US PRESIDENT'S MALARIA INITIATIVE ACTION TO REINFORCE MALARIA VECTOR CONTROL PROGRAM IN BENIN

Monitoring and Evaluation of the efficacy of the third year of Indoor Residual Spraying in Alibori and Donga, northern Benin, West Africa

Final report

Final report: September 2018- September 2019

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Abbreviations

Ace-1	Acetylcholinesterase
CDC	U.S. Centers for Disease Control and Prevention
CREC	Centre de Recherche Entomologique de Cotonou / Entomological Research Center of Cotonou
CS	Capsulated suspension
CSP	Circumsporozoite protein
DCO	Djougou-Copargo-Ouake
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
GST	Glutathione-S-transferase
HBR	Human Biting Rate
HLC	Human Landing Catch
HZ	Health zones
IRS	Indoor Residual Spraying
ITN	Insecticide-treated net
KGS	Kandi-Gogounou-Segbana
Kdr	Knock-down resistance
M&E	Monitoring and Evaluation
MFO	Mixed Function Oxidase
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction – Restriction Fragment Length Polymorphism
PSC	Pyrethrum Spray Catch
PM	Pirimiphos-Methyl
PMI	U.S. President's Malaria Initiative
SI	Sporozoite index
s.l.	Sensu lato
s.s.	Sensu stricto
WHO	World Health Organization

Executive summary

This annual report presents the evolution of entomological indicators in the IRS treated and control districts after the 2019 Indoor Residual Spray (IRS) campaign in northern Benin.

With the technical and financial support of the U.S. President's Malaria Initiative (PMI), Benin's National Malaria Control Program has been implementing Indoor Residual Spraying (IRS) in various parts of the country from 2008 to 2019. In 2019, IRS was conducted in 6 districts located in the Alibori department (Gogounou, Kandi, and Segbana district), and Donga department (Copargo, Djougou, and Ouake district). The PMI funded project, VectorLink[1], implemented the IRS campaign, using the insecticide, pirimiphos-methyl (Brand name: Actellic 300 CS). The Centre de Recherches Entomologiques de Cotonou (CREC) conducted an entomological evaluation to determine the impact of the IRS campaign on entomological indicators. Data was collected on mosquito behavior and entomological transmission indicators in IRS districts and compared the results with control areas (Bembereke and Kouande district) from September 2018 to September 2019.

To better assess the impact of IRS on malaria transmission, entomological indicators were compared not only between treated and control (untreated) areas but also during two different periods:

i. Period before the 2019 IRS intervention (from November 2018 to March 2019);
ii. Bio-efficacy period of Actellic 300 CS (from June 2019 to August 2019, when delayed mortality in Kisumu 24h bioassay ≥80%).

Twelve visits were made from November 2018 to August 2019 to collect mosquitoes, conduct advanced laboratory testing on *Anopheles gambiae* species collect and evaluate the efficacy of the spraying against the Kisumu strain of *An. gambiae* after the walls were treated.

The report includes the following data:

- Efficacy control of the spraying: Cone/Wall bioassay.
- Residual activity of pirimiphos-methyl
- Vector identification (species and molecular forms of *Anopheles gambiae* s.l.)
- Density of mosquitoes inside bedrooms of IRS areas compared to control areas
- Mosquito blood-feeding behaviors (endophagy, exophagy behaviors)
- Human Biting Rate (HBR)
- Entomological Inoculate Rate (EIR)

[1] https://pmivectorlink.org/about/the-pmi-vectorlink-project/

- Results of insecticide susceptibility tests
- Identification of mosquito genetic mutations that confer resistance (Kdr, Ace-1)

The main findings of the evaluation were as follows:

1. Average mosquito mortality at one- and two-months post-spray during wall bioassays was 100%, suggesting good spray quality. However, the residual activity only lasted up to four-month on cement and mud substrates, after which residual activity fell below the WHO recommended threshold of ≥80%.
2. The predominant malaria vector found in the collection was *Anopheles gambiae* s.l. comprising ~26% of mosquitoes caught.
3. Before the IRS campaign *An. gambiae* s.l. had higher rates of capture indoors in IRS districts; however, after IRS, *An. gambiae* s.l. had higher rates of capture outdoors. In control areas, *An. gambiae* s.l. had higher capture rates indoors before and after the IRS campaign.
4. Peak mosquito biting generally occurred in July and August indoor and outdoor across IRS and control sites ranging from 2 bites/person/night to 55 bites/person/night during those two months of collection.
5. Hourly HBR generally peaks between 11:00 pm and 3:00 am.
6. Significant differences in blood-feeding rate were not detected between IRS and control districts before or after the campaign.
7. Blood meals in *An. gambiae* s.l. from the study sites only comprised of human and bovine blood. In control areas, 97% of mosquitoes had a human blood meal, while in treatment areas 73% of mosquitoes had a human blood meal and 12% had mixed blood meals (human and bovine).
8. No difference in parity was observed between IRS districts (70.59%) and control districts (69.70%) before the campaign ($p= 0.972$). However, after the campaign, parity in IRS districts (41.36%) was significantly lower than the control district (65.78%) ($p<0.001$).
9. After the campaign, IRS sites had significantly lower HBR, sporozoite rates, and entomological inoculation rates (EIR) compared to control sites. However, apart from HBR, sporozoites rates and EIR were already higher in control sites than in IRS sites before the campaign.
10. After the IRS campaign, it was estimated that 75.2% of the malaria transmission, measured by EIR, originated outdoors and 24.8% originated outdoors in IRS areas. The outdoor-to-

indoor EIR risk ratio was 2.75% (95% CI: 0.31 – 79) in the IRS areas. For the control areas, it was estimated that 38.0% of the malaria transmission, measured by EIR, originated outdoors and 62% originated outdoors. The outdoor-to-indoor EIR risk ratio was 0.61% (95% CI: 0.34 – 1.08) in the control areas.

11. Resistance testing in Copargo, Djouguou, Gogounou, and Kandi showed that *An. gambiae* s.l. were susceptible pirimiphos-methyl in all sites. Resistance to bendiocarb was shown in Copargo, Djouguou, Gogounou (range: 82.55% to 88.57%), while in Kandi mosquitoes were completely susceptible. *An. gambiae* s.l. were resistant to deltamethrin in all sites with rates ranging from 26.37% in Gogounou and Kandi to 34.44% in Djougou.
12. Overall *An. gambiae* s.l. species were identified to be *An. gambiae* s.s. (74.42%), *An. coluzzi* (21.17%) and, *An. arabiensis* (4.4%).
13. There was a high frequency of the kdr gene in all *An. gambiae* s.s., *An. coluzzi*, *An. arabiensis* ranging from 50.00% to 89.29%. The Ace-I frequency ranged from 0 to 3.81%

The implications of these results are discussed in the remainder of the report.

1 Introduction

Implementation of Indoor Residual Spraying (IRS) in Benin since 2008 was accompanied by a drastic reduction in Entomological Inoculation Rate (EIR). After 6 years of the IRS in the Atacora region, Benin decided to temporarily stop this intervention in certain districts to avoid the emergence of insecticide resistance and to extend IRS in other regions [2]. As part of Benin's Insecticide Resistance Management strategy, IRS was withdrawn after 6 years of implementation. The temporary stopping of IRS may reduce the emergence of insecticide resistance by limiting the selection pressures on mosquitoes carrying resistance genes. Another reason for Indoor Residual Spraying withdrawal from Atacora was to offer an opportunity for other communities to be covered by IRS.

Since May 2017, eight districts were retained in Atacora, Alibori, and Donga regions for entomological monitoring of the IRS campaign. During the first and second year of the IRS, a significant reduction in Entomological Inoculation Rate (EIR) and a change in biting behavior of the main vector was observed in sprayed areas.

In 2019, IRS was renewed in 6 districts in Alibori and Donga with the complete withdrawal of this intervention from Atacora (Kerou and Pehunco). The main objective of this evaluation is to collect data on mosquito behavior and malaria transmission in IRS districts and compare the results with those obtained in the control areas (Bembereke and Kouande) during the period September 2018 to September 2019.

1.1 Objectives

- Evaluate the spray efficacy using the Kisumu strain of *An. gambiae* s.s. one week after the walls were treated;
- Assess the monthly pirimiphos-methyl decay rates on cement and mud walls using wall bioassay (cone test);
- Identify the different species in *Anopheles gambiae* s.l. populations by molecular assay
- Evaluate the density of vectors in IRS-targeted areas compared to control areas.
- Determine the sporozoite indices (SI) and the Entomological Inoculation Rate (EIR);
- Compare the density of mosquitoes inside bedrooms in IRS areas and control areas
- Mosquito blood-feeding behaviors (endophagy, exophagy behaviors)
- Evaluate the susceptibility of vectors to various classes of insecticides

[2] Akogbeto MC, Aikpon R, Azondekon R, Padonou G, Osse R, Agossa FR, Raymond Beach, Michel Sèzonlin. Six years of experience in entomological surveillance of indoor residual spraying against malaria transmission in Benin: lessons learned challenges and outlooks. Malar J. 2015; 14:242.

- Identification of mosquito genetic mutations that confer resistance (Kdr, Ace-1).

2 Methodology

2.1 Study areas

Two health zones (HZ) were protected by IRS in 2019 (Figure 1):

- HZ Copargo, Djougou, Ouake (Donga region)
- HZ Gogounou, Kandi, Segbana (Alibori region)

In total, 6 districts were used for monitoring and evaluation (M&E):

- IRS M&E districts
    - Copargo and Djougou in Donga,
    - Gogounou and Kandi in Alibori.
- Control districts
    - Bembereke, the closest district from Alibori, was used as the regional non-IRS control district for the Alibori region.
    - Kouande was selected to serve as the regional non-IRS control district for the Donga region because it is the only district near Copargo and Djougou.

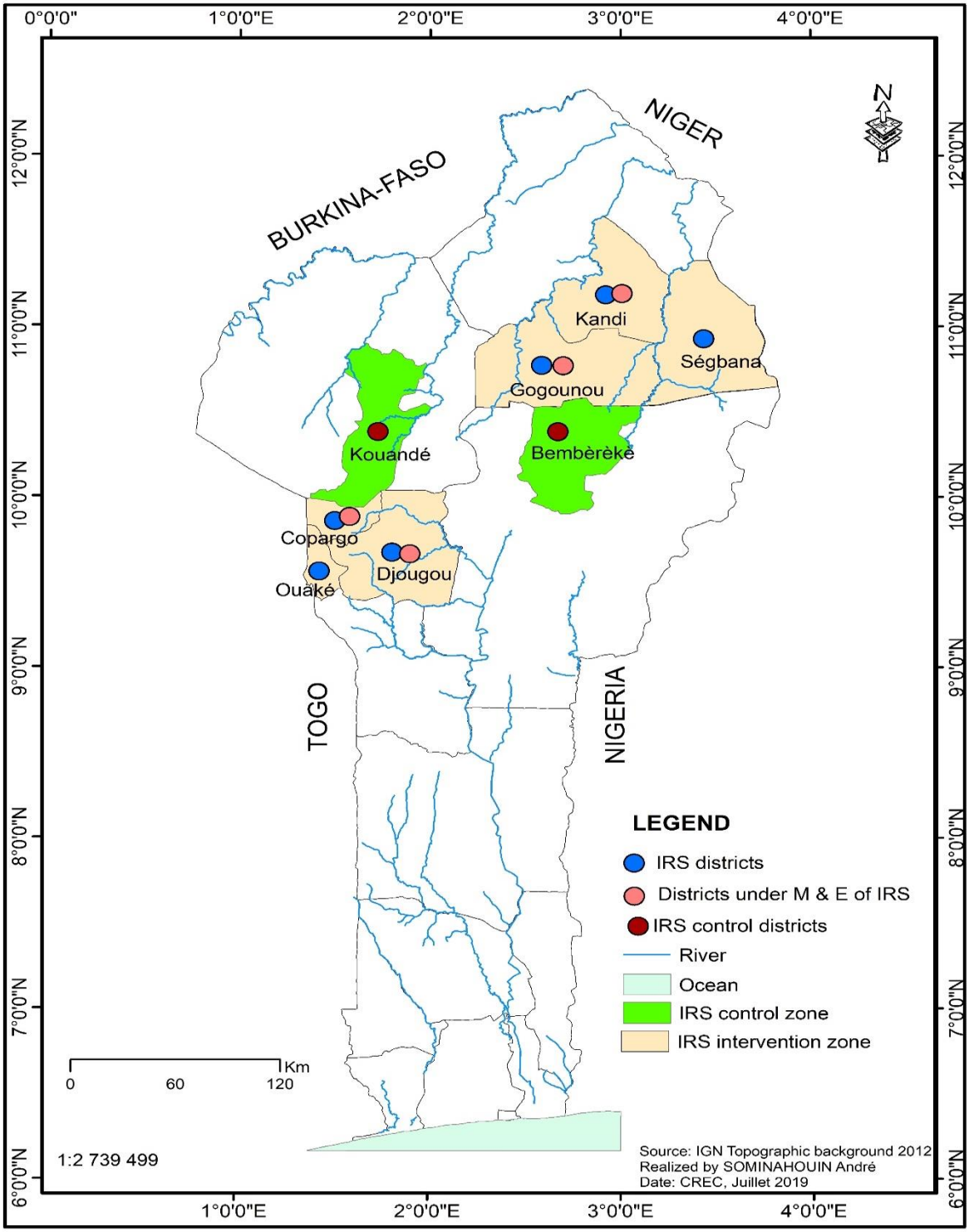


Figure 1. *Map showing the IRS evaluation areas*

Figure 2 provides the evolution of the overall number of monthly malaria cases from December 2018 to September 2019[3]. The Djougou-Copargo-Ouake (DCO) health zone had a higher number

[3] Malaria data from the National Malaria Control Program

of malaria cases compared to Kandi-Gogounou-Segbana (KGS). Figure 3 shows the monthly seasonal climate (rainfall and temperature) patterns in the Alibori and Donga regions in 2019[4].

In both health zones, the rainiest months are from June to September with the rainfall peak in August. Malaria cases are higher in the rainy season than in the dry season.

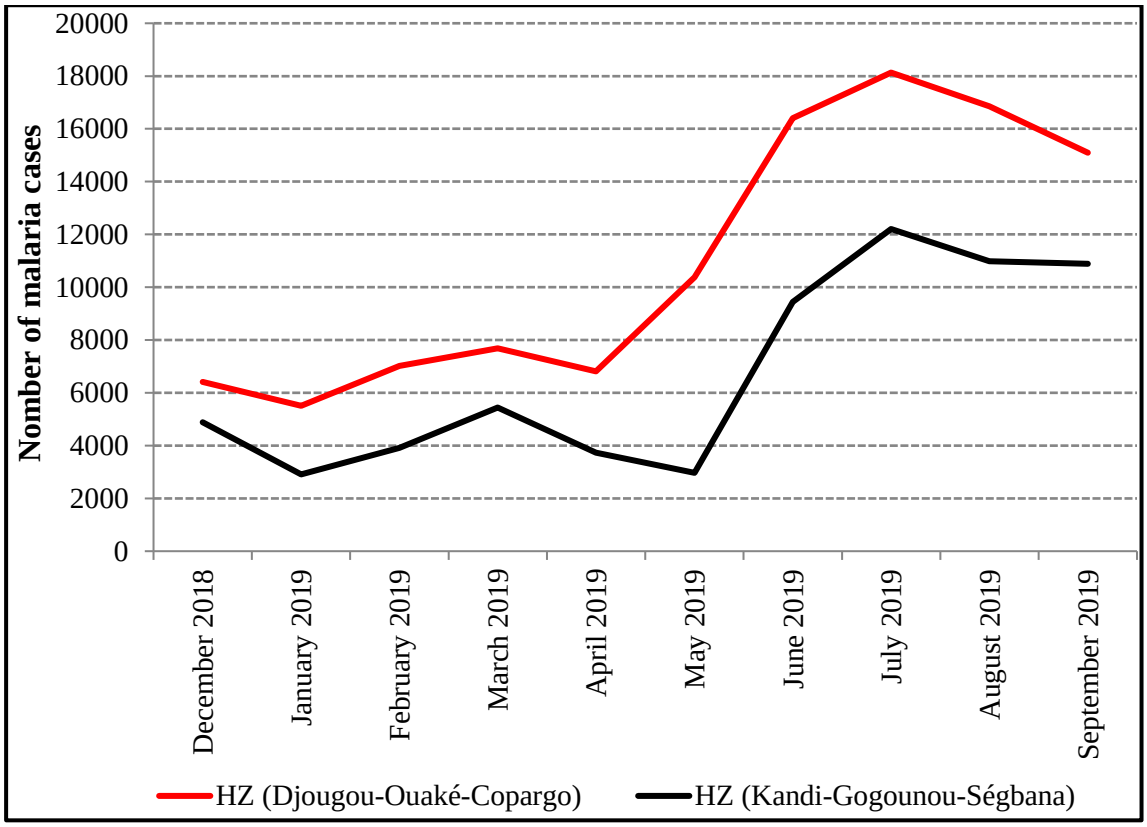


Figure 2. *Malaria cases (number tested positive) in Kandi-Gogounou-Segbana (KGS) and Djougou-Copargo-Ouake (DCO) health's zones*

[4] Climate data from World Bank Group, Climate Change Knowledge Portal(https://climateknowledgeportal.worldbank.org/country/benin/climate-data-historical)

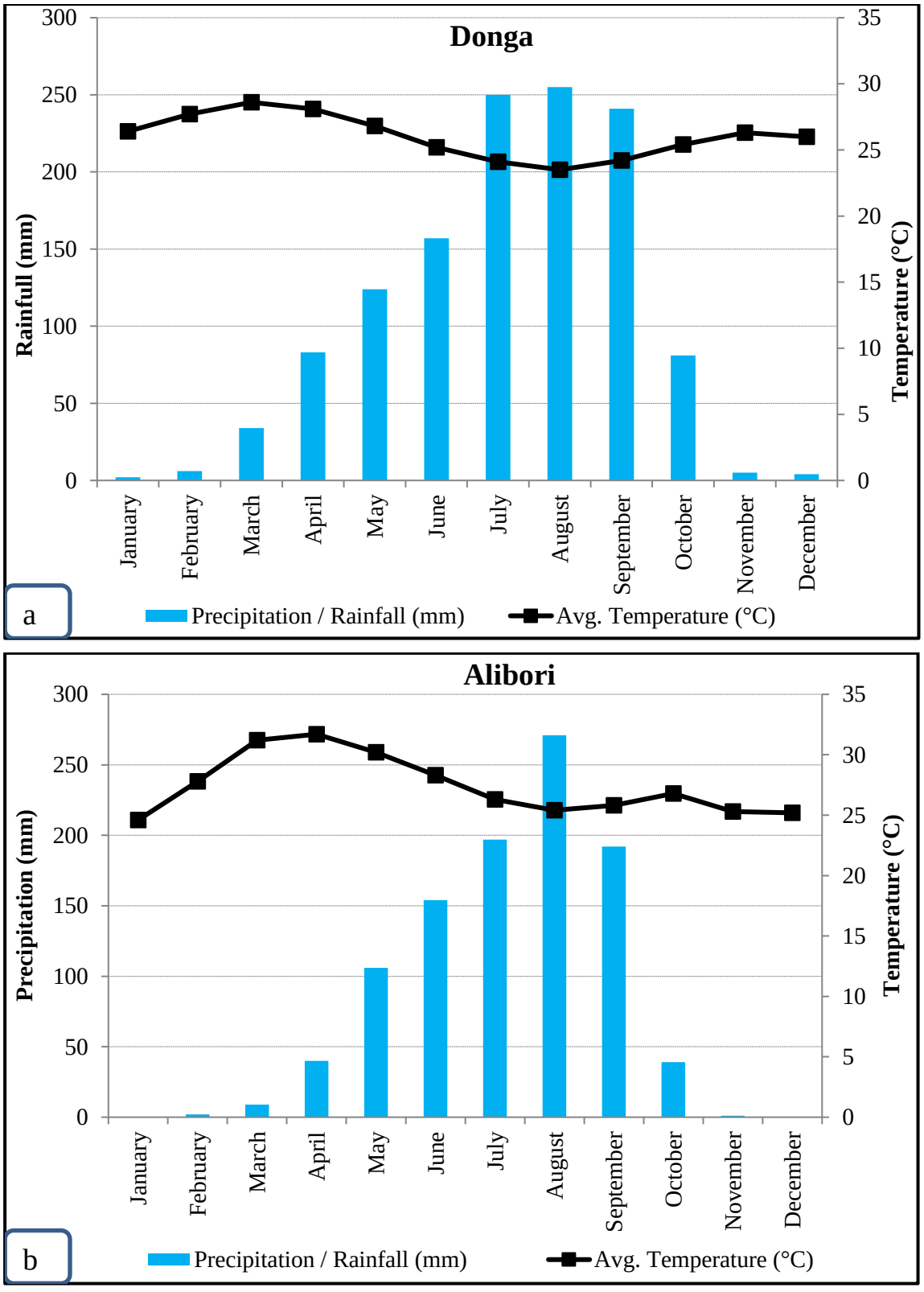


Figure 3. *Climate patterns (temperature and rainfall) for: a) Alibori region and b) Donga region*

2.2 Bioassay cone tests for residual activity on walls

A laboratory colony of *An. gambiae* s.s. Kisumu strain which is fully susceptible to all insecticides was used for the bioassays. WHO cone bioassays (WHO, 2006) [5] were conducted seven days post-spray (T0) in May 2019 in Djougou and Copargo districts to assess the quality of treatment in both districts. After the initial bioassay, residual activity monitoring was carried out every month in the treated districts to evaluate the persistence of the insecticide used on the wall surface. Using a mouth aspirator, 15 females *An. gambiae* Kisumu aged 2–5 days-old were carefully introduced into each cone, fixed at four different heights (0.5 m; 1.0 m; 1.5 m; 2.0 m) of the treated walls. Mosquitoes were exposed to the sprayed walls for 30 min; then removed from the cones and transferred to labeled sterile cups and provided with 10% sugar solution. After 24 h of observation at a temperature of 27 ± 2 °C and relative humidity of 80 ± 10%, the mortality rate was determined (Figure 4). When the control mortality was between 5–20%, the corrected mortality was performed accordingly using Abbott's formula [6]; when the control mortality was higher than 20%, the bioassay was considered invalid and repeated.

Figure 4. *Exposure for 30 minutes to cement and mud walls treated with pirimiphos-methyl and mortality reading after 24 hours of observation*

2.3 Sampling of malaria vectors

Mosquito sampling was carried out in the six districts:

- IRS districts: Djougou, Copargo, Kandi, and Gogounou
- Control areas: Kouande and Bembereke.

[5] World Health Organization 2006 Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets (https://www.who.int/whopes/resources/who_cds_ntd_whopes_gcdpp_2006.3/en/)
[6] Abbott WSA. Method of computing of insecticide effectiveness. J Econ Entomol. 1925; 18:265–7.

2.3.1 Human landing catches (HLCs)

Mosquitoes were collected by human landing catch (HLCs) in two villages per district, with one village located in the center of the district, and one village located at the periphery. For each village, mosquitoes were collected in 2 houses by 4 mosquito collectors: 2 mosquito collectors indoors and 2 outdoors. In total, 48 mosquito collectors were used for one round of collection. Two rounds of sampling were done per month. Two teams of eight mosquito collectors in each village worked inside and outside the selected dwellings, from 1900 to 0000 hours (7:00 PM to 12:00 AM) for the first team and from 0000 to 0700 hours (12:00 AM to 7:00 PM) for the second team. Mosquito collectors were rotated indoors and outdoors every hour through the different dwellings to avoid biases related to their mosquito sampling ability or individual attractiveness.

2.3.2 Indoor resting density

To estimate the density of mosquitoes per room, 10 houses per village were selected [7]. The bedrooms were sprayed with pyrethrum (mixed with water) and a white canvas was placed on the floor to collect knocked-down mosquitoes. After 15 minutes, all fallen mosquitoes were collected from the floor and placed in Petri dishes to determine the number of mosquitoes in the room and to estimate indoor behaviors.

Vector species that were collected and identified were transported to CREC's laboratory for dissection using a light microscope to determine the parous rates. The heads/thoraces of the vector species were analyzed by the ELISA method to look for circumsporozoite protein (CSP) antigens. Abdomens of female *An. gambiae* s.l. were used in PCR analyses to identify sibling species and molecular forms.

2.4 Insecticide resistance testing

2.4.1 Mosquito larval collections

Anopheles gambiae s.l. larvae were collected from natural mosquito larval habitats during the rainy seasons (August 2019). The mosquito larvae collected were transported in labeled plastic bottles to the insectary of the Centre de Recherche Entomologique de Cotonou (CREC) where they were maintained at 27 ± 2° C and 72 ± 5% relative humidity. The larvae were morphologically identified and separated for rearing. Adults obtained were provided with 10% sugar solution on cotton wool.

2.4.2 Phenotypic insecticide susceptibility tests

Unfed 2-5-day old *An. gambiae* s.l. adults from the larval collections were used for the WHO susceptibility test using various classes of insecticides. The susceptibility status of the population

[7] These houses were different from the houses used in the HLC collection

was graded according to the WHO protocol[8]. Dead and surviving mosquitoes from these bioassays were kept separately in Eppendorf tubes containing silica gel and stored at −20°C for further molecular analysis.

2.4.3 PCR detection of *Kdr* and *Ace-1* mutations

The PCR-RFLP diagnostic test was used to detect the presence of L1014F mutation (*Kdr*) and G119S mutation (*Ace 1R* gene).

2.5 Molecular species identification

Mosquitoes from HLCs, indoor resting density catches, and larval collection were analyzed using PCR according to the protocol of Santolamazza et al.[9] to determine species within the *An. gambiae* (s.l.) complex. The same mosquitoes were genotyped for the kdr L1014F, kdr L1014S, and G119S Ace-1 mutations, according to the protocols of Martinez-Torres et al.[10], Ranson et al.[11] , and Weill et al.[12], respectively.

3 Data analysis and entomological indicators

We calculated the following malaria transmission indicators:

Indicator	Source
Human biting rate (HBR)	HLC
Indoor resting density	PSC
Blood feeding rate	PSC
Parity rate	HLC
Sporozoite index (SI)	HLC

[8] WHO 2018 Test procedures for insecticide resistance monitoring in malaria vector mosquitoes – 2nd ed. (https://www.who.int/malaria/publications/atoz/9789241511575/en/)

[9] Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. Malar J. 2008;7:163.

[10] Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Bergé JB, Devonshire AL, Guillet P, Pasteur N. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. Insect Mol Biol.1998; 7: 179–184.

[11] Ranson H, Jensen B, Vulule J, Wang X, Hemingway J, Collins F. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect Mol Biol. 2000; 9:491–7.

[12] Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, Raymond M. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. *Insect* Mol Biol. 2004; 13: 1–7.

Data were analyzed with the statistical R software, version 2.8. using the stats package [13]. The Poisson method[14] was used to estimate and compare the confidence intervals of indoor vector density and entomological inoculation rates (EIRs) of *An. gambiae* (s.l.). The rate ratio method by unconditional maximum likelihood estimation (Wald) was used to compare the biting rate of *An. gambiae* (s.l.) and *Culex quinquefasciatus* early and late at night. The χ^2-test of comparison of proportions was used to compare the proportion of *An. gambiae* (s.l.) indoors and outdoors, blood-feeding rate, sporozoite index, and parity rate of *An. gambiae* (s.l.). These different parameters were compared before and after IRS and then between the treated and control areas. We calculated the % reduction in EIR in IRS areas compared to control areas using the Mulla's formula[15]:

$$\%R = 100 - [(C_1/T_1) \times (T_2/C_2)] \times 100;$$

where C_1 = pre-treatment EIR in unsprayed control area, C_2 = post-treatment EIR in unsprayed control area, T_1 = pre-treatment EIR in the sprayed area, and T_2 = post-treatment EIR in sprayed area.

4 Results

4.1 Residual effect of pirimiphos-methyl in-wall bioassays

Pirimiphos-methyl (Actellic CS) decay rates on treated cement and mud walls were evaluated for four months in 2019. At T0 (7 days after wall treatment; May 2019), there was 100% mortality in *An. gambiae* Kisumu exposed to walls treated with pirimiphos-methyl CS, regardless of the substrate (cement or mud) and wall height (Figure 5). This suggests good spray quality and evidence that the insecticide was available on walls at the lethal dose. Residual activity was above 80% for four months until September in 2019 IRS campaigns (Figure 5 and Table 1). Previous assessments in Benin have shown that the residual activity of Actellic 300 CS only lasts for four months[16]. Therefore, residual efficacy monitoring was only done up to five months of post spray.

[13] . R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2018.

[14] Rothman KJ. Epidemiology: an introduction. Oxford: Oxford University Press; 2012.

[15] Mulla MS, Norland RL, Fanara DM, Darwezeh HA, McKean DW. 1971. Control of chironomid midges in recreational lakes. J. Econ. Entomol. 64:300–307.

[16] https://www.pmi.gov/docs/default-source/default-document-library/implementing-partner-reports/benin-2017-entomological-monitoring-final-report.pdf

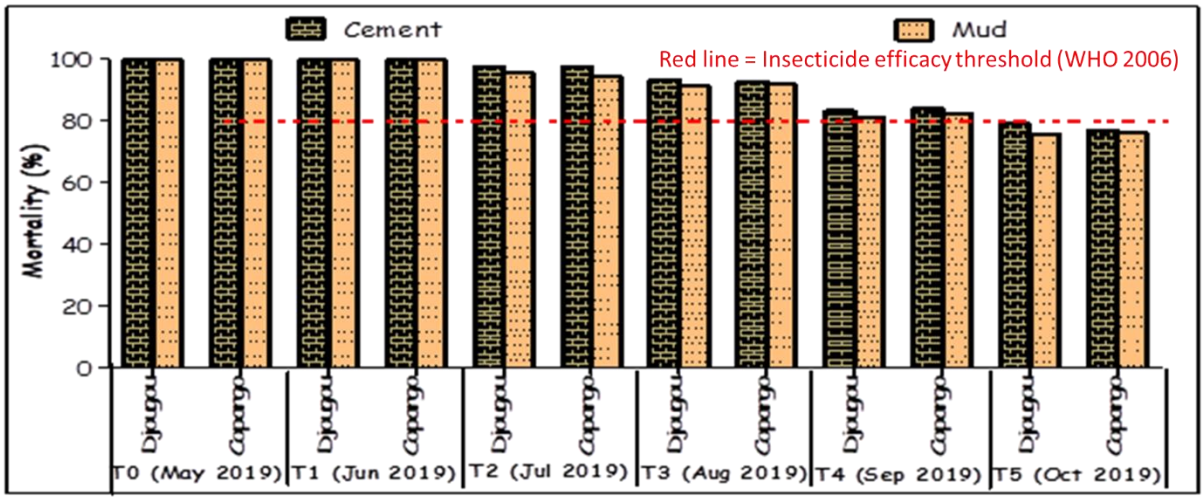


***Figure 5**. Residual activity of Actellic 300 CS on different surfaces (cement and mud) in Djougou and Copargo (IRS 2019 campaign).*

***Table 1**. Spraying quality and residual effect of pirimiphos-methyl 300 CS five months after IRS 2019 campaign.*

	T0 (May)		T1 (Jun)		T2 (Jul)		T3 (Aug)		T4 (Sep)		T5 (Oct)	
Wall type	Djougou	Copargo	Djougou	Copargo	Djougou	Copargo	Djougou	Copargo	Djougou	Copargo	Djougou	Copargo
Cement	100	100	100	100	97.83	97.85	93.58	92.93	83.33	84.23	79.18	77.08
Mud	100	100	100	100	95.73	94.58	91.76	91.89	81.32	82.46	75.99	76.50

4.2 Mosquito species composition in IRS and controls sites

During this evaluation, a total of 15,222 human-biting mosquitoes belonging to four genera (*Anopheles*, *Aedes*, *Culex*, *Mansonia*) and 14 species were collected in IRS and controls sites (Table 2). Out of the 14 species, *An. gambiae* s.l. was the second most abundant species collected (23.61% of the total of mosquitoes; 3,595 of 15,222) after *Culex quinquefasciatus* (Table 1). The two major malaria vectors collected were *An. gambiae* s.l. and *An. funestus*, albeit at low frequency.

Culex quinquefasciatus (74.05%; 11,272 of 15,222) and other *Culex* species were found. *Cx. quinquefasciatus* was the most abundant mosquito collected in all sites except Copargo. *Mansonia africana* (0.62%; 114 of 15,222) and *Aedes aegypti* (0.58%; 89 of 15,222) were collected but in a low proportion. The disparity between the frequency of Anophelinae and Culicinae in each site is explained by the ecological characteristics of the environment. The relative abundance of *Culex quinquefasciatus* may be due to the presence of larval habitats polluted (sewers, abandoned wells, and cisterns) with organic matters in urban areas. Such larval sites are choices of preference for the development of larvae of *Cx. quinquefasciatus*.

While many of the mosquitoes collected do not transmit malaria in Benin, they still have medical importance. *Culex quinquefasciatus* transmits *bancroftian filariasis* and West Nile Virus. *An. pharoensis* and *Mansonia africana* are important in the transmission of the Rift Valley Fever virus. *Aedes aegypti* transmits yellow fever and dengue fever with recent cases in southern Benin (Abomey –Calavi).

Table 2. *Mosquito species composition in IRS and controls sites (November 2018-August 2019)*

Species	Djougou	Copargo	Kandi	Gogounou	Bembereke	Kouande	Total
An. gambiae s.l.	434	346	493	309	1638	375	3595
An. funestus	3	3	3	4	6	10	29
An. pharoensis	0	0	3	1	5	1	10
An. paludis	0	0	0	0	1	1	2
An. ziemani	1	1	0	1	0	0	3
Culex quinquefasciatus	1500	90	2319	1044	5288	1031	11272
Culex nebulosus	6	24	2	1	2	10	45
Culex descens	0	19	0	1	2	3	25
Culex tigripes	2	7	0	0	0	0	9
Mansonia africana	85	12	4	3	3	7	114
Mansonia uniformis	0	0	0	0	1	0	1
Aedes aegypti	28	11	7	13	9	21	89
Aedes luteocephalus	1	0	0	0	4	0	5
Aedes vitatus	1	10	1	6	3	2	23
Total	2061	523	2832	1383	6962	1461	15222

4.3 Mosquito blood-feeding behaviors

4.3.1 Human Biting Rate (HBR) of *An. gambiae* s.l. indoors versus outdoors in treated and untreated houses

A total of 3,595 *An. gambiae* s.l. were caught from November 2018 to August 2019 in treated districts (Djougou, Copargo, Kandi, and Gogounou) and control districts (Bembereke and Kouande). Table 3, Figures 6 and 7 shows the proportion of *An. gambiae* s.l. indoors compared to outdoors in these districts. In Table 3 and Figures 6 and 7, two observations can be made:

1. Before the 2019 IRS campaign (the period from November 2018 to March 2019), the density of *An. gambiae* s.l. is low compared to the period from June 2019 to August 2019. During this period (November 2018 to March 2019), *An. gambiae* s.l. were collected more indoors in Bembereke, Kouande, Copargo, and Gogounou. Indoor and outdoor biting behavior in Djougou (Table 3) was similar. Globally, 52.82% (150/284) of *An. gambiae* s.l. were collected indoors in houses designated for IRS treatment compared to 47.18% (134/284) outdoors (p=0.208). In contrast, in houses designated as controls, 69% (69/100) were collected indoors versus 31% (31/100) outdoors (Figure 6). The low density of *An.*

gambiae s.l. in all localities during this period would be due to the harmattan season and the dry conditions which characterize it. This similar biting behavior was observed in *An. gambiae* s.l. inside and outside treated houses during this period is believed to be due to the decrease in the effect of the insecticide used in May 2018.

2. During the bio-efficacy period of Actellic CS (June 2019 to August 2019), the proportion of *An. gambiae* s.l. collected is significantly lower indoors compared to outdoors in all treated houses (P<0.001), except Gogounou (P = 0.138). In contrast, in untreated houses (Bembereke and Kouande), we recorded the opposite situation with a higher biting rate indoors (Table 3). Globally, 41.45% (538/1298) of *An. gambiae* s.l. was collected indoors in treated houses compared to 58.55% (760/1298) outdoors. In contrast, in untreated houses, 55.93% (1070/1913) were collected indoor versus 44.07% (843/1913) outdoors (Figure 7). This shows that treated houses pirimiphos-methyl on the walls has significantly reduced vectors indoors.

Tables 4 and 5 below present the details of the human biting rate (HBR) of *An. gambiae* s.l. indoors and outdoors in treated districts and control.

Table 3. *Number and proportion of* An. gambiae *s.l. caught indoors and outdoors before and after the IRS intervention (2019) in treated districts* vs *control*

Districts	Before 2019 IRS (Nov. 2018 – Mar. 2019)		P-value*	Actellic bio-efficacy period (Jun. 2019 – Aug. 2019)		P-value*
	Indoors	Outdoors		Indoors	Outdoors	
	nb (%)	nb (%)		nb (%)	nb (%)	
Djougou	12 (50.00)	12 (50.00)	1.000	172 (41.95)	238 (58.05)	<0.001
Copargo	31 (67.27)	18 (36.73)	0.015	119 (40.07)	178 (59.93)	<0.001
Kouande (control)	30 (69.77)	13 (30.23)	<0.001	196 (59.04)	136 (40.96)	<0.001
Kandi	33 (40.74)	48 (59.26)	0.027	165 (40.05)	247 (59.95)	<0.001
Gogounou	74 (56.92)	56 (43.08)	0.034	82 (45.81)	97 (54.19)	0.138
Bembereke (control)	39 (68.42)	18 (31.58)	<0.001	874 (55.28)	707 (44.72)	<0.001
Districts under IRS	150 (52.82)	134 (47.18)	0.208	538 (41.45)	760 (58.55)	<0.001
Control	69 (69.00)	31 (31.00)	<0.001	1070 (55.93)	843 (44.07)	<0.001

nb: number of *An. gambiae* s.l.; %: the proportion of *An. gambiae* s.l.; * P-value of comparison of the proportion of *An. gambiae* s.l. indoors and outdoors in the same district (Test used: Chi-square test)

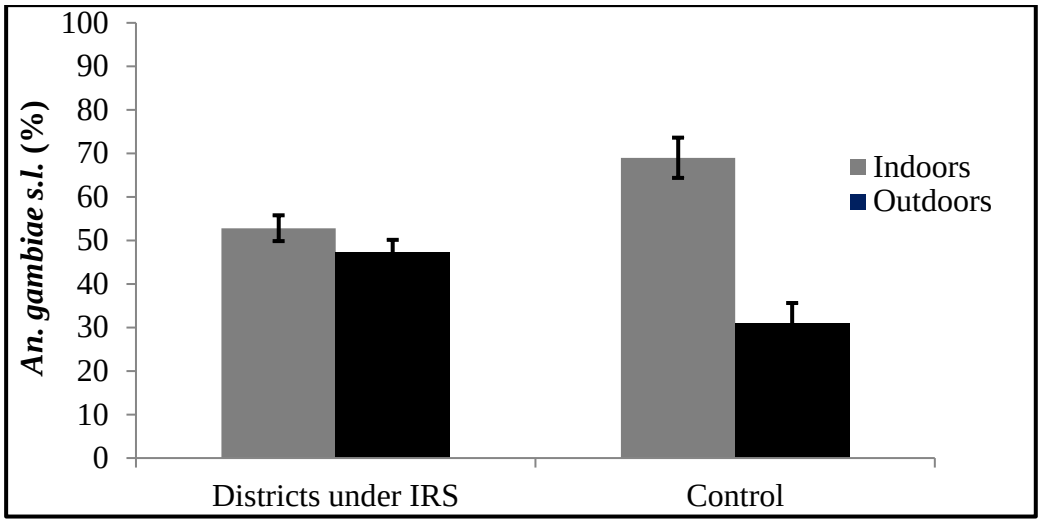


Figure 6*. The overall percentage of* An. gambiae *s.l. collected using HLC indoors and outdoors (before 2019 IRS) (period November 2018 to March 2019) in treated and untreated houses*

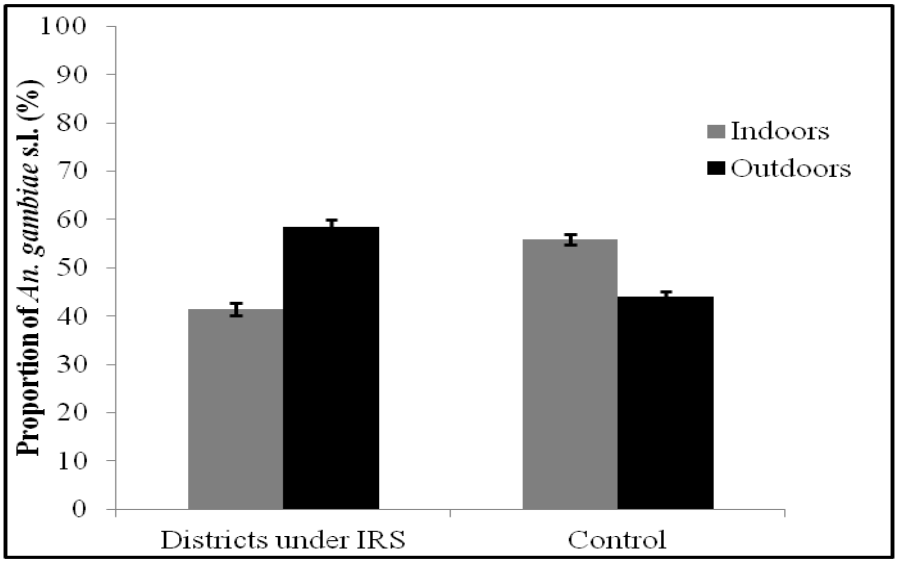


Figure 7. *The overall percentage of* An. gambiae *s.l. collected using HLC indoors and outdoors after 2019 IRS intervention (bio-efficacy period of Actellic 300CS: June 2019 to August 2019) in treated and untreated house.*

Table 4. *Human Biting Rate (HBR) of* An. gambiae *s.l. indoor and outdoor in treated districts (Alibori and Donga) and control district (Kouande).*

Districts	Position	Indicators	November 2018	January 2019	March 2019	June 2019	July 2019	August 2019
Djougou	Inside	Total Mosquitoes	3	6	3	33	80	59
		nb human catches	8	8	8	8	8	8
		HBR/night	0.375	0.750	0.375	4.125	10.000	7.375
	Outside	Total Mosquitoes	4	7	1	60	95	83
		nb human catches	8	8	8	8	8	8
		HBR/night	0.500	0.875	0.125	7.500	11.875	10.375
Copargo	Inside	Total Mosquitoes	5	22	4	19	44	56
		nb human catches	8	8	8	8	8	8
		HBR/night	0.625	2.750	0.500	2.375	5.500	7.000
	Outside	Total Mosquitoes	4	13	1	26	44	108
		nb human catches	8	8	8	8	8	8
		HBR/night	0.500	1.625	0.125	3.250	5.500	13.500
Kouande (control)	Inside	Total Mosquitoes	13	4	13	33	38	125
		nb human catches	8	8	8	8	8	8
		HBR/night	1.625	0.500	1.625	4.125	4.750	15.625
	Outside	Total Mosquitoes	7	1	5	13	19	104
		nb human catches	8	8	8	8	8	8
		HBR/night	0.875	0.125	0.625	1.625	2.375	13.000

Table 5. *Human Biting Rate (HBR) of* An. gambiae *s.l. indoors and outdoors in treated districts (Alibori) and control district (Bembereke).*

Districts	Position	Indicators	November 2018	January 2019	March 2019	June 2019	July 2019	August 2019
Kandi	Inside	Total Mosquitoes	4	19	10	4	55	106
		nb human catches	8	8	8	8	8	8
		HBR/night	0.500	2.375	1.250	0.500	6.875	13.250
	Outside	Total Mosquitoes	17	23	8	15	71	161
		nb human catches	8	8	8	8	8	8
		HBR/night	2.130	2.880	1.000	1.880	8.880	20.130
Gogounou	Inside	Total Mosquitoes	3	7	64	16	29	37
		nb human catches	8	8	8	8	8	8
		HBR/night	0.375	0.875	8.000	2.000	3.625	4.625
	Outside	Total Mosquitoes	5	11	40	23	29	45
		nb human catches	8	8	8	8	8	8
		HBR/night	0.625	1.375	5.000	2.875	3.625	5.625
Bembereke (control)	Inside	Total Mosquitoes	16	12	11	92	424	358
		nb human catches	8	8	8	8	8	8
		HBR/night	2	1.500	1.375	11.500	53.000	44.750
	Outside	Total Mosquitoes	12	2	4	60	352	295
		nb human catches	8	8	8	8	8	8
		HBR/night	1.500	0.250	0.500	7.500	44.000	36.875

4.4 Hourly night-time human biting rate (HBR) of *An. gambiae* (*s.l.*) and *Culex quinquefasciatus* from November 2018 to August 2019)

The hourly human biting rate (HBR) of *An. gambiae* s.l. and *Culex quinquefasciatus* was monitored in the treated districts (Kandi, Gogounou, Djougou, Copargo) and the control districts (Bembereke and Kouande).

The hourly HBR of *An. gambiae* s.l. and *Culex quinquefasciatus* was similar in treated and untreated (Bembereke and Kouande) houses (Figures 8, 9, 10, and 11), but the biting behavior (indoors vs outdoors) varied according to species and area (treated or untreated): *Culex quinquefasciatus* was collected more outdoors in treated and untreated houses from 7 p.m. to 7 a.m. unlike *An. gambiae* s.l. where mosquitoes were collected more outdoors in the treated areas and more mosquitoes were collected indoors in the control area. This cycle can be divided into 2 main periods:

i. During the period of 7 pm to 11 pm, the hourly biting rate of *An. gambiae* (s.l.) and *Culex quinquefasciatus* was low in both areas (treated and control area) but increases constantly to their peak between 12 a.m. and 2 a.m. (Figures 8a, 8b, 10a, and 10b).

ii. The biting rate of *An. gambiae* between 7 p.m. and 11 p.m. was significantly high indoors in IRS areas (p (Wald) = 0.029 for Rate ratio test) and control (p (Wald) = 0.001), whereas

between 11 p.m. and 7 a.m., the rate was significantly high outdoors the treated houses (p (Wald) < 0.001) (Figures 8a, 8b). In contrast, *Culex quinquefasciatus* showed a significantly high biting rate outdoors the houses in both areas between 7 pm and 11 pm and again between 11 pm and 7 am (p (Wald) < 0.05).

iii. After 2 a.m., the hourly biting rate gradually decreases until the early morning (Figures 9a, 9b, 9a, and 9b).

In summary, the period between 7 p.m. and 11 p.m. reflects a low biting rate, while between 11 p.m. and 7 a.m. a very high biting rate of *An. gambiae* (s.l.) is observed in both treated and untreated areas) (Figures 4b and 6b). Tables 6 and 7 show the number of *An. gambiae* (s.l.) and *Culex quinquefasciatus* in all treated districts and control districts.

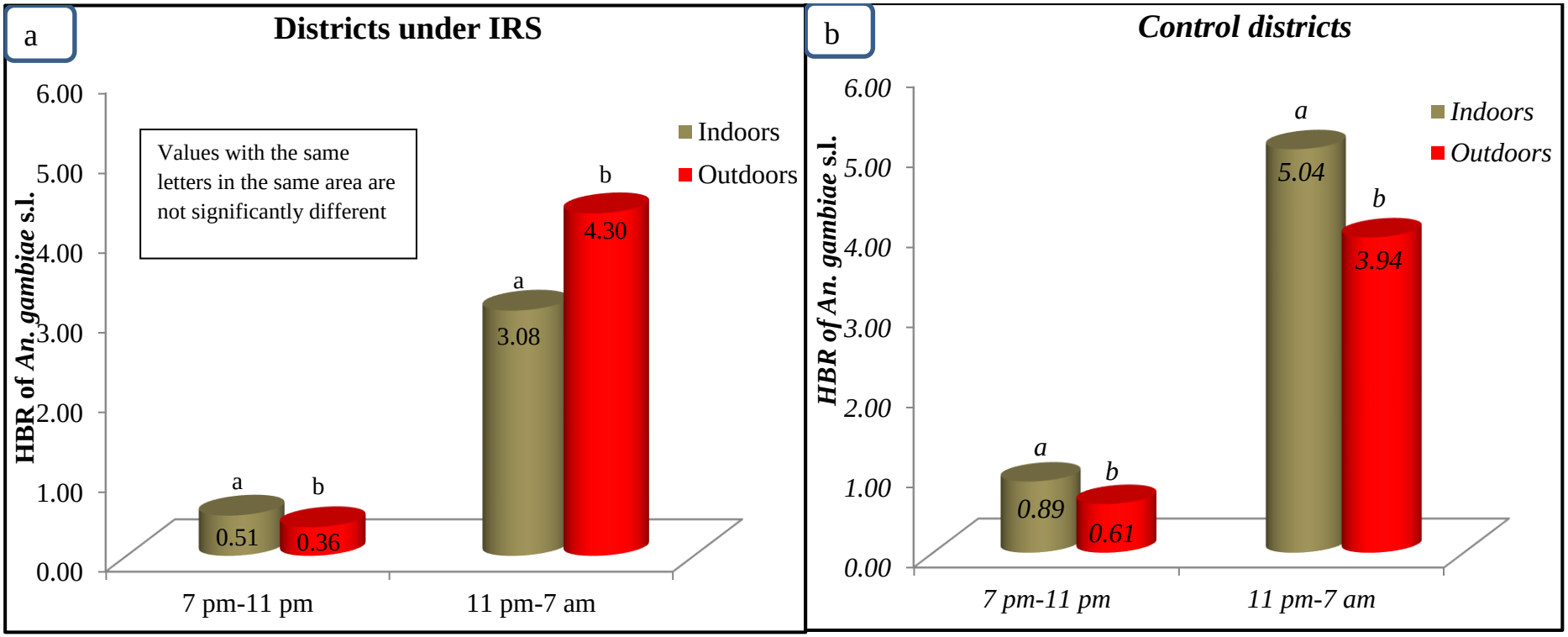


***Figure 8**. Biting location of An. gambiae* (s.l.) *early (7 pm-11 pm) and late at night in treated (a) and in control areas (b) (period November 2018 – August 2019).*

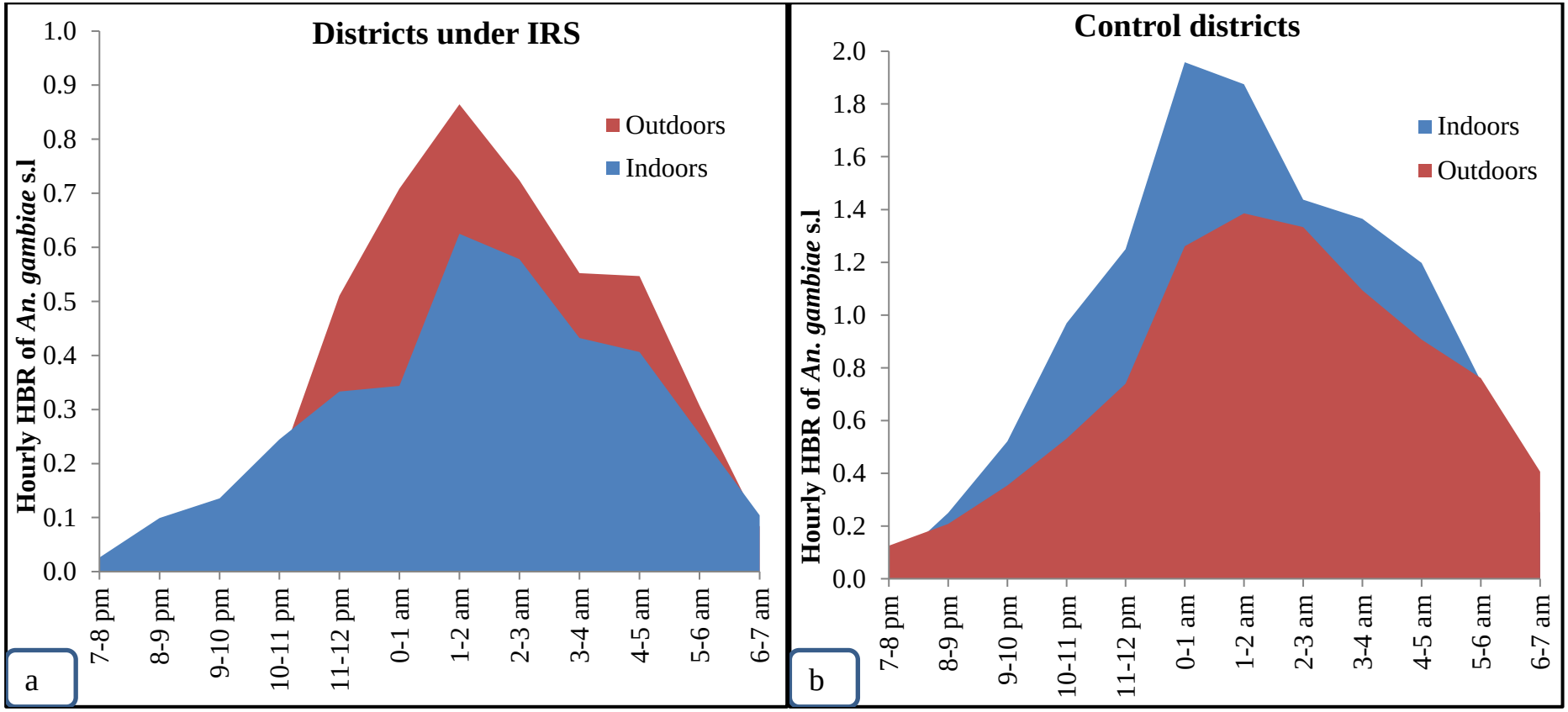


***Figure 9**. Hourly HBR of* An. gambiae *s.l. in all treated districts (a) and control districts (b) (November 2018 – August 2019)*

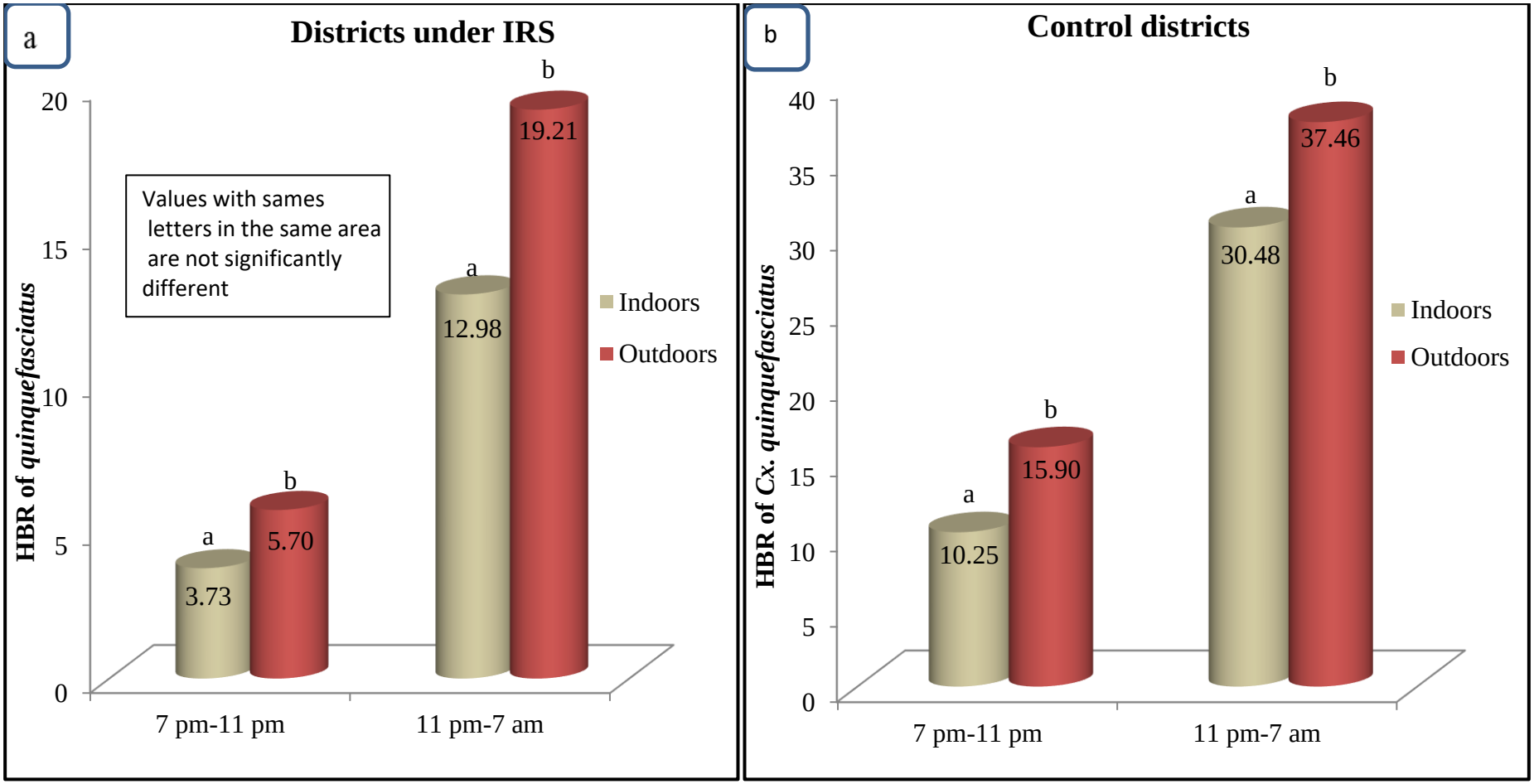


Figure 10. *Biting location of* Culex quinquefasciatus *early (7 pm-11 pm) and late at night in treated (a) and in control areas (b) (period June 2019 – August 2019).*

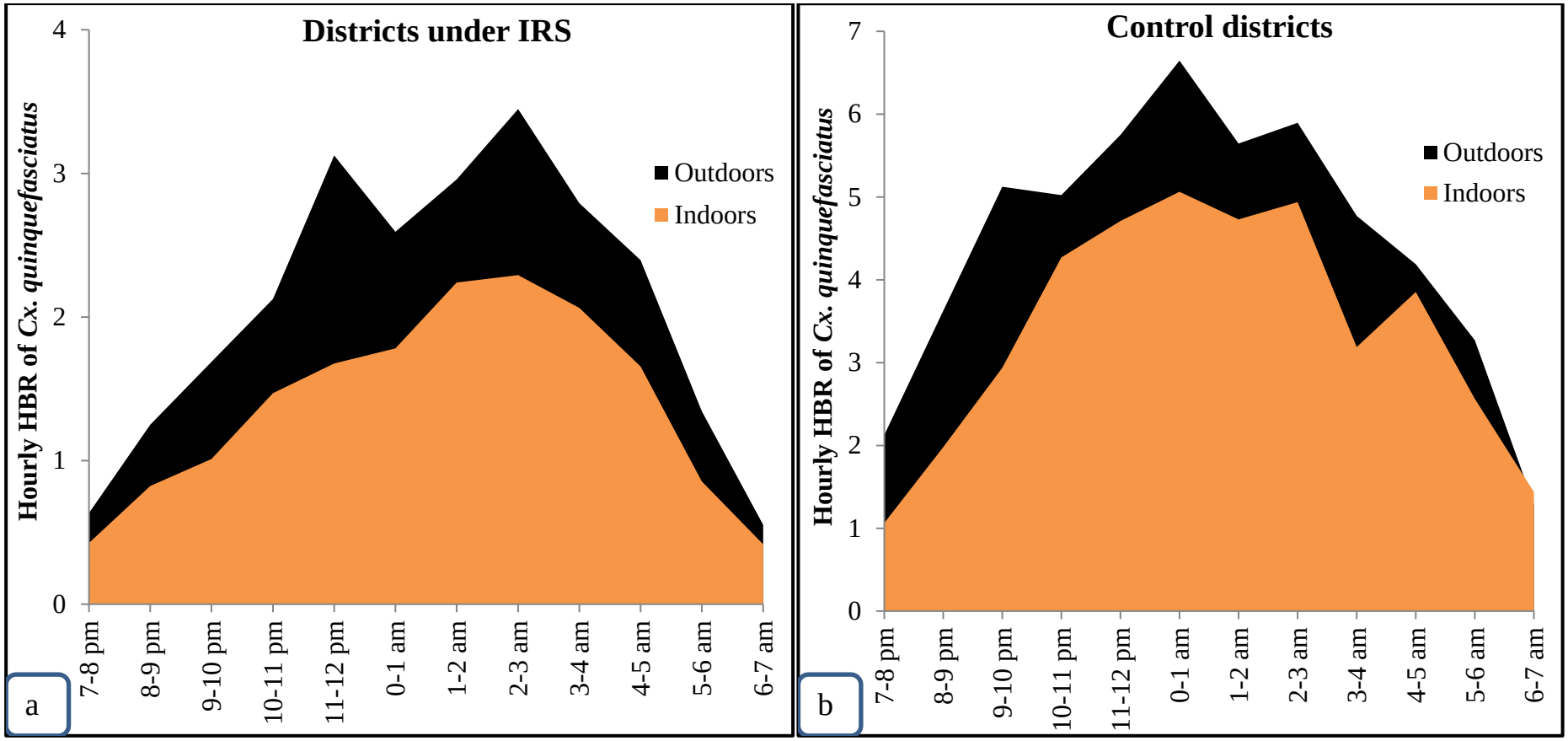


Figure 11. *Hourly HBR of* Culex quinquefasciatus *in all treated districts (a) and control districts (b) (June 2019 –August 2019)*

Table 6. *Hourly biting rate of* An. gambiae *s.l. in all treated and control districts (period November 2018-August 2019)*

	Treated districts												
	7-8 pm	**8-9 pm**	**9-10 pm**	**10-11 pm**	**11-12 pm**	**12-1 am**	**1-2 am**	**2-3 am**	**3-4 am**	**4-5 am**	**5-6 am**	**6-7 am**	**Total**
Indoor	5	19	26	47	64	66	120	111	83	78	49	20	688
Outdoor	4	7	20	38	98	136	166	139	106	105	59	16	894
Total	9	26	46	85	162	202	286	250	189	183	108	36	1582
	Control districts												
Indoor	4	24	50	93	120	188	180	138	131	115	72	24	1139
Outdoor	12	20	34	51	71	121	133	128	105	87	73	39	874
Total	16	44	84	144	191	309	313	266	236	202	145	63	2013

Table 7*. Hourly biting rate of* Culex quinquefasciatus *in all treated districts (period June-August 2019)*

	Treated districts												
	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**	**6-7 am**	**Total**
Indoor	41	79	97	141	161	171	215	220	198	159	82	40	1604
Outdoor	61	120	162	204	300	249	284	331	268	230	129	53	2391
Total	102	199	259	345	461	420	499	551	466	389	211	93	3995
	Control districts												
Indoor	51	95	141	205	226	243	227	237	153	185	123	69	1955
Outdoor	102	174	246	241	276	319	271	283	229	201	157	62	2561
Total	153	269	387	446	502	562	498	520	382	386	280	131	4516

4.5 Indoor resting density and blood-feeding rates of *An. gambiae s.l.* collected in IRS and control districts from November 2018 to August 2019

Before the 2019 IRS campaign (November 2018 –March 2019), approximately 0.39 specimens of *An. gambiae* s.l. per room were collected early in the morning (7 AM - 9 AM) after PSCs in IRS zone (Alibori and Donga) against 0.48 *An. gambiae* s.l. per room in the control areas (p=0.291) (Table 8). Similarly, the blood-feeding rates of *An. gambiae* s.l. was similar in treated (70.21%) and the control areas (83.72%) (p=0.141).

After 2019 IRS implementation (June-August 2019), the density of *An. gambiae* s.l. was significantly reduced in IRS areas compared to the control areas (Table 8). This density is respectively 0.30 mosquitoes/room in treated houses versus 2.59 mosquitoes/room in the control areas (p<0.001). Despite the reduction of the indoor resting density observed in treated areas in this period (June-August 2019), the blood-feeding rates of *An. gambiae* s.l. was still high in the treated (73.97%) and the control (80.26%) areas (p=0.326) (Table 8).

Table 8. *Residual density and blood-feeding rates of* An. gambiae *s.l. collected before May 2019 IRS implementation and during the bio-efficacy period of Actellic CS*

Period	Districts	Nb of rooms	Nb *An. gambiae* (s.l.) collected	Density/ Room	Unfed	Fed	Gravid	Half-Gravid	Blood feeding Rate (%)	*P-value
Pre-IRS evaluation: November 2018-March 2019	Kandi	60	23	0.38	2	18	3	0	78.26	1
	Gogounou	60	56	0.93	6	38	10	2	71.43	0.642
	Bembèrèkè (control)	60	16	0.27	0	13	3	0	81.25	-
	Djougou	60	2	0.03	0	0	1	1	50.00	0.763
	Copargo	60	13	0.22	2	4	4	3	53.85	0.080
	Kouande (control)	30	27	0.90	2	23	2	0	85.19	-
	Total treated districts	240	94	0.39	10	60	18	6	70.21	0.141
	Total control districts	90	43	0.48	2	36	5	0	83.72	-
Post-IRS evaluation: June 2019-August 2019	Kandi	60	13	0.22	4	9	0	0	69.23	0.611
	Gogounou	60	12	0.20	1	11	0	0	91.67	0.549
	Bembèrèkè (control)	60	94	1.57	9	69	10	6	79.79	-
	Djougou	60	21	0.35	7	12	0	2	66.67	0.243
	Copargo	60	27	0.45	7	19	0	1	74.07	0.613
	Kouande (control)	30	139	4.63	6	91	21	21	80.58	-
	Total treated districts	240	73	0.30	19	51	0	3	73.97	0.326
	Total control districts	90	233	2.59	15	160	31	27	80.26	-

*P-value: Comparison of the blood-feeding rate of *An. gambiae* s.l. between the treated and control districts (Test used: Chi-square test)

4.6 Origin of blood-meal observed in *Anopheles gambiae* s.l. in treated and in control areas (June-August 2019)

The origin of mosquito blood meal in treated and untreated districts is shown in Figure 12. below. Despite significantly reduced mosquito density in the treated areas compared to the control areas, an average of 73.17% of *An. gambiae* s.l. collected by PSC in treated areas were positive with human blood, 14.3% were positive for bovine blood and 12.20% were positive for both human and bovine. On the other hand, 97.14% of *An. gambiae* s.l. collected in control area were positive for human blood (X^2 = 182.93; *df*=1; *P*< 0.001) (Figure 12).

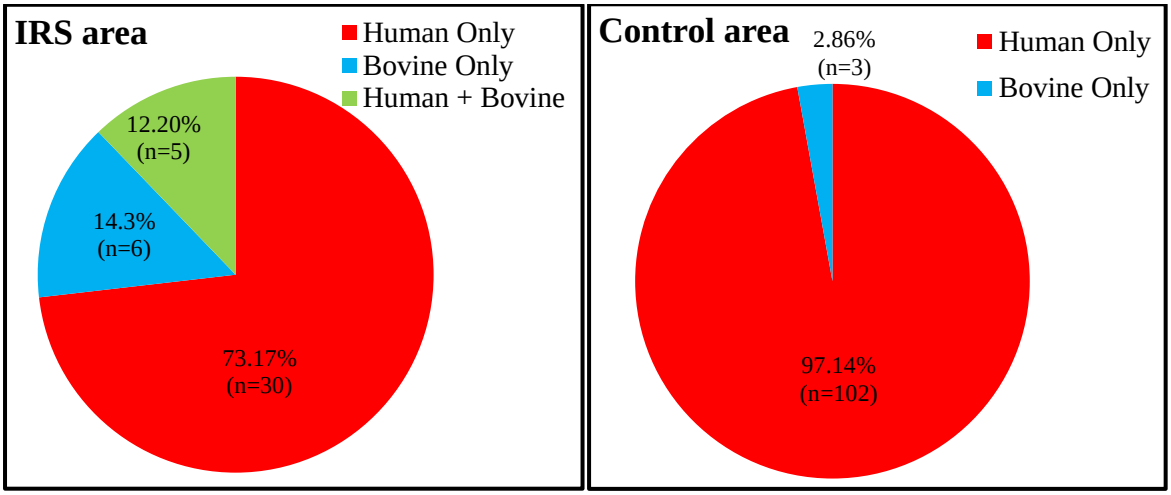


***Figure 12**. Origin of blood meal observed in* Anopheles gambiae s.l. *in treated and control areas*

4.7 Parous rate observed in *An. gambiae* s.l. in districts under IRS and control districts

Table 9 below shows the impact of the IRS on the longevity of *An. gambiae* in terms of the proportion of mosquitoes that have laid at least once.

Before the 2019 IRS intervention (November 2018 to March 2019), the parous rate of *An. gambiae* registered in treated districts (Alibori and Donga) was estimated at 70.59% (180/255) compared to 69.70% (69/99) in controls districts (Bembereke and Kouande) (p=0.972).

After the 2019 IRS implementation (June-August 2019), this parous rate of *An. gambiae* s.l. was significantly reduced in IRS areas compared to the control areas (Table 9). This rate is respectively 41.36% (371/897) in treated districts versus 65.78% (569/865) in the control areas (p<0.001).

Table 9. *Parous rate of* An. gambiae *s.l. in districts under IRS and control districts before 2019 IRS implementation and during the bio-efficacy period of Actellic CS*

Period	Districts	Nb of *An. gambiae* s.l. dissected	Nb of parous	Parity rate (%)	P-value*
November 2018- March 2019	Kandi	77	52	67.53	0.337
	Gogounou	107	78	72.90	0.750
	Bembèrèkè (control)	56	40	71.43	-
	Djougou	23	13	56.52	0.541
	Copargo	48	37	77.08	0.427
	Kouande (control)	43	29	67.44	-
	Total treated districts	255	180	70.59	0.972
	Total control districts	99	69	69.70	-
June 2019- August 2019	Kandi	227	94	41.41	0.001
	Gogounou	200	81	40.50	0.004
	Bembèrèkè (control)	551	362	65.70	-
	Djougou	229	93	40.61	<0.001
	Copargo	241	103	42.74	0.004
	Kouande (control)	314	207	65.92	-
	Total treated districts	897	371	41.36	<0.001
	Total control districts	865	569	65.78	-

Nb: Number; P-value: P-value of comparison of the parity rate of *An. gambiae* s.l. between the treated and control districts; *P-value based on χ^2-test

4.8 Sporozoite index (SI) of *Plasmodium falciparum* and Entomological Inoculation Rate (EIR) of *An. gambiae* s.l. in districts under IRS and control districts.

Tables 10 and 11 summarize the human biting rates (HBR), sporozoite index (SI), and entomological inoculation rate (EIR) recorded before and during the bio-efficacy period of Actellic CS in treated and untreated districts from November 2018 to August 2019.

CS-ELISAs were done in *An. gambiae* s.l. before the 2019 IRS campaign (the period from November 2018 to March 2019). An average sporozoite positivity rate of 1.05% in treated districts (Alibori and Donga) was observed (3 positive mosquitoes for *Plasmodium falciparum* antigen out of a total of 284 *An. gambiae* s.l. head-thoraces analyzed). In the control districts (Bembereke and Kouande), an average sporozoite positivity rate of 8.00% was observed (8 positive mosquitoes for *Plasmodium falciparum* antigen out of a total of 100 *An. gambiae* s.l. head-thoraces analyzed) (Table 10).

CS-ELISA performed after the IRS campaign showed that during the bio-efficacy period of Actellic CS (period June 2019 to August 2019), the sporozoite positivity rate was 0.31% (4 thoraces positive of 1,298 tested) in districts under IRS and 2.6% (50 thoraces positive of 1,913 tested) in control district (p=0.0008) (Table 10). This result shows that, despite the loss of the residual activity of pirimiphos-methyl, 6 to 9 months after the treatment of the walls, the positivity of *An. gambiae* s.l. for *P. falciparum* circumsporozoite antigen was low in the districts covered by IRS before and after the IRS campaign.

In parallel, before the period of the residual activity of pirimiphos-methyl (2019 IRS) (Nov 2018 -March 2019), EIR was 5.31 times lower in the districts under intervention (0.47 infected bites of *An. gambiae* per human per month) compared to the control districts (2.6 infected bites of *An. gambiae* per human per month), which means a reduction of 81.2% (p=0.008) (Table 10). Similarly, during the period of residual activity of pirimiphos-methyl in 2019, the reduction of EIR in treated districts was important as well: 96.03% (0.62 infected bites /human/month against 15.62) (Table 11).

Figure 13 and 14 shows the dynamics of HBR and EIRs from May 2016 to August 2019. The lowest HBR and EIRs were observed during the dry periods (January 2017 to April 2017, November 2017 to March 2018, and November 2018 to March 2019) in both treated and control areas. After IRS implementation, lower monthly HBR and EIRs were observed in the treated areas compared to the control areas between June and October 2017, 2018 and 2019, which equals to 4 months of impact each year (Figure 13 and 14).

Table 10. *Statistical comparison of Human Biting Rate (HBR), Sporozoite Index (SI %), Entomological Inoculation Rate (EIR) in* Anopheles gambiae *s.l. in districts under IRS and control districts according to the period of the residual effect of pirimiphos-methyl (PM) on treated walls.*

Periods	Indicators	Districts under IRS (Alibori, Donga)	Control district (Bembereke, Kouande)	P-value*
Before IRS 2019 (Nov 2018- Mar 2019)	HBR/night	1.48	1.04	0.002
	SI (%)	1.05 (3/284)	8.00 (8/100)	0.001
	EIR/month	0.47	2.50	0.008
Period of residual effect of Actellic CS (Jun 2019-Aug 2019)	HBR/night	6.76	19.93	<0.001
	SI (%)	0.31 (4/1,298)	2.60 (50/1,913)	0.008
	EIR/month	0.62	15.62	<0.001

*P-value based on χ^2-analysis

Table 11. *Percentage reduction of the Human Biting Rate (HBR), sporozoites index (SI), and entomological inoculation rate (EIR) of* An. gambiae *s.l. before and during the bio-efficacy period of Actellic CS in each district*

Periods	Period before 2019 IRS (Nov 2018- March 2019)				Bio-efficacy period of Actellic CS (June 2019-August 2019)			
Zone	HBR/night	SI (%)	EIR/month	% reduction of EIR	HBR/night	SI (%)	EIR/month	% reduction of EIR
Kandi	1.69	0.00	0.00	100.00	8.58	0.00	0.00	100.00
Gogounou	2.71	0.77	0.62	66.84	3.73	1.11	1.25	89.46
Bembereke (control)	1.19	5.26	1.87	-	32.94	1.20	11.87	-
Djougou	0.50	0.00	0.00	100.00	8.54	0.24	0.62	96.79
Copargo	1.02	4.08	1.25	59.93	6.19	0.33	0.62	96.79
Kouande (control)	0.90	11.62	3.12	-	6.91	9.33	19.37	-
Total districts under IRS	1.48	1.05	0.47	81.20	6.76	0.31	0.62	96.03
Total districts control	1.04	8.00	2.50	-	19.93	2.6	15.62	-

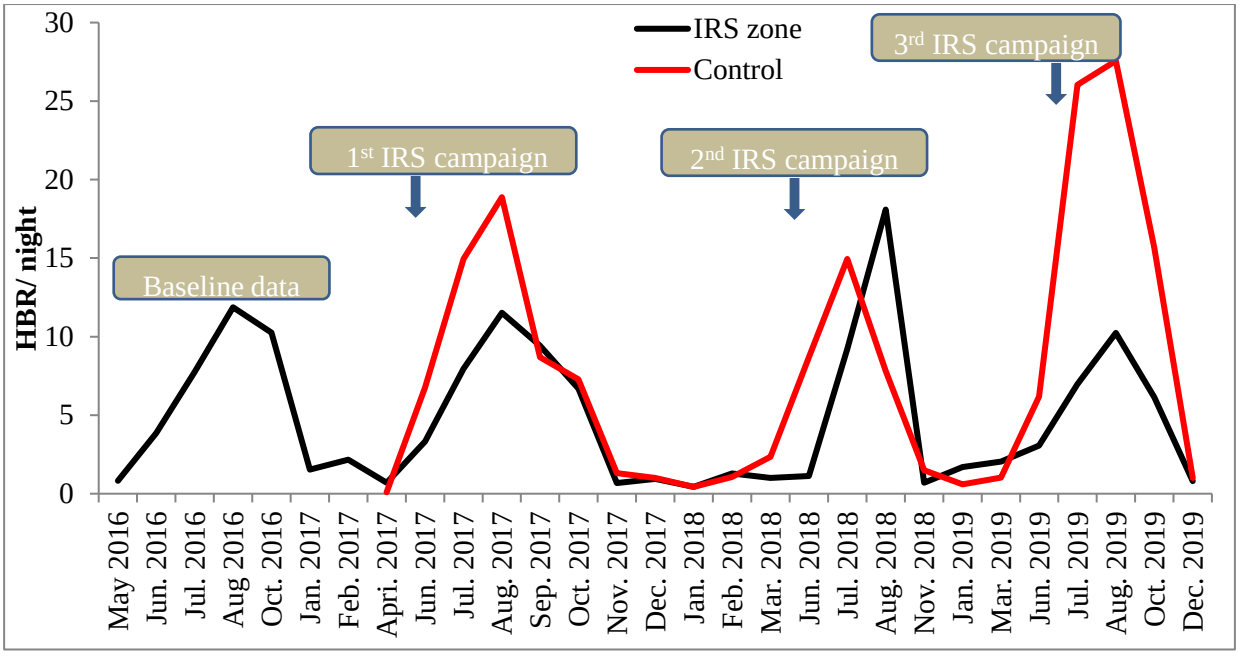


Figure 13. *Dynamic of Human biting rate in IRS and control areas from May 2016 to December 2019*

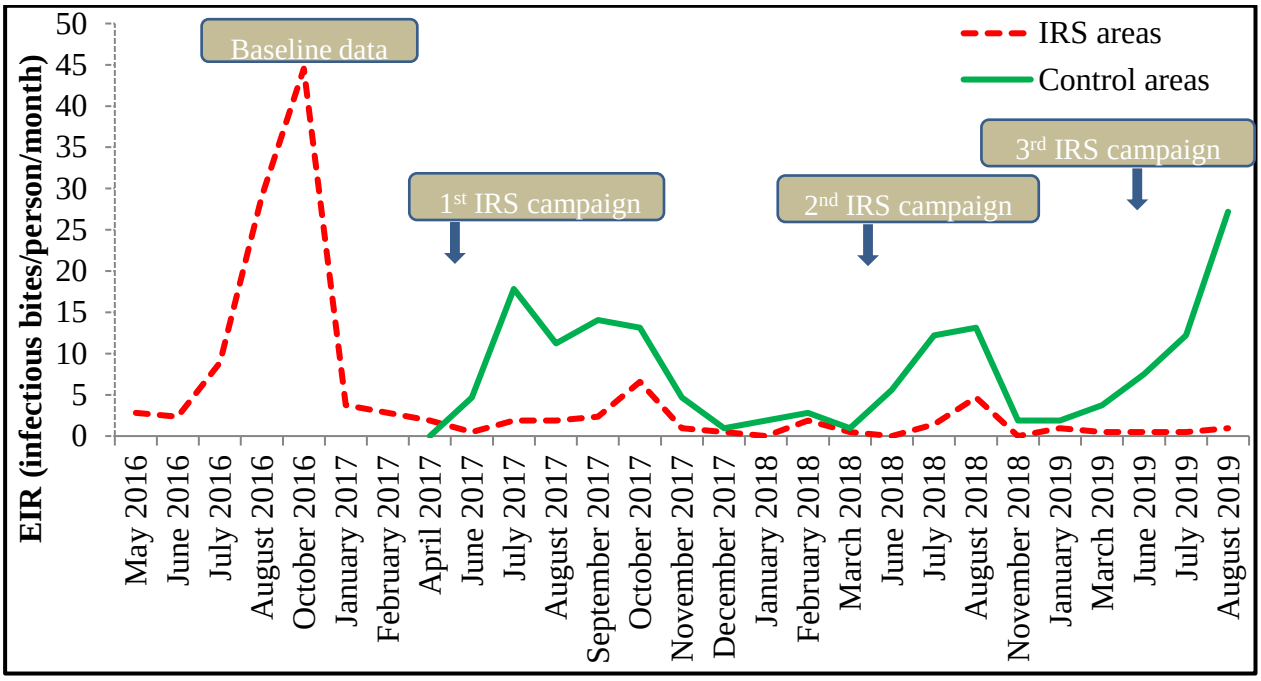


Figure 14. *Dynamics of EIR in the treated area (Alibori, Donga) and in the control area (Bembereke, Kouande) from May 2016 to August 2019.*

4.8.1 SI and EIR indoors and outdoors (period June 2019 to August 2019)

A total of 3211 head-thorax of *An. gambiae* (s.l.) were analyzed by ELISA CSP in the treated and control areas throughout the period from June 2019 to August 2019). The average infectivity rate

of *An. gambiae* (s.l.) in treated areas was 0.31% [95% CI: 0.098 - 0.84] (4 positive/1,298 tested) compared to 2.6% [95% CI: 1.96 -3.45] (50 positive /1913 tested) in the control area (p<0.0001; χ^2=23.48; df=1) (Table 12 and 13). This rate was similar indoors and outdoors the houses in both the treated and control areas. It was 0.39% [95% CI: 0.1 - 1.2] (3 positive//760 tested) outdoors the treated houses compared to 0.18% [95% CI: 0.098 - 0.2] (1 positive /538 tested) indoors (p=0.872; χ^2=0.025; df=1) (Table 12 and 13). The trend was the same in the control area: 2.25% [95% CI: 1.4 - 3.5] (19 positive thoraces of 843 thoraces tested) outdoors houses versus 2.9% [95% CI: 2.00 - 4.13] indoors (p=0.464; χ^2=0.53; df=1).

Overall, the average Entomological Inoculation Rate in the control area was 15.62 infective bites/person/month compared to 0.63 infective bites/person/month in the treated area, a 95.96% reduction (p<0.001). A low EIR of *An. gambiae* s.l. is observed indoors (0.31 infective bites/person/month) of treated houses compared to outdoors (0.94 infective bites/person/month) but without any significant difference (p=0.625). Thus, 24.8% of malaria transmission occurred indoors treated houses compared to 75.2% outdoors (Table 12). In contrast, in the control area, 62% (19.38 infective bites/person/month) of malaria transmission occurred indoors compared to 38% (11.88 infective bites/person/month) outdoors (p=0.118) households (Table 13).

Table 12. *Human Biting Rate (HBR), Sporozoite Index (SI) and the Entomological Inoculation Rate (EIR) in IRS areas three months after the 2019 IRS campaign*

Districts	Location	Indicators	June 2019	July 2019	August 2019	Period (June-August) 2019	Percentage of EIR by location (%)	RR [95% CI]	P-value (Wald)
IRS zone	**Inside**	Total tested	72	208	258	538	24.8	2.75 [0.31 - 79]	0.317
		nb Thorax+	0	0	1	1			
		SI (%)	0.00	0.00	0.39	0.18			
		HBR/night	2.25	6.50	8.06	5.60			
		EIR/night	0.00	0.00	0.03	0.01			
		EIR/month	0.00	0.00	0.94	0.31			
	Outside	Total tested	124	239	397	760	75.2		
		nb Thorax+	1	1	1	3			
		SI (%)	0.81	0.42	0.25	0.39			
		HBR/night	3.88	7.47	12.41	7.92			
		EIR/night	0.03	0.03	0.03	0.03			
		EIR/month	0.94	0.94	0.94	0.94			
	Total	Total tested	196	447	655	1298			
		nb Thorax+	1	1	2	4			
		SI (%)	0.51	0.22	0.31	0.31			
		HBR/night	3.06	6.98	10.23	6.76			
		EIR/night	0.02	0.02	0.03	0.02			
		EIR/month	0.47	0.47	0.94	0.63			

RR = Rate ratio; Thorax+ = mosquitoes positive for circumsporozoite protein in the mosquitoes' thorax

***Table 13**. Human Biting Rate (HBR), Sporozoite Index (SI), and the Entomological Inoculation Rate (EIR) in control areas three months after the 2019 IRS campaign*

Districts	Location	Indicators	June 2019	July 2019	August 2019	Period June-August 2019	Percentage of EIR by location (%)	RR [95%]	P-value
Control	**Inside**	Total tested	125	462	483	1070	62.0	0.61 [0.34 - 1.08]	0.09
		nb Thorax+	5	9	17	31			
		SI (%)	4	1.95	3.52	2.90			
		HBR/night	7.81	28.87	30.19	22.29			
		EIR/night	0.31	0.56	1.06	0.65			
		EIR/month	9.38	16.88	31.88	19.38			
	Outside	Total tested	73	371	399	843	38.0		
		nb Thorax+	3	4	12	19			
		SI (%)	4.11	1.08	3.01	2.25			
		HBR/night	4.56	23.19	24.94	17.56			
		EIR/night	0.19	0.25	0.75	0.40			
		EIR/month	5.63	7.50	22.50	11.88			
	Total	Total tested	198	833	882	1913			
		nb Thorax+	8	13	29	50			
		SI (%)	0.04	0.02	0.03	2.60			
		HBR/night	6.19	26.03	27.56	19.93			
		EIR/night	0.25	0.41	0.91	0.52			
		EIR/month	7.50	12.19	27.19	15.62			

RR = Rate ratio; Thorax+ = mosquitoes positive for circumsporozoite protein in the mosquitoes' thorax

The lowest Entomological Inoculation Rates were observed between 7 pm and 11 pm and the highest between 11 pm and 7 am in both the treated and control areas (Figure 15). Indeed, between 7 pm and 11 pm, everyone received 0.31 infective bites respectively outside the treated houses while the EIR was zero inside (P=0.398) (Figure 15). On the other hand, between 11 p.m. and 7 a.m., the Entomological Inoculation Rates were respectively 0.31 infective bites/person/month indoors versus 0.63 outdoors, i.e. an outdoor EIR 3 times higher than that of the interior of the treated houses (Figure 15). During the same period, the EIRs in the control area were respectively 3.75 infective bites/person/month indoors versus 3.13 outdoors between 7 pm -11 pm (p=1) and 15.63 infective bites/person/month indoors versus 8.75 outdoors between 11 pm and 7 am (P=0.108) (Figure 15)

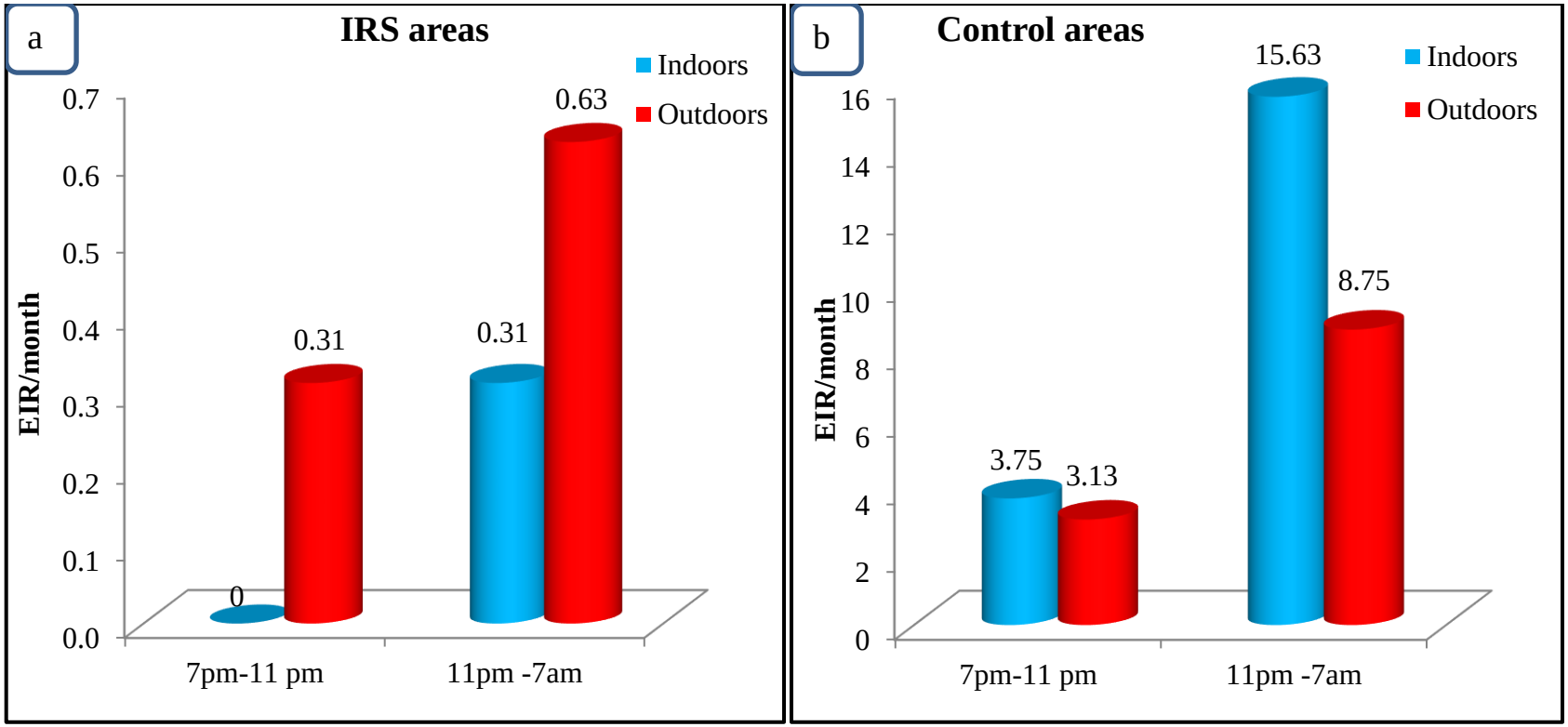
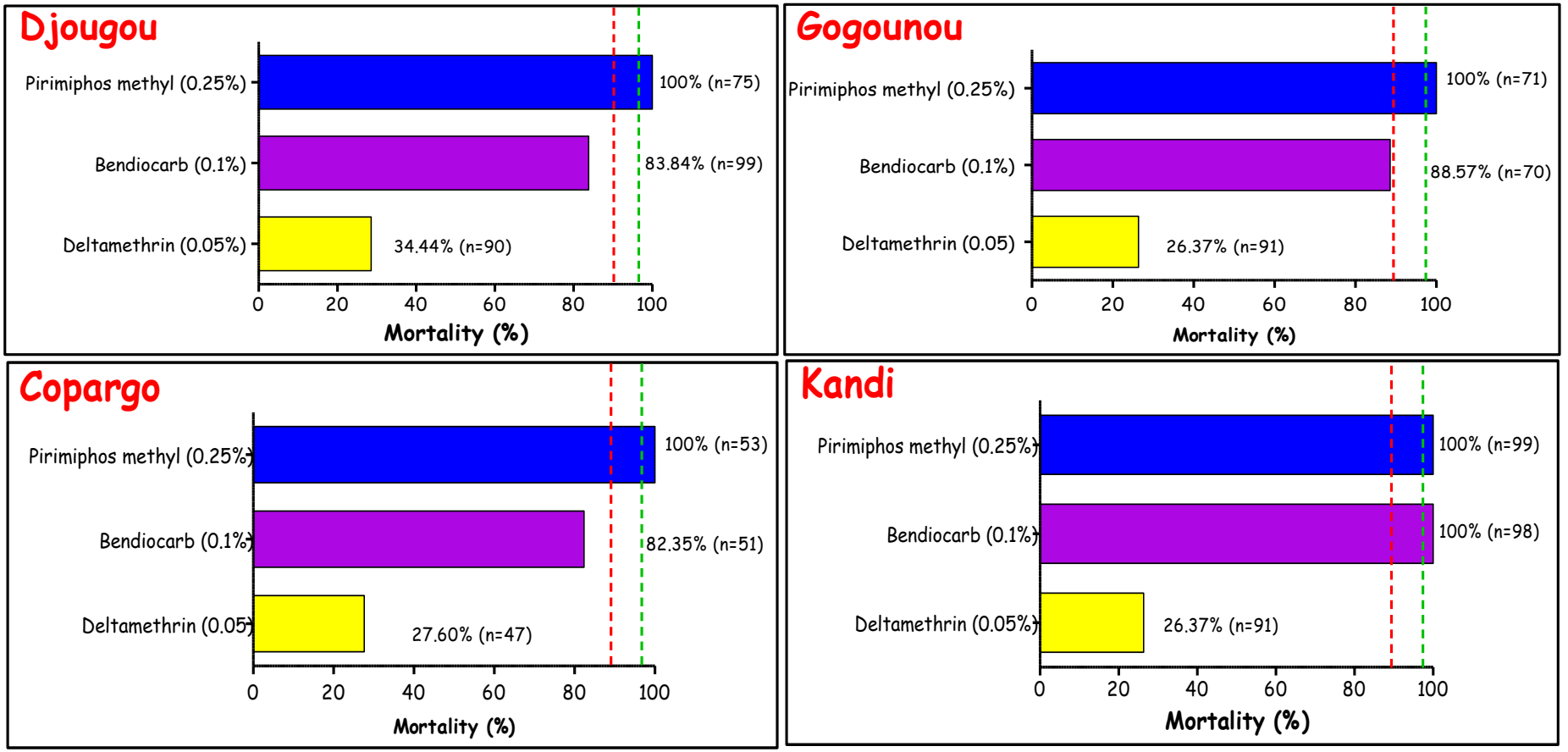


Figure 15. *Indoor and outdoor EIR of* An. gambiae *s.l. early and late at night measured in treated (a) and control areas (b).*

4.9 Insecticide susceptibility tests

Figure 16 below summarizes the susceptibility level of local vectors to different insecticides (bendiocarb, pirimiphos-methyl, and deltamethrin). All mosquito populations tested were susceptible to pirimiphos-methyl (mortality > 98%). However, these same vector populations showed a decrease in susceptibility to bendiocarb (mortality between 90 and 97%; suspected resistance) except Kandi where mortality was 100% for those mosquitoes. For deltamethrin, *An. gambiae* s.l. was resistant in all the districts (mortality< 90%) (Figure 16).



Based on World Health Organization criteria, the area below broken red lines indicates insecticide resistance (<90%); the area in between the broken red and green lines indicate the possible resistance (90% to 97%); the area above the green broken line indicates insecticide susceptibility (≥98%)

Figure 16. *Susceptibility of Anopheles gambiae s.l. to bendiocarb 0.1%, pirimiphos-methyl 0.25%, permethrin 0.75%, and deltamethrin 0.05% in four districts under IRS during the period July 2019 - August 2019.*

4.10 Distribution of *An. gambiae* complex species in districts under IRS and control

Of the 477 specimens of *An. gambiae* s.l. analyzed by PCR over the whole study period, three sibling species [*An. gambiae* s.s. (74.42%, *n* = 355), *An. coluzzii* (21.17%, *n* = 101) and *An. arabiensis* (4.4%, *n* = 21)] were detected. Overall, the same trend (i.e. the predominance of *An. gambiae* s.s.) was observed in all localities (treated and control) (Figure 17). Seasonal variation in the frequency of *An. gambiae* s.s. and *An. coluzzii* was observed during the study (Figure 18). Overall, out of a total of 749 mosquito specimens analyzed in the dry season, 75.56% (*n* = 566) of *An. coluzzii* were detected *vs* 23.46% (*n* = 183) of *An. gambiae* s.s. In contrast, in the rainy season, *An. gambiae* s.s. was predominant (80.81%; 1,934 of 2,393) compared to *An. coluzzii* (19.18%; 459 of 2,393) (Figure 18).

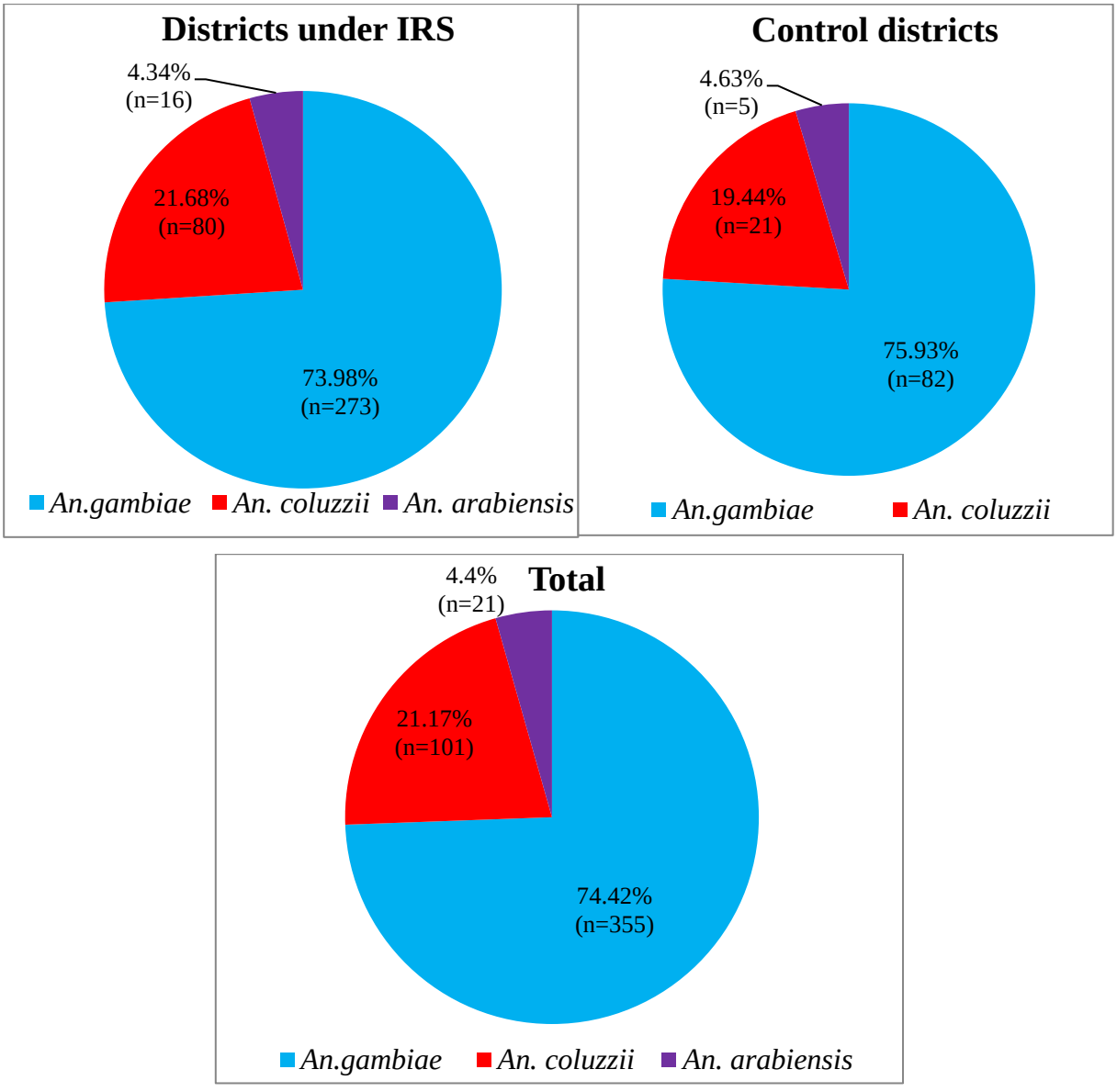
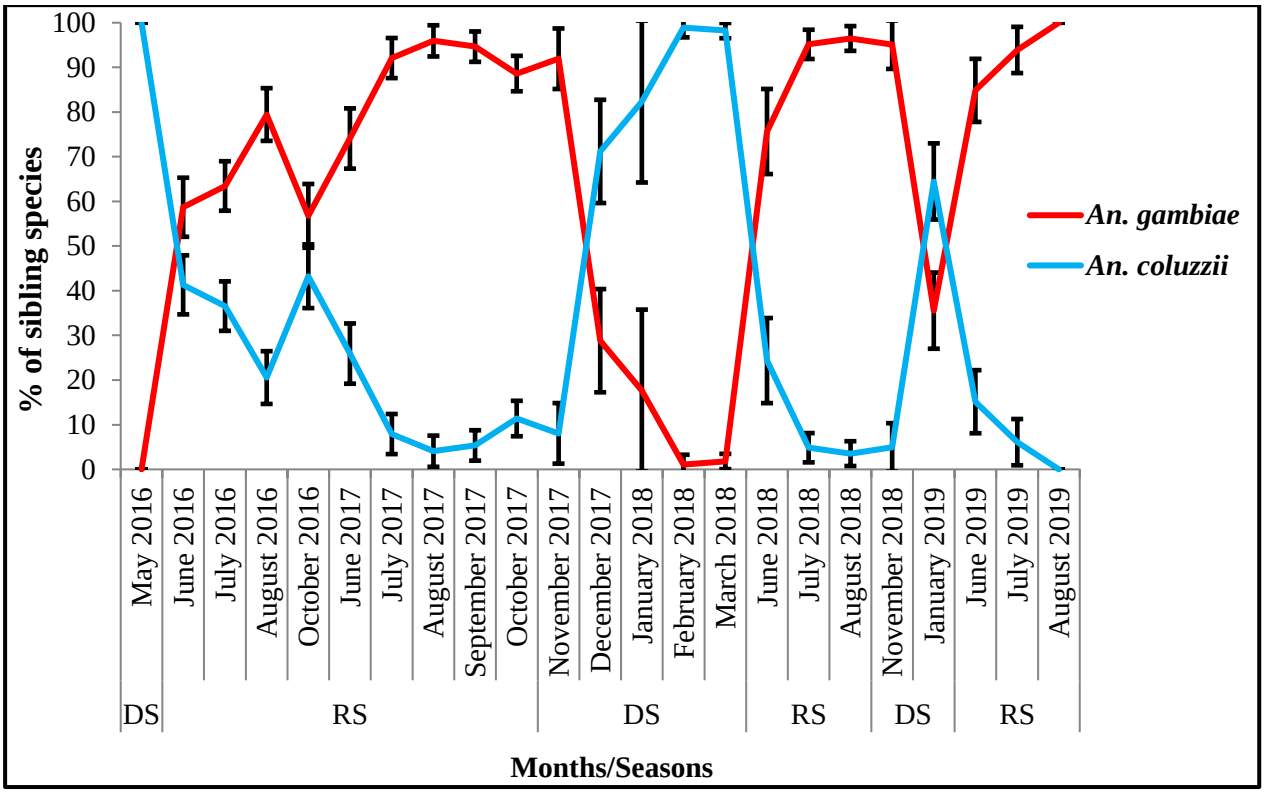


Figure 17. *Distribution of* An. gambiae s.l. *species in districts under IRS and control*



Abbreviations: DS, dry season; RS, rainy season

***Figure 18**. Seasonal variation of sibling species (*An. coluzzii *and* An. gambiae*) in the study area.*

4.11 Multiple insecticide resistance mechanisms in *An. gambiae* s.l. (*Kdr, Ace-1*)

Data presented in Tables 14 and 15 show the distribution of Knock-down and *Ace*-1 resistance among *An. gambiae* complex species collected. Results from this study showed that the *Kdr* (1014F) mutation was present at high frequency (80.71% on average) in all *An. gambiae* populations collected from a different district. This frequency is 80.22% in districts under IRS compared to 82.41% in control. The highest frequency *Kdr* (1014F) was recorded in the Djougou district (85.48%) and the lowest frequency was recorded in *An. gambiae* s.l. strains from Gogounou (72.32%) (Figure 19). Data presented in Table 14 shows the distribution of *Kdr* (1014F) resistance among *An. gambiae* complex species collected between November 2018 and August 2019. *Kdr* (1014F) frequency is higher in *An. gambiae* than in other species except for Kandi.

As for *Ace-1R* mutation associated with carbamates and organophosphate resistance was identified in all sites but with very low frequencies (1.3% to 3.6%) (Figure 20). Data presented in Tables 14 and 15 show the distribution of Knock-down and *Ace-1* resistance among *An. gambiae* complex species collected between November 2018 and August 2019.

***Table 14**. Distribution of Knock-down resistance (*Kdr*) frequencies between malaria vectors and localities*

Localities	Species	Number tested	RR	RS	SS	Freq. 1014F (%)
Kandi	*An. gambiae*	72	49	14	9	77.78
	An. coluzzii	32	17	12	3	71.88
	An. arabiensis	14	12	1	1	89.29
Gogounou	*An. gambiae*	45	32	5	8	76.67
	An. coluzzii	20	9	7	4	62.50
Djougou	*An. gambiae*	51	43	4	4	88.24
	An. coluzzii	11	6	4	1	72.73
Copargo	*An. gambiae*	105	84	14	7	86.67
	An. coluzzii	17	11	3	3	73.53
	An. arabiensis	2	1	0	1	50.00
Bembèrèkè	*An. gambiae*	82	66	8	8	85.37
	An. coluzzii	21	14	3	4	73.81
	An. arabiensis	5	3	1	1	70.00

SS = homozygous susceptible; RS = hybrid resistant and susceptible; RR = homozygous resistant; F = Frequency.

***Table 15.** Distribution of* Ace-1R *frequency between species*

Localities	Species	Number tested	RR	RS	SS	Freq. 119S (%)
Kandi	*An. gambiae*	72	0	2	70	1.39
	An. coluzzii	32	0	1	31	1.56
	An. arabiensis	14	0	0	14	0
Gogounou	*An. gambiae*	45	0	1	44	1.11
	An. coluzzii	20	0	1	19	2.50
Djougou	*An. gambiae*	51	0	3	48	2.94
	An. coluzzii	11	0	0	11	0.00
Copargo	*An. gambiae*	105	0	8	97	3.81
	An. coluzzii	17	0	1	16	2.94
	An. arabiensis	2	0	0	2	0
Bembèrèkè	*An. gambiae*	82	0	5	77	3.05
	An. coluzzii	21	0	0	21	0.00
	An. arabiensis	5	0	0	5	0

SS = homozygous susceptible; RS = hybrid resistant and susceptible; RR = homozygous resistant; F = Frequency

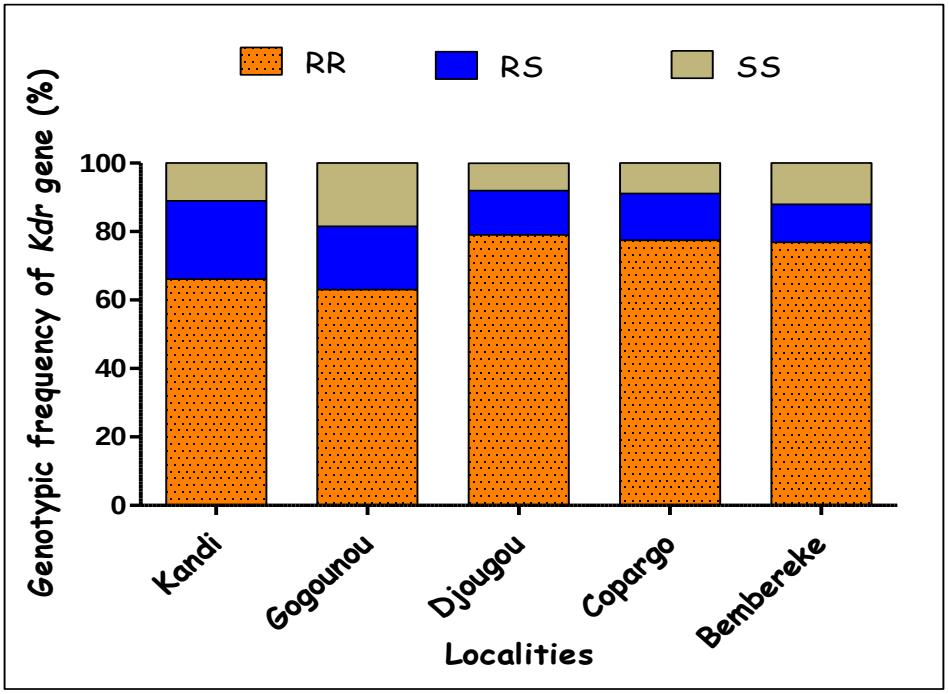


Figure 19. *Genotypes frequency of* Kdr *gene in the populations of* An. gambiae *s.l.*

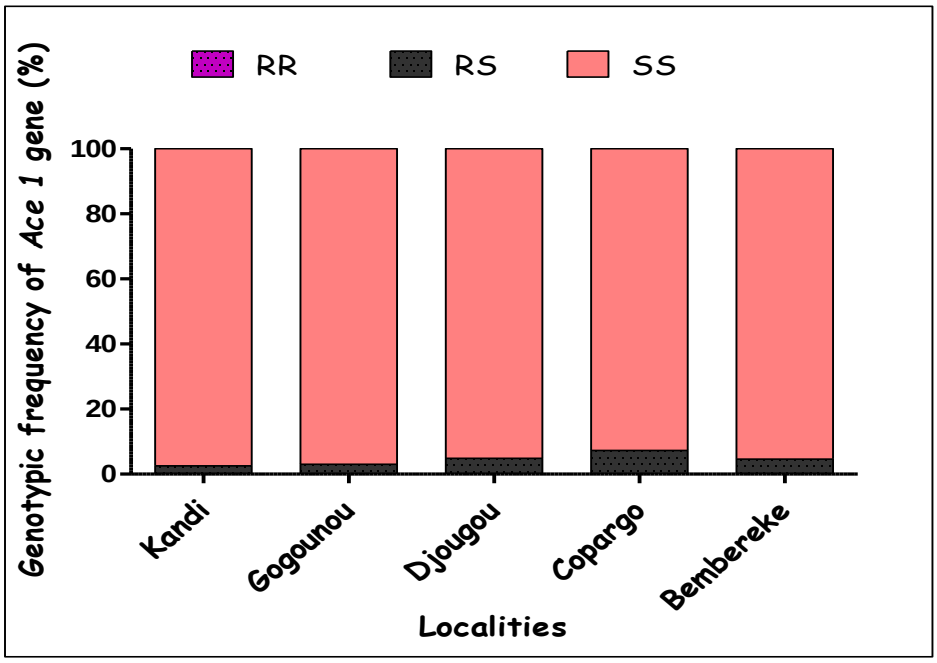


Figure 20. *Genotypes frequency of* Ace-1 *gene in the populations of* An. gambiae *s.l.*

5 Conclusions

All targets set during deliverable covering the period from September 2017 to September 2018 are met. Monitoring and evaluation of the 3rd indoor residual spray campaign carried out from September 2018 to September 2019 in Alibori and Donga continues to demonstrate the impact of the IRS strategy on the reduction of malaria transmission. From the evaluation of the region of

Albori and Donga, we observed a significant difference between the entomological indicators from the districts under IRS and controls districts.

Bioassays on treated walls have shown that pirimiphos-methyl (Actellic CS) remains effective for up to four months (May-September) after spraying. During this period of bio-efficiency of Actellic CS, we observed a significant reduction of some indicators such as indoor resting density, vector longevity, sporozoite index, and EIR in most of the treated districts compared to control areas. Strong exophagy of *An. gambiae* (s.l.) was also observed in the treated districts compared to the controls, presumably due to IRS. However, there was still IRS impact on some indicators even though the persistence of pirimiphos-methyl fell below the 80% efficacy threshold at four-month post-spray. IRS did not affect the blood-feeding rate; blood-feeding in treated and control districts were not significantly different and relatively high.

With regards to vector susceptibility, *An. gambiae* (s.l.) is sensitive to pirimiphos-methyl in all sites but is experiencing a decrease in susceptibility to bendiocarb and widespread resistance to pyrethroids in all localities.

6 Difficulties encountered and recommendations

During monitoring and evaluation of the 2019 IRS campaign (September 2018 to September 2019), the rarity of positive *Anopheles* larval sites in some treated localities limited execution of susceptibility tests on more insecticide classes. However, enough mosquitoes were available to do tests on pirimiphos-methyl and deltamethrin. These two insecticides are priorities since they are the ones used for IRS and ITNs in the region.

Insecticide susceptibility testing will be continued in the upcoming rainy season when *Anopheles* larvae are available.

7 Activities planned for the next 3 months (October - December)

The same monitoring will continue in the same districts and this data will serve as a control for the next May 2020 spraying campaign.