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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-Evaluation of the efficacy of the sixth round  
of Indoor Residual Spraying (IRS) in Atacora, Benin,  
West Africa.

## **PRELIMINARY REPORT**

**Activities carried out from May to August 2016**

**Doc/CREC/PMI/USAID/August 2016**

# Summary

The sixth round of the IRS campaign in Atacora has led on May 2016. At the beginning of this campaign, the quality and effectiveness control study was carried out through WHO cone bioassay test and insecticide quantification, to verify the efficacy and homogeneity of insecticide applied on the walls. Moreover, we have also measured the pH of wall substratum to better understand the impact of pH on insecticide decay. After the campaign operation, the monitoring of IRS impact on malaria transmission was assessed through the primary and secondary entomological indicators.

The current report shows entomological indicators measured respectively in districts treated with pirimiphos methyl CS compared to the control district, one, two and three months following 2016 IRS campaign in Atacora department.

During the first month of IRS, all mosquitoes exposed to the treated walls were killed. Three months after IRS, the mortality rate was still beyond the threshold of 80% mortality for both types of walls, cement and mud.

During the period from June to August, the monthly entomological monitoring shows a high decrease of *An. gambiae* density in treated districts compared to control. The decrease was higher indoor treated houses compared to outdoor. In addition, we observed a decrease of Human Biting Rate (HBR) of mosquitoes indoor and outdoor houses in districts under IRS. When we compared Anopheles density recorded in treated houses to the control, we noticed more than 80% of reduction in treated districts.

In the control area, each inhabitant receives 0.792 infected bites of *An. gambiae* per night or **23.75** infected bites per person per month. In the districts under IRS, the EIR was relatively low: It was between 0.63 and 1.25 infective bites of *An. gambiae* per person per month. That means 94.74 % of reduction of EIR in IRS districts compared to the control.

During the study period (June - August), the mean parity rate of *An. gambiae* was 68% in the control against 22% in the districts under IRS.

Regarding the blood feeding rate, it was 30.65% in average in the IRS districts against 90.97% in the control.

As far as insecticide resistance is concerned, *Anopheles gambiae* appears susceptible to pirimiphos methyl, but resistant to bendiocarb, deltamethrin and permethrin

In conclusion, the sixth IRS campaign in Atacora had a significant impact on entomological malaria transmission indicators the three following months the spray operation.

### **Monitoring-Evaluation of the efficacy of the sixth round of Indoor Residual Spraying (IRS) in Atacora, the three months following the spraying operation in Atacora, Benin, West Africa.**

#### **© Study area**

For mosquitoes collection through HLC and PSC, five districts were randomly selected for the study: four (04) districts sprayed with Pirimiphos methyl CS ( Tanguiéta, Kouandé, Natitingou and Toukountouna) and Copargo as the control district.

#### **© Activities planned for the period**

- Quality control and insecticide decay assessment
- 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
- Impact of IRS on physiological age grading (parity rate) of *An. gambiae*
- Vectors resistance assessment

## © Protocol

### 1. Mosquito sampling

#### 1.1. Human Landing Catches (HLC) for malaria transmission evaluation

In each district under IRS selected for M&E, the sampling of mosquitoes was done in 2 villages: 1 village in the central part of the district and 1 village at the periphery. *Anopheles* mosquitoes were dissected to determine the parity rate. The heads/thoraxes of the vector species were analyzed using ELISA method to determine CSP antigens. Abdomens of females of the vector species were used for PCR analyses, to identify sibling species.

#### 1.2. Indoor Pyrethrum Spray Catches.

The indoor Pyrethrum Spray Catches was performed to estimate mosquito density in the treated houses compared to the control houses.

### 2. Bioassay cone tests for the study of the residual effect of bendiocarb and pirimiphos methyl

#### 2.1. Mosquitoes and material tested

*Anopheles gambiae* Kisumu, a susceptible colony from insectary of CREC was used for bioassay cone tests. Various treated walls (walls made with cement, and mud) were tested. Bioassays were performed each month after IRS according to the WHO procedures.

#### 2.2. Quality control and bioassay cone test

The quality control of the 2016 IRS campaign was carried out in 3 districts: Tounkountounan, Tanguieta and Natitingou. In each district, two villages were selected (one in central area and the next in peripheral area) Bioassays were performed in Tanguiéta and Natitingou, and insecticide quantification in Natitingou and Toukountounan. Below are the selected villages for bioessai cone test:

District of Tanguieta: Taiacou and Fnta

District of Natitingou: Perporiyakou and Bérécingou

### **2.2.a. Mosquitoes and material tested**

Because of the scarcity of the local strain of *Anopheles gambiae* larvae at the end of dry season, only the susceptible colony from laboratory (*Anopheles gambiae* Kisumu) was tested for bioassay cone tests for T<sub>0</sub>.

Various treated walls (walls made with cement, and mud) were bioassayed. Bioassays were performed a week after IRS according to the WHO procedures.

### **2.2. b. Bioassays Protocol**

Bioassays were done according to the WHO procedures for test cones. Cones were placed on the treated walls. About 10 females of *An. gambiae* Kisumu 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 and dead at the end of 24 hours holding period were registered. The number of the houses and GPS coordinates are registered.

### **2.2.c. pH measure**

Two days before spraying operation, about 5 g of substratum of the walls internal surface were deducted. The deduction aim is measuring the wall pH so as to see an eventual impact of the pH on insecticide half-life on the wall.

### **2.2. d. Quantification of insecticide active material per surface unit**

Before the spraying operation we have labelled the walls with wattman papers ( 5 X 5 cm<sup>2</sup>) at different locations (0.5m ; 1m ; 1,5m ; 2m ). 24hours after spray operation, the labels were picked up. A quantification of active material of insecticide applied on labels will be done in relationship with WHO.



**Picture 1** : Labelling wall in Ourbouga, in Natitingou district

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### **3. Insecticide susceptibility assessment**

#### **3.1. Mosquito collection**

In August 2016, mosquito larvae were collected at all stages in water collections across Tanguiéta and Natitingou districts. In the field laboratory, larvae were transferred into distilled water and reared separately at room temperature. Pupa were collected in cups and placed inside cages covered with netting for emergence.

#### **3.2. Insecticide susceptibility test**

Insecticide susceptibility tests were conducted using 2-4 days old females emerging from larvae collected on the field. Unfed *An. gambiae s.l* females were exposed to 0.1% bendiocarb, 0.05% deltamethrin and 0.25 pirimiphos methyl following WHO procedures.

## **© Results**

### **1. Insecticide decay rates using wall bioassay cone test**

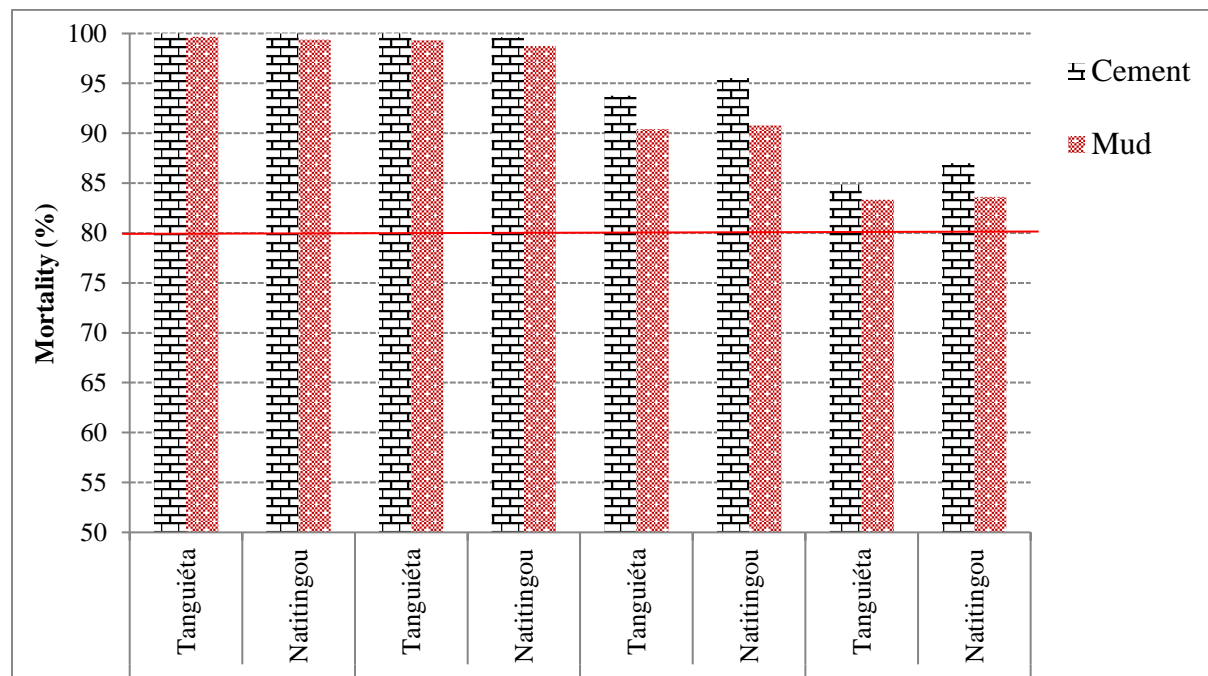
The initial cone bioassay test was conducted 24 hrs after houses were sprayed. Subsequent tests were done on a monthly basis (13-14 June, 13-14 July, 13-14 August) to determine the decay of insecticide applied on the walls. The number of mosquitoes dead after 24 hours was

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 pirimiphos methyl CS on the walls after 2015 IRS campaign in Atacora. During the first month of IRS, all mosquitoes exposed to the treated walls were killed. Three months after IRS, the mortality rate was still beyond the threshold of 80% mortality for both types of walls, cement and mud.

**Table 1.** Cone Bioassay mean mortalities obtained for different sprayed surface three months after spray operation in Atacora.

Kind of surface	T0 (May 2016)		T1 (June 2016)		T2 (July 2016)		T3 (August 2016)	
	Tanguiéta	Natitingou	Tanguiéta	Natitingou	Tanguiéta	Natitingou	Tanguiéta	Natitingou
Cement	100	100	100	99,62	93,75	95,53	84,88	87,00
Mud	99.66	99.38	99.32	98.75	90.44	90.79	83.33	83.62



**Figure 1:** Quality of the spray and residual effect of pirimiphos methyl CS, 1 2, 3 months after 2016 IRS campaign in Atacora.

## 2. Density and Human Biting Rate (HBR) of mosquitoes in districts under IRS compared to the control

A total of 702 *An gambiae s.l* were collected from June to August from IRS districts and the control district. We have noticed a high decrease of the density of *An gambiae* collected by HLC in treated houses compared to the control. In the control, the majority of *An. gambiae* was collected indoor houses (Table 2 and Figure 2), which is in accordance with the behaviour of this species more endophilic/endophagic. In the districts under IRS, a contrary situation was observed: very few mosquitoes were collected indoor houses.

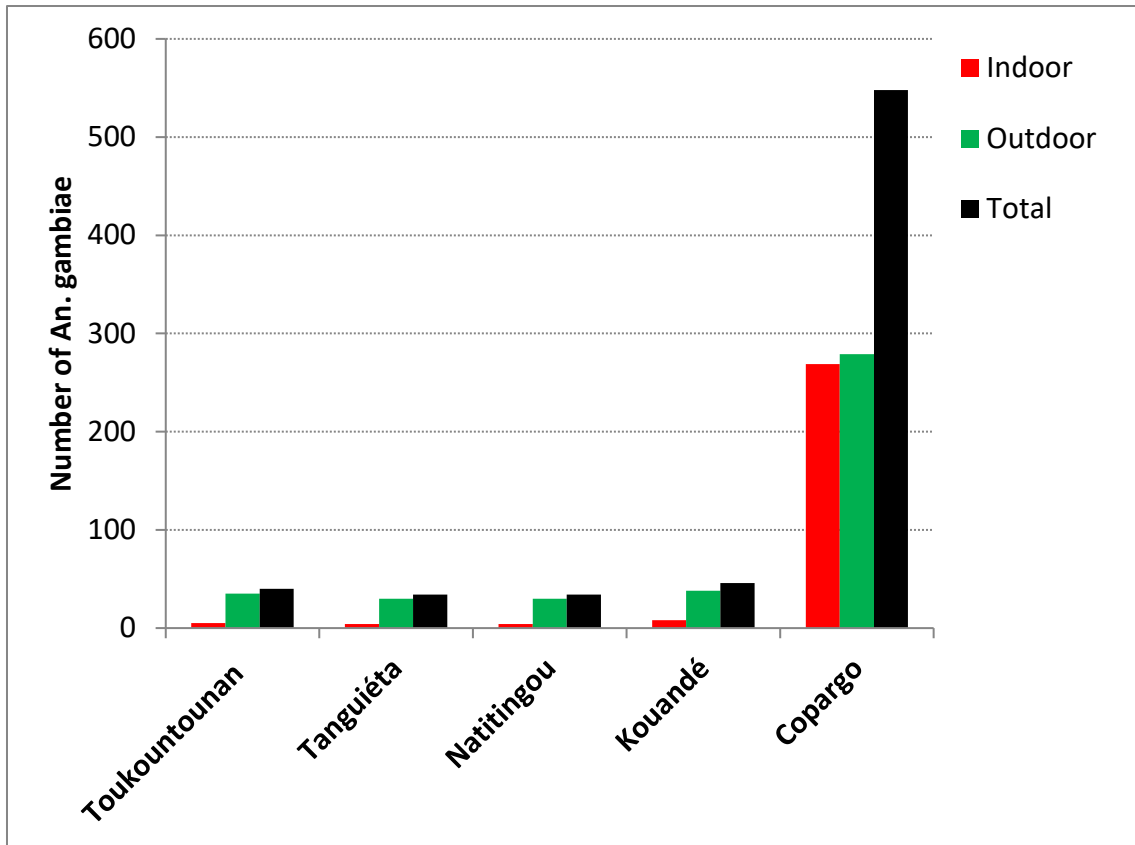
The human biting rate was calculated using mosquitoes collected by Human Land Catch. In the IRS districts, the mean HBR was 0.67 bites/night (129/192) or 20.15 bites/month during the period of June to August, against 162.5 bites/month in the control districts. The tables 3 and 4 show monthly details about the HBR (indoor Vs outdoor, and the mean HBR per district) district by district.

In conclusion, during the 3 months following the IRS, low density of *An. gambiae* was observed in districts under IRS. In addition, the low indoor biting was observed compared to outdoor.

**Table 2:** *An. gambiae* density indoor Vs outdoor from June to August 2016.

<b>Districts</b>	<b>Indoor</b>	<b>Outdoor</b>	<b>Total</b>
Toukountounan	5	35	40
Tanguiéta	4	30	34
Natitingou	4	30	34
Kouandé	8	38	46
Copargo	269	279	548
<b>Total</b>	290	412	702





**Figure 2:** Number of *An. gambiae* caught indoor Vs outdoor from June to August 2016

**Table 3:** *An.gambiae* biting rate indoor Vs Outdoor per district from June to August 2016.

			June- 2016	July- 2016	August- 2016	Total
<b>Toukountounan</b>	Inside	Total Mosquitoes	1	1	3	5
		nb human cathes	8	8	8	24
		HBR/night	<b>0,13</b>	<b>0,13</b>	<b>0,38</b>	<b>0,21</b>
	Outside	Total Mosquitoes	9	11	15	35
		nb human cathes	8	8	8	24
		HBR/night	<b>1,13</b>	<b>1,38</b>	<b>1,88</b>	<b>1,46</b>
<b>Tanguiéta</b>	Inside	Total Mosquitoes	1	1	2	4
		nb human cathes	8	8	8	24
		HBR/night	<b>0,13</b>	<b>0,13</b>	<b>0,25</b>	<b>0,17</b>
	Outside	Total Mosquitoes	6	8	16	30
		nb human cathes	8	8	8	24
		HBR/night	<b>0,75</b>	<b>1</b>	<b>2</b>	<b>1,25</b>
<b>Natitingou</b>	Inside	Total Mosquitoes	1	2	1	4
		nb human cathes	8	8	8	24
		HBR/night	<b>0,13</b>	<b>0,25</b>	<b>0,13</b>	<b>0,17</b>
	Outside	Total Mosquitoes	8	9	13	30
		nb human cathes	8	8	8	24
		HBR/night	<b>1,00</b>	<b>1,13</b>	<b>1,63</b>	<b>1,25</b>
<b>Kouandé</b>	Inside	Total Mosquitoes	1	3	4	8
		nb human cathes	8	8	8	24
		HBR/night	<b>0,13</b>	<b>0,38</b>	<b>0,50</b>	<b>0,33</b>
	Outside	Total Mosquitoes	7	14	17	38
		nb human cathes	8	8	8	24
		HBR/night	<b>0,88</b>	<b>1,75</b>	<b>2,13</b>	<b>1,58</b>
<b>Copargo</b>	Inside	Total Mosquitoes	61	88	120	269
		nb human cathes	8	8	8	24
		HBR/night	<b>7,625</b>	<b>11</b>	<b>15</b>	<b>11,208</b>
	Outside	Total Mosquitoes	52	81	146	279
		nb human cathes	8	8	8	24
		HBR/night	<b>6,50</b>	<b>10,13</b>	<b>18,25</b>	<b>11,63</b>

**Table 4.** *An.gambiae* mean biting rate per district from June to August 2016

		June- 2016	July- 2016	August- 2016	Total
	Total Mosquitoes	10	12	18	40
<b>Toukountounan</b>	nb human cathes	16	16	16	48
	HBR/night	<b>0,63</b>	<b>0,75</b>	<b>1,13</b>	<b>0,83</b>
	Total Mosquitoes	7	9	18	34
<b>Tanguiéta</b>	nb human cathes	16	16	16	48
	HBR/night	<b>0,44</b>	<b>0,56</b>	<b>1,13</b>	<b>0,71</b>
	Total Mosquitoes	9	11	14	34
<b>Natitingou</b>	nb human cathes	16	16	16	48
	HBR/night	<b>0,56</b>	<b>0,69</b>	<b>0,88</b>	<b>0,71</b>
	Total Mosquitoes	8	17	21	46
<b>Kouandé</b>	nb human cathes	16	16	16	48
	HBR/night	<b>0,50</b>	<b>1,06</b>	<b>1,31</b>	<b>0,96</b>
	Total Mosquitoes	113	170	266	549
<b>Copargo</b>	nb human cathes	16	16	16	48
	HBR/night	<b>7,06</b>	<b>10,63</b>	<b>16,63</b>	<b>11,44</b>

#### 4. Sporozoitic index and Entomological Inoculation Rate (EIR)

##### 4.1. Sporozoitic index of *P.falciparum* in *An. gambiae* in districts under IRS

In the control area, 548 thoraces were analyzed by ELISA CSP from June to August. The sporozoitic index was 6.934%. (38 thoraces+ for circum-sporozoitic antigen). This index was relatively lower in the districts under IRS (between 2.5% and 4.35%) during the same period (see details in table 5).

##### 4.2. Entomological Inoculating Rate in IRS districts compared to the control.

Table 5 below shows the Entomological Inoculation Rate (EIR) from June to August for each intervention area and control area. In the control area, each inhabitant receives 0.792 infected bites of *An. gambiae* per night or **23.75** infected bites per person per month. In the districts under IRS, the EIR was relatively low: It was between 0.63 and 1.25 infective bites of *An.*

*gambiae* per person per month. That means 94.74 % of reduction of EIR in IRS districts compared to the control.

**Table 5:** Infection rate for *P.falciparum* calculated by circumsporozoite protein (CSP) ELISA from the head and thoraces of *An.gambiae* and EIR from June to August 2016.

		June 2016	July 2016	August 2016	Total
<b>Toukountounan</b>	Thorax	10	12	18	40
	Thorax +	1	0	0	1
	IS	<b>0,1</b>	<b>0,000</b>	<b>0,000</b>	<b>0,025</b>
	HBR/night	0,625	0,750	1,125	0,833
	EIR	<b>0,0625</b>	<b>0,000</b>	<b>0,000</b>	<b>0,021</b>
<b>Tanguiéta</b>	Thorax	7	9	18	34
	Thorax +	0	0	1	1
	IS	<b>0</b>	<b>0</b>	<b>0,056</b>	<b>0,029</b>
	HBR/night	0,4375	0,5625	1,125	0,708
	EIR	<b>0</b>	<b>0</b>	<b>0,063</b>	<b>0,021</b>
<b>Natitingou</b>	Thorax	9	11	14	34
	Thorax +	1	0	0	1
	IS	<b>0,1111</b>	<b>0,000</b>	<b>0</b>	<b>0,029</b>
	HBR/night	0,5625	0,688	0,875	0,708
	EIR	<b>0,0625</b>	<b>0,000</b>	<b>0</b>	<b>0,021</b>
<b>Kouandé</b>	Thorax	8	17	21	46
	Thorax +	0	1	1	2
	IS	<b>0</b>	<b>0,059</b>	<b>0,0476</b>	<b>0,043</b>
	HBR/night	0,5	1,063	1,3125	0,958
	EIR	<b>0</b>	<b>0,063</b>	<b>0,0625</b>	<b>0,042</b>
<b>Copargo</b>	Thorax	113	170	266	548
	Thorax +	2	11	25	38
	IS	<b>0,018</b>	<b>0,065</b>	<b>0,094</b>	<b>0,069</b>
	HBR/night	7,063	10,630	16,625	11,417
	EIR	<b>0,125</b>	<b>0,688</b>	<b>1,563</b>	<b>0,792</b>

## 5. Physiological age of *An. gambiae* in the districts under IRS

Table 6 shows the parity rate observed in treated districts and the control. During the study period (June - August), the mean parity rate of *An. gambiae* was 68% in the control against 22% in the districts under IRS.

**Table 6:** Parity rate of *An.gambiae* caught by HLC in June and August 2016.

Districts	June 2016			July 2016			August 2016			Total		
	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)	n tested	Parous	Parous rate (%)
<b>Toukountounan</b>	10	3	<b>30</b>	12	3	<b>25</b>	18	2	<b>11,1</b>	40	8	<b>20</b>
<b>Tanguiéta</b>	7	2	<b>28,6</b>	9	2	<b>22,2</b>	18	3	<b>16,7</b>	34	7	<b>21</b>
<b>Natitingou</b>	9	2	<b>22,2</b>	11	3	<b>27,3</b>	14	2	<b>14,3</b>	34	7	<b>21</b>
<b>Kouandé</b>	8	3	<b>37,5</b>	17	3	<b>17,6</b>	21	6	<b>28,6</b>	46	12	<b>26</b>
<b>Total IRS districts</b>	34	10	<b>29,4</b>	49	11	<b>22,4</b>	71	13	<b>18,3</b>	154	34	<b>22</b>
<b>Copargo (Control)</b>	113	86	<b>76,1</b>	112	67	<b>59,8</b>	110	76	<b>69,1</b>	335	229	<b>68</b>

### 6. Blood feeding rate of *An. gambiaes.sl*

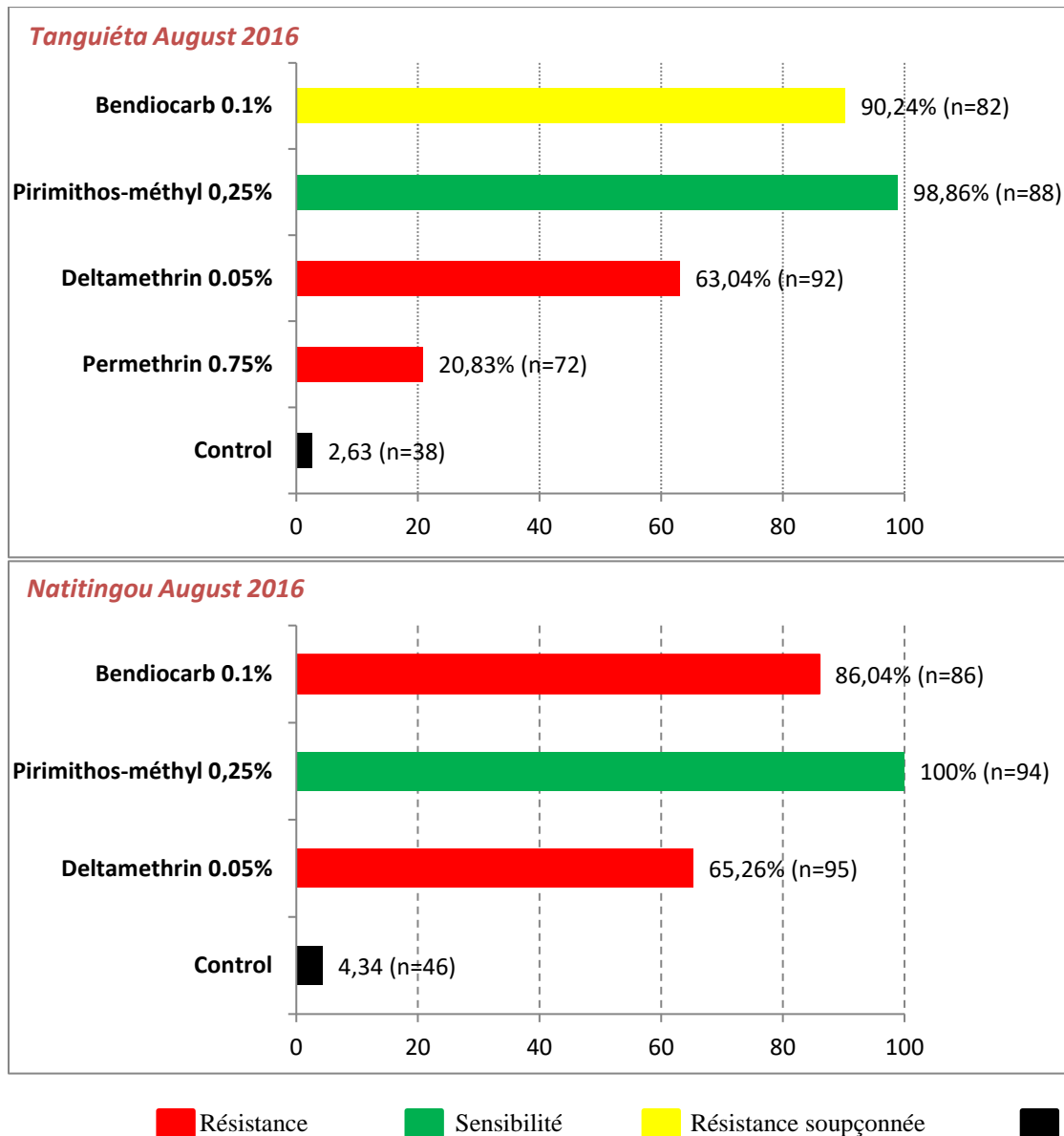
The blood feeding rate is measured considering the number of fed mosquitoes collected in the morning in the houses by Pyrethrum Spray Catch. The blood feeding rate was 30.65% in average in the IRS districts against 90.97% in the control.(Table 7).

**Table 7:** Blood feeding rate of *An. gambiae*

Districts	June 2016			July 2016			August 2016			Total		
	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate	Total	n feed	Blood feeding rate
<b>Toukountounan</b>	4	1	<b>25</b>	4	1	<b>25</b>	11	5	<b>45,45</b>	19	7	<b>36,84</b>
<b>Tanguiéta</b>	3	0	<b>0</b>	2	0	<b>0</b>	8	3	<b>37,5</b>	13	3	<b>23,08</b>
<b>Natitingou</b>	3	0	<b>0</b>	3	1	<b>33,3</b>	6	2	<b>33,33</b>	12	3	<b>25</b>
<b>Kouandé</b>	5	1	<b>20</b>	4	1	<b>25</b>	9	4	<b>44,44</b>	18	6	<b>33,33</b>
<b>Total IRS districts</b>	15	2	<b>13,33</b>	13	3	<b>23,1</b>	34	14	<b>41,18</b>	62	19	<b>30,65</b>
<b>Copargo (Control)</b>	87	61	<b>70,11</b>	133	133	<b>100</b>	79	78	<b>98,73</b>	299	272	<b>90,97</b>

## 7. *An. gambiaes.sl* susceptibility to insecticides

Figure 3 below shows *An. gambiaes.sl* susceptibility status to various insecticides in Tanguiéta and Natitingou in August 2016.



**Figure 3:** *An. gambiaes.sl* susceptibility to insecticides

### Conclusion

The sixth IRS campaign in Atacora had a significant impact on entomological malaria transmission indicators the three following months the spray operation. The results show that in intervention areas, Anopheles density and its human biting rate dropped significantly compared to the control. A reduction of 94.74% in the entomological inoculation rate was recorded during this period, due to the decline in the lifespan of *An. gambiae* induced by

pirimiphos methyl CS. As far as insecticide resistance is concerned, *Anopheles gambiae* appears susceptible to pirimiphos methyl, but resistant to bendiocarb, deltamethrin and permethrin. The next coming activities will be focused on vector resistance mechanism monitoring add to entomological malaria indicators monitoring in IRS districts.