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U.S. PRESIDENT'S MALARIA INITIATIVE ACTION TO REINFORCE MALARIA VECTOR CONTROL IN BENIN

PMI/USAID/IRS OR ACTIVITIES IN BENIN

Monitoring and Evaluation of the efficacy of the fifth round of Indoor Residual Spraying (IRS) in Atacora, Benin, West Africa.

FINAL REPORT

2015 IRS Campaign

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1. Introduction

The entomological evaluation has currently almost become the only one way to appreciate the operational effectiveness of numerous current struggling tools against malaria

The current report is a global report of the monitoring of 2015 IRS campaign. It integrates collected data from March to December 2015. To well assess the impact of IRS, we have compared the entomological indicators (PMI primary and secondary indicators) not only between the IRS districts and the control (untreated district), but also the indicators have been compared among different periods such as :

- Before campaign period (Baseline data ; March-April)
- Bioefficacy period of Actellic CS (from June to August, when 24h mortality of Kisumu for cone bioassay ≥80%)
- Period beyond the bioefficacy of Actellic CS (from September to December, when 24h mortality of Kisumu tested using cone bioassay <80%)

CREC's entomological monitoring activities provide baseline data and informations about the following entomological indicators required by PMI to follow IRS:

- Malaria Vector Species identification
- Vector distribution and seasonality (Vector density)
- Mosquito behavior: biting (endophagy or exophagy), vector feeding time and resting (endophily or exophily)
- Sporozoite rates, Entomological Inoculate Rate (EIR)
- Parity rates (age grading)
- Vector susceptibility to insecticides

2. Study area

Five districts were randomly selected for the study:

- Four districts sprayed with Pirimiphos methyl CS: Tanguiéta, Kouandé, Natitingou and Toukountouna
- Control district: Copargo.

3. Activities planned and indicators evaluated for the period from March to December 2015

3.1 Before campaign period: Baseline data from March to April

- Preparation for Entomological Monitoring for 2015 IRS Campaign. Ensure all entomological surveillance staff and materials are ready. Assure that all sentinel surveillance sites are in working order
- Human Biting Rate of anopheles and Entomological Inoculation Rate (EIR)
- Endophily and exophily (exit rate) behaviour of anopheles
- Physiological age grading (parous rate) of *An. gambiae*

3.2. Post-campaign period (from July to December)

- Dynamics of mosquitoes in districts under IRS intervention and control district
- Variation of Human Biting Rate (HBR) of anopheles and Entomological Inoculation Rate (EIR) in districts under IRS intervention and control district
- Indoor and outdoor biting rate behaviour of anopheles in districts under IRS intervention and control district
- Exophily (exit rate) behaviour of anopheles in districts under IRS
- Impact of IRS on physiological age grading (parity rate) of *An. gambiae*
- Assessment of vector susceptibility to various insecticides

4. Protocol

4.1. Human Landing Catches and Mosquito Collection

In each district selected for M&E, the sampling of mosquitoes was done in 4 villages: 2 villages in the central part of the district and 2 villages at the periphery. In each village, two houses were chosen for human landing catches activities. Human landing catches were used to determine mosquitoes biting time, location of biting (inside and/or outside), and monitor the behavior of mosquitoes. Adult mosquitoes were collected for 2 consecutive nights, every month, using human landing catches with one collector (human acting as a landing catch) placed indoor, and another collector placed outdoor in each of the houses used for mosquito collection. All Anopheles mosquitoes caught during the night collections were identified for

their species. Any vector species that are collected and identified was transported to a small laboratory on the field 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.

4.2. Window Exit Trap Catches and Indoor Pyrethrum Spray Catches.

The Window Exit Trap Catches and the Indoor Pyrethrum Spray Catches were completed to estimate the total density of mosquito species in the houses and the proportion of female mosquitoes exiting from the houses. Eight bedrooms per districts from separate houses (not from houses that were used for human landing catches) were selected for window exit trap catches and indoor pyrethrum spray catches. This activity takes place over two nights, with mosquito collection occurring in the morning. This activity occurs twice per month. The Window Exit Trap activity measures exit rate of Anopheles. For this activity, exit traps were positioned over the windows of each study bedroom for two nights per month. All mosquitoes escaping the study bedrooms via the windows were thereby trapped. Mosquito collections were completed the following morning, using a mouth aspirator. Mosquitoes that are still alive were transferred into plastic cups supplied with 10% honey solution, with mortality recorded over the next 24 hours. After window exit trap collections, Indoor Pyrethrum Spray Catches were organized. The study bedrooms were sprayed with Pyrethrum (mixed with water) and a white canvas placed on the floor. After 10 minutes, all fallen mosquitoes were collected from the floor and placed in petri dishes, to measure the number of mosquitoes in the room, and develop data for endophily behavior.

4.3. Bioassay cone tests for the study of the residual effect of pirimiphos methyl CS

a. Mosquitoes and material tested

Anopheles gambiae Kisumu, a susceptible colony from insectary of CREC were used for bioassay cone tests. Various treated walls (walls made with cement, and mud) were tested. The initial cone bioassay test was conducted 24 hours after houses were spayed. Subsequent tests were done on a monthly basis according to the WHO procedures.

b. Bioassay cone test

Bioassays were done according to the WHO procedures for test cones. Cones were placed on the treated walls. About 10 females of Kisumu susceptible laboratory *An. gambiae* were

introduced per cone and exposed for 30 minutes. For each house, the cones are put at 4 locations: 0.5 m, 1 m, 1.5 m and 2 m. During the tests, the temperature and the relative humidity of exposure and holding periods were registered. The number of mosquitoes knocked down after 30 min exposure period and dead at the end of 24 hours holding period were registered. The house number and the household name are registered as well as GPS coordinates.

4.4. Insecticide susceptibility test

Female mosquitoes aged 2-5 days old from the districts under IRS were exposed to diagnostic doses of various insecticides for susceptibility tests using insecticide-impregnated papers, as described by the standard WHO testing protocol. The following insecticides were tested during the period: bendiocarb (0.1%) and pirimiphos methyl (0.25%)

5. Results

5.1. Insecticide decay rates using wall bioassay cone test

The resisdual efficacy of Actellic CS on tested substrates was monitored for a period of 6 months. The initial cone bioassay test was conducted 24 hours after houses were sprayed. Subsequent tests were done on a monthly basis (May, June, July, August, September, November) 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-day 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 September showed 24 h mortality to be $\leq 80\%$.

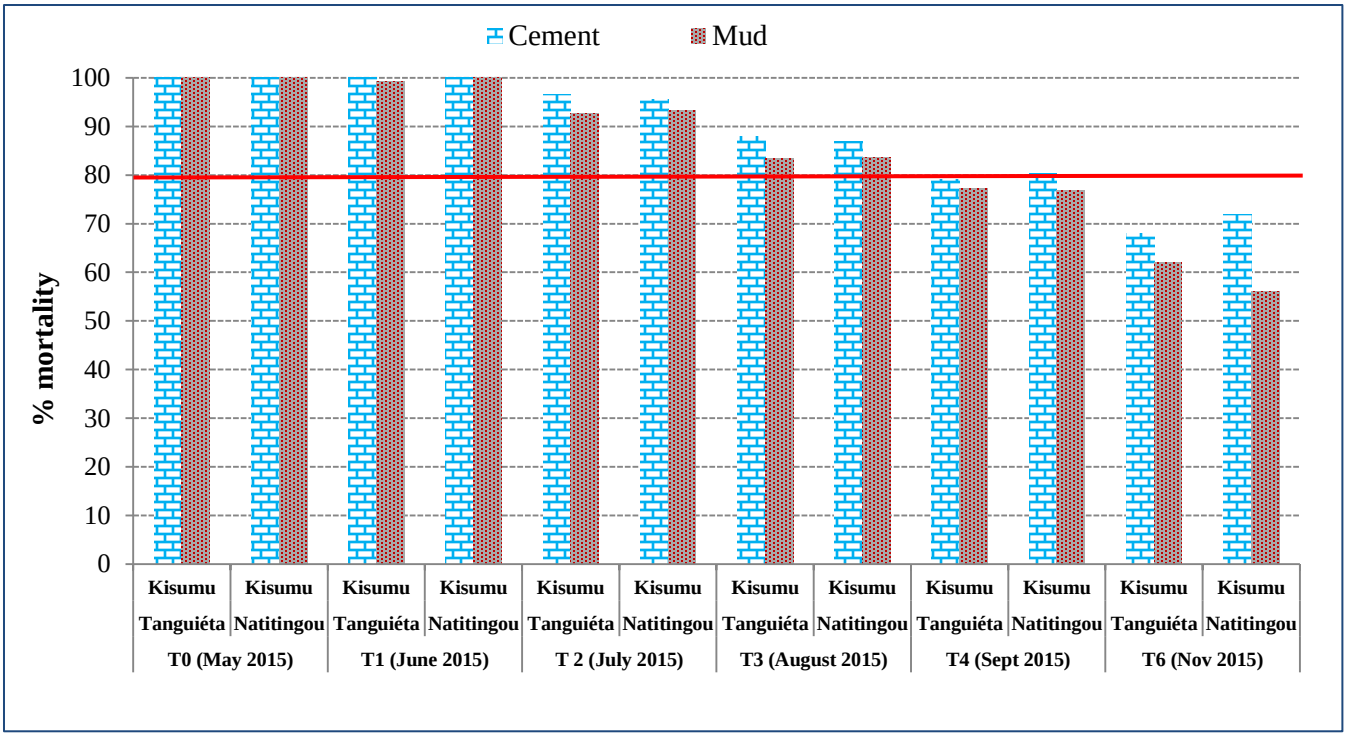


Figure 1: Mean mortalities obtained for different sprayed surface after the six following months after spray operation in Atacora using WHO cone test

Table I. Efficacy of the spray and residual effect of pirimiphos methyl CS 6 months after 2015 IRS campaign

	T0 (May 2015)		T1 (June 2015)		T 2 (July 2015)		T3 (August 2015)		T4 (Sept 2015)		T6 (Nov 2015)	
	Tanguiéta Kisumu	Natitingou Kisumu	Tanguiéta Kisumu	Natitingou Kisumu	Tanguiéta Kisumu	Natitingou Kisumu	Tanguiéta Kisumu	Natitingou Kisumu	Tanguiéta Kisumu	Natitingou Kisumu	Tanguiéta Kisumu	Natitingou Kisumu
Cement	100	100	100	100	96.7	96	88	87	79.1	80	68	72
Mud	100	100	99.2	100	92.7	93.20	83.33	83.62	77.24	76.80	62	56

5.2. Density of mosquitoes indoor and outdoor in districts under IRS *Vs* control

A total of 808 *An gambiae s.l* were collected from march to December in treated districts (Toukountounan Tanguiéta, Kouandé and Natitingou) and the control district (Copargo). The table II and figure 2 show *An gambiae* density indoor and outdoor in districts under IRS *Vs* control. From reading the figure 2, we can make three observations:

Before the IRS campaign (baseline data; March-April), *An. gambiae* density is low compared to the others periods, and *An. gambiae* were endophagic during this period (more vectors caught indoor than outdoor). Indeed, a total of 121 *An. gambiae* were caught of which 85 indoor. During this period, there is no significant difference between the number of vector caught in the control district compared to the other districts. The low density of *An. gambiae* collected during March and April, before IRS, is due to the period (dry season)

- **During the period of bioefficacy of Actellic CS** (June-August, when 24h mortality of Kisumu ≥80% for cone test), a total of 389 *An. gambiae* were caught of which 260 in the control district (Copargo). We have noticed a reduction of 95.65% to 97.51% of *An. gambiae* indoor. *An. gambiae* were found exophagic during this period (more vectors caught outdoor than indoor). This shows that the presence of the pirimiphos methyl considerably decreases the entry of vectors in houses.

- **During the period beyond the bioefficacy of Actellic CS** (Sept-Dec, when 24h mortality of Kisumu <80%for cone bioassay), a total of 298 *An. gambiae* were caught of which 150 in the control district (Copargo). We continue to notice a differed impact of IRS as far as mosquitoes density is concerned when we compare data from the treated districts against the control

Table 2: *An. gambiae* density, before during and after the 2015 IRS campaign

Districts	Before campaign period (Baseline data ; March-April)		Period of bioefficacy of Actellic CS (June-August ; 24h mortality ≥80%)		Period beyond the bioefficacy of Actellic CS (Sept-Dec ; 24h mortality <80%)	
	Inside	Outside	Inside	Outside	Inside	Outside
Toukountounan	14	2	7	33	17	20
Tanguiéta	16	8	4	21	15	21
Natitingou	18	7	5	24	12	24
Kouandé	17	6	6	29	19	20
Copargo	20	13	161	99	98	52

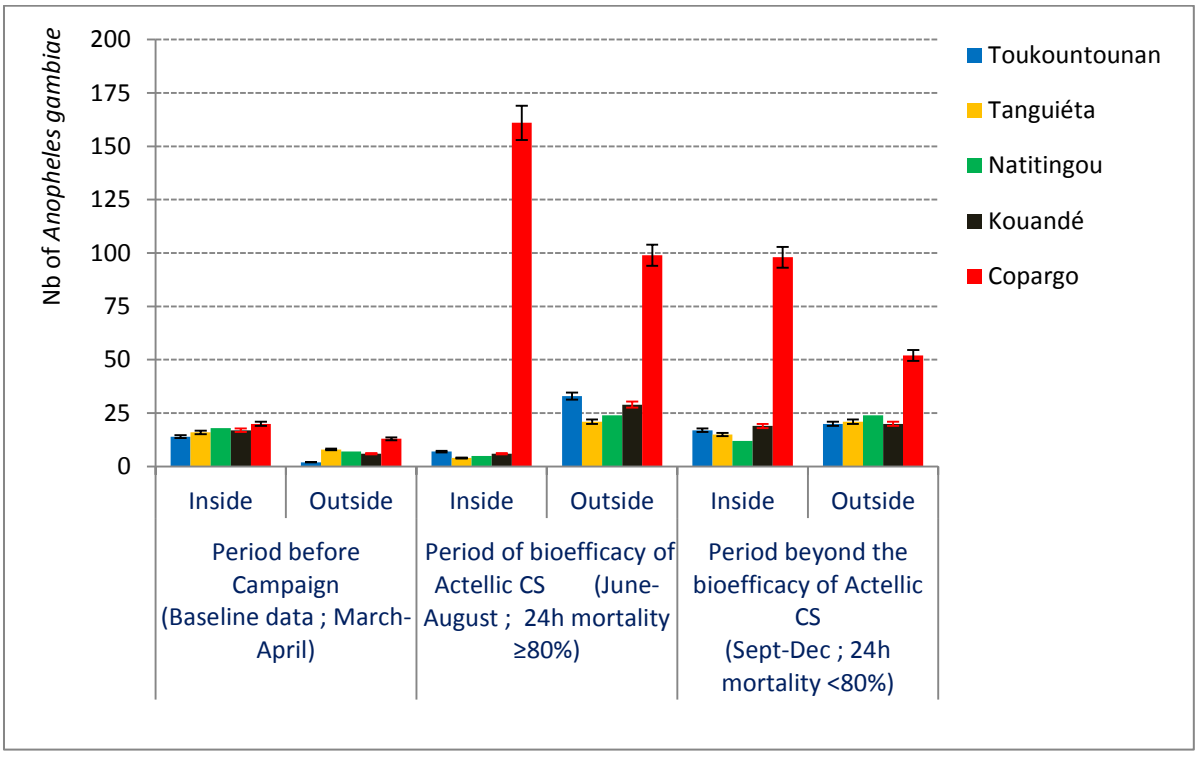


Figure II: *An. gambiae* density, before, during and after the 2015 IRS campaign

5.3. Human Biting Rate (HBR) of *An. gambiae* and Entomological Inoculation Rate (EIR)

Table 3 summarizes the Human biting rate (HBR), the sporozoitic index (IS) and Entomological Inoculation Rate (EIR) recorded before, during and after 2015 IRS campaign. The HBR follows the same trend as *An. gambiae* density dynamic described above. As far as Entomological Inoculation Rate is concerned (figure 3), we can make three observations:

- Before the 2015 IRS campaign (March-April), each inhabitant received between 3.75 and 5,625 infected bites/months in IRS districts against 2.813 infected bites/month in the control
- From June to August we have noticed a significant decrease of EIR in treated districts compared to the control. Indeed, a reduction of 96.61% to 98.97% has been observed during this period in districts under IRS.
- During the period beyond the bioefficacy of Actellic CS (Sept-Dec), the EIR was relatively low (between 0- 1.25 ib/month against 1.87 in the control)

Figure 4 shows the dynamic of EIR from March to December 2015.

Table 3: Human Biting Rate (HBR) of *An. gambiae* and Entomological Inoculation Rate (EIR) before, during and after 2015 IRS campaign.

Districts	**Before Campaign period (Baseline data ; March-April)**			**Period of bioefficacy of Actellic CS (June-August ; 24h mortality ≥80%)**			**Period beyond the bioefficacy of Actellic CS (Sept-Dec ; 24h mortality <80%)**		
	HBR/ night	IS	EIR/ month	HBR/ night	IS	EIR/ month	HBR/ night	IS	EIR/ month
Toukountounan	0.50	0.25	**3.75**	0.830	0.025	**0.6225**	0.771	0.027	**0.625**
Tanguiéta	0.75	0.17	**3.75**	0.521	0.040	**0.625**	0.750	0.000	**0**
Natitingou	0.78	0.20	**4.688**	0.604	0.021	**0.3776**	0.750	0.028	**0.625**
Kouandé	0.72	0.26	**5.625**	0.729	0.057	**1.25**	0.813	0.051	**1.25**
Copargo	1.03	0.09	**2.813**	5.417	0.227	**36.875**	3.125	0.020	**1.875**

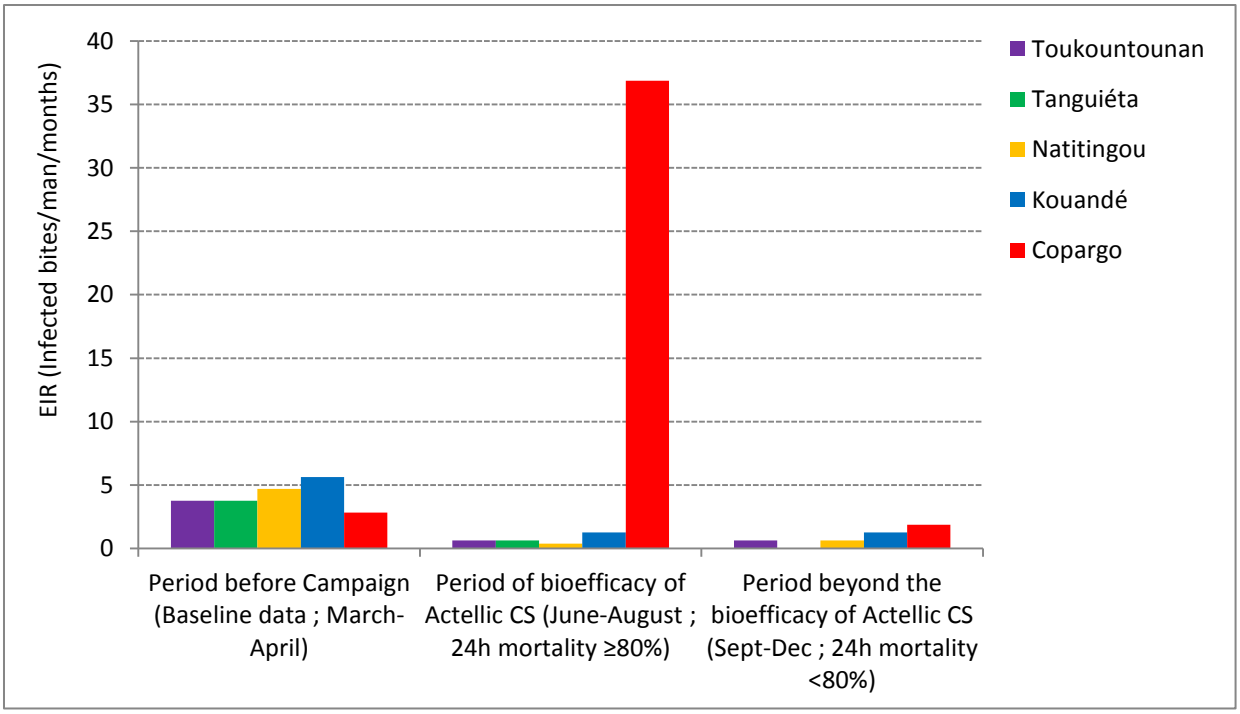


Figure 3: Entomological Inoculation Rate (EIR) before, during and after 2015 IRS campaign.

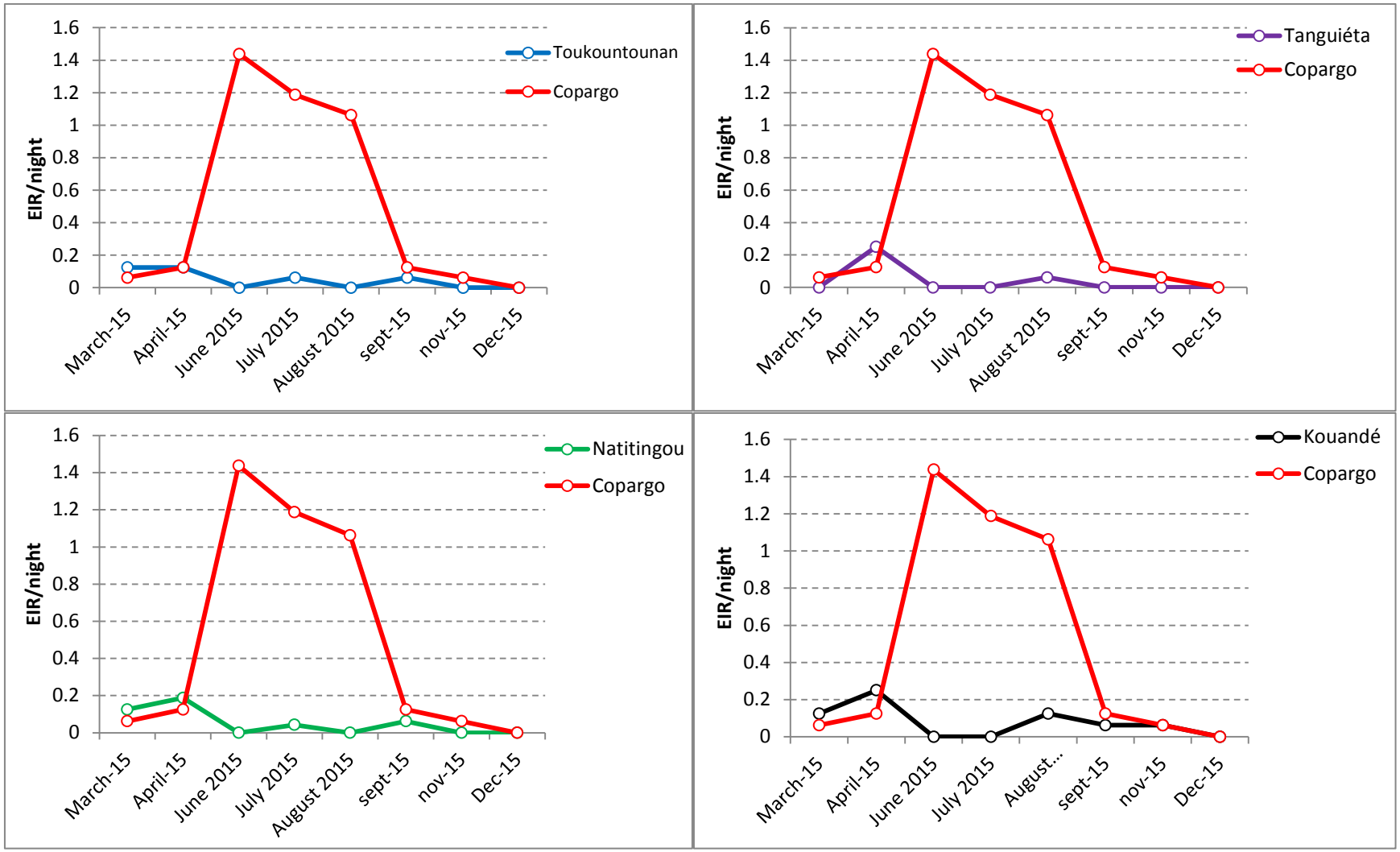


Figure 4: Dynamic of Entomological Inoculation Rate (EIR) from March to December

5.4. Exophily induced by pirimiphos methyl CS IRS on *An. gambiae* in the districts under IRS *Vs* Control

To determine mosquitoes staying indoor the houses and the part of them exiting from the rooms at day break, we prepared exit traps at the windows of 40 bedrooms. Mosquitoes that entered the houses at night were retained in the traps while exiting at dawn and then caught by our team in the morning. The rest of them were then caught after spraying a non-residual insecticide inside the houses.

The table 4 shows the number of mosquitoes collected by Exit Window Traps and Pyrethrum Spray Catch recorded before, during and after 2015 IRS campaign . From June to August, in the treated districts, *An.gambiae* has shown a high exophily rate compared to the control. During this period, between 75 and 87.5% of *An.gambiae* which have entered in the treated houses can't stay in and are obliged to run away. During the period of September to December, the average exophily in IRS districts was between 40.91% and 63.64% against 16.67% in the control**.** However, before IRS campaign, the exophily rate was very low (between 0-13.33%). That means that, before IRS campaign, *An.gambiae* preferred to rest indoor (endophilic behavior).

Table 4. Exophily induced by pirimiphos methyl CS IRS on *An. gambiae*, before, during and after 2015 IRS campaign.

Districts	Before Campaign period (Baseline data ; March-April)			Period of bioefficacy of Actellic CS (June-August ; 24h mortality ≥80%)			Period beyond the bioefficacy of Actellic CS (Sept-Dec ; 24h mortality <80%)		
	PSC	EWT	Exophiy rate	PSC	EWT	Exophily rate	PSC	EWT	Exophily rate
Toukountounan	14	0	**0**	4	13	**76.47**	11	14	**63.64**
Tanguiéta	14	1	**6.667**	4	12	**75**	11	14	**63.64**
Natitingou	12	1	**7.692**	3	9	**75**	10	12	**60**
Kouandé	13	2	**13.33**	1	7	**87.5**	11	9	**40.91**
Copargo	24	0	**0**	101	32	**24.06**	66	22	**16.67**

5.5. 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 in the houses by Exit Window Traps and by Pyrethrum Spray Catch.

Even though IRS have reduced the blood feeding rate, it's important to realize that this reducing still appears low and an important proportion of vectors could still blood feed in treated houses (Table 5). However, the high rates of blood feeding were recorded before IRS campaign (March-April). It's then important to strengthen the complementary use of mosquito nets at this period.

Table 5. Blood feeding rate of *An. gambiae s.l* before, during and after 2015 IRS campaign.

Districts	Before campaign period (Baseline data ; March-April)			Period of bioefficacy of Actellic C (June-August ; 24h mortality ≥80%)			Period beyond the bioefficacy of Actellic CS (Sept-Dec ; 24h mortality <80%)		
	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate
Toukountounan	14	13	**92.86**	17	13	**76.5**	25	15	**60**
Tanguiéta	15	13	**86.67**	16	10	**62.5**	25	13	**52**
Natitingou	13	9	**69.23**	12	8	**66.7**	22	11	**50**
Kouandé	15	13	**86.67**	8	4	**50**	20	11	**55**
Copargo	24	23	**95.83**	133	122	**91.7**	88	76	**86.36**

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

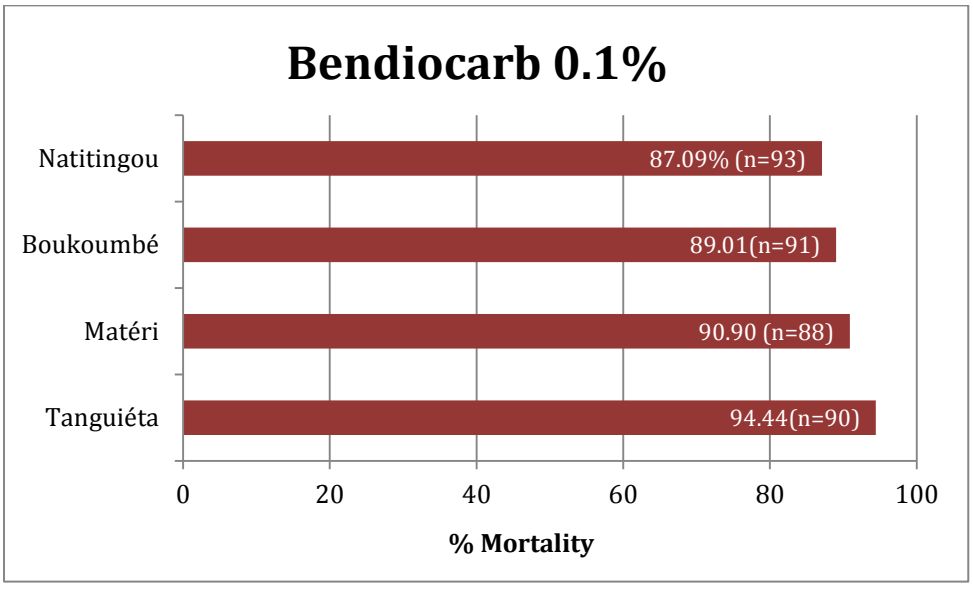
Table 6 below shows the impact of IRS on the physiological age of *An. gambiae* in terms of parous rate. From March to April, there is no significant difference between the vector longevity when we compare the districts under IRS to the control. During this period, the main parity rate of *An. gambiae* has varied from 35.3% to 44% in the districts under IRS against 33.3% in the control. However from June to August, we have observed an impact of IRS on this indicator by reducing of parous rate in districts under IRS (45.6% in the control against 20.7%-30% in districts under IRS). The same trend has been observed during the period from September to December **(**54% in the control against 27.8% - 40.5% in districts under IRS (Table 6).

Table 6. Physiological age in terms of parous rate of *An. gambiae* in the districts under IRS *Vs* Control

Districts	Period before Campaign (Baseline data ; March-April)			Period of bioefficacy of Actellic CS (June-August ; 24h mortality ≥80%)			Period beyond the bioefficacy of Actellic CS (Sept-Dec ; 24h mortality <80%)		
	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)
Toukountounan	17	6	35.3	40	12	**30**	37	15	**40.5**
Tanguiéta	24	10	41.7	25	6	**24**	36	12	**33.3**
Natitingou	25	11	44	29	6	**20.7**	36	10	**27.8**
Kouandé	23	9	39.1	35	9	**25.7**	39	12	**30.8**
Copargo	33	11	33.3	90	41	**45.6**	63	34	**54**

5.7. Vector susceptibility

Figure 5 below summarizes the findings of the vector susceptibility testing that was undertaken on local malaria vectors (*Anopheles gambiae* s.l) against various insecticides (bendiocarb and pirimiphos methyl). *Anopheles* mosquitoes tested were susceptible to pirimiphos methyl in all the districts (mortality >98%). However the same populations of *Anopheles* were still resistant to bendiocarb.



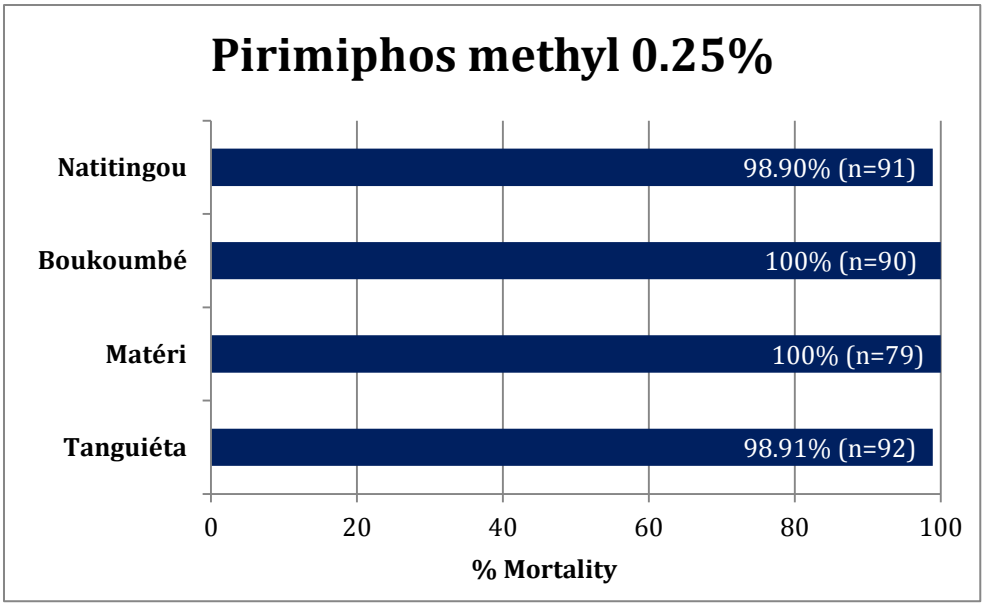


Figure 5: Susceptibility of *Anopheles gambiae* s.l to Bendiocarb 0.1% and Pirimiphos methyl 0.25% in four districts under IRS in October 2015.

6. Conclusion and overvew

The monitoring of 2015 IRS campaign in Atacora region has shown once more the impact of this tool on malaria transmission dwindling. It comes out of this monitoring that, before the IRS campaign, there was no huge difference between the IRS districts and the control. Elsewhere, before IRS campaign, some indicators like blood feeding rate, parous rate and Entomological Inoculating Rate (EIR) were high. The results of the bioassay testing showed that Pirimiphos methyl remains effective for up to four months post-IRS (May-August). During the bioefficacy period of Actellic CS (from June to August ; 24h mortality of Kisumu for cone bioassay ≥80%), we obseverd a spectacular reduction of some indicators like mosquitoes density and EIR, and a high exophily rate was also observed. This impact has been differed although the pirimiphos CS decay went down 80% threshold. However, the IRS impact was not been so visible on some indicators like blood feeding rate which stayed relatively high in IRS districts and this, no matter how the period was. As far as vector susceptibility to insecticides is concerned, *An. gambae* is still susceptible to pirimiphos methyl in evaluated regions.

As satisfactoring, this results may be, there still some challenges in terms of data quality improvement for the coming IRS campaigns monitoring in Benin. That is why, for the 2016 IRS camapaign monitoring, we have planned added to what has already been doing to:

- Enhance the study of quality of the IRS control. Indeed, the improved quality control is necessary to try to identify the origin of the rapid deterioration of the insecticides we use.

Therefore, we will replace 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 and the determination of the amount of insecticide deposited on the walls by the sprayers agents.

- Performe Insecticide quantification test

- Establish a database on the length of the high malaria transmission period and the status of resistance of vectors to insecticides in future potential communes of IRS extension.