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

Entomological and sociological study in Ouémé-Plateau, Benin, after cessation of indoor residual spraying. Results of activities carried out in December 2011- January 2012

### **FINAL REPORT**

By  
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#### **Summary**

After cessation of Indoor Residual Spraying in Oueme Plateau (Adjohoun, Dangbo, Misserete, Seme) in 2010, the Ministry of Health decided to replace this intervention by the universal coverage of Long Lasting Insecticidal Nets to avoid malaria outbreaks happening. It is also decided continued entomological surveillance in the 4 districts to allow prompt detection of any rebound in malaria transmission

This report shows the results of activities planned with the National Malaria Control Program and Abt Associates for December 2011 and January 2012. All these activities were carried out. A sociological investigation done in January 2012 has shown that the coverage rate of OlysetNets distributed to the communities in the 4 districts is what was universally decided and expected by the National Malaria Control Program (NMCP). In Adjohoun and Misserete, the use is more than 1 Olyset per 1.9 person. In spite this good score, the rate is low in Dangbo district (1 Olyset for 2.1 persons) and very low in Seme (1 Olyset for 3.4 persons). By other hand, the ownership rate of use of these nets is good: 81.76% of persons interviewed regularly use their nets. The rate of use is high in all the localities visited except Misserete

where it is less than 80%. People using NMCP Olyset nets are those who declared slept under these nets the last night before our questionnaire. To calculate this proportion, we have divided the number of respondents who declared sleeping under Olyset NMCP by the total of respondents who declared received Olyset NMCP.

We have studied the effectiveness of Olyset nets distributed by the NMCP after 6 months of use and some Permanet which have been using since 1-2 years (2009-2010) using bioassay cone test analyze. Cone test results show that all the PernaNet® were found ineffective (Mortality of *An. gambiae* Kisumu less than 80%). Concerning Olyset, 1 net was found ineffective among 10 tested (10%).

Only 49 *An. gambiae* were collected in the 5 districts in December 2011. The low density of malaria vectors is due to the dry season. During this period, most of the breeding sites of *An. gambiae* are dry. The human biting rate (HBR) registered is less than 1 bite per man per night. But, the parous rate (physiological age grading of *An. gambiae*) observed is high: more than 80%. All *An. gambiae* collected were analyzed for their infectivity for *Plasmodium falciparum*. Among 6 *An. gambiae* collected in the districts previously under IRS, no CS+ was found, but in the control district, 1 CS+ was registered among 42 analyzed.

The evolution of *An. gambiae* resistance to insecticides in districts previously under IRS is an important issue for the National Malaria Control Program to verify if 3 years use of bendiocarb at large-scale in Oueme-Plateau has increased or decreased the susceptibility of *An. gambiae* to insecticides. Data obtained in 2010 and in 2011, particularly in December 2011 show:

(i). After 3 years of use of bendiocarb, we have registered no resistance to this insecticide. The susceptibility tests performed in December 2011 have confirmed this result.

(ii). A very high resistance to DDT was registered during the whole period of the IRS intervention until today.

(iii). Before 2010, *An. gambiae* was fully susceptible to deltamethrin. But from 2010-2011, the susceptibility tests performed show a decrease of susceptibility or a fully resistance of *An. gambiae* to deltamethrin. The emergence of *An. gambiae* resistance to deltamethrin in 2011 was observed not only in areas previously under IRS but also everywhere in Benin. Before 2010, WHO susceptibility tests performed have shown everywhere mortalities of *An. gambiae* more than 90%. But, from 2010-2011, this rate is less than 90% in many localities ( 14.28% and 73.8% in July 2010 respectively in Adjohoun and Dangbo and 86.90% and 88.10% in November-December 2011 respectively in Adjohoun and Dangbo).

(iv). *An. gambiae* has developed a very high resistance to permethrin in Benin and particularly in Oueme, what was observed since 1999. But in December 2011, this high resistance has decreased in intensity. We suggest to follow this phenomenon next year.

(v). After 3 years of use of IRS in Oueme-Plateau, the massive use of bendiocarb has not induced the development of the ACHE mechanism by increasing Ace-1<sup>R</sup> mutation. The frequency of Ace-1<sup>R</sup> is zero everywhere in 2010 and 2011 except in the control area (Adjara: 0.02)

The main resistance mechanism observed is Kdr mutation which frequency is very high, around 80% in 2010 and 2011.

## Introduction/Background

Since 2007, the US President's Malaria Initiative (PMI) has been supporting the Malaria Vector Control Program in Benin, implementing the Indoor Residual Spraying (IRS) strategy in the Ouémé-Plateau department, particularly in 4 districts: Adjohoun, Dangbo, Akpro-Misséréte and Sèmè-Kpodji. In 2008 and 2009, one round of bendiocarb IRS was carried out per year. In 2010, two rounds of IRS were implemented using the same insecticide to cover the whole period of transmission.

Although Ouémé-Plateau department is characterized by high resistance of *Anopheles gambiae* to pyrethroids, and the majority of house walls are made with mud, this first experience of IRS implemented by the National Malaria Control Program (NMCP) with the support of PMI was a success (Akogbeto *et al.*, AJTMH, 2011; Padonou *et al.*, JPVB, 2011; JPHE, 2011). In the four districts under IRS, the density of *An. gambiae* (human biting rate) and the inoculation rate (EIR) drastically decreased by 94.4%. This reduction due to the lethal action of bendiocarb was observed in all districts. No positive *An. gambiae* for *Plasmodium falciparum* CS+ was found during the evaluation period. During the same period, managers of health centers observed a 70% reduction in cases of severe malaria. For this reason, the National Malaria Control Program (NMCP) decided to continue to implement the IRS operations in Benin. However, in 2011, IRS was stopped in Ouémé-Plateau department and was moved to Atacora, in the northern Benin. The main reason for this is as follows. One round of IRS per year is not sufficient to control malaria morbidity due to the short residual effect of carbamates (bendiocarb) and to the long period of the malaria transmission (April-November) in Ouémé-Plateau. However, in Atacora department, the malaria transmission period is relatively short (June-November), allowing one round of IRS per year to be

sufficient. In terms of cost-effectiveness, those areas characterized by a short malaria transmission are of higher priority for IRS compared to those characterized by two peaks of malaria transmission per year.

Nevertheless, certain accompanying measures were taken in Ouémé-Plateau to continue the fight against malaria. These accompanying measures were also taken to avoid the communities to express their dissatisfaction. Two measures are as follows:

- (i) Universal distribution of long lasting insecticide-treated nets (LLIN) in the 4 districts (Adjohoun, Dangbo, Akpro-Misséréte and Sèmè-Kpodji)
- (ii) Continued entomological surveillance in the 4 districts to allow prompt detection of any rebound in malaria transmission. This monitoring includes: (1) insecticide resistance surveillance; (2) assessment of IRS insecticide decay rates for LLINs and IRS-treated surfaces; and (3) impact of IRS cessation on vector taxonomy, density, behavior and entomological inoculation rate (EIR).

**In December 2011 and January 2012, three activities were carried out:**

- (i).Efficacy and durability under field condition of OlysetNets distributed in July 2011 by the National Malaria Control Program after 6 months of use
- (ii) Impact of cessation of IRS on the dynamics of malaria vectors, malaria transmission and physiological age grading of *An. gambiae*
- (iii) Evolution (increase or decrease) of vector resistance in districts previously under IRS.

Razaki Osse, PhD student, which thesis is related to operational research activities in Ouémé-Plateau, is responsible for field and laboratory activities under our supervision.

## Objectives

1. Assess permethrin decay rates on OlysetNets universally distributed to communities in the four districts in July 2011 by the NMCP.
2. Study the impact of IRS cessation on mosquito population dynamics and behavior.
3. Study the impact of IRS cessation on entomological inoculation rate (EIR) of *An. gambiae*, and compare it to data observed during IRS implementation.
4. Study the evolution of insecticide vector resistance in the four districts after IRS cessation.
5. Study the evolution of vector resistance mechanisms to verify if the massive use of bendiocarb in Ouémé-Plateau has induced the development of the ACHE mechanism by increasing Ace-1<sup>R</sup> mutation.

# Materials and methods

## 1. Study areas

Four districts previously under IRS in department of Ouémé-Plateau: Adjohoun, Sèmè-Kpodji, Dangbo and Akpro-Missérété, and one control district: Adjara

## 2. Activities

### *2.1. Sociological investigation on the use of mosquito nets in the 4 districts previously under IRS intervention*

#### **Sampling method**

The objective of the sociological investigation is to provide information on the coverage and use of LLINs and particularly OlysetNets distributed by the NMCP in July 2011 and to assess the level of bioeffectiveness of these LLINs after 6 months, 1 and 2 years of use.

The investigation was carried out in the 4 districts (communes: Adjohoun, Dangbo, Misserete and Seme). A sampling by level has been set up by associating an uniform weighting for each district. In each district, we have randomly selected 2 district boroughs (arrondissements). In each district borough, another level of selection has randomly taken into account 2 villages with the same uniform weighting.

#### **Sampling size**

Taking into account our objectives and our desire to have a good estimation of parameters to measure, we decided to visit 96 households per village with a margin of 5% to compare for contingencies such as non-response due to registration errors and others. Thus 100 households per village were visited per district, 400 households per district and in total 1 600 for the entire study area. This sample is representative of the population of the four districts.

#### **Principle of the survey**

In each household, all categories of nets under using by the people in the households (Olyset distributed by the NMCP in July 2011, other nets like Permanet and Olyset and others donated by the NMCP and partners) are identified.

#### **Evaluation of the coverage of Olyset distributed by the NMCP in July 2011**

To assess the coverage of Olyset distributed by the NMCP in July 2011, in each district borough, we have determined the number of people who declared received the NMCP Olyset and the number of people who have not received them. According to the principle of one net for 2 people, the coverage rate is determined by the ratio of the number of nets available in the households by the number needed.

## **Evaluation of the use of Olyset distributed by the NMCP in July 2011**

People using NMCP Olyset nets are those who declared slept under these nets the last night before our questionnaire. To calculate this proportion, we have divided the number of respondents who declared sleeping under Olyset NMCP by the total of respondents who declared received Olyset NMCP. Any net installed or not folded row and that users admit to having used it during the night before the survey in a household is considered as in using.

### **Investigation**

In January 2012, a survey team organized by CREC has moved from household to household to conduct the sociological questionnaire in the 4 districts, the use rate of nets, the types of the nets met in the 4 districts: impregnated nets, unimpregnated nets, Olyset, Permanets...etc. used by communities. The physical aspect of each net is assessed. Number and size of holes in the nets are recorded and categorized. Observed holes and rips were categorized as either: (1) smaller in size than a hole that would allow a thumb to pass through it; (2) larger in size than a hole described in (1) but not large enough to allow a fist to pass through it; (3) hole bigger than a fist(closed hand). Informations obtained are registered on a sociological investigation form. The team identifies, counts and records on the survey form nets of each type that are met. The purpose of the sociological investigation is to appreciate the state of the nets in terms of efficiency and rate of utilization, and to assess the physical durability of netting samples.

The interviewees were the heads of households (men and women) volunteers. They are free to participate in the investigation. They are also free to stop the investigation at any time.

### ***2.2. Insecticide decay of LLINs (Olyset) distributed in July 2011 after cessation of IRS***

Bioassays using WHO cones were performed. The percentage of Olyset under threshold of efficacy (mortality of *An. gambiae* Kisumu <80%) was registered

To evaluate residual activity of permethrin on the OlysetNets, WHO cone bioassay was undertaken. Females of a susceptible laboratory strain of *An. gambiae* (Kisumu) aged 3-5 days were introduced into cones attached to nets for 3 min exposure as per WHO guidelines (WHO, 2006). Honey solution was provided during 24 hours holding period at the room temperature.

### ***2.3. Impact of IRS cessation on mosquito population dynamics and behavior and on malaria transmission***

In each district, 2 mosquito sampling points were randomly selected and 2 houses chosen per sampling point for mosquito collections to monitor malaria transmission. Adult mosquitoes

were collected twice a month using human landing catches with one collector placed indoor and another outdoor at each collection point. In total 8 mosquito collectors were used for a night of mosquito collection per districts, so 16 mosquito human catch per month per district and 64 for the 4 districts previously under IRS per month. Mosquitoes were collected by human landing catch to monitor vector species and densities in previous IRS target areas and the control area, inside and outside of houses. Anopheles collected were dissected to determine the parturity rate (physiological age grading) of *An. gambiae*. Heads and thoraces were analyzed using ELISA/CSP protocol to determine the infectivity rate of *Plasmodium falciparum* (CS+) for Anopheles. The abdomens were used for PCR (species, form). The EIR was calculated using the formula:  $EIR = ma \times s$  (number of infected bites of Anopheles received per man per night or per a determined period).

Exit window traps were installed in 4 bedrooms per district to determine the exophily induced by the impregnated nets on the mosquitoes.

#### ***2.4. Evolution of insecticide vector resistance and resistance mechanisms after IRS cessation***

*An. gambiae* larvae were collected in the previously IRS targeted districts and in a control district and reared to perform susceptibility tests using WHO test tubes and impregnated papers. After the tests, dead and alive mosquitoes were stored for PCR analysis (ACHE mechanism: Ace-1<sup>R</sup> mutation and kdr mutation)

##### **Insecticide susceptibility test**

The insecticide susceptibility test was performed using mosquitoes aged 2-5 days old exposed to diagnostic doses of various insecticides-impregnated papers, as described by the standard WHO testing protocol. For each district, five test tubes were used: one untreated paper as a control and four treated papers to expose mosquitoes. Control tubes contained filter papers impregnated with silicon oil (insecticide carrier) only, whereas treated papers were impregnated with diagnostic doses of insecticide plus carrier. An average of twenty-five mosquitoes was introduced into each tube. Females of *An. gambiae* used in this study were exposed for one hour to insecticide-treated papers and monitored at different time intervals (10, 15, 20, 30, 45, 60 minutes) to record the “knock-down” times. After one-hour exposure, mosquitoes were transferred into holding tubes and provided with cotton wool wetted with a 10% honey solution. Mortalities were recorded after 24 hours and the susceptibility status of the population was graded according to the WHO recommended protocol. Dead and survived

mosquitoes from this bioassay were separately kept in Carnoy solution at -20°C for molecular characterization.

### **Molecular characterization: PCR detection of the *Kdr* and *Ace.1* mutations**

Polymerase chain reaction diagnostic test for detection of *kdr* “Leu-phe” mutations was carried out on *An. gambiae* mosquitoes as described by Martinez-Torres et al. (1998, 1999). The PCR-RFLP diagnostic test was used to detect the presence of G119S mutation (*Ace.1* gene) as described by Weill et al. (2004).

## **Results**

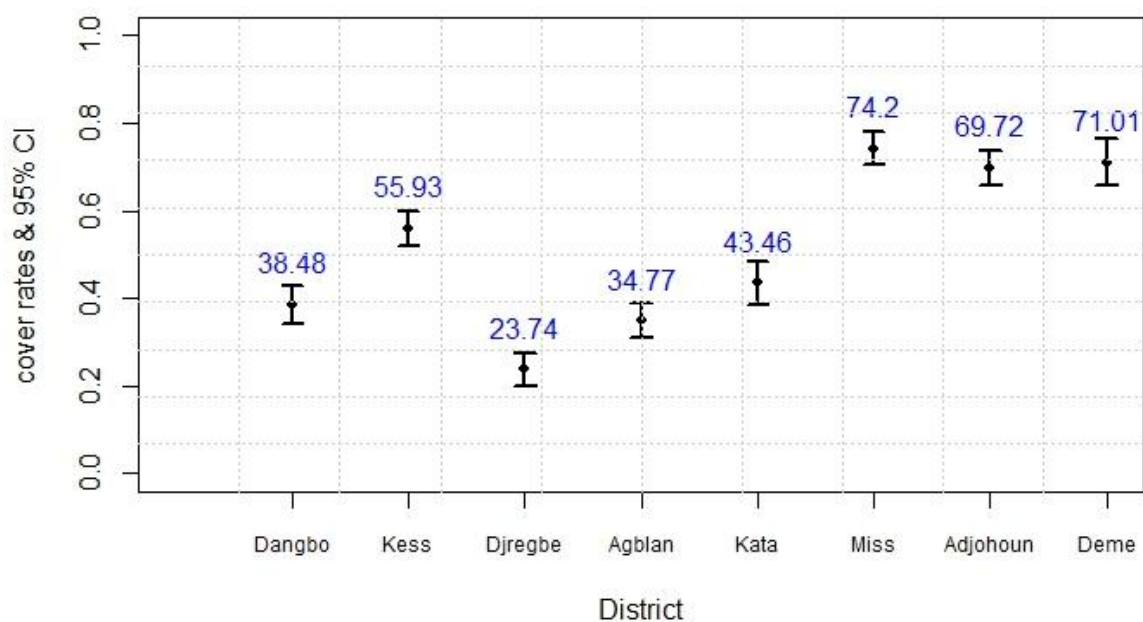
### **1. Efficacy and durability of LLINs distributed to communities in the four districts previously under IRS.**

#### ***1.1. Nature of LLINs met and rate of distribution of OlysetNets per district***

During our investigation in the 4 districts, various types of nets were found. The majority of Long Lasting Insecticidal Nets found were the OlysetNet® distributed in July 2011 by the National Malaria Control Program (59.3%: 998 Olyset among a total of 1682 registered) (table I, Figure I) and other OlysetNets (2%). Other LLINs were found: PermaNet® (20.9%) and other nets (Olyset, Permanet, Interceptor, Safinet and non-impregnated nets donated by the NMCP before 2011 or by different partners or bought by the owners: 17.8). The OlysetNets distributed in July 2011 were retained for permethrin decay analysis to appreciate their efficacy and durability after 6 months of use. The decay of deltamethrin of some Permanet were also analysed. According to the families, the other mosquito nets (other Olyset, Permanet and not identified nets were donated in 2009 and 2010 (1-2 years of use). In the district of Seme, the distribution rate of OlysetNets by the NMCP is low: 15.6% (156/998) against 29.6% (296/998) in Adjohoun, 26.1% (261/998) in Dangbo and 28.5% (285/998) in Misserete (Table I, Figure 1).

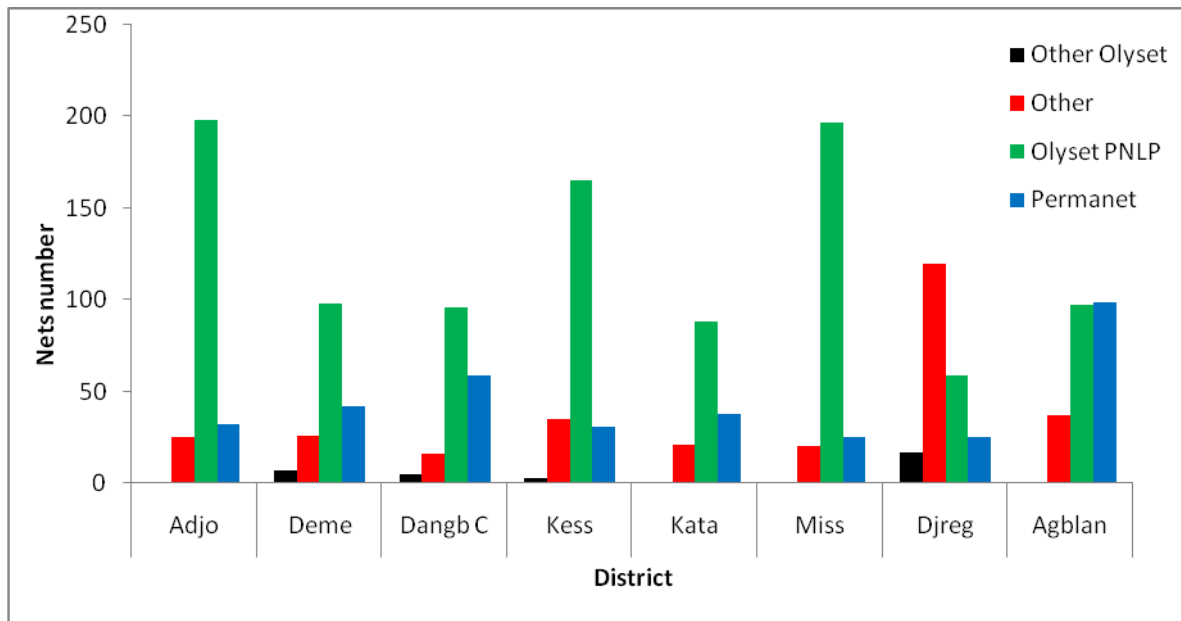


### District cover rates



**Table I:** Number of types of nets /district

Districts	Borough	Olyset NMCP	Other Olyset	Permanet	Other	Total	Total/district
ADJOHOUN	Adjohoun	198	1	32	25	256	429
	Deme	98	7	42	26	173	
	Dangbo						
DANGBO	Centre	96	5	59	16	176	410
	Kessounou	165	3	31	35	234	
MISSERETE	Katagon	88	0	38	21	147	389
	Missérété	197	0	25	20	242	
SEME	Djregbe	59	17	25	120	221	454
	Agblangandan	97	0	99	37	233	
Total		998	33	351	300	1682	1682



**Figure 1:** Type of nets recorded per district

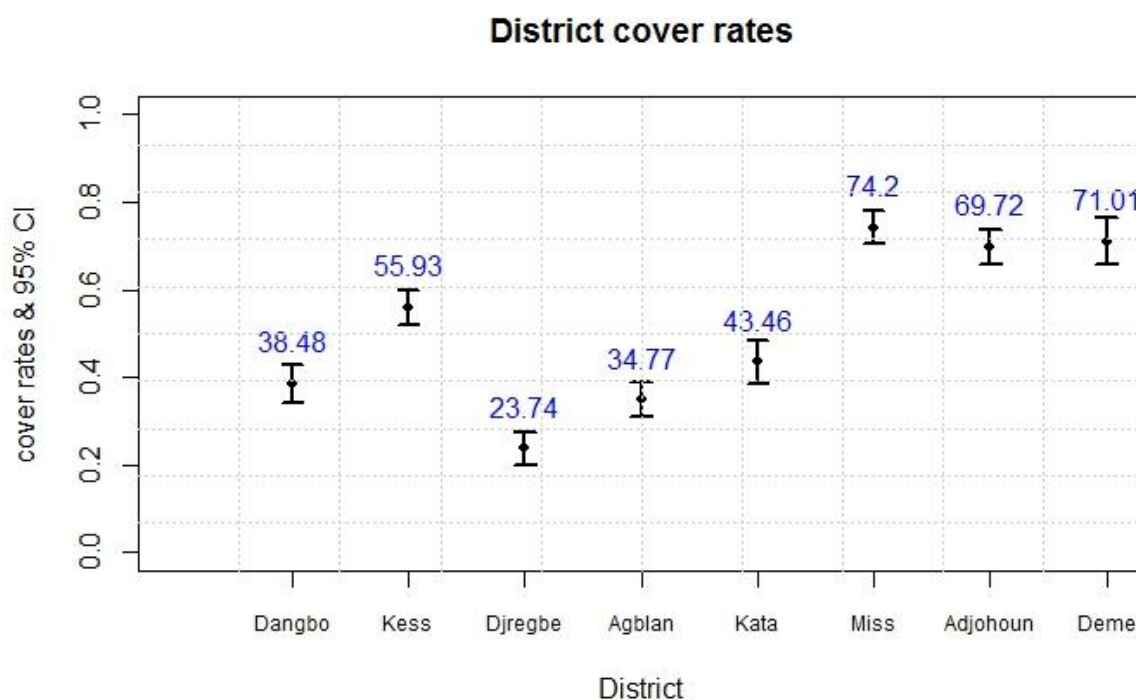
### *1.2. Coverage rate of mosquito nets per district*

The table II shows the coverage rate of OlysetNets distributed in July 2011 in the 4 districts. In total, 1996 OlysetNets were distributed for 3924 people (infants and adults). That means 50.87% of this respondents have globally received new OlysetNets, either one Olyset net per 1.9 person. This coverage rate is what was universally decided and expected by the NMCP. In Adjohoun and Missere, this rate is more than 1 Olyset per 1.9 person, respectively, 70.14% (1 Olyset for 1.4 person) and 60.90% (1 Olyset for 1.6 person). In spite this good score, the coverage rate of Olyset distributed in July 2011 is low in Dangbo district (47.93%: 1 Olyset for 2.1 person) and very low in Seme (29.57%: 1 Olyset for 3.4 person). But in these 2 districts, if we take into account the other nets available before the distribution campaign of July 2011, the coverage rate is improved: 1 mosquito net for 2.3 persons in Seme (1055/454). The coverage number of Olyset distributed in July 2011 per household is 2.96 Olyset per household in Adjohoun, 2.61 in Dangbo, 2.85 in Missere and 1.47 in Seme.

**Table II:** Coverage rate of Olyset NMCP per district

Districts	Borough	Olyset		Other nets			People		Cover rate (%)&95% CI	Coverage rate (%) /district
		Other	NMCP	Other	Permanet	Total	Visited number	Nb.received Olyset NMCP		
ADJOHOUN	Adjohoun	1	198	25	32	256	568	396	69,72 [66 73]	70,14
	Deme	7	98	26	42	173	276	196	71,01 [65 76]	
DANGBO	Dangbo Centre	5	96	16	59	176	499	192	38,48 [34 42]	47,93
	Kessounou	3	165	35	31	234	590	330	55,93 [52 60]	
MISSERETE	Katagon	0	88	21	38	147	405	176	43,46 [39 48]	60,90
	Misséréte	0	197	20	25	242	531	394	74,20 [70 77]	
SEME	Djregbe	17	59	120	25	221	497	118	23,74 [20 27]	29,57
	Agblangandan	0	97	37	99	233	558	194	34,77 [31 38]	
<b>Total</b>		33	998	300	351	1682	3924	1996	50,87 [49 52]	

Other: nets (Olyset, Permanet, Interceptor, Safinet and non-impregnated nets donated by the NMCP before 2011 or by different partners or bought by the owners)



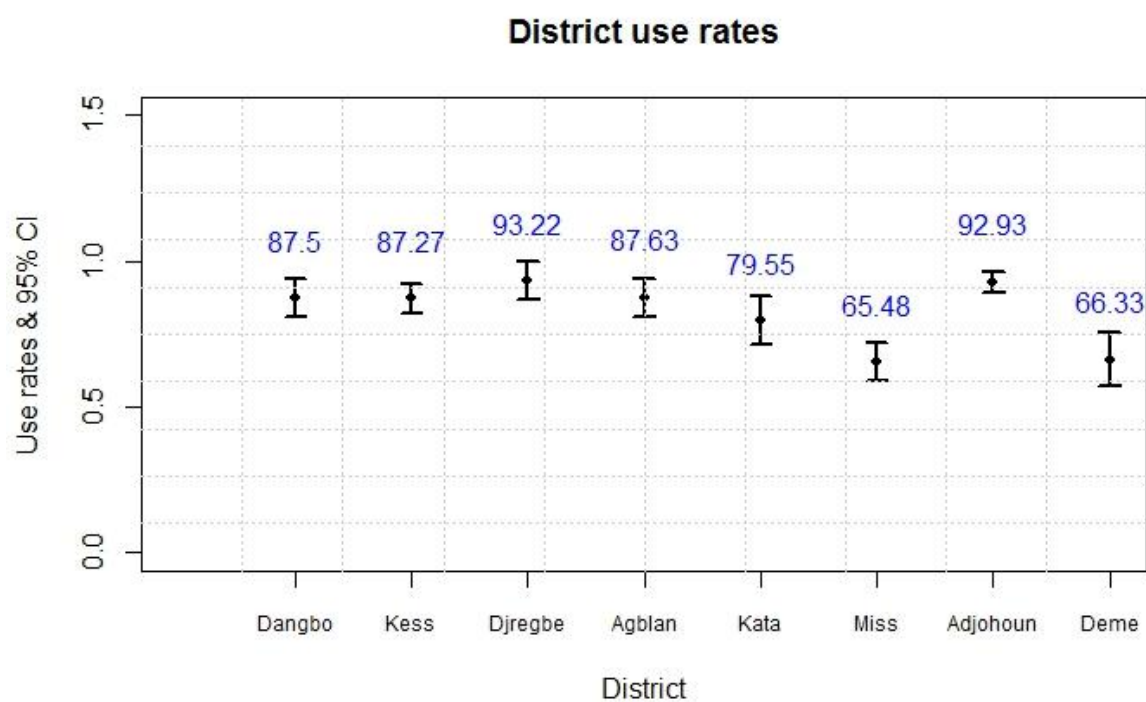
**Figure 2\_:** Coverage rate of Olyset net/district

### 1.3. Utilization rate of nets

The table III shows the rate of the use of OlysetNets distributed. Among 998 Olyset distributed, 816 were regularly used. The rate of use is 81.76%. This rate is high in all the localities visited except Misserete where it is less than 80% (Table III, Figure III).

**Table III:** Rate of use of Olyset NMCP per district

Districts	Borough	Olyset		Other nets		Total	Olyset NMCP number		Utilization rate (%)	Utilization rate (%) / district
		Other	NMCP	Other	Permanet		Found	Declared to be used		
ADJOHOUN	Adjohoun	1	198	25	32	256	198	184	92,93 [89 96]	84,12
	Deme	7	98	26	42	173	98	65	66,33 [57 75]	
DANGBO	Dangbo Centre	5	96	16	59	176	96	84	87,5 [81 94]	87,36
	Kessounou	3	165	35	31	234	165	144	87,27 [82 92]	
MISSERETE	Katagon	0	88	21	38	147	88	70	79,55 [71 87]	69,82
	Misséréte	0	197	20	25	242	197	129	65,48 [58 72]	
SEME	Djregbe	17	59	120	25	221	59	55	93,22 [87 99]	89,74
	Agblangandan	0	97	37	99	233	97	85	87,63 [81 94]	
<b>Total</b>		33	998	300	351	1682	998	816	81,76 [79 84]	



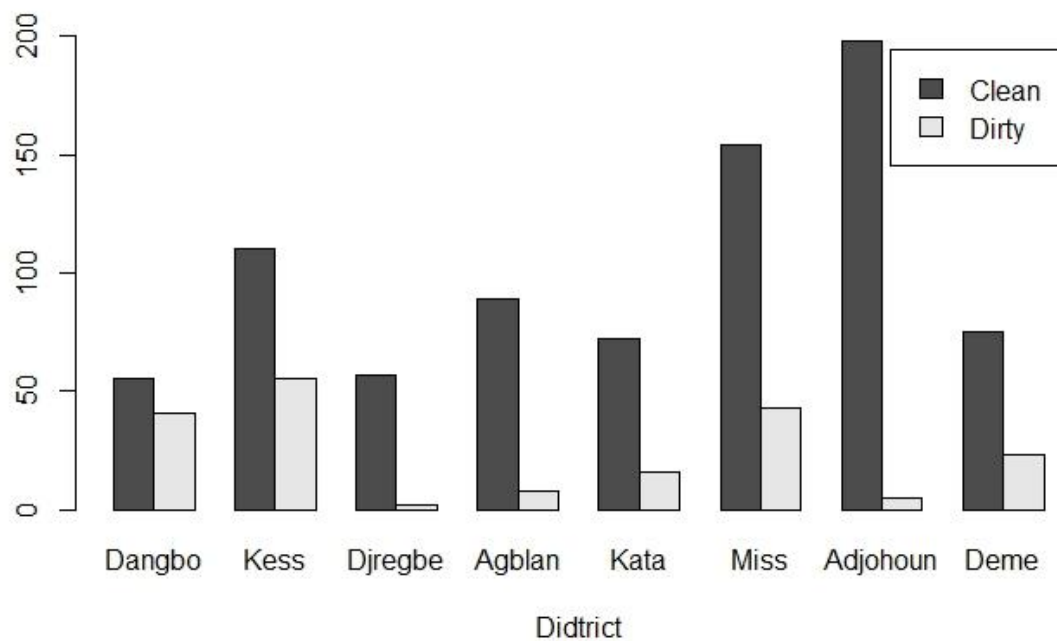
**Figure 3 :** Rate of use of Olyset NMCP per district

#### 1.4. Percentage of OlysetNets found cleaned and dirty

Most of OlysetNets observed were found cleaned (80.6%), but 19.4% of them must be considered as dirty and some of them very dirty with residues of oil, blood, dust and urine (Table IV, Figure IV)

**Table IV:** Number of Olyset found cleaned and dirty during the investigation

<b>Olyset NMCP</b>				
<b>Districts</b>	<b>Borough</b>	<b>Total</b>	<b>Clean</b>	<b>Dirty</b>
ADJOHOUN	Adjohoun	198	193	5
	Deme	98	75	23
DANGBO	Dangbo Centre	96	55	41
	Kessounou	165	110	55
MISSERETE	Katagon	88	72	16
	Misséréte	197	154	43
SEME	Djregbe	59	57	2
	Agblangandan	97	89	8
<b>Total</b>		998	805	193



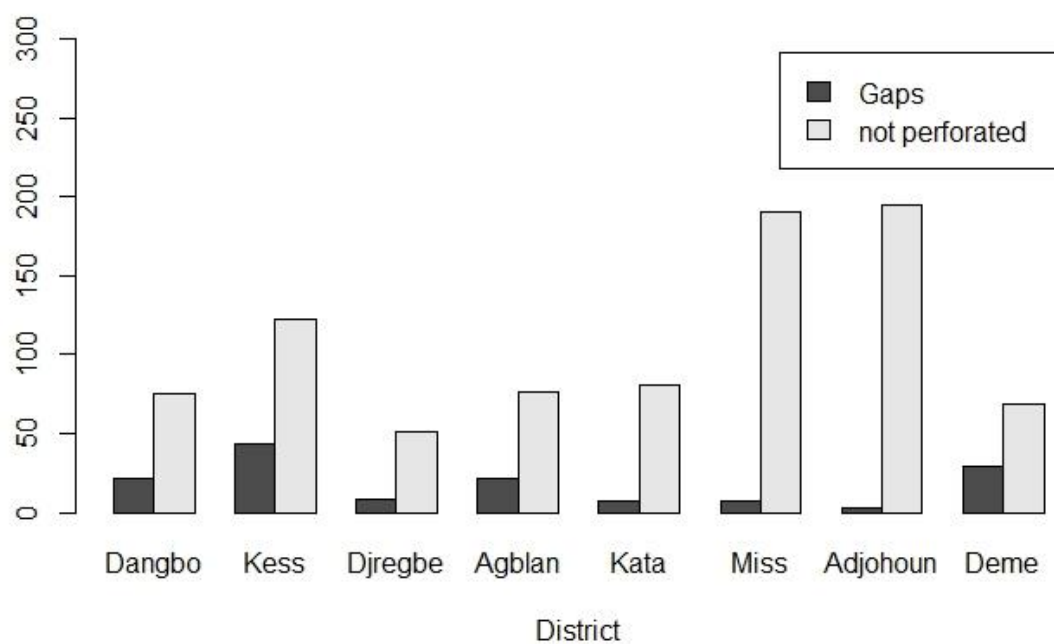
**Figure 4 :** Cleaned Olyset NMCP

### ***1.5. Durability state of Olyset after 6 months of use***

14% of Olysets were found with rips and holes after 6 months of use (Table V, Figure V).

**Table V:** Number of Olyset with holes

<b>Olyset NMCP</b>				
<b>Districts</b>	<b>Borough</b>	<b>Total</b>	<b>With holes</b>	<b>Not perforated</b>
ADJOHOUN	Adjohoun	198	3	195
	Deme	98	29	69
DANGBO	Dangbo Centre	96	21	75
	Kessounou	165	43	122
MISSERETE	Katagon	88	7	81
	Misséréte	197	7	190
SEME	Djregbe	59	8	51
	Agblangandan	97	21	76
<b>Total</b>		998	139	859



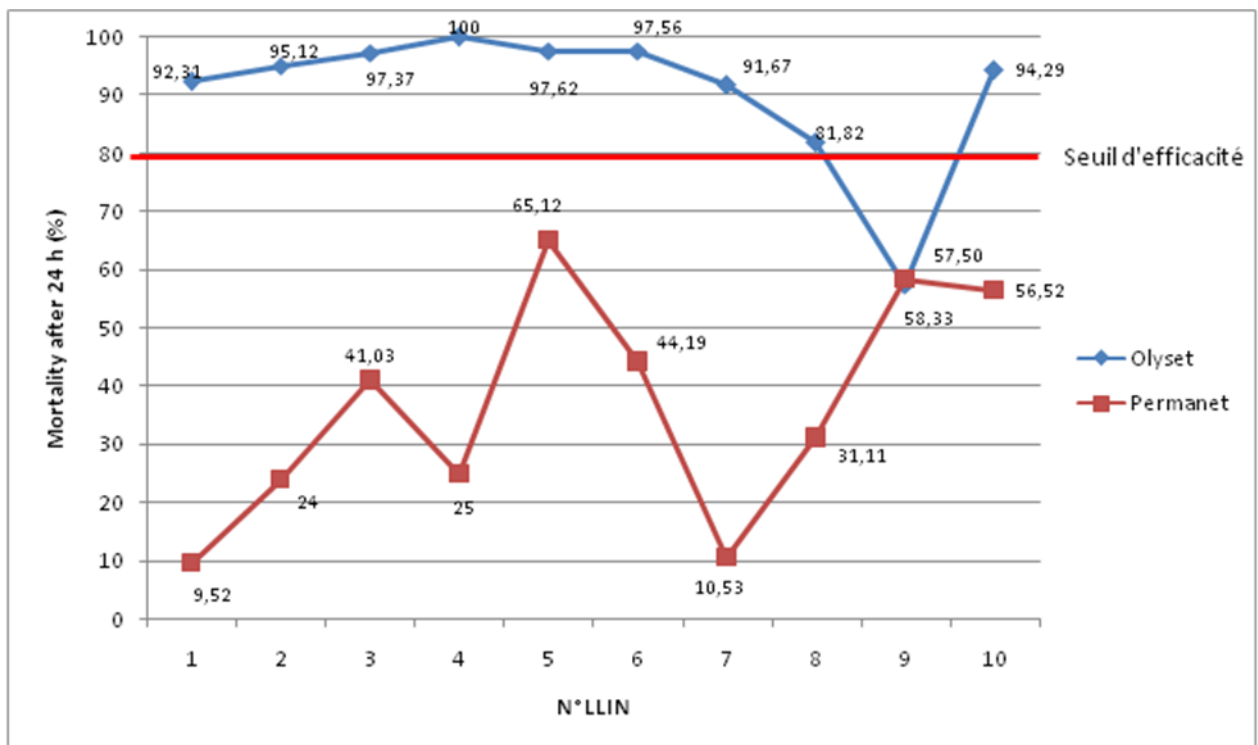
**Gaps: holes from the burning of nets by lanterns or the tears by artinales oyster beds**

**Figure 5:** Number of holed Olyset after 6 months of use

### ***1.6. Effectiveness of Olyset net after 6 months of use***

20 impregnated nets (10 Olyset distributed in July 2011 and 10 Permanet which have been using since 1-2 years: 2009-2010) were randomly selected to perform bioassay cone test. 4 other nets (2 not impregnated nets, 1 new Olyset and 1 new Permanet never used) were also tested and used as negative and positive controls. This study is a quick investigation in

addition to the results of another project ongoing at CREC supported by USAID on the effectiveness and durability of Olyset distributed in July 2011 by the NMCP in 4 sentinel sites in Benin: Kessounou, Allada, Kandi and Malanville. We can increase the sample size more than 10 Olyset and 10 Permanet, but these kinds of studies need too much *An. gambiae* for bioassay. Cone test results show that all the 10 PernaNet® were found ineffective (Mortality of *An. gambiae* Kisumu less than 80%). Concerning Olyset, 1 net was found ineffective among the 10 tested (10%) (see figure 6 below)



**Figure 6 :** Mortality rate observed after 24h of *An.gambiae* kisumu (susceptible strain) after 3 min exposure to Olyset NMCP and Permanet removed from areas previously under IRS

## 2. Entomological data

### Malaria transmission data

Only 49 *An. gambiae* were collected in the 5 districts: 42 in the control area, 4 in Missérété, 1 in Adjohoun, 1 in Sèmè and 1 in Dangbo. The low density of vectors is due to the dry season. During this period, most of the breeding sites of *An. gambiae* are dry. The table I shows the distribution of malaria vectors and other mosquitoes collected in December 2011.

The human biting rate (HBR) is less than 1 bite per man per night:

Adjara (control):  $HBR = 42/16 = 2.6$  bites of *An. gambiae* per night = 78 bites for the month of December (theorically)

Adjohoun, Dangbo and Seme: HBR = 1/16 = 0.06 bite of *An. gambiae* per night = 1.87 bite for the month of December

Missereete: HBR = 4/16 = 0.25 bite of *An. gambiae* per night = 7.5 bites for the month of December

All *An. gambiae* collected were analyzed for their infectivity for *Plasmodium falciparum*.

Among 6 *An. gambiae* collected in the districts previously under IRS, no CS+ was found, but in the control district, 1 CS+ was registered among 42 analyzed.

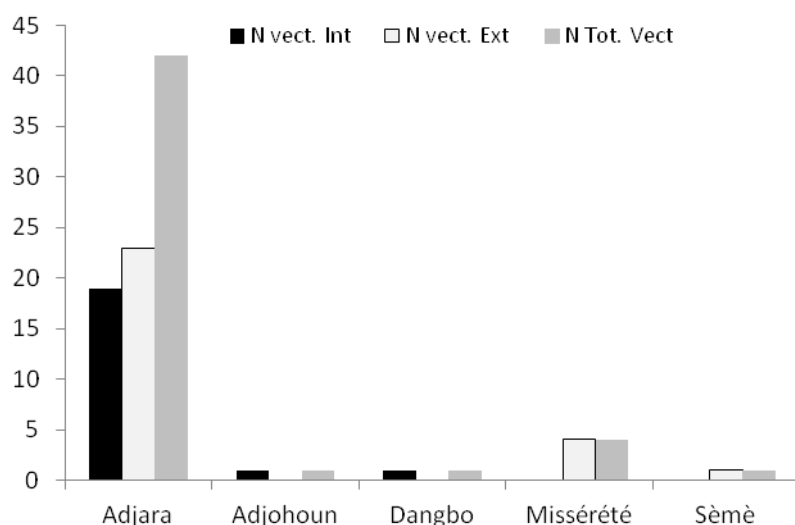
The ovaries of 43 *An. gambiae* were dissected and read according to Detinova method. The parturity rate is very high: 85.7-100% (Table III)

### Behaviour of mosquitoes

63 mosquitoes were collected using exit window traps. There was no malaria vector, then the exophly rate was not calculated. The distribution of these mosquitoes is shown in table IV.

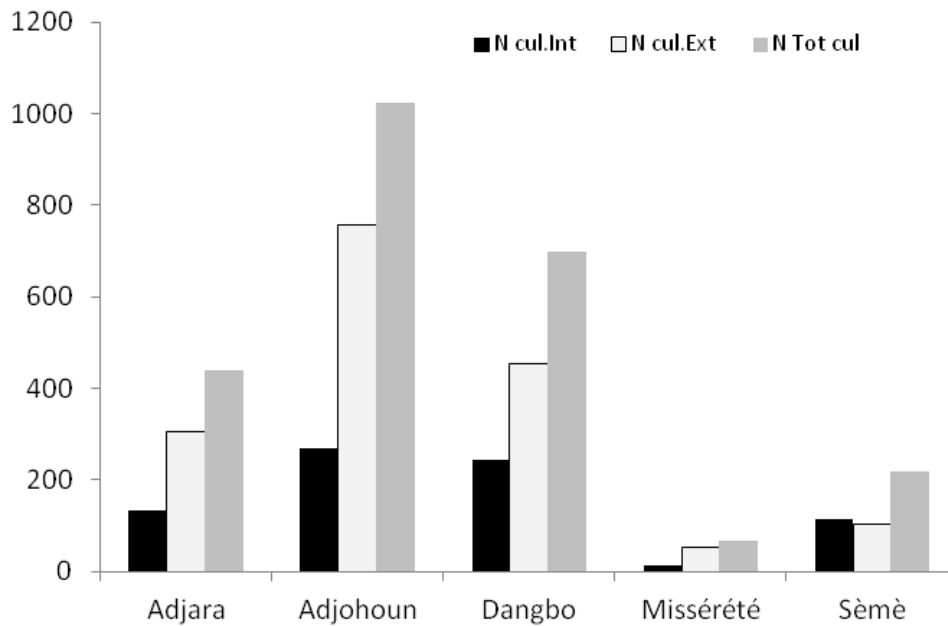
**Table I:** Distribution of malaria vectors and other mosquitoes collected in December 2011

Communes	An. gambiae			An. funestus			Autres An.			Cx. quinque			Aedes aegypti			Autres			Total		
	Int	Ext	Tot	Int	Ext	Tot	Int	Ext	Tot	Int	Ext	Tot	Int	Ext	Tot	Int	Ext	Tot	Int	Ext	Tot
Adjara	19	23	42	0	0	0	9	79	88	21	75	96	0	0	0	85	129	214	134	306	440
Adjohoun	1	0	1	0	0	0	5	6	11	77	239	316	0	0	0	185	511	696	268	756	1024
Dangbo	1	0	1	0	0	0	4	7	11	8	61	69	0	0	0	232	387	619	245	455	700
Misséréte	0	4	4	0	0	0	0	0	0	2	30	32	0	0	0	10	20	30	12	54	66
Sèmè	0	1	1	0	0	0	0	4	4	19	7	26	0	0	0	97	92	189	116	104	220
<b>Total</b>	21	28	49	0	0	0	18	96	114	127	412	539	0	0	0	609	1139	1748	775	1675	2450



**Figure 1:** Number of malaria vectors (*An. gambiae*) per district





**Figure 2:** Number of total of mosquitoes (Culicidae) per district

**Tableau II:** Distribution of mosquitoes collected per area

Communes	Zones	<i>An. gambiae</i>			Culex spp.			Mansonia spp.			Aedes spp.			Total		
		Int	Ext	Tot	Int	Ext	Tot	Int	Ext	Tot	Int	Ext	Tot	Int	Ext	Tot
<b>Adjarra</b>	Plateau	0	0	0	14	33	47	3	0	3	0	0	0	19	33	52
	Marécageuse	19	23	42	15	80	95	74	91	165	0	0	0	115	273	388
<b>Adjohoun</b>	Plateau	1	0	1	27	110	137	56	120	176	0	0	0	86	231	317
	Marécageuse	0	0	0	52	142	194	123	371	494	4	7	11	182	525	707
<b>Dangbo</b>	Plateau	0	0	0	3	10	13	4	9	13	0	0	0	8	20	28
	Marécageuse	1	0	1	8	62	70	225	367	592	0	0	0	237	435	672
<b>Missérété</b>	Plateau	0	2	2	2	29	31	0	5	5	0	0	0	2	36	38
	Marécageuse	0	2	2	1	2	3	9	14	23	0	0	0	10	18	28
<b>Sèmè</b>	Plateau	0	0	0	4	9	13	15	47	62	0	0	0	19	60	79
	Marécageuse	0	1	1	19	2	21	78	41	119	0	0	0	97	44	141

**Tableau III:** Physiological age grading (parturity rate) of *An. gambiae*

Communes	<i>An. gambiae</i>		
	N disséqués	N pares	Taux de pare (%)
<b>Adjara</b>	42	36	85,71
<b>Adjohoun</b>	1	1	100
<b>Dangbo</b>	1	1	100
<b>Misséréte</b>	4	4	100
<b>Sèmè</b>	1	1	100

**Tableau IV:** Distribution of mosquitoes collected using exit window traps and clay pots

Communes	<i>An. gambiae</i>				<i>An. funestus</i>				Autres An.				<i>Cx. quinque</i>				<i>Aedes aegypti</i>				Autres				Total							
	Sp		Fp		Pot		Tot		Sp		Fp		Pot		Tot		Sp		Fp		Pot		Tot		Sp		Fp		Pot		Tot	
	Sp	Fp	Pot	Tot	Sp	Fp	Pot	Tot	Sp	Fp	Pot	Tot	Sp	Fp	Pot	Tot	Sp	Fp	Pot	Tot	Sp	Fp	Pot	Tot	Sp	Fp	Pot	Tot				
<b>Adjara</b>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	36	37	0	0	0	0	4	0	0	4	5	0	36	41				
<b>Adjohoun</b>	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0	0	3	0	0	3	5	0	0	5				
<b>Dangbo</b>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
<b>Misséréte</b>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1				
<b>Sèmè</b>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	14	0	1	15	15	0	1	16				
<b>Total</b>	0	0	0	0	0	0	0	0	0	0	0	0	5	0	36	41	0	0	0	0	21	0	1	22	26	0	37	63				

Sp: spray      Fp= exit window trap;      Pot : Clay pot

### 3. Evolution of *An. gambiae* resistance to insecticides after cessation of IRS

In 2010 and 2011, we have organized three investigations on the susceptibility of *An. gambiae* in areas where Indoor Residual Spraying was implemented from 2008 to 2010 in order to determine the impact of IRS on the evolution of vector resistance. The goal of the investigations is to verify if 3 years use of bendiocarb at large-scale in Oueme-Plateau has increased or decreased the susceptibility of *An. gambiae* to insecticides. The surveillance of susceptibility is done every year since the beginning of IRS in 2008. This report shows here data obtained last year (2010) and in 2011, particularly in December 2011.

#### Bendiocarb

One of the criteria of the selection of bendiocarb for IRS in Oueme-Plateau is due the fully susceptibility of *An. gambiae* to this insecticide. After 4 years of use of bendiocarb, we have registered no resistance to this insecticide. The susceptibility tests performed in December

2011 have confirmed this result (Tables XII-XVI, Figures 13a-13d). But a decrease of susceptibility found in Adjohoun (mortality rate = 89.9%) will be followed.

### **DDT**

A very high resistance was registered to DDT during the whole period of the IRS intervention until today. This high resistance was mentioned since 1999 (Akogbeto and Yacoubou, 1999).

### **Deltamethrin**

Before 2010, *An. gambiae* was fully susceptible to deltamethrin. But from 2010-2011, the susceptibility tests performed show a decrease of susceptibility or a fully resistance of *An. gambiae* to deltamethrin. The emergence of *An. gambiae* resistance to deltamethrin in 2011 was observed not only in areas previously under IRS but also everywhere in Benin.

### **Permethrin**

*An. gambiae* has developed a very high resistance to permethrin in Benin and particularly in Oueme, what was observed since 1999. But in December 2011, this high resistance has decreased in intensity. As shown in tables XII-XVI and Figures 13a-13d, the mortality rates of the specimens analyzed in Adjohoun, Dangbo, Seme and Missereté were lower in 2010 than in November-December 2011. We suggest to follow this phenomenon next year.

## **4. Evolution of vector resistance mechanisms**

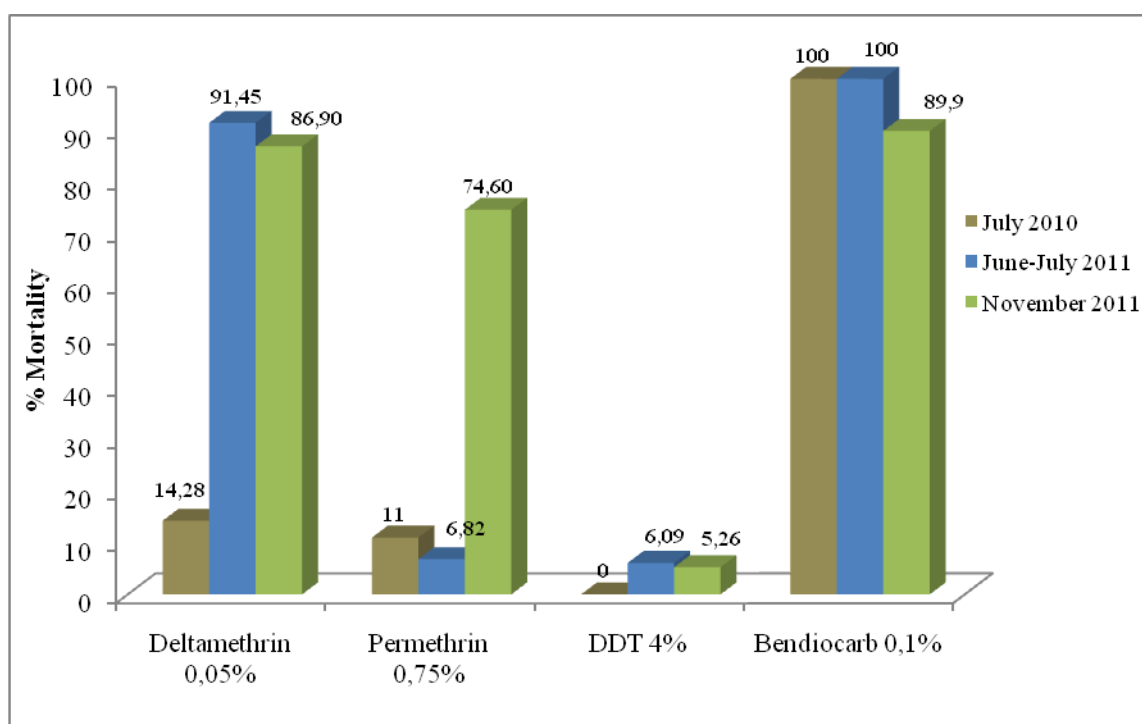
After 4 months of use of IRS in Oueme-Plateau, the massive use of bendiocarb has not induced the development of the ACHE mechanism by increasing Ace-1<sup>R</sup> mutation. The frequency of Ace-1<sup>R</sup> is zero everywhere in 2010 and 2011 except in the control area (Adjara): 0.02.

The main resistance mechanism observed is Kdr mutation which frequency is very high, around 80% in 2010 and 2011.

**Table XII:** Percentage of dead *An. gambiae* observed after one hour exposure to papers treated with various insecticides during IRS implementation (April-December 2010) and after cessation of IRS (April-December 2011) in Adjohoun district.

Locality	Insecticides	Years	Number of mosquitoes exposed	Mortality (%)	Status
Adjohoun	Deltamethrin 0,05%	July 2010	28	14,28	R
		June-July 2011	117	91,45	R
		Nov-Dec 2011	84	86,90	R
	Permethrin 0,75%	July 2010	53	11	R
		June-July 2011	88	6,82	R
		Nov-Dec 2011	63	74,60	R
	DDT 4%	July 2010	35	0	R
		June-July 2011	82	6,09	R
		Nov-Dec 2011	19	5,26	R
	Bendiocarb 0,1%	July 2010	34	100	S
		June-July 2011	84	100	S
		Nov-Dec2011	89	89,9	r

**NB:** For all susceptibility tests the mortality of control mosquitoes is less than 5%

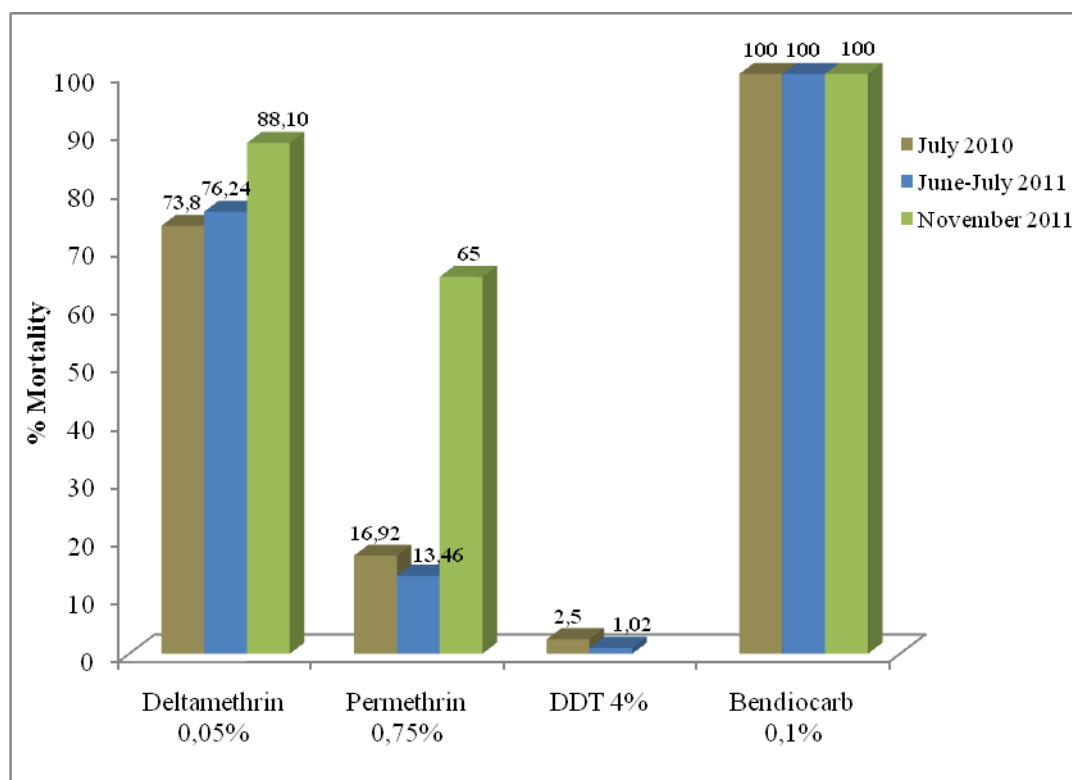


**Figure 13a:** Diagram of percentage of dead *An. gambiae* observed after one hour exposure to papers treated with various insecticides during IRS implementation (April-December 2010) and after cessation of IRS (April-December 2011) in Adjohoun district

**Table XIII:** Percentage of dead *An. gambiae* observed after one hour exposure to papers treated with various insecticides during IRS implementation (April-December 2010) and after cessation of IRS (April-December 2011) in Dangbo district.

Locality	Insecticides	years	Number	Mortality (%)	Status
Dangbo	Deltamethrin 0,05%	July 2010	84	73,8	R
		June-July 2011	101	76,24	R
		Nov-Dec 2011	42	88,10	R
	Permethrin 0,75%	July 2010	65	16,92	R
		June-July 2011	104	13,46	R
		Nov-Dec 2011	20	65	R
	DDT 4%	July 2010	48	2,5	R
		June-July 2011	98	1,02	R
		Nov-Dec 2011			
	Bendiocarb 0,1%	July 2010	45	100	S
		June-July 2011	112	100	S
		Nov-Dec 2011	40	100	S

100 mosquitoes were planned to be analyzed. But, due to the unavailability of breeding sites, we did not reach this number.

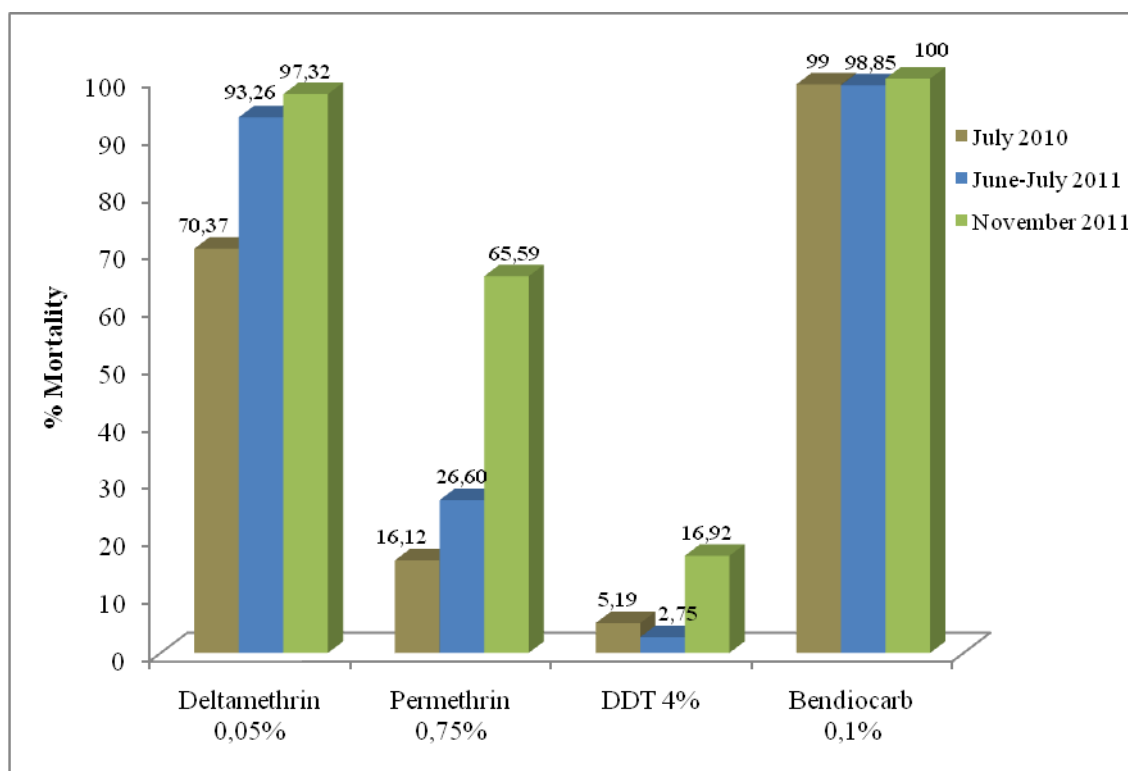


**Figure 13b:** Diagram of percentage of dead *An. gambiae* observed after one hour exposure to papers treated with various insecticides during IRS implementation (April-December 2010) and after cessation of IRS (April-December 2011) in Dangbo district

**Table XIV:** Percentage of dead *An. gambiae* observed after one hour exposure to papers treated with various insecticides during IRS implementation (April-December 2010) and after cessation of IRS (April-December 2011) in Missérété district.

Locality	Insecticides	years	Number	Mortality (%)	Status
Missérété	Deltamethrin 0,05%	July 2010	54	70,37	R
		June-July 2011	89	93,26	R
		Nov-Dec 2011	112	97,32	R
	Permethrin 0,75%	July 2010	62	16,12	R
		June-July 2011	94	26,60	R
		Nov-Dec 2011	93	65,59	R
	DDT 4%	July 2010	77	5,19	R
		June-July 2011	109	2,75	R
		Nov-Dec 2011	65	16,92	R
	Bendiocarb 0,1%	July 2010	100	99	S
		June-July 2011	87	98,85	S
		Nov-Dec 2011	112	100	S

100 mosquitoes were planned to be analyzed. But, due to the unavailability of breeding sites, we did not reach this number.

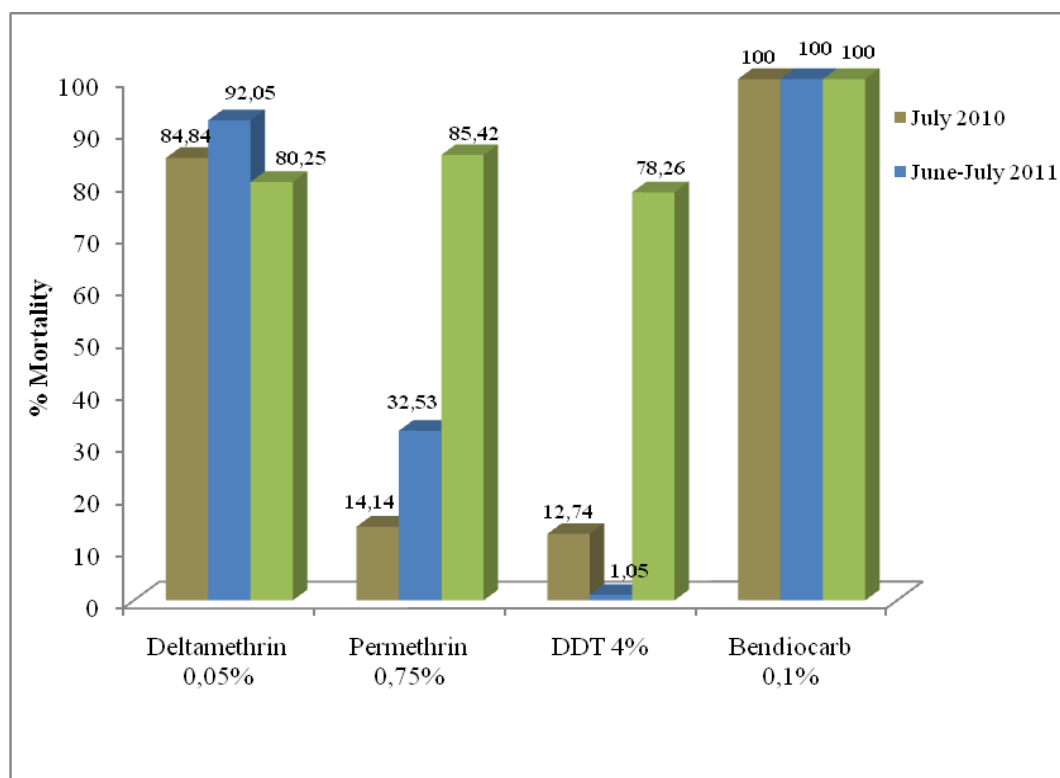


**Figure 13c:** Diagram of percentage of dead *An. gambiae* observed after one hour exposure to papers treated with various insecticides during IRS implementation (April-December 2010) and after cessation of IRS (April-December 2011) in Missérété district

**Table XV:** Percentage of dead *An. gambiae* observed after one hour exposure to papers treated with various insecticides during IRS implementation (April-December 2010) and after cessation of IRS (April-December 2011) in Sèmè district.

Locality	Insecticides	years	Number	Mortality (%)	Status
Sèmè	Deltamethrin 0,05%	July 2010	99	84,84	R
		June-July 2011	88	92,05	R
		Nov-Dec 2011	81	80,25	R
	Permethrin 0,75%	July 2010	99	14,14	R
		June-July 2011	83	32,53	R
		Nov-Dec 2011	48	85,42	R
	DDT 4%	July 2010	102	12,74	R
		June-July 2011	95	1,05	R
		Nov-Dec 2011	46	78,26	R
	Bendiocarb 0,1%	July 2010	49	100	S
		June-July 2011	119	100	S
		Nov-Dec 2011	49	100	S

100 mosquitoes were planned to be analyzed. But, due to the unavailability of breeding sites, we did not reach this number.



**Figure 13d:** Diagram of percentage of dead *An. gambiae* observed after one hour exposure to papers treated with various insecticides during IRS implementation (April-December 2010) and after cessation of IRS (April-December 2011) in Sèmè district.

**Table XVI:** Kdr and Ace-1 mutation with *An.gambiae* collected during IRS implementation and after cessation of IRS in Oueme

Localities	Years	Tested Number	Species		Kdr mutation				Ace-1 mutation			
			Am	Ag	RR	RS	SS	F (Kdr)	RR	RS	SS	F (Ace 1R)
Adjohoun	2010	24	0	24	16	8	0	0,83	0	0	24	0
	2011	23	0	23	12	8	0	0,80	0	0	23	0
Dangbo	2010	25	0	25	19	6	0	0,88	0	0	25	0
	2011	20	0	20	10	5	0	0,83	0	0	20	0
Misséréte	2010	22	0	22	18	4	0	0,91	0	0	22	0
	2011	26	0	26	15	6	0	0,86	0	0	26	0
Sèmè	2010	25	0	25	20	5	0	0,9	0	0	25	0
	2011	28	0	28	18	7	0	0,86	0	0	28	0
Adjara (Ctrl)	2010	27	0	27	21	6	0	0,89	0	0	27	0
	2011	27	0	27	25	2	0	0,96	0	1	26	0,02