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

Activity 6: M&E of IRS

Results of the M&E of the 1st round Indoor Residual Spraying (IRS) in Atacora-Alibori-Donga

Deliverable 6B2

Quarterly report: May-December 2017

Coordinator: Professor Martin C. Akogbéto

Assistants: Albert S. Salako, MSc., PhD student, CREC/UAC

Collaboration: National Malaria Control Program (NMCP)

CREC/NMCP/USAID Doc/12/2017

1. Introduction

Since May 2017, 8 districts of the departments of Atacora, Donga and Alibori (Figure 1) were under Indoor Residual Spraying (IRS). The main goal of the current study (deliverable 6 C) is to collect data on mosquito behavior and malaria transmission in the districts under M&E and compare results obtained during the period of October-December to those previously registered.

2. Study areas (see map below)

Three health zones (HZ) were protected by IRS in 2017:

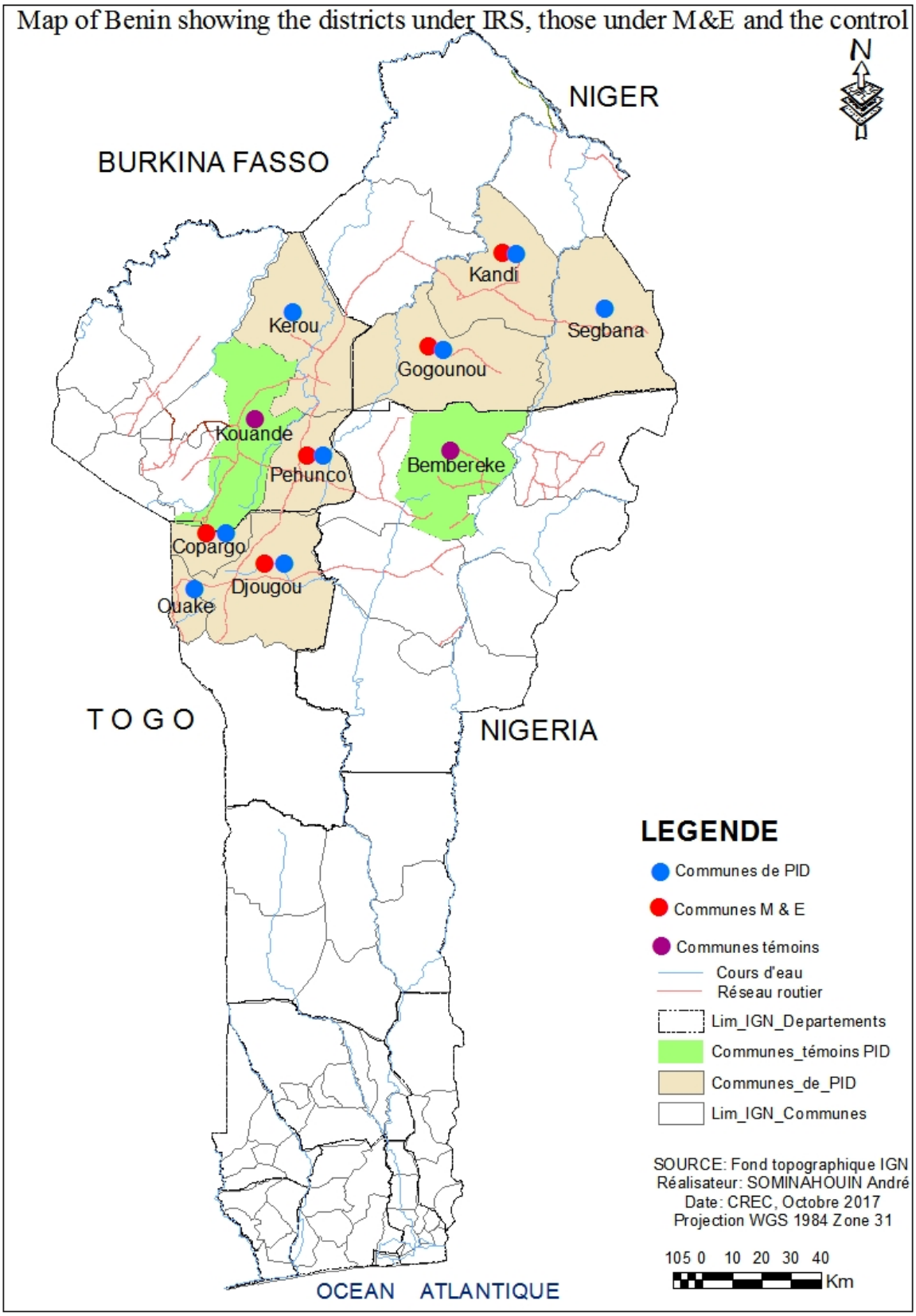
- HZ Djougou, Copargo, Ouaké (Donga region)
- HZ Kandi, Gogounou, Segbana (Alibori region)
- HZ 2KP: Pehunco, Kerou (Atacora region)

In total, 8 districts among which Djougou and Copargo were used for M&E in Donga, Kandi and Gogounou in Alibori and Pehunco in the HZ 2KP.

- Bimbèrèkè, the closest district from Alibori is the control of the districts under IRS in Alibori.
- Kouandé is chosen for control for 2KP districts under IRS and for Donga districts because it is the alone district near Pehunco and Copargo/Djougou.

Whatever, data collected in all treated districts during the bio-efficacy period of Actellic CS (June-September, when delayed mortality of Kisumu 24h ≥80% in bioessay) was compared those registered during Beyond the bio-efficacy period of Actellic CS (October-December, when the delayed 24h mortality of Kisumu <80% in bioassay).

Map of Benin showing the districts under IRS, those under M&E and the control



3. Data retained in the deliverable

• Residual activity of pirimiphos methyl

• Vector identification (species and molecular forms of *Anopheles gambiae*)

• 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)

4. Organization of the report

Two visits were done during the period of October and December 2017 to collect mosquitoes, conduct advanced laboratory testing on captured *Anopheles gambiae* species and assess susceptibility of mosquitoes to various insecticides.

This quarterly report should be based on the data collected during October to December. But, for a best understanding of the evolution of the indicators since the 2017 IRS was implemented, the report was presented in a cumulative way taking into account data registered from May, the beginning of IRS.

5. Bioassay cone tests

Bioassays were carried out according to WHO protocol (WHO, 2006) to assess the residual effect of pirimiphos methyl. The details of the protocol were described in the previous report (see deliverable 6B).

6. Sampling of malaria vectors and study of PMI malaria transmission indicators

Mosquitoes were collected by human landing catch 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 56 mosquito collectors were used for one round of collection. Two rounds are planned per month.

In the same villages selected for human landing catches, 20 additional houses per village were selected to have pyrethrum spray catches performed to determine the number of mosquitoes in the room, estimate the density of mosquitoes, and estimate indoor behaviors. Two pyrethrum spray catches are planned per month in each of the 20 houses/rooms, for a

goal of 40 total rooms per village sampled each month. However, because household members are not always present when study personnel visit them, the number of houses/rooms in which pyrethrum spray catches are performed varies each month.

Vector species that are collected and identified were transported to CREC's laboratory for 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 females of the vector species were used for PCR analyses, to identify sibling species and molecular forms.

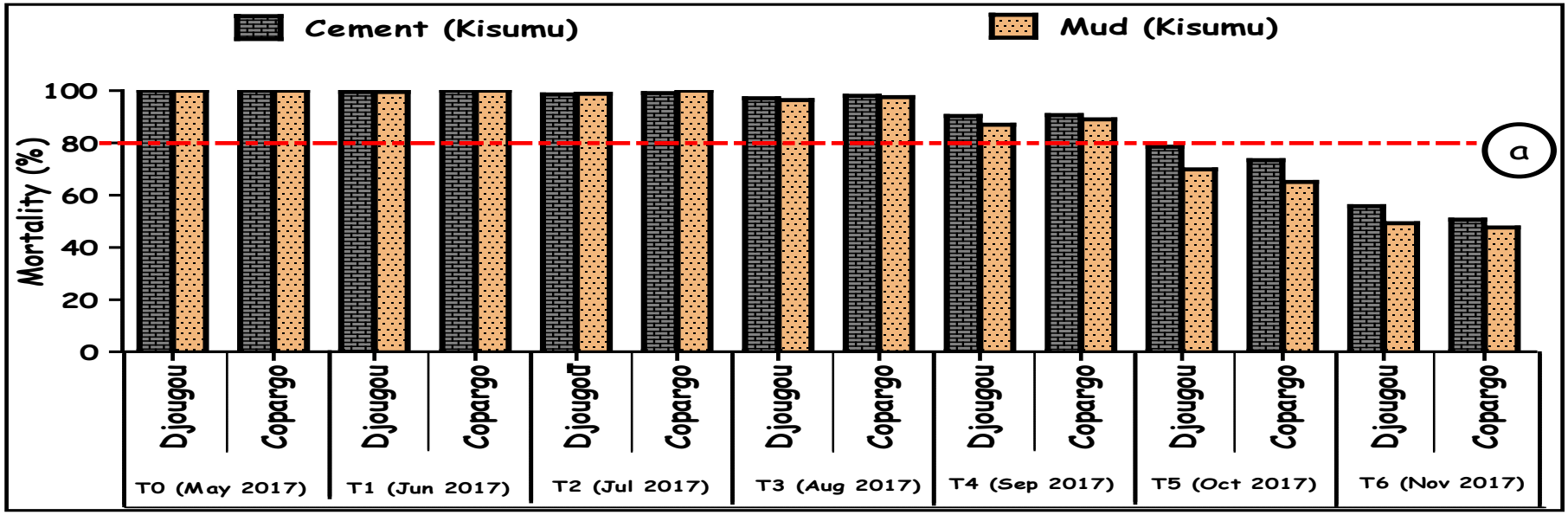
7. Mosquito collections and insecticide susceptibility tests, Species identification and PCR detection of Kdr and Ace-1 mutations and metabolic resistance

Unfed 2-5 days old *An. gambiae s.l* adults from larvae collected on the field were used for WHO susceptibility test using various classes of insecticides. Susceptibility status of the population was graded according to the WHO protocol. Dead and surviving mosquitoes from this bioassay were kept separately in Eppendorf tubes containing silica gel and stored at −20°C for further molecular analysis. The PCR-RFLP diagnostic test was used to detect the presence of L1014F mutation (Kdr) and G119S mutation (Ace.1R gene). Metabolic resistance (esterases, oxidases, GST) was analyzed by spectrophotometer using separated mosquitoes not in contact with insecticides (the control mosquitoes).

8. Results

8.1. Residual effect of pirimiphos methyl on cement and mud walls treated during the first IRS campaign

A good residual effect of pirimiphos methyl was observed during the 4 months of follow-up (Table I). However, after September the delayed Kisumu mortality was well below the 80% efficacy threshold. Six months after spraying (November), the mortality rate of *An. gambiae* Kisumu exposed to the treated walls was 55.8% on cement and 49.3% on mud in Djougou compared to 50.6% and 47.6% respectively in Coprgo (Table I, Figure 2).



a=Insecticide efficacy threshold of Kisumu (WHO)

Figure 2: Mortality rate of *Anopheles gambiae* Kisumu (susceptible strain) after 30 minutes exposure to cement and mud walls treated with pirimiphos methyl and 24 hours of holding period.

Table I: Mortality rate of *Anopheles gambiae* Kisumu (susceptible strain) after 30 minutes exposure to cement and mud walls traited with pirimiphos methyl and 24 hours of holding period.

	To (May 2017)		T1 (June 2017)		T2 (July 2017)		T3 (August 2017)		T4 (September2017)		T5 (October 2017)		T6 (November2017)	
	Djougou Kisumu	Copargo Kisumu	Djougou Kisumu	Copargo Kisumu	Djougou Kisumu	Copargo Kisumu	Djougou Kisumu	Copargo Kisumu	Djougou Kisumu	Copargo Kisumu	Djougou Kisumu	Copargo Kisumu	Djougou Kisumu	Copargo Kisumu
Cement	100	100	100	100	98,59	99,06	97,08	98,08	90,34	90,65	78,55	73,38	55,8	50,63
Mud	100	100	100	100	98,81	100	96,4	97,58	87	89,07	69,93	65,06	49,3	47,62

8.2. Mosquito blood feeding behaviors

8.2.1. Human Biting Rate (HBR) of An gambiae indoor versus outdoor in treated and untreated houses.

A total of 3 774 *An. gambiae s.l* were collected from June 2017 to December 2017 in the localities treated (Djougou, Copargo, Kandi, Gogounou and Pehunco) and in the control districts (Bembereke and Kouande) (Table II). Figures 3a and 3b show the percentage of *Anopheles gambiae* biting rate on man indoor compared to outdoor in these districts. From these figures we can make two observations:

- **During the bio-efficacy period of Actellic CS** (June-September, when delayed mortality of Kisumu 24h ≥80% in bioessay); the percentage of mosquitoes collected is very low indoor compared to outdoor in all treated districts. In Bembereke and Kouande chosen as control, we registered a contrary situation with higher HBR indoor compared to outdoor (Figures 3a and 3b).

Globally, 32% of *An. gambiae* (563/1756) were collected indoor in treated houses against 68% (1193/1756) outdoor. In untreated houses, 60.32% (678/1124) were collected against 39.68% (446/1124) (Figure 4).

- **Beyond the bio-efficacy period of Actellic CS** (October-December, when the delayed 24h mortality of Kisumu <80% in bioassay); during this period, we continue to note an impact of the IRS in terms of reducing the density of *An. gambiae* inside the houses compared to the outside in the treated localities compared to the control (Figures 3a, 3b and 4).

Table II: Number of *An. gambiae* collected during and after the bio-efficacy period of Actellic CS (IRS campaign 2017)

Districts	Bio-efficacy period of Actellic CS (Jun-Sep; mortality ≥80%)		Period beyond the bio-efficacy of Actellic CS (Oct-Dec, 24h mortality <80%)	
	Indoor	Outdoor	Indoor	Outdoor
Djougou	133	271	29	55
Copargo	155	372	41	84
Pehunco	19	43	25	39
Kouande (control)	139	82	55	40
Kandi	126	302	32	76
Gogounou	130	205	96	112
Bembereke (control)	539	364	154	56

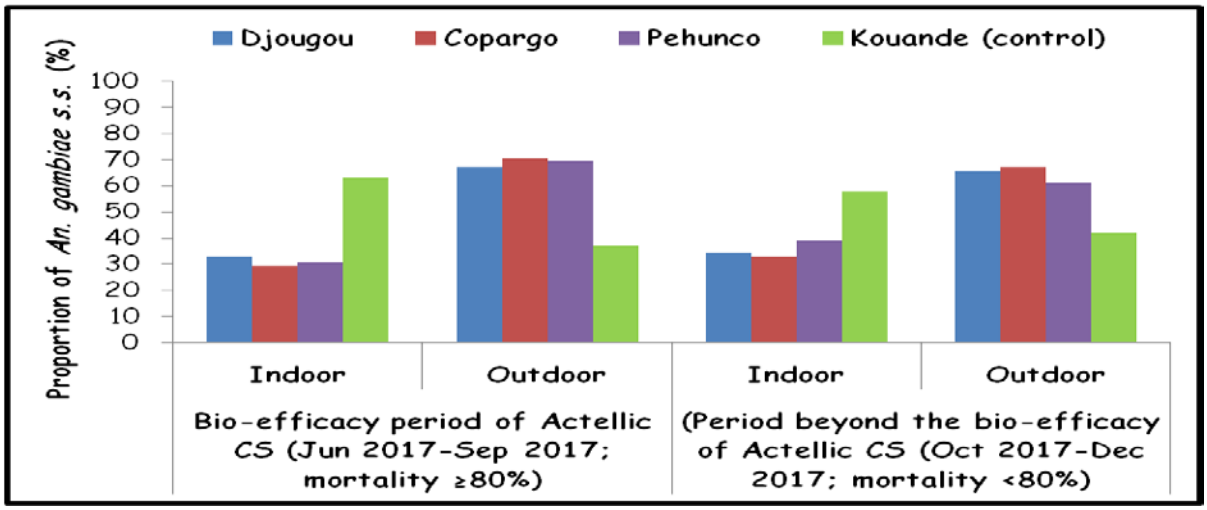


Figure 3a: Proportion of *An. gambiae s.s* during and after the bio-efficacy period of Actellic CS (IRS campaign 2017) in Atacora and Donga

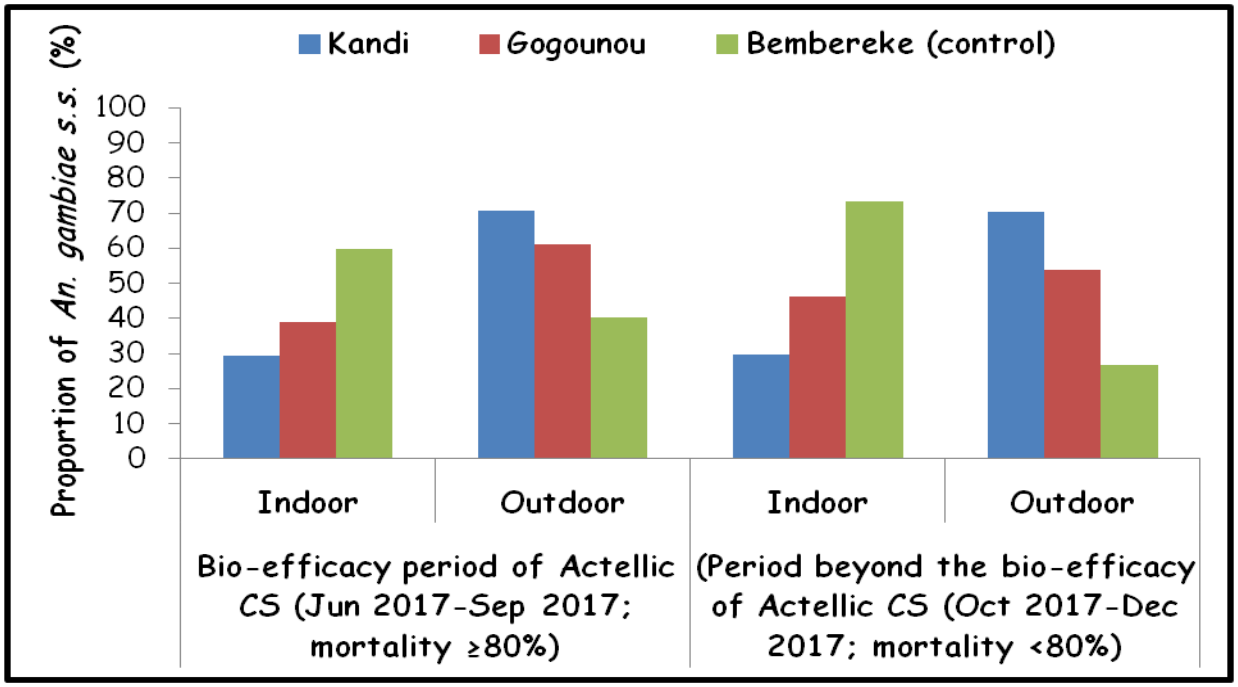


Figure 3b: Proportion of *An. gambiae s.s* during and after the bio-efficacy period of Actellic CS (IRS campaign 2017) in Alibori

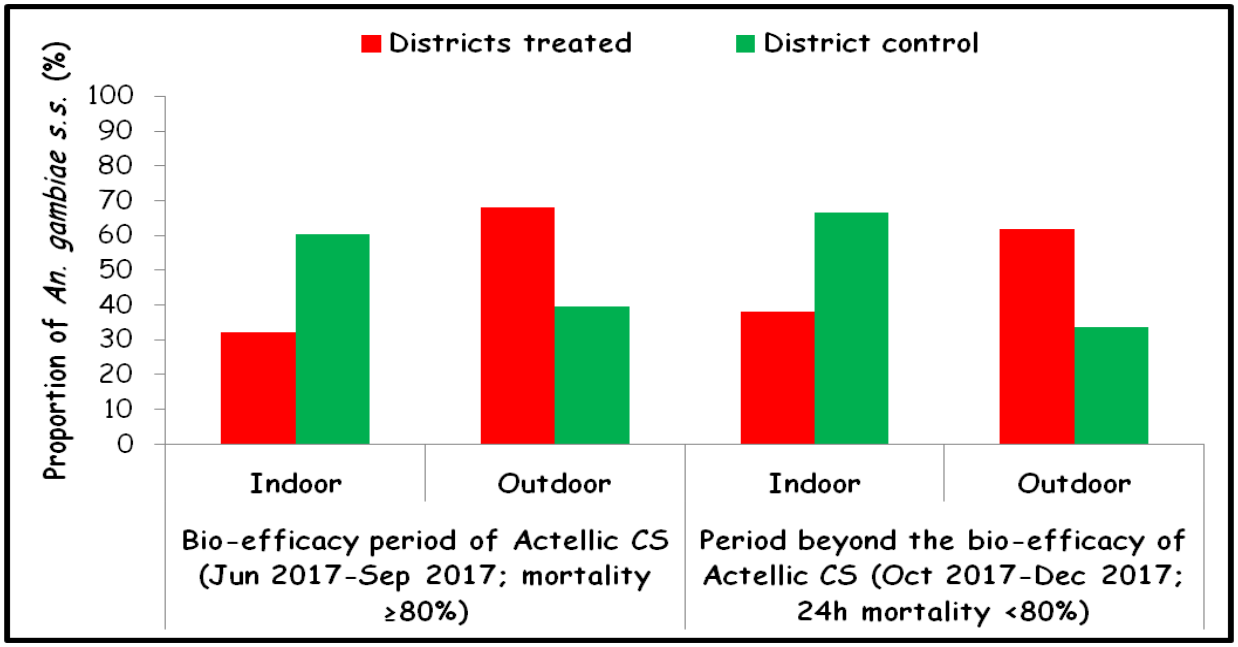


Figure 4: Overall percentage of *An. gambiae* collected using HLC indoor and outdoor during and after the bio-efficacy period of Actellic CS (IRS campaign 2017) in treated and untreated houses

8.2.2. Human Biting Rate (HBR) of Culex quinquefasciatus indoor versus outdoor in treated and untreated houses.

A total of 2 144 *Culex quinquefasciatus* were collected from June 2017 to December 2017 in the localities treated (Djougou, Copargo, Kandi, Gogounou and Pehunco) and in the control districts (Bembereke and Kouande) (Table III). Figures 5a and 5b show the percentage of *Culex quinquefasciatus* biting on man indoor compared to outdoor in these districts. When we read these figures, we make two observations:

- **During the bio-efficacy period of Actellic CS** (June-September, when delayed mortality of Kisumu 24h ≥80% in bioessay), the percentage of mosquitoes collected is very low indoor compared to outdoor in all treated districts. In Bembereke and Kouande chosen as control, we registered a contrary situation with higher HBR indoor compared to outdoor (Figures 3a and 3b).

Globally, 30.76% (308/1001) of *Culex quinquefasciatus* were collected indoor in treated houses against 69.23% (693/1001) outdoor. In untreated houses, contrary to what was noted with *An. gambiae*, the HBR is also lower in *Cx. quinquefasciatus* indoor (40.40%) (Figures 5a, 5b and 6), probably because this species is less anthropophilic and more exophilic than *An. gambiae.*

.

- **Beyond the bio-efficacy period of Actellic CS** (October-December, when the delayed 24h mortality of Kisumu <80% in bioassay); during this period, we continue to note an impact of the IRS in terms of reducing the density of *Culex quinquefasciatus* inside the houses compared to the outside in the treated localities compared to the control (Figures 5a, 5b and 6).

Table III: Number of *Culex quinquefasciatus* collected during and after the bio-efficacy period of Actellic CS (IRS campaign 2017)

Districts	Bio-efficacy period of Actellic CS (Jun-Sep; mortality ≥80%)		Period beyond the bio-efficacy of Actellic CS (Oct-Dec, 24h mortality <80%)	
	Indoor	Outdoor	Indoor	Outdoor
Djougou	84	161	47	95
Copargo	7	16	14	22
Pehunco	15	74	25	45
Kouande (control)	90	85	42	58
Kandi	129	254	142	285
Gogounou	73	188	58	102
Bembereke (control)	373	598	250	347

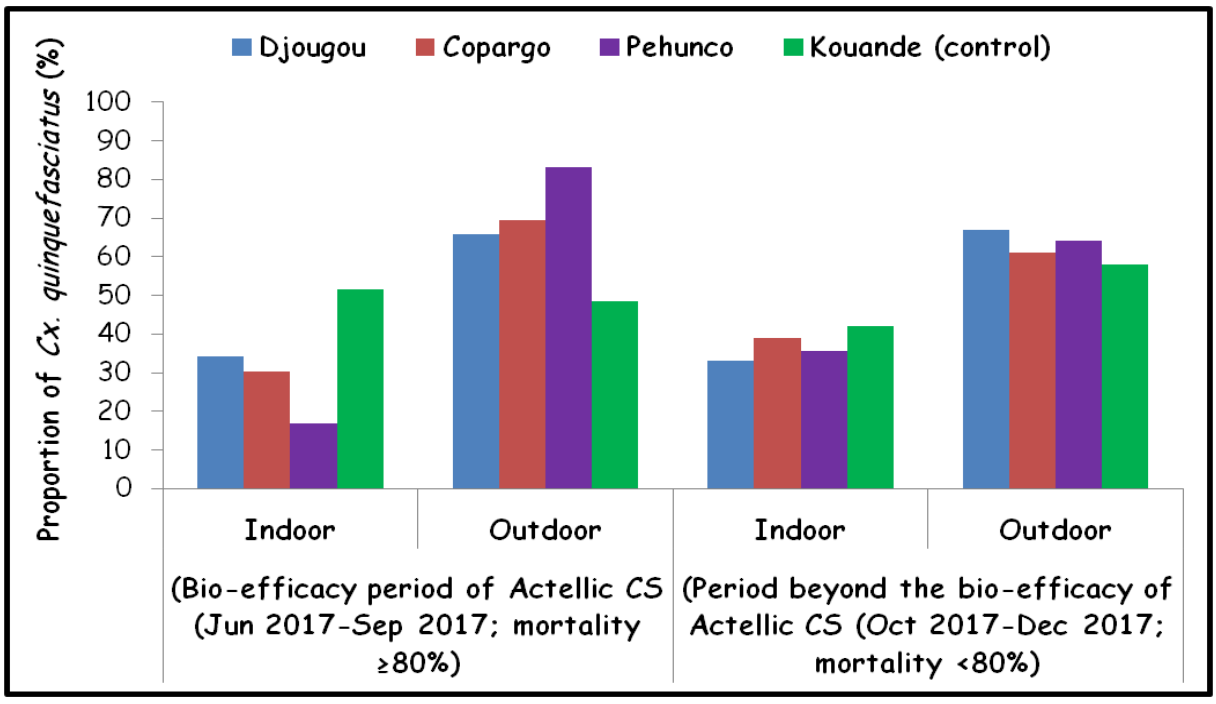


Figure 5a: Proportion of *Culex quinquefasciatus* during and after the bio-efficacy period of Actellic CS (IRS campaign 2017) in Atacora and Donga

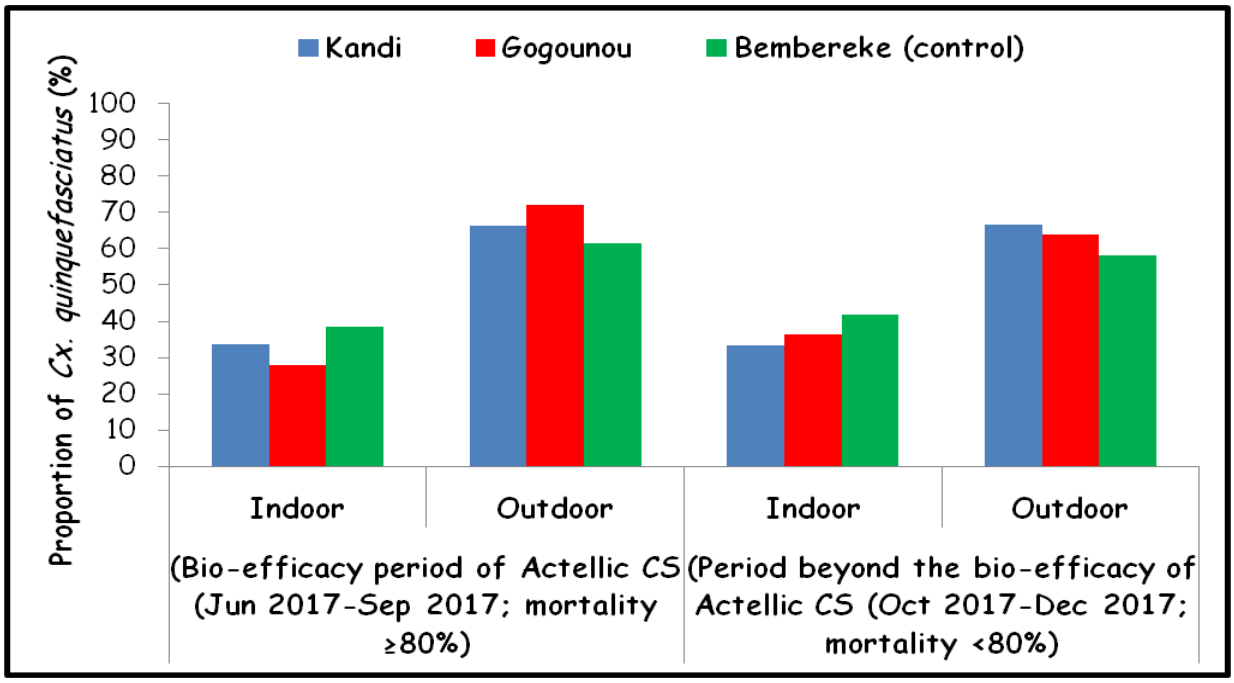


Figure 5b: Proportion of *Culex quinquefasciatus* during and after the bio-efficacy period of Actellic CS (IRS campaign 2017) in Alibori

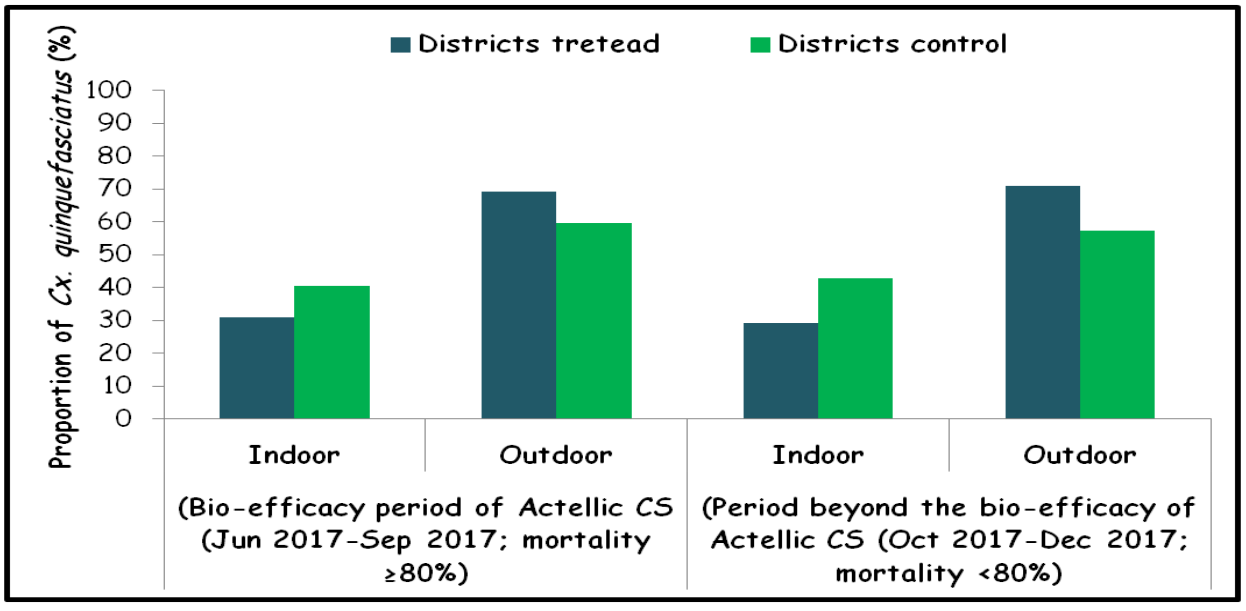


Figure 6: Overall percentage of *Culex quinquefasciatus* collected using HLC indoor and outdoor during and after the bio-efficacy period of Actellic CS (IRS campaign 2017) in treated and untreated houses

8.2.3. Room density and blood feeding rate within An. gambiae and Cx. quinquefasciatusin districts under IRS and in control districts

During the bio-efficacy period of Actellic CS (June-September), about 8.91 is the average density of *Anopheles gambiae s.l.* in 20 rooms (ie an average of 0.41 *Anopheles gambiae s. l.* per room) using by Pyrethrum Spray Catch (PSC) in Alibori, Atacora and Donga (treated). Beyond the bio-efficacy period of Actellic CS (October-December), the room density of *An. gambiae* has not changed too much and is 7.56 *Anopheles gambiae s.l.* in 20 rooms (ie an average of 0.38 *Anopheles gambiae* per room) (Table II). In parallel, 61.57% of these mosquitoes were found fed and half-fed during the bio-efficacy period of Actellic CS against 70.94% beyond the bio-efficacy period of Actellic CS ($p<0.05$) (Table IV).

For *Cx. quinquefasciatus*, the feeding rate is estimated for 59.92% and the room density for 8.97 *Culex quinquefasciatus* in 20 rooms (ie an average of 0.45 *Culex quinquefasciatus* per room) during the bio-efficacy period of Actellic CS. Beyond the bio-efficacy period of Actellic CS, the feeding rate increased to 61.16% and the room density to 18.64 *Culex quinquefasciatus* in 20 rooms (ie an average of 0.93 *Culex quinquefasciatus* per room) (Table V). In conclusion, 2.02% of increase of the blood feeding rate and 51.87% of increase of the room density of *Cx. quinquefasciatus*were registered beyond the bio-efficacy period of Actellic CS.

In some districts, the room density and the blood feeding rate of *An. gambiae* and *Cx. quinquefasciatus* are higher beyond the bio-efficacy period of Actellic CS than during the bio-efficacy period of Actellic CS (Table IV and V) and than control district (Kouande) (Figures 7a). But in Gogounou and Copargo, the difference in blood feeding rate of *An. gambiae* is significant compared to the controls (Table VI).

Tableau IV: Room density and feeding rate of *An. gambiae* collected by PSC (Pyrethrum Spray Catch) between the Actellic CS bio-efficacy period (June-september; mortality ≥80%) and the period beyond the bio-efficacy of Actellic CS (October-December; mortality <80%).

	Bio-efficacy period of Actellic CS								Period beyond the bio-efficacy of Actellic CS								
	Period (jun 2017 -sep 2017;mortality ≥80%)								Period (Oct 2017 -Dec 2017)								
Districts	Number of *An. gambiae* collected	Un feed	Feed	Gravid	Half-gravid	Nb of roomss	**Density for 20 rooms**	**Feeding rate (%)**	Number of *An. gambiae* collected	Un feed	Feed	Gravid	Half-gravid	Nb of rooms	**Density for 20 rooms**	**Feeding rate (%)**	P-value
Kandi	35	12	23	0	0	142	4.93	**65,71**	12	4	8	0	0	104	2,4	**66,67**	**1**
Gogounou	75	30	44	0	1	138	10,87	**60**	39	15	23	1	0	101	7,8	**58,97**	**1**
Djougou	43	19	24	0	0	148	5,81	**55,81**	11	2	9	0	0	75	3	**81,82**	0.217
Copargo	55	17	38	0	0	123	8.94	**69,09**	29	4	23	0	2	80	7,2	**86,21**	0.145
Pehunco	21	10	11	0	0	30	14	**52,38**	26	4	18	4	0	30	17,4	**69,23**	0.379

P-value: P-value of comparison of the feeding rate of *An. gambiae* between the Actellic CS bio-efficacy period (June-september; mortality ≥80%) and the period beyond the bio-efficacy of Actellic CS (October-December; mortality <80%)

Tableau V: Room density and feeding rate of *Culex quinquefasciatus* collected by PSC (Pyrethrum Spray Catch) between the Actellic CS bio-efficacy period (June-september; mortality ≥80%) and the period beyond the bio-efficacy of Actellic CS (October-December; mortality <80%).

	Bio-efficacy period of Actellic CS								Period beyond the bio-efficacy of Actellic CS								
	Period (jun 2017 -sep 2017;mortality ≥80%)								Period (Oct 2017 -Dec 2017)								
Districts	Number of *Culex quinquefasciatus* collected	Unfeed	Feed	Gravid	Half-gravid	Nb of room	**Density for 20 rooms**	**Feeding rate (%)**	Number of *Culex quinquefasciatus* collected	Unfeed	Feed	Gravid	Half-gravid	Nb of room	**Density for 20 rooms**	**Feeding rate (%)**	P-value
Kandi	84	29	48	6	1	142	11,8	**58,33**	97	30	56	11	0	104	18,6	**57,73**	1
Gogounou	100	33	61	3	3	138	14,4	**64**	146	33	91	22	0	101	28,9	**62,33**	0.894
Djougou	54	20	28	4	2	148	7,2	**55,56**	94	14	62	16	2	75	25,06	**68,09**	0.178
Copargo	14	6	7	0	1	123	2,2	**57,14**	8	0	0	8	0	80	2	**0**	0.026

P-value: P-value of comparison of the feeding rate of *Culex quinquefasciatus* between the Actellic CS bio-efficacy period (June-september; mortality ≥80%) and the period beyond the bio-efficacy of Actellic CS (October-December; mortality <80%)

VI: Comparative table of the blood feeding rate of *An. gambiae* between the treated districts and the control during the period beyond the bio-efficacy of Actellic CS (October-December; mortality <80%)

Districts	Period beyond the bio-efficacy of Actellic CS							
	Period (Oct 2017 -Dec 2017)							
	Number of *An. gambiae* collected	Unfeed	Feed	Gravid	Half-gravid	Density for 20 rooms	Feeding rate (%)	P-value
Bembereke (control)	20	0	19	1	0	5	**95**	-
Kandi	12	4	8	0	0	2,4	**66,67**	0,102
Gogounou	39	15	23	1	0	7,8	**58,97**	0,0096
Kouande (control)	56	10	32	14	0	37,4	**57,14**	-
Djougou	11	2	9	0	0	3	**81,82**	0,231
Copargo	29	4	23	0	2	7,2	**86,21**	0,0139
Pehunco	26	4	18	4	0	17,4	**69,23**	0,423

P-value: P-value of comparison of the blood feeding rate of *An. gambiae* between the treated districts and the control beyond the bio-efficacy of Actellic CS (October-December; mortality <80%)

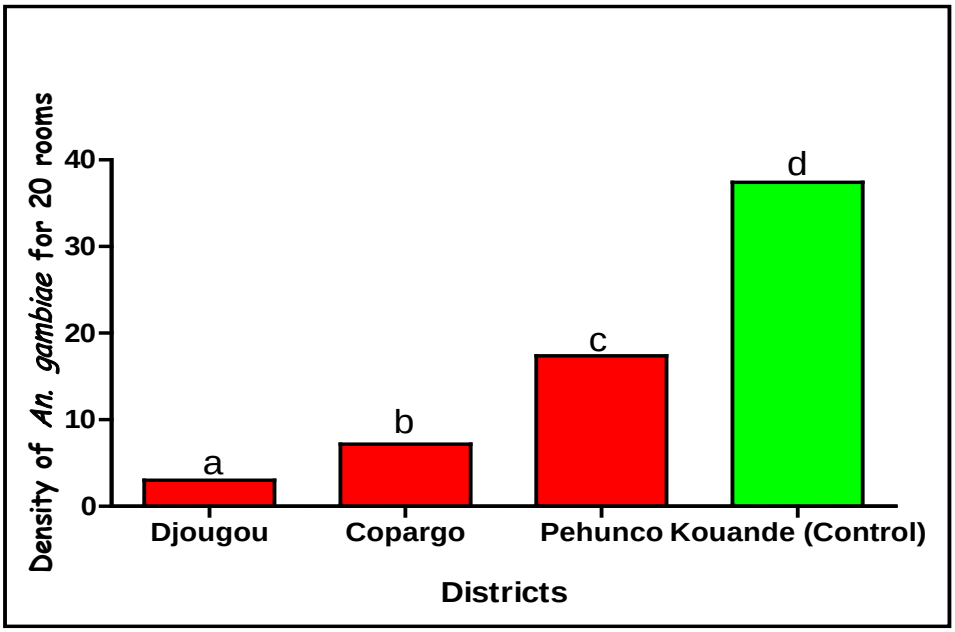


Figure 7a: Room density of *An. gambiae* collected in treated and in control rooms (Atacora and Donga) beyond the bio-efficacy period of Actellic CS

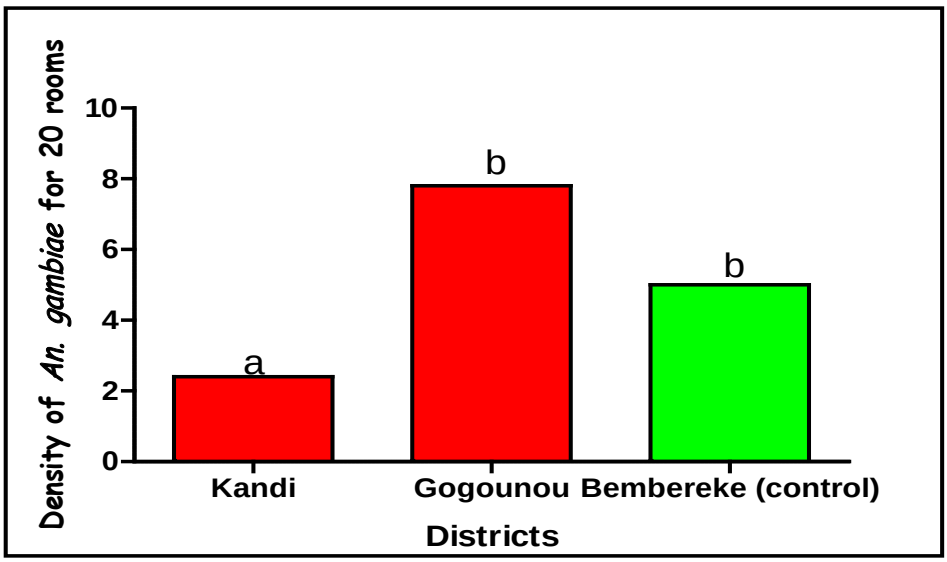


Figure 7b: Room density of *An. gambiae* collected in treated and in control rooms (Alibori) beyond the bio-efficacy period of Actellic CS

8.3. Parous rate observed in An. gambiae in districts under IRS and in control districts.

During the bio-efficacy period of Actellic CS, the overall parous rate registered in *An. gambiae* in Alibori, Atacora and Donga was estimated at 35.51% (376/1059) (Table VII, figure 8). Beyond the bio-efficacy period of Actellic CS (October – December), the parous rate of *An. gambiae* increases to 42.78% (160/374). This rate is lower than what was found in the control districts (56.56%: 112/198) (p = 0.0022) (Table III).

Table VII: Comparaison of the parous rate of *Anopheles gambiae* recorded in 2016 -and 2017 during the same period.

Districts	Bio-efficacy period of Actellic CS			Period beyond the bio-efficacy of Actellic CS			
	Period (jun 2017 -sep 2017;mortality ≥80%)			Period (Oct 2017 -Dec 2017 ; mortality<80%)			
	Number tested	Parous	Parous rate (%)	Number tested	Parous	Parous rate (%)	P-value
Kandi	227	75	33.04	77	37	48.05	0.026
Gogounou	191	64	33.51	90	26	28.89	0.523
Djougou	316	118	37.34	47	39	82.98	0.00009
Copargo	260	107	41.15	90	39	43.33	0.812
Pehunco	65	12	18.46	70	19	27.14	0.320

P-value: P-value of comparison of the parous rate of *An. gambiae* between the Actellic CS bio-efficacy period (June-september; mortality ≥80%) and the period beyond the bio-efficacy of Actellic CS (October-December; mortality <80%)

Table VIII: Comparison of the parous rate of *Anopheles gambiae* recorded during the period October- December 2017 in the treated areas and controls.

Districts	Period beyond the bio-efficacy of Actellic CS			
	Period (Oct 2017 -Dec 2017)			
	Number tested	Parous	Parous rate (%)	P-value
Bembereke (Control)	94	69	73,40	-
Kandi	77	37	48,05	0.0011
Gogounou	90	26	28,89	0.00038
Kouande (Control)	104	43	41,35	-
Djougou	47	39	82,98	0.00046
Copargo	90	39	43,33	0.893
Pehunco	70	19	27,14	0.078

P-value: P-value of comparison of parous rate of *An. gambiae* between the treated districts and the control during the period beyond the bio-efficacy of Actellic CS (October-December; mortality <80%)

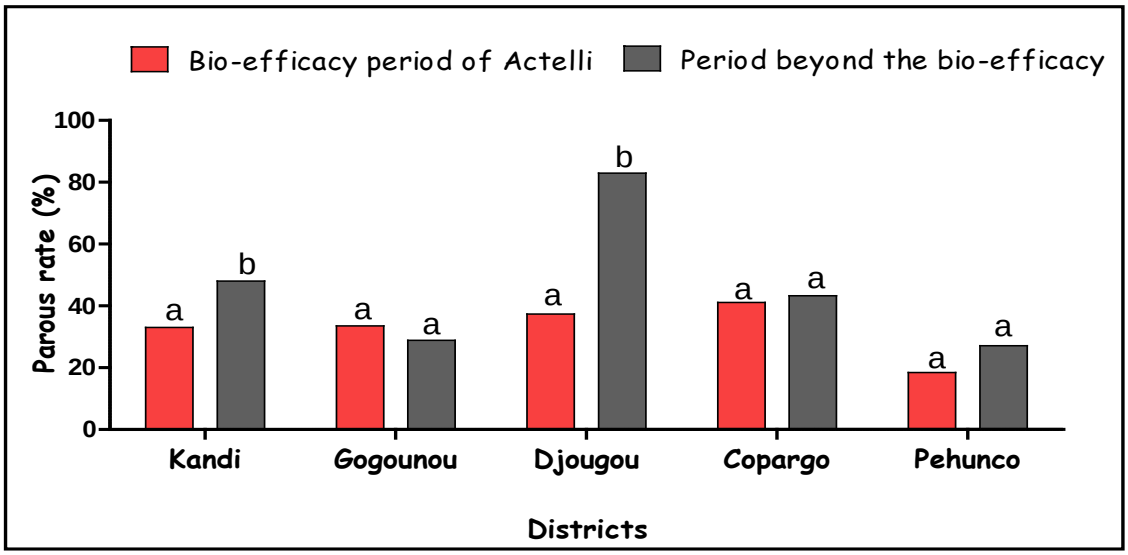


Figure 8: Parous rate of *Anopheles gambiae* observed during and beyond the bio-efficacy period of Actellic CS in Alibori (Kandi, Gogounou), Donga (Djougou, Copargo) and Atacora (Pehunco)

8.4. Sporozoite index (%CS+) of Plasmodium falciparum and Entomological Inoculation Rate (EIR) of An. gambiae in districts under Indoor Residual Spraying (IRS) and in control districts.

During the bio-efficacy period of Actellic CS (June-Septembe), 1 981 *An. gambiae* collected in Alibori, Atacora and Donga were analyzed by PCR-CSP and 30 were found positive for *Plasmodium falciparum*, that means 1.51% of CS+. Beyond the bio-efficacy period of Actellic CS, the %CS+ increased to 3.08% (21 thorax+/680 thorax). In parallel, the Entomological Inoculation Rate (EIR) decreased from 2.92 infected bites/month to 2.26 for the period of beyond bio-efficacy of Actellic CS (22.6% of reduction). This reduction was observed in Pehunco (71.45% of reduction) and Gogounou (19.84% of reduction) (Table IX). On the other hand, there is an increase in the EIR in the other localities (Table IX and Figure 9).

There was also a reduction in EIR in the treated districts compared to controls during the period June-December except Kandi during the period November - December (Figures 10 and 11).

Tableau IX: Sporozoite index (Is) (%) of *Plasmodium falciparum* and Entomological Inoculation Rate (EIR) of *An. gambiae* during and beyond the bio-efficacy period of Actellic CS

Districts	Bio-efficacy period of Actellic CS			Period beyond the bio-efficacy of Actellic CS		
	Period (jun 2017 -sep 2017;mortality ≥80%)			Period (Oct 2017 -Dec 2017; mortality<80%)		
	HBR	Is (%)	EIR/month	HBR	Is (%)	EIR/month
Kandi	7,64	1,08	**2,47**	2,500	3,33	**2,50**
Gogounou	5,98	0,732	**1,31**	4,330	0,81	**1,05**
Djougou	7,21	0,89	**1,94**	1,750	5,26	**2,76**
Copargo	9,41	1,2	**3,4**	2,60	4,55	**3,55**
Pehunco	1,29	16,9	**6,55**	1,33	4,69	**1,870**

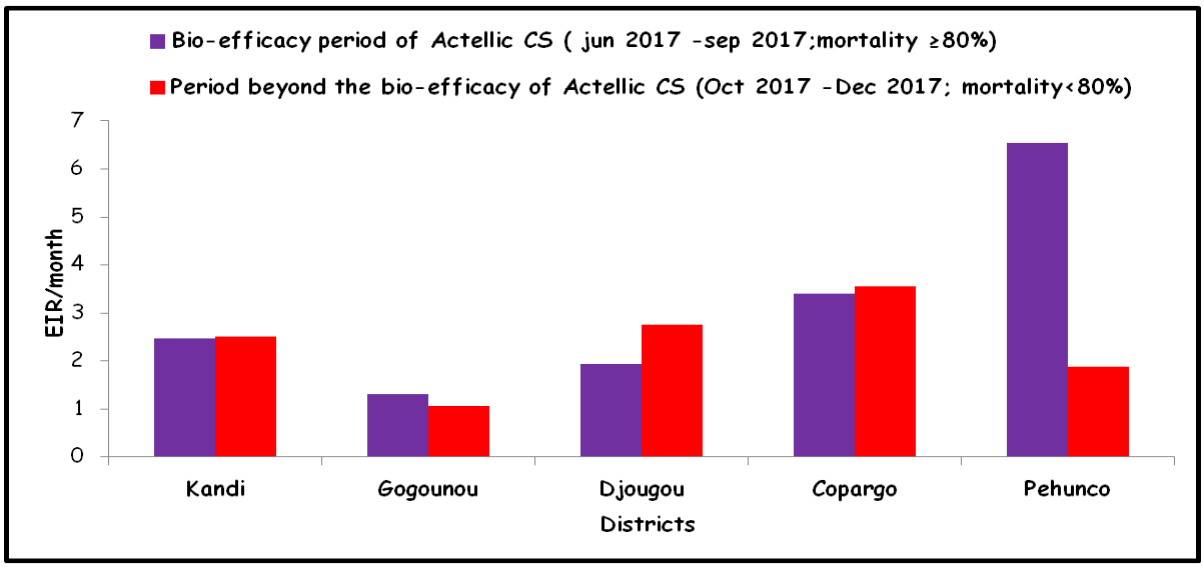


Figure 9: Entomological Inoculation Rate (EIR) of *An. gambiae* during and beyond the bio-efficacy period of Actellic CS in Alibori, Donga and Atacora

Tableau IX: Human Biting Rate (HBR), Sporozoite index (Is) (%) of *Plasmodium falciparum* and Entomological Inoculation Rate (EIR) of *An. gambiae* from June to December 2017

District		June 2017	July 2017	August 2017	sept-17	IS/period (%)	EIR/ Period	Oct 17	Nov 17	Dec 2017	(Oct-Dec)	IS/period (%)	EIR/Period
Kandi	**Thorax**	56	89	112	206	1,08000	2,47	88	14	18	120	3,33333	2,50
	Thorax+	1	1	2	1			1	2	1	4		
	Is	0,018	0,011	0,02	0,0049			0,011	0,143	0,056	0,0333		
	HBR	3,06	5,25	13,50	11,69			4,75	0,8750	1,1250	2,500		
	EIR	0,05	0,06	0,24	0,06			0,054	0,1250	0,063	0,0833		
	EIR/month	**1,64**	**1,77**	**7,23**	**1,70**			**1,62**	**3,750**	**1,88**	2,500		
Gogounou	**Thorax**	7	65	134	204	0,732	**1,31**	234	6	7	247	0,8097	**1,05**
	Thorax+	0	0	1	2			2	0	0	2		
	Is	0,00	0,00	0,01	0,01			0,0085	0,00	0,00	0,0081		
	HBR	0,31	3,25	14,62	10,06			12,56	0,1875	0,2500	4,330		
	EIR	0,00	0,00	0,11	0,10			0,1074	0,00	0,00	0,0351		
	EIR/month	**0,00**	**0,00**	**3,27**	**2,96**			**3,22**	**0,00**	**0,00**	1,052		
Djougou	**Thorax**	120	119	70	138	0,839	**1,94**	76	6	13	95	5,263	**2,76**
	Thorax+	0	2	0,00	2,00			5	0,00	0,00	5		
	Is	0,00	0,0168	0,00	0,01			0,0658	0,0000	0,0000	0,0526		
	HBR	8,00	7,19	7,87	8,00			4,187	0,3125	0,7500	1,750		
	EIR	0,00	0,12	0,00	0,12			0,275	0,00	0,00	0,0921		
	EIR/month	**0,00**	**3,62**	**0,00**	**3,48**			**8,26**	**0,00**	**0,00**	2,763		

Copargo	**Thorax**	75	276	89	142	1,203	**3,40**	95	30	29	154	4,545	**3,55**
	Thorax+	1	1	2	3			7	0	0	7		
	Is	0,013	0,004	0,02	0,02			0,0737	0,000	0,000	0,0455		
	HBR	3,81	16,13	10,12	7,94			5,25	1,31	1,25	2,60		
	EIR	0,05	0,06	0,23	0,17			0,39	0,00	0,00	0,1182		
	EIR/month	**1,53**	**1,75**	**6,82**	**5,03**			**11,61**	**0,00**	**0,00**	3,545		
Bembèrèkè	**Thorax**	213	417	181	256	3,187	**15,41**	209	8	13	230	1,739	**2,23**
	Thorax+	5	15	6	8			3	1	0	4		
	Is	0,0235	0,0360	0,03	0,03			0,01435	0,12500	0,00000	0,0174		
	HBR	8,94	24,06	18,87	14,00			11,81	0,50	0,81	4,27		
	EIR	0,21	0,87	0,63	0,44			0,17	0,06	0,00	0,0743		
	EIR/month	**6,29**	**25,97**	**18,77**	**13,13**			**5,09**	**1,88**	**0,00**	2,228		
Kouandé	**Thorax**	74	93		82	9,5	13,11	44	34	19	97	17,53	10,62
	Thorax +	2	5		14			12	4	1	17		
	IS	0,027	0,054		0,2593			0,273	0,118	0,053	0,175		
	HBR	4,625	5,813		3,375			2,75	2,12	1,18	2,02		
	EIR	0,124875	0,313902		0,875			0,75	0,249	0,062	0,354		
	EIR/month	3,74625	9,41706		26,25			22,50	7,48	1,86	10,62		
Pehunco	**Thorax**	17	22		40	16,9	6,55	40	15	9	64	4,69	1,870
	Thorax +	0	1		10			2	1	0	3		
	IS	0	0,045		0,385			0,05	0,06667	0	0,0469		
	HBR	1,0625	1,375		1,625			2,5	0,937	0,562	1,33		
	EIR	0	0,061875		0,625			0,125	0,0625	0	0,0623		
	EIR/month	0	1,85625		18,75			3,75	1,874	0	1,870		

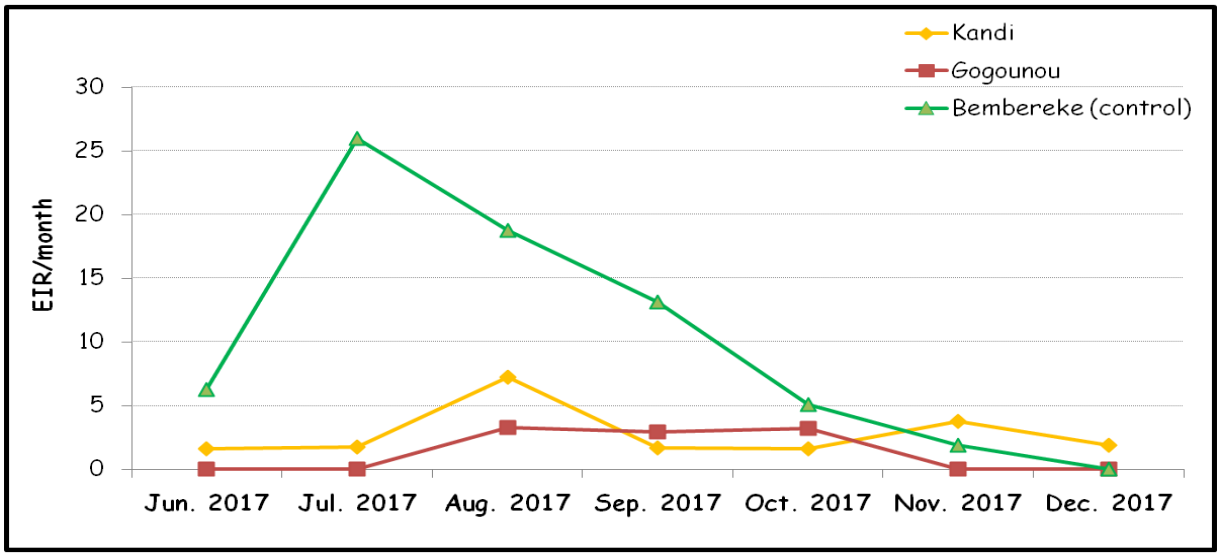


Figure 10: Variation of the Entomological Inoculation Rate (EIR) of *An. gambiae* in districts under Indoor Residual Spraying (IRS) and in control district (Alibori)**.**

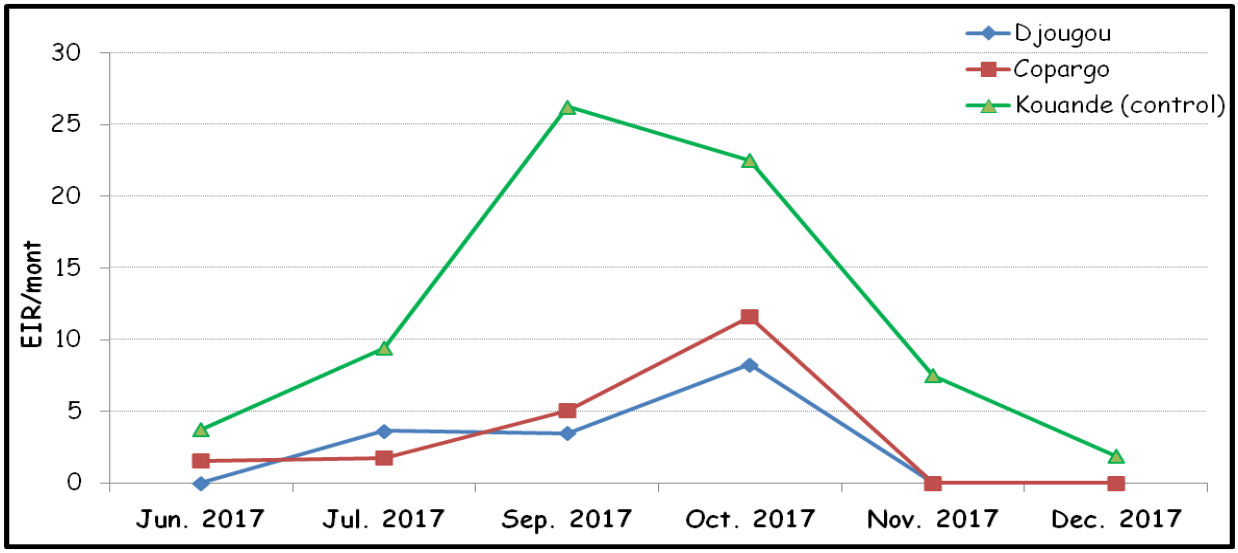


Figure 11: Variation of the Entomological Inoculation Rate (EIR) of *An. gambiae* in districts under Indoor Residual Spraying (IRS) and in control district (Atacora and Donga)

8.5. Insecticide susceptibility tests

Figure 12 summarizes the susceptibility of local vectors to various insecticides (bendiocarb, pirimiphos methyl and deltamethrin in November 2017. *Anopheles* mosquitoes tested were susceptible to pirimiphos methyl in Djougou (98%) (Mortality =98%). However, the same population of *Anopheles gambiae* showed a resistance to bendiocarb (62%) and the Gogounou population showed resistance to deltamethrin (pyrethroids) (29.76%) (<90% mortality).

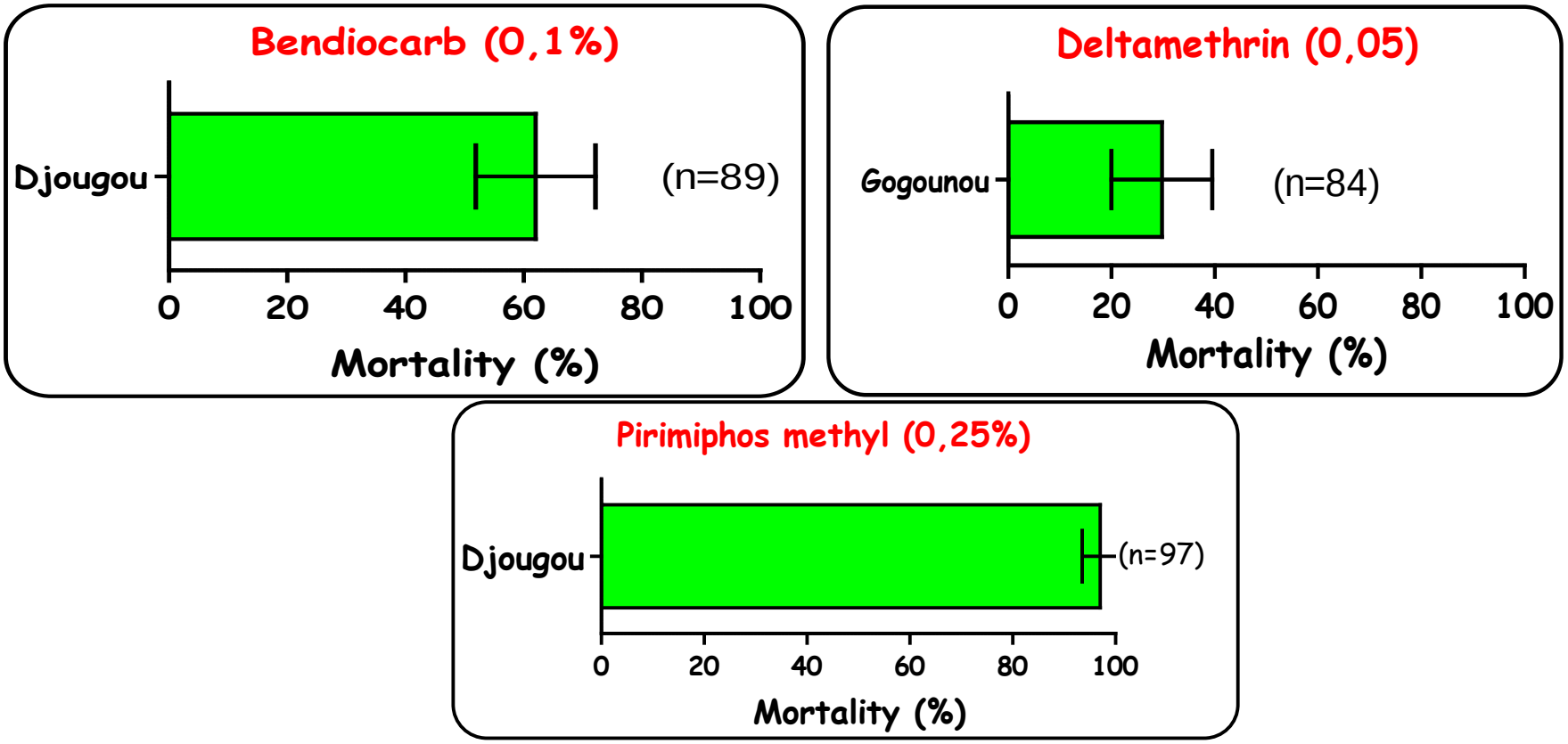


Figure 12: Susceptibility of *Anopheles gambiae s.l* to Bendiocarb 0.1%, Pirimiphos methyl 0.25% and Deltamethrin 0.05% in tow districts under IRS in November 2017.

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

Data presented in Table X and XI show the distribution of Knock-down and Ace 1 resistance among *An. gambiae* complex species collected in October and November 2017. Two species of the *An. gambiae* complex were found in all localities: *An. gambiae* which is the predominant species and *An. coluzzii* poorly represented. Knock-down resistance frequencies were high in *An. gambiae* and *An. coluzzii* encountered in all the sites (P>0.05) (Table X).

Ace-1R mutation associated with carbamate and organophosphate resistance was identified in all localities in *An. gambiae* at very low frequencies (4%) (Table XI). In *An. coluzzii*, these frequencies are zero in all localities excepted Copargo (3%) (Table XI).

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

Localities	Species	Number tested	RR	RS	SS	F (Kdr)	p-value
Kandi	*An. gambiae*	57	45	9	3	0,87	0.38
	An. coluzzii	8	6	0	2	0,75	
Gogounou	*An. gambiae*	75	56	14	5	0,84	0.857
	An. coluzzii	4	3	0	1	0,75	
Djougou	*An. gambiae*	35	28	5	2	0,87	1
	An. coluzzii	6	5	1	0	0,91	
Copargo	*An. gambiae*	58	46	8	4	0,86	1
	An. coluzzii	7	5	2	0	0,85	
Bembereke	*An. gambiae*	50	44	4	2	0,92	1
	An. coluzzii	8	7	1	0	0,94	

Table XI: Distribution of Knock-down resistance (*Ace1*) frequencies between malaria vectors and localities

Localities	Species	Number tested	RR	RS	SS	F (Ace 1)	p-value
Kandi	*An. gambiae*	57	0	5	52	0,04	0.872
	An. coluzzii	8	0	0	8	0	
Gogounou	*An. gambiae*	75	0	6	69	0,04	1
	An. coluzzii	4	0	0	4	0	
Djougou	*An. gambiae*	35	0	2	33	0,03	1
	An. coluzzii	6	0	0	6	0	
Copargo	*An. gambiae*	58	0	5	53	0,04	0.0047
	An. coluzzii	7	0	4	3	0,3	
Bembereke	*An. gambiae*	50	0	5	45	0,05	0.801
	An. coluzzii	8	0	0	8	0	

9. Conclusion

All objectives set during deliverable 6B2 covering the period from October to December 2017 are met. During this period, we continue to observe a reduction of some indicators like the EIR and a strong exophagy of *An. gambiae* and *Cx. quinquefasciatus* and a gradual increase of other indicators such as the blood feeding rate and the parous rate. With respect to vector susceptibility, *An. gambae s. l.* is sensitive to pirimiphos methyl but suspected resistance to bendiocarb and resistant to deltamethrin were registered in localities evaluated.

10. Difficulties encountered

During the period November to December characterized by intense drought and harmattan, the rarity of positive Anopheles larvae breeding sites was a handicap to perform susceptibility tests in all the districts and for all insecticide classes.

11. Activities planned for the next 3 months (January and March)

The same monitoring will continue in the same districts and this data will serve as a witness for the next May 2018 spraying campaign.