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PMI VECTORLINK CÔTE D'IVOIRE 2020 ANNUAL ENTOMOLOGICAL REPORT JANUARY 2020–DECEMBER 2020

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PMI VECTORLINK CÔTE D'IVOIRE 2020 ANNUAL ENTOMOLOGICAL REPORT JANUARY 2020–DECEMBER 2020

CONTENTS

List of Tables

List of Figures

ACRONYMS

Ace-1	Acetylcholinesterase
b/p/h	Bites per Person per Hour
b/p/n	Bites per Person per Night
CDC	Centers for Disease Control and Prevention
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
f/r/d	Females per Room per Day
HBR	Human Biting Rate
HLC	Human Landing Catch
ib/p/n	Infective Bites per Person per Night
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Net
kdr	Knockdown Resistance
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SOP	Standard Operating Procedure
WHO	World Health Organization

EXECUTIVE SUMMARY

To strengthen its vector control activities, which until 2020 focused on countrywide distribution of insecticide-treated nets (ITNs), Cote d'Ivoire implemented its first-ever large-scale indoor residual spraying (IRS) campaign in two districts using two clothianidin-based insecticides: SumiShield in Nassian and Fludora Fusion in Sakassou. The U.S. President's Malaria Initiative (PMI) VectorLink Project in Côte d'Ivoire supported the 2020 IRS campaign from August 10 to September 12, 2020. The project sprayed a total of 53,949 of the 58,682 structures found by spray operators in targeted districts, resulting in a coverage rate of 91.9%.

Alongside the IRS campaign, the PMI VectorLink Cote d'Ivoire project conducted vector bionomics surveillance, insecticide resistance monitoring, assessment of IRS quality, and insecticide residual efficacy.

Vector surveillance was conducted from January to December 2020 in two sentinel sites within both sprayed districts and two control sites, Béoumi and Dabakala, except in April and May 2020 when the COVID-19 pandemic and restrictions prevented mosquito collection activities. Monthly longitudinal monitoring was conducted using three different methods, human landing catch, pyrethrum spray catch, and Centers for Disease Control and Prevention (CDC) light traps. The entomological parameters assessed were vector composition, seasonality, distribution, biting and resting behavior, sporozoite infection, parity, and entomological inoculation rate (EIR).

Insecticide susceptibility tests, resistance intensity tests, and synergist assays were also conducted in 18 sites distributed across the country, including the four vector surveillance sites and 14 insecticide resistance monitoring-only sites (Abengourou, Abidjan, Aboisso, Adzopé, Bouaké, Bouna, Daloa, Gagnoa, Jacqueville, Korhogo, Man, Odienné, San Pedro, and Yamoussoukro). World Health Organization (WHO) susceptibility tube tests were used to test the resistance status, resistance intensity, and piperonyl butoxide (PBO) synergism of alpha-cypermethrin, deltamethrin, permethrin, pirimiphos-methyl, bendiocarb, and clothianidin insecticides against the locally collected malaria vectors. CDC bottle assays were used to test vector susceptibility to chlorfenapyr.

Spray quality was assessed within one week of IRS in one village in Nassian and two villages in Sakassou in remote villages where IRS started based on the established progress plan. The test was repeated during the third week of spraying in a second set of selected villages (Adjekro, Kpatanou, and Kpetebonou in Sakassou and Lande, Nassian city, and Parhadi, in Nassian) that were more easily accessible for the follow up cone bioassay where the residual life of the sprayed insecticides was monitored monthly.

Species composition across all longitudinal monitoring sites showed that *An. gambiae* s.l. was the predominant malaria vector, representing 94.8% (n=52,076) of the total *Anopheles* collected. *Anopheles gambiae* s.l. mean indoor resting densities (IRDs) were highest in Sakassou (0.8 to 29.4 female per room per day (f/r/d)), followed by Dabakala (0.2 to 9.4 f/r/d); the lowest was observed in Nassian (0 to 6.1 f/r/d). The overall *An. gambiae* s.l. mean IRD decreased after IRS in both IRS and control sites, from 8.2 to 3.4 f/r/d in sprayed sites and from 4.3 to 2.9 f/r/d in control sites. The overall *An. gambiae* s.l. female biting activities were highest between 12:00 p.m. and 5:00 a.m., both indoors and outdoors, in all sites. The *An. gambiae* s.l. mean biting rate decreased after IRS in sprayed sites from 260.7 bites per person per night (b/p/n) to 157.2 b/p/n, while it increased in control sites from 44.2 to 81.8 b/p/n in the same time frame. Parity rates were high in all sites, ranging from 51.8% in Béoumi to 92.5% in Nassian. Similarly, *An. gambiae* s.l. parity rates in the sprayed and control sites after IRS was 9.5% and 4.2% more parous female mosquitoes, respectively. Nassian recorded the highest sporozoite infection rate (0.067) followed by Dabakala (0.060) and Béoumi (0.046). After IRS, fewer mosquitoes were infected in sprayed sites, with a 60.3% reduction in the EIR compared to the control sites, which recorded a 19% reduction in the EIR

In contrast, *An. funestus* s.l. IRD increased after IRS from 0.32 to 2.29 f/r/d in sprayed sites and decreased from 0.18 to 0.08 in control sites. The mean human biting rate of *An. funestus* s.l. increased after IRS in sprayed sites (from 1.4 to 1.7 b/p/n) compared to control sites, where it decreased (from 0.9 to 0.4 b/p/n) in the same period. The parity rate decreased by 8.7% after IRS in sprayed sites..

Resistance was observed in *An. gambiae* s.l. to the diagnostic dose of all pyrethroids and bendiocarb in all sites surveyed for insecticide resistance. High pyrethroid resistance was observed in all sites tested except for alpha-cypermethrin in Aboisso, deltamethrin in Daloa and Jacqueville, and permethrin in Adzopé, where moderate pyrethroid resistance was observed. Susceptibility to the diagnostic dose of pirimiphos-methyl was observed in 14

sites, while the remaining four other sites recorded low resistance. Pre-exposure to PBO did not yield full susceptibility to pyrethroids but induced a substantial increase in mortality in nine of the 18 sites. *An. gambiae* s.l. was susceptible to chlorfenapyr in 10 of the 18 sites at the dose of 100 µg/bottle, and in 17 of the sites at the dose of 200 µg/bottle. For clothianidin, susceptibility was observed in nine of 18 sites seven days post-exposure. Resistance to bendiocarb was also observed in all sites.

Molecular species identification of mosquitoes collected for insecticide susceptibility testing indicated that *An. coluzzii* represented the predominant species in 13 sites, and *An. gambiae* s.s. was predominant in the other five. A few hybrids of both species were identified in Abidjan.

Both knockdown resistant (*kdr*)-West and -East mutations were present in all sites except Nassian and Odienné. That is consistent with the high resistance to pyrethroids commonly observed in Côte d'Ivoire. The acetylcholinesterase (*Ace-1*) target site mutation observed in all sites was also consistent with phenotypic resistance to bendiocarb in all sites and to pirimiphos-methyl in some sites.

One hundred percent (100%) mortality of *An. gambiae* Kisumu exposed to cement and mud walls sprayed with SumiShield and to cement walls sprayed with Fludora Fusion was recorded within a week after IRS in both sprayed districts, showing that the 2020 IRS application was not underdosed. The monthly insecticide residual life assessment conducted from September to March 2021 showed that both insecticides sprayed were effective on all cement and mud surfaces for at least the seven months in which testing took place post spraying.

The vector surveillance data confirm all the site population density, biting, and behavior trends per month, as observed in previous years and can be considered for IRS timing. A slight decrease in density was observed after IRS compared to the control sites during the same post-IRS period. The findings also suggest that clothianidin-based insecticides are still appropriate for IRS in both sites. Based on the susceptibility status of vector populations, pirimiphos-methyl-based products could be considered for IRS in certain districts, including in Nassian, as part of an insecticide rotation strategy to mitigate resistance. Furthermore, the data confirmed that the decision made to procure PBO ITNs and dual-active-ingredient ITNs (e.g., Interceptor G2) for the 2021 ITN distribution campaign in the country was appropriate and will further contribute to insecticide resistance management.

I. INTRODUCTION

Malaria is a leading cause of morbidity and mortality in Côte d'Ivoire. It accounts for about 33% of outpatient visits in health facilities, with an incidence of 134 per 1000 cases in the general population and 247 per 1,000 among children under 5, according to the 2017 National Malaria Control Program (NMCP) report. To reduce the malaria burden, the main malaria vector control method used in Côte d'Ivoire has historically been the distribution and use of insecticide-treated nets (ITNs) through mass campaigns and routine distribution. Since the NMCP started ITN mass and routine distributions, only pyrethroid-based ITNs have been distributed. The National Malaria Strategic Plan 2016–2020 prioritized indoor residual spraying (IRS) as an additional vector control method to reduce malaria morbidity and mortality. From August 10 to September 12, 2020, the U.S. President's Malaria Initiative (PMI) VectorLink Project conducted IRS in Nassian and Sakassou targeting 597,625 structures using clothianidin-based insecticides (SumiShield and Fludora Fusion). The project sprayed a total of 53,949 structures out of 58,682 structures found by spray operators in targeted districts, resulting in a coverage rate of 91.9%.

Entomological surveillance is a key component of IRS programming, providing information on the impact of IRS on malaria vector density and behavior in sites where IRS was implemented compared to non-IRS sites. In 2020, VectorLink Côte d'Ivoire, in collaboration with the Centre Suisse de Recherches Scientifiques (the subcontractor) and all local entomological research institutes, conducted longitudinal entomological surveillance in four sites selected by the NMCP and generated data on key entomological indicators including malaria vector species composition, density, feeding behavior, parity, and infection rates in the four districts. In addition, VectorLink Côte d'Ivoire conducted insecticide susceptibility tests in 18 sites across the country, assessed the quality of spray during the IRS campaign, and monitored the residual life of the insecticides after IRS. These data support the NMCP and the malaria vector control stakeholders (including PMI VectorLink) in determining the optimal timing and insecticides for IRS and inform the selection of ITNs for distribution campaigns.

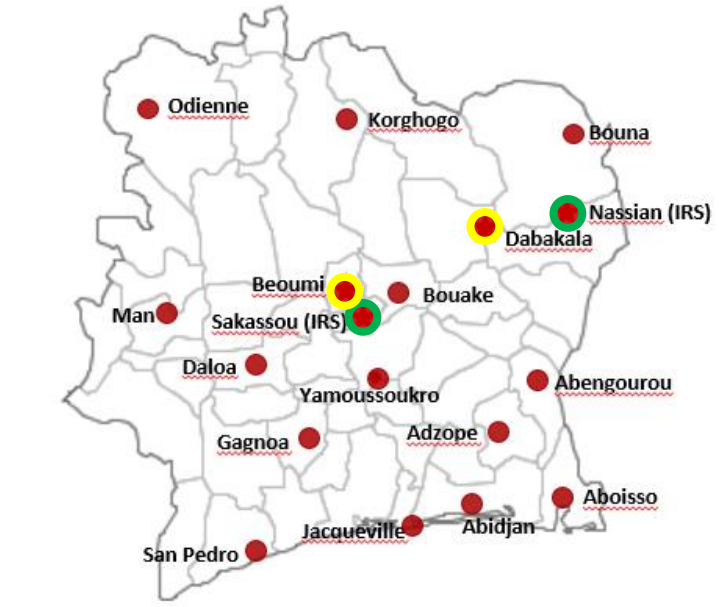
2. METHODOLOGY

From January through December 2020, VectorLink Côte d'Ivoire conducted longitudinal entomological surveillance in four NMCP-selected sites and insecticide resistance monitoring in 18 sites including the four longitudinal monitoring sites.

2.1 ENTOMOLOGICAL MONITORING SITES

VectorLink Côte d'Ivoire conducted comprehensive monitoring (vector surveillance and insecticide resistance monitoring) in two IRS sites in 2020 (Nassian and Sakassou) and two control sites (Béoumi and Dabakala), and insecticide resistance monitoring only in 14 additional sites including Abengourou, Abidjan, Aboisso, Adzopé, Bouaké, Bouna, Daloa, Gagnoa, Jacqueville, Korhogo, Man, Odienné, San Pedro, and Yamoussoukro. IRS quality assessment and longitudinal residual efficacy monitoring were done at the two sprayed sites. Figure 1 shows the location of the sentinel sites in their respective districts.

FIGURE 1: MAP OF CÔTE D'IVOIRE SHOWING THE 2020 PMI VECTORLINK ENTOMOLOGICAL MONITORING SITES



Comprehensive vector monitoring sites with IRS (2)
Comprehensive vector monitoring sites without IRS (2)
Insecticide resistance monitoring sites (18 including the 4 IRS sites)

2.2 VECTOR BIONOMICS MONITORING

Adult mosquitoes were collected using human landing catch (HLC), pyrethrum spray catch (PSC), and Centers for Disease Control and Prevention (CDC) light trap methods. The HLCs were conducted during two consecutive nights in four houses (two urban and two rural, at a minimum of five km from the town) per site per month. The PSCs were conducted in 30 houses (15 urban and 15 rural) per site per month. The CDC light trap collections were performed in four houses (two urban and two rural, and other than those used for the HLCs) during two consecutive nights per site per month. The same houses were maintained for HLC and CDC light trap collections throughout

the longitudinal monitoring, while randomly selected houses were used each month for PSC collections depending on the availability of households. Collections were conducted every month from January through December 2020 except in April and May, when no collection activities could take place due to COVID-19 pandemic restrictions; thus, 10 months of collection were completed. Table 1 summarizes the collection times and sampling methods. All entomological data were collected following the PMI VectorLink standard operating procedures (SOPs).[1]

TABLE 1: LONGITUDINAL MONITORING COLLECTION METHODS

Collection Method	Time	Frequency	Sample
HLC	6:00 p.m. to 6:00 a.m.	Two nights per site per month	Four houses per site (two urban and two rural); same houses each month
PSC	6:00 am to 8:00 a.m.	Two days per site per month	30 houses per site (15 urban and 15 rural)
CDC light trap	6:00 p.m. to 6:00 a.m.	Two nights per site per month	Four houses per site (two urban and two rural; same houses each month)

HLCs were performed indoors and outdoors to collect adult mosquitoes landing on human bait (mosquito collectors) following SOP 02/01. With legs exposed to attract host-seeking mosquitoes, one human bait was seated indoors and another one outdoors in each house for two consecutive nights per month for a total of eight person-nights indoors and eight person-nights outdoors per site per month. The collectors switched between indoors and outdoors on an hourly basis to control for potential differences in attractiveness. The doors of the houses were kept closed when collections were underway. The collectors used flashlights and hemolysis tubes to collect mosquitoes that landed on their legs before they could be bitten. The tubes were covered with cotton after individual collection of mosquitoes. The teams transferred the mosquitoes hourly to custom-made labelled bags over 12 hours.

The PSCs were carried out between 6:00 a.m. and 8:00 a.m. during two days per site per month following SOP 03/01. White sheets were placed on the floor from wall to wall in sampled rooms. The rooms were sprayed with the commercial aerosol made of pyrethroid insecticides and PBO, after closing the house's windows and doors and removing drinking water and food items from the room. For houses with open eaves, collectors sprayed from the outside through the eaves before entering and spraying indoors. Ten minutes after spraying, all mosquitoes knocked down by the chemical were collected from the white sheets using forceps. The mosquitoes were kept in Petri dishes and then sorted by species using a morphological identification key (Gillies and Coetzee 1987, Coetzee 2020). The abdominal status of all female *Anopheles* was determined and sorted into four categories: unfed, blood-fed, half-gravid, and gravid.

CDC light traps were installed indoors in selected houses where people slept under an ITN following SOP 01/01. The CDC light traps were suspended in a bedroom 1.5 meters above the floor, near the sleeper's legs. Traps were set 15-30mins before 6:00 pm to enable the collection starting from about 6:00 p.m. to 6:00 a.m. to ensure that surveillance was conducted during the suspected peak host-seeking periods.

All mosquitoes collected through each method were morphologically identified to genus. *Anopheles* mosquitoes were identified to species or species complex by microscope, using identification keys (Gillies and Coetzee 1987, Coetzee 2020). The identification was done by a team of well-trained technicians from research institutes and by VectorLink Côte d'Ivoire staff. A subsample of *An. gambiae* s.l. from each site was dissected to estimate parity rate. All mosquitoes were preserved on silica gel in Eppendorf tubes for further laboratory processing to identify sibling species, resistance mechanisms, plasmodium infection, and source of blood meal, using polymerase chain reaction and enzyme-linked immunosorbent assay (ELISA).

The indicators listed in Table 2 were calculated based on the number of mosquitoes collected through each collection method.

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

TABLE 2: VECTOR SURVEILLANCE INDICATORS PER COLLECTION METHOD

Collection Method	Indicator	Definition
HLC	HBR	Mean number of b/p/n
	Peak biting time	Hour of highest HBR
	Parity rate	Percentage of parous mosquitoes/total dissected
	Exophagic rate	Percentage of mosquitoes biting outside
	Endophagic rate	Percentage of mosquitoes biting inside
PSC	Indoor resting density	Mean number of mosquitoes/house/day
	% of fed females	Number of fed mosquitoes/total collected by PSC
CDC light trap	Indoor density	Mean number of mosquitoes collected indoors/trap/night

Note: HBR=human biting rate, b/p/n=bites per person per night

2.3 INSECTICIDE RESISTANCE MONITORING

From August through November 2020, VectorLink Côte d'Ivoire completed insecticide resistance monitoring in 18 sites across the country including the four longitudinal monitoring sites (see above Figure 1, Section 2.1). Larvae and pupae of *An. gambiae* s.l. were collected in each site from several larval habitats, pooled, and reared to adulthood in the field laboratory. Insecticide susceptibility tests were conducted on two- to five-day-old adult females using World Health Organization (WHO) tube tests (SOP 06/01) and CDC bottle assays (SOP 04/01).

For each tube test, about 80–100 female *An. gambiae* s.l. were tested against the insecticide (in four batches of 20–25) and an additional 40–50 were tested in two control tubes (20–25 each) in parallel.

The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), bendiocarb (0.1%), and pirimiphos-methyl (0.25%) were tested in all sites.

When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested at 5 and 10 times the diagnostic concentration of permethrin, deltamethrin, alpha-cypermethrin, bendiocarb, and pirimiphos-methyl.

Clothianidin 2% papers were treated locally using a protocol designed by PMI VectorLink. The susceptibility testing was conducted as described above, and the mortality was recorded up to seven days post-exposure.

Synergist assays with piperonyl butoxide (PBO) were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol to determine the involvement of cytochrome P450s in pyrethroid resistance. A high percent mortality and/or reversal of susceptibility when pre-exposed to PBO indicates probable involvement of enzymes such as P450s in the resistance mechanism.

CDC bottle assays were conducted using chlorfenapyr at the doses of 100µg/bottle and 200µg/bottle with one-hour exposure, and mortality was recorded every 24 hours up to three days (72 hours). For all tests, resistance and intensity were defined following the WHO criteria (WHO 2016):

Resistance status at diagnostic doses:

- 98% or greater mortality indicates susceptibility.
- Between 90 and 97% mortality indicates possible resistance.
- Less than 90% mortality indicates confirmed resistance.

Resistance intensity with mortality at 5x and or mortality at 10x:

- 98–100% at 5x: Low resistance
- <98% at 5x and 98–100% at 10x: moderate resistance
- <98% at 10x: high resistance

2.4 Molecular Characterization

Insecticide resistance in mosquitoes can be related to target-site mutations. Among these, resistance to pyrethroids and dichlorodiphenyltrichloroethane (i.e., DDT) is described as a substitution of the amino acid leucine with either phenylalanine (L1014F, referred to as knockdown rate (*kdr)*-West) or serine (L1014S, referred to as *kdr*-East) at the position 1014 in the sodium channel gate. For organophosphate and carbamate insecticides, the target site mechanism, known as acetylcholinesterase (*Ace-1*), is a substitution of an amino acid glycine to serine at position 119. About 50 *An. gambiae* s.l. mosquitoes were randomly selected from each of the 18 sites among the dead and surviving mosquitoes from the WHO susceptibility tests, and further analyzed to assess molecular markers of insecticide resistance. The presence of *kdr*-West and *kdr*-East was characterized using the Taqman protocol described by Bass et al. (2007).

In the four longitudinal monitoring sites, a subsample of about 400, 100, and 100 females per site preserved from the HLC, PSC, and CDC light trap collections, respectively, were used to determine subspecies of *An. gambiae* s.l. The DNA of each individual mosquito was extracted using the protocol designed by Collins et al. (1987). *An. gambiae* complex species were identified as *An. gambiae*, *An. coluzzii,* or hybrids of the two species, following the Short-Interspersed Element protocol described by Santolamazza et al. (2008).

The sporozoite infection status of a subsample of mosquitoes collected by HLC from each site was determined using ELISA for the identification of *Plasmodium falciparum* Circumsporozoite infection.

2.5 Quality Assurance of IRS and Insecticide Decay Rate

Cone bioassays using susceptible *An. gambiae* Kisumu strain mosquitoes were conducted during the IRS campaign to confirm the quality of spray in Nassian and Sakassou (SOP 09/01). During the first week of the IRS campaign, the spray quality assessment was conducted in one village (Brombiredouo) in Nassian and two (Mamela and Kpatanou) in Sakassou. A separate group of more centrally located sentinel villages were selected for subsequent monthly cone bioassay (the first time point, T0, was collected immediately after IRS was delivered in these sites in the final week of the spray campaign) to enable easy access and favorable conditions for the mosquitoes.[2] In Nassian, the tests were conducted in Parhadi, Lande, and Nassian city on both mud and cement surfaces found in the area. Three cement houses were selected in each of the three villages and two additional mud wall houses were selected in Parhadi and Nassian for a total of nine cement houses and four mud houses. In Sakassou, the villages of Adjekro, Kpatanou, and Kpetebonou were selected. Three cement houses (the only substrate used in the district) were selected in each of the three villages. In addition to the sprayed structures, one unsprayed structure (ineligible for IRS due to food storage) was used as control bioassays in each of the sites. The cone bioassays were repeated monthly until mosquito mortality dropped below 80% for two consecutive months (Table 3).

The cones were placed on the treated walls at 0.5m, 1m, and 1.5m above the ground. Ten female *An. gambiae* Kisumu or wild collected *An. gambiae* s.l. (specific to Sakassou) were introduced per cone and exposed for 30 minutes. After the 30 minutes, the exposed mosquitoes were retrieved back in the corresponding disposable cups, and the cups were then placed in a rack covered with a damp towel to create favorable humidity for the mosquitoes until laboratory where they were held for up to five days due to the slow-acting nature of the insecticides. The number of mosquitoes knocked down after 30 minutes and 60 minutes and the number dead after every 24 hours of holding were recorded up to five days. When the mortality of the control was between 5 and 20%, corrected mortality was determined using Abbot's formula.

[2] In 2021, IRS quality assurance will be conducted in the first week of spray operations in the same sites that will be monitored for residual life monitoring.

To assess the fumigant (airborne) effect of the insecticide, two replicates of 10 mosquitoes were placed in a small cage 1.0 meter above the floor and about 0.1 meter from the sprayed wall. After 30 minutes of exposure, the mosquitoes were transferred to insecticide-free paper cups supplied with 10% sugar solution and mortality was recorded every 24 hours and up to five days.

TABLE 3: QUALITY ASSURANCE AND INSECTICIDE RESIDUAL EFFICACY ACTIVITIES

Activity	Frequency	Sample
Quality assurance of IRS	Once within a week of spraying	Thirteen houses in Nassian (three sprayed cement per village and two sprayed mud in two villages) Nine houses in Sakassou (three sprayed cement in each of the three villages)
Monitoring of insecticide: residual efficacy on walls	Monthly, until exposed mosquito mortality falls below 80% for two consecutive months	Thirteen houses in Nassian (three sprayed cement per village and two sprayed mud in two villages) Nine houses in Sakassou (three sprayed cement in each of the three villages)
Monitoring of insecticide: fumigation effect	Monthly, until exposed mosquito mortality falls below 50% for two consecutive months	Five houses in Nassian (one cement per village and two sprayed mud in two villages) Three houses in Sakassou (one per village)

2.6 DATA PRESENTATION AND STATISTICAL ANALYSIS

The District Health Information Software (DHIS2)-based VectorLink Collect database was used for entomological data management in Côte d'Ivoire for the first time in 2020. The PMI VectorLink home office staff trained VectorLink Côte d'Ivoire entomologists and database managers on updated data workflows, including field paper collections, technical reviews, data entry, data cleaning, and analytics, to support the generation and use of high-quality entomological data. All entomological data collected in Côte d'Ivoire in 2020 were analyzed in VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. In 2021, key stakeholders, including NMCP, the scientific working group (SWG), Swiss Centre of Scientific Research (CSRS), and PMI, will all have ongoing access to these results dashboards to support timely decision making.

Control and IRS site entomological parameters including species composition, human blood index, sporozoite rate, and entomological inoculation rate (EIR) data were categorized as IRS (sprayed) or control (non-sprayed) and compared a month before IRS (August 2020) and after IRS from September to December 2020. We considered five main parameters: indoor resting density (IRD), the HBR, the proportion of gravid and fed vectors, the parity rate, and the EIR with separate analyses for *An. gambiae* s.l. and for *An. funestus* s.l. Quantitative data on IRD, HBR, and EIR were compared using a mixed linear model. A statistically significant difference was observed when p-value <0.05.

3. RESULTS

3.1 VECTOR BIONOMICS MONITORING

Vector bionomics activities were conducted in the two IRS and two control sites from January to December 2020. Activities were suspended in April and May in compliance with the PMI VectorLink Project's Mitigation Measures and Modifications for Vector Control Monitoring Activities in the Context of COVID-19.

3.1.1 SPECIES COMPOSITION

3.1.1.1 OVERALL SPECIES COMPOSITION

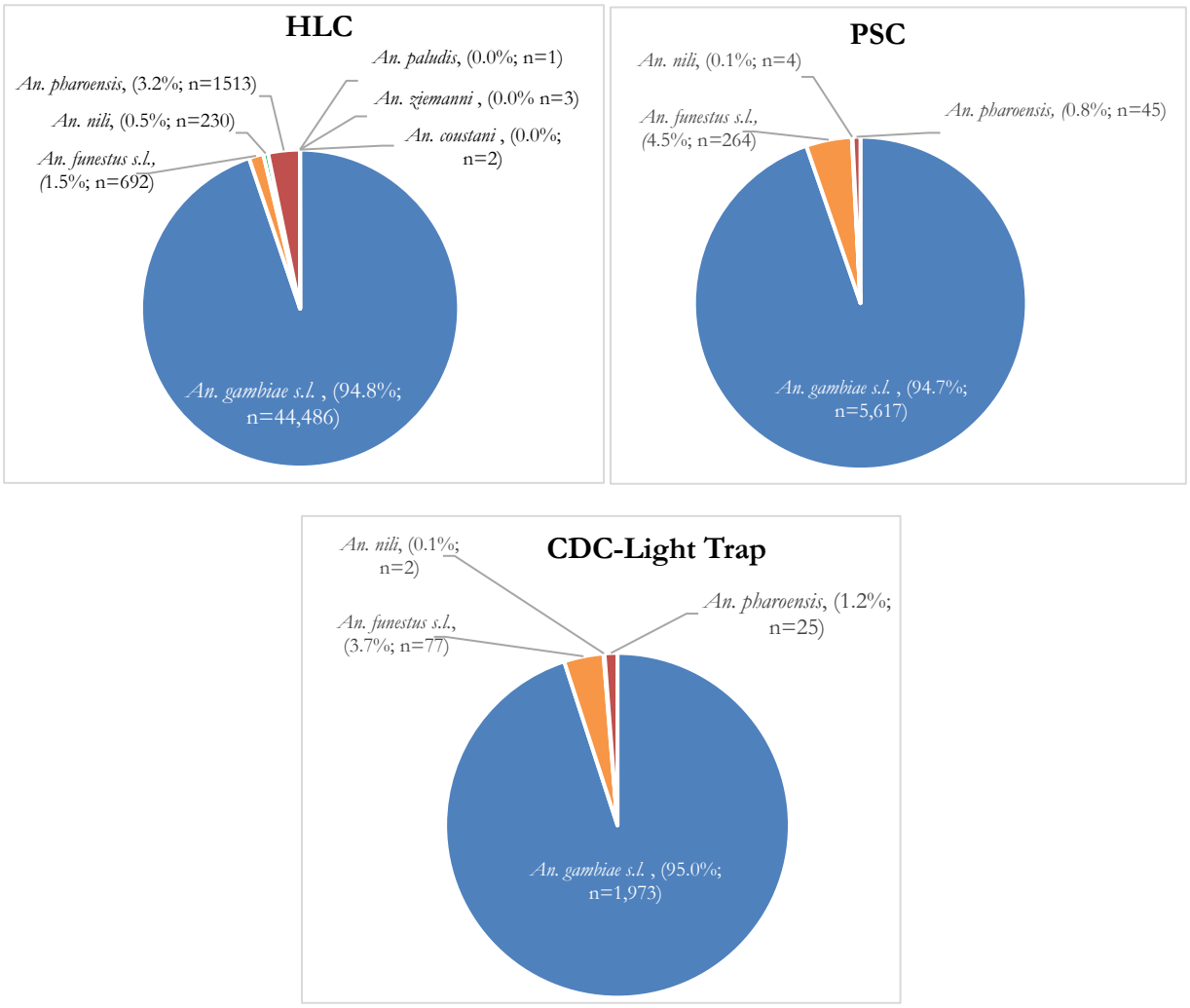
Over the longitudinal monitoring period, a total of 67,810 mosquitoes were collected in the four sentinel sites using the three collection methods: HLC, PSC, and CDC light trap. Nineteen percent (n=12,876) were culicines and 81% (n=54,934) were *Anopheles* mosquitoes (Table 4). Seven *Anopheles* species were collected across the surveyed sites. *An. gambiae* s.l. (n=52,076; 76.8% of all mosquitoes collected) was the predominant malaria vector species, representing 94.8% of the total *Anopheles* mosquitoes collected across all sites and methods. Two secondary malaria vectors were recorded among the total *Anopheles* collected: *An. funestus* s.l. (1.5%; 1,033) and *An. nili* (0.3%; n=236). The four non-malaria vectors collected represented 2.3% (n=1,589) of *Anopheles* species; the majority was *An. pharoensis* (n=1,583), as well as *An. coustani* (n=2), *An. ziemanni* (n=3), and *An. paludis* (n=1).

All seven *Anopheles* species collected were found through HLCs. Of all 54,934 *Anopheles* species collected, 86.8% (47,678) were caught using this method. The *Anopheles* were predominantly *An. gambiae* s.l. (94.8%; n=44,486) (Figure 2). Additional species consisting of *An. funestus* s.l. (1.5%; n=692) and *An. nili* (0.5%; n=230); *An. pharoensis* (n=1,513), *An. coustani* (2), *An. ziemanni* (3), and *An. paludis* (1) represented about 3.2% of *Anopheles* caught by HLC. PSCs collected 5,930 *Anopheles* mosquitoes (10.8% of the total collected). As with the HLC collection, *An. gambiae* s.l. (94.7%; n=5,617) was the main vector collected. *An. funestus* s.l. (4.5%; n=264) and *An. nili* (0.1%; n=4) were the other malaria vector species collected using PSC. The only other *Anopheles* species found was *An. pharoensis* (0.8%; n=45). Few *Anopheles* (3.8%; n=2077) were collected using CDC light traps. Again, *An. gambiae* s.l. was predominant (95.0%; n=1,973); *An. funestus* s.l. and *An. nili* accounted for 3.7% (77) and 0.1% (2), respectively (Annex Tables A1–A3).

TABLE 4: NUMBER OF MOSQUITOES COLLECTED IN ALL SITES USING ALL COLLECTION METHODS

	Béoumi	Dabakala	Nassian	Sakassou	Total	Species % of Total
An. gambiae s.l.	4,241	7,730	1,787	38,318	52,076	76.8
An. funestus s.l.	115	225	610	83	1,033	1.5
An. nili	0	189	20	27	236	0.3
An. pharoensis	376	255	11	941	1,583	2.3
An. coustani	0	0	0	2	2	0.0
An. paludis	0	1	0	0	1	0.0
An. ziemanni	0	2	0	1	3	0.0
Culicine	3,155	6,671	545	2,505	12,876	19.0
Total	7,887	15,073	2,973	41,877	67,810	

FIGURE 2: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN THE FOUR SITES USING HLC, PSC, AND CDC LIGHT TRAPS FROM JANUARY TO DECEMBER 2020

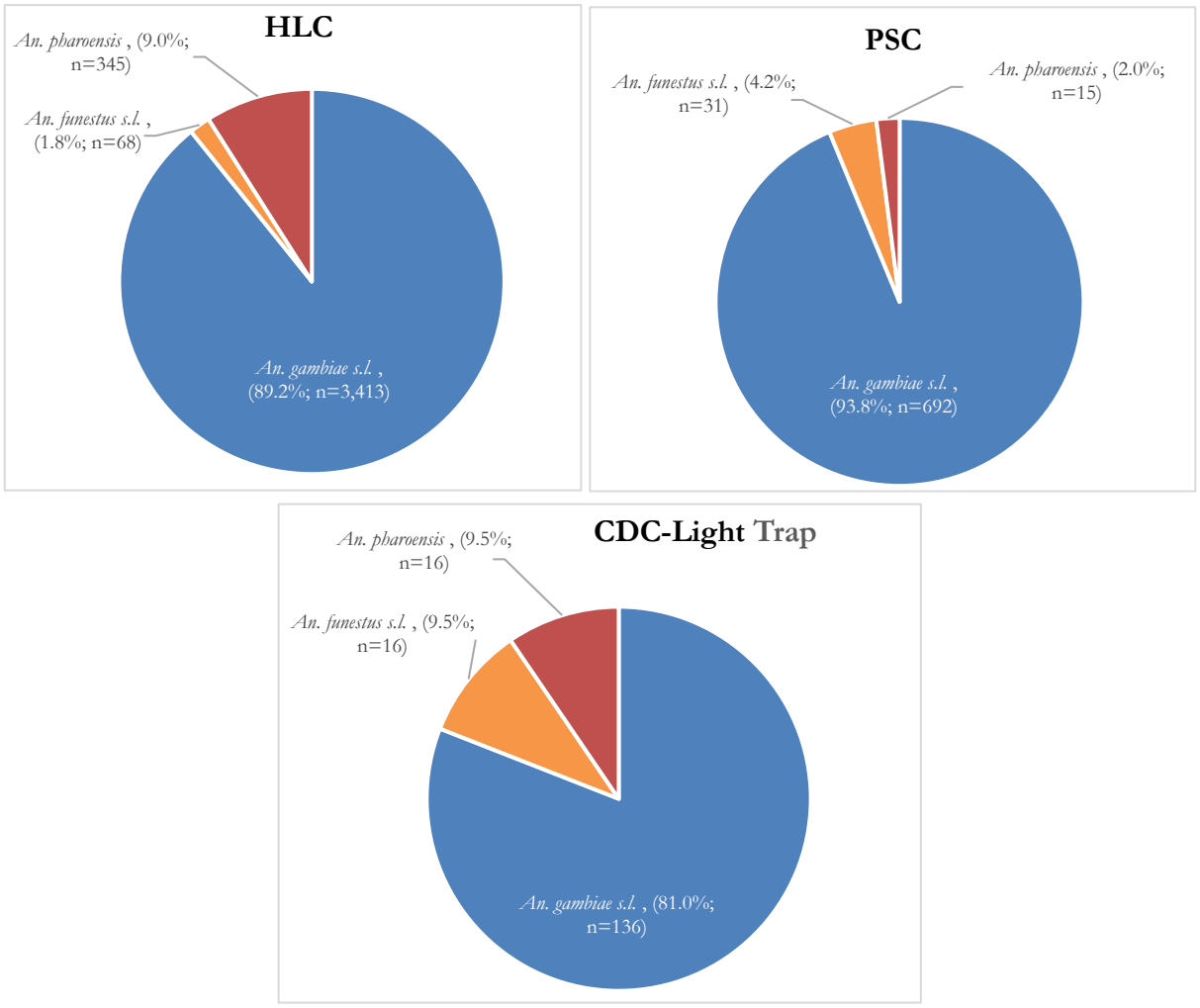


3.1.1.2 SPECIES COMPOSITION IN BÉOUMI

A total of 4,732 *Anopheles* mosquitoes were caught in Béoumi over the 10 months using all three collection methods, representing 8.6% (4,732/54,934) of *Anopheles* mosquitoes collected across all sites. *An. gambiae* s.l. was the predominant malaria vector species collected (89.6%, n=4,241), followed by *An. funestus* s.l. (2.4%; n=115). *An. pharoensis* (7.9%; n=376) represented the only additional and non-vector *Anopheles* species collected through all the collection methods.

About 80.9% (n=3,826) of the *Anopheles* mosquitoes collected were caught using HLC and 89.2% (n=3,413) were *An. gambiae* s.l. A few *An. funestus* s.l. (1.8%; n=68) and *An. pharoensis* (9.0%, n=345) were collected by HLCs (Figure 3. PSC yielded 738 *Anopheles* mosquitoes overall, representing 15.6% of the total collected in Béoumi. *An. gambiae* s.l. (93.8%; n=692) was the predominant *Anopheles* vector collected by PSC, followed by *An. funestus* s.l. (4.2%; n=31). The other *Anopheles* species found was *An. pharoensis* (2.0%; n=15). With CDC light traps, 168 (3.6%) *Anopheles* mosquitoes were collected, including 136 *An. gambiae* s.l. (81.0%), 16 *An. funestus* s.l. (9.5%), and 16 *An. pharoensis* (9.5%) (Annex Tables A1–A3).

FIGURE 3: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN BÉOUMI USING HLC, PSC, AND CDC LIGHT TRAP FROM JANUARY TO DECEMBER 2020

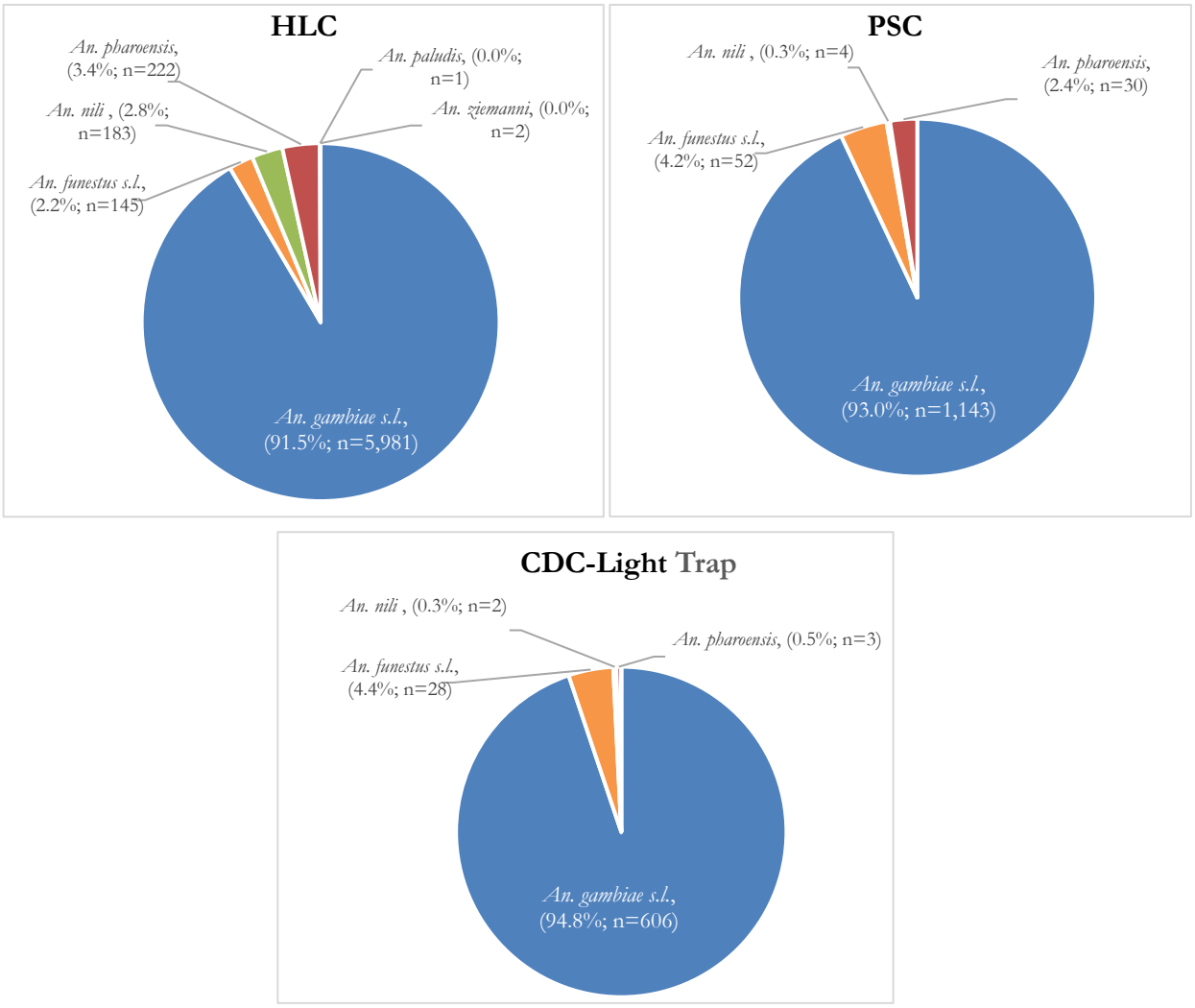


3.1.1.3 SPECIES COMPOSITION IN DABAKALA

In Dabakala, a total of 8,402 *Anopheles* mosquitoes (15.3%; 8,402/54,934) of the overall collection were collected using the three methods. *An. gambiae* s.l. was the predominant malaria vector species collected (92.0%; n=7,730). *An. funestus* s.l. and *An. nili* represented the other malaria vectors found, at 2.7% (n=225) and 2.2% (n=189) respectively. A higher number of *An. nili* was collected in Dabakala than in the three other sites. *An. pharoensis* represented 3.1% (n=255) of all *Anopheles* collected in the sites.

A total 6,531 *Anopheles* mosquitoes were collected by HLC (77.7% of total); 91.5% of them were *An. gambiae* s.l. (n=5,981), 2.2% were *An. funestus* s.l. (n=145), and 2.8% were *An. nili* (n=183) (Figure 4). The single *An. paludis* (n=1) recorded over the 10 months was found in Dabakala. The other species included *An. pharoensis* (3.4%; n=222) and *An. ziemanni* (n=2). PSC yielded 1,894 *Anopheles* mosquitoes representing 22.5% of the *Anopheles* collected in Dabakala. *An. gambiae* s.l. was the predominant species collected (93.0%; n=1143) followed by *An. funestus* s.l. (4.2%, n=52). Only four *An. nili* were collected (0.2%); 30 were *An. pharoensis* (2.4%). CDC light trap collections recorded 636 *Anopheles* mosquitoes (7.6%), mostly *An. gambiae* s.l. (94.8%; n=606) and a small number of *An. funestus* s.l. (4.4%; n=28) and *An. nili* (0.3%; n=2) (Annex Tables A1–A3).

FIGURE 4: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN DABAKALA USING HLC, PSC, AND CDC LIGHT TRAPS FROM JANUARY TO DECEMBER 2020

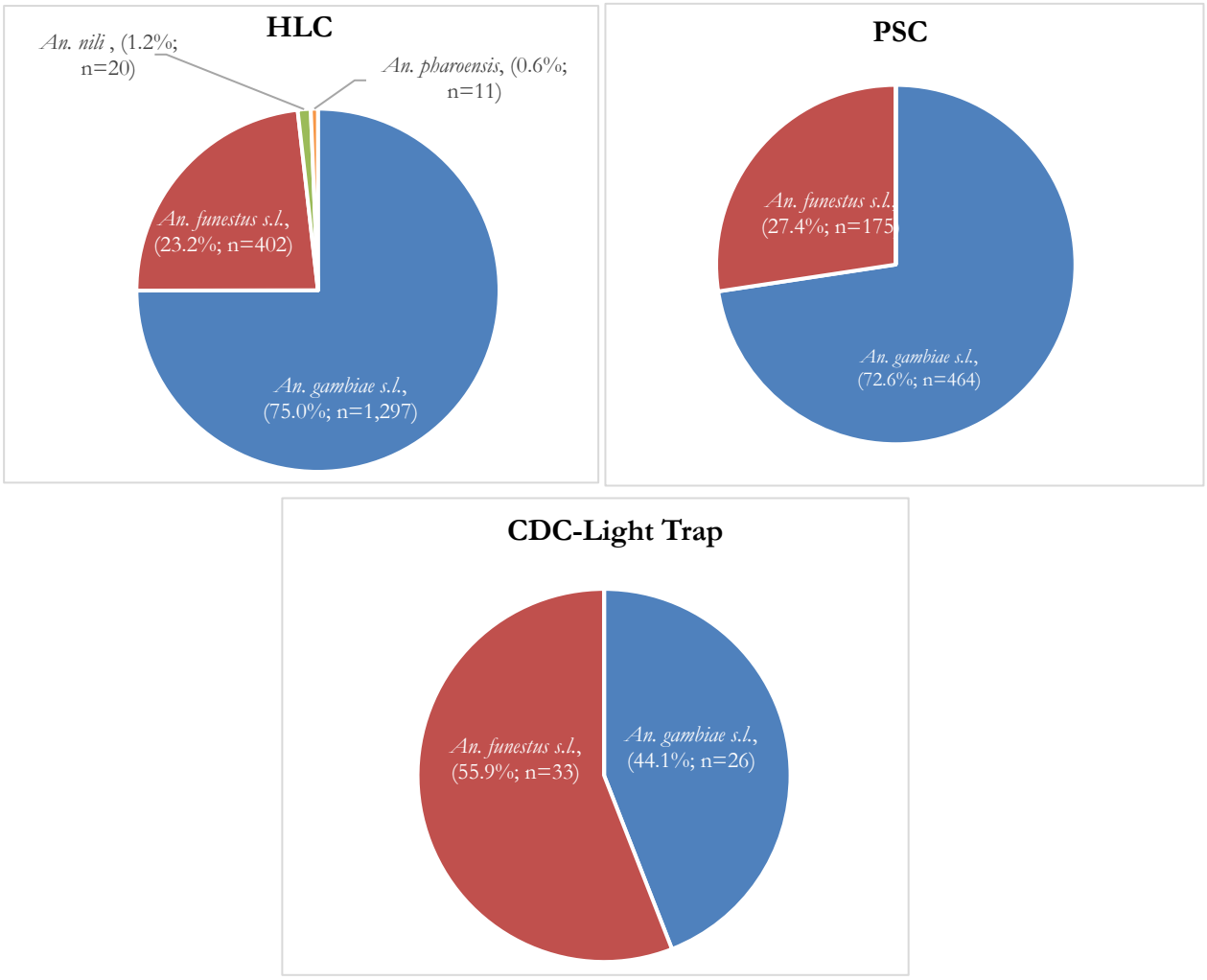


3.1.1.4 SPECIES COMPOSITION IN NASSIAN

In Nassian, the three collection methods yielded a total of 2,428 *Anopheles* mosquitoes, representing 4.4% of all *Anopheles* mosquitoes recorded at all sentinel sites. *An. gambiae* s.l. was the main vector species collected (73.6%; n=1,787). Nassian was the site that recorded the largest proportion (59%) of the total *An. funestus* s.l. caught. After *An. gambiae* s.l., *An. funestus* s.l. represented the next largest proportion (25.1%; n=610). A few *An. nili* (1.2%; n=20) and *An. pharoensis* (0.6%; n=11) were collected, all by HLC (Figure 5). Overall, HLC yielded 1,719 *Anopheles* mosquitoes, of which 1,297 were *An. gambiae* s.l. (75.0%) and 402 were *An. funestus* s.l. (23.2%).

An. gambiae s.l. and *An. funestus* s.l. were the only *Anopheles* mosquitoes collected by PSC and CDC light trap: 464 *An. gambiae* s.l. (72.6%) and 175 *An. funestus* s.l. (27.4%) constituted the 639 *Anopheles* mosquitoes collected using PSC, while 26 *An. gambiae* s.l. (44.1%) and 33 *An. funestus* s.l. (55.9%) were collected by CDC light trap (n=59) (Annex Tables A1–A3).

FIGURE 5 : SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN NASSIAN USING HLC, PSC, AND CDC LIGHT TRAPS FROM JANUARY TO DECEMBER 2020



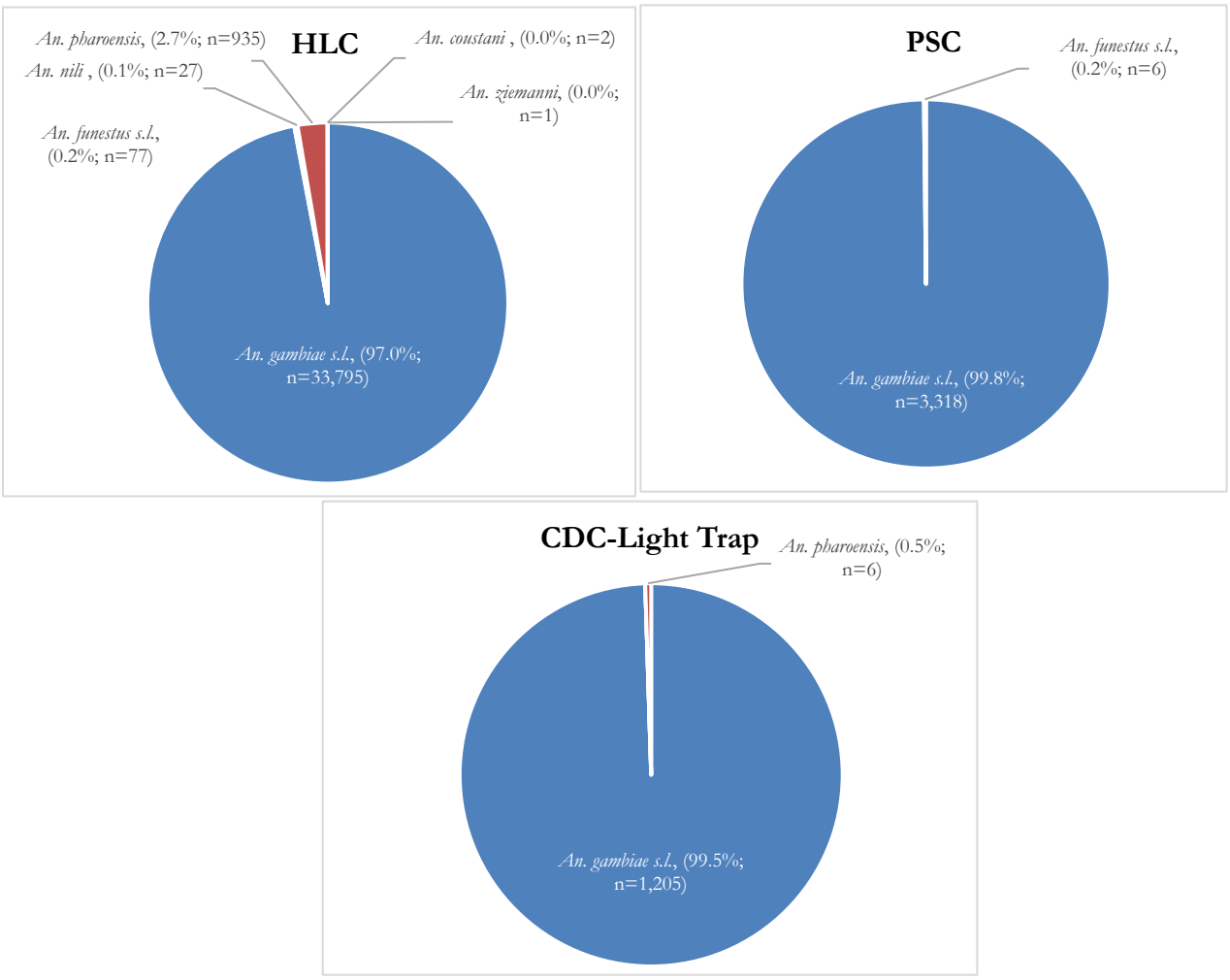
3.1.1.5 SPECIES COMPOSITION IN SAKASSOU

Sakassou was the most productive of the four sites where longitudinal vector surveillance was conducted. A total of 39,372 *Anopheles* mosquitoes were collected over the 10 months using all three collection methods, representing 75.8% of the year's overall collection. *An. gambiae* s.l. was the main vector species collected (97.3%; n=38,318) with a few *An. funestus* s.l. (0.2%; n=83), *An. nili* (0.1%; n=27), *An. pharoensis* (2.4%; n=9,41), *An. coustani* (n=2), and *An. ziemanni* (n=1).

HLC remained the most effective method for collecting *Anopheles* mosquitoes. In Sakassou, 34,834 *Anopheles* were collected, mostly *An. gambiae* s.l. (97%; n=33,795), *An. funestus* s.l. (0.2%; n=77), and *An. nili* (0.1%; n=27) (Figure 6). The other species found were *An. pharoensis* (2.7%; n=935), *An. coustani* (2). and *An. ziemanni* (1).

A total of 3,324 *Anopheles* mosquitoes were collected by PSC. *An. gambiae* s.l. represented 99.8% (n=3,318). A few *An. funestus* s.l. (0.2%; n=6) also were collected. CDC light traps recorded the lowest number of *Anopheles* mosquitoes collected (1,479). *An. gambiae s.l.* was the only malaria vector collected by CDC light trap (99.5%; n=1,205). *An. pharoensis* was the other *Anopheles* species collected (0.5%; n=6) (Annex Tables A1–A3).

FIGURE 6: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN SAKASSOU USING HLC, PSC, AND CDC-LIGHT TRAP FROM JANUARY TO DECEMBER 2020



3.2 VECTOR DENSITY AND BEHAVIOR

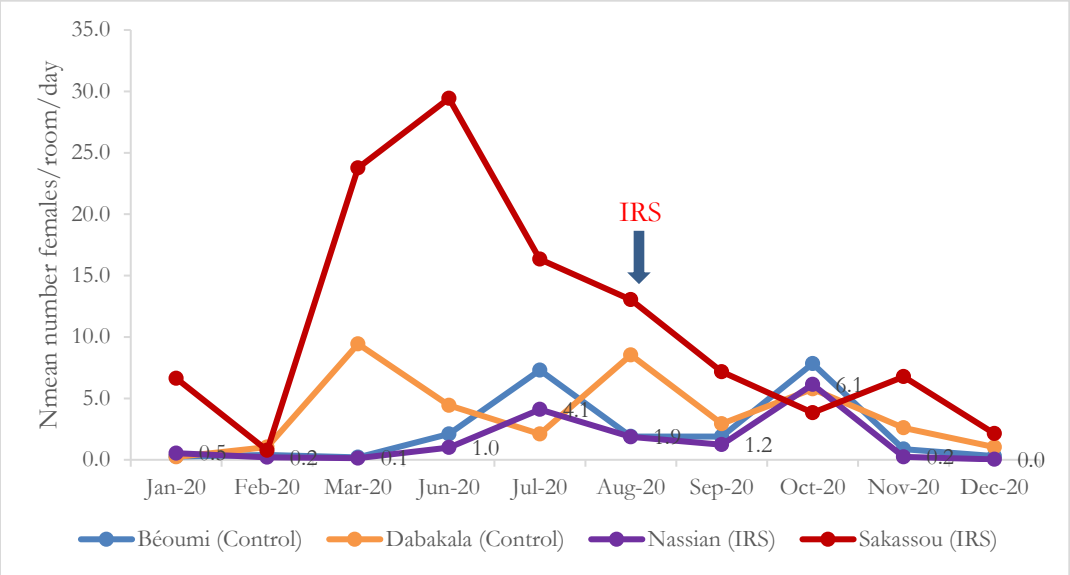
3.2.1 IRD OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY PSC

3.2.1.1 AN. GAMBIAE S.L.

An. gambiae s.l. mean IRDs, measured using PSCs, recorded several peaks in the control sites. Mean IRDs were between 0.2 females/room/day (f/r/d) and 9.4 f/r/d in Dabakala and 0.2 and 7.8 f/r/d in Béoumi (Figure 7). Dabakala had three IRD peaks, and Béoumi had two. Dabakala's highest peak was in March 2020 (9.4 f/r/d) followed by two additional peaks in August (8.5 f/r/d) and October 2020 (5.8 f/r/d). Béoumi's two peaks were recorded in July (7.3 f/r/d) and October 2020 (7.8 f/r/d).

In the IRS site of Sakassou, monthly IRDs of *An. gambiae* s.l. rose from 0.8 f/r/d in February 2020 to a single major peak at 29.4 f/r/d in June 2020. IRDs of *An. gambiae* s.l. decreased from 13.0 f/r/d in August (after IRS) to 2.1 f/r/d in December 2020 after a slight peak of 6.8 f/r/d in November 2020. In contrast, Nassian recorded its IRD peak (6.1 f/r/d) after IRS, in October 2020, and then decreased to 0.0 f/r/d during the final two months of the year (Annex Table A4).

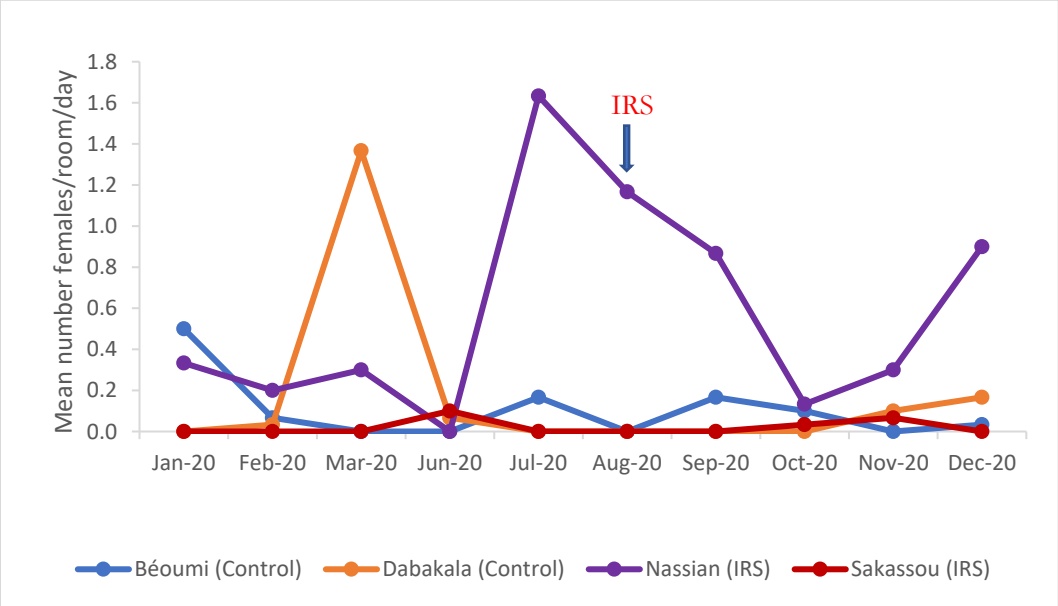
FIGURE 7: MEAN IRD OF *AN. GAMBIAE* S.L. BY MONTH IN CONTROL AND IRS SITES BY MONTH



3.2.1.2 *AN. FUNESTUS* S.L.

Consistent with the observation that Nassian is the site with the largest presence of *An. funestus* s.l. throughout the year, Nassian recorded the highest mean IRD with 0.6 f/r/d followed by Dabakala (0.2 f/r/d) and Béoumi (0.1 f/r/d) (Figure 8 and Annex Table A4). Sakassou (0.02 f/r/d) recorded the lowest density of *An. funestus* s.l. In Nassian, the IRD of *An. funestus s.l.* decreased from 1.2 f/r/d in August 2020 to 0.1 f/r/d in October 2020, two months after IRS, before increasing between November and December 2020 from 0.3 to 0.9 f/r/d. In Dabakala, IRD fell from 1.4 f/r/d in March to less than 0.2 f/r/d, were it stayed from May to December 2020 (0.0 from July to October). In Béoumi, IRD fluctuated between 0.2 f/r/d in July and September 2020 and 0.0 f/r/d in November and December 2020 (Annex Table A4). *An. funestus* s.l. IRD decreased in both control and IRS sites after IRS, between August and December 2020, but in contrast to *An. gambiae* s.l., it was lower in sprayed sites than in control sites.

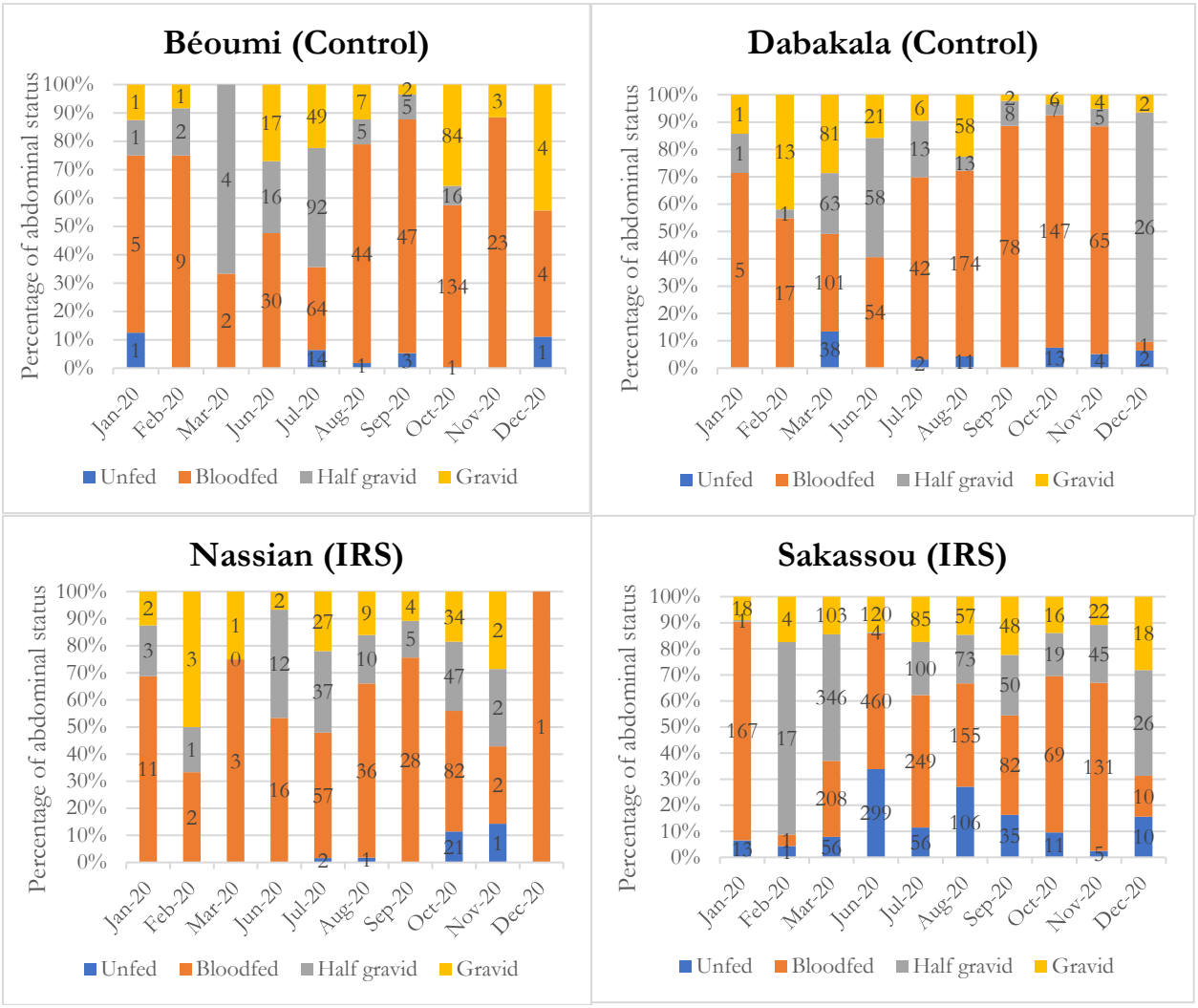
FIGURE 8: MEAN IRD OF *AN. FUNESTUS* S.L. BY MONTH IN CONTROL AND IRS SITES BY MONTH



3.2.2 ABDOMINAL STATUS OF *AN. GAMBIAE S.L.* AND *AN. FUNESTUS* S.L. COLLECTED BY PSCS

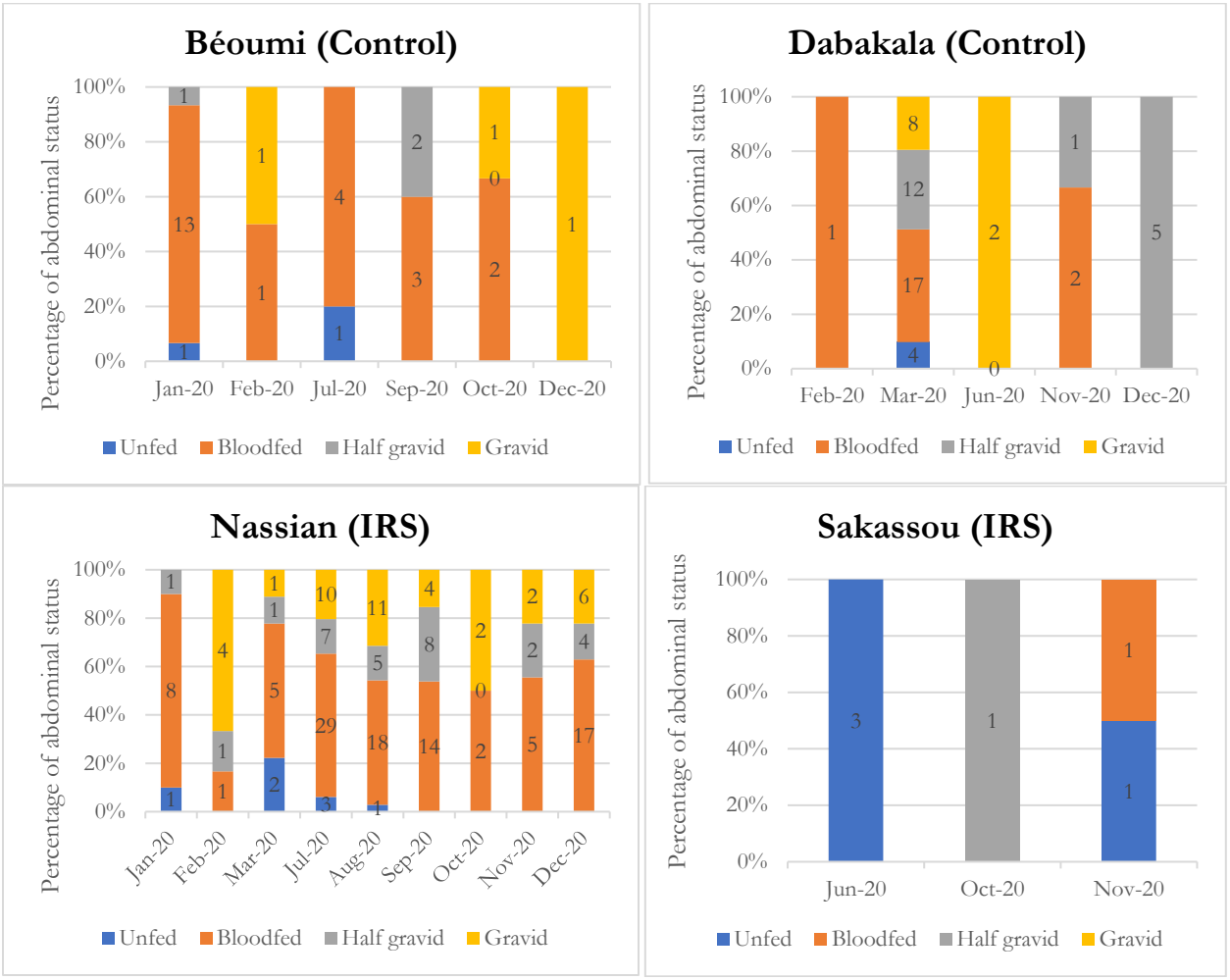
Figures 9 and 10 show the abdominal status of *An. gambiae* s.l. and *An. funestus* s.l. collected indoors by PSCs from sprayed and control sites. The percentages of unfed, fed/half gravid, and gravid was determined for 5,617 *An. gambiae* s.l. (3,782 from sprayed sites and 1,835 from control sites) and 264 *An. funestus* s.l. (181 from sprayed sites and 83 from control sites). Overall, the percentages of gravid *An. gambiae* s.l. mosquitoes were 15.2% in the sprayed sites and 19.7% in the control sites, while the percentages of gravid *An. funestus* s.l. were 22.1% in the sprayed sites and 15.7% in the control sites. The proportions of fed/half gravid *An. gambiae* s.l. were 67.9% in the sprayed sites and 75.3% in the control sites, while the percentages of fed/half gravid *An. funestus* s.l. were 71.8% in the sprayed sites and 77.1% in the control sites. No drastic change in blood-feeding ability was observed in both IRS sites, post spraying (Annex Table A4).

FIGURE 9: PROPORTION OF ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. BY SITE AND BY MONTH



Note: The number in the bars represents the number of females collected per month and per abdominal status.

FIGURE 10: PROPORTION OF ABDOMINAL STATUS OF *AN. FUNESTUS* S.L. BY SITE AND BY MONTH WHEN ANY *AN. FUNESTUS* WERE COLLECTED



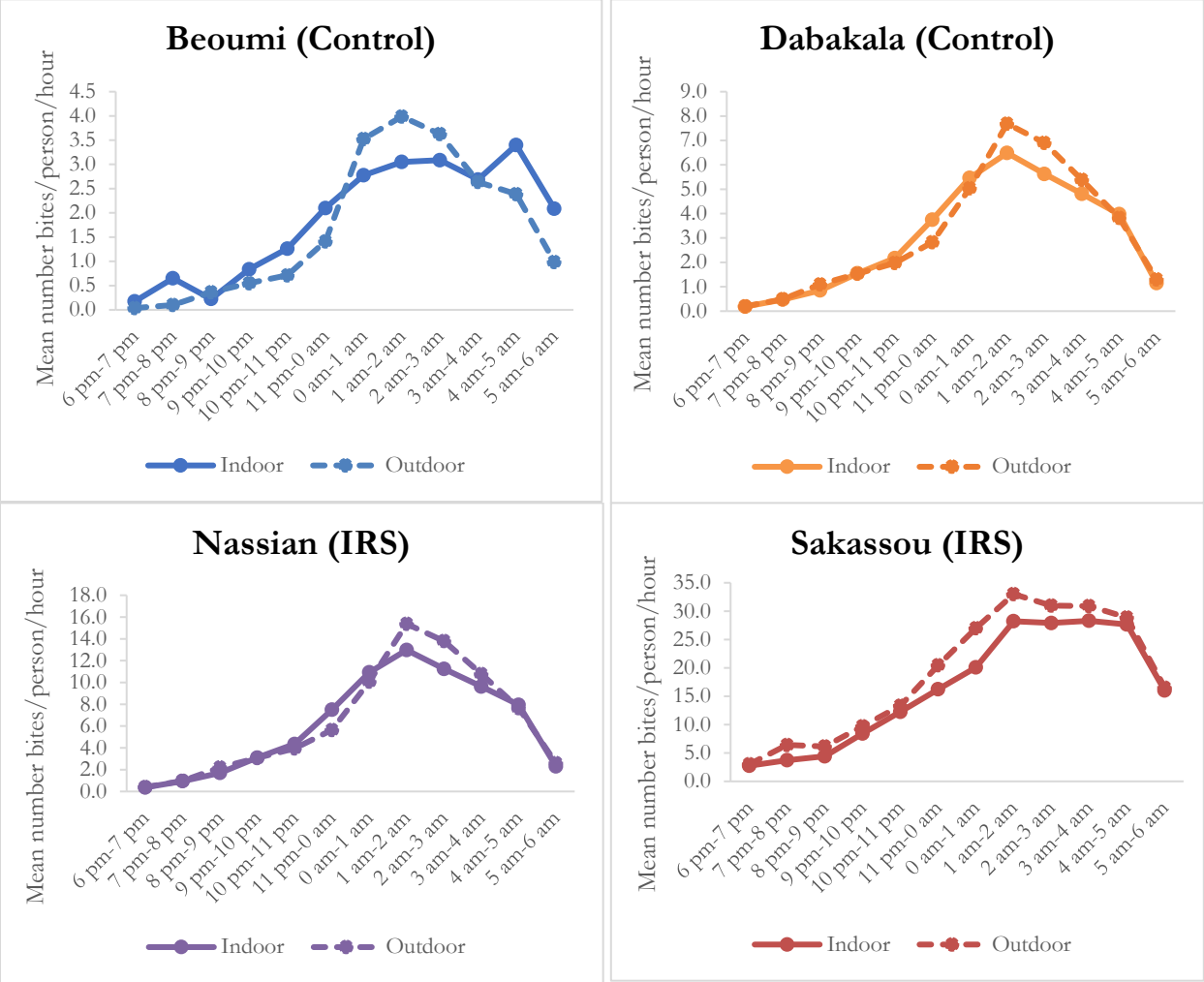
*The number in the bars represents the number of females collected per month and per abdominal status.

3.2.3 BITING TIME OF *AN. GAMBIAE* S.L.

The hourly and monthly collections using HLC methods permitted the determination of the mean biting time and biting cycle of the main malaria vector, *An. gambiae* s.l., within the four longitudinal monitoring sites.

An. gambiae s.l. biting time was similar at all sites with the peak biting observed between 1:00 am and 2:00 am, both indoors and outdoors at all sites, except in Béoumi where the indoor peak was recorded between 4:00 am and 5:00 am. The overall highest indoor and outdoor densities were recorded between midnight and 5:00 am for all sites (Figure 11). The peak hourly biting was up to 4.0 bites per person per hour (b/p/h) outdoors and 3.4 b/p/h indoors in Béoumi, and 7.7 b/p/h outdoors and 6.5 b/p/h indoors in Dabakala. Nassian recorded its peak hourly biting of 15.4 b/p/h outdoors and 13.0 b/p/h indoors between 1:00 am to 2:00 am. Sakassou yielded the highest number of *An. gambiae* s.l. collected hourly with a peak observed at the same time as other sites, both outdoors (33.0 b/p/h) and indoors (28.2 b/p/h) (Annex Table A5).

FIGURE 11: MEAN INDOOR AND OUTDOOR BITING TIMES OF *AN. GAMBIAE* S.L. COLLECTED BY HLC BY SITE



3.2.4 HBR, BITING CYCLE, AND BEHAVIOR OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY HLC

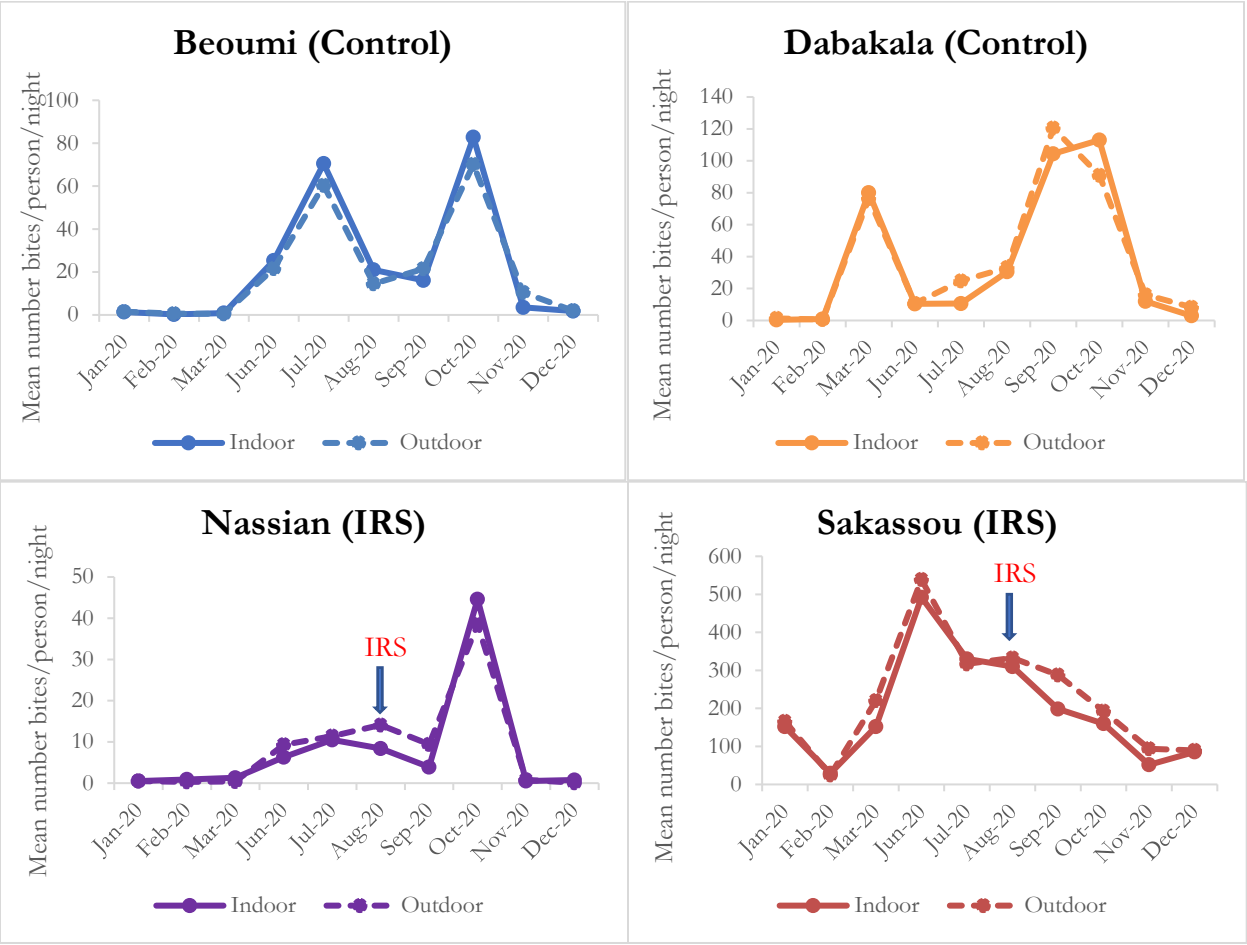
3.2.4.1 AN. GAMBIAE S.L.

Both control sites (Béoumi and Dabakala) recorded two human biting peaks over the year and both sites' second peak occurred around the same time (September/October 2020) (Figure 12). In Béoumi, HBRs peaked in July 2020, both indoors (60.4 b/p/n) and outdoors (70.5 b/p/n), and in October 2020, again both indoors (82.8 b/p/n) and outdoors (70.1 b/p/n). Similar trends were observed in Dabakala with a first HBR peak of 80.0 b/p/n and 76.4 b/p/n indoors and outdoors, respectively, recorded in March 2020. A second peak was recorded in September 2020 outdoors (120.5 b/p/n) and in October 2020 indoors (113 b/p/n).

The HBRs of *An. gambiae s.l.* were very high overall in the IRS site of Sakassou. The lowest biting rates were observed in February 2020 at 29.3 b/p/n indoors and 24.9 b/p/n outdoors, but then rose to a single peak in June 2020, both indoors (492.0 b/p/n) and outdoors (539.0 b/p/n). However, after IRS, a steady and significant decrease was observed, from 198.4 b/p/n to 85.3 b/p/n indoors and 287.8 b/p/n to 89.5 b/p/n outdoors in September 2020 and after IRS respectively, therefore dropping drastically the peak of densities observed in Sakassou during previous year's collections. Nassian recorded the lowest overall *An. gambiae* s.l. HBRs; the mean was 0.43 b/p/n indoors and 41.6 b/p/n outdoors. A single peak biting was observed in October 2020 for both indoors (44.6 b/p/n) and

outdoors (38.5 b/p/n), two months after IRS was conducted. However, there then was a dramatic drop in November and December to less than 1.0 b/p/n both indoors and outdoors (Annex Table A6).

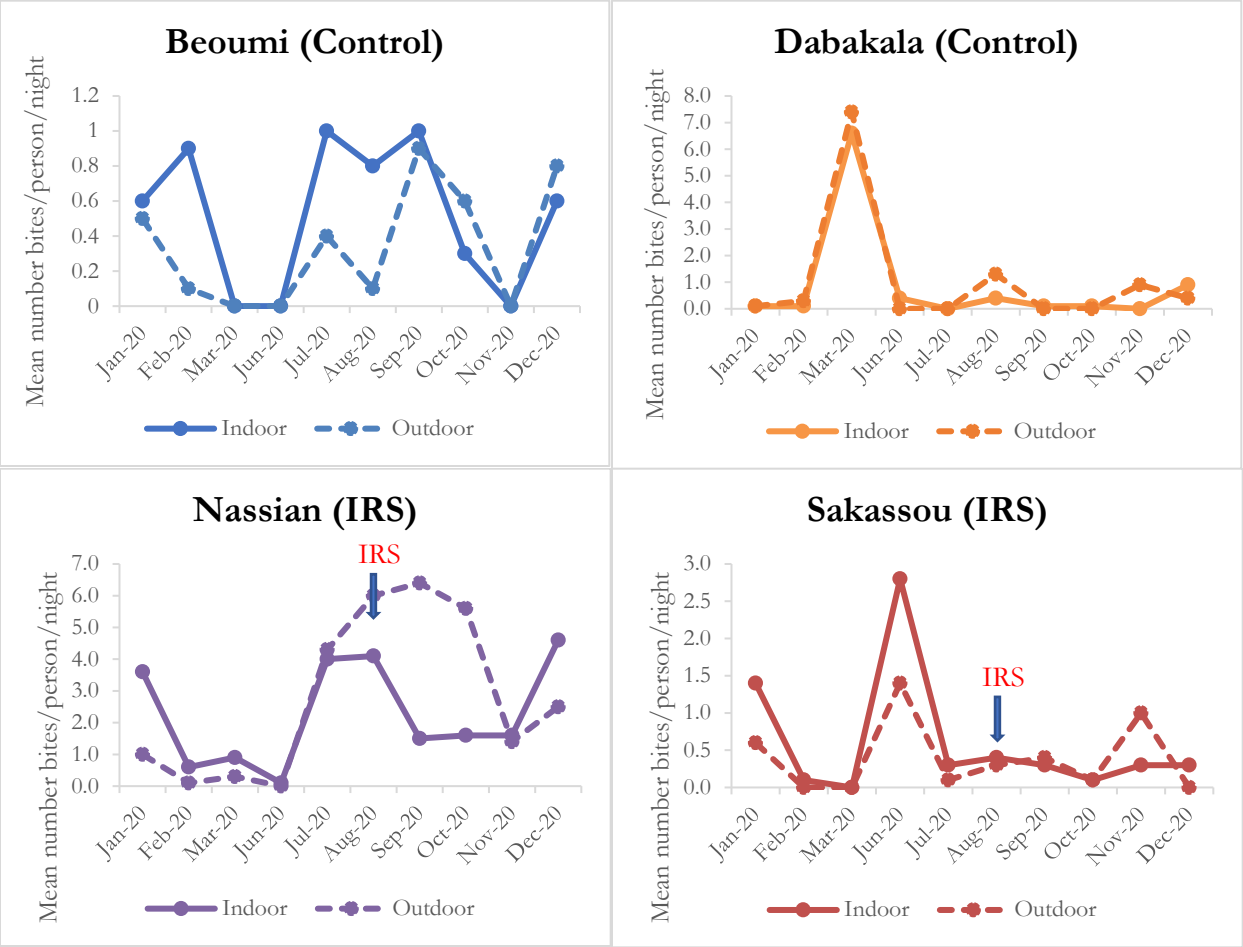
FIGURE 12: AN. GAMBIAE S.L. INDOOR AND OUTDOOR HBRS BY SITE AND BY MONTH



3.2.4.2 *AN. FUNESTUS S.L.*

The *An. funestus* s.l. indoor and outdoor HBRs in Béoumi recorded four peaks over the year, though the densities were low with a mean between 0 and 1 b/p/n (Figure 13). This was the lowest among all sites surveyed.
In Dabakala, a single peak was observed, in March 2020, both indoors (6.6 b/p/n) and outdoors (7.4 b/p/n). All other months of collection recorded less than 2.0 b/p/n in all collection sites (Annex Table A6).
The highest HBRs of *An. funestus* s.l. were observed in Nassian with an overall (indoor and outdoor) rate ranging from 0.1 in July to 5.1 b/p/n in September. The highest outdoor HBRs were recorded between August 2020 and October 2020; indoors, there was a decrease from the peak HBR in August (4.1 b/p/n) to 1.4 b/p/n in November 2020. A slight increase in indoor HBRs was noted in December 2020 (4.6 b/p/n). Sakassou recorded a single HBR peak indoors (2.8 b/p/n) and outdoors (1.4 b/p/n) in June 2020. The densities were low from July to October 2020 with less than 1.0 b/p/n indoors and outdoors before slightly increasing in November and December 2020, two months after IRS.

FIGURE 13: *AN. FUNESTUS* S.L. INDOOR AND OUTDOOR HBRS BY SITES AND BY MONTH



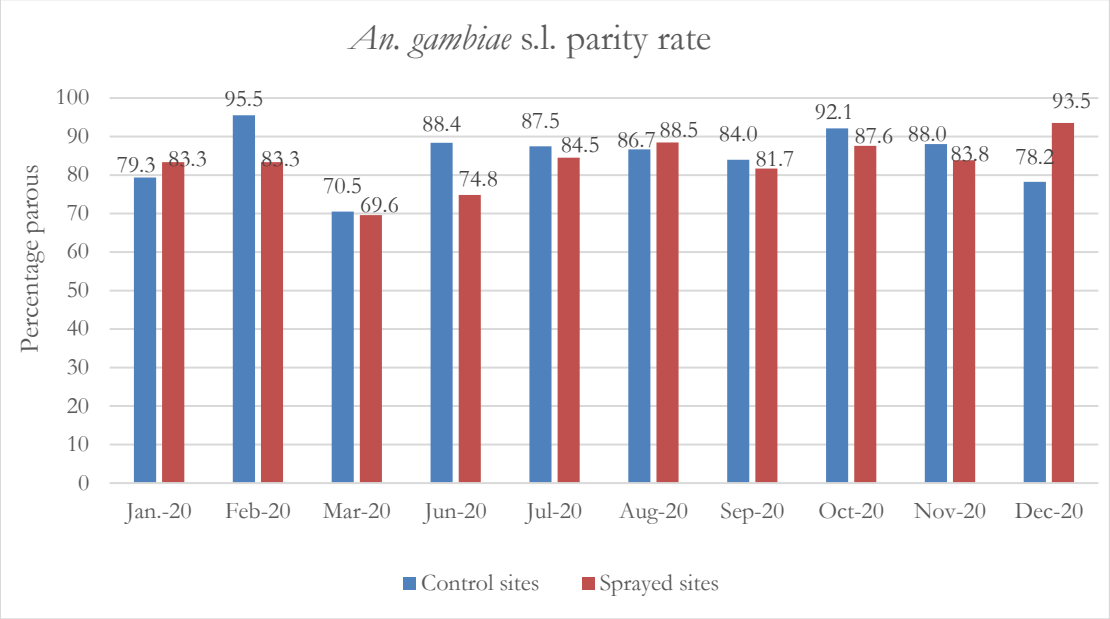
3.3 PARITY RATE

3.3.1 *AN. GAMBIAE* S.L.

A total of 8,688 *An. gambiae* s.l. collected using HLCs (1,776 in Béoumi, 2,205 in Dabakala, 881 in Nassian, and 3,826 in Sakassou) were dissected to determine parity across the four sites. Of these, 920 (51.8%) were found to be parous in Béoumi, 1,253 (56.8%) in Dabakala, 957 (92.5%) in Nassian, and 3,257 (72.8%) in Sakassou (Annex Table A7). Overall, the parity rate was similar for mosquitoes collected indoors and outdoors (Annex Table A8).

Figure 14 shows mean monthly parity rates for *An. gambiae* s.l. Mean parity rates for *An. gambiae* s.l. at the sprayed sites were 86.7% in August versus 85.6% after IRS (September–December 2020); at the control sites, the rates were 88.5% versus 86.7 at the same points in time. The mean parity rates of *An. gambiae* s.l. at the sprayed sites were similar to those observed in the control sites (p=0.123, and p=0.106, respectively).

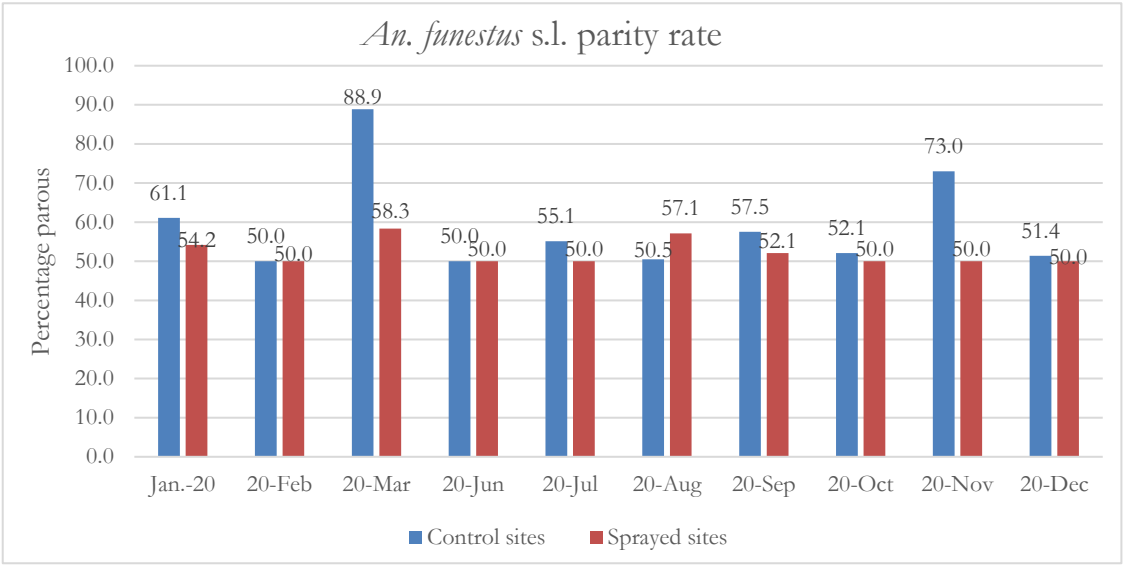
FIGURE 14: PARITY RATE OF *AN. GAMBIAE* S.L. COLLECTED USING HLC IN CONTROL AND IRS SITES BY MONTH



3.3.2 *AN. FUNESTUS* S.L.

A total of 245 *An. funestus s.l.* females were dissected for parity: 26 in Béoumi, 17 in Dabakala, 186 in Nassian, and 16 in Sakassou. Of these, 18 (69.2%) were parous in Béoumi, 10 (58.8%) in Dabakala, 159 (85.5%) in Nassian, and 15 (93.8%) in Sakassou (Figure 15). No significant difference was observed between control and sprayed sites (p=0.619).

FIGURE 15: PARITY RATE OF *AN. FUNESTUS* S.L. COLLECTED USING HLC IN CONTROL AND IRS SITES BY MONTH



3.4 *PLASMODIUM FALCIPARUM* SPOROZOITE AND EIRS

Table 5 shows the overall infection rate and EIRs for *An. gambiae* s.l. and *An. funestus s.l.* collected by HLC in the four districts. Nassian recorded the highest *Plasmodium falciparum* sporozoite infection rate among *An. gambiae* s.l. (0.07), followed by Dabakala (0.06) and Béoumi (0.05). The lowest rate (0.012, or 1.2%) was recorded in Sakassou. The highest number of *An. gambiae* s.l. infected bites were recorded between June and August in all four districts (Figure 16).

As also shown in Table 5, Sakassou recorded the highest EIR (2.53 infective bites per person per night (ib/p/n)) of *An. gambiae* s.l., followed by Dabakala (2.23 ib/p/n) and Béoumi (0.99 ib/p/n). Nassian recorded the lowest EIR, 0.54 ib/p/n. The monthly EIR of *An. gambiae* s.l. decreased in Sakassou, from 9.6 ib/p/n in August 2020 to 2.4 ib/p/n in September 2020, one month after IRS (Figure 16). The EIR was 0.0 ib/p/n during October and November 2020 before increasing to 0.9 ib/p/n in December 2020, four months after IRS was implemented. In Nassian, the EIR decreased from 0.9 ib/p/n in August to 0.0 ib/p/n in September 2020, then increased to 0.9 ib/p/n in October 2020 and again fell to 0.0 ib/p/n in November and December 2020, three and four months after IRS. Similar EIR fluctuations were observed in the control sites, in Béoumi from 2.7 ib/p/n in July to 0.0 ib/p/n in September 2020 to a peak of 1.5 ib/p/n in October, and in Dabakala from 2.5 ib/p/n in July to 0.3 ib/p/n in November and December 2020.

In Nassian, *An. funestus* s.l. was analyzed for infection throughout the year, and it reported an overall EIR of 0.13 ib/p/n among the *An. funestus* s.l. (Table 5). The highest number of infected bites was observed in December and July 2020, with a peak of 0.13 ib/p/n in December (Figure 17) (Annex Table A9).

TABLE 5: VECTOR SPOROZOITE INFECTION RATE IN AND EIR OF MALARIA VECTORS COLLECTED USING HLC IN CONTROL AND IRS SITES

	***An. gambiae* s.l.**						***An. funestus* s.l.**					
	TC	TA	P	SR	HBR (b/p/n)	EIR (ib/p/n)	TC	TA	P	SR	HBR (b/p/n)	EIR (ib/p/n)
Béoumi (Control)	4,241	883	41	0.046	22.33	0.99	115	12	1	0.083	0.575	0.048
Dabakala (Control	7730	939	56	0.06	37.4	2.23	225	0	0	0	0.23	0
Nassian (IRS)	1787	599	40	0.067	8.1	0.541	610	218	11	0.05	2.51	0.1277
Sakassou (IRS)	38318	990	12	0.012	211.2	2.534	83	0	-	-	-	-

Note: TC=total collected; TA=total analyzed; P=positive; SR=sporozoite rate

FIGURE 16: *AN. GAMBIAE* S.L. EIRS AT CONTROL AND IRS SITES BY MONTH

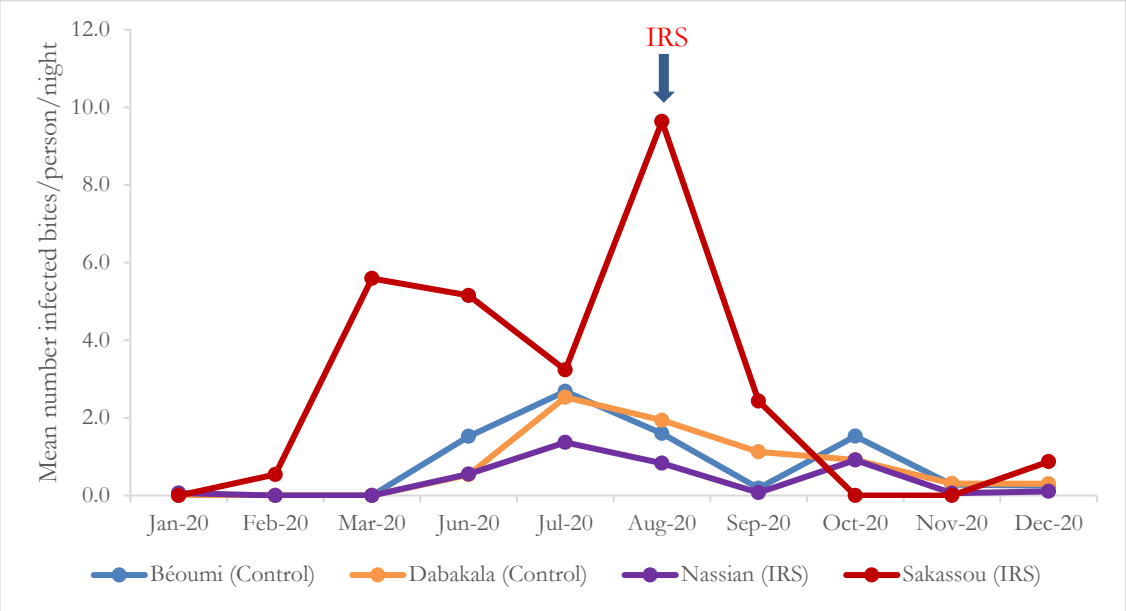
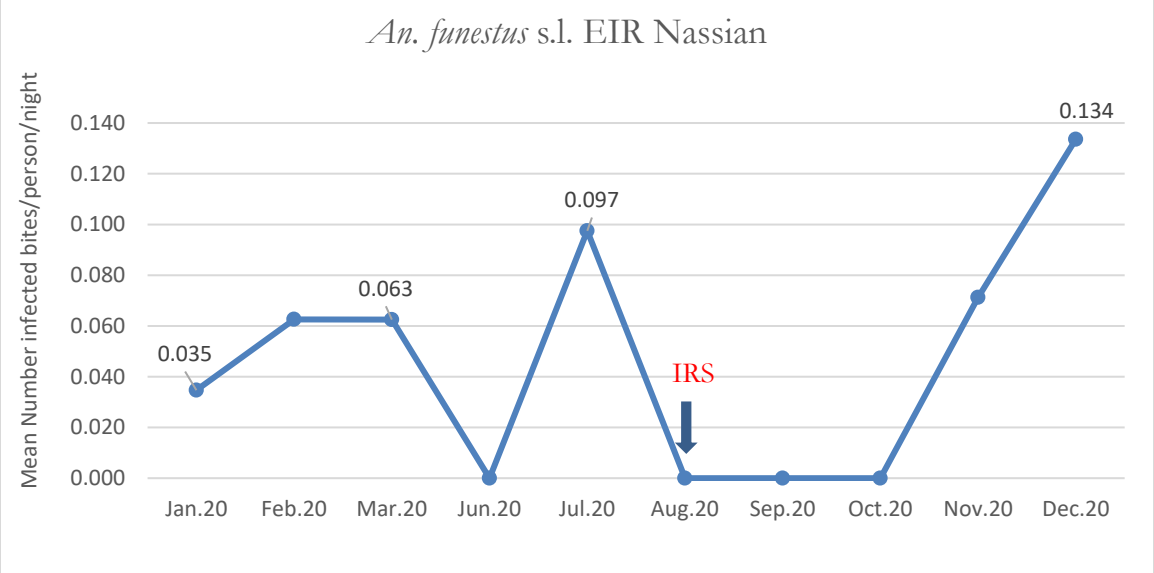


FIGURE 17: *AN. FUNESTUS* S.L. EIRS IN NASSIAN BY MONTH



3.5 INSECTICIDE RESISTANCE MONITORING

Figures 18–20 show the resistance status to the different insecticides tested against *An. gambiae* s.l. collected from the 18 different sites. All insecticides were tested in all sites except Aboisso and Jacqueville, where pirimiphos-methyl and bendiocarb were not tested due to a mosquito larvae shortage.

Resistance was observed to the diagnostic dose of all pyrethroids and bendiocarb in all sites surveyed. Pre-exposure of mosquitoes to PBO before exposure to deltamethrin, permethrin, and alpha-cypermethrin did not reverse the resistance status of the *An. gambiae* s.l. population but yielded an important increase in the mortality in all sites. Deltamethrin showed the highest increase in mortality among the pyrethroids when combined with PBO in all sites, with full restitution of vector susceptibility in Abidjan (Figure 18). The intensity of resistance was high in all sites for all three pyrethroids (deltamethrin, permethrin, and alpha-cypermethrin), except in Abidjan, Daloa, and Jacqueville. Deltamethrin resistance was moderate in Daloa and Jacqueville, and permethrin resistance was moderate in Adzopé and Daloa. For alpha-cypermethrin only Aboisso recorded a moderate resistance intensity (Figure 19). Susceptibility to pirimiphos-methyl was recorded at the diagnostic dose in 12 sites, while resistance was observed in six of remaining sites, but at a low intensity (Figure 20) (Annex Table A10).

FIGURE 18: PYRETHROID INSECTICIDE SYNERGIST TEST RESULTS IN 18 SITES

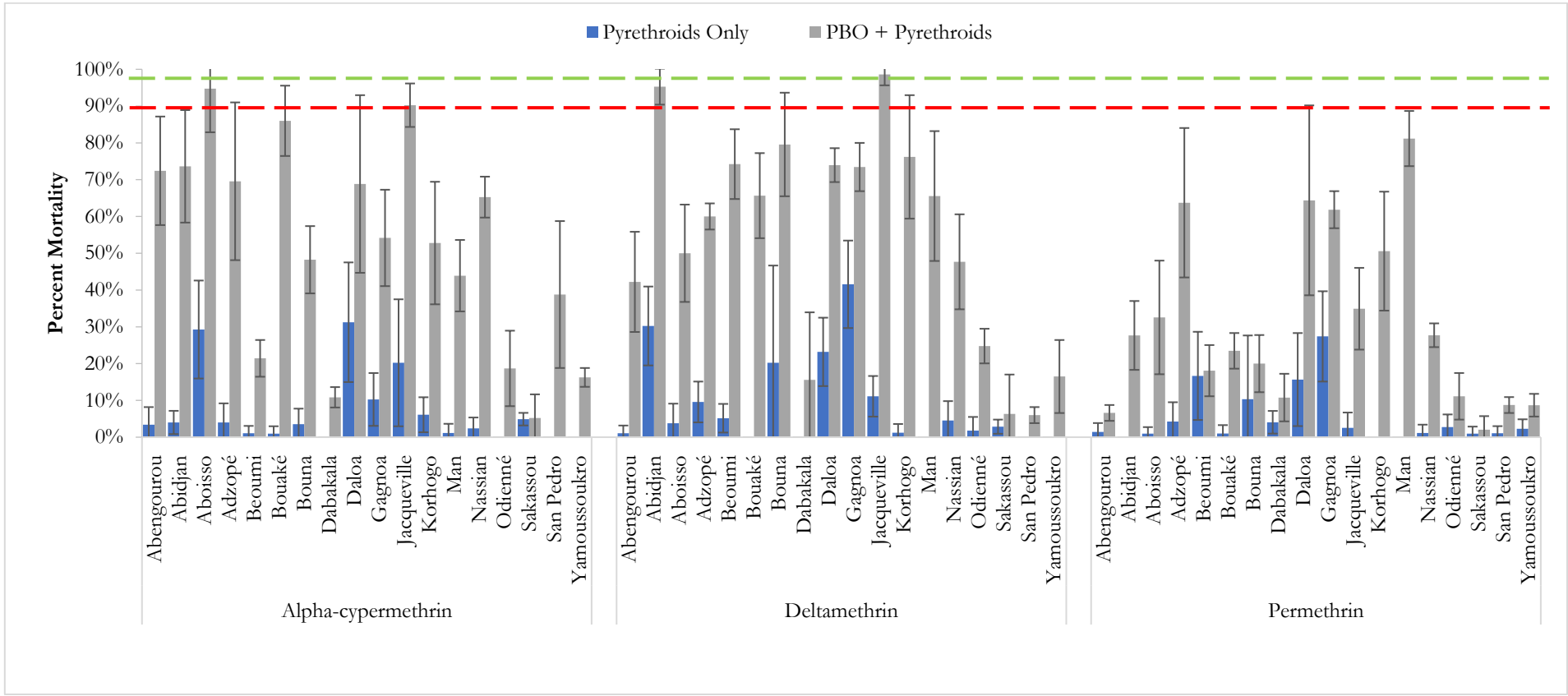


FIGURE 19: INTENSITY OF RESISTANCE TO PYRETHROID INSECTICIDES IN 18 SITES

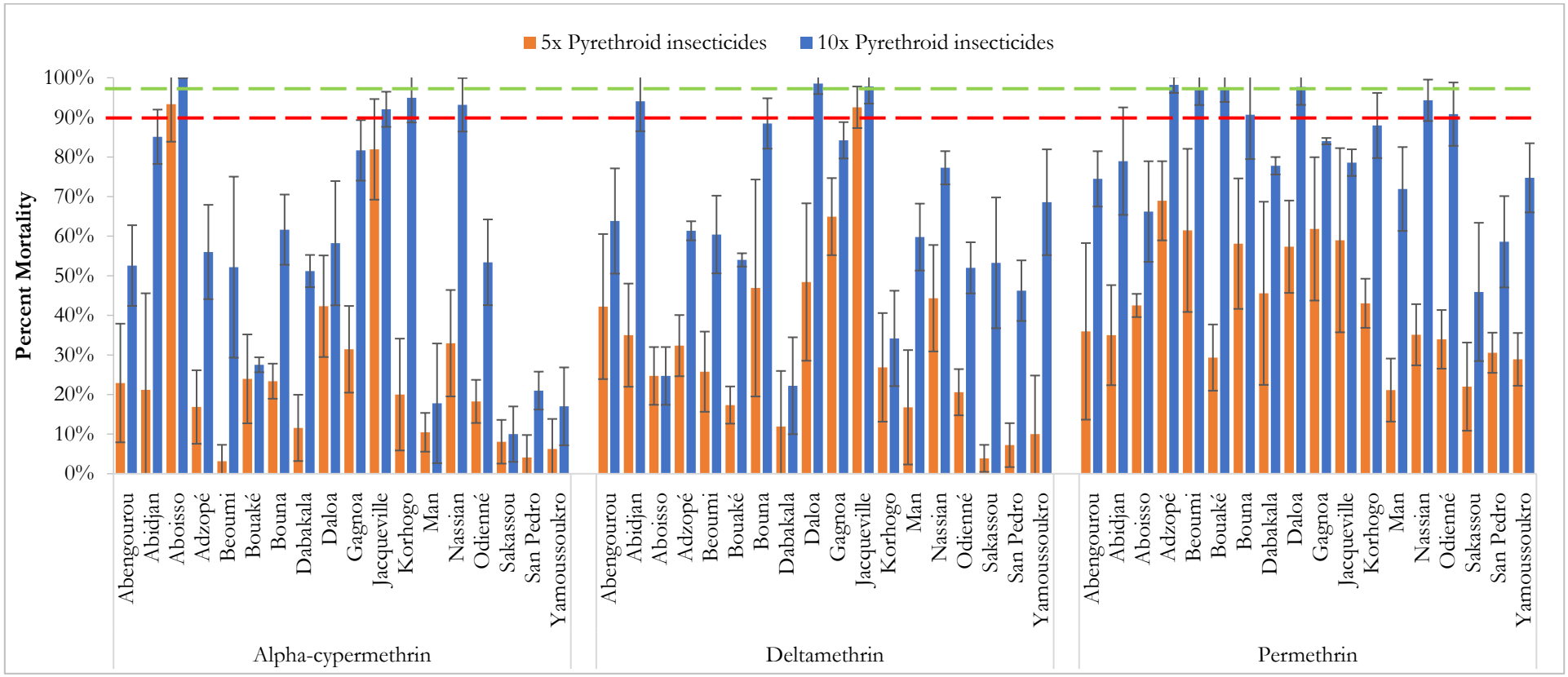
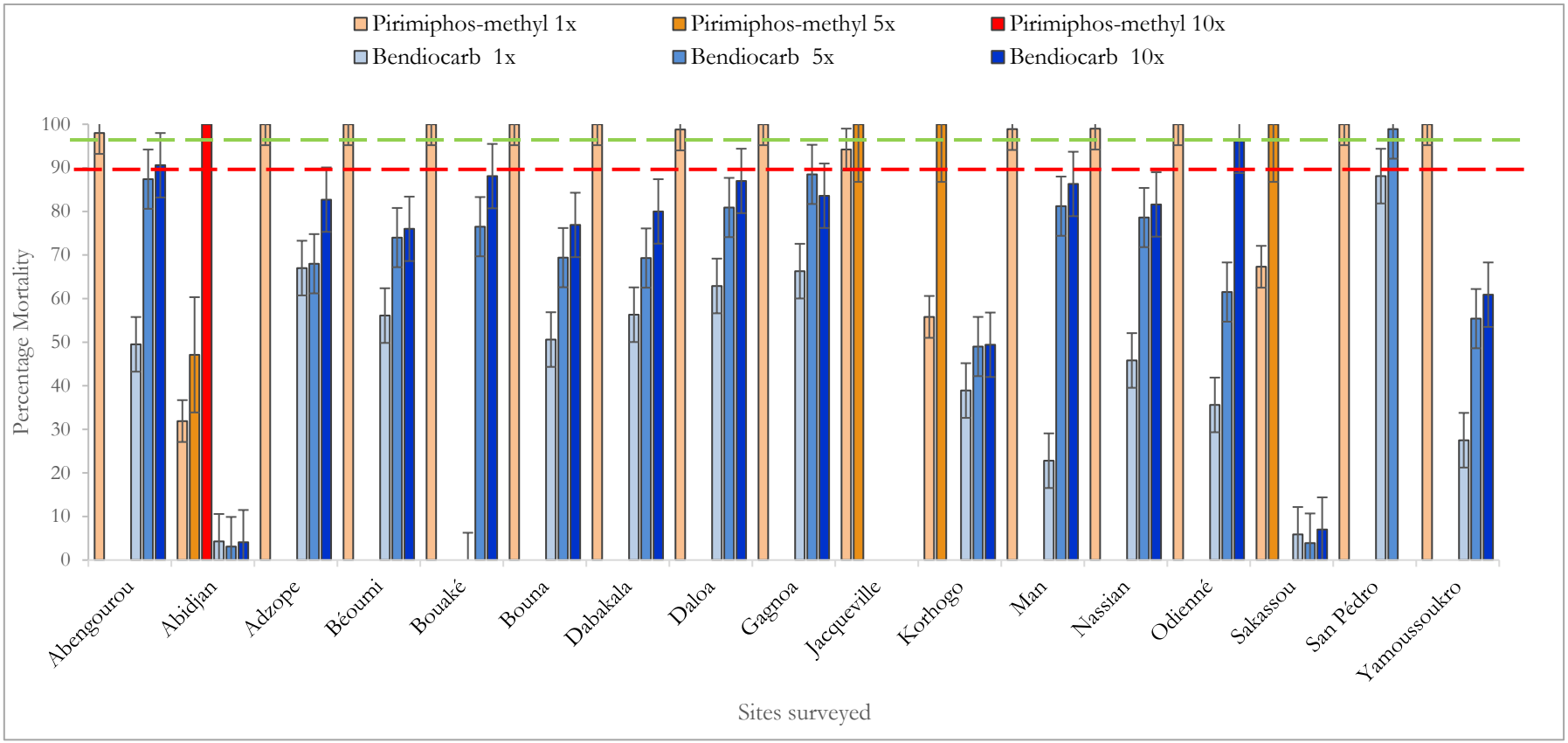


FIGURE 20: INTENSITY OF RESISTANCE TO PIRIMIPHOS-METHYL AND BENDIOCARB IN 17 SITES



The results of the CDC bottle assays using chlorfenapyr and WHO susceptibility tests using clothianidin are shown in Figures 21–23. The horizontal dashed red line represents the 90% threshold for resistance, and the green line represents the 98% threshold for susceptibility.

Susceptibility to chlorfenapyr was observed at the dose of 100 µg/bottle after observing mortality for 72 hours in 10 of the surveyed sites. Mosquitoes from Béoumi, Bouaké, Dabakala, Jacqueville, Nassian, San Pedro, and Yamoussoukro were resistant to the 100 µg/bottle dose (Figure 21). The lowest mortality rate was recorded in Sakassou. Susceptibility to chlorfenapyr at 200µg/bottle was recorded in 17 sites. Only Sakassou showed resistance at the dose of 200µg/bottle (Figure 22).

For clothianidin, full susceptibility was observed in nine out of the 18 sites (Figure 23).

FIGURE 21: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 100µG/BOTTLE BY SITE

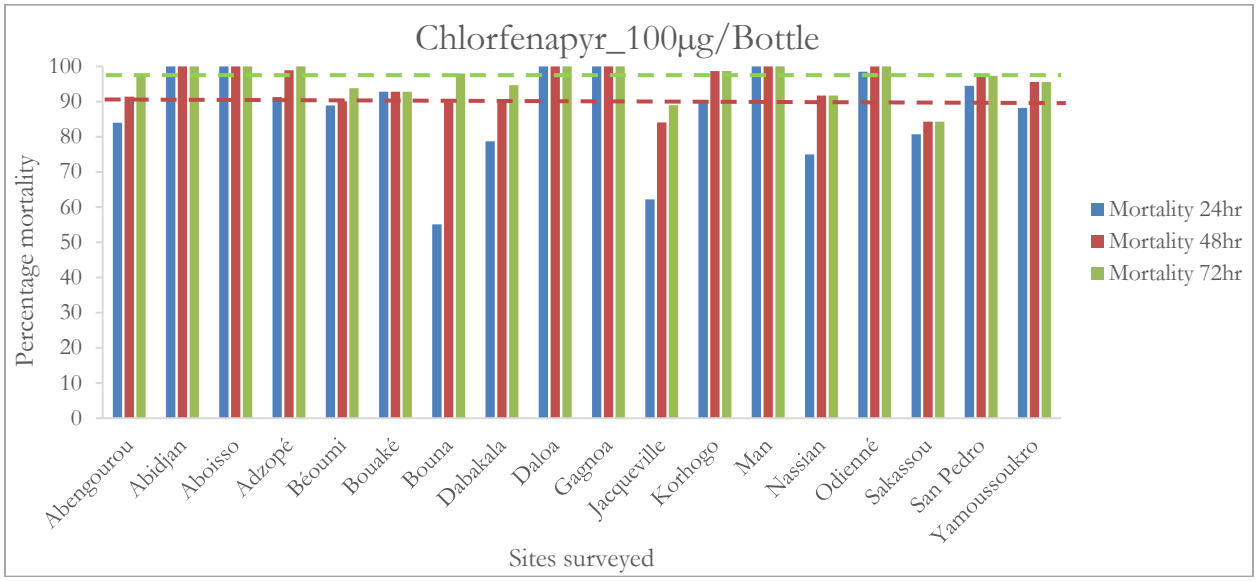


FIGURE 22: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 200µG/BOTTLE BY SITE

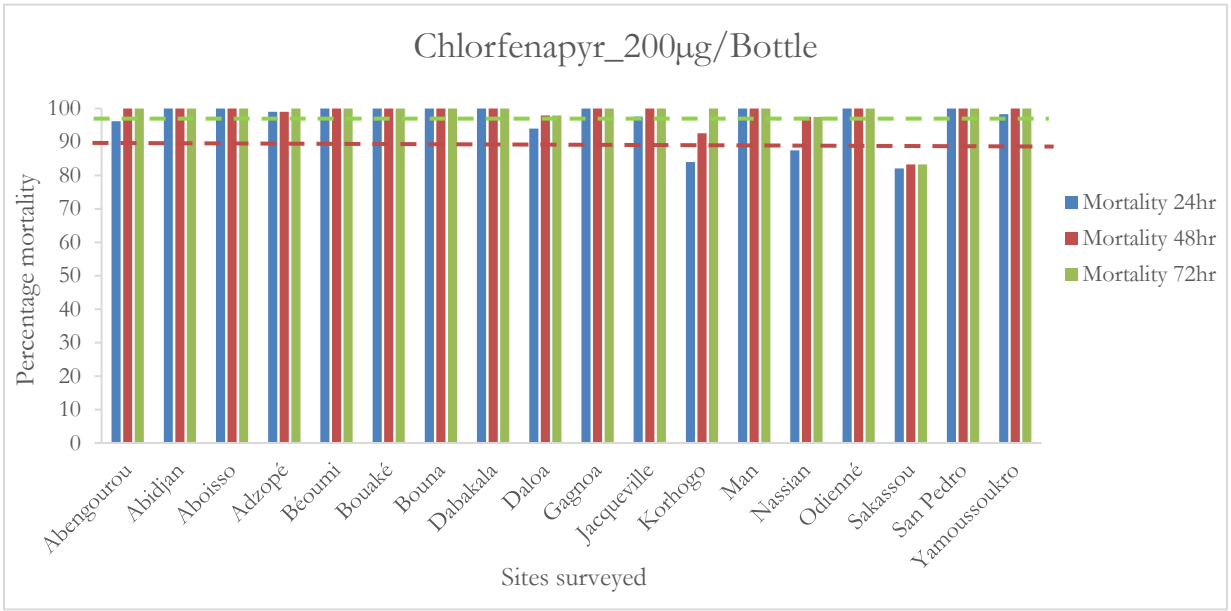
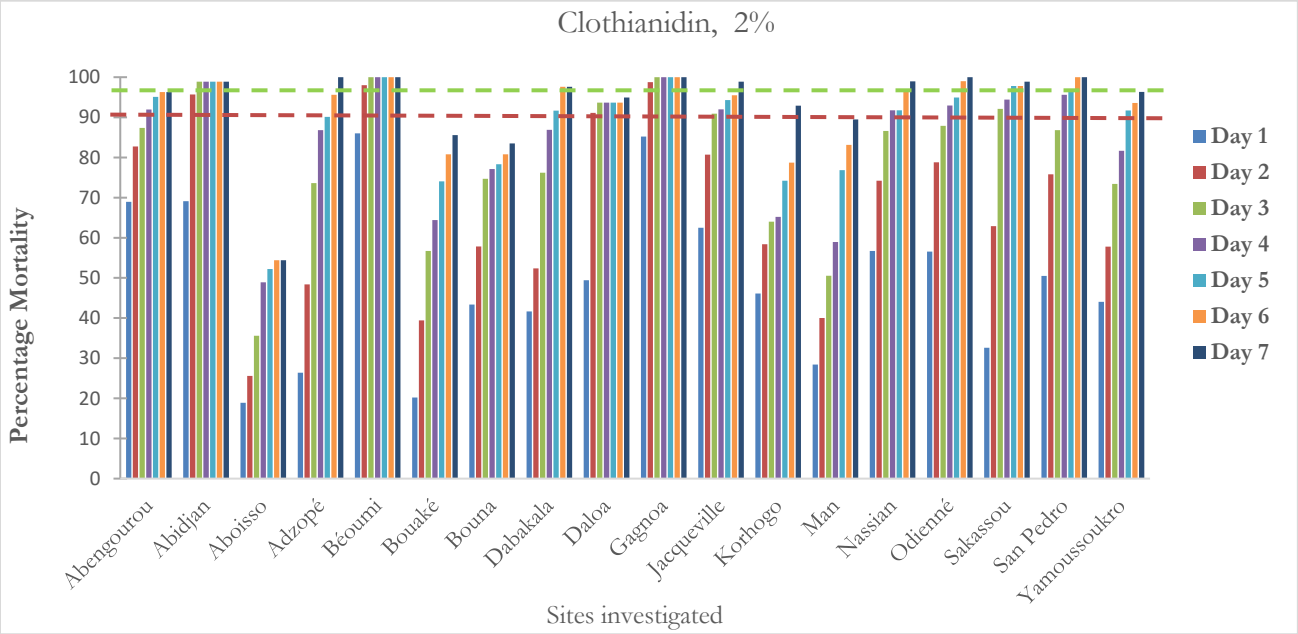


FIGURE 23: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN BY SITE

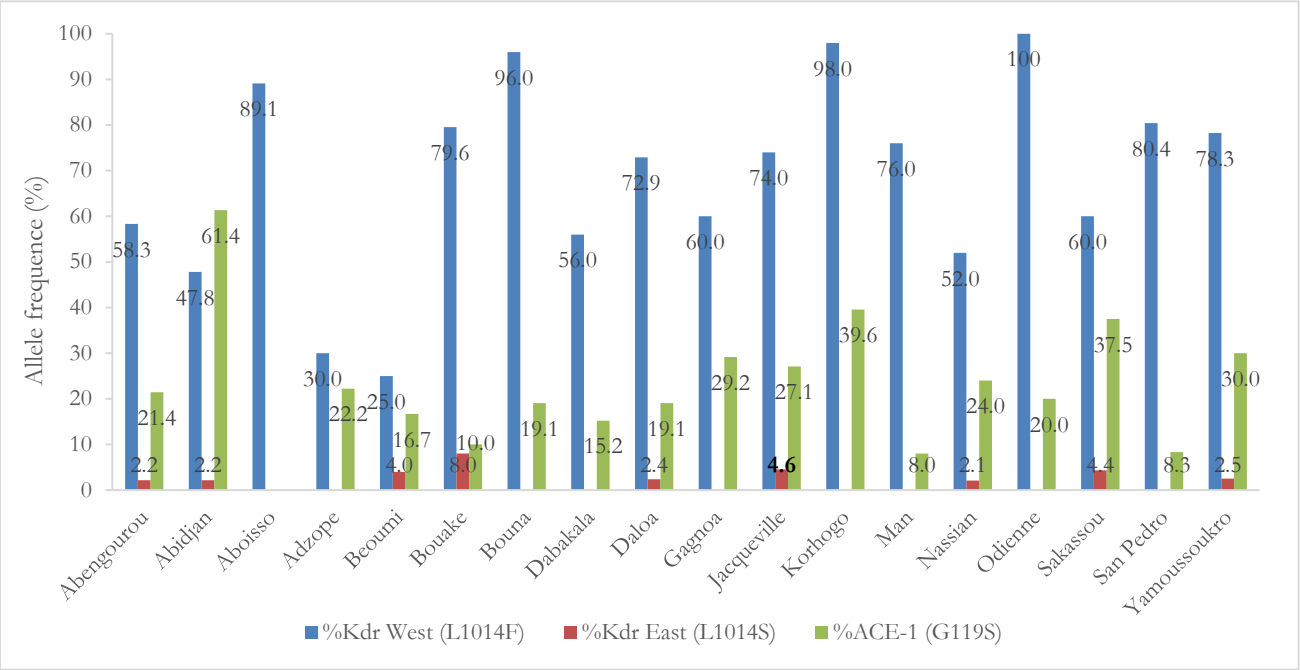


3.6 MOLECULAR MARKERS OF RESISTANCE

Figure 24 shows the distribution of *kdr*-West and *kdr*-East in *An. gambiae sl.* from the 18 sites. Overall, *kdr*-West mutations were frequently present (range: 25–100%) in all study sites. The *kdr*-East mutation was found at low frequency in all sites, the highest being in Bouaké (8%). In other locations, *kdr*-East was present at frequencies that ranged between 0% and 4.6%. The presence of both *kdr* mutations in the country is consistent with the high phenotypic resistance observed to pyrethroid insecticides and represents a significant threat to pyrethroid-based vector control tools.

Similar to *kdr*, the *Ace-1* mutation was found in all sites at frequencies ranging from around 8.0% in Man and San Pedro to 61.4% in Abidjan (Annex Tables A12–A14).

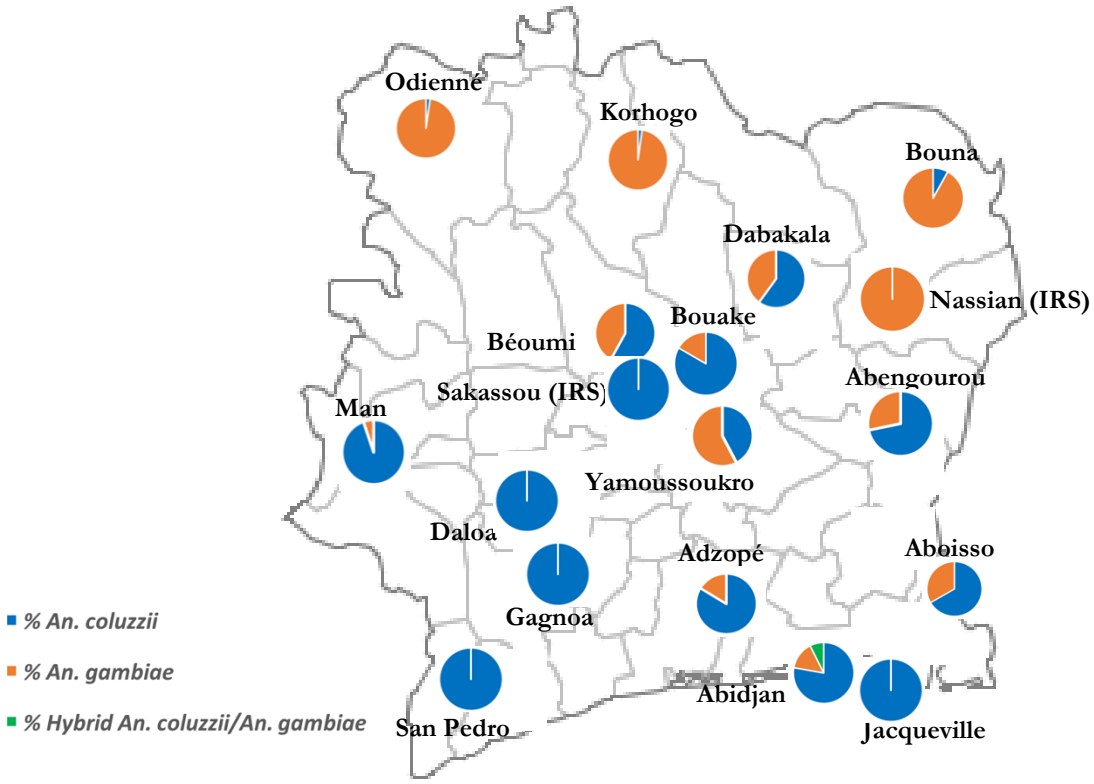
FIGURE 24: DISTRIBUTION OF TARGET SITE MUTATIONS IN *AN. GAMBIAE* S.L IN ALL SITES TESTED FOR INSECTICIDE RESISTANCE IN CÔTE D'IVOIRE



3.6.1 MOLECULAR SPECIES IDENTIFICATION OF ADULT *AN. GAMBIAE* S.L. TESTED FOR SUSCEPTIBILITY

Figure 25 shows the species composition of *An. gambiae* s.l. used for susceptibility testing per site. *An. coluzzii* represented the predominant species in Abengourou, Abidjan, Aboisso, Adzopé, Béoumi, Daloa, Dabakala, Man, and Sakassou and the entire population in Daloa, Gagnoa, Jacqueville, San Pedro, and Sakassou. *An. gambiae s.s.* represented the predominant species in Bouna, Korhogo, Odienné, and Yamoussoukro, and it was the entire population in Nassian. A few hybrids of the two species were characterized in Abidjan (7.4%).

FIGURE 25: PROPORTION OF *AN. GAMBIAE* S.L. USED FOR SUSCEPTIBILITY TEST ACROSS THE 18 DISTRICTS



3.7 QUALITY ASSURANCE OF IRS AND INSECTICIDE DECAY RATE

3.7.1 QUALITY ASSURANCE

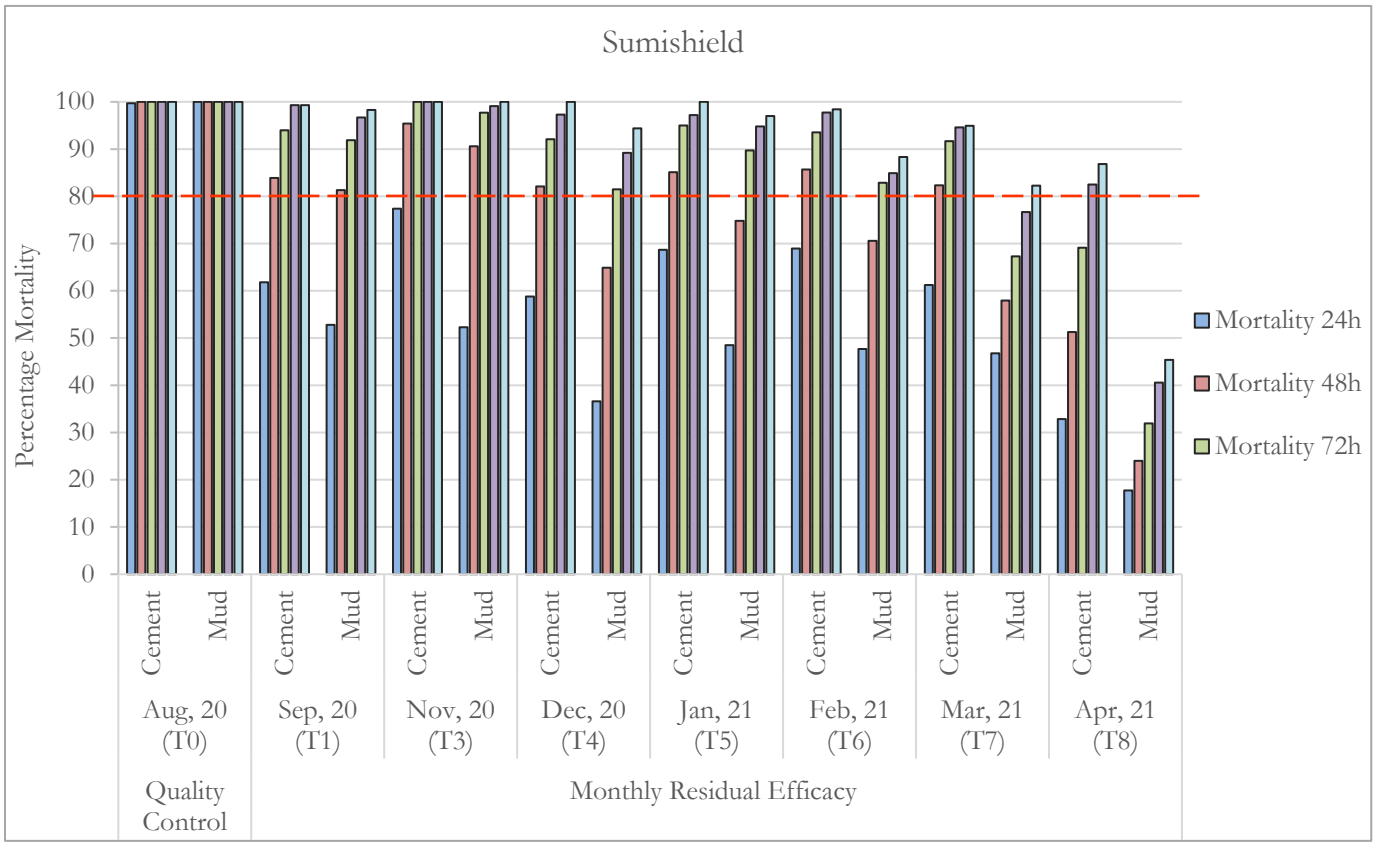
One hundred percent (100%) mortality of *An. gambiae* Kisumu exposed to cement and mud walls sprayed with SumiShield and to cement walls sprayed with Fludora Fusion was recorded between 24h and 48h after exposure during the initial tests conducted within a week after IRS in both sprayed districts. The repeat of the quality assurance conducted in a total of 22 houses (nine cement and four mud in Nassian, and nine cement in Sakassou) yielded identical results in all the sites and wall types tested.

3.7.2 INSECTICIDE DECAY RATE

For insecticide residual life monitoring, wall cone bioassays conducted monthly from September 2020 to April 2021 showed five-day mortality of *An. gambiae* Kisumu above 80% (efficacy threshold) through April, representing a minimum of seven months of residual efficacy for both SumiShield in Nassian on cement walls. On mud walls, the mortality dropped below 80% (45.4%), for the first time in the eighth month post IRS (Figures 26–28). [3] Fludora Fusion in Sakassou continued to cause five-day mortality above 98% after eight months using the susceptible strain *An. gambiae* Kisumu. However, the residual efficacy against wild (resistant) *An. gambiae* s.l. from Sakassou dropped below the threshold (to 72.7%) in the seventh and eighth month, consecutively, post-IRS.

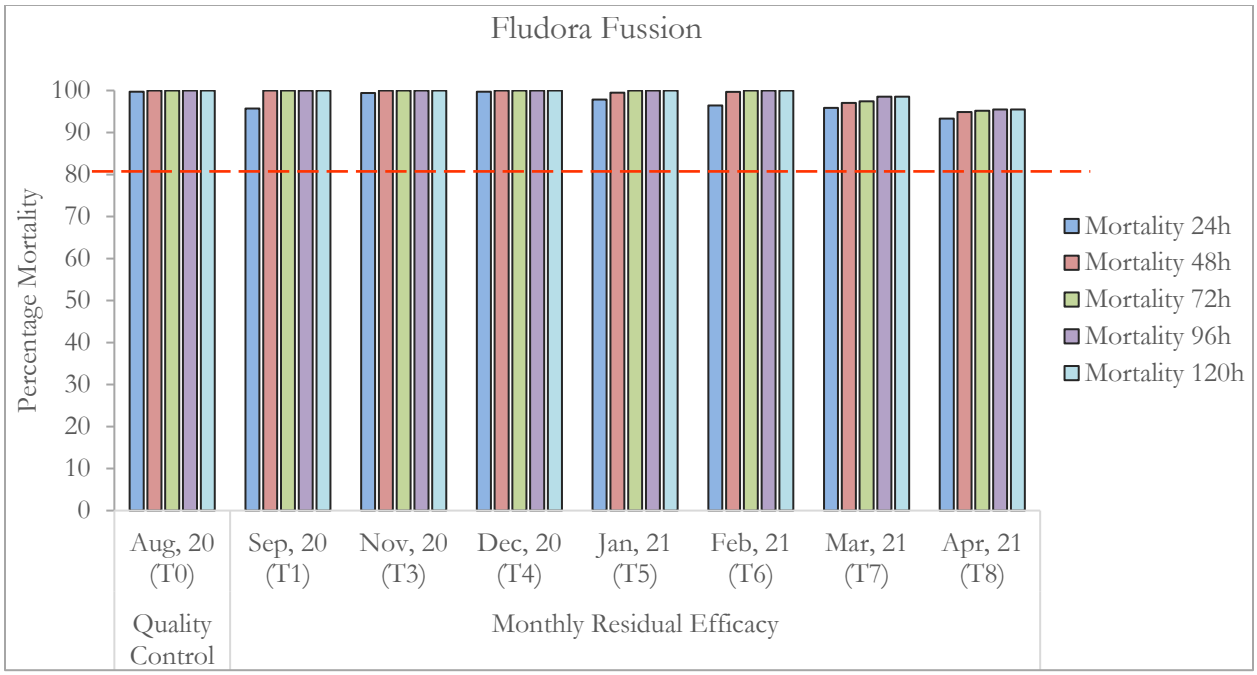
[3] The insecticide decay rate is reported though March 2021 using the available data at the time this report was submitted.

FIGURE 26: QUALITY CONTROL AND RESIDUAL EFFICACY: MORTALITY OF *AN. GAMBIAE* KISUMU STRAIN ON FOUR MUD AND NINE CEMENT SURFACES SPRAYED WITH SUMISHIELD IN NASSIAN



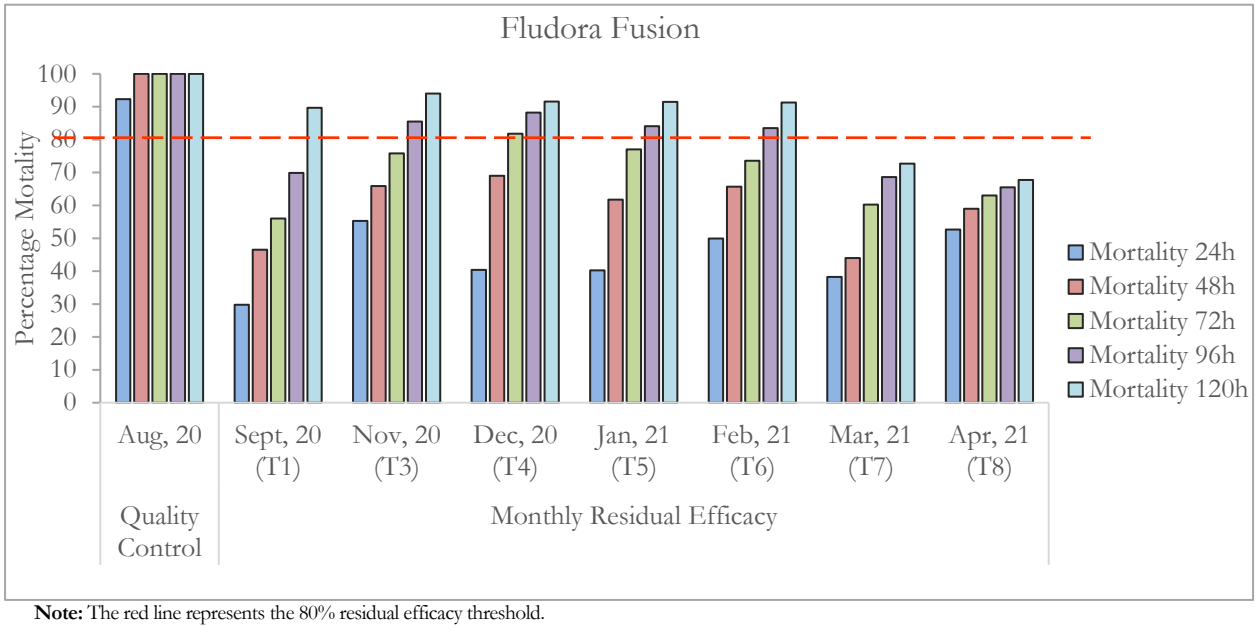
Note: The red line represents the 80% residual efficacy threshold.

FIGURE 27: QUALITY CONTROL AND RESIDUAL EFFICACY: MORTALITY OF *AN. GAMBIAE* KISUMU STRAIN ON NINE CEMENT SURFACES SPRAYED WITH FLUDORA FUSION IN SAKASSOU



Note: The red line represents the 80% residual efficacy threshold.

FIGURE 28: QUALITY CONTROL AND RESIDUAL EFFICACY: MORTALITY OF WILD COLLECTED *AN. GAMBIAE* S.L. ON NINE CEMENT SURFACES SPRAYED WITH FLUDORA FUSION IN SAKASSOU

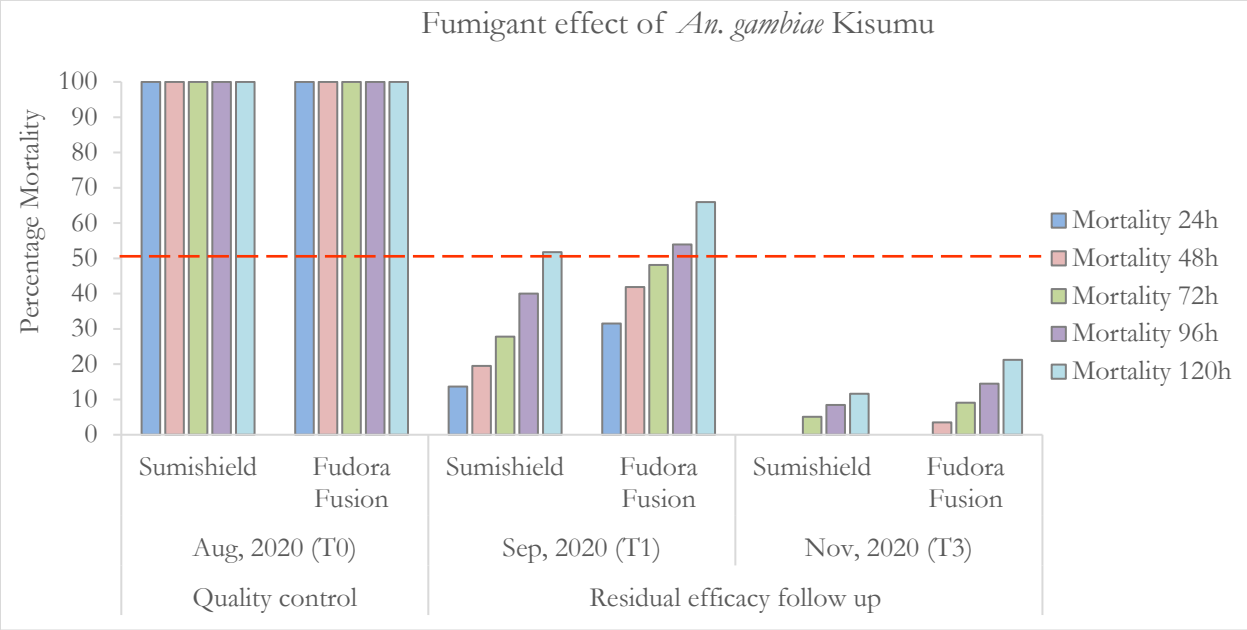


Note: The red line represents the 80% residual efficacy threshold.

3.7.3 FUMIGANT EFFECT

Fumigant effects were observed for both the Sumishield- and Fludora Fusion-sprayed walls on susceptible *An. gambiae* Kisumu in Nassian and Sakassou, respectively. During the quality control, 100% mortality was observed in both sites and insecticides. The effect dropped to 51.7% and 65.9% for SumiShield and Fludora Fusion, respectively, one month after IRS, before reaching less than 50% mortality, the threshold for the fumigant effect (Figure 29).

FIGURE 29: FUMIGATION EFFECT MORTALITY *AN. GAMBIAE* S.L. KISUMU ON CEMENT SURFACES SPRAYED WITH SUMISHIELD IN NASSIAN AND FLUDORA FUSION IN SAKASSOU



Note: The red line represents the 50% residual efficacy threshold.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 VECTOR BIONOMICS

An. gambiae s.l. remains the predominant *Anopheles* species and predominant malaria vector in all the surveillance sites. The other notable *Anopheles* species are *An. funestus* s.l., *An. nili*, and *An. pharoensis*. The three malaria vectors were collected in similar proportions in 2020 (94.8%, 1.5%, and 0.3%, respectively) as in 2019 (94%, 2%, and 0.7%, respectively). *An. coluzzii* and *An. gambiae* s.s. were the two species of the complex recorded in all sites. Similar to the 2019 collection, *An. coluzzii* was predominant in Béoumi, Dabakala, and Sakassou. *An. gambiae* s.s. represented the main vector only in Nassian.

IRDs of *An. gambiae* s.l. vectors were considerably reduced after IRS in Sakassou (15 to 4.9 f/r/d, a 67.3% reduction), while it increased in Nassian (1.3 to 1.9 f/r/, a 31.6% increase). However, there was 48% decrease in the IRD after IRS compared to the 2019 collection. In unsprayed sites 33% reduction (4.3 to 2.9 f/r/d) was observed in the same period. The post-spray period represents the second rainy season, when the peak IRD was observed in all sites in 2019.

The HBR of *An. gambiae* s.l. decreased after IRS in Sakassou (255.5 to 144.9 b/p/n), while it increased in Nassian (5.3 to 12.3 b/p/n), but it nevertheless was lower than in 2019.

Anopheles gambiae s.l. biting activity peaked betweenmidnight and 5:00 a.m., both indoors and outdoors, in all four sites. We also observed that *An. gambiae* s.l. bite at similar rates outdoors and indoors in the sprayed sites (indoor biting ranged from 46.4% in Sakassou to 48.8% in Nassian).

The proportion of gravid *An. gambiae* s.l. was similar one month before IRS to the four months after IRS in all sites (14.6% to 22.4%). Furthermore, there were similar numbers of parous *An. gambiae* s.l. in the sprayed sites (86.7% and 85.6%) as in the control sites (88.5% and 86.7%) one month before IRS. Different trends were observed for *An. funestus* s.l., the predominant vector collected in Nassian. The proportion of gravid mosquitoes decreased from September to December 2020 in the sprayed sites (from 23.2% to 11.7%), while a slight increase was noted over the same period in the control sites (16.7% to 20.3%). Similarly, the parity rate was similar in sprayed sites and in control sites (53.4% to 50.6%) after IRS. .

In the four months after IRS, the sporozoite infection rate decreased in Sakassou (from 1.76% to 0.5%), while it increased in Nassian (from 6.7% to 9.4%). However, there was a 31% decrease of the infection rate after IRS, in Nassian, compared to the 2019 collection. The EIR of *An. gambiae* s.l. decreased from 2.25 ib/p/n to 0.56 ib/p/n (75%) in sprayed sites, while it decreased from 0.90 ib/p/n to 0.61 ib/p/n (32%) in control sites.

The maximum potential protective effect of IRS may not have been achieved due tothe postponement of the 2020 IRS campaign (by four months in Sakassou and two months in Nassian) as a result of the COVID-19 pandemic and other conflicting NMCP priorities in 2020. These entomological findings, which are consistent with the trends observed in 2019, combined with available epidemiological data support the recommendation to implement IRS in April in Sakassou and in June in Nassian to maximize to potential protective effect. The increase in both the proportion of gravid vectors and parity rates after IRS, however, requires further investigation, with careful attention to the slow-acting nature of clothianidin-based insecticides.

4.2 INSECTICIDE SUSCEPTIBILITY

High pyrethroid and carbamate resistance was observed in all 18 sites monitored for susceptibility of *An. gambiae* s.l., emphasizing the potentially limited efficacy of pyrethroid-only interventions in Cote d'Ivoire.

Pre-exposure to PBO before exposure to deltamethrin, permethrin, and alpha-cypermethrin yielded partial but substantial increases in mortality of the *An. gambiae* s.l. populations in all sites, particularly with deltamethrin, with a full restitution of vector susceptibility in one site (Abidjan). This suggests that enzymes such as P450s might be

involved in insecticide resistance, but further molecular analysis is needed to confirm the extent of enzyme involvement.
Susceptibility to pirimiphos-methyl was observed in 12 sites, while low intensity resistance was observed in the remaining six sites.
Susceptibility to clothianidin was observed in nine out of 18 sites within seven days of exposure, including the two IRS sites.
Chlorfenapyr at the dose of 100µg/bottle yielded full susceptibility in 10 sites, while the 200 µg/bottle dose did so in 17 sites. As the insecticide is still being assessed for diagnostic concentration, the "resistance" already observed in a few sites in the country highlights the need for continuous monitoring to determine its extent and contribute to the WHO prequalification team's determination of the appropriate diagnostic dose for this insecticide. These results support the inclusion of Interceptor G2 nets, which contain chlorphenapyr, in a stratified distribution of ITNs for malaria vector control and resistance management in Côte d'Ivoire.

4.3 RESIDUAL EFFICACY OF SUMISHIELD AND FLUDORA FUSION

SumiShield and Fludora Fusion were effective on both mud and cement walls with residual efficacy of at least seven months at the time of this report. The observed mortality showed that the sprayed walls were not underdosed during the campaign. As of March 2021, monthly data collected on the residual efficacy of both insecticides on mud and cement sprayed walls recorded more than 80% mortality of the susceptible *An. gambiae* strain, indicating adequate residual efficacy of both insecticides in the seven months of observation.
The mortality recorded against the wild collected mosquitoes from Sakassou (where Fludora Fusion was sprayed) dropped below the efficacy threshold of 80% after six months, which suggests that the continued residual efficacy against the susceptible colony may be attributable to the deltamethin content. The IRD results suggest that mosquitoes rest longer on treated walls than the 30 minutes of controlled exposure for cone bioassays, though, potentially extending the residual efficacy in uncontrolled settings. These results support the recommendation of conducting IRS in April in Sakassou, as the insecticide is expected to remain effective long enough to cover both peak malaria transmission periods.
The fumigant effects of both insecticides (SumiShield and Fludora Fusion) were observed only for one month post IRS in Nassian and Sakassou. The expected fumigant effect of the neonicotinoid-based clothianidin insecticides is unknown and currently being examined across several countries.
The consistently high pyrethroid resistance confirms the need for strategic deployment of PBO and/or dual insecticide ITNs across the country.
As pyrethroid resistance mechanisms are widespread and target site mutation alleles were characterized in the vector populations for several years, targeting new additional resistance markers is advisable.
Both clothianidin-based insecticides showed good residual efficacy, so their use could continue in the upcoming IRS campaigns. In addition, the susceptibility observed against pirimiphos-methyl in Nassian could support an insecticide rotation plan for IRS, taking into account PMI's insecticide procurement policy and the residual life of pirirmiphos-methyl based insecticide, which has been shown to last for at least six months in neighboring countries such as Ghana, Burkina Faso, and Benin (PMI VectorLink Annual Reports, 2016-2018).

ANNEX

TABLE A1: SPECIES COMPOSITION OF MOSQUITOES COLLECTED AT ALL SITES USING HLC (4 COLLECTION HOUSES DURING 2 CONSECUTIVE NIGHTS OF COLLECTION INDOOR AND OUTDOOR PER MONTH)

Sites	Data / Period	January 2020	February 2020	March 2020	June 2020	July2020	August2020	September 2020	October 2020	November 2020	December 2020	Total
Beoumi	*An. funestus s.l.*	9	8	0	0	11	7	15	7	0	11	68
	An. gambiae s.l.	23	7	11	375	1047	284	301	1223	112	30	3413
	An. pharoensis	8	0	0	17	9	4	4	230	34	39	345
	Culicine	89	271	449	188	108	31	80	78	32	43	1369
	Sub-Total	129	286	460	580	1175	326	400	1538	178	123	5195
Dabakala	*An. funestus s.l.*	2	3	112	3	0	13	1	1	7	3	145
	An. gambiae s.l.	13	12	1251	168	283	509	1799	1632	225	89	5981
	An. nili	0	0	9	4	8	43	12	105	1	1	183
	An. paludis	0	0	0	0	0	0	0	1	0	0	1
	An. pharoensis	1	0	114	2	2	24	31	35	7	6	222
	An. ziemanni	0	0	0	1	0	0	0	0	1	0	2
	Culicine	83	788	969	814	545	481	331	290	212	406	4919
	Sub-Total	99	803	2455	992	838	1070	2174	2064	453	505	11453
Nassian	*An. funestus s.l.*	37	6	9	1	66	81	63	58	24	57	402
	An. gambiae s.l.	8	9	13	124	175	180	106	665	10	7	1297
	An. nili	0	0	0	0	0	1	0	19	0	0	20
	An. pharoensis	0	0	0	3	0	0	1	7	0	0	11
	Culicine	17	25	23	30	36	30	41	64	24	85	375
	Sub-Total	62	40	45	158	277	292	211	813	58	149	2105
Sakassou	*An. coustani*	0	0	0	0	2	0	0	0	0	0	2
	An. funestus s.l.	16	1	0	33	3	5	5	2	10	2	77
	An. gambiae s.l.	2541	433	2983	8248	5177	5141	3889	2821	1164	1398	33795
	An. nili	0	0	0	1	16	2	0	8	0	0	27
	An. pharoensis	10	0	0	371	183	162	69	124	9	7	935
	An. ziemanni	0	0	0	0	0	0	0	1	0	0	1
	Culicine	163	257	464	290	175	184	100	121	66	22	1842
	Sub-Total	2730	691	3447	8943	5556	5494	4063	3077	1249	1429	36679
Total		3020	1820	6407	10673	7846	7182	6848	7492	1938	2206	55432

TABLE A2: SPECIES COMPOSITION OF MOSQUITOES COLLECTED AT ALL SITES USING PSC (30 HOUSES PER MONTH)

PSC												
Sites	Data / Period	January 2020	February 2020	March 2020	June 2020	July 2020	August 2020	September 2020	October 2020	November 2020	December 2020	Total
Beoumi	*An. gambiae s.l*	8	12	6	63	219	57	57	235	26	9	692
	An. funestus s.l.	15	2	0	0	5	0	5	3	0	1	31
	An. nili	0	0	0	0	0	0	0	0	0	0	0
	An. pharoensis	0	0	0	0	0	0	0	15	0	0	15
	Culicine	81	116	320	51	100	76	51	26	42	65	928
		104	130	326	114	324	133	113	279	68	75	1666
Dabakala	*An. gambiae s.l*	7	31	283	133	63	256	88	173	78	31	1143
	An. funestus s.l.	0	1	41	2	0	0	0	0	3	5	52
	An. nili	0	0	4	0	0	0	0	0	0	0	4
	An. pharoensis	0	0	29	0	0	0	0	0	1	0	30
	Culicine	41	201	58	243	116	139	41	128	94	66	1127
		48	233	415	378	179	395	129	301	176	102	2356
Nassian	*An. gambiae s.l*	16	6	4	30	123	56	37	184	7	1	464
	An. funestus s.l.	10	6	9	0	49	35	26	4	9	27	175
	An. nili	0	0	0	0	0	0	0	0	0	0	0
	An. pharoensis	0	0	0	0	0	0	0	0	0	0	0
	Culicine	0	1	7	17	1	6	3	10	45	18	108
		26	13	20	47	173	97	66	198	61	46	747
Sakassou	*An. gambiae s.l*	199	23	713	883	507	396	215	115	203	64	3318
	An. funestus s.l.	0	0	0	3	0	0	0	1	2	0	6
	An. nili	0	0	0	0	0	0	0	0	0	0	0
	An. pharoensis	0	0	0	0	0	0	0	0	0	0	0
	Culicine	157	73	102	20	11	12	3	5	4	8	395
		356	96	815	906	518	408	218	121	209	72	3719
Total		534	472	1576	1445	1194	1033	526	899	514	295	8488

TABLE A3: SPECIES COMPOSITION OF MOSQUITOES COLLECTED AT ALL SITES USING CDC LIGHT TRAPS (4 TRAPS INDOORS FOR 2 CONSECUTIVE NIGHTS PER MONTH)

CDC Light Trap												
Sites	Data / Period	January 2020	February 2020	March 2020	June 2020	July 2020	August 2020	September 2020	October 2020	November 2020	December 2020	Total
Beoumi	*An. gambiae s.l.*	1	1	3	6	32	11	28	50	3	1	136
	An. funestus s.l.	3	4	4	0	4	0	0	1	0	0	16
	An. nili	0	0	0	0	0	0	0	0	0	0	0
	An. pharoensis	1	0	0	0	0	0	1	14	0	0	16
	Culicine	128	103	129	18	103	143	109	78	23	24	858
		133	108	136	24	139	154	138	143	26	25	1026
Dabakala	*An. gambiae s.l.*	0	1	153	45	1	12	183	196	9	6	606
	An. funestus s.l.	0	0	25	0	0	0	0	2	1	0	28
	An. nili	0	0	2	0	0	0	0	0	0	0	2
	An. pharoensis	0	0	1	1	1	0	0	0	0	0	3
	Culicine	1	8	3	153	90	220	55	36	44	15	625
		1	9	184	199	92	232	238	234	54	21	1264
Nassian	*An. gambiae s.l.*	0	0	0	0	8	0	2	16	0	0	26
	An. funestus s.l.	1	0	0	0	20	3	7	1	0	1	33
	An. nili	0	0	0	0	0	0	0	0	0	0	0
	An. pharoensis	0	0	0	0	0	0	0	0	0	0	0
	Culicine	1	0	1	0	6	15	25	4	5	5	62
		2	0	1	0	34	18	34	21	5	6	121
Sakassou	*An. gambiae s.l.*	39	206	495	99	119	64	113	25	26	19	1205
	An. funestus s.l.	0	0	0	0	0	0	0	0	0	0	0
	An. nili	0	0	0	0	0	0	0	0	0	0	0
	An. pharoensis	0	0	0	0	6	0	0	0	0	0	6
	Culicine	73	43	27	39	42	22	9	6	3	4	268
		112	249	522	138	167	86	122	31	29	23	1479
Total		248	366	843	361	432	490	532	429	114	75	3890

TABLE A4: ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. COLLECTED USING PSC

ABDOMINAL STATUS												
Sites	Data / Period	January 2020	February 2020	March 2020	June 2020	July 2020	August 2020	September 2020	October 2020	November 2020	December 2020	Total
Beoumi	Unfed *An. gambiae* s.l.	1	0	0	0	14	1	3	1	0	1	21
	Blood fed *An. gambiae* s.l	5	9	2	30	64	44	47	134	23	4	362
	Half-Gravid *An. gambiae* s.l.	1	2	4	16	92	5	5	16	0	0	141
	Gravid *An. gambiae* s.l.	1	1	0	17	49	7	2	84	3	4	168
	Unfed An. funestus s.l.	1	0	0	0	1	0	0	0	0	0	2
	Blood fed *An. funestus* s.l	13	1	0	0	4	0	3	2	0	0	23
	half- gravid *An. funestus* s.l.	1	0	0	0	0	0	2	0	0	0	3
	Gravi *An. funestus* s.l.	0	1	0	0	0	0	0	1	0	1	3
		23	14	6	63	224	57	62	238	26	10	723
Dabakala	Unfed *An. gambiae* s.l.	0	0	38	0	2	11	0	13	4	2	70
	Blood fed *An. gambiae* s.l	5	17	101	54	42	174	78	147	65	1	684
	Half-Gravid *An. gambiae* s.l.	1	1	63	58	13	13	8	7	5	26	195
	Gravid *An. gambiae* s.l.	1	13	81	21	6	58	2	6	4	2	194
	Unfed An. funestus s.l.	0	0	4	0	0	0	0	0	0	0	4
	Blood fed *An. funestus* s.l	0	1	17	0	0	0	0	0	2	0	20
	half- gravid *An. funestus* s.l.	0	0	12	0	0	0	0	0	1	5	18
	Gravi *An. funestus* s.l.	0	0	8	2	0	0	0	0	0	0	10
		7	32	328	135	63	256	88	173	81	36	1199
Nassian	Unfed *An. gambiae* s.l.	0	0	0	0	2	1	0	21	1	0	25
	Blood fed *An. gambiae* s.l	11	2	3	16	57	36	28	82	2	1	238
	Half-Gravid *An. gambiae* s.l.	3	1	0	12	37	10	5	47	2	0	117
	Gravid *An. gambiae* s.l.	2	3	1	2	27	9	4	34	2	0	84
	Unfed An. funestus s.l.	1	0	2	0	3	1	0	0	0	0	7
	Blood fed *An. funestus* s.l	8	1	5	0	29	18	14	2	5	17	99
	half- gravid *An. funestus* s.l.	1	1	1	0	7	5	8	0	2	4	29
	Gravi *An. funestus* s.l.	0	4	1	0	10	11	4	2	2	6	40
		26	12	13	30	172	91	63	188	16	28	639
Sakassou	Unfed *An. gambiae* s.l.	13	1	56	299	56	106	35	11	5	10	592
	Blood fed *An. gambiae* s.l	167	1	208	460	249	155	82	69	131	10	1532
	Half-Gravid *An. gambiae* s.l.	1	17	346	4	100	73	50	19	45	26	681
	Gravid *An. gambiae* s.l.	18	4	103	120	85	57	48	16	22	18	491
	Unfed An. funestus s.l.	0	0	0	3	0	0	0	0	1	0	4
	Blood fed *An. funestus* s.l	0	0	0	0	0	0	0	0	1	0	1
	half- gravid *An. funestus* s.l.	0	0	0	0	0	0	0	1	0	0	1
	Gravi *An. funestus* s.l.	0	0	0	0	0	0	0	0	0	0	0
		199	23	713	886	490	391	215	116	205	64	3302
Total		255	81	1060	1114	949	795	428	715	328	138	5863

TABLE A5: BITING CYCLE OF *AN. GAMBIAE* S.L. USING HLC

					An. gambiae s.l.								
Sites	Collection area	18-19: *An. gambiae* s.l.	19-20: *An. gambiae* s.l.	20-21: *An. gambiae* s.l.	21-22: *An. gambiae* s.l.	22-23: *An. gambiae* s.l.	23-24: *An. gambiae* s.l.	24-1: *An. gambiae* s.l.	1-2: *An. gambiae* s.l.	2-3: *An. gambiae* s.l.	3-4: *An. gambiae* s.l.	4-5: *An. gambiae* s.l.	5-6: *An. gambiae* s.l.
Beoumi	Indoor	0.09	0.33	0.11	0.42	0.63	1.05	1.39	1.53	1.54	1.34	1.70	1.04
Dabakala	Indoor	0.09	0.24	0.43	0.78	1.09	1.88	2.73	3.24	2.81	2.41	1.99	0.58
Nassian	Indoor	0.03	0.02	0.05	0.09	0.18	0.31	0.64	0.61	0.61	0.41	0.57	0.35
Sakassou	Indoor	1.38	1.86	2.21	4.22	6.13	8.12	10.04	14.11	13.96	14.15	13.83	8.01
Total	Indoor	1.59	2.44	2.80	5.51	8.03	11.36	14.81	19.49	18.92	18.31	18.08	9.98
Sites	Collection area	18-19: *An. gambiae* s.l.	19-20: *An. gambiae* s.l.	20-21: *An. gambiae* s.l.	21-22: *An. gambiae* s.l.	22-23: *An. gambiae* s.l.	23-24: *An. gambiae* s.l.	24-1: *An. gambiae* s.l.	1-2: *An. gambiae* s.l.	2-3: *An. gambiae* s.l.	3-4: *An. gambiae* s.l.	4-5: *An. gambiae* s.l.	5-6: *An. gambiae* s.l.
Beoumi	Outdoor	0.02	0.05	0.18	0.28	0.36	0.71	1.76	1.99	1.81	1.32	1.19	0.49
Dabakala	Outdoor	0.10	0.24	0.55	0.77	0.99	1.41	2.52	3.84	3.45	2.69	1.91	0.65
Nassian	Outdoor	0.00	0.01	0.07	0.09	0.23	0.39	0.70	0.74	0.62	0.58	0.46	0.36
Sakassou	Outdoor	1.51	3.21	3.08	4.86	6.67	10.23	13.50	16.50	15.49	15.45	14.44	8.26
Total	Outdoor	1.63	3.51	3.88	5.99	8.24	12.74	18.48	23.08	21.38	20.04	18.00	9.76
					An. funestus s.l.								
Sites	Collection area	18-19: *An. funestus* s.l.	19-20: *An. funestus* s.l.	20-21: *An. funestus* s.l.	21-22: *An. funestus* s.l.	22-23: *An. funestus* s.l.	23-24: *An. funestus* s.l.	24-1: *An. funestus* s.l.	1-2: *An. funestus* s.l.	2-3: *An. funestus* s.l.	3-4: *An. funestus* s.l.	4-5: *An. funestus* s.l.	5-6: *An. funestus* s.l.
Beoumi	Intdoor	0.00	0.01	0.00	0.00	0.02	0.01	0.03	0.05	0.05	0.03	0.04	0.03
Dabakala	Intdoor	0.00	0.01	0.01	0.05	0.04	0.02	0.06	0.10	0.03	0.03	0.03	0.02
Nassian	Intdoor	0.01	0.03	0.03	0.04	0.06	0.05	0.19	0.26	0.13	0.18	0.12	0.05
Sakassou	Intdoor	0.02	0.00	0.02	0.03	0.04	0.06	0.01	0.03	0.03	0.03	0.01	0.02
Total	Intdoor	0.03	0.05	0.05	0.12	0.15	0.13	0.29	0.44	0.24	0.27	0.19	0.11
Sites	Collection area	18-19: *An. funestus* s.l.	19-20: *An. funestus* s.l.	20-21: *An. funestus* s.l.	21-22: *An. funestus* s.l.	22-23: *An. funestus* s.l.	23-24: *An. funestus* s.l.	24-1: *An. funestus* s.l.	1-2: *An. funestus* s.l.	2-3: *An. funestus* s.l.	3-4: *An. funestus* s.l.	4-5: *An. funestus* s.l.	5-6: *An. funestus* s.l.
Beoumi	Outdoor	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.03	0.04	0.03	0.03	0.00
Dabakala	Outdoor	0.01	0.01	0.01	0.03	0.03	0.06	0.07	0.06	0.11	0.10	0.02	0.02
Nassian	Outdoor	0.01	0.03	0.06	0.02	0.05	0.09	0.15	0.18	0.18	0.23	0.18	0.21
Sakassou	Outdoor	0.00	0.01	0.01	0.01	0.03	0.03	0.00	0.03	0.01	0.03	0.02	0.03
Total	Outdoor	0.02	0.04	0.09	0.06	0.11	0.18	0.24	0.30	0.33	0.39	0.24	0.26

TABLE A6: MONTHLY BITING RATE RESULTS USING HLC

Sites	Period / Data	Indoor human biting rate: *An. gambiae* s.l.	Outdoor human biting rate: *An. gambiae* s.l.	Indoor human biting rate : *An. funestus* s.l.	Outdoor human biting rate: *An. funestus* s.l.	Total
Beoumi	January 2020	1.4	1.5	0.6	0.5	4
	February 2020	0.25	0.6	0.9	0.1	1.9
	March 2020	0.88	0.5	0	0	1.4
	June 2020	25.3	21.6	0	0	46.9
	July 2020	70.5	60.4	1	0.4	132.3
	August 2020	21	14.5	0.8	0.1	36.4
	September 2020	16.1	21.5	1	0.9	39.5
	October 2020	82.8	70.1	0.3	0.6	153.8
	November 2020	3.5	10.5	0	0	14
	December 2020	1.8	2	0.6	0.8	5.1
		223.4	203.2	5.2	3.4	435.2
Dabakala	January 2020	0.38	1.3	0.1	0.1	1.9
	February 2020	0.88	0.6	0.1	0.3	1.9
	March 2020	80	76.4	6.6	7.4	170.4
	June 2020	10.4	10.6	0.4	0	21.4
	July 2020	10.6	24.8	0	0	35.4
	August 2020	30.5	33.1	0.4	1.3	65.3
	September 2020	104.4	120.5	0.1	0	225
	October 2020	113	91	0.1	0	204.1
	November 2020	12	16.1	0	0.9	29
	December 2020	2.9	8.3	0	0.4	11.6
		365	382.7	7.8	10.4	765.9
NASSIAN	January 2020	0.5	0.5	3.6	1	5.6
	February 2020	0.88	0.3	0.6	0.1	1.9
	March 2020	1.3	0.4	0.9	0.3	2.8
	June 2020	6.3	9.3	0.1	0	15.7
	July 2020	10.5	11.4	4	4.3	30.2
	August 2020	8.4	14.1	4.1	6	32.6
	September 2020	3.9	9.4	1.5	6.4	21.2
	October 2020	44.6	38.5	1.6	5.6	90.3
	November 2020	0.5	0.8	1.6	1.4	4.3
	December 2020	0.75	0.1	4.6	2.5	7.9
		77.5	84.8	22.6	27.6	212.5
SAKASSOU	January 2020	151.8	165.9	1.4	0.6	319.6
	February 2020	29.3	24.9	0.1	0	54.3
	March 2020	152.1	220.8	0	0	372.9
	June 2020	492	539	2.8	1.4	1035.2
	July 2020	330.1	317	0.3	0.1	647.5
	August 2020	310	332.6	0.4	0.3	643.3
	September 2020	198.4	287.8	0.3	0.4	486.9
	October 2020	159.8	192.9	0.1	0.1	352.9
	November 2020	51.8	93.8	0.3	1	146.9

TABLE A7: MONTHLY PARITY RATES OF DISSECTED *AN. GAMBIAE* S.L. PER SITE

		An. gambiae **s.l.**			*An. funestus* **s.l.**		
District	***Period / Data***	***Examined***	***Parous***	***Parity rate***	***Examined***	***Parous***	***Parity rate***
Béoumi	Jan-20	21	8	38.1	4	3	75.0
	Feb-20	5	3	60.0	5	0	0.0
	Mar-20	11	3	27.3	0	0	
	Jun-20	335	129	38.5	0	0	
	Jul-20	402	172	42.8	1	0	0.0
	Aug-20	221	128	57.9	4	1	25.0
	Sep-20	285	189	66.3	7	6	85.7
	Oct-20	357	184	51.5	2	1	50.0
	Nov-20	109	84	77.1	0	0	
	Dec-20	30	20	66.7	5	5	100.0
	Total	1776	920	51.8	26	18	69.2
Dabakala	Jan-20	12	9	75.0	1	0	0.0
	Feb-20	9	4	44.4	0	1	
	Mar-20	367	180	49.0	4	3	75.0
	Jun-20	152	80	52.6	2	0	0.0
	Jul-20	275	194	70.5	0	0	
	Aug-20	363	220	60.6	7	2	28.6
	Sep-20	338	161	47.6	1	0	0.0
	Oct-20	421	260	61.8	1	0	0.0
	Nov-20	185	88	47.6	0	2	
	Dec-20	83	57	68.7	0	3	
	Total	2205	1253	56.8	17	10	58.8
Nassian	Jan-20	8	6	75.0	26	7	26.9
	Feb-20	8	2	25.0	4	1	25.0
	Mar-20	13	1	7.7	7	2	28.6
	Jun-20	96	61	63.5	0	0	
	Jul-20	160	101	63.1	30	23	76.7
	Aug-20	175	118	67.4	46	32	69.6
	Sep-20	102	74	72.5	49	12	24.5
	Oct-20	302	200	66.2	20	5	25.0
	Nov-20	10	5	50.0	13	11	84.6
	Dec-20	7	2	28.6	37	20	54.1
	Total	881	570	64.7	186	159	85.5
Sakassou	Jan-20	259	104	40.2	9	4	44.4
	Feb-20	346	197	56.9	1	0	0.0
	Mar-20	516	338	65.5	0	0	
	Jun-20	421	226	53.7	0	1	
	Jul-20	319	179	56.1	2	0	0.0
	Aug-20	436	262	60.1	2	2	100.0
	Sep-20	432	225	52.1	0	0	
	Oct-20	398	242	60.8	0	0	
	Nov-20	410	249	60.7	8	1	12.5

		An. gambiae **s.l.**			*An. funestus* **s.l.**		
District	***Period / Data***	***Examined***	***Parous***	***Parity rate***	***Examined***	***Parous***	***Parity rate***
	Dec-20	289	191	66.1	1	0	0.0
	Total	3826	2213	57.8	16	15	93.8

TABLE A8: MONTHLY PARITY RATES OF DISSECTED MOSQUITOES FROM INDOOR AND OUTDOOR COLLECTIONS

		Outdoor			Indoor		
District	**Month**	**Dissected**	**Parous**	**Parity Rate**	**Dissected**	**Parous**	**Parity Rate**
Béoumi	May 19	144	118	81.9	88	78	88.6
	June 19	84	81	96.4	60	58	96.7
	July 19	178	149	83.7	206	170	82.5
	August 19	138	122	88.4	284	258	90.8
	September 19	125	104	83.2	147	127	86.4
	October 19	464	406	87.5	480	410	85.4
	November 19	231	216	93.5	176	167	94.9
	December 19	163	150	92.0	74	65	87.8
Dabakala	May 19	255	169	66.3	164	120	73.2
	June 19	118	118	100.0	150	150	100.0
	July 19	220	67	30.5	202	77	38.1
	August 19	270	216	80.0	200	144	72.0
	September 19	158	17	10.8	150	22	14.7
	October 19	166	124	74.7	132	94	71.2
	November 19	285	235	82.5	124	107	86.3
	December 19	93	84	90.3	39	37	94.9
Nassian	May 19	19	18	94.7	7	7	100.0
	June 19	18	15	83.3	16	15	93.8
	July 19	29	27	93.1	33	27	81.8
	August 19	22	22	100.0	27	26	96.3
	September 19	80	80	100.0	87	85	97.7
	October 19	77	69	89.6	104	95	91.3
	November 19	97	84	86.6	200	169	84.5
	December 19	65	64	98.5	154	154	100.0
Sakassou	April 19	214	184	86.0	152	134	88.2
	May 19	239	127	53.1	232	132	56.9
	June 19	247	214	86.6	243	205	84.4
	July 19	238	202	84.9	341	292	85.6
	August 19	299	254	84.9	295	243	82.4
	September 19	344	276	80.2	272	193	71.0
	October 19	240	169	70.4	218	166	76.1
	November 19	221	102	46.2	207	80	38.6
	December 19	244	146	59.8	226	138	61.1

TABLE A9: MONTHLY SPOROZOITE AND EIRS OF THE FOUR DISTRICTS OF BIONOMIC SURVEY

District	Month	Total *An. gambiae* s.l. Tested	Number of Circum-sporozoite Positive	Sporozoite Rate	HBR (b/p/n)	EIR (ib/p/n)
Béoumi	Jan 20	15	0	0	1.45	0
	Feb 20	6	0	0	0.425	0.000
	Mar 20	8	0	0	0.69	0.000
	Jun 20	154	10	0.065	23.45	1.524
	Jul 20	271	11	0.041	65.45	2.683
	Aug 20	100	9	0.09	17.75	1.598
	Sep 20	100	1	0.01	18.8	0.188
	Oct 20	99	2	0.02	76.45	1.529
	Nov 20	100	4	0.04	7	0.280
	Dec 20	30	4	0.133	1.9	0.253
	Total	883	41	0.046	109.031	5.015
Dabakala	Jan 20	10	0	0	0.84	0
	Feb 20	8	0	0	0.74	0
	Mar 20	101	0	0	78.2	0
	Jun 20	99	5	0.051	10.5	0.536
	Jul 20	258	37	0.143	17.7	2.531
	Aug 20	99	6	0.061	31.8	1.940
	Sep 20	103	1	0.01	112.45	1.125
	Oct 20	108	1	0.009	102	0.918
	Nov 20	89	2	0.022	14.05	0.309
	Dec 20	74	4	0.054	5.6	0.302
	Total	939	56	0.06	187.5	11.250
Nassian	Jan 20	8	1	0.125	0.5	0.063
	Feb 20	12	0	0	0.59	0.000
	Mar 20	7	0	0	0.85	0.000
	Jun 20	112	8	0.071	7.8	0.554
	Jul 20	128	16	0.125	10.95	1.369
	Aug 20	136	10	0.074	11.25	0.833
	Sep 20	91	1	0.011	6.65	0.073
	Oct 20	90	2	0.022	41.55	0.914
	Nov 20	11	1	0.091	0.65	0.059
	Dec 20	4	1	0.25	0.425	0.106
	Total	599	40	0.067	39.969	2.678
Sakassou	Jan 20	100	0	0	158.85	0.000
	Feb 20	100	2	0.02	27.1	0.542
	Mar 20	101	3	0.03	186.45	5.594
	Jun 20	100	1	0.01	515.5	5.155
	Jul 20	98	1	0.01	323.55	3.236
	Aug 20	100	3	0.03	321.3	9.639

District	Month	Total *An. gambiae* s.l. Tested	Number of Circum-sporozoite Positive	Sporozoite Rate	HBR (b/p/n)	EIR (ib/p/n)
	Sep 20	100	1	0.01	243.1	2.431
	Oct 20	91	0	0	176.35	0.000
	Nov 20	100	0	0	72.8	0.000
	Dec 20	100	1	0.01	87.4	0.874
Sakassou	Total	990	12	0.012	1055.125	12.662
Béoumi	Oct 20	1	0	0	0.45	0
	Dec 20	11	1	0.091	0.7	0.031
	Total	12	1	0.083	0.575	0.048
Dabakala	Oct-20	1	0	0	0.05	0
	Nov-20	2	0	0	0.45	0
	Dec 20	1	0	0	0.2	0
	Total	4	0	0	0	0
Nassian	Jan 20	33	1	0.03	2.3	0.035
	Feb 20	5	1	0.2	0.35	0.063
	Mar 20	4	1	0.25	0.6	0.063
	Jun 20	1	0	0	0.05	0
	Jul 20	42	2	0.048	4.15	0.097
	Aug 20	18	0	0	5.05	0
	Sep 20	31	0	0	3.95	0
	Oct 20	10	0	0	3.6	0
	Nov 20	21	2	0.095	1.5	0.071
	Dec 20	53	4	0.075	3.55	0.134
	Total	218	11	0.050	2.51	0.127

TABLE A10: INSECTICIDE SUSCEPTIBILITY TEST RESULTS FROM THE 18 SITES

		Abengouro u	Abidjan	Aboisso	Adzopé	Beoumi	Bouaké	Bouna	Dabakala	Daloa	Gagnoa	Jacquevill e	Korhogo	Man	Nassian	Odienné	Sakassou	San Pedro	Yamoussoukr o
Insecticide Tested	Concent ration	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Alpha-cypermethrin 1x	0.05%	88 (3.4)	100 (4.0)	82 (29.3)	100 (4.0)	94 (1.1)	103 (1.0)	85 (3.5)	69 (0.0)	80 (31.3)	78 (10.3)	89 (20.2)	82 (6.1)	88 (1.1)	84 (2.4)	102 (0.0)	102 (4.9)	101 (0.0)	91 (0.0)
PBO+Alpha-cypermethrin	0.05%	87 (72.4)	91 (73.6)	76 (94.7)	92 (69.6)	98 (21.4)	100 (86.0)	85 (48.2)	83 (10.8)	77 (68.8)	72 (54.2)	82 (90.2)	72 (52.8)	82 (43.9)	95 (65.3)	107 (18.7)	96 (5.2)	98 (38.8)	80 (16.3)
Alpha-cypermethrin 5x	0.25%	96 (22.9)	85 (21.2)	90 (93.3)	89 (16.9)	95 (3.2)	96 (24.0)	77 (23.4)	95 (11.6)	78 (42.3)	70 (31.4)	83 (81.9)	80 (20.0)	86 (10.5)	91 (33.0)	104 (18.3)	99 (8.1)	97 (4.1)	80 (6.3)
Alpha-cypermethrin 10x	0.50%	97 (52.6)	94 (85.1)	81 (100.0)	100 (56.0)	92 (52.2)	109 (27.5)	73 (61.6)	84 (51.2)	79 (58.2)	80 (61.3)	88 (92.0)	79 (94.9)	90 (17.8)	88 (93.2)	103 (53.4)	100 (10.1)	100 (21.0)	94 (17.0)
Deltamethrin 1x	0.05%	92 (1.1)	86 (30.2)	79 (3.8)	94 (9.6)	97 (5.2)	102 (0.0)	89 (20.2)	72 (0.0)	69 (17.5)	77 (41.6)	90 (11.1)	83 (1.2)	88 (0.0)	88 (4.5)	111 (1.8)	105 (2.9)	101 (0.0)	94 (0.0)
PBO+Deltamethrin	0.05%	90 (42.2)	85 (95.3)	84 (50.0)	100 (60.0)	97 (74.2)	99 (65.7)	93 (79.6)	77 (15.6)	96 (72.1)	64 (73.4)	71 (98.6)	84 (76.2)	90 (65.6)	86 (47.7)	109 (24.8)	95 (6.3)	106 (5.7)	97 (16.5)
Deltamethrin 5x	0.25%	90 (42.2)	100 (35.0)	85 (24.7)	102 (32.4)	97 (25.8)	98 (17.3)	81 (46.9)	67 (11.9)	95 (44.7)	77 (64.9)	94 (92.6)	67 (26.9)	143 (16.8)	97 (44.3)	102 (20.6)	103 (3.9)	83 (7.2)	80 (10.0)
Deltamethrin 10x	0.50%	94 (63.8)	84 (94.0)	85 (24.7)	88 (61.4)	96 (60.4)	100 (54.0)	78 (88.5)	63 (22.2)	68 (98.4)	76 (84.2)	92 (97.8)	79 (34.2)	87 (59.8)	88 (77.3)	100 (52.0)	92 (53.3)	93 (46.2)	70 (68.6)
Permethrin 1x	0.75%	139 (1.4)	95 (0.0)	105 (1.0)	94 (4.3)	96 (16.7)	99 (1.0)	87 (10.3)	74 (4.1)	83 (15.7)	73 (27.4)	79 (2.5)	70 (0.0)	83 (0.0)	88 (1.1)	109 (2.8)	105 (1.0)	93 (1.1)	88 (2.3)
PBO+Permethrin	0.75%	91 (6.6)	94 (27.7)	86 (32.6)	91 (63.7)	94 (18.1)	98 (23.5)	75 (20.0)	65 (10.8)	73 (64.4)	76 (61.8)	63 (34.9)	87 (50.6)	85 (81.2)	83 (27.7)	99 (11.1)	99 (2.0)	103 (8.7)	92 (8.7)
Permethrin 5x	3.75%	89 (36.0)	80 (35.0)	80 (42.5)	103 (68.9)	96 (61.5)	75 (29.3)	74 (58.1)	79 (45.6)	75 (57.3)	76 (61.8)	39 (59.0)	72 (43.1)	71 (21.1)	94 (35.1)	109 (33.9)	100 (22.0)	72 (30.6)	90 (28.9)
Permethrin 10x	7.50%	94 (74.5)	95 (78.9)	74 (66.2)	112 (98.2)	99 (97.0)	109 (97.2)	75 (90.7)	90 (77.8)	87 (97.7)	75 (84.0)	42 (78.6)	83 (88.0)	89 (71.9)	88 (94.3)	98 (90.8)	98 (45.9)	99 (58.6)	95 (74.7)
Bendiocarb 1x	0.10%	97 (44.3)	93 (2.2)	x	103 (67.0)	98 (56.1)	109 (89.0)	77 (50.6)	87 (56.3)	97 (62.9)	77 (69.2)	x	72 (38.9)	92 (22.8)	83 (45.8)	104 (35.6)	102 (5.9)	101 (88.1)	91 (27.5)
Bendiocarb 5x	0.50%	95 (87.4)	97 (3.1)	x	103 (68.0)	96 (74.0)	98 (76.5)	85 (69.4)	88 (69.3)	89 (80.9)	75 (88.5)	x	102 (49.0)	85 (81.2)	84 (78.6)	104 (61.5)	102 (3.9)	95 (98.9)	83 (55.4)
Bendiocarb 10x	1.00%	96 (90.6)	97 (6.2)	x	98 (82.7)	96 (76.0)	109 (88.1)	91 (76.9)	95 (80.0)	77 (87.0)	79 (83.7)	x	80 (50.0)	95 (86.3)	76 (81.6)	105 (96.2)	100 (7.0)	x	92 (60.9)
Pirimiphos-methyl 1x	0.25%	98 (98.0)	91 (31.9)	x	100 (100.0)	96 (100.0)	106 (100.0)	83 (100.0)	76 (100.0)	84 (98.8)	74 (100.0)	69 (94.2)	80 (86.3)	93 (98.9)	100 (99.0)	101 (100.0)	101 (67.3)	101 (100.0)	89 (100.0)
Pirimiphos-methyl 5x	1.25%	x	87 (47.1)	x	x	x	x	x	x	x	x	45 (100.0)	86 (55.8)	x	x	x	103 (56.3)	x	x
Pirimiphos-methyl 10x	2.50%	x	98 (100.0)	x	x	x	x	x	x	x	x	x	85 (100.0)	x	x	x	107 (72.9)	x	x

X represents the tests that were not completed, either because not enough mosquitoes were collected or because these mosquitoes were not needed for the intensity assay.

Resistance confirmed Suspected resistance Susceptible

TABLE A11: FREQUENCIES OF *KDR*-WEST IN MOSQUITOES

District	Total (n)	RR	RS	SS	% *kdr*-West (L1014F)
Abengourou	24	11	6	7	58.33
Abidjan	23	8	6	9	47.83
Aboisso	46	37	8	1	89.13
Adzope	25	4	7	14	30.00
Béoumi	24	2	8	14	25.00
Bouaké	22	17	1	4	79.55
Bouna	25	23	2	0	96.00
Dabakala	25	3	22	0	56.00
Daloa	24	14	7	3	72.92
Gagnoa	25	9	12	4	60.00
Jacqueville	25	13	11	1	74.00
Korhogo	25	24	1	0	98.00
Man	25	14	10	1	76.00
Nassian	25	1	24	0	52.00
Odienné	25	25	0	0	100.00
Sakassou	20	4	16	0	60.00
San Pedro	23	14	9	0	80.43
Yamoussoukro	23	15	6	2	78.26
TOTAL	454	238	156	60	69.60

Note:
RR: Homozygous with two resistant Kdr West alleles;
RS : Heterozygous with one resistant Kdr West allele and one susceptible allele;
SS: Homozygous with two susceptible alleles.

TABLE A12: FREQUENCIES OF *KDR*-EAST IN MOSQUITOES

District	Total (n)	RR	RS	SS	% *kdr*-East (L1014S)
Abengourou	23	0	1	22	2.17
Abidjan	23	0	1	22	2.17
Aboisso	50	0	0	50	0.00
Adzope	25	0	0	25	0.00
Béoumi	25	0	2	23	4.00
Bouaké	25	0	4	21	8.00
Bouna	24	0	0	24	0.00
Dabakala	23	0	0	23	0.00
Daloa	21	0	1	20	2.38
Gagnoa	24	0	0	24	0.00
Jacqueville	22	0	2	20	4.55
Korhogo	25	0	0	25	0.00
Man	24	0	0	24	0.00
Nassian	24	0	1	23	2.08
Odienné	25	0	0	25	0.00
Sakassou	23	0	2	21	4.35
San Pedro	24	0	0	24	0.00
Yamoussoukro	20	0	1	19	2.50
TOTAL	450	0	15	435	1.67

Note:
RR: Homozygous with two resistant Kdr East alleles.
RS : Heterozygous with one resistant Kdr East allele and one susceptible allele;
SS: Homozygous with two susceptible alleles.

TABLE A13: FREQUENCIES OF *ACE*-1 IN MOSQUITOES

District	Total (n)	RR	RS	SS	% *kdr*-East (L1014S)
Abengourou	23	0	1	22	2.17
Abidjan	23	0	1	22	2.17
Aboisso	50	0	0	50	0.00
Adzope	25	0	0	25	0.00
Béoumi	25	0	2	23	4.00
Bouaké	25	0	4	21	8.00
Bouna	24	0	0	24	0.00
Dabakala	23	0	0	23	0.00
Daloa	21	0	1	20	2.38
Gagnoa	24	0	0	24	0.00
Jacqueville	22	0	2	20	4.55
Korhogo	25	0	0	25	0.00
Man	24	0	0	24	0.00
Nassian	24	0	1	23	2.08
Odienné	25	0	0	25	0.00
Sakassou	23	0	2	21	4.35
San Pedro	24	0	0	24	0.00
Yamoussoukro	20	0	1	19	2.50
TOTAL	450	0	15	435	1.67

Note:
RR: Homozygous with two resistant Kdr East alleles.
RS : Heterozygous with one resistant Kdr East allele and one susceptible allele;
SS: Homozygous with two susceptible alleles.

TABLE A14: FREQUENCIES OF *ACE*-1 IN MOSQUITOES

District	Total (n)	RR	RS	SS		% Ace-1 (G119S)
Abengourou	21	3	3	15		21.43
Abidjan	22	7	13	2		61.36
Aboisso	0	0	0	0		0!
Adzope	18	0	8	10		22.22
Béoumi	24	0	8	16		16.67
Bouaké	25	0	5	20		10.00
Bouna	21	0	8	13		19.05
Dabakala	23	0	7	16		15.22
Daloa	21	0	8	13		19.05
Gagnoa	24	0	14	10		29.17
Jacqueville	24	0	13	11		27.08
Korhogo	24	2	15	7		39.58
Man	25	0	4	21		8.00
Nassian	25	0	12	13		24.00
Odienné	20	2	4	14		20.00
Sakassou	24	1	16	7		37.50
San Pedro	24	0	4	20		8.33
Yamoussoukro	20	0	12	8		30.00
TOTAL	365	15	142	208		23.56

Note:
RR: Homozygous with two resistant Ace-1 (G119S) alleles;
RS : Heterozygous with one resistant Ace-1G119Sallele and one susceptible allele;
SS: Homozygous with two susceptible alleles.

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