US PRESIDENT'S MALARIA INITIATIVE ACTION TO REINFORCE

MALARIA VECTOR CONTROL PROGRAM IN BENIN

Impact of Indoor Residual Spraying on mosquito behavior and malaria transmission in Alibori, Atacora and Donga, Benin, West Africa

Final report

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Table of Contents

List of Figures

List of Tables

Abbreviations

2KP	Kérou-Kouandé-Pehunco
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
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
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
PMI	U.S. President's Malaria Initiative
SI	Sporozoite index
s.l.	Sensu lato
s.s.	Sensu stricto
WHO	World Health Organization

1. Introduction

In Benin, Indoor Residual Spraying (IRS) was introduced due to the emergence and expansion malaria vectors resistance to pyrethroid insecticides[1], which is the only family of insecticides registered by WHO for use in LLINs. Implementation of IRS in Benin since 2008 was accompanied by a drastic reduction in Entomological Inoculation Rate (EIR)[2]. After six years of IRS implementation in the Atacora region, the National Malaria Control Program (NMCP), in agreement with various partners, decided to partially withdraw the intervention from some districts of this region and relocate it to two other regions (Alibori and Donga). This decision falls within the framework of the implementation of the national insecticide resistance management plan and provides the opportunity for coverage in two high burden regions which have never benefitted from this intervention. The temporary stopping of IRS may reduce the emergence of the insecticide resistance by limiting the selection pressures on mosquitoes carrying resistance genes.

In preparation for this relocation, prior studies have shown that *An. gambiae* (*s.l.*), the main malaria vector in the two target regions, was susceptible to pirimiphos-methyl, an organophosphate insecticide (Actellic® 300 CS) and potential candidate for the IRS campaigns in Alibori and Donga.

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

In 2018, IRS was renewed in 6 districts (Kerou, Pehunco, Djougou, Copargo, Kandi and Gogounou). The present report shows the results of the IRS entomological monitoring conducted in both regions. Thus, the impact of the strategy on key entomological indicators of malaria transmission as well as, the residual efficacy duration of Actellic 300CS on the different type of sprayed walls were evaluated.

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 control districts (Bembereke and

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[1] Chandre F, Darriet F, Manga L, Akogbeto M, Faye O, Mouchet J, Guillet P. Situation de la résistance aux pyréthrinoïdes chez *Anopheles gambiae* sensu lato. Bull World Health Organ. 1999; 77: 230-234.
Corbel V, N'guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, Akogbéto M, Hougard JM, Rowland M. Multiple insecticide mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta Tropica. 2007; 101: 207-216.

[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.

Kouande) during the pre-IRS period or baseline period (January 2018 to March 2018) and post-IRS period (June 2018 to November 2018).

To better assess the IRS impact on malaria transmission, we compared the entomological indicators not only between treated and control (untreated) areas, but also between the pre-IRS period (January 2018 to March 2018) and post-IRS period (from June 2018 to November 2018).

2. Material and methods

2.1. Study areas

The map below (Figure 1) provides the three health zones (HZ) that were protected by IRS after the 2018 IRS campaign:

- HZ of Djougou, Copargo, Ouaké (Donga department)
- HZ of Kandi, Gogounou, Segbana (Alibori department)
- Pehunco and Kerou districts (Atacora department)

In total, 7 districts were used for entomological monitoring and evaluation (M&E) of the IRS intervention:

M&E sites:

- In the Donga department – the districts of Djougou and Copargo
- In the Alibori department – the districts of Kandi and Gogounou
- In the Atacora department (HZ 2KP) – Pehunco (Fig. 1)

Control sites:

- Bembereke district was selected because it was the closest district from the districts receiving IRS in Alibori department;
- Kouande district was selected because of its location; it serves as a control for the district of Pehunco, the 2KP district, and the district of Copargo and Djougou, the Donga department

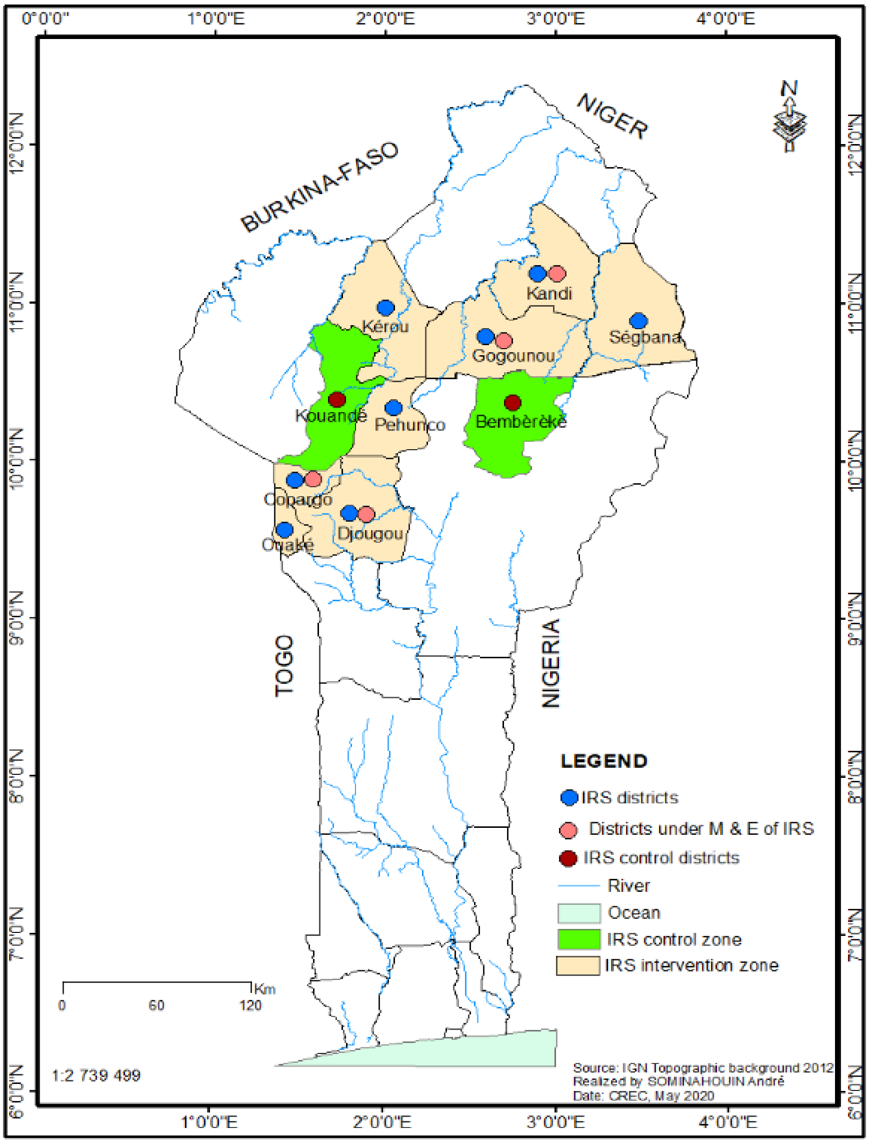


Figure 1. *Map showing the IRS evaluation areas*

The climate in the sites is Sudano-Guinean in the Donga region, Sudanese in the Alibori region and Atacorian in Atacora region. These two regions are dry savannah areas, with six months rainy season (mid- April to mid-October) and a dry season which spans the remainder of the year. Overall, average annual rainfall ranges between 700–1200 mm in Alibori and 1200–1300 mm in Donga.

Figure 2. shows the evolution of rainfall and temperature in the Alibori and Donga regions in 2018. The rainiest months were from June to October in three regions with the rainfall peak occurring in August.

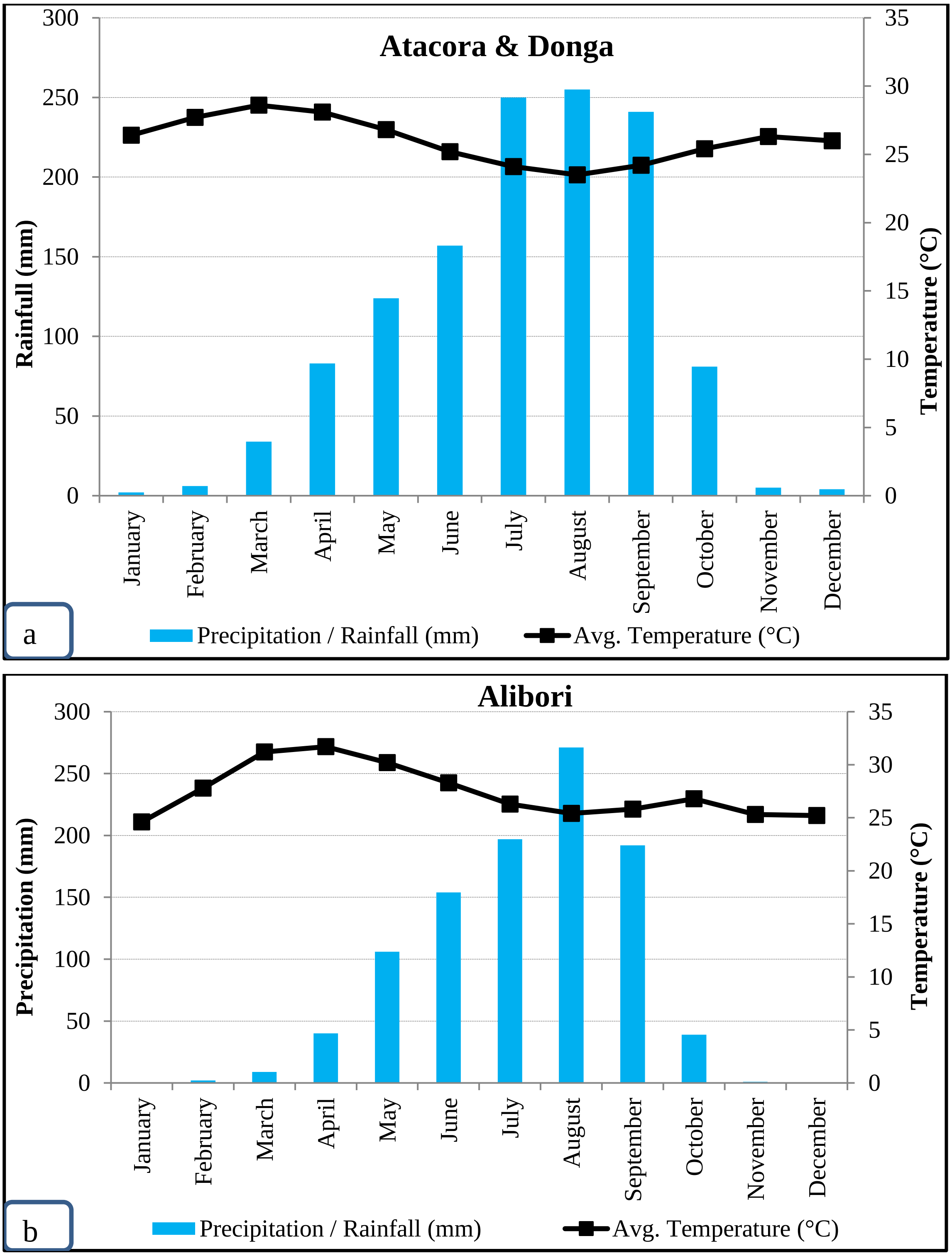


Figure 2. *Monthly climate data in the Atacora, Alibori and Donga regions*[3].

Malaria is the leading cause of mortality among children under five years of age and morbidity among adults in Benin. Malaria accounts for 40% of outpatient consultations and 25 %t of all

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

hospital admissions. Malaria places an enormous economic strain on Benin's development. The incidence of uncomplicated and severe malaria in 2016 was 26.4% in Donga region and 13.3% in Alibori.

2.2. Indicators measured

Activities planned for 2017 and 2018 provided data and information about the following entomological indicators required by PMI and the NMCP:

- Efficacy control of the spraying: cone/wall bioassay.
- Residual activity of pirimiphos methyl
- Vector identification (species and molecular forms of *Anopheles gambiae*)
- Vector density
- Mosquito behavior: biting (endophagy or exophagy) and resting (endophily or exophily)
- Entomological Inoculate Rate (EIR)
- Vector resistance to insecticides and resistance mechanisms
- Altered target-site resistance: knockdown resistance (Kdr), acetylcholinesterase (Ace-1)
- Metabolic resistance: oxidases, esterases, and glutathione S-transferase (GST)

2.3. WHO wall bioassays

A laboratory colony of *An. gambiae* s.s Kisumu strain which is fully susceptible to all insecticides was used for the bioassays. Among the districts chosen for the entomological monitoring of the 2018 IRS campaign, Kandi and Gogounou were selected to evaluate the persistence of pirimiphos methyl on cement and mud walls. Sprayed houses were selected randomly. WHO wall bioassays[4] were conducted on treated walls of 40 houses randomly selected (20 treated houses /district) seven days (T0) after May 2018 IRS campaigns in Kandi and Gogounou districts. This test allowed us to assess the quality of treatment in both districts. Every month, residual activity monitoring was carried out in the treated districts for six months or until <80% mortality is recorded for two consecutive months. This test allowed us to evaluate the persistence of the insecticide used on the wall surface. Using a mouth aspirator, 15 females of *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. Mosquitoes exposed to

[4] 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/)

untreated wall surfaces were used as controls. After 24 h of observation at a temperature of 27 ± 2 °C and a relative humidity of 80 ± 10%, the mortality rate was determined (Fig. 3). When the control mortality was between 5–20%, corrected mortality was calculated using Abott's formula[5]. If mortality in the controls was >20%, the assay was repeated. If mortality was <5% no correction was needed.

Bioassays on the walls were done at the following time points:

- T0: 7 days after the spraying date (May 2018);
- T1: 1 month after the spraying date (June 2018);
- T2: 2 months after the spraying date (July 2018);
- T3: 3 months after the spraying date (August 2018);
- T4: 4 months after the spraying date (September 2018).

Figure 3. *Exposure for 30 minutes to cement and mud walls treated with pirimiphos-methyl and mortality reading after 24 hours of observation*

2.4. Sampling of malaria vectors and evaluation of entomological indicators

Mosquito sampling was conducted in 7 districts selected for IRS M&E: Djougou, Copargo, Pehunco, Kandi and Gogounou under IRS and 2 controls (Kouande and Bembereke).

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. Table 1 shows the mosquito sampling sites and their geographic coordinates. For each village, mosquitoes were collected in 2 houses by 4 mosquito collectors – 2 mosquito collectors indoors and 2 outdoors. In total, 56 local mosquito collectors were used for one round of collection. Two rounds of sampling

[5] Abbott WSA. Method of computing of insecticide effectiveness. J Econ Entomol. 1925; 18:265–7.

were done per month. Two teams of four mosquito collectors in each village worked inside and outside the selected dwellings, from 19:00 to 00:00 hours (7:00 PM to 12:00 AM) for the first team and from 00:00 to 07:00 hours (12:00 AM to 7:00PM) for the second team. Mosquito collectors rotated through the different dwellings to avoid biases related to their trapping ability or individual attractiveness. The collection was done with the hemolysis tubes.
To estimate the indoor resting density of mosquitoes per room, 10 houses per village were selected[6]. 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 determine the blood feeding stage (unfed, fed, half-gravid and gravid).
Vector species that were collected and identified were transported to the Centre de Recherche Entomologique de Cotonou (CREC) laboratory for ovary dissection using a microscope to determine the parous rates. The heads/thoraxes of the vector species were analyzed by ELISA method to look for CSP antigens. Abdomens of female vector species were used for PCR analyses to identify sibling species and molecular forms.

Table 1. *Mosquito sampling sites and their geographic coordinates.*

District	Village	Latitude/Latitude
Djougou	Zountori	09°42′10″N, 01°40′55″W)
	Bariénou	
Copargo	Kparakouna	09°50′19″N, 01°32′39″W)
	Kataban	
Pehunco	Gbeba	10° 13′ 42″, 2° 00′ 07″
	Sinaorou	
Kandi	Kossarou	11°07′29″N, 2°56′9″W)
	Sonsoro	
Gogounou	Gogounou centre	10°50′30″N, 2°50′20″W
	Gounarou	
Kouande	Mahi	10°19′54″N, 1°41′29″W)
	Niaroson	
Bembereke	Kossou	10°13′30″N, 02°40′05″W
	Gamia	

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

2.5. Species identification and insecticide susceptibility testing

Anopheles gambiae s.l. larvae were collected from natural breeding sites during the rainy seasons in districts under IRS (Djougou, Copargo, Kandi and Gogounou). The mosquito larvae collected were transported in well labeled plastic bottles to the CREC insectary 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 a cotton wool. Unfed 2-5-day old *An. gambiae* s.l. adults were used for WHO susceptibility test using various classes of insecticides. Susceptibility status of the population was graded according to the WHO protocol.

- Bioassays with mortality rate between 98–100%, the mosquito population was considered susceptible to the tested insecticide.
- Bioassays with mortality rate between 90–97%, the mosquito population was suspected of being resistant to the tested insecticide.
- Bioassays with mortality rate below 90%, the mosquito population was considered resistant to the tested insecticide.

Dead and surviving mosquitoes from this bioassay were kept separately in Eppendorf tubes containing silica gel and stored at −20°C for further molecular analysis. The PCR-RFLP diagnostic test was used to detect the presence of L1014F mutation (Kdr) and G119S mutation (Ace-1 gene). Metabolic resistance (esterases, oxidases, GST) was analyzed by spectrophotometer using the control mosquitoes in the WHO susceptibility test (i.e. the non-insecticide exposed, field-collected mosquitoes). These mosquitoes were compared to insecticide susceptible colony-reared *An. gambie* s.s. Kisumu strain.

3. Data analysis

Data were analyzed with the statistical R software, version 2.8. using the stats package[7]. The mortality rates of *An. gambiae* Kisumu strain exposed to different wall types and heights were compared using a Chi-square test determine the association of proportions. The Chi-square test of comparison of proportions was also used to determine if there was an association between the areas receiving IRS and the following indicators: proportion of *An. gambiae* (s.l.) indoors and outdoors, blood-feeding rate, sporozoite index, and parity rate of *An. gambiae* (*s.l.*). The association of the different indicators were compared before and after the 2018 IRS campaign and between the treated and

[7] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2018). Available online at https://www.R-project.org/.

control areas. The Poisson test[8] was used to estimate the rate ratio (RR) and compare the confidence intervals of indoor vector density and EIRs of *An. gambiae* (s.l.) between treated and untreated areas. Comparative measure of mean enzyme activities between the study sites was performed by one-way analysis of variance (ANOVA) using SPSS (ver 18). Tukey's test was used to separate means in significant ANOVAs, $P<0.05$. Independent-samples t-test was performed to indicate significant increase in mean differences (Table 13).

4. Results

4.1. Residual effect of pirimiphos methyl in wall bioassays

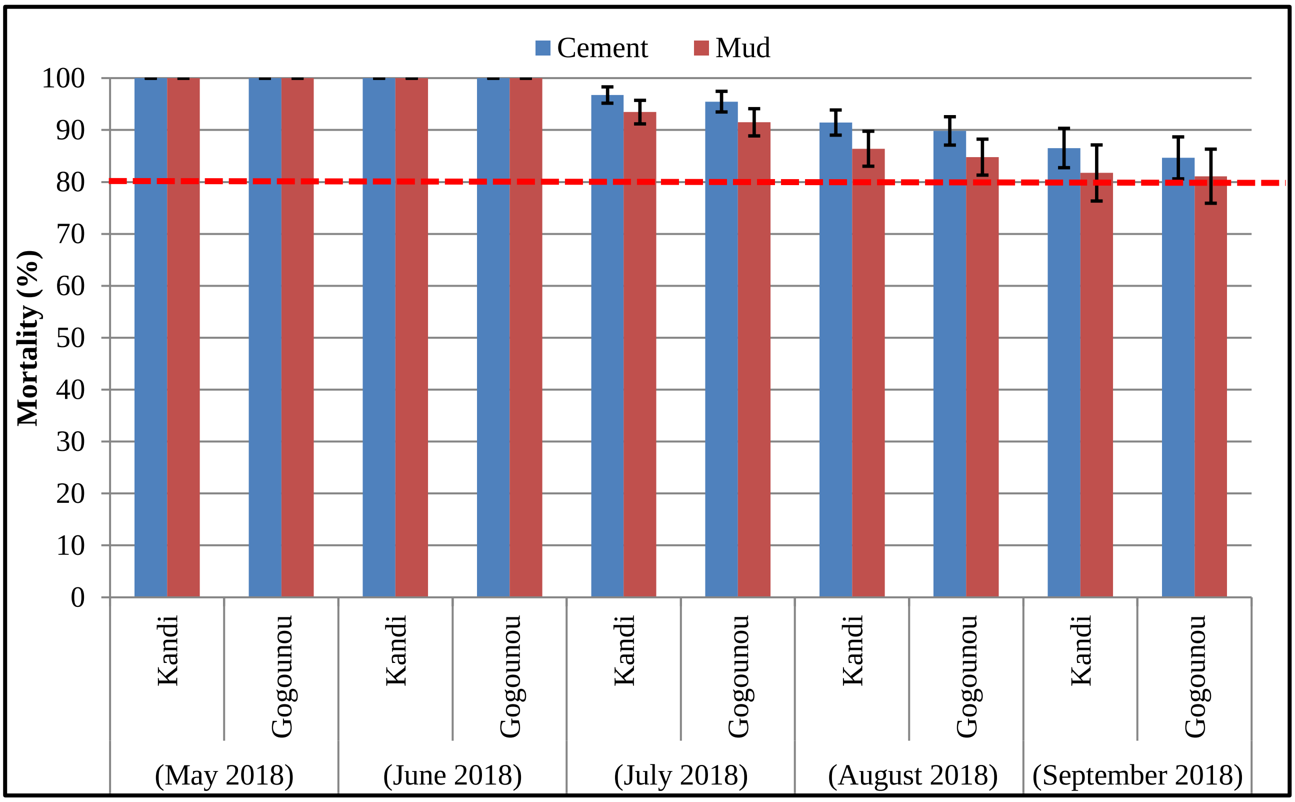
Pirimiphos methyl (Actellic CS) decay rates on treated cement and mud walls were monitored for four months in 2018. At T0 (May 2018; 7 days after treatment of the walls), there was 100% mortality in *An. gambiae* s.s. Kisumu strain exposed to pirimiphos methyl CS-treated walls irrespective of wall-type (cement or mud) and wall height (Fig. 4). This suggests good quality of the treatment and the availability of the insecticide's lethal dose on the walls. Good persistence was observed for 4 months (up to September) in the 2018 IRS campaigns (Table 1; Figure 2). No major difference in mortality was observed between wall type and wall. However, the wall bioassays were stopped due to the discovery of contamination in the strain of *An. gambiae* (Kisumu) used for wall bioassays[9]. Therefore, accurate determination of the residual effectiveness of Actellic 300CS on wall after September 2018 is not possible. However, it was reported in the 2017 entomological monitoring report[10] that mortality rates of *An. gambiae* Kisumu strain fell well below the 80% efficiency threshold in October 2017, giving effectiveness of pirimiphos methyl-CS a duration of 4–5 months in Benin.

Figure 4. *Mortality of mosquitoes and standard error bars of residual activity of Actellic 300 CS on different surfaces (cement and mud) in Kandi and Gogounou (IRS 2018 campaign)*

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

[9] This colony was destroyed due to the discovery of contamination from a wild strain kept concurrently with the Kisumu strain the CREC laboratory in September.

[10] Centre de Recherche Entomologique de Cotonou (CREC) Results of the M&E of the 1stround Indoor Residual Spraying (IRS) in Atacora-Alibori-Donga (2017) Available online at https://www.pmi.gov/



Red line = Insecticide efficacy threshold of Kisumu[11]

Table 2. *Mortality of mosquitoes (± standard error) to measure quality of the spray and residual effect of pirimiphos-methyl 300 CS four months after the 2018 IRS campaign*

	IRS 2018									
	T0 (May 2018)		T1 (June 2018)		T2 (July 2018)		T3 (August 2018)		T4 (September 2018)	
	Kandi	Gogounou	Kandi	Gogounou	Kandi	Gogounou	Kandi	Gogounou	Kandi	Gogounou
Cement	100 (± 0)	100 (± 0)	100 (± 0)	100 (± 0)	96.74 (± 1.57)	95.47 (± 1.99)	91.44 (± 2.42)	89.83 (± 2.73)	86.54 (± 3.79)	84.64 (± 4.04)
Mud	100 (± 0)	100 (± 0)	100 (± 0)	100 (± 0)	93.45 (± 2.27)	91.49 (± 2.62)	86.40 (±3.37)	84.78 (± 3.46)	81.73 (± 5.40)	81.11 (± 5.21)

4.2. Mosquito blood feeding behaviors

4.2.1. Indoor and outdoor Human Biting Rate (HBR)

A total of 2,547 *An. gambiae* s.l. were caught from January 2018 to November 2018 in treated districts (Djougou, Copargo, Kandi, Gogounou and Pehunco) and in control area (Bembereke and Kouande). Table 2 and Figures 5 and 6 show the proportion of *An. gambiae s.l.* indoors compared to outdoors in these districts. Before the 2018 IRS campaign (period from January 2018 to March

[11] WHO 2006. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. Geneva. Available at : http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.3_eng.pdf.

2018), the density of *An. gambiae s.l.* was low compared to the period from June 2018 to November 2018. During this period (January 2018 to March 2018), *An. gambiae s.l.* were collected more indoors in Bembereke, Kouande, Kandi, Pehunco and Copargo. Indoor and outdoor biting behavior in Djougou and Gogounou (Table 3) was similar. Globally, 64.50% (109/169) of *An. gambiae* s.l. were collected indoors in houses designated for IRS treatment compared to 35.50% (60/169) outdoors (P<0.001). Similarly, in houses designated as controls, 64.66% (75/116) were collected indoors versus 35.34% (41/116) outdoors (Fig. 5). After the 2018 IRS campaign (June 2018 to November 2018), the proportion of *An. gambiae s.l* collected was significantly lower indoors compared to outdoors in all treated houses, except Copargo and Pehunco (Table 3). In contrast, in untreated houses (Bembereke and Kouande), we recorded the opposite situation with higher biting rates indoors (Table 3). Globally, 47.79% (637/1333) of *An. gambiae s.l* was collected indoors in treated houses compared to 52.21% (696/1333) in outdoors (X^2 = 5.04; *df*=1; *P* =0.024). In contrast, in untreated houses, 64.26% (597/929) were collected indoor versus 35.74% (332/929) outdoors (X^2 = 150.05; *df*=1; *P* <0.001) (Table 3; Fig. 6). Tables 4 and 5 below present the details of 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 IRS intervention in treated and control districts*

Districts	Pre-2018 IRS period (Jan 2018-Mar 2018) Indoors	Outdoors		Post-2018 IRS period (June 2018 - Nov 2018) Indoors	Outdoors	
	nb (%)	nb (%)	p-value	nb (%)	nb (%)	p-value
Djougou	35 (57.38)	26 (42.62)	0.147	152 (44.84)	187 (55.16)	0.009
Copargo	27 (72.97)	10 (27.03)	<0.001	194 (47.09)	218 (52.91)	0.109
Pehunco	8 (88.89)	1 (11.11)	0.004	23 (50.00)	23 (50.00)	1
Kouande (control)	16 (88.89)	2 (11.11)	<0.001	59 (64.13)	33 (35.87)	<0.001
Kandi	22 (70.97)	9 (29.03)	<0.001	69 (34.85)	129 (65.15)	<0.001
Gogounou	17 (54.84)	14 (45.16)	0.611	199 (58.88)	139 (41.12)	<0.001
Bembereke (control)	59 (60.20)	39 (39.80)	0.006	538 (64.28)	299 (35.72)	<0.001
Total districts under IRS	109 (64.50)	60 (35.50)	<0.001	637 (47.79)	696 (52.21)	0.024
Total (control)	75 (64.66)	41 (35.34)	<0.001	597 (64.26)	332 (35.74)	<0.001

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

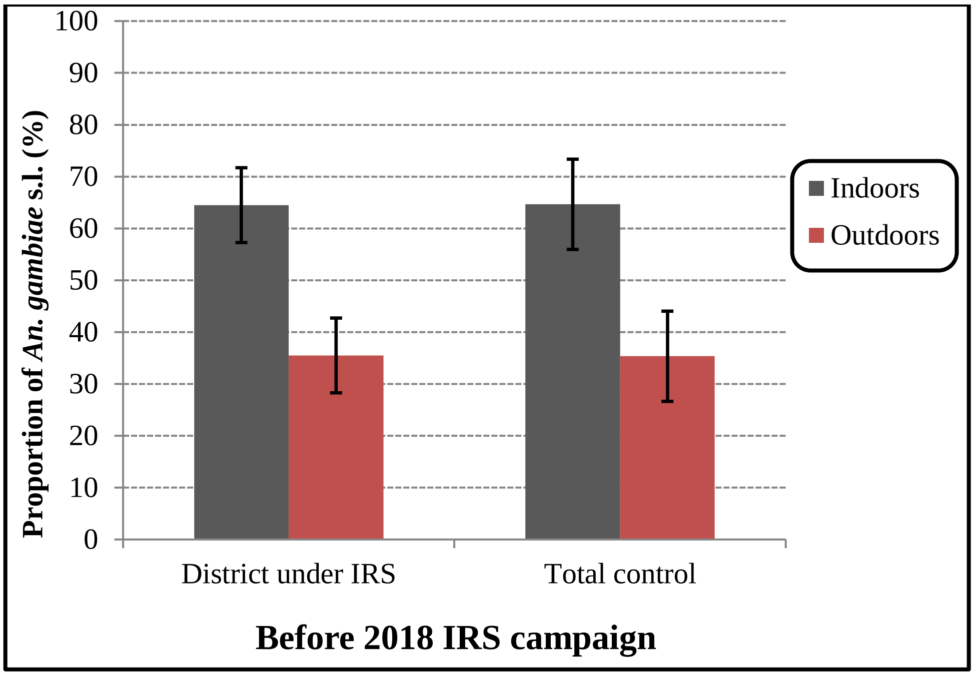


Figure 5. *Overall percentage of* An. gambiae *s.l. collected using HLC indoors and outdoors (before 2018 IRS) (period January 2018 to March 2018) in treated and untreated houses*

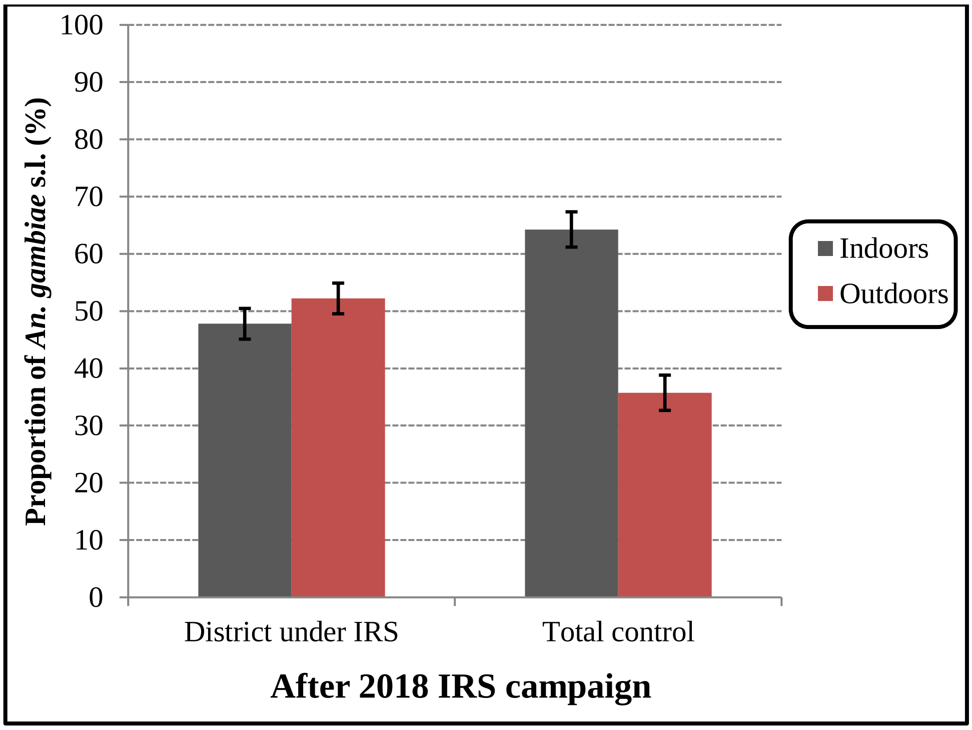


Figure 6. *Overall percentage of* An. gambiae *s.l. collected using HLC indoors and outdoors (after 2018 IRS) (period June 2018 to November 2018) in treated and untreated houses.*

Figure 7. shows the dynamics of HBR from May 2016 to November 2018. The lowest HBR were observed during the dry periods (January 2017 to April 2017, November 2017 to March 2018 and

November 2018) in both treated and control areas. After IRS implementation, lower monthly HBR was observed in the treated areas compared to the control areas between June and October 2017 & 2018, which equals to 4 months of impact each year (Fig. 7).

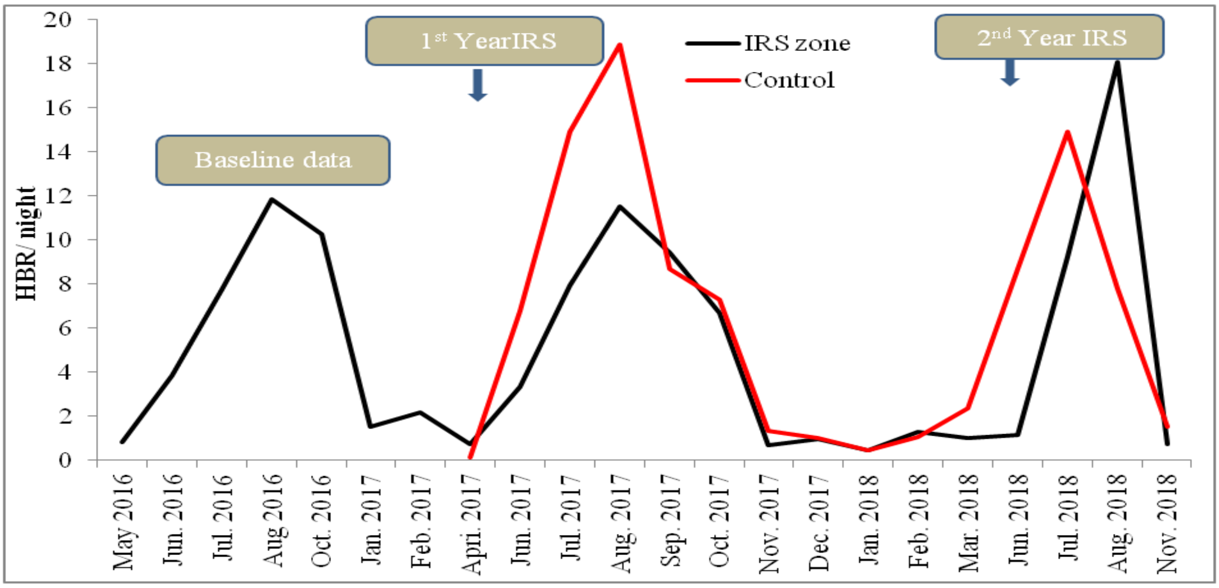


Figure 7. *Dynamic of Human biting rate in IRS and control areas from May 2016 to November 2018*

Table 4. *Biting rates of* An. gambiae *s.l. indoor and outdoor in treated districts (Atacora and Donga) and in control (Kouande)*

Districts	Position	Indicators	Jan 2018	Feb 2018	Mar 2018	June 2018	July 2018	Aug 2018	Nov 2018
Djougou	Inside	Total Mosquitoes	3	20	12	6	99	44	3
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.75	2.50	1.50	0.75	12.38	11.00	0.38
	Outside	Total Mosquitoes	2	14	10	13	108	62	4
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.50	1.75	1.25	1.63	13.50	15.50	0.50
Copargo	Inside	Total Mosquitoes	3	20	4	15	47	127	5
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.75	2.50	0.50	1.88	5.88	31.75	0.63
	Outside	Total Mosquitoes	2	7	1	30	65	118	4
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.50	0.88	0.13	3.75	8.13	29.50	0.50
Pehunco	Inside	Total Mosquitoes	2	-	6	2	11	7	3
		nb human catches	4	-	8	8	8	4	8
		HBR/night	0.50	-	0.75	0.25	1.38	1.75	0.38
	Outside	Total Mosquitoes	0	-	1	5	10	5	3

Districts	Position	Indicators	Jan 2018	Feb 2018	Mar 2018	June 2018	July 2018	Aug 2018	Nov 2018
		nb human catches	4	-	8	8	8	4	8
		HBR/night	0.00	-	0.13	0.63	1.25	1.25	0.38
		Total Mosquitoes	4	4	8	13	20	13	13
	Inside	nb human catches	4	8	8	8	8	4	8
Kouande (control)		HBR/night	1.00	0.50	1.00	1.63	2.50	3.25	1.63
		Total Mosquitoes	0	1	1	6	14	6	7
	Outside	nb human catches	4	8	8	8	8	4	8
		HBR/night	0.00	0.13	0.13	0.75	1.75	1.50	0.88

HBR: Human biting rate; nb: number; " - " means no data.

Table 5. *Biting rates of* An. gambiae *s.l. indoor and outdoor in treated districts (Alibori) and in control (Bembereke)*

Districts	Position	Indicators	Jan 2018	Feb 2018	Mar 2018	June 2018	July 2018	Aug 2018	Nov 2018
Kandi	Inside	Total Mosquitoes	2	5	15	0	26	39	4
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.50	0.63	1.88	0.00	3.25	9.75	0.50
	Outside	Total Mosquitoes	0	1	8	4	57	51	17
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.00	0.13	1.00	0.50	7.13	12.75	2.13
Gogounou	Inside	Total Mosquitoes	1	9	7	3	116	77	3
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.25	1.13	0.88	0.38	14.50	19.25	0.38
	Outside	Total Mosquitoes	1	6	7	1	72	61	5
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.25	0.75	0.88	0.13	9.00	15.25	0.63
Bembereke (control)	Inside	Total Mosquitoes	2	17	40	146	306	70	16
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.50	2.13	5.00	18.25	38.25	17.50	2.00
	Outside	Total Mosquitoes	1	12	26	113	138	36	12
		nb human catches	4	8	8	8	8	4	8
		HBR/night	0.25	1.50	3.25	14.13	17.25	9.00	1.50

HBR: Human biting rate; nb: number

4.2.2. Indoor resting density and blood feeding rate of *An. gambiae* s.l

Prior to the 2018 IRS campaign (January 2018 –March 2018), approximately 0.69 *An. gambiae s.l* per room were collected early in the morning (7AM - 9AM) after PSCs in IRS zone (Alibori, Atacora and Donga), while 1.83 *An. gambiae* s.l. per room were collected in control areas (p=0) (Table 7). The blood-feeding rates of *An. gambiae* s.l. were similarly high in treated (77.97%) and the control areas (67.88%) (p <0.001) (Table6). After 2018 IRS campaign (June-November 2018), the density of *An. gambiae s.l* was significantly reduced in IRS areas compared to the control areas

(Table 7). This density was 0.38 *An. gambiae* s.l./room in treated houses and 1.44 *An. gambiae* s.l./room in the control areas (p<0.001) (Table 7). Despite the reduction of room density observed in treated areas during this period (June-November 2018), the blood feeding rates of *An. gambiae* s.l. remained high in treated (77.04%) and the control (80.77%) areas (p=0.525) after the IRS campaign (Table 6). Figure 8 and 9 shows the dynamics of blood feeding rate and room density of *An. gambiae* s.l. in treated and control districts from May 2016 to November 2018.

Table 6. *Blood feeding rates of* An. gambiae s.l *collected (PSCs data).*

Period	Districts	Nb of rooms	Nb of *An. gambiae* (s.l.) collected	Nb Blood fed	Blood feeding rate (%)	P-value
Pre-IRS evaluation: Jan - Mar 2018	Bembèrèkè (control)	60	101	73	72.28	-
	Kandi	58	66	57	86.36	0.050
	Gogounou	60	36	26	72.22	1.000
	Kouande (control)	30	64	39	60.94	-
	Djougou	60	28	21	75.00	0.286
	Copargo	60	41	29	70.73	0.414
	Pehunco	20	6	5	83.33	0.519
	Total control districts	90	165	112	67.88	-
	Total treated districts	258	177	138	77.97	<0.001
Post-IRS evaluation: June - Nov 2018	Bembèrèkè (control)	78	126	101	80.16	-
	Kandi	80	28	23	82.14	1
	Gogounou	80	21	17	80.95	1
	Kouande (control)	30	30	25	83.33	-
	Djougou	82	22	19	86.36	1
	Copargo	80	50	34	68.00	0.212
	Pehunco	30	14	11	78.57	1
	Total control districts	108	156	126	80.77	-
	Total treated districts	352	135	104	77.04	0.525

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

Table 7. *Indoor resting density of* An. gambiae s.l *collected (PSCs data).*

Period	Districts	Nb of rooms	Nb *An. gambiae* (s.l.) collected	Indoor resting density	RR [95% CI]	P-value (Wald)
Pre-IRS evaluation: Jan - Mar 2018	Bembèrèkè (control)	60	101	1.68	1	-
	Kandi	58	66	1.14	0.67 [0.49- 0.92]	0.012
	Gogounou	60	36	0.60	0.35 [0.24- 0.52]	<0.001
	Kouande (control)	30	64	2.13	1	-
	Djougou	60	28	0.47	0.21 [0.14- 0.34]	<0.001
	Copargo	60	41	0.68	0.32 [0.21- 0.47]	<0.001

Period	Districts	Nb of rooms	Nb *An. gambiae* (s.l.) collected	Indoor resting density	RR [95% CI]	P-value (Wald)
	Pehunco	20	6	0.30	0.14 [0.05- 0.30]	<0.001
	Total control districts	90	165	1.83	1	-
	Total treated districts	258	177	0.69	0.37 [0.30- 0.46]	<0.001
Post-IRS evaluation: Jun- Nov 2018	Bembèrèkè (control)	78	126	1.62	1	-
	Kandi	80	28	0.35	0.21 [0.14- 0.32]	<0.001
	Gogounou	80	21	0.26	0.16 [0.10- 0.25]	<0.001
	Kouande (control)	30	30	1.00	1	-
	Djougou	82	22	0.27	0.26 [0.15- 0.46]	<0.001
	Copargo	80	50	0.63	0.62 [0.39- 0.99]	0.039
	Pehunco	30	14	0.47	0.46 [0.24- 0.87]	0.015
	Total control districts	108	156	1.44	1	-
	Total treated districts	352	135	0.38	0.26 [0.21- 0.33]	<0.001

P-value: comparison of the indoor resting density of *An. gambiae* s.l. between the treated and control districts (Test used: Poisson.test).

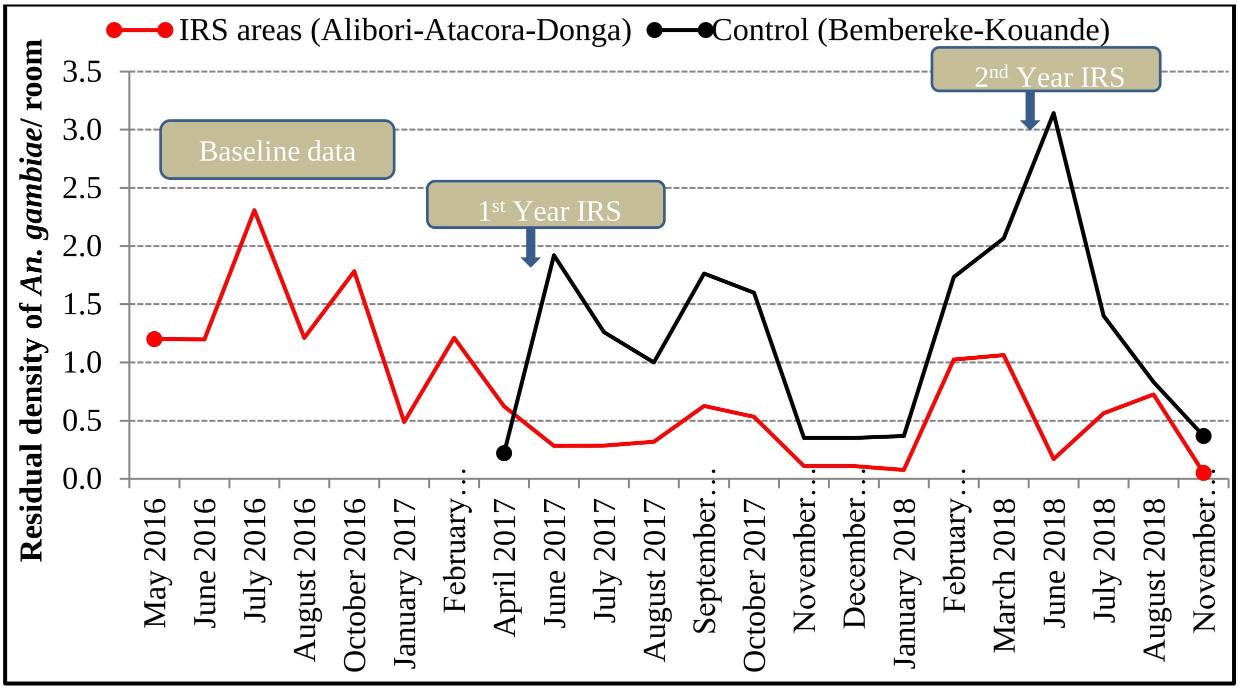


Figure 8. *Dynamics of residual density* An. gambiae s.l *in treated and control districts from May 2016 to November 2018 (PSCs data)*

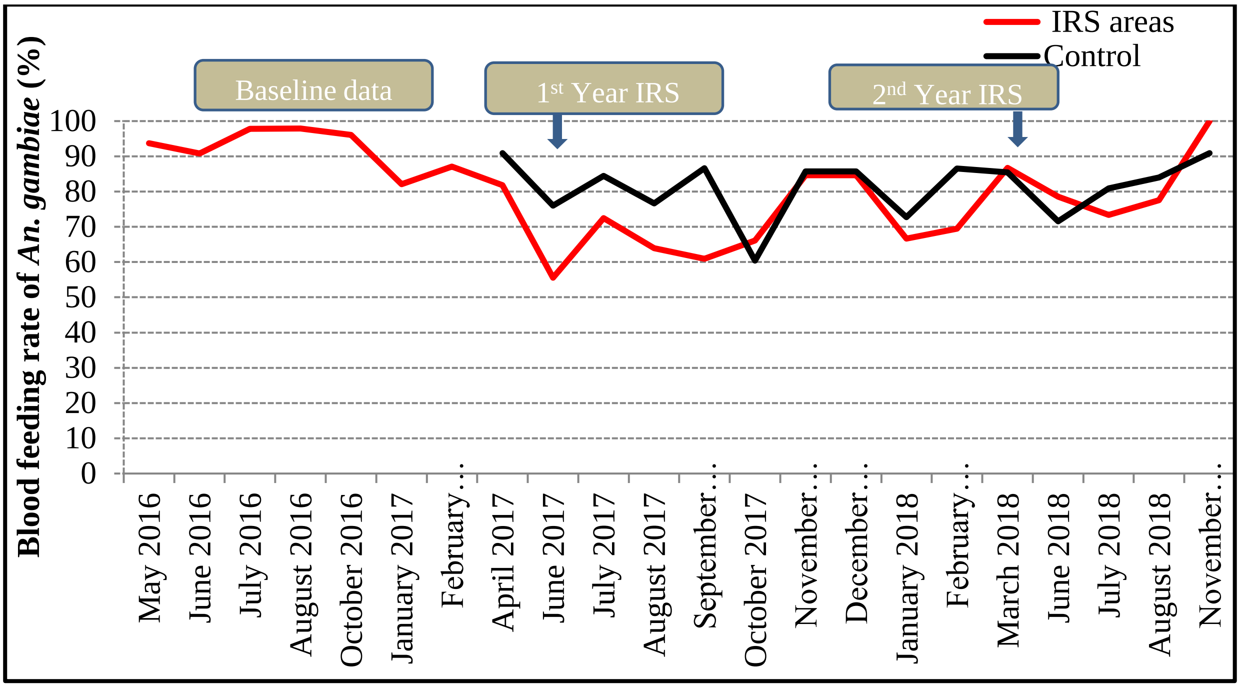


Figure 9. *Dynamics of blood feeding rate* An. gambiae *s.l. in treated and control districts from May 2016 to November 2018 (PSCs data)*

4.2.3. Blood meal analysis of *Anopheles gambiae* s.l.

The origin of blood meal of mosquitoes in the treated and untreated districts is presented in the Table 8. Despite the significantly reduced indoor resting density of vector, an average of 76.81% of *An. gambiae* s.l. caught in the treated rooms was fed with human blood compared to 87.71% in the control area ($\chi 2$ = 1.81; *df* = 1; *P* =0.178).

Table 8. *Blood-meal analysis of* Anopheles gambiae s.l. *in treated and control districts (PSCs data: period June 2018 – August 2018)*

District	Total analyzed	Human only	Bovine only	Human + pig	Bovine + goat	Human + bovine
Kandi	22	18	0	0	0	4
Gogounou	10	4	5	0	0	1
Djougou	11	11	0	0	0	0
Copargo	26	20	0	0	0	6
Total district under IRS	69	53 (76.81%)	5 (7.25%)	0 (0.00%)	0 (0.00%)	11 (15.94%)
Bembereke (control)	57	50 (87.72%)	5 (8.77%)	1 (1.75%)	1 (1.75%)	0 (0.00%)

4.3. Parous rate of *An. gambiae* s.l.

Table 9. shows the impact of the IRS on the longevity of *An. gambiae* based on proportion of mosquitoes that have laid at least once; mosquitoes from HLCs were used. Before 2018 IRS intervention (November 2018 to March 2018), the parous rate of *An. gambiae* in the treated districts (Alibori,

Atacora and Donga) was 76.40% (123/161) compared to 71.70% (76/106) in controls districts (Bembereke and Kouande) (X^2 = 0.51; *df*=1; p=0.472). After the 2018 IRS campaign (June-November 2018), the parous rate of *An. gambiae s.l* was significantly reduced in IRS areas compared to the control areas (Table 9). In treated districts, the rate was 47.56% (389/818) while in the control areas, the rate was 63.64% (322/506) (X^2 = 31.87; *df*=1; *P*< 0.001).

Table 9. *Parous rate of* An. gambiae s.l *in IRS and control districts before and after the 2018 IRS campaign*

Period	Districts	Nb *An. gambiae* (s.l.) dissected	Number of parous	Parous rate (%)	P-value
Pre-IRS evaluation: Jan - Mar 2018	Kandi	30	22	73.33	1
	Gogounou	31	24	77.42	0.879
	Bembèrèkè (control)	88	65	73.86	-
	Djougou	54	42	77.78	0.279
	Copargo	37	29	78.38	0.304
	Pehunco	9	6	66.67	1
	Kouande (control)	18	11	61.11	-
	Total treated districts	161	123	76.40	0.472
	Total control districts	106	76	71.70	-
Post-IRS evaluation: Jun 2018- Nov 2018	Kandi	164	87	53.05	0.003
	Gogounou	247	128	51.82	0.0002
	Bembèrèkè (control)	414	276	66.67	-
	Djougou	147	62	42.18	0.294
	Copargo	214	98	45.79	0.581
	Pehunco	46	14	30.43	0.045
	Kouande (control)	92	46	50.00	-
	Total treated districts	818	389	47.56	<0.001
	Total control districts	506	322	63.64	-

Nb: Number; P-value: comparison of the parity rate of *An. gambiae* s.l. between the treated and control districts (Test used: Chi-square test).

4.4. Sporozoite index and Entomological Inoculation Rate (EIR) of *An. gambiae* s.l.

Tables 10, 11, 12 summarize biting rates (HBR), sporozoite index (SI) and entomological inoculation rate (EIR) recorded before and after the 2018 IRS campaign in the treated and untreated districts.

Before the 2018 IRS campaign (period from January 2018 to March 2018), a total of 340 head-thoraces of *An. gambiae s.l.* were analyzed by *Plasmodium falciparum* CS-ELISA in the treated districts (Alibori and Donga); this resulted in a sporozoite positivity of 2.94% (10/340). In the control districts (Bembereke and Kouande), the sporozoite positivity was 3.94% (9/288). Before the IRS campaign, there was no statistically significant difference between sporozoite positivity in the treated and control districts (X^2 = 0.172; *df*=1; p=0.677) (Table 10). However, the EIR was 2.14 times lower in the treated districts (0.80 infectious bites of *An. gambiae* s.l. per human per month) compared to the control districts (1.71 infectious bites of *An. gambiae* s.l. per human per month) (p<0.001) (Table 10). After 2018 IRS campaign (June- November 2018), the EIR in treated districts was 5.22 times lower in the treated districts (1.27 infectious bites /human/month) compared to control districts (6.64 infectious bites /human/month) (p<0.001) (Table 8).

Tables 11 and 12 shows the monthly biting rates (HBR), sporozoite index (SI) and entomological inoculation rate (EIR) recorded before and after 2018 IRS intervention in treated and untreated districts. From January 2018 to March 2018, EIR was relatively low in treated and control areas while from June 2018 to November 2018, we found a significant decrease in EIR in treated districts compared to controls localities.

Table 10. *Sporozoite index (SI) and entomological inoculation rate (EIR) of* An. gambiae *before and after the 2018 IRS campaign in treated and control districts.*

Districts	Period before 2018 IRS intervention			After 2018 IRS intervention		
	Period (Jan 2018-Mar 2018)			Period (Jun 2018-Nov 2018)		
	SI (%)	HBR/night	EIR/month	SI (%)	HBR/night	EIR/month
Kandi	1.03	0.77	0.24	0.00	5.73	0.00
Gogounou	1.49	0.77	0.35	0.27	6.03	0.50
Bembereke (control)	2.03	2.45	1.49	1.04	14.94	4.67
Djougou	4.49	1.52	2.06	1.38	6.05	2.51
Copargo	3.85	0.92	1.07	1.09	7.33	2.40
Pehunco	11.11	0.44	1.46	3.77	0.82	0.92
Kouandé (control)	16.13	0.44	2.18	15.44	1.64	7.61
Total treated districts	2.94	0.91	0.80	0.89	4.75	1.27
Total controls districts	3.94	1.45	1.71	2.67	8.29	6.64

Table 11. *Monthly sporozoite index (SI), human biting rate (HBR) and entomological inoculation rate (EIR) of* An. gambiae *s.l. in treated areas (Alibori) and in control (Bembereke).*

Districts	Indicators	Jan 2018	Feb 2018	Mar 2018	June 2018	July 2018	Aug 2018	Nov 2018
	SI	0.00	0.00	1.39	0.00	0.00	0.00	0.00
	HBR/night	0.25	0.375	1.44	0.25	5.19	11.25	1.31
Kandi	EIR/night	0.00	0.00	0.02	0.00	0.00	0.00	0.00
	SI	0.00	2.60	0.00	0.00	0.00	0.704	0.00
	HBR/night	0.125	0.94	0.88	0.25	11.75	17.25	0.5
Gogounou	EIR/night	0.00	0.02	0.00	0.00	0.00	0.121	0.00
	SI	0.00	5.33	0.00	0.59	1.05	1.67	3.57
Bembereke	HBR/night	0.375	1.81	4.125	16.19	27.75	13.25	1.75
(control)	EIR/night	0.00	0.097	0.00	0.096	0.292	0.221	1.87

Table 12. *Monthly sporozoite index (SI), biting rate (HBR) and entomological Inoculation rate (EIR) of* An. gambiae *s.l. in treated area (Atacora-Donga) and in control (Kouande).*

Districts	Indicators	Jan 2018	Feb 2018	Mar 2018	June 2018	July 2018	Aug 2018	Nov 2018
	SI	0.00	5.17	4.17	0.00	0.96	2.42	0.00
	HBR/night	0.63	2.13	1.38	1.19	12.94	13.25	0.43
Djougou	EIR/night	0.00	0.11	0.06	0.00	0.12	0.32	0.00
	SI	0.00	2.27	7.41	0.00	1.50	1.13	0.00
	HBR/night	0.63	1.69	0.31	2.81	7.00	30.63	0.56
Copargo	EIR/night	0.00	0.04	0.02	0.00	0.105	0.34	0.00
	SI	0.00	NA	14.29	0.00	4.00	6.67	0.00
	HBR/night	0.13	NA	0.44	0.44	1.31	1.5	0.37
Pehunco	EIR/night	0.00	NA	0.06	0.00	0.05	0.10	0.00
	SI	15.38	22.22	11.11	10.71	17.78	23.33	5.00
Kouande	HBR/night	0.50	0.31	0.56	1.19	2.13	2.38	1.25
(control)	EIR/night	0.07	0.07	0.06	0.13	0.38	0.55	1.87

NA: Not applicable

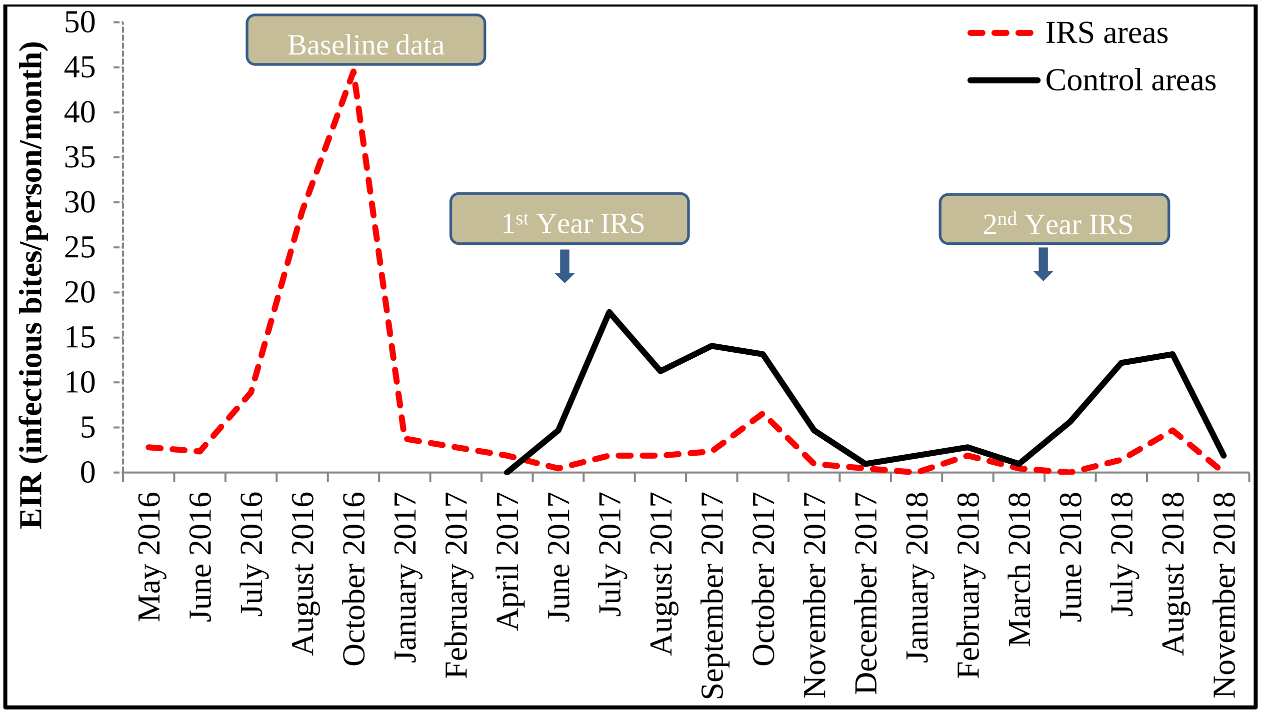


Figure 10. *Dynamics of EIR in treated area (Alibori, Donga) and in control area (Bembereke, Kouande) from May 2016 to November 2018.*

4.5. Insecticide susceptibility tests

Figure 11 summarizes the susceptibility level of local vectors to different insecticides (bendiocarb, pirimiphos methyl, fenitrothion, alpha-cypermethrin, permethrin and deltamethrin). All mosquito populations tested were susceptible to pirimiphos methyl and fenitrothion (mortality > 98%). However, these same vectors populations showed a decrease in susceptibility to bendiocarb (mortality between 90 and 97%) (suspected resistance) except Kandi (100%). For pyrethroids (deltamethrin, permethrin and alpha-cypermethrin), *An. gambiae* s.l. was resistant in all the districts (mortality< 90%) (Fig. 11).

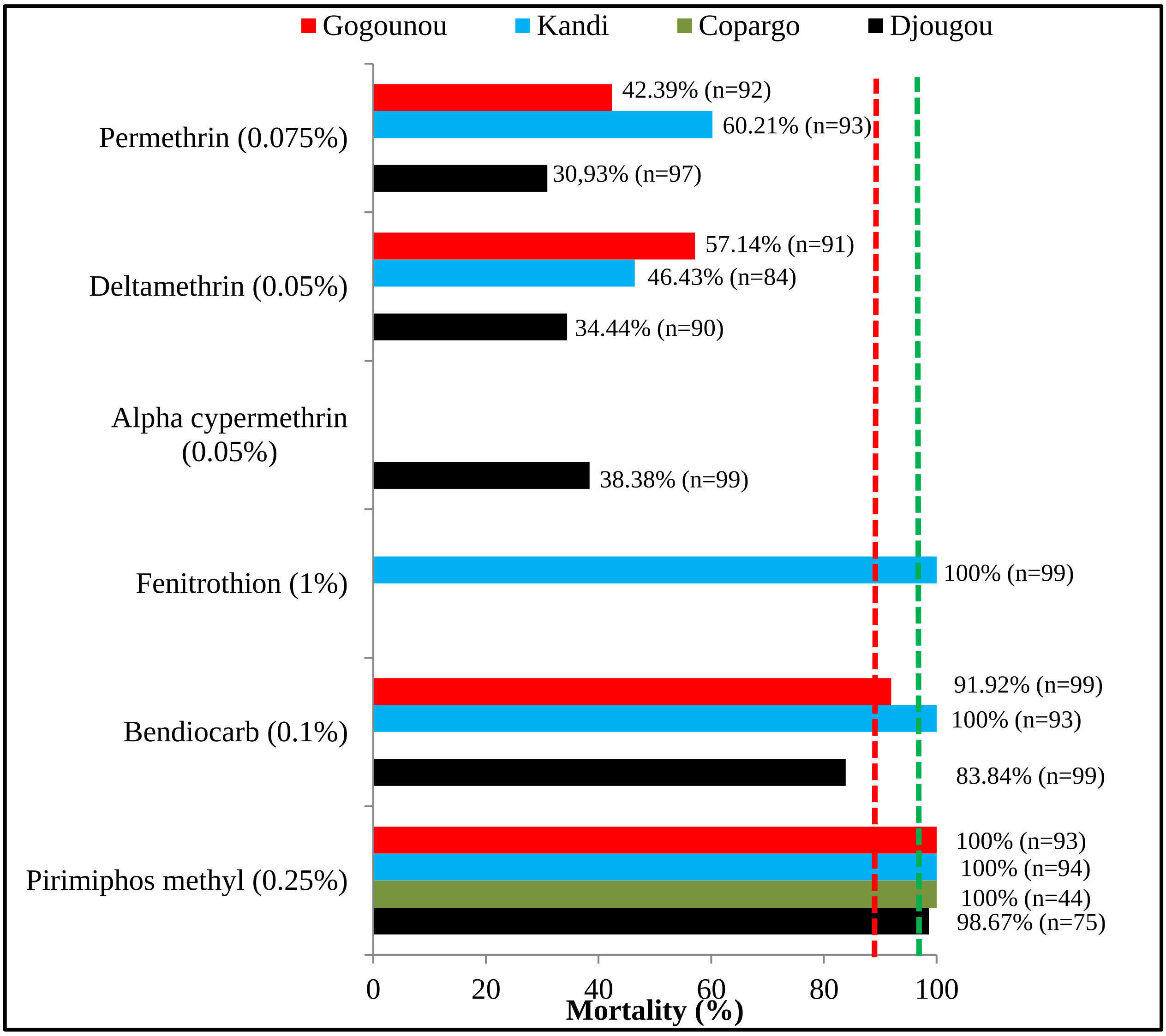


Figure 11. *Mortalities observed 24 hours after mosquito exposure to bendiocarb 0.1%, pirimiphos methyl 0.25%, fenitrothion 01%, permethrin 0.75%, alpha cypermethrin 0.05% and deltamethrin 0.05% in four districts under IRS during period June 2018 -August 2018 (based on World Health Organization criteria the area below broken red lines indicates insecticide resistance; the area in between the broken red and green lines indicate possible resistance; the area above the green broken line indicates insecticide susceptibility)* [12]

5.6. Multiple insecticide resistance mechanisms in *An. gambiae* s.l.

Data presented in Table 13 and 14 show the frequency and distribution of *kdr* and *Ace 1* resistance genes among *An. gambiae* complex species collected. *An. gambiae* s.s. was predominant vector (93.04%; 321/345) in all localities; *An. coluzzii* (4.05%; 14/345) and *An. arabiensis* (2.89%: 10/345) were only found in Alibori and Bembereke but at a very low frequency. *Kdr* mutation was detected

[12] WHO 2013. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: World Health Organization.

in high frequency in all species of the *An. gambiae* complex within all the localities. *Kdr* varied from 66.67% in *An. arabiensis* to 100% in *An. coluzzii* (Table 13 and Fig. 12). *Ace-1R* mutation associated with carbamates and organophosphate resistance was identified in all sites but with very low frequencies (0 to 11%). It varied from 0 to 2.9% in *An. gambiae* and *An. coluzzii* but zero in *An. arabiensis* (Table 14 and Fig. 13).

In all localities, the biochemical assays showed relative high activity of at least one detoxifying enzyme in local populations of *An. gambaie* s.l. compared to *An. gambiae* s.s. Kisumu strain (susceptible strain) (Table 15 and Figures 14 to 17). Figure 14 shows the average level of oxidases activity (MFO) in different populations tested. Cytochrome P450 activity was significantly elevated in Djougou, Gogounou and Kandi compared to the *An. gambiae* Kisumu strain (Table 13). The highest glutathione-S-transferase (GST) activities were observed in the Copargo and Gogounou populations with a significant difference compared to the Kisumu strain (Table 13). The activity of α esterase's was higher in the populations of Djougou, Copargo and Kandi compared to the Kisumu strain (Table 15). Significantly elevated β esterase activities were observed in Djougou, Copargo and Kandi compared to the Kisumu strain (Table 15).

Table 13. *Distribution of Knock-down resistance (*Kdr*) frequencies between malaria vectors and localities*

Localities	Species	Number tested	RR	RS	SS	F (*Kdr*) (%)
Kandi	*An.gambiae*	65	50	9	6	83.85
	An. coluzzii	2	2	0	0	100.00
	An. arabiensis	3	2	0	0	66.67
Gogounou	*An.gambiae*	69	54	10	5	85.51
	An. coluzzii	3	1	2	0	66.67
	An. arabiensis	2	1	1	0	75.00
Djougou	*An.gambiae*	64	45	15	4	82.03
Copargo	*An.gambiae*	71	60	10	1	91.55
	An. arabiensis	2	1	1	0	75.00
Bembereke (control)	*An.gambiae*	52	40	8	4	84.62
	An. coluzzii	9	6	2	1	77.78
	An. arabiensis	3	3	0	0	100.00

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

Table 14. *Distribution of* Ace-1 *frequency between species*

Localities	Species	Number tested	RR	RS	SS	F (Ace-1) (%)
Kandi	*An. gambiae*	65	0	2	63	1.54
	An. coluzzii	2	0	1	1	25.00

Localities	Species	Number tested	RR	RS	SS	F (Ace-1) (%)
	An. arabiensis	3	0	0	3	0.00
Gogounou	*An. gambiae*	69	0	4	65	2.90
	An. coluzzii	3	0	0	3	0.00
	An. arabiensis	2	0	0	2	0.00
Djougou	*An. gambiae*	64	0	3	61	2.34
Copargo	*An. gambiae*	71	0	3	68	2.11
	An. arabiensis	2	0	0	2	0.00
Bembereke (control)	*An. gambiae*	52	0	0	52	0.00
	An. coluzzii	9	0	2	6	11.11
	An. arabiensis	3	0	0	3	0.00

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

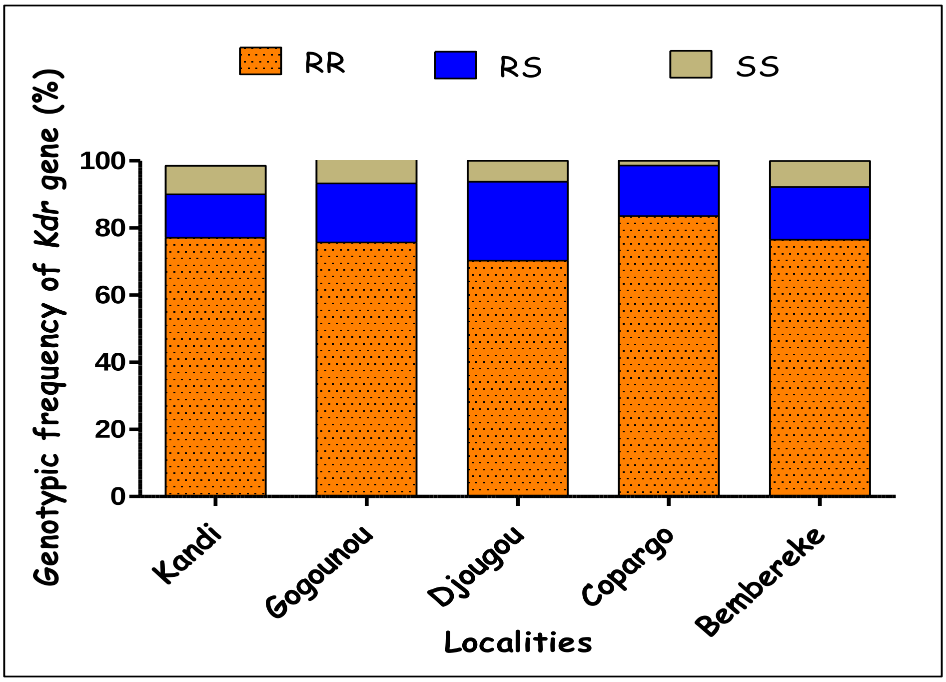


Figure 12. *Genotypes frequency of* Kdr *gene in the populations of* An. gambiae *(s.l.)*

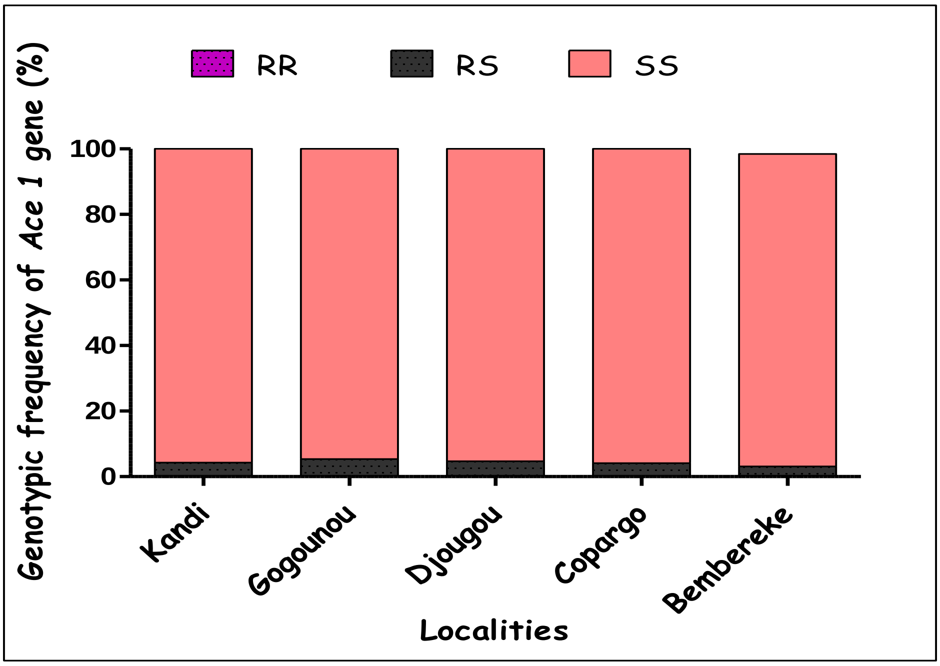


Figure 13. *Genotypes frequency of* Ace-1 *gene in the populations of* An. gambiae *(s.l.)*

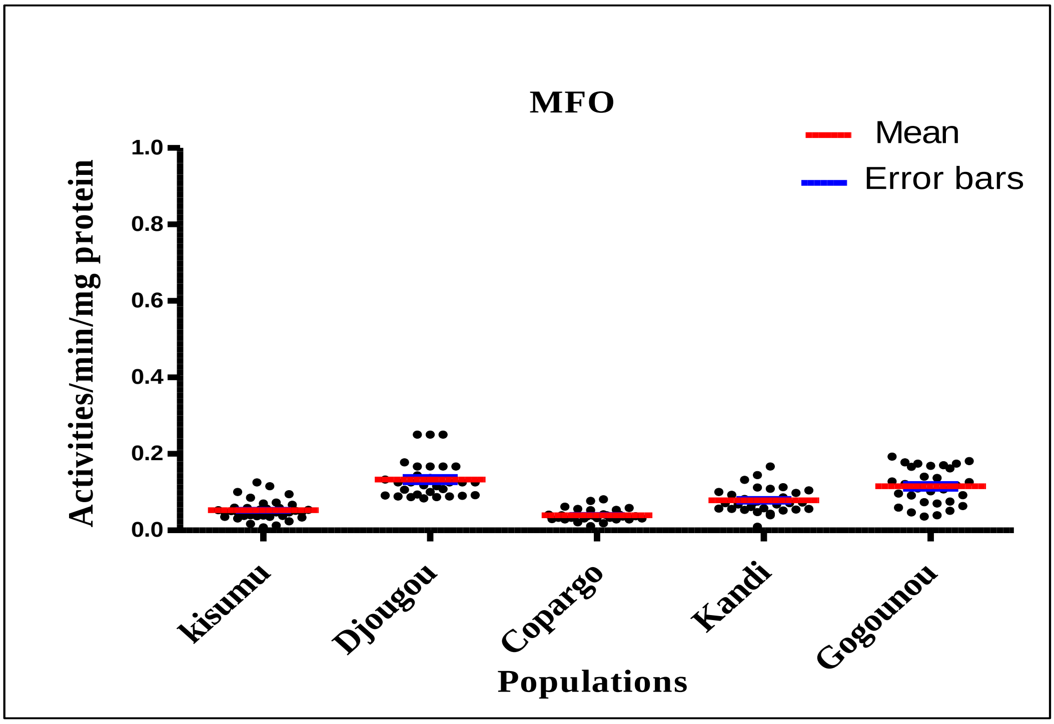


Figure 14. *Mixed functional oxidase (MFO) activities in* Anopheles gambiae sensu lato *collected.*

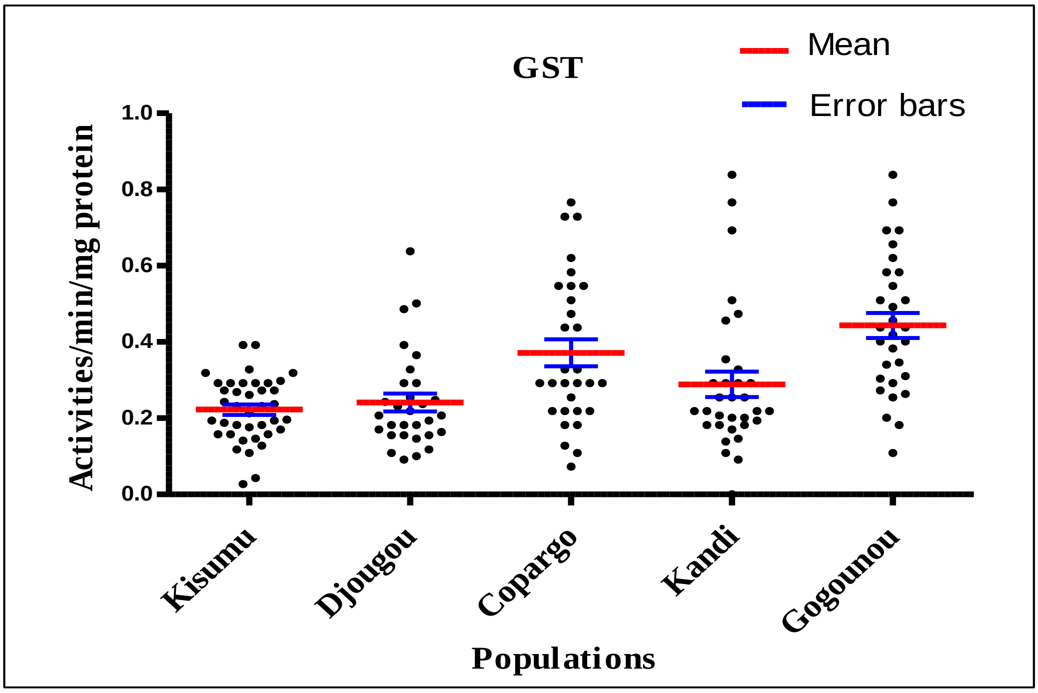


Figure 15. Glutathione-S-transferase activities in Anopheles gambiae sensu lato *collected*

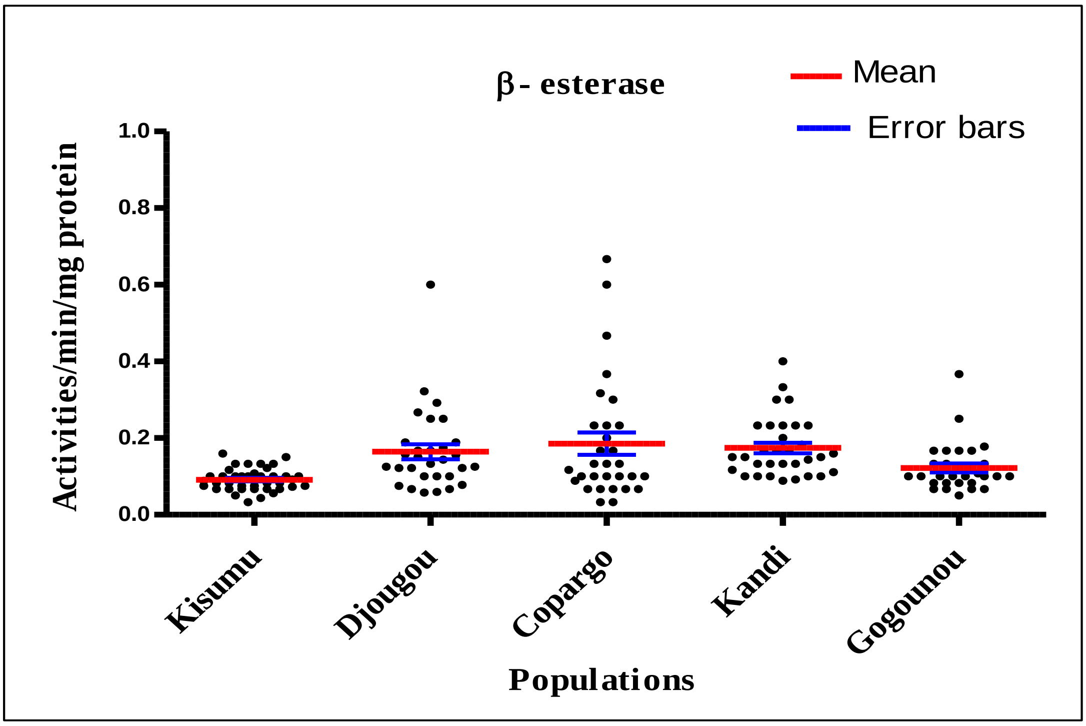


Figure 16. β-esterases activities in Anopheles gambiae sensu lato *collected*

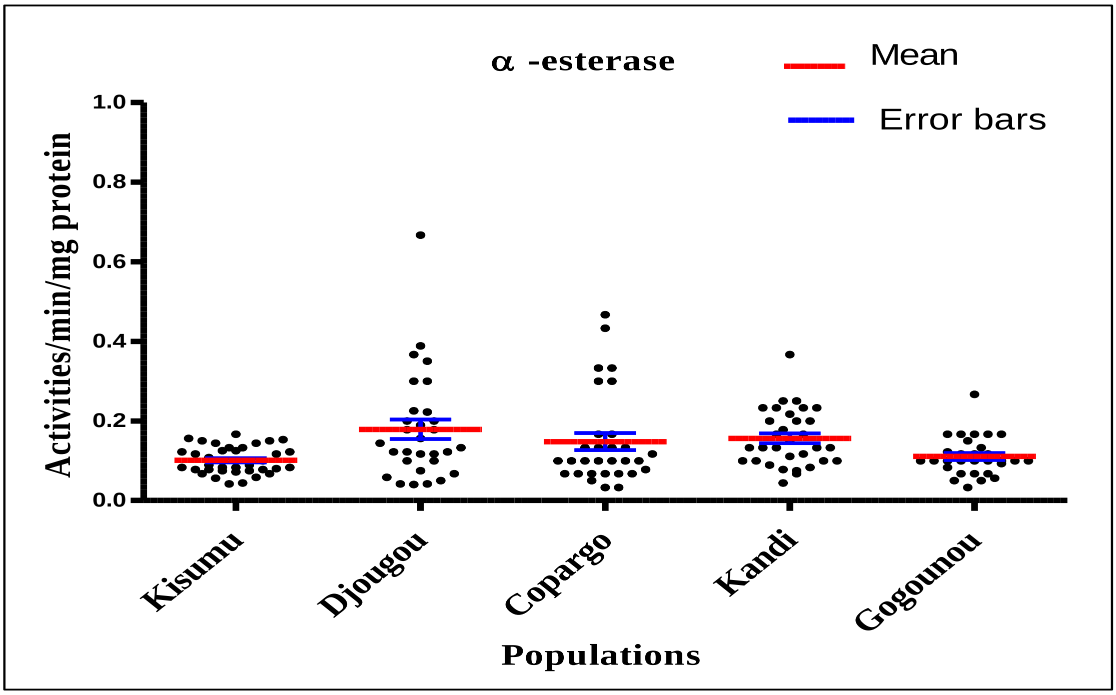


Figure 17. *α-esterases activities in* Anopheles gambiae sensu lato *collected*

Table 15. *Mean (± SE) mixed function oxidases, glutathione-S-transferases and esterases activities in An. gambiae s.l. populations.*

Strain	MFO (nmol/P450/min/mg protein)	GST (ηmol/GSH conj/min/mg protein)	α-Esterase (µmol α Naph/ /min/mg protein)	β-Esterase (µmol β Naph /min/mg protein)
Kisumu	0.05±0.003	0.22±0.01	0.10±0.005	0.09±0.004
Djougou	*0.13±0.008[a]	0.24±0.02[a]	*0.18±0.02[a]	0.16±0.01[a]
Copargo	0.004±0.002[b]	*0.37±0.03[b]	*0.15±0.02[a]	0.18±0.02[a]
Kandi	*0.08±0.005[a]	0.28±0.03[b]	*0.16±0.01[a]	0.17±0.01[a]
Gogounou	*0.11±0.008[a]	*0.44±0.03[c]	0.11±0.008[b]	0.12±0.01[b]
One Way ANOVA	F=38.80; df=5, 156; P<0.0001	F=11.05; df=5, 156; P<0.0001	F=4.57; df=5, 156; P=0.0016	F=5.98; df=5, 156; P=0.0002

MFO = mixed function oxidases, GST = glutathione-S-transferase; Mean followed by a different letter were significantly different, P<0.05, Tukey's test. *Significant increase in mean differences compared to the laboratory reference strain, P<0.05, t-test

5. Conclusions

All entomological monitoring targets set during deliverable covering the period from January 2018 to November 2018 were met. Monitoring and evaluation of the 2nd year of indoor residual spraying campaign carried out from January 2018 to November 2018 in Alibori, Atacora and Donga has shown once more the impact of this strategy on the reduction of entomological indicators. From evaluation of this campaign, we can note a significant difference between the entomological indicators of treated districts and those controls districts.

The low density of *An. gambiae* s.l. in all localities during this period is likely due to the harmattan season and the dry conditions which characterize it. This higher biting behavior of *An. gambiae* s.l. indoors compared to outdoors of treated houses during this period is likely due to the complete decrease in the effect of the insecticide used in May 2017.

Bioassays on treated walls have shown that pirimiphos methyl (Actellic CS) remains effective for up to four months (May-September) after spraying date. During this period of bio-efficiency of Actellic CS, we observed a spectacular reduction of some indicators like the residual density, vector longevity, sporozoite index and EIR and strong exophagy of *Anopheles gambiae* in most treated districts compared to control areas. However, IRS impact is not so visible on some indicators such as the blood feeding rate; this particular indicator appeared relatively high in treated and control districts.

With regard 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 2018 IRS campaign, the rarity of positive *Anopheles* larvae breeding sites in some treated localities was a handicap to carry out susceptibility tests in all localities and for all insecticide classes. The search for larvae will continue in these areas in 2019.
- We stopped monitoring the study of the availability of Actellic 300 CS on treated walls after 4 months due to the contamination of the susceptible strain (Kisumu) in the CREC insectarium. Then, we were obliged to destroy our colony and develop a new one.

Mosquito catches on human volunteers will now be reorganized in time slots from 7 p.m. to 7 a.m. for the study of the correlation of entomological and socio-anthropological data.

8. Activities planned for the next 3 months (January – March 2019)

The same monitoring will continue in the same districts and this data will be used to guide the next May 2019 spraying campaign.