International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 10 No. 1 Jan. 2015, pp. 8-14

http://www.ijias.issr-journals.org/

Cotton production in Benin: a cause of the emergence of insecticide resistance in populations of *Anopheles gambiae* in Benin

A. Yadouléton[1-2], C. Vodounon[2], C. Chabi[3], R. Agbanrin[1], K. Badirou[1], R. Attolou[1], F. Ursins[1], H. Allagbé[4], and M. Akogbéto[1]

[1]Centre de Recherche Entomologique de Cotonou (CREC), 06 BP 2604 Cotonou, Benin

[2]Ecole Normale Supérieure de Natitingou, Université de Parakou, Benin

[3]Université de Parakou, Faculté de Médecine, Benin

[4]Université d'Abomey-Calavi, Département d'Aménagement du Territoire Recherche, Benin

ABSTRACT: *Background:* Agricultural pesticides may play a profound role in selection of resistance in field populations of mosquito vectors. The objective of this study is to investigate possible links between agricultural pesticides use and development of resistance to insecticides by the major malaria vector *Anopheles gambiae* from cotton field.

Method: Susceptibility to 4% DDT, 0.05% deltamethrin, 0.75% permethrin, 0.1% bendiocarb was assessed using the WHO standard procedures for adult mosquitoes from cotton field. Tests were carried out with two to three days-old, non-engorged female mosquitoes. The *An. gambiae* Kisumu strain was used as a reference. Knockdown effect was recorded every 5 min and mortality scored 24 h after exposure. Mosquitoes were identified to species and molecular form by PCR-RFLP and genotypes at the *knock down resistance (kdr*) and, *acetylcholinesterase* mutations were determined in surviving specimens.

Results: During this survey, full susceptibility to bendiocarb was recorded in all samples. WHO diagnostic tests showed high frequency of resistance in *An. gambiae* to permethrin (ranging from 3% to 4% mortality), deltamethrin (13% to 22%), DDT (1.01% to 2%) in the seven selected areas. The *Kdr* gene seemed the main target- site resistance mechanism detected at the rates ranging from ranging from 65 to 71%.

The frequency of *ace-1R* gene was found but at very low frequency (< 0.1).

Conclusion: This investigation of malaria vector susceptibility to insecticides revealed a strong resistance to pyrethroid insecticides (permethrin and deltamethrin).

This Pyrethroid resistance may seriously jeopardize the efficacy of of IRS and LLINs on which, most African countries including Benin, rely to reduce malaria transmission.

The current findings will help for decision making in the National Malaria control program particularly in the choice of insecticide to use during campaigns of Indoor residual spraying in this part of Benin.

KEYWORDS: Cotton; *Anopheles gambiae*; Insecticide; Resistance; Banikoara; Benin.

1 INTRODUCTION

Cotton production is an important crop in West and Central Africa [**1**]. It has long been seen as an engine of development in West Africa [**2**]. A recent report from the Food and Agriculture Organization (FAO) showed more than 10 million people depend on cotton production to earn enough cash to pay for the food they eat [**3**]. It is the most important source of export revenue in Burkina-Faso, Mali, Cameroon, Senegal and Benin [3].

However, Cotton is vulnerable to insect pest's attack, especially when grown in a monoculture. The agrochemicals used to control these insect pests are often acutely toxic, with potential to cause serious adverse health and environmental impacts.

In West and Central Africa, cotton crop protection represents 90% of the insecticide use against insect pests [**1**]. The control strategies implemented against cotton pest especially *Helicoverpa armigera*, the main pest of cotton required a regular repeated applications of insecticides during the cotton plant growing cycle. In Benin, as recommended by the Institut National des Recherches Agronomiques du Benin (INRAB), six consecutive treatments are applied at two weeks interval to protect the crop against bollworms, leafworms and sucking pests [**4**].These insecticides are essentially composed of organochlorines, organophosphates, carbamates and pyrethroids which are also the main classes used in public health for the impregnation of long lasting insecticide-treated bed nets (LLINs) and indoor residual spraying (IRS) [**5**]. All four of these classes are nerve poisons and either target acetylcholinesterase in the synapses or the voltage-gated sodium channel on the insect neurones.

According to Yadouleton et al. [4], in Benin, insecticide treatments against cotton pests are applied twice a month, for a timeframe of three consecutive months (between July and October) each year. These treatment periods coincide with the rainy season and correspond to the period of high mosquito densities. During the treatments, insecticide residues in cotton fields are washed downwards into mosquito breeding sites thus affecting larval population where they exert a huge selection pressure on larval stages of *An. gambiae* s.l [**6**]. For the last ten years, to increase the yield of cotton, farmers have received considerable attention by the government support. This has led to the use of insecticide in an improper manner to control cotton pests

It therefore crucial to investigate the status of insecticide resistance in *An. gambiae* from the cotton field Benin, because pyrethroid resistance has been reported with a clear evidence of reduced efficacy of ITNs and IRS in experimental huts [**7-8**]. In addition to the knock down resistance (kdr) mutation, which is the main mechanism of resistance to pyrethroids, it's important to address also the presence of the Acethylchonesterase (*Ace-1*) mutation that causes resistance to organophosphates and carbamates.

The present study proposes to assess the resistance status of malaria vectors to carbamates, pyrethroids and organochlorine and assessed the implications for vector control strategy at Banikora in the northern Benin.

2 METHODS

DESCRIPTION OF STUDY SITES

The study was conducted in a cotton field located at Banikoara (2°59 E, 11°31 N) in the northern Benin (Figure 1). The first reason which justifies the choice of this area is that Banikoara is the most productive area of cotton in Benin [**4**]. Moreover, much quantity of the four classes of insecticides is used widely by farmers for pests control [**4**].

The annual mean rainfall recorded at Banikoara ranging from 723.8 to1280.7 mm yearly and this area is characterized by a Sudanian climate with one rainy season (middle of June to October) and one dry season (November-May).

KAP STUDY ON THE USE OF INSECTICIDES IN COTTON FARMS

To generate adequate information on the use of insecticide on cotton fields, Knowledge Attitude-Practice (KAP) surveys were organized in the study site. Following the protocol used by Yadouleton et al. [**4**], leaders of farmer's organizations in seven sites at Banikoara were interviewed using semi-structured questionnaires that focused on the treatment strategies, and the different type of insecticides in the farms. Further, qualitative data was collected through direct observations, in-depth interviews and focus group discussions.

MOSQUITO COLLECTIONS

Mosquitoes were collected during the rainy season (from July to October) across seven selected points at Banikoara in northern Benin. All larvae collected using the dipping on breeding sites from each location were pooled together and reared locally until emergence. Emerging adults were provided with a 10% sugar solution. Adult mosquitoes were sexed and identified morphologically [**9**]

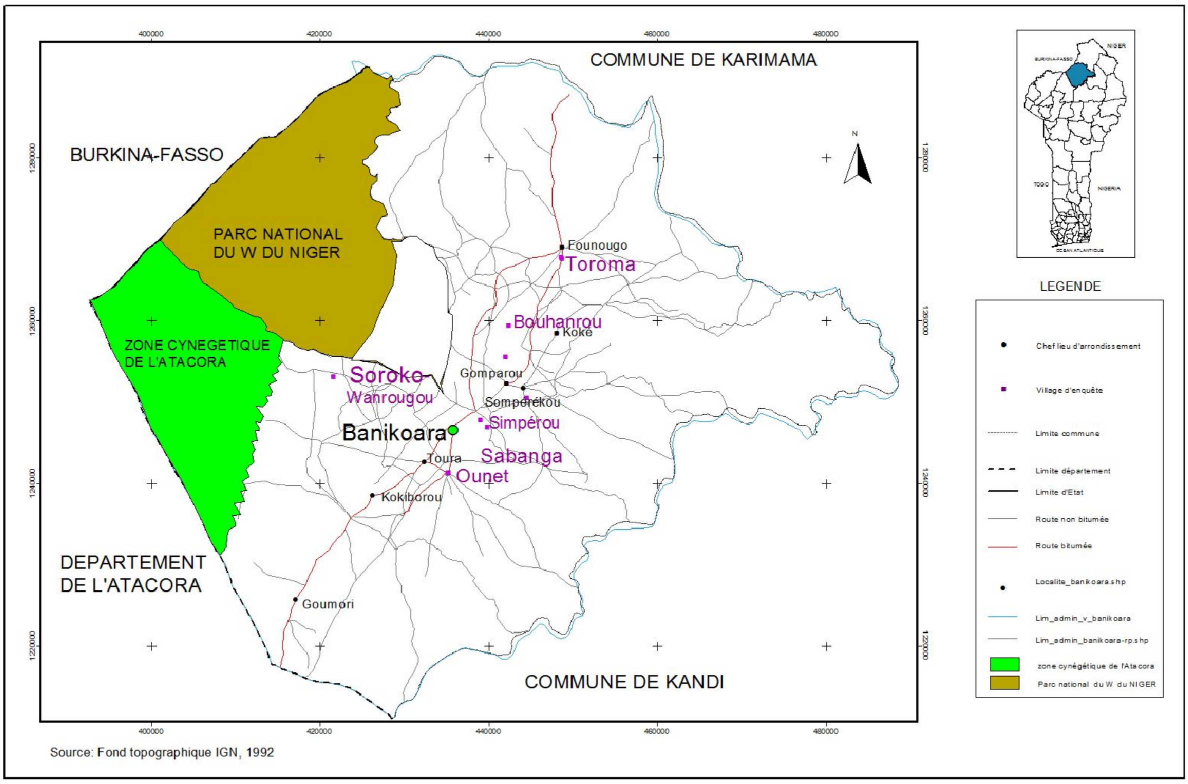

Figure 1: Map of Benin showing the study sites

INSECTICIDE SUSCEPTIBILITY TESTS

Females mosquitoes aged 2-5 days old were exposed to diagnostic doses of various insecticides for susceptibility tests using insecticide-impregnated papers, as described by the standard WHO testing protocol [**10**]. Impregnated papers with recommended diagnostic concentrations of 4% DDT, 0.05% deltamethrin, 0.75% permethrin, 0.1% bendiocarb were used. For each insecticide, 4 batches of 20 to 25 females were exposed to impregnated papers for 1 h. Control tests consisted of a group of 25 mosquitoes exposed to untreated papers.

The *An. gambiae* Kisumu strain was used as the reference strain and tested concomitantly. The number of knockdown mosquitoes was recorded every 10 minutes during the exposure period. Mosquitoes were then transferred into holding tubes and supplied with a 10% sugar solution. Mortality was recorded 24 h after exposure.

The tests results were discarded if mortality in the control group was over 20%. If it was between 5 and 20%, mortality rates were corrected using Abbott's formula [**11**]. Dead and alive mosquitoes were kept separately in 1.5 ml tubes with silica gel and stored at -20°C for molecular analysis.

MOLECULAR IDENTIFICATION AND DETECTION OF THE *KDR AND ACE-1* MUTATIONS

A random sample of 150 mosquitoes from each point of unexposed mosquitoes (bioassays controls) were identified using the PCR-RFLP technique described by Fanello *et al* [**12**] after DNA extraction according to Collins *et al* [**13**]. The last series of PCRs were based on mosquitoes surviving the bioassays to determine the presence of kdr and *Ace-1R* mutations using respectively the methods of Martinez-Torres etal. [**14**] and Weill et al [**15**].

DATA ANALYSIS

The resistance/susceptibility status of the tested populations was determined for each insecticide according to WHO criteria [**10**]. By the said criteria, a resistant population is defined by mortality rates less than 80% after the 24 h observation period while mortality rates greater than 98% are indicative of susceptible populations. Mortality rates between 80–98% suggest a possibility of resistance (suspected resistance) that requires confirmation.

3 RESULTS

KNOWLEDGE-ATTITUDE -PRACTICE (KAP) INVESTIGATIONS

Results from our KAP investigations showed that about 6 pesticide treatments were applied by farmers 45 days after seeding and at two week intervals from flowering. Endosulfan or Tihan® (mixture of spirotetramat + flubendiamide) were sprayed in the first two treatments followed by the mixtures of cyfluthrin + chlorpyrifos ethyl for the 3rd and the 4th treatment and then cypermethrin + dimethoate applied for the last two treatments (Table 1)

Table 1: Pesticides commonly used in cotton field at the seven study sites

Commercial name	Active ingredient	Family name
Tihan 175 O-TEQ	Flubendiamide (100 g/l) + Spirotetramate (75 g/l)	Organophosphate
Emir 88EC	Cyperméthrine (72 g/l) + Acétamipride (16 g/l)	Organophosphate
Cotofan EC	Endosulfan	Organophosphate
Thionex 350EC	Endosulfan	Organophosphate
Nurelle D 236 CE	Cypermethrin (200 g/l) + Chlorpyrifos- methyl (36 g/l) Cypermethrin (200 g/l)	pyrethroid + Organophosphate
Decis 25	Deltamethrin (25 g/l)	pyrethroid
Thunder 145 OD	Betacyfluthrin (45 g/l) + Imidaclopride (100 g/l)	pyrethroid + Organophosphate

RESISTANCE STATUS

Table 2 shows that the reference strain Kisumu and the wild population of *An. gambiae* from the seven sites were fully susceptible (100% mortality) to DDT, deltamethrin , permethrin and bendiocarb.

However, *An. gambiae s.l.* populations at the 7 points at Banikoara were classified as resistant to DDT with mortality rates ranging from 1 to 2% (Table 2). With the two pyrethroids tested, WHO diagnostic tests showed also high frequency of resistance in *An. gambiae* to permethrin (ranging from 3% to 4% mortality), deltamethrin (13% to 22%).

Table 2: Mortality of the wild populations of An. gambiae from the seven study sites after exposure to organochlorine (DDT = 4%), pyrethroids (permethrin = 0.75% and deltamethrine 0.05%) and carbamate (bendiocarb = 0.1%).

	sites	Insecticides	N	% Mortality	Resistance status
	Torama	DDT	100	1.01	R
		Permethrin	100	3	R
		Deltamethrin	100	16	R
		Bendiocarb	100	100	S
	Bouhanrou	DDT	95	2.10	R
		Permethrin	98	4.08	R
		Deltamethrin	100	13	R
		Bendiocarb	100	100	S
Banikoara	Soroko	DDT	100	1	R
		Permethrin	100	4	R
		Deltamethrin	100	18	R
		Bendiocarb	100	100	S
	Wanrougou	DDT	100	2	R
		Permethrin	100	3	R
		Deltamethrin	100	15	R
		Bendiocarb	100	100	S
	Simperou	DDT	100	2	R
		Permethrin	100	4	R
		Deltamethrin	100	17	R
		Bendiocarb	100	100	S
	Sabanga	DDT	100	1	R
		Permethrin	100	4	R
		Deltamethrin	100	18	R
		Bendiocarb	100	100	S
	Ounet	DDT	100	1	R
		Permethrin	100	3	R
		Deltamethrin	100	22	R
		Bendiocarb	100	100	S
An. gambiae	Control	DDT	100	98	S
Kisumu		Permethrin	100	100	S
		Deltamethrin	100	100	S
		Bendiocarb	100	100	S

R = Resistance; S= Susceptible

IDENTIFICATION OF MOLECULAR FORMS OF *ANOPHELES GAMBIAE* S.S

An average of 80 mosquitoes from each site at Banikoara was successfully analyzed by species, molecular forms. PCR on mosquito's populations revealed the presence of two sub-species of *An. gambiae*: *An. gambiae* sensu stricto (*s.s)* and *Anopheles arabiensis* with a predominance of *An. gambiae* s.s (68%). The M form was predominant over the S form (S = 70%). (Table 3)

DETECTION OF RESISTANCE GENES

A total sample of 420 mosquitoes (80 per site at Banikoara) was genotyped both for the 1014 *kdr* and *Ace.1R* mutation. Results from this PCR showed that the *kdr* mutation was present in all *An. gambiae* populations collected in the seven points at Banikoara with frequency ranging from 0.65 to 0.72. There is no difference among the frequency of *Kdr* mutation found in *An. gambiae* populations from the seven points at Banikoara (P>0.05). (Table 3).

The Ace-1 mutation was found but at very low frequency (<0.1) (Table 3)

Table 3: Species and molecular forms identification within Anopheles gambiae complex and the frequency of Kdr and Ace-1R mutations in Anopheles gambiae s.s. in Benin

	Species[a]		Mol. Form		*Kdr* mutation				*Ace.1* mutation			
Localities	**%Aa**	**%Ag**	**%M**	**%S**	**SS**	**RS**	**RR**	**F(R)**	**SS**	**RS**	**RR**	**F(R)**
Torama (90)	20	70	5	85	10	35	45	0.69	84	06	0	0.03
Bouhanrou (90)	18	72	12	78	10	40	40	0.67	86	04	0	0.02
Soroko (92)	25	70	6	86	7	45	40	0.68	85	07	0	0,04
Wanrougou (80)	30	50	10	70	6	34	40	0.71	75	05	0	0.03
Simperou (90)	25	65	18	72	15	25	50	0.69	84	06	0	0.03
Sabanga (100)	30	70	15	85	10	36	54	0.72	92	08	0	0.04
Ounet (100)	28	72	20	80	18	34	48	0.65	90	10	0	0.05
Banikoara (96)	26	70	16	80	12	36	48	0.69	88	8	0	0.04

4 DISCUSSION

The information collected during interviews with cotton farmers and the observations made in cotton fields has confirmed the use an improper manner of insecticides in cotton fields at Banikoara. These insecticides used for pests control belonging unfortunately to the two main classes recommended for vector control in public health: organophosphates and pyrethroids. In West Africa, pyrethroid-treated beds nets remain are the only class approved for use on

insecticide-treated netting owing to their relatively low human toxicity, excito-repellent properties, rapid rate of knock-down and killing effects [**16**], and are being increasingly deployed in indoor residual spraying (IRS) programs in Africa [**5**]. Pyrethroids are also widely used in the control of agricultural pests worldwide [**17**]. The extensive use of pyrethroids particularly in agriculture has increased the selection pressure on the major malaria vectors, which have inevitably developed resistance. In the last decade, the emergence of resistance in populations of Anopheles to common classes of insecticides used in public health has been reported in many African countries including Kenya [18], Benin [**19**].

In Benin, pyrethroids have been extensively introduced in agriculture since 1980s [**6**]. This factor is probably one of the causes of the selection of strong resistance in *An. gambiae* population from cotton field at Banikoara to permethrin and deltamethrin.

According to Yadouleton et al. [**4**], in Benin, insecticide treatments against cotton pests are applied twice a month, for a timeframe of three consecutive months (between July and October) each year. These treatment periods coincide with the rainy season and correspond to the period of high mosquito densities. During the treatments, insecticide residues in cotton fields are washed downwards into mosquito breeding sites thus affecting larval population where they exert a huge selection pressure on larval stages of *An. gambiae* s.l [**6**].

There is clear evidence on the implication of agricultural use of insecticides in the selection of resistance in the major malaria vectors.

Moreover, with pyrethroid resistance spreading in *Anopheles gambiae*, and the introduction of carbamate, bendiocarb as an alternative to pyrethroids for Indoor Residual Spraying (IRS) since six years in Benin, resistance to carbamate has been reported in Benin **[20**]. Therefore, the massive use of these insecticides belongs to carbamate family in cotton fields by farmers can explain the presence of *Ace-1R* mutation in *An. gambiae* populations from the seven sites of Banikoara.

5 CONCLUSION

With the high level of *Kdr* allele frequency in *An. gambiae* populations, pyrethroid resistance resistance may seriously jeopardize the efficacy of of IRS and LLINs on which, most African countries including Benin, rely to reduce malaria transmission.

The current findings will help for decision making in the National Malaria control program particularly in the choice of insecticide to use during campaigns of Indoor residual spraying in this part of Benin.

ACKNOWLEDGEMENTS

This work was financially supported by the Ecole Normale Supérieure de Natitingou. I am grateful to CREC's staff particularly Géraldo Houndéton for technical assistance during laboratory bioassays

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

[1] Dagris (2005). Production cotonnière africaine– Campagne 2004/05. *Note de conjoncture n°8, novembre*, Paris.

[2] Zoundi J.S., Hussein K., Hitimana L.(2006). Libéralisation de la filière coton et innovation agricole en Afrique de l'Ouest. *Cahiers Agriculture*, vol. 15, n°1, janvier-février 2006.

[3] UEMOA(2003). Étude d'identification et de promotion d'unités industrielles régionales dans la filière coton de l'UEMOA, UEMOA mars 2003.

[4] Yadouleton A, Martin T, Padonou G, Chandre F, Asidi A, Djogbenou L, Dabiré R, Aïkpon R, Boko M, Glitho I, Akogbeto M, 2011. Cotton pest management practices and the selection of pyrethroid resistance in *Anopheles gambiae* population in Northern Benin. Parasit Vectors , 4:60.

[5] Ranson, H., H. Abdallah, A. Badolo, W.M. Guelbeogo, C. Kerah-Hinzoumbe, E. Yangalbé-Kalnoné, N.F. Sagnon, F. Simard and M. Coetzee (2009). Insecticide resistance in *Anopheles gambiae*: Data from the first year of a multi-country study highlight the extent of the problem. *Malar. J.*, 8: 299.

[6] Akogbéto M, Djouaka R, Noukpo H (2005).Use of agricultural insecticides in Benin. *Bull Soc Pathol Exot*. 98:400-405.

[7] Magesa SM, Wilkest J, Minzawa AEP, Myamba J, Philip MD (1990).Trial of pyrethroid impregnated bed nets in area of Tanzania hole endemic of malaria. Part II. Effects on vector population. *Acta Trop.* 49:97-108.

[8] N'Guessan R, Corbel V, Akogbeto M, Rowland M (2007). Reduced efficacy of insecticide treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg Infect Dis*. 13:199-206.

[9] Gillies MT, De Meillon B (1968). The Anophelinae of Africa South of the Sahara. *South Africa Institute of Medical Research Johannesburg, South Africa*.

[10] WHO:World Malaria Report (2008).World Health Organzation, Geneva.

[11] Abbott WS (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*,18, 265–267

[12] Fanello C, Petrarca V, Della Torre A, Santolamazza F, Dolo G, Coulibaly M, Alloueche A, Curtis CG, Toure YT, Coluzzi M (2003). The pyrethroid knock-down resistance gene in the Anopheles gambiae complex in Mali and further indication of incipient speciation within *An. gambiae s.s*. *Insect Mol Biol*. 12:241-245.

[13] Collins FH, Finnerty V, Petrarca V (1988). Ribosomal DNA probes differentiate five cryptic species in the *Anopheles gambiae* complex. *Parassitologia*. 30(2-3):231-240

[14] Martinez TD, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL,Guillet P, Pasteur N, Pauron D (1998). Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae s.s*. *Insect Mol Biol*. 7:179-184.

[15] Weill M, Malcolm C, Chandre , Mogensen K, Berthomieu A, Marquine M, Raymond M (2004). The unique mutation inace-1giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Mol*. 9:1-7

[16] Zaim M, Aitio A & Nakashima N (2000). Safety of pyrethroid-treated mosquito nets. *Medical and Veterinary Entomology*, 14,1–5.

[17] Santolamazza F et al. (2008). Distribution of knock-down resistance mutations in Anopheles gambiae molecular forms in west and west central Africa. *Malaria Journal* 7, 74.

[18] Vulule JM, Beach RF, Atieli FK et al. (1999) Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin-impregnated nets. *Medical and Veterinary Entomology*. 13, 239–244.

[19] Djouaka, R., H. Irving, Z. Tukur and C.S. Wondji (2011). Exploring mechanisms of multiple insecticide resistance in a population of the malaria vector *Anopheles funestus* in Benin. *PLoS ONE*, 10.1371.

[20] Aïkpon R, Agossa R, Ossè R, Oussou Ol, Aïzoun N, Oké-Agbo F, Akogbéto Martin (2013). Bendiocarb resistance in *Anopheles gambiae s.l*. populations from Atacora department in Benin, West Africa: a threat for malaria vector control. *Parasit Vectors*. ; 6: 192.