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

Activity 2B: Mosquito behaviors and malaria transmission after the withdrawal of IRS in HZ NTB

Title of the study:
Mosquito behaviour and malaria transmission in the Health Zone "Natitingou-Boukombé-Toucountouna" after the withdrawal of Indoor Residual Spraying.

Deliverable 2B-1

Quarterly Report

September 2018

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Collaboration: National Malaria Control Program (NMCP)

1. Introduction

After 6 years of the Indoor Residual Spraying (IRS) in the Atacora region, Benin decided to withdraw this intervention from the most of the districts to avoid the emergence of the vector resistance and to extend IRS in other regions (Alibori and Donga). To achieve this, only the health zone Kouandé-Kérou-Pehunco continues to receive IRS. Concerning the health zones Natitingou-Boukombé-Toucountouna and Tanguieta-Materi-Cobli, IRS was alternated with an extensive use of LLINs. At the same time, continued entomological surveillance in the health zone Natitingou-Boukombé-Toucountouna is carried out to allow prompt detection of any rebound in malaria transmission.

The current study is focused on the mosquito behaviour and malaria transmission in this health zone after the withdrawal of Indoor Residual Spraying. The goal is to verify if the withdrawal of the IRS is accompanied by a rebound in malaria transmission. The current report is for the period from September 2017 to September 2018.

2. Study area

Health zone: Natitingou, Toucountouna, Boukombé
Control: Pehunco as treated district.

3. Data retained in the deliverable

- Vector identification (species and molecular forms of *Anopheles gambiae*)
- Density of mosquitoes inside bedrooms
- Mosquito blood feeding behaviors (endophagy, exophagy behaviors)
- Human Biting Rate (HBR)
- Entomological Inoculate Rate (EIR)
- Results of insecticide susceptibility tests
- Identification of mosquito genetic mutations that confer resistance

4. Organization of the report

Ten visits were done during the period from September 2017 toSeptember 2018 to collect mosquitoes, conduct advanced laboratory testing on captured *Anopheles gambiae* species and assess susceptibility of mosquitoes and mechanisms involved in vector resistance.

5. Protocol

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

Mosquitoes were collected by human landing catch in two villages per district, with one village located in the center of the district, and one village located at the periphery. For each village, mosquitoes were collected in 2 houses by 4 mosquito collectors, 2 mosquito collectors

indoor and 2 outdoor. In total 32 mosquito collectors were used for one round of collection. Two rounds of mosquito collections are planned per month.

In addition, 10 other houses per village were selected to estimate the density of mosquitoes per room to determine the number of mosquitoes in the room and to estimate indoor behaviors.

Vector species that are collected and identified were transported to CREC's laboratory for dissection using a microscope to determine the parous rates. The heads/thoraxes of the vector species were analyzed by ELISA method to look for CSP antigens. Abdomens of females of the vector species were used for PCR analyses, to identify sibling species and molecular forms.

5.2. Mosquito collections, insecticide susceptibility tests and resistance mecanisme assessement.

Unfed 2-5 days old *An. gambiae s.l* adults from larvae collected on the field were used for WHO susceptibility test using various classes of insecticides. Susceptibility status of the population was graded according to the WHO protocol. Dead and surviving mosquitoes from this bioassay were kept separately in Eppendorf tubes containing silica gel and stored at −20°C for further molecular analysis. The PCR-RFLP diagnostic test was used to detect the presence of L1014F mutation (Kdr) and G119S mutation (Ace.1R gene).

6. Results

6.1. *Human Biting Rate (HBR) of An gambiae indoor versus outdoor and* blood feeding behaviors

The Human Biting Rate (HBR) is calculated indoor and outdoor through Human Landing catch (HLC) in NTB HZ compared to the treated control district (Pehunco) (figure 1). The reading of this figure leads to two remarks. The first is that the HBR is higher inside than outside in the BNT HZ compared to the control where the opposite trend is observed. The second remark is that the global vector aggressiveness is stronger in NTB HZ compared to control (figure 2). This allows to conclude an induced exophay in the control that could be attributable to the IRS. (Details on HBR estimation is indexed in table I)

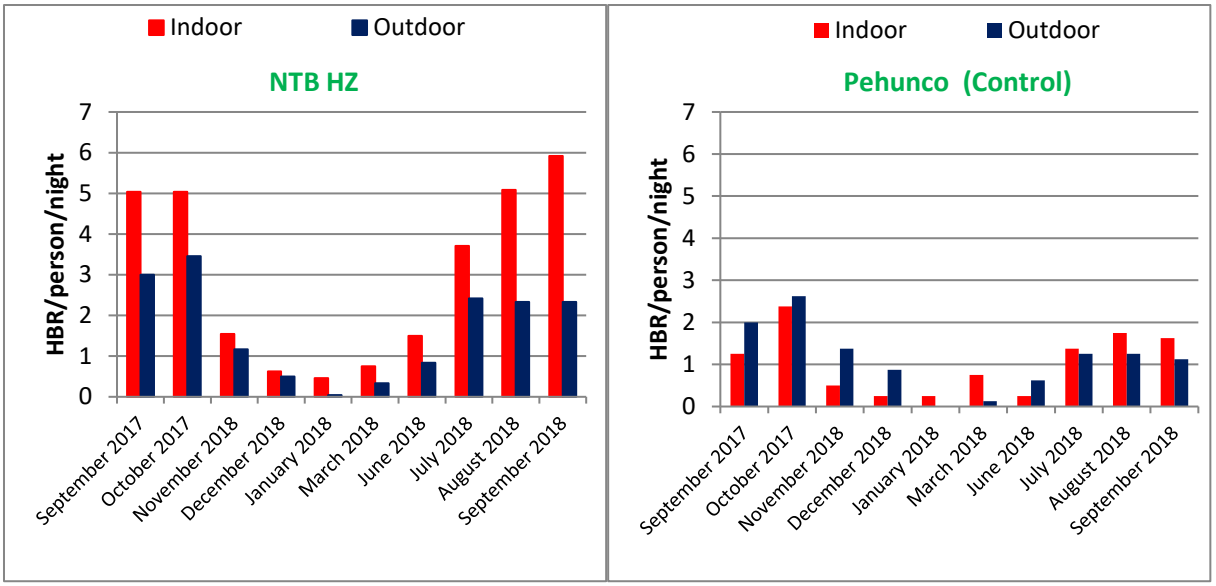


Figure 1: Human biting rate indoor *Vs* outdoor in NTB HZ compared to the control

Figure 2: Dynamic of global HBR in NTB HZ compared to the control.

6.2. Room density and blood feeding rate within An. gambiae

- Room density

The *An. gambiae* room density was evaluated through the pyrethrum spray. We notice that the average density of *An. gambiae* is relatively low in control than in the other (NTB) districts during the period from September 2017 to September 2018. The Figure 3 shows the dynamic of room density of *An. gambiae* in BNT HZ compared to the control.

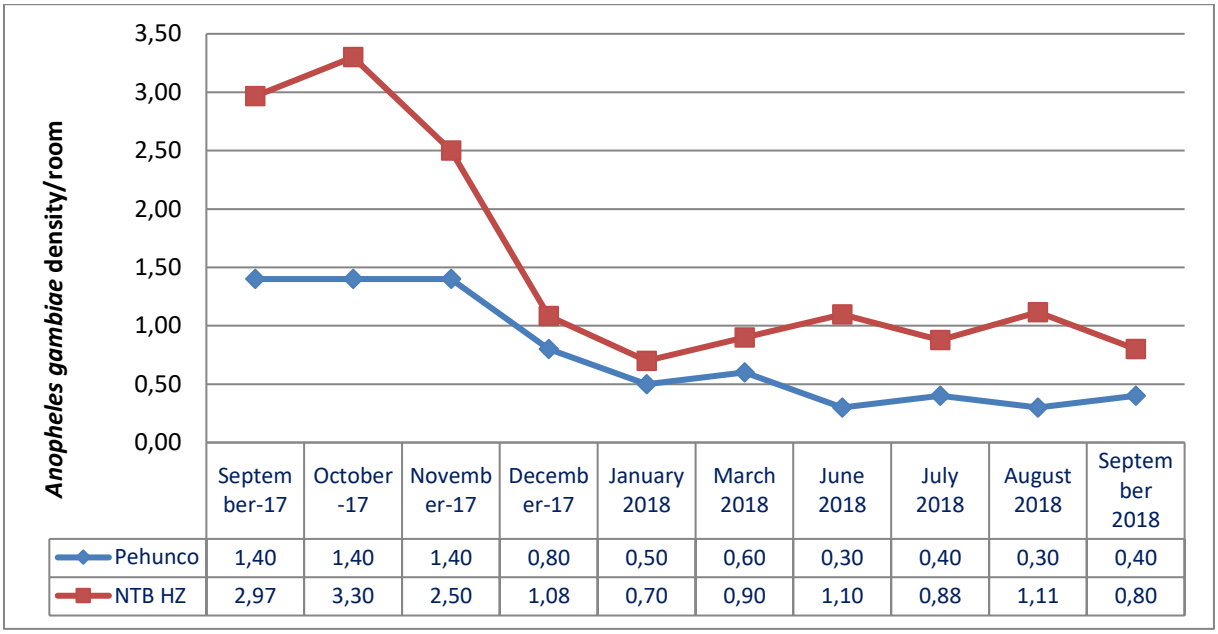


Figure 3: Dynamic of room density of *An. gambiae* in NTB HZ compared to the control (Pehunco).

- blood feeding rate

The figure 4 shows the dynamic of the blood feeding rate in BNT HZ (untreated) *Vs* Pehunco (treated). It's noticed that the blood feeding rate is high in the control district as in NTB HZ during September 2017 to September 2018.

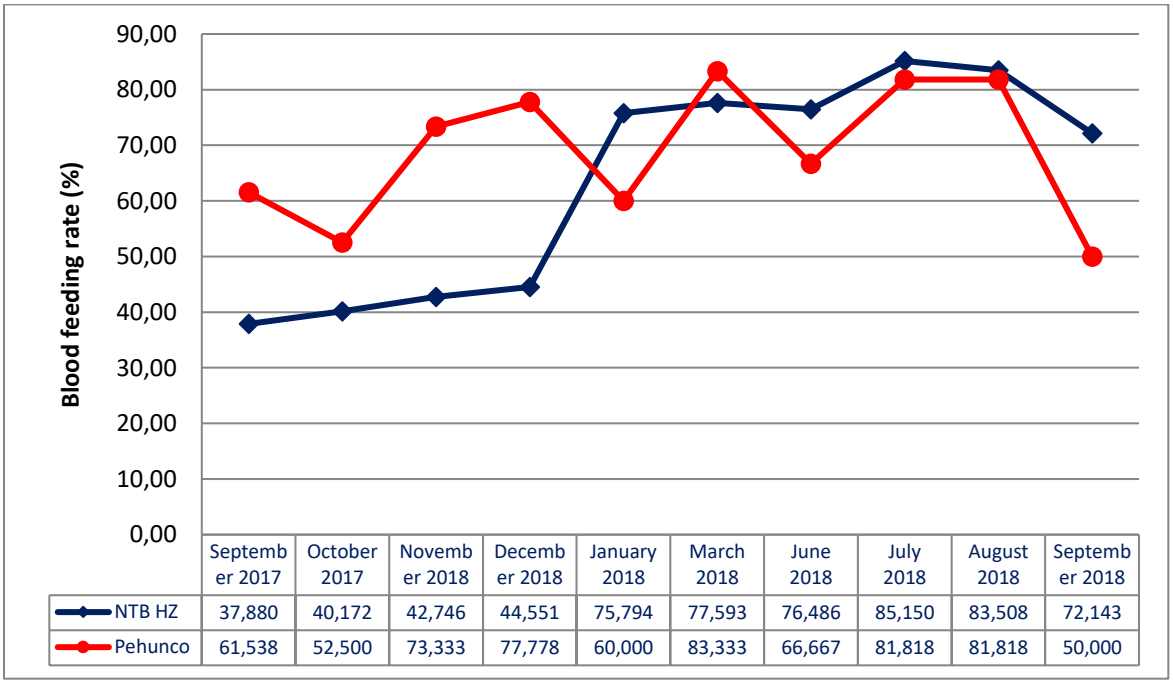
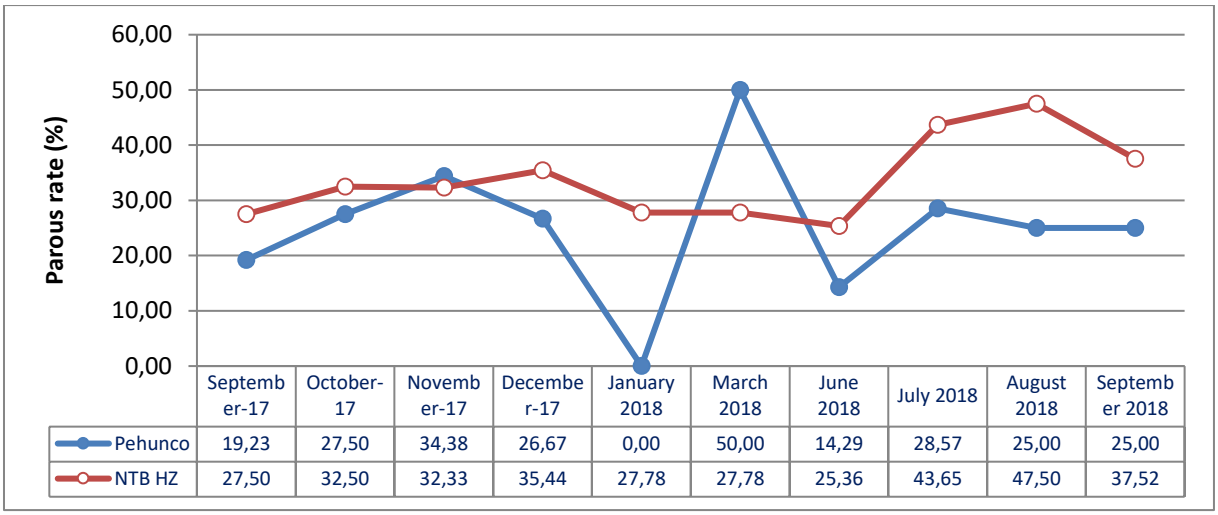


Figure 4: Dynamic of blood feeding rate in NTB HZ (untreated) *Vs* Pehunco (treated)

6.3. Parous rate observed in An. gambiae.

The Figure 5 shows the dynamic of parous rate of *An. gambiae* in NTB HZ compared to the control. The basic remark here is that there is no significant difference of the parous rate between the NTB HZ and the treated control (Pehunco).



	Septemb er-17	October- 17	Novemb er-17	Decembe r-17	January 2018	March 2018	June 2018	July 2018	August 2018	Septemb er 2018
Pehunco	19,23	27,50	34,38	26,67	0,00	50,00	14,29	28,57	25,00	25,00
NTB HZ	27,50	32,50	32,33	35,44	27,78	27,78	25,36	43,65	47,50	37,52

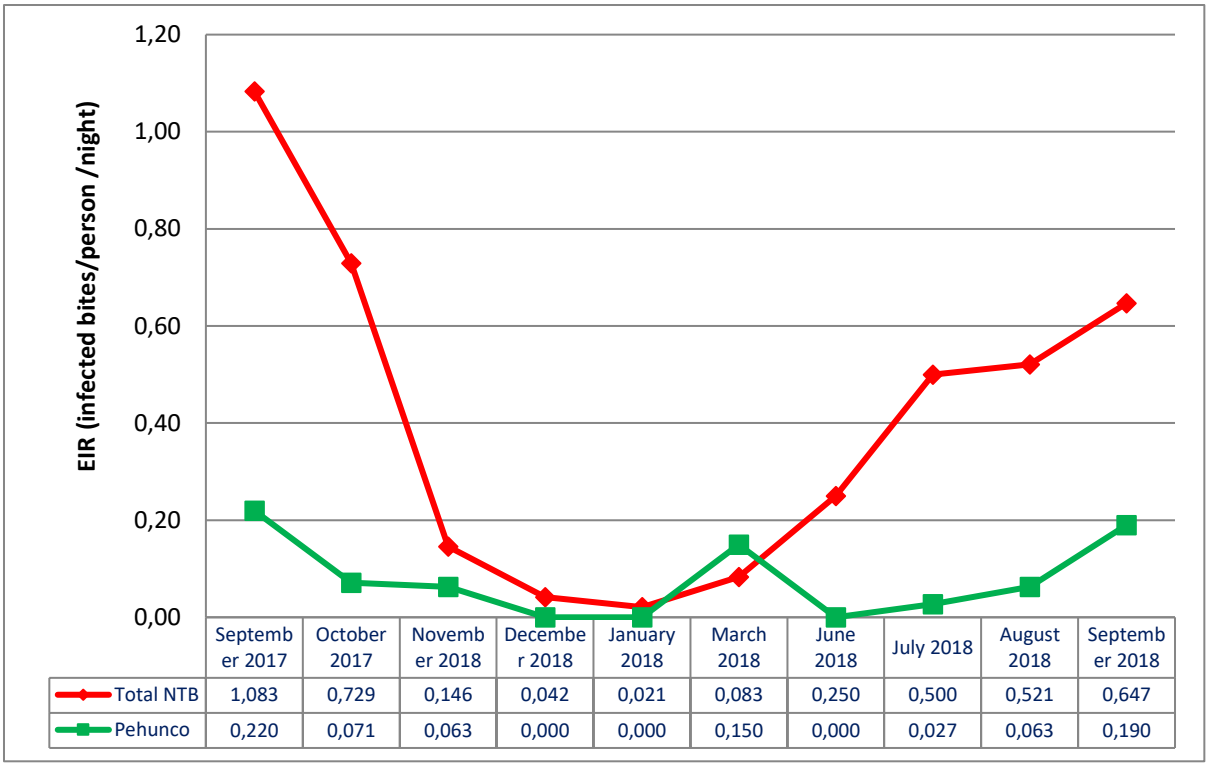
Figure 5: Dynamic of parous rate of *An. gambiae* in NT HZ compared to the control

6.4. Sporozoite index (%CS+) of Plasmodium falciparum and Entomological Inoculation Rate (EIR) of An. gambiae.

The table 2 (and Figure 5 show the infectivity as well as the Entomological Inoculation Rate (EIR) of *An. gambiae*. The high EIR is observed in NTB HZ from September 2017 to September 2018. The highest EIRs were observed in September 2017, where it ranged from 2.02 to 1.23 infected bites/ person/night in NTB HZ, against 0.22 infected bites/ person/night in the treated control (Pehunco).

Table 2: Sporozoite index (%CS+) of Plasmodium falciparum and Entomological Inoculation Rate (EIR) of *An. gambiae.*

Districts	Paramaters	sept-17	Oct-17	Nov-17	Dec-17	Jan-18	Mar-18	Jun-18	Jul-18	Aug-18	sept-18
Toukountouna	Thorax	78	85	26	13	4	7	21	53	64	63
	Thorax +	17	13	3	1	0	1	5	11	10	12
	IS	0,41	0,27	0,18	0,17	0,00	0,14	0,45	0,24	0,21	0,19
	HBR/night	4,88	5,31	1,63	0,81	0,25	0,44	1,31	3,31	4,00	3,94
	EIR	2,02	1,41	0,29	0,14	0,00	0,06	0,60	0,81	0,83	0,75
Boukoumbé	Thorax	74	70	22	8	6	11	24	49	66	72
	Thorax +	9	8	2	1	1	2	5	8	9	11
	IS	0,35	0,20	0,13	0,11	0,17	0,18	0,71	0,38	0,38	0,15
	HBR/night	4,63	4,38	1,38	0,50	0,38	0,69	1,50	3,06	4,13	4,50
	EIR	1,60	0,88	0,18	0,06	0,06	0,13	1,07	1,17	1,55	0,69
Natitingou	Thorax	41	49	17	6	2	8	11	45	48	63
	Thorax +	26	14	2	0	0	1	2	5	6	8
	IS	0,48	0,32	0,06	0,00	0,00	0,13	0,11	0,15	0,17	0,13
	HBR/night	2,56	3,06	1,06	0,38	0,13	0,50	0,69	2,81	3,00	3,94
	EIR	1,23	0,97	0,06	0,00	0,00	0,06	0,07	0,41	0,51	0,50
Total NTB	Thorax	193	204	65	27	12	26	56	147	178	198
	Thorax +	52	35	7	2	1	4	12	24	25	31
	IS	0,27	0,17	0,11	0,07	0,08	0,15	0,21	0,16	0,14	0,16
	HBR/night	4,02	4,25	1,35	0,56	0,25	0,54	1,17	3,06	3,71	4,13
	EIR	1,08	0,73	0,15	0,04	0,02	0,08	0,25	0,50	0,52	0,65
Pehunco (Control; treated)	Thorax	26	40	15	9	2	7	7	21	24	22
	Thorax +	10	2	1	0	0	1	0	1	1	3
	IS	0,14	0,03	0,07	0,00	0,00	0,14	0,00	0,02	0,04	0,14
	HBR/night	1,63	2,50	0,9375	0,56	0,13	1,06	0,44	1,31	1,5	1,38
	EIR	0,220	0,071	0,063	0,000	0	0,15	0	0,027	0,063	0,19



	Septemb er 2017	October 2017	Novemb er 2018	Decembe r 2018	January 2018	March 2018	June 2018	July 2018	August 2018	Septemb er 2018
Total NTB	1,083	0,729	0,146	0,042	0,021	0,083	0,250	0,500	0,521	0,647
Pehunco	0,220	0,071	0,063	0,000	0,000	0,150	0,000	0,027	0,063	0,190

Figure 5: Dynamic of Entomological Inoculation Rate (EIR) of *An. gambiae.*

6.5. Insecticide susceptibility of An. gambiae

The WHO insecticide susceptibility tests were performed in Boukoumbé and Natitingou districts. Three insecticides were tested (Pirimiphos methyl 0.25%; Bendiocarb 0.1%; deltamethrin 0.05%) (figures 6,7). The trend in the two evaluated districts is the same with a vector sensitivity to pirimiphos-methyl and resistance to bendiocarb and deltamethrin.

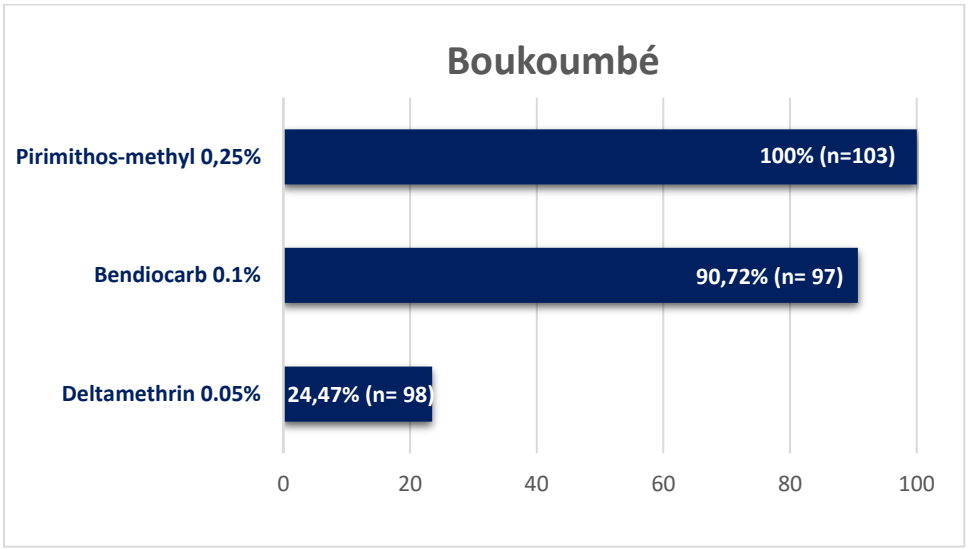


Figure 6: susceptibility test results in Boukoumbé district

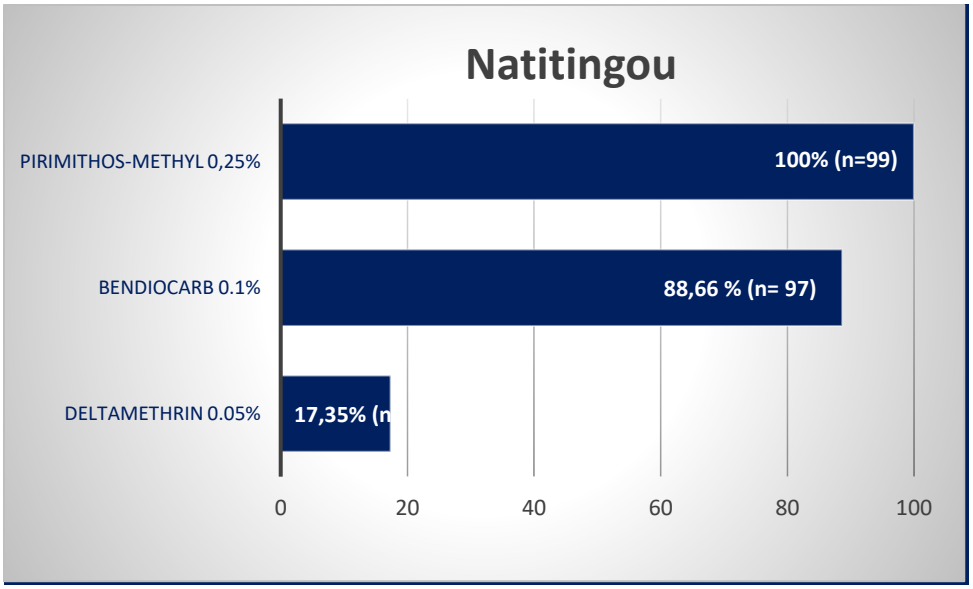


Figure 7: Insecticide susceptibility test results in Natitingou district

6.6. Vector resistance: Identification of mosquito genetic mutations that confer resistance (Kdr, Ace-1).

Kdr and *Ace-1* resistance mechanisms were evaluated through the PCR. A total of 90 mosquitoes were tested, including 30 in each district (Table 3). There is a high allelic frequency of the Kdr gene (86-90%). However, the allelic frequency of the *Ace-1* gene remains between 20 and 21.7%. Over 88 % of the Anopheles mosquitoes caught were *Anopheles gambiae* s.s. as shown in Table 3 below.

Table 3. *Ace-1*^{R} and *Kdr* mutation frequency and species composition in *Anopheles gambiae* from NTB HZ

Districts	**Nb tested**	**Espèces**		***Kdr mutation***				***Ace-1 mutation***			
		An. gambiae s.s	***An. coluzzii***	**RR**	**RS**	**SS**	**F(kdr)**	**RR**	**RS**	**SS**	**F(Ace.1)**
Natitingou	30	27	3	24	6	0	**0.900**	3	7	20	**0.217**
Toukountouna	30	24	6	23	6	1	**0.867**	1	10	19	**0.200**
Boukoumbé	30	29	1	22	8	0	**0.867**	0	13	17	**0.217**

7. Conclusion

After the withdrawal of IRS in some districts in Atacora the monitoring of entomological malaria indicators is usefull to follow what happened after IRS discontinuance in this area. If

the monitoring doesn’t present a significant difference among the entomological malaria indicators during the dry season (January-Mars), we can notice a huge increase of EIR mainly in NTB HZ compared to Pehunco which continues to be treated. These entomological malaria transmission have to be followed over the time to better understand the evolution tendency.