/30)	80.0 (24/30)	77.8	90.0 (27/30)	80.0 (24/30)	83.3 (25/30)	84.4	77.2
T7																	
T8																	

Lira and Otuke, T0 = June 2020

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.

TABLE 3 A2: FUMIGANT EFFECT OF ACTELLIC 300CS AND FLUDORA FUSION SPRAYING ON MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE* S.S. AND *AN. GAMBIAE* S.L. (WILD STRAIN) IN BUGIRI DISTRICT, EASTERN UGANDA

Insecticide sprayed	Sub-county	Date sprayed	Species	Type of wall surface	Number of mosquitoes tested in sprayed houses/mois	Jul-20 % Mortality	Aug-20 % Mortality	Sep-20 % Mortality	Oct-20 % Mortality
Pirimiphos-methyl CS	Buleesa	20-Mar	Susceptible *An. gambiae* s.s.	Plaster painted	10	100	100	60	30
				Plain brick	10	100	90	60	0
				Mud	10	100	100	0	0
		20-Mar	Wild collected *An. gambiae s.l.*	Plaster painted	10	100	100	80	20
				Plain brick	10	100	100	70	20
				Mud	10	100	90	60	10
Fludora Fusion	Kapyanga	20-Mar	Susceptible *An. gambiae* s.s.	Plaster painted	10	50	20		
				Plain brick	10	50	0		
				Mud	10	50	0		
			Wild collected *An. gambiae s.l.*	Plaster painted	10	100	30		
				Plain brick	10	100	20		
				Mud	10	20	10		

TABLE 4 A2: FUMIGANT EFFECT OF SUMISHIELD AND FLUDORA FUSION SPRAYING ON MORTALITY OF *AN. GAMBIAE* S.S. IN LIRA AND OTUKE DISTRICTS, NORTHERN UGANDA

Insecticide sprayed	District	Sub-county	Date sprayed	Type of wall surface	Number of *An. gambiae* s.s tested in sprayed houses/mois	Jul-20	Aug-20	Sep-20	Oct-20
						% Mortality	% Mortality	% Mortality	% Mortality
SumiShield	Lira	Adeke-kwok	June	Plaster painted	10	80	60	80	30
				Plain brick	10	80	50	80	20
				Mud	10	100	60	70	20
	Otuke	Alango	June	Plaster painted	10	100	80	60	20
				Plain brick	10	90	80	70	10
				Mud	10	90	60	60	10
Fludora fusion	Lira	Adeke-kwok	June	Plaster painted	10	80	100	100	50
				Plain brick	10	80	80	100	30
				Mud	10	90	60	100	20
	Otuke	Otuke T.C.	June	Plaster painted	10	90	70	100	50
				Plain brick	10	80	100	80	40
				Mud	10	80	90	70	20

ANNEX C: INSECTICIDE SUSCEPTIBILITY

TABLE 1 A3: PERCENT 24 HOUR HOLDING MORTALITY OF *ANOPHELES GAMBIAE* S.L. AFTER EXPOSURE TO 6 INSECTICIDES, SEPTEMBER-OCTOBER 2020 (RESULTS FROM ADULTS REARED FROM LARVAE)

Insecticide	Bugiri	Hoima	Gulu	Kamwenge	Katakwi	Kitgum	Lira	Nakaseke	Soroti	Tororo	Wakiso
	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)	# Tested (Mortality)
Organophosphate:											
Pirimiphos-methyl 0.25%	105 (100.0%)	100 (100.0%)	100 (100.0%)	100 (100.0%)	104 (100.0%)	100 (97.0%)	ND	100 (100.0%)	100 (100.0%)	102 (100.0%)	100 (100.0%)
Carbamate:											
Bendiocarb 0.10%	109 (100.0%)	-	-	30 (86.7%)	-	-	-	100 (100.0%)	106 (100.0%)	102 (100.0%)	100 (100.0%)
Pyrethroids:											
Deltamethrin 0.05%	100 (39.0%)	100 (49.0%)	100 (4.0%)	100 (19.0%)	94 (45.7%)	-	100 (83.0%)	100 (89.0%)	-	89 (85.4%)	100 (22.0%)
PBO + Deltamethrin 0.05%	100 (100.0%)	-	100 (96.0%)	-	-	-	100 (100.0%)	100 (100.0%)	-	103 (100%)	100 (100.0%)
Permethrin 0.75% (x1)	105 (55.2%)		100 (0.0%)	100 (15.0%)	103 (72.8%)	-	100 (67.0%)	-	103 (23.3%)	100 (71.0%)	100 (21.0%)
PBO + Permethrin 0.75%	-	-	-	-	-	-	100 (100%)	-	-	-	100 (85.0%)
Alpha-cypermethrin 0.05%	103 (18.4%)	100 (32.0%)	100 (6.0%)	100 (7.0%)	99 (12.1%)	100 (74.0%)	100 (84.0%)	100 (20.0%)	105 (80.0%)	100 (40.0%)	100 (11.0%)
PBO + Alpha-cypermethrin 0.05%	110 (100.0%)	100 (85.0%)	100 (73.0%)	100 (85.0%)	101 (79.2%)	100 (99.0%)	100 (93.0%)	100 (100.0%)	103 (98.0%)	100 (100.0%)	100 (64.0%)
Alpha-cypermethrin 0.25% (x5)	-	-	100 (14.0%)	-	-	-	-	-		-	100 (44.0%)
Alpha-cypermethrin 0.5% (x10)	-	-	100 (56.0%)	-	-	-	-	-	-	-	-
KEY :	**Confirmed resistance**				**Probable resistance**				**Susceptible**		

Key: - = Tests not conducted due to limited number of *An. gambiae* s.l.

TABLE 2 A3: CLOTHIANIDIN DELAYED MORTALITY DURING SUSCEPTIBILITY STUDIES, SEP TO OCT 2020 (RESULTS FOR ADULTS REARED FROM LARVAE)

District	Clothianidin 2% mortality at days post-exposure						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Bugiri	33	78	79	83.3	92.2	93.2	94.2
Hoima	89	98	100				
Gulu	92	96	99.5	100			
Kamwenge	77	88	93	96	98	98	**100**
Katakwi	99	100					
Kitgum	89	92	93	98	98	100	
Lira	93	96	100				
Nakaseke	51	81	100				
Soroti	92	94	98	98	99	99	99
Tororo	91	93	99	100			
Wakiso	88	96	100				