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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Collaboration: National Malaria Control Program (NMCP)

Doc/CREC/PMI/USAID/Nov 2019

1. Introduction

Implementation of Indoor Residual Spraying (IRS) in Benin since 2008 was accompanied by a drastic reduction in Entomological Inoculation Rate (EIR) (Padonou *et al*., 2011, Aïkpon *et al*., 2013, Akogbeto *et al.,* 2015). 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 the vector resistance and to extend IRS in other regions. As part of Benin's Insecticide Resistance Management strategy, IRS was withdrawn after 6 years of implementation.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 the insecticide resistance by limiting the selection pressures on mosquitoes carrying resistance genes. Another reason for Indoor Residual Spraying withdrawal from Atacora is 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, 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 (Alibori and Donga) with complete withdrawal of this intervention from Atacora (Kerou and Pehunco). The main objective of this evaluation is to collect data on mosquito behaviour 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.

2. Study areas (see map)

Three health zones (HZ) were protected by IRS in 2019:

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

In total, 6 districts among which Djougou and Copargo were used for M&E in Donga, Kandi and Gogounou in Alibori.

- Bembereke, the closest district from Alibori is the control of the districts under IRS in Alibori region.
- Kouande is chosen to serve as a control for IRS districts in the Donga region because it is the only district near Copargo and Djougou (Figure 1).

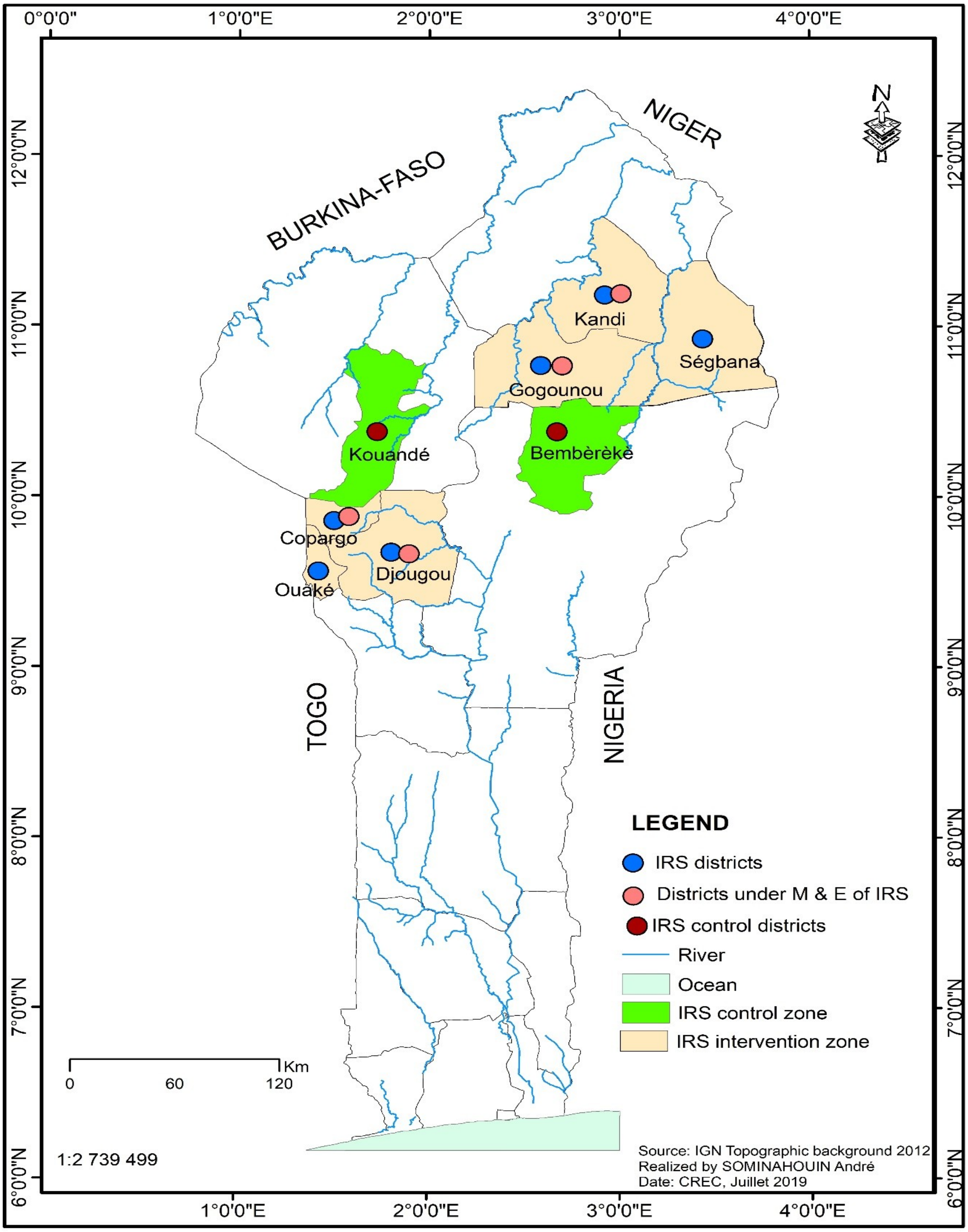
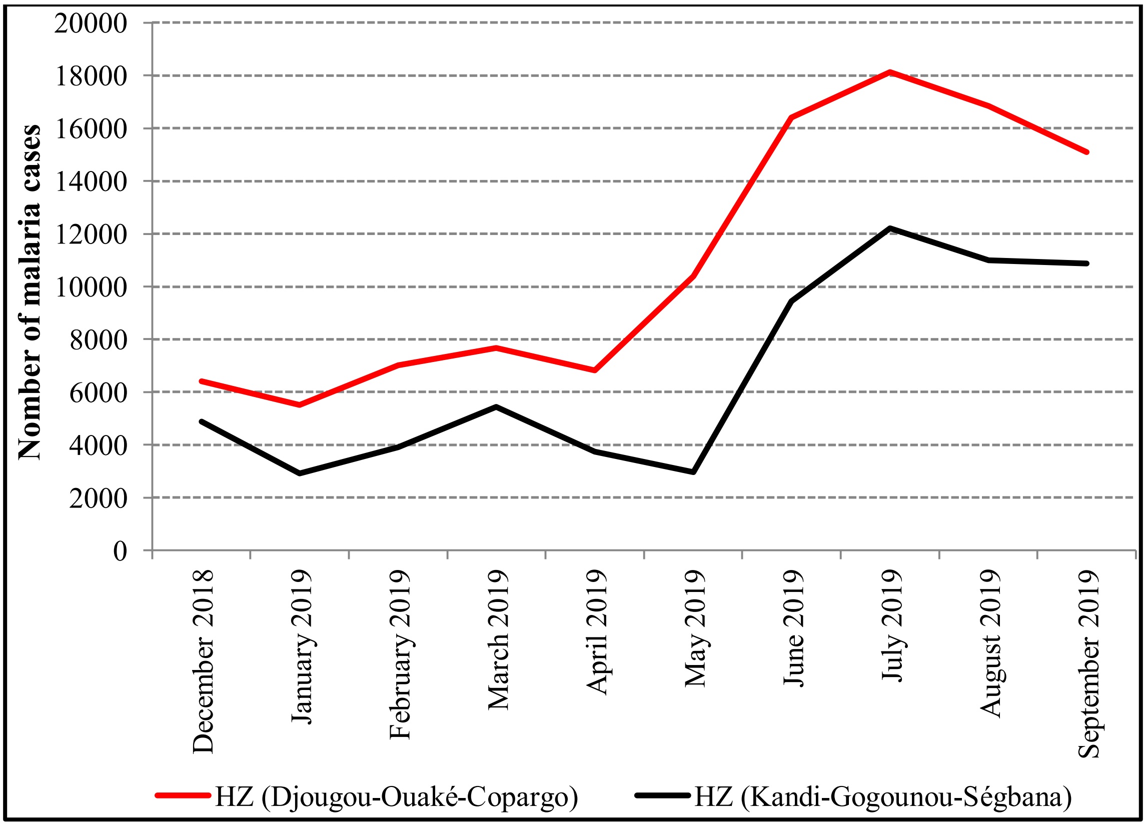
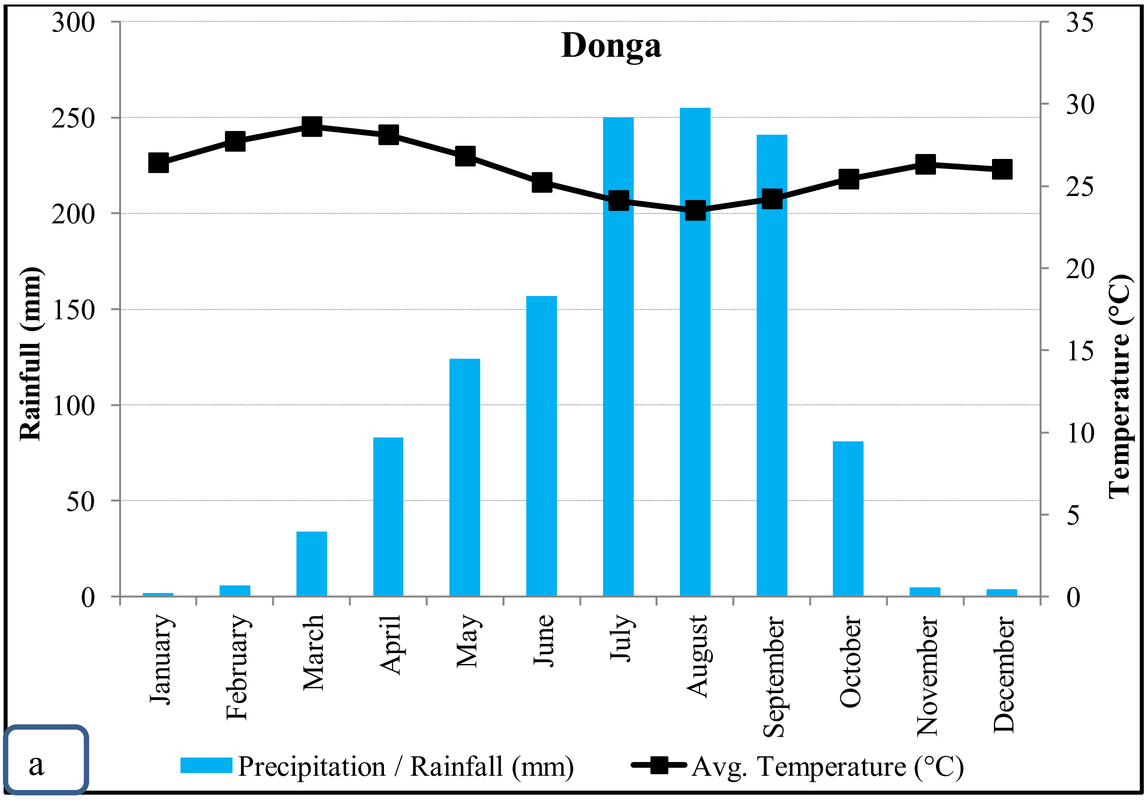


Figure 1: Map showing the IRS evaluation areas

Figure 2 shows the evolution of the number of malaria cases (number tested positive) in Kandi-Gogounou-Segbana (KGS) and Djougou-Copargo-Ouake (DCO) health's zones and the evolution of rainfall and temperature in the Alibori and Donga regions in 2019.The number of malaria cases is higher in the DCO health zone compared to KGS. This number is higher in the rainy season than in the dry season.

The rainiest months are June, July, August and September in both regions with the rainfall peak in August.





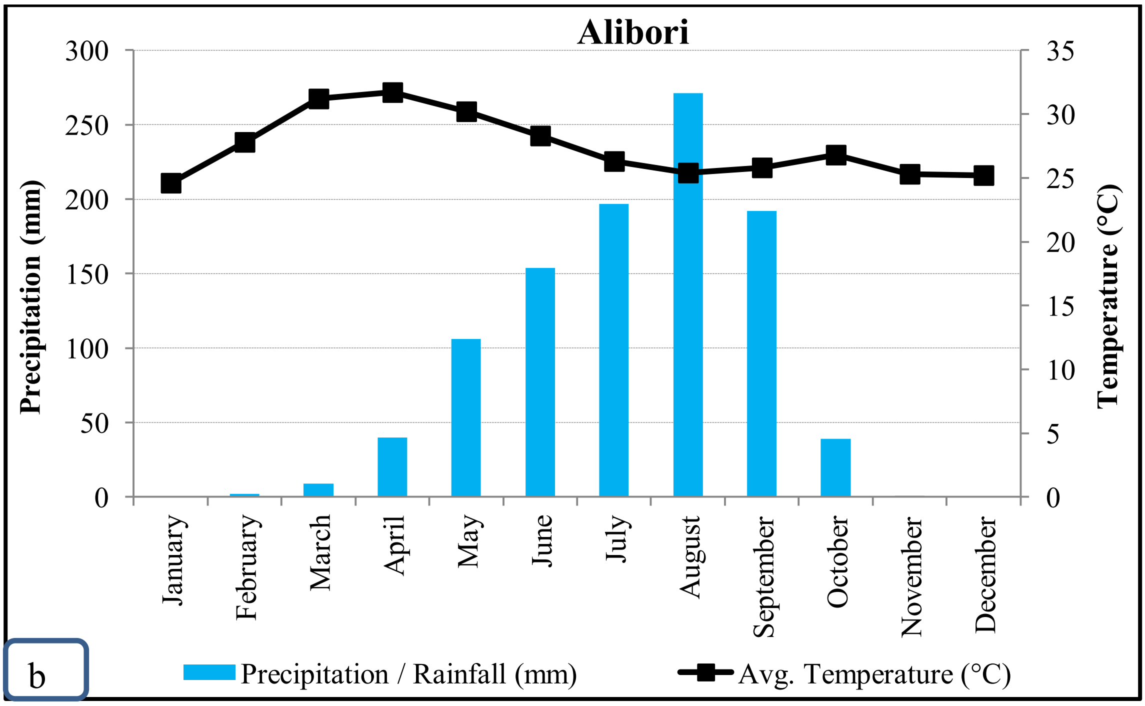


Figure 2: Monthly malaria cases[1] and monthly climate data in the Alibori and Donga regions[2].

3. 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
- Identification of mosquito genetic mutations that confer resistance (Kdr, Ace-1, Oxidases, esterases, GST).

4. Organization of the report

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[1] Malaria data from the National Malaria Control Program

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

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 Kisumu strain of *An. gambiae* after the walls were treated.
This annual report presents the evolution of entomological indicators in the treated and control districts.
To better assess the impact of IRS on malaria transmission, we compared entomological indicators 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%).

The report includes the following data retained in the protocol:

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

5. Bioassay cone tests

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)[3] were conducted seven days (T0) May 2019 IRS campaigns Djougou and Copargo 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. This test allowed us 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

[3] 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/)

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 a relative humidity of 80 ± 10%, the mortality rate was determined. When the control mortality was between 5–20%, corrected mortality was performed accordingly using Abott's formula[4]; when the control mortality was higher than 20%, the bioassay was considered invalid and repeated.

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

6. Sampling of malaria vectors to determine malaria transmission indicators

Mosquito sampling is being carried out in the six districts:

Districts selected for IRS M&E: Djougou, Copargo, Kandi, Gogounou under IRS and two control areas (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. For each village, mosquitoes were collected in 2 houses by 4 mosquito collectors, 2 mosquito collectors indoor and 2 outdoor. 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 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 ability or individual attractiveness.

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

To estimate the density of mosquitoes per room, 10 houses per village were selected[5]. 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 microscope to determine the parous rates. The heads/thoraces of the vector species were analyzed by 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.

7. Mosquito larval collections and insecticide susceptibility tests, Species identification and PCR detection of *Kdr* and *Ace-1* mutations and metabolic resistance

Anopheles gambiae s.l. larvae were collected from natural breeding sites 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 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[6]. 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. The PCR-RFLP diagnostic test was used to detect the presence of L1014F mutation (*Kdr*) and G119S mutation (*Ace 1R* gene). Metabolic resistance (esterases, oxidases, GST) was analyzed by biochemical assays and a spectrophotometer using the non-exposed control mosquitoes from the WHO susceptibility test and comparing this to *An. gambiae* s.s. Kisumu strain.

8. Data analysis

Data were analyzed with the statistical R software, version 2.8. using the stats package[7]. The Poisson method[8] was used to estimate and compare the confidence intervals of indoor vector

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

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

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

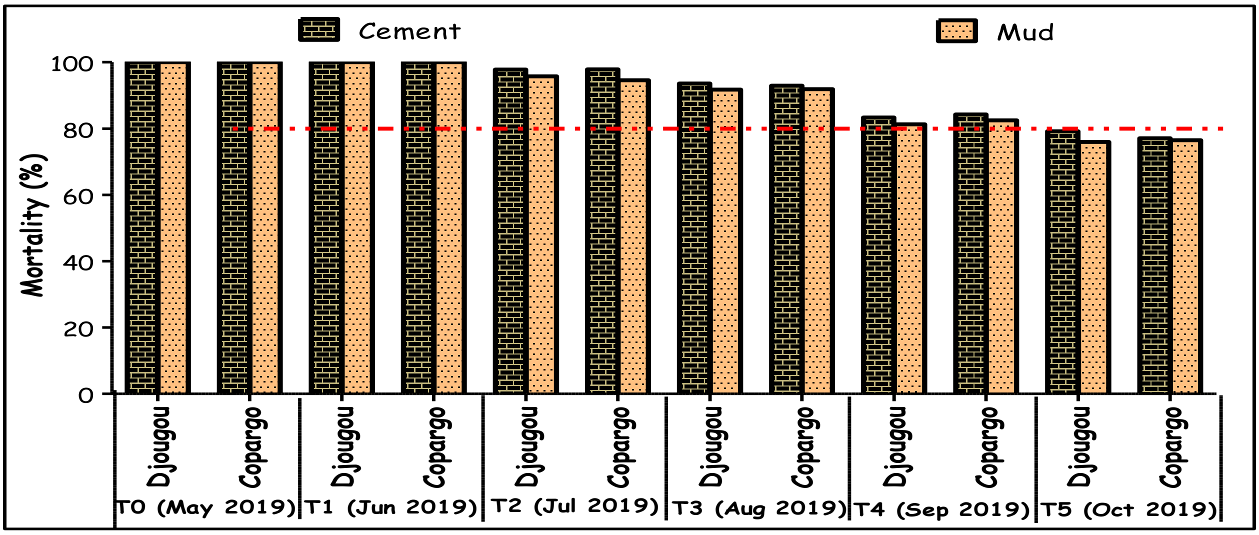
density and EIRs of *An. gambiae* (s.l.). The Chi-square test of comparison of proportions was used to compare proportion of *An. gambiae* (s.l.) indoors and outdoors, blood-feeding rate, sporozoite index, and parity rate of *An. gambiae* (*s.l.*). These different parameters were compared before and after IRS and then between the treated and control areas.

We calculated the % reduction in EIR in IRS areas compared to control areas using this formula: % reduction of EIR in Sprayed areas compared to control areas = (number of infectious bites/month in Control area - number of infectious bites/month in Sprayed areas / (number of infectious bites/month in Control area) * 100

9. Results

9.1. Residual effect of pirimiphos methyl on cements and mud walls treated during the 3rd (2019) campaign years of IRS in Benin.

Pirimiphos-methyl (Actellic CS) decay rates on treated cement and mud walls were evaluated for four months in 2019. At T0 (May 2019) (7 days after wall treatment), 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 2). This suggests the good quality of treatment and evidence that the insecticide is available on walls and at a lethal dose. Residual activity was above 80% for four months until September in 2019 IRS campaigns (Figure 4 and Table 1).



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

Red line = Insecticide efficacy threshold of Kisumu (WHO 2006)

Figure 4: 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 2019)		T1 (June 2019)		T2 (July 2019)		T3 (August 2019)		T4 (September 2019)		T5 (October 2019)	
	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

9.2. ***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. gambie* 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. gambie* 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 caracteristics 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 breeding sites are choices of preference for the development of larvae of *Cx. quinquefasciatus*.

While many of the mosquitoes collected do not transmit malaria, they have their importance in medical entomology. *Culex quinquefaciatus* transmits *bancroftian filariasis* and West Nile Virus. *An. pharoensis* and *Mansonia africana* are important in transmission of 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

9.3. Mosquito blood feeding behaviors

9.3.1. 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 in control (Bembereke and Kouande). Table 2, Figures 5a and 5b shows the proportion of *An. gambiae s.l.* indoors compared to outdoors in these districts. In Table 3 and Figures 5a and 5b, two observations can be made:

1. Before 2019 IRS campaign (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 behaviour 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 5a). 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 behaviour observed of *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 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) in outdoors. In contrast, in untreated houses, 55.93% (1070/1913) were collected indoor versus 44.07% (843/1913) outdoors (Figure 5b). This shows that treated houses pirimiphos methyl on the walls have significantly reduced vectors indoors.

Tables 4a and 4b 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 indoor and outdoor before and after IRS intervention (2019) in treated districts *vs* control

Ditricts	Before 2019 IRS (November 2018-March 2019) Indoors nb (%)	Before 2019 IRS (November 2018-March 2019) Outdoors nb (%)	p-value	Bio-efficacy period of Actellic CS (June 2019-August 2019) Indoors nb (%)	Bio-efficacy period of Actellic CS (June 2019-August 2019) Outdoors nb (%)	p-value
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.; %: proportion of *An. gambiae* s.l.; p-value: p-value of comparison of the proportion of *An. gambiae* s.l indoors and outdoors in the same district (Test used: Chi-square test)

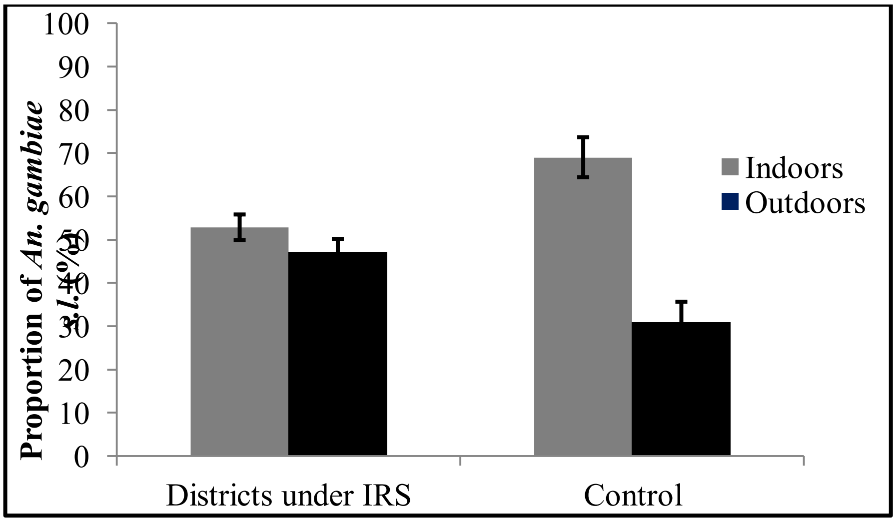


Figure 5a: Overall percentage of *An. gambiae* s.l. collected using HLC indoors and outdoors (before 2019 IRS) (period November 2018 to March 2019) in treated and untreated houses

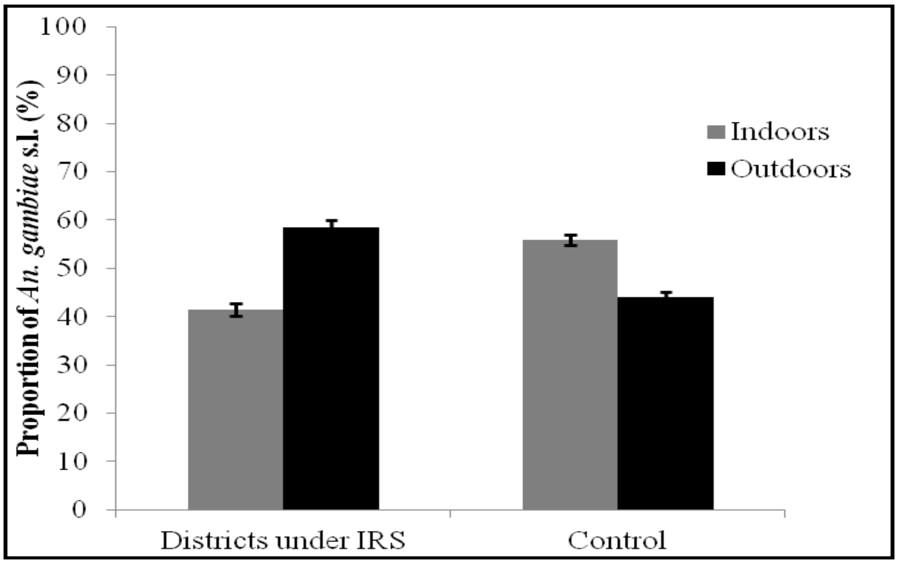


Figure 5b: Overall percentage of *An. gambiae* s.l. collected using HLC indoors and outdoors after 2019 IRS intervention (bio-efficacy period of Actellic 300CS: June 2019 to August 2019) in treated and untreated house.

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

Distics	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	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 4b: Details biting rates of *An. gambiae* s.l. indoor and outdoor in treated districts (Alibori) and in control (Bembereke).

Disticts	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 cathes	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 cathes	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 cathes	8	8	8	8	8	8
		HBR/night	1.500	0.250	0.500	7.500	44.000	36.875

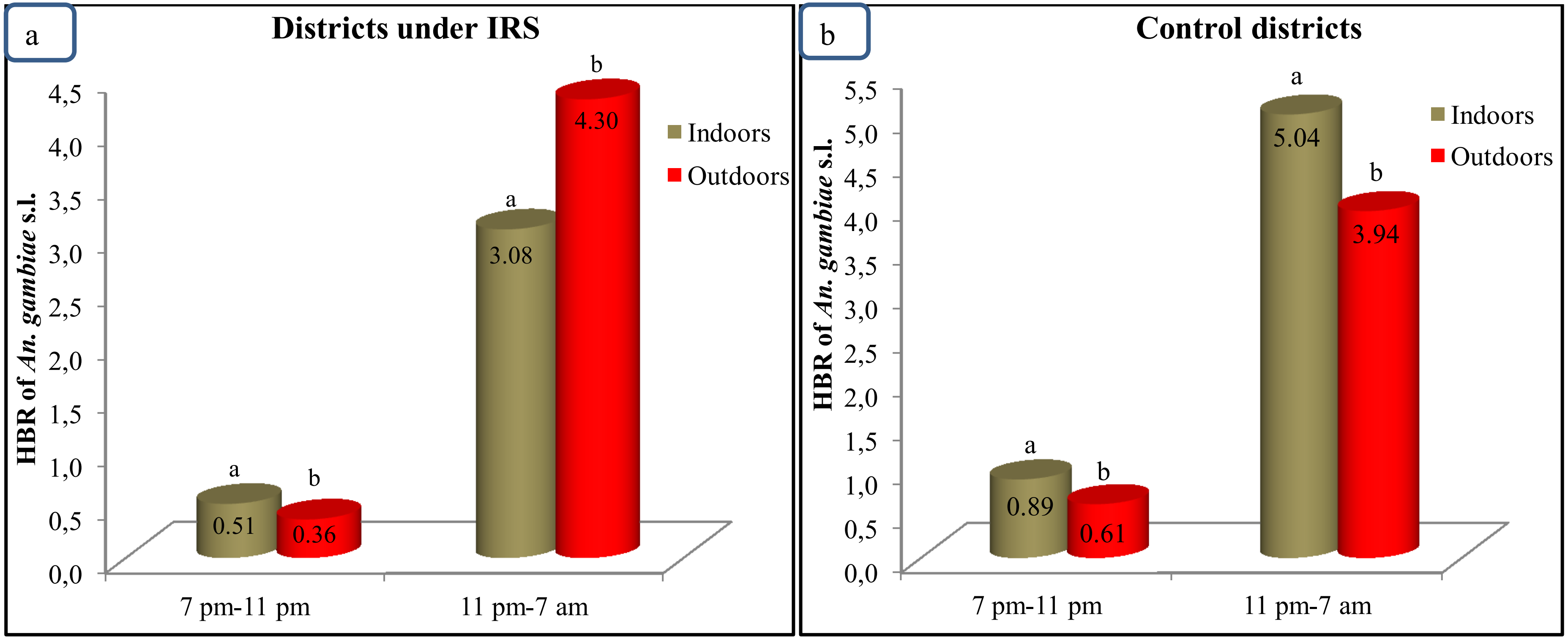
9.3.2. Hourly Human Biting Rate of *An. gambiae* (*s.l.*) and *Culex quinquefasciatus* during the night (period November 2018 - August 2019)

The hourly biting rate of *An. gambiae s.l.* and *Culex quinquefasciatus* was monitored in treated districts (Kandi, Gogounou, Djougou, Copargo) and in control (Bembereke and Kouande). The hourly HBR of *An. gambiae s.l.* and *Culex quinquefasciatus* appears to be the same in treated (Figures 7a and 9a) and untreated (Bembereke and Kouande) houses (Figures 7b and 9b), but the biting behaviour (indoors or outdoors) varies 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. From 7 p.m. to 11 p.m., hourly biting rate of *An. gambiae s.l.* and *Culex quinquefasciatus* increases constantly to its peak between 12 a.m. and 2 a.m. hours (Figures 7a, 7b, 9a and 9b). During this time period (7 pm to 11 pm), biting rate of *An. gambiae* (s.l.) and *Culex quinquefasciatus* /person is low in both areas (treated and control area) (Figures 6a and 8a).

ii. After 2 a.m., hourly biting rate gradually decreases until the early morning (Figures 7a, 7b, 9a and 9b). Between 11 p.m and 7 a.m., biting rate of *An. gambiae* (s.l.) is very high in both areas (treated and untreated areas) (Figures 6b and 8b).

Tables 5a, 5b, 6a and 6b show the number of *An. gambiae* (s.l.) and *Culex quinquefasciatus* in all treated districts and in control.



HBR with the same letters in the same area are not significantly different

Figure 6: Biting location of *An. gambiae* (s.l.) early (7pm-11pm) and late at night in treated (a) and in control areas (b) (period November 2018 – August 2019).

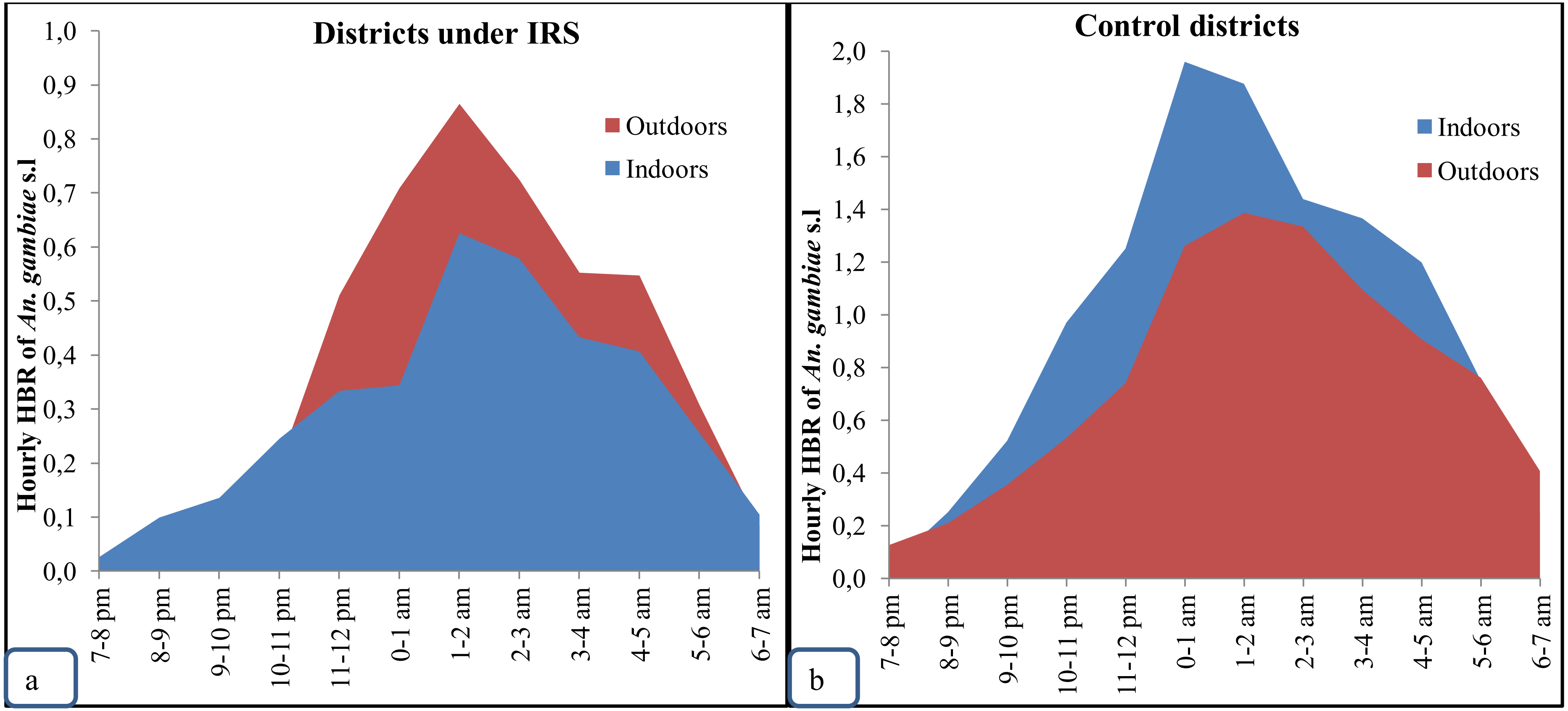
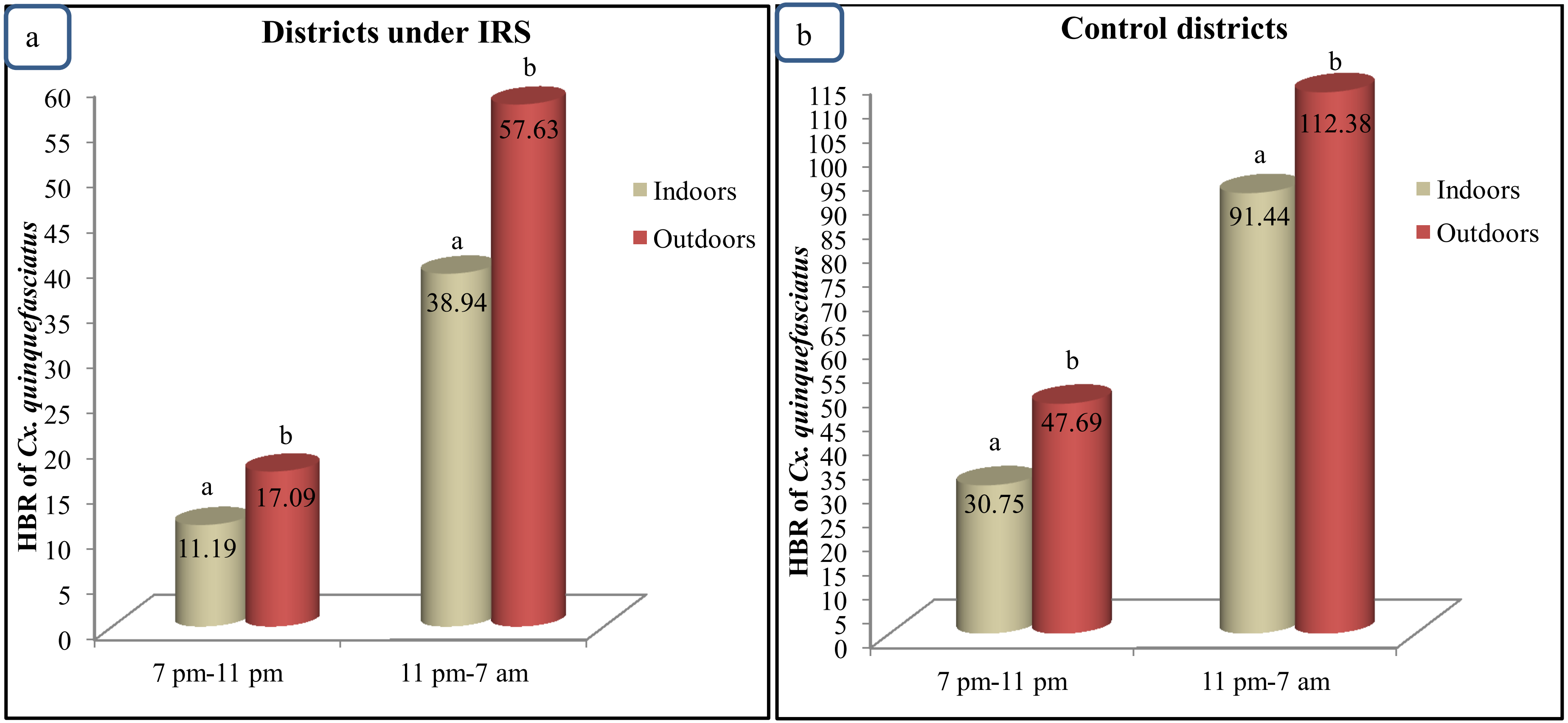


Figure 7: Hourly HBR of *An. gambiae s.l.* in all treated districts (a) and in control districts (b) (November 2018 – August 2019)



HBR with the same letters in the same area are not significantly different

Figure 8: Biting location of *Culex quinquefasciatus* early (7pm-11pm) and late at night in treated (a) and in control areas (b) (period June 2019 – August 2019).

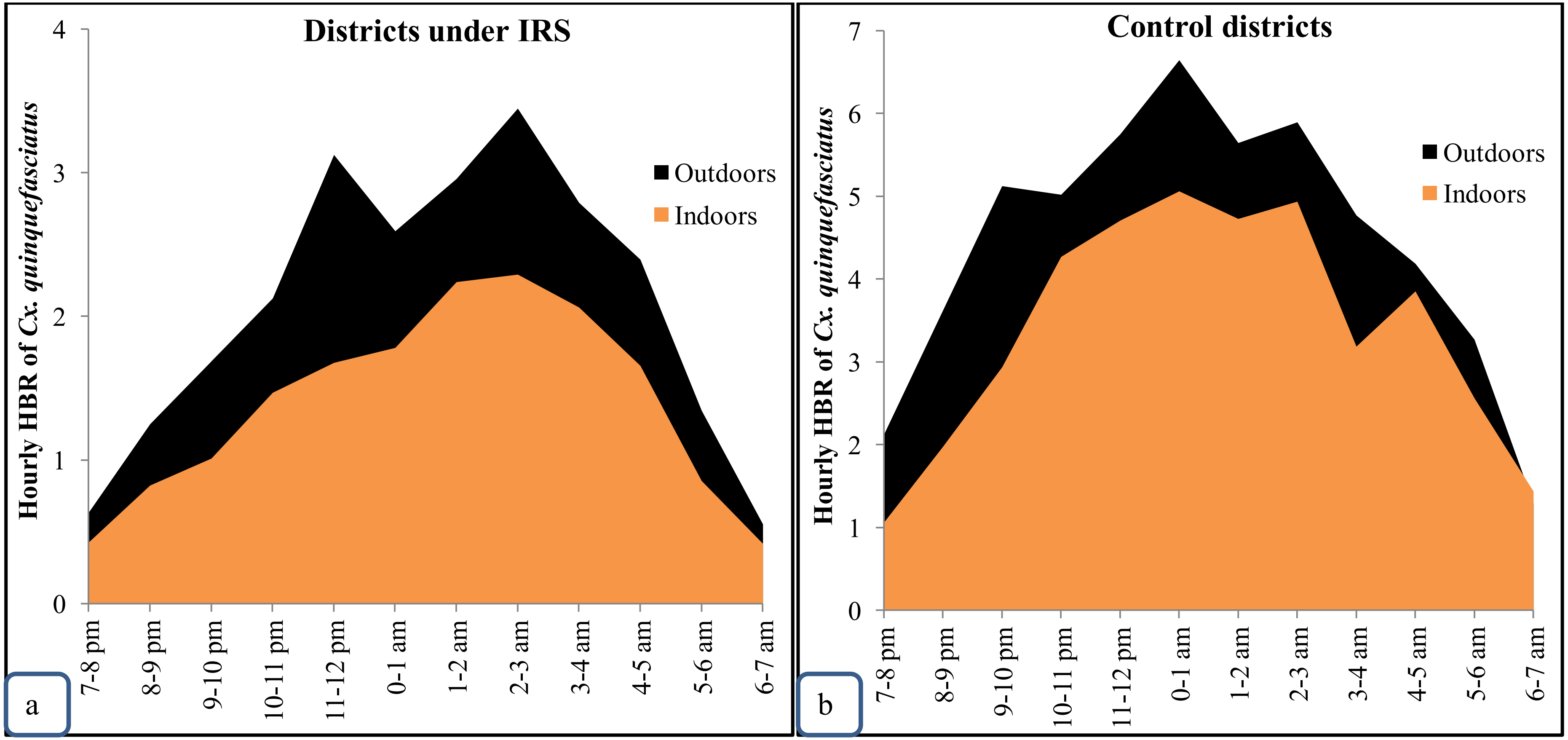


Figure 9: Hourly HBR of *Culex quinquefasciatus* in all treated districts (a) and in control districts (b) (June 2019 –August 2019)

Table 5a: Biting rate of *An. gambiae* s.l. in all treated districts (period November 2018-August 2019)

	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	6h-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

Table 5b: Biting rate of *An. gambiae* s.l. in all control districts (period November 2018-August 2019)

	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	6h-7 am	Total
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 6a: Biting rate of *Culex quinquefasciatus* in all treated districts (period June-August 2019)

	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	6h-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

Table 6b: Biting rate of *Culex quinquefasciatus* in all control districts (period June-August 2019)

	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	6h-7 am	Total
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

9.3.3. Residual density and blood feeding rates of *An. gambiae s.l* collected in districts under IRS and control districts during the period November 2018 to August 2019 in Alibori and Donga

Before 2019 IRS (November 2018 –March 2019), approximately 0.39 specimens of *An. gambiae s.l* per room were collected early in the morning (7AM - 9AM) 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 7). Similarly, the blood feeding rates of *An. gambiae* s.l. is similar in treated (70.21%) and the control areas (83.72%) (p=0.141). In parallel, 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 7). 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 residual density observed in treated areas in this period (June-August 2019), the blood feeding rates of *An. gambiae* s.l. is high in treated (73.97%) and the control (80.26%) areas (p=0.326) (Table 7).

Table 7: 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 room	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: P-value of comparison of the blood feeding rate of *An. gambiae* s.l. between the treated and control districts (Test used: Chi-square test)

9.3.4. 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 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, 14.63% 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 10).

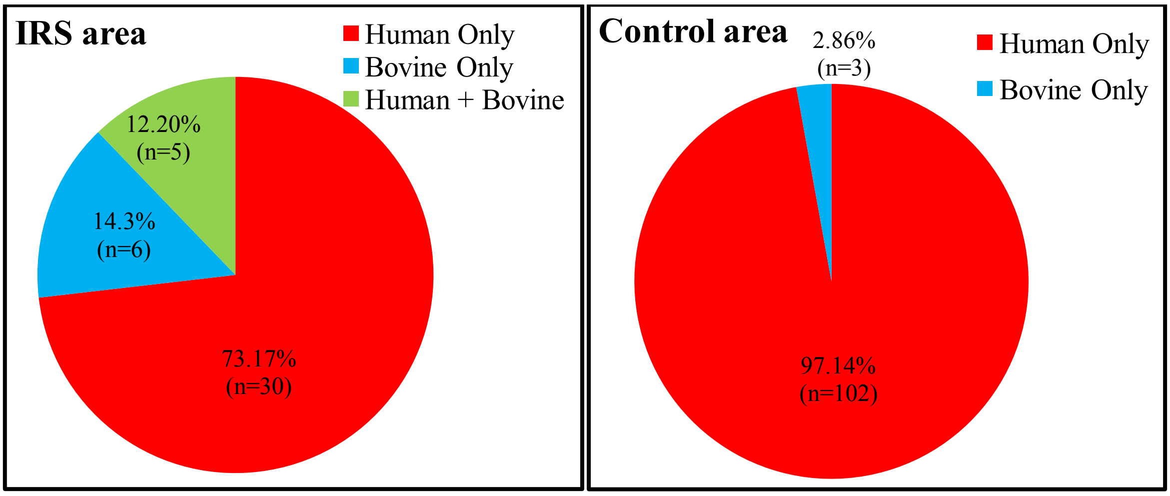


Figure 10: Origin of blood meal observed in *Anopheles gambiae s.l.* in treated and in control areas

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

Table 8 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 2019 IRS intervention (Novmber 2018 to March 2019), the parous rate of *An. gambiae* registered in treated districts (Alibori, Atacora and Donga) was estimated at 70.59% (180/255) compared to 69.70% (69/99) in controls districts (Bembereke and Kouande) (p=0.972). In parallel, after 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 8). This rate is respectively 41.36% (371/897) in treated districts versus 65.78% (569/865) in the control areas (p<0.001).

Table 8: 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

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

Tables 9 and 10 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.

Before 2019 IRS campaign (period from November 2018 to March 2019), a total of 284 head-thoraces of *An. gambiae s.l.* were analyzed by CS-ELISA in treated districts (Alibori and Donga), and approximately 3 were found positive for *Plasmodium falciparum* antigen, an average sporozoite positivity of 1.05% versus 8.00% (8 thoraces positive of 100 thoraces tested) in control districts (Bembereke and Kouande) (p=0.001) (Table 9). 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. Similar results were observed during bio-efficacy period of Actellic CS (period June 2019 to August 2019): 0.31% (4 thoraces positive of 1,298 tested) in districts under IRS against 2.6% (50 thoraces positive of 1,913 tested) in control district (p=0.008) (Table 9).

In parallel, before 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 9 & 12). Similarly, during 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 9 & 10).

Figure 11 & 12 shows the dynamics of HBR and EIRs from May 2016 to August 2019. The lowest HBR & 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 11 & 12).

Table 9: Human Biting Rate (HBR), Sporozoite Index (SI %), Entomological Inoculation Rate (EIR) in *Anopheles gambiae* in districts under IRS and control districts according to the period of 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 effet 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

Table 10: Detail of Sporozoites index (SI) and entomological inoculation rate (EIR) of *An. gambiae* s.1 before and during the bio-efficacy period of Actellic CS in each districts

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	-

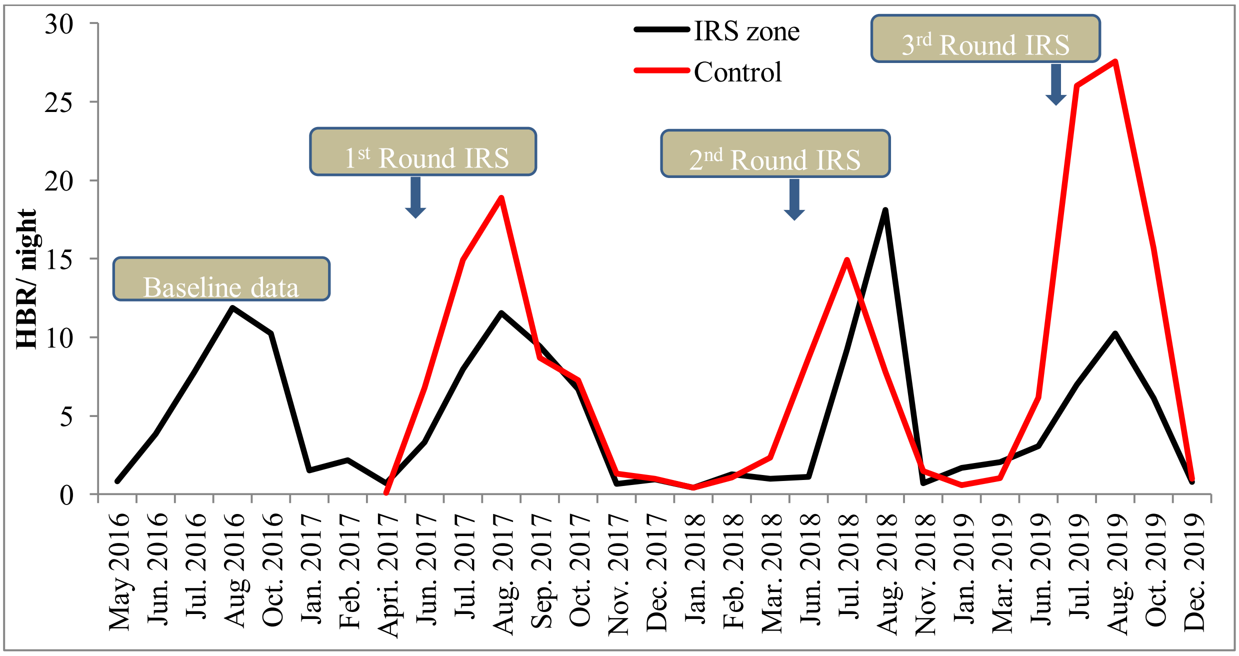


Figure 11: Dynamic of Human biting rate in IRS and control areas from May 2016 to December 2019

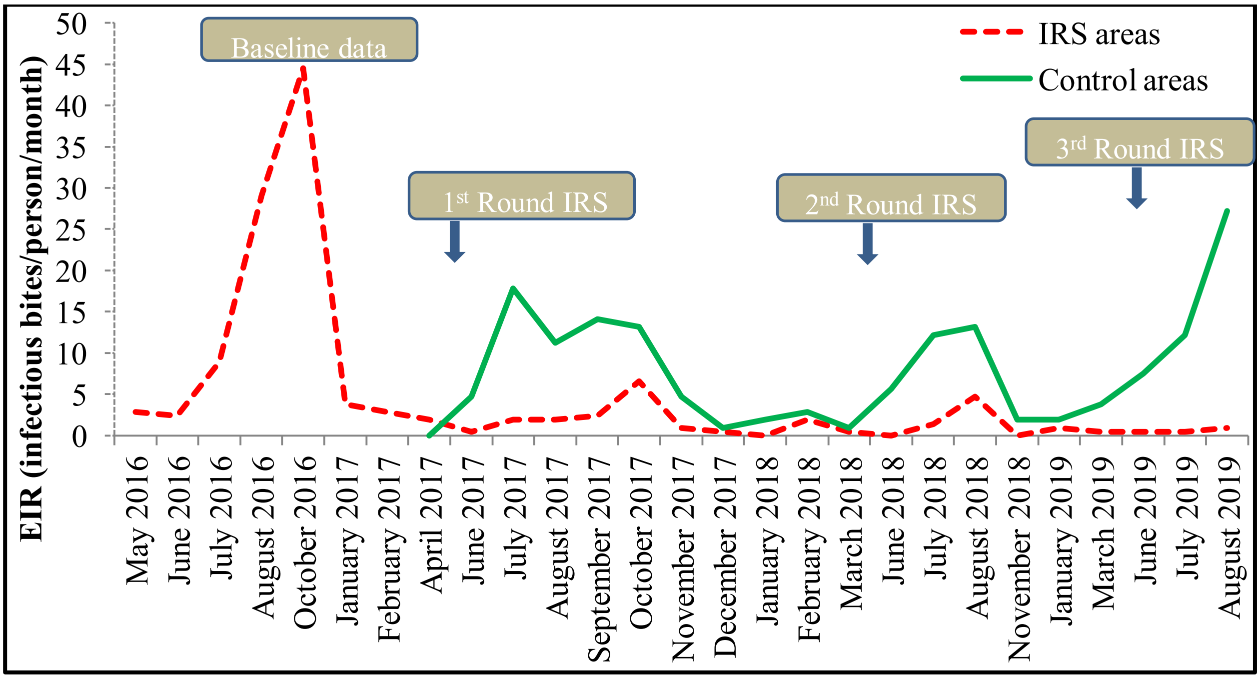


Figure 12: Dynamics of EIR in treated area (Alibori, Donga) and in control area (Bembereke, Kouande) from May 2016 to August 2019.

9.5.1. 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% [0.098 - 0.84] (4th+/1298th) compared to 2.6% [1.96 -3.45] (50 th+/1913th) in the control area (p<0.0001; $\chi 2$=23.48; df=1) (Table 11 & 12). This rate was similar indoors and outdoors the houses in both the treated and control areas. It was 0.39% [0.1 - 1.2] (3th+//760th) outdoors the treated houses compared to 0.18% [0.098 - 0.2] (1th+ /538th) indoors (p=0.872; $\chi 2$=0.025; df=1) (Table 11 & 12). The trend was the same in the control area: 2.25% [1.4 - 3.5] (19 positive thoraces of 843 thoraces tested) outdoors houses versus 2.9% [2.00 - 4.13] indoors (p=0.464; $\chi 2$=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 11). 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 12).

Table 11: Human biting rate, Sporozoite Index and the Entomological Inoculation Rate in IRS areas

Districts	Location	Indicators	June 2019	July 2019	August 2019	Period (June-August) 2019	Percentage of EIR insured (%)	RR [95% CI]	p-value (Wald)
IRS zone	**Inside**	Total tested	72	208	258	538			
		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	24.8	2.75 [0.31 - 79]	0.317
	Outside	Total tested	124	239	397	760			
		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	75.2		
	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

Table 12: HBR, SI and, EIR in control areas during three months after IRS 2019

Districts	Location	Indicators	June 2019	July 2019	August 2019	Period June-August 2019	Percentage of EIR insured (%)	RR [95%]	P-value
Control	**Inside**	Total tested	125	462	483	1070		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	62		
	Outside	Total tested	73	371	399	843			
		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	38		
	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

The lowest Entomological Inoculation Rates were observed between 7pm and 11pm and the highest between 11pm and 7am in both the treated and control areas (Figure 13a & b). Indeed, between 7pm and 11pm, each individual received 0.31 infective bites respectively outside the treated houses while the EIR was zero inside (P=0.398) (Figure 13a). 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 13a). During the same period, the EIRs in the control area were respectively 3.75 infective bites/person/month indoors versus 3.13 outdoors between 7pm -11pm (p=1) and 15.63 infective bites/person/month indoors versus 8.75 outdoors between 11pm and 7am (P=0.108) (Figure 13b)

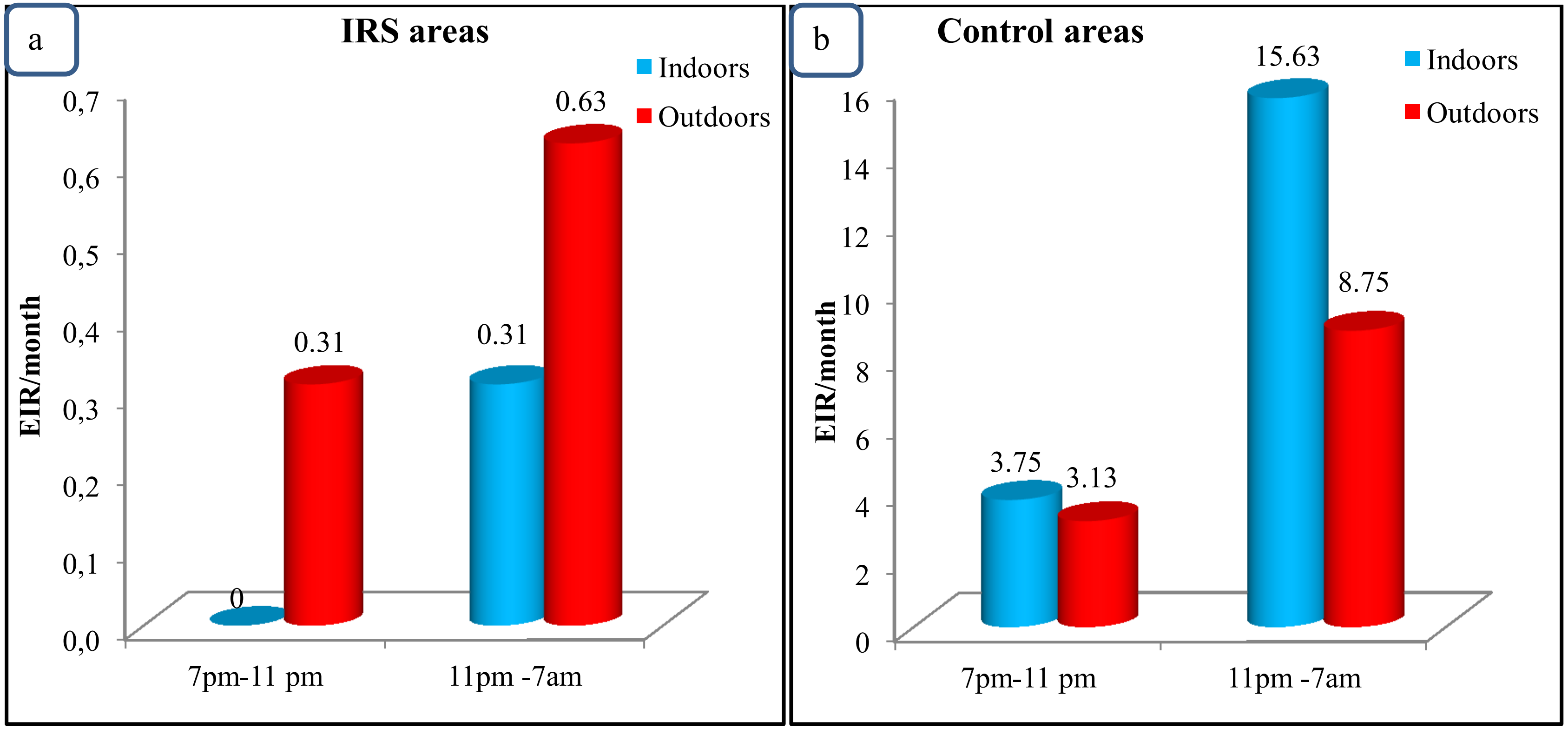


Figure 13: Indoor and outdoor EIR of *An. gambiae* s.l. early and late at night measured in treated (a) and control areas (b).

9.6. Insecticide susceptibility tests

Figure 14 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 vectors 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 14).

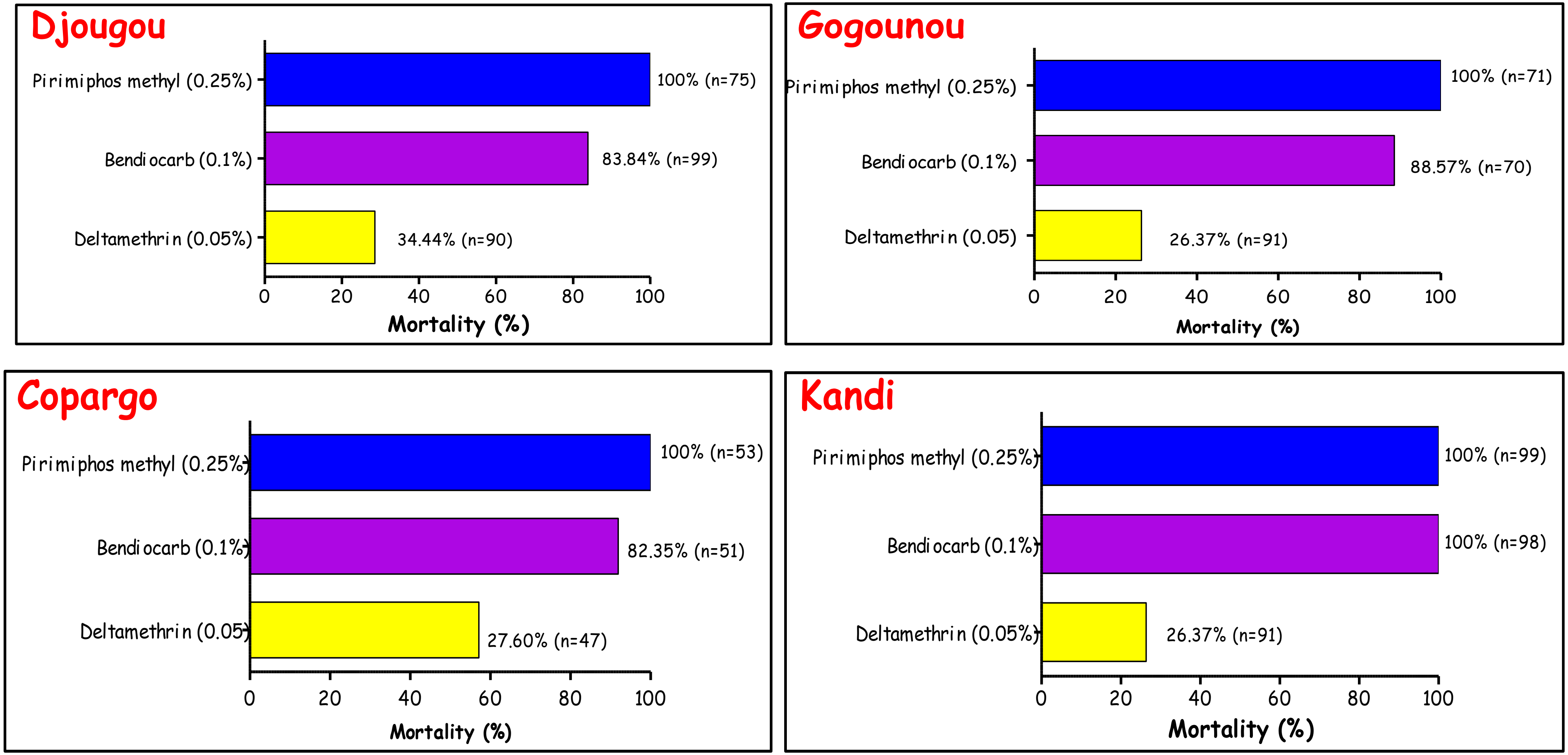


Figure 14: Susceptibility of *Anopheles gambiae s.l* to bendiocarb 0.1%, pirimiphos methyl 0.25%, permethrin 0.75%, and deltamethrin 0.05% in four districts under IRS during period July 2019 - August 2019.

9.7. 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 15). Seasonal variation in the frequency of *An. gambiae* s.s. and *An. coluzzii* was observed during the study (Figure 16). 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 16).

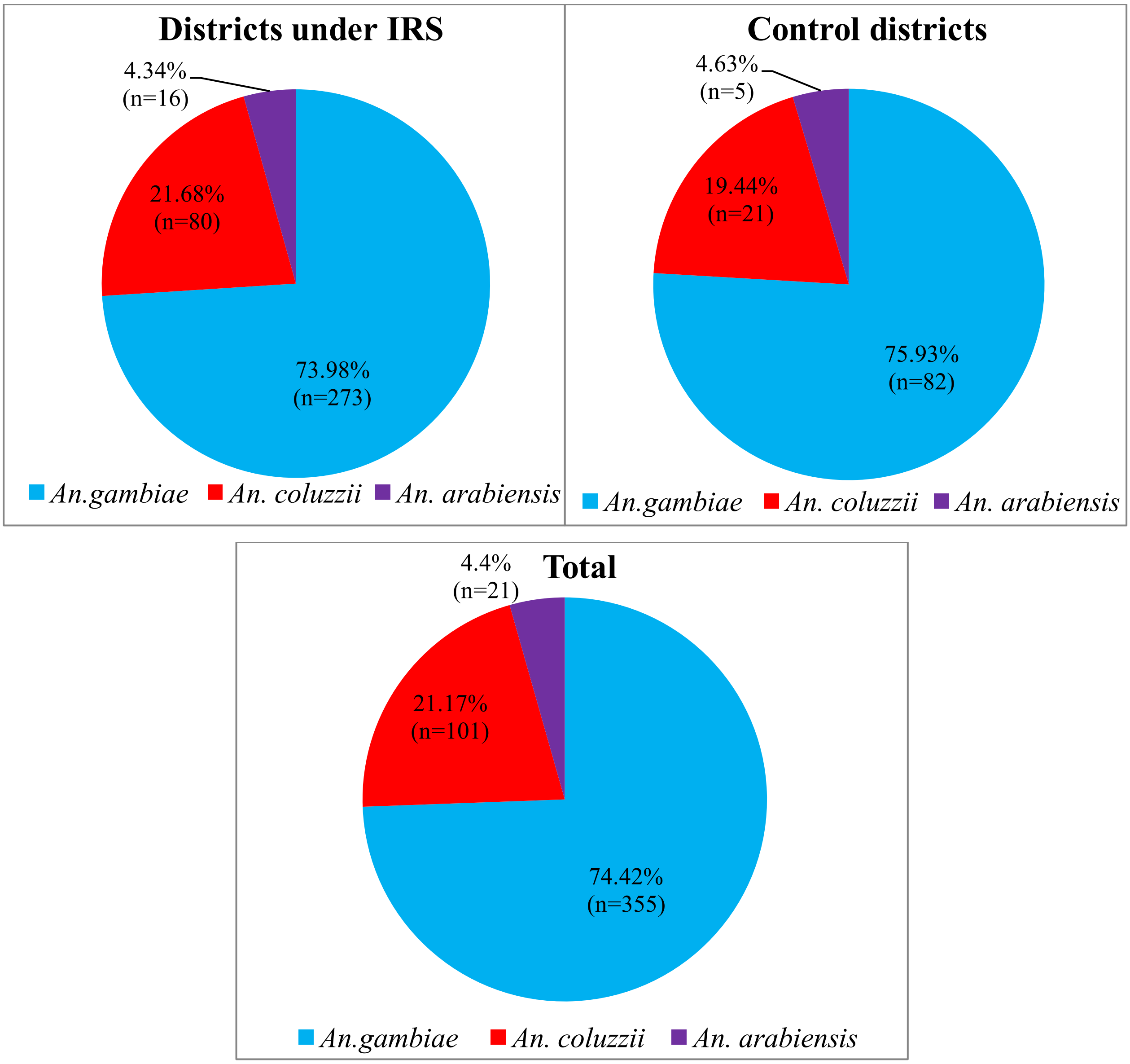
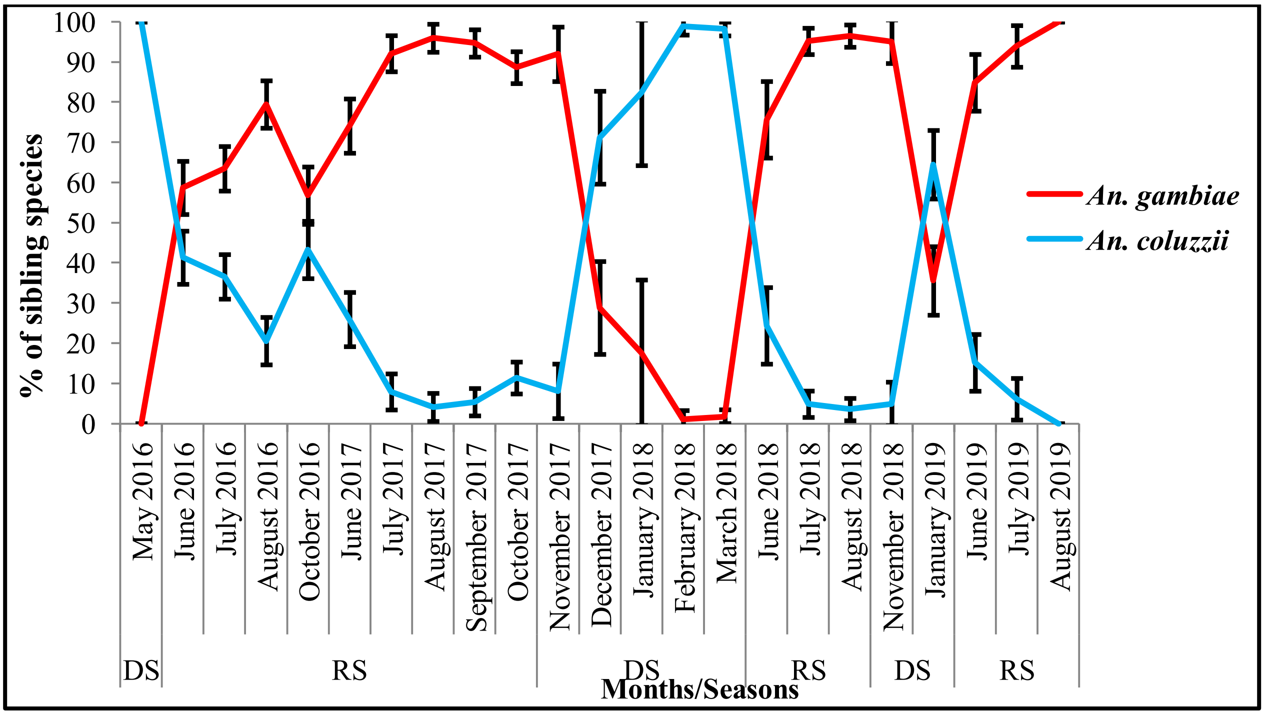


Figure 15: Distribution of *An. gambiae s.l.* species in districts under IRS and control



Abbreviations: DS, dry season; RS, rainy season

Figure 16. Seasonal variation of sibling species (*An. coluzzii* and *An. gambiae*) in the study area.

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

Data presented in table 13 and 14 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% in average) in all *An. gambiae* populations collected from 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 Djougou district (85.48%) and the lowest frequency was recorded in *An. gambiae* strains from Gogounou (72.32%) (Figure 17). Data presented in table 11 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 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 18). Data presented in table 13 & 14 shows the distribution of Knock-down and *Ace*[1] resistance among *An. gambiae* complex species collected between November 2018 and August 2019.

Table 13: 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	62.5
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
Bembèrèkè	*An. gambiae*	82	66	8	8	85.37
	An. coluzzii	21	14	3	4	73.81
	An. arabiensis	5	3	1	1	70

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

Table 14: 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	0.00
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

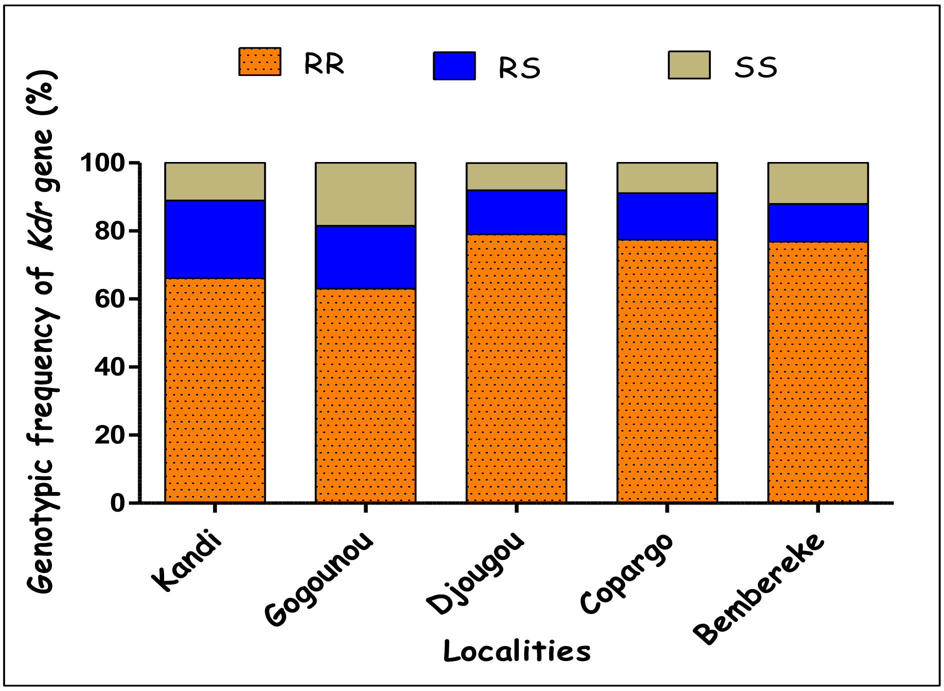


Figure 17: Genotypes frequency of *Kdr* gene in the populations of *An. gambiae s.l*

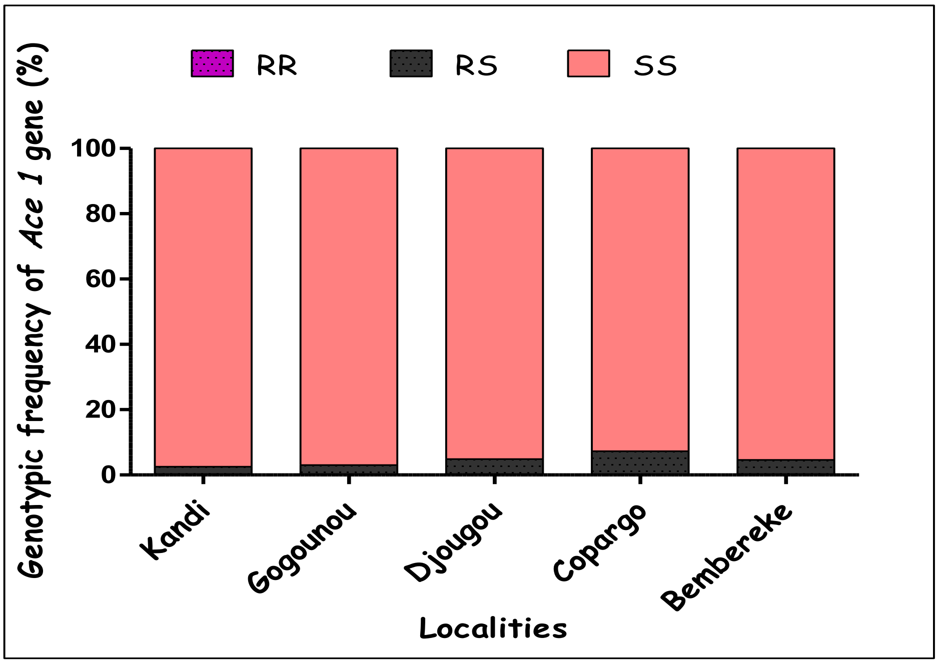


Figure 18: Genotypes frequency of *Ace1* gene in the populations of *An. gambiae s.l.*

10. Conclusions

All targets set during deliverable covering the period from September 2017 to September 2018 are met. Monitoring and evaluation of 3rd rounds of indoor residual spraying campaign carried out from September 2018 to September 2019 in Alibori and Donga has shown once more again demonstrated the impact of this strategy on the reduction of malaria transmission. From evaluation of these two campaigns, we can note a significant difference between the indicators of districts under IRS and those controls districts.

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 observe a spectacular reduction of some indicators like the residual density, vector longevity, sporozoitic index and EIR and strong exophagy of *An. gambiae* (*s.l.*) in most treated districts compared to control areas. However, there was still a delayed IRS impact on some indicators even though the persistence of pirimiphos methyl has been below the 80% efficacy threshold. However, IRS impact is not so visible on some indicators such as the blood feeding rate that appears relatively high in treated districts and in controls.

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.

11. Difficulties encountered

During monitoring and evaluation of 2019 IRS campaign (September 2018 to September 2019), the rarity of positive *Anopheles* larvae breeding sites in some treated localities was a handicap to carry out susceptibility tests for all insecticides classes.

12. 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 witness for the next May 2020 spraying campaign.