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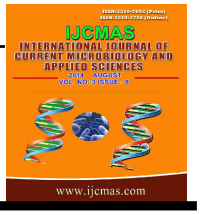

Original Research Article

Exploring Glutathione S-transferases involved in dichlorodiphenyltrichloroethane (DDT) and permethrin cross-resistance in *Anopheles gambiae s.l* populations in the south-north transect Benin, West Africa

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Keywords

Cross-resistance, synergist ETAA, insecticide, vectors, Glutathione *S*-transferases, Benin

A B S T R A C T

Because of the free-insecticide treated net (OlysetNets) distribution by Beninese National Malaria Control Programme since July 2011 throughout the country to increase coverage of LLINs, we investigated the distribution of insecticide resistance and of metabolic resistance mechanisms in *Anopheles gambiae s.l* mosquitoes in the south-north transect Benin. Larvae and pupae of *Anopheles gambiae s.l.* mosquitoes were collected from the breeding sites in Zou and Borgou departments. CDC susceptibility tests were conducted on unfed female mosquitoes aged 2-5 days old with stock solutions of permethrin (21.5µg per bottle) and DDT (100µg per bottle). CDC biochemical assays using synergist ETAA were also carried out to detect any increase in the activity of enzyme typically involved in insecticide metabolism. CDC diagnostic tests showed high frequency of cross-resistance in *An. gambiae* to permethrin and DDT in both areas surveyed. Mortality rates observed with permethrin were higher than the one observed with DDT in both *Anopheles gambiae s.l* tested populations and may be likely explained by the presence of an additional resistance mechanism in Benin (e.g. "Leu-Ser" mutation). Pyrethroid and DDT resistance was widespread in malaria vector in Benin but Glutathione *S*-transferases may play no role in *An. gambiae* Parakou and Bohicon resistant to DDT.

Introduction

The intense use of DDT in agricultural settings and during the WHO malaria eradication programme in the 1950s and 1960s were suspected to be the main factors selecting for pyrethroids and DDT resistance in *An. gambiae* populations (Akogbéto *et al.*, 2005). Pyrethroids are the only option for net treatment due to their relative safety for humans at low dosage, excito-repellent properties, rapid rate of knock-down and killing effects (Zaim *et al.*, 2000). However widespread reports of pyrethroid resistance in *An. gambiae* in West and East Africa (Vulule *et al.*, 1999; Chandre *et al.*, 1999)

and its cross-resistance with DDT are major challenges to its adoption for vector control purposes. Resistance to the insecticide DDT in the mosquito vectors of malaria has severely hampered efforts to control this disease and has contributed to the increase in prevalence of malaria cases. Over 90% of the 300–500 million annual cases of malaria occur in Africa, where the major vector is *Anopheles gambiae s.l* (Ranson *et al.*, 2000a).

The two primary causes of insecticide resistance are alterations in the target sites and increases in the rate of insecticide metabolism. Three major enzyme families, the esterases, glutathione S-transferases (GSTs) and mono-oxygenases, are primarily involved in insecticide metabolism and the activity of one or more of these families is often elevated in resistant insects (Hemingway & Ranson, 2000).

Elevated levels of GST activity have been found to be associated to insecticide resistance in many insects. For instance, GSTs are found to be elevated in *Ae. aegypti* resistant to DDT (Grant & Hammock, 1992; Grant & Matsumura, 1989). In mosquitoes, the metabolic resistance based on GST is the major mechanism of DDT resistance (Hemingway & Ranson, 2000). In Benin, very few studies have shown the involvement of glutathione S-transferases (GSTs) in *An. gambiae* resistance to DDT. However, the cross-resistance to DDT and pyrethroids in *An. gambiae s.l.* with strong geographic variations in a south-north transect was recently reported (Djogbénou *et al.*, 2009; Djègbé *et al.*, 2011). In addition, the Beninese

National Malaria Control Programme has implemented large-scale and free distribution of LLIN (OlysetNets) since July 2011 throughout the entire country to increase coverage of LLINs. It is crucial that information on current status of *An. gambiae s.l.* resistance to permethrin and DDT being investigated. This will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate resistance management strategies.

Although it was shown by Ranson *et al.* (2000b), that a leucine–phenylalanine substitution at position 1014 of the voltage-gated sodium channel is associated with resistance to permethrin and DDT in many insect species, including *Anopheles gambiae s.l* from West Africa, a recent study has shown that target site mutation (*kdr*) was not responsible for DDT and permethrin resistance in *An. arabiensis* populations, a major malaria vector in Nigeria. This study has likely suggested the involvement of metabolic resistance mechanisms in this resistance (Oduola *et al.*, 2010).

The main goal of this study was to explore the involvement of glutathione S-transferases (GST) in cross-resistance to DDT and permethrin in *Anopheles gambiae s.l.* in the south-north transect Benin using classical synergist.

Materials and Methods

Study areas

The study was carried out in some localities; following a south-north transect. Two contrasting localities of Benin were selected for mosquito collection on the basis of variation in agricultural production, use of insecticides and/or ecological settings. The localities were: Bohicon located in the central part of the country, where the farmers used significant amounts of pyrethroids and organophosphates for cotton protection or to control agricultural pests.

Parakou is an urban vegetable growing area located in the north of Benin.The central part of the country is characterized by a sudano-guinean climate with an average rainfall of 1,000 mm per year. The northern zone (Parakou) is characterized by a sudanian climate with only one rainy season per year (May to October) and one dry season (November-April). The temperature ranged from 22 to 33°C with the annual mean rainfall which is 1,300 mm.

Mosquito collection

An. gambiae s.l. mosquitoes were collected from April to June 2013 during the first rainy season in Bohicon district selected in the central part of Benin. We have also collected *An. gambiae s.l.* mosquitoes from May to June 2013 during the rainy season (May to October) in Parakou district selected in north Benin. Larvae and pupae were collected using the dipping on breeding sites and then kept in separated labeled bottles related to each locality. The samples were reared up to adult emergence at the CREC (Centre de Recherche Entomologique de Cotonou, Benin) insectary. *An. gambiae* Kisumu, a reference susceptible strain was used as a control for the bioassay tests. Susceptibility tests were done following CDC protocol on unfed females mosquitoes aged 2-5 days old reared from larval and pupal collections. All susceptibility tests were conducted in the CREC laboratory at 25+/-2°C and 70 to 80% relative humidity.

CDC protocol

The principle of CDC bottle bioassay is to determine the time it takes an insecticide to penetrate an arthropod, traverse its intervening tissues, get to the target site, and act on that site relative to a susceptible control. Anything that prevents or delays the compound from achieving its objective of killing the arthropods contributes to resistance. Diagnostic doses that were applied in the current study were the doses recommended by CDC (Brogdon & Chan, 2010).These doses were checked on the *An. gambiae* Kisumu susceptible reference strain before being applied to field populations. For *An. gambiae s.l.*, the diagnostic dose of 21.5µg per bottle for permethrin was used for a diagnostic exposure time of 30 minutes whereas the diagnostic dose of 100µg per bottle for DDT was used for a diagnostic exposure time of 45 minutes. The choice of permethrin was justified by the insecticide used on OlysetNets that are distributed free by the NMCP in July 2011 across all the country. DDT was tested because of its intensive use in the past as well as to assess cross-resistance with permethrin in districts surveyed. The solutions were prepared and the bottles coated according to the CDC protocol (Brogdon & Chan, 2010). Fifteen to 20 unfed female mosquitoes aged 2-5 days were introduced into four 250 ml Wheaton bottles coated with insecticide and one control bottle coated with acetone only. The number of dead or alive mosquitoes was monitored at different time intervals (15, 30, 35, 40, 45, 60, 75, 90, 105, 120 minutes). This allowed us to determine the percentage of total mortality (Y axis) against the exposure time (X axis) for all replicates using a linear scale.

Biochemical assays using synergists

Synergists were used according to the protocol described by CDC (Brogdon & McAllister, 1998; Brogdon & Chan, 2010) following the procedure outlined in Fig 1. Samples that showed high resistance to DDT in Bohicon and Parakou districts were exposed to the effects of synergist: Ethacrynic acid (ETAA or EA) (80µg/bottle), which inhibits glutathione S-transferases activity.

Approximately 125 mosquitoes were used for each synergist assay. The number of dead or alive mosquitoes was monitored at different time intervals (0, 15, 30, 35, 40, 45, 60, 75, 90, 105, 120 minutes). This test allowed us to compare the obtained percentages of dead mosquitoes (Y axis) against time (X axis) before the addition of the synergist to those obtained after the addition of the synergist (Figure 1).

Figure 1. Diagram for performing the CDC bottle bioassay with synergists [CDC: Methods in *Anopheles* Research, 2010].

Statistical analysis

The resistance status of mosquito samples was determined according to the CDC criteria (Brogdon & McAllister 1998; Brogdon & Chan, 2010). The susceptibility thresholds at the diagnostic time of 30 minutes for pyrethroids and 45 minutes for organochlorines are:

- Mortality rate = 100%: the population is fully susceptible
- Mortality rate < 100%: the population is considered resistant to the tested insecticides.

Abbott's formula was not used in this study for the correction of mortality rates in test-bottles because the mortality rate in all controls was always less than 5% (Abbott, 1987).

To appreciate the effects of synergist EA on *An. gambiae* Parakou and Bohicon populations resistant to permethrin and DDT, we used a Kruskal-Wallis test. The knockdown times for 50% and 95% of tested mosquitoes (kdt_{50} and kdt_{95}) were estimated using SPSS version 16.0 (SPSS Inc., Chicago, IL).The significance level was set at 5%.

Results and Discussion

Susceptibility of *An. gambiae s.l.* populations to permethrin and DDT

Kisumu strain (control) confirmed its susceptibility status as a reference strain. All female mosquitoes of *Anopheles gambiae s.l* Kisumu which were exposed to CDC bottles treated with permethrin 21.5µg/bottle and DDT 100µg/bottle were knocked-down after 30 minutes which represents susceptibility threshold time or diagnostic time clearly defined by CDC protocol. That confirmed this strain was fully susceptible to both products (Table 1 and Table 2). A non neglected proportion of *An. gambiae* Parakou and Bohicon populations; 7.5% and 12.5%, respectively after 30 minutes exposure to CDC bottles treated with permethrin, continue again to fly in these bottles. That showed these populations were resistant to this product (Table 1). A similar pattern was observed when these populations were exposed to DDT; 32.84% and 68.06% of *An. gambiae* Parakou and Bohicon populations respectively continue again to fly in these bottles. That also showed these populations were highly resistant to this product (Table 2).

Table 1. Susceptibility status and permethrin resistance levels in *Anopheles gambiae s.l s.l.* populations.

Table 2. Susceptibility status and DDT resistance levels in *Anopheles gambiae s.l s.l.* populations.

Correlation between resistance level and 'knocked-down' time

The analysis of table 3 shows that after 10.91 minutes exposure to CDC bottles treated with DDT, 50% of *Anopheles gambiae s.l* Kisumu tested populations were

knocked-down (Kdt_{50}) and 95% were knocked-down after 16.38 minutes (Kdt_{95}). Regarding *Anopheles gambiae s.l* Parakou and Bohicon populations, the Kdt_{50} and Kdt_{95} obtained were high. After 53.67 minutes and 121.86 minutes exposure to CDC bottles treated with DDT; 50% of tested mosquitoes were knocked-down respectively (Kdt_{50}) and 95% were knocked-down after 139.69 minutes and 291.75 minutes respectively (Kdt_{95}). These results showed that there is a correlation between the resistance degree of a mosquito to an insecticide and the time that this mosquito takes to react to the product (Table 3).

Table.3 Correlation between resistance level to DDT and 'knocked-down' time

Effects of synergist ETAA on *Anopheles gambiae s.l* Parakou populations resistant to DDT

The analysis of table 4 and table 5 shows that after the addition of synergist EA in CDC bottles treated with DDT, the KdT_{50} value obtained with *Anopheles gambiae s.l* Parakou populations was 53.09 minutes. This value was slightly lower than the one obtained with DDT alone which was 53.67minutes. A similar pattern was observed with KdT_{95} value obtained with these same populations which was 105.08 minutes after the addition of synergist EA. This value was also lower than the one obtained with DDT alone which was 139.69 minutes. Synergist Ratio (SR) (Before addition of EA/ after addition of EA) was 1.01 for KdT_{50} whereas Synergist Ratio (SR) (Before addition of EA/after addition of EA) of these same populations was 1.32 for KdT_{95}.

Table 4. Knockdown Time KdT_{50} (minutes) of *Anopheles gambiae s.l* Parakou populations to DDT and DDT + ETAA.

Table 5. Knockdown Time KdT_{95} (minutes) of *Anopheles gambiae s.l* Parakou populations to DDT and DDT + ETAA.

The analysis of figure 2 shows that after the addition of synergist EA to DDT 100µg/bottle, the percentage of dead mosquitoes from Parakou is lower than the one obtained with DDT alone. The use of synergist EA in bottles treated with DDT 100µg/bottle did not eliminate DDT resistance, and the mortality rate decreased from 67.16% to 39.68% ($P<0.05$). These results show that GSTs may play no role in *An. gambiae* Parakou resistance to DDT.

Figure.2 Effects of synergist ETAA on *Anopheles gambiae s.l* Parakou populations resistant to DDT.

Effects of synergist ETAA on *Anopheles gambiae s.l* Bohicon populations resistant to DDT

The analysis of table 6 and table 7 shows that after the addition of synergist EA in CDC bottles treated with DDT, the KdT_{50} value obtained with *Anopheles gambiae s.l* Bohicon populations was 121.86 minutes. This value was the same as the one obtained with DDT alone. A similar pattern was observed with KdT_{95} value obtained with these same populations which was 291.75 minutes after the addition of synergist EA. This value was the same as the one obtained with DDT alone. Synergist Ratio (SR) (Before addition of EA/ after addition of EA) was 1 for KdT_{50} and for KdT_{95}.

Table 6. Knockdown Time KdT_{50} (minutes) of *Anopheles gambiae s.l* Bohicon populations to DDT and DDT + ETAA.

Table 7. Knockdown Time KdT_{95} (minutes) of *Anopheles gambiae s.l* Bohicon populations to DDT and DDT + ETAA.

The analysis of figure 3 shows that after the addition of synergist EA to DDT100µg/bottle, the percentage of dead mosquitoes from Bohicon is lower than the one obtained with DDT alone. The use of synergist EA in bottles treated with DDT 100µg/bottle did not eliminate DDT resistance, and the mortality rate decreased from 31.94% to 3.12% ($P<0.05$).These results also show that GSTs may play no role in *An. gambiae* Bohicon resistance to DDT.

Figure 3. Effects of synergist ETAA on *Anopheles gambiae s.l* Bohicon populations resistant to DDT.

Anopheles gambiae s.l natural populations have developed high resistance to both DDT and permethrin in the different bio-climatic areas surveyed in the south-north transect Benin. This cross-resistance was also observed in the different ecological settings surveyed.

The mortality rate to permethrin observed in *Anopheles gambiae s.l* Parakou was higher than the one observed with *Anopheles gambiae s.l* Bohicon. A similar pattern was also observed with DDT with these same *Anopheles gambiae s.l* populations. These results showed that the resistance level recorded in *Anopheles gambiae s.l* from cotton growing areas was higher than that recorded in *Anopheles gambiae s.l* from vegetable growing areas. Similar results were already reported in Burkina Faso and in Côte-d'Ivoire where it was shown that resistance to DDT and pyrethroids may be selected by cotton treatments (Diabaté *et al.*, 2002; Chandre *et al.*, 1999).

The higher mortality rates observed with permethrin compared to DDT in all *Anopheles gambiae s.l* tested populations may be explained by the presence of an additional resistance mechanism in Benin (e.g. "Leu-Ser" mutation) which might confer higher resistance to DDT than to permethrin (Ranson *et al.*, 2000b; Martinez-Torres *et al.*, 1999). Djègbé *et al.* (2011) have recently showed a first evidence of the presence of *L1014S kdr* mutation in very few *Anopheles gambiae s.l* mosquitoes from West Africa.

Knock down effect is a characteristic of pyrethroids. It happens immediately after the insects are exposed to pyrethroids (Coats, 1982).Therefore, if the time need for insects to be knocked down increases, it indicates that the insects may be resistant to the insecticide (Cochran, 1994).When insects are exposed to pyrethroids, they fall down but will not die immediately. For susceptible insects, they will eventually die. But for resistant insects, after they are knocked down for a while, they will recover and soon be able to fly again after the pyrethroids entering their bodies are detoxified by their metabolism (Cochran, 1994). A correlation between resistance level to pyrethroids and ''knock-down'' time has already been shown in a study on *An. gambiae s.l.* resistance to pyrethroids in Africa (Akogbéto & Yakoubou, 1999). But, the Knock down effect is not only a characteristic of pyrethroids. It is also a characteristic of DDT. This pattern was obtained with *Anopheles gambiae s.l* Parakou and Bohicon populations resistant to DDT in the current study. After the addition of synergist EA on *Anopheles gambiae s.l* Parakou and Bohicon populations resistant to DDT, the mortality rates decreased and Glutathione *S*-transferases therefore may play no role in *An. gambiae* Parakou and Bohicon resistance to DDT. In some cases, the use of synergists at the same time as the application of insecticide could inhibit the

penetration of the insecticide through the cuticle, therefore reducing the amount of insecticide entering the insect's body (Martin *et al.*, 1997), the result of which was that the toxicity effect would also be reduced.

The current study clearly shows that increasing incidence of DDT and pyrethroid resistance in *Anopheles* mosquitoes is seen as a limiting factor for malaria vector control in Benin. More attention should be paid to the pyrethroid and DDT cross-resistance in *Anopheles gambiae s.l.* in the country for the implementation and management of current and future malaria vector control programs. Glutathione *S*-transferases may play no role in *An. gambiae* Parakou and Bohicon resistant to DDT.

Figure.1 Diagram for performing the CDC bottle bioassay with synergists [CDC: Methods in *Anopheles* Research, 2010].

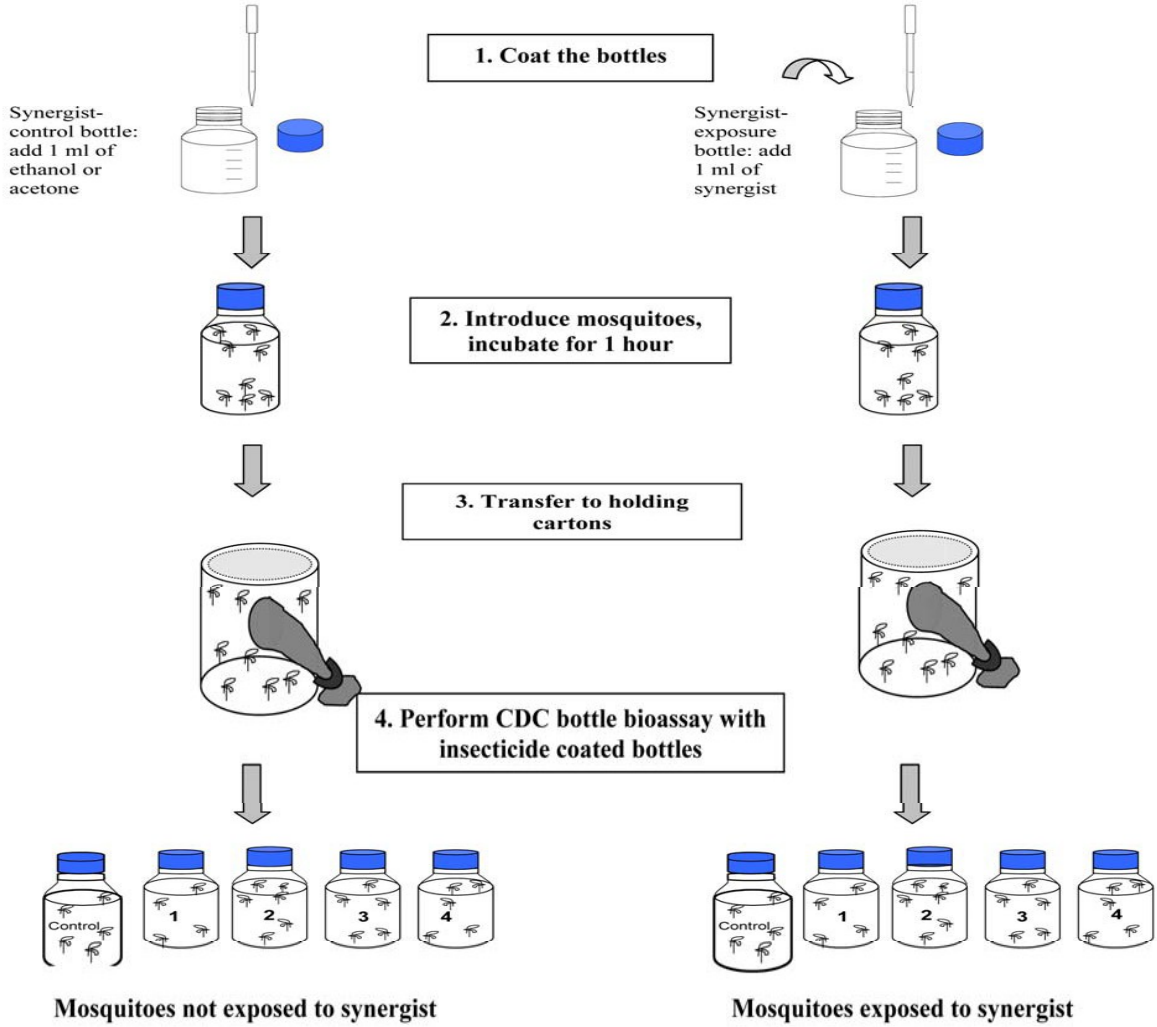

Figure.2 Effects of synergist ETAA on *Anopheles gambiae s.l* Parakou populations resistant to DDT

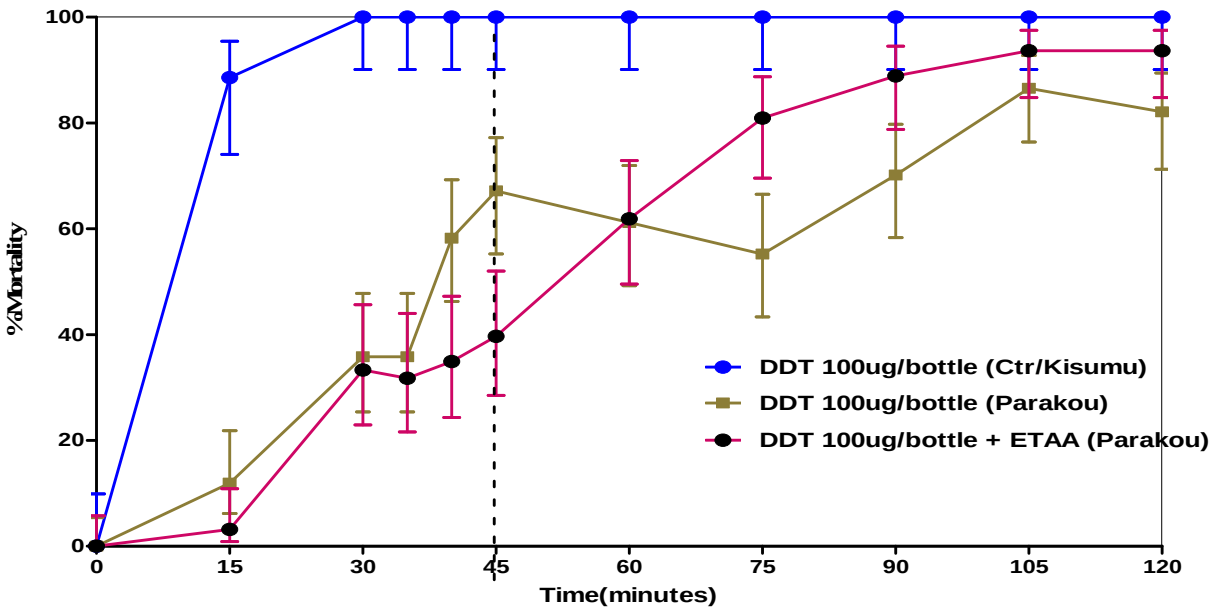

Figure.3 Effects of synergist ETAA on *Anopheles gambiae s.l* Bohicon populations resistant to DDT

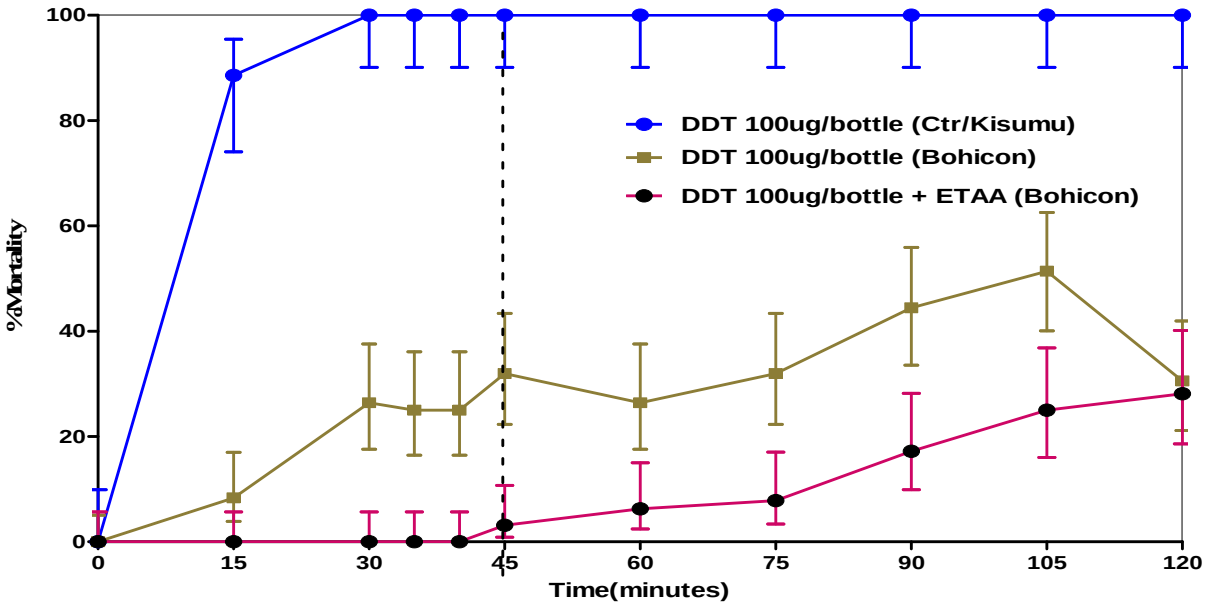

Table.1 Susceptibility status and permethrin resistance levels in *Anopheles gambiae s.l s.l.* populations

		Permethrin		
Bio-climatic areas	Locality	Number tested	% Mortality	Resistance Status
	Kisumu	37	100	S
Sudanian	Parakou	40	92.5	R
Sudano-guinean	Bohicon	64	87.5	R

Table.2 Susceptibility status and DDT resistance levels in *Anopheles gambiae s.l s.l.* populations

		DDT		
Bio-climatic areas	Locality	Number tested	% Mortality	Resistance status
	Kisumu	35	100	S
Sudanian	Parakou	67	67.16	R
Sudano-guinean	Bohicon	72	31.94	R

Table.3 Correlation between DDT resistance level and 'knocked-down' time

Population	Kdt50 (min)	Kdt95 (min)
Kisumu	10.91	16.38
Parakou	53.67	139.69
Bohicon	121.86	291.75

Table.4 Knockdown Time KdT50 (minutes) of *Anopheles gambiae s.l* Parakou populations to DDT and DDT + ETAA

	Without ETAA		With ETAA		
Population	Number tested	KDT50(min)	Number tested	KDT50(min)	SR
Parakou	125	53.67	125	53.09	1.01

Table.5 Knockdown Time KdT95 (minutes) of *Anopheles gambiae s.l* Parakou populations to DDT and DDT + ETAA

	Without ETAA		With ETAA		
Population	Number tested	KDT95(min)	Number tested	KDT95(min)	SR
Parakou	125	139.69	125	105.08	1.32

Table.6 Knockdown Time KdT50 (minutes) of *Anopheles gambiae s.l* Bohicon populations to DDT and DDT + ETAA

	Without ETAA		With ETAA		
Population	Number tested	KDT50(min)	Number tested	KDT50(min)	SR
Bohicon	125	121.86	125	121.86	1

Table.7 Knockdown Time KdT95 (minutes) of *Anopheles gambiae s.l* Bohicon populations to DDT and DDT + ETAA

	Without ETAA		With ETAA		
Population	Number tested	KDT95(min)	Number tested	KDT95(min)	SR
Bohicon	125	291.75	125	291.75	1

References

Abbott, W.S. 1987. A method of computing the effectiveness of an insecticide. *J Am Mosq Control Assoc*, 3(2):302–303.

Akogbeto, M., Yakoubou, S. 1999. Résistance des vecteurs du paludisme vis-à-vis des pyréthrinoïdes utilisés pour l'imprégnation des moustiquaires au Bénin, Afrique de l'Ouest. *Bull Soc Pathol Exot*, 92: 123 -130.

Akogbeto, M.C., Djouaka, R., Noukpo, H. 2005. L'utilisation des insecticides en agriculture au Bénin. *Bull Soc Pathol Exot*, 98:400–405.

Brogdon, W.G. & McAllister, J.C. 1998. Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. *J Am Mosq Control Assoc*, 14(2):159–164.

Brogdon, W., & Chan, A. 2010. *Guidelines for Evaluating Insecticide Resistance in Vectors using the CDC Bottle Bioassay/ Methods in anopheles research.* Second edition. CDC Atlanta USA: CDC technical report; 2010:343.

Chandre, F., Darriet, F., Manga, L., Akogbeto, M., Faye, O., Mouchet, J., Guillet, P. 1999. Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. *Bull World Health Organ*, 77(3)**:**230-234.

Coats, J.R. 1982. Insecticide Mode of Action. *Academic Press* London.

Cochran, D.G. 1994. Effects of three synergists on Pyrethroid Resistance in the German Cockroach (Dictyoptera: Blatellidae). *J Econ Entomol*, 87(4):879-884.

Diabaté, A., Baldet, T., Chandre, F., Guiguemde, T.R., Guillet, P. *et al*. 2002. First report of the *kdr* mutation in *Anopheles gambiae* M form from Burkina Faso, West Africa. *Parassitologia*. 44:157-158.

Djègbé, I., Boussari, O., Sidick, A., Martin, T., Ranson, H., Chandre, F., Akogbéto, M., and Corbel, V. 2011. Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S *kdr* mutation in *Anopheles gambiae* from West Africa. *Malar J*, 10: 261.

Djogbénou, L., Pasteur, N., Akogbéto, M., Weill, M., Chandre, F. 2009. Insecticide resistance in the *Anopheles gambiae* complex in Benin: a nationwide survey. *Med Vet Entomology*, 69: 160-164.

Grant, D.F., Matsumura, F. 1989. Glutathione S-transferase 1 and 2 in susceptible and insecticide resistant *Aedes aegypti*. *Pestic Biochem Physiol*, 33:132–43.

Grant, D.F., Hammock, B.D. 1992. Genetic and molecular evidence for a trans-acting regulatory locus controlling glutathiones transferase-2 expression in *Aedes aegypti***.** *Mol Gen Genet*, 234:169–76.

Hemingway, J., Ranson, H. 2000. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol*, 45: 371-391.

Martin, S.H., Ottea, J.A., Leonard, B.R., Graves, J.B., Burris, E., Micinski, S., and Church, G.E. 1997. Effects of selected synergists and insecticide toxicity in tobacco budworms (Lepidoptera: Noctuidae) in laboratory and field studies. *J Econ Entomol*, (3):723-721.

Martinez-Torres, D., Chevillon, C., Brun-Barale, A., Bergé, J.B., Pasteur, N., Pauron, D. 1999. Voltage-dependent

Na+ channels in pyrethroid-resistant *Culex pipiens* L mosquitoes. *Pestic Sci*, 55: 1012–1020.

Oduola, O.A., Olojede, B.J., Ashiegbu, O.C., Adeogun, O.A., Otubanjo, A.O. and Awolola, S.T. 2010. High level of DDT resistance in the malaria mosquito: *Anopheles gambiae* from rural, semi-urban and urban communities in Nigeria. *J Rural Trop Public Health*, 114 120.

Ranson, H., Jensen, B., Wang, X., Prapanthadara, L., Hemingway, J. and Collins, H.F. 2000a. Genetic mapping of two loci affecting DDT resistance in the malaria vector *Anopheles gambiae Insect Mol Biol*, 9(5): 499–507.

Ranson, H., Jensen, B., Vulule, J.M., Wang, X., Hemingway, J., Collins, F.H. 2000b. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect Mol Biol*, 9(5): 491-497.

Vulule, J.M., Beach, R.F., Atieli, F.K., Mount, D.L., Roberts, J.M. and Mwangu, R.W. 1994. Reduced Susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin impregnated bednets and curtains in Kenya. *Med Vet Entomol*, 8: 71-5.

Zaim, M., Aitio, A., Nakashima, N. 2000. Safety of pyrethroid-treated mosquito nets. *Med Vet Entomol* 14:1-5.