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

CREC/Final report 2020

Title of activity:

Monitoring & Evaluation of the efficacy of the fourth year of Indoor Residual Spraying (IRS) in Alibori and Donga, northern Benin, West Africa

Final Report

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Abbreviations

Ace-1	Acetylcholinesterase
CDC	U.S. Centers for Disease Control and Prevention
CREC	Centre de Recherche Entomologique de Cotonou/Entomological Research Center of Cotonou
CS	Capsulated suspension
CSP	Circumsporozoite protein
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
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
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

With the support of the USAID/PMI, Benin's National Malaria Control Program (NMCP) has been has been extending indoor residual spraying (IRS) in the Alibori and Donga departments in northern Benin since 2017. Implementation of IRS in Alibori and Donga regions since 2017 was accompanied by a drastic reduction in Entomological Inoculation Rate (EIR), but the residual activity of the Actellic 300 CS used is short[1].

After three years of IRS with Actellic 300 CS, in April 2020, IRS was renewed in 6 districts: Donga region (Djougou, Copargo and Ouake); Alibori region (Kandi, Gogounou and Segbana) with Fludora ® Fusion, a new-generation insecticide with two different modes of action. This final 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 Fludora ® Fusion 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 Bassila) during the pre-IRS period or baseline period (February to March 2020) and post-IRS period (June to December 2020).

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 (February- March 2020) and post-IRS period (June –December 2020).

February to December 2020, we proceeded with mosquito sampling to cross the nighttime socio-behavioral surveillance data of the household members (activity 4) with those of entomological parameters, for the pre-IRS period (February- March 2020) (dry season) (hot period) (February-March 2020) and post-IRS (June- December 2020) in northern Benin. For this reason, mosquito sampling was organized from 7:00 pm to 7:00 am. The anopheles samples collected in February and March 2020 are not sufficient to make the correlation we expect. Indeed, we pooled the mosquitoes collected every hour for the whole of the treated and control areas to determine the trend in the hourly human biting rate for *Anopheles gambiae* (s.l.). However, pre-IRS period (March 2020), data were not collected in the two control areas (Bassila and Bembereke) due to the restrictive measures taken by the government to limit the spread of COVID-19, these measures were taken at the time of mosquito collection in these two localities.

[1] Salako AS, Dagnon F, Sovi A, Padonou GG, Aïkpon R, Ahogni I, Syme T, Govoétchan R, et al. Efficacy of Actellic 300 CS-based indoor residual spraying on key entomological indicators of malaria transmission in Alibori and Donga, two regions of northern Benin. Parasit Vectors 2019. 12:612

2. Material and methods

2.1. Study areas

The map below (Fig. 1) shows the two health zones (HZ) that will be protected by IRS in April 2020:

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

A total, 6 districts will be 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

Control sites:

- Bembereke district was selected because it was the closest to the district receiving IRS in Alibori department.
- Bassila district was selected because it was the closest to the district receiving IRS in Donga department.

However, mosquito collections were not conducted in March 2020 (pre-IRS period) in the two control areas (Bassila and Bembereke) and in all sites in May, September, October and November 2020 for two main reasons:

✓ Restrictive measures taken by the government to limit the spread of COVID-19, these measures were taken in March 2020 at the time of mosquito collection in some localities.

✓ Delay in the acquisition of funding until the end of the contract in September.

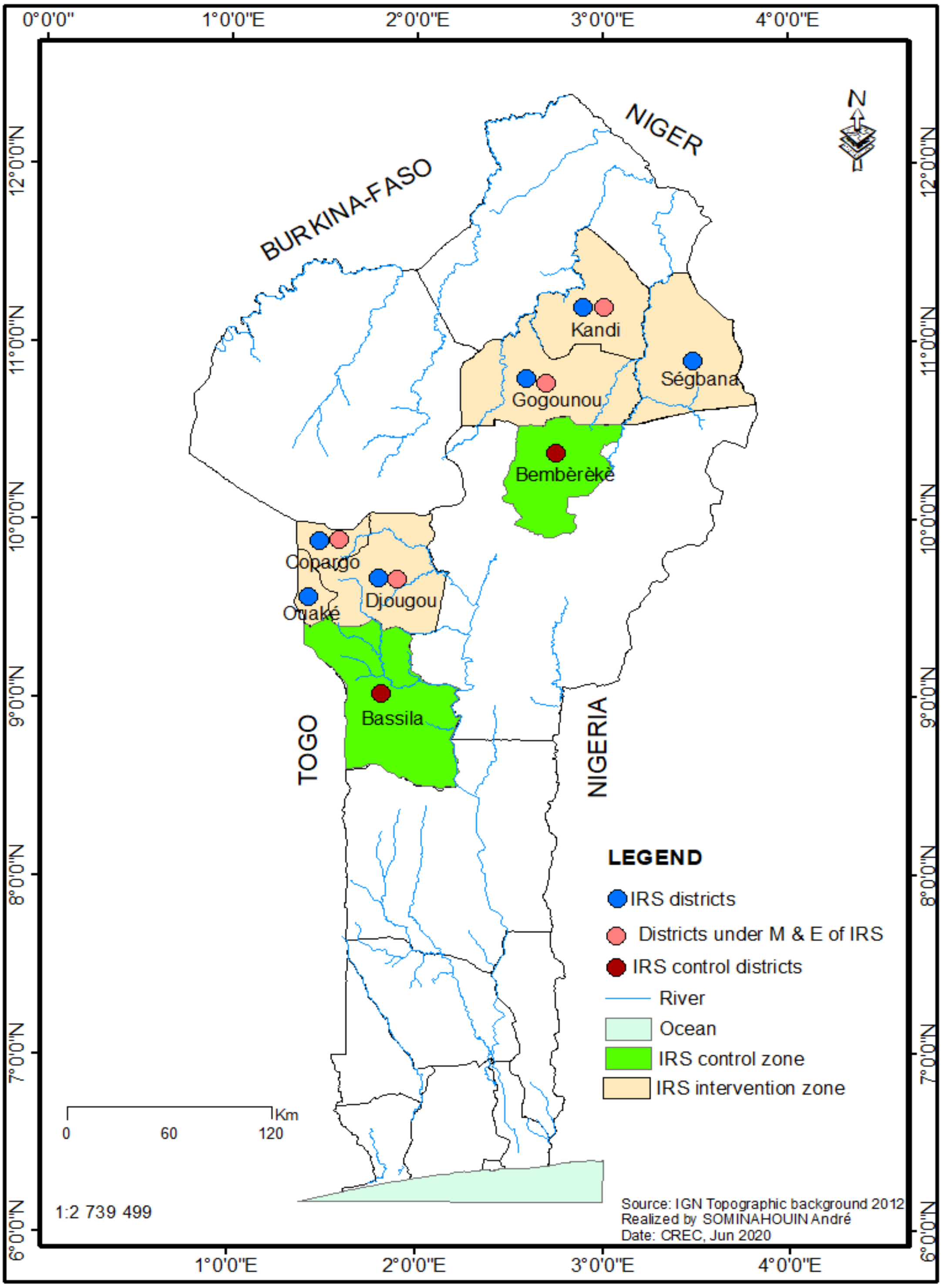

Figure 1*. Map showing the IRS evaluation areas*

The climate in the sites is Sudano-Guinean in the Donga and Sudanese in the Alibori regions. 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 2020. The rainiest months were from June to October in both regions with the rainfall peak occurring in August.

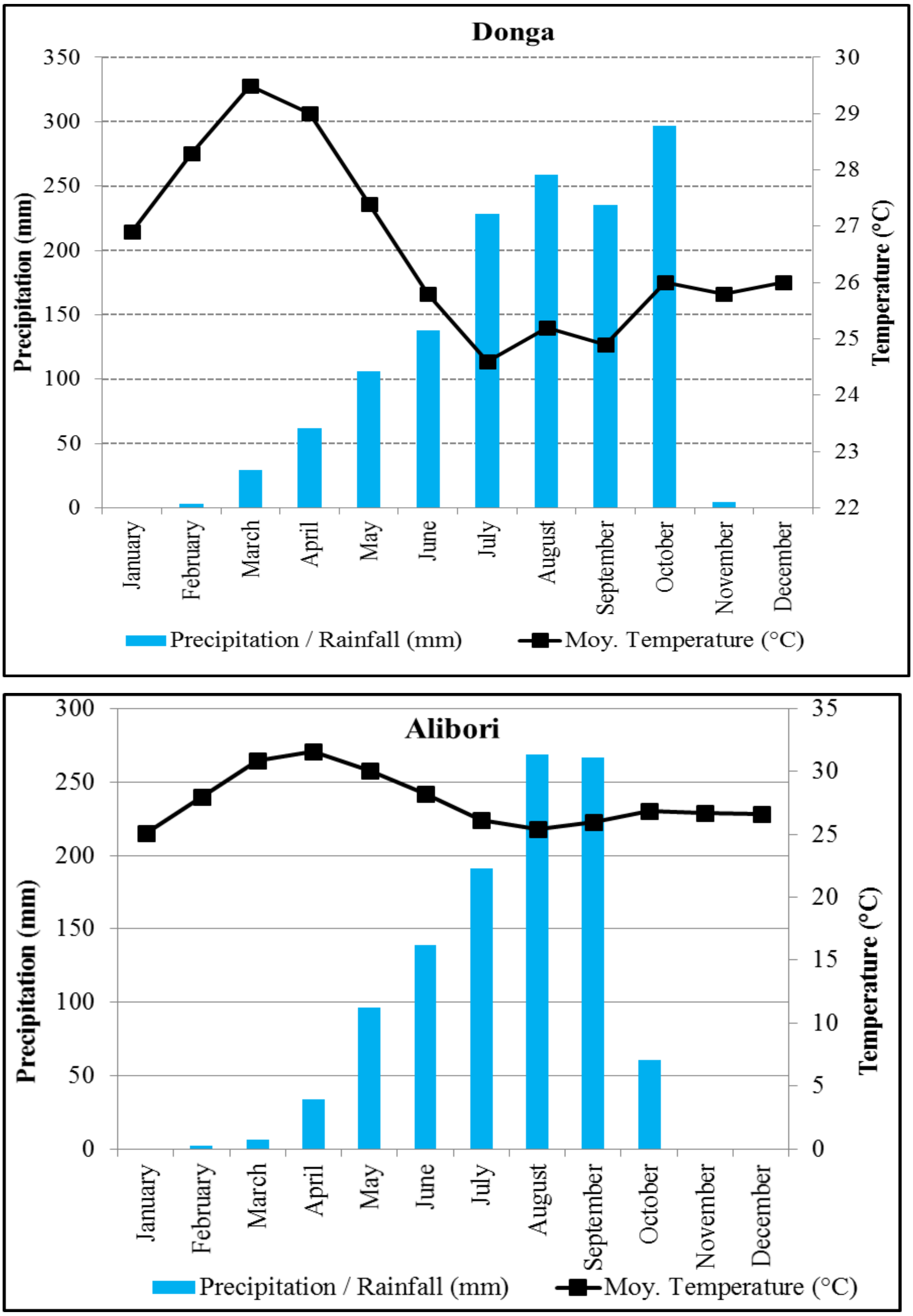

Figure 2. *Monthly climate data in the Alibori and Donga regions*[2].

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

2.2. Indicators measured

Activities planned between February and December 2020 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 Fludora ® Fusion
- Mosquitoes composition
- 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)

Table 1. *Summary of indicators by collection method*

Collection method	Indicator	Definition
	Indoor resting density	# mosquitoes / house / day
PSC	% of fed females	# fed mosquitoes / Total collected by PSC
	HBR	# bites / person / night
	Parity rate	Percentage of parous mosquitoes
	Exophagic rate	Percentage of mosquitoes biting outside
HLC	Endophagic rate	Percentage of mosquitoes biting inside

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 2020 IRS campaign, Djougou and Copargo were selected to evaluate the persistence of Fludora ® Fusion on cement and mud smooth walls. Sprayed houses were selected randomly. WHO wall bioassays[3] were conducted on treated walls of 40 houses randomly selected (20 treated houses /district) seven days (T0) after April 2020 IRS campaigns in Djougou and Copargo. 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 of *An. gambiae* Kisumu aged 2–5 days-old were carefully introduced into each cone,

[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/)

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. Respectively, five cement walls and five smooth mud walls were selected in each of the four villages. Four untreated walls (02 cement and 02 mud walls) were also selected for control. Mosquitoes exposed to untreated wall surfaces were used as controls (Fig. 3). Based on the delayed effect of clothianidin on mosquito mortality as demonstrated in previous studies[4], mortality was recorded every 24 hours up to 120 hours post-exposure and observation at a temperature of 27 ± 2 °C and a relative humidity of 80 ± 10 %. 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 (1week) (April 2020);
- T1: 1 month after the spraying date (May 2020);
- T2: 2 months after the spraying date (June 2020);
- T3: 3 months after the spraying date (July 2020);
- T4: 4 months after the spraying date (August 2020);
- T5: 5 month after the spraying date (September 2020);
- T6: 6 months after the spraying date (October 2020);
- T7: 7 months after the spraying date (November 2020);
- T8: 8 months after the spraying date (December 2020).

[4] Ngufor C, Fongnikin A, Rowland M, N'Guessan R. Indoor residual spraying with a mixture of clothianidin (a neonicotinoid insecticide) and deltamethrin provides improved control and long residual activity against pyrethroid resistant Anopheles gambiae s.l. in Southern Benin. PLoS One. 2017;12:e0189575.

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

Figure 3. *Exposure for 30 minutes to cement and mud walls treated with Fludora ® Fusion mortality reading after respectively 24 hours, 48 hours,72 hours, 96 hours and 120 hours of observation*

2.4. Adult mosquito collections

Mosquito sampling was conducted in 6 districts selected for IRS M&E: Djougou, Copargo, Kandi and Gogounou under IRS and 2 controls (Bassila 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 2 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, 48 local mosquito collectors were used for one round of collection. Two rounds of sampling 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.

The central urban sites of Djougou and Kandi were not treated in 2020, due to the refusal of intervention observed in these large cities (Table 2).

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

Table 2. *Mosquito sampling sites and their geographic coordinates.*

District	Village	Treated or untreated site in April 2020	Latitude/Longitude
Djougou	Taïfa	Untreated	09°41′39″N, 01°41′57″E
	Serou	Treated	09°39′59″N, 01°41′57″E
Copargo	Toungouli	Treated	09°50′19″N, 01°32′59″E
	Fowa	Treated	09°53′49″N, 01°32′59″E
Kandi	Gansosso	Untreated	11°07′51″N, 2°55′36″E
	Koffoïssa	Treated	11°14′55″N, 2°59′55″E
Gogounou	Gbanin	Treated	10°50′06″N, 2°50′10″E
	Bantansouè	Treated	10°55′05″N, 2°51′33″E
Bembereke	Bembereke centre	Untreated	10°13′30″N, 02°40′05″E
	Gamia	Untreated	10°18′05.78″N, 03°29′11″E
Bassila	Bassila I	Untreated	9°12'54.7"N 1°58'07.7"E
	Penessoulou	Untreated	9°20'24.05"N 2°37'40.1"E

2.5. Mosquito identification and processing

2.5.1. Morphological identification of vectors species

After each collection, mosquitoes were counted and morphologically identified using the taxonomic key of Gillies & Meillon[7]. All *Anopheles* vectors captured through HLC were dissected to assess their physiological age[8]. Each specimen was then stored in a labeled Eppendorf tube containing silica gel and cotton for further molecular analyses.

2.5.2. Molecular analyses

Vector species identified were transported to the Centre de Recherche Entomologique de Cotonou (CREC) to detect the presence of *P. falciparum*. The heads/thoraxes of all females *An. gambiae* (*s.l.*) were analyzed by ELISA CSP according to the protocol described by Wirtz et al[9]. The abdomens, legs and wings of specimens of *An. gambiae* (*s.l.*) captured through HLC was analyzed by PCR according to the protocol of Santolamazza et al[10], for molecular species identification. The same mosquitoes were genotyped for the *kdr* L1014F, *kdr* L1014S and G119S

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[7] Gillies MT, De Meillon B. The Anophelinae of Africa south of the Sahara. S Afr Inst Med Res. 1968;54:1–343.

[8] Detinova TS, Gillies MT. Observations on the determination of the age composition and epidemiological importance of populations of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in Tanganyika. Bull World Health Organ. 1964;30:23–8.

[9] Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. Bull World Health Organ. 1987; 65(1): 39-45.

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

Ace-1 mutations, according to the protocols of Martinez-Torres et al[11], Ranson et al[12] and Weill et al[13], respectively.

2.5.3. 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, 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.

3. Data analysis

Data were analyzed with the statistical R software, version 2.8. using the stats package[14]. 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, parity rate of *An. gambiae* (*s.l.*) and allelic frequencies of kdr L1014F and G119S Ace-1. The Poisson test[15] was

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

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

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

[14] 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/.

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

used to estimate the risk ratio (RR) and compare the confidence intervals of indoor vector density and EIRs of *An. gambiae* (s.l.) between treated areas.

We calculated the % reduction in EIR in IRS areas compared to control areas using the Mulla's formula[16]:

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

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

4. Results

4.1. Residual effect of Fludora ® Fusion in wall bioassays (2020 IRS campaign).

Fludora ® Fusion decay rates on treated cement and mud walls were monitored for 8 months after 2020 IRS campaign. At T0 (1 week) (April 2020; 07 days after treatment of the walls), there was 100% mortality in *An. gambiae* s.s. Kisumu strain exposed to Fludora ® Fusion-treated walls irrespective of wall-type (cement or mud) and wall height (Tables 3 & 4; Fig. 4 & 5). This suggests good quality of the treatment and the availability of the insecticide's lethal dose on the walls. Eight months after IRS 2020, mortality rates of susceptible mosquitoes is approximately 80% (WHO efficacy threshold) after 24 hours of observation on both cement and mud smooth walls in Djougou and Copargo (Tables 3 & 4; Figs. 4 & 5). However, there was an increase in mortality rates during the different observation periods, demonstrating the delayed lethal effect of Fludora ® Fusion as a function of time. This mortality varies between 80 and 82% after 24 hours of observation versus 95 and 97% after 120 hours of observation, eight months after 2020 IRS campaign.

The detailed results showing the killing effect with time of Fludora ® Fusion against Kisumu are provided (Tables 3 and 4).

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

Table 3. *Efficacy represented by mortality rate and Knock down 30 min after exposition per time and per wall substrate of Fludora ® Fusion in Djougou*

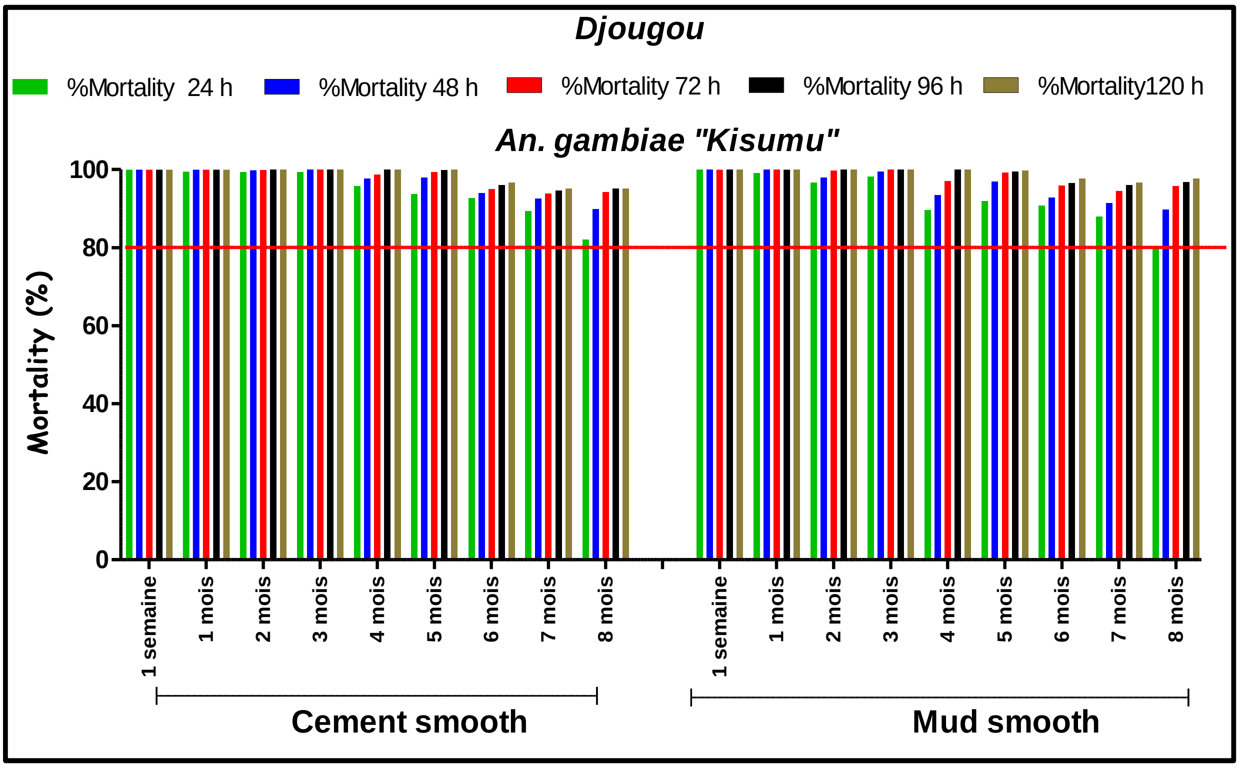
Type of walls	Period after IRS	KD 30 min	Death 24 h	Death 48 h	Death 72 h	Death 96 h	Death 120h	Total tested	%KD min	%Mortality 24 h	%Mortality 48 h	%Mortality 72 h	%Mortality 96 h	%Mortality 120 h
Smooth cement	1 week	441	452	452	452	452	452	452	97.57	100.00	100.00	100.00	100.00	100.00
	1 month	313	368	370	370	370	370	370	84.59	99.46	100.00	100.00	100.00	100.00
	2 month	224	463	465	465	466	466	466	48.07	99.36	99.79	99.79	100.00	100.00
	3 month	158	449	452	452	452	452	452	34.96	99.34	100.00	100.00	100.00	100.00
	4 month	151	454	463	468	474	474	474	31.86	95.78	97.68	98.73	100.00	100.00
	5 month	61	443	463	470	472	473	473	12.90	93.66	97.89	99.37	99.79	100.00
	6 month	69	444	450	455	460	463	479	14.41	92.69	93.95	94.99	96.03	96.66
	7 month	65	423	438	444	448	450	473	13.74	89.43	92.60	93.87	94.71	95.14
	8 month	62	169	185	194	196	196	206	30.10	82.04	89.81	94.17	95.15	95.15
Smooth mud	1 week	401	428	428	428	428	428	428	93.69	100.00	100.00	100.00	100.00	100.00
	1 month	320	411	415	415	415	415	415	77.11	99.04	100.00	100.00	100.00	100.00
	2 month	214	434	440	448	449	449	449	47.66	96.66	98.00	99.78	100.00	100.00
	3 month	128	450	456	458	458	458	458	27.95	98.25	99.56	100.00	100.00	100.00
	4 month	182	492	513	533	549	549	549	33.15	89.62	93.44	97.09	100.00	100.00
	5 month	58	331	349	357	358	359	360	16.11	91.94	96.94	99.17	99.44	99.72
	6 month	102	445	455	470	473	479	490	20.82	90.82	92.86	95.92	96.53	97.76
	7 month	107	400	416	430	437	440	455	23.52	87.91	91.43	94.51	96.04	96.70
	8 month	45	172	193	206	208	210	215	20.93	80.00	89.77	95.81	96.74	97.67

KD 30 min: Knock down 30 min; %: Percentage

Tableau 4. *Efficacy represented by mortality rate and Knock down 30 min after exposition per time and per wall substrate of Fludora ® Fusion in Copargo*

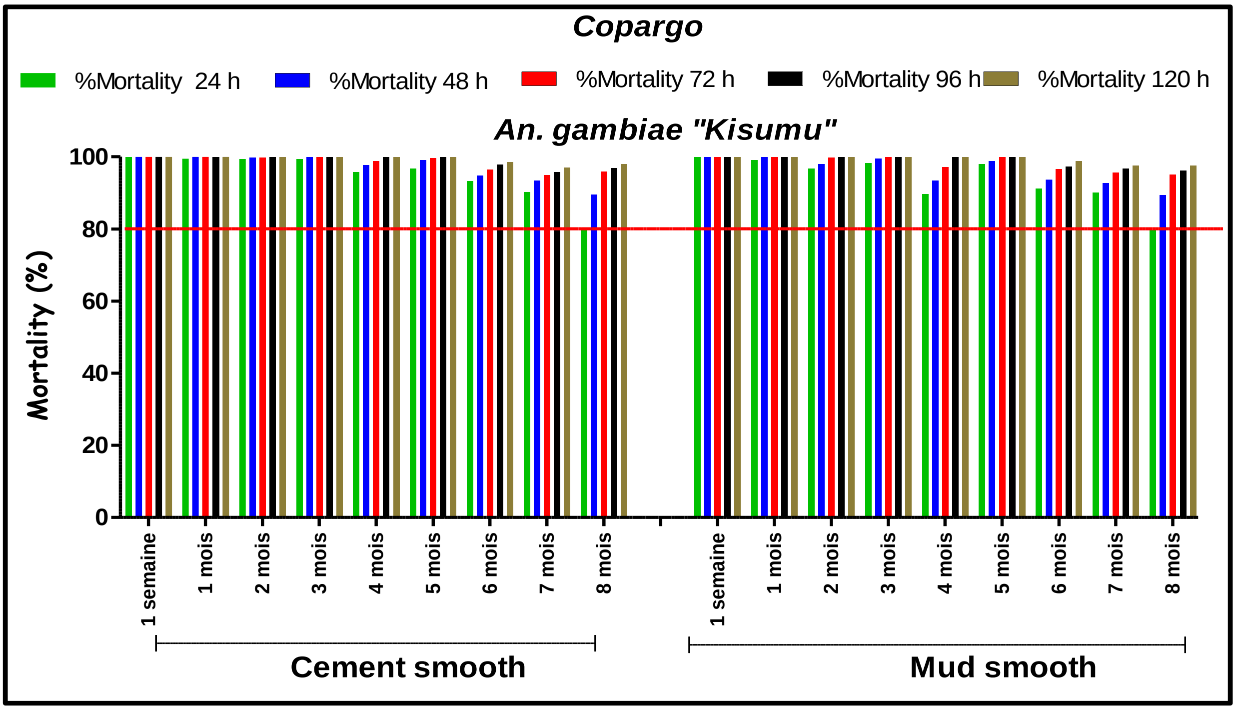
Type of walls	Period after IRS	KD 30 min	Death 24 h	Death 48 h	Death 72 h	Death 96 h	Death 120h	Total tested	%KD min	%Mortality 24 h	%Mortality 48 h	%Mortality 72 h	%Mortality 96 h	%Mortality 120 h
Smooth cement	1 week	441	452	452	452	452	452	452	97.57	100.00	100.00	100.00	100.00	100.00
	1 month	313	368	370	370	370	370	370	84.59	99.46	100.00	100.00	100.00	100.00
	2 month	224	463	465	465	466	466	466	48.07	99.36	99.79	99.79	100.00	100.00
	3 month	158	449	452	452	452	452	452	34.96	99.34	100.00	100.00	100.00	100.00
	4 month	151	454	463	468	474	474	474	31.86	95.78	97.68	98.73	100.00	100.00
	5 month	103	299	306	308	309	309	309	33.33	96.76	99.03	99.68	100.00	100.00
	6 month	193	447	454	462	469	472	479	40.29	93.32	94.78	96.45	97.91	98.54
	7 month	65	427	442	449	453	459	473	13.74	90.27	93.45	94.93	95.77	97.04
	8 month	88	276	308	330	333	337	344	25.58	80.23	89.53	95.93	96.80	97.97
Smooth mud	1 week	401	428	428	428	428	428	428	93.69	100.00	100.00	100.00	100.00	100.00
	1 month	320	411	415	415	415	415	415	77.11	99.04	100.00	100.00	100.00	100.00
	2 month	214	434	440	448	449	449	449	47.66	96.66	98.00	99.78	100.00	100.00
	3 month	128	450	456	458	458	458	458	27.95	98.25	99.56	100.00	100.00	100.00
	4 month	182	492	513	533	549	549	549	33.15	89.62	93.44	97.09	100.00	100.00
	5 month	110	399	402	407	407	407	407	27.03	98.03	98.77	100.00	100.00	100.00
	6 month	198	448	460	474	478	485	491	40.33	91.24	93.69	96.54	97.35	98.78
	7 month	107	410	422	435	440	444	455	23.52	90.11	92.75	95.60	96.70	97.58
	8 month	72	352	395	420	425	431	442	16.29	79.64	89.37	95.02	96.15	97.51

KD 30 min: Knock down 30 min; %: Percentage



The red line indicates the WHO efficacy threshold (mortality of 80%) of an insecticide

Figure 4. *Quality of the spray and residual effect of Fludora ® Fusion 8 months after 2020 IRS campaign in Djougou.*



The red line indicates the WHO efficacy threshold (mortality of 80%) of an insecticide

Figure 5*. Quality of the spray and residual effect of Fludora ® Fusion 8 months after 2020 IRS campaign in Copargo*

4.2. Mosquitoes composition before and after 2020 IRS campaign

During the five months of mosquito collection (February to December 2020), a total of 18,146 human-biting mosquitoes belonging to four genera (Anopheles, Aedes, Culex, Mansonia) and 15 species were collected in IRS and control areas (Table 5). Out of the 15 species, *Anopheles gambie* s.l. was the second most abundant species collected (29.60% of the total of mosquitoes: 5,372 of 18,146) after *Culex quinquefasciatus* (67.49% of the total of mosquitoes; 12,246 of 18,146) (Table 5). The two major malaria vectors collected were *An. gambie* s.l. and *An. funestus*, albeit at low frequency (0.46%: 84 *An. funestus* /18,146) in this period. *An. nili*, a local vector, was found only in Djougou.

Overall, we noted a high abundance of *An. gambiae* s.l. in the Donga region (Djougou: 50.54%; Copargo: 43.70%; Bassila: 35.10%) than in the Alibori region (Kandi: 21.24%; Gogounou: 17.42%) and Bembereke (21.80%).

The relative abundance of *Culex quinquefasciatus* may be due to the presence of larval habitats polluted (sewers, abandoned wells, and cisterns) with organic matters at this time of year (dry season and beginning of the rainy season) and in urban areas. Such breeding sites are choices of preference for the development of larvae of *Cx. quinquefasciatus*.

Table 5. *Mosquitoes species composition (February to August 2020).*

Species	Djougou	Copargo	Kandi	Gogounou	Bembereke	Bassila	Total
	% (Nb)	% (Nb)	% (Nb)	% (Nb)	% (Nb)	% (Nb)	
An. gambiae s.l.	50.54(1344)	43.70(610)	21.24(790)	17.42(468)	21.80(882)	35.10(1278)	29.60
An. funestus	1.17(31)	0.29(4)	0.05(2)	0.11(3)	0.02(1)	1.18(43)	0.46
An. nili	0.15(4)	0.00(0)	0.00(0)	0.00(0)	0.00(0)	0.00(0)	0.02
An. pharoensis	0.00(0)	0.07(1)	0.05(2)	0.00(0)	0.30(12)	0.00(0)	0.08
An. ziemani	0.00(0)	0.00(0)	0.03(1)	0.00(0)	0.00(0)	0.03(1)	0.01
An. coustani	0.00(0)	0.07(1)	0.05(2)	0.00(0)	0.00(0)	0.00(0)	0.02
Cx. quinquefasciatus	45.20(1202)	50.00(698)	76.53(2846)	81.65(2193)	76.93(3112)	60.29(2195)	67.49
Cx. nebulosus	0.68(18)	1.65(23)	0.08(3)	0.26(7)	0.02(1)	0.80(29)	0.45
Cx. descens	0.00(0)	0.00(0)	0.00(0)	0.07(2)	0.07(3)	0.03(1)	0.03
Cx. tigripes	0.00(0)	0.21(3)	0.00(0)	0.00(0)	0.00(0)	0.03(1)	0.02
M. africana	0.98(26)	2.36(33)	1.64(61)	0.22(6)	0.12(5)	2.39(87)	1.20
M. uniformis	0.00(0)	0.14(2)	0.00(0)	0.00(0)	0.00(0)	0.00(0)	0.01
A. aegypti	1.28(34)	1.50(21)	0.30(11)	0.22(6)	0.67(27)	0.16(6)	0.58
A. luteocephalus	0.00(0)	0.00(0)	0.03(1)	0.04(1)	0.02(1)	0.00(0)	0.02
A. vitatus	0.00(0)	0.00(0)	0.00(0)	0.00(0)	0.02(1)	0.00(0)	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Nb: number of specie; %: percentage

A total of 607 *An. gambiae* s.l. were analysed by PCR for species identification: 327 (53.87%) were *An. coluzzii* and 280 (46.13%) were *An. gambiae* (p=0.008) (Fig. 6). Both of the sibling species were present throughout the dry and rainy seasons in both regions (Fig. 6). During the dry season (February to March), *An. coluzzii* predominated (88.61%) in both the Alibori and Donga regions. However, during the rainy season, we observed a predominance of *An. gambiae* (76.07%) in both regions (Fig. 6).

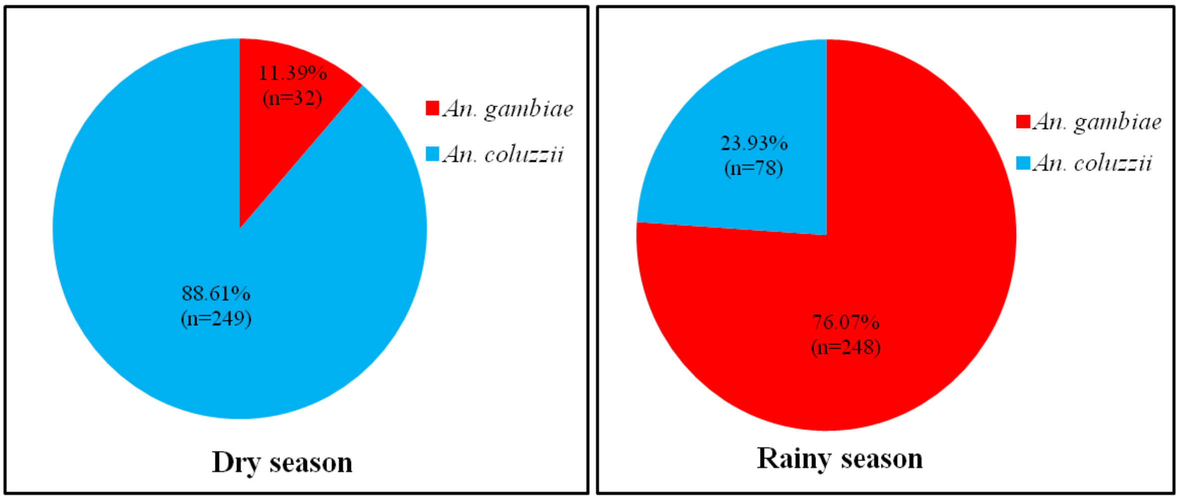


Figure 6. *Distribution of* An. gambiae s.l. *species in districts under IRS and control per season*

4.3. Mosquito blood feeding behaviors

4.3.1. Human Biting Rate (HBR) of An. gambiae s.l. *indoor versus outdoor in districts*

A total of 5,372 *An. gambiae* s.l. were caught from February 2020 to December 2020 in treated districts (Djougou, Copargo, Kandi and Gogounou) and in control area (Bembereke and Bassila). Table 6 shows the proportion of *An. gambiae s.l.* indoors compared to outdoors in these districts. Before the 2020 IRS campaign (period from February 2020 to March 2020), the density of *An. gambiae s.l.* was low compared to the period from June 2020 to December 2020. During this period (February to March 2020), the proportion of *An. gambiae* s.l. collected is similar indoors and outdoors in some houses designated for IRS treatment and control sites except Bassila (p>0.05). Globally, 51.34% (115/224) of *An. gambiae* s.l. were collected indoors in houses designated for IRS treatment compared to 48.66% (109/224) outdoors (P=0.636). Similarly, in houses designated as controls, 43.55% (27/62) were collected indoors versus 56.45% (35/62) outdoors. After the 2020 IRS campaign (June 2020 to December 2020), the proportion of *An. gambiae s.l* collected was significantly lower indoors compared to outdoors only in treated houses in Djougou, but similar indoors and outdoors in some treated houses in Gogounou and Copargo (Table 6). Similarly, in untreated houses (Bembereke), we recorded the opposite situation with higher biting rates indoors (Table 6), but higher outdoor biting rates were observed in Bassila (control). Globally, 47.86% (1430/2988) of *An. gambiae s.l* was collected indoors in treated houses compared to 52.14% (1558/2988) in outdoors (X^2 = 10.79;

df=1; *P* =0.001). Similarly, in untreated houses, 48.43% (1016/2098) were collected indoor versus 51.57% (1082/2098) outdoors (X^2 = 4.02; *df*= 1; *P* =0.044) (Table 6).

Tables 7 and 8 below present the details of biting rate (HBR) of *An. gambiae s.l.* indoors and outdoors in treated districts and control.

Table 6. *Number and proportion of* An. gambiae s.l. *caught indoors and outdoors before IRS intervention (February- March 2020) and after IRS intervention (June-December 2020) in treated and control districts.*

Districts	Pre-2020 IRS period (Feb 2020-Mar 2020) Indoors	Outdoors		Post-2020 IRS period (June 2020 - December 2020) Indoors	Outdoors	
	nb (%)	nb (%)	p-value	nb (%)	nb (%)	p-value
Djougou	19 (63.33)	11 (36.67)	0.07	557 (42.39)	757 (57.61)	<0.001
Copargo	11 (55)	9 (45)	0.751	288 (48.81)	302 (51.19)	0.449
Bassila (control)	13 (36.11)	23 (63.89)	0.033	493 (39.69)	749 (60.31)	<0.001
Kandi	53 (50.96)	51 (49.04)	0.889	377 (54.96)	309 (45.04)	<0.001
Gogounou	32 (45.71)	38 (54.29)	0.398	208 (52.26)	190 (47.74)	0.228
Bembereke (control)	14 (53.85)	12 (46.15)	0.781	523 (61.10)	333 (38.90)	<0.001
Total districts under IRS	115 (51.34)	109 (48.66)	0.636	1430 (47.86)	1558 (52.14)	0.001
Total (control)	27 (43.55)	35 (56.45)	0.208	1016 (48.43)	1082 (51.57)	0.044

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)

Table 7. *Biting rates of* An. gambiae *s.l. indoor and outdoor in treated districts (Donga) and in control (Bassila)*

Disticts	Position	Indicator	Feb 2020	Mar 2020	Jun 2020	Jul 2020	Aug 2020	Dec 2020	Pre-2020 IRS (Fev-Mar 2020)	Post-2020 IRS (Jun-Dec 2020)
Djougou	Inside	Total Mosquitoes	1	18	274	255	23	5	19	557
		nb human cathes	8	8	8	8	4	8	16	28
		HBR/night	0.13	2.25	34.25	31.88	5.75	0.63	1.19	19.89
	Outside	Total Mosquitoes	3	8	364	338	44	11	11	757
		nb human cathes	8	8	8	8	4	8	16	28
		HBR/night	0.38	1.00	45.50	42.25	11.00	1.38	0.69	27.04
Copargo	Inside	Total Mosquitoes	0	11	144	91	44	9	11	288
		nb human cathes	8	8	8	8	4	8	16	28
		HBR/night	0.00	1.38	18.00	11.38	11.00	1.13	0.69	10.29
	Outside	Total Mosquitoes	1	8	110	155	31	6	9	302
		nb human cathes	8	8	8	8	4	8	16	28
		HBR/night	0.13	1.00	13.75	19.38	7.75	0.75	0.56	10.79
Bassila (control)	Inside	Total Mosquitoes	13	NA	269	200	24	0	13	493
		nb human cathes	8	NA	8	8	4	8	8	28
		HBR/night	1.63	NA	33.63	25.00	6.00	0.00	1.63	17.61
	Outside	Total Mosquitoes	23	NA	345	363	40	1	23	749
		nb human cathes	8	NA	8	8	4	8	8	28
		HBR/night	2.88	NA	43.13	45.38	10.00	0.13	2.88	26.75

NA: Not applicable; Pre-2020 IRS period: February, March**; Post-2020 IRS period:** June, July, August, and December.

Table 8. *Biting rates of* An. gambiae *s.l. indoor and outdoor in treated districts (Alibori) and in control (Bembereke)*

Disticts	Position	Indicator	Feb 2020	Mar 2020	Jun 2020	Jul 2020	Aug 2020	Dec 2020	Pre-2020 IRS (Fev-Mar 2020)	Post-2020 IRS (Jun-Dec 2020)
		Total Mosquitoes	44	9	63	190	113	11	53	377
	Inside	nb human cathes	8	8	8	8	4	8	16	28
		HBR/night	5.50	1.13	7.88	23.75	28.25	1.38	3.31	13.46
Kandi						0				
	Outside	Total Mosquitoes	20	31	58	151	96	4	51	309
		nb human cathes	8	8	8	8	4	8	16	28
		HBR/night	2.50	3.88	7.25	18.88	24.00	0.50	3.19	11.04
		Total Mosquitoes	31	1	21	124	62	1	32	208
	Inside	nb human cathes	8	8	8	8	4	8	16	28
		HBR/night	3.88	0.13	2.63	15.50	15.50	0.13	2.00	7.43
Gogounou										
		Total Mosquitoes	33	5	20	87	75	8	38	190
	Outside	nb human cathes	8	8	8	8	4	8	16	28
		HBR/night	4.13	0.63	2.50	10.88	18.75	1.00	2.38	6.79
		Total Mosquitoes	14	NA	175	307	39	2	14	523
	Inside	nb human cathes	8	NA	8	8	4	8	8	28
		HBR/night	1.75	NA	21.88	38.38	9.75	0.25	1.75	18.68
Bembereke (control)										
		Total Mosquitoes	12	NA	121	195	16	1	12	333
	Outside	nb human cathes	8	NA	8	8	4	8	8	28
		HBR/night	1.50	NA	15.13	24.38	4.00	0.13	1.50	11.89

NA: Not applicable; Pre-2020 IRS period: February, March**; Post-2020 IRS period:** June, July, August, and December.

Figure 7 shows the dynamics of HBR from May 2016 to December 2020. The lowest HBR were observed during the dry periods (January 2017 to April 2017, November 2017 to March 2018, November 2018 to March 2019, December 2019 to March 2020 and December 2020) 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 & 2019 and June to August 2020, which equals to 4 months of impact each year (Fig. 7).

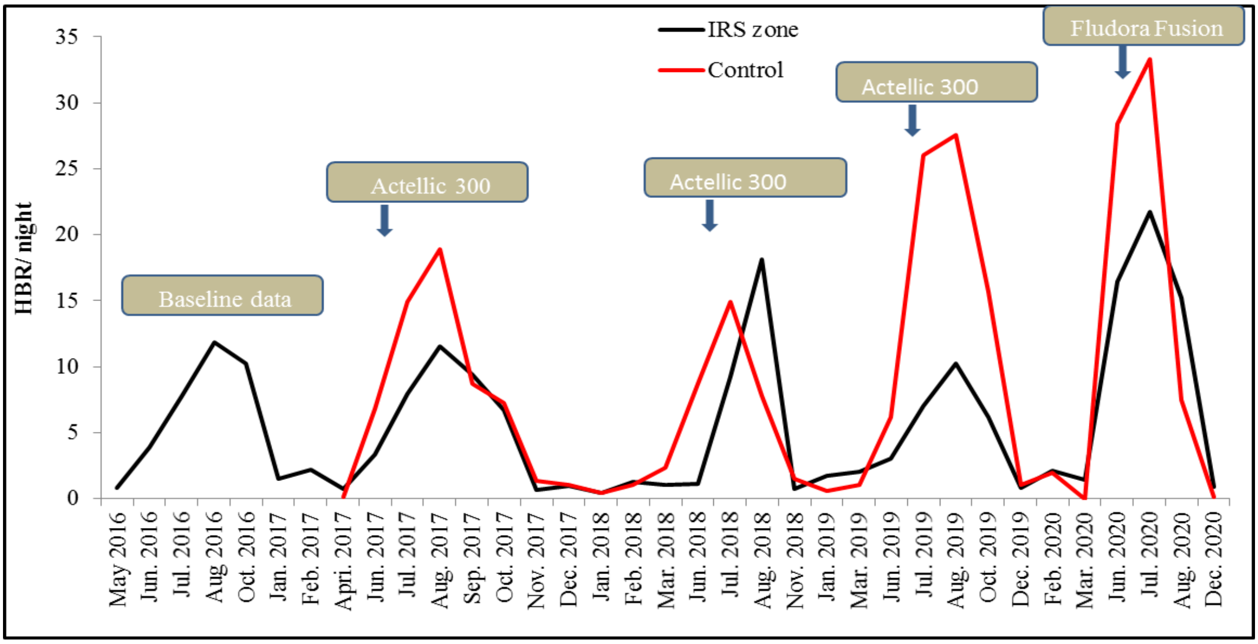


Figure 7. *Dynamic of Human biting rate in IRS and control areas from May 2016 to December 2020.*

4.3.2. Hourly Human Biting Rate of An. gambiae (s.l.) during the night before IRS intervention (February- March 2020) (a) and after IRS intervention (June-December 2020) (b).

This study was conducted to better understand the biting behavior of *An. gambiae s.l* before IRS intervention (February- March 2020) and after IRS intervention (June-December 2020). The biting cycle of *An. gambiae* observed is similar to what is mentioned in many papers: a constantly increase of biting from 7:00PM to reach a peak between 0:00AM and 3:00AM followed by a gradually drop until early morning (Fig. 8 & 9). Outdoor bites are most noticeable early at night and early in the morning (Fig. 8 & 9). *An. gambiae s.l.* appears to be collected more outside of house in the middle of night (Tables 9 and 10). This could be due to the excito-repellent effect of the delthamethrin contained in Fludora ® Fusion used to treat houses.

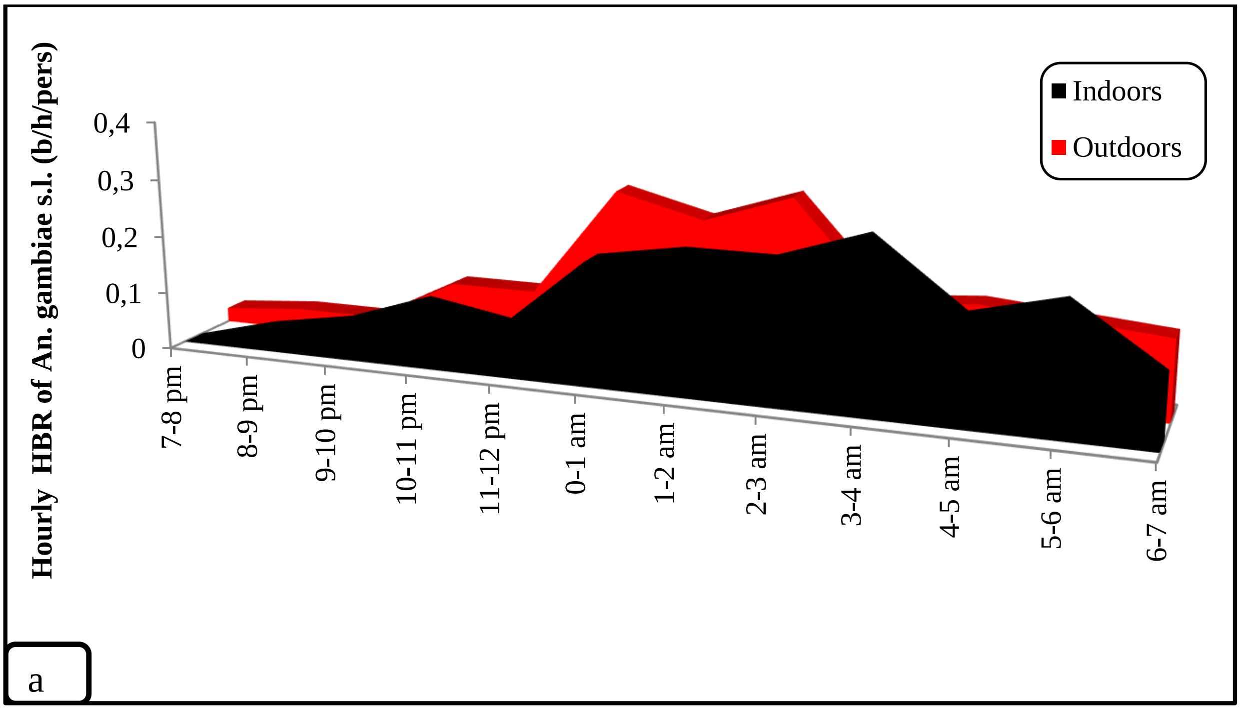


Figure 8. *Hourly HBR of* An. gambiae s.l. *in all treated districts) in dry season (February-March 2020) (a).*

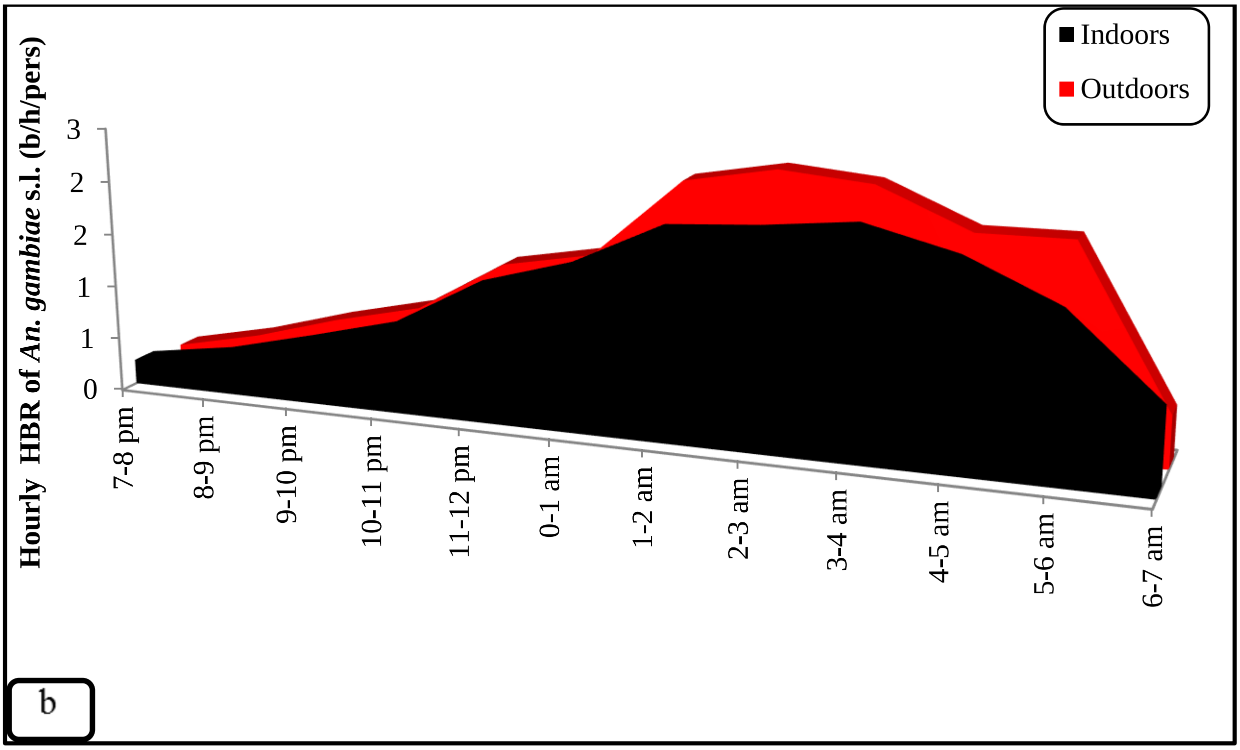


Figure 9. *Hourly HBR of* An. gambiae s.l. *in all treated and control districts after IRS intervention (June-December 2020) (b).*

Table 9. *Number of* An. gambiae s.l. *caught in all treated districts in dry season (February-March 2020)*

Position	7-8 pm	8-9 pm	9-10 pm	10-11 pm	11-12 pm	0-1 am	1-2 am	2-3 am	3-4 am	4-5 am	5-6 am	6-7 am	Total
Indoors	0	3	5	9	7	17	19	19	23	14	17	9	142
Outdoors	2	3	3	9	9	24	21	25	12	13	12	11	144
Total	2	5	8	18	15	39	40	38	30	22	26	20	286

Table 10. *Number of* An. gambiae s.l. *caught in all treated and control districts in rainy season (June-December 2020).*

	7-8 pm	8-9 pm	9-10 pm	10-11 pm	11-12 pm	0-1 am	1-2 am	2-3 am	3-4 am	4-5 am	5-6 am	6-7 am	Total
Indoors	39	60	94	130	208	249	318	328	344	309	246	121	2446
Outdoors	28	58	98	131	214	241	367	394	382	322	324	81	2640
Total	67	118	192	261	422	490	685	722	726	631	570	202	5086

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

Prior to the 2020 IRS campaign (February-March 2020), approximately 0.44 *An. gambiae s.l* per room were collected early in the morning (7AM - 9AM) after PSCs in IRS zone (Alibori and Donga), while 0.10 *An. gambiae* s.l. per room were collected in control areas (p=0.0006) (Table 11). The blood-feeding rates of *An. gambiae* s.l. were similarly high in treated (100%) and the control areas (71.43%) (p =0.501) (Table 12). After 2020 IRS campaign (June-August 2020), the density of *An. gambiae s.l* was significantly reduced in IRS areas compared to the control areas (Table 11). This density was 0.92 *An. gambiae* s.l./room in treated houses and 3.25 *An. gambiae* s.l./room in the control areas (p<0.001) (Table 11). Despite the reduction of room density observed in treated areas during this period (June-August 2020), the blood feeding rates of *An. gambiae* s.l. remained high in treated (69.09%) and the control (69.23%) areas (X^2 = 7.64; *df*= 1; *p=1*) after the IRS campaign (Table 12).

Table 11. *Indoor resting density of* An. gambiae s.l *collected (PSCs data) before and after 2020 IRS intervention*

Period	Districts	Nb of rooms	Nb of *An. gambiae* s.l. collected	Indoor resting density	RR [95% CI]	P-value (Wald)
Pre-IRS evaluation: February-March 2020	Bembereke (control)	20	2	0.10	1	-
	Kandi	40	44	1.10	0.1 [0.01 -0.34]	< 0.001
	Gogounou	40	15	0.38	0.26 [0.03 -1.14]	0.07
	Bassila (control)	20	2	0.10	1	-
	Djougou	40	10	0.25	0.4 [0.04 - 1.87]	0.358
	Copargo	38	1	0.03	3.8 [0.19 - 224.18]	0.274
	Total control districts	40	4	0.10	1	-
	Total treated districts	158	70	0.44	0.22 [0.06- 0.6]	0.0006
Post-IRS evaluation: June- August 2020	Bembereke (control)	60	189	3.15	1	-
	Kandi	60	78	1.30	2.42 [1.85- 3.19]	<0.001
	Gogounou	60	24	0.40	7.87 [5.13- 12.59]	<0.001
	Bassila (control)	60	201	3.35	1	-
	Djougou	60	63	1.05	3.19 [2.39- 4.30]	<0.001
	Copargo	60	55	0.92	3.65 [2.69- 5.01]	<0.001
	Total control districts	120	390	3.25	1	-
	Total treated districts	240	220	0.92	3.54 [2.99- 4.20]	<0.001

RR: rate ratio; p (wald): p-value of the Wald test; [IC 95%]: 95% confidence interval

Table 12. *Blood feeding rates of* An. gambiae s.l *collected (PSCs data) before and after 2020 IRS intervention.*

Period	Districts	Nb of *An. gambiae* s.l. collected	Nb of blood feed	Blood feeding rate (%)	P-value
Pre-IRS evaluation: February-March 2020	Bembereke (control)	2	2	100	-
	Kandi	44	29	65.91	0.814
	Gogounou	15	13	86.67	1
	Bassila (control)	2	2	100	-
	Djougou	10	8	80	1
	Copargo	1	0	0	0.665
	Total control districts	4	4	100	-
	Total treated districts	70	50	71.43	0.501
Post-IRS evaluation: June- August 2020	Bembereke (control)	189	130	68.78	-
	Kandi	78	61	78.21	0.160
	Gogounou	24	14	58.33	0.424
	Bassila (control)	201	140	69.65	-
	Djougou	63	42	66.67	0.771
	Copargo	55	35	63.64	0.492
	Total control districts	390	270	69.23	-
	Total treated districts	220	152	69.09	1

4.4. Parous rate observed in An. gambiae *before and after the 2020 IRS campaign*

Table 13. 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 2020 IRS intervention (February to March 2020), the parous rate of *An. gambiae* in the treated districts (Alibori and Donga) was 53.20% (108/203) compared to 47.37% (27/57) in controls districts (Bembereke and Kouande) ($X^2 = 0.39$; df=1; p=0.529). After the 2020 IRS campaign (June-July 2020), the parous rate of *An. gambiae s.l* was significantly reduced in Alibori IRS areas compared to untreated area (Bembereke) (Table 13) ($p<0.05$). In contrast, the opposite situation was observed in the treated areas in Donga, with higher parous rates compared to untreated district (Bassila) ($p<0.05$). Globally, in treated districts, the rate was 65.86% (382/580) while in the control areas, the rate was 60.09% (256/426) ($X^2 = 3.27$; df= 1; P= 0.07).

Table 13. *Parous rate of* An. gambiae s.l *in IRS and control districts before and after the 2020 IRS campaign*

Period	Districts	Nb of *An. gambiae* (s.l.) dissected	Number of parous	Parous rate (%)	P-value
Pre-IRS evaluation: February-March 2020	Kandi	96	50	52.08	0.892
	Gogounou	64	29	45.31	1
	Bembèrèkè (control)	23	11	47.83	-
	Djougou	25	17	68.00	0.279
	Copargo	18	12	66.67	0.290
	Bassila (control)	34	16	47.06	1
	Total treated districts	203	108	53.20	0.529
	Total control districts	57	27	47.37	-
Post-IRS evaluation: June- July 2020	Kandi	153	70	45.75	0.003
	Gogounou	84	36	42.86	0.004
	Bembèrèkè (control)	179	112	62.57	-
	Djougou	170	129	75.88	0.0003
	Copargo	173	147	84.97	<0.001
	Bassila (control)	247	144	58.30	-
	Total treated districts	580	382	65.86	0.07
	Total control districts	426	256	60.09	-

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).

4.5. Sporozoite index (%CS+) of Plasmodium falciparum *and entomological inoculation rate (EIR) of* An. gambiae s.l.

Tables 14, 15, 16, 17 and 18 summarize biting rates (HBR), sporozoite index (SI) and entomological inoculation rate (EIR) recorded before and after the 2020 IRS campaign in the treated and untreated districts.

Before the 2020 IRS campaign (February- March 2020), a total of 224 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 0.89% (2/224). No mosquito tested positive for CSP antigen of *P. falciparum* in untreated districts (Bembereke and Bassila) (Table 14). However, the EIR was low in some houses designated for IRS treatment during this period (0.47 infectious bites of *An. gambiae* s.l. per human per month) (Table 14). After 2020 IRS campaign (June- December 2020), the EIR in treated districts was 3.37 times lower in the treated districts (2.54 infectious bites/human/month) compared to control districts (8.57 infectious bites /human/month), represents a reduction of 70.37% of the EIR (Table 14).

Overall, like the parous rate, the entomological inoculation rate (EIR) of *An. gambiae* (s.l.) was relatively low in Kandi (0.05ib/pers/night) and Gogounou (0.04 ib/pers/night) districts but high in Djougou (0.11 ib/pers/night) and Copargo (0.14 ib/pers/night) (Tables 15 &16).

Tables 17 and 18 shows the monthly biting rates (HBR), sporozoite index (SI) and entomological inoculation rate (EIR) recorded indoors and outdoors before and after 2020 IRS intervention in treated and untreated districts. Figure 10 shows dynamics of EIR in treated area (Alibori, Donga) and in control area (Bembereke, Bassila) from May 2016 to December 2020. From December 2019 to March 2020, EIR was relatively low in treated and control areas while from June 2020 to August 2020, we found a significant decrease in EIR in treated districts compared to controls localities.

Table 14. *Sporozoite index (SI) (%) of* Plasmodium falciparum *and entomological inoculation rate (EIR) of* An. gambiae s.l. *before and after 2020 IRS intervention*

Areas	Period before 2020 IRS intervention			After 2020 IRS intervention		
	Period (February- March 2020)			Period (June -December 2020)		
	SI (%)	HBR/night	EIR/month	SI (%)	HBR/night	EIR/month
IRS area	0.89	1.75	0.47	0.64	13.34	2.54
Controls	0.00	1.94	0.00	1.53	18.73	8.57

Table 15. *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	Feb 2020	Mar 2020	June 2020	July 2020	Aug 2020	Dec 2020	Before IRS (Feb-Mar) 2020	After IRS (Jun-Dec) 2020
Kandi	SI	0.000	0.000	0.000	0.006	0.005	0.004	0.000	0.004
	HBR/night	4.00	2.50	7.56	21.31	26.13	12.25	3.25	12.25
	EIR/night	0.00	0.00	0.00	0.13	0.13	0.05	0.00	0.05
Gogounou	SI	0.000	0.000	0.000	0.005	0.007	0.005	0.000	0.005
	HBR/night	4.00	0.38	2.56	13.19	17.13	7.11	2.19	7.11
	EIR/night	0.00	0.00	0.00	0.06	0.13	0.03	0.00	0.04
Bembereke (control)	SI	0.000	NA	0.007	0.008	0.091	0.01	0.000	0.013
	HBR/night	1.63	NA	18.50	31.38	6.88	10.53	1.63	15.28
	EIR/night	0.00	NA	0.13	0.25	0.63	0.14	0.00	0.19

Table 16. *Monthly sporozoite index (SI), human biting rate (HBR) and entomological inoculation rate (EIR) of* An. gambiae s.l. *in treated areas (Donga) and in control (Bassila).*

Districts	Indicators	Feb 2020	Mar 2020	June 2020	July 2020	Aug 2020	Dec 2020	Before IRS (Feb-Mar) 2020	After IRS (Jun-Dec) 2020
Djougou	SI	0.000	0.038	0.003	0.003	0.030	0.004	0.033	0.004
	HBR/night	0.25	1.63	39.88	37.06	8.38	22.46	0.94	23.46
	EIR/night	0.00	0.06	0.13	0.13	0.25	0.11	0.03	0.11
Copargo	SI	0.000	0.053	0.012	0.012	0.027	0.01	0.050	0.014
	HBR/night	0.06	1.19	15.88	15.38	9.38	10.53	0.63	10.53
	EIR/night	0.00	0.06	0.19	0.19	0.25	0.14	0.03	0.14
Bassila (control)	SI	0.000	NA	0.013	0.014	0.078	0.01	0.000	0.02
	HBR/night	2.25	NA	38.38	35.19	8.00	22.17	2.25	22.17
	EIR/night	0.00	NA	0.50	0.50	0.63	0.37	0.00	0.37

NA: Not applicable

Table 17. *Monthly sporozoite index (SI), human biting rate (HBR) and entomological inoculation rate (EIR) of* An. gambiae s.l. *indoors and outdoors in treated areas (Alibori) and in control (Bembereke).*

Districts	Location	Indicators	February 2020	March 2020	June 2020	July 2020	August 2020	December 2020
Bembereke (control)	**Inside**	Total tested	14	NA	175	307	39	2
		nb Thorax+	0	NA	0	3	3	0
		SI	0.00	NA	0.00	0.01	0.08	0.00
		HBR/night	1.75	NA	21.88	38.38	9.75	0.25
		EIR/night	0.00	NA	0.00	0.38	0.75	0.00
		EIR/month	0.00	NA	0.00	11.25	22.50	0.00
	Outside	Total tested	12	NA	121	195	16	1
		nb Thorax+	0	NA	2	1	2	0
		SI	0.00	NA	0.02	0.01	0.13	0.00
		HBR/night	1.50	NA	15.13	24.38	4.00	0.13
		EIR/night	0.00	NA	0.25	0.13	0.50	0.00
		EIR/month	0.00	NA	7.50	3.75	15.00	0.00
Kandi	**Inside**	Total tested	44	9	63	190	113	11
		nb Thorax+	0	0	0	0	1	0
		SI	0.00	0.00	0.00	0.00	0.01	0.00
		HBR/night	5.50	1.125	7.875	23.75	28.25	1.375
		EIR/night	0.00	0.00	0.00	0.00	0.25	0.00
		EIR/month	0.00	0.00	0.00	0.00	7.50	0.00
	Outside	Total tested	20	31	58	151	96	4
		nb Thorax+	0	0	0	2	0	0
		SI	0.00	0.00	0.00	0.01	0.00	0.00
		HBR/night	2.5	3.88	7.25	18.88	24.00	0.50
		EIR/night	0.00	0.00	0.00	0.25	0.00	0.00
		EIR/month	0.00	0.00	0.00	7.50	0.00	0.00
Gogounou	**Inside**	Total tested	31	1	21	124	62	1
		nb Thorax+	0	0	0	1	0	0
		SI	0.00	0.00	0.00	0.01	0.00	0.00
		HBR/night	3.88	0.13	2.63	15.50	15.50	0.13
		EIR/night	0.00	0.00	0.00	0.13	0.00	0.00
		EIR/month	0.00	0.00	0.00	3.75	0.00	0.00

	Outside	Total tested	33	5	20	87	75	8
		nb Thorax+	0	0	0	0	1	0
		SI	0.00	0.00	0.00	0.00	0.01	0.00
		HBR/night	4.13	0.63	2.50	10.88	18.75	1.00
		EIR/night	0.00	0.00	0.00	0.00	0.25	0.00
		EIR/month	0.00	0.00	0.00	0.00	7.50	0.00

NA: Not applicable

Table 18. *Monthly sporozoite index (SI), biting rate (HBR) and entomological Inoculation rate (EIR) of* An. gambiae *s.l. indoors and outdoors in treated area (Donga) and in control (Bassila).*

Districts	Location	Indicators	February 2020	March 2020	June 2020	July 2020	August 2020	December 2020
Bassila (control)	**Inside**	Total tested	13	NA	269	200	24	0
		nb Thorax+	0	NA	4	7	4	0
		SI	0.00	NA	0.01	0.04	0.17	0.00
		HBR/night	1.63	NA	33.63	25.00	6.00	0.00
		EIR/night	0.00	NA	0.50	0.88	1.00	0.00
		EIR/month	0.00	NA	15.00	26.25	30.00	0.00
	Outside	Total tested	23	NA	345	363	40	1
		nb Thorax+	0	NA	4	1	1	0
		SI	0.00	NA	0.01	0.00	0.03	0.00
		HBR/night	2.88	NA	43.13	45.38	10.00	0.13
		EIR/night	0.00	NA	0.50	0.13	0.25	0.00
		EIR/month	0.00	NA	15.00	3.75	7.50	0.00
Djougou	**Inside**	Total tested	1	18	274	255	23	5
		nb Thorax+	0	1	1	1	1	0
		SI	0.00	0.06	0.00	0.00	0.04	0.00
		HBR/night	0.13	2.25	34.25	31.88	5.75	0.63
		EIR/night	0.00	0.13	0.13	0.13	0.25	0.00
		EIR/month	0.00	3.75	3.75	3.75	7.50	0.00
	Outside	Total tested	3	8	364	338	44	11
		nb Thorax+	0	0	1	1	1	0
		SI	0.00	0.00	0.00	0.00	0.02	0.00
		HBR/night	0.38	1.00	45.50	42.25	11.00	1.38
		EIR/night	0.00	0.00	0.13	0.13	0.25	0.00
		EIR/month	0.00	0.00	3.75	3.75	7.50	0.00
Copargo	**Inside**	Total tested	0	11	144	91	44	9
		nb Thorax+	0	0	3	2	0	0
		SI	0.00	0.00	0.02	0.02	0.00	0.00
		HBR/night	0.00	1.38	18.00	11.38	11.00	1.13
		EIR/night	0.00	0.00	0.38	0.25	0.00	0.00
		EIR/month	0.00	0.00	11.25	7.50	0.00	0.00
								0
	Outside	Total tested	1	8	110	155	31	6
		nb Thorax+	0	1	0	1	2	0
		SI	0.00	0.13	0.00	0.01	0.06	0.00
		HBR/night	0.13	1.00	13.75	19.38	7.75	0.75
		EIR/night	0.00	0.13	0.00	0.13	0.50	0.00
		EIR/month	0.00	3.75	0.00	3.75	15.00	0.00

NA: Not applicable

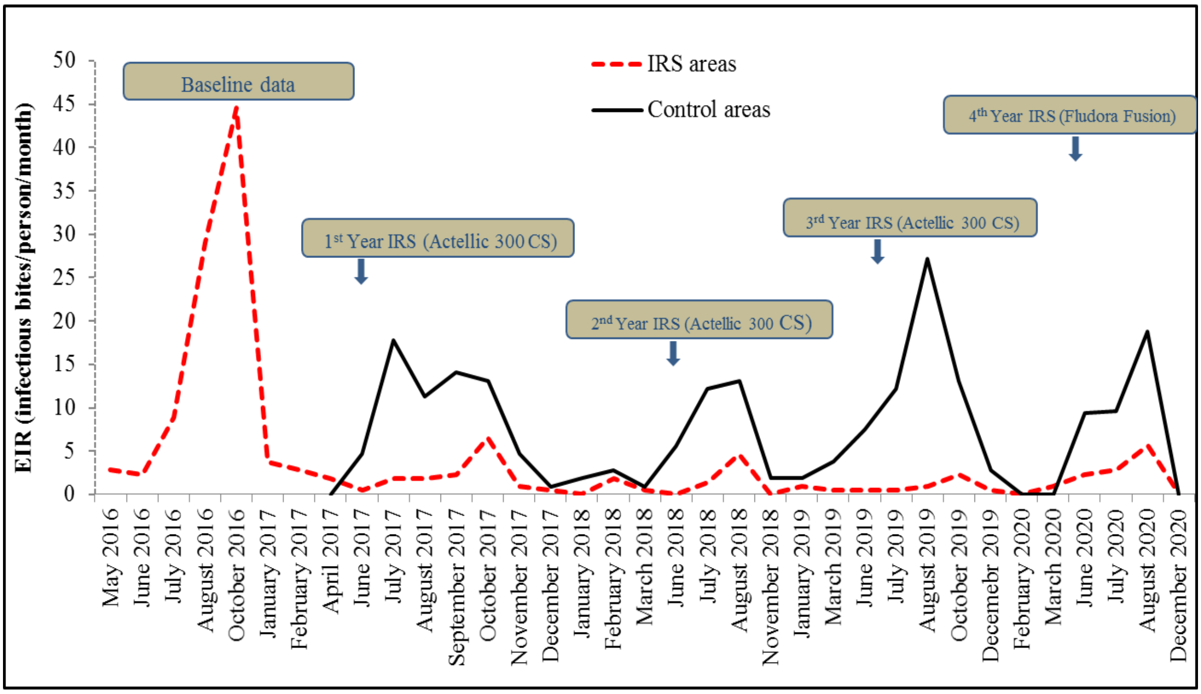


Figure 10. *Dynamics of EIR in treated area (Alibori, Donga) and in control area (Bembereke, Bassila) from May 2016 to December 2020.*

4.6. Insecticide susceptibility tests

Figure 11 summarizes the susceptibility level of local vectors to different insecticides (bendiocarb, pirimiphos methyl, permethrin and deltamethrin). All mosquito populations tested were susceptible to pirimiphos methyl (mortality > 98%). However, Gogounou vectors populations showed a resistance to bendiocarb (mortality < 90) but in Kandi, vectors are susceptible to Bendiocarb (100%). For pyrethroids (deltamethrin and permethrin), *An. gambiae* s.l. was resistant in all the districts (mortality< 90%) (Fig. 11).

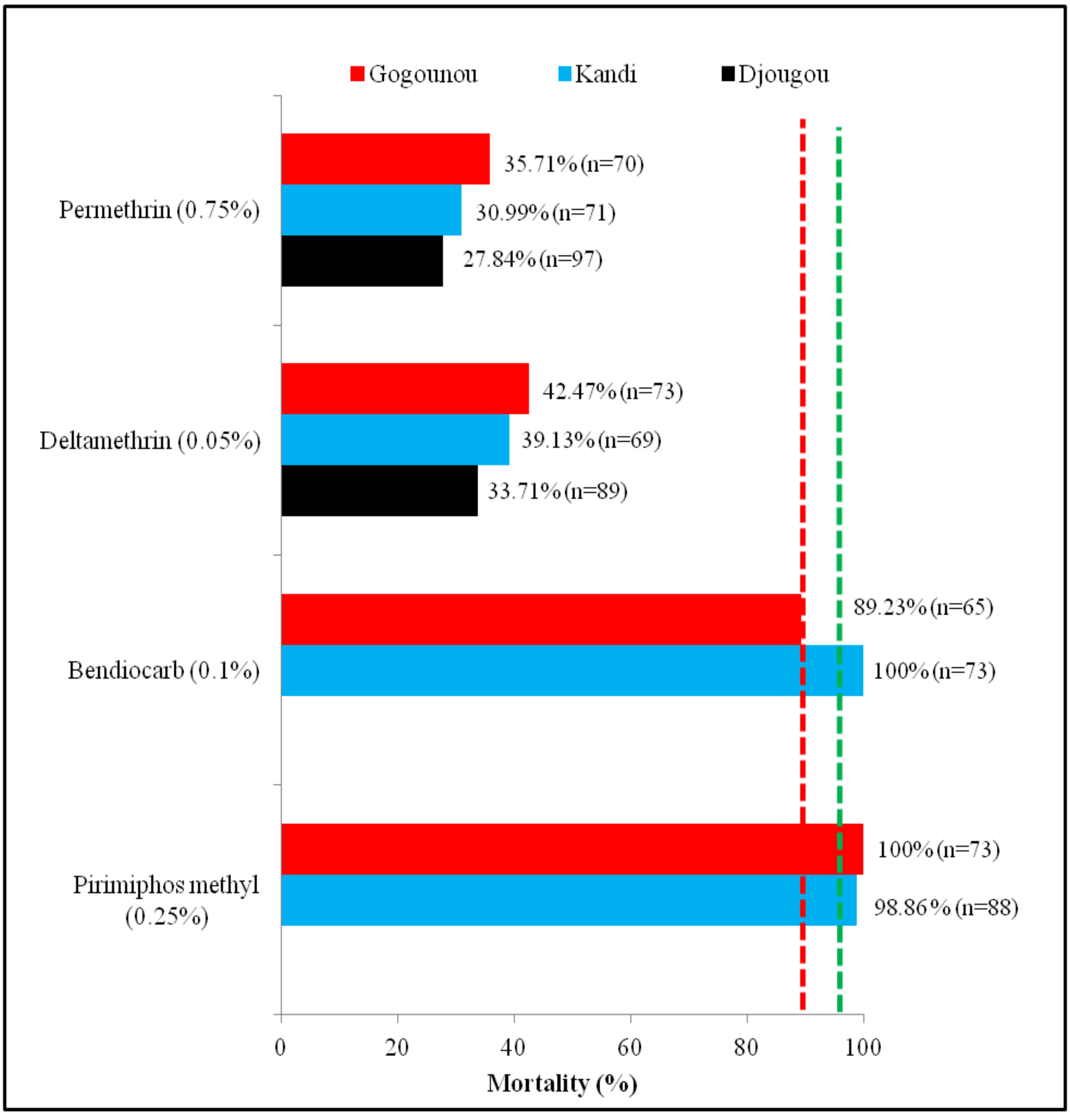


Figure 11. *Mortalities observed 24 hours after mosquito exposure to bendiocarb 0.1%, pirimiphos methyl 0.25%, permethrin 0.75% and deltamethrin 0.05% in four districts under IRS during period June -August 2020 (based on World Health Organization criteria the area below broken red lines indicates insecticide resistance; the area in between the broken red and green lines indicate possible resistance; the area above the green broken line indicates insecticide susceptibility)*

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

Data presented in Table 20 and 22 show the frequency and distribution of *kdr* L1014F and *Ace 1* resistance genes among *An. gambiae* complex species collected. *Kdr* mutation was detected in high frequency in all species of the *An. gambiae* complex within all the localities. Globally, *kdr* L1014F mutation was detected at a significantly higher frequency in *An. gambiae* (77.70% in IRS districts and 86.73% in controls) compared to *An. coluzzii* (54.94% in IRS districts and 46.62% in controls) (Table 20). *Kdr* L1014F mutation frequency was similar between the IRS and control areas (p=0.386) (Table 19).

Table 19. *Frequencies* Kdr *L1014F of* An. gambiae s.l. *in IRS zone and control*

Localities	Number tested	RR	RS	SS	Freq. 1014F (%)	P-value
Kandi	224	115	43	66	60.94	-
Gogounou	111	62	21	28	65.32	0.309
Djougou	69	43	13	13	71.74	0.027
Copargo	80	52	16	12	75.00	0.001
Bembereke (control)	70	37	15	18	63.57	0.645
Bassila (control)	53	29	7	17	61.32	1
Total IRS districts	484	272	93	119	65.81	-
Total untreated districts	123	66	22	35	62.60	0.386

Table 20. *Distribution of Knock-down resistance (Kdr) frequencies between malaria vectors and localities*

Localities	Species	Number tested	RR	RS	SS	Freq. L1014F (%)	P-value
Kandi	*An. gambiae*	80	50	12	18	70.00	0.004
	An. coluzzii	144	65	31	48	55.90	
Gogounou	*An. gambiae*	35	32	2	1	94.00	<0.001
	An. coluzzii	76	30	19	27	51.97	
Djougou	*An. gambiae*	51	35	9	7	77.45	0.021
	An. coluzzii	18	8	4	6	55.56	
Copargo	*An. gambiae*	65	45	12	8	78.46	0.061
	An. coluzzii	15	7	4	4	60.00	
Bembereke (control)	*An. gambiae*	33	25	6	2	84.85	<0.001
	An. coluzzii	37	12	9	16	44.59	
Bassila (control)	*An. gambiae*	16	14	1	1	90.63	0.0001
	An. coluzzii	37	15	6	16	48.65	
Total districts under IRS	*An. gambiae*	231	162	35	34	77.70	<0.001
	An. coluzzii	253	110	58	85	54.94	
Total districts control	*An. gambiae*	49	39	7	3	86.73	<0.001
	An. coluzzii	74	27	15	32	46.62	

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

Ace-1R mutation associated with carbamates and organophosphate resistance was identified in all sites but with very low frequencies (2.03 in untreated areas and 3.10 in IRS zone) (Table 21). It ranged from 3.06% in *An. gambiae* to 1.35% in *An. coluzzii* (Table 22).

Table 21. *Frequencies* Ace-1R *G119S of* An. gambiae s.l. *in IRS zone*

Localities	Number tested	RR	RS	SS	Freq. 119S (%)	P-value
Kandi	224	0	14	210	3.13	-
Gogounou	111	0	5	105	2.25	0.694
Djougou	69	0	6	60	4.35	0.671
Copargo	80	0	5	75	3.13	1
Bembereke (control)	70	0	3	67	2.14	0.751
Bassila (control)	53	0	2	51	1.89	0.717
Total IRS districts	484	0	30	450	3.10	-
Total untreated districts	123	0	5	118	2.03	0.496

Table 22. *Distribution of* Ace-1R *frequencies between malaria vectors and localities*

Localities	Species	Number tested	RR	RS	SS	Freq. G119S (%)	P-value
Kandi	*An. gambiae*	80	0	6	74	3.75	0.776
	An. coluzzii	144	0	8	136	2.78	
Gogounou	*An. gambiae*	35	0	1	33	1.42	0.940
	An. coluzzii	76	0	4	72	2.63	
Djougou	*An. gambiae*	51	0	5	46	4.90	0.950
	An. coluzzii	18	0	1	14	2.78	
Copargo	*An. gambiae*	65	0	4	61	3.08	1
	An. coluzzii	15	0	1	14	3.33	
Bembereke (control)	*An. gambiae*	33	0	2	31	3.03	0.920
	An. coluzzii	37	0	1	36	1.35	
Bassila (control)	*An. gambiae*	16	0	1	15	3.13	1
	An. coluzzii	37	0	1	36	1.35	
Total districts under IRS	*An. gambiae*	231	0	16	214	3.46	0.660
	An. coluzzii	253	0	14	236	2.77	
Total districts control	*An. gambiae*	49	0	3	46	3.06	0.639
	An. coluzzii	74	0	2	72	1.35	

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

5. Conclusion

All entomological monitoring targets set during deliverable covering the period from February 2020 to August 2020 were met. Monitoring and evaluation of the 4th year of indoor residual spraying campaign with Fludora ® Fusion carried out from February 2020 to December 2020 in Alibori 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 (February to March 2020) is likely due to the harmattan season and the dry conditions which characterize it. Similar biting behavior of *An. gambiae* s.l. indoors and outdoors of treated houses during this period (February to March 2020) is likely due to the complete decrease in the effect of the insecticide used in May 2019.

Bioassays on treated walls have shown that Fludora ® Fusion remains effective with a mortality rate above 80% on the susceptible strain Kisumu, 8 months after spraying date.

During this period of bio-efficiency of Fludora ® Fusion, we observed a spectacular reduction of some indicators like the indoors resting density, 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. was susceptible to pirimiphos methyl in all sites but is experiencing a resistance to bendiocarb in Gogounou and widespread resistance to pyrethroids in all localities.

6. Difficulties encountered and recommendations

- Mosquitoes were not collected in the two control areas (Bassila and Bembèrèkè) in March 2020 due to the restrictive measures taken by the government to limit the spread of COVID-19, these measures were taken at the time of mosquito collection in these two localities.
- The mosquitoes were not collected in May 2020 due to the restrictive measures taken by the government to limit the spread of COVID-19 and the temporary cessation of some activities required by a USAID decision.

Since June when activities resumed, the different actors involved in field data collection have been complying with the measures required by the government of Benin to prevent the spread of COVID-19: masks and gloves are provided to the mosquito collectors by the Centre de Recherche Entomologique de Cotonou.

- During the period June -August 2020, 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 July 2021.

7. Activities planned for the next 3 months (January –March 2021)

The same monitoring will continue in the same districts and this data will be used to guide the next April 2021 spraying campaign.