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# PMI VECTORLINK CÔTE D'IVOIRE 2022 ANNUAL ENTOMOLOGICAL REPORT

## JANUARY 2022–DECEMBER 2022

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Abt Associates Inc. | 4550 Montgomery Avenue | Suite 800 North | Bethesda, Maryland 20814 | T. 301.347.5000 | F. 301.913.9061 | www.abtassociates.com

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# ACRONYMS

| | |
|---|---|
| ***Ace-1*** | Acetylcholinesterase |
| **b/p/h** | Bites per Person per Hour |
| **b/p/n** | Bites per Person per Night |
| **CDC** | Centers for Disease Control and Prevention |
| **cDNA** | Complementary DNA |
| **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 |
| ***kdr*** | knockdown resistance |
| **NMCP** | National Malaria Control Program |
| **PBO** | Piperonyl Butoxide |
| **PCR** | Polymerase Chain Reaction |
| ***Pf*** | *Plasmodium falciparum* |
| **PMI** | U.S. President's Malaria Initiative |
| **PSC** | Pyrethrum Spray Catch |
| **qPCR** | Quantitative Polymerase Chain Reaction |
| **SOP** | Standard Operating Procedure |
| **WHO** | World Health Organization |

# EXECUTIVE SUMMARY

In May-June 2022, the U.S. President's Malaria Initiative VectorLink Côte d'Ivoire Project conducted its third consecutive indoor residual spraying (IRS) campaign in the districts of Nassian and Sakassou, switching clothianidin-based insecticide formulations between districts (to SumiShield in Sakassou and Fludora Fusion in Nassian) from the two previous IRS campaigns. The project assessed the spray quality and residual efficacy of IRS insecticides in sprayed districts.

To assess trends in entomological indicators, the VectorLink project conducted longitudinal vector surveillance in the two IRS districts and two nearby unsprayed districts (Beoumi and Dabakala). The project also monitored insecticide resistance in 18 sites across the country, including the four sites listed above..

Adult mosquito collections were conducted using human landing catches, pyrethrum spray catches, and Centers for Disease Control and Prevention (CDC) light traps. The entomological parameters assessed included vector composition, seasonality, distribution, biting and resting density , parity, sporozoite infection, and entomological inoculation rate, which enabled comparison of trends observed since the implementation of IRS began.

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, and pirimiphos-methyl, as well as resistance intensity and PBO synergism as needed. WHO bottle assays were used to test vector susceptibility to chlorfenapyr (100 µg/bottle) and clothianidin (4 µg/bottle).

Spray quality was assessed within a week of spraying in three villages per district, which were subsequently monitored monthly to determine residual efficacy using wall cone bioassays.

*An. gambiae* s.l. was the predominant malaria vector, representing 93.2% of the total *Anopheles* collected. *An. gambiae* s.l. mean indoor resting densities were highest in Sakassou (mean: 4.9 females per room per day [f/r/d]), followed by Dabakala (3.7 f/r/d), Beoumi (1.3 f/r/d), and Nassian (0.7 f/r/d). The overall *An. gambiae* s.l. mean indoor resting densities were similar in IRS and unsprayed sites.

Overall, *An. gambiae* s.l. biting was highest between 0.00 a.m. and 4:00 a.m., at all sites, both indoors and outdoors. The mean human biting rate was highest in Sakassou (123 bites per person per night [b/p/n]), followed by Dabakala (35.2 b/p/n), Beoumi (16.1 b/p/n), and Nassian (9.8 b/p/n).

The mean entomological inoculation rate was highest in Dabakala (0.764 infective bites per person per night [ib/p/n]), followed by Sakassou (0.603 ib/p/n), Beoumi (0.294 ib/p/n), and Nassian (0.190 ib/p/n). Both *An. gambiae* s.s. and *An. coluzzii* live in sympatry in Beoumi and Dabakala, while *An. gambiae* s.s. and constituted the main species in Nassian (99% *An. gambiae s.s.* and 1% *An. coluzzii*) and *An. coluzzii* in Sakassou (100%)y. Furthermore, both species were equally plasmodium sporozoite infected. Overall, there was a decrease in vector density, parity, and transmission in both IRS districts.

*An. 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 Divo, Bongouanou, and Seguela, where resistance intensity to deltamethrin and permethrin was moderate (mortality >98% at 10× the diagnostic dose). Pre-exposure to PBO partially restored susceptibility to pyrethroids in 11 of the 18 sites and fully restored susceptibility to deltamethrin in two sites. Susceptibility to pirimiphos-methyl was observed in 12 sites, though low-intensity resistance was observed in five sites, and one site was unable to conduct intensity tests due to a limited number of larvae. *An. gambiae* s.l. was susceptible to chlorfenapyr at 100 µg/bottle in six sites. Susceptibility to clothianidin 4 µg/bottle was recorded in only one site out of the 17 tested using WHO bottle bioassay methods.
The knockdown resistance (*kdr*)-West mutation was present in all sites, and the *kdr*-East mutation was present in 10 sites. This is consistent with the high pyrethroids resistance often observed in Côte d'Ivoire. The acetylcholinesterase

(*Ace-1*) mutation observed in all sites was also consistent with phenotypic resistance to pirimiphos-methyl in 5 of the sites.

One hundred percent (100%) mortality of *An. gambiae* Kisumu exposed to walls sprayed with Fludora Fusion and SumiShield was recorded within a week of spraying in both IRS districts, confirming the good quality of the spraying. The insecticide residual efficacy assessment conducted monthly from June 2022 to February 2023 showed that both insecticides were efficacious against the wild population of *An. gambiae* s.l. from Sakassou for at least ten months post-spraying.

Overall, there was a decrease in vector indoor resting density and entomological inoculation rate after implementing IRS compared to before IRS. Insecticide resistance monitoring results show improvement in mortality using the PBO synergist and new molecules such as chlorfenapyr. These findings suggest that IRS is an appropriate intervention for malaria vector control in Cote d'Ivoire and may also help guide the NMCP's choice of insecticide-treated nets and inform on strategic stratification of the net distribution across the country.

# I. INTRODUCTION

Malaria is a leading cause of morbidity and mortality in Côte d'Ivoire. It accounts for about 33% of outpatient visits in health facilities, with an incidence of 230.94 per 1000 cases in the general population and 493.65 per 1,000 among children under 5 years of age, according to the 2020 National Malaria Control Program (NMCP) report (MSHPCMU 2021). 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 distributions. Before 2021, only pyrethroid-based ITNs were distributed across the country as the sole malaria vector control tool. The National Malaria Strategic Plan 2016–2020 initiated and prioritized indoor residual spraying (IRS) as a complementary vector control method to reduce malaria morbidity and mortality.

To support the NMCP's malaria control efforts, the U.S. President's Malaria Initiative (PMI) VectorLink Project conducted IRS from May 16 to June 20, 2022, in Nassian and Sakassou targeting 62,551 structures using clothianidin-based insecticides (Fludora Fusion and SumiShield). The project sprayed a total of 70,392 of the 71,474 structures found by spray operators in targeted districts, resulting in a coverage rate of 98.5%.

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 2022, VectorLink Côte d'Ivoire, subcontracted with the *Centre Suisse de Recherches Scientifiques* which collaborated with three other local entomological research institutes (*Institut Pierre Richet* , *Centre d'Entomologie Medicale et Veterinaire* , and *Institut National d'Hygiene Publique*), to conduct longitudinal entomological surveillance in the four sites selected by the NMCP and generated 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 including 14 newly selected sites to extend the coverage of insecticide susceptibility data over the country. The project assessed the quality of spray during the IRS campaign and monitored the residual efficacy of the insecticides after IRS. These data will continue to support the NMCP and malaria vector control stakeholders in determining the appropriate timing and insecticides for IRS and inform the selection of ITNs for distribution campaigns.

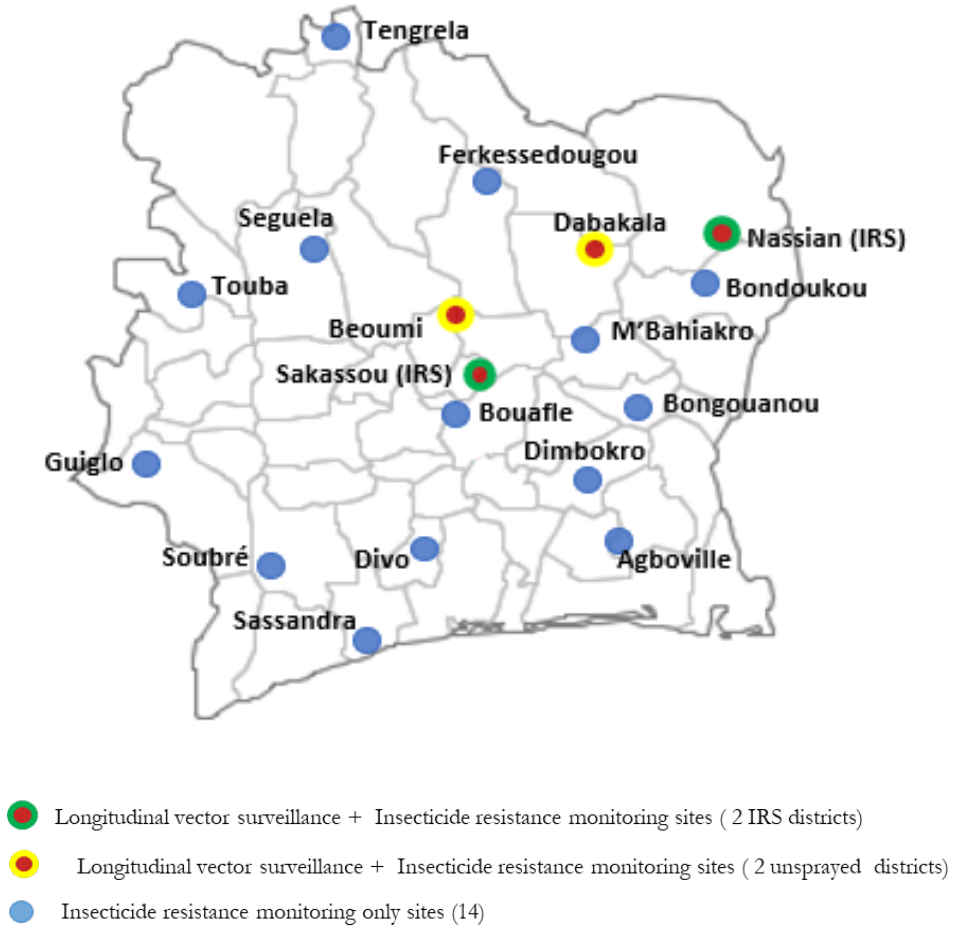
# 2. METHODOLOGY

From January through December 2022, 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 (including monthly vector feeding and resting surveillance and annual insecticide resistance monitoring in the two IRS sites (Nassian and Sakassou) and the two unsprayed sites (Beoumi and Dabakala), and insecticide resistance monitoring only in 14 additional sites: Agboville, Bouafle, Bondoukou, Bongouanou, Divo, Dimbokro, Ferkessedougou, Guiglo, M'Bahiakro, Sassandra, Seguela, Soubre, Tingrela, and Touba (Figure 1). During the countrywide 2021 ITN distribution campaign, all four comprehensive vector monitoring sites were provided with deltamethrin-only treated ITNs, allowing comparison of data collected in IRS sites versus unsprayed sites. IRS quality assessment and residual efficacy monitoring was done at the two sprayed sites.

**FIGURE 1: MAP OF CÔTE D'IVOIRE SHOWING THE 2022 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 5 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 monthly from January through December 2022. Table 1 summarizes the collection times, frequency, and sampling methods. All entomological data were collected following the PMI standard operating procedures (SOPs).[1]

**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 indoor | 6:00 am to 8:00 a.m. | Two days per site per month | 30 houses per site (15 urban and 15 rural) random selection each month |
| CDC light trap indoor | 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 (SOPs)[1]. 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 (SOPs)[1].

All mosquitoes collected through each method were morphologically identified to genus. *Anopheles* mosquitoes were identified to species or species complex using microscope and 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. collected using HLC method 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).

[1] https://pmivectorlink.org/resources/tools-and-innovations/

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 bites/person/nights |
| | Peak biting time | Hour of highest HBR |
| | Parity rate | Percentage of parous mosquitoes/total dissected |
| | Exophagic rate | Percentage of mosquitoes biting outside |
| | Endophagic rate | Percentage of mosquitoes biting inside |
| PSC | Indoor resting density | Mean number of mosquitoes/houses |
| | % of fed females | Number of fed mosquitoes/totals 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 in June, 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 WHO bottle assays (SOP 04/01).

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 was also tested using 5 and 10 times the diagnostic concentration. All impregnated papers were received from the Universiti Sains Malaysia.

Synergist assays with 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.

WHO bottle bioassays were used to test susceptibility to chlorfenapyr at the doses of 100 µg/bottle with one-hour exposure, and mortality was recorded daily for up to three days (72 hours); and to test clothianidin 4 µg/bottle active ingredient following one-hour exposure and 24-hour mortality. For all tests, resistance status 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 suspected 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

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 either *An. gambiae s.s, An. coluzzii,* or hybrids of the two species, following the protocol described by Santolamazza et al. (2008).

About 50 *An. gambiae* s.l. mosquitoes were randomly selected from each of the 18 sites among the dead and surviving mosquitoes from the WHO susceptibility tests, and further analyzed to assess molecular markers of insecticide resistance. The presence of *kdr*-West, *kdr*-East, and *Ace-1* mutations was revealed using the Taqman protocol described by Bass et al. (2007).

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* (*Pf*) circumsporozoite infection as in Burkot et al. (1994). All *Pf* positive specimen were boiled and retested for confirmation and to detect false positives according to Durnez et al. (2011).

For insecticide resistance monitoring sites, 30 adult female mosquitoes were randomly selected among the surviving of pyrethroid susceptibility tests 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 RNAlater 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.

The gene expression patterns of key detoxification genes in *An. coluzzii* and *An. gambiae* populations were assessed in each site. Four cytochrome P450s genes (CYP6P3, CYP6P5, CYP6P4, and CYP6M2) were assessed using quantitative real-time-PCR. RNA was extracted from three biological replicates (pool of 10 specimen) resistant and control unexposed mosquitoes, and *An. gambiae* Kisumu were used to assess the relative fold change in the level of expression of each gene. 1 µg total RNA from each of the three biological replicates from field resistant, unexposed control, and susceptible mosquitoes were used as the template for complementary DNA (cDNA) synthesis using Superscript III (Invitrogen, US) with oligo-dT20 and RNase. A serial dilution of cDNA was used to establish standard curves for each gene to assess PCR efficiency and quantitative differences between samples. The qPCR amplification was carried out in a Biorad CFX qPCR system (BioRad Technologies) using Brilliant III Ultra-Fast SYBR Green qPCR Master Mix and following the protocol of Kwiatkowska *et al*, 2013. The relative 6 expression and fold change of each target gene was calculated according to the 2-ΔΔCT method, incorporating PCR efficiency after normalization with the housekeeping genes RSP7 (ribosomal protein S7, AGAP010592) and actin (Edi et al. 2014).

## 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 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).

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 and test conducted according to SOP x.

**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 three sprayed muds in two villages) and one control mud and cement<br>Nine houses in Sakassou (three sprayed cement in each of the three villages) and one 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 three sprayed muds in two villages) and one control mud and cement<br>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 muds in three villages) and one control mud and cement<br>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 2022 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 for entomological parameters from each site (IRS or unsprayed). We considered five main indicators for *An. gambiae* s.l. between sites: 1) indoor resting density (IRD), 2) the human biting rate (HBR), 3) the proportion of gravid and fed vectors, 4) the parity rate, 5) the sporozoite rate, and 6) the entomological inoculation rate (EIR). Separate analyses were done for *An. funestus* s.l. in Nassian. P-values of $<0.05$ for the different variables tested indicated statistically significant difference.

For enzyme activity comparison, the Graph Pad Prism 5 software was used to compare enzyme fold changes and plotting of the graphs.

# 3. RESULTS

## 3.1 VECTOR BIONOMICS MONITORING

### 3.1.1 SPECIES COMPOSITION

#### *OVERALL SPECIES COMPOSITION*

A total of 60,448 adult mosquitoes were collected in the four sentinel sites from January to December 2022, using all three collection methods: HLC, PSC, and CDC light trap. *Anopheles* mosquitoes represent 70.1% (n=42,363) and culicines 29.9% (n=18,085) of all mosquitoes collected (Table 4). Four *Anopheles* species were identified across the four sites. *An. gambiae* s.l. (n=39,485) was the predominant malaria vector species, representing 93.2% of the total *Anopheles* mosquitoes collected across all sites and methods. *An. funestus* s.l. (1.8%; n=768) and *An. nili* (1.7%; n=706) were the second most-abundant of the known vectors in Côte d'Ivoire. *An. pharoensis* (3.3%; n=1,404) represented the other *Anopheles* species collected and not detected as malaria vector in the sites surveyed in Côte d'Ivoire. Sakassou recorded more than half of the total *Anopheles* mosquitoes collected (50.4%, n=30,452).

The HLC method yielded 37,983 *Anopheles* mosquitoes representing 89.7% of the total of 42,363 *Anopheles* collected of all methods. *An. gambiae* s.l. was the predominant vector species (93.0%; n=35,335) followed by *An. nili* (1.9%; n=706) and *An. funestus* s.l. (1.5%; n=584). The other *Anopheles* species was *An. pharoensis* (3.6%; n=1,358).

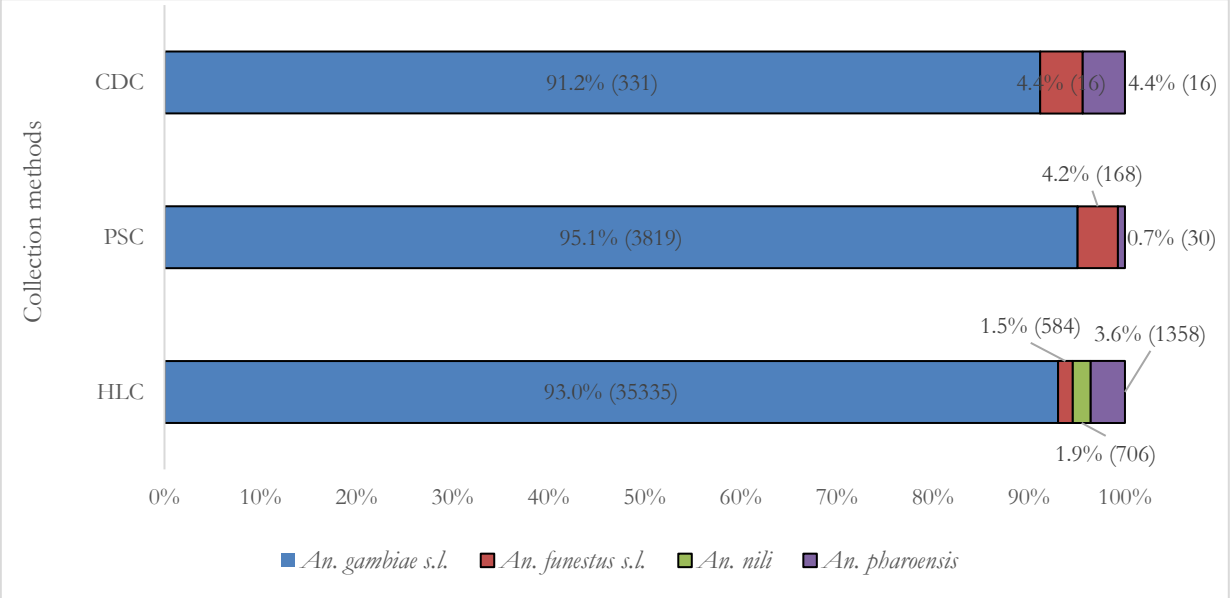
Using PSCs 4,017 (9.5%) *Anopheles* mosquitoes were collected. *An. gambiae* s.l. (95.1%; n=3,819) still represented the main vector collected followed by *An. funestus* s.l. (4.2%; n=168) and *An. pharoensis* (0.7%; n=30).

CDC light traps collected fewer *Anopheles* mosquitoes (0.86%; n=363). *An. gambiae* s.l. still represents the predominant species (91.2%; n=331) followed by *An. funestus* s.l. (4.4%; n=16) and *An. pharoensis* (4.4%; n=16). A summary of results is shown in Figure 2, and detailed results are included in the Annex, in Tables A-1–A-3.

**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. | 3,587 | 8,306 | 2,125 | 25,467 | 39,485 | 65.3 | 93.2 |
| *An. funestus* s.l. | 99 | 40 | 563 | 66 | 768 | 1.3 | 1.8 |
| *An. nili* | 0 | 700 | 0 | 6 | 706 | 1.2 | 1.7 |
| *An. pharoensis* | 331 | 160 | 4 | 909 | 1,404 | 2.3 | 3.3 |
| Culicines | 6,079 | 7,584 | 418 | 4,004 | 18,085 | 29.9 | 0.0 |
| **Total** | 10,096 | 16,790 | 3,110 | 30,452 | **60,448** | 100.0 | 100.0 |
| **% per site** | 16.7 | 27.8 | 5.1 | 50.4 | 100 | | |

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




**Note:** The number in the bars represents the percentage and number per species collected.

## SPECIES COMPOSITION BY SITE

A summary of results is shown in Figure 3, and detailed results are included in Annex Tables A-1–A-3.

In Beoumi, a total of 4,017 *Anopheles* mosquitoes were caught over the 12 months using all three collection methods, representing 39.8% of the total mosquitoes collected in Beoumi and 9.5% of the total *Anopheles* mosquitoes collected across all sites. *An. gambiae* s.l. was the most collected malaria vector species (89.3%, n=3,587) followed by *An. funestus* s.l. (2.5%; n=99). *An. pharoensis* (8.2%; n=331) was the only other *Anopheles* species.

Using HLCs, about 80.5% (n=3,455) *Anopheles* mosquitoes were collected in Beoumi, including 89.7% *An. gambiae* s.l. (n=3,098), 1.9% *An. funestus* s.l. (n=66), and 8.4% *An. pharoensis* (n=291). PSCs yielded 508 *Anopheles* mosquitoes overall, including 91.7% *An. gambiae* s.l. (n=466), 3.5% *An. funestus* s.l. (n=18), and 4.7% *An. pharoensis* (n=24). With CDC light traps, 54 *Anopheles* mosquitoes were collected, including 23 *An. gambiae* s.l. (42.6%), 15 *An. funestus* s.l. (27.8%), and 16 *An. pharoensis* (29.6%).

In Dabakala, a total of 9,206 *Anopheles* mosquitoes were collected, which is 54.8% of the total mosquitoes collected in Dabakala and about 15.2% of the total collections. *An. gambiae* s.l. was the predominant malaria vector species collected (90.2%; n=8,306). *An. funestus* s.l. (0.4%, n=40) and *An. nili* (7.6%, n=700) 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 was *An. pharoensis* (1.7%; n=160).

Using HLC, a total 7,649 (56.1%) *Anopheles* mosquitoes were collected. *An. gambiae* s.l. was the major malaria vector representing 88.3% (n=6,753) of the total *Anopheles* collected, followed by *An. funestus* s.l. (0.5%; n=38) and *An. nili* (9.2%; n=700). The other species was *An. pharoensis* (2.1%; n=158). Using PSC, 1,346 *Anopheles* mosquitoes were collected representing 14.6% of the *Anopheles* collected in Dabakala. *An. gambiae* s.l. was the predominant species collected (99.7%; n=1,342) and a few *An. funestus* s.l. and *An. pharoensis* (0.1%; n=2). CDC light trap collections recorded 211 *Anopheles* mosquitoes representing 7.8% of *Anopheles* collected in Dabakala only composed of *An. gambiae* s.l. (100%).

In Nassian, the collections yielded a total of 2,692 *Anopheles* mosquitoes, representing 86.6% of the total *Anopheles* in Nassian and 4.5% of all *Anopheles* mosquitoes recorded at all four sites. *An. gambiae* s.l. was the main vector species collected (78.9%; n=2,125). Nassian was the site that recorded the largest percentage (73.3%) of the total *An. funestus* s.l. caught;

this represented 20.9% (n=563) of the total *Anopheles* collected in Nassian. A few *An. pharoensis* (0.1%; n=4) were collected. Overall, HLC yielded 2,297 *Anopheles* mosquitoes, of which 1,876 (81.7%) were *An. gambiae* s.l., 417 (18.2%) *An. funestus* s.l., and 4 (0.1%) *An. pharoensis*.

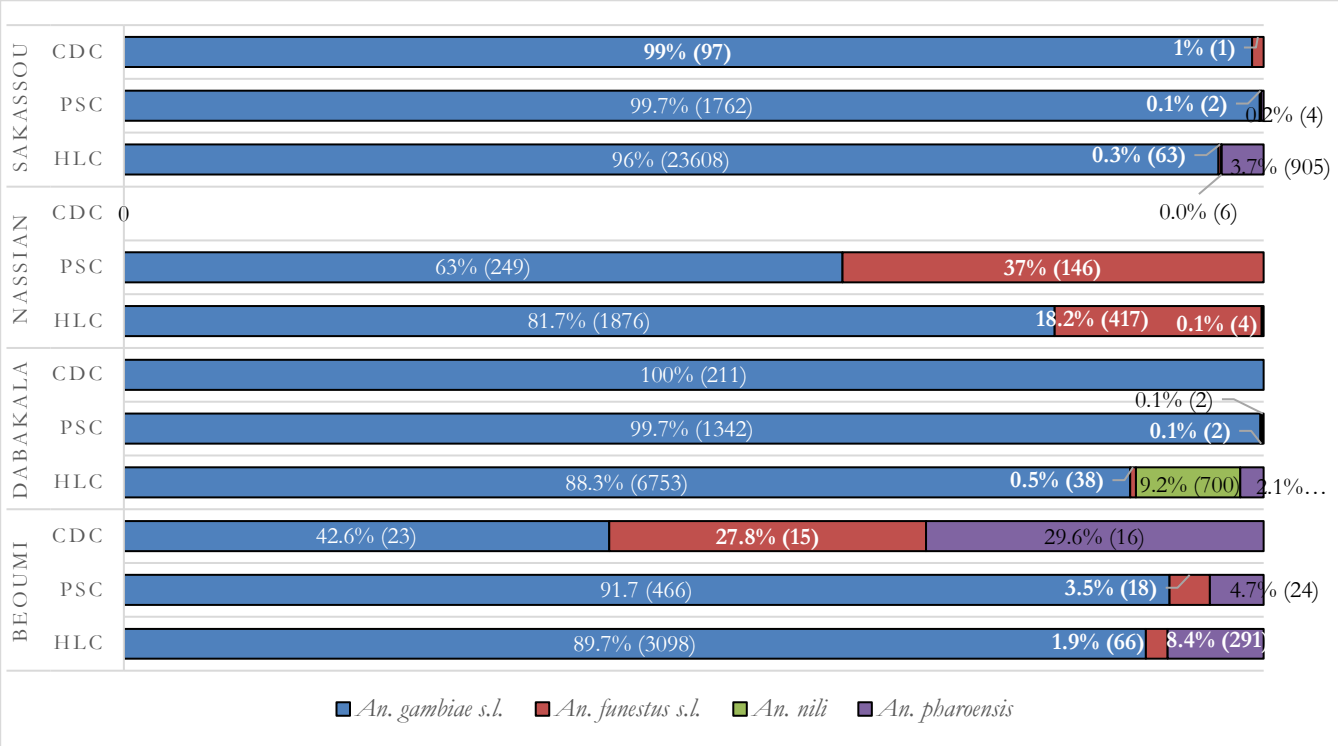
Using PSC, 395 *Anopheles* were caught. *An. gambiae* s.l. again had the highest percentage (63.0%; n=249) followed by *An. funestus* s.l. (37.0%; n=146). The CDC light trap yielded 10 Culicines. No *Anopheles* was collected using CDC light traps.

Sakassou was the most productive of the four sites where longitudinal vector surveillance was conducted. A total of 26,448 *Anopheles* mosquitoes were collected over the 12 months using all three collection methods, representing 86.9% of the total mosquitoes collected in Sakassou and 43.8% of the overall collection. *An. gambiae* s.l. was the main vector species collected (96.3%; n=25,467) with a few An. *funestus* s.l. (0.2%; n=66) and *An. nili* (2.3%; n=6). *An. pharoensis* was the other *Anopheles* species collected (3.4%; n=909).

HLC remained the highest-yield method for adult *Anopheles* mosquito collections. In Sakassou, 24,582 *Anopheles* were collected through HLC and comprised predominantly *An. gambiae* s.l. (96.0%; n=23,608) followed by *An. funestus* s.l. (0.3%; n=63), and *An. nili* (0.0%; n=6). The other species found were *An. pharoensis* (3.7%; n=905).

A total of 1,768 *Anopheles* mosquitoes were collected using PSC. *An. gambiae* s.l. represented 99.7% (n=1,762) while a few *An. funestus* s.l. (0.1%; n=2) and *An. pharoensis* (2.3%; n=4) were collected. CDC light traps recorded the lowest number of *Anopheles* mosquitoes collected (98) in Sakassou. *An. gambiae* s.l. was predominant (99.0%; n=97); and only one *An. funestus* s.l. (1.0%; n=1) was found.

**FIGURE 3: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED BY METHOD, BY SITE, FROM JANUARY TO DECEMBER 2022**




**Note:** The number in the bars represents the percentage and number per species collected

## 3.2 VECTOR DENSITY AND BEHAVIOR

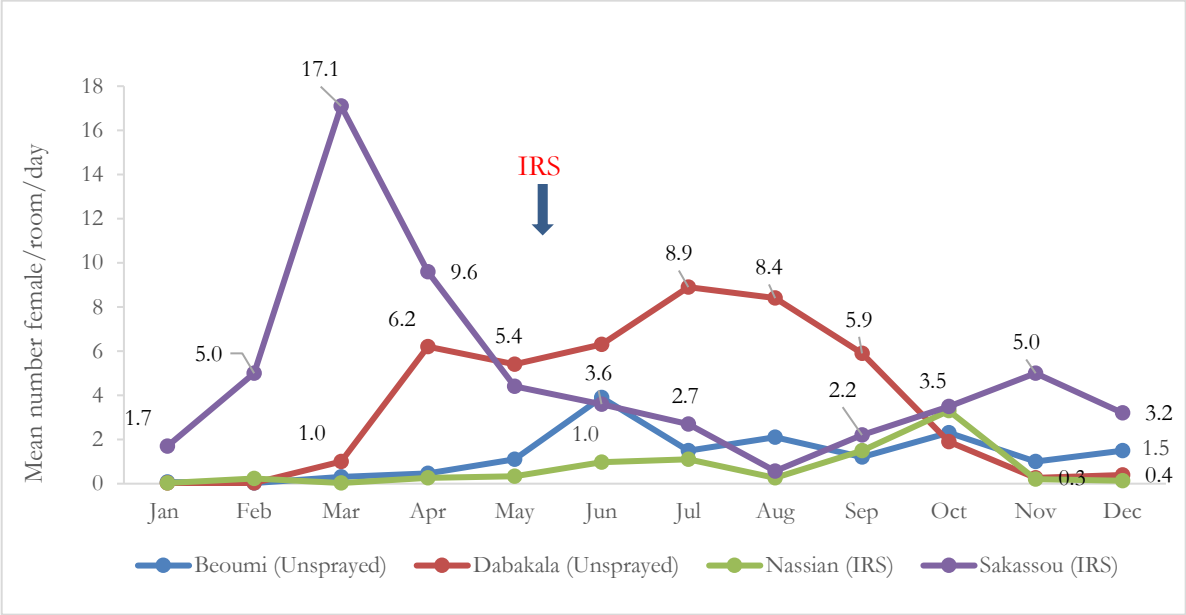
### 3.2.1 IRD OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY PSCS

#### *AN. GAMBIAE S.L.*

*An. gambiae* s.l. mean monthly IRDs that were estimated using PSCs recorded different peak period of mosquito density in the four sites over the year. Mean monthly IRDs were between 0.6 and 17.1 females/room (f/r) in Sakassou, between 0 and 8.9 f/r in Dabakala, between 0 and 3.6 f/r in Beoumi, and between 0 and 3.3 f/r in Nassian (Figure 4). There was significant difference in IRD between each of the four sites (p=0.0012). In Sakassou, the peak IRD was recorded in March (17.1 f/r) . In Dabakala, peak IRD was recorded in July (8.9 f/r). In Beoumi, the IRD peaked in June (3.9 f/r), and in Nassian, the peak occurred in October (3.3 f/r).

The overall mean IRDs of Sakassou and Nassian (IRS sites) over the 12 months were 4.9 and 0.7 f/r/d, respectively, while in Beoumi and Dabakala (unsprayed sites) the mean IRD was 1.3 and 3.7 f/r/d, respectively. (Figure 4, Annex Table A-4).

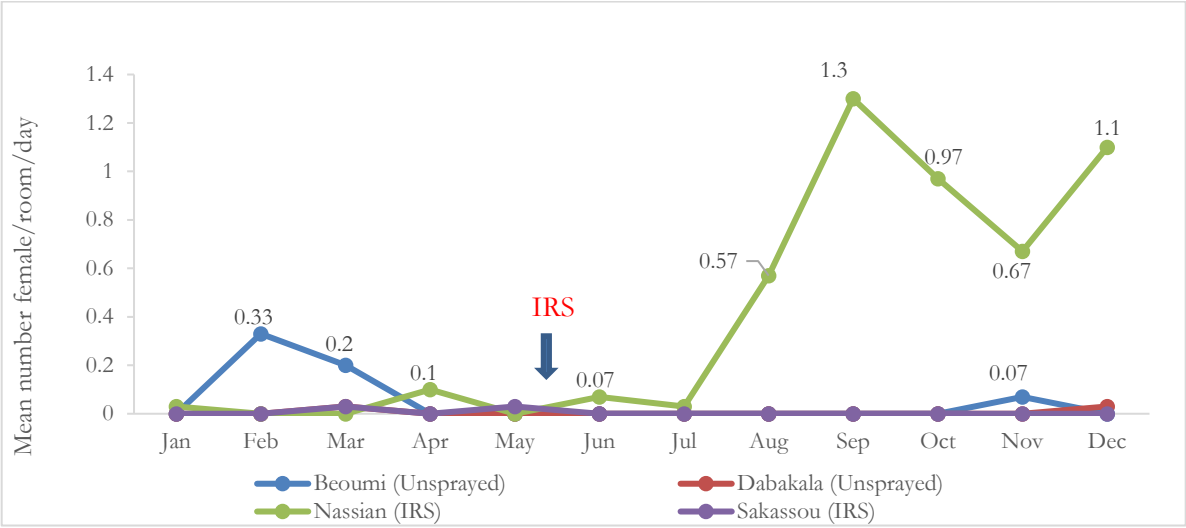
**FIGURE 4: MEAN IRD OF *AN. GAMBIAE* S.L. BY MONTH IN SPRAYED AND UNSPRAYED SITES BY MONTH**




#### *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 mean number of *An. funestus* s.l. (0.4 f/r), with the highest peak observed in September (1.3 f/r). There was also a sporadic collection of *An. funestus s.l.* in Beoumi in February, March, and November, in Sakassou in March and May, and in Dabakala in May and December (Figure 5, Annex Table A-4).

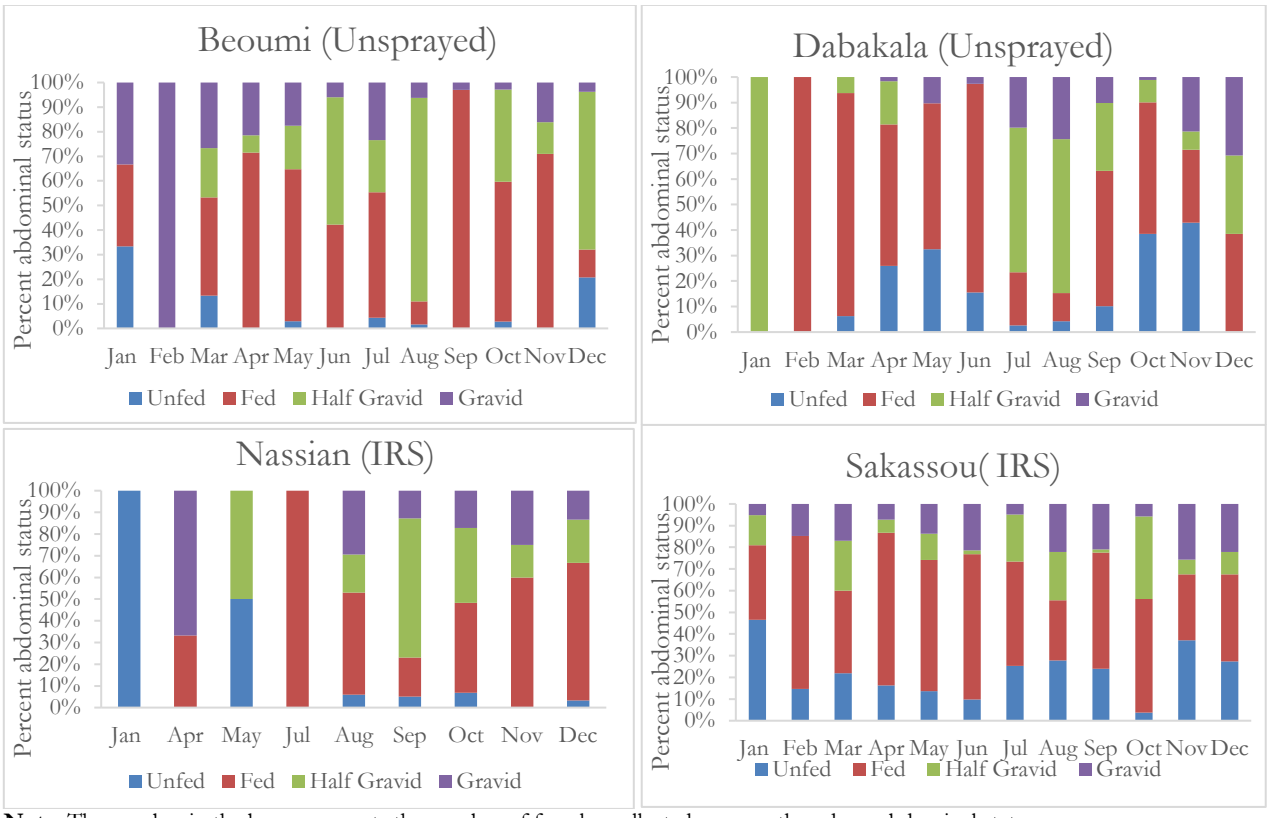
**FIGURE 5: MEAN IRD OF *AN. FUNESTUS* S.L. BY MONTH IN SPRAYED AND UNSPRAYED SITES BY MONTH**




### 3.2.2 ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. COLLECTED BY PSC

Figures 6 shows the abdominal status of *An. gambiae* s.l. collected indoors by PSCs, by site. The percentages of unfed, fed, half gravid, and gravid was determined for 2,423 *An. gambiae* s.l. (2,003 from sprayed sites and 420 from unsprayed sites) due to higher density of mosquitoes collected in Sakassou compared to the other sites. Overall, similar percentages of gravid and blood fed *An. gambiae* s.l. mosquitoes were recorded at all four sites (12.1% of gravid in IRS sites vs 10.3% in the unsprayed sites and 76% of blood fed in IRS sites vs 88.4% in unsprayed sites (*p=0.1518*) (Annex Table A-5).

**FIGURE 6: PERCENTAGE COMPOSITION OF ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. BY SITE AND BY MONTH**



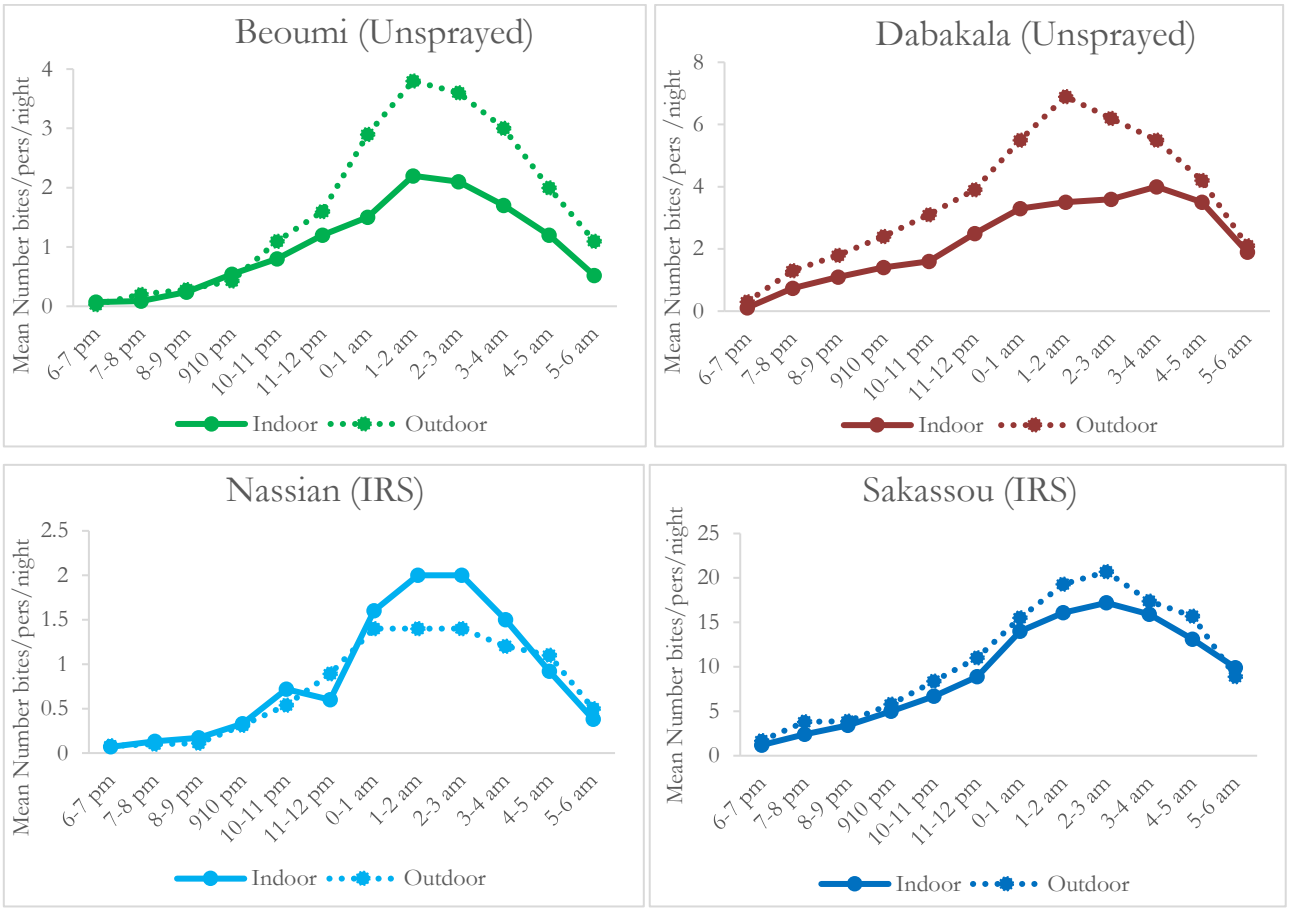

**Note:** The number in the bars represents the number of females collected per month and per abdominal status.

### 3.2.3 BITING TIME OF *AN. GAMBIAE* S.L. COLLECTED BY HLC

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 (indoors and outdoors) and Dabakala (outdoors); between 3:00 and 4:00 in Dabakala (indoors); between 1:00 and 3:00 in Nassian (indoors and outdoors); and between 2:00 and 3:00 (indoors and outdoors) in Sakassou. Sakassou yielded the highest *An. gambiae* s.l. hourly biting rates with a peak of 20.7 bite/person/hour (b/p/h) outdoors and 17.2 b/p/h indoors. Nassian recorded its peak hourly biting of 1.4 b/p/h outdoors and 2.0 b/p/h indoors. The peak hourly biting was 3.8 b/p/h outdoors and 2.2 b/p/h indoors in Beoumi, and 6.9 b/p/h outdoors and 4.0 b/p/h indoors in Dabakala (Figure 7, Annex Table A-6).

**FIGURE 7: MEAN INDOOR AND OUTDOOR BITING TIMES OF *AN. GAMBIAE* S.L. COLLECTED BY HLC BY SITE**



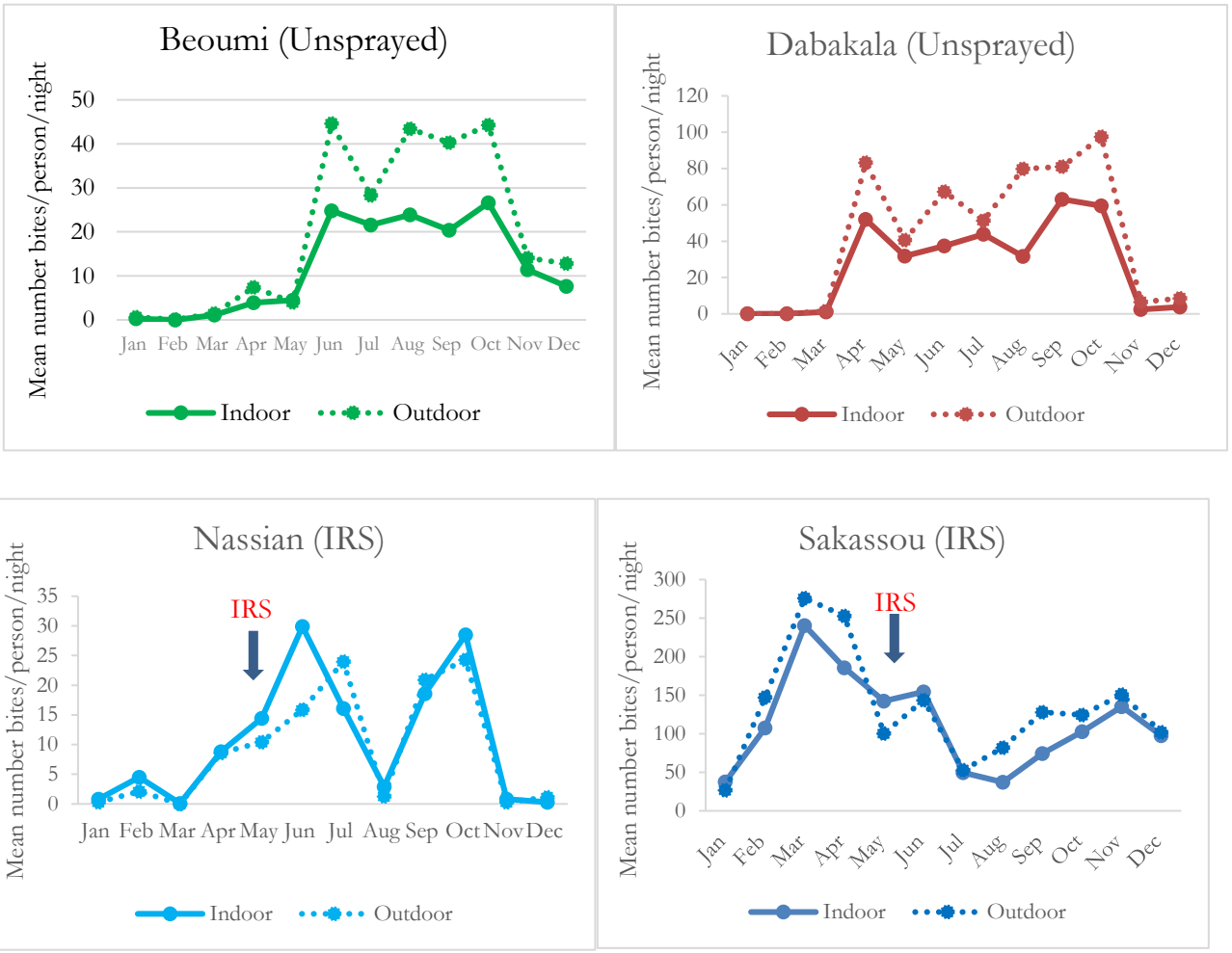

### 3.2.4 HBR, BITING CYCLE, AND BEHAVIOR OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY HLC

#### *AN. GAMBIAE S.L.*

There were different trends in HBRs in the four sites over the course of the year. In Beoumi, three HBR peaks were observed outdoors and indoors in June (44.6 and 24.8 bites per person per night [b/p/n]), August (43.4 and 23.9 b/p/n), and October (44.3 and 26.6 b/p/n). In Nassian, the HBR peaks were in July outdoors (24 b/p/n), in June indoors (29.9 b/p/n), and in October outdoors (24.3 b/p/n) and indoors (28.5 b/p/n). In Dabakala, four HBR peaks were recorded

outdoors in April (83.3 b/p/n), June (67.3 b/p/n), August (79.9 b/p/n), and October (97.4 b/p/n). The indoors HBRs peaked in April (52 b/p/n), July (51.3 b/p/n), and September (63.1 b/p/n). In Sakassou, the HBR peaked in March outdoors (275.9 b/p/n) and indoors (240.4 b/p/n) and in November outdoors (150.8 b/p/n) and indoors (135.1 b/p/n). The annual mean HBR was highest in Sakassou (123.0 b/p/n), followed by Dabakala (35.2 b/p/n) and Beoumi (16.1 b/p/n). Nassian recorded the lowest HBR with 9.8 b/p/n recorded over the year (p=0.0001). *An. gambiae* s.l. tended to be more exophilic in Sakassou (53.7%) and endophilic in Nassian (53.5%) (Figure 8, Annex Table A-7).

**FIGURE 8: INDOOR AND OUTDOOR HBRS OF *AN. GAMBIAE* S.L. BY SITE AND BY MONTH**

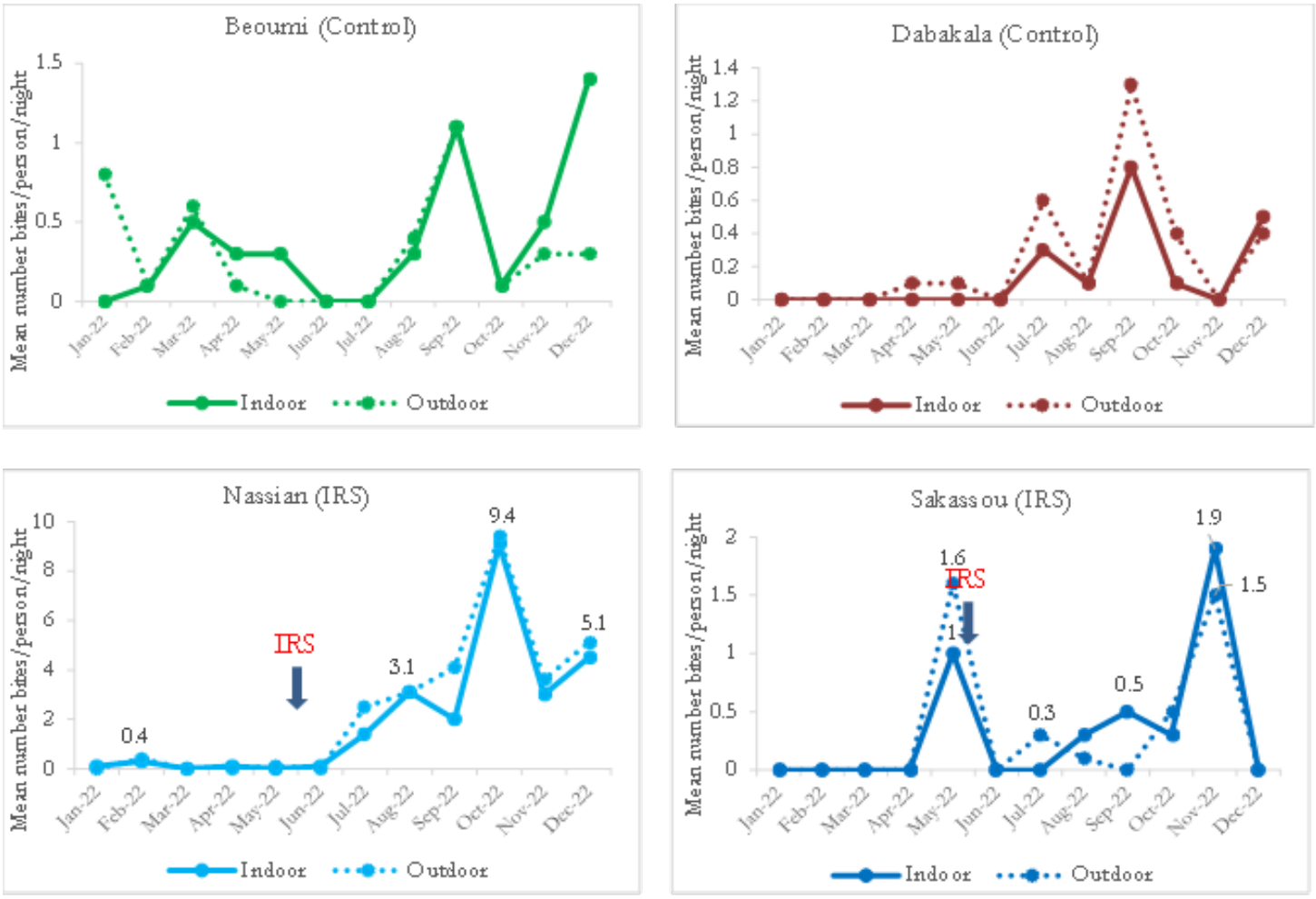



## *AN. FUNESTUS S.L.*

The *An. funestus* s.l. HBRs in Beoumi peaked in September outdoors and indoors (1.1 b/p/n) and in December indoors (1.4 b/p/n) (Figure 9, Annex Table A-7). Dabakala also had two peaks, one in July outdoors (0.6 b/p/n) and indoors (0.3 b/p/n) and the other in September outdoors (1.3 b/p/n) and indoors (0.8 b/p/n).

The highest HBRs of *An. funestus* s.l. were observed in Nassian with an overall (indoor and outdoor) rate ranging from 0 b/p/n in March to 9.4 b/p/n in October. A slight increase in indoor HBRs was noted in December 2022 (4.8 b/p/n). Sakassou recorded two HBR peaks, in May outdoors (1.6 b/p/n) and indoors (1.0 b/p/n) and in November outdoors (1.5 b/p/n) and indoors (1.9 b/p/n). The annual mean HBR was highest in Nassian (2.2 b/p/n), followed by Beoumi (0.4 b/p/n) and Sakassou (0.3 b/p/n). Dabakala recorded the lowest HBR with 0.2 b/p/n recorded over the year. However, there is no significant difference in the *An. funestus* s.l. HBR between sites (*p=0.089*). In all the sites, the density of *An. funestus* s.l. increased during the end of the rainy season from October to December.

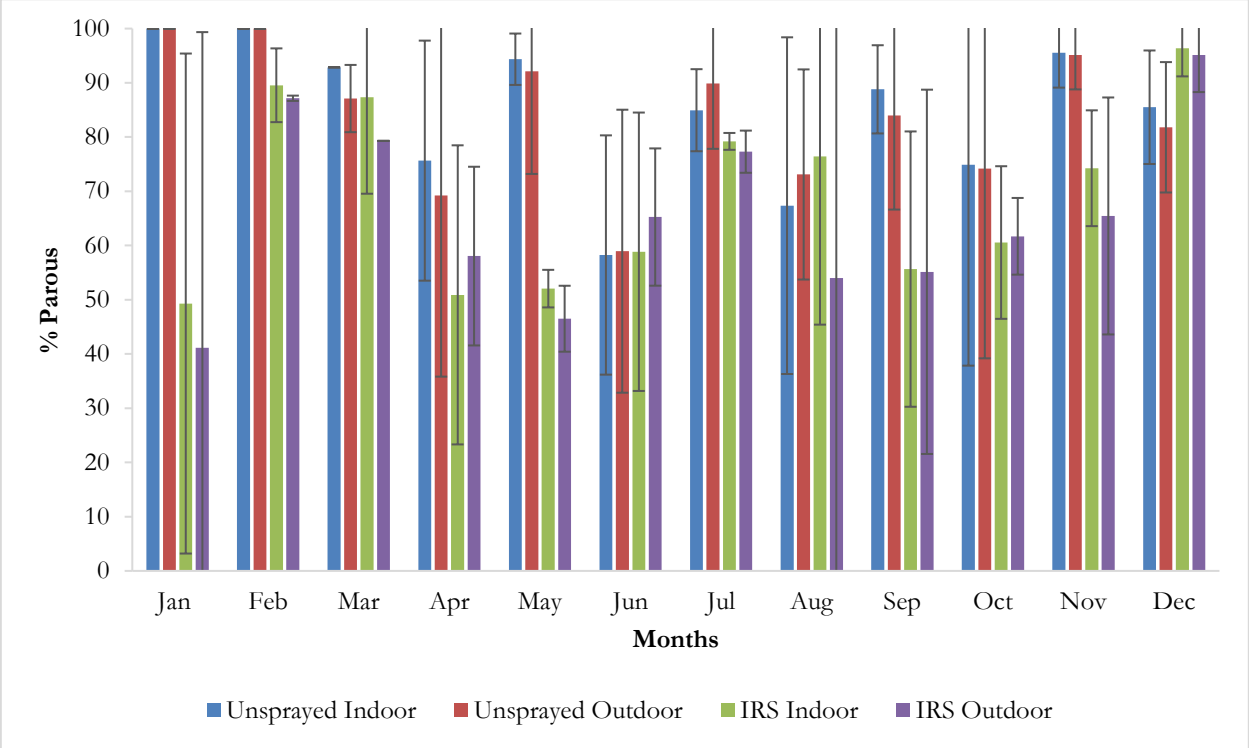
**FIGURE 9: INDOOR AND OUTDOOR HBRS OF *AN. FUNESTUS* S.L. BY SITE AND BY MONTH**




## 3.3 PARITY RATE

A total of 7,356 *An. gambiae* s.l. collected using HLCs (1,614 in Beoumi, 1,985 in Dabakala, 1,255 in Nassian, and 2,502 in Sakassou) were dissected, and their ovaries examined to determine parity rates across the four sites. Of these, 1,122 (69.5%) were parous in Beoumi, 1,720 (86.6%) in Dabakala, 643 (51.2%) in Nassian, and 1,953 (78.1%) in Sakassou. Overall, the parity rate was similar for mosquitoes collected indoors and outdoors in both IRS and unsprayed sites, though the mean parity rates for *An. gambiae* s.l. in the sprayed sites (66.7%) was lower than that of the unsprayed sites 82.9% (*p=0.0004*) showing that younger vector population was recorded in IRS sites than unsprayed sites (Figure 10, Annex Tables A-8 and A-9).

**FIGURE 10: MEAN PARITY RATE OF *AN. GAMBIAE* S.L. COLLECTED USING HLC IN SPRAYED AND UNSPRAYED SITES BY MONTH**




Error bars represent the standard deviations.

## 3.4 *PLASMODIUM FALCIPARUM* SPOROZOITE INFECTION AND 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. Dabakala had the highest *Pf* sporozoite infection rate among *An. gambiae* s.l. (0.046), followed by Nassian (0.033) and Beoumi (0.014). The lowest *Pf* sporozoite rate (0.006) was recorded in Sakassou.

The highest *An. gambiae* s.l. EIRs (infected bites per person per night [ib/p/n]) were recorded between June and November at all four districts. Dabakala recorded the highest EIR (0.764 ib/p/n) of *An. gambiae* s.l., followed by Sakassou (0.603 ib/p/n) and Nassian (0.294 ib/p/n). Beoumi recorded the lowest EIR, 0.190 ib/p/n, but the difference between the four sites was not statistically significant (p=0.4424) (Figure 11, Annex Table A-10).

In Nassian, *An. funestus* s.l. was analyzed for infection throughout the year, with an overall EIR of 0.218 ib/p/n. (Table 5). The highest number of infected bites per person per night was observed in December (0.662 ib/p/n) (Figure 12, Annex Table A-10).

**TABLE 5: VECTOR SPOROZOITE INFECTION RATE IN AND EIR OF MALARIA VECTORS COLLECTED USING HLC IN SPRAYED AND UNSPRAYED SITES**

| | *An. gambiae* s.l. | | | | | | *An. funestus* sl | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TC | TA | P | SR | HBR | EIR | TC | TA | P | SR | HBR | EIR |
| Beoumi (Unsprayed) | 3,587 | 876 | 14 | 0.014 | 16.2 | 0.190 | 99 | 17 | 0 | 0 | 0.4 | 0 |
| Dabakala (Unsprayed) | 8,306 | 910 | 23 | 0.046 | 35.2 | 0.864 | 40 | 1 | 0 | 0 | 0.2 | 0 |
| Nassian (IRS) | 2,125 | 724 | 25 | 0.033 | 9.8 | 0.294 | 563 | 156 | 8 | 0.075 | 2.2 | 0.218 |
| Sakassou (IRS) | 25,467 | 1,195 | 7 | 0.006 | 123 | 0.603 | 66 | 0 | - | - | 0.3 | - |

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

**FIGURE 11: MONTHLY EIR OF *AN. GAMBIAE* S.L. AT SPRAYED AND UNSPRAYED SITES**

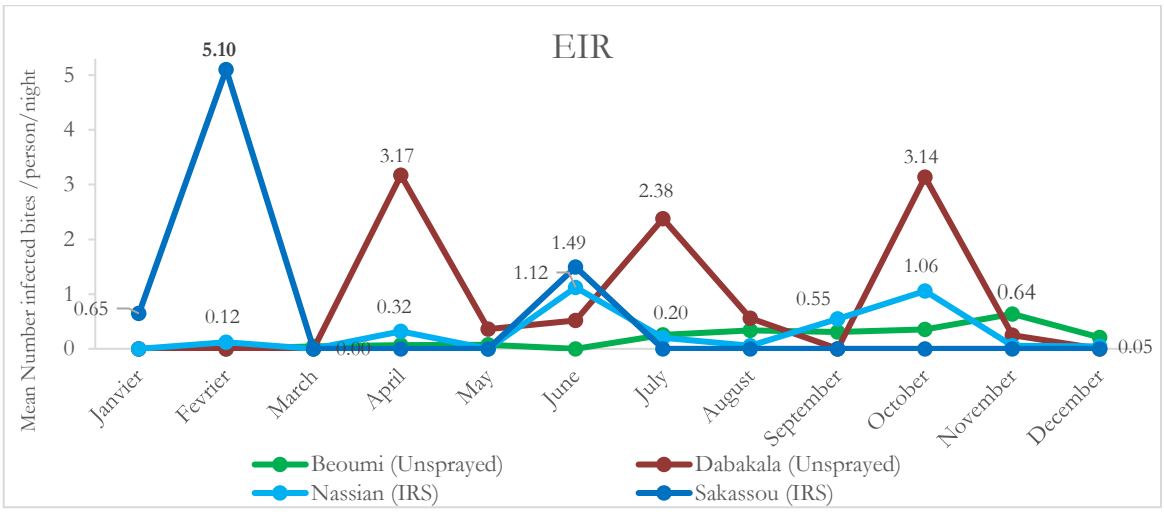
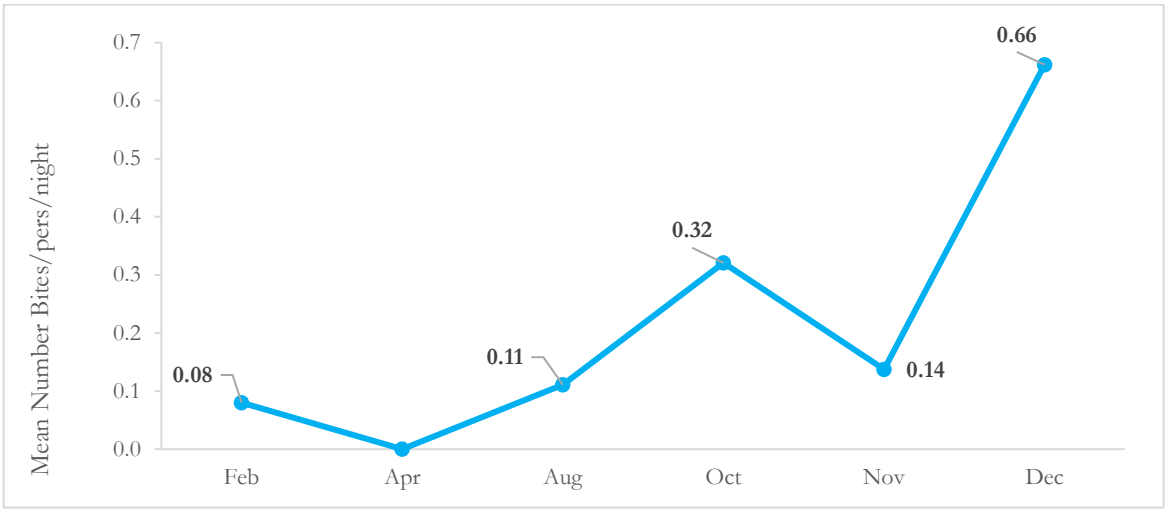



**FIGURE 12: MONTHLY EIR OF *AN. FUNESTUS* S.L. IN NASSIAN**




The *An. gambiae* s.l. *Pf* infection rate was furthermore described for each species of the complex. All infected *An. gambiae* complex species in Nassian and Sakassou were *An. gambiae* s.s.and *An. coluzzii* respectively both species composed the population of each site. However, in Beoumi and Dabakala, where both species (*An. gambiae* s.s. and *An. coluzzii)* live in sympatry, infection was slightly equal among the species in both sites. For *An. gambiae* s.s., the sporozoite rate was 6.0% and 10.3% in Beoumi and Dabakala respectively while that of *An. coluzzii* was 3.8% and 8.9% in both districts respectively (Table 6)

**TABLE 6: *AN. GAMBIAE* S.S. AND *AN. COLUZZII* SPOROZOITE INFECTION RATE ACROSS SITES**

| | Beoumi | | Dabakala | | Nassian | | Sakassou | |
|---|---|---|---|---|---|---|---|---|
| | *An. gambiae* s.s. | *An. coluzzii* | *An. gambiae* s.s. | *An. coluzzii* | *An. gambiae* s.s. | *An. coluzzii* | *An. gambiae* s.s. | *An. coluzzii* |

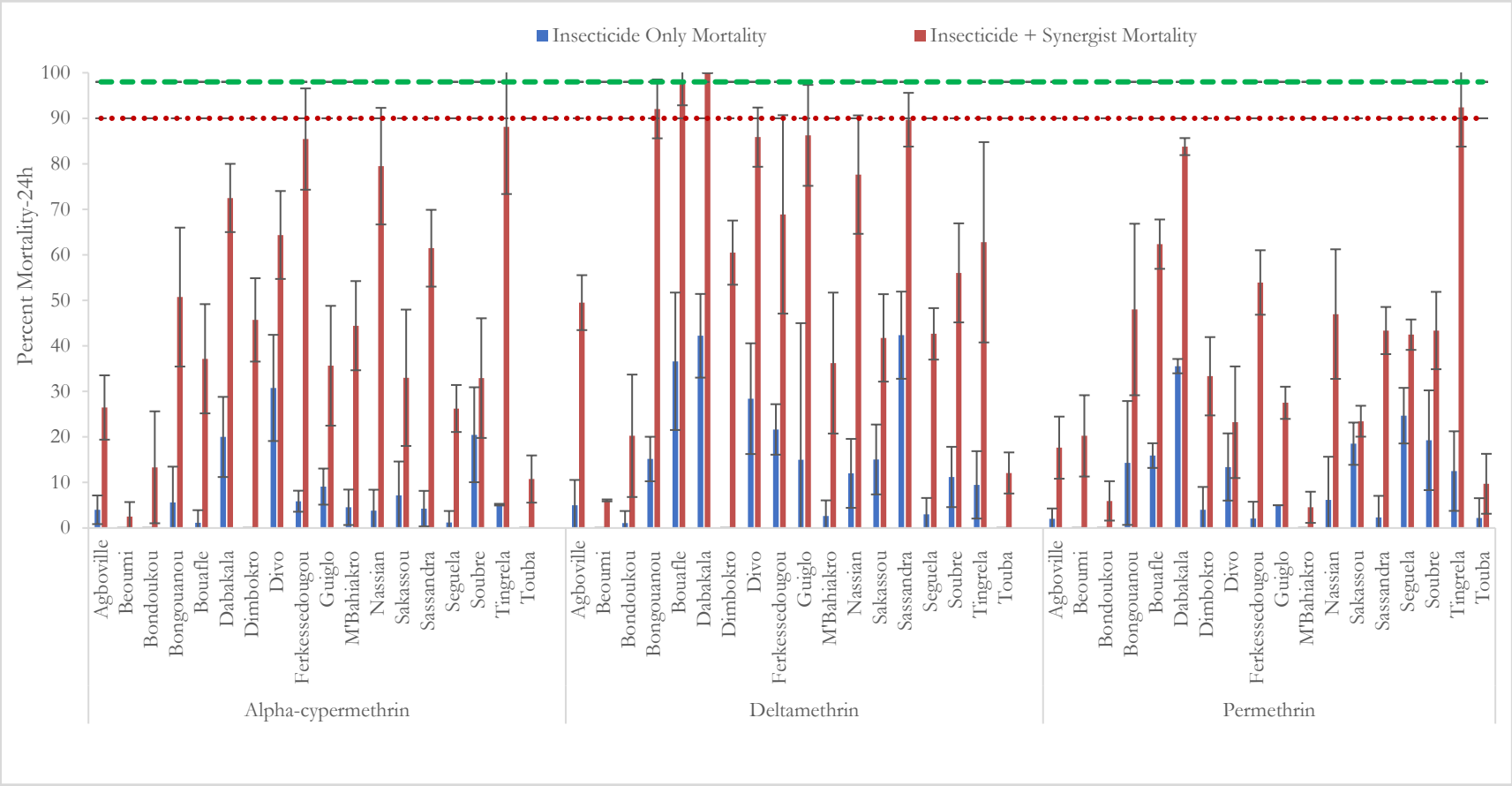
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Total Tested** | 116 | 186 | 97 | 146 | 232 | 3 | 0 | 373 |
| **Total Infected** | 7 | 7 | 10 | 13 | 25 | 0 | 0 | 7 |
| **Sporozoite rate** | 6.0% | 3.8% | 10.3% | 8.9% | 10.8% | 0.0% | 0.0% | 1.9% |

## 3.5 INSECTICIDE RESISTANCE MONITORING

Figures 13–17 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 Beoumi and Dabakala, where clothianidin was not tested due limited number of mosquitoes. Also, pirimiphos-methyl 0.25% was not tested at Dabakala 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's formula was used to correct the overall mortality of the pyrethroids.

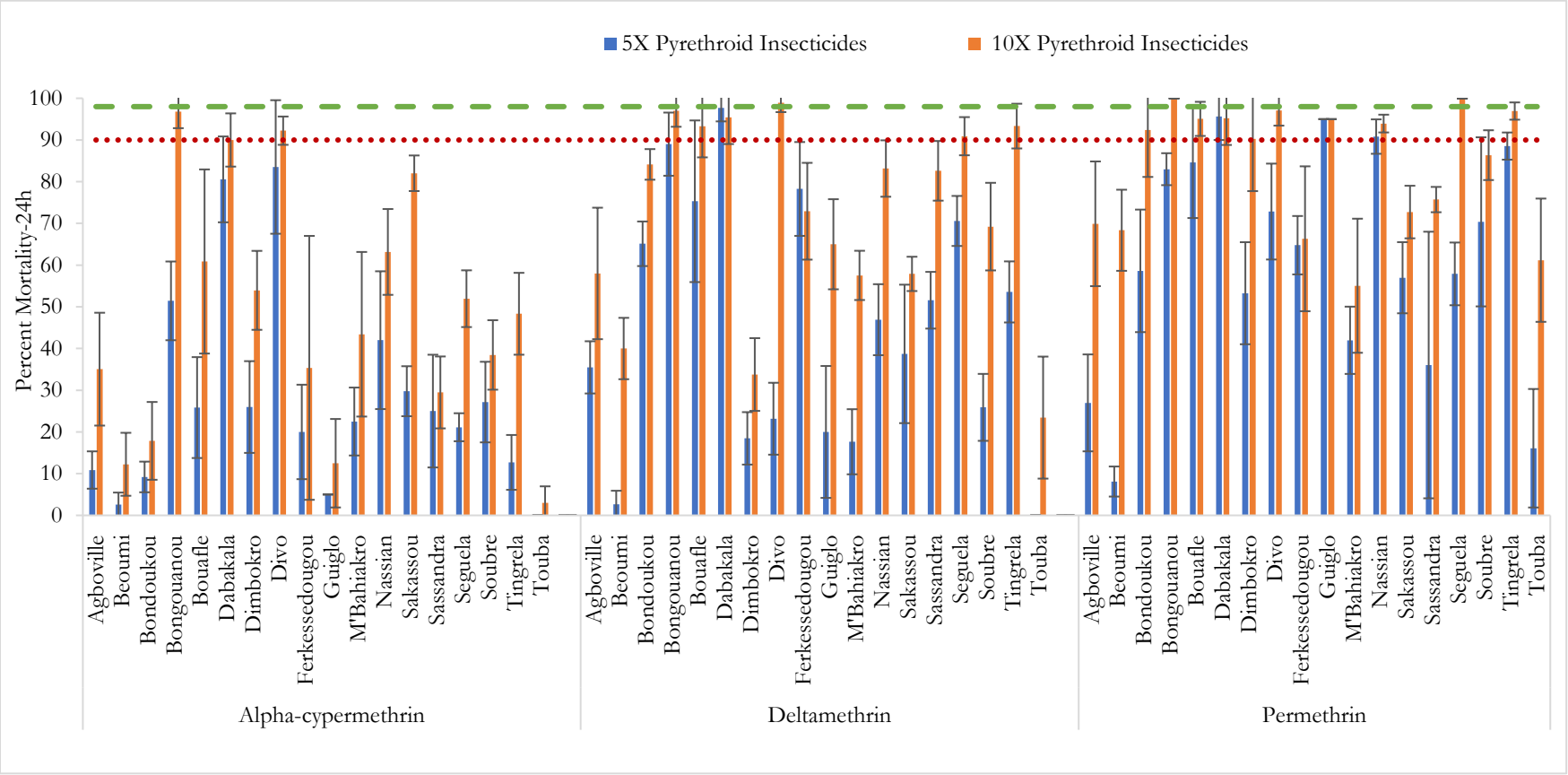
Resistance was observed to all pyrethroids in all sites surveyed. Pre-exposure of mosquitoes to PBO before exposure to deltamethrin, permethrin, and alpha-cypermethrin yielded a substantial increase of mortality in all sites. Deltamethrin showed the highest increase in mortality among the pyrethroids when combined with PBO in all sites, with full restoration of vector susceptibility in Bouafle and Dabakala (Figure 13). The intensity of resistance was high in all sites for all three pyrethroids, except in Divo and Bongouanou and Seguela where deltamethrin and permethrin resistance was moderate, respectively (Figure 14). Susceptibility to pirimiphos-methyl was recorded at 12 out of 17 sites tested, and low resistance intensity was observed in the remaining sites (Figure 15, Annex Table A-11).

## FIGURE 13: PYRETHROID INSECTICIDE SYNERGIST TEST RESULTS IN 18 SITES



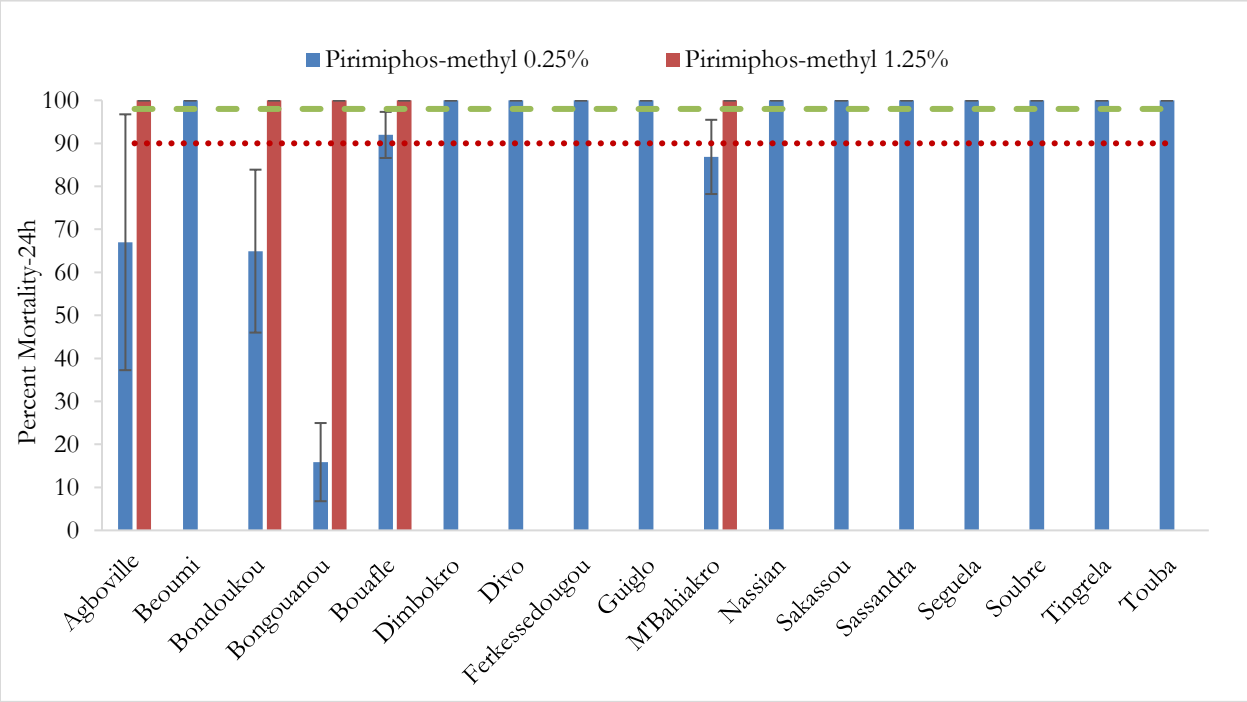

Error bars represent the standard deviation.

## FIGURE 14: INTENSITY OF RESISTANCE TO PYRETHROID INSECTICIDES IN 18 SITES



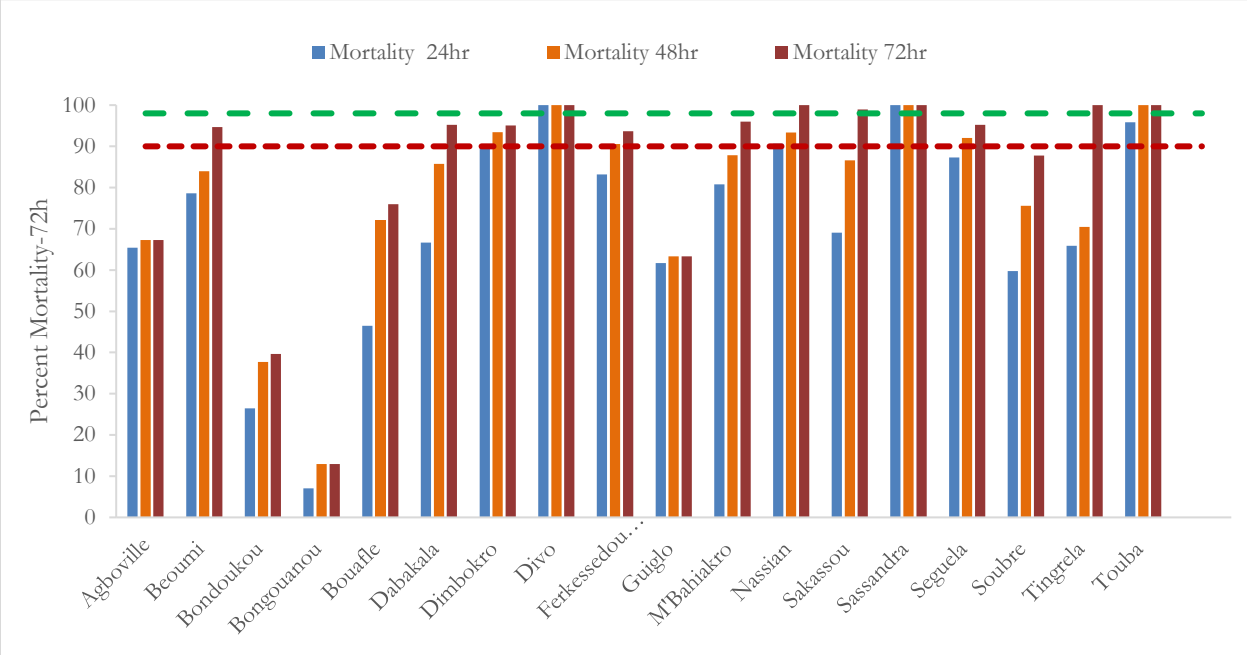

Error bars represent the standard deviation.

**FIGURE 15: SUSCEPTIBILITY AND RESISTANCE INTENSITY TO PIRIMIPHOS-METHYL IN 17 SITES**



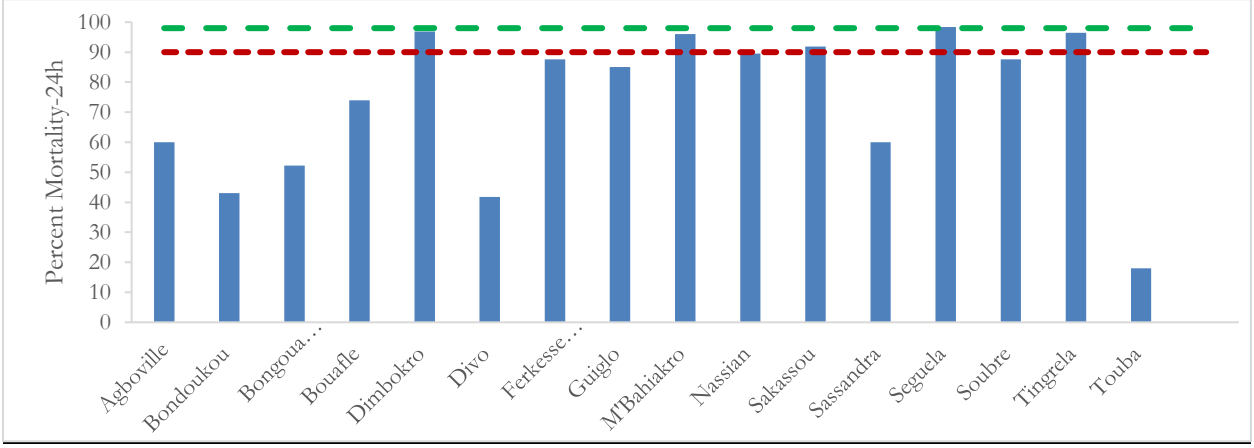

Susceptibility to chlorfenapyr at the dose of 100 µg/bottle was observed after observing mortality for 72 hours at six sites (Divo, Nassian, Sakassou, Sassandra, Tingrela, and Touba). Mosquitoes from Agboville, Beoumi, Bondoukou, Bongouanou, Bouafle, Dabakala, Dimbokro, Ferkessedougou, Guiglo, M'Bahiakro, Seguela, and Soubre were resistant to the 100 µg/bottle dose The lowest mortality rate was recorded in Bongouanou (Figure 16, Annex Table A-11).

**FIGURE 16: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 100µG/BOTTLE BY SITE**



The results of the WHO bottle assays and WHO susceptibility test using clothianidin are shown in Figures 17. For clothianidin 4 µg/bottle, out of the 16 sites tested, susceptibility was only observed in Seguela (Figure 17) (Figure 22, Annex Table 11).

**FIGURE 17: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN USING WHO BOTTLE ASSAY IN 16 SITES**

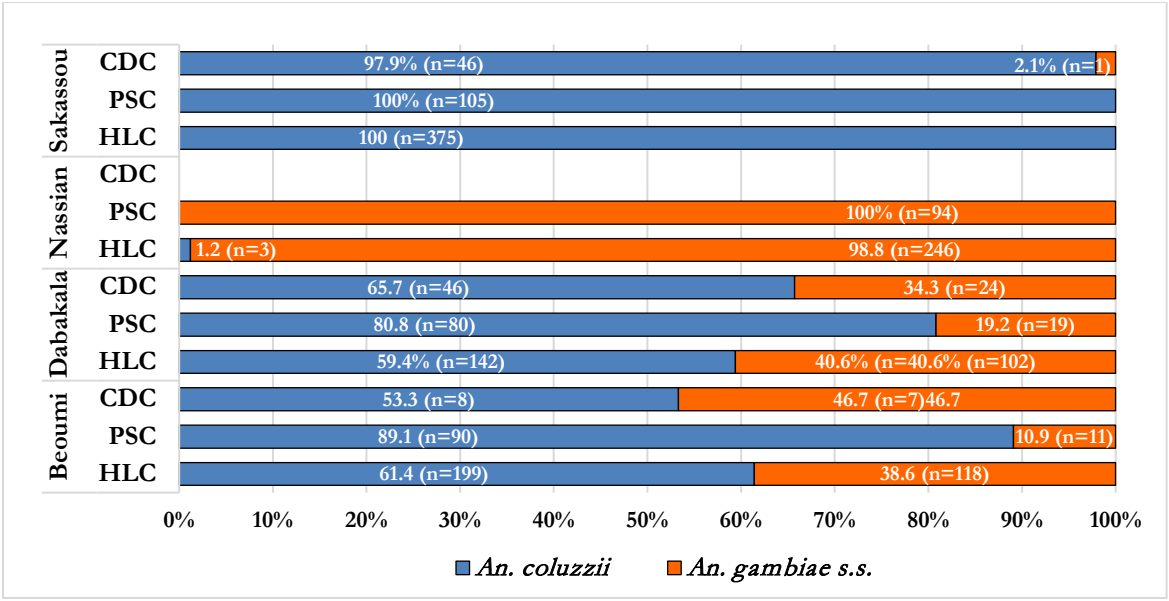



## 3.6 MOLECULAR ANALYSIS

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

A subset of 422 *An. gambiae* s.l. from Beoumi, 420 from Dabakala, 343 from Nassian, and 527 from Sakassou were molecularly identified to the sub-species. Four hundred (400) were targeted for species with adjustment made for the low density in Nassian. *An. coluzzii* represented the predominant species in Beoumi (67.9%) and Dabakala (68.6%) (all collection methods included) and was almost the only species found in Sakassou (99.3%). In Nassian (99.4%), *An. gambiae* s.s. was the predominant species collected and almost the only vector species captured using HLC (Figure 18, Annex Table A-12).

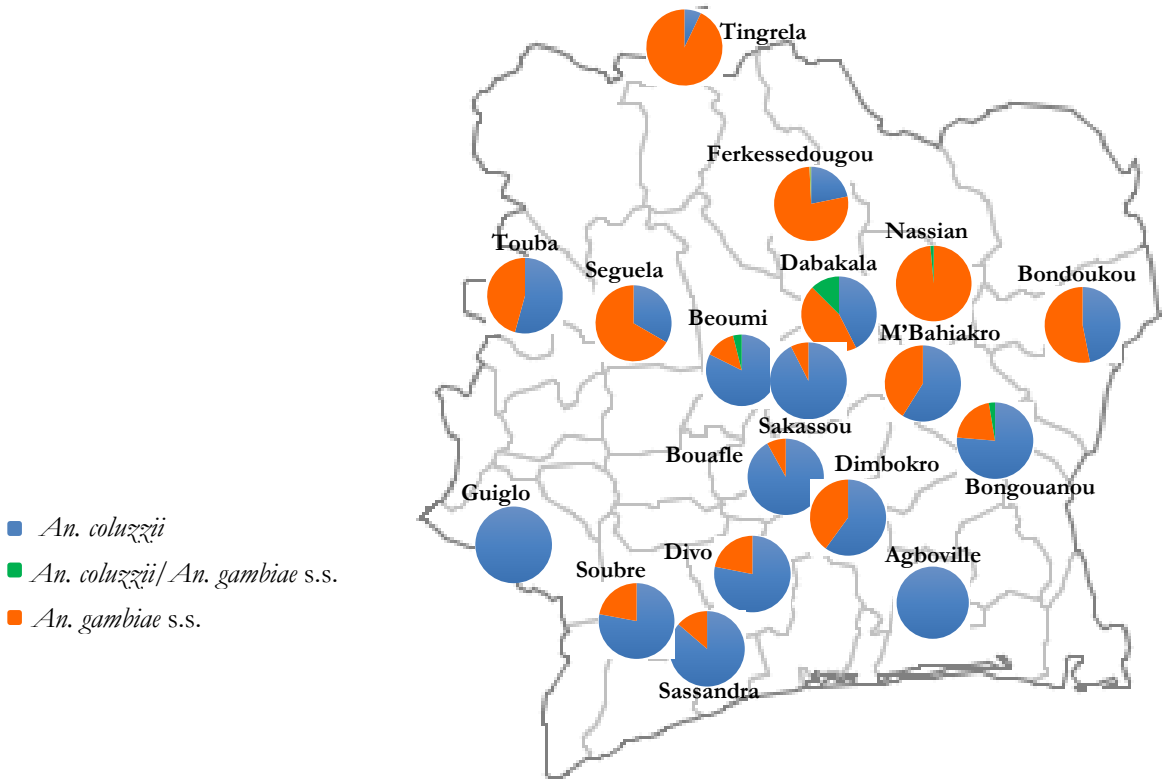
**FIGURE 18: PERCENTAGE *AN. GAMBIAE* SPECIES PER COLLECTION METHODS IN THE BIONOMIC MONITORING SITES IN 2022**



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

Figure 19 shows the species composition of *An. gambiae* s.l. used for susceptibility testing per site. *An. coluzzii* represented the predominant species in Beoumi (82.4%; n=51), Bongouanou (76.3%; n=58), Bouafle (92%; n=149), Dimbokro (60%; n=42), Divo (78.1%; n=57), M'Bahiakro (58.9%; n=63), Sakassou (92.5%; n=62), Sassandra (86.3%; n=44), Soubre (77.9%; n=67), and Touba (54.3%; n=38) as well as the entire population in Agboville (100%; n=60) and Guiglo (100%; n=64). *An. gambiae* s.s. represented the predominant species in Bondoukou (53.1%; n=51), Dabakala (45%; n=18), Ferkessedougou (77.4%; n=103), Nassian (98.6%; n=69), Seguela (66.7%; n=34), and Tingrela (92.9%; n=65). Eleven hybrid samples of the two species were found in Beoumi (3.9%; n=2), Bongouanou (2.6%; n=2), Dabakala (12.5%; n=5), Ferkessedougou (0.8%; n=1), and Nassian (1.4%; n=1) (Annex Table A-13).

**FIGURE 19: SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. USED FOR SUSCEPTIBILITY TEST ACROSS THE 18 DISTRICTS**




### 3.6.3 MOLECULAR MARKERS OF RESISTANCE

Subsets of 488 An. *gambiae* s.l. (418 alive and 69 dead), 450 *gambiae* s.l. (380 alive and 70 dead), 397 *gambiae* s.l. (52 alive and 345 dead) from the susceptibility tests were analyzed for *kdr*-West, *kdr*-East and *Ace*-1 mutations, respectively (Table 7). Figure 20 shows the distribution of *kdr*-West, *kdr*-East, and *Ace*-1 mutation in *An. gambiae* s.l. from the 18 sites. The *kdr*-West mutation was found at all sites surveyed with frequencies ranging between 4% in Tingrela to 78% in Agboville. Agboville, Bongouanou, Dabakala, Dimbokro and Seguela recorded the highest frequencies of the mutation (>60%). The *kdr*-East mutation was found at low frequency in only 10 sites, the highest being in Sakassou (14.6%). In other locations, *kdr*-East was present at frequencies that ranged between 2.1% in M'Bahiakro and 14% in Touba.

Similar to *kdr*-West, the *Ace*-1 mutation was found in all sites tested for pirimiphos-methyl at frequencies ranging from 27.1% in Man and Sassandra to 55% in Divo (Annex Tables A-14–A15).

Overall, the mean frequency of mutation was overall 0.43 and 0.04 for the *kdr*-West and East respectively and was similar in the live mosquitoes than dead for both *kdr*-West and East mutations. The overall *Ace*-1 was 0.46 and same within dead and live tested mosquitoes (0.46 for both status) (Table 7).

**FIGURE 20: DISTRIBUTION OF TARGET SITE MUTATIONS IN *AN. GAMBIAE* S.L. IN ALL SITES TESTED FOR INSECTICIDE RESISTANCE IN CÔTE D'IVOIRE**

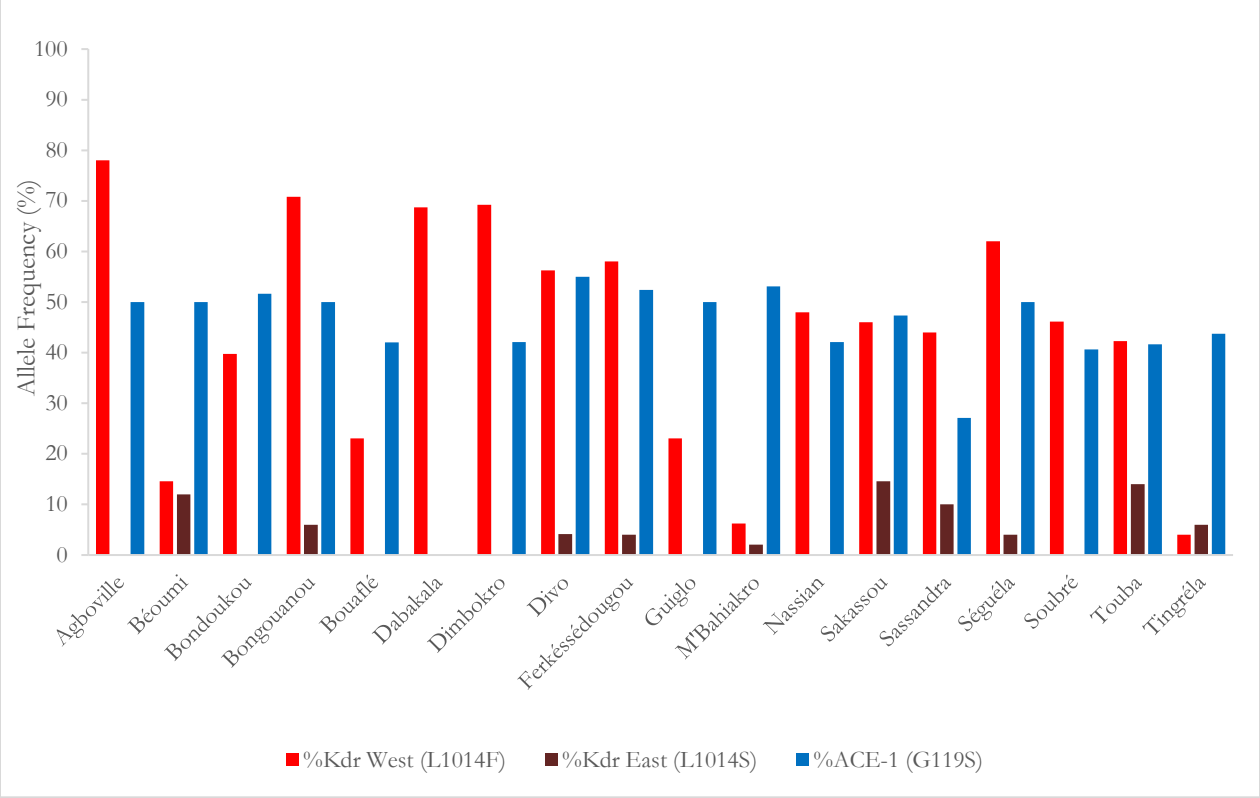



Table 7: Frequency of *kdr*-West, *kdr*-East and *Ace*-1 mutations in alive and dead *An. gambiae* s.l. tested for susceptibility in all sites

| Status | *Kdr-West* | | | | | *Kdr-East* | | | | | *Ace*-1 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Total** | **SS** | **RS** | **RR** | **Freq** | **Total** | **SS** | **RS** | **RR** | **Freq** | **Total** | **SS** | **RS** | **RR** | **Freq** |
| **Dead** | 69 | 14 (2.9) | 44 (9.0) | 12 (2.5) | 0.53 | **70** | 65 (14.4) | 5 (1.1) | 0 (0) | 0.04 | **345** | 39 (9.8) | 293 (73.8) | 13 (3.3) | 0.46 |
| **Alive** | 418 | 146 (29.9) | 194 (39.8) | 78 (16.0) | 0.42 | **380** | 353 (78.4) | 21 (4.7) | 6 (1.3) | 0.04 | **52** | 4 (1.0) | 48 (12.1) | 0 (0) | 0.46 |
| **Total** | **488** | **160 (32.8)** | **238 (48.8)** | **90 (18.5)** | **0.43** | **450** | **418 (92.9)** | **26 (5.8)** | **6 (1.3)** | **0.04** | **397** | **43 (10.8)** | **341 (85.9)** | **13 (3.3)** | **0.46** |

### 3.6.4 DETECTION OF METABOLIC RESISTANCE IN *AN. GAMBIAE* S.L.

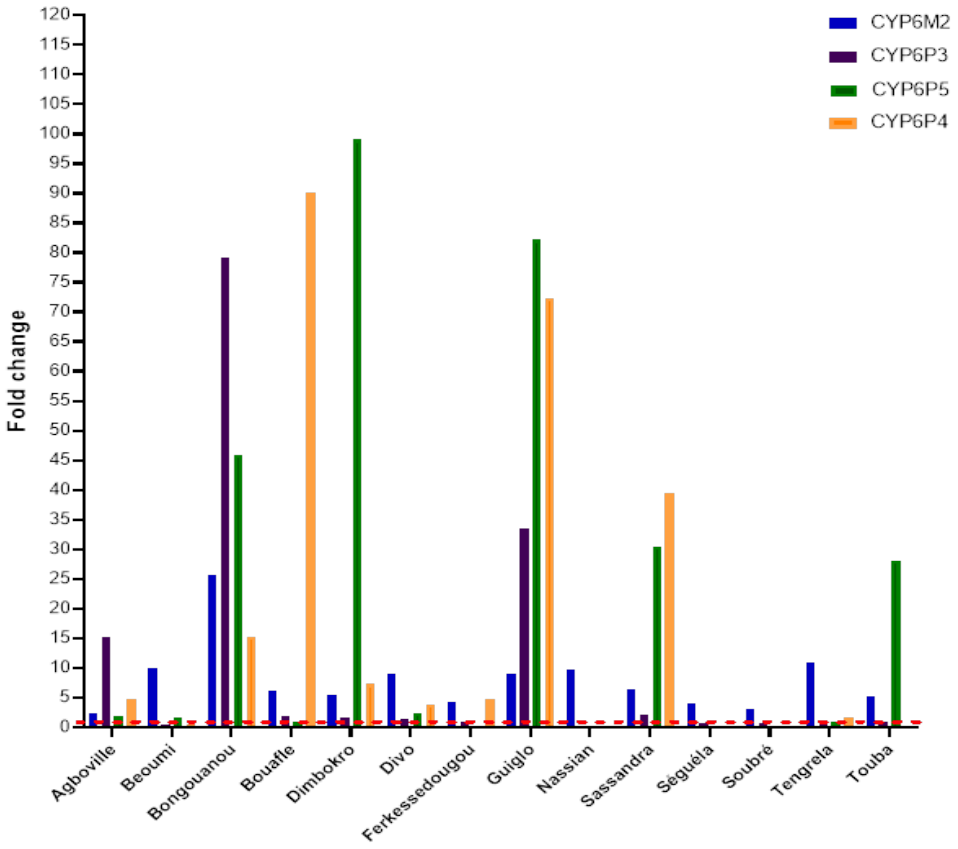
Fourteen out of the 18 sites surveyed were successfully tested for metabolic resistance enzymes (Figure 21). Samples could not be secured for four sites. Overall, the level of expression of three CYP genes was significantly higher in *An. gambiae* s.l. of all sites with specific enzymes in each of them (Annex Figure A-1 and Table A-16). In Agboville, CYP6P3 and CYP6P4 were 15-fold and 4.7-fold, respectively, expressed in the wild resistant populations. The expression of CYP6M2, a cytochrome gene known to induce a cross resistance across different classes of insecticides, is 2-fold significantly higher than that of *An. gambiae* Kisumu. CYP6P5 was not significantly expressed (1.8-fold). In Beoumi, the expression of CYP6M2 (10-fold), was significantly higher than the susceptible strain. The other enzymes (CYP6P3, CYP6P5, CYP6P4) were not significantly expressed. In Bouafle, the level of expression of CYP6P4 (90-fold) and CYP6M2 (6-fold) were, respectively, higher in *An. gambiae* s.l. compared to the fully susceptible lab strain. CYP6P3 was 1.9-fold and 1-fold for CYP6P5 respectively and not significantly expressed.

In Bongouanou, CYP6P3, CYP6P5, CYP6P4 were all above 10-fold and CYP6M2, was highly expressed ranginging between 15- and 79-fold. In Dimbokro, CYP6P5 was highly expressed (99-fold), while CYP6M2 was 5.6-fold CYP6P5, followed by CYP6P4 (7-fold). CYP6P3 (1.7-fold) was similarly expressed as *An. gambiae* Kisumu. In Divo, CYP6M2 was 9-fold, significantly higher than the susceptible strain. CYP6P5 (2.5-fold) and CYP6P4 (3.8-fold) are highly expressed, respectively, in *An. gambiae* compared to the susceptible strain. CYP6P3 (1.5-fold) was not significantly expressed in the population.

In Ferkessedougou, CYP6P4 (4.8-fold) and CYP6M2 (4.3-fold) were the highest expressed enzymes, while CYP6P5 and CYP6P3 were under-expressed (less than 1-fold). In Guiglo, all four enzymes were highly expressed. CYP6P5 was 82-fold higher, CYP6M2 9-fold, and CYP6P3 and CYP6P4 were 72-fold and 33.6-fold, respectively, higher than in the susceptible strain. In Nassian, all enzymes were under-expressed, except for CYP6M2, which was 10-fold higher than the susceptible strain. In Sassandra, the level of expression of all CYP genes was significantly higher in the wild population compared to Kisumu with CYP6P5 at 39.4-fold, CYP6M2 at 6.4-fold, CYP6P4 at 30.5-fold, and CYP6P3 at 2.1-fold higher than in the susceptible strain. In Seguela, only CYP6M2 was 4-fold, significantly higher than the susceptible strain. CYP6P3, CYP6P5, and CYP6P4 were not significantly expressed in the local population compared to Kisumu. In Soubre, the level of expression of CYP6M2 was 3.2-fold, significantly higher than the susceptible strain. CYP6P3, CYP6P5, and CYP6P4 were not significantly expressed. In Touba, CYP6M2 (5.2-fold) and CYP6P5 (28-fold) were the two cytochromes with higher expression than in the susceptible strain, while CYP6P3 and CYP6P4 were not significantly expressed. In Tingrela, only the level of expression of CYP6M2 (11-fold) was significantly higher than the susceptible strain. CYP6P3, CYP6P5, and CYP6P4 were not significantly expressed in the wild vector population.

For all figures, the intercept corresponding to the level of expression of the gene in the susceptible strain are represented by red dotted line and error bars are 95% confidence intervals.

**FIGURE 21: OVERALL DISTRIBUTION OF CYP 450S GENES ACROSS THE SURVEYED DISTRICTS IN CÔTE D'IVOIRE IN 2022**


CYP6M2
CYP6P3
CYP6P5
CYP6P4
Fold change
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Agboville
Beoumi
Bongouanou
Bouafle
Dimbokro
Divo
Ferkessedougou
Guiglo
Nassian
Sassandra
Séguéla
Soubré
Tengrela
Touba

## 3.7 Quality Assurance of IRS and Insecticide Decay Rate

### 3.7.1 Quality Assurance

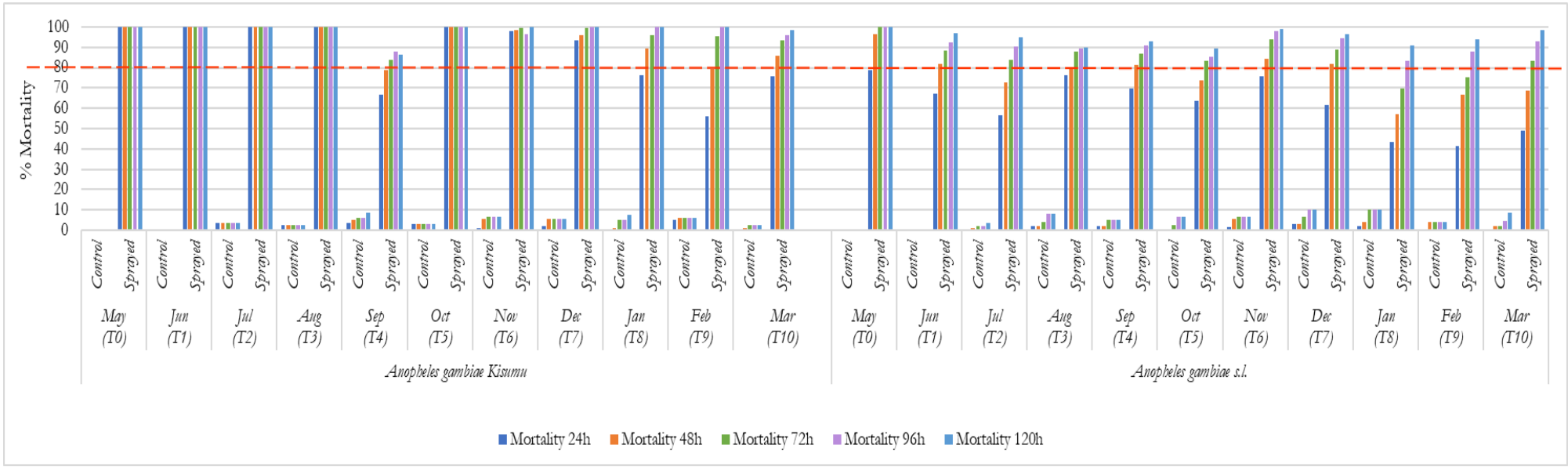
Quality assurance assessments using WHO wall bioassays conducted in a total of 21 houses (nine cement and three mud in Nassian, and nine cement in Sakassou) and compared to the six control houses (three in Nassian and three in Sakassou) in May 2022, yielded identical results in all the sites and wall types tested. One hundred percent (100%) mortality of *An. gambiae* Kisumu exposed to cement and mud walls sprayed with Fludora Fusion and to cement walls sprayed with SumiShield was recorded between 24h and 48h after exposure during the initial tests, conducted within a week after IRS in both sprayed districts.

### 3.7.2 Insecticide Residual Efficacy

For insecticide residual efficacy monitoring, wall cone bioassays conducted monthly from June 2022 to February 2023 showed mortality of *An. gambiae* Kisumu above 80% (efficacy threshold) through March, representing a minimum of ten months of residual efficacy for SumiShield in Nassian on both cement and mud walls (Figures 22–24).[2] Fludora Fusion in Sakassou continued to cause mortality above 80% after ten months using the susceptible strain *An. gambiae* Kisumu as well as the wild strain from Sakassou.

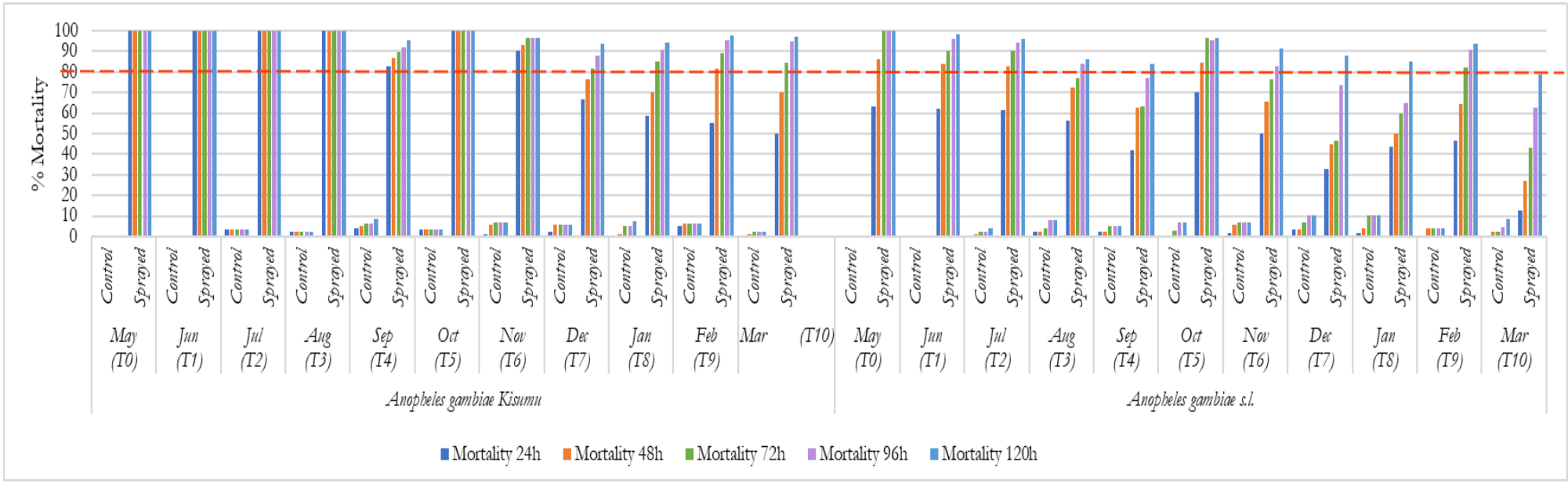
[2] The insecticide residual efficacy is reported through February 2022 using the available data at the time this report was submitted.

## FIGURE 22: QUALITY CONTROL AND RESIDUAL EFFICACY: MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE* KISUMU AND WILD COLLECTED *AN. GAMBIAE* S.L. ON NINE CEMENT SURFACES SPRAYED WITH FLUDORA FUSION IN NASSIAN



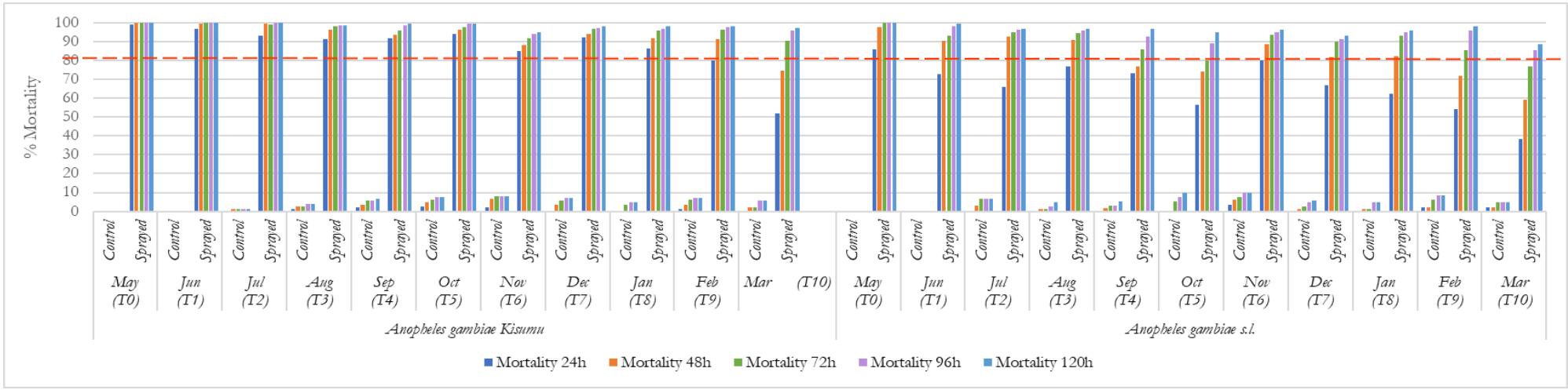

Note: Overall mortality adjusted using Abbott's formula for all control mortality above 5%. The red dotted line represents the 80% residual efficacy threshold.

**FIGURE 23: QUALITY CONTROL AND RESIDUAL EFFICACY: MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE* KISUMU AND WILD COLLECTED AN. GAMBIAE S.L. ON THREE MUD SURFACES SPRAYED WITH FLUDORA FUSION IN NASSIAN**




Note: Overall mortality adjusted using Abbott's formula for all control mortality above 5%. The red dotted line represents the 80% residual efficacy threshold.

## FIGURE 24: QUALITY CONTROL AND RESIDUAL EFFICACY: MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE* KISUMU AND WILD COLLECTED *AN. GAMBIAE* S.L. ON NINE CEMENT SURFACES SPRAYED WITH SUMISHIELD IN SAKASSOU

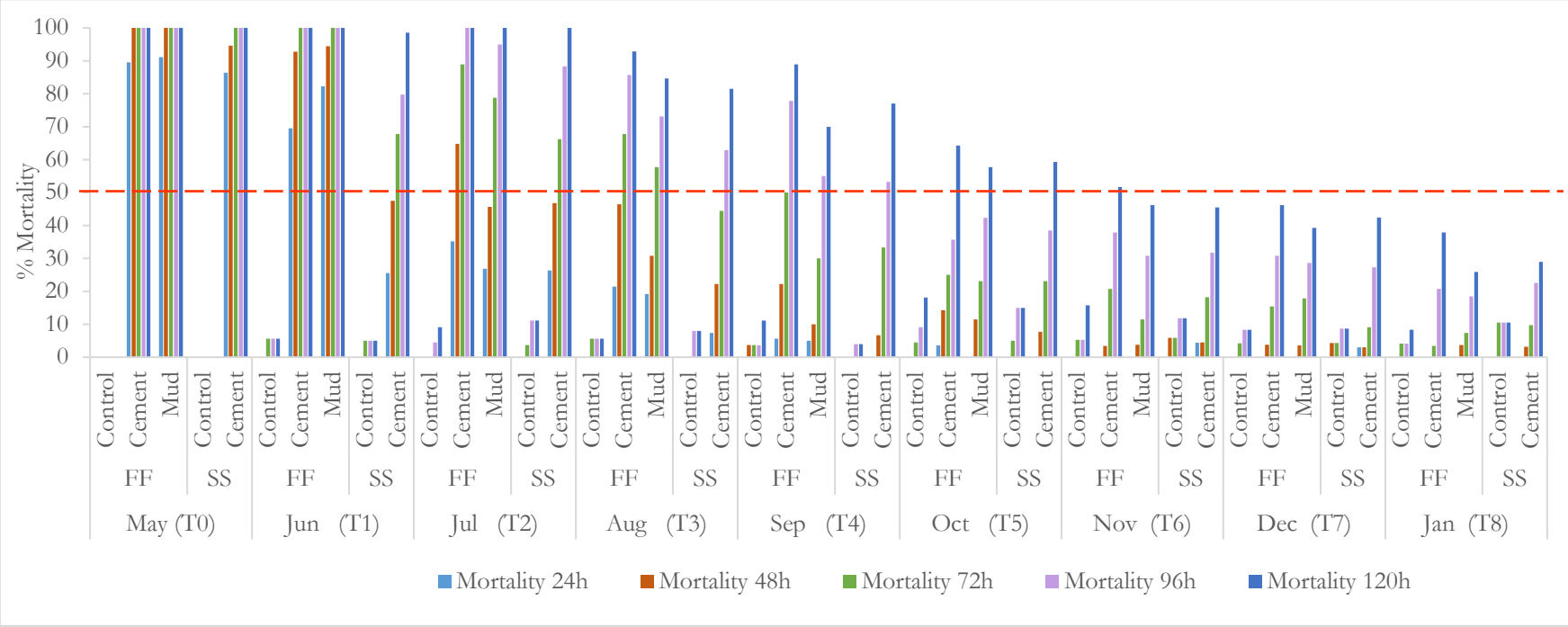



Note: Overall mortality adjusted using Abbott's formula for all control mortality above 5%. The red dotted line represents the 80% residual efficacy threshold.

### 3.7.3 FUMIGANT EFFECT

Fumigant effects were observed for both the SumiShield and Fludora Fusion-sprayed walls on susceptible *An. gambiae* Kisumu in Nassian and Sakassou, respectively. During the quality control, 100% mortality was observed in both sites and insecticides. The fumigant effect was observed on Fludora Fusion up to six months post IRS on cement (51.7%) and up to five months on mud (57.7%) in Nassian, while in Sakassou, the fumigant effect of SumiShield sprayed cement was observed up to five months (59.3%), after which it dropped below the 50% threshold (Figure 25).

**FIGURE 25: FUMIGANT EFFECT MORTALITY USING *AN. GAMBIAE* S.L. KISUMU ON CEMENT SURFACES SPRAYED WITH FLUDORA FUSION IN NASSIAN AND SUMISHIELD IN SAKASSOU**




Note: Overall mortality adjusted using Abbott's formula for all control mortality above 5%. The red dotted line represents the 50% fumigant effect cut off point. FF = Fludora Fusion; SS = SumiShield

# 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*. There was less species diversity in the 2022 collection compared to the 2020 and 2021 collections. *An. gambiae* s.l. remains the major vector observed over years of monitoring; this was consistent at all sites except in Nassian, where a population replacement by *An. funestus* s.l., considered as a secondary vector in the country, was observed after the rainy season ended. This is related to the habitat preference of *An. funestus* s.l., which is favored by the vegetation around the river that is in the area during the dry season.

*An. coluzzii* and *An. gambiae* s.s. were the two species of the complex recorded in all sites, similar to previous years. *An. coluzzii* was predominant in Beoumi and Dabakala, and essentially the only species collected in Sakassou while *An. gambiae* s.s. represented the main vector only in Nassian as reported in previous years. This is related to the fact Sakassou has permanent and irrigated rice fields known to be suitable for *An. coluzzii* development, while Nassian has transient, rain-dependant breeding sites such as puddles and small rivers that are replenished during rainy season and favorable to *An. gambia*e s.s.

The mean IRD of *An. gambiae* s.l. was relatively low at all sites, which could be explained by IRS in sprayed sites and the scale-up of ITNs in unsprayed sites. There was a recent mass ITN distribution in the country.

The HBRs of *An. gambiae* s.l. declined significantly in Sakassou (mean of 50%) during the months after IRS, while in Nassian and unsprayed sites, there was no notable decrease. It is important to note that Sakassou had the highest vector biting density, which enables to estimate the potential vector density reduction caused by IRS over the three years of IRS implementation compared to Nassian.

*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, which is consistent with the findings of previous VectorLink reports. Interestingly, the vector bites similarly indoors and outdoors in the IRS sites, whereas in the unsprayed sites, higher outdoor biting was recorded. These data demonstrate that IRS has not changed the vector's feeding and resting density. Along with IRS, it's important to include a social behavior campaign to promote and improve the use of ITN in order to reduce vector humancontact and biting.

Overall, the percentages of gravid *An. gambiae* s.l. mosquitoes were similar in all sites whether sprayed or not. The percentage of combined fed and half-gravid *An. gambiae* s.l. were 76.1% in the sprayed sites and 76.5% in the unsprayed sites. No difference in abdominal status was observed between IRS and unsprayed sites, suggesting that the vectors could have bitten a host and remained indoors in all settings. Given that the NMCP conducted a mass ITN distribution campaign in April 2021, it is important to better understand ITN coverage, use, and night time human behavior.

Mean parity rates for *An. gambiae* s.l. was significantly lower at the sprayed sites (66.7%) than at the unsprayed sites (82.9%) showing the relative impact of IRS on vector longevity in sprayed sites. The reduction of parous mosquitoes is indicative of a younger vector population and a reduction in the likelihood of malaria transmission.

The mean sporozoite rate of *An. gambiae* s.l. was similar in sprayed (0.019) and unsprayed sites (0.03)., similar EIRs of *An. gambiae* s.l. were also recorded in sprayed (0.449) and unsprayed (0.53) sites. However, the mean EIR observed

in unsprayed sites showed a stability of transmission when compared to previous year's data. In contrast, a continuous decrease of the number of infective bites was observed in the IRS sites, indicating that IRS reduces malaria transmission intensity regardless of the biting intensity that was observed every year in Sakassou.

The 2022 data collected showed a continuous decrease of malaria transmission in IRS sites since implementation of IRS began. These results are consistent with those of an epidemiological impact evaluation of the 2020 and 2021 IRS campaigns, indicating that IRS is effective in reducing malaria infection in Côte d'Ivoire.

## 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 ITNs interventions in Côte d'Ivoire.

Pre-exposure to PBO with 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 Bouafle and Dabakala. Furthermore, the metabolic enzyme detections showed high enzyme activities, confirming the involvement of P450s in insecticide resistance to pyrethroids and neonicotinoids in particular at all sites surveyed. These results comport with the decision made by the country to introduce PBO ITNs in selected districts during the recent mass ITN distribution campaign and may support a countrywide distribution of PBO ITNs.

Susceptibility to pirimiphos-methyl was observed in 12 sites, while low intensity of the resistance was observed in the five sites where resistance was confirmed and intensity tests completed.

Susceptibility to 4 µg/bottle of clothianidin tested in CDC bottle assays yielded less susceptible sites than did paper-based tests. Only one site showed susceptibility to this concentration of clothianidin. The trend will need to be confirmed during the next collection as there could be a bottle coating variability among sites, and to enable deciding on the appropriate protocol to be considered for clothianidin testing, though this may require discussion and decision at a high level, such as the WHO Vector Control Working Group.

Chlorfenapyr at the dose of 100 µg/bottle yielded full susceptibility in six 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. Further investigation will be required to reconfirm the resistance observed in the other sites, though tests were mostly conducted in parallel with a susceptible mosquito strain to support the quality of the coated bottles.

Furthermore, both *kdr* (West and East) mutations were present in Côte d'Ivoire, which correlate with the high phenotypic resistance observed to pyrethroid insecticides and represents a significant threat to the use of pyrethroid-only ITNs. The frequencies of all target sites mutations were similar within the dead and surviving mosquitoes after exposure to insecticides, showing that the mutations may be fixed in most of the tested population as the phenotypic status did not impact the observed frequencies. The data are similar to previous results the country has reported and may not require annual laboratory resistance mechanism characterization from the same site during successive years

## 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 ten months at the time of this report. The observed mortality showed that the sprayed walls were not underdosed during the campaign. As of March 2023, 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 ten months of observation.

The mortality recorded against the wild collected mosquitoes was above the efficacy threshold of 80% after ten months showing that the sprayed insecticides lasted enough to cover the peak transmission period in both sites.

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

## 4.4 RECOMMENDATIONS

Based on these results, the recommendations are as follows:

### 4.4.1 TECHNICAL

- The high density of *An. funestus* s.l. population collected in Nassian may need further investigation for insecticide resistance monitoring and probable enzymes (CypP6P9a/b) involved in any resistance of that vector to understand and ensure that the insecticides used in all vector control tools implemented in the district will be effective on killing the species.
- The resistance status of the vectors to new molecules will need follow-up investigation on the mechanisms driving the trends. Though both molecules were tested using WHO bottle test methods, the particularity of clothianidin data require further consideration. Reported data from other countries should be checked for decision making on the appropriateness of the protocol used for testing.

### 4.4.2 STRATEGIC

- The consistently high pyrethroid resistance confirms the need for PBO and/or dual insecticide ITNs across the country.
- Dual AI (chlorfenapyr + pyrethroids) and PBO ITNs should continue to be deployed on a mosaic basis considering the similarity of the PBO synergism and chlorfenapyr test results between the formerly surveyed and the new selected sites across the country.
- The observed spatial and temporal variability in indoor and outdoor biting of malaria vectors in Côte d'Ivoire necessitates 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. This will require the implementation of a human behavior observation through a survey-based approach to collect data on how ITNs are used and how people spend their nights. 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 any IRS implementation in the country. In addition, the susceptibility observed against pirimiphos-methyl in Nassian and other districts that were tested for susceptibility could support a strategic insecticide rotation plan for IRS.

# ANNEX

**TABLE A-1: 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)**

| Site | Species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Beoumi | *An. gambiae* s.l. | 7 | 1 | 21 | 90 | 68 | 555 | 399 | 538 | 485 | 567 | 204 | 163 | 3098 |
| | *An. funestus* s.l. | 6 | 2 | 9 | 3 | 2 | | | 5 | 18 | 2 | 6 | 13 | 66 |
| | *An. pharoensis* | | | | 2 | 4 | 3 | 94 | 12 | | 31 | 130 | 15 | 291 |
| | Culicine | 160 | 527 | 796 | 1099 | 164 | 152 | 203 | 111 | 772 | 109 | 80 | 175 | 4348 |
| Dabakala | *An. gambiae* s.l. | 0 | 2 | 21 | 1082 | 579 | 837 | 761 | 892 | 1154 | 1255 | 72 | 98 | 6753 |
| | *An. funestus* s.l. | | | | 1 | 1 | | 7 | 2 | 16 | 4 | | 7 | 38 |
| | *An. nili* | | | | | 11 | 5 | 163 | 30 | 487 | 2 | 2 | | 700 |
| | *An. pharoensis* | | | 1 | 5 | 14 | 19 | 47 | 18 | 35 | 10 | 4 | 5 | 158 |
| | Culicine | 486 | 859 | 194 | 1338 | 1068 | 365 | 502 | 230 | 188 | 186 | 303 | 274 | 5993 |
| | Total | **486** | **861** | **216** | **2426** | **1673** | **1226** | **1480** | **1172** | **1880** | **1457** | **381** | **384** | **13642** |
| Nassian | *An. gambiae* s.l. | 8 | 53 | 1 | 139 | 198 | 366 | 321 | 33 | 316 | 422 | 8 | 11 | 1876 |
| | *An. funestus* s.l. | 1 | 5 | | 1 | 1 | 1 | 31 | 50 | 49 | 148 | 53 | 77 | 417 |
| | *An. pharoensis* | | | | 1 | | | 3 | | | | | | 4 |
| | Culicine | 0 | 15 | 9 | 5 | 16 | 28 | 109 | 33 | 37 | 24 | 12 | 14 | 302 |
| | Total | **9** | **73** | **10** | **146** | **215** | **395** | **464** | **116** | **402** | **594** | **73** | **102** | **2599** |
| Sakassou | *An. gambiae* s.l. | 516 | 2040 | 4130 | 3506 | 1942 | 2386 | 819 | 952 | 1618 | 1817 | 2287 | 1595 | 23608 |
| | *An. funestus* s.l. | | | | | 21 | | 2 | 3 | 4 | 6 | 27 | | 63 |
| | *An. nili* | | | | | 3 | | | 2 | | 1 | | | 6 |
| | *An. pharoensis* | 5 | 39 | 50 | 125 | 108 | 109 | 66 | 71 | 152 | 91 | 53 | 36 | 905 |
| | Culicine | 47 | 929 | 627 | 386 | 261 | 138 | 176 | 194 | 124 | 183 | 86 | 136 | 3287 |
| | Total | **52** | **968** | **677** | **511** | **2335** | **247** | **242** | **1222** | **276** | **2098** | **139** | **172** | **8939** |

## TABLE A-2: SPECIES COMPOSITION OF MOSQUITOES COLLECTED IN 2021 AT ALL SITES USING PSC (30 HOUSES PER MONTH)

| Site | Species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Beoumi | *An. gambiae* s.l. | 2 | 1 | 9 | 14 | 33 | 116 | 45 | 64 | 37 | 70 | 31 | 44 | 466 |
| | *An. funestus* s.l. | 0 | 10 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 18 |
| | *An. pharoensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 21 | 1 | 24 |
| | *An. nili* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Culicine | 175 | 121 | 156 | 233 | 213 | 89 | 145 | 48 | 144 | 21 | 48 | 100 | 1493 |
| | Total | **177** | **132** | **171** | **247** | **246** | **205** | **190** | **112** | **181** | **93** | **102** | **145** | **2001** |
| Dabakala | *An. gambiae* s.l. | 1 | 1 | 31 | 185 | 161 | 189 | 268 | 253 | 177 | 56 | 8 | 12 | 1342 |
| | *An. funestus* s.l. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| | *An. pharoensis* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | *An. nili* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Culicine | 201 | 87 | 63 | 329 | 119 | 133 | 68 | 44 | 38 | 119 | 125 | 123 | 1449 |
| | Total | **202** | **88** | **95** | **514** | **281** | **322** | **336** | **297** | **215** | **175** | **134** | **136** | **2795** |
| Nassian | *An. gambiae* s.l. | 1 | 7 | 1 | 8 | 10 | 29 | 32 | 8 | 45 | 98 | 6 | 4 | 249 |
| | *An. funestus* s.l. | 1 | 0 | 0 | 3 | 0 | 2 | 1 | 17 | 39 | 29 | 20 | 34 | 146 |
| | *An. pharoensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *An. nili* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Culicine | 4 | 1 | 1 | 7 | 6 | 9 | 3 | 15 | 18 | 19 | 13 | 10 | 106 |
| | Total | **6** | **8** | **2** | **18** | **16** | **40** | **36** | **40** | **102** | **146** | **39** | **48** | **501** |
| Sakassou | *An. gambiae* s.l. | 52 | 150 | 514 | 288 | 132 | 109 | 82 | 17 | 67 | 105 | 151 | 95 | 1762 |
| | *An. funestus* s.l. | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | *An. pharoensis* | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 4 |
| | *An. nili* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Culicine | 124 | 176 | 56 | 74 | 4 | 5 | 18 | 3 | 25 | 39 | 66 | 12 | 602 |
| | Total | **176** | **326** | **571** | **362** | **137** | **114** | **103** | **20** | **92** | **145** | **217** | **107** | **2370** |

## TABLE A-3: SPECIES COMPOSITION OF MOSQUITOES COLLECTED IN 2021 AT ALL SITES USING CDC LIGHT TRAPS (4 TRAPS INDOORS FOR 2 CONSECUTIVE NIGHTS PER MONTH)

| Site | Species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Beoumi | *An. gambiae* s.l. | 1 | 0 | 6 | 0 | 1 | 0 | 2 | 0 | 0 | 2 | 1 | 10 | 23 |
| | *An. funestus* s.l. | 0 | 11 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| | *An. pharoensis* | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 6 | 1 | 16 |
| | Culicine | 10 | 17 | 123 | 2 | 24 | 0 | 19 | 9 | 22 | 2 | 1 | 9 | 238 |
| | Total | **11** | **28** | **133** | **2** | **25** | **0** | **30** | **9** | **22** | **4** | **8** | **20** | **292** |
| Dabakala | *An. gambiae* s.l. | 0 | 0 | 1 | 62 | 73 | 31 | 0 | 2 | 0 | 35 | 6 | 1 | 211 |
| | Culicine | 4 | 1 | 0 | 15 | 16 | 16 | 17 | 36 | 14 | 2 | 15 | 6 | 142 |
| | Total | **4** | **1** | **1** | **77** | **89** | **47** | **17** | **38** | **14** | **37** | **21** | **7** | **353** |
| Nassian | Culicine | 0 | 0 | 0 | 1 | 0 | 2 | 5 | 0 | 1 | 0 | | 1 | 10 |
| | Total | **0** | **0** | **0** | **1** | **0** | **2** | **5** | **0** | **1** | **0** | | **1** | **10** |
| Sakassou | *An. gambiae* s.l. | 6 | 6 | 33 | 17 | 7 | 3 | 2 | 1 | 0 | 0 | 0 | 22 | 97 |
| | *An. funestus* s.l. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Culicine | 7 | 40 | 6 | 4 | 0 | 11 | 12 | 9 | 11 | 0 | 4 | 11 | 115 |
| | Total | **13** | **46** | **39** | **21** | **8** | **14** | **14** | **10** | **11** | **0** | **4** | **33** | **213** |

## TABLE A-4: INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED IN 2021 USING PSC

| Species | Site | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *An. gambiae* s.l. | Beoumi (Unsprayed) | 0.1 | 0.0 | 0.3 | 0.5 | 1.1 | 3.9 | 1.5 | 2.1 | 1.2 | 2.3 | 1.0 | 1.5 |
| | Dabakala (Unsprayed) | 0.0 | 0.0 | 1.0 | 6.2 | 5.4 | 6.3 | 8.9 | 8.4 | 5.9 | 1.9 | 0.3 | 0.4 |
| | Nassian (IRS) | 0.0 | 0.2 | 0.0 | 0.3 | 0.3 | 1.0 | 1.1 | 0.3 | 1.5 | 3.3 | 0.2 | 0.1 |
| | Sakassou (IRS) | 1.7 | 5.0 | 17.1 | 9.6 | 4.4 | 3.6 | 2.7 | 0.6 | 2.2 | 3.5 | 5.0 | 3.2 |
| | | | | | | | | | | | | | |
| *An. funestus* s.l. | Beoumi (Unsprayed) | 0 | 0.33 | 0.2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.07 | 0 |
| | Dabakala (Unsprayed) | 0 | 0 | 0.03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.03 |
| | Nassian (IRS) | 0.03 | 0 | 0 | 0.1 | 0 | 0.07 | 0.03 | 0.57 | 1.3 | 0.97 | 0.67 | 1.1 |
| | Sakassou (IRS) | 0 | 0 | 0.03 | 0 | 0.03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

## TABLE A-5: 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 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Beoumi | Unfed | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 1 | 5 |
| | Fed | 0 | 0 | 1 | 5 | 14 | 32 | 0 | 3 | 33 | 25 | 17 | 2 | 132 |
| | Half-gravid | 0 | 0 | 0 | 0 | 3 | 28 | 1 | 17 | 0 | 13 | 1 | 24 | 87 |
| | Gravid | 1 | 1 | 1 | 3 | 4 | 4 | 0 | 3 | 1 | 0 | 2 | 1 | 21 |
| | **Total** | **1** | **1** | **2** | **8** | **22** | **64** | **1** | **24** | **37** | **40** | **20** | **28** | **248** |
| Dabakala | Unfed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | Fed | 0 | 0 | 6 | 8 | 14 | 47 | 1 | 4 | 18 | 13 | 2 | 1 | 114 |
| | Half-gravid | 0 | 0 | 0 | 1 | 0 | 0 | 10 | 12 | 9 | 6 | 1 | 0 | 39 |
| | Gravid | 0 | 0 | 0 | 0 | 4 | 1 | 2 | 0 | 10 | 0 | 3 | 1 | 21 |
| | **Total** | **0** | **0** | **6** | **9** | **18** | **48** | **13** | **16** | **38** | **19** | **6** | **2** | **175** |
| Nassian | Unfed | 0 | 1 | 0 | 0 | 0 | 4 | 2 | 0 | 1 | 7 | 0 | 0 | 15 |
| | Fed | 1 | 6 | 1 | 5 | 8 | 4 | 19 | 3 | 20 | 57 | 5 | 3 | 132 |
| | Half-gravid | 0 | 0 | 0 | 2 | 1 | 19 | 3 | 3 | 21 | 29 | 1 | 1 | 80 |
| | Gravid | 0 | 0 | 0 | 1 | 1 | 2 | 8 | 2 | 3 | 5 | 0 | 0 | 22 |
| | **Total** | **1** | **7** | **1** | **8** | **10** | **29** | **32** | **8** | **45** | **98** | **6** | **4** | **249** |
| Sakassou | Unfed | 21 | 22 | 95 | 32 | 17 | 11 | 21 | 5 | 16 | 4 | 55 | 15 | 314 |
| | Fed | 20 | 105 | 201 | 212 | 84 | 73 | 38 | 4 | 36 | 55 | 45 | 42 | 915 |
| | Half gravid | 8 | 0 | 126 | 18 | 17 | 2 | 18 | 4 | 1 | 40 | 10 | 12 | 256 |
| | Gravid | 3 | 23 | 92 | 22 | 14 | 23 | 4 | 4 | 14 | 6 | 38 | 26 | 269 |
| | **Total** | **52** | **150** | **514** | **288** | **132** | **109** | **82** | **17** | **67** | **105** | **151** | **95** | **1762** |

## TABLE A-6: 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 | 0.03 | 0.2 | 0.28 | 0.43 | 1.1 | 1.6 | 2.9 | 3.8 | 3.6 | 3 | 2 | 1.1 |
| Dabakala | Outdoor | 0.3 | 1.3 | 1.8 | 2.4 | 3.1 | 3.9 | 5.5 | 6.9 | 6.2 | 5.5 | 4.2 | 2.1 |
| Nassian | Outdoor | 0.08 | 0.1 | 0.11 | 0.31 | 0.54 | 0.89 | 1.4 | 1.4 | 1.4 | 1.2 | 1.1 | 0.5 |
| Sakassou | Outdoor | 1.7 | 3.8 | 3.9 | 5.8 | 8.4 | 11 | 15.5 | 19.3 | 20.7 | 17.4 | 15.7 | 8.9 |
| | **Total Outdoor** | **2.11** | **5.4** | **6.09** | **8.94** | **13.14** | **17.39** | **25.3** | **31.4** | **31.9** | **27.1** | **23** | **12.6** |
| Beoumi | Indoor | 0.07 | 0.09 | 0.24 | 0.54 | 0.8 | 1.2 | 1.5 | 2.2 | 2.1 | 1.7 | 1.2 | 0.52 |
| Dabakala | Indoor | 0.11 | 0.74 | 1.1 | 1.4 | 1.6 | 2.5 | 3.3 | 3.5 | 3.6 | 4 | 3.5 | 1.9 |
| Nassian | Indoor | 0.07 | 0.13 | 0.17 | 0.33 | 0.72 | 0.6 | 1.6 | 2 | 2 | 1.5 | 0.92 | 0.38 |
| Sakassou | Indoor | 1.2 | 2.4 | 3.4 | 5 | 6.7 | 8.9 | 14 | 16.1 | 17.2 | 15.9 | 13.1 | 9.9 |
| | **Total Indoor** | **1.45** | **3.36** | **4.91** | **7.27** | **9.82** | **13.2** | **20.4** | **23.8** | **24.9** | **23.1** | **18.72** | **12.7** |

## TABLE A-7: MONTHLY BITING RATE RESULTS USING HLC

| Site | Month | Indoor HBR; *An. gambiae* s.l. | Outdoor HBR; *An. gambiae* s.l. | Indoor HBR; *An. funestus* s.l. | Outdoor HBR; *An. funestus* s.l. |
|---|---|---|---|---|---|
| Beoumi | Jan-22 | 0.3 | 0.6 | 0 | 0.8 |
| | Feb-22 | 0 | 0.1 | 0.1 | 0.1 |
| | Mar-22 | 1.1 | 1.5 | 0.5 | 0.6 |
| | Apr-22 | 3.9 | 7.4 | 0.3 | 0.1 |
| | May-22 | 4.5 | 4 | 0.3 | 0 |
| | Jun-22 | 24.8 | 44.6 | 0 | 0 |
| | Jul-22 | 21.6 | 28.3 | 0 | 0 |
| | Aug-22 | 23.9 | 43.4 | 0.3 | 0.4 |
| | Sep-22 | 20.4 | 40.3 | 1.1 | 1.1 |
| | Oct-22 | 26.6 | 44.3 | 0.1 | 0.1 |
| | Nov-22 | 11.4 | 14.1 | 0.5 | 0.3 |
| | Dec-22 | 7.6 | 12.8 | 1.4 | 0.3 |
| | Total | **146.1** | **241.4** | **4.6** | **3.8** |
| Dabakala | Jan-22 | 0 | 0 | 0 | 0 |
| | Feb-22 | 0.1 | 0.1 | 0 | 0 |
| | Mar-22 | 1 | 1.6 | 0 | 0 |
| | Apr-22 | 52 | 83.3 | 0 | 0.1 |
| | May-22 | 31.8 | 40.6 | 0 | 0.1 |
| | Jun-22 | 37.4 | 67.3 | 0 | 0 |
| | Jul-22 | 43.9 | 51.3 | 0.3 | 0.6 |
| | Aug-22 | 31.6 | 79.9 | 0.1 | 0.1 |
| | Sep-22 | 63.1 | 81.1 | 0.8 | 1.3 |
| | Oct-22 | 59.5 | 97.4 | 0.1 | 0.4 |
| | Nov-22 | 2.5 | 6.5 | 0 | 0 |
| | Dec-22 | 3.8 | 8.5 | 0.5 | 0.4 |
| | Total | **326.7** | **517.6** | **1.8** | **3** |
| Nassian | Jan-22 | 0.8 | 0.3 | 0.1 | 0 |
| | Feb-22 | 4.5 | 2.1 | 0.3 | 0.4 |
| | Mar-22 | 0.1 | 0 | 0 | 0 |
| | Apr-22 | 8.8 | 8.6 | 0.1 | 0 |
| | May-22 | 14.4 | 10.4 | 0 | 0.1 |
| | Jun-22 | 29.9 | 15.9 | 0.1 | 0 |
| | Jul-22 | 16.1 | 24 | 1.4 | 2.5 |
| | Aug-22 | 2.9 | 1.3 | 3.1 | 3.1 |
| | Sep-22 | 18.6 | 20.9 | 2 | 4.1 |
| | Oct-22 | 28.5 | 24.3 | 9.1 | 9.4 |
| | Nov-22 | 0.8 | 0.3 | 3.0 | 3.6 |
| | Dec-22 | 0.3 | 1.1 | 4.5 | 5.1 |
| | Total | **125.7** | **109.2** | **23.7** | **28.3** |
| Sakassou | Jan-22 | 37.8 | 26.8 | 0 | 0 |
| | Feb-22 | 107.8 | 147.3 | 0 | 0 |
| | Mar-22 | 240.4 | 275.9 | 0 | 0 |
| | Apr-22 | 185.6 | 252.6 | 0 | 0 |
| | May-22 | 142.5 | 100.3 | 1 | 1.6 |
| | Jun-22 | 154.5 | 143.8 | 0 | 0 |
| | Jul-22 | 49.5 | 52.9 | 0 | 0.3 |
| | Aug-22 | 37.1 | 81.9 | 0.3 | 0.1 |
| | Sep-22 | 74.4 | 127.9 | 0.5 | 0 |
| | Oct-22 | 102.9 | 124.3 | 0.3 | 0.5 |
| | Nov-22 | 135.1 | 150.8 | 1.9 | 1.5 |
| | Dec-22 | 97.5 | 101.9 | 0 | 0 |
| | Total | **1365.1** | **1586.4** | **4.0** | **4.0** |

## TABLE A-8: MONTHLY PARITY RATES OF DISSECTED *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. PER SITE

| Site | Month | Dissected | Parous | Parity Rate (%) |
|---|---|---|---|---|
| Beoumi | Jan-22 | 7 | 7 | 100.0 |
| | Feb-22 | 0 | | |
| | Mar-22 | 20.00 | 19 | 95.0 |
| | Apr-22 | 89 | 79 | 88.8 |
| | May-22 | 55 | 49 | 89.1 |
| | Jun-22 | 236 | 92 | 39.0 |
| | Jul-22 | 185 | 156 | 84.3 |
| | Aug-22 | 241 | 127 | 52.7 |
| | Sep-22 | 235 | 191 | 81.3 |
| | Oct-22 | 181 | 92 | 50.8 |
| | Nov-22 | 203 | 184 | 90.6 |
| | Dec-22 | 162 | 126 | 77.8 |
| | **Total** | **1614** | **1122** | **69.5** |
| Dabakala | Jan-22 | 0 | | |
| | Feb-22 | 2 | 2 | 100.0 |
| | Mar-22 | 19 | 16 | 84.2 |
| | Apr-22 | 247 | 133 | 53.8 |
| | May-22 | 263 | 258 | 98.1 |
| | Jun-22 | 246 | 193 | 78.5 |
| | Jul-22 | 317 | 290 | 91.5 |
| | Aug-22 | 294 | 264 | 89.8 |
| | Sep-22 | 206 | 187 | 90.8 |
| | Oct-22 | 233 | 229 | 98.3 |
| | Nov-22 | 71 | 71 | 100.0 |
| | Dec-22 | 87 | 77 | 88.5 |
| | **Total** | **1985** | **1720** | **86.6** |
| Nassian | Jan-22 | 8 | 1 | 12.5 |
| | Feb-22 | 52 | 48 | 92.3 |
| | Mar-22 | 1 | 1 | 100.0 |
| | Apr-22 | 139 | 54 | 38.8 |
| | May-22 | 198 | 92 | 46.5 |
| | Jun-22 | 246 | 115 | 46.7 |
| | Jul-22 | 177 | 144 | 81.4 |
| | Aug-22 | 32 | 13 | 40.6 |
| | Sep-22 | 240 | 83 | 34.6 |
| | Oct-22 | 144 | 77 | 53.5 |
| | Nov-22 | 8 | 5 | 62.5 |
| | Dec-22 | 10 | 10 | 100.0 |
| | **Total** | **1255** | **643** | **51.2** |
| Sakassou | Jan-22 | 284 | 233 | 82.0 |
| | Feb-22 | 313 | 269 | 85.9 |
| | Mar-22 | 207 | 160 | 77.3 |
| | Apr-22 | 220 | 154 | 70.0 |
| | May-22 | 258 | 136 | 52.7 |
| | Jun-22 | 207 | 156 | 75.4 |
| | Jul-22 | 2070 | 160 | 77.3 |
| | Aug-22 | 212 | 208 | 98.1 |
| | Sep-22 | 200 | 154 | 77.0 |
| | Oct-22 | 204 | 140 | 68.6 |
| | Nov-22 | 198 | 161 | 81.3 |
| | Dec-22 | 199 | 182 | 91.5 |
| | **Total** | **2502** | **1953** | **78.1** |

## TABLE A-9: MONTHLY PARITY RATES OF DISSECTED *AN. GAMBIAE* S.L. FROM INDOOR AND OUTDOOR COLLECTIONS

| Site | Month | 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-22 | 2 | 2 | 100.0 | 5 | 5 | 100.0 |
| | Feb-22 | 0 | | | 0 | | |
| | Mar-22 | 9.00 | 9 | 100.0 | 11.00 | 10.00 | 90.9 |
| | Apr-22 | 30 | 27 | 90.0 | 59 | 52 | 88.1 |
| | May-22 | 34 | 31 | 91.2 | 21 | 18 | 85.7 |
| | Jun-22 | 79 | 30 | 38.0 | 157 | 62 | 39.5 |
| | Jul-22 | 71 | 58 | 81.7 | 114 | 98 | 86.0 |
| | Aug-22 | 80 | 35 | 43.8 | 161 | 92 | 57.1 |
| | Sep-22 | 98 | 88 | 89.8 | 137 | 103 | 75.2 |
| | Oct-22 | 87 | 45 | 51.7 | 94 | 47 | 50.0 |
| | Nov-22 | 90 | 82 | 91.1 | 113 | 102 | 90.3 |
| | Dec-22 | 60 | 49 | 81.7 | 102 | 77 | 75.5 |
| | **Total** | **640** | **456** | **71.3** | **974** | **666** | **68.4** |
| Dabakala | Jan-22 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Feb-22 | 1 | 1 | 100.0 | 1 | 1 | 100.0 |
| | Mar-22 | 7 | 6 | 85.7 | 12 | 10 | 83.3 |
| | Apr-22 | 80 | 49 | 61.3 | 167 | 84 | 50.3 |
| | May-22 | 118 | 115 | 97.5 | 145 | 143 | 98.6 |
| | Jun-22 | 93 | 73 | 78.5 | 153 | 120 | 78.4 |
| | Jul-22 | 127 | 112 | 88.2 | 190 | 178 | 93.7 |
| | Aug-22 | 110 | 100 | 90.9 | 184 | 164 | 89.1 |
| | Sep-22 | 82 | 72 | 87.8 | 124 | 115 | 92.7 |
| | Oct-22 | 106 | 104 | 98.1 | 127 | 125 | 98.4 |
| | Nov-22 | 20 | 20 | 100.0 | 51 | 51 | 100.0 |
| | Dec-22 | 28 | 25 | 89.3 | 59 | 52 | 88.1 |
| | **Total** | **772** | **677** | **87.7** | **1213** | **1043** | **86.0** |
| Nassian | Jan-22 | 6 | 1 | 16.7 | 2 | 0 | 0.0 |
| | Feb-22 | 36 | 34 | 94.4 | 16 | 14 | 87.5 |
| | Mar-22 | 1 | 1 | 100.0 | | | |
| | Apr-22 | 70 | 22 | 31.4 | 69 | 32 | 46.4 |
| | May-22 | 115 | 57 | 49.6 | 83 | 35 | 42.2 |
| | Jun-22 | 150 | 61 | 40.7 | 96 | 54 | 56.3 |
| | Jul-22 | 66 | 53 | 80.3 | 111 | 91 | 82.0 |
| | Aug-22 | 22 | 12 | 54.5 | 10 | 1 | 10.0 |
| | Sep-22 | 122 | 46 | 37.7 | 118 | 37 | 31.4 |
| | Oct-22 | 77 | 39 | 50.6 | 67 | 38 | 56.7 |
| | Nov-22 | 6 | 4 | 66.7 | 2 | 1 | 50.0 |
| | Dec-22 | 1 | 1 | 100.0 | 9 | 9 | 100.0 |
| | **Total** | **672** | **331** | **49.3** | **583** | **312** | **53.5** |
| Sakassou | Jan-22 | 160 | 131 | 81.9 | 124 | 102 | 82.3 |
| | Feb-22 | 131 | 111 | 84.7 | 182 | 158 | 86.8 |
| | Mar-22 | 91 | 68 | 74.7 | 116 | 92 | 79.3 |
| | Apr-22 | 98 | 69 | 70.4 | 122 | 85 | 69.7 |
| | May-22 | 134 | 73 | 54.5 | 124 | 63 | 50.8 |
| | Jun-22 | 87 | 67 | 77.0 | 120 | 89 | 74.2 |
| | Jul-22 | 105 | 82 | 78.1 | 102 | 78 | 76.5 |
| | Aug-22 | 64 | 63 | 98.4 | 148 | 145 | 98.0 |
| | Sep-22 | 72 | 53 | 73.6 | 128 | 101 | 78.9 |
| | Oct-22 | 105 | 74 | 70.5 | 99 | 66 | 66.7 |
| | Nov-22 | 88 | 72 | 81.8 | 110 | 89 | 80.9 |
| | Dec-22 | 96 | 89 | 92.7 | 103 | 93 | 90.3 |
| | **Total** | **1126** | **870** | **77.3** | **1376** | **1083** | **78.7** |

## TABLE A-10: 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-22 | 11 | 0 | 0.000 | 0.45 | 0.000 |
| | Feb-22 | 5 | 0 | 0.000 | 0.05 | 0.000 |
| | Mar-22 | 31 | 1 | 0.032 | 1.30 | 0.042 |
| | Apr-22 | 84 | 1 | 0.012 | 5.65 | 0.067 |
| | May-22 | 58 | 1 | 0.017 | 4.25 | 0.073 |
| | Jun-22 | 90 | 0 | 0.000 | 34.70 | 0.000 |
| | Jul-22 | 99 | 1 | 0.010 | 24.95 | 0.252 |
| | Aug-22 | 100 | 1 | 0.010 | 33.65 | 0.337 |
| | Sep-22 | 99 | 1 | 0.010 | 30.35 | 0.307 |
| | Oct-22 | 100 | 1 | 0.010 | 35.45 | 0.355 |
| | Nov-22 | 100 | 5 | 0.050 | 12.75 | 0.638 |
| | Dec-22 | 99 | 2 | 0.020 | 10.20 | 0.206 |
| Dabakala | Jan-22 | 3 | 1 | 0.333 | 0.00 | 0.000 |
| | Feb-22 | 2 | 0 | 0.000 | 0.10 | 0.000 |
| | Mar-22 | 16 | 0 | 0.000 | 1.30 | 0.000 |
| | Apr-22 | 128 | 6 | 0.047 | 67.65 | 3.171 |
| | May-22 | 100 | 1 | 0.010 | 36.20 | 0.362 |
| | Jun-22 | 101 | 1 | 0.010 | 52.35 | 0.518 |
| | Jul-22 | 100 | 5 | 0.050 | 47.60 | 2.380 |
| | Aug-22 | 100 | 1 | 0.010 | 55.75 | 0.558 |
| | Sep-22 | 100 | 0 | 0.000 | 72.10 | 0.000 |
| | Oct-22 | 100 | 4 | 0.040 | 78.45 | 3.138 |
| | Nov-22 | 73 | 4 | 0.055 | 4.50 | 0.247 |
| | Dec-22 | 87 | 0 | 0.000 | 6.15 | 0.000 |
| Nassian | Jan-22 | 1 | 0 | 0.000 | 0.55 | 0.000 |
| | Feb-22 | 54 | 2 | 0.037 | 3.30 | 0.122 |
| | Mar-22 | 1 | 0 | 0.000 | 0.05 | 0.000 |
| | Apr-22 | 55 | 2 | 0.036 | 8.70 | 0.316 |
| | May-22 | 97 | 0 | 0.000 | 12.40 | 0.000 |
| | Jun-22 | 102 | 5 | 0.049 | 22.90 | 1.123 |
| | Jul-22 | 100 | 1 | 0.010 | 20.05 | 0.201 |
| | Aug-22 | 36 | 1 | 0.028 | 2.10 | 0.058 |
| | Sep-22 | 72 | 2 | 0.028 | 19.75 | 0.549 |
| | Oct-22 | 100 | 4 | 0.040 | 26.40 | 1.056 |
| | Nov-22 | 30 | 3 | 0.100 | 0.55 | 0.055 |
| | Dec-22 | 76 | 5 | 0.066 | 0.70 | 0.046 |
| Sakassou | Jan-22 | 100 | 2 | 0.020 | 32.30 | 0.646 |
| | Feb-22 | 100 | 4 | 0.040 | 127.55 | 5.102 |
| | Mar-22 | 93 | 0 | 0.000 | 258.15 | 0.000 |
| | Apr-22 | 100 | 0 | 0.000 | 219.10 | 0.000 |
| | May-22 | 107 | 0 | 0.000 | 121.40 | 0.000 |
| | Jun-22 | 100 | 1 | 0.010 | 149.15 | 1.492 |
| | Jul-22 | 100 | 0 | 0.000 | 51.20 | 0.000 |
| | Aug-22 | 100 | 0 | 0.000 | 59.50 | 0.000 |
| | Sep-22 | 100 | 0 | 0.000 | 101.15 | 0.000 |
| | Oct-22 | 100 | 0 | 0.000 | 113.60 | 0.000 |
| | Nov-22 | 100 | 0 | 0.000 | 142.95 | 0.000 |
| | Dec-22 | 95 | 0 | 0.000 | 99.70 | 0.000 |

| Site | Month | Number *An. funestus* s.l. Tested | Number Infected | Sporozoite Rate | HBR (b/p/n) | EIR (ib/p/n) |
|---|---|---|---|---|---|---|
| Beoumi | Jan-22 | 5 | 0 | 0.000 | 0.4 | 0 |
| | Feb-22 | 4 | 0 | 0.000 | 0.1 | 0 |
| | Mar-22 | 4 | 0 | 0.000 | 0.55 | 0 |
| | Apr-22 | 2 | 0 | 0.000 | 0.2 | 0 |
| | Sep-22 | 2 | 0 | 0.000 | 1.1 | 0 |
| Dabakala | Dec-22 | 1 | 0 | 0.000 | 0.45 | 0 |
| Nassian | Feb-22 | 5 | 1 | 0.200 | 0.4 | 0.080 |
| | Apr-22 | 1 | 0 | 0.000 | 0.1 | 0.000 |
| | Aug-22 | 28 | 1 | 0.036 | 3.1 | 0.111 |
| | Oct-22 | 29 | 1 | 0.034 | 9.3 | 0.321 |
| | Nov-22 | 24 | 1 | 0.042 | 3.3 | 0.138 |
| | Dec-22 | 29 | 4 | 0.138 | 4.8 | 0.662 |

## TABLE A-11: INSECTICIDE SUSCEPTIBILITY: MORTALITY PERCENTAGE FROM TEST RESULTS FROM THE 18 SITES

| Insecticide | Abg oville | Beoumi | Bond oukou | Bongou anou | Bouafle | Daba kala | Dimb okro | Divo | Ferkesse dougou | Guiglo | M'Bah iakro | Nassian | Saka ssou | Sass andra | Seguela | Soubre | Tingrela | Touba |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alpha-cypermethrin | 4.0 (100) | 0.0 (71) | 0.0 (87) | 5.6 (89) | 1.1 (88) | 20.0 (45) | 0.0 (78) | 30.8 (104) | 5.9 (102) | 9.1 (99) | 4.5 (88) | 3.8 (79) | 7.1 (98) | 4.3 (94) | 1.2 (81) | 20.5 (83) | 5.1 (39) | 0.0 (101) |
| PBO+Alpha-cypermethrin | 26.5 (102) | 2.5 (80) | 13.3 (75) | 50.7 (69) | 37.2 (78) | 72.5 (40) | 45.7 (70) | 64.4 (87) | 85.4 (103) | 35.6 (101) | 44.4 (90) | 79.5 (78) | 33.0 (100) | 61.5 (96) | 26.3 (80) | 32.9 (82) | 88.1 (42) | 10.8 (93) |
| Alpha-cypermethrin 5X | 10.9 (92) | 2.6 (78) | 9.2 (76) | 51.4 (70) | 25.8 (89) | 80.6 (36) | 26.0 (77) | 83.5 (85) | 20.0 (95) | 5.0 (40) | 22.5 (80) | 42.0 (100) | 29.8 (84) | 25.0 (81) | 21.1 (71) | 27.2 (92) | 12.7 (63) | 0.0 (85) |
| Alpha-cypermethrin 10X | 35.1 (97) | 12.2 (98) | 17.9 (84) | 96.8 (94) | 60.9 (92) | 90.0 (40) | 53.9 (76) | 92.2 (103) | 35.4 (82) | 12.5 (40) | 43.4 (76) | 63.2 (95) | 82.0 (89) | 29.5(95) | 51.9 (77) | 38.5 (91) | 48.3 (60) | 3.0 (100) |
| Deltamethrin | 5.0 (100) | 0 (77) | 1.1 (91) | 15.2 (66) | 36.6 (71) | 42.2 (45) | 0.0 (86) | 28.4 (88) | 21.6 (97) | 15.0 (80) | 2.6 (76) | 12.0 (75) | 15.1 (93) | 42.4 (85) | 3.0 (67) | 11.2 (116) | 9.5 (95) | 0.0 (91) |
| PBO + Deltamethrin | 49.5 (101) | 6.1 (66) | 20.3 (79) | 92.1 (63) | 97.6 (84) | 100.0 (45) | 60.5 (81) | 85.9 (99) | 68.9 (90) | 86.3 (80) | 36.2 (69) | 77.6 (76) | 41.8 (91) | 89.7 (97) | 42.6 (68) | 56.0 (91) | 62.8 (94) | 12.1 (91) |
| Deltamethrin 5X | 35.5 (93) | 2.7 (75) | 65.1 (86) | 89.0 (100) | 75.3 (81) | 97.7 (43) | 18.5 (65) | 23.2 (82) | 78.3 (92) | 20.0 (80) | 17.6 (85) | 46.9 (81) | 38.7 (93) | 51.6 (95) | 70.6 (68) | 25.9 (112) | 53.6 (84) | 0.0 (96) |
| Deltamethrin 10X | 58.0 (100) | 40.0 (75) | 84.1 (82) | 97.0 (100) | 93.3 (89) | 95.5 (44) | 33.8 (77) | 99.0 (96) | 72.9 (96) | 65.0 (80) | 57.5 (73) | 83.2 (95) | 57.9 (95) | 82.6 (92) | 90.9 (77) | 69.2 (91) | 93.3 (90) | 23.4 (64) |
| Permethrin | 2.0 (99) | 0.0 (86) | 0.0 (80) | 14.3 (70) | 15.9 (80) | 35.6 (45) | 4.0 (75) | 13.4 (97) | 2.1 (97) | 5.0 (40) | 0.0 (85) | 6.2 (81) | 18.5 (81) | 2.3 (87) | 24.7 (77) | 19.3 (83) | 12.5 (96) | 2.2 (91) |
| PBO + Permethrin | 17.6 (102) | 20.2 (84) | 6.0 (84) | 48.0 (75) | 62.4 (85) | 83.8 (37) | 33.3 (72) | 23.2 (99) | 53.9 (89) | 27.5 (40) | 4.5 (88) | 47.0 (83) | 23.5 (81) | 43.4 (83) | 42.5 (73) | 43.4 (83) | 92.4 (92) | 9.7 (103) |
| Permethrin 5X | 27.0 (89) | 8.1 (74) | 58.6 (87) | 83.0 (100) | 84.6 (78) | 95.7 (46) | 53.3 (92) | 72.8 (81) | 64.8 (88) | 95.0 (40) | 90.8 (98) | 42.0 (81) | 57.0 (86) | 36.0 (86) | 57.9 (76) | 70.4 (81) | 88.5 (96) | 16.1 (87) |
| Permethrin 10X | 69.9 (103) | 68.4 (79) | 92.4 (92) | 100.0 (97) | 95.1 (81) | 95.2 (42) | 90.2 (82) | 97.1 (102) | 66.3 (95) | 95.0 (40) | 93.9 (99) | 55.1 (89) | 72.7 (88) | 75.7 (70) | 100.0 (76) | 86.4 (88) | 96.9 (98) | 61.2 (85) |
| Pirimiphos-methyl 1X | 67.0 (100) | 100.0 (83) | 64.9 (77) | 15.9 (63) | 92.0 (87) | NC | 100.0 (75) | 100.0 (85) | 100.0 (100) | 100.0 (80) | 86.8 (76) | 98.8 (85) | 98.7 (76) | 100.0 (90) | 100.0 (94) | 100.0 (70) | 100.0 (97) | 100.0 (90) |
| Pirimiphos-methyl 5X | 100.0 (104) | NA | 100.0 (93) | 100.0 (98) | 100.0 (77) | NA | NA | NA | NA | NA | 100.0 (86) | NA | NA | NA | NA | NA | NA | NA |
| Chlorfenapyr | 67.3 (52) | 94.6 (56) | 39.6 (53) | 12.9 (85) | 76.3 (71) | 95.2 (42) | 95.1 (61) | 100.0 (66) | 93.7 (95) | 63.3 (60) | 96.0 (99) | 100.0 (75) | 99.0 (97) | 100.0 (63) | 95.2 (63) | 87.8 (82) | 100.0 (88) | 100.0 (48) |
| Clothianidin | 60.0 (55) | NC | 43.1 (65) | 52.2 (69) | 74.0 (100) | NC | 96.9 (65) | 41.8 (79) | 87.6 (97) | 85.0 (60) | 96.0 (75) | 89.5 (76) | 91.8 (49) | 60.0 (65) | 98.4 (63) | 87.6 (97) | 96.4 (84) | 17.9 (39) |

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's formula for pyrethroids

Resistance  confirmed  Suspected resistance Susceptible

## TABLE A-12: NUMBER AND PERCENTAGE OF *AN. GAMBIAE* SPECIES COLLECTED BY HLC, PSC, AND CDC LIGHT TRAP METHODS

| Site | Collection Method | Number Tested | *An. coluzzii (%)* | *An. gambiae* s.s. *(%)* |
|---|---|---|---|---|
| Beoumi | HLC | 306 | 188 (61.4) | 118 (38.6) |
| | PSC | 101 | 90 (89.1) | 11 (10.9) |
| | CDC | 15 | 8 (53.3) | 7 (46.7) |
| Dabakala | HLC | 251 | 149 (59.4) | 102 (40.6) |
| | PSC | 99 | 80 (80.8) | 19 (19.2) |
| | CDC | 70 | 46 (65.7) | 24 (34.3) |
| Nassian | HLC | 249 | 3 (1.2) | 246 (98.8) |
| | PSC | 94 | 0 (0) | 94 (100) |
| | CDC | 0 (0) | 0 (0) | 0 (0) |
| Sakassou | HLC | 375 | 375 (100) | 0 (0) |
| | PSC | 105 | 105 (100) | 0 (0) |
| | CDC | 47 | 46 (97.9) | 1 (2.1) |

## TABLE A-13: NUMBER AND PERCENTAGE OF *AN. GAMBIAE* SPECIES TESTED FOR RESISTANCE MONITORING

| Site | Tested | *An. coluzzii* | *An. gambiae* s.s. | *An. coluzzii/ An. gambiae* s.s. |
|---|---|---|---|---|
| Agboville | 60 | 60 (100) | 0 (0) | 0 (0) |
| Beoumi | 51 | 42 (82.4) | 7 (13.7) | 2 (3.9) |
| Bondoukou | 96 | 45 (46.9) | 51 (53.1) | 0 (0) |
| Bongouanou | 76 | 58 (76.3) | 16 (21.1) | 2 (2.6) |
| Bouafle | 162 | 149 (92) | 13 (8) | 0 (0) |
| Dabakala | 40 | 17 (42.5) | 18 (45) | 5 (12.5) |
| Dimbokro | 70 | 42 (60) | 28 (40) | 0 (0) |
| Divo | 73 | 57 (78.1) | 16 (21.9) | 0 (0) |
| Ferkéssédougou | 133 | 29 (21.8) | 103 (77.4) | 1 (0.8) |
| Guiglo | 64 | 64 (100) | 0 (0) | 0 (0) |
| M'Bahiakro | 107 | 63 (58.9) | 44 (41.1) | 0 (0) |
| Nassian | 70 | 0 (0) | 69 (98.6) | 1 (1.4) |
| Sakassou | 67 | 62 (92.5) | 5 (7.5) | 0 (0) |
| Sassandra | 51 | 44 (86.3) | 7 (13.7) | 0 (0) |
| Seguela | 51 | 17 (33.3) | 34 (66.7) | 0 (0) |
| Soubre | 86 | 67 (77.9) | 19 (22.1) | 0 (0) |
| Tingrela | 70 | 5 (7.1) | 65 (92.9) | 0 (0) |
| Touba | 70 | 38 (54.3) | 32 (45.7) | 0 (0) |

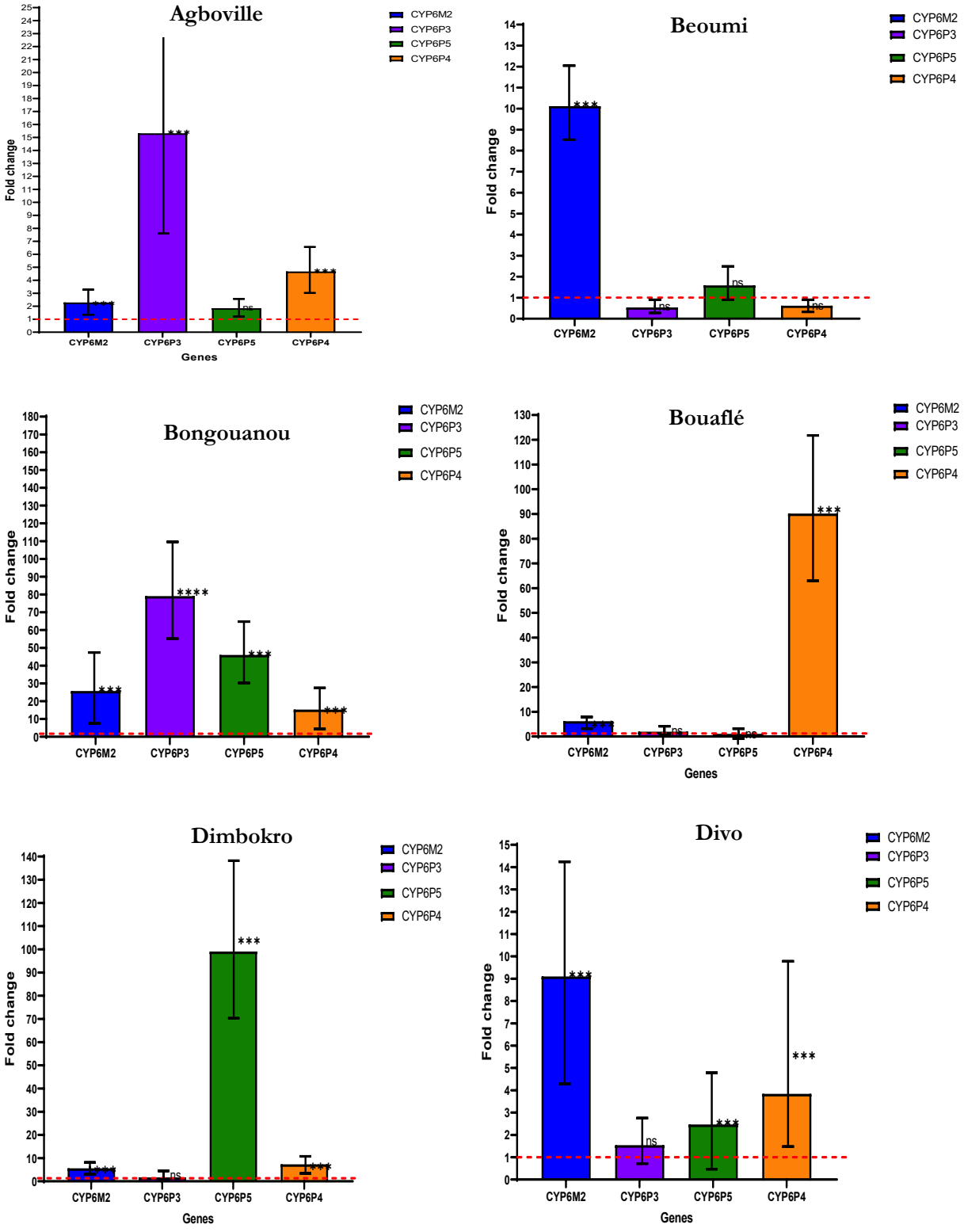
## TABLE A-14: NUMBER AND FREQUENCIES OF KDR-WEST AND KDR-EAST IN AN. GAMBIAE S.L. TESTED FOR RESISTANCE MONITORING

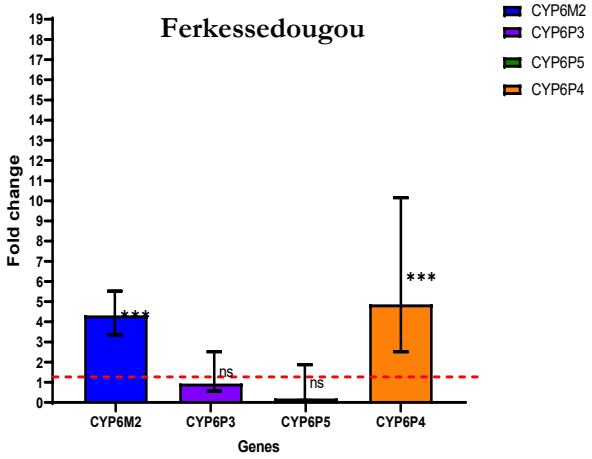
| Site | *kdr*-West L1014F | | | | | *kdr*-East L1014S | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total (n) | RR | RS | SS | % *kdr*-West (L1014F) | Total (n) | RR | RS | SS | % *kdr*-East (L1014S) |
| Béoumi | 48 | 5 | 4 | 39 | 14.58 | 25 | 1 | 4 | 20 | 12.0 |
| M'Bahiakro | 24 | 0 | 3 | 21 | 6.25 | 24 | 0 | 1 | 23 | 2.1 |
| Bongouanou | 24 | 13 | 8 | 3 | 70.83 | 25 | 0 | 3 | 22 | 6.0 |
| Guiglo | 26 | 0 | 12 | 14 | 23.08 | 25 | 0 | 0 | 25 | 0.0 |
| Touba | 26 | 1 | 20 | 5 | 42.31 | 25 | 1 | 5 | 19 | 14.0 |
| Séguéla | 25 | 6 | 19 | 0 | 62.00 | 25 | 1 | 0 | 24 | 4.0 |
| Bondoukou | 39 | 7 | 17 | 15 | 39.74 | 27 | 0 | 0 | 27 | 0.0 |
| Ferkéssédougou | 25 | 7 | 15 | 3 | 58.00 | 25 | 0 | 2 | 23 | 4.0 |
| Divo | 24 | 3 | 21 | 0 | 56.25 | 24 | 1 | 0 | 23 | 4.2 |
| Soubré | 26 | 3 | 18 | 5 | 46.15 | 25 | 0 | 0 | 25 | 0.0 |
| Sassandra | 25 | 1 | 20 | 4 | 44.00 | 25 | 1 | 3 | 21 | 10.0 |
| Sakassou | 25 | 2 | 19 | 4 | 46.00 | 24 | 0 | 7 | 17 | 14.6 |
| Bouaflé | 26 | 1 | 10 | 15 | 23.08 | 26 | 0 | 0 | 26 | 0.0 |
| Dimbokro | 26 | 11 | 14 | 1 | 69.23 | 26 | 0 | 0 | 26 | 0.0 |
| Agboville | 25 | 14 | 11 | 0 | 78.00 | 25 | 0 | 0 | 25 | 0.0 |
| Tingréla | 25 | 0 | 2 | 23 | 4.00 | 25 | 1 | 1 | 23 | 6.0 |
| Nassian | 25 | 6 | 12 | 7 | 48.00 | 25 | 0 | 0 | 25 | 0.0 |
| Dabakala | 24 | 10 | 13 | 1 | 68.75 | 24 | 0 | 0 | 24 | 0.0 |
| **Total** | **488** | **90** | **238** | **160** | **42.83** | **450** | **6** | **26** | **418** | **4.22** |

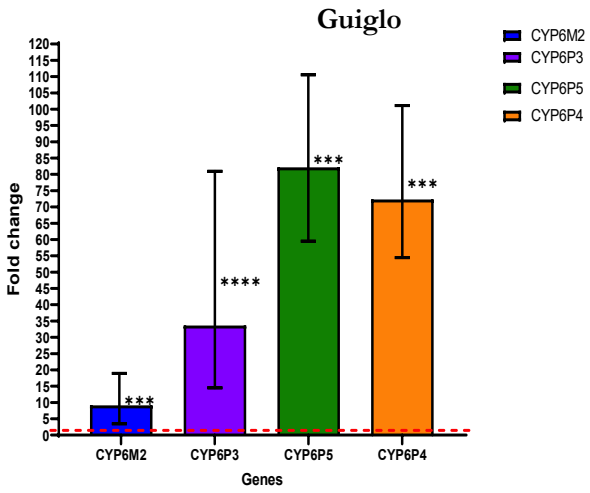
## TABLE A-15: NUMBER AND FREQUENCIES OF *ACE-1* IN *AN. GAMBIAE* S.L. TESTED FOR RESISTANCE MONITORING

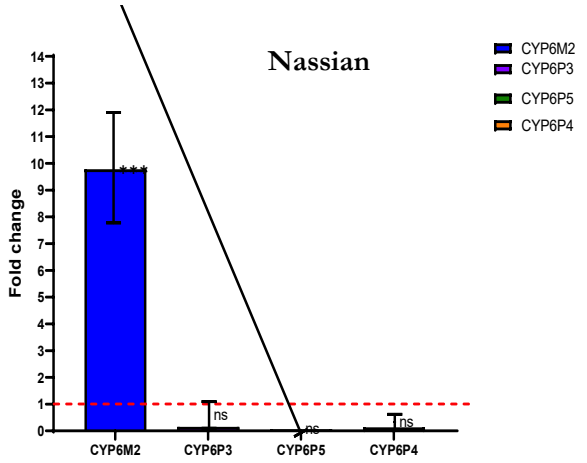
| Site | *Ace-1* G119S | | | | |
|---|---|---|---|---|---|
| | Total (n) | RR | RS | SS | %ACE-1 (G119S) |
| Béoumi | 20 | 0 | 20 | 0 | 50.00 |
| M'Bahiakro | 32 | 2 | 30 | 0 | 53.13 |
| Bongouanou | 40 | 1 | 38 | 1 | 50.00 |
| Guiglo | 20 | 1 | 18 | 1 | 50.00 |
| Touba | 18 | 1 | 13 | 4 | 41.67 |
| Séguéla | 16 | 2 | 12 | 2 | 50.00 |
| Bondoukou | 30 | 1 | 29 | 0 | 51.67 |
| Ferkéssédougou | 21 | 1 | 20 | 0 | 52.38 |
| Divo | 20 | 2 | 18 | 0 | 55.00 |
| Soubré | 32 | 0 | 26 | 6 | 40.63 |
| Sassandra | 24 | 0 | 13 | 11 | 27.08 |
| Sakassou | 19 | 0 | 18 | 1 | 47.37 |
| Bouaflé | 44 | 0 | 37 | 7 | 42.05 |
| Dimbokro | 19 | 2 | 12 | 5 | 42.11 |
| Agboville | 7 | 0 | 7 | 0 | 50.00 |
| Tingréla | 16 | 0 | 14 | 2 | 43.75 |
| Nassian | 19 | 0 | 16 | 3 | 42.11 |
| **Total** | 397 | 13 | 341 | 43 | **46.22** |

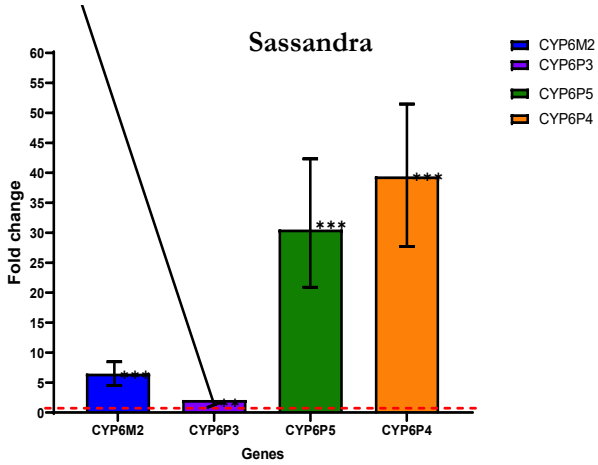
## FIGURE A1: CYP GENE AND FREQUENCY OF *AN. GAMBIAE* S.L. FOLD CHANGES AT EACH SITE SURVEYED

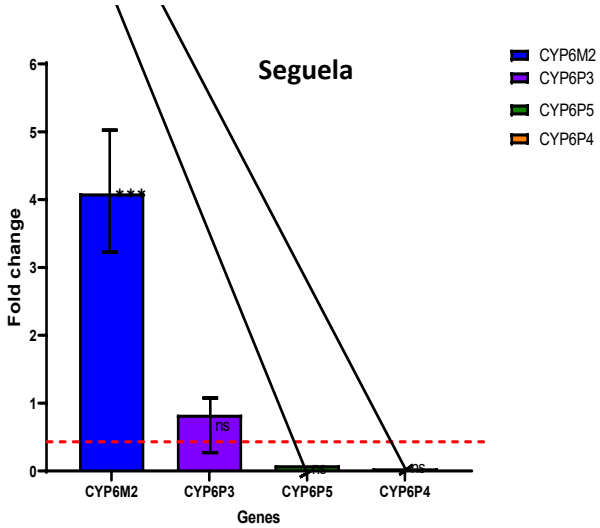



Ferkessedougou
Fold change
Genes
CYP6M2
CYP6P3
CYP6P5
CYP6P4
***
ns
ns
***


Guiglo
Fold change
Genes
CYP6M2
CYP6P3
CYP6P5
CYP6P4
***
****
***
***


Nassian
Fold change
Genes
CYP6M2
CYP6P3
CYP6P5
CYP6P4
***
ns
ns
ns


Sassandra
Fold change
Genes
CYP6M2
CYP6P3
CYP6P5
CYP6P4
***
***
***


Seguela
Fold change
Genes
CYP6M2
CYP6P3
CYP6P5
CYP6P4
***
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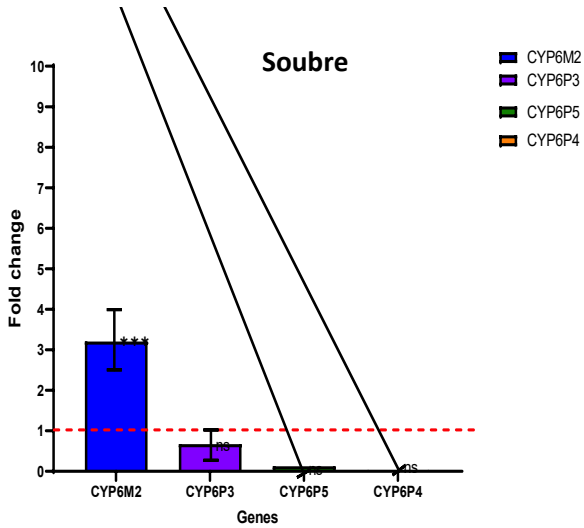
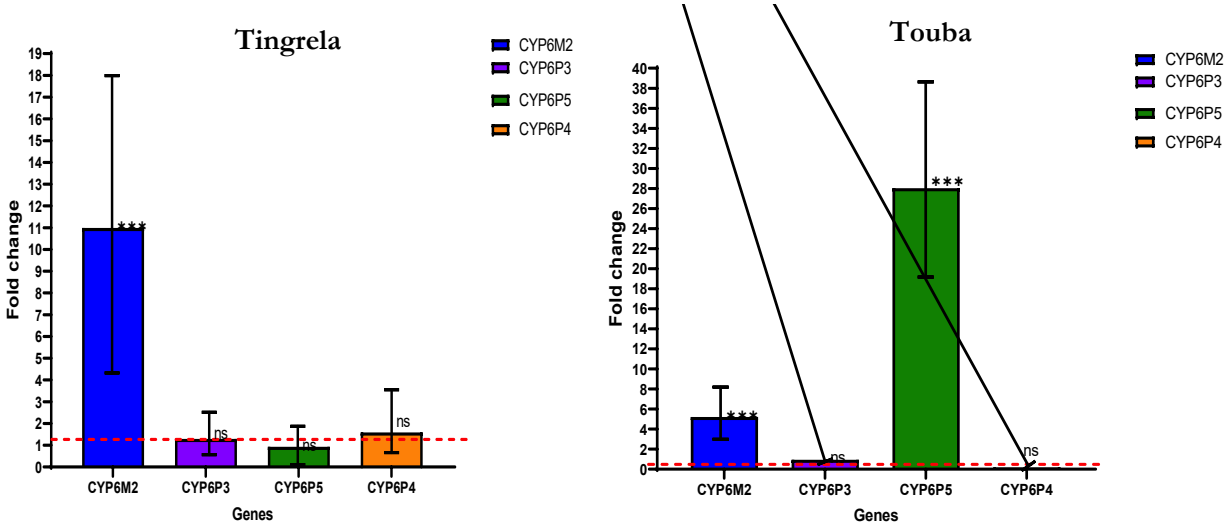
ns
ns


Soubre
Fold change
Genes
CYP6M2
CYP6P3
CYP6P5
CYP6P4
***
ns
ns
ns




## TABLE A-16: CYTOCHROME ENZYME DETECTION AND ANALYSIS

| | GENE NAME | FOLD CHANGE | CONFIDENCE INTERVAL | P-VALUE |
|---|---|---|---|---|
| AGBOVILLE | CYP6M2 | 2.29 | 1.47- 3.11 | P < 0.0001 |
| | CYP6P3 | 15.32 | 10.87- 21.59 | P < 0.0001 |
| | CYP6P5 | 1.85 | 1.25- 2.46 | P = 0.6648 |
| | CYP6P4 | 4.68 | 2.89- 6.46 | P < 0.0001 |
| BEOUMI | CYP6M2 | 10.1208833 | 8.28-11.96 | P < 0.0001 |
| | CYP6P3 | 0.5387330 | 0.46-0.62 | P = 0.6653 |
| | CYP6P5 | 1.5835118 | 0.78-2.39 | P = 0.7473 |
| | CYP6P4 | 0.6169676 | 0.41- 0.83 | P = 0.7370 |
| BONGOUANOU | CYP6M2 | 25.77 | 3.40-48.13 | P < 0.0001 |
| | CYP6P3 | 79.12 | 56.30 -111.18 | P < 0.0001 |
| | CYP6P5 | 46.00 | 23.55- 68-44 | P < 0.0001 |
| | CYP6P4 | 15.26 | 3.71- 26.80 | P < 0.0001 |
| BOUAFLE | CYP6M2 | 6.18 | 4.13- 8.23 | P < 0.0001 |
| | CYP6P3 | 1.96 | 1.65- 2.26 | P = 0.6355 |
| | CYP6P5 | 1.07 | 0.35- 1.79 | P = 0.9620 |
| | CYP6P4 | 90.16 | 64.15- 126.67 | P < 0.0001 |
| DIMBOKRO | CYP6M2 | 5.58 | 2.28- 8.87 | P < 0.0001 |
| | CYP6P3 | 1.70 | 0.04- 3.37 | P = 0.2180 |
| | CYP6P5 | 99.08 | 70.51-139.19 | P < 0.0001 |
| | CYP6P4 | 7.30 | 2.82- 11.77 | P < 0.0001 |
| DIVO | CYP6M2 | 9.10 | 3.64- 14.56 | P < 0.0001 |
| | CYP6P3 | 1.54 | 0.67- 2.41 | P = 0.7611 |
| | CYP6P5 | 2.47 | 0.42- 5.36 | P < 0.0001 |
| | CYP6P4 | 3.84 | 1.92-9.61 | P < 0.0001 |
| FERKESSEDOUGOU | CYP6M2 | 4.32 | 3.74- 4.89 | P < 0.0001 |
| | CYP6P3 | 0.93 | 0.58- 1.29 | P = 0.9593 |
| | CYP6P5 | 0.20 | 0.005- 0.40 | P = 0.2621 |
| | CYP6P4 | 4.86 | 2.33- 12.06 | P < 0.0001 |
| GUIGLO | CYP6M2 | 9.12845274 | 1.96- 16.29 | P < 0.0001 |
| | CYP6P3 | 33.637501 | 14.02- 81.29 | P < 0.0001 |
| | CYP6P5 | 82.1617861 | 58.47-115.45 | P < 0.0001 |
| | CYP6P4 | 72.2878696 | 51.44-101.59 | P < 0.0001 |
| NASSIAN | CYP6M2 | 9.78 | 7.73-11.83 | P < 0.0001 |
| | CYP6P3 | 0.15 | 0.08-1.07 | P = 0.1880 |

| | Gene Name | Fold Change | Confidence Interval | P-Value |
|---|---|---|---|---|
| | CYP6P5 | 0.06 | 0.01-0.16 | P = 0.0565 |
| | CYP6P4 | 0.13 | 0.04-0.75 | P = 0.1579 |
| Sassandra | CYP6M2 | 6.48 | 3.38- 9.58 | P < 0.0001 |
| | CYP6P3 | 2.11 | 1.72-2.50 | P < 0.0001 |
| | CYP6P5 | 30.54 | 21.71- 42.96 | P < 0.0001 |
| | CYP6P4 | 39.41 | 28.02- 55.41 | P < 0.0001 |
| Seguela | CYP6M2 | 4.09 | 3.27- 4.92 | P < 0.0001 |
| | CYP6P3 | 0.83 | 0.55- 1.11 | P = 0.8957 |
| | CYP6P5 | 0.09 | 0.02- 0.15 | P = 0.0983 |
| | CYP6P4 | 0.04 | 0.02- 0.06 | P = 0.4419 |
| Soubre | CYP6M2 | 3.20 | 2.41- 4.00 | P < 0.0001 |
| | CYP6P3 | 0.67 | 0.43- 0.91 | P = 0.7784 |
| | CYP6P5 | 0.13 | 0.009- 0.24 | P = 0.1579 |
| | CYP6P4 | 0.03 | 0.004- 0.06 | P = 0.0220 |
| Tingrela | CYP6M2 | 10.99 | 3.71- 18.26 | P < 0.0001 |
| | CYP6P3 | 1.29 | 0.48- 2.10 | P = 0.8577 |
| | CYP6P5 | 0.93 | 0.08- 1.95 | P = 0.9593 |
| | CYP6P4 | 1.59 | 0.63- 3.81 | P = 0.7440 |
| Touba | CYP6M2 | 5.22 | 2.71- 7.72 | P < 0.0001 |
| | CYP6P3 | 0.94 | 0.55- 1.34 | P = 0.7589 |
| | CYP6P5 | 28.04 | 19.92- 39.44 | P < 0.0001 |
| | CYP6P4 | 0.18 | 0.13-0.24 | P = 0.2328 |

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