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

Results of the M&E of the 6th Indoor Residual Spraying (IRS) in Atacora and database on entomological surveillance in two regions (Alibori and Donga) proposed for extension of IRS

LE 26

Final Report

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Collaboration: National Malaria Control Program (NMCP)

Introduction

One of the strengths in the Indoor Residual Spraying (IRS) campaigns in Benin is the setting up a monitoring and evaluation on an ongoing basis. It is this follow-up that has allowed, in 2015, to bring out the strong points of the IRS in Benin, but also and especially the challenges (Akogbeto et al., 2015). Four strengths have been identified by these authors: (i) a massive support of the intervention from the population, (ii) the skill and expertise of the international NGOs responsible for implementation, (iii) the high coverage of protected households exceeding 80% of the sleeping quarters, and (iv) a drastic reduction in malaria transmission.

For the 6th round campaign, we offered through this study, that the M&E be pursued as since the beginning. Indeed, it is unnecessary to embark on the implementation of an intervention of vector control based on the use of chemicals without taking some precautions. The assessment allowed addressing three fundamental questions, among others, for the future of the IRS in Benin.

(i) What are the results achieved during the IRS of the previous years in terms of reduction of malaria transmission are maintained?

(ii) What is the status of the resistance of vectors to the bendiocarb in 2016 after we have stopped the use of this product since 2013?

(iii) Does the pyrimiphos methyl always preserve its efficacy on wild populations of *Anopheles gambiae* in Atacora? In other words, do the populations of *An. gambiae* Atacora still preserve their susceptibility to pyrimiphos methyl after three consecutive years of use of this product?

Furthermore, during this evaluation, in 2016, we enhance the study of quality of the IRS control. We replaced control of effectiveness that we carry out 24 hours after treatment of houses (bioassay after 24 hours) by a bioassay a week after treatment followed by analysis of the pH of the walls of treated houses. By other hand, we collected an amount of insecticide deposited on the walls by the sprayer agents for analysis. The improved quality control is necessary to try to identify the origin of the rapid deterioration of the insecticides we use (bendiocarb and pyrimophos methyl). In case this delay shows no change, in 2017, we will check for the airborne effect of Actellic in Alibori and Donga by holding mosquitoes in sprayed rooms for one night.

At the same time, a database study on the length of the high malaria transmission period in districts recently proposed for IRS extension (Djougou, Copargo, Ouaké in the department of Donga and Kandi, Gogounou and Segbana in the department of Alibori: see map) was carried. The goal of the study is to prevent from relapsing in the mistakes made in the past with the choice of Oueme in 2008 and Atacora in 2011 during the implementation of the IRS. Indeed, these two regions were chosen without sufficient entomological database. During this study, attention is taken to Culex mosquitoes, particularly *Culex quinquefasciatus* a mosquito of high nuisance and also a vector of *lymphatic filarasis*. The impact of pirimiphos methyl on Anopheles and Culex mosquitoes will be assessed after IRS.

In addition, a small investigation on sleeping behavior and ITN use of people of Alibori and Donga is carried out. The objective of this investigation is to identify practices which could reduce the effect of IRS intervention. The investigation was organized at night (9-11 PM) during dry and rainy season using a direct observation during site visits. Our intention is to verify if sleeping behavior by the majority of people of Alibori and Donga are in concordance with good practices recommended by the National Malaria Control Program. In case sleeping outdoor is a practice of the majority of people that could reduce the impact of IRS in facilitating the contact between people and anopheles, and then increasing outdoor malaria transmission.

We know difficulties, problems and challenges of this kind of data. People may start the night outside and go in after 11 pm but this would not be captured by the survey. By other hand, even if people are bitten outside, if the mosquitoes go inside to rest even for a little bit, IRS would still be effective. This is why, now, we are elaborating another questionnaire. We need assistance from USAID

This database study is what was expected from the map road elaborated before implementation of IRS in Alibori and Donga. Data obtained will be correlated with those of epidemiological component.

The third activity carried out in 2016/2017 was focused on database on the susceptibility of mosquitoes to insecticides in Alibori and Donga. Different classes of insecticides were concerned:

- Pyrethroids (deltamethrin, permethrin and alphacypermethrin) used for the treatment of the nets distributed in 2014 and probably to be purchased for the mass distribution campaign of Long Lasting Insecticidal Nets planned for 2017
- Organophosphates and carbamates used for IRS campaigns in Benin.

The *Kdr* L1014F mutation responsible of pyrethroid resistance in *An. gambiae* in West Africa and *Ace*-^{1} mutation responsible of organophosphate/carbamate resistance and biochemical mechanisms were analysed.

We also tested papers impregnated with the standard dose of pyrethroid + a synergist (PBO) anticipating on the results of the use of LLINs of new generation (nets treated with an insecticide + a synergist).

PMI Indicators measured

Activities planned in 2016 provided data and information about the following entomological indicators required by PMI and the NMCP:

• Quality Control of the 6th round of IRS in Atacora

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

• Vector density (Human Biting Rate)

• Mosquito behavior: biting (endophagy or exophagy)

• Entomological Inoculate Rate (EIR)

• Vector resistance to insecticides and resistance mechanisms (Kdr, Ace-1, Oxidases, esterases, GST)

• Percentage of families which practices are not appropriate to a good effectiveness of vector control tools

Activities and Tasks

1. Atacora

1.1. Surfaces/substrates preparation

The pH of cement and mud walls was tested 2 days before spraying. This was done by using a scalpel blade to scrape a small quantity (5g) of substrates from the wall surface into a Petri dish. The substrates were dissolved in distilled water,then the pH meter submerged into the concrete or mud solution for reading.

1.2. Assessment of the quality of treatment

In order to ensure that the recommended dose has been accurately applied to the substrate, samples of treated surface was subjected to chemical assay according to WHOPES Guidelines for testing mosquito adulticides for Indoor Residual Spraying and treatment of mosquito nets (WHO 2006). In this study, to assess the accuracy of indoor spraying, 4 filter papers (Whatman Ni. 1) 5 cm x 5 cm was attached to the selected surface of some houses randomly

selected before spraying. Samples was placed individually in labeled aluminum foil prior to dispatch. Papers was suspended from the walls on pins to ensure that product running down the wall does not soak into the paper and give misleading results.

1.3. IRS quality Assurance and Monitoring Decay Rates of pirimiphos methyl sprayed

A strain of mosquito larvae (*Anopheles gambiae* Kisumu) was reared in CREC's insectary and used for bioassays. All types of material walls sprayed by the insecticide were bioassayed using WHO cones within one week after spraying. Kisumu was used to determine the residual effect of the insecticide on the walls. The threshold of efficacy is 80% mortality of mosquitoes exposed to the treated walls.

Bioassays were performed according to the WHO procedures. Cones were placed on the walls at the bottom, the middle and the top of the walls (500cm, 1000cm, 1500cm, 2000cm). Ten females of *An. gambiae* susceptible reference strain Kisumu were introduced per cone and exposed for 30 minutes. Mortality was checked after 24 hours holding period.

2. Atacora, Alibori and Donga

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

In each of the villages selected for mosquito collection, Indoor Pyrethrum Spray Catches was carried out to estimate the total density of mosquito species in the treated houses and the room density. In each selected village, ten bedrooms were selected for this activity. The selected bedrooms were sprayed with Pyrethrum (mixed with water) and a white canvas was placed on the floor to collect killed mosquitoes. After 15 minutes, all fallen mosquitoes were collected from the floor and placed in Petri dishes, to measure the number of mosquitoes in the room and to estimate the endophily behavior.

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 Circumsporozoit (CSP) antigens. Abdomens of females of the vector species were used for PCR analyses, to identify sibling species and molecular forms.

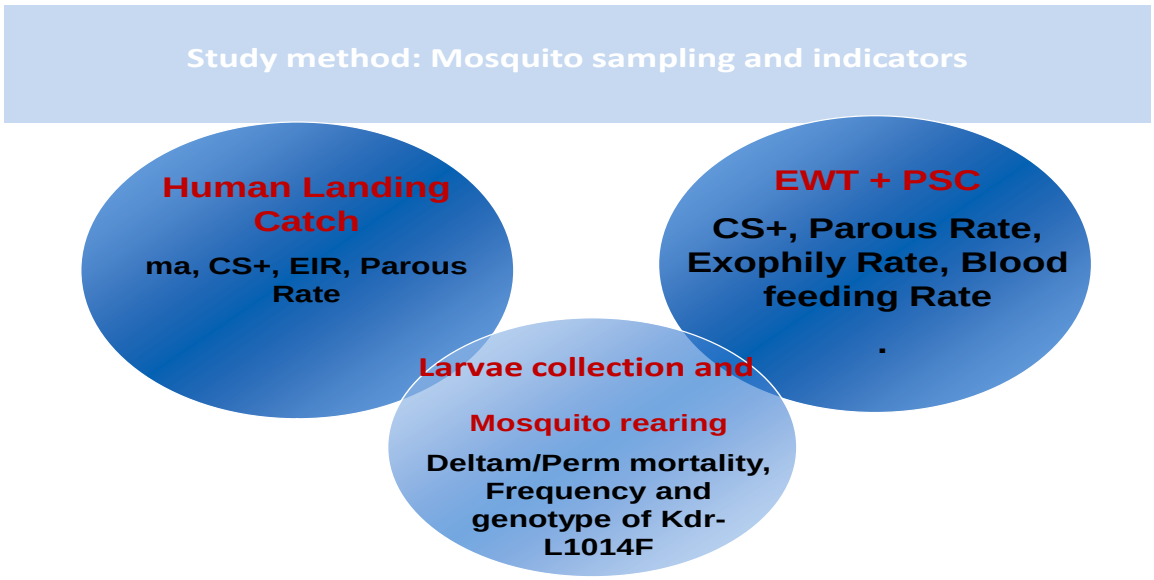
2.2. Mosquito collections and insecticide susceptibility tests, detection of Kdr and Ace-1 mutations and metabolic resistance

Anopheles gambiae s.l. larvae were collected during the rainy seasons and transported in well 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.ladults were used

for WHO susceptibility test using various classes of insecticides. Susceptibility status of the populations 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 molecular analysis. The PCR-RFLP diagnostic test were used to detect the presence of L1014F mutation (Kdr) and G119S mutation (ace.1R gene). Metabolic resistance (esterases, oxidases, GST) were analyzed by spectrophotometer using separated mosquitoes not in contact with insecticides (the control mosquitoes)

2.3. Sleeping behavior and ITN use investigation

Sleeping behavior and ITN use investigation was carried out using questions administrated to a subset of 40 adult head of household per month per district by direct observations on ITN use and sleeping behavior. The survey was conducted each month during the dry and rainy. The survey was conducted at night from 9 PM to 11 PM when people were supposed to sleep. The study allowed us to see at what time children, women and men go to bed, the outdoor sleeping practices during the dry season, the main measures used against mosquito bites and particularly the location of mosquito nets found in the rooms (proportion of ITN hung on



Results

1. M&E of the 6th IRS in Atacora

1.1. Insecticide decay rates using wall bioassay cone test

The residual efficacy of Actellic CS on tested substrates was monitored for a period of 5 months. The initial cone bioassay test was conducted one week after houses were sprayed. Subsequent tests were done on a monthly basis (May, June, July, August, October) to determine the decay of insecticide applied on the walls. The number of mosquitoes dead after 24 hours was registered (table I, figure 1) in all houses where the tests have been done. For the control, the percentage of dead mosquitoes at the end of the test was less than 5% for all the tests and correction formula was not used. Figure 1 shows the variability of bio-availability of Actellic CS on the walls after IRS. Baseline bioassay tests conducted one-week post-IRS revealed 100% mortality on all sprayed surfaces. A good residual efficacy of Actellic CS was maintained on all sprayed around 4 months. However, the bioefficacy tests conducted in October showed 24 h mortality to be ≤ 80%.

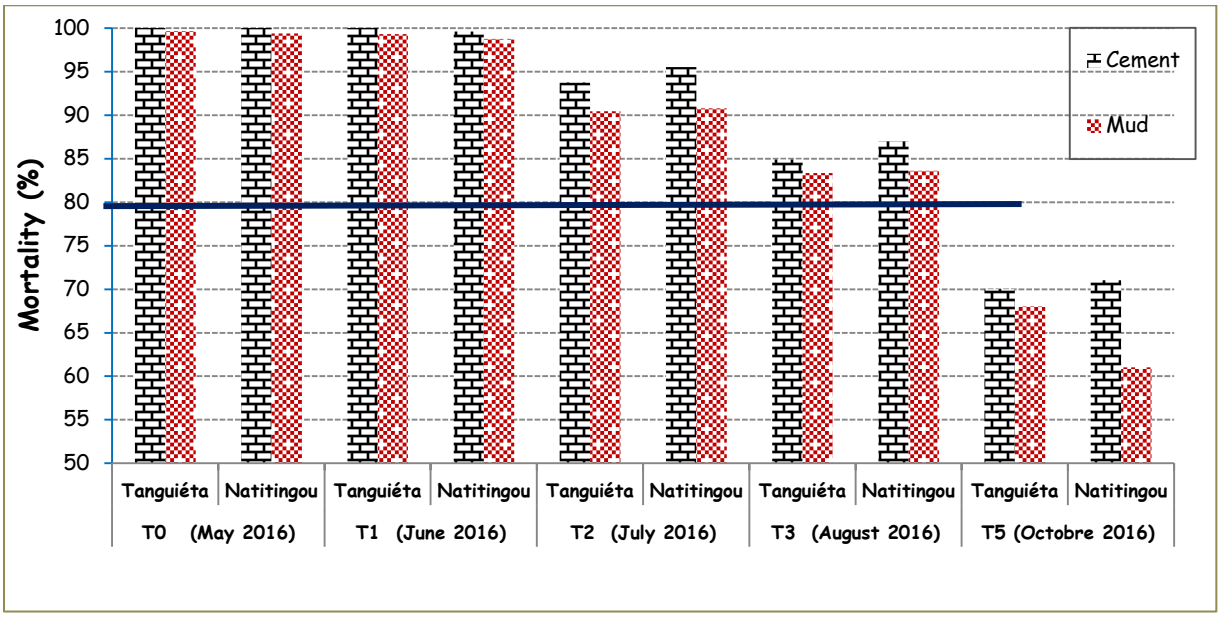


Figure 1: Mean mortalities obtained for different sprayed surface after the five following months after spray operation in Atacora using WHO cone test

Table I. Quality of the spray and residual effect of pirimiphos methyl CS five after 2016 IRS campaign

	T0 (May 2016)		T1 (June 2016)		T2 (July 2016)		T3 (August 2016)		T5 (Octobre 2016)	
	Tanguiéta	Natitingou	Tanguiéta	Natitingou	Tanguiéta	Natitingou	Tanguiéta	Natitingou	Tanguiéta	Natitingou
Cement (%)	100	100	100	99,62	93,75	95,53	84,88	87,00	70	71
Mud (%)	99,66	99,38	99,32	98,75	90,44	90,79	83,33	83,62	68	61,00

1.2. Vector Density and Human Biting Rate in districts under IRS*Vs*control

A total of 1130 *Angambiae s.l* were collected from June 2016 to February 2017 in treated districts (Toukountounan, Tanguiéta, Kouandé and Natitingou) and the control district (Copargo). Figure 2 shows the dynamic of *An. gambiae*global density in districts under IRS *Vs*control. Two main remarks can be done. Firstly, we noticed a high vector density in the control compared to the treated districts during the period from June to October. Secondly, anopheles density trend seems similar (low) in both treated and control districts from January to February.

Table II shows details about indoor *Vs* outdoor vectors density, in treated districts and the control. During the period from June to October,*An. gambiae*were found exophagic in treated districts (more vectors caught outdoor than indoor). This shows that the presence of the pirimiphos methyl considerably decreases the density of vectors in treated area.

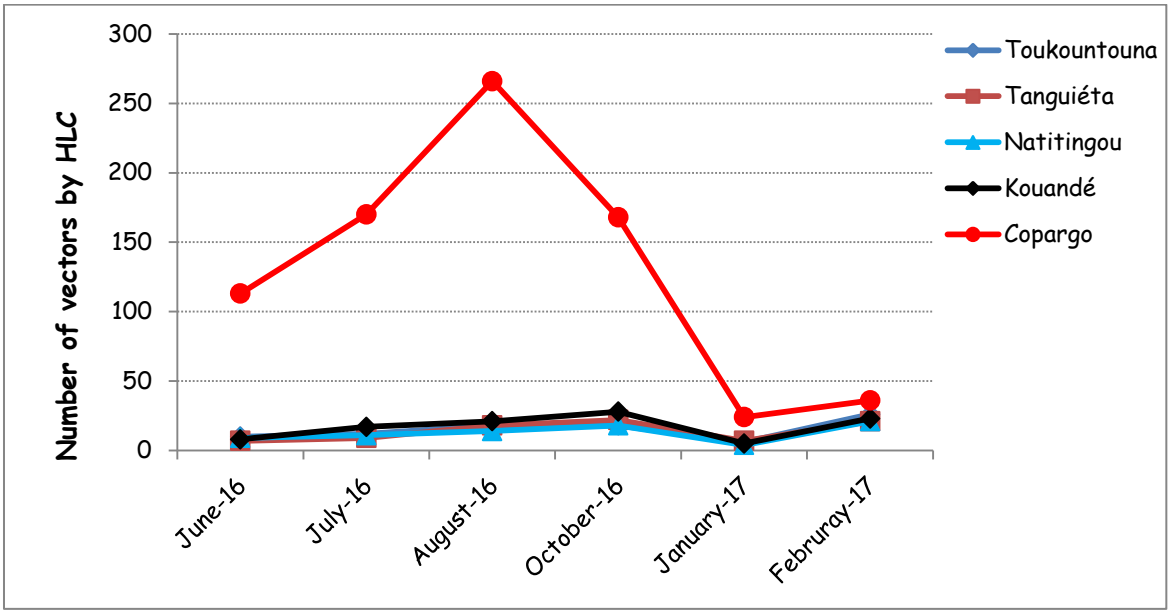


Figure 2: Dynamic of global density of *An.gambiae s.l* in treated districts *Vs* control.

Table II: Details about vector density as well as human biting rate indoor *Vs* outdoor in treated districts compared to the control.

			June 2016	July 2016	August 2016	October 2016	January 2017	February 2017
Toukountounan	**Inside**	Total Mosquitoes	1	1	3	8	5	17
		nb human cathes	8	8	8	8	8	8
		HBR/night	0,13	0,13	0,38	1,00	0,63	2,13
	Outside	Total Mosquitoes	9	11	15	10	1	9
		nb human cathes	8	8	8	8	8	8
		HBR/night	1,13	1,38	1,88	1,25	0,13	1,13
Tanguiéta	**Inside**	Total Mosquitoes	1	1	2	9	6	13
		nb human cathes	8	8	8	8	8	8
		HBR/night	0,13	0,13	0,25	1,13	0,75	1,63
	Outside	Total Mosquitoes	6	8	16	13	1	8
		nb human cathes	8	8	8	8	8	8
		HBR/night	0,75	1	2	1,625	0,125	1
Natitingou	**Inside**	Total Mosquitoes	1	2	1	6	3	14
		nb human cathes	8	8	8	8	8	8
		HBR/night	0,13	0,25	0,13	0,75	0,38	1,75
	Outside	Total Mosquitoes	8	9	13	12	1	7
		nb human cathes	8	8	8	8	8	8
		HBR/night	1,00	1,13	1,63	1,50	0,13	0,88
Kouandé	**Inside**	Total Mosquitoes	1	3	4	10	5	15
		nb human cathes	8	8	8	8	8	8
		HBR/night	0,13	0,38	0,50	1,25	0,63	1,88
	Outside	Total Mosquitoes	7	14	17	18	0	8
		nb human cathes	8	8	8	8	8	8
		HBR/night	0,88	1,75	2,13	2,25	0,00	1,00
Copargo (Control)	**Inside**	Total Mosquitoes	61	88	120	20	17	25
		nb human cathes	8	8	8	8	8	8
		HBR/night	7,625	11	15	2,5	2,125	3,125
	Outside	Total Mosquitoes	52	81	146	148	7	11
		nb human cathes	8	8	8	8	8	8
		HBR/night	6,50	10,13	18,25	18,50	0,88	1,38

1.3.Entomological Inoculation Rate (EIR)

Table III summarizes the Human biting rate (HBR), the sporozitic index (IS) and Entomological Inoculation Rate (EIR) in treated districts and the control after 2016 IRS campaign. The HBR follows the same trend as *An. gambiae* density dynamic described above.

As far as the dynamic of Entomological Inoculation Rate is concerned (figure 3), we can make two main observations:

- FromJune to October we have noticedasignificantdecrease of EIR in treated districts compared to the control.
- From January to Februry, the EIR wasrelativelylow in both treated and control districts

Table III: Human Biting Rate (HBR) of *An. gambiae* and Entomological Inoculation Rate (EIR/night) in treated districts and the control after 2016 IRS campaign.

Districts	Indicators	June 2016	July 2016	August 2016	October 2016	January 2017	February 2017
Toukountouna	IS	0,100	0,000	0,000	0,111	0,167	0,000
	HBR/night	0,625	0,750	1,125	1,130	0,380	1,625
	EIR	0,063	0,000	0,000	0,126	0,063	0,000
Tanguiéta	IS	0,000	0,000	0,056	0,136	0,143	0,048
	HBR/night	0,438	0,563	1,125	1,380	0,440	1,313
	EIR	0,000	0,000	0,063	0,188	0,063	0,063
Natitingou	IS	0,111	0,000	0,000	0,167	0,000	0,000
	HBR/night	0,563	0,688	0,875	1,130	0,250	1,313
	EIR	0,063	0,000	0,000	0,188	0,000	0,000
Kouandé	IS	0,000	0,059	0,048	0,143	0,000	0,043
	HBR/night	0,500	1,063	1,313	1,750	0,310	1,438
	EIR	0,000	0,063	0,063	0,250	0,000	0,063
Copargo (Control)	IS	0,018	0,065	0,094	0,126	0,125	0,000
	HBR/night	7,063	10,630	16,625	10,880	1,500	2,250
	EIR	0,125	0,688	1,563	1,376	0,188	0,000

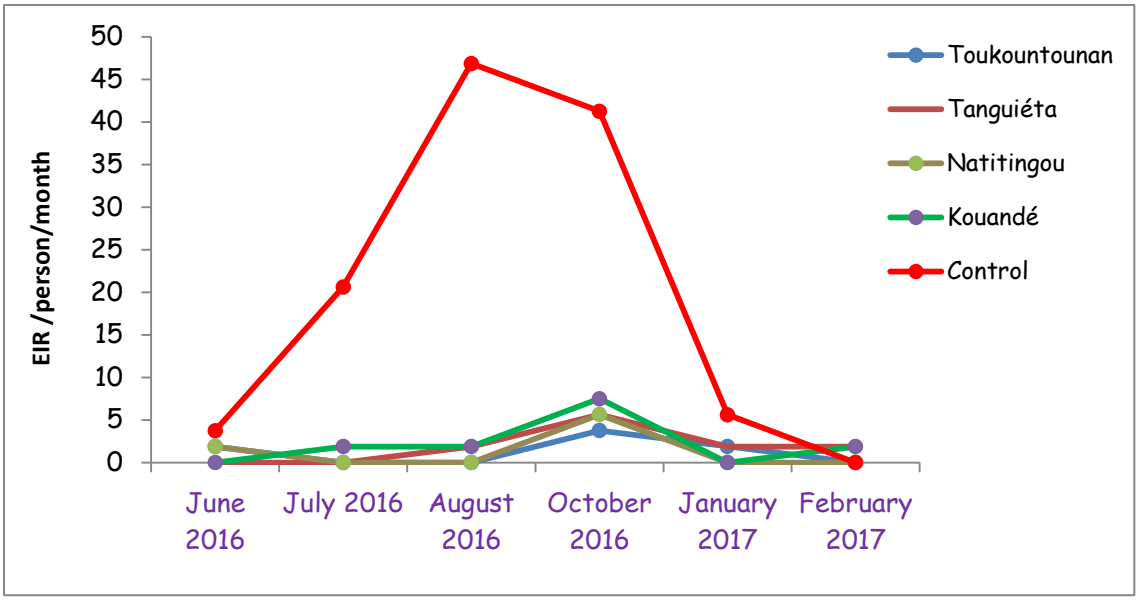


Figure 3: Dynamic of EIR from June 2016 to February 2017

1.3. Blood feeding rate of *An. gambiae* in the districts under IRS *Vs* Control

The blood feeding rate is measured considering the number of fed mosquitoes collected in the morning by Pyrethrum Spray Catch.

Even though IRS have reduced the blood feeding rate, it's important to realize that this reducing still ears low and an important proportion of vectors could still blood feed in treated houses after IRS campaign (Table IV). However, the high rates of blood feeding were recorded in January and February in all the districts. Figure 4 shows the dynamic of

Table IV. Blood feeding rate of *An. gambiae s.l* from June 2016 to February 2017

Districts	June 2016			July 2016			August 2016			October 2016			January 2017			February 2017		
	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate
Toukountounan	4	1	25	4	1	25	11	5	45,45	14	6	43	7	6	85,71	13	11	84,62
Tanguiéta	3	0	0	2	0	0	8	3	37,5	13	8	62	8	6	75	16	14	87,5
Natitingou	3	0	0	3	1	33,3	6	2	33,33	6	4	67	6	5	83,33	11	10	90,91
Kouandé	5	1	20	4	1	25	9	4	44,44	11	6	55	11	9	81,82	14	12	85,71
Total treated districts	15	2	13,33	13	3	23,1	34	14	41,18	44	24	55	32	26	81,25	54	47	87,04
Copargo (Control)	87	61	70,11	133	133	100	79	78	98,73	51	50	98	15	13	86,67	32	31	96,88

Table IV- a: *An. gambiae s.l* density per room per night (mosquitoes caught for PSCs

Months	Districts	Number of sprayed houses	Number of sleepers	Total *An. gambiae*	Average density/room
June 2016	sprayed districts	39	59	15	0,38
	Control	39	145	87	2,23
July 2016	sprayed districts	39	59	15	0,38
	Control	40	127	133	3,33
August 2016	sprayed districts	39	59	24	0,62
	Control	40	155	79	1,98
October 2015	sprayed districts	40	62	44	1,10
	Control	40	109	51	1,28
January 2017	sprayed districts	78	242	32	0,41
	Control	40	138	15	0,38

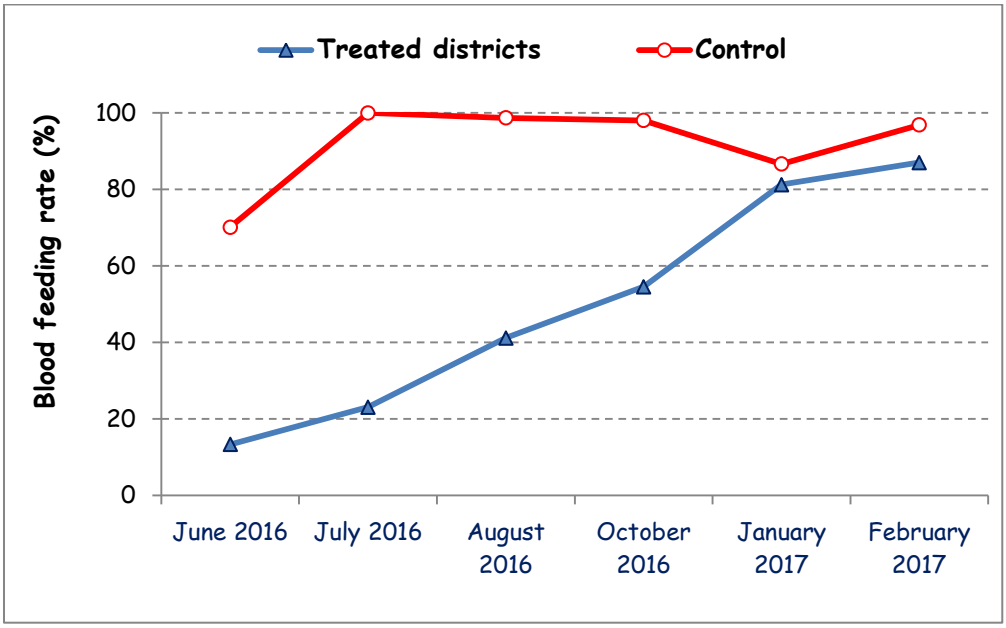


Figure 4: Dynamic ofblood feeding rate of *An. gambiae s.l* in the districts under IRS *Vs* Control from June 2016 to February 2017

1.4. Physiological age of *An. gambiae* in the districts under IRS *Vs* Control

Table V below shows the impact of IRS on the physiological age of *An. gambiae* in terms of parous rate. During the period from June 2016 to February 2017, the main parity rate of *An. gambiae* is constantly high in the control compared to the treated districts. However it has

increased significantly in treated districts from January to February. Figure 5 shows the dynamic of parous rate from June 2016 to February 2017.

Table V. Physiological age in terms of parous rate of *An. gambiae* in the districts under IRS *Vs* Control

Districts	June 2016			July 2016			August 2016			October 2016			January 2017			February 2017		
	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)
Toukountounan	10	3	30	12	3	25	18	2	11,1	18	5	27,78	4	1	25	13	10	76,9
Tanguiéta	7	2	28,6	9	2	22,2	18	3	16,7	22	7	31,82	2	0	0	16	13	81,3
Natitingou	9	2	22,2	11	3	27,3	14	2	14,3	18	4	22,22	2	0	0	11	9	81,8
Kouandé	8	3	37,5	17	3	17,6	21	6	28,6	28	6	21,43	6	2	33,3	14	12	85,7
Total treated districts	34	10	29,4	49	11	22,4	71	13	18,3	86	22	25,58	14	3	21,4	54	44	81,5
Copargo (Control)	113	86	76,1	112	67	59,8	110	76	69,1	83	59	71,08	18	17	94,4	34	32	94,1

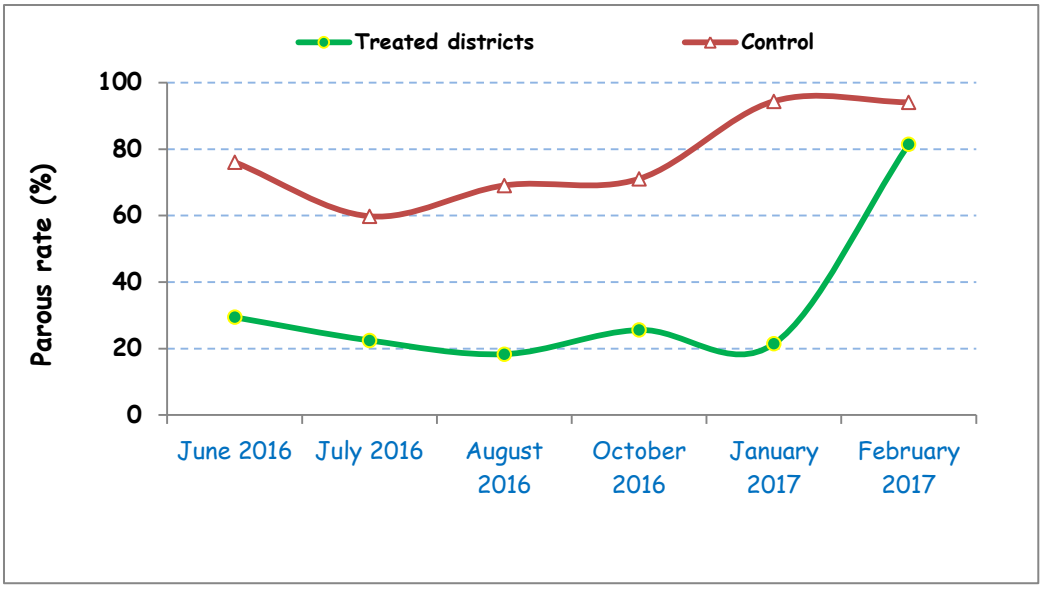


Figure 5: Dynamics oftheparous rate of *An. gambiae* in the districts under IRS *Vs* Control

1.5. Vector susceptibility

Figure 6 below summarizes the findings of the vector susceptibility testing that was undertaken on local malaria vectors (*Anopheles gambiae* s.l) against various insecticides (bendiocarb, pirimiphos methyl, permethrin and deltamethrin). *Anopheles* mosquitoes tested were susceptible to pirimiphos methyl in all the districts (mortality >98%). However the same populations of *Anopheles* were resistant to pyrethroids (permethrin and deltamethrin). As far as Bendiocarb is concerned, *An. gambiae* is restistant in Natitingou but this resistance is suspected in Tanguiéta.

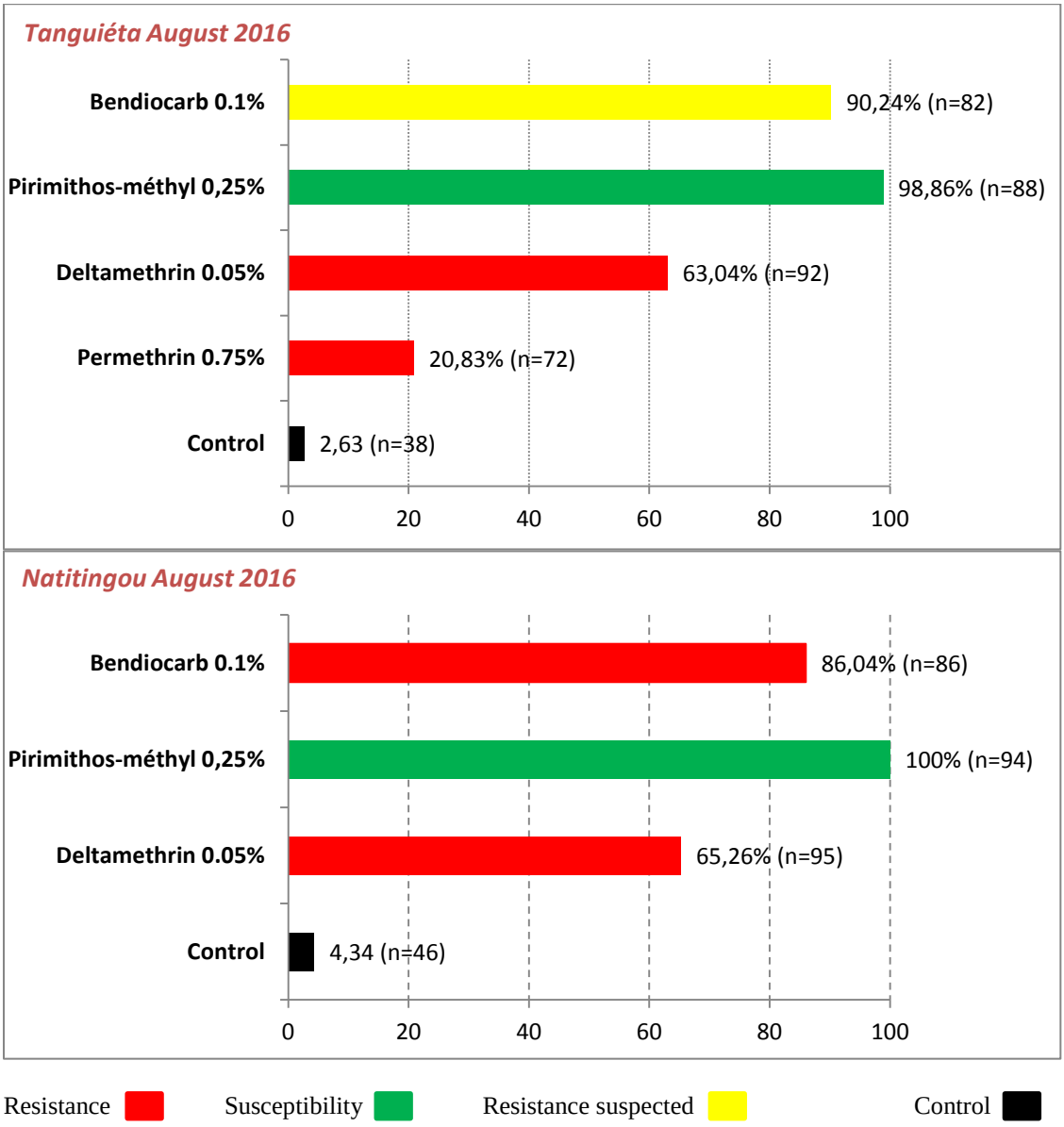


Figure 6: *An. gambiaes.sl* susceptibility to various insecticides in Natitingou and Tanguiéta in August 2016

1.6. Conclusion

The monitoring of 2016 IRS campaign in Atacora region has shown once more the impact of this intervention on malaria transmission. It comes out of this monitoring that, the bioassay testing showed that Pirimiphos methyl remains effective for up to four months post-IRS. During the months following the IRS operation(from June to October) we observed a substantial reduction of some indicators like vector density, vector longevity, blood feeding rate and EIR. As far as vector susceptibility to insecticides is concerned, *An. gambae* is still susceptible to pirimiphos methyl in Atacora.

2. Entomological surveillance in Alibori and Donga (database collection before IRS)

2.1. Anopheles mosquito Population Dynamics

2.1.1. Composition of the mosquito species in the six districts

During the study, a total of 10 367 man-biting mosquitoes belonging to four genera (*Anopheles*, *Aedes*, *Culex*, *Mansonia*) and 15 species were collected in the 6 sites (table I below). Two main malaria vectors were collected: *Anopheles gambiae s.l* (which is found everywhere and represents 40.38% of the total mosquitos: 4187/10367) and *Anopheles funestus* (found mainly in Djougou, Ouake and Copargo: 0.98%). *An. gambiae s.l* is the second most abundant species after *Culex quinquefasciatus* in all sites except Copargo (Figure 2). *An. nili* a local vector, was also collected, but at very low density in Ouake (0.14%). *An. pharoensis*, *An. ziemanni*, *An. paludis* and *An. coustani* were also collected but they don't transmit malaria in Benin.

Culex quinquefasciatus and the other Culex were found. *Cx quinquefasciatus* is the most abundant mosquito collected in Segbana (81.15%), Gogounou (75.67), Kandi (56.76), Ouake (55.72) and Djougou (49.53%) but relatively abundant in Copargo (20.61%) (Figure 2). *Mansonia africana* was also found at a very low density. The disparity between the frequency of Anophelinae and Culicinae in each site is explained by the ecological characteristics of the environment. In the selected districts, some areas are characterized by the presence of many polluted larvae breeding places generally favorable for the development of *Culex quinquefasciatus*. Many mosquitoes collected don't transmit malaria, but they have their importance in medical entomology. *Culex quinquefaciatus* is a very nuisance mosquito but also transmits *bancroftian filariasis* and West Nile virus.

The specific mosquito richness observed at Copargo (11) is not significantly higher than that of Kandi and Gogounou (10) (P> 0.05) but significantly higher than that of Djougou (8) (P

<0.05). The number of species collected at Segbana (7) and Ouake (6) is relatively low and is due to the sampling method (PSC) which does not allow a good diversity of culicidae fauna. Indeed, capture inside houses does not take into account exophilic mosquitoes that come out early in the morning from human habitations or mosquitoes that bite outdoor and rest in the outer shelters.

Table I. Diversity of Culicidae collected using HLC and PSC from May 2016 to February 2017

Species	**Djougou**		**Copargo**		**Kandi**		**Gogounou**		**Ségbana**		**Ouaké**	
	ni	**Pi (%)**	ni	**Pi (%)**	ni	**Pi (%)**	ni	**Pi (%)**	ni	**Pi (%)**	ni	**Pi (%)**
Anopheles gambiae s.l	**1087**	**46,14**	**1188**	**76,74**	**869**	**41,80**	**649**	**22,62**	**148**	**17,77**	**246**	**36,07**
Anopheles funestus	**60**	**2,55**	**8**	**0,52**	**4**	**0,19**	**1**	**0,03**	**2**	**0,24**	**27**	**3,96**
Anopheles nili	0	0	0	0	0	0	0	0	0	0	1	0,15
Anopheles pharoensis	0	0	2	0,13	0	0	1	0,03	0	0	0	0
Anopheles ziemanni	0	0	1	0,06	1	0,05	0	0	0	0	0	0
Anopheles coustani	1	0,04	8	0,52	0	0	0	0	0	0	0	0
Anopheles paludis	0	0	1	0,06	0	0	0	0	0	0	0	0
Aedes aegypti	14	0,59	8	0,52	11	0,53	15	0,52	2	0,24	5	0,73
Aedes vitatus	0	0	0	0	1	0,05	0	0	0	0	0	0
Aedes luteocephalus	0	0	0	0	1	0,05	1	0,03	0	0	0	0
Cx.quinquefasciatus	**1167**	**49,53**	**319**	**20,61**	**1180**	**56,76**	**2171**	**75,67**	**676**	**81,15**	**380**	**55,72**
Culex gr decens	3	0,13	1	0,06	1	0,05	3	0,10	1	0,12	0	0
Culex nebulosus	17	0,72	9	0,58	8	0,38	18	0,63	3	0,36	23	3,37
Culex fatigans	0	0	0	0	0	0	1	0,03	0	0	0	0
Mansonia Africana	7	0,3	3	0,19	3	0,14	9	0,31	1	0,12	0	0
Individuals	**2356**	**100**	**1548**	**100**	**2079**	**100**	**2869**	**100**	**833**	**100**	**682**	**100**
Taxas_S	**8**		**11**		**10**		**10**		**7**		**6**	

NB: Taxa_S = number of species found (Simpson, 1949); Pi = the proportion of species i in the study population.

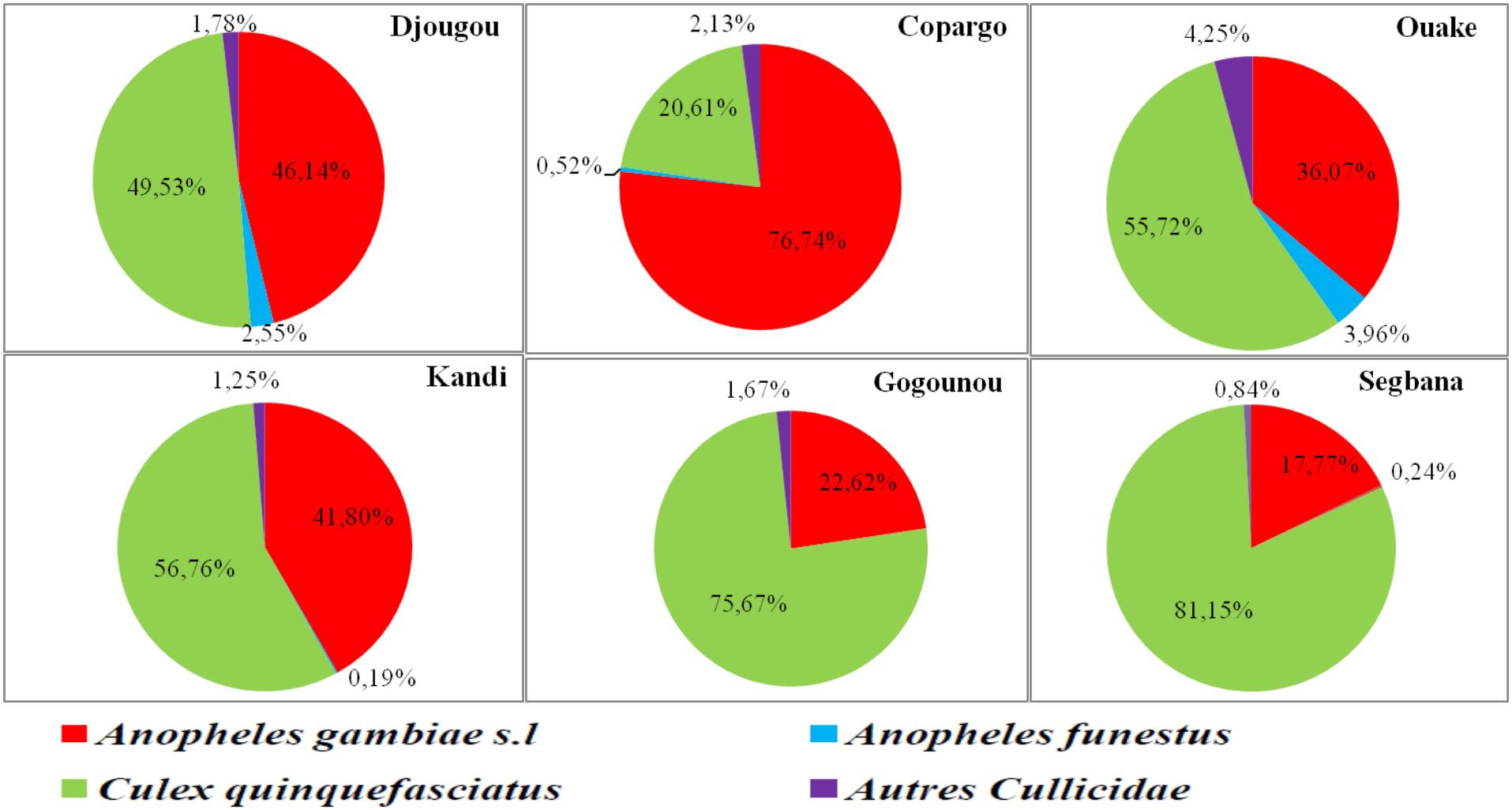


Figure 2: Frequency (%) of *An. gambiae s. l*, *An. funestus*, *Cx quinquefasciatus* and other *Culicidae*

2.1.2. Distribution of *Anopheles gambiae* species complex

In total, 1497 *An. gambiae s.l.* were analyzed by PCR for species identification. Two species of the *An. gambiae* complex were identified: *Anopheles coluzzii* and *An. gambiae*. *Anopheles coluzzii* represented 56 % (n = 835) of mosquitoes tested against 44% (n = 662) of *An. gambiae*. The predominant mosquito of the two species is different from a region to another: in the Alibori, *Anopheles coluzzii* represented 69 % (n = 452) of the 2 species tested against 31 % (n = 206) for *An. Gambiae*, in Donga, *An. gambiae* is the most abundant 54% (n= 456). The two species are present throughout the transmission season at all sites ($p < 0.0001$) (Figure 1). *An. Coluzzii* predominates from May to June and from February to January (dry season) on the three sites of Donga (Djougou, Copargo and Ouake) while *An. gambiae* predominated from June to October (rainy season) (Figure 1). *An. coluzzii* is predominant in Kandi and Gogounou from May to July and from mid-October to February (dry season) ($p < 0.05$) while *An. gambiae* predominated only in August (rainy season) ($p < 0, 05$). In Segbana in the department of Alibori, *An. gambiae* predominated in June and August while *An. coluzzii* predominated during the rest of the transmission period ($p < 0.05$).

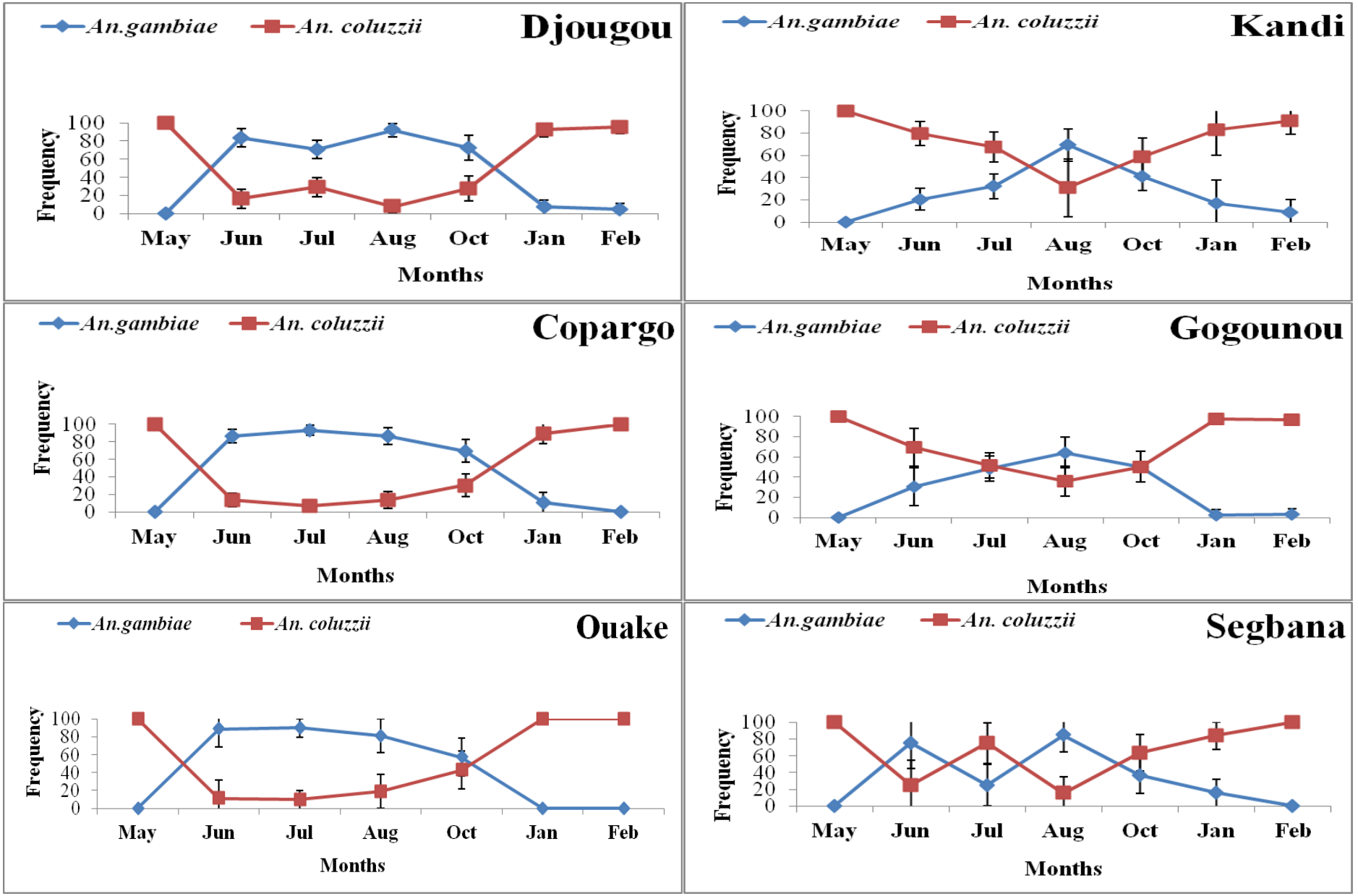


Figure 3: Spatio-temporal distribution of species of the *Anopheles gambiae* complex in Alibori and Donga

2.1.3. Dynamics of An. gambiae s.s populaion

2.3.1. Proportion of *An. gambiae s.s.* collected indoor and outdoor per district

The frequency of *An. gambiae s.s.* is significantly higher indoor than outdoor ($p<0.05$) at all sites (Figure 2). This behavior is normal and expected and justifies the endophagous nature of *An. gambiae s.s.*

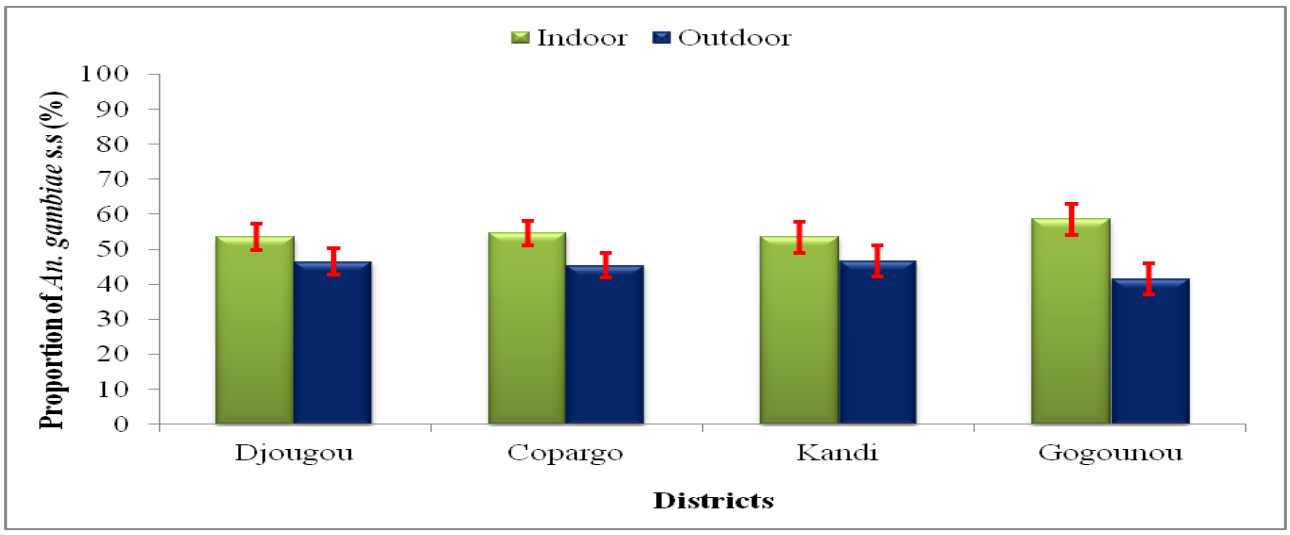


Figure 4: Frequency of *An. gambiae s.s* collected indoor and outdoor per district

2.3.2. Seasonal variation of *An. gambiae s.s* per district using HLC and PSC methods

Anopheles gambiae s.s frequency is significantly higher ($p<0.05$) in rainy season than in dry season in all sites except the Segbana site (Figures 5 and 6). The abundance of *An. gambiae s.s* during the rainy season is due to the multiplication of its breeding sites during this period.

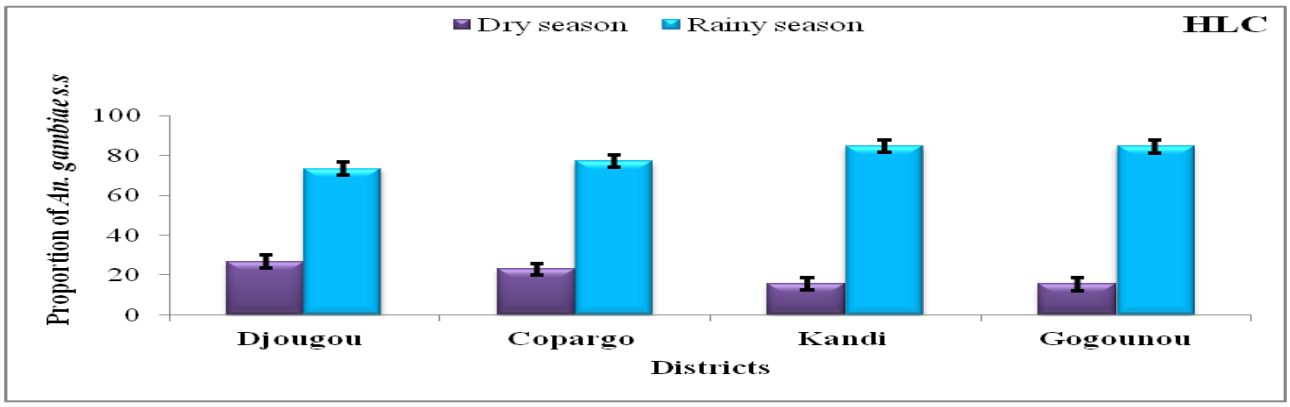


Figure 5: Seasonal variation of *An. gambiae s.s* (frequency of *An. gambiae s.s* collected) per district using HLC method

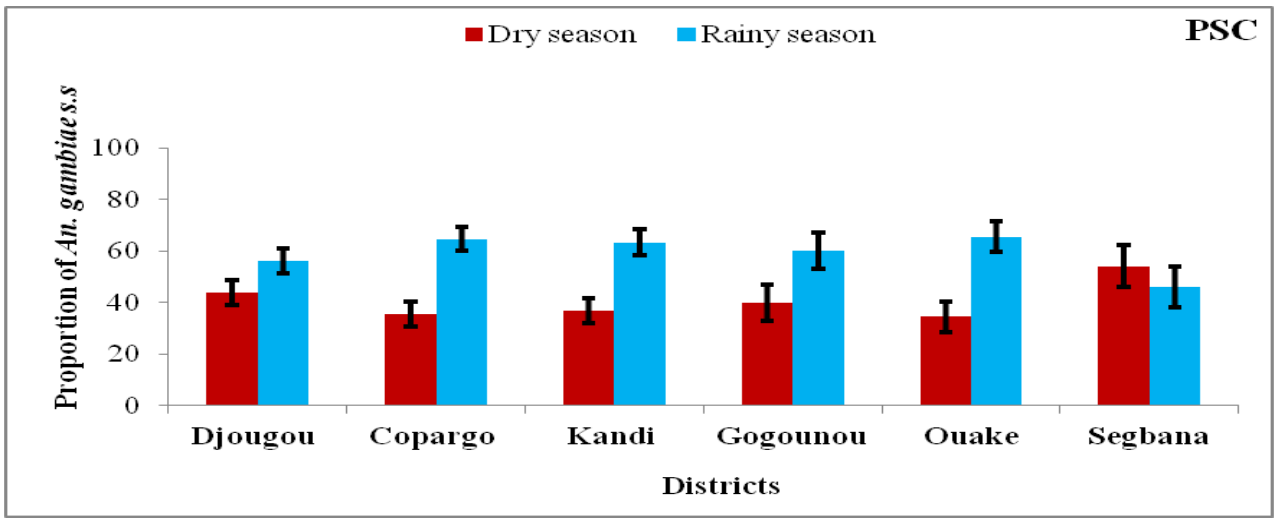


Figure 6: Seasonal variation of *An. gambiae s.s* in the rooms per district using PSC method

2.1.4. Frequency of *An. gambiae s.s* collected using HLC and PSC methods in urban and rural areas

The frequency of *An. gambiae s.s* is higher in rural than in urban areas (Figures 7 and 8). However, in Gogounou and Ouake, there was no significant difference between the proportion of *An. gambiae s.s* in the rural and urban areas with the PSC sampling method (Figure 6). The observed disparity between the frequency of *An. gambiae s.s* in rural and urban areas is explained by the ecological characteristics of the rural environment that are suitable for the development of anopheline mosquitoes. In rural areas, unlike in urban areas, breeding sites are less polluted and are conducive to the production of Anopheles, hence producing higher anopheline fauna in rural areas. Whereas in rural areas the means of protection against bites of these Anopheles are often lacking because of poverty, which justifies the high number of cases of malaria in rural areas.

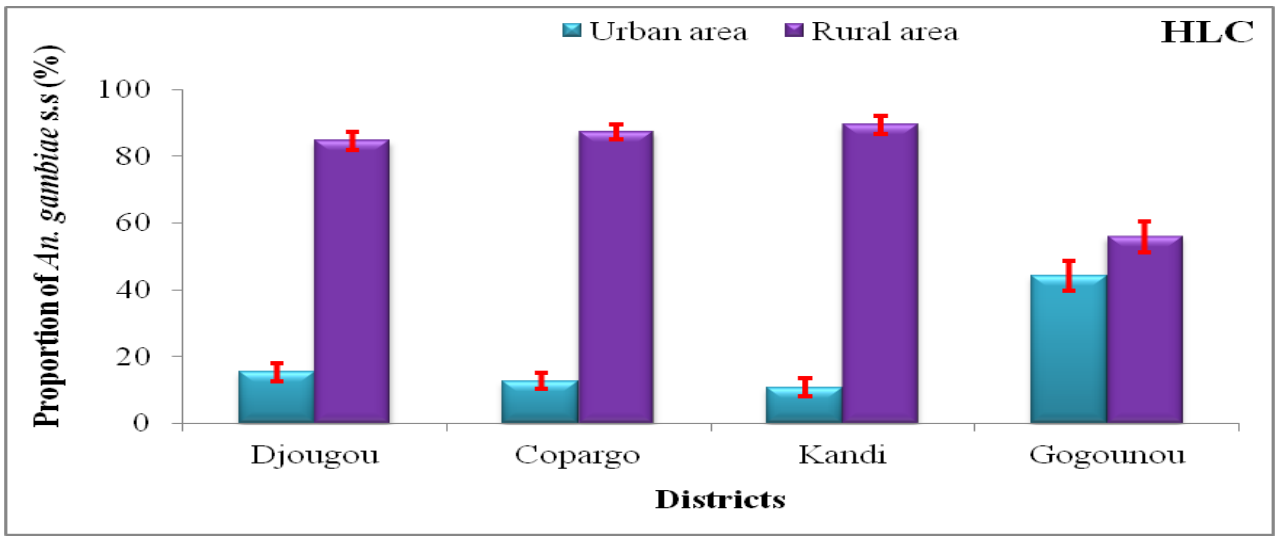


Figure 7: Frequency of *An. gambiae s.s* collected using HLC method in urban and rural areas

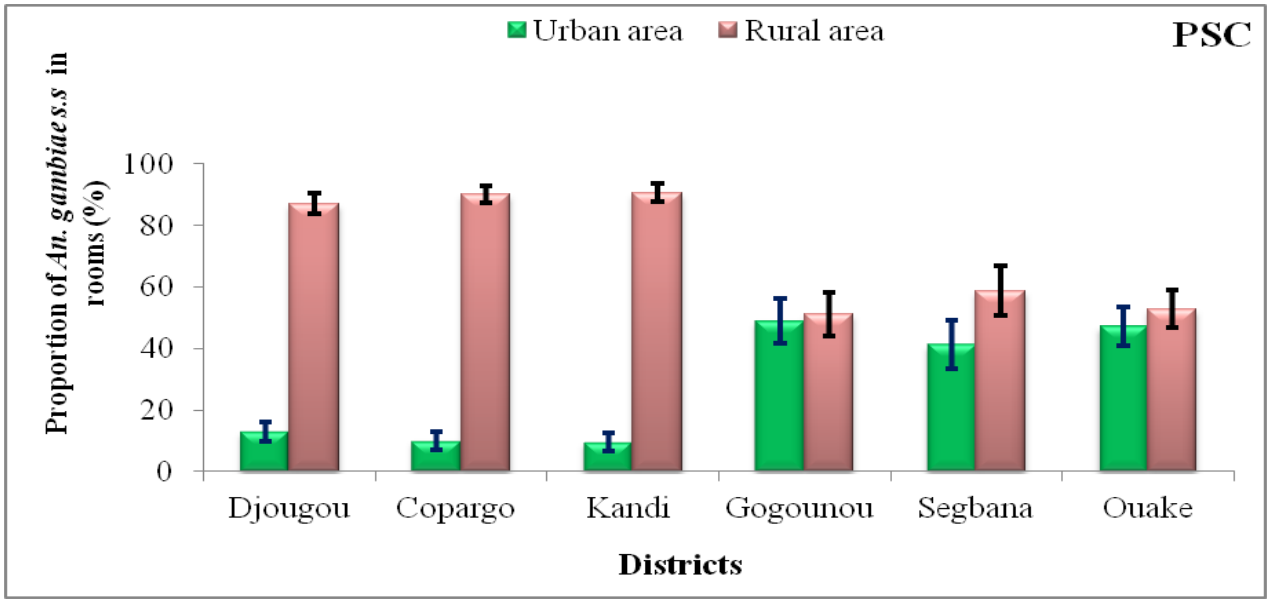


Figure 8: Frequency (room density) of *An. gambiae s.s* collected in the rooms using PSC method in urban and rural areas

2.2. *Culex quinquefasciatus* population dynamics

2.2.1. Proportion of *Culex quinquefasciatus* collected indoor and outdoor per district by HLC method

Unlike *An. gambiae s.s, Culex quinquefasciatus* biting behavior is similar outdoor and indoor in Djougou and Copargo (P>0, 05). But in Kandi and Gogounou, the trophic activity of this species is higher outside than inside. As a matter of fact, *Cx quinquefasciatus* is less anthropophilic than *Anopheles.*

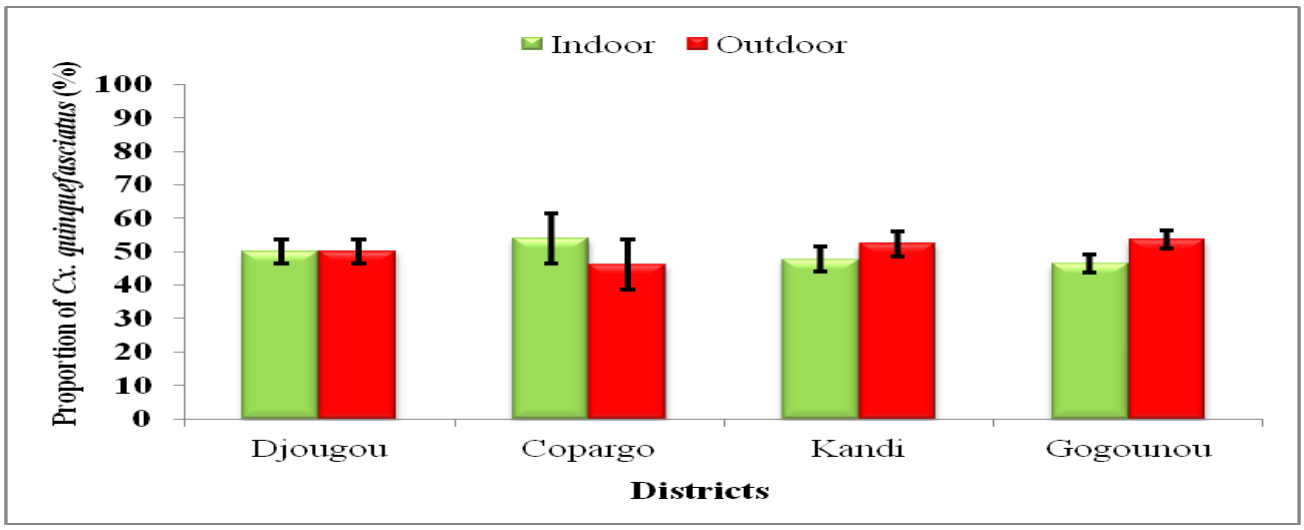


Figure 9: Proportion of *Culex quinquefasciatus* collected indoor and outdoor per district

2.2.2. Proportion of *Culex quinquefasciatus* collected by HLC per district in urban and rural areas

Except Gogounou, *Culex quinquefasciatus* is higher in urban areas compared to rural areas (Figure 10). This situation is classic. Inside cities, the majority of breeding sites is polluted and is appropriated for the development of Culex mosquitoes and not for Anophelines. In Gogounou, the frequency of *Cx. quinquefasciatus* in rural areas is higher than in urban areas because of the similar ecological characteristics of the two areas. Gogounou is a small district and the urban area is not developed.

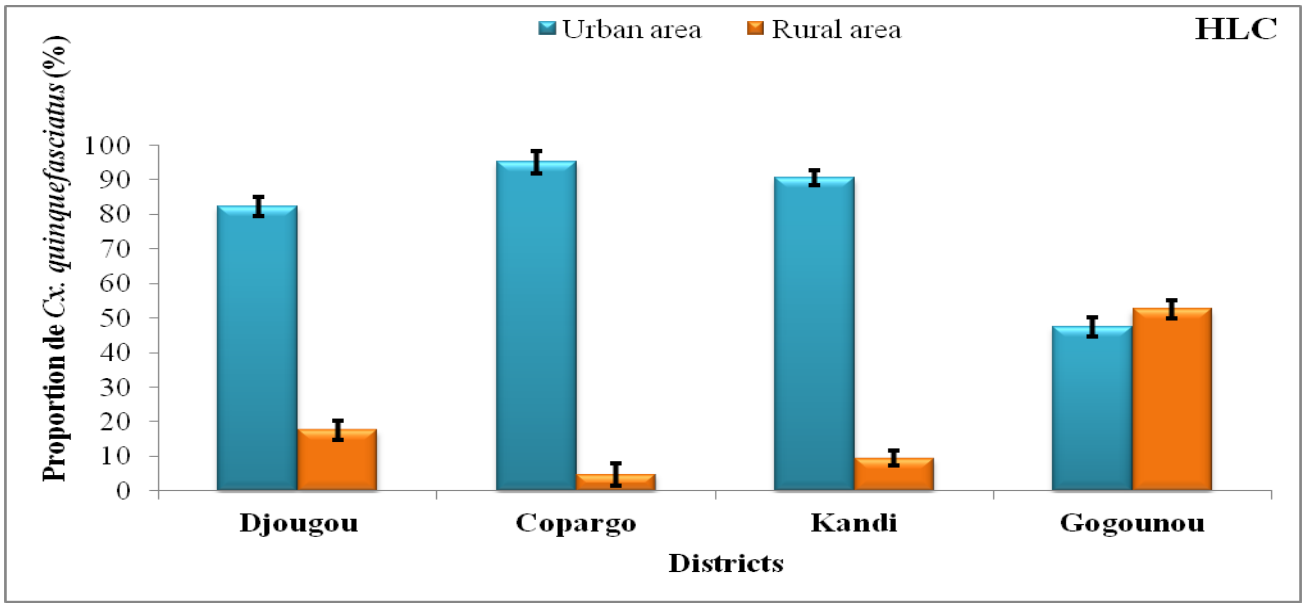


Figure 10: Proportion of *Cx quinquefasciatus* collected by HLC per district in urban and rural areas

2.2.3. Seasonal variation of *Cx. quinquefascitus* per district using HLC method

Unlike *An. gambiae s.s*, *Culex quinquefasciatus* is more abundant during the dry season. During this period of the year, the rarity of the rains causes the pollution of the permanent mosquito breeding sites which become more favorable to the development of larvae of the *Culex quinquefasciatus.*

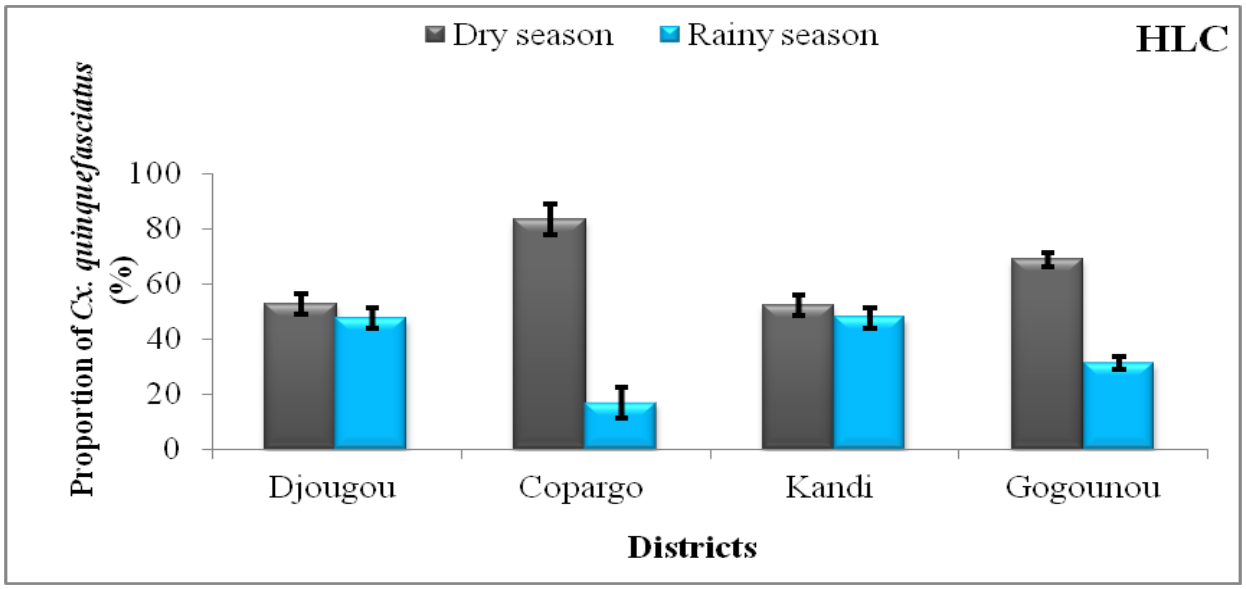


Figure 11: Seasonal distribution of *Culex quinquefasciatus* per district

2.2.4. Variability of Human Biting Rate (HBR) of *An. gambiae s.s* and *Culex quiquefasciatus*
The HBR of *An. gambiae s.s* varies significantly from one department to another ($p < 0.001$). In Kandi and Gogounou sites in the department of Alibori, the HBR of *An. gambiae s.s* is 132-143 bites per month. It is significantly higher in Djougou and Copargo (Donga) (180-210) (Table 2). HBR is four times higher in rural than in urban areas (Rate Ratio = 4.27; $p = 0$). (60 bites per month in urban against 270 in rural areas). Similarly, HBR of *An. gambiae s.s.* is higher inside than outside the dwellings (180 bites/man /month against 150 bites; $p = 0.001$). In the dry season, the average HBR observed is about 60 bites per month (Table 2). It significantly increases up to 300 bites in the rainy season ($p = 0$) (Table 2). During the hot dry season (May and June), the bites are higher outdoor than indoor in the Alibori region. The same situation is observed in Donga (Figure 12). This change in bite habit observed in *An. gambiae* could mean this species is anthropophilic and follows man in his movement

.

Table 2: Variability of *An. gambiae s.s* biting rate

Variables	Modality	HBR	CI-95%	RR&CI-95%	P (Wald)
Donga	**Djougou**	**6,55** [a]	[6,07 - 7,06]	1	
	Copargo	**7,51** [b]	[7,00 - 8,06]	1,15[1,03 - 1,27]	**0,0089**
Alibori	**Kandi**	**4,78** [c]	[4,37 - 5,22]	0,73[0,65 - 0,82]	**<0,001**
	Gogounou	**4,4** [c]	[4 - 4,83]	0,67[0,59 - 0,76]	**<0,001**
Urbanization	**Urban area**	**2,18** [a]	[1,99 - 2,39]	1	
	Rural area	**9,42** [b]	[9,01- 9,85]	4,27[3,85 - 4,75]	**0**
Position	**Indoor**	**6,37** [a]	[6,03- 6,72]	1	
	Outdoor	**5,25** [b]	[4,95- 5,58]	0,83[0,76 - 0,89]	**<0,001**
Season	**Dry season**	**2,27** [a]	[2,07- 2,47]	1	
	Rainy season	**9,95** [b]	[9,51- 10,40]	4,38[3,97 - 4,83]	**0**

The HBR of *Culex quinquefasciatus* in Kandi and Gogounou (Alibori) (respectively 210 and 390 bites/man/month) was significantly higher than that of *An. gambiae s.s* in the same districts (p <0.05) (Table 3). It is similar to that of *An. gambiae s.s* in Djougou and significantly lower than that of *An. gambiae s.s* at Copargo (Table 3). Unlike *An. gambiae s.s*, aggressive densities of *Culex quinquefasciatus* were significantly higher in urban areas compared to peripheral sites (P = 0). (300 bites per month in urban areas versus 120 bites in peripheral areas. However, *Culex quinquefasciatus* more frequent outdoor than indoor (p = 0.0378) with an endophagy index of 0.48 versus 0.52. In the dry season, its bites are the most observed in communities (p = 0.001) (Table 3).

Table 3: Variability of *Culex quinquefasciatus* biting rate

Variables	Modality	HBR	CI-95%	RR&CI-95%	P (Wald)
Donga	**Djougou**	**7,03** [a]	[6,53 - 7,56]	1	
	Copargo	**1,6** [b]	[1,37 - 1,86]	0,23[0,19 - 0,27]	**0**

Alibori	**Kandi**	**6,71** [a]	[6,22 - 7,22]	0,95[0,85 - 1,06]	**0,369**
	Gogounou	**12,6** [c]	[11,93 - 13,30]	1,79[1,63 - 1,96]	**<0,001**
Urbanization	**Urban area**	**9,69** [a]	[9,27 - 10,13]	1	
	Rural area	**4,28** [b]	[4,00 - 4,57]	0,44[0,40 - 0,48]	**0**
Position	**Indoor**	**6,72** [a]	[6,37 - 7,08]	1	
	Outdoor	**7,26** [a]	[6,89 - 7,63]	1,08[1 - 1,16]	**0,0378**
Season	**Dry season**	**7,99** [a]	[7,62 - 8,37]	1	
	Rainy season	**5,81** [b]	[5,48 - 6,16]	0,73[0,67 - 0,78]	**<0,001**

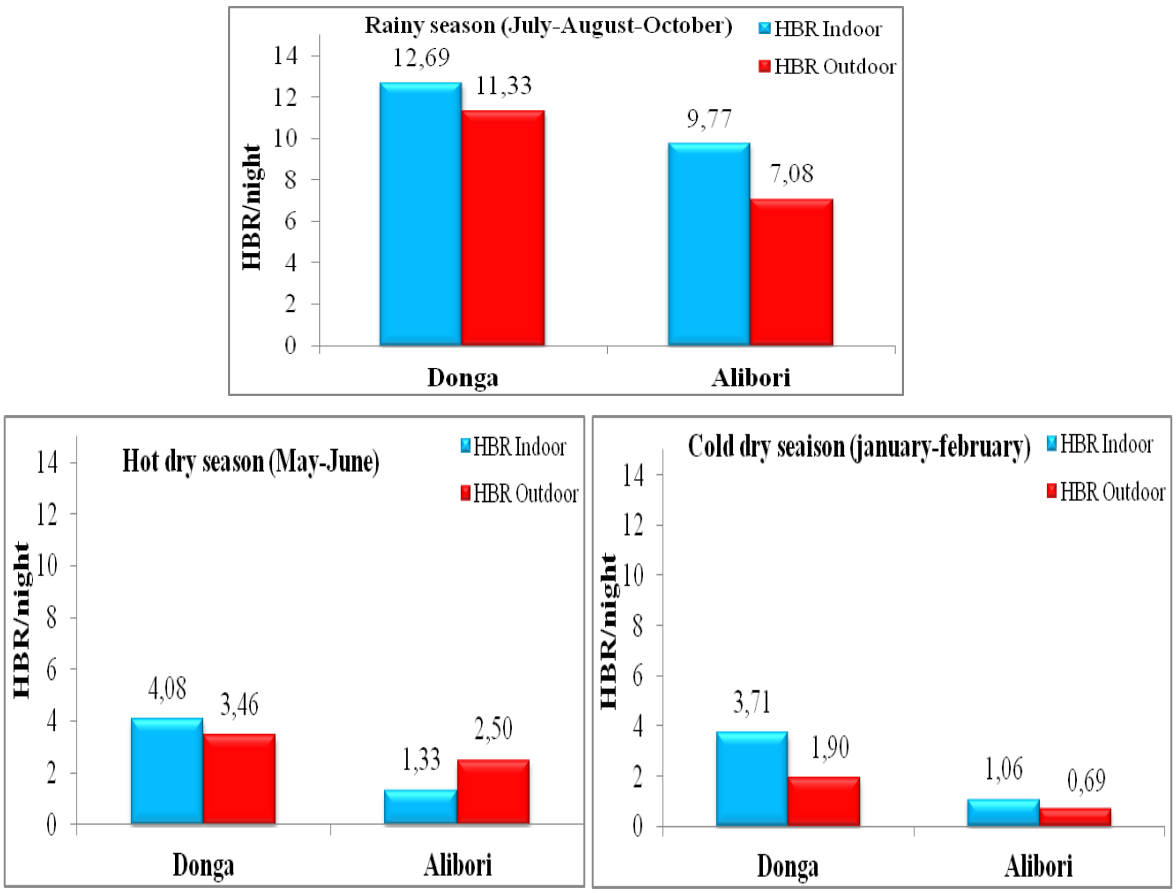


Figure 12: Variability of *An. gambiae s.s* biting rate during the rainy, cold and hot seasons in Alibori and Donga (HLC sampling method)

2.2.4. Sporozoitic index and Entomological Inoculation Rate of *Anopheles gambiae*

After 7 missions in Alibori and Donga, from May to February 2017, we have analyzed 4183 thoraces of *An. gambiae* s.s. collected in 06 districts by HLC and by PSC using ELISA method from which 365 thoraces were found positive for circum-sporozoitic antigen of

Plasmodium falciparum. The mean CS+ rate is 8.72%. This rate is the same in most of the districts: Gogounou (8.35%: 54 th+/646), Djougou (8.6%: 93 th+/1086), Copargo (8%: 94/1188). The 2 highest rates are found in Ouake (17.5%: 43 th+/246) and Segbana (10.81%: 16 th+/148).

The two tables below show the distribution of the Entomological Inoculation Rate (EIR) in each district according to each method of vector sampling. The EIR of *An. gambiae* is very high in the Donga area: 18 and 16 infected bites per man per month during the study period, respectively at Copargo and Djougou (Table 4). These EIR are very high. The risk of malaria transmission is very high in these localities. This is probably due to the absence or very limited use of protective measures such as long-lasting impregnated nets (LLINs) and inadequate health facilities for effective management of clinical malaria cases. The period of malaria transmission is much longer in Donga (Djougou and Copargo): August and October are the 2 months of peak (Figures 10). This is due to two factors: (i) the multiplication of *Anopheles gambiae* breeding sites created during the period of heavy rains, (ii) Djougou and Copargo are crossed and watered by several rivers which creates habitats favorable to the development of larvae of *An. gambiae* and *An. funestus*. On the other hand, the Alibori (Kandi, Gogounou) is characterized by a short period of malaria transmission: October is the one month of peak transmission (Figure 10). Lower inoculation rates than Donga were observed in Alibori: Kandi (12 infected bites/ man /month) and Gogounou (11 infected bites/ man /month) during the study period (Table 4).

We are not used to use data obtained with PSC collection to calculate the EIR. We did it here just for comparison with the HLC method. The lowest entomological inoculation rates are observed at Ouake (2 infected bites/ man /month) and Segbana (0, 49 infected bites/ man /month) despite the high infectivity rates observed in the residual fauna in these districts. This disparity is in fact related to the method of indirect calculation of HBR (Human Britain Rate) with the PSC sampling method (Table 5).

Table 4: Monthly variation of HBR, Sporozoitic Index and EIR in each site (HLC sampling method)

District		May	June	July	August	October	Jan-17	Feb-17	Period (may-february
Kandi	Thorax	1	67	108	140	173	4	5	**498**
	Thorax+	0	1	1	3	36	0	0	**41**
	SI	0,00	0,01	0,01	0,02	0,21	0	0	**0,0823**
	nb of mosquitoes collectors	8	16	16	16	16	16	16	**104**
	HBR	0,13	4,19	6,75	8,75	10,81	0,25	0,31	**4,79**
	EIR	0	0,06	0,06	0,19	2,25	0	0	**0,39**
	EIR/month	**0**	**1,88**	**1,94**	**5,81**	**69,75**	**0**	**0**	**11,83**
Gogounou	Thorax	3	21	73	144	170	32	15	**458**
	Thorax+	0	1	3	9	19	3	2	**37**
	SI	0	0,05	0,04	0,06	0,11	0,09	0,13	**0,0808**
	nb of mosquitoes collectors	8	16	16	16	16	16	16	**104**
	HBR	0,38	1,31	4,56	9	10,63	2,00	0,94	**4,404**
	EIR	0	0,06	0,19	0,56	1,19	0,19	0,13	**0,3558**
	EIR/month	**0**	**1,88**	**5,81**	**17,44**	**36,81**	**5,81**	**3,5**	**10,67**
Djougou	Thorax	16	46	146	209	145	38	82	**682**
	Thorax+	3	1	4	24	18	2	4	**56**
	SI	0,19	0,02	0,03	0,11	0,12	0,05	0,05	**0,0821**
	nb of mosquitoes collectors	8	16	16	16	16	16	16	**104**
	HBR	2	2,88	9,13	13,06	9,06	2,38	5,13	**6,558**
	EIR	0,38	0,06	0,25	1,50	1,13	0,13	0,25	**0,5385**
	EIR/month	**11,63**	**1,88**	**7,75**	**46,50**	**34,88**	**3,88**	**7**	**16,15**
Copargo	Thorax	6	113	169	266	168	24	36	**782**
	Thorax+	0	2	11	26	22	3	0	**64**
	SI	0	0,02	0,07	0,10	0,13	0,13	0	**0,0818**
	nb of mosquitoes collectors	8	16	16	16	16	16	16	**104**
	HBR	0,75	7,06	10,56	16,63	10,50	1,50	2,25	**7,519**
	EIR	0	0,13	0,69	1,63	1,38	0,19	0	**0,6154**
	EIR/month	**0**	**3,75**	**21,31**	**50,38**	**42,63**	5,81	0	**18,46**

SI=Sporozoïte Index

Table 5: Monthly variation of HBR, Sporozoitic Index and EIR in each site (PSC sampling method)

District		May 2016	Jun 2016	Jul 2016	Aug 2016	Oct 2016	Jan 2017	Feb 2017	SI/ Period (%)	EIR/ Period
Kandi	**Thorax**	44	56	73	42	120	8	28		
	Thorax+	6	5	8	1	2	2	0		
	Is	0,14	0,09	0,11	0,02	0,02	0,25	0	**6,4**	
	ma	0,48	0,38	0,67	0,30	0,88	0,05	0,17		
	TIE	0,07	0,03	0,07	0,01	0,01	0,01	0		
	TIE/mois	**2,04**	**1,01**	**2,29**	**0,22**	**0,45**	**0,38**	**0**		**0,807**
Gogounou	**Thorax**	4	10	24	21	68	24	37		
	Thorax+	0	1	0	3	13	0	0		
	Is	0	0	0	0,14	0,19	0,00	0	**9,0**	
	ma	0,05	0,07	0,14	0,14	0,54	0,17	0,18		
	TIE	0	0,01	0	0,02	0,10	0	0		
	TIE/mois	**0**	**0,20**	**0**	**0,64**	**3,18**	**0**	**0**		**0,543**
Djougou	**Thorax**	40	11	138	48	41	31	95		
	Thorax+	12	3	3	6	0	2	11		
	Is	0,30	0,27	0,02	0,13	0	0,06	0,12	**9,2**	
	ma	**0,43**	0,20	1,08	0,44	**0,34**	0,24	0,71		
	TIE	0,13	0,05	0,02	0,06	0	0,02	0,08		
	TIE/mois	**4,03**	**1,61**	**0,73**	**1,71**	**0**	**0,48**	**2,30**		**1,427**
Copargo	**Thorax**	8	87	132	79	51	15	34		
	Thorax+	0	6	5	15	4	0	0		
	Is	0	0,07	0,04	0,19	0,08	0	0	**7,4**	
	ma	0,14	0,54	1,05	0,50	0,46	0,11	0,25		
	TIE	0	0,04	0,04	0,10	0,04	0	0		
	TIE/mois	**0**	**1,11**	**1,23**	**2,96**	**1,12**	**0**	**0**		**1,079**
Ouake	**Thorax**	34	9	76	54	31	18	24		
	Thorax+	8	1	7	12	10	2	3		
	Is	0,24	0,11	0,09	0,22	0,32	0,11	0,13	**17,5**	
	ma	0,44	0,19	0,71	0,63	0,38	0,18	0,23		
	TIE	0,10	0,02	0,07	0,14	0,12	0,02	0,03		

	TIE/mois	**3,18**	**0,64**	**2,03**	**4,31**	**3,83**	**0,61**	**0,805**		**2,028**
Segbana	**Thorax**	26	8	12	15	41	26	20	**10,8**	
	Thorax+	5	0	0	2	9	0	0		
	Is	0,19	0	0	0,13	0,22	0	0		
	ma	0,31	0,05	0,07	0,09	0,25	0,20	0,13		
	TIE	0,06	0	0	0,01	0,05	0	0		
	TIE/mois	**1,83**	**0**	**0**	**0,38**	**1,67**	**0**	**0**		**0,489**

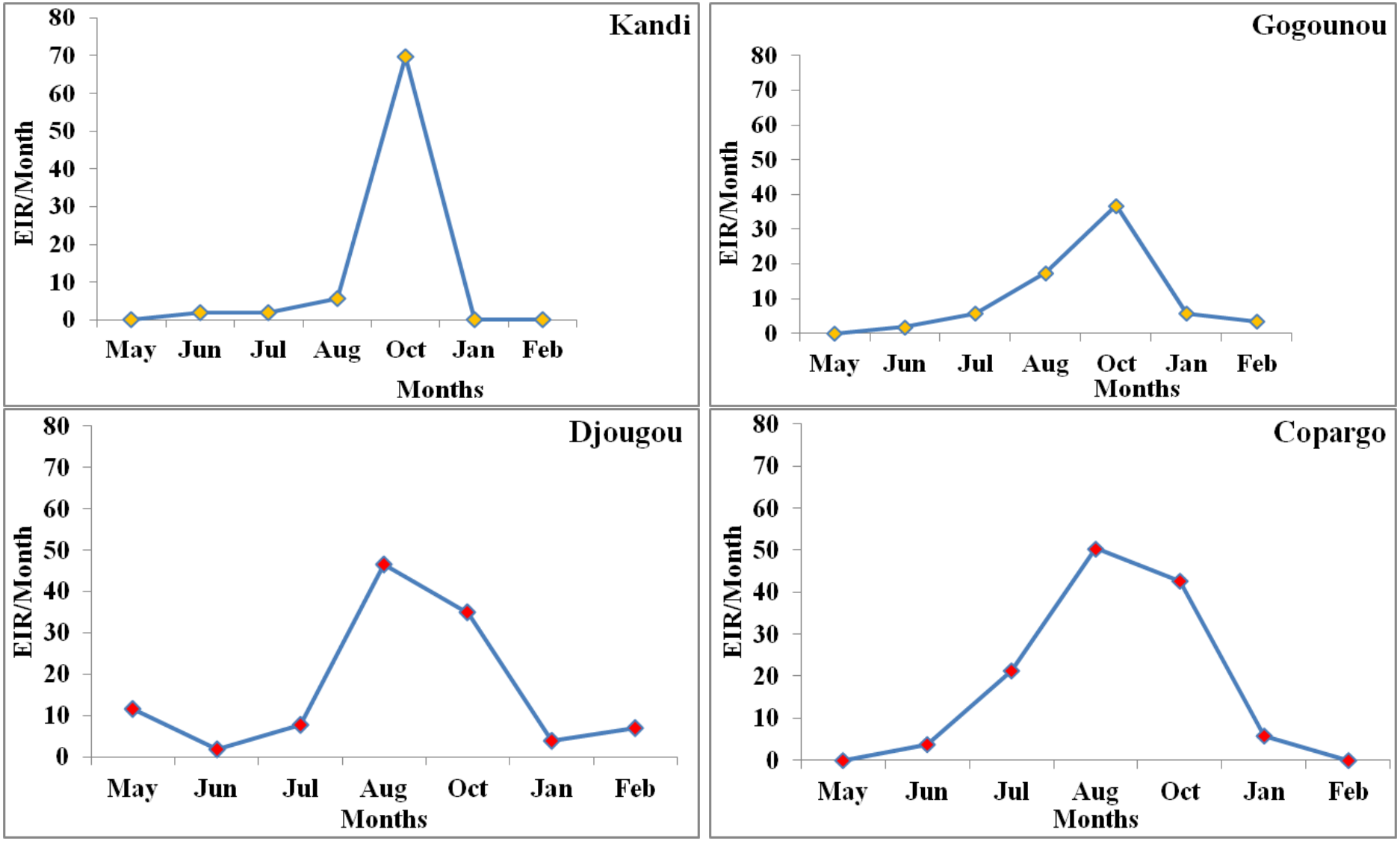


Figure 13: Entomological inoculation rate per month in each study site

2.2.5. Physiological age grading (parous rate) of An. gambiae s.s

A total of 1640 *An. gambiae* were dissected during the study (May 2016 - February 2017) wit h a parous rate average of 72, 25% (see Table below). A significant variation in this rate was observed during the dry season (78.16%) and during the rainy season (69.91%) (p= 0.00094). The rate during the rainy season is low due to the high density of *An. gambiae* during this peri od

Table 3: Parity rates of *Anopheles gambiae*

	Dry season			**Rainy season**		
	An. gambiae			*An. gambiae*		
Districts	Number dissected	Parous	**Parous Rate (%)**	Number dissected	Parous	**Parous Rate (%)**
Djougou	151	120	**79,47**	349	247	**70,77**
Copargo	171	139	**81,29**	295	202	**68,47**
Kandi	78	53	**67,95**	289	204	**70,59**
Gogounou	67	53	**79,10**	240	167	**69,58**
Total	467	365	**78,16**	1173	820	**69,91**

2.2.6. Blood fed mosquitoes collected in bedrooms

The blood feeding rate of mosquitoes observed per site is presented in Table below. The rates observed in mosquitoes collected after PSC in the morning are high (94.14% for *An. gambiae s.s* and 73.13% for *Cx quinquefasciatus).*

Table 2: Blood fed mosquitoes collected in bedrooms

	An. gambiae s.s						***Culex quinquefasciatus***					
	Total		**N. fed**		**Blood feeding rate (%)**		**Total**		**N. fed**		**Blood feeding rate (%)**	
Districts	Dry season	Rainy season	Dry season	Rainy season	**Dry season**	**Rainy season**	**Dry season**	**Rainy season**	**Dry season**	**Rainy season**	**Dry season**	**Rainy season**
Kandi	138	236	125	225	**90,58**	**95,34**	219	263	164	215	**74,89**	**81,75**
Gogounou	75	113	56	110	**74,67**	**97,35**	531	329	349	276	**65,73**	**83,89**
Segbana	81	68	72	64	**88,89**	**94,12**	200	476	117	348	**58,50**	**73,11**
Ouake	85	188	79	184	**92,94**	**97,87**	161	219	99	139	**61,49**	**63,47**
Djougou	177	241	167	234	**94,35**	**97,10**	281	154	206	140	**73,31**	**90,91**
Copargo	144	263	126	261	**87,50**	**99,24**	74	78	57	73	**77,03**	**93,59**
Total	700	1109	625	1078	**89,29**	**97,20**	1466	1519	992	1191	**77,03**	**93,59**

Table 3: Density of *An. gambiae s.l.* /room/night for each month for each district

District		May	June	July	August	October	January	February
Kandi	Nb of *An. gambiae*	44	56	73	42	120	8	28
	Nb sleepers	83	140	104	133	131	162	139
	Nb of rooms	20	38	39	39	38	40	40
	Density/rooms/night	**2,2**	**1,47**	**1,87**	**1,08**	**3,16**	**0,2**	**0,7**
Gogounou	Nb of *An. gambiae*	4	10	24	21	68	24	37
	Nb sleepers	73	137	160	146	123	140	160
	Nb of rooms	20	40	40	38	40	40	40
	Density/rooms/night	**0,2**	**0,25**	**0,6**	**0,55**	**1,7**	**0,6**	**0,925**
Segbana	Nb of *An. gambiae*	26	8	12	15	41	26	20
	Nb sleepers	78	173	137	162	159	130	143
	Nb of rooms	20	40	40	40	40	40	40
	Density/rooms/night	**1,3**	**0,2**	**0,3**	**0,375**	**1,03**	**0,65**	**0,5**
Ouake	Nb of *An. gambiae*	34	9	76	54	31	18	24
	Nb sleepers	71	47	114	107	94	101	102
	Nb of rooms	20	20	40	40	40	39	40
	Density/rooms/night	**1,7**	**0,45**	**1,9**	**1,35**	**0,78**	**0,46**	**0,6**
Djougou	Nb of *An. gambiae*	40	11	139	48	41	31	95
	Nb sleepers	90	56	128	111	140	129	124
	Nb of rooms	20	20	40	40	39	40	40
	Density/rooms/night	**2**	**0,55**	**3,475**	**1,2**	**1,051**	**0,775**	**2,375**
Copargo	Nb of *An. gambiae*	8	87	133	79	51	15	34
	Nb sleepers	49	145	127	155	109	138	111
	Nb of rooms	20	39	40	40	40	40	40
	Density/rooms/night	**0,4**	**2,23**	**3,325**	**1,975**	**1,275**	**0,375**	**0,85**

2.2.7. Infectivity rates of *An. coluzzii and An. gambiae for Plasmodium falsciparum*

In Alibori and Donga, a total of 662 *An. gambiae* and 835 *An. coluzzii* were identified using PCR method from which 191 and 172 were respectively found positive for circumsporozoite (CS) protein of *P. falciparum*. The CS+ is 28.85% (191 thorax + / 662) for *An. gambiae* and 20.59% (172 thorax + / 835 h) for *An. coluzzii*. There is a seasonal contribution of each species in malaria transmission. *An. coluzzii* alone ensures transmission during the periods of May to June and from January to February (dry season) and both species during the period of July to October (rainy season) (Figure 14).

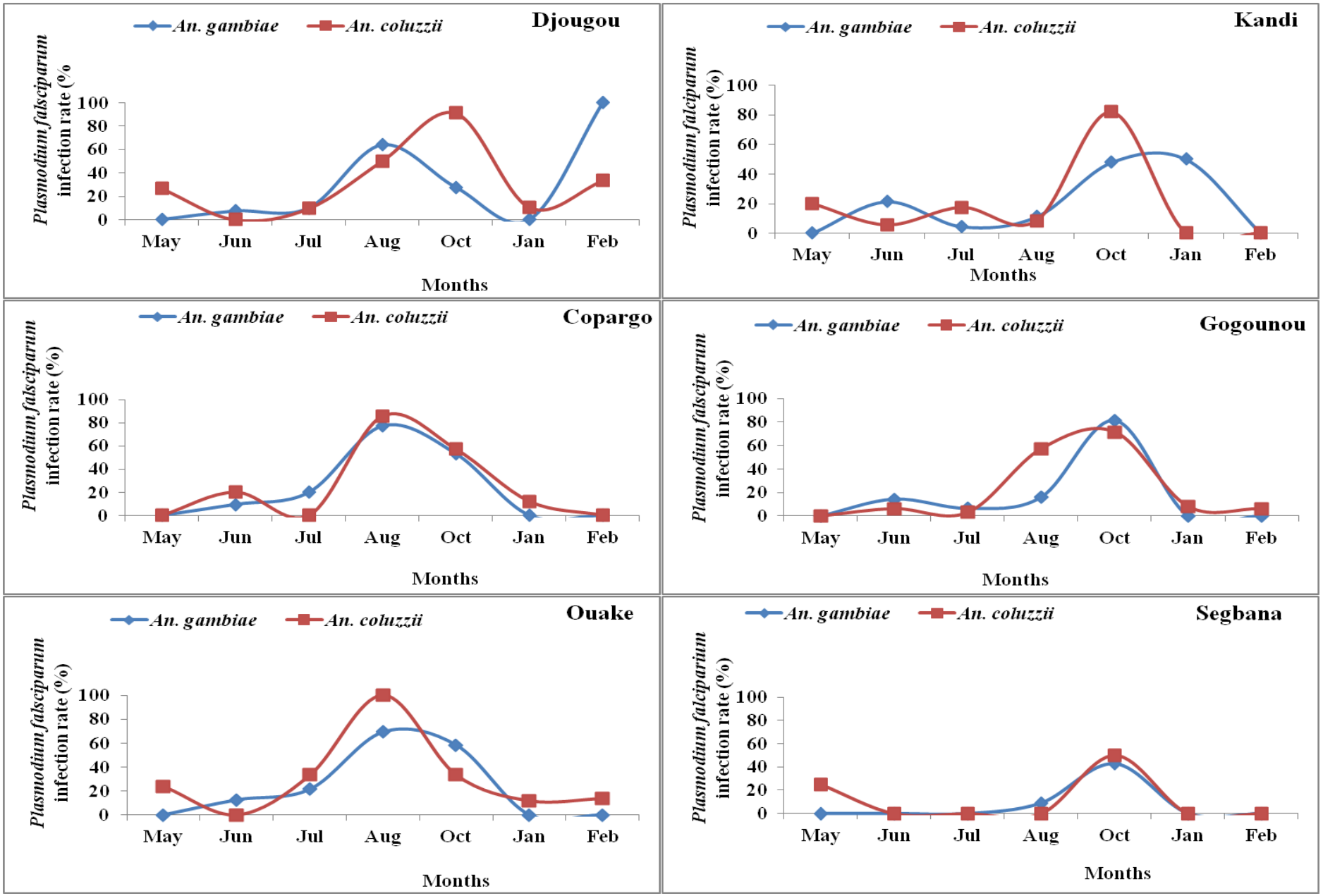


Figure 14: Variation in infectivity rates for *Plasmodium falsciparum* from *An. Coluzzi* and *An. gambiae* during the study period

Table 4: Variation in infectivity rates for *Plasmodium falsciparum* from *An. Coluzzi* and *An. gambiae* during the study period

Localities	Species		May	June	July	August	October	January	February
Kandi	*An. gambiae*	Number	0	14	22	27	23	2	2
		Positif	0	3	1	3	11	1	0
		Is	**0**	**21,43**	**4,55**	**11,11**	**47,83**	**50**	**0**
	An. coluzzii	Number	35	54	46	12	33	10	21
		Positif	7	3	8	1	27	0	0
		Is	**20**	**5,56**	**17,39**	**8,33**	**81,82**	**0**	**0**
Gogounou	*An. gambiae*	Number	0	7	31	25	21	1	1
		Positif	0	1	2	4	17	0	0
		Is	**0**	**14,29**	**6,45**	**16**	**80,95**	**0**	**0**
	An. coluzzii	Number	7	16	33	14	21	37	32
		Positif	0	1	1	8	15	3	2
		Is	**0**	**6,25**	**3,03**	**57,14**	**71,43**	**8,11**	**6,25**
Segbana	*An. gambiae*	Number	0	6	3	11	7	3	0
		Positif	0	0	0	1	3	0	0
		Is	**0**	**0**	**0**	**9,09**	**42,86**	**0**	**0**
	An. coluzzii	Number	20	2	9	2	12	16	20
		Positif	5	0	0	0	6	0	0
		Is	**25**	**0**	**0**	**0**	**50**	**0**	**0**
Djougou	*An. gambiae*	Number	0	41	51	47	29	3	2
		Positif	0	3	5	30	8	0	2
		Is	**0**	**7,32**	**9,80**	**63,83**	**27,59**	**0**	**100**
	An. coluzzii	Number	56	8	21	4	11	38	39
		Positif	15	0	2	2	10	4	13
		Is	**26,79**	**0**	**9,52**	**50**	**90,91**	**10,53**	**33,33**
Copargo	*An. gambiae*	Number	0	63	80	44	32	3	0
		Positif	0	6	16	34	17	0	0
		Is	**0**	**9,52**	**20**	**77,27**	**53,13**	**0**	**0**
	An. coluzzii	Number	14	10	6	7	14	25	41
		Positif	0	2	0	6	8	3	0
		Is	**0**	**20**	**0**	**85,71**	**57,14**	**12**	**0**
Ouaké	*An. gambiae*	Number	0	8	28	13	12	0	0
		Positif	0	1	6	9	7	0	0
		Is	**0**	**12,50**	**21,43**	**69,23**	**58,33**	**0**	**0**
	An. coluzzii	Number	34	1	3	3	9	17	22
		Positif	8	0	1	3	3	2	3
		Is	**23,53**	**0**	**33,33**	**100**	**33,33**	**11,76**	**13,64**

2. 3. Sleeping behaviour and ITN use in Alibori and Donga

During the dry season, people of Alibori and Donga are not protected against mosquito bites. They also benefit very few protection from the nets. Indeed, over a population of 667 children and adults visited in the Alibori and 493 in Donga between May and June (hot period dry season), about 41.52% and 42.19% respectively, are found sleeping outside houses between 9 PM and 11 PM in search of fresh air. However, during the cold period (January and February) of the same season, out of a population of 472 and 430 children and adults visited, only 1.05 % and 9.53% of people are found lying outdoor compared with 98.94% and 90.46% indoor due to intense freshness respectively in Alibori and Donga (Table 1 and 2). There is therefore a significant difference between the sleeping habits during these two periods in the same season ($P < 0.05$). The behavior of spending a good part of his time outside is less marked during the rainy season. Indeed, in July, August and October, only 10.12% and 8.94% of the population remain outside a part of the night before returning to the room respectively in the Alibori as Donga. By comparing the data stored in the dry season and rainy season, it appears that the sleeping habits are dependent on temperature. During the dry season, mosquitoes have easy access to a part of the population without mosquito protection. The high exposure of the population to mosquito bites is also noted among those who sleep inside. About 47,72% in the Alibori and 8,94% in the Donga sleep indoor without mosquito nets even though these nets are available against 25.64% and 38.17% during the rainy season (Table 1 and 2).

These results show once again that climate conditions influence the use of mosquito nets.

Tableau 1: Proportions of children and adults sleeping inside or outside rooms and LLINs use according to sleeping habits

ALIBORI						
	Dry season			Dry season	Rainy season	
	Hot Period (May-June)	Cold Period (Jan-Fev)	P-value		(Jul-Aug-Oct)	P-value
Persons sleeping indoor (%)	390 (58,47)	467 (98,94)	< 0,001	857(75,24)	772 (89,87)	< 0,001
Persons sleeping outdoor (%)	277 (41,52)	5 (1,05)	< 0,001	282(24,75)	87 (10,12)	< 0,001
Persons indoor with MILD (%)	229 (58,71)	219 (46,89)	0,0007	448(52,27)	574 (74,35)	< 0,001
Persons indoor without MILD(%)	161 (41,28)	248 (53,10)	0,0007	409(47,72)	198 (25,64)	< 0,001
Persons outdoor with MILD(%)	103 (37,18)	0 (0)	0,214	103(36,52)	57 (65,51)	< 0,001
Persons outdoor without MILD(%)	174 (62,81)	5 (100)	0,214	179(63,47)	30 (34,48)	< 0,001

Tableau 2: Proportions of persons sleeping inside or outside rooms and LLINs use according to sleeping habits

Donga						
	Dry season			Dry season	Rainy season	
	Hot Period (May-June)	Cold Period (Jan-Fev)	P-value		(Jul-Aug-Oct)	P-value
Persons sleeping indoor	285 (57,80)	389 (90,46)	< 0,001	674(73,02)	723 (91,05)	< 0,001
Persons sleeping outdoor	208 (42,19)	41 (9,53)	< 0,001	249(26,97)	71 (8,94)	< 0,001
Persons indoor with MILD	127 (44,56)	263 (67,60)	< 0,001	390(57,86)	447 (61,85)	0,146
Persons indoor without MILD	158 (55,43)	126 (32,39)	< 0,001	284(42,13)	276 (38,17)	0,146
Persons outdoor with MILD	37 (17,78)	7 (17,07)	1	44(17,67)	7 (9,85)	0,161
Persons outdoor without MILD	171 (82,21)	34 (82,92)	1	205(82,32)	64 (90,14)	0,161

Photo 1: Sleeping habit and LLINs use during the hot dry season (May-June 2016) in Alibori

2.4. Conclusion

Anopheles gambiae s.s. is the second most abundant species found in Alibori and Donga after *Culex quinquefasciatus*. The human biting rate of this species is higher indoor compared to outdoor, what is appropriate to the implementation of IRS in the study area. *An. gambiae* and *An. coluzzii* are practically the only members of the complex that ensure the full transmission of malaria. The high rate of CS antigen positivity testifies to the vulnerability of populations to malaria and calls not only for strengthening pre-existing control strategies (LLINs), but also the use of other complementary control strategies such as IRS.

3. Status of vector resistance in Alibori and Donga

Table: Summary of WHO susceptibility test results

		July 2016				
	Susceptibility test					Kno
Tested insecticide	Localities	Tested number	Number dead/24h	Mean Mortality rate	10min	15min
Deltamethrin 0.05%	Kandi	99	41	41,41	0	0
	Djougou	97	1	1,03	0	0
	Copargo	101	80	79,2	0	0
	Segbana	100	60	60	0	0
Permethrin 0.75%	Kandi	99	9	9.09	0	0
	Djougou	100	2	2	0	0
	Copargo	98	54	55.1	0	0
	Segbana	100	28	28	0	0
Permethrin 0.75% +PBO 4%	Copargo	103	102	99.02	0	0
Deltamethri 0.05% +PBO 4%	Copargo	107	106	99.06	4	7
		August 2016				
Permethrin 0.75%	Djougou	99	6	6,06	0	0
Deltamethri 0.05%	Djougou	101	20	20	0	0
Permethrin 0.75% +PBO 4%	Gogounou	103	85	82,52	0	0
Deltamethri 0.05% +PBO 4%	Gogounou	102	100	98,03	0	0
Alphacypermethrin 0.05%	Copargo	95	7	7,36	0	0
Alphacypermethrin 0.05%	Djougou	94	6	6,38	0	0
Pirimiphos methyl 0,25%	Kandi	89	89	100	0	0
	Djougou	102	102	100	0	0
	Copargo	92	92	100	9	32
	Gogounou	100	100	100	0	0
	Ouake	101	101	100	0	0
Bendiocarb 0.1%	kandi	90	89	100	1	4
	Djougou	105	100	95,23	0	3
	Copargo	102	97	95,09	56	78
	Gogounou	85	79	93	0	0
	Ouake	95	88	93	0	11
	Segbana	103	97	94.17	0	0

		October 2016				
	Susceptibility test				Knock- down or Immdiate mortali	
Tested insecticide	**Localities**	Tested number	Number dead/24h	Mean Mortality rate(%)	10min	15min
Deltamethrin 0.05%	**Djougou**	91	23	25.27	1	1
	Gogounou	89	74	83.14	0	1
Permethrin 0.75%	**Gogounou**	95	42	44.21	1	8
Permethrin 0.75% +PBO 4%	**Kandi**	99	57	57.57	1	23
	Djougou	99	93	93.93	0	1
Deltamethrin 0.05% + PBO 4%	**Kandi**	**103**	65	63.10	1	25
	Djougou	103	96	93.20	4	34
	Segbana	105	90	85.71	0	1
Alphacypermethrin 0.05%	**Kandi**	96	50	52.08	22	41
	Gogounou	101	47	46.53	3	18
Pirimiphos methyl 0,25%	**Segbana**	101	101	100	0	0
Permethrin 0.75% +PBO 4%	**Gogounou**	**103**	85	83	0	5
Deltamethri 0.05% +PBO 4%	**Gogounou**	101	100	98	0	0

3.1-Effect of the pirimiphos methyl on mosquitoes

Immediate mortality induced by pirimiphos methyl varied significantly between the different mosquito populations tested ($p < 0.05$) (Figure 1). It is after the thirtieth minute that the effect of pirimiphos methyl was more noticeable on all mosquito populations tested except Gogounou (after the fiftieth minute) (Figure 1). The observed immediate mortalities range from 22.41-79.21% at Kandi, Ségbana, Djougou, Copargo and Ouake. On the other hand, in Gogounou, no immediate mortalitie was found (Figure 1). Mortalities noted after 24 hours of observation were 100% suggesting a complete susceptibility of all mosquito populations tested with pirimiphos methyl (Figure 2).

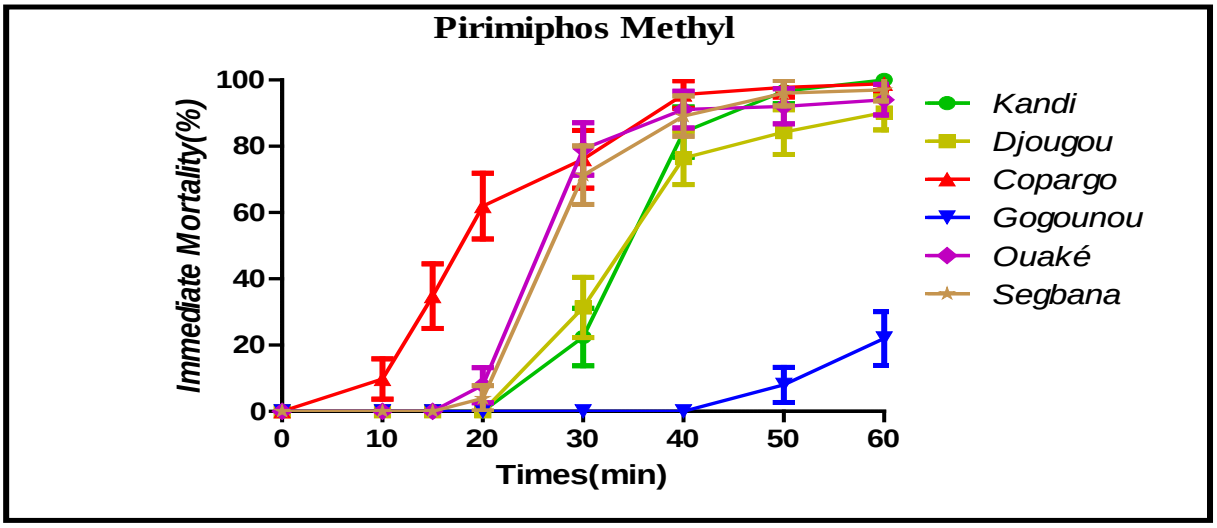


Figure 1: Immediate mortalities observed during mosquitoes exposure to pyrimiphos methyl

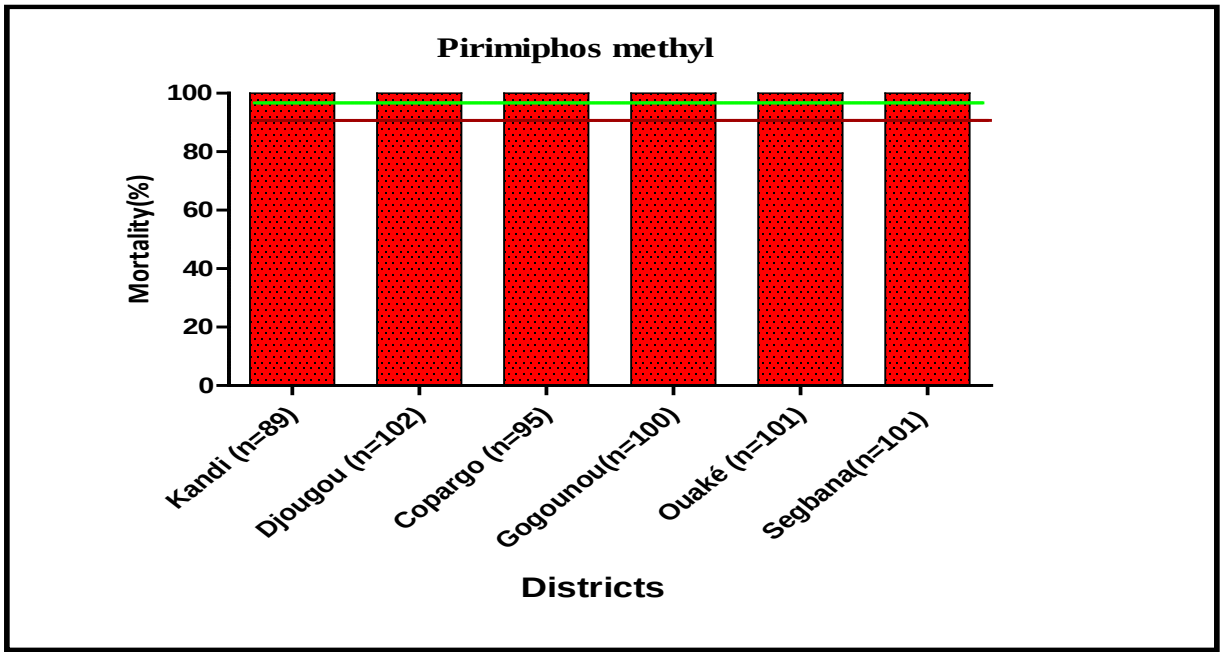


Figure 2: Mortalities observed with Pyrimiphos methyl

3.2-Effect of the bendiocarb on mosquitoes

The proportion of dead mosquitoes after 30 minutes exposure to bendiocarb is 12 to 91%. After 60 minutes of exposure, more than 93% of the mosquitos tested died excepted Gogounou (Figure 3). The mortality rate observed after 24 hours in Kandi is 100%, which shows a sensitivity of this population of mosquitoes to bendiocarb. On the other hand, in the other localities, the mortality rates observed are between 90-97%. This suggests a decrease in susceptibility in these different populations of mosquitoes (suspected resistance) (Figure 4).

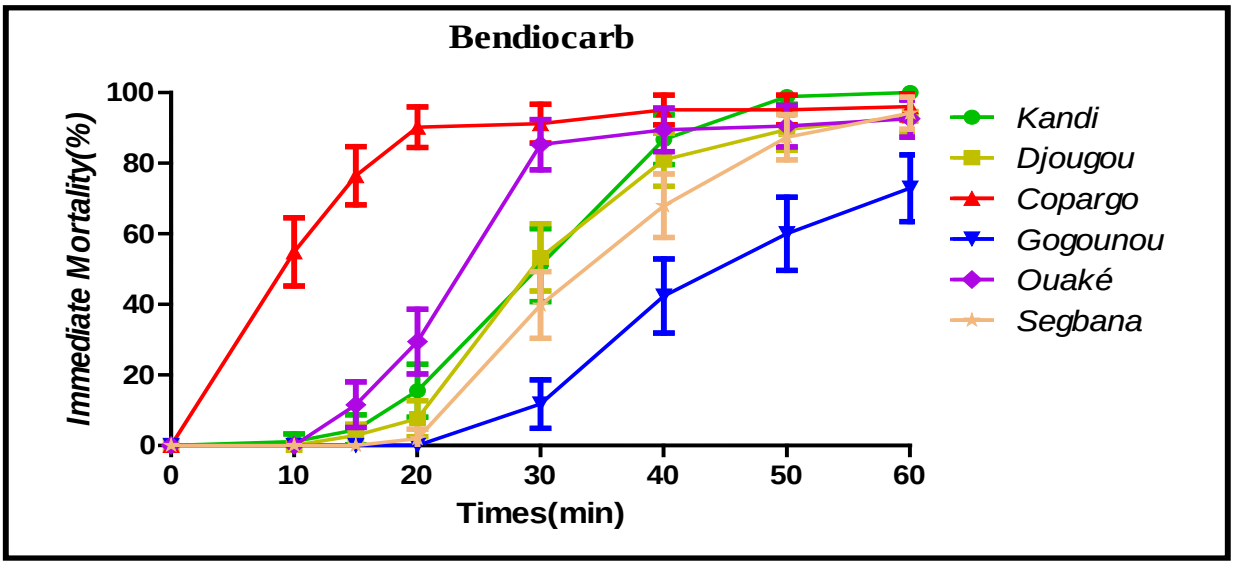


Figure 3: Immediate mortalities observed during mosquitoes exposure to Bendiocarb

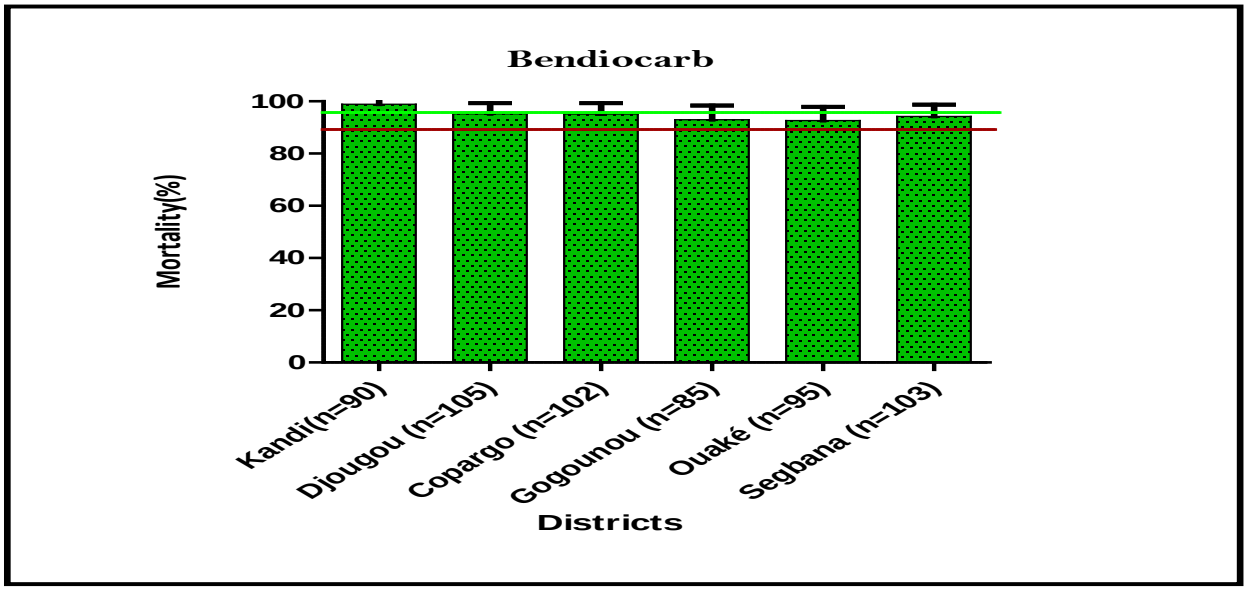


Figure 4: Mortalities observed with Bendiocarb

3.3-Knock-down effect of permethrin alone and permethrin + PBO on An. gambiae

The knock-down effect of permethrin on mosquitoes is very low, compared to that of permethrin + PBO. After 30 minutes of exposure, the proportion of mosquitoes "knocked down" under the effect of permethrin is between 0 and 17% *versus* 1 and 86% under the effect of permethrin + PBO. After 60 min, the proportion of mosquitoes knocked-down is between 0 and 50% with permethrin alone and between 54 and 98% with permethrin + PBO (figure 5). The PBO synergist has therefore strengthened the knock-down effect of permethrin on resistant mosquitoes.

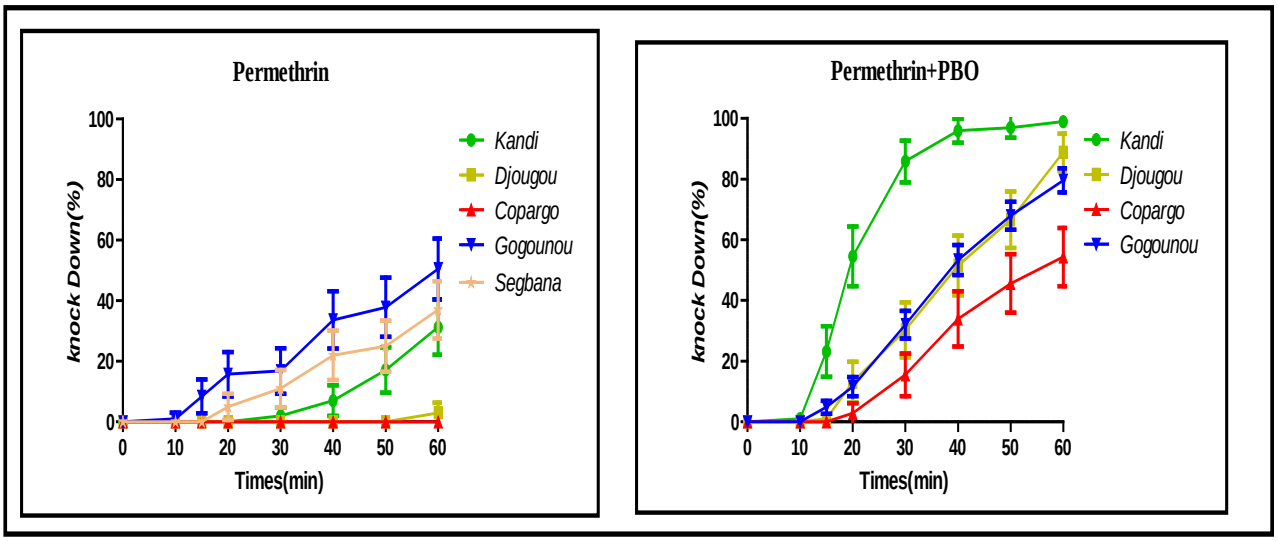


Figure 5: Percentage of *An. gambiae s.l.* "knocked-down" function time during its exposure to permethrin alone and permethrin + PBO

3.4-Lethal effect of permethrin alone and permethrin + PBO on mosquitoes

After 24 hours, all populations of *An. gambiae* tested showed a mortality rate less than 80% with permethrin (6.06 to 55.1%) confirming the resistance to this pyrethrinoid. This mortality rate was improved under the effect of permethrin + PBO (57.57 and 99.02%) (Figure 6). The PBO synergist has therefore strengthened the lethal effect of permethrin on resistant mosquitoes. This suggests the presence of biochemical resistance in these different mosquito populations.

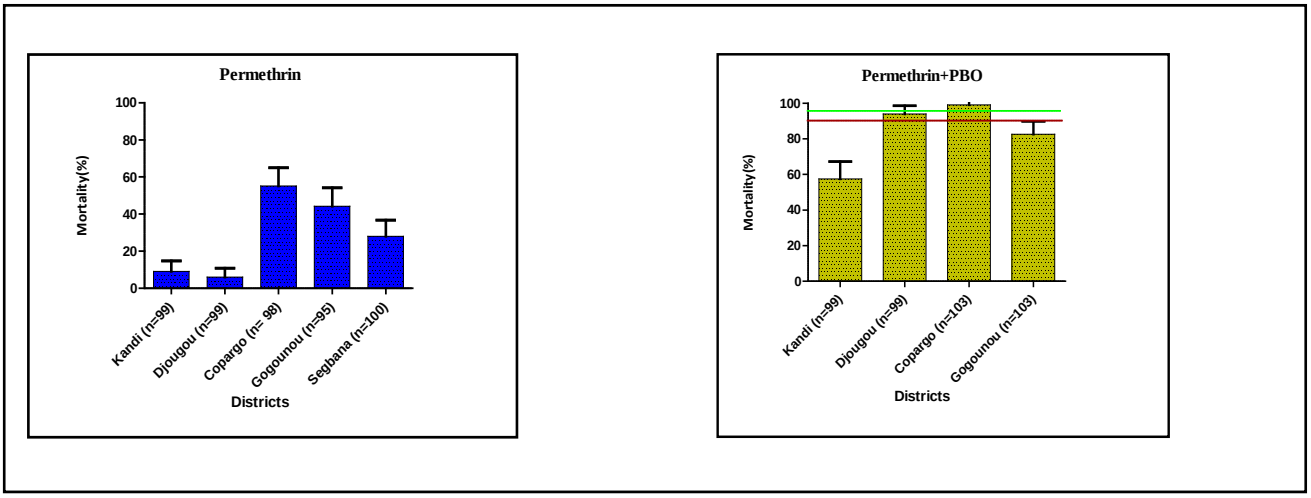


Figure 6: Mortalities observed with permethrin and permethrin + PBO

3. 5-Knock-down effect of deltamethrin alone and deltamethrin + PBO on mosquitoes

The knock-down effect of deltamethrin on mosquitoes is very low compared to deltamethrin + PBO effect. After 15 minutes, the mosquito proportion knocked-down under the effect of deltamethrin was between 0 and 1.1% *versus* 0 and 33.01% under the effect of deltamethrin + PBO. After 30 minutes of exposure to deltamethrin, the mosquito proportion knocked-down is low, below 2% in Kandi, less than 10% in Gogounou and Copargo and around 15% in Djougou and Segbana. On the other hand, deltamethrin + PBO showed a significantly higher Knock-down effect on the same mosquito populations (P <0.05) except Gogounou. After 60 min, the mosquito proportion fell down on the back is between 33.33 and 82.02% with deltamethrin alone and between 89 and 100% with deltamethrin + PBO (figure 7). The PBO synergist has therefore strengthened the knock-down effect of deltamethrin on resistant mosquitoes.

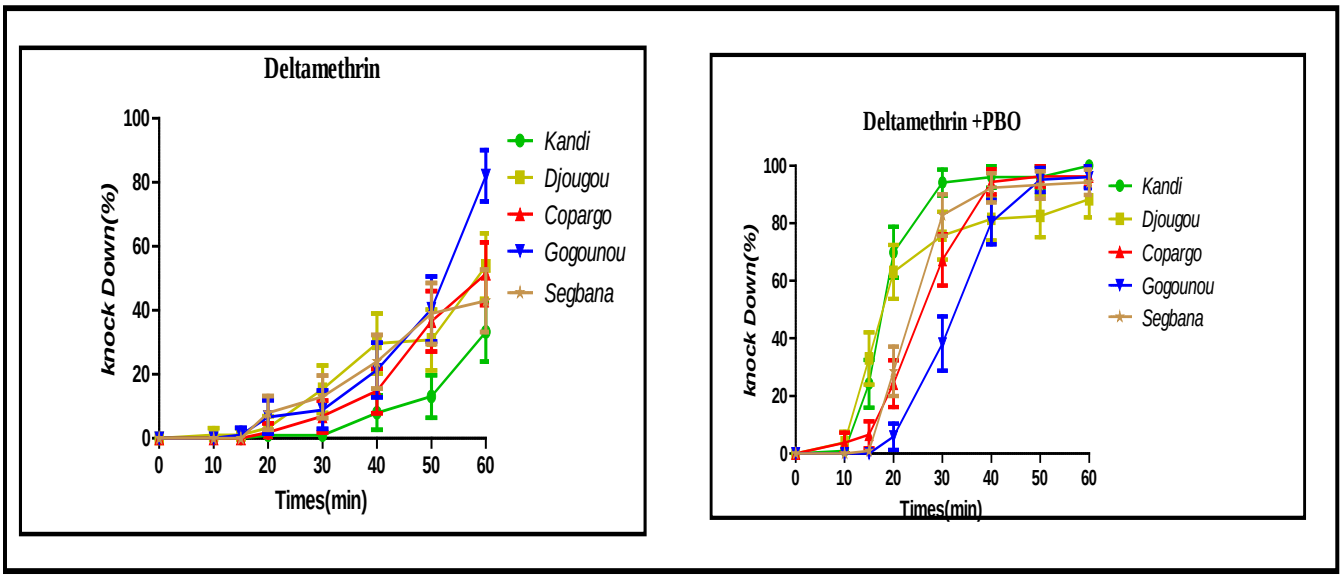


Figure 7: Percentage of *An. gambiae s.l.* "knocked-down" function time during its exposure to permethrin alone and deltamethrin + PBO

3. 6-Lethal effect of deltamethrin alone and deltamethrin + PBO on mosquitoes

After 24 hours, all populations of *An. gambiae* tested showed a mortality rate less than 90% with deltamethrin (25.27 to 83.14%) confirming the resistance to this pyrethrinoid. On the other hand, the mortality rate of same vector populations was improved with the effect of deltamethrin + PBO (63.10 to 99.06%) (Figure 8). The PBO synergist has therefore strengthened the lethal effect of deltamethrin on resistant mosquitoes. These results suggest an implication of biochemical resistance in these different mosquito populations.

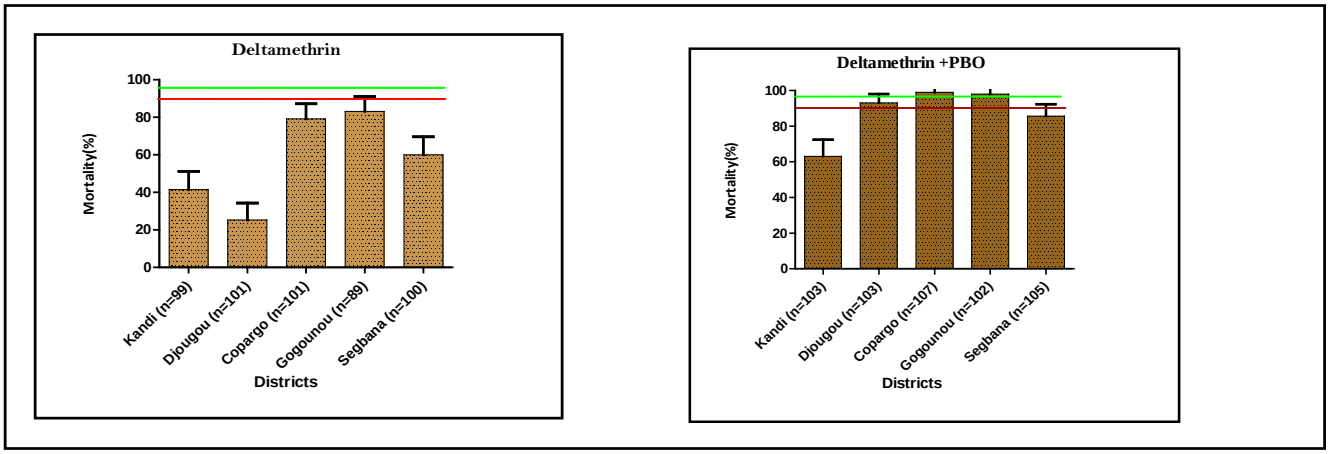


Figure 8: Mortalities observed with only deltamethrin and deltamethrin + PBO

3. 7-Eeffect of the alphacypermethrin on mosquitoes

The knock-down effect of alphacypermethrin on mosquitoes varies significantly from one district to another (P <0.05). It is very low in Djougou and Copargo districts and relatively high in Kandi and Gogounou (figure 9). After 24 hours, all populations of *An. gambiae* showed a mortality rate less than 90% (6.38% and 52.08%), suggesting resistance of all mosquito populations tested to this pyrethroid. This mortality was too low in Djougou and Copargo localities.

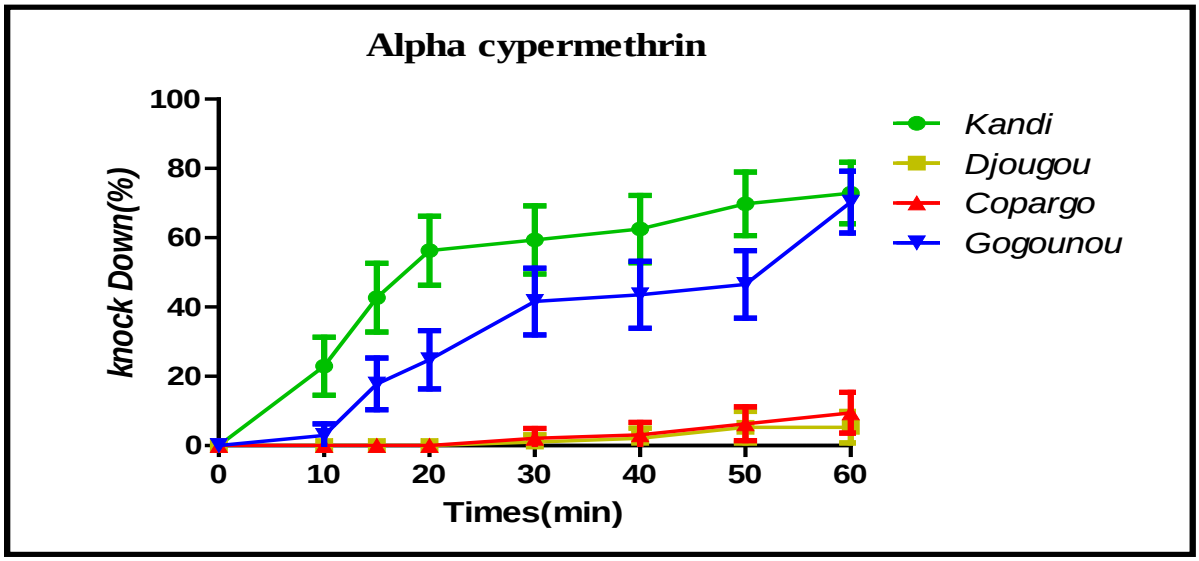


Figure 9: Percentage of *An. gambiae s.l.* exposed to alphacypermethrin impregnated papers "knocked-down

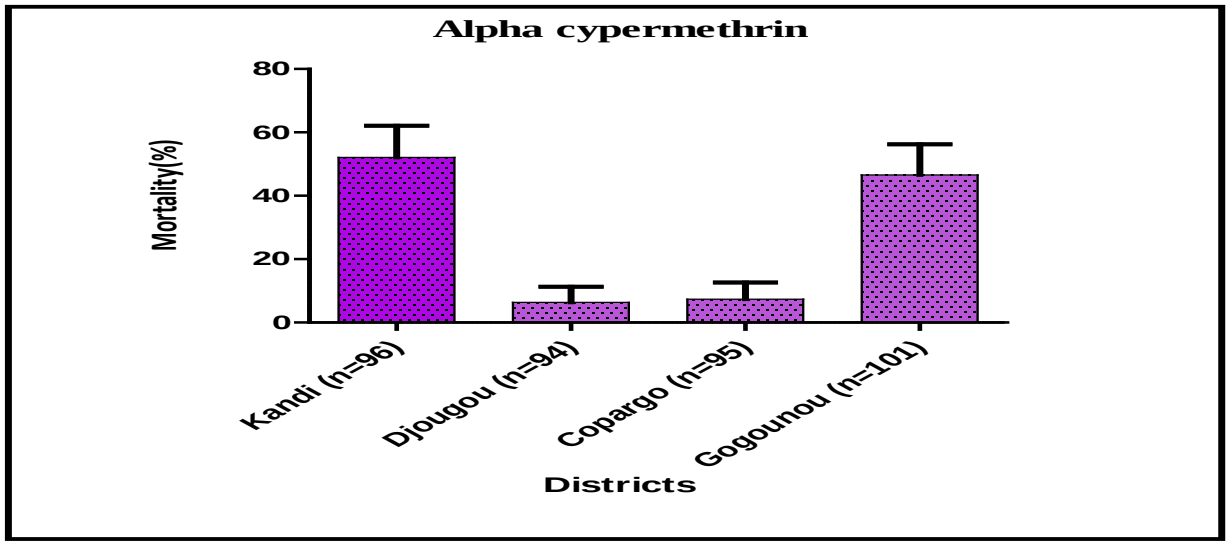


Figure 10: Mortalities observed with alpha cypermethrin

3.8- Mechanisms of molecular resistance to insecticides detected in Anopheles gambiae s.s

The *Kdr mutation* appears to be the main mechanism of resistance in *An. gambiae s.s* populations. The mean allelic frequency of the *kdr* gene is 0.77. A variation of the kdr frequencies is observed in the species of *An. gambiae* complex. They were significantly higher in *An. gambiae* than in *An. coluzzii* in all districts (P <0.05) except Copargo district. *Ace-1* mutation was identified at very low frequencies (1-4%) in all districts. It is higher in *An. gambiae* (3-4%) and very low or zero in *An. coluzzii* (table I).

Table 1: Distribution of Knock-down resistance (*Kdr*) and *Ace-1R* frequencies in *An. gambiae* and *An. coluzzii* in Alibori and Donga

Localities	Species	Number tested	RR	RS	SS	F (Kdr)	RR	RS	SS	F (Ace -1)
Kandi	***An.gambiae***	**86**	57	19	10	**0,77**	0	4	82	**0,02**
	An. coluzzii	**180**	86	64	30	**0,66**	0	5	175	**0,01**
Gogounou	***An.gambiae***	**84**	66	15	3	**0,88**	0	6	78	**0,04**
	An. coluzzii	**91**	57	26	8	**0,77**	0	2	89	**0,01**
Segbana	***An.gambiae***	**27**	15	9	3	**0,72**	0	3	24	**0,06**
	An. coluzzii	**45**	22	16	7	**0,67**	0	0	45	**0,00**
Djougou	***An.gambiae***	**168**	114	41	13	**0,80**	0	10	158	**0,03**
	An. coluzzii	**101**	63	23	15	**0,74**	0	4	97	**0,02**
Copargo	***An.gambiae***	**219**	158	52	9	**0,84**	0	10	209	**0,02**
	An. coluzzii	**51**	39	11	1	**0,87**	0	0	51	**0,00**
Ouake	***An.gambiae***	**61**	44	11	6	**0,81**	0	2	59	**0,02**
	An. coluzzii	**50**	27	15	8	**0,69**	0	1	49	**0,01**

3.9- Mechanisms of biochemical resistance in Anopheles gambiae s.s

Biochemical assays showed significantly high activities of glutathione-s-transferase in the Djougou and Gogounou populations compared to the susceptible Kisumu strain ($p < 0.001$) (Figure 14). On the other hand, there were no significant differences in the activities of the esterases and oxidases of the two populations (Figures 1, 2 and 3).

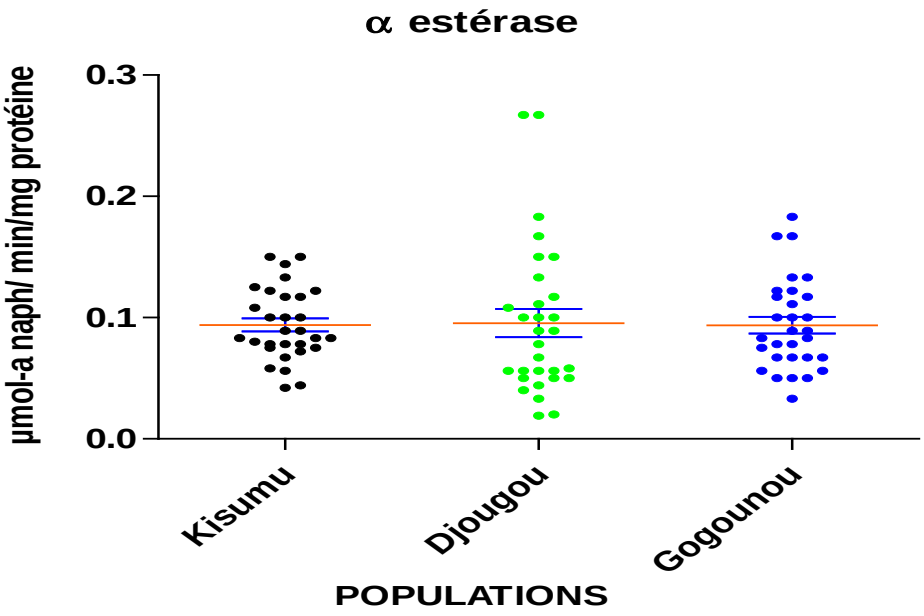


Figure 11: α-esterases activities of populations of *An. gambiae* of Djougou and Gogounou.

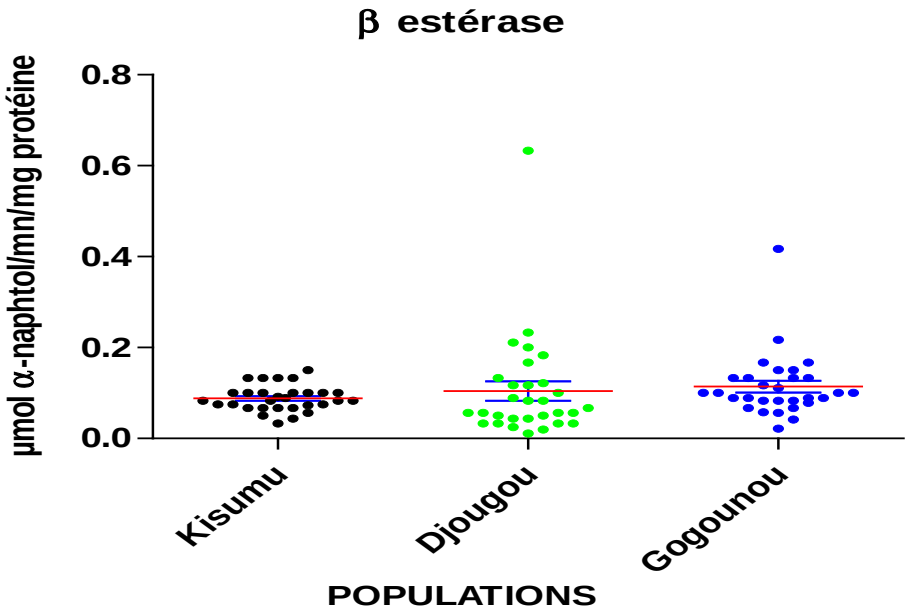


Figure 12: β-esterases activities of populations of *An. gambiae* of Djougou and Gogounou.

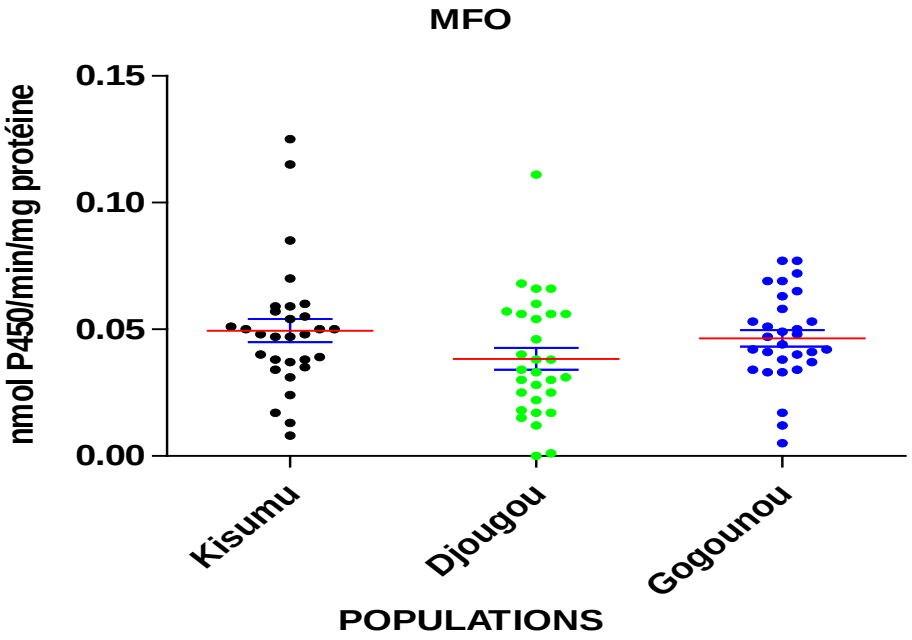


Figure 13: Mono-oxygenase activities of populations of *An. gambiae* of Djougou and Gogounou.

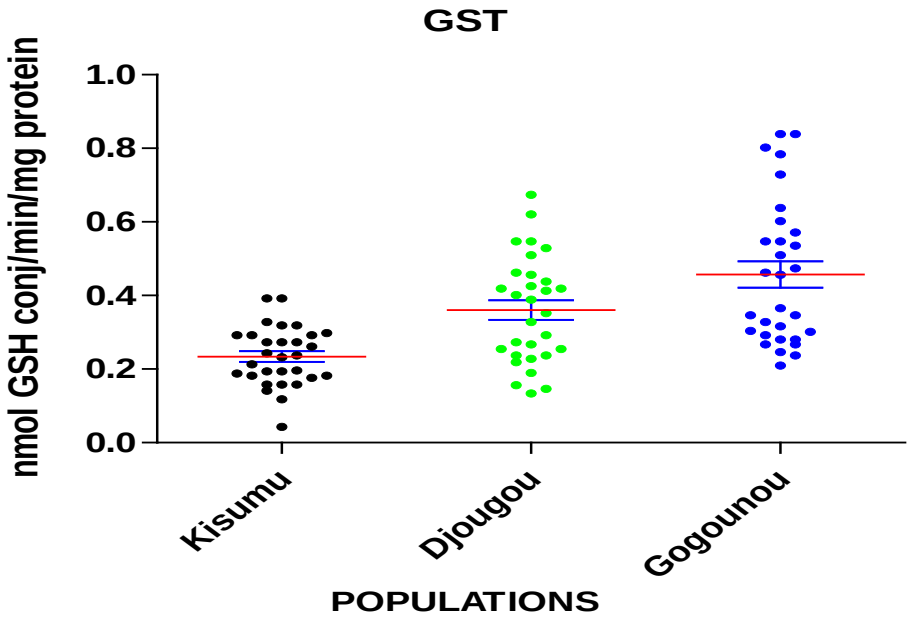


Figure 14: Glutathion-S-transferase activities of populations of *An. gambiae* of Djougou and Gogounou

3. 10. Conclusion

The present study revealed confirmed resistance of *An. gambiae s.s* to deltamethrin and permethrin (pyrethroids) throughout the IRS extension zone (Alibori and Donga). Even if the *Leu-Phe kdr* mutation is the most important resistance mechanism in these *An. gambiae s.s.* populations, metabolic resistance conferred by detoxifying enzymes is also an indication of phenotypic resistance to pyrethroids in Alibori and Donga. The effect of PBO synergists on pyrethroid-resistant vectors is encouraging and clearly shows that new mosquito nets impregnated with both pyrethroids and PBO could be a promising tool for controlling malaria transmission and resistance management. Besides resistance to pyrethroids, we noted a decrease in the vectors' susceptibility (suspected resistance) to carbamates (bendiocarb). It is associated with low frequencies of the *Ace-1R* gene detected in the districts concerned. The *Ace-1* mutation alone could not fully explain this reduced susceptibility to bendiocarb observed and strongly suggests involving other resistance mechanisms such as metabolic detoxification. All populations of *An. gambiae s.s* are still susceptible to organophosphate (pirimiphos methyl) with the faster susceptibility to the effect of this insecticide (faster immediate mortality). This organophosphate is the potential candidate insecticide for the next IRS in Alibori and Donga.