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PMI/IRS OR ACTIVITIES IN BENIN

ENTOMOLOGICAL MONITORING-EVALUATION IN DISTRICTS UNDER INDOOR RESIDUAL SPRAYING IN ATACORA DEPARTMENT, BENIN, WEST AFRICA

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Summary

Adhesion of the communities of Atacora to IRS

Before implementing Indoor Residual Spraying in Atacora, a sociological survey was conducted to collect information on the communities' perception about malaria, the practices of these communities about the prevention of malaria and acceptability/refusal, or possible rejection of IRS, their point of view about IRS. The goal of this investigation is to get the adhesion and the participation of these communities for the implementation of this strategy.

More than 97% of 1058 respondents agree with the implementation of IRS in their areas. Participants to the Focus Group discussion organized in Tanguieta automatically applauded for the implementation of IRS in Atacora. However, 28 out of 1058 respondents do not support the implementation of the strategy because they consider that insecticides are toxic for human.

Decay of bendiocarb

In Atacora, the residual activity of bendiocarb is good for 3 months. Beyond 3 months, the efficiency of bendiocarb has drastically decreased below the threshold of efficacy (80% mortality of *An. gambiae*) as well for the susceptible strain of *An. gambiae* (Kisumu) as for the local resistant strain. (see figure 3).

Compared to what we have observed in the department of Oueme, the decay of bendiocarb in Atacora is rapid. This situation is particularly due to the quality of the walls. In Atacora, the majority of the walls are mad with mud. Therefore, the mud is of a porous structure and insecticide can easily go inside. The consequence would be a decrease of insecticide on the surface of the walls. Considering the high percentage of houses made with mud in the rural areas, we recommend to the manufacturers to develop formulations of insecticide which can cover the surface of walls as a solid and adhesive film after spraying.

Decrease of Hunan Biting Rate of mosquitoes in districts under IRS

The results show a decrease of human biting rate (HBR) in districts under IRS compared to the control, particularly Materi, Natitingou and Kouande where each inhabitant receives,

respectively 51.90 and 111 bites from *An gambiae* per month, during the rainy season. At the same time, the human biting rate is high in Pehunco, the control area (381bites/man/month), either more than 75% of reduction of human biting rate thanks to IRS strategy using bendiocarb in the 3 districts under IRS. However, in the 4th district under IRS (Tanguieta), a little reduction was found: 205 bites per man per month from *An. gambiae* against 381 in the control area (46.6% of reduction). Compared to what we have obtained in Oueme, the decrease of HBR is lower.

Decrease of Entomological Inoculation Rate (EIR) in districts under IRS

In the control area, each inhabitant receives a mean of 23.8 infected bites per month during the study period. In areas under IRS, this rate was drastically reduced: 2.54 infected bites per month. When we compare the EIR observed in the control area to those of districts under IRS, the decline is very high: 90%. But, in spite of this decrease, this rate of EIR is high compare to the results of Oueme campaign.

Decrease of the lifespan of *An. gambiae* in districts under IRS

In the control district, physiological age grading is high: 84.3%. But in the districts under IRS, it is low: 52.38, 52.78, 56.34 and 34.85, respectively in Tanguieta, Materi, Natitingou and Kouande.

Impact of IRS on blood feeding of *An. gambiae*

In spite of IRS implementation, the proportion of mosquitoes which succeed to go into bedrooms also succeed in feeding on human. The blood feeding index is as high in the control district as in districts under IRS. This situation was found in 2007 in experimental huts when we were evaluating various insecticides in Akron (see Akogbeto *et al*., 2010, *Malaria journal*) and during the first IRS campaign in Oueme (Akogbeto et al, 2011, *AJTMH*). For us, the implementation of IRS should not exclude the use of LLINs by the community. Indeed, some families, particularly husbands and wives are used to sleep under bednets. For these families, bednets are considered as intimate tool. Inspite implementation of IRS, these families would be encouraged to use their bednets. The best strategy is the joint use of IRS + LLIN. This combination not only protects homes from the invasion of mosquitoes, it also prevents contact between humans and mosquitoes. Such a strategy implicates an increase of the cost of malaria prevention and can't be implemented everywhere. It must be reserved only for areas of highest malaria transmission.

Increase of exophily of *An.gambiae* in areas under IRS

We organized mosquito sampling using exit window traps and pyrethrum spray catches to measure exophily induced by the presence of bendiocarb on treated walls. The natural exophily is 50%. In intervention districts, the rate is higher: 71.18%% (Materi), 79.48% (Natitingou). The high induced exophily indicates bendiocarb is not conducive to mosquito survival for a long time.

Evolution of *An. gambiae* resistance to bendiocarb in Atacora: *Anopheles gambiae is losing its susceptibility to bendiocarb*

We have compared the data obtained on the susceptibility test performed in 2010 to what we have observed in 2011. *An. gambiae* is losing its susceptibility to bendiocarb. In October 2010, the percentages of dead *An.gambiae* observed after one hour exposure to papers treated with bendiocarb were 98%, 95%, 97%, 98%, respectively in Matéri, Tanguiéta, Natitingiu and Kouandé. Then, *An. gambiae* was susceptible to bendiocarb. One year later (October 2011), the situation has changed: the percentages of mortality have decreased, respectively to 87%, 93%, 85% and 89% in the 4 districts. *An. gambiae* is progressively developing resistance to the carbamates in Atacora. The development of resistance to carbamates in Atacora is shown by the presence of some *Ace.1* mutation

In conclusion, it is important to stop the use of bendiocarb for IRS in Atacora to avoid to increase the level of resistance to carbamates.

INTRODUCTION

After the success of Indoor Residual Spraying (IRS) strategy implemented from 2008 to 2010 by the National Malaria Control Program (NMCP) in department of Oueme with the support of PMI, the NMCP has decided to extend the implementation of this strategy to other regions of Benin. The department of Atacora has been secondly proposed by Benin NMCP. The President's Malaria Initiative (PMI) of US Government has accepted to give support for the Monitoring-Evaluation (M&E) of this intervention. The goal of this M&E is to evaluate the entomological and sociological impact of the intervention.

The following information was expected:

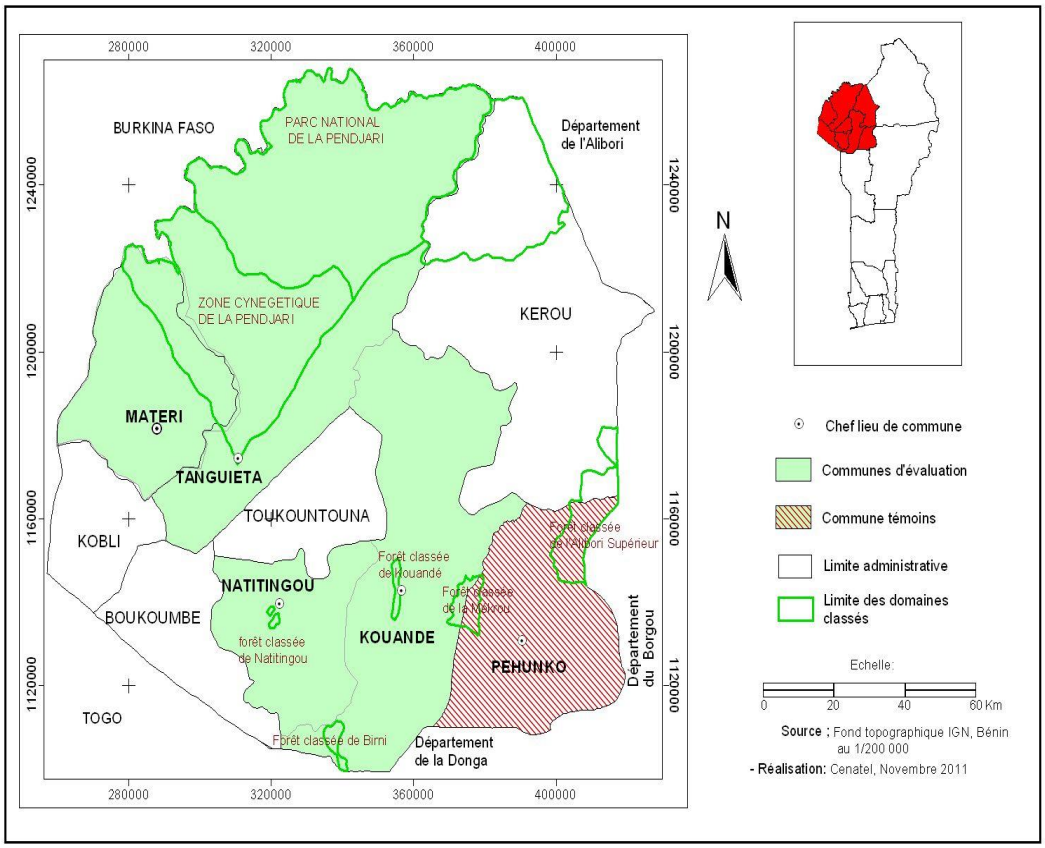
● Decay of bendiocarb treated on walls in a context of high temperature and relatively low humidity compared to what was observed in southern Benin (Oueme-Plateau)

● Evolution of pyrethroid resistance and involved mechanisms in districts under IRS

● Behavioral changes of mosquitoes (physiological age grading, indoor/outdoor feeding, exophilic rate), dynamics of M and S forms of *An. gambiae* and Entomological Inoculation Rate (EIR) in districts under IRS compared to the control district.

● Capacity building

Seven districts out of nine in Atacora were retained for this intervention. Four of them were randomly selected for the monitoring-evaluation (Kouande, Materi, Tanguieta and Natitingou). One district (Pehunco) not far from Kouande and not concerned by IRS was used as control.



CARTE DE LA ZONE D'ETUDE MONTRANT LES COMMUNES D'EVALUATION

Figure 1. Study area showing the 9 districts (communes) belonging to Atacora department, the districts under IRS and under M&E (in green) and the control district (Pehunco)

ACTIVITIES CARRIED OUT DURING

1. Sociological investigation before IRS implementation

1. 1. Protocol

A sociological survey was conducted to collect information on the community's perception about malaria, their practices about the prevention of malaria and acceptability/refusal, or possible rejection of IRS, their point of view about IRS.

The general objective of this investigation is to collect information on the perception of communities on the implementation of indoor residual spraying.

More than 1000 houses (1058) were randomly selected. The survey was conducted with emphasis on individual perceptions and focus group discussions addressed to the head of households particularly women and local leaders. Discussions were based on a quantitative and qualitative questionnaire. These discussions were completed by a direct field observation. The protocol was articulated on getting sociological data on:

-Communities knowledge on the relationship between mosquitoes and malaria

-Communities knowledge on the relationship between mosquitoes and disease

-Prevention measures against mosquito bites

-Advantages/Disadvantages of IRS

-Percentage of people who accept IRS implementation in Atacora

1.2. Results

Communitie knowledge on the relationship between mosquitoes and malaria

Malaria is an old disease known by everybody in Atacora. However, 1 person out of 4 (27%) ignores it is transmitted by mosquitoes (Fig. 1).

Communities knowledge on the relationship between mosquitoes and disease

All the respondents recognized that mosquitoes are vectors of disease. Among them, 64% indicate that the main transmitted disease by mosquitoes is malaria (Fig. 2)

Prevention measures against mosquito bites

Different control methods are used in Atacora to protect against mosquito bites: bed nets, aerosols, mosquito coils, plants. More than 92% of our respondents believe that it is not possible to sleep at night without any protection against mosquitoes, especially during the rainy season. However, 84 persons out of 1058 (7.23%) take no precaution against mosquitoes. Bednets are the main tool used to control malaria (90.82%) (Fig. 3)

Acceptability of IRS

Population of Atacora had no information on vector control strategy based on indoor residual spraying. We had to explain the principle by indicating that the method is similar to aerosols that some of them use in their bedrooms at night. Plus, IRS is for a large scale and a long-term efficacy. This information has positively led 97.42% of 1058 respondents to agree on the implementation of IRS in their areas. Participants to the Focus Group discussion held in Tanguieta automatically applauded the implementation of IRS in Atacora. They stated the advantages that could be generated by IRS strategy (reduction of malaria, insects, diseases…). Among those who support the implementation of IRS in their area, some acknowledged that the insecticides are toxic and smelly (Fig. 5). However, 28 out of 1058 respondents do not support the implementation of the strategy because they consider that insecticides are toxic for human.

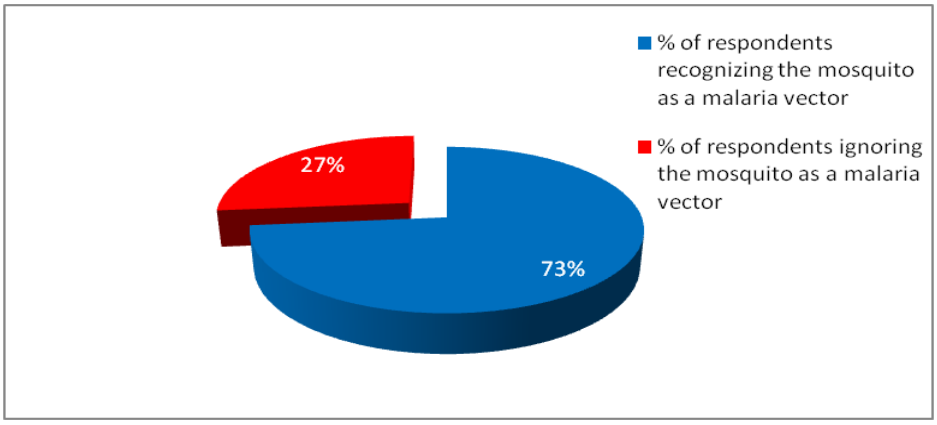


Fig.1: Communities knowledge on the relationship between mosquitoes and malaria

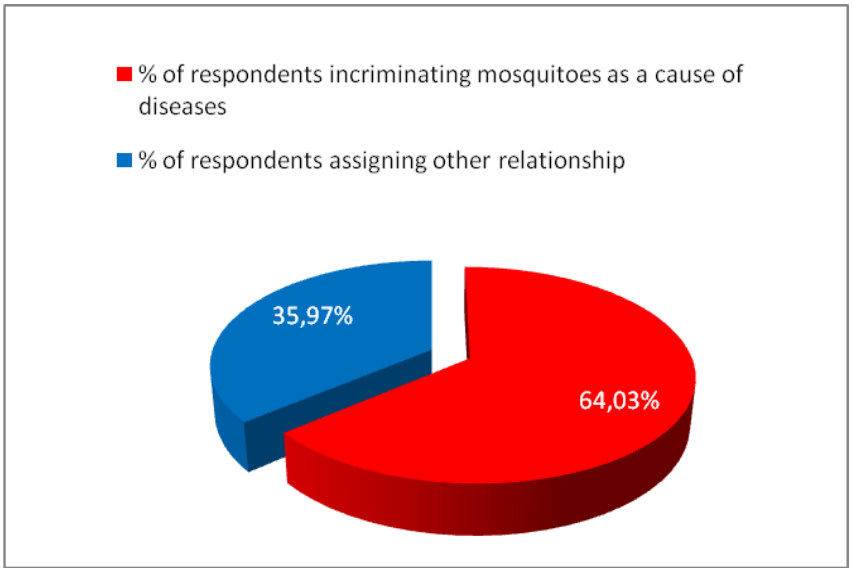


Fig.2: Percentage of respondents on the relationship between mosquitoes and disease

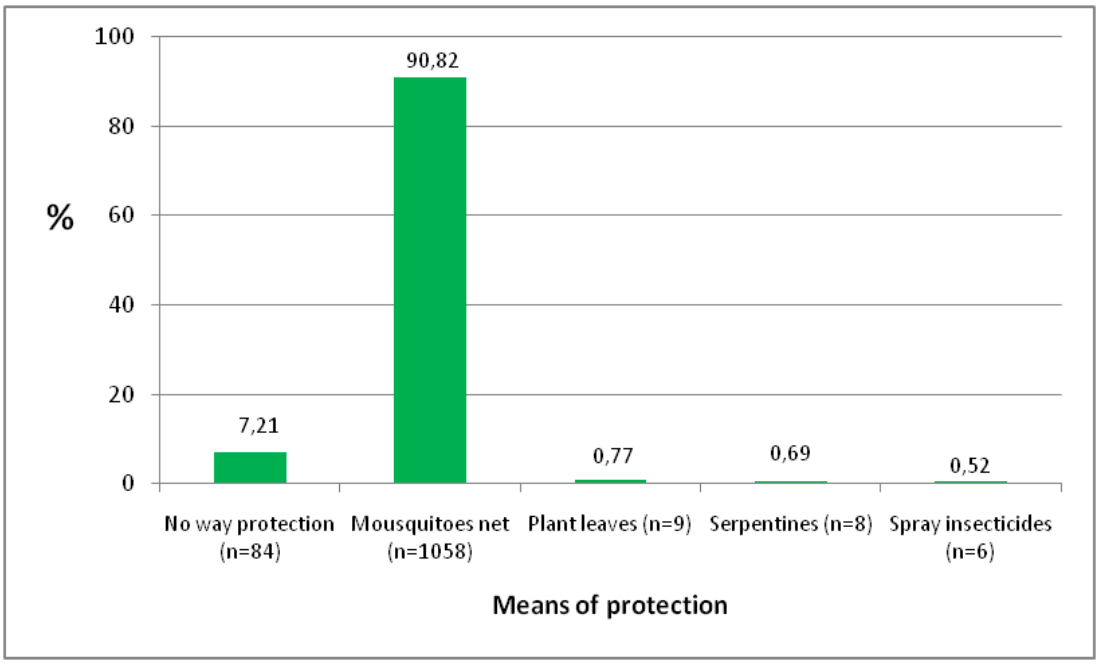


Fig.3: Mean measures of prevention against mosquito bites

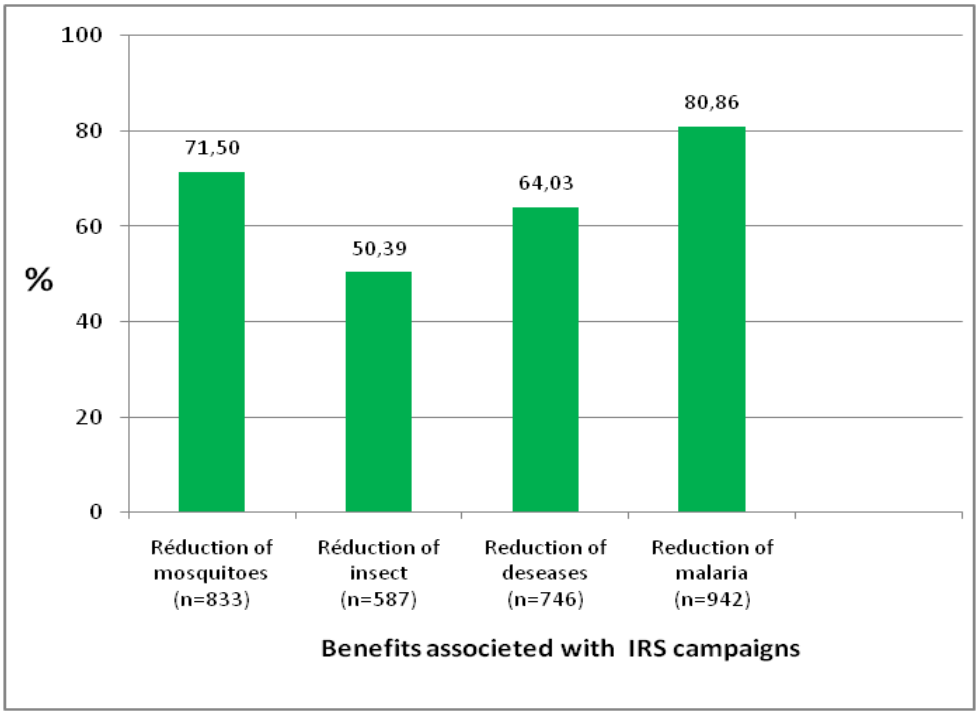


Fig. 4: Benefits associated with IRS campaigns

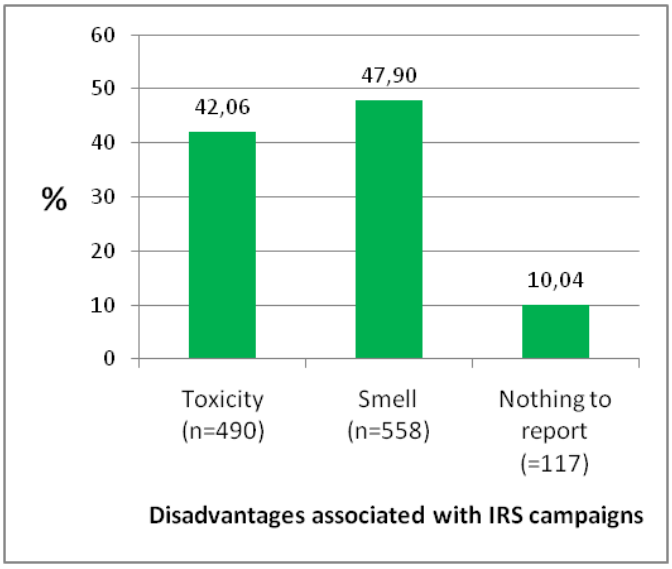


Fig.5: Disadvantages associated with IRS campaigns

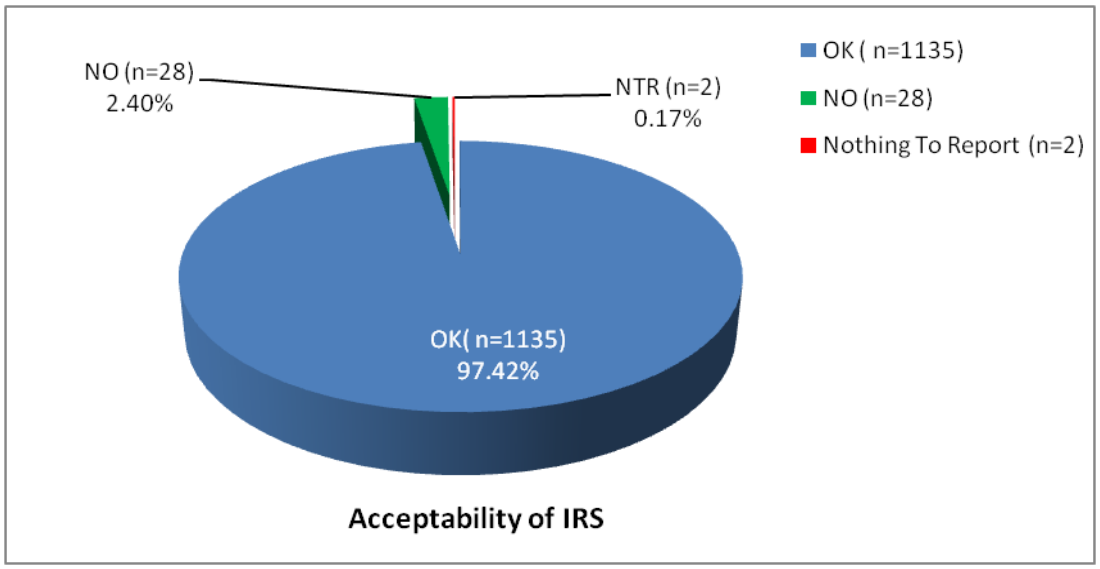


Fig.6-a: Percentage of people involved in IRS implementation

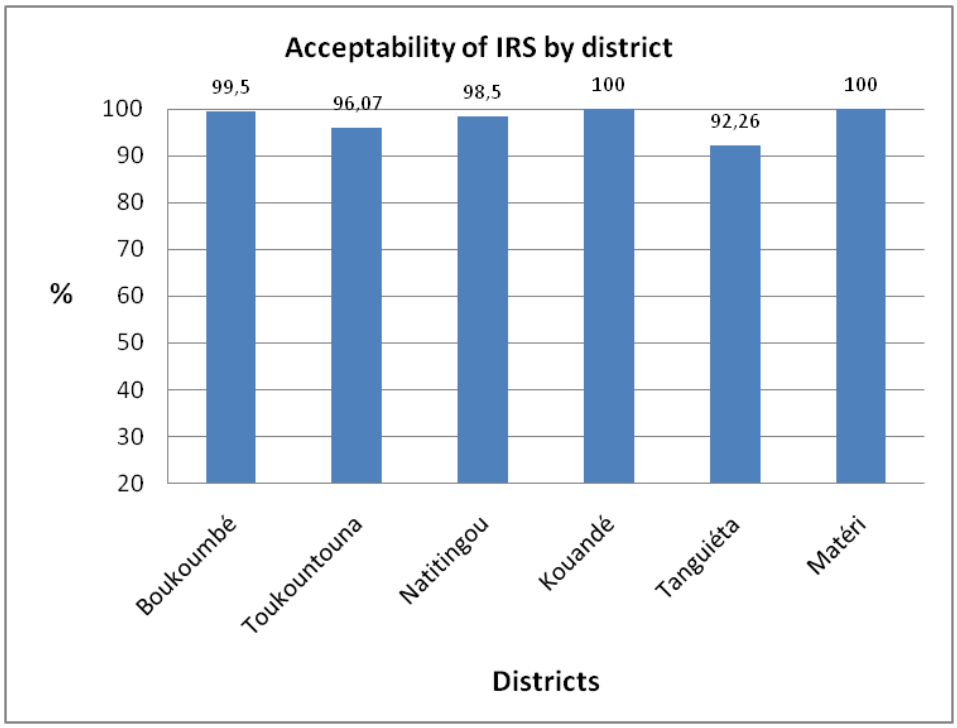


Fig.6-b: Percentage of people involved in IRS implementation in each district

2. Decay of bendiocarb: residual effect on various materials after 1, 2, 3, 4 months after IRS.

2.1. Protocol

In September and October 2010, a quick entomological study was carried out before implementing Indoor Residual Spraying (IRS) in department of Atacora in Bénin. This study has shown that the walls of the majority of houses were made in plaster mud, in simple mud or in plaster cement. The residual effect of bendiocarb on these 3 materials was followed according to the WHO procedures using cone tests. Cones were placed at 3 locations of the walls: bottom, middle, top. About 10 females of *Anopheles gambiae* susceptible reference strain Kisumu and wild females were introduced per cone and exposed for 30 minutes to treated walls. Bioassays were replicated, then 20 mosquitoes were tested per cone and therefore 60 mosquitoes of each type (susceptible and resistant) were tested per treated material. Mosquitoes analysed were young 2-5 days *Anopheles gambiae*. The wild mosquitoes were larvae of *An. gambiae* collected in the breeding sites of the study area (Atacora) and reared.

2.2. Results

During the first round of IRS in Atacora, all mosquitoes tested 2 weeks after IRS were killed. The mortality was 100% for all locations of WHO cones on treated materials. Figure 3 shows 100% mortality of the 2 strains of tested mosquitoes.

For the residual effect of bendiocarb, we have carried out one bioassay per month:

T1: 30-60 days after IRS

T2: 61-90 days after IRS

T3: 91-120 days after IRS

The results obtained are presented on figure 3. They show that the residual activity of bendiocarb is good for 3 months. During this period, the mortality rate was more than 90% for all treated materials. Beyond 3 months, the efficiency of bendiocarb has drastically decreased below the threshold of efficacy (80% mortality of *An. gambiae*) as well for the susceptible strain of *An. gambiae* (Kisumu) as for the local resistant strain. (see figure 3).

Compared to what we have observed in the department of Oueme, the decay of bendiocarb in Atacora is rapid. This situation is particularly due to the quality of the treated walls. In Atacora, the majority of the walls are mad with mud. Therefore, the mud is porous structure and insecticide can easily go inside. The consequence would be a decrease of insecticide on the surface of the walls. Considering the high percentage of houses made with mud in the rural areas, we recommend to the manufacturers to develop formulations of insecticide which can cover the surface of walls as a solid and adhesive film after spraying.

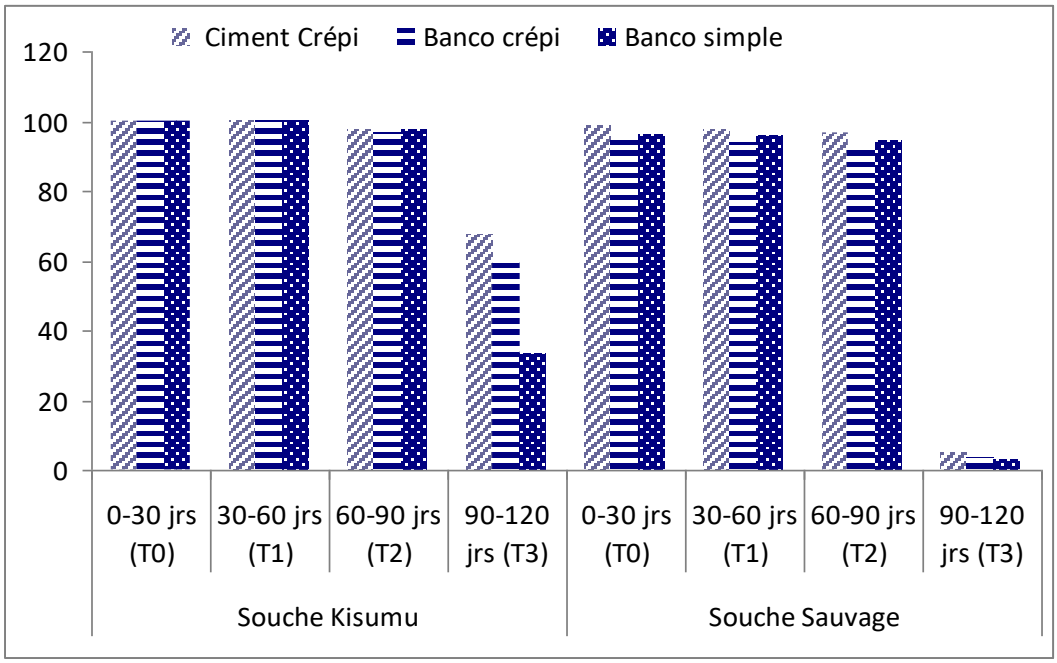


Figure 2: Percentage of mortality of *Anopheles gambiae* KISUMU (susceptible strain) and *An.gambiae* (wild stump) after 30 minutes exposure to various treated materials 1, 2, 3 months after IRS in Matéri and Tanguiéta.

N.B.
Ciment crépi = plaster cement
Banco crépi = plaster mud
Banco simple = not plaster mud
Souche Kisumu = Kisumu strain
Souche sauvage = wild strain

3. Dynamics of mosquitoes in districts under IRS

3.1. Protocol

3.1. 1. Species of mosquitoes, Human Biting Rate (HBR), Entomological Inoculation Rate (EIR) and physiological age grading (parous rate)

a). Mosquito sampling by landing catch

Out of the seven districts of Atacora under IRS intervention, four of them were randomly selected for the monitoring-evaluation (Kouande, Materi, Tanguieta and Natitingou). One district (Pehunco) not far from Kouande and not concerned by IRS was used as control. In each district, 2 villages were selected: one in the central part, one at the periphery. Two houses were chosen per village for mosquito collections. Adult mosquitoes were collected 2 consecutive days each 2 months using human landing catches with one collector placed

indoor and another outdoor at every collection point (house) resulting in 16 night collections for a total number of 80 night collections in the 5 districts per each 2 months. All Anopheles mosquitoes caught during the night were identified to species. Vector species were dissected using microscope to determine the physiological age grading (parous rate) and the heads/thoraxes were stored separately in Eppendorf tubes with silicagel at -20°C for ELISA method analyses to look for CSP antigens. Abdomens of females were also stored for PCR analyses to identify mosquito species and molecular forms of An. gambiae.

b). Expected parameters

Five entomological parameters were expected from landing catch collections:

- Species and molecular forms within *An. gambiae s.l.*
- Physiological age grading (parous rate) of *An. gambiae*
- Human Biting Rate (HBR) inside and outside
- Infectivity of Anopheles: % CS+
- Entomological Inoculation Rate (EIR)

3.1.2. Behavioral changes of mosquitoes in districts under IRS

a) Mosquito sampling using exit traps

Four bedrooms were selected for mosquito collection in the morning. Exit traps were positioned over the windows of every bedroom retained for this study. We expect all mosquitoes escaping the insecticidal effects to be trapped while exiting through the window. The collections from the window traps were done in the morning using a mouth aspirator. Alive mosquitoes were transferred into plastic cups supplied with 10% honey solution and mortality recorded after 24 hours holding. In the meantime, morning catches within bedrooms were operated using pyrethrum spray and white canvas lined up on the floor to collect fallen mosquitoes. These two sampling methods were led to an accurate estimation of the total density of mosquito species in the treated houses and the proportion of female mosquitoes exiting from the houses.

This mosquito sampling was carried out to complete the landing catch

b). Expected parameters

Density of mosquitoes per room in districts under IRS and in the control district

Exophily induced by IRS

Percentage of mosquitoes collected fed, unfed and gravid

3.2. Results

3.2.1. Diversity of mosquitoes met in Atacora

Various species of mosquitoes were collected at the beginning of the implementation of IRS. The list is shown below. Out of 16 species, *An. gambie* is the most abundant (56.6%) (table1). Some mosquitoes may disapear after IRS, this is why it is important to get this list.

Table I: Mosquitoes species caught from July to October 2011

Species	Int	Ext	Total	%
Anopheles gambiae	334	334	668	56,66
Anopheles funestus	15	15	30	2,54
Anopheles nili	13	17	30	2,54
Anopheles pharoensis	0	1	1	0,08
Anopheles ziemanni	6	26	32	2,71
Anopheles broheri	0	0	0	0,00
Anopheles coustani	0	0	0	0,00
Autres Anopheles	0	0	0	0,00
Aedes aegypti	1	3	4	0,34
Aedes vittatus	6	11	17	1,44
Aedes luteocephalus	0	2	2	0,17
Aedes longipalpis	1	3	4	0,34
Aedes fucifer	1	0	1	0,08
Aedes gr. palpalis	0	1	1	0,08
Aedes gr. tarsalis	1	0	1	0,08
Autres Aedes	0	0	0	0,00
Culex quinquefasciatus	139	96	235	19,93
Culex gr decens	1	0	1	0,08
Culex nebulosus	2	1	3	0,25
Culex tigripes	0	2	2	0,17
Culex fatigans	85	18	103	8,74
Culex annulioris	1	0	1	0,08
Autres Culex	0	0	0	0,00
Mansonia africana	18	25	43	3,65
TOTAL	**624**	**555**	**1179**	

3.2.2. Comparison of Anopheles density and other mosquitoes in the districts under IRS and in the control.

Two main disease vectors were collected in the study area: *An. gambiae* and *Culex*

quinquefasciatus. In the control area, the density of *An. gambiae* (70.6%) is higher than *Cx quinquefasciatus* (29.4%) one. In Materi, Natitingou and Kouande under IRS, the density of Cx quinqefasciatus is too low, respectively, 11.1% (4/45), 0% and 0% (Table II). *Cx quinquefasciatus* is not collected in Materi and Kouandé from July to October. We can't not affirm that the absence of this species is due to the effect of IRS, because, we have no database about it before IRS.

Table II: Comparaison of Anophele density and others mosquitoes in the communes under IRS and control from July to October 2011.

		July - October 2011		
		N	Inside	Outside
Pehunco (control)	*An.gambiae*	305	141	164
	An.funestus	5	1	4
	Other anoplelinae	4	0	4
	Culex quinquefasciatus	127	70	57
	Aedes aegypti	0	0	0
	Other culicinae	112	89	23
Tanguiéta	*An.gambiae*	164	95	69
	An.funestus	8	4	4
	Other anoplelinae	11	7	4
	Culex quinquefasciatus	107	68	39
	Aedes aegypti	2	1	1
	Other culicinae	34	11	23
Matéri	*An.gambiae*	41	22	19
	An.funestus	15	9	6
	Other anoplelinae	6	0	6
	Culex quinquefasciatus	4	0	4
	Aedes aegypti	1	0	1
	Other culicinae	17	7	10
Natitingou	*An.gambiae*	72	36	13
	An.funestus	1	1	0
	Other anoplelinae	12	12	0
	Culex quinquefasciatus	0	0	0
	Aedes aegypti	2	0	2
	Other culicinae	8	5	3
Kouandé	*An.gambiae*	89	39	50

An.funestus	1	0	1
Other anoplelinae	1	0	1
Culex quinquefasciatus	6	1	5
Aedes aegypti	0	0	0
Other culicinae	10	4	6

3.2.3. Decrease of Hunan Biting Rate of mosquitoes in districts under IRS

After IRS, 3 missions of 20 days each are organized in Atacora from July to October to collect mosquitoes and to follow the decrease of human biting rate. Data obtained are shown in table III. The results show a decrease of human biting rate (HBR) in districts under IRS compared to the control, particularly Maeri, Natitingou and Kouande where each inhabitant receives, respectively 51.90 and 111 bites from *An gambiae* per month during the rainy season. At the same time, the human biting rate is high in Pehunco, the control area (381bites/man/month), either more than 75% of reduction of human biting rate thanks to IRS strategy using bendiocarb in the 3 districts under IRS listed above. However, in the 4th district under IRS (Tanguieta), a little reduction was found: 205 bites per man per month from *An. gambiae* against 381 in the control area (46.6% of reduction): table III.

The decrease of HBR was more important in July when the houses were recently treated. In fact, during our first mission (2 weeks after IRS), the human biting rate (HBR) of *An. gambiae* has drastically decreased to more than 95%. HBR was 388 bites of *An. gambiae* per man per month in the control district (Pehunco). But in the districts under IRS, it was too low: 5.62, 1.8, 9.37 and 76.9, respectely in Tanguieta, Natitingou, Materi and Kouande. But, two months after IRS, the reduction decreased to 65.5% in Natitingou and Kouande.

Compared to what we have obtained in Oueme, the decrease of HBR is lower probably because of the difference in term of residual effect in Oueme and Atacora as mentioned in the paragraph 2 related to decay of bendiocarb.

Table III: Comparaison of HBR of *An.gambiae* observed inside and outside in control area and in areas under IRS

		July-October 2011			
		Total Mosquitoes	nb human cathes	HBR/night	HBR/month
Pehunco	Inside	141	24	5,9	176

(control)					
	Outside	164	24	6,8	205
	Total	305	24	13	381
Tanguiéta	Inside	95	24	4	119
	Outside	69	24	2,9	86
	Total	164	24	6,8	205
Matéri	Inside	36	24	1,5	45
	Outside	19	24	0,8	24
	Total	41	24	1,7	51
Natitingou	Inside	36	24	1,5	45
	Outside	13	24	0,5	16
	Total	72	24	3	90
Kouandé	Inside	39	24	1,6	49
	Outside	50	24	2,1	63
	Total	89	24	3,7	111

3.2.4. Decrease of Entomological Inoculation Rate (EIR) in districts under IRS

2. Decline of EIR in districts under IRS

In areas under IRS, 166 thoraces of *An. gambiae* were analysed using ELISA/CSP technique among which 11 were found positive for *Plasmodium falciparum* circumsporozoit (CS) antigen. The mean of sporozoit index is 0.06. In the control area, 32 thoraces were found positive on a total of 378 (Is = 0.084) (Table IV).

In the control area, each inhabitant receives a mean of 23.8 infected bites per month during the study period. In areas under IRS, this rate was drastically reduced: 2.54 infected bites per month. When we compare the EIR observed in the control area to those of districts under IRS, the decline is very high: 90%. But, in spite of this decrease, this rate of EIR is high compare to the results of Oueme campaign.

Table IV: Comparaison of EIR from June to July

		June-July 2011	August-September 2011
Pehunco (Control)	Thorax	283,00	95,00
	Thorax +	26,00	6,00
	IS	0,09	0,06
	HBR/night	12,94	5,94
	EIR	1,16	0,38
Tanguiéta	Thorax	6,00	54,00

	Thorax +	2,00	2,00
	IS	0,33	0,04
	HBR/night	0,19	3,38
	EIR	0,06	0,13
Matéri	Thorax	6,00	18,00
	Thorax +	0,00	1,00
	IS	0,00	0,06
	HBR/night	0,06	1,13
	EIR	0,00	0,06
Natitingou	Thorax	6,00	20,00
	Thorax +	1,00	3,00
	IS	0,17	0,15
	HBR/night	0,31	1,25
	EIR	0,05	0,19
Kouandé	Thorax	17,00	39,00
	Thorax +	2,00	0,00
	IS	0,12	0,00
	HBR/night	2,56	2,44
	EIR	0,31	0,00

3.2.5. Decrease of the lifespan of *An. gambiae* in districts under IRS

In the control district, physiological age grading is high: 84.3%. But in the districts under IRS, it is low: 52.38, 52.78, 56.34 and 34.85, respectively in Tanguieta, Materi, Natitingou and Kouande (table V).

Table V: Comparison of parous rate

	July-October 2011		
	N tetsed	Parous	Parous rate (%)
Pehunco (Control)	191	161	84,29
Tanguiéta	147	77	52,38
Matéri	36	19	52,78
Natitingou	71	40	56,34

Kouandé	66	23	34,85

3.2. 6. Impact of IRS on the changes of *An. gambiae* behaviour

At each intervention area, 4 bedrooms were selected for mosquito collection in the morning. Exit traps were positioned over the windows of every bedroom retained for this study. We expect all mosquitoes escaping the insecticidal effects to be trapped while exiting through the window. The collections from the window traps were done in the morning using a mouth aspirator. Alive mosquitoes were transferred into plastic cups supplied with 10% honey solution and mortality recorded after 24 hours holding. In the meantime, morning catches within bedrooms were operated using pyrethrum spray and white canvas lined up on the floor to collect fallen mosquitoes. These two sampling methods were led to an accurate estimation of the total density of mosquito species in the treated houses and the proportion of female mosquitoes exiting from the houses.

a). Impact of IRS on blood feeding of *An. gambiae*

In spite of IRS implementation, the proportion of mosquitoes which succeed to go into bedrooms also succeed in feeding on human. The blood feeding index is as high in the control district as in districts under IRS (Table VI). This situation was found in 2007 in experimental huts when we were evaluating various insecticides in Akron (see Akogbeto *et al*., 2010, Malaria journal) and during IRS first campaign in Oueme (Akogbeto et al, 2011, AJTMH). For us, the implementation of IRS should not exclude the use of LLINs by the community. Indeed, some families, particularly husbands and wives are used to sleep under bednets. For these families, bednets are considered as intimate tool. Inspite implementation of IRS, these families would be encouraged to use their bednets. The best strategy is the joint use of IRS + LLIN. This combination not only protects homes from the invasion of mosquitoes, it also prevents contact between humans and mosquitoes. Such a strategy implicates an increase of the cost of malaria prevention and can't be implemented everywhere. It must be reserved only for areas of highest malaria transmission. For example, in areas where EIR is near 300 Anopheles infected bites per year, 90% reduction is not sufficient to significantly reduce malaria prevalence.

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Table VI : Blood feeding rate

	July - October 2011					
	An.gambiae	***Culicidés***				
	Fp+Sp	Fp+Sp				
	Total	N, feed	Blood feeding rate	Total	N, feed	Blood feeding rate
Pehunco	86	63	73	154	104	68
Tanguiéta	168	101	60	246	157	64
Matéri	59	55	93	64	58	91
Natitingou	39	33	85	48	42	88
Kouandé	7	3	43	16	12	75

b). Induced exophily of *An.gambiae* in areas under IRS

We organized mosquito sampling using exit window traps and pyrethrum spray catches to measure exophily induced by the presence of bendiocarb on treated walls. In intervention districts the rate of exophily is higher: 71.18%% (materi), 79.48% (Natitingou). Compared to the natural exophily of *An. gambiae*, the difference is significant**.** The high induced exophily indicates bendiocarb is not conducive to mosquito survival for a long time.

Table VII:Exophiliy rate of mosquitoes observed in control area and in areas under bendiocarb IRS.

July-October 2011	

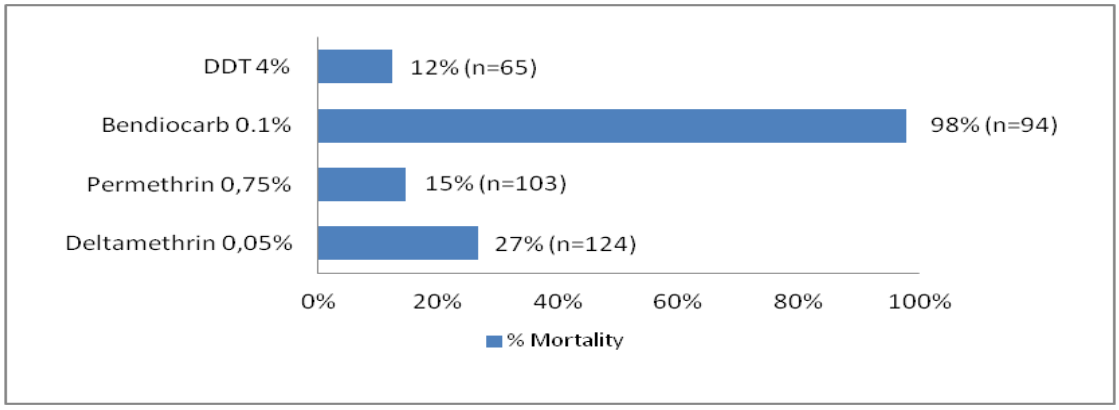
	An.gambiae		
	Exit window trap	PSC	Exophilie rate (%)
Pehunco	43	43	50
Tanguiéta	94	74	55,95
Matéri	42	17	71,18
Natitingou	31	8	79,48
Kouandé	0	7	0

3.3. Evolution of *An. gambiae* resistance to bendiocarb in Atacora

We have compared the data obtained on the susceptibility test performed in 2010 to what we have observed in 2011. *An. gambiae* is losing its susceptibility to bendiocarb. In October 2010, the percentages of dead *An.gambiae* observed after one hour exposure to papers treated with bendiocarb were 98%, 95%, 97%, 98%, respectively in Matéri, Tanguiéta, Natitingiu and Kouandé (Figures 1a, a' to 4d, d'). Then, *An. gambiae* was susceptible to bendiocarb. By other hand, the percentage of dead *An. gambiae* observed after two hours exposure to CDC bottles treated with bendiocab confirms the good susceptibility of *An. gambiae* to bendiocarb in southern Benin in 2010. One year later (October 2011), the situation has changed: the percentages of mortality have decreased, respectively to 87%, 93%, 85% and 89% in the 4 districts. *An. gambiae* is progressively developing resistance to the carbamates in Atacora. The development of resistance to carbamates in Atacora is shown by the presence of *Ace.1* mutation (table3.1). Contrary to Atacora, in other departments, this mutation was not found (table 3.2).

In conclusion, it is important to stop the use of bendiocarb for IRS in Atacora to avoid to increase the level of resistance to carbamates.

a. Matéri October 2010



a'. Matéri October 2011

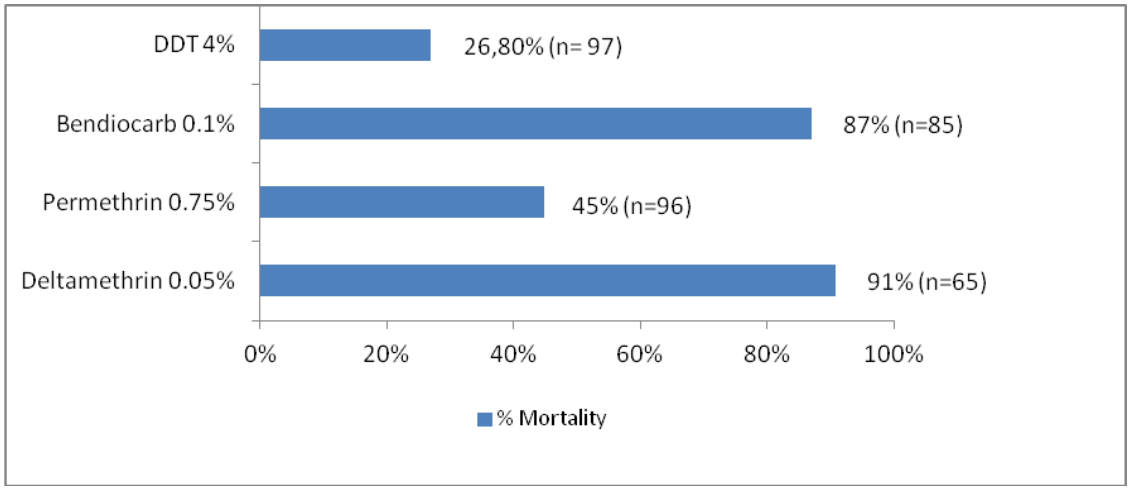
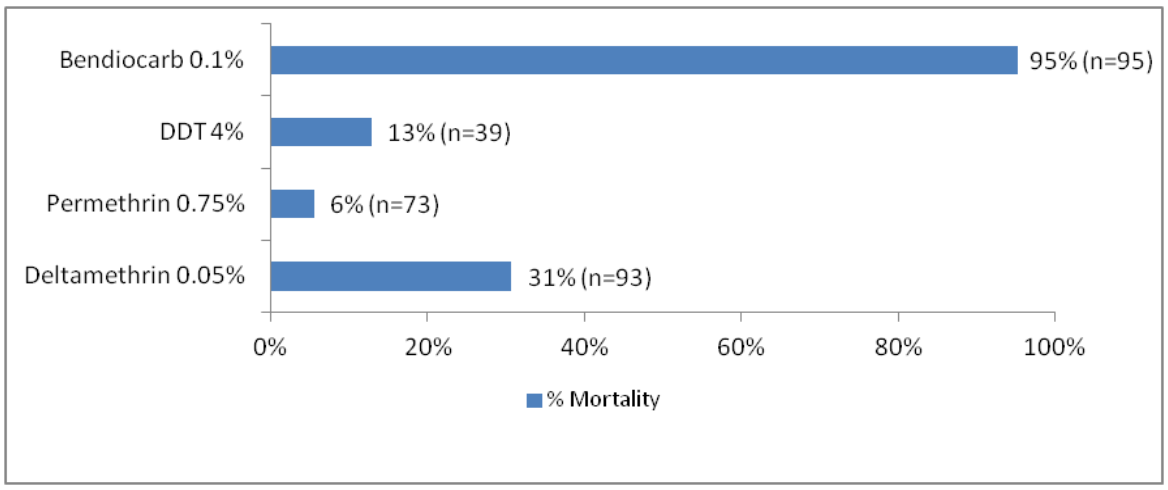


Figure 1: Percentage of dead *An.gambiae* observed after one hour exposure to papers treated with various insecticides in Matéri

b.Tanguiéta October 2010



b. Tanguiéta October 2011

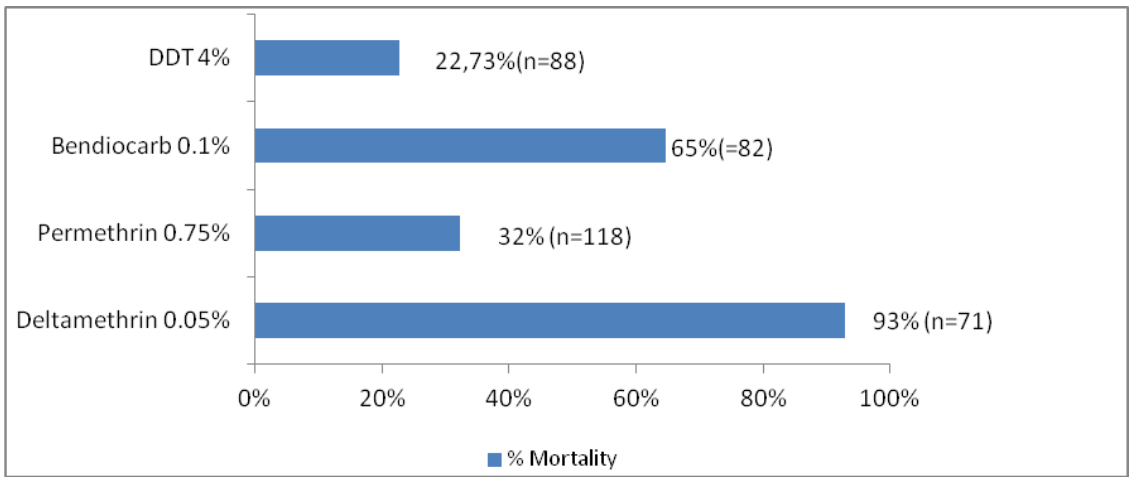
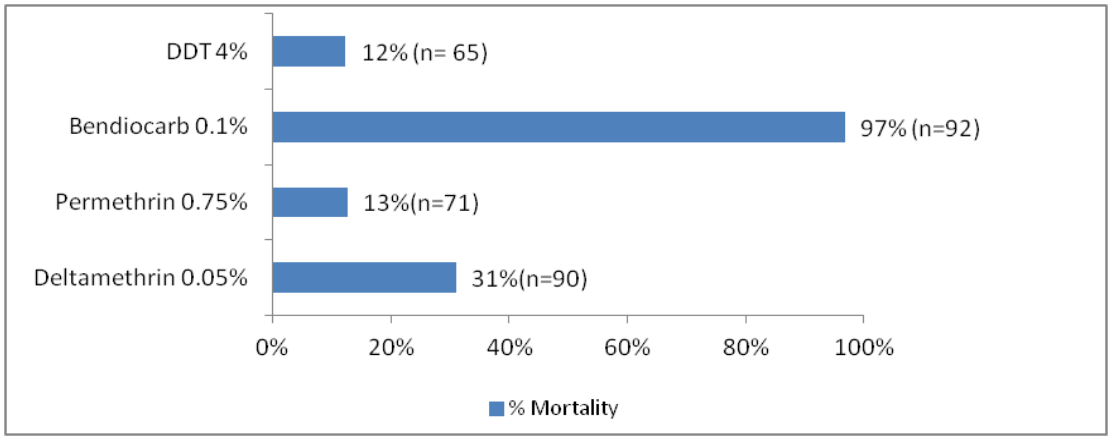


Figure 2: Percentage of dead *An.gambiae* observed after one hour exposure to papers treated with various insecticides in Tanguiéta.

c . Natitingou, October 2010



c. Natitingou, October 2011

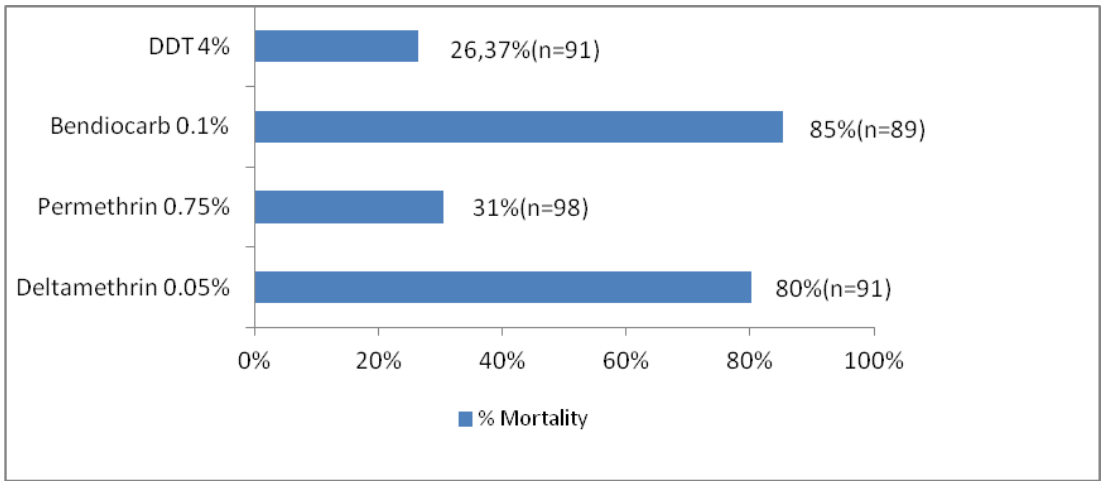
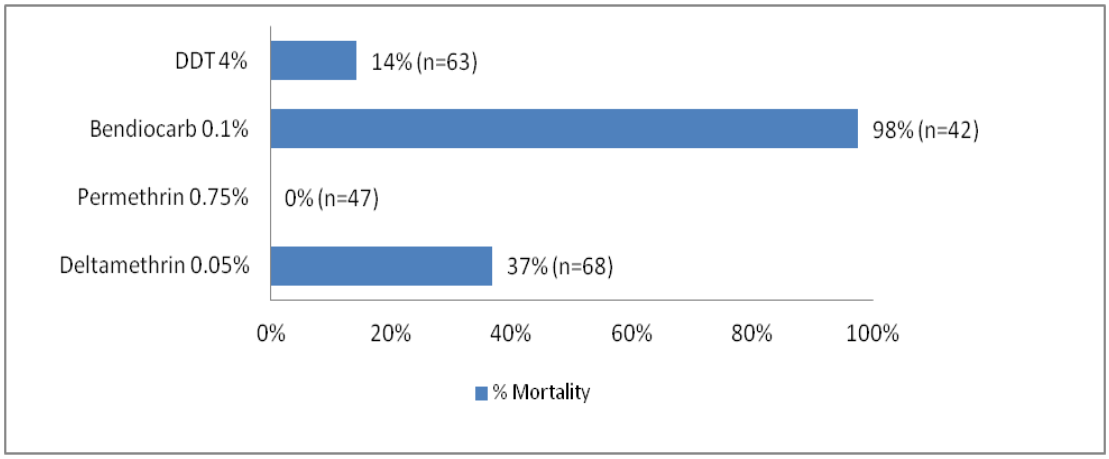


Figure 3: Percentage of dead *An.gambiae* observed after one hour exposure to papers treated with various insecticides in Natitingou.

d . Kouandé, October 2010



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d. Kouandé, October 2011

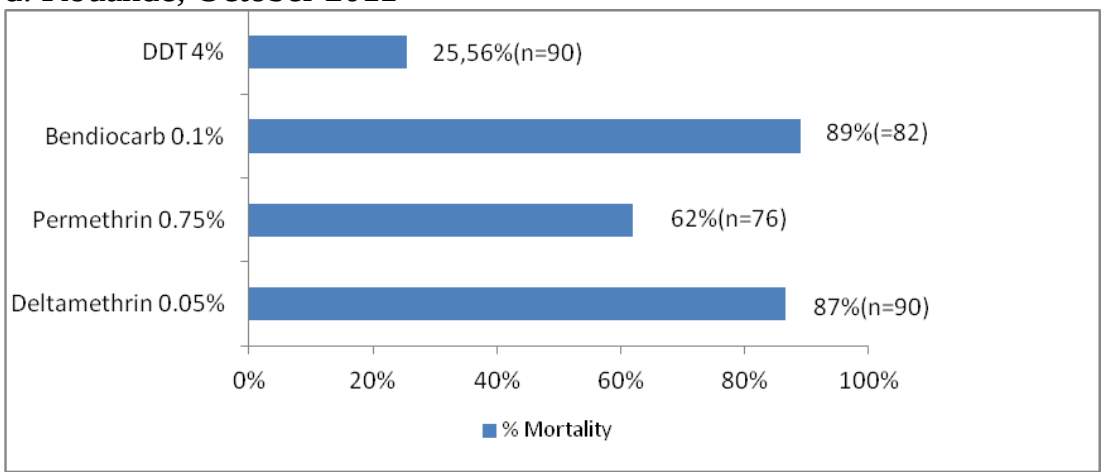

Figure 4: Percentage of dead *An.gambiae* observed after one hour exposure to papers treated with various insecticides inKouandé

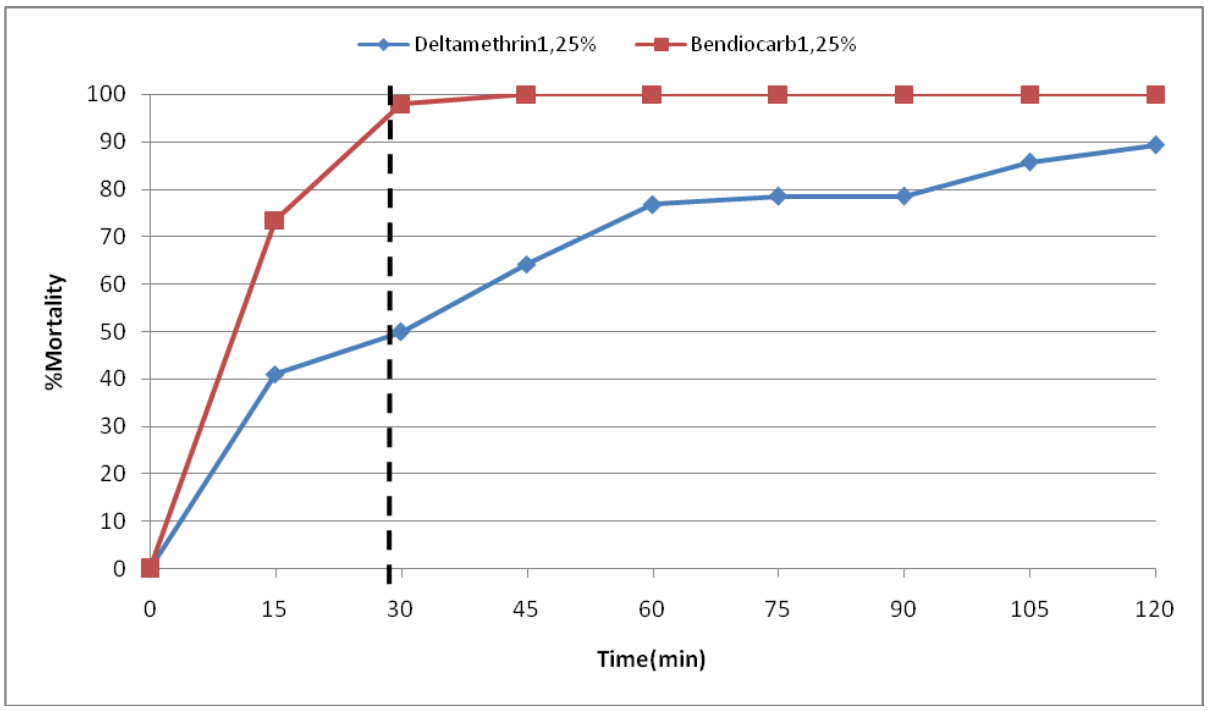

Figure 5: Percentage of dead *An. gambiae* observed after two hours exposure to CDC bottles treated with various insecticides in Control (2010).

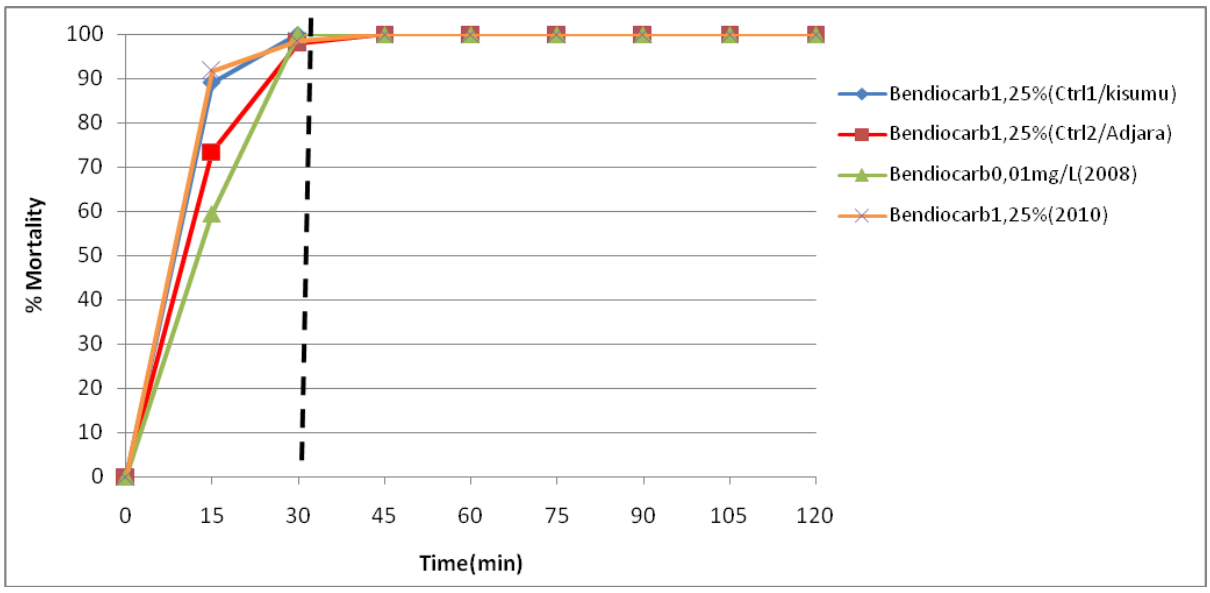

Figure 6: Percentage of dead *An. gambiae* observed after two hours exposure to CDC bottles treated with various insecticides in Dangbo.

Table 3.1. Species identification, molecular forms and frequency of the *kdr*, *Ace.1* alleles and génotypes in *An.gambiae s.l* from Atacora.

	Species		Molecular forms		*kdr* mutation	*Ace-1* mutation							
	Aa	Ag		M	S	RR	RS	SS	F(Ace.1)		RR	RS	SS	F(Ace.1)
Pehunco	0	28		1	27	1	18	0	0,68		0	2	26	0,04
Kouandé	0	27		0	27	2	1	0	0,98		0	1	26	0,02
Cobly	0	12		2	19	2	1	0	0,98		0	1	20	0,02
Boukoumbé	0	27		0	27	1	17	0	0,69		0	0	27	0,00
Tanguiéta	0	62		6	20	2	4	2	0,85		0	0	26	0,00
Toukountounan	0	26		0	26	1	11	2	0,71		0	1	25	0,02
Natitingou	0	9		0	9	3	3	3	0,50		0	1	8	0,06
Matéri	0	17		0	17	1	5	2	0,75		0	1	16	0,03
Kérou	1	16		2	14	1	2	3	0,75		0	6	10	0,19

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