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PMI VECTORLINK CÔTE D'IVOIRE 2021 ANNUAL ENTOMOLOGICAL REPORT

JANUARY 2021–DECEMBER 2021

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PMI VECTORLINK CÔTE D'IVOIRE 2021 ANNUAL ENTOMOLOGICAL REPORT JANUARY 2021–DECEMBER 2021

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ACRONYMS

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	Females per Room
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
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PMI	**U.S.** President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SOP	Standard Operating Procedure
WHO	World Health Organization

EXECUTIVE SUMMARY

In August of 2021, the U.S. President's Malaria Initiative (PMI) VectorLink Côte d'Ivoire Project conducted its second consecutive indoor residual spraying (IRS) campaign in the districts of Nassian and Sakassou using the same insecticides as in 2020 (SumiShield and Fludora Fusion respectively) shortly after the nationwide insecticide-treated net (ITN) distribution campaign. To assess entomological indicator trends before and after IRS, the VectorLink project conducted longitudinal vector surveillance monitored insecticide resistance and assessed the quality and residual efficacy of IRS insecticides.

Methods

Longitudinal vector surveillance was conducted monthly from January to December 2021 in two sprayed districts (Nassian and Sakassou) and two control districts (Béoumi and Dabakala). Adult mosquito collections were conducted using human landing catch (HLC), pyrethrum spray catch (PSC), and Centers for Disease Control and Prevention (CDC) light traps. The entomological parameters assessed included vector composition, seasonality, distribution, biting and resting behavior, sporozoite infection, parity, and entomological inoculation rate (EIR) which enabled comparison of trends observed since the implementation of IRS.

Insecticide susceptibility tests, resistance intensity tests, and synergist assays were also conducted in 18 sites distributed across the country (including 4 sites listed above). The World Health Organization (WHO) susceptibility test kits were used to test the resistance status of wild *An. gambiae* s.l. against the diagnostic concentration of alpha-cypermethrin, deltamethrin, permethrin, pirimiphos-methyl, and clothianidin (2%), as well as resistance intensity and piperonyl butoxide (PBO) synergism as needed. CDC bottle assays were used to test vector susceptibility to chlorfenapyr (100 and 200 µg/bottle) and clothianidin (4 µg/bottle) per the new WHO-approved diagnostic dose and testing protocol.

IRS efficacy was assessed within one week of spraying in three villages per district, which were subsequently monitored monthly to determine residual efficacy.

Results

Anopheles gambiae s.l. was the predominant malaria vector, representing 94% of the total *Anopheles* collected. *Anopheles gambiae* s.l. mean indoor resting densities (IRDs) were highest in Sakassou (mean: 7.1 females per room [f/r]), followed by Dabakala (mean: 5.7 f/r), Beoumi (mean: 3.0 f/r) and Nassian (mean: 1.8 f/r/n). The overall *An. gambiae* s.l. mean IRDs were similar in IRS sites and control sites. Furthermore, the mean IRD in Sakassou decreased from 7.4 f/r before IRS (January-August 2021) to 6.5 f/r after IRS (September-December 2021) while the IRD in unsprayed Beoumi increased from 2.9 f/r to 3.2 f/r in the same time frame. In contrast, the IRD in Nassian increased from 0.7 to 4.6 f/r while in Dabakala, the control site, the IRD decreased from 6.3 to 4.6 f/r.

Overall, *An. gambiae* s.l. biting was highest between 1:00 p.m. and 3:00 a.m., at all sites, both indoors and outdoors. The mean human biting rate (HBR) was highest in Sakassou (161.6 bites per person per night [b/p/n]), followed by Dabakala (44.4 b/p/n), Beoumi (33.2 b/p/n) and Nassian (13.6 b/p/n).

The mean parity rates for *An. gambiae* s.l. was 85.8% in the sprayed sites and 88.5% in control sites. The mean EIR was highest in Sakassou (0.950 infective b/p/n [ib/p/n]), followed by Dabakala (0.678 ib/p/n), Beoumi (0.444 ib/p/n). and Nassian (0.150 ib/p/n). Overall, there was a continuous decrease in vector density, endophily, and transmission in both IRS districts and particularly in Sakassou after IRS..

Anopheles gambiae s.l. was resistant to all pyrethroids in all sites surveyed. High resistance intensity (mortality <98% at 10× the diagnostic doses) was observed against all pyrethroids in all sites except in Abengourou, Daloa, Nassian, and Yamoussoukro where moderate permethrin resistance (mortality >98% at 10× the diagnostic dose) was recorded. Susceptibility to pirimiphos-methyl was observed in 10 sites, seven sites recorded low resistance (mortality >98% at 5× the diagnostic dose), and one site was unable to conduct intensity tests

due to larvae shortage. Pre-exposure to PBO partially restored susceptibility to pyrethroids in 11 of the 18 sites and fully restored susceptibility to deltamethrin in three sites. *Anopheles gambiae* s.l. was susceptible to chlorfenapyr at 100 µg/bottle in four sites and 200 µg/bottle in 12 sites. Susceptibility to clothianidin (2%)was observed in 15 of 17 sites, seven days post-exposure while clothianidin 4 µg/bottle recorded susceptibility in seven out of the 17 sites tested.

One hundred percent (100%) mortality of *An. gambiae* Kisumu exposed to walls sprayed with SumiShield and Fludora Fusion was recorded within a week of spraying in both IRS districts. The monthly insecticide residual efficacy assessment conducted from September 2021 to February 2022 showed that both insecticides were efficacious on for at least seven months. Furthermore, Fludora Fusion remained effective against the wild population of *An. gambiae* s.l. in Sakassou at six months post-spraying.

Conclusion

Overall, the data recorded in the two IRS sites showed that there was a decrease in vector indoor resting density and entomological inoculation rate after implementing IRS. While these results may support continued advocacy in implementing and expanding IRS in the country, particularly targeting high transmission districts, evaluation of epidemiological trends pre- and post-IRS would be needed before making a definitive decision. Insecticide resistance monitoring results show improvement in mortality using PBO synergist and new molecules such as chlorfenapyr. These findings should continue to guide the NMCP's choice of ITNs and inform the strategy of stratified ITN distribution across the country per the resistance profile and levels.

1. INTRODUCTION

Malaria is still 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 five years of age, according to the 2020 National Malaria Control Program (NMCP) report (RASS 2020). 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. Prior to 2021, only pyrethroid-based ITNs were distributed. The National Malaria Strategic Plan 2016–2020 prioritized indoor residual spraying (IRS) as a complementary vector control method to reduce malaria morbidity and mortality.

To support the NMCP malaria control efforts, the U.S. President's Malaria Initiative (PMI) VectorLink Project, conducted IRS campaign from August 2 to September 4, 2021 in Nassian and Sakassou targeting 58,695 structures using clothianidin-based insecticides (SumiShield and Fludora Fusion). The project sprayed a total of 60,496 of the 62,551 structures found by spray operators in targeted districts, resulting in a coverage rate of 96.7%. PMI, through the VectorLink project, also supported and assisted the NMCP in distributing 3,097,087 piperonyl butoxide (PBO)-ITNs in 11 (non-IRS) districts.

Entomological surveillance is a key component of integrated vector control programming, providing information on malaria vector density and behavior in sites where vector control interventions are implemented. In 2021, VectorLink Côte d'Ivoire, subcontracted with the *Centre Suisse de Recherches Scientifiques* (CSRS) and collaborated with three other local entomological research institutes (*Institut Pierre Richet* [IPR], *Centre d'Entomologie Médicale et Véterinaire* [CEMV] and *Institut National d'Hygiène Publique* [INHP]), to conduct longitudinal entomological surveillance in four sites selected by the NMCP and generate data on key entomological indicators including malaria vector species composition, density, feeding behavior, parity, and sporozoite infection rates in mosquitoes in the four districts. In addition, the project conducted insecticide susceptibility tests in 18 sites selected by the NMCP, assessed the quality of spray during the IRS campaign, and monitored the residual efficacy of the insecticides after IRS. These data will continuously support the NMCP and the malaria vector control stakeholders in determining the optimal timing and insecticides for IRS and inform the selection of ITNs for distribution campaigns.

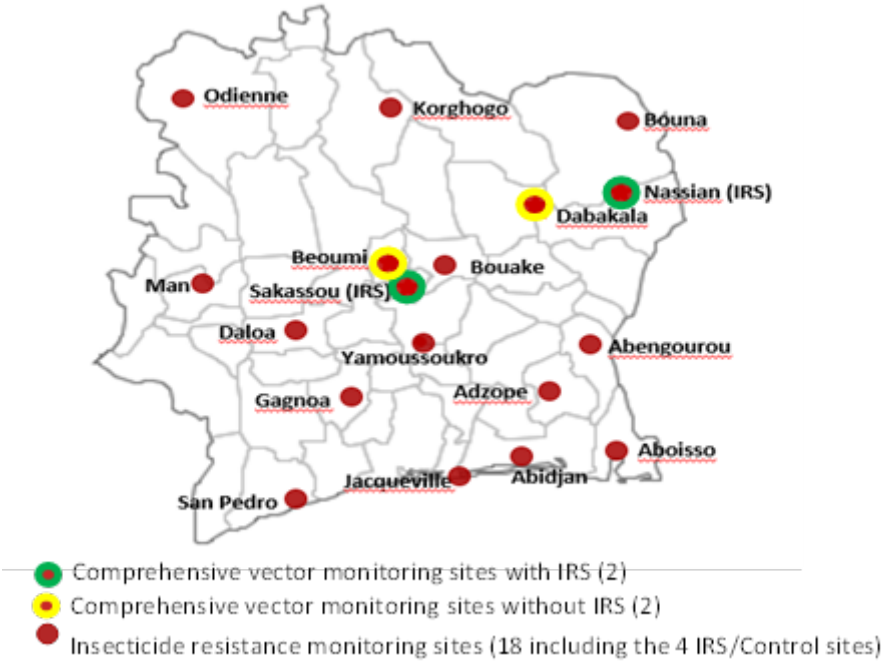
2. METHODOLOGY

From January through December 2021, VectorLink Côte d'Ivoire conducted longitudinal entomological vector surveillance in four sentinel 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 vector monitoring (vector surveillance and insecticide resistance monitoring) in the two IRS sites (Nassian and Sakassou) and the two control sites (Béoumi and Dabakala), and insecticide resistance monitoring only in 14 additional sites: Abengourou, Abidjan, Aboisso, Adzopé, Bouaké, Bouna, Daloa, Gagnoa, Jacqueville, Korhogo, Man, Odienné, San Pedro, and Yamoussoukro. During the countrywide 2021 ITN distribution campaign, all four comprehensive vector monitoring sites were provided with deltamethrin-only treated ITNs, allowing continuous comparison of data collected in IRS sites versus unsprayed sites. IRS quality assessment and longitudinal residual efficacy monitoring were done at the two sprayed sites. Figure 1 shows the location of the sentinel sites across the country.

FIGURE 1: MAP OF CÔTE D'IVOIRE SHOWING THE 2021 PMI VECTORLINK ENTOMOLOGICAL MONITORING 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 urban site per site per month. The PSCs were conducted in 30 houses (15 urban and 15 rural) within two days 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 houses were randomly selected each month for PSC collections depending on the availability of households. Collections were conducted every month from January through December 2021. Table 1 summarizes the collection times, frequency, and sampling methods. All entomological data were collected following the PMI standard operating procedures (SOPs) (PMI, 2020).

TABLE 1: LONGITUDINAL MONITORING COLLECTION METHODS

Collection Method	Time	Frequency	Sample
HLC (indoor and outdoor)	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) for two consecutive days per month. The PSCs were carried out between 6:00 p.m. and 8:00 a.m. during two days per site per month. White sheets were placed on the floor from wall to wall in sampled rooms. The rooms were sprayed with a locally available 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. The mosquitoes were kept in Petri dishes and then sorted by species using a morphological identification key (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. The CDC light traps were suspended in a bedroom 1.5 meters above the floor, near the sleeper's legs. To ensure that surveillance was conducted during the suspected peak host-seeking periods, CDC light collection started from about 6:00 p.m. to 6:00 a.m. with traps set 15-30 mins before 6:00 pm to assure the first hour of collection was fully represented.

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 (Coetzee 2020). The identification was done by a team of well-trained technicians from collaborating research institutes and by the 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 (PCR) and enzyme-linked immunosorbent assay (ELISA).

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

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

Collection Method	Indicator	Definition
PSC	Indoor resting density	Mean number of mosquitoes/house
	% of fed females	Number of fed mosquitoes/total collected by PSC
CDC light trap	Indoor trap 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

Starting from July, 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 per tube) and an additional 40–50 was tested in two control tubes (20–25 each per tube) in parallel.

The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), and pirimiphos-methyl (0.25%) were tested in all sites. When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested using 5 and 10 times the diagnostic concentration. All impregnated papers were received from the Universiti Sains Malaysia (USM) except for clothianidin 2% impregnated papers that were prepared locally using a protocol designed by PMI VectorLink and the 300 mg ai/m^2 formulated SumiShield 50WG product. The susceptibility test of clothianidin 2% was conducted as described above, and the mortality was recorded daily for 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 used to test susceptibility to chlorfenapyr at the doses of 100 µg/bottle and 200 µg/bottle with one-hour exposure, and mortality was recorded daily for up to three days (72 hours) and clothianidin 4 µg/bottle active ingredient following one-hour exposure and up to 24-hour mortality. For all tests, resistance and intensity were defined following the WHO criteria (WHO 2016):

Resistance status at diagnostic dose:

- 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 5× or 10× the diagnostic dose:

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

2.4 Molecular Characterization

Thirty adult female mosquitoes reared from larval collection were selected within the cages used for susceptibility test per site and genotyped by quantitative polymerase chain reaction (qPCR) for the detection of enzymes such as CYP6s, involved in the metabolic resistance of the vectors. The samples were preserved in RNA (ribonucleic acid) later in the field, brought to the laboratory and stored at -80°C prior to analysis. The susceptible laboratory strain (Kisumu) was used to assess the relative fold-change in the level of expression of each enzyme.

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 first week of the IRS campaign to confirm the quality of spray in Nassian and Sakassou (SOP 09/01). In Nassian, three cement houses were selected in each of the three villages (Parhadi, Lande, and Nassian town) 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, where cement is the only substrate used, three houses were selected in each of three villages (Adjekro, Kpatanou, and Kpetebonou) for a total of nine cement houses. In addition to the sprayed structures, one unsprayed structure (ineligible for IRS due to food storage) was used for control bioassays in each of the six 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; due to difficulty to find *An. gamibae* s.l. breeding sites at the time of the survey) were introduced per cone and exposed for 30 minutes. After the 30 minutes, the mosquitoes were transferred back to disposable cups, which were then placed on a rack covered with a damp towel to create favorable humidity for the mosquitoes until reaching the laboratory. The number of mosquitoes knocked down was recorded after 30- and 60-minutes post wall exposure and the mortality was recorded every 24 hours for up to five days.

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. For both bioassays.

For both bioassays, the mortality was corrected using Abbot's formula when the mortality of the control was between 5 and 20%.

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) and one control mud and cement Nine houses in Sakassou (three sprayed cement in each of the three villages) and control cement
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) and one control mud and cement Nine houses in Sakassou (three sprayed cement in each of the three villages) and one control cement
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) and one control mud and cement Three houses in Sakassou (one per village) and one control cement

2.6 Data Presentation and Statistical Analysis

The District Health Information Software (DHIS2)-based VectorLink Collect database has been used for entomological data management in Côte d'Ivoire since 2020. The PMI VectorLink Côte d'Ivoire entomologists and database managers adopted 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 2021 were summarized in VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results.

Using the Kruskal-Wallis equality-of-population rank test in STATA Statistical software version 13 (College Station, TX), an exploratory statistical analysis was done of entomological parameters from the IRS (sprayed) sites and the control (non-sprayed) sites to compare the period before IRS (January to August 2021) and after IRS (September to December 2021) to assess the impact of IRS on entomological parameters. We considered five main parameters: 1) indoor resting density (IRD), 2) the HBR, 3) the proportion of gravid and fed vectors, 4) the parity rate, and 5) the entomological inoculation rate (EIR) with separate analyses for *An. gambiae* s.l. and for *An. funestus* s.l. P-values of <0.05 for the different variables tested indicated statistically significant difference.

3. RESULTS

3.1 VECTOR BIONOMICS MONITORING

Vector bionomics monitoring was conducted monthly in the two IRS and two control sites from January to December 2021.

3.1.1 SPECIES COMPOSITION

3.1.1.1 OVERALL SPECIES COMPOSITION

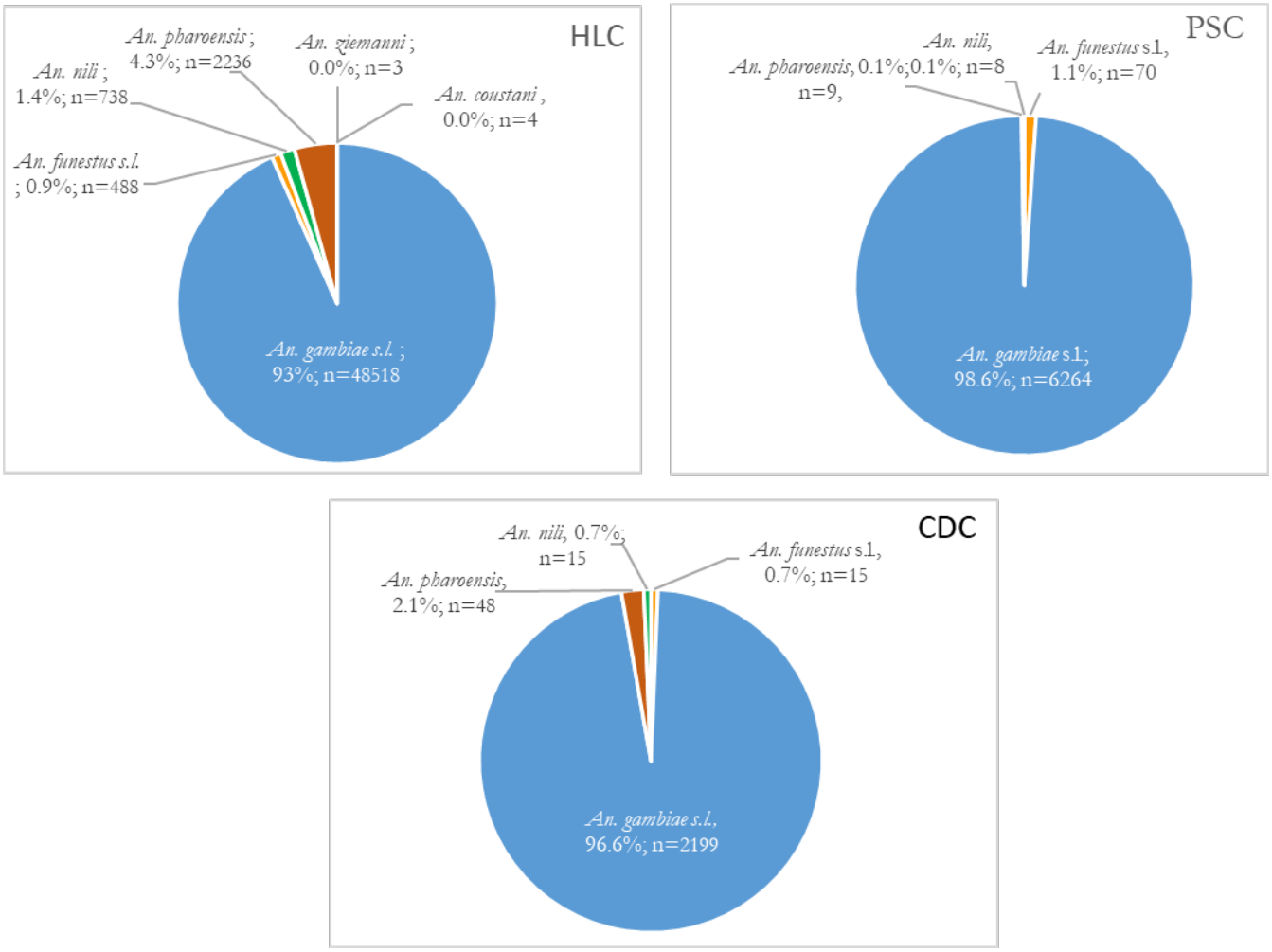
A total of 74,049 adult mosquitoes were collected in the four sentinel sites from January to December 2021, using all three collection methods: HLC, PSC, and CDC light trap. *Anopheles* mosquitoes represent 81.9% (n=60,615) and Culicines 18.1% (n=13,434) of all mosquitoes collected (Table 4). Six *Anopheles* species were identified across the four sites. *An. gambiae* s.l. (n=56,981) was the predominant malaria vector species, representing 94.0% of the total *Anopheles* mosquitoes collected across all sites and methods. *An. funestus* s.l. (0.9%; n=573) and *An. nili* (1.3%; n=761) were the second most-abundant of the known vectors in Cote d'Ivoire. *Anopheles pharoensis* (3.8%; n=2,293), *An. coustani* (<0.1%; n=4) and *An. ziemanni* (<0.1%; n=3) represented the other *Anopheles* species collected not usually implicated in malaria transmission in Cote d'Ivoire.

The HLC method yielded 51,987 *Anopheles* mosquitoes representing 85.8% of the total *Anopheles* collected. *Anopheles gambiae* s.l. was the predominant species (93.3%; n=48,518) followed by *An. funestus* s.l. (0.9%; n=488) *An. nili* (1.4%; n=738); *An. pharoensis* (n=4.3%; n= 22 36), *An. coustani* (4), and *An. ziemanni* (3). Using PSCs 5,930 (10.8%) *Anopheles* mosquitoes were collected. *An. gambiae* s.l. (94.7%; n=5,617) still represented the main vector collected followed by *An. funestus* s.l. (1.1%; n=70) and *An. nili* (0.1%; n=8). The other *Anopheles* species found was *An. pharoensis* (0.1%; n=95). CDC light traps collected fewer *Anopheles* mosquitoes with an overwhelming majority of *An. gambiae* s.l. (96.6%; n=2,199) and a few *An. funestus* s.l. (0.7%; n=15) and *An. nili* (0.7; n=15) collected (Annex Tables A1–A3).

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

Species/District	Beoumi	Dabakala	Nassian	Sakassou	Total	Species % of Total	Species % of total *Anopheles*
An. gambiae s.l.	8,079	11,606	3,310	33,986	56,981	77.0	94.0
An. funestus s.l.	93	198	251	31	573	0.8	0.9
An. nili	0	749	10	2	761	1.0	1.4
An. pharoensis	898	175	1	1,219	2,293	3.1	4.3
An. coustani	0	4	0	0	4	0.0	0.0
An. ziemanni	0	1	0	2	3	0.0	0.0
Culicines	3,793	5,370	671	3,600	13,434	18.1	-
Total	**12,863**	**18,103**	**4,243**	**38,840**	**74,049**	**100**	**100**

FIGURE 2: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN ALL FOUR SITES USING HLC, PSC, AND CDC LIGHT TRAPS FROM JANUARY TO DECEMBER 2021

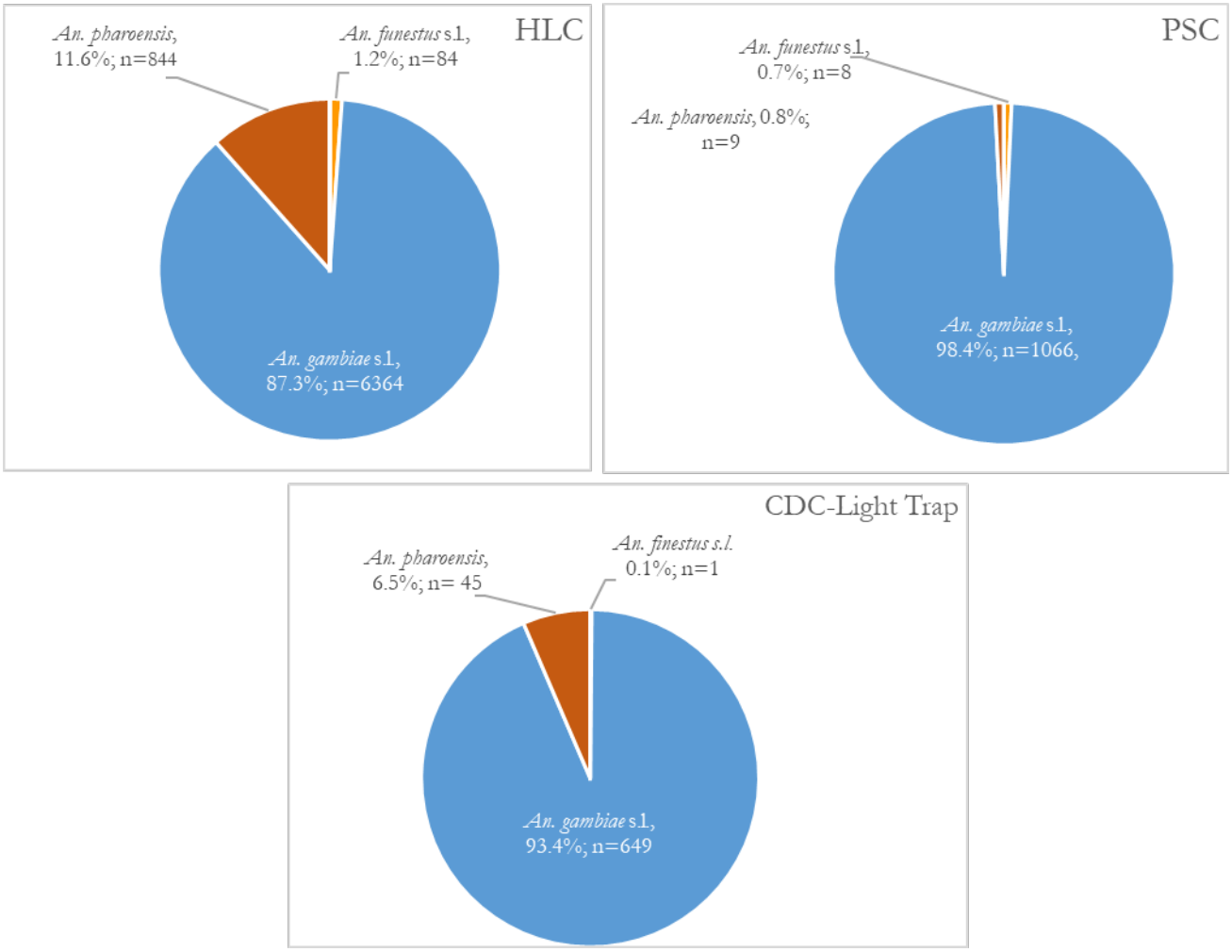


3.1.1.2 SPECIES COMPOSITION IN BÉOUMI

In Beoumi, a total of 9,070 *Anopheles* mosquitoes were caught over the 12 months using all three collection methods, representing 15% of the total *Anopheles* mosquitoes collected across all sites. *An. gambiae* s.l. was the most collected malaria vector species (89.1%, n=8,079). Only a few *An. funestus* s.l. were collected. (1%; n=93). *An. pharoensis* (9.9%; n=898) was the only other *Anopheles* species.

Using HLC, about 80.5% (n=7,292) *Anopheles* mosquitoes were collected in Béoumi, including 87.3% *An. gambiae* s.l. (n=6,364), 1.2% *An. funestus* s.l. (n=84), and 11.6% *An. pharoensis* (n=844) (Figure 3). PSC yielded 1,083 *Anopheles* mosquitoes overall, representing 12% of the total collected in Béoumi. *An. gambiae* s.l. (98.4%; n=1,066) was the predominant *Anopheles* vector collected by PSC, followed by *An. funestus* s.l. (0.7%; n=8). The other *Anopheles* species found was *An. pharoensis* (0.8%; n=9). With CDC light traps, 695 (7.7%) *Anopheles* mosquitoes were collected, including 649 *An. gambiae* s.l. (93.4%) and 1 *An. funestus* s.l. (0.1%) and 45 *An. Pharoensis* (6.5%) (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 2021

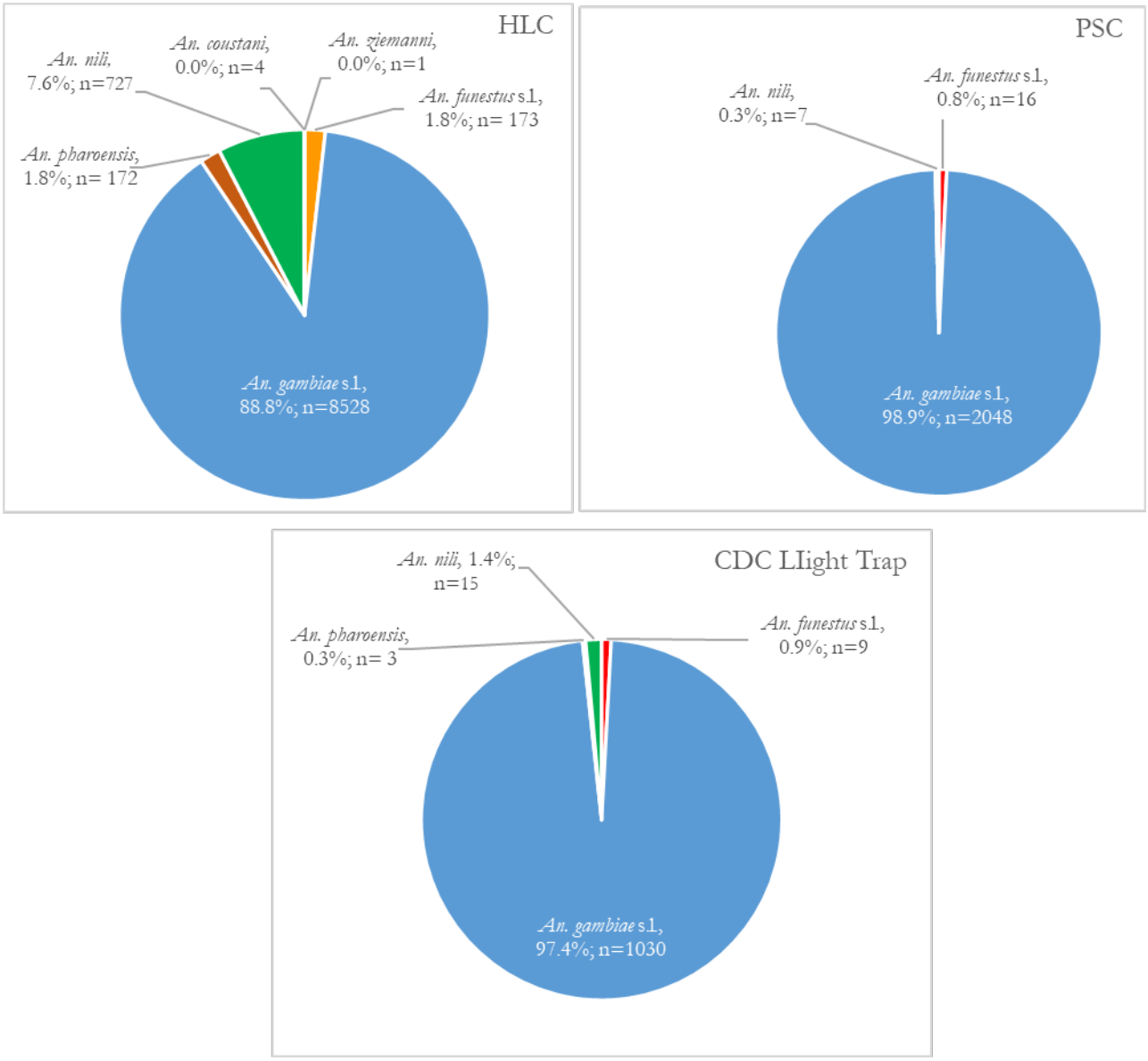


3.1.1.3 SPECIES COMPOSITION IN DABAKALA

In Dabakala, a total of 12,733 *Anopheles* mosquitoes were collected, which is about 21% of the total collections. *An. gambiae* s.l. was the predominant malaria vector species collected (91.2%; n=11,606). *An. funestus* s.l. (1.6%, n=198) and *An. Nili* (5.9%, n=749) represented the other malaria vectors found. A higher number of *An. nili* was collected in Dabakala than in the three other sites. The other *Anopheles* species collected were *An. pharoensis* (1.4%; n=175), *An. coustani* (0.03%; n=4) and *An. ziemanni* (0.01%; n=1).

Using HLC, a total 9,605 (75.4%) *Anopheles* mosquitoes were collected. *An. gambiae* s.l. was the major malaria vector representing 88.8% (n=8,528) of the total *Anopheles* collected, followed by *An. funestus* s.l. (1.8%; n=173), *An. nili* (7.6%; n=727) (Figure 4). The other species included *An. pharoensis* (1.8%; n=172), *An. coustani* (0.01%; n=4) and *An. ziemanni* (n=1). Using PSC, 2,071 *Anopheles* mosquitoes were collected representing 16.2% of the *Anopheles* collected in Dabakala. *An. gambiae* s.l. was the predominant species collected (98.9%; n=2,048) followed by *An. funestus* s.l. (0.8%, n=16) and *An. nili* (7). CDC light trap collections recorded 1,057 *Anopheles* mosquitoes (8.3%), composed of *An. gambiae* s.l. (97.4%; n=1,030) and a few of *An. funestus* s.l. (0.9%; n=9), *An. nili* (1.4%; n=15) and *An. pharoensis* (3) (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 2021

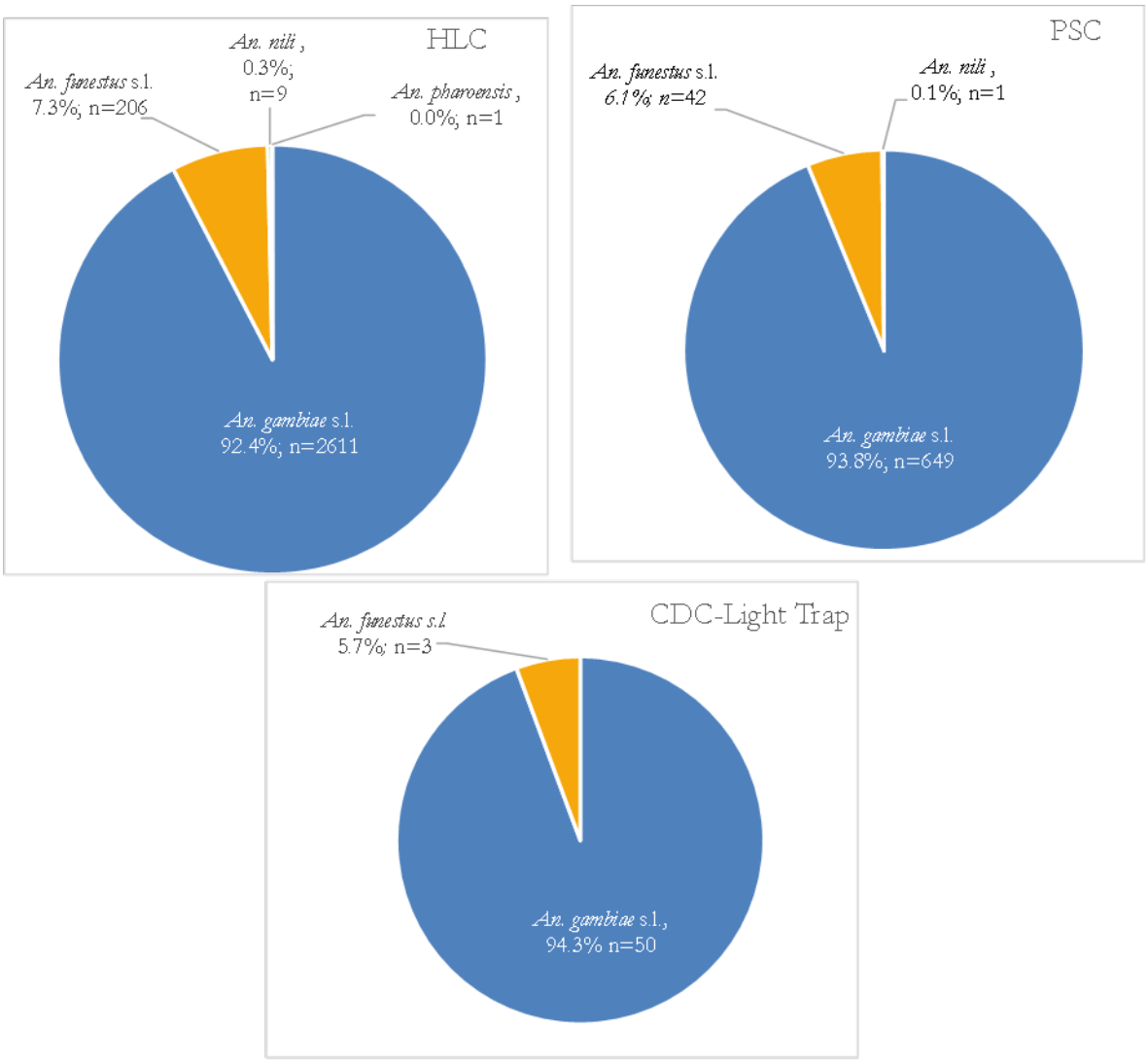


3.1.1.4 SPECIES COMPOSITION IN NASSIAN

In Nassian, the collections yielded a total of 3,572 *Anopheles* mosquitoes, representing 5.9% of all *Anopheles* mosquitoes recorded at all four sites. *An. gambiae* s.l. was the main vector species collected (92.7%; n=3,310). Nassian was the site that recorded the largest percentage (43.8%) of the total *An. funestus* s.l. caught and represented 7.0% (n=251) of the total *Anopheles* collected in Nassian. A few *An. nili* (0.3%; n=10) and *An. pharoensis* (0.0%; n=1) were collected (Figure 5). Overall, HLC yielded 2,827 *Anopheles* mosquitoes, of which 2,611 (92.4%) were *An. gambiae* s.l., 206 (7.3%) *An. funestus* s.l. and 9 (0.3%) *An. nili*.

Using PSC, 692 *Anopheles* were caught. *An. gambiae* s.l. still had the highest percentage (93.8%; n=649) followed by *An. funestus* s.l. (6.1%; n=42). Only one (1; 0.1%) *An. nili* was collected. The CDC-light trap yielded 53 *Anopheles* composed of 50 (94.3%) *An. gambiae* s.l. and 2 (5.7%) *An. nili* (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 2021



3.1.1.5 SPECIES COMPOSITION IN SAKASSOU

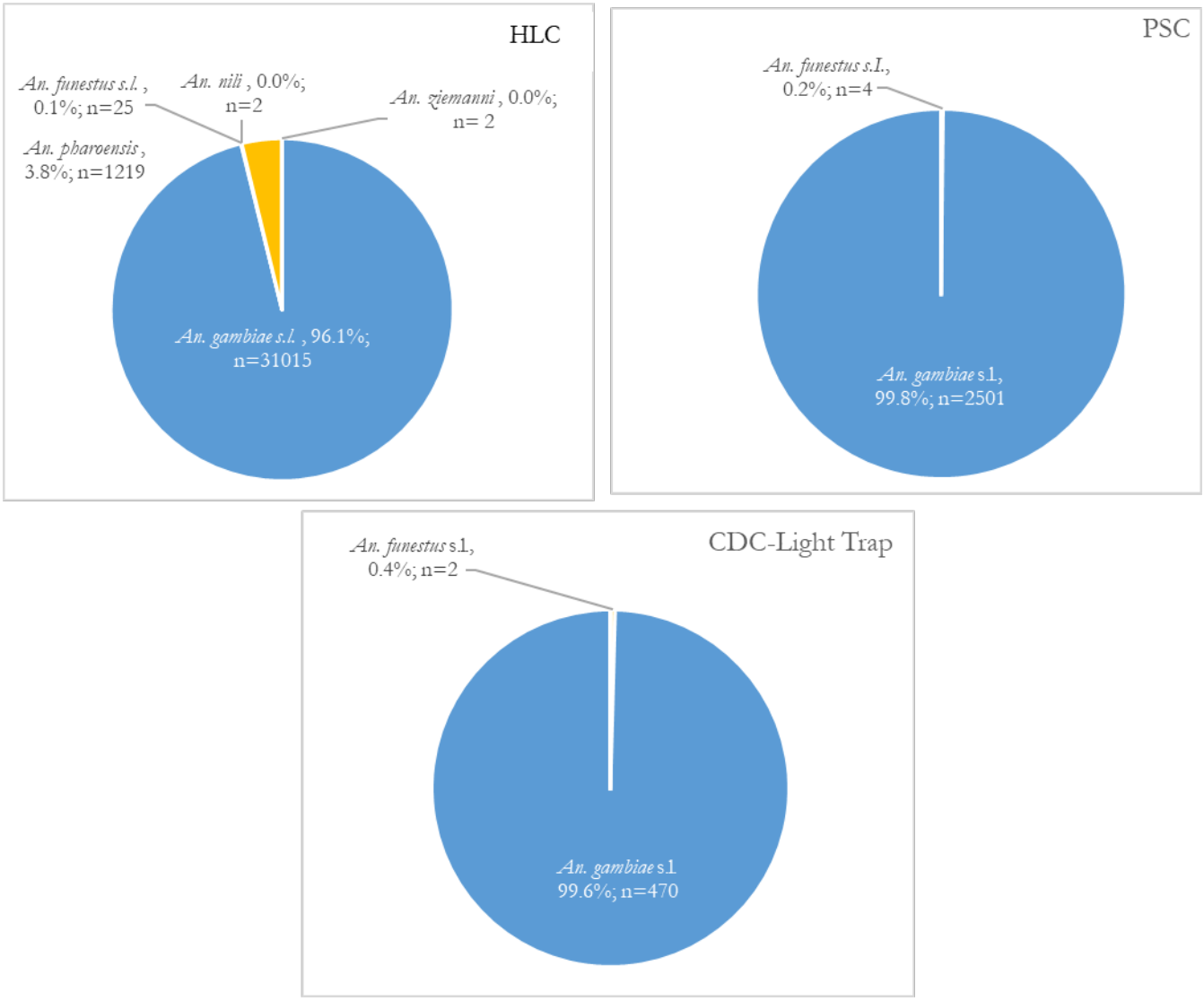
Sakassou was the most productive of the four sites where longitudinal vector surveillance was conducted. A total of 35,240 *Anopheles* mosquitoes were collected over the 12 months using all three collection methods, representing 58.1% of the overall collection. *An. gambiae* s.l. was the main vector species collected (96.4%; n=33,986) and An. *funestus* s.l. (0.1%; n=31), *An. nili* (0.0%; n=2), *An. pharoensis* (3.5%; n=1,219) and *An. ziemanni* (0.0%; n=n=2).

HLC remained the highest-yield method for adult *Anopheles* mosquito collections. In Sakassou, 32,263 *Anopheles* were collected through HLC and mainly *An. gambiae* s.l. (96.1%; n=31,015), *An. funestus* s.l. (0.1%; n=25), and *An. nili* (0.0%; n=2) (Figure 6). The other species found were *An. pharoensis* (3.87%; n=1,219) and *An. ziemanni* (0.0%; n=2).

A total of 2,505 *Anopheles* mosquitoes were collected using PSC. *An. Gambiae* s.l. represented 99.8% (n=2,501) while a few *An. Funestus* s.l. (0.2%; n=4) were collected. CDC light traps recorded the lowest number of

Anopheles mosquitoes collected (472) in Sakassou. *An. gambiae s.l.* was predominantly collected (99.6%; n=470), and only two *An. funestus* s.l. (0.4%; n=2) was found. (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 2021



3.1.2 VECTOR DENSITY AND BEHAVIOR

3.1.2.1 INDOOR RESTING DENSITY (IRD) OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY PYRETHRUM SPRAY CATCHES (PSCS)

3.1.2.1.1 AN. GAMBIAE S.L.

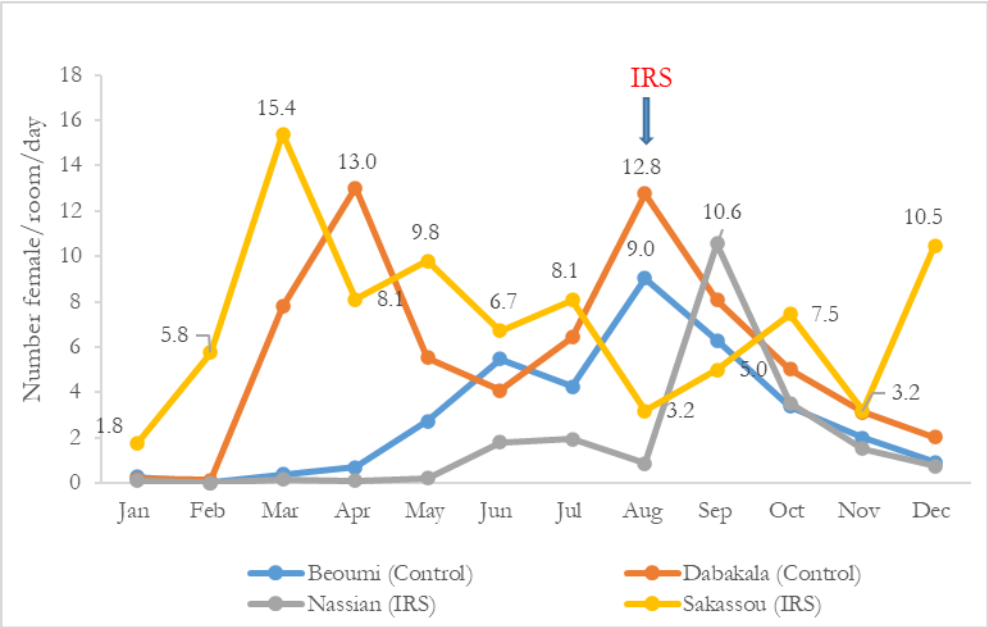
An. gambiae s.l. mean monthly IRDs, estimated using PSCs recorded several peaks of mosquito density in the four sites over the year. Mean monthly IRDs were between 1.8 and 15.4 females/room (f/r) in Sakassou, between 0.1 and 13 f/r in Dabakala, 0 and 9 f/r in Beoumi, and between 0.1 and 10.6 f/r in Nassian (Figure 7). There was significant difference of IRD between each of the four sites (p=0.002). In Sakassou, peak IRDs were recorded in March (15.4 f/r) and December (10.5 f/r). In Dabakala, peak IRDs were recorded in April

(13.0 f/r) and August (12.8 f/r). In Béoumi, the IRD peaked in August (9 f/r), and in Nassian the peak occurred in September (10.6 f/r).

The overall mean IRD of Sakassou and Nassian (IRS sites) over the 12 months were 7.1 and 2.5 f/r respectively, while in Beoumi and Dabakala (control sites) the mean IRD was 3.0 and 5.7 f/r, respectively. The overall combined mean of the *An. gambiae* s.l. IRDs were 4.4 f/r in IRS sites and 4.3 f/r in control sites and were similar (p=0.9015), which is lower than the mean IRD observed in 2019 (9.2 f/r and 9.7 f/r, respectively) (Annex Table B1).

The mean IRD in Sakassou decreased from 7.4 f/r before IRS (January-August) to 6.5 f/r after IRS (September-December) while in Beoumi it increased from 2.9 f/r to 3.2 f/r at the same periods. The reverse was observed for Nassian where the IRD increased from 0.7 to 4.6 f/r while in Dabakala, the control site, it decreased from 6.3 to 4.6 f/r (Figure 7 and Annex Table A4)

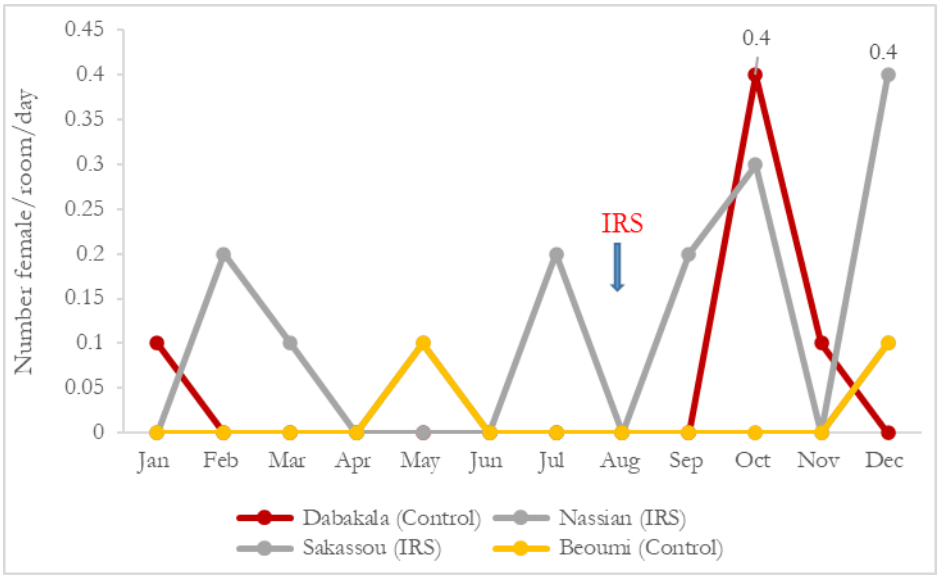
FIGURE 7: MEAN IRD OF *AN. GAMBIAE* S.L. BY MONTH IN CONTROL AND IRS SITES BY MONTH



3.1.2.1.2 AN. FUNESTUS S.L.

The mean IRD of *An. funestus* s.l. was also observed differently in the four sites throughout the year. Nassian had the highest number of *An. funestus* s.l. (0.12 f/r), showing five peaks with the highest observed in December 0.40 females were collected per room per day. There was also a sporadic detection of *An. funestus s.l.* in Dabakala in January, October, and November, in Beoumi in May and December and in Sakassou in December. In the three sites, the values were low with the highest IRD detected in October (0.4 f/r) in Dabakala, in May (0.1 f/r) in Beoumi and in December (0.1 f/r) in Sakassou (Figure 8 and Annex Table A4).

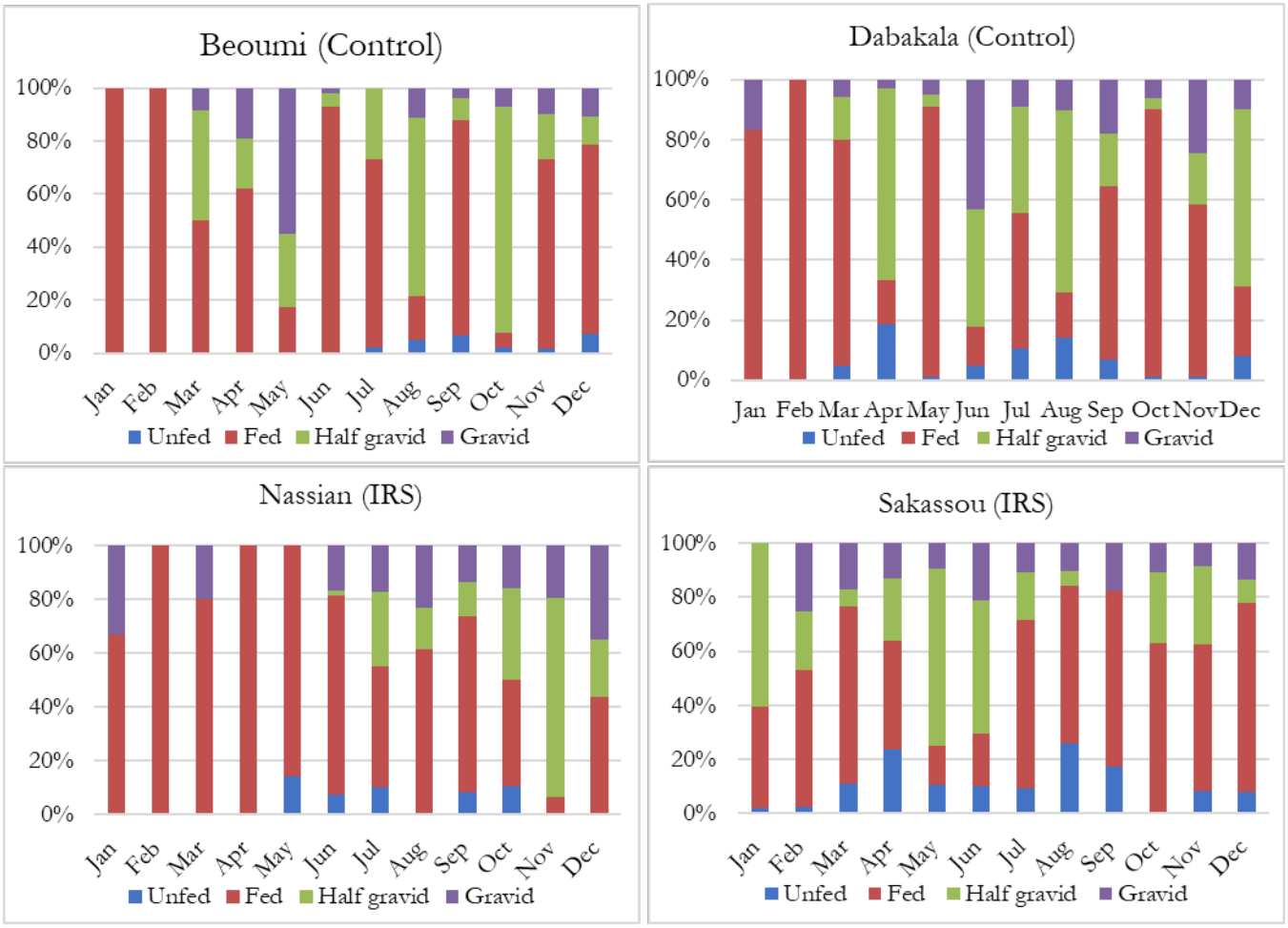
FIGURE 8: MEAN IRD OF *AN. FUNESTUS* S.L. BY MONTH IN CONTROL AND IRS SITES BY MONTH



3.1.2.2 ABDOMINAL STATUS OF *AN. GAMBIAE 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 6,262 *An. gambiae* s.l. (3,150 from sprayed sites and 3,112 from control sites). Overall, the percentages of gravid *An. gambiae* s.l. mosquitoes were 14.5% in the sprayed sites and 11.3% in the control sites (p= 0.403). The percentage of fed *An. gambiae* s.l. was not significantly different between the sprayed sites (74.9%) compared to the control sites (81.5%) (p=0.6573) (Annex Table A5, Table B2 & B3).

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



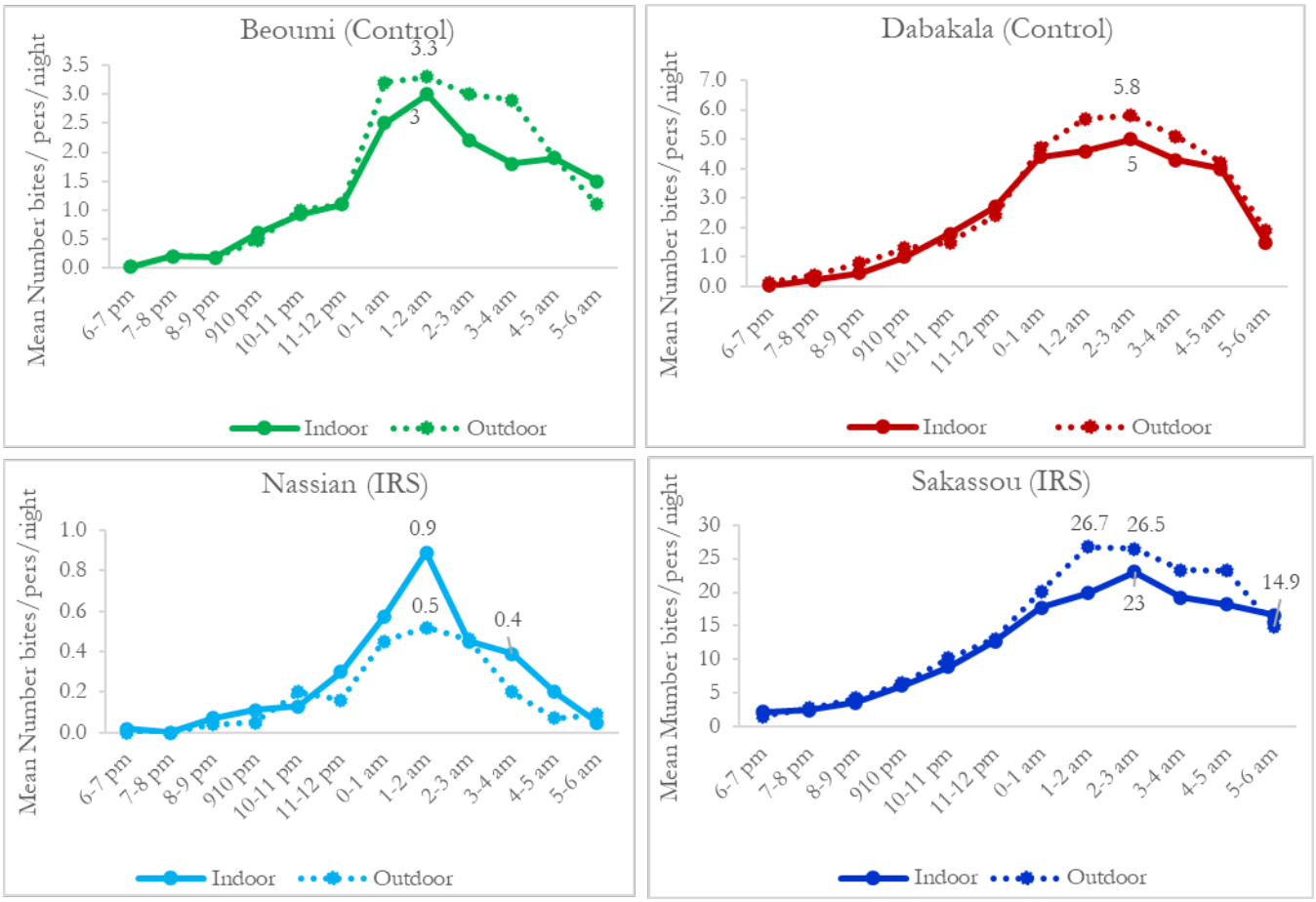
Note: The number in the bars represents the number of females collected per month and per abdominal status.

3.1.2.3 BITING TIME OF *AN. GAMBIAE S.L.* COLLECTED BY HUMAN LANDING CATCHES (HLCS)

The hourly mosquito collections using the HLC methods enabled the estimation of the mean peak biting time of the main malaria vector, *An. gambiae* s.l., of the four longitudinal monitoring sites.

An. gambiae s.l. biting time was similar in all sites with the peak biting observed between 0:00 am and 4:00 am, both indoors and outdoors. The overall highest densities were recorded between 1:00 and 2:00 in Beoumi and Nassian, (indoors and outdoors), between 2:00 and 3:00 in Dabakala (indoors and outdoors) and between 2:00 and 3:00 (indoors) and 1:00 and 2:00 (outdoors) in Sakassou (Figure 10). Sakassou yielded the highest *An. gambiae* s.l. hourly biting rates with a peak of 26.7 b/p/n outdoors and 23 b/p/h indoors. Nassian recorded its peak hourly biting of 0.89 b/p/h outdoors and 0.52 b/p/h indoors The peak hourly biting was 3.3 bites per person per hour (b/p/h) outdoors and 3.0 b/p/h indoors in Béoumi, and 5.8 b/p/h outdoors and 5.0 b/p/h indoors in Dabakala (Annex Table A6).

FIGURE 10: MEAN INDOOR AND OUTDOOR BITING TIMES OF *AN. GAMBIAE* S.L. COLLECTED BY HLC BY SITE

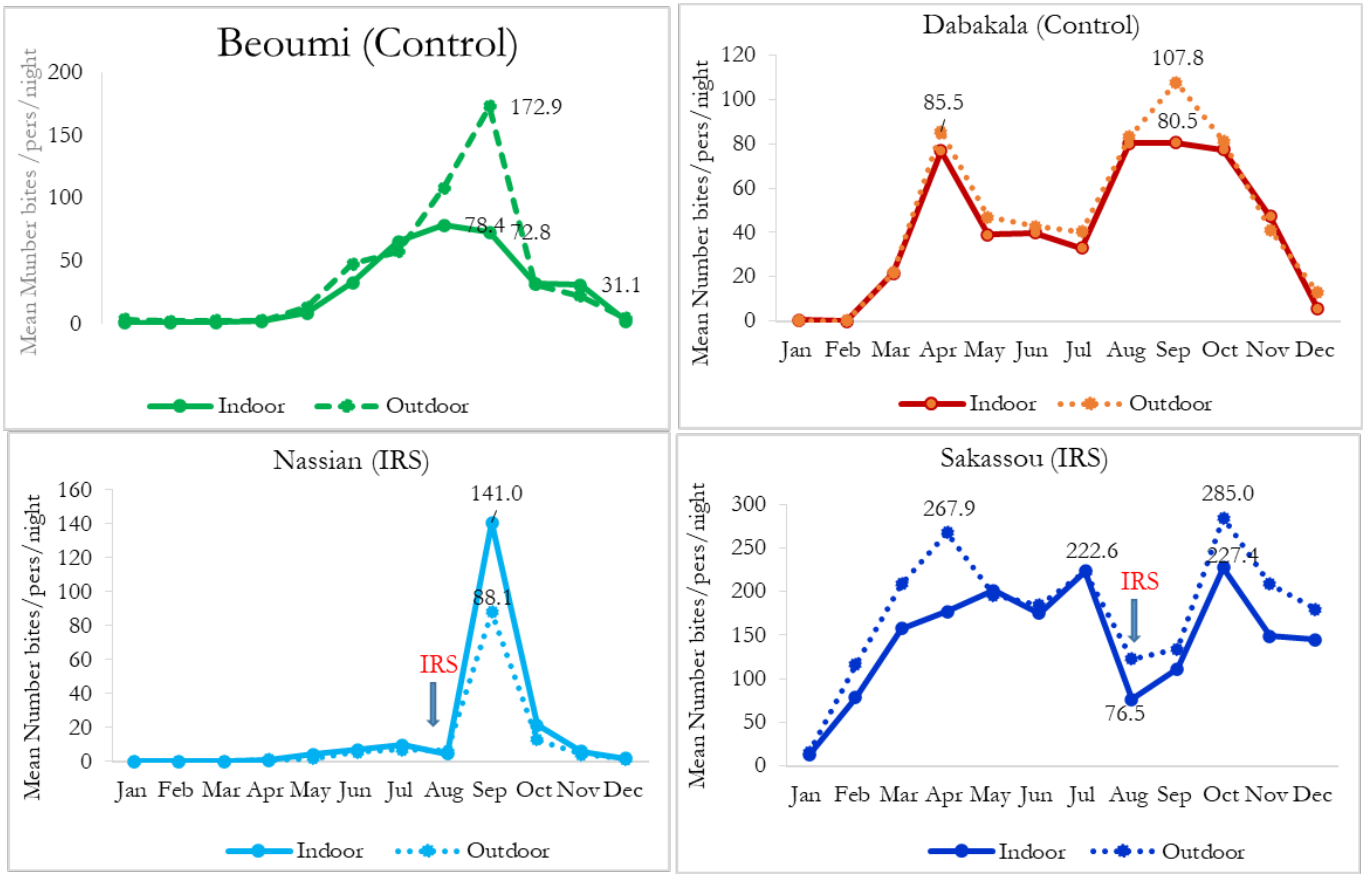


3.1.2.4 HUMAN BITING RATE (HBR), BITING CYCLE, AND BEHAVIOR OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY HLC

3.1.2.4.1 AN. GAMBIAE S.L.

There were different HBR trends in the four sites over the year. In Beoumi, the HBR peaked outdoors (172.9 bites per person per night [b/p/n]) in September and indoors 78.4 b/p/n) in August. In Nassian, one HBR peak was recorded indoors (141 b/p/n) and outdoors (88.1 b/p/n) in September. In Dabakala, two HBR peaks were recorded; one in April indoors (76.9 b/p/n) and outdoors (85.5 b/p/n), and the other in September outdoors (107.8 b/p/n) and indoors (80.5 b/p/n). In Sakassou, the HBR peaked only outdoors (267.9 b/p/n) in April, indoors (223.8 b/p/n) and outdoors (222.6 b/p/n), and indoors (227.4 b/p/n) and outdoors (285 b/p/n). The annual mean HBR was highest in Sakassou (161.6 b/p/n), followed by Dabakala (44.4 b/p/n) and Beoumi (33.2 b/p/n). Nassian recorded the lowest HBR with 13.6 b/p/n recorded over the year ($p<0.0157$) (Annex Table B4). *An. gambiae* s.l. tended to be more exophilic in Sakassou (55.2%) and endophilic in Nassian (60.4%).

FIGURE 11: INDOOR AND OUTDOOR HBRS OF *AN. GAMBIAE* S.L. BY SITE AND BY MONTH



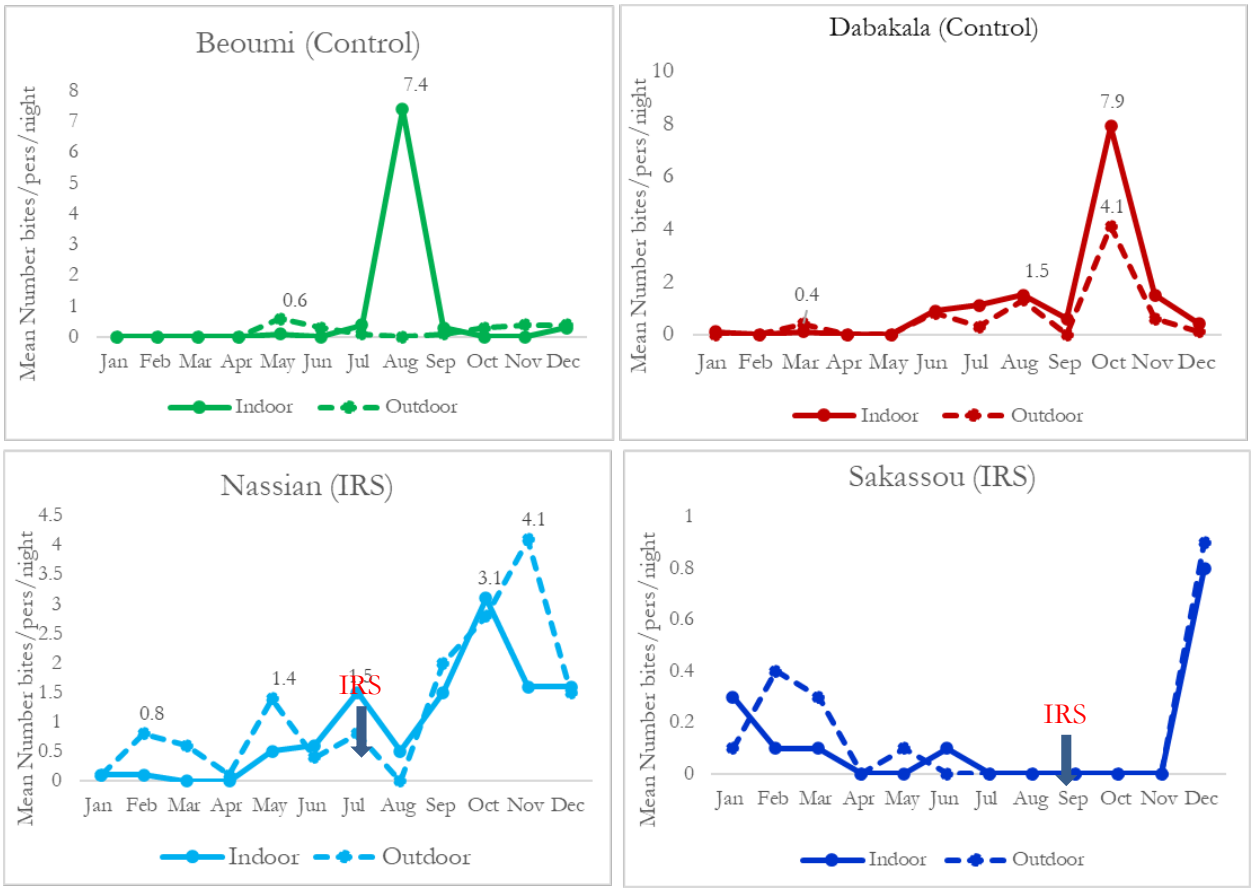
3.1.2.4.2 *AN. FUNESTUS* S.L.

The *An. funestus* s.l. HBRs in Béoumi had one peak indoors in May (0.6 b/p/n) and outdoors in August (7.4 b/p/n). The mean density was 0.4 b/p/n (Figure 12).

Dabakala also had a single peak indoors (4.1 b/p/n) and outdoors (7.9 b/p/n) in October 2021. The mean HBR density was 0.9 b/p/n. (Annex Table A7).

The highest HBRs of *An. funestus* s.l. were observed in Nassian with an overall (indoor and outdoor) rate ranging from 0.1 b/p/n in July to 3 b/p/n in October. The highest outdoor HBRs were recorded in October (3.1 b/p/n) and highest indoor in November 2021 (4.1 b/p/n). A slight increase in indoor HBRs was noted in December 2021 (4.6 b/p/n). Sakassou recorded a single HBR peak indoors (0.8 b/p/n) and outdoors (0.9 b/p/n) in December, though it was the lowest among all sites surveyed.

FIGURE 12: INDOOR AND OUTDOOR HBRS OF *AN. FUNESTUS* S.L. BY SITES AND BY MONTH

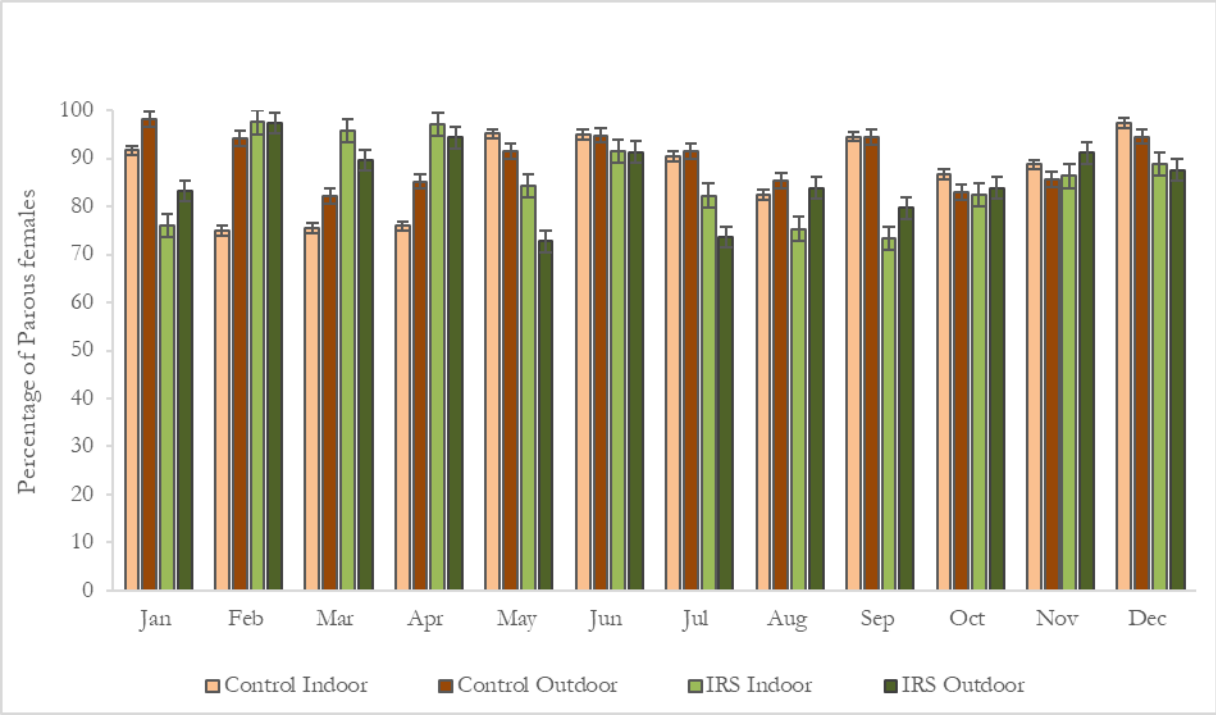


3.1.2.5 PARITY RATE

A total of 10,988 *An. gambiae* s.l. collected using HLCs (2,829 in Béoumi, 3,102 in Dabakala, 974 in Nassian, and 4,083 in Sakassou) were dissected, and their ovaries were examined to determine parity rates across the four sites. Of these, 2,518 (89.1%) were parous in Béoumi, 2,785 (89.8%) in Dabakala, 801 (82.2%) in Nassian, and 3,547 (86.9%) in Sakassou (Annex Table A8). Overall, the parity rate was similar for mosquitoes collected indoors and outdoors in both IRS (p=0.964) and control sites (p=0.999) (Annex Table A9).

Figure 13 shows mean monthly parity rates for *An. gambiae* s.l. The mean parity rates for *An. gambiae* s.l. in the sprayed sites were 85.8% versus 88.5% at the control sites (p=0.162) (Annex Table B5).

FIGURE 13: PARITY RATE OF *AN. GAMBIAE* S.L. COLLECTED USING HLC IN CONTROL AND IRS SITES BY MONTH



Error bars represent the standard errors.

3.1.2.6 PLASMODIUM FALCIPARUM SPOROZOITE AND ENTOMOLOGICAL INOCULATION RATES (EIRS)

Table 5 shows the overall infection rates and EIRs for *An. gambiae* s.l. and *An. funestus* s.l. collected by HLC in the four districts. Nassian had the highest *Plasmodium falciparum* (Pf) sporozoite infection rate among *An. gambiae* s.l. (0.037), followed by Dabakala (0.017) and Béoumi (0.011). The lowest Pf sporozoite rate (0.006) was recorded in Sakassou.

The highest *An. gambiae* s.l. EIRs (infected bites per person per night [b/p/n]) were recorded between June and November at all four districts (Figure 14). Sakassou recorded the highest EIR (0.950 infective b/p/n [ib/p/n]) of *An. gambiae* s.l., followed by Dabakala (0.678 ib/p/n) and Nassian (0.150 ib/p/n). Béoumi recorded the lowest EIR, 0.365 ib/p/n, but the difference between the four sites was not statistically significant (p=0.3132) (Annex Table B6).

In Nassian, *An. funestus* s.l. was analyzed for infection throughout the year, with an overall EIR of 0.057 ib/p/n. (Table 5). The highest number of infected bites per person per night was observed in November (0.3 ib/p/n) (Figure 15; Annex Table A10).

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)	8,079	1,181	13	0.011	33.2	0.444	93	9	0	0	0.2	0.000
Dabakala (Control)	11,606	1,194	20	0.017	44.4	0.678	198	4	0	0	0.2	0.000
Nassian (IRS)	3,310	597	22	0.037	13.6	0.150	251	116	6	0.052	1.1	0.057
Sakassou (IRS)	33,986	1,201	7	0.006	161.6	0.950	31	0	-	-	-	-

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

FIGURE 14: MONTHLY EIR OF *AN. GAMBIAE* S.L. AT CONTROL AND IRS SITES

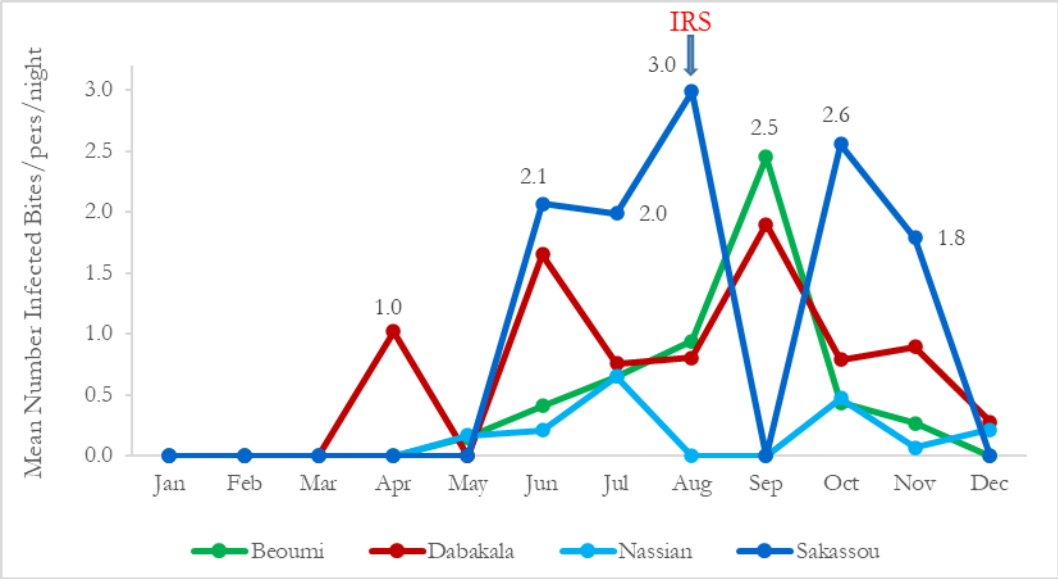
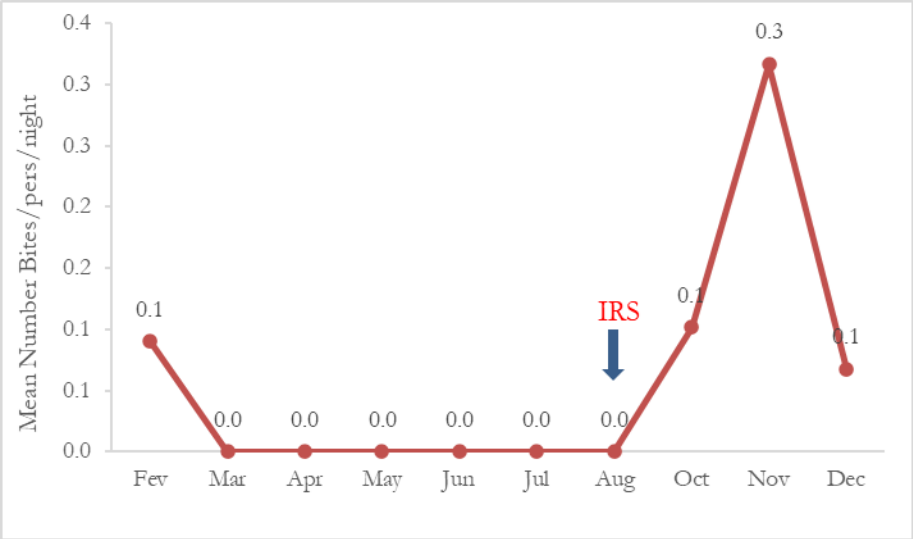


FIGURE 15: MONTHLY EIR OF *AN. FUNESTUS* S.L. IN NASSIAN

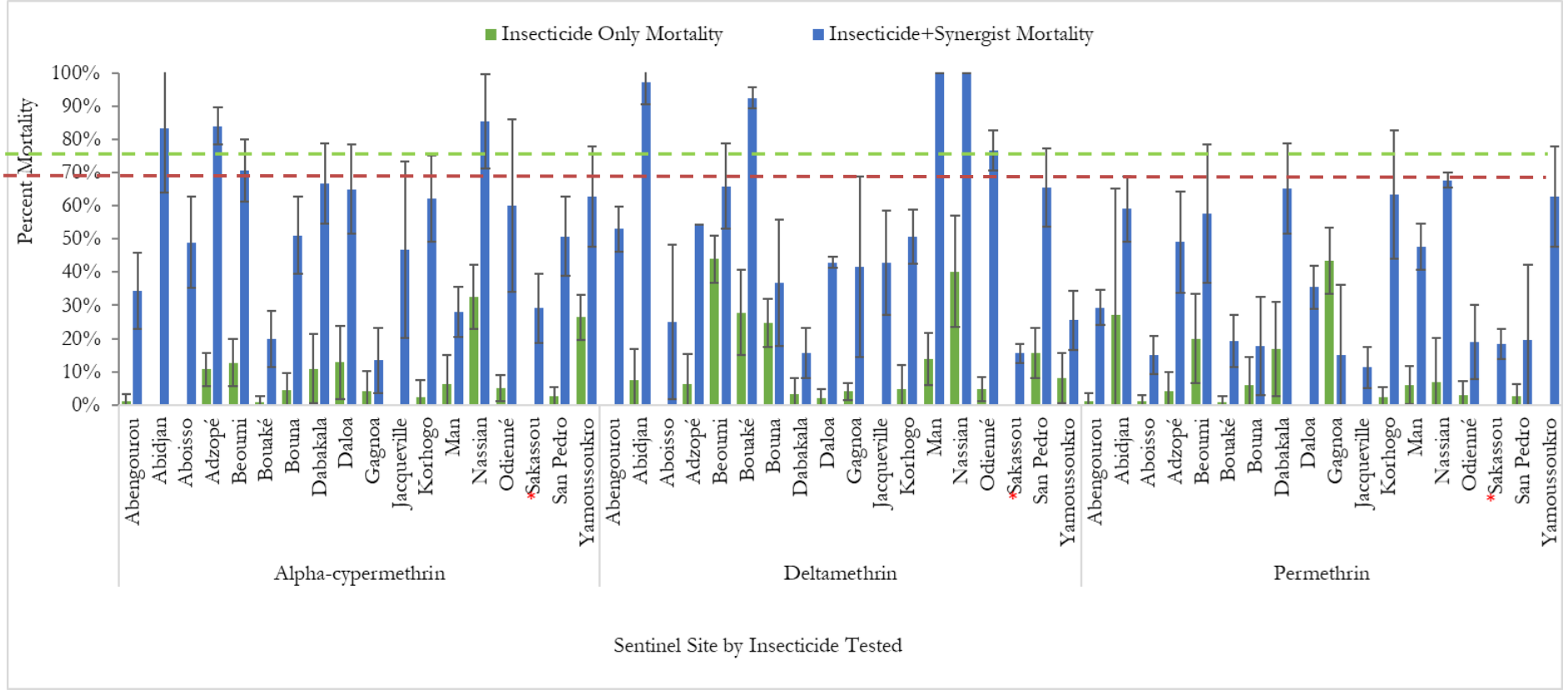


3.2 INSECTICIDE RESISTANCE MONITORING

Figures 16–22 show the resistance status, resistance intensity and synergism to the different insecticides tested against *An. gambiae* s.l. collected from the 18 different sites. All insecticides were tested in all sites except in Aboisso and Adzopé, where pirimiphos-methyl was not tested due limited number of mosquitoes. Also, pirimiphos-methyl 0.25% was not tested at Jacqueville due to insufficient number mosquito larvae at the time of testing. For all figures, the horizontal dashed red line represents the 90% threshold for resistance, and the green line represents the 98% threshold for susceptibility. Furthermore, all recorded control mortalities were below 5% except in Sakassou where Abbott formula was used to correct the overall mortality of the pyrethroids.

Resistance was observed to all pyrethroids in all sites surveyed. Pre-exposure of mosquitoes to PBO before exposure to deltamethrin, permethrin, and alpha-cypermethrin yielded a substantial increase of mortality in all sites. Deltamethrin showed the highest increase in mortality among the pyrethroids when combined with PBO in all sites, with full restoration of vector susceptibility in Abidjan, Man and Nassian (Figure 16). The intensity of resistance was high in all sites for all three pyrethroids, except in Abengourou, Daloa, Nassian and Yamoussoukro where permethrin resistance was moderate (Figure 17). Susceptibility to pirimiphos-methyl was recorded at 10 out of 17 sites, and low resistance intensity was observed in the remaining sites (Figure 18) (Annex Table A11).

FIGURE 16: PYRETHROID INSECTICIDE SYNERGIST TEST RESULTS IN 18 SITES



Error bars represent the standard deviation

* Percentage mortality corrected using Abbott's formula

FIGURE 17: INTENSITY OF RESISTANCE TO PYRETHROID INSECTICIDES IN 18 SITES

Error bars represent the standard errors.

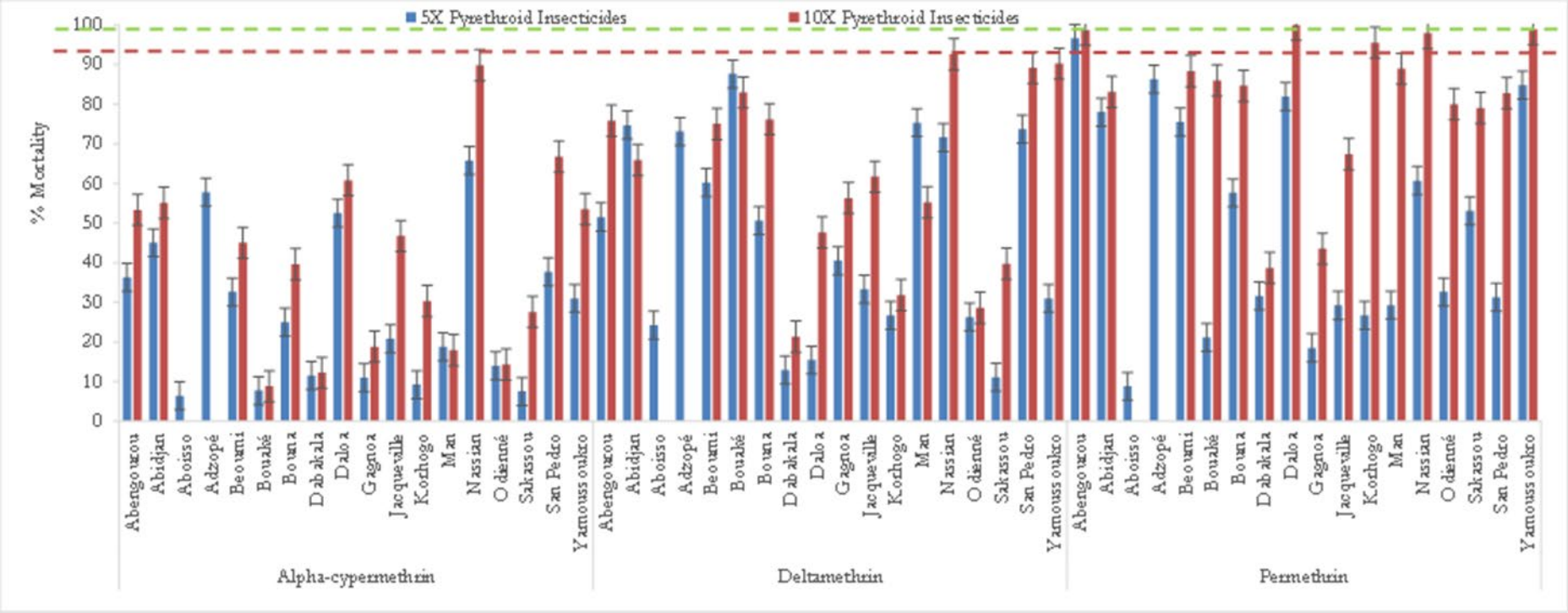
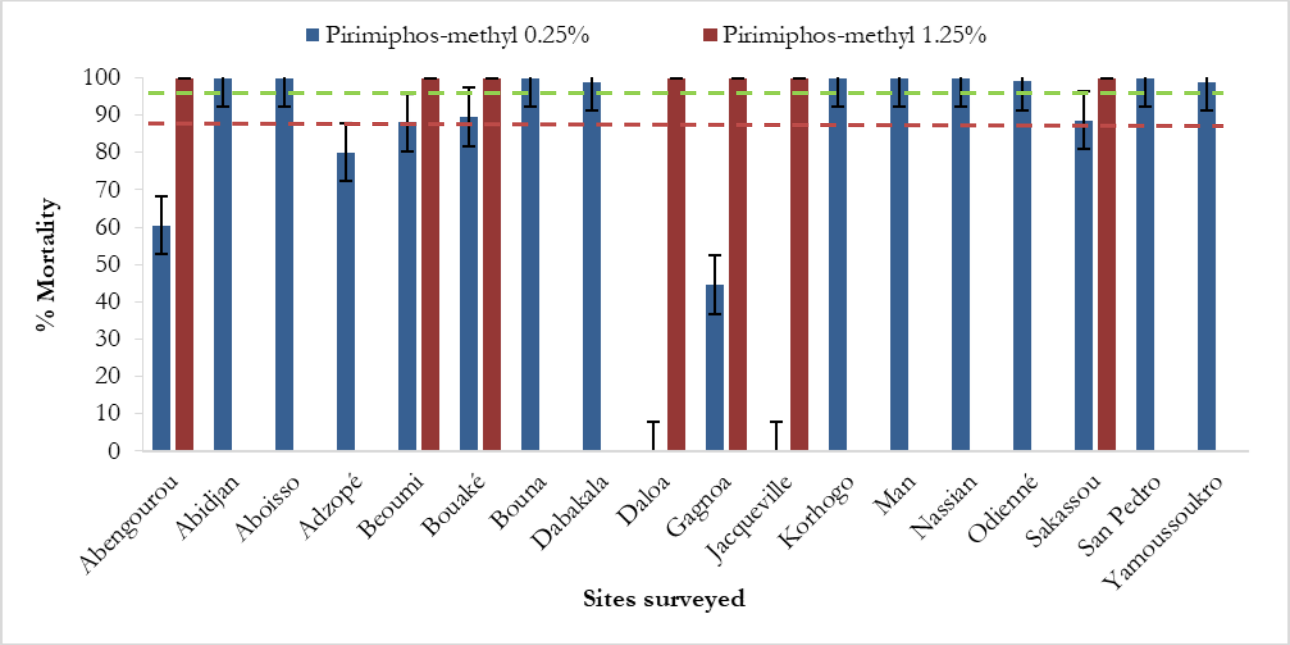


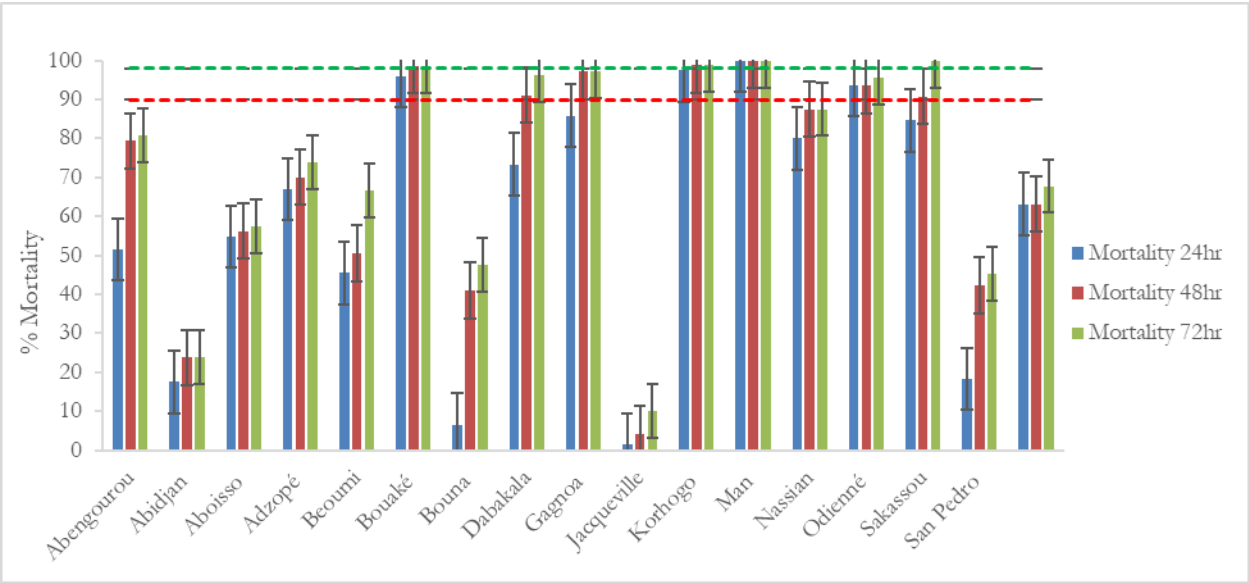
FIGURE 18: SUSCEPTIBILITY AND RESISTANCE INTENSITY TO PIRIMIPHOS-METHYL IN 17 SITES



Error bars represent the standard errors

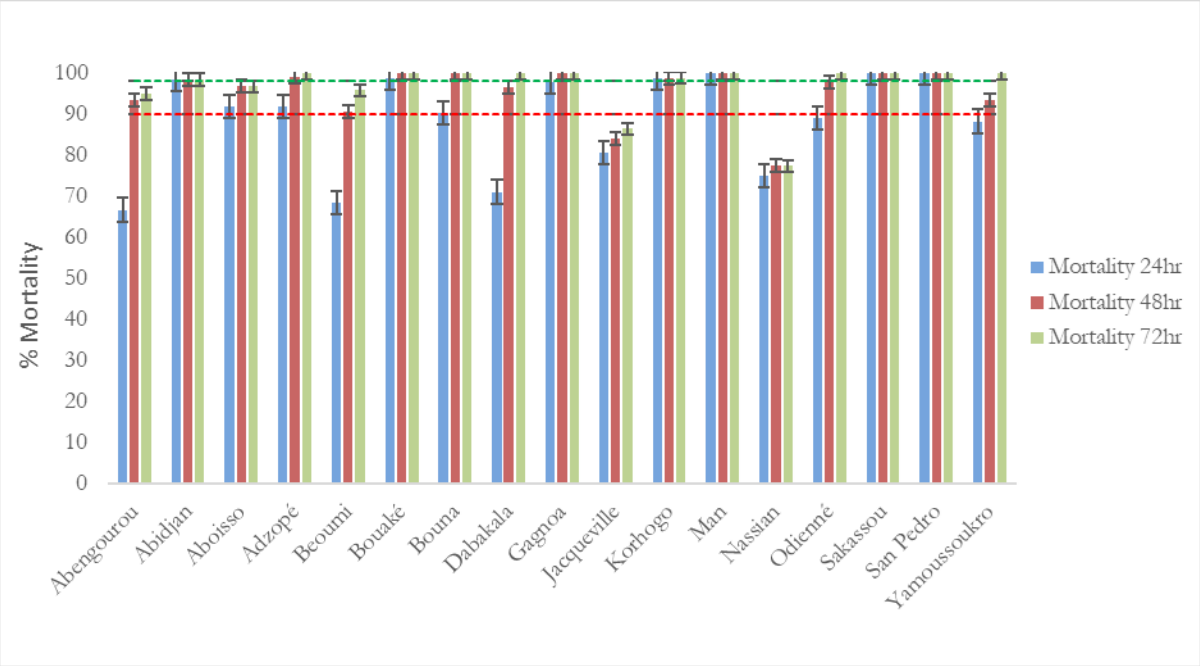
Susceptibility to chlorfenapyr was observed at the dose of 100 µg/bottle after observing mortality for 72 hours in four sites (Bouake, Korhogo, Man, and Sakassou). Mosquitoes from Béoumi, Bouaké, Dabakala, Jacqueville, Nassian, San Pedro, and Yamoussoukro were resistant to the 100 µg/bottle dose (Figure 19). The lowest mortality rate was recorded in Jacqueville. Susceptibility to chlorfenapyr at 200µg/bottle was recorded in 12 sites. Abengourou, Aboisso, Beoumi, Jacqueville and Nassian showed resistance at the dose of 200µg/bottle (Figure 20).

FIGURE 19: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 100µG/BOTTLE BY SITE



Error bars represent the standard errors

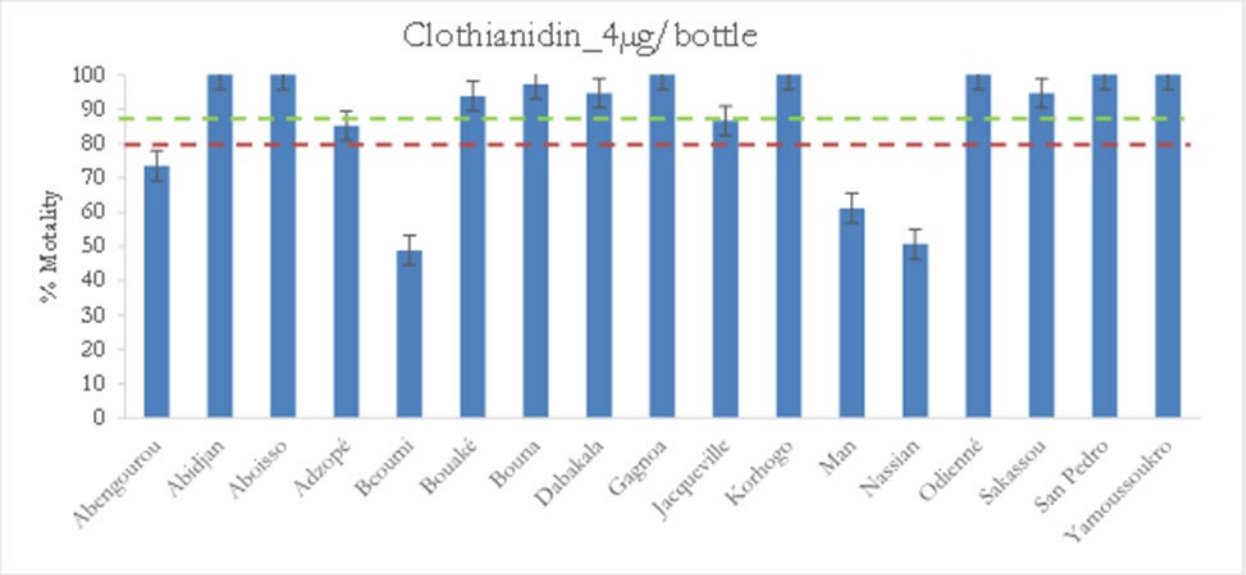
FIGURE 20: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 200µG/BOTTLE BY SITE



Error bars represent the standard errors

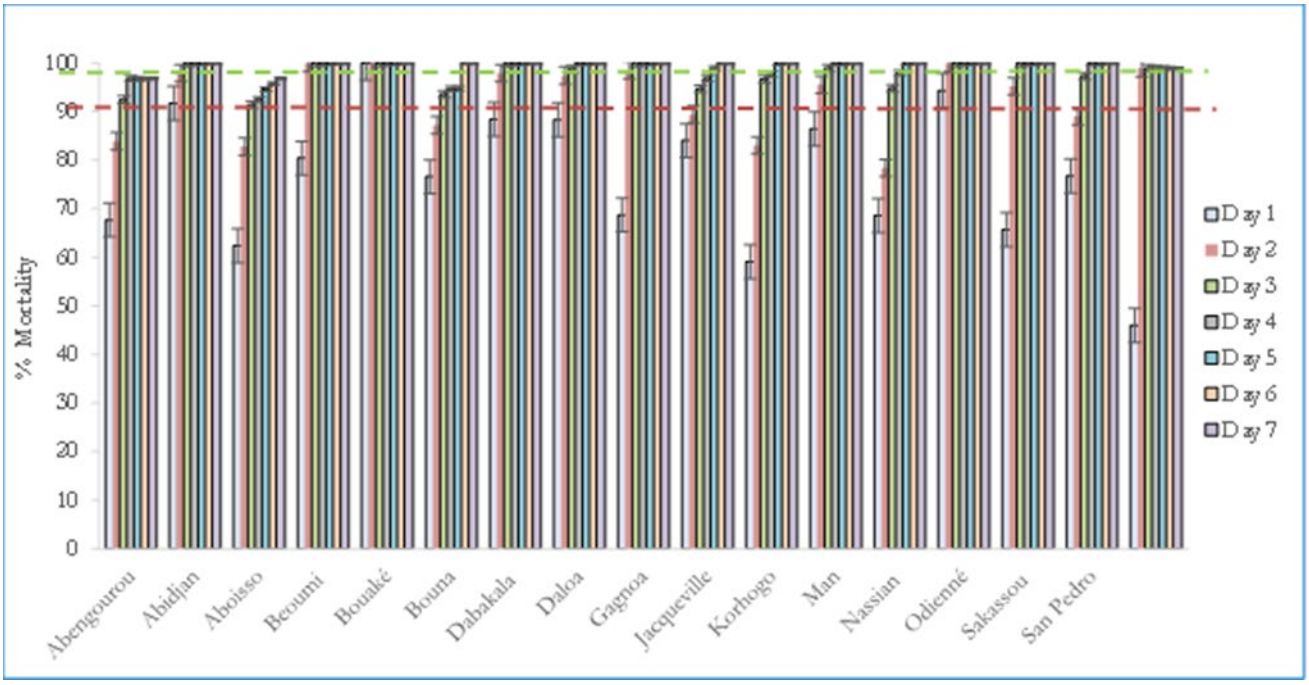
The results of the CDC bottle assays and WHO susceptibility test using clothianidin are shown in Figures 21–22. For clothianidin 4 µg/bottle, susceptibility was observed in 7 of the 17 sites tested (Figure 21) while full susceptibility was observed in 15 out of the 17 sites tested for the 2% clothianidin tested in WHO tube (Figure 22).

FIGURE 21: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN USING CDC BOTTLE ASSAY IN 17 SITES



Error bars represent the standard errors

FIGURE 22: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN 2% USING WHO TUBE TEST BY SITE



Error bars represent the standard errors.

3.3 MOLECULAR ANALYSIS

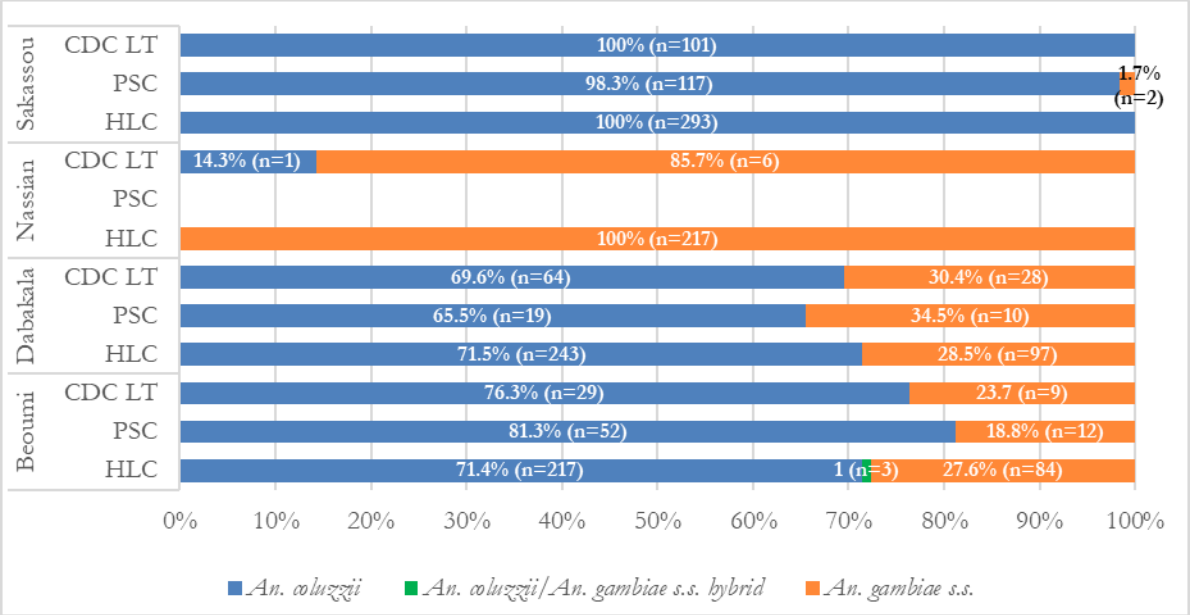
3.3.1 SPECIES IDENTIFICATION OF ADULT *AN. GAMBIAE* S.L. COLLECTED FROM BIONOMIC STUDY

A subset of 406 *An. gambiae* s.l. from Béoumi 461 from Dabakala, 224 from Nassian, and 513 from Sakassou were molecularly identified to the sub-species. *An. coluzzii* represented the predominant species in Béoumi and Dabakala (all collection methods included) and was the only species found in Sakassou. In Nassian, *An. gambiae* s.s. was the predominant species collected and the only vector species captured using HLC. Few hybrids of the two species were characterized in the HLC samples from Béoumi (1.0%; n=3) (Figure 23 and Annex Table A12).

3.3.2 DETECTION OF ENZYMES INVOLVED IN INSECTICIDE RESISTANCE *AN. GAMBIAE* S.L.

The analyses are still underway due to delayed reagent delivery. Data will be shared when available through an addendum.

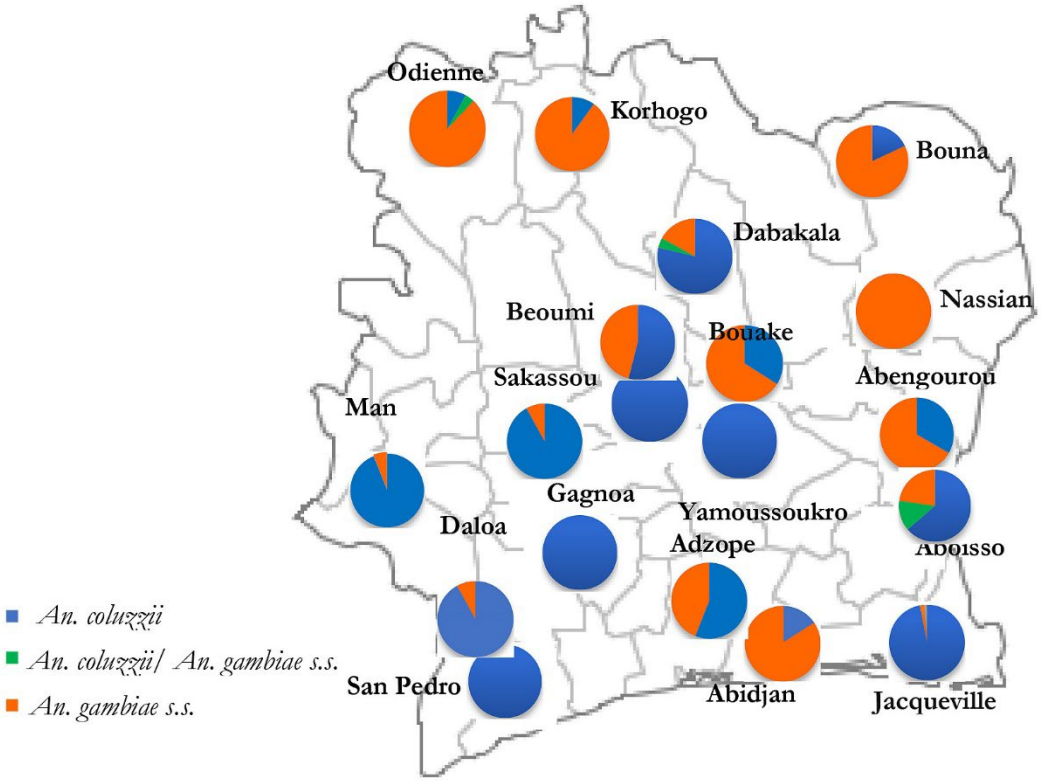
FIGURE 23: PERCENTAGE *AN. GAMBIAE* SPECIES PER COLLECTION METHODS IN THE BIONOMIC MONITORING SITES IN 2021



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

Figure 24 shows the species composition of *An. gambiae* s.l. used for susceptibility testing per site. *An. coluzzii* represented the predominant species in Aboisso (54%; n=50), Adzopé (56%; n=28), Béoumi (54%; n=27), Dabakala (79%; n=37), Daloa (92%; n=46), Jacqueville (97%; n=97) and Man (94%; n=47) and the entire population in, Gagnoa (100%; n=49), San Pedro (100%; n=66), Yamoussoukro (100%; n=48) and Sakassou (100%; n=50). *An. gambiae s.s.* represented the predominant species in Abengourou (67%; n=32), Abidjan (84; n=42), Bouake (66%; n=33), Bouna (82%; n=41), Korhogo (90%; n=45) and Odienné (88%; n=44) and it was the entire population in Nassian (100%; n=50). A few hybrids of the two species were characterized in Odienné (4.0%; n=2), Dabakala (4.3%; n=2), Aboisso (13.6%; n=6) and Jacqueville (2%; n=2) (Annex Table A13).

FIGURE 24: SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. USED FOR SUSCEPTIBILITY TEST ACROSS THE 18 DISTRICTS



3.4 QUALITY ASSURANCE OF IRS AND INSECTICIDE DECAY RATE

3.4.1 QUALITY ASSURANCE

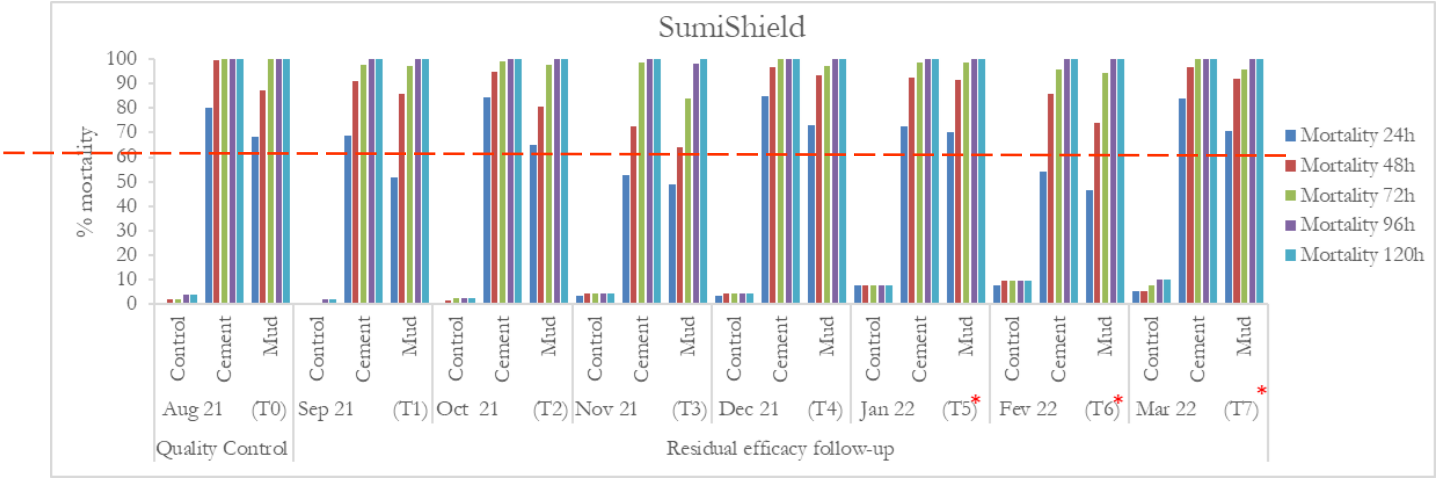
Quality assurance assessments using WHO wall bioassays conducted in a total of 22 houses (nine cement and four mud in Nassian, and nine cement in Sakassou) compared to the six control houses (three in Nassian and Sakassou) yielded identical results in all the sites and wall types tested; one hundred 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.

3.4.2 INSECTICIDE RESIDUAL EFFICACY

For insecticide residual efficacy monitoring, wall cone bioassays conducted monthly from September 2021 to March 2022 showed five-day mortality of *An. gambiae* Kisumu above 80% (efficacy threshold) through March, representing a minimum of six months of residual efficacy for SumiShield in Nassian on both cement and mud walls (Figures 25–26).[1] Fludora Fusion in Sakassou continued to cause five-day mortality above 80% after six months using the susceptible strain *An. gambiae* Kisumu as well as the wild strain from Sakassou.

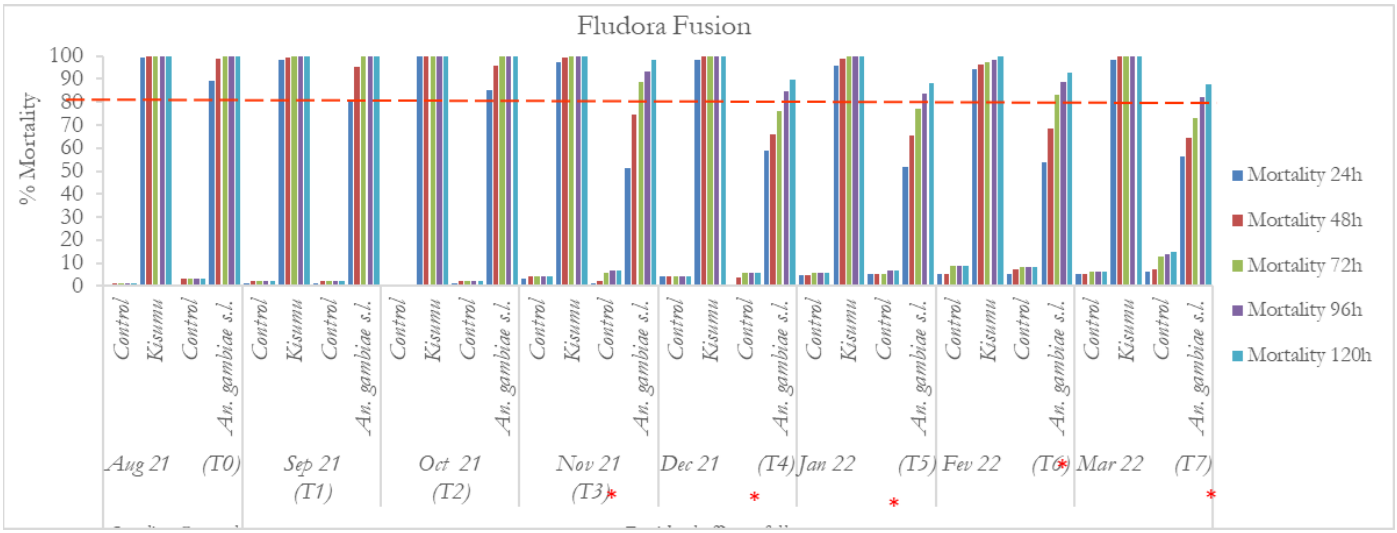
[1] The insecticide decay rate is reported through February 2022 using the available data at the time this report was submitted.

FIGURE 25: QUALITY CONTROL AND RESIDUAL EFFICACY: MORTALITY OF *AN. GAMBIAE* KISUMU STRAIN ON FOUR MUD AND NINE CEMENT SURFACES SPRAYED WITH SUMISHIELD IN NASSIAN



Overall mortality adjusted using Abbott formula **Note:** The red line represents the 80% residual efficacy threshold.

FIGURE 26: QUALITY CONTROL AND RESIDUAL EFFICACY: MORTALITY OF *AN. GAMBIAE* KISUMU AND WILD COLLECTED *AN. GAMBIAE* S.L. ON NINE CEMENT SURFACES SPRAYED WITH FLUDORA FUSION IN SAKASSOU



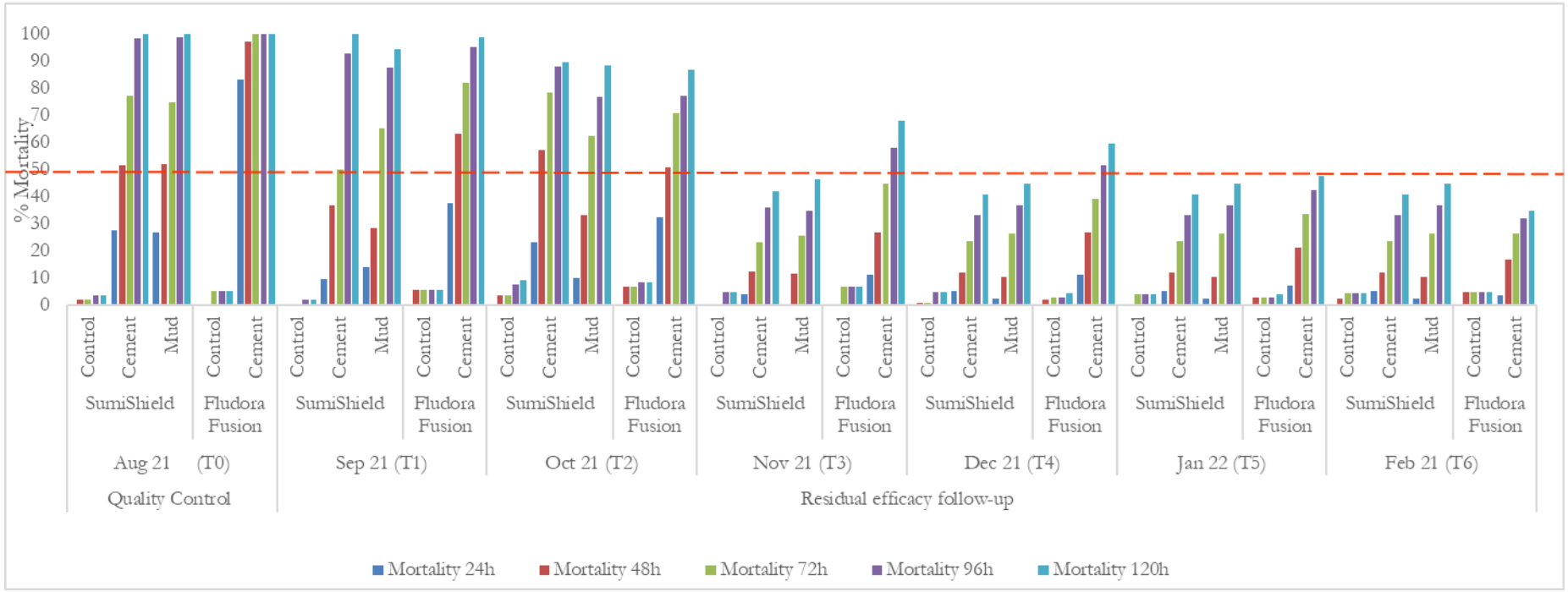
Overall mortality adjusted using Abbott formula

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

3.4.3 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 fumigant effect was observed on SumiShield treated cement and mud up to 2 months post IRS in Nassian (90.7% and 89.4%, respectively) while in Sakassou, the fumigant effect of Fludora Fusion sprayed cement was observed at T4 (59.6%) after which it dropped below the 50% threshold (47.7%) (Figure 27).

FIGURE 27: FUMIGATION EFFECT MORTALITY USING *AN. GAMBIAE* S.L. KISUMU ON CEMENT SURFACES SPRAYED WITH SUMISHIELD IN NASSIAN AND FLUDORA FUSION IN SAKASSOU



Overall mortality adjusted using Abbott formula

Note: The red line represents the 50% fumigant effect cut off.

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 caught were *An. funestus* s.l., *An. nili*, and *An. pharoensis*. The three malaria vectors were collected in similar proportions in 2021 (94.7%; 1.1% and 0.1%) as in 2020 (94.8%, 1.5%, and 0.3%, respectively) and in 2019 (94%, 2%, and 0.7%, respectively) (Annex Figures B1-B3).

An. coluzzii and *An. gambiae* s.s. were the two species of the complex recorded in all sites. Similar to the 2019 and 2020 collection, *An. coluzzii* was predominant in Béoumi, Dabakala, and the only species collected in Sakassou. *An. gambiae* s.s. represented the main vector only in Nassian.

The mean IRD of *An. gambiae* s.l. were 7.1 f/r/d in Sakassou showing a slight and significant decrease in endophilic mosquitoes in Sakassou compared to the previous years (10 f/r in 2020 and 15.9 f/r in 2019). However, the mean IRDs in Nassian (2.5 f/r) increased from 1.6 f/r in 2020. The IRDs in both control sites increased in Beoumi (3 f/r) and Dabakala (5.7 f/r) in 2021 compared to 2020 (2.3 f/r in Beoumi and 3.8 f/r in Dabakala).

The HBRs of *An. gambiae* s.l. were significantly reduced in all sites from 48.5 to 33.2 b/p/n (representing 31.6 % of percentage reduction) in Beoumi, from 81.4 to 44.4 b/p/n (45.4% percentage reduction) in Dabakala, from 26.6 to 13.6 b/p/n (48.9% percentage reduction) in Nassian and from 323.4 to 161.6 b/p/n (50.2% percentage reduction) in Sakassou after IRS period.

However, both IRD and HBR recorded in IRS sites in 2021 after spraying were still higher than those recorded in 2020 after IRS. These high densities could be attributed to the heavy rains that immediately occurred after the last week of 2021 IRS campaign.

An. gambiae s.l. biting time was similar at all sites with the peak biting observed between 0:00 am and 4:00 am, both indoors and outdoors at all sites. We also observed that there was a slight change in biting behavior of *An. gambiae* s.l. in the sprayed sites in 2021 and particularly in Nassian (mean outdoor biting of 55.2% in Sakassou and 39.6% in Nassian) compared to 2020 where both sites outdoor biting was similar (46.4% in Sakassou and 48.8% in Nassian) showing that the vector population in Nassian may be biting more outdoors than indoors. Therefore, the vector outdoor biting trend observed in Nassian may need to be closely monitored to understand the relationship between the implementation of IRS and the vector behavior as both sites host different species (*An. coluzzii* in Sakassou and *An. gambiae* s.s. in Nassian); this will allow estimation any behavior change of any of the species, following IRS.

Overall, the percentages of gravid *An. gambiae* s.l. mosquitoes were 14.5% in the sprayed sites and 11.3% in the control sites. The percentage of combined fed and half gravid *An. gambiae* s.l. were 74.9% in the sprayed sites and 81.5% in the control sites. No difference in abdominal status was observed between both IRS and control sites, suggesting that the vectors could have bitten a host and remained indoors in all settings. This may be a cause of concerns as the NMCP conducted a recent mass ITN distribution campaign across the country. The need to assess the ITN use is crucial.

Mean parity rates for *An. gambiae* s.l. at the sprayed sites were 85.8% versus 88.5% at the control sites. The mean parity rates of *An. gambiae* s.l. at the sprayed sites were similar to those observed in the control sites.

The mean EIR of *An. gambiae* s.l. recorded in Sakassou (0.950 ib/p/n) in 2021 was less than that observed in 2020 (2.53 ib/p/n) and before implementing IRS in 2019 (3.42 ib/p). Similar to Sakassou, the EIR of *An. gambiae* s.l. recorded in Nassian (0.150 ib/p/n) in 2021 was less than that observed in 2020 (0.54 ib/p/n) and

before implementing IRS in in 2019 (0.60 ib/p/n). In the control sites, the mean EIR increased from 0.27 ib/p/n in 2019 to 0.99 ib/p/n in 2020 and decreased to 0.44 ib/p/n in 2021 in Beoumi and in Dabakala, increased from 0.73 ib/p/n in 2019 to 2.23 ib/p/n in 2020 and decreased in 2021 (0.68 ib/p/n). The data showed a reduction in malaria transmission parameters such as the EIR in Sakassou than Nassian, even though the transmission in Nassian was reduced compared to the control unsprayed sites (Table Annex B2, B4 and B6).

The 2021 data collected showed a continuous decrease of malaria transmission in IRS sites from 2019 before the first IRS campaign to the post second year of IRS in 2021. Based on these results, a retrospective and prospective epidemiological evaluation may be useful for exploring expansion of IRS sites and continuous implementation of the strategy in the country targeting high transmission areas.

4.2 Insecticide Susceptibility

High pyrethroid resistance was observed in all 18 sites monitored for vector susceptibility, underlining the potential limited efficacy of pyrethroid-only interventions in Côte d'Ivoire.

Pre-exposure to PBO before exposure to deltamethrin, permethrin, and alpha-cypermethrin yielded 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 Abidjan, Man and Nassian. This suggests that enzymes such as P450s might be involved in insecticide resistance. Additionally, the results are in line with the choice to introduce PBO ITNs in the recent mass distribution campaign of ITNs in selected districts across the country and may support a countrywide distribution of PBO ITNs. The molecular analysis to characterize any oxidase involved will help confirm the extent of enzyme involvement and confirm the choice made on procuring PBO ITNs for the recent mass distribution campaign.

Susceptibility to pirimiphos-methyl was observed in 10 sites, while low intensity of the resistance was observed in the 7 sites where resistance was confirmed, and intensity test completed.

Susceptibility to clothianidin 2% impregnated papers was observed in fifteen out of the 17 sites tested within seven days of exposure, including the two IRS sites. However, the CDC bottle assays conducted with the active ingredient at 4 µg/bottle yielded less susceptible sites compared to the paper-based tests. The trend will need to be confirmed during the next collection as there could be a bottle coating variability among sites, and also to enable deciding on the appropriate protocol to be considered for clothianidin testing.

Chlorfenapyr at the dose of 100 µg/bottle yielded full susceptibility in only four sites. With the 200 µg/bottle, susceptibility was observed in 12 of the tested sites. These results support the inclusion of Interceptor G2 nets, which contain chlorfenapyr, 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 six months at the time of this report. The observed mortality showed that the sprayed walls were not underdosed during the campaign. As of February 2022, 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 was above the efficacy threshold of 80% after six months showing that the sprayed insecticides lasted enough to cover the peak transmission period in both sites.

The umigant effects of both insecticides (SumiShield and Fludora Fusion) were observed only for two months post IRS in Nassian and four in Sakassou when using a 50% mortality threshold.

4.4 RECOMMENDATIONS

Based on these results, the recommendations are as follows:

4.4.1. TECHNICAL

- Conduct advanced statistical analysis to assess entomological monitoring trends and the impact of the IRS on malaria transmission compared to control sites, adjusted for seasonal and other relevant factors.
- Conduct an evaluation of epidemiological trends pre- and post-IRS to determine if there is an association between IRS deployment and malaria cases.
- Closely follow-up on entomological trends in Nassian to assess the hypothesis of vector behavior change post-IRS.
- As WHO has now defined the 100 µg/bottle as a diagnosis dose of chlorfenapyr, consider improving testing conditions by implementing parallel testing with the susceptible *An. gambiae* Kisumu strain to confirm all resistance trends observed in the surveyed sites.

4.4.2. STRATEGIC

- The consistently high pyrethroid resistance confirms the need for advocacy and strategic deployment of PBO and/or dual insecticide ITNs across the country.
- The use of Interceptor G2 and PBO ITN should continue to be considered in a mosaic deployment considering the results of the insecticide resistance monitoring
- The observed spatial and temporal variability in indoor and outdoor biting of malaria vectors in Cote d'Ivoire necessities an investigation of human behavior during the night to estimate the risk of being bitten and determine the gap in protection from ITNs and IRS. Results from these studies may inform social and behavior change communication campaigns ensuring vector control tools are maximized.
- Both SumiShield and Fludora Fusion showed good residual efficacy, enabling their continuous use in the upcoming IRS campaigns. In addition, the susceptibility observed against pirimiphos-methyl in Nassian could support an insecticide rotation plan for IRS, though the country is aiming to switch both SumiShield and Fludora Fusion between both IRS sites. However, it is important to note that per PMI guidance[2] switching between SumiShield and Fludora Fusion does not constitute a real insecticide rotation as these two brands of insecticide still contain clothianidin.

[2] PMI FY 2023 Technical Guidance, https://www.pmi.gov/resources/technical-documents/

ANNEX A

TABLE A1: SPECIES COMPOSITION OF MOSQUITOES COLLECTED AT ALL SITES USING HLC IN 2021 (4 COLLECTION HOUSES DURING 2 CONSECUTIVE NIGHTS OF COLLECTION INDOOR AND OUTDOOR PER MONTH)

District	Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Beoumi	*An. gambiae* s.l.	33	27	30	34	176	644	983	1492	1965	506	425	49	6364
	An. funestus s.l.		0			6	2	4	59	3	2	3	5	84
	An. pharoensis	1	1		6	7	9	28	1	129	189	370	103	844
	Culicine	62	175	257	193	115	179	150	74	134	157	469	281	2246
	Total	**96**	**203**	**287**	**233**	**304**	**834**	**1165**	**1626**	**2231**	**854**	**1267**	**438**	**9538**
Dabakala	*An. gambiae* s.l.	5	1	348	1299	688	661	588	1308	1506	1270	709	145	8528
	An. funestus s.l.	1		4			13	11	22	5	96	17	4	173
	An. nili			1			84	17	41	405	164	14	1	727
	An. pharoensis	1		7	11	24	31	7	16		46	29		172
	An. coustani					2			1		1			4
	An. ziemanni											1		1
	Culicine	486	787	417	230	350	389	383	117	164	191	322	294	4130
	Total	**493**	**788**	**777**	**1540**	**1064**	**1178**	**1006**	**1505**	**2080**	**1768**	**1092**	**444**	**13735**
Nassian	*An. gambiae* s.l.	0	0	2	18	49	101	133	88	1833	276	82	29	2611
	An. funestus s.l.	2	7	5	1	15	8	18	4	28	47	46	25	206
	An. nili			1						8				9
	An. pharoensis										1			1
	Culicine	10	16	41	20	29	41	63	84	25	71	46	18	464
	Total	**12**	**23**	**49**	**39**	**93**	**150**	**214**	**176**	**1894**	**395**	**174**	**72**	**3291**
Sakassou	*An. gambiae* s.l.	225	1554	2932	3560	3180	2881	3571	1595	1960	4099	2862	2596	31015
	An. funestus s.l.	3	4	3		1	1						13	25
	An. nili											2		2
	An. pharoensis		43	179	120	127	71	65	41	58	278	130	107	1219
	An. ziemanni						2							2
	Culicine	61	217	211	277	256	367	361	122	170	111	149	188	2490
	Total	**289**	**1818**	**3325**	**3957**	**3564**	**3322**	**3997**	**1758**	**2188**	**4488**	**3143**	**2904**	**34753**

TABLE A2: SPECIES COMPOSITION OF MOSQUITOES COLLECTED IN 2021 AT ALL SITES USING PSC (30 HOUSES PER MONTH)

District	Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Beoumi	*An. gambiae* s.l.	8	1	12	21	82	164	128	271	189	102	60	28	1066
	An. funestus s.l.	0	0	0	0	4	0	0	0	0	0	0	4	8
	An. pharoensis	0	0	0	1	1	0	0	0	3	1	3	0	9
	Culicine	94	29	167	108	84	218	78	120	13	34	31	45	1021
	Total	102	30	179	130	171	382	206	391	205	137	94	77	2104
Dabakala	*An. gambiae* s.l.	6	4	234	391	166	123	193	383	242	151	94	61	2048
	An. funestus s.l.	2	0	0	0	0	0	0	0	0	12	2	0	16
	An. nili	0	0	0	0	0	0	0	0	0	7	0	0	7
	Culicine	33	170	182	53	51	110	67	55	22	29	92	83	947
	Total	41	174	416	444	217	233	260	438	264	199	188	144	3018
Nassian	*An. gambiae* s.l.	3	1	5	3	7	54	58	26	317	106	46	23	649
	An. funestus s.l.	0	6	3	0	2	0	6	0	5	8	1	11	42
	An. nili	1	0	0	0	0	0	0	0	0	0	0	0	1
	Culicine	7	3	8	7	13	3	9	6	8	27	8	17	116
	Total	11	10	16	10	22	57	73	32	330	141	55	51	808
Sakassou	*An. gambiae* s.l.	53	173	462	243	294	202	242	96	150	224	48	314	2501
	An. funestus s.l.	0	0	0	0	0	0	0	0	0	0	0	4	4
	Culicine	19	36	38	78	67	288	37	21	6	15	52	285	942
	Total	72	209	500	321	361	490	279	117	156	239	100	603	3447

TABLE A3: SPECIES COMPOSITION OF MOSQUITOES COLLECTED IN 2021 AT ALL SITES USING CDC LIGHT TRAPS (4 TRAPS INDOORS FOR 2 CONSECUTIVE NIGHTS PER MONTH)

District	Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Beoumi	*An. gambiae* s.l.	0	0	0	0	5	21	26	345	201	21	23	7	649
	An. funestus s.l.	0	0	0	1	0	0	0	0	0	0	0	0	1
	An. nili	0	0	0	0	0	0	0	0	0	0	0	0	0
	An. pharoensis	0	0	0	0	0	0	0	0	3	9	14	19	45
	Culicine	16	42	34	33	18	71	31	21	37	70	53	100	526
	Total	16	42	34	34	23	92	57	366	241	100	90	126	1221
Dabakala	*An. gambiae* s.l.	0	0	19	148	17	27	161	306	267	33	47	5	1030
	An. funestus s.l.	0	0	2	0	0	1	0	0	0	6	0	0	9
	An. nili	0	0	0	0	0	5	3	4	0	3	0	0	15
	An. pharoensis	0	0	1	0	0	0	1	1	0	0	0	0	3
	Culicine	9	4	4	14	36	25	60	71	18	8	32	12	293
	Total	9	4	26	162	53	58	225	382	285	50	79	17	1350
Nassian	*An. gambiae* s.l.	0	0	0	0	0	0	2	1	45	0	0	2	50
	An. funestus s.l.	0	0	0	0	0	0	0	0	3	0	0	0	3
	Culicine	1	0	2	14	5	13	3	20	8	6	11	8	91
	Total	1	0	2	14	5	13	5	21	56	6	11	10	144
Sakassou	*An. gambiae* s.l.	3	3	185	37	5	21	102	35	31	17	2	29	470
	An. funestus s.l.	0	0	0	2	0	0	0	0	0	0	0	0	2
	Culicine	3	7	31	5	10	10	50	24	20	5	2	1	168
	Total	6	10	216	44	15	31	152	59	51	22	4	30	640

TABLE A4: INDOOR RESTING DENSITY OF *AN. GAMBIAE S.L.* AND *AN. FUNESTUS S.L.* COLLECTED IN 2021 USING PSC

Species	Sites	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
An. gambiae s.l.	Beoumi (Control)	0.3	0.0	0.4	0.7	2.7	5.5	4.3	9.0	6.3	3.4	2.0	0.9
	Dabakala (Control)	0.2	0.1	7.8	13.0	5.5	4.1	6.4	12.8	8.1	5.0	3.1	2.0
	Nassian (IRS)	0.1	0.0	0.2	0.1	0.2	1.8	1.9	0.9	10.6	3.5	1.5	0.8
	Sakassou (IRS)	1.8	5.8	15.4	8.1	9.8	6.7	8.1	3.2	5.0	7.5	3.2	10.5
An. funestus s.l.	Beoumi (Control)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	Dabakala (Control)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.1	0.0
	Nassian (IRS)	0.0	0.2	0.1	0.0	0.1	0.0	0.2	0.0	0.2	0.3	0.0	0.4
	Sakassou (IRS)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1

TABLE A5: ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. COLLECTED IN 2021 USING PSC

Site	Abdominal status	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Beoumi	Unfed	0	0	0	0	0	0	2	13	13	2	1	2
	Fed	8	1	6	13	14	152	76	45	153	6	43	20
	Half gravid	0	0	5	4	23	9	29	182	16	87	10	3
	Gravid	0	0	1	4	45	3	21	31	7	7	6	3
	Total	**8**	**1**	**12**	**21**	**82**	**164**	**128**	**271**	**189**	**102**	**60**	**28**
Dabakala	Unfed	0	0	11	73	2	6	20	54	17	2	1	5
	Fed	5	4	176	57	149	16	86	57	139	134	54	14
	Half gravid	0	0	34	250	7	48	68	233	43	6	16	36
	Gravid	1	0	13	11	8	53	17	39	43	9	23	6
	Total	**6**	**4**	**234**	**391**	**166**	**123**	**191**	**383**	**242**	**151**	**94**	**61**
Nassian	Unfed	0	0	0	0	1	4	6	0	26	11	0	0
	Fed	2	1	4	3	6	40	26	16	207	42	3	10
	Half gravid	0	0	0	0	0	1	16	4	41	36	34	5
	Gravid	1	0	1	0	0	9	10	6	43	17	9	8
	Total	**3**	**1**	**5**	**3**	**7**	**54**	**58**	**26**	**317**	**106**	**46**	**23**
Sakassou	Unfed	1	4	50	57	31	20	22	25	26	21	4	25
	Fed	20	88	303	98	43	40	151	56	98	128	26	220
	Half gravid	32	37	30	57	193	99	43	5	0	53	14	27
	Gravid	0	44	79	31	27	43	26	10	26	22	4	42
	Total	**53**	**173**	**462**	**243**	**294**	**202**	**242**	**96**	**150**	**224**	**48**	**314**

TABLE A6: BITING CYCLE OF *AN. GAMBIAE* S.L. USING HLC

Site	Location	6-7 pm	7-8 pm	8-9 pm	9-10 pm	10-11 pm	11-12 pm	0-1 am	1-2 am	2-3 am	3-4 am	4-5 am	5-6 am
Beoumi	Outdoor	0.02	0.21	0.18	0.48	1.00	1.10	3.20	3.30	3.00	2.90	1.90	1.10
Dabakala	Outdoor	0.13	0.39	0.80	1.30	1.50	2.40	4.70	5.70	5.80	5.10	4.20	1.90
Nassian	Outdoor	0.00	0.00	0.04	0.05	0.20	0.16	0.45	0.52	0.46	0.20	0.07	0.09
Sakassou	Outdoor	1.60	2.80	4.30	6.50	10.20	13.10	20.10	26.70	26.50	23.3	23.20	14.90
	Total Outdoor	**1.75**	**3.40**	**5.32**	**8.33**	**12.90**	**16.76**	**28.45**	**36.22**	**35.76**	**31.50**	**29.37**	**17.99**
Beoumi	Indoor	0.02	0.21	0.18	0.61	0.93	1.10	2.50	3.00	2.20	1.80	1.90	1.50
Dabakala	Indoor	0.02	0.23	0.45	1.00	1.80	2.70	4.40	4.60	5.00	4.30	4.00	1.50
Nassian	Indoor	0.02	0.00	0.07	0.11	0.13	0.30	0.57	0.89	0.45	0.39	0.20	0.05
Sakassou	Indoor	2.20	2.50	3.60	6.10	8.90	12.70	17.70	19.90	23.00	19.20	18.20	16.60
	Total Indoor	**2.26**	**2.94**	**4.30**	**7.82**	**11.76**	**16.80**	**25.17**	**28.39**	**30.65**	**25.69**	**24.30**	**19.65**

TABLE A7: MONTHLY BITING RATE RESULTS USING HLC

Site	Month and year	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
Beoumi	January 21	0.8	3.4	0.0	0.0
	February 21	1.3	2.1	0.0	0.0
	March 21	1.3	2.5	0.0	0.0
	April 21	1.9	2.4	0.0	0.0
	May 21	8.6	13.4	0.6	0.1
	June 21	32.9	47.6	0.3	0.0
	July 21	65.4	57.5	0.1	0.4
	August 21	78.4	108.1	0.0	7.4
	September 21	72.8	172.9	0.1	0.3
	October 21	32.0	31.3	0.3	0.0
	November 21	31.1	22.0	0.4	0.0
	December 21	1.9	4.3	0.4	0.3
	Total	**328.4**	**467.5**	**2.2**	**8.5**
Dabakala	January 21	0.5	0.1	0.0	0.1
	February 21	0.0	0.1	0.0	0.0
	March 21	21.5	22.0	0.4	0.1
	April 21	76.9	85.5	0.0	0.0
	May 21	39.0	47.0	0.0	0.0
	June 21	39.8	42.9	0.8	0.9
	July 21	33.1	40.4	0.3	1.1
	August 21	80.4	83.1	1.3	1.5
	September 21	80.5	107.8	0.0	0.6
	October 21	77.3	81.5	4.1	7.9
	November 21	47.4	41.3	0.6	1.5
	December 21	5.4	12.8	0.1	0.4
	Total	**501.8**	**564.5**	**7.6**	**14.1**
Nassian	January 21	0.0	0.0	0.1	0.1
	February 21	0.0	0.0	0.8	0.1
	March 21	0.3	0.0	0.6	0.0
	April 21	1.0	1.3	0.1	0.0
	May 21	4.4	1.8	1.4	0.5
	June 21	6.9	5.8	0.4	0.6
	July 21	9.8	6.9	0.8	1.5
	August 21	4.5	6.5	0.0	0.5
	September 21	141.0	88.1	2.0	1.5
	October 21	21.5	13.0	2.8	3.1
	November 21	5.9	4.4	4.1	1.6
	December 21	2.0	1.6	1.5	1.6
	Total	**197.3**	**129.4**	**14.6**	**11.1**

Site	Month and year	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
Sakassou	January 21	12.6	15.5	0.1	0.3
	February 21	78.4	115.9	0.4	0.1
	March 21	157.9	208.6	0.3	0.1
	April 21	177.1	267.9	0.0	0.0
	May 21	201.4	196.1	0.1	0.0
	June 21	175.3	184.9	0.0	0.1
	July 21	223.8	222.6	0.0	0.0
	August 21	76.5	122.9	0.0	0.0
	September 21	111.1	133.9	0.0	0.0
	October 21	227.4	285.0	0.0	0.0
	November 21	148.8	209.0	0.0	0.0
	December 21	144.8	179.8	0.9	0.8
	Total	**1735.1**	**2142.5**	**1.8**	**1.4**

TABLE A8: MONTHLY PARITY RATES OF DISSECTED *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. PER SITE

Site	Month and year	Total Dissected *An. gambiae* s.l.	Total Parous *An. gambiae* s.l.	Parity rate (%)	Total Dissected *An. funestus* s.l.	Total Parous *An. funestus* s.l.	Parity rate (%)
Beoumi	Jan 21	32	30	89.8	0	0	
	Feb 21	25	22	84.6	0	0	
	Mar 21	30	23	75.0	0	0	
	Apr 21	27	19	70.1	0	0	
	May 21	176	159	91.1	6	6	100.0
	Jun 21	455	421	92.6	2	2	100.0
	Jul 21	441	382	86.6	3	3	100.0
	Aug 21	384	370	95.9	59	59	100.0
	Sep 21	447	430	97	1	1	100.0
	Oct 21	477	369	77.4	2	2	100.0
	Nov 21	286	246	86.6	3	3	100.0
	Dec 21	49	47	97.1	5	5	100.0
	Total	**2829**	**2518**	**87.0**	**81**	**81**	**100.0**
Daabakala	Jan 21	5	5	100.0	1	1	100.0
	Feb 21	1	0	0.0	0	0	
	Mar 21	224	185	82.6	1	0	0.0
	Apr 21	311	283	91.1	0	0	
	May 21	324	310	95.6	0	0	
	Jun 21	449	436	97.2	6	6	100.0
	Jul 21	364	347	95.4	9	9	100.0
	Aug 21	419	301	71.9	22	15	66.7
	Sep 21	203	186	92.0	5	5	100.0
	Oct 21	407	376	92.3	22	22	100.0
	Nov 21	261	229	87.8	17	17	100.0
	Dec 21	134	127	94.9	4	4	100.0
	Total	**3102**	**2785**	**83.4**	**87**	**79**	**85.2**
Nassian	Jan 21	0	0		2	0	0.0
	Feb 21	0	0		7	5	41.7
	Mar 21	1	1	100.0	5	5	100.0
	Apr 21	18	18	100.0	1	1	100.0
	May 21	48	35	68.3	15	8	60.3
	Jun 21	101	88	87.2	8	6	73.4
	Jul 21	133	99	73.7	18	18	100.0
	Aug 21	88	70	78.9	4	3	75.0
	Sep 21	215	159	74.0	0	0	
	Oct 21	266	233	87.1	44	44	100.0
	Nov 21	76	72	95.6	41	39	96.6
	Dec 21	28	26	92.8	23	22	95.5
	Total	**974**	**801**	**85.7**	**168**	**151**	**76.6**

Site	Month and year	Total Dissected *An. gambiae* s.l.	Total Parous *An. gambiae* s.l.	Parity rate (%)	Total Dissected *An. funestus* s.l.	Total Parous *An. funestus* s.l.	Parity rate (%)
Sakassou	Jan 21	205	164	79.7	3	3	100.0
	Feb 21	348	339	97.5	4	4	100.0
	Mar 21	451	408	90.5	1	1	100.0
	Apr 21	418	383	91.5	0	0	
	May 21	412	366	88.9	1	1	100.0
	Jun 21	419	401	95.7	1	1	100.0
	Jul 21	417	342	82.3	0	0	
	Aug 21	237	191	80.3	0	0	
	Sep 21	266	211	79.2	0	0	
	Oct 21	325	258	79.1	0	0	
	Nov 21	298	244	81.9	0	0	
	Dec 21	287	240	83.6	2	2	100.0
	Total	**4083**	**3547**	**85.8**	**12**	**12**	**100.0**

TABLE A9: MONTHLY PARITY RATES OF DISSECTED MOSQUITOES FROM INDOOR AND OUTDOOR COLLECTIONS

Site	Month and year	INDOOR			OUTDOOR		
		Total Dissected *An. gambiae* s.l.	Total Parous *An. gambiae* s.l.	Parity rate (%)	Total Dissected *An. gambiae* s.l.	Total Parous *An. gambiae* s.l.	Parity rate (%)
Beoumi	**Jan 21**	6	5	83.3	26	25	96.2
	Feb 21	8	6	75.0	17	16	94.1
	Mar 21	10	7	70.0	20	16	80.0
	Apr 21	13	8	61.5	14	11	78.6
	May 21	69	65	94.2	107	94	87.9
	Jun 21	184	171	92.9	271	250	92.3
	Jul 21	207	178	86.0	234	204	87.2
	Aug 21	154	144	93.5	230	226	98.3
	Sep 21	152	151	99.3	295	279	94.6
	Oct 21	235	187	79.6	242	182	75.2
	Nov 21	121	109	90.1	165	137	83.0
	Dec 21	15	15	100.0	34	32	94.1
	Total	**1174**	**1046**	**85.5**	**1655**	**1472**	**88.5**
Dabakala	**Jan 21**	4	4	100.0	1	1	100.0
	Feb 21	0	0		1	0	0.0
	Mar 21	114	96	84.2	110	89	80.9
	Apr 21	146	134	91.8	165	149	90.3
	May 21	123	117	95.1	201	193	96.0
	Jun 21	182	177	97.3	267	259	97.0
	Jul 21	169	162	95.9	195	185	94.9
	Aug 21	196	142	72.4	223	159	71.3
	Sep 21	87	82	94.3	116	104	89.7
	Oct 21	184	167	90.8	223	209	93.7
	Nov 21	118	104	88.1	143	125	87.4
	Dec 21	40	38	95.0	94	89	94.7
	Total	**1363**	**1223**	**91.4**	**1739**	**1562**	**83.0**
Nassian	**Jan 21**	0	0		0	0	
	Feb 21	0	0		0	0	
	Mar 21	1	1	100.0	0	0	
	Apr 21	8	8	100.0	10	10	100.0
	May 21	34	27	79.4	14	8	57.1
	Jun 21	55	48	87.3	46	40	87.0
	Jul 21	78	61	78.2	55	38	69.1
	Aug 21	36	27	75.0	52	43	82.7
	Sep 21	119	88	73.9	96	71	74.0
	Oct 21	166	148	89.2	100	85	85.0
	Nov 21	45	41	91.1	31	31	100.0
	Dec 21	16	15	93.8	12	11	91.7
	Total	**558**	**464**	**86.8**	**416**	**337**	**83.0**

Site	Month and year	INDOOR			OUTDOOR		
		Total Dissected *An. gambiae* s.l.	Total Parous *An. gambiae* s.l.	Parity rate (%)	Total Dissected *An. gambiae* s.l.	Total Parous *An. gambiae* s.l.	Parity rate (%)
Sakassou	**Jan 21**	92	70	76.1	113	94	83.2
	Feb 21	158	154	97.5	190	185	97.4
	Mar 21	210	192	91.4	241	216	89.6
	Apr 21	223	210	94.2	195	173	88.7
	May 21	205	183	89.3	207	183	88.4
	Jun 21	208	199	95.7	211	202	95.7
	Jul 21	197	170	86.3	220	172	78.2
	Aug 21	111	84	75.7	126	107	84.9
	Sep 21	129	94	72.9	137	117	85.4
	Oct 21	147	111	75.5	178	147	82.6
	Nov 21	135	110	81.5	163	134	82.2
	Dec 21	123	103	83.7	164	137	83.5
	Total	**1938**	**1680**	**85.0**	**2145**	**1867**	**86.7**

TABLE A10: MONTHLY SPOROZOITE AND EIRS OF THE FOUR DISTRICTS OF BIONOMIC SURVEY

Site	Month	Number *An. gambiae* s.l. Tested	Number infected	Sporozoite Rate	HBR (b/p/n)	EIR (ib/p/n)
Beoumi	Jan	13	0	0.000	2.1	0.000
	Feb	15	0	0.000	1.7	0.000
	Mar	23	0	0.000	1.9	0.000
	Apr	16	0	0.000	2.2	0.000
	May	142	2	0.014	11.0	0.155
	Jun	294	3	0.010	40.3	0.411
	Jul	187	2	0.011	61.5	0.657
	Aug	99	1	0.010	93.3	0.942
	Sep	100	2	0.020	122.9	2.457
	Oct	144	2	0.014	31.7	0.440
	Nov	98	1	0.010	26.6	0.271
	Dec	50	0	0.000	3.1	0.000
	Total	**1181**	**13**	**0.011**	**33.2**	**0.365**
Dabakala	Jan	5	0	0.000	0.3	0.000
	Feb				0.1	0.000
	Mar	162	0	0.000	21.8	0.000
	Apr	238	3	0.013	81.2	1.024
	May				43.0	0.000

Site	Month	Number *An. gambiae* s.l. Tested	Number infected	Sporozoite Rate	HBR (b/p/n)	EIR (ib/p/n)
	Jun	100	4	0.040	41.4	1.654
	Jul	193	4	0.021	36.8	0.762
	Aug	101	1	0.010	81.8	0.809
	Sep	99	2	0.020	94.2	1.902
	Oct	100	1	0.010	79.4	0.794
	Nov	99	2	0.020	44.4	0.896
	Dec	97	3	0.031	9.1	0.281
	Total	**1194**	**20**	**0.017**	44.4	0.744
Nassian	Jan				0.0	0.000
	Feb				0.0	0.000
	Mar	1	0	0.000	0.2	0.000
	Apr	19	0	0.000	1.2	0.000
	May	36	2	0.056	3.1	0.172
	Jun	90	3	0.033	6.4	0.212
	Jul	102	8	0.078	8.4	0.655
	Aug	69	0	0.000	5.5	0.000
	Sep				114.6	0.000
	Oct	182	5	0.027	17.3	0.474
	Nov	73	1	0.014	5.2	0.071
	Dec	25	3	0.120	1.8	0.216
	Total	**597**	**22**	**0.037**	13.6	0.502
Sakassou	Jan	111	0	0.000	14.1	0.000
	Feb	100	0	0.000	97.2	0.000
	Mar	100	0	0.000	183.3	0.000
	Apr	101	0	0.000	222.5	0.000
	May	89	0	0.000	198.8	0.000
	Jun	87	1	0.011	180.1	2.070
	Jul	112	1	0.009	223.2	1.993
	Aug	100	3	0.030	99.7	2.991
	Sep	101	0	0.000	122.5	0.000
	Oct	100	1	0.010	256.2	2.562
	Nov	100	1	0.010	178.9	1.789
	Dec	100	0	0.000	162.3	0.000
	Total	**1201**	**7**	**0.006**	161.6	0.942

Site	Month	Number *An. funestus* s.l. Tested	Number infected	Sporozoite Rate	HBR (b/p/n)	EIR (ib/p/n)
Beoumi	Jun	2	0	0.000	0.15	0.000
	Oct	1	0	0.000	0.15	0.000
	Nov	2	0	0.000	0.20	0.000
	Dec	4	0	0.000	0.35	0.000
	Total	**9**	**0**	**0.000**	**0.21**	0.000
Dabakala	Jan	1	0	0.000	0.05	0.000
	Sep	1	0	0.000	0.3	0.000
	Dec	2	0	0.000	0.25	0.000
	Total	**4**	**0**	0.000	0.2	0.000
Nassian	Fev	5	1	0.200	0.45	0.090
	Mar	3	0	0.000	0.3	0.000
	Apr	1	0	0.000	0.05	0.000
	May	6	0	0.000	0.95	0.000
	Jun	6	0	0.000	0.5	0.000
	Jul	14	0	0.000	1.15	0.000
	Aug	2	0	0.000	0.25	0.000
	Oct	29	1	0.034	2.95	0.102
	Nov	27	3	0.111	2.85	0.317
	Dec	23	1	0.043	1.55	0.067
	Total	**116**	**6**	**0.052**	**1.1**	0.057

TABLE A11: INSECTICIDE SUSCEPTIBILITY: MORTALITY PERCENTAGE FROM TEST RESULTS FROM THE 18 SITES

	Aben gourou	Abidjan	Aboisso	Adzopé	Beoumi	Bouaké	Bouna	Dabakala	Daloa	Gagnoa	Jacque ville	Korhogo	Man	Nassian	Odienné	*Sakassou	San Pedro	Yamous soukro
Alpha-cypermethrin 1×	1.1 (93)	0 (85)	0 (96)	10.7 (56)	12.8 (86)	0.95 (105)	4.6 (87)	10.9 (101)	12.8 (78)	4.2 (71)	0 (98)	2.5 (2.5)	6.5 (93)	32.5 (83)	5.2 (96)	0 (95)	2.6 (78)	26.4 (91)
PBO+Alpha-cypermethrin	34.4 (93)	83.1 (77)	48.9 (92)	84 (50)	70.6 (85)	19.8 (106)	51 (96)	66.7 (87)	64.9 (77)	13.5 (74)	46.7 (45)	62.2 (90)	28 (100)	85.4 (82)	60 (95)	29.2 (96) *	50.7 (71)	62.6 (91)
Alpha-cypermethrin 5×	36.3 (80)	45 (80)	6.4 (78)	57.8 (45)	32.6 (95)	7.7 (104)	25 (92)	11.5 (61)	52.5 (80)	11 (73)	20.8 (48)	9.2 (87)	18.8 (80)	65.8 (76)	14 (100)	7.5 (107)	37.7 (69)	31 (87)
Alpha-cypermethrin 10×	53.3 (75)	55.1 (78)	NC	NC	45 (100)	8.8 (102)	39.6 (101)	12.2 (98)	60.8 (79)	18.8 (69)	46.7 (45)	30.3 (89)	17.9 (78)	89.8 (98)	14.3 (91)	27.6 (98)	66.7 (72)	53.5 (86)
Deltamethrin 1×	0 (97)	7.6 (79)	0 (83)	6.3 (48)	38.5 (91)	27.8 (97)	24.7 (81)	3.2 (93)	2 (51)	4.1 (74)	0 (102)	4.8 (83)	13.8 (94)	40.2 (82)	4.8 (104)	0 (87)	15.7 (83)	8.2 (98)
PBO+Deltamethrin	52.9 (102)	97 (101)	25 (84)	54.2 (24)	62.6 (88)	92.5 (106)	36.8 (76)	15.7 (89)	42.9 (49)	41.7 (72)	42.9 (49)	50.6 (81)	100 (77)	100 (82)	76.7 (103)	15.6 (96) *	65.5 (84)	25.5 (98)
Deltamethrin 5×	51.5 (103)	74.7 (83)	24.2 (91)	73.1 (52)	60.2 (88)	87.6 (105)	50.6 (87)	12.9 (93)	15.4 (39)	40.5 (74)	33.3 (87)	26.7 (86)	75.3 (85)	71.6 (81)	26.3 (99)	11.1 (90)	73.7 (76)	31 (87)
Deltamethrin 10×	75.8 (91)	65.9 (82)	NC	NC	75 (84)	82.9 (105)	76.2 (105)	21.3 (89)	47.6 (42)	56.3 (71)	61.7 (47)	31.8 (85)	55.2 (58)	92.6 (95)	28.6 (98)	39.7 (78)	89.2 (74)	90.2 (92)
Permethrin 1×	1.1 (88)	27.3 (88)	1.1 (95)	4.2 (48)	20 (95)	0.95 (105)	6 (84)	16.9 (83)	0 (82)	43.5 (69)	0 (104)	2.4 (84)	6 (83)	6.9 (58)	3.1 (96)	0 (97)	2.8 (72)	0 (91)
PBO+Permethrin 1×	29.3 (92)	59 (78)	14.9 (87)	49 (51)	57.6 (92)	19.3 (109)	17.7 (96)	65.2 (69)	35.4 (79)	15.2 (66)	11.3 (53)	63.4 (82)	47.5 (61)	67.7 (96)	19.1 (89)	18.5 (92) *	19.7 (71)	62.6 (91)
Permethrin 5×	96.6 (87)	78 (82)	8.8 (91)	86.3 (51)	75.5 (98)	21.1 (90)	57.6 (85)	31.6 (79)	81.9 (72)	18.5 (65)	29.2 (96)	26.7 (86)	29.3 (92)	60.7 (89)	32.6 (95)	53.1 (98)	31.3 (67)	84.8 (99)
Permethrin 10×	98.7 (77)	83.1 (83)	NC	NC	88.3 (94)	86 (100)	84.6 (104)	38.7 (93)	100 (55)	43.5 (69)	67.4 (46)	95.5 (88)	88.9 (72)	97.9 (95)	80 (105)	79 (100)	82.8 (64)	98.9 (88)
Pirimiphos-methyl 1×	60.5 (86)	100 (87)	100 (87)	80 (50)	88.1 (90)	89.5 (95)	100 (97)	98.9 (88)	0 (61)	44.6 (83)	0 (98)	100 (98)	100 (100)	100 (95)	99 (100)	88.6 (105)	100 (70)	98.9 (93)
Pirimiphos-methyl 5×	100 (102)	NA	NA	NC	100 (98)	100 (96)	100 (100)	NA	100 (69)	100 (87)	100 (52)	100 (96)	100 (90)	NA	NA	100 (93)	NA	NA

NC= Not completed due to limited number of larvae; number in parenthesis are the number of mosquitoes tested

NA= Not applicable * Mortality adjusted with abbott formula for pyrethroids

Resistance confirmed Suspected resistance Susceptible * Percentage mortality corrected using Abbott's

TABLE A12: NUMBER AND PERCENTAGE OF *AN. GAMBIAE* SPECIES COLLECTED BY HLC, PSC AND CDC-LIGHT TRAP METHODS

Site	Collection method	Number tested	*An. coluzzii* (%)	*An. coluzzii*/ *An. gambiae* s.s. (%)	*An. gambiae* s.s. (%)
Beoumi	HLC	304	217 (71.4)	3 (1)	84 (27.6)
	PSC	64	52 (81.3)		12 (18.8)
	CDC LT	38	29 (76.3)	0 (0)	9 (23.7)
Dabakala	HLC	340	243 (71.5)	0 (0)	97 (28.5)
	PSC	29	19 (65.5)		10 (34.5)
	CDC LT	92	64 (69.6)		28 (30.4)
Nassian	HLC	217	0 (0)	0 (0)	217 (100)
	PSC				
	CDC LT	7	1 (14.3)	0 (0)	6 (85.7)
Sakassou	HLC	293	293 (100)	0 (0)	0 (0)
	PSC	119	117 (98.3)	0 (0)	2 (1.7)
	CDC LT	101	101 (100)	0 (0)	0 (0)

TABLE A13: NUMBER AND PERCENTAGE OF *AN. GAMBIAE* SPECIES TESTED FOR RESISTANCE MONITORING

Site	Number Tested	*An. coluzzii* (%)	*An. coluzzii*/ *An. gambiae* s.s. (%)	*An. gambiae* s.s. (%)
Abengourou	48	16 (33.3)	0 (0)	32 (66.7)
Abidjan	50	8 (16.0)	0 (0)	42 84.0
Aboisso	44	28 (63.6)	6 (13.6)	10 (22.7)
Adzope	50	28 (56.0)	0 (0)	22 (44.0)
Beoumi	50	27 (54.0)	0 (0)	23 (46.0)
Bouake	50	17 (34.0)	0 (0)	33 (66.0)
Bouna	50	9 (18.0)	0 (0)	41 (82.0)
Dabakala	47	37 (78.7)	2 (4.3)	8 (17.0)
Daloa	50	46 (92.0)	0 (0)	4 (8.0)
Gagnoa	49	49 (100)	0 (0)	0 (0)
Jacqueville	100	97 (97.0)	2 (2.0)	1 (1.0)
Korhogo	50	5 (10.0)	0 (0)	45 (90.0)
Man	50	47 (94.0)	0 (0)	3 (6.0)
Nassian	50	0 (0)	0 (0)	50 (100)
Odienne	50	4 (8.0)	2 (4.0)	44 (88.0)
Sakassou	50	50 (100)	0 (0)	0 (0)
San Pedro	66	66 (100)	0 (0)	0 (0)
Yanoussoukro	48	48 (100)	0 (0)	0 (0)

ANNEX B: STATISTICAL OUTPUT AND GRAPHS

TABLE B1: INDOOR RESTING DENSITY OF AN. GAMBIAE S.L COLLECTED BY PSC

Site	Comparison	Mean IRD (first group)	Mean IRD (second group)	Kruskal-Wallis chi-squared	p-value
All	Control vs Sprayed	4.3	4.4	0.015	0.9015
All sprayed	Pre-IRS vs Post-IRS	9.4	4.4	3.631	0.0567
All control	Pre-IRS vs Post-IRS	9.7	4.3	3.629	0.0568
Sakassou**	Beoumi vs Sakassou	5.1	15.5	8.333	0.0039
Nassian**	Dabakala vs Nassian	14.3	2.5	7.467	0.0063
Sakassou	Beoumi vs Sakassou	2.9	7.1	7.056	0.0079
Nassian	Dabakala vs Nassian	5.7	1.8	6.753	0.0094*
Beoumi	Pre-IRS vs Post-IRS	5.1	2.9	2.381	0.1228
Dabakala	Pre-IRS vs Post-IRS	14.3	5.7	2.149	0.1427
Nassian	Pre-IRS vs Post-IRS	2.5	1.8	1.931	0.1646
Sakassou	Pre-IRS vs Post-IRS	15.5	7.1	7.687	0.0056

* Indicates statistical significance at 0.05%; ** Indicate rows for the 2019 data only

TABLE B2: FED AN. GAMBIAE S.L. VECTORS COLLECTED BY PSC

Site	Comparison	Mean proportion fed first group	Mean proportion fed second group	Kruskal-Wallis chi-squared	p-value
All	Control vs Sprayed	57.9%	56.8%	0.197	0.6573
All sprayed	Pre-IRS vs Post-IRS	59.9%	56.8%	0.168	0.6816
All control	Pre-IRS vs Post-IRS	51.0%	57.9%	0.882	0.3478
Sakassou	Beoumi vs Sakassou	60.6%	49.7%	1.614	0.2039
Nassian	Dabakala vs Nassian	55.2%	64.0%	0.214	0.6438
Beoumi	Pre-IRS vs Post-IRS	69.1%	60.6%	0.381	0.5369
Dabakala	Pre-IRS vs Post-IRS	42.9%	55.2%	0.596	0.4402
Nassian	Pre-IRS vs Post-IRS	59.0%	64.0%	0.073	0.787
Sakassou	Pre-IRS vs Post-IRS	61.6%	49.8%	2.313	0.1283

TABLE B3: GRAVID AN. GAMBIAE S.L. COLLECTED BY PSC

Site	Comparison	Mean proportion gravid (first group)	Mean proportion gravid (second group)	Kruskal-Wallis chi-squared	p-value
All	Control vs Sprayed	12.2%	14.6%	2.529	0.1118
All sprayed	Pre-IRS vs Post-IRS	14.5%	14.6%	0.254	0.6145
All control	Pre-IRS vs Post-IRS	19.2%	12.2%	3.028	0.0818
Sakassou	Beoumi vs Sakassou	12.0%	13.0%	1.474	0.2247
Nassian	Dabakala vs Nassian	12.5%	16.2%	0.754	0.3853
Beoumi	Pre-IRS vs Post-IRS	14.2%	12.0%	0.597	0.4397
Dabakala	Pre-IRS vs Post-IRS	24.3%	12.5%	2.881	0.896
Nassian	Pre-IRS vs Post-IRS	18.0%	16.2%	0.096	0.7566
Sakassou	Pre-IRS vs Post-IRS	10.2%	13.5%	1.704	0.1918

TABLE B4: HUMAN BITING RATES OF AN. GAMBIAE S.L. COLLECTED BY HLC

Site	Comparison	Mean HBR† (first group)	Mean HBR (second group)	Kruskal-Wallis chi-squared	p-value
All	Control vs Sprayed	38.8	87.6	1.263	0.2611
All sprayed	Pre-IRS vs Post-IRS	184.3	87.6	3.051	0.0807
All control	Pre-IRS vs Post-IRS	64.9	38.8	2.93	0.087
Sakassou**	Beoumi vs Sakassou	48.5	324.4	12	0.0005
Nassian**	Dabakala vs Nassian	81.4	26.7	5.835	0.0157
Sakassou	Beoumi vs Sakassou	33.2	161.6	13.23	0.0003*
Nassian	Dabakala vs Nassian	44.5	13.6	5.883	0.0153*
Beoumi	Pre-IRS vs Post-IRS	48.5	33.2	1.339	0.2472
Dabakala	Pre-IRS vs Post-IRS	81.4	44.5	2.881	0.0896
Nassian	Pre-IRS vs Post-IRS	26.7	13.6	1.007	0.3157
Sakassou	Pre-IRS vs Post-IRS	324.4	161.6	9.338	0.0022*

* Indicates statistical significance at 0.05%; ** Indicate rows for the 2019 data only; † Mean mosquito bites per person per night

TABLE B5: PARITY RATES OF AN. GAMBIAE S.L. COLLECTED BY HLC

Site	Comparison	Mean proportion parous (first group)	Mean proportion parous (second group)	Kruskal-wallis chi-squared	p-value
All	Control vs Sprayed	85.2%	85.8%	0.793	0.373
All sprayed	Pre-IRS vs Post-IRS	81.9%	85.8%	0.072	0.7879
All control	Pre-IRS vs Post-IRS	78.2%	85.2%	2.348	0.1254
Sakassou	Beoumi vs Sakassou	87.0%	85.9%	0.27	0.6033
Nassian	Dabakala vs Nassian	83.4%	85.8%	0.245	0.6204
Beoumi	Pre-IRS vs Post-IRS	88.8%	87.0%	0.006	0.9385
Dabakala	Pre-IRS vs Post-IRS	87.6%	83.4%	2.754	0.097
Nassian	Pre-IRS vs Post-IRS	93.0%	85.8%	1.236	0.2662
Sakassou	Pre-IRS vs Post-IRS	72.1%	85.8%	3.414	0.646

TABLE B6: ENTOMOLOGICAL INOCULATION RATES OF AN. GAMBIAE S.L. COLLECTED BY HLC

Site	Comparison	Mean EIR† (first group)	Mean EIR (second group	Kruskal-Wallis chi-squared	p-value
All	Control vs Sprayed	0.561	0.55	1.017	0.3132
All sprayed	Pre-IRS vs Post-IRS	3.39	0.55	8.311	0.0039*
All control	Pre-IRS vs Post-IRS	0.757	0.561	0.162	0.6875
Sakassou**	Beoumi vs Sakassou	0.34	5.5	6.213	0.0127
Nassian**	Dabakala vs Nassian	1.173	1.02	0.045	0.8312
Sakassou	Beoumi vs Sakassou	0.444	0.95	0.024	0.8774
Nassian	Dabakala vs Nassian	0.677	0.15	4.151	0.0416*
Beoumi	Pre-IRS vs Post-IRS	0.34	0.444	0.491	0.4835
Dabakala	Pre-IRS vs Post-IRS	1.174	0.677	0.056	0.8131
Nassian	Pre-IRS vs Post-IRS	1.02	0.15	3.361	0.0668
Sakassou	Pre-IRS vs Post-IRS	5.497	0.95	7.099	0.0077

* Indicates statistical significance at 0.05%; ** Indicate rows for the 2019 data only; † Mean infectious bites per person per night

FIGURE 14: MEAN IRD OF *AN. GAMBIAE* S.L. BY SITE FROM 2019 TO 2021

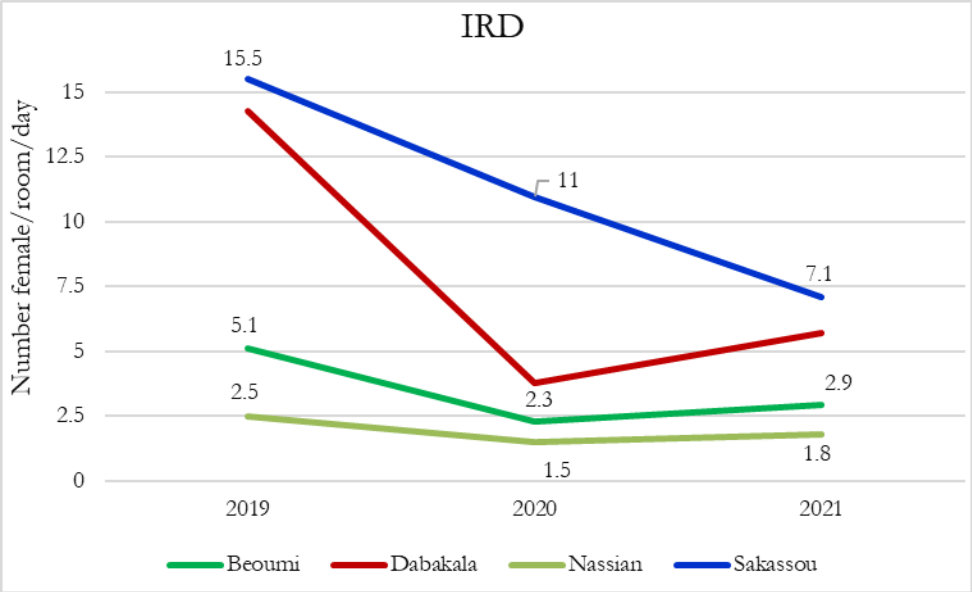


FIGURE 15: MEAN HBRS OF *AN. GAMBIAE* S.L. BY SITE FROM 2019 TO 2021

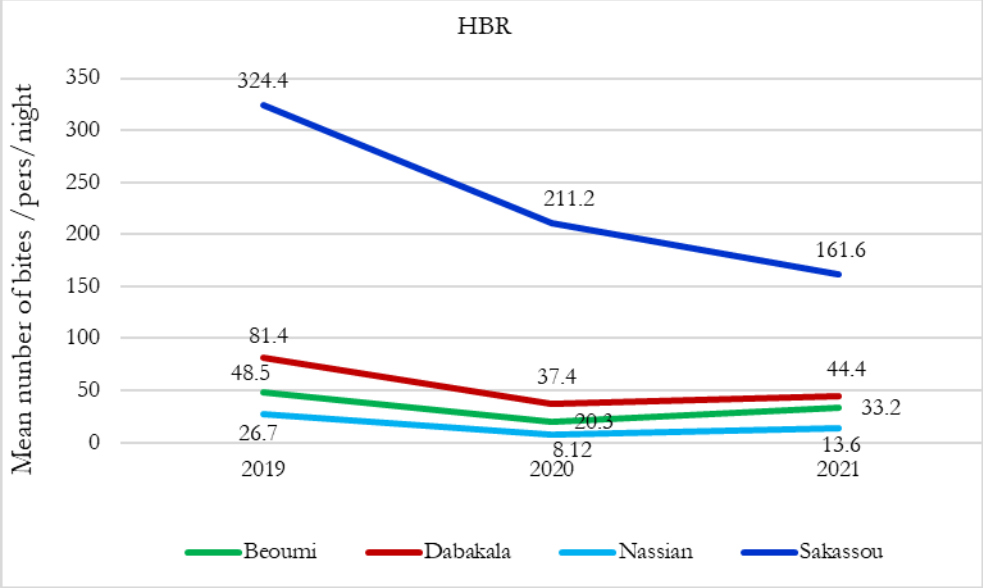
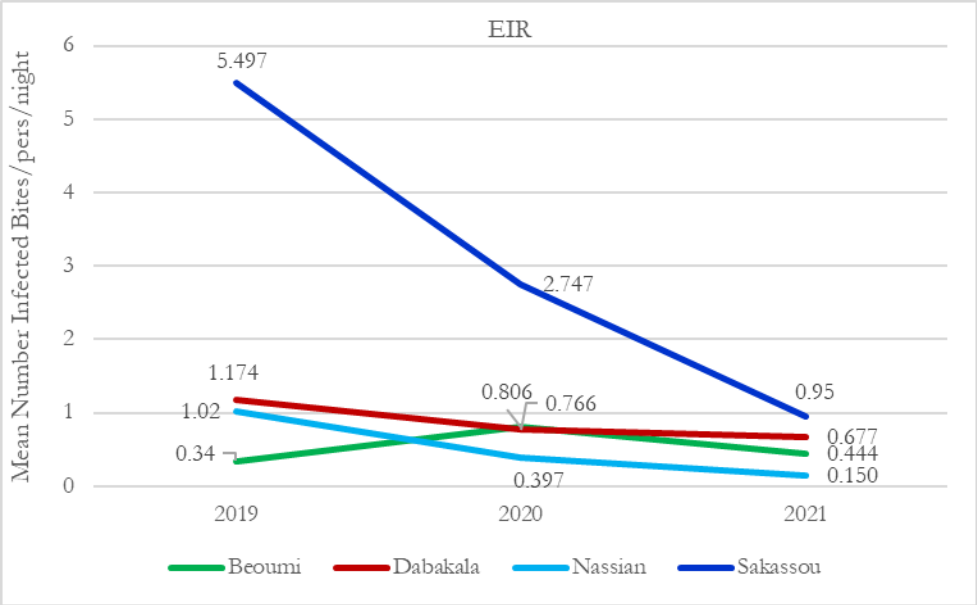


FIGURE 16: MEAN EIR OF *AN. GAMBIAE* S.L. BY SITE FROM 2019 TO 2021



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