US PRESIDENT'S MALARIA INITIATIVE ACTION TO REINFORCE

MALARIA VECTOR CONTROL PROGRAM IN BENIN

Activity 5: Quantification of the intensity of insecticide vector resistance

Title of the study. Quantification study and evolution of the insecticide resistance in *Anopheles gambiae* s.l in Benin. Implication of high vector resistance in *Anopheles gambiae* s.l for vector control

Deliverable 5-2

Quarterly Report

December 2020

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Abreviations

***al.* :** Collaborators

ASS: Health Statistics Yearbook

s. l : Sensu lacto (sens large)

***Ace-1* :** Acethylcholinesterase

***Ace-1R* :** Acethylcholinesterase Resistant

***An. gambiae* :** *Anopheles gambiae*

***Kdr* :** knock-down resistance

AChE : L'acetylcholinesterase

ADN : Deoxyribonucleic acid

CREC : Centre de Recherche Entomologique de Cotonou

MILD : Long Lasting Impregnated Mosquito Net

WHO : World Heath Organisation

CDC: Center for disease control

IRS : Indoor residuel spray

PNLP : National Malaria Control Program

MS : Ministry of Health

GST : Glutathion S-Transférase

PCR : Polymerase Chain Reaction

1. Background

In September 2017 we implemented a study on the quantification of the intensity of vector resistance with the support of USAID. During two years, the exposition of various populations of *Anopheles gambiae s.l.* to doses of insecticides (permethrin, delatamethrin) 2 and 5 times higher than the diagnostic dose did not kill the total number of mosquitoes tested. On the other hand, the combination of the diagnostic dose with PBO has only partially abolished the resistance showing a very high resistance in *An. gambiae s.l.* in Benin. The second step we have investigated in 2020 is to determine what could be the direct implications of this high intensity of insecticide resistance on vector control management. We have evaluated the response of mosquitoes survived after exposure to high concentrations of insecticides and to the combined insecticides + PBO to different types of LLINs. Results we obtained will serve as database for the utilization of LLINs of new generations in Benin.

2. Objectives

- Perform *An. gambiae* susceptibility to the diagnostic dose of permethrin, deltamethrin and alphacypermethtrin and doses 2 and 5 times higher using WHO and CDC bioassys;
- Determine the mechanisms associated to insecticide resistance within *An. gambiae* in the various ecological zones;
- Asses the efficacy of the CDC bottles or WHO papers with combined “pyrethroid + PBO” against resistant mosquitoes for each ecological zone in relation with the mechanisms of insecticide resistance;
- Determine the response of *An. gambiae* survived after exposure to high concentrations of insecticides and combined insecticides + PBO to different types of LLINs

3. Study methods

In 2018 (IL #24) and 2019 (IL #31), *Anopheles gambiae* collected in 13 districts was tested for susceptibility to permethrin, deltamethrin and alphacypermethrin. The same populations were exposed to diagnostic doses of the three insecticides and doses 2 and 5 times higher than the initial dose to assess the survival rate of resistant mosquitoes after exposure. On the other hand, the same populations of mosquitoes were exposed to the three insecticides combined with Synergist piperonylbutoxide (PBO) using CDC bottles and WHO papers bioassays. Mosquitoes not killed after exposure to the high concentrations of insecticides or to combination insecticide + PBO were exposed to different types of Long Lasting Insecticide Nets (LLINs).

The three insecticides tested are those used to treated the majority of LLINs distributed in Benin: Olyset treated with permethrin, PermaNet 3.0 and PermaNet 2.0 and other nets treated with deltamethrin. Recently (2020), three types of LLINs (Royal Guard, Interceptor and Interceptor G2) were distributed in three communes in southern Benin as part of the mass distribution of the National Malaria Control

Program and the New Nets Project (Unitaid Project) supported by Bill and Melinda Gates. These three types of nets are impregnated with alphacypermethrin combined or not with another product.

3.1. Study area

The study areas are chosen in various ecological zones (Figure 1):

- **Cotton production area** (Kandi, N'Dali and Parakou): this area is characterized by a high use of pesticides against cotton pests. Intensive cotton cultivation is practiced, combined with the use of several families of insecticides.
- **Rice growing area** (Malanville): Malanville rice area is about 70 hectare. Two rice crops are grown per year.
- **Urban vegetable production area**: characterized by high use of impregnated mosquito nets, various aerosol insecticides, smoke coils and various pesticides against vegetable pests (Cotonou and Porto-Novo).
- **Cereal area:** districts of Missérété (Ouémé department), Bantè (Collines department), Ouidah and Allada (Atlantique department), Bohicon (Zou department)
- **Hilly area:** Dassa et Savè in the central part.

Table I : *GPS coordinates of the thirteen study data collection sites*

	Departement	Communes	Latitude/ Longitude
Zone urbaine et maraîchère	Littoral	Cotonou	06°22′26.76″N, 02°27′32.4″E
	Ouémé	Porto-Novo	06°28′19.64″N, 02°37′24.632″E
Zone cotonnière	Alibori	Kandi	11°1′34.68″N, 02°55′37.2″E
	Borgou	N'Dali	09°52′21.108″N, 02°37′24.632″E
	Borgou	Parakou	09°33′47.633″N, 02°25′3026″E
Zone rizicole	Alibori	Malanville	11°51′9.781″N, 02°25′30.26″E
Zone céréalière	Ouémé	Missérété	06°38′17.275″N, 02°33′54.837″E
	Atlantique	Allada	06°38′9.92″N, 02°0′0″E
	Atlantique	Ouidah	06°19′29.279″N, 02°14′14.279″E
	Collines	Bantè	08°24′37.312″N, 01°53′11.38″E
Zone forestière	Zou	Bohicon	07°10′51553″N, 01°53′11.40″E
Zone des collines	Collines	Dassa	07°45′19.634″N, 02°11′40.261″E
	Collines	Savè	07°48′12.562″N, 02°35′2.953″E

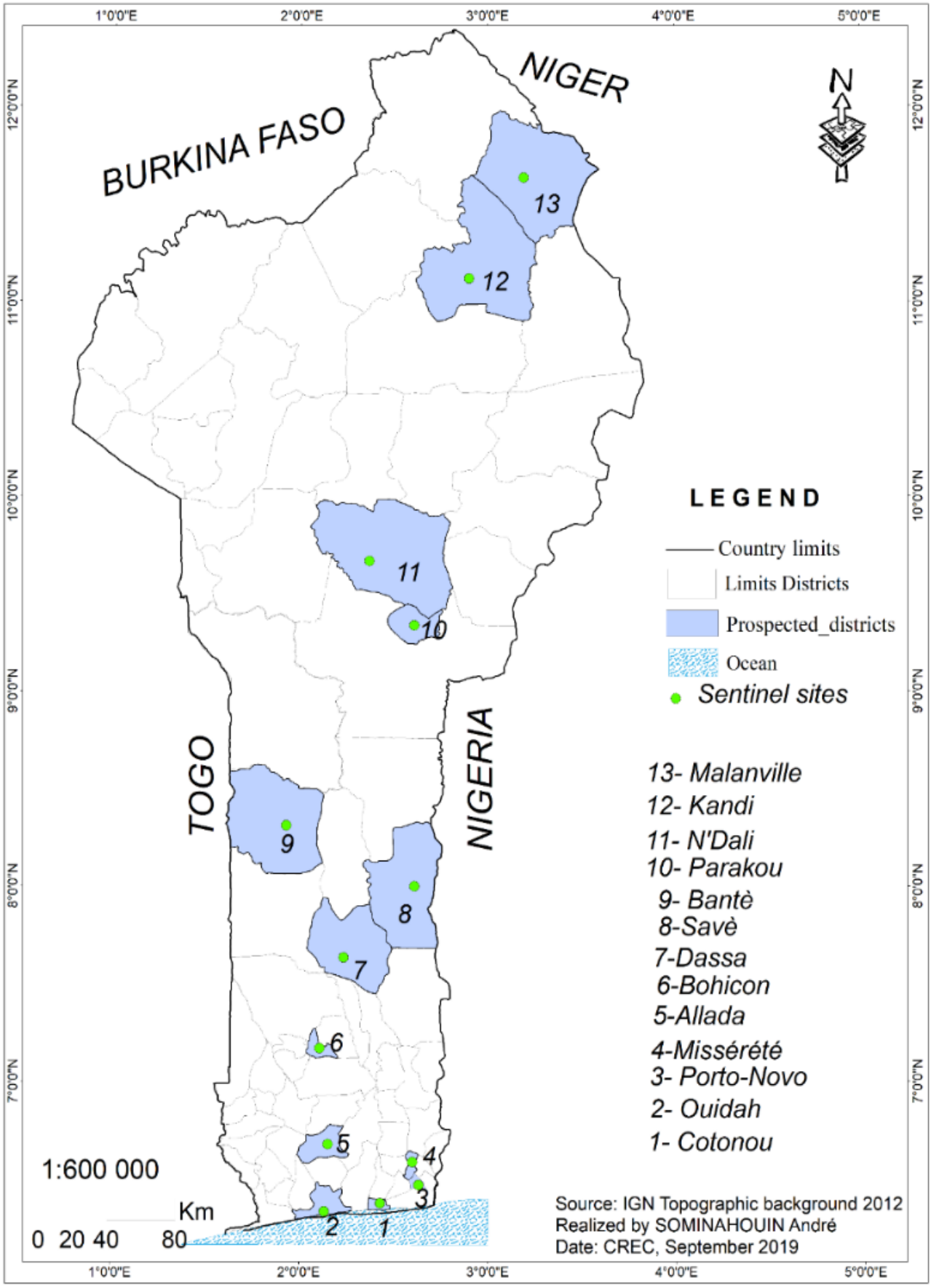

Figure 1: *Map of Benin showing the communes where larvae of Anopheles gambiae were collected to perform susceptibility tests.*

The study sites from the northern Benin (Kandi, N'dali, Parakou, Malanville) are characterized by dry savannah areas, with six months rainy season (mid- April to mid-October) and a dry season which spans the remainder of the year. Overall, average annual rainfall ranges between 700–1200 mm in Kandi and Malanville region and 1200–1300 mm in Parakou and N'dali region.

The districts of Missérété (Ouémé department), Bantè (Collines department), Ouidah and Allada (Atlantique department), Bohicon (Zou department) are characterized by a long rainy season (March-July), a long dry season (December-February), a short rainy season (September-November) and a short dry season (August-September).

Malaria is the leading cause of mortality among children under five years of age and morbidity among adults in Benin. Malaria accounts for 40% of outpatient consultations and 25 %t of all hospital admissions. Malaria places an enormous economic strain on Benin's development. The incidence of uncomplicated and severe malaria in 2016 was 26.4% in Donga region and 13.3% in Alibori.

3.2. Long Lasting Insecticide Nets tested

Seven different nets were used for their efficacy against *Anopheles gambiae* characterized by a high intensity of vector control:

- PermaNet 2.0 (polyester + deltamethrin)
- PermaNet 3.0 (polyester + deltamethrin + PBO)
- Olyset Net (polyethylene + permethrin)
- Olyset Net Plus (polyethylene + permethrin +PBO)
- Alphacypermethrin Net (polyethylene + Alphacypermethrin +PBO)
-Yorkol (polyester + deltamethrin)
-Dawan (polyester + deltamethrin)
- Net not treated (control)

3.3. Protocol of CDC bottles and WHO susceptibility tests and mechanisms associated to insecticide resistance

a. *WHO insecticide susceptibility tests*

The susceptibility tests are performed according to the WHO protocol (WHO, 2013). At the end of the tests, live and dead specimens are used for species identification and determination of resistance mechanisms (*Kdr* L1014F and G119S *Ace-1R*) using PCR method (protocols already described: see previous reports, IL 24 and IL 31).

b. CDC bottle bioassay

The CDC bottle bioassay is conducted using *An. gambiae* s.l collected and the laboratory strain *An. gambiae* s.s Kisumu, according to the CDC protocol (Brogdon and Chan, 1998; CDC, 2014) (protocol already described: see IL 24 and IL 31).

c. Insecticide resistance mechanism tests

Three methods already described (see previous reports, IL 24 and IL 31) were used:

- Identification of the species of *An. gambiae* s.l. tested and molecular characterization of the Kdr L1014F and G119S Ace-1R resistance genes;
- Biochemical analyzes by spectrophotometry;
- Biochemical analyzes by pre-exposing *Anopheles gambiae* s.l. to synergist PBO.

d. WHO cone test method

The WHO cone test measures knock down and mortality of mosquitoes in a standard WHO cone to a piece of treated netting for exposure time. Five non-blood-fed, 2–5-day-old female *Anopheles gambiae* s.l. mosquitoes were exposed to netting materials (25 cm x 25 cm) for 3 minutes under standard WHO cones after which they were held for 24 h with access to sugar solution. Five cones were used for each of 5 surfaces of the net. 5 mosquitoes were used for each cone and 2 replicates were carried out. In total, 50 mosquitoes are needed for one net. Mosquitoes exposed to untreated nets were used as negative control. Bioassays were carried out at 27 ± 2 °C and 75% ± 10% relative humidity. Knock-down (KD) was recorded 60 minutes after exposure and mortality after 24 h. For susceptible mosquitoes, the net is declared efficacious when 95% of mosquitoes survive to the knock down effect or mortality rate >= 80%.

3.4. Statistical analysis

The resistance status of malaria vectors is determined according to WHO criteria (WHO, 2013). When the mortality rate of the An. gambiae s.l. population after 24 hours is between [98;100], the An. gambiae s.l. population is susceptible, when it is between [90;97], the An. gambiae s.l. population is suspected of resistance and this resistance remains to be confirmed, and finally it is qualified as resistant when the mortality rate is less than 90%.

The One-way analysis of variance tests coupled with the Tukey's Multiple Comparison test allowed us to compare the mean values of enzyme activities (esterases, oxidases and GST) between the field strains and between the reference Kisumu strain and the field strains. Mortality rates and allelic frequencies of the kdr-ouest and ace-1R genes were analyzed to assess their variability across different populations.Statistical analyses were performed using the R 2.15

4. Results

Susceptibility tests in WHO tubes and CDC bottles were performed on the thirteen (13) malaria vector populations (*An. gambiae* s.l.) collected in the different districts communes: Cotonou, Malanville, Parakou, Kandi, Porto-Novo, Bohicon, Missérété, Allada, Ouidah, Savè, Dassa, Bantè and N'Dali. The susceptibility results obtained and the resistance status are summarized in the tables below.

a. Resistance status of An. gambiae s.l. to 1x and 2x doses of deltamethrin, permethrin, alpha cypermethrin and bendiocarb according to WHO and CDC test methods.

The results of susceptibility of *An. gambiae* s.l. using the WHO tube test method at 1 and 2 doses of permethrin, deltamethrin and alpha cypermethrin showed widespread resistance in the thirteen communes where larval surveys were carried out (Table I and Figures 2, 3). When we associated PBO with these insecticides, a clear increase in the mortality rate of these vectors was observed, but resistance was still present. For Bendiocarb, only Anopheles from the cotton communes were found to be resistant. Most of the other communes tested were suspected to be resistant. The same trends were observed with CDC bottle tests. However, the addition of the synergist DEF to bendiocarb in the CDC bottled tests significantly increased mortality rates in all communes except in the commune of Missérété where we recorded a mortality rate of less than 90% (80.95%) despite the addition of this synergist (Table II, Table III and Figures 2, 3, 4, 5, 6 et 7).

Tableau II: *Mortality rate after exposure of thirteen populations of An. gambiae s.l. to multiples of diagnostic concentrations of pyrethroids with and without PBO and bendiocarb using WHO bioassay from different area.*

Method	Locality	Insecticides	Delta 1x	Delta 1x+ PBO	Delta 2x	p-value	Per 1x	Per 1x + PBO	Per 2x	Alpha 1x	p-value	Bendio 1x
WHO test	Kandi	Mortality rate	17.34±7.5^{a}	67.36±9.7^{b}	46.53±9.4^{c}	<0.0001	16.16±7.5^{a}	46.80±10.1^{a}	34.73±9.6a	31.76±9.9a	>0.0001	92±5.3
	Parakou	Mortality rate	40±9.9^{a}	90.81±5.7^{b}	44.68±10.1^{a}	<0.0001	23.95±8.5^{a}	58.51±10^{b}	38.04±9.9^{a}	35.10±9.6^{a}	<0.0001	96.84±3.5
	Bohicon	Mortality rate	29.89±9.1a	70.2±8.8^{c}	45.45±9.8^{b}	<0.0001	24.1±9^{a}	55.32±10.1^{b}	43.33±10.2^{b}	45.83±10^{b}	<0.0001	96.96±3.4
	Cotonou	Mortality rate	18.4±7,7^{a}	76.24±8.3^{c}	41±9.6^{b}	<0.0001	10.4±6.1^{a}	43.30±9.9^{b}	35.22±10^{b}	42.42±9.7^{b}	<0.0001	73.52±8.6
	Malanville	Mortality rate	41.7±9.9^{a}	77.01±8.8^{b}	54.44±10.3^{a}	<0.0001	31.9±9.4^{a}	66.66±9.3^{b}	39.02±10.6^{a}	41.93±10^{a}	<0.0001	91.48±5.6
	Missérété	Mortality rate	29.78±9.2^{a}	82.65±7.5^{b}	41.83±9.8^{a}	<0.0001	23.86±8,9^{a}	64.51±9,7^{b}	48.91±10.2^{b}	45.45±9.8^{b}	<0.0001	97.72±3.1
	Porto-Novo	Mortality rate	21.11±8.4^{a}	83.52±7.9^{c}	60.20±9.7^{b}	<0.0001	23.95±8,5^{a}	79.56±8,2^{b}	66.66±10.1^{b}	33.67±9.4^{a}	<0.0001	96.87±3.5
	Allada	Mortality rate	26.04±8.8^{a}	85.85±6.9^{c}	43.75±9.9^{b}	<0.0001	15.00±7.2^{a}	78.78±8.1^{c}	36.26±9.9^{b}	12.08±6.7^{a}	<0.0001	97.95±3.3
	Ouidah	Mortality rate	20.00±7.8^{a}	90.81±5.7^{b}	47.00±10.2^{a}	<0.0001	19.00±7.7^{a}	92.00±5.3^{c}	50.60±10.8^{b}	21.00±8^{a}	<0.0001	97.00±3.3
	Bantè	Mortality rate	44.56±10,2^{a}	97.91±2.9^{b}	48.38±10.2^{a}	<0.0001	23.15±8.5^{a}	58.00±9.7^{b}	64.77±10^{b}	26.66±9.1^{a}	<0.0001	96.96±3.4
	Dassa	Mortality rate	51.25±11^{a}	96.70±3.7^{c}	67.90±10.2^{b}	<0.0001	46.42±10.7^{a}	85.55±7.3^{c}	63.75±10.5^{b}	34.04±9.6^{a}	<0.0001	86.66±8.6
	Savè	Mortality rate	19.38±7.8^{a}	82.65±7.5^{b}	29.16±9.1^{a}	<0.0001	15.30±7.1^{a}	88.77±6,2^{c}	58.82±10.5^{b}	10.10±5.9^{a}	<0.0001	93.20±4.9
	N'Dali	Mortality rate	30.00±9^{a}	95.95±3.9^{c}	46.73±10.2^{b}	<0.0001	26.73±8.6^{a}	89.53±6.5^{c}	55.00±10.9^{b}	28.12±9^{a}	<0.0001	93.93±4.7

m±n = Mortality rate (%); ±se; P: p-value of comparison between localities; In colomn rate with same latter are not statistically different.

Tableau III: *Mortality rate after exposure of thirteen populations of An. gambiae s.l collected in seven districts to diagnostic concentration of deltamethrin, permethrin with and without PBO and bendiocarb with and without DEF using CDC bioassay.*

Method	Locality	Insecticides	Delta 1x	Delta 1x + PBO	Per 1x	Per 1x + PBO	Bendio 1x	Bendio 1x + DEF
CDC test	Malanville	Mortality rate	79±8,9a	96,3±4,1b	67,1±10,4a	97,6±3,3b	95,1±4,8a	98,7±2,5a
	Porto-Novo	Mortality rate	78,8±8,7a	87,8±7,1a	30,9±10,1a	52,5±10,9b	87,7±7,7a	94±5,3a
	Kandi	Mortality rate	38,3±17,1a	86,7±7,8b	51,9±15,3a	97,5±3,5b	96,5±4a	100±0a
	Parakou	Mortality rate	69±9,7a	97,9±2,9b	36,3±10,5a	84,1±7,9b	96,6±3,9a	100±0a
	Missérété	Mortality rate	42,7±16,4a	60,8±13,8b	36,1±17,2a	61,2±13,2b	76,7±10,2a	81±9,3a
	Cotonou	Mortality rate	49,4±11a	97,6±3,3b	34,2±10,7a	87,5±7,2b	97,6±3,4a	100±0a
	Bohicon	Mortality rate	49,4±11a	97,6±3,3b	67,1±12,7a	97,6±3,4b	95,1±4,8a	98,7±2,5a
	Allada	Mortality rate	65,4±10,6a	76,5±9,2a	55,6±10,8a	68,8±10,2a	96,2±4,4a	100±0a
	Ouidah	Mortality rate	63±10,5a	77,5±9,2a	50,6±10,9a	69,5±10b	91±6,6a	100±0b
	Dassa	Mortality rate	39,2±10,8a	67,5±10,3b	37,3±10,4a	66,7±10,3b	87,8±7,6a	96,3±4,2a
	Bantè	Mortality rate	65,4±10,6a	78,8±9a	52,4±10,8a	67,5±10,3a	85,5±8,6a	97,5±3,4b
	Savè	Mortality rate	43,4±11,1a	65,8±10,5b	22±19,1a	63±10,5b	84,4±8,8a	95,1±4,8a
	N'dali	Mortality rate	61,2±10,4a	80,2±8,4b	38,8±10,7a	72,2±9,9b	85,5±8,2a	96,2±4,3b
p-value			<0,001	<0,001	<0,001	<0,001	<0,001	<0,001

P:p-value for comparison of mortality rates between localities for the formulation of the same insecticide; in each locality and for each insecticide, mortalities with the same letter are not significantly different.

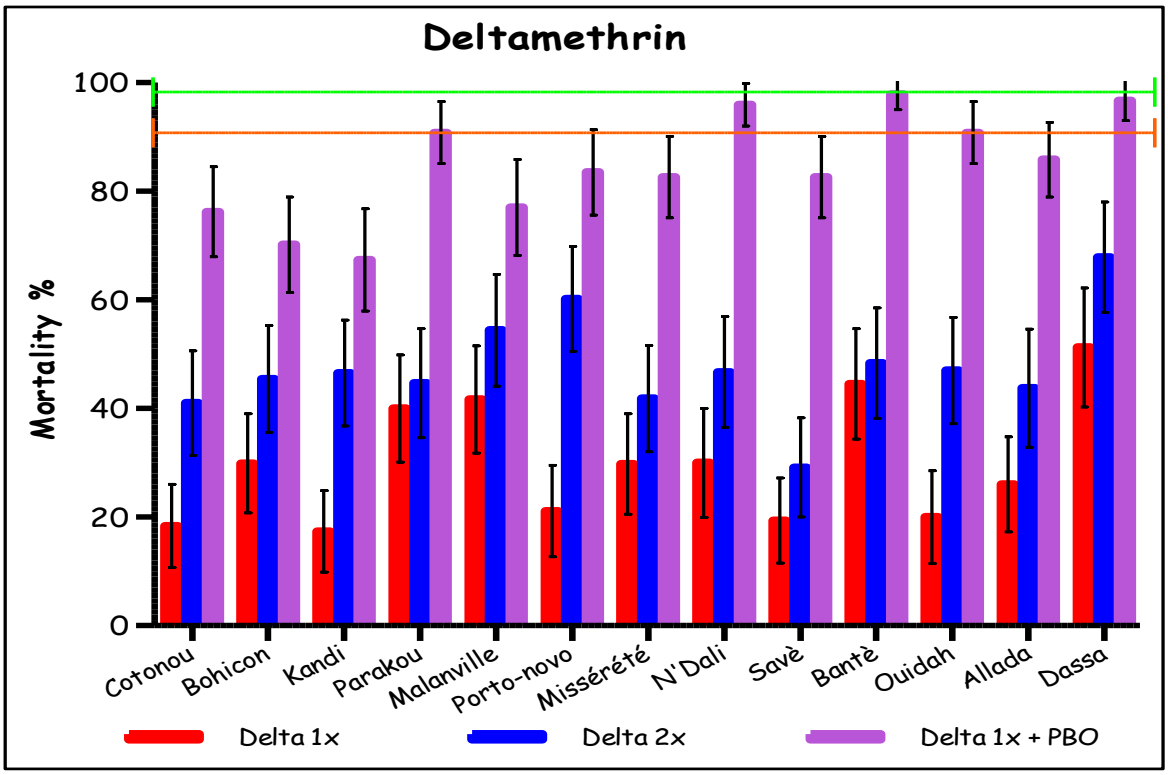

Figure 2: *Mortality rate after exposure of thirteen populations of An. gambiae s.l. to multiple diagnostic concentrations of deltamethrin with PBO using WHO bioassay from different area.*

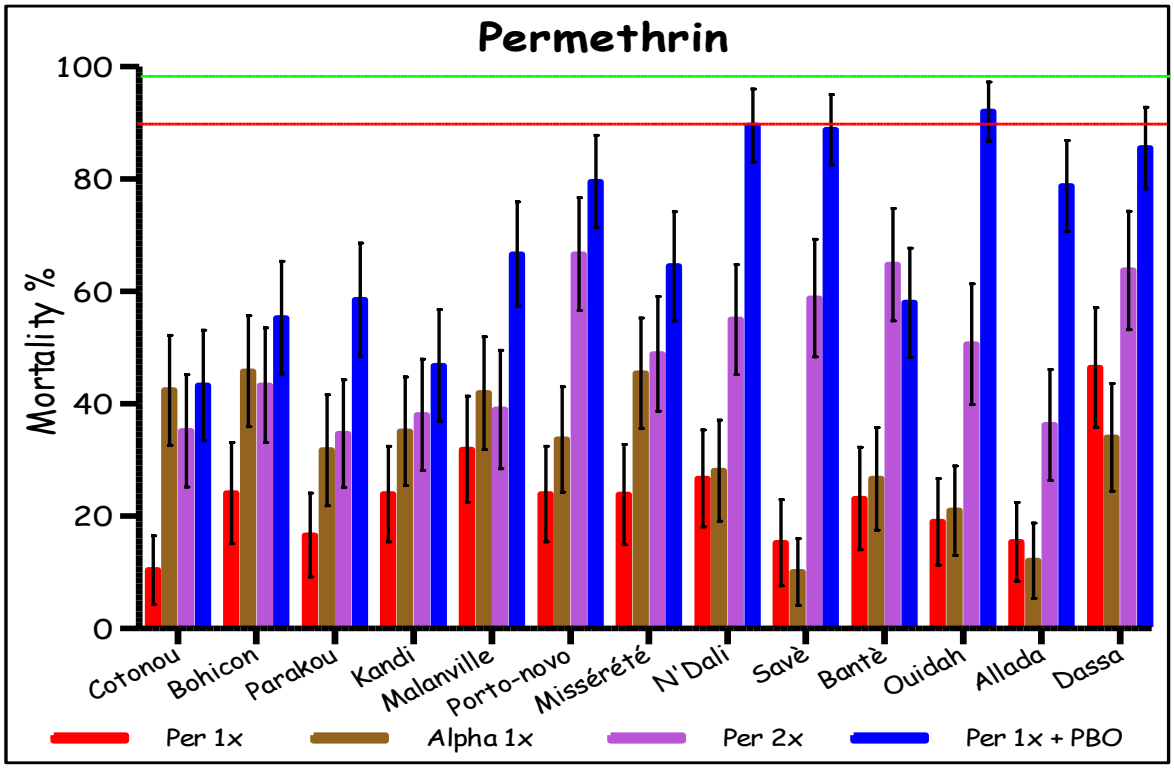

Figure 3: *Mortality rate after exposure of thirteen populations of An. gambiae s.l. to multiple diagnostic concentrations of permethrin and alphacypermethrinwith PBO using WHO bioassay from different area.*

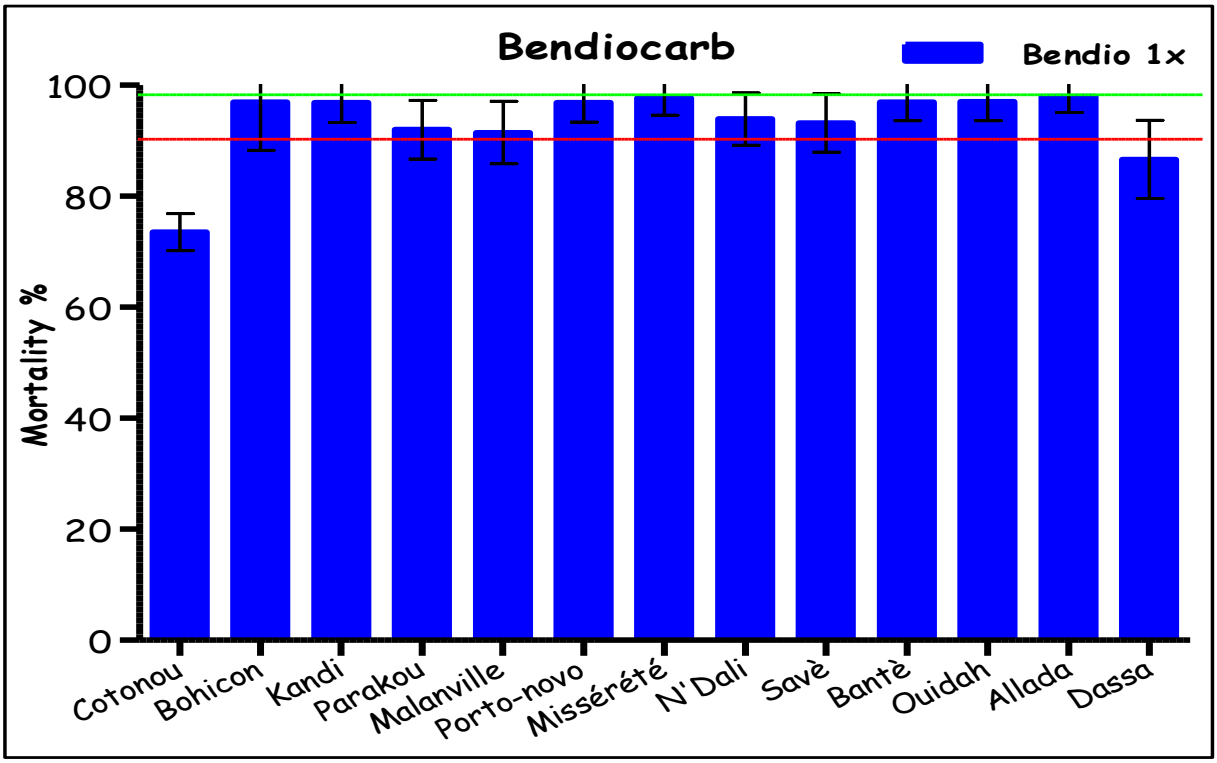

Figure 4: *Mortality rate after exposure of thirteen populations of An. gambiae s.l. to diagnostic concentrations of alpha bendiocarb using WHO bioassay from different area.*

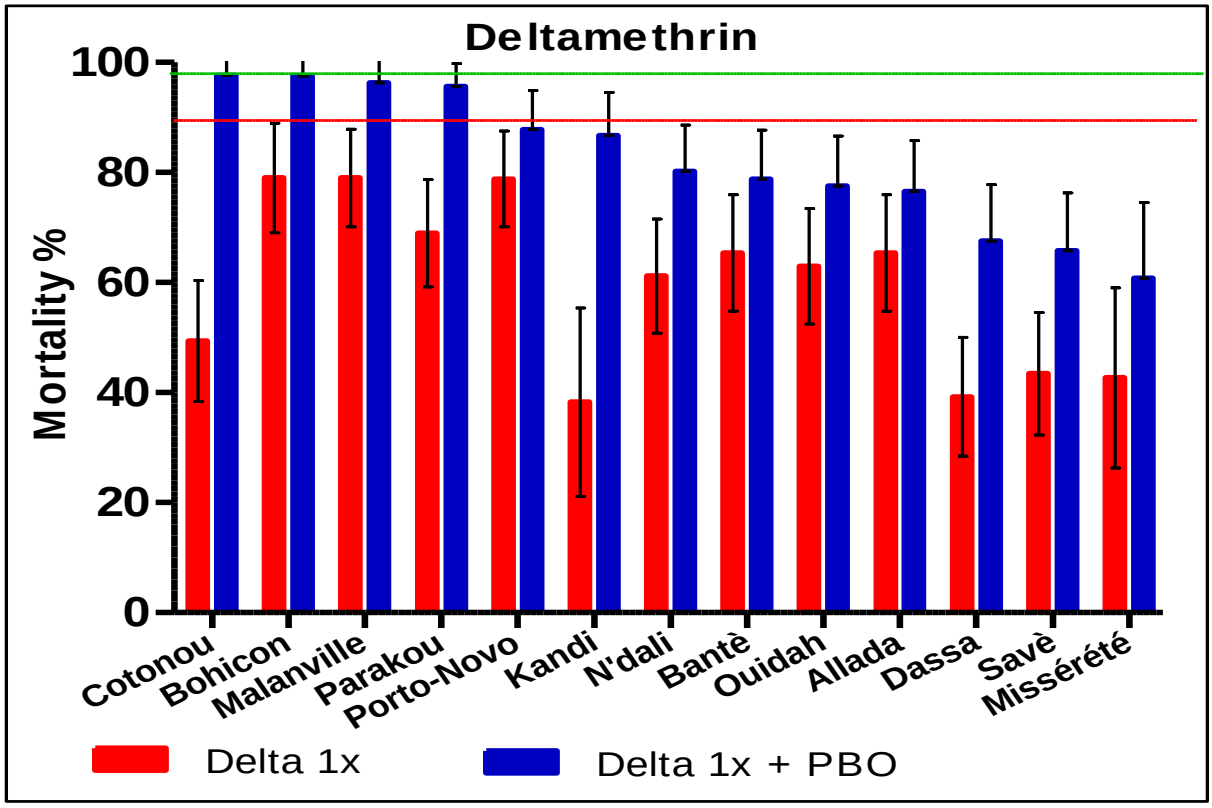

Figure 5: *Mortality rate after exposure of thirteen populations of An. gambiae s.l. to diagnostic dose of deltamethrin associated with the PBO synergist using CDC bottle bioassays.*

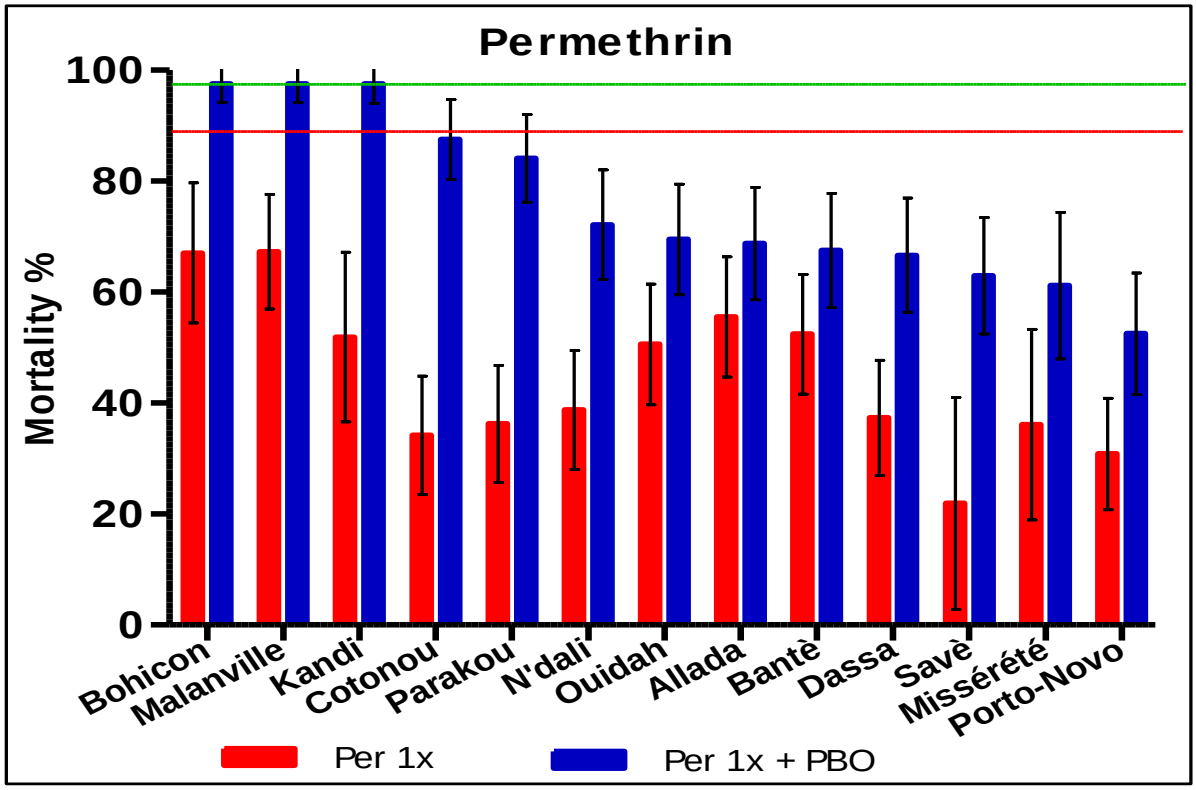

Figure 6: *Mortality rate after exposure of thirteen populations of An. gambiae s.l. to diagnostic dose of permethrin associated with the PBO synergist using CDC bottle bioassays.*

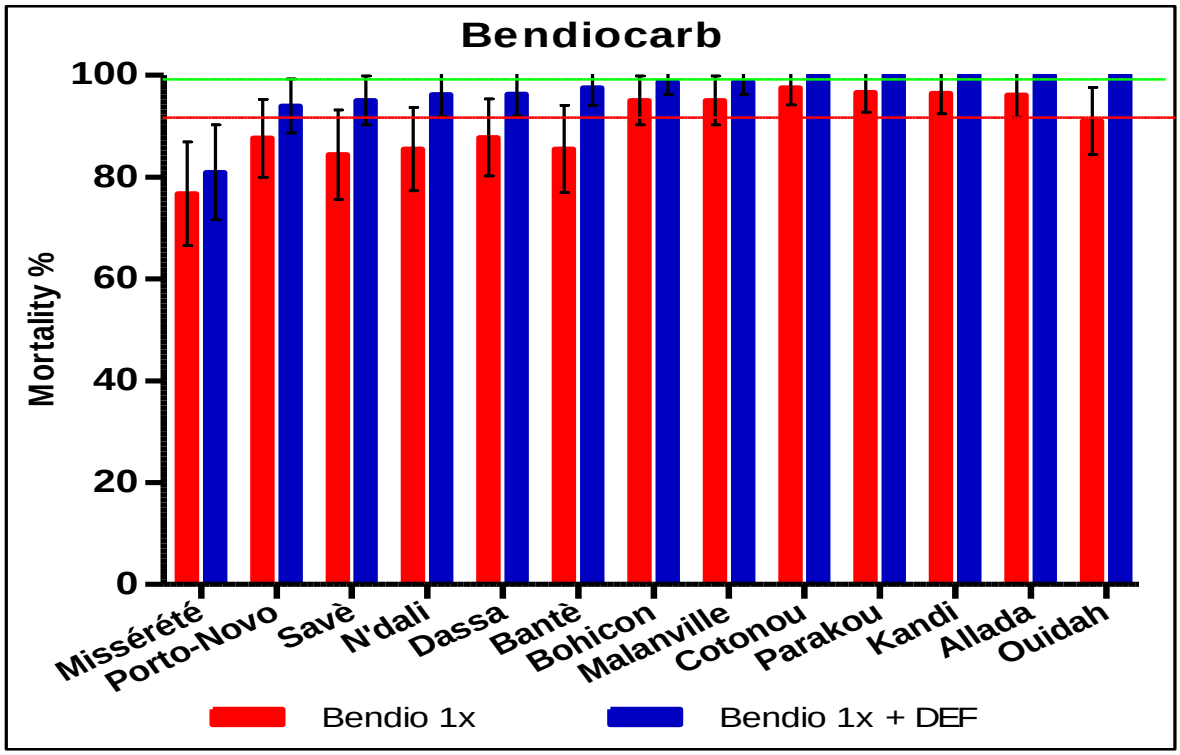

Figure 7: *Mortality rate after exposure of thirteen populations of An. gambiae s.l. to diagnostic dose of bendiocarb associated with the DEF synergist using CDC bottle bioassays.*

b- Implication of An. gambiae s.l. resistance for impregnated materials (ITMs)

Efficacy tests carried out with seven types of mosquito nets (PermaNet 2.0, PermaNet 3.0, Olyset, Olyset Plus, Dawa, Yorkol and Aspirationel) on mosquitoes resistant to 1x and 2x doses of pyrethrinoids once again showed that these vectors are resistant to all the nets tested except the Olyset Plus and PermanNet 3.0. Olyset Plus and PermaNet 3.0 are new generation mosquito nets, impregnated with pyrethrinoids associated with the PBO synergist. The recorded mortality rate varied from 91.04% to 100% for Olyset Plus and is 100% for PermaNet 3.0 in all the localities surveyed. No dead mosquitoes were recorded after exposure of these resistant mosquitoes to the non-insecticide-treated net (control) (Table III).

Table IV: *Mortality rate after exposure of thirteen resistance populations of An. gambiae s.l. to seven different net using cone bioassay from different area.*

	LLNs test							
Locality	*Net not treated*	Olyset Net	Olyset Net Plus	Permanet 2.0	Permanet 3.0	Yorkol	Dawa	Aspirationel
Kandi	0.00%	83.33%	100%	07.14%	100%	39.21%	-	05.55%
Parakou	0.00%	76.92%	100%	03.63%	100%	38.46%	94.82%	03.70%
Bohicon	0.00%	66.15%	97.01%	42.10%	100%	03.70%	79.62%	86.20%
Cotonou	0.00%	96.66%	100%	08.77%	100%	14.54%	66.66%	43.39%
Malanville	0.00%	68.51%	91.07%	19.29%	100%	21.15%	25%	31.37%
Missérété	0.00%	35.29%	98.21%	14.43%	100%	26.41%	50%	23.52%
Porto-Novo	0.00%	46%	100%	12%	100%	29.41%	60.37%	13.72%
Allada	0.00%	60.00%	98.70%	12.69%	93.75%	71.21%	100%	85.13%
Ouidah	0.00%	75.00%	100%	34.48%	93.44%	76.78%	100%	72.00%
Bantè	0.00%	96.15%	100%	61.90%	100%	83.33%	93.33%	36.66%
Dassa	0.00%	42.00%	100%	26.31%	96.07%	40.67%	90.00%	38.33%
Savè	0.00%	31.03%	100%	13.69%	100%	21.21%	87.65%	28.75%
N'Dali	0.00%	91.54%	100%	31.66%	97.14%	29.62%	100%	43.54%

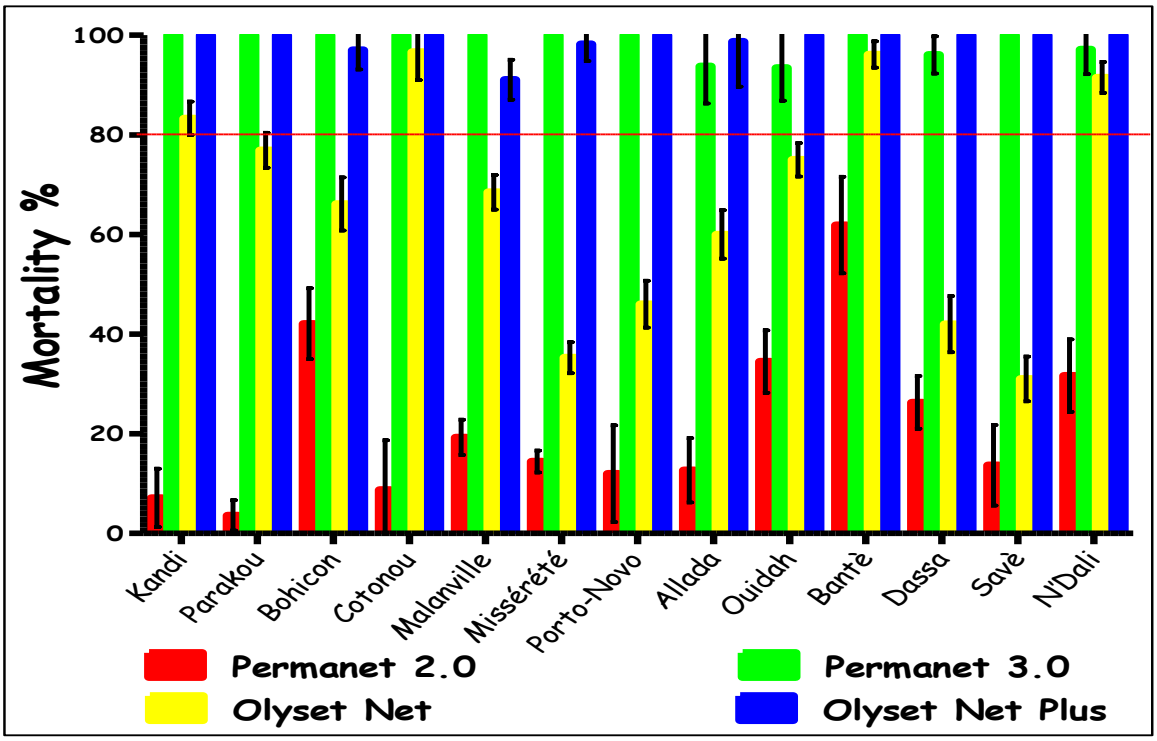

Figure 8: *Mortality rate after exposure of thirteen resistance populations of An. gambiae s.l. to Permanet 2.0, PermaNet 3.0, Olyset and Olyset Plus using cone bioassay from different areas.*

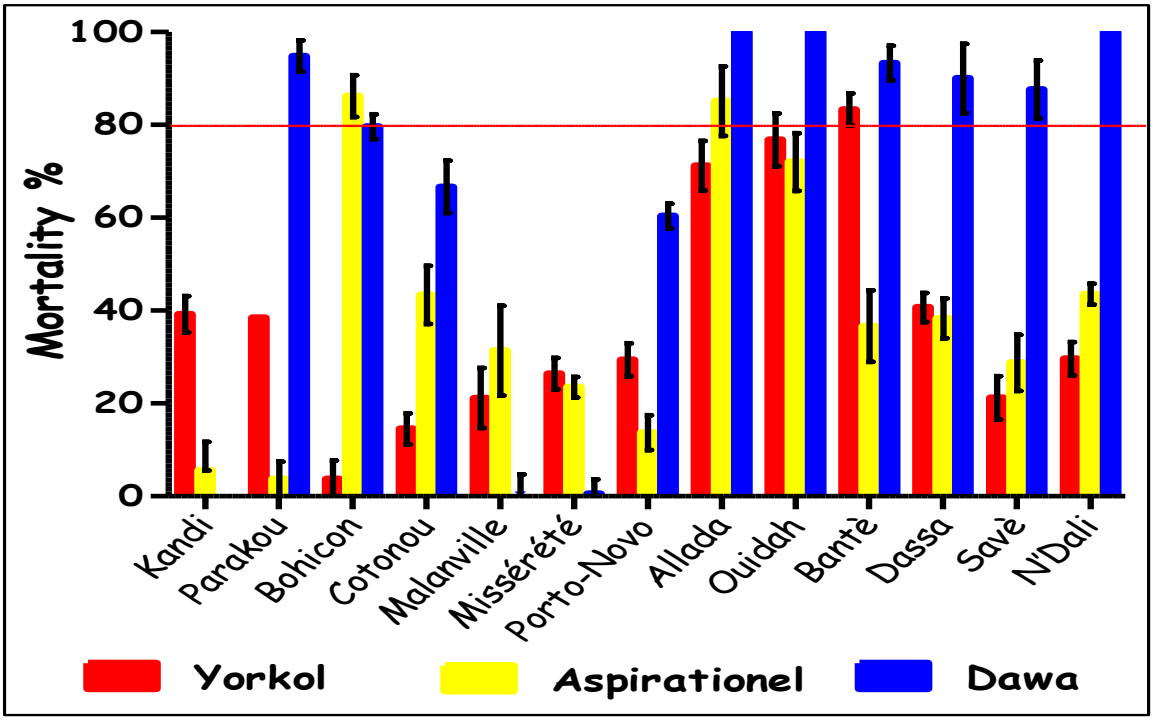

Figure 9: *Mortality rate after exposure of thirteen resistance populations of An. gambiae s.l. to Yorkol, Aspirational and Dawa Plus using cone bioassay from different area.*

c- Characterization of molecular forms and resistance genes (Kdr and Ace-1r) in the thirteen communes

The distribution of *An. gambiae* s.l. showed the presence of three main species: *An. gambiae, An. Coluzzii* and *An. arabiensis* with very high proportions for *An. gambiae* in almost localities except Malanville where all Anopheles analysed were *An. Coluzzii*. As for the distribution of resistance genes, the frequency of the L1014F allele of the *Kdr* gene was high in all localities. The lowest frequency of the *Kdr* resistance gene was observed in Malanville (0.57) and the highest in Cotonou (0.84). Unlike the *Kdr* resistance gene, the frequency of the G119S allele of the Ace-1 gene was very low in all the localities surveyed (Table V).

Table V: *Distribution of resistance genes in An. gambiae, An. coluzzii and An. arabiensis in the thirteen communes.*

	Molecularly form			Mutation *Kdr*					Mutation *Ace-1R*				
Locality	*An. Arabiensis*	*An. Coluzzii*	*An. gambiae*	RR	RS	SS	F. *Kdr*	P. Value	RR	RS	SS	F. *Ace-1*	P. Value
Cotonou	00	00	50	36	12	02	**0.84[a]**		00	04	46	**0.04[a]**	
Porto-Novo	00	37	13	31	15	04	**0.77[a]**		00	01	49	**0.01[a]**	
Bohicon	00	33	17	31	15	04	**0.77[a]**		**00**	01	49	**0.01[a]**	
Missérété	00	07	43	35	12	03	**0.82[a]**		00	06	44	**0.06[a]**	
Parakou	00	03	47	33	14	03	**0.80[a]**		00	02	48	**0.02[a]**	
Kandi	06	00	44	31	15	04	**0.77[a]**		00	00	50	**0.00[a]**	
Malanville	00	50	00	16	25	09	**0.57[b]**	**<0,0001**	00	02	48	**0.02[a]**	**0,091**
Allada	00	43	00	32	09	02	**0.84[a]**		00	02	41	**0.02[a]**	
Ouidah	00	46	00	31	09	06	**0.77[a]**		00	01	45	**0.01[a]**	
Savè	2	18	27	34	10	03	**0.82[a]**		00	01	46	**0.01[a]**	
Bantè	00	12	32	29	11	04	**0.78[a]**		00	01	45	**0.01[a]**	
Dassa	04	06	36	30	10	06	**0.76[a]**		00	01	45	**0.01[a]**	
N'Dali	01	13	32	23	13	10	**0.64[a]**		00	04	42	**0.04[a]**	

In the same column, the values indexed to the same letter are not significantly different.

d- Enzyme activities (MFO, esterase and GST) between the thirteen wild populations and Kisumu

The activity of non-specific esterases (α and β esterases) is higher in the populations of Kandi, Parakou, Porto-Novo, Bohicon, Missérété, Porto-Novo, Dassa, Bante, N'dali, Ouidah, and Cotonou than in the Kisumu strain ($p < 0.05$) (Table VI, Figure 8). Mixed-function oxidase activity is higher in the Cotonou populations than in those of Kisumu ($p < 0.0001$) (Table VI, Figure 9). The highest glutathione S-transferase (GST) activity was observed in the Cotonou, Kandi, Parakou, Bohicon, and Save populations compared to the Kisumu strain ($p < 0.0001$) (Table VI, Figure 10).

Table VI: *Mean (± SE) mixed function oxidases, glutathione-S-transferases and esterases activities in An. gambiae s.l. populations.*

Strain	MFO (nmol/P450/min/mg protein)	GST (nmol/GSH conj/min/mg protein)	α-Esterase (µmol α Naph/ /min/mg protein)	β-Esterase (µmol β Naph /min/mg protein)
kisumu	0.060±0.008[a]	0.222±0.027[a]	0.101±0.010[a]	0.091±0.009[a]
Cotonou	0.116±0.011[b]	0.388±0.035[b]	0.129±0.027[a]	0.160±0.012[b]
Malanville	0.070±0.009[a]	0.305±0.044[a]	0.083±0.011[b]	0.084±0.012[a]
Porto-Novo	0.083±0.013[a]	0.255±0.025[a]	0.135±0.008[a]	0.123±0.008[a]
Missérété	0.083±0.011[a]	0.341±0.028[a]	0.105±0.020[a]	0.131±0.022[b]
Bohicon	0.046±0.005[a]	0.410±0.037[b]	0.154±0.013[a]	0.159±0.015[b]
Parakou	0.057±0.007[a]	0.333±0.045[a]	0.141±0.012[a]	0.147±0.013[b]
Kandi	0.079±0.013[a]	0.405±0.054[b]	0.127±0.014[a]	0.133±0.016[b]
Dassa	0.061±0.009[a]	0.330±0.039[a]	0.114±0.013[a]	0.123±0.013[b]
Savè	0.051±0.007[a]	0.438±0.086[b]	0.067±0.007[b]	0.078±0.010[a]
Allada	0.060±0.012[a]	0.348±0.051[a]	0.084±0.010[b]	0.085±0.009[a]
Ouidah	0.044±0.008[a]	0.212±0.109[a]	0.093±0.014[b]	0.094±0.013[a]
Bantè	0.049±0.009[a]	0.313±0.037[a]	0.127±0,008[a]	0.130±0.008[b]
N'dali	0.072±0.012[a]	0.318±0.070[a]	0.110±0.010[a]	0.118±0.022[a]
One Way ANOVA	F=12.47; df=13, 574; P<0.0001	F=06.29; df=13, 574; P<0.0001	F=14.06; df=13, 574; P<0.0001	F=14.24; df=13, 574; P<0.0001

MFO= mixed function oxidases, GST = glutathione-S-transferase; Mean followed by a different letter were significantly different, P<0.05, Tukey's test. *Significant increase in mean differences compared to the laboratory reference strain, P<0.05, t-test. In the same column, the values indexed to the same letter are not significantly different.

✓ **Non-specific esterase activity (α and β esterases)**

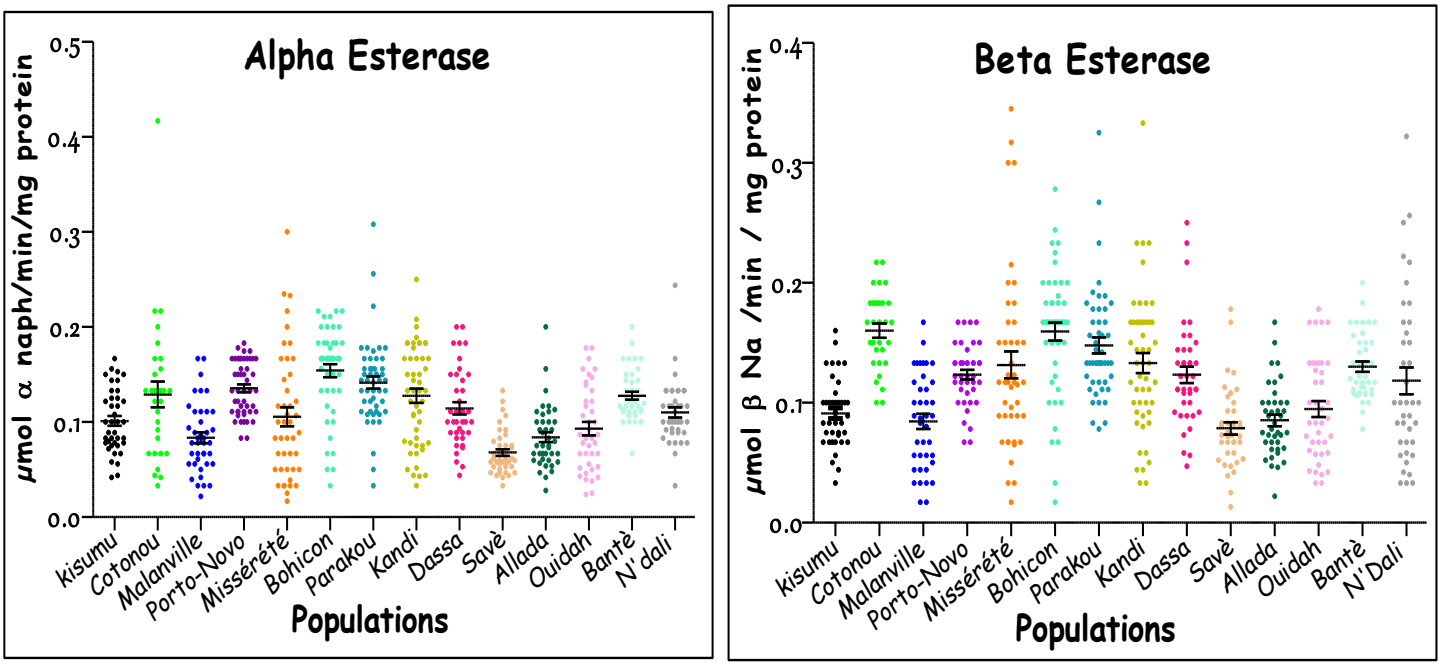

Figure10: *Non-specific esterase activity*

✓ **Oxidase activity**

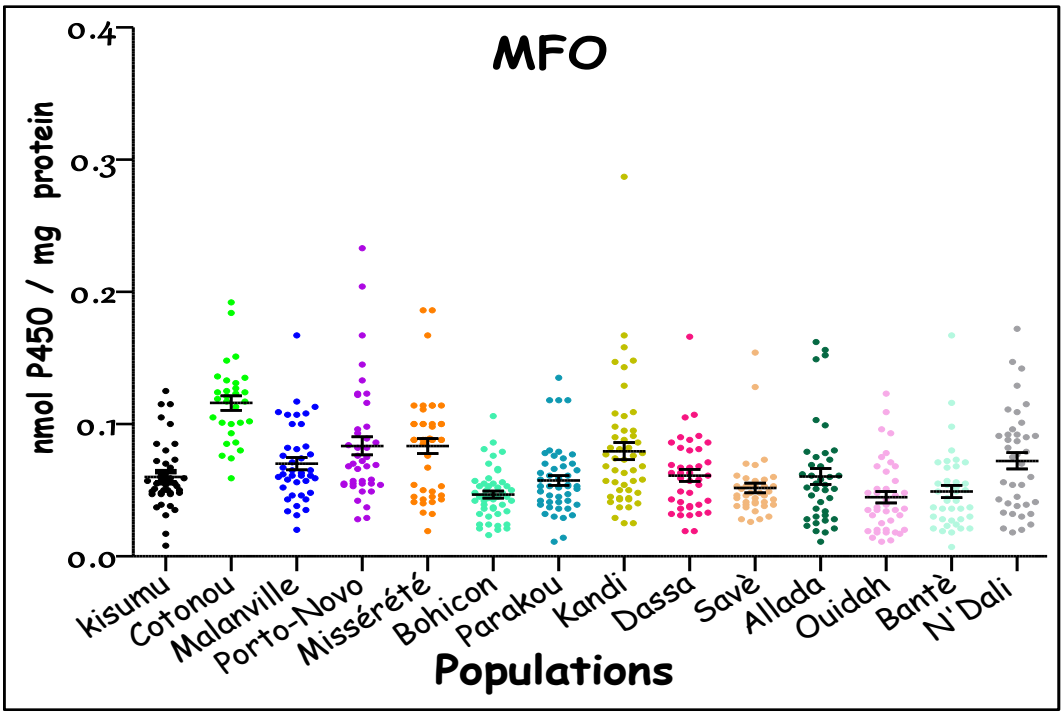

Figure 11: *Oxidase activity (MFO)*

✓ **Glutathione S-transferase activity (GST)**

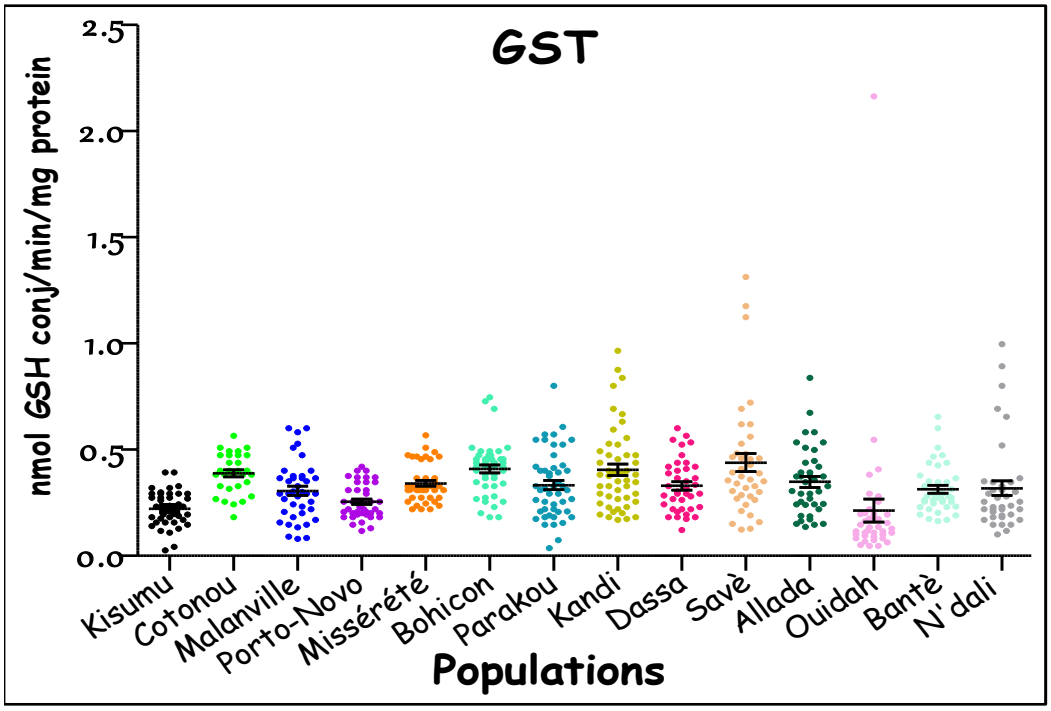

Figure 12: *Glutathione S-transferase activity (GST)*

Conclusion

Mosquito susceptibility to permethrin, deltamethrin, alphacypermethrin and bendiocarb followed the same trend in the thirteen different communes: a strong resistance to these insecticides due to several mechanisms: L1014F kdr, overexpression of oxidases, esterases and glutathione S-transferase. However, mosquitoes resistant to these different insecticides were susceptible to pyrethroid-impregnated nets combined with BPO (PermaNet 3.0; Olyset Plus). This result is a good news for the NMCP in its efforts to manage insecticide resistance in malaria vectors.

Challenges

Our next challenge is to identify the other mechanisms involved in the resistance of these vectors including the level of expression of resistance genes through QPCR. The achievement of these results will allow the NMCP to define other vector resistance management strategies in the effective fight against malaria in Benin.

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