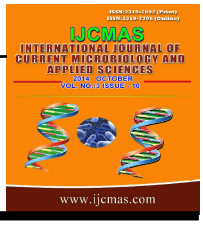
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 878-884**
http://www.ijcmas.com



Original Research Article

Establishment of the correlations between resistance level to permethrin and DDT and 'knocked-down' time in two *Anopheles gambiae* sensu lato populations from the Sudano Guinean area in the central part of Benin, West Africa

Nazaire Aïzoun[1,2]*, Roseric Azondekon[1,3], and Martin Akogbéto[1,2]

[1]Centre de Recherche Entomologique de Cotonou (CREC), 06 BP 2604, Cotonou, Bénin
[2]Faculté des Sciences et Techniques, Université d'Abomey Calavi, Calavi, Bénin
[3]University of Massachusetts Amherst, Amherst, Massachusetts, USA
**Corresponding author*

Keywords

Cross-Resistance, 'Knocked-Down' Time, Insecticide, Vectors, Malaria, Benin.

A B S T R A C T

Monitoring of insecticide resistance is a necessary element of any medium-scale or large-scale deployment of an insecticidal intervention. The current study aimed at an establishment of the correlation between the resistance level to an insecticide and the 'knocked-down' time. Larvae and pupae of *Anopheles gambiae s.l.* mosquitoes were collected from the breeding sites in Zou and Collines 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 diagnostic tests showed high frequency of cross-resistance in *An. gambiae s.l.* 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 and there is a correlation between the resistance degree of a mosquito to permethrin and DDT and the time that this mosquito takes to react to these products.

Introduction

Monitoring of insecticide resistance is a necessary element of any medium-scale or large-scale deployment of an insecticidal intervention. In 2010, 78 countries reported that they were carrying out insecticide resistance monitoring (WHO, 2011).

The main mechanisms that enable insects to resist the action of insecticides can be grouped into two distinct categories: Target site resistance and metabolic resistance. Target site resistance is based on alterations of amino acids in the site of action where the insecticide is supposed to bind, causing the insecticide to be less effective or ineffective at all. Knock down resistance (*Kdr*) occurs due to a single or multiple substitutions in the sodium channel (Martinez-Torres *et al.*,

1998; Ranson *et al.,* 2000); and alteration in acetylcholinesterase results in decreased sensitivity to insecticides (Mutero *et al.,* 1994). Metabolic resistance usually involves over-expression of enzymes capable of detoxifying insecticides or modifications in the amino acid sequences that cause alterations in the levels and activity of detoxifying proteins.

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.

The main goal of this study was to establish the correlations between the resistance level to permethrin and DDT and the 'knocked-down' time in two *Anopheles gambiae sensu lato* populations from the Sudano Guinean area in the central part of Benin.

Materials and Methods

Study area

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: Bamè, a rice growing area located in Zagnanado district in Zou department, in the central part of the country. Savè is a cereal (maize, ground-nut and so on) growing area located in Collines department, in the central part of the country too. The central part of the country is characterized by a sudano-guinean climate with two rainy seasons (March–July and August) with an average rainfall of 1,000 mm per year.

Mosquito collection

An. gambiae s.l. mosquitoes were collected from March to July 2013 during the first rainy season in Savè and Zagnanado districts selected in the central part of the country. Larvae and pupae were collected in Savè district within both padding and village using the dipping method on several breeding sites (brick pits, pools, marshes, streams, ditches, pits dug for plastering traditional huts, puddles of water, water pockets caused by the passage of cattle and gutters). *Anopheles* pre-imaginal stages (L1 to L4 instars) were also collected via ladles within rice farms from Bamè. Due to that the farms are irrigated, breeding sites are present throughout the year and we therefore assumed that the larvae collected in the study period were representative of the population that could be found during other periods of the year. Larvae collected from multiple breeding sites were pooled together then re-distributed evenly in development trays containing tap water. Larvae were provided access to powdered TetraFin® fish food, and were reared to adults under insectary conditions of 25+/-2°C and 70 to 80% relative humidity at Centre de Recherche Entomologique de Cotonou (CREC) located in Akpakpa, in Cotonou district. Larvae and pupae collected in Savè were also reared to adults under insectary conditions at CREC. *An. Gambiae s.l* Kisumu, a reference susceptible strain was used as a control for the bioassay tests. We used Kisumu more precisely to confirm the quality of treated or impregnated papers and then to calculate the resistance ratio. Susceptibility tests were done following WHO 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 and Chan, 2010). These doses were checked on the *An. gambiae s.l.* 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 and Chan, 2010). Fifteen to twenty 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.

Statistical analysis

The resistance status of mosquito samples was determined according to the CDC criteria (Brogdon and McAllister, 1998; Brogdon and 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).

The knockdown times for 50% and 95% of tested mosquitoes (kdt50 and kdt95) were estimated using SPSS version 16.0 (SPSS Inc., Chicago, IL). The resistance ratio (RR50) was determined relative to the Kisumu susceptible strain. This was obtained by dividing the kdt50 of wild strain to the kdt50 of the susceptible strain. The software R-2.15.2. (R Development Core Team, 2011) was used for the statistical analysis. The significance level was set at 5%.

Ethical approval

This study was approved by the Ministry of Health and the Center for Entomological Research of Cotonou, Benin.

Results and Discussion

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 and 45 minutes respectively, which represent susceptibility threshold times or diagnostic times 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 s.l.* populations from Savè and Zagnanado; 36.85% and 5.67% 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; 52.78% and 87.18% of *An. gambiae s.l.* populations from Savè and Zagnanado 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.* populations

	Permethrin		
Locality	Number tested	% Mortality	Resistance status
Kisumu	37	100	S
Savè	38	63.15	R
Zagnanado	53	94.33	R

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

	DDT		
Locality	Number tested	% Mortality	Resistance status
Kisumu	35	100	S
Savè	36	47.22	R
Zagnanado	39	12.82	R

Correlation between resistance level to DDT 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.* populations from Savè and Zagnanado, the Kdt_{50} and Kdt_{95} obtained were high. After 45.18 minutes and 93.87 minutes exposure to CDC bottles treated with DDT; 50% of tested mosquitoes were knocked-down respectively (Kdt_{50}) and 95% were knocked-down after 135.26 minutes and 173.34 minutes respectively (Kdt_{95}). These results showed that there is a correlation between the resistance degree of a mosquito to DDT and the time that this mosquito takes to react to this product (Table 3).

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

Population	Kdt_{50} (min)	Kdt_{95} (min)
Kisumu	10.91	16.38
Savè	45.18	135.26
Zagnanado	93.87	173.34

Correlation between resistance level to permethrin and 'knocked-down' time

The analysis of table 4 shows that after 9.12 minutes exposure to CDC bottles treated with permethrin, 50% of *Anopheles gambiae s.l.* Kisumu tested populations were knocked-down (Kdt_{50}) and 95% were knocked-down after 12.41 minutes (Kdt_{95}). Regarding *Anopheles gambiae s.l.* populations from Savè and Zagnanado, the Kdt_{50} and Kdt_{95} obtained were high. After 38.18 minutes and 15.93 minutes exposure to CDC bottles treated with permethrin; 50% of tested mosquitoes were knocked-down respectively (Kdt_{50}) and 95% were knocked-down after 108.51 minutes and 58.52 minutes respectively (Kdt_{95}).

These results showed that there is a correlation between the resistance degree of a mosquito to permethrin and the time that this mosquito takes to react to this product (Table 4).

Table.4 Correlation between resistance level to permethrin and 'knocked-down' time

Population	Kdt_{50} (min)	Kdt_{95} (min)
Kisumu	9.12	12.41
Savè	38.18	108.51
Zagnanado	15.93	58.52

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

The higher mortality rates observed with permethrin compared to DDT in both *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 (Martinez-Torres *et al.*, 1999; Ranson *et al.*, 2000). Similar pattern was also recently reported by Aïzoun *et al.*, (2014) with *Anopheles gambiae s.l.* populations from Akron, Suru-léré and Bamè resistant to permethrin and DDT in 2008 and in 2013. In addition, Djègbé *et al.*, (2011) have recently showed a first evidence of the presence of *L1014S kdr* mutation in very few *Anopheles gambiae* 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).

There is a correlation between the resistance degree of a mosquito to permethrin and the time that this mosquito takes to react to this product. The 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 Benin (Akogbéto and Yakoubou, 1999). There is also a correlation between the resistance degree of a mosquito to DDT and the time that this mosquito takes to react to this product. So, 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.* populations from Savè and Zagnanado resistant to DDT in the current study.

The current study clearly shows that pyrethroid and DDT resistance was widespread in malaria vector in Benin and there is a correlation between the resistance degree of a mosquito to permethrin and DDT and the time that this mosquito takes to react to these products.

Acknowledgements

We are grateful to the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique (MESRS) of Benin which financially supported this study and the doctoral training of Nazaire. We are also grateful to the President's Malaria Initiative (PMI) of the U.S. Government through USAID which financially supported certain of research activities of Nazaire in the framework of his doctoral training. We would like to thank Dr. William G. BROGDON from CDC Atlanta, USA who supplied us the reagents used for CDC bioassays. The authors would also like to thank Frederic OKE-AGBO for statistical analysis and Damien TODJINOU for providing technical assistance. Tel: (229) 95317939.

References

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

Aïzoun, N., Azondekon, R., Aïkpon, R., Anagonou, R., Gnanguenon, V., Akogbéto, M., 2014. Dynamics of insecticide resistance and exploring biochemical mechanisms involved in pyrethroids and dichloro diphenyl trichloroethane (DDT) cross-resistance in *Anopheles gambiae s.l.* populations from Benin, West Africa. *J. Cell Anim. Biol.*, 8(3): 41–50.

Akogbéto, M., Yakoubou, S., 1999. Resistance of Malaria vectors to pyrethroids used for impregnated bednets, Bénin, West Africa. *Bull. Soc. Path. Exot.*, 92: 123–130.

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. 2nd edn, CDC Atlanta, USA: CDC technical report, 2010: 343.

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.

Djègbé, I., Boussari, O., Sidick, A., Martin, T., Ranson, H., Chandre, F., Akogbéto, M., 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.

Martinez-Torres, D., Chandre, F., Williamson, M.S., Darriet, F., Bergé, J.B., Devonshire, A.L., 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(2): 179–184.

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.

Mutero, A., Pralavorio, M., Bride, J.M., Fournier, D. 1994. Resistance-associated point mutations in insecticide-insensitive acetylcholin esterase. *Proc. Natl. Acad. Sci.*, 91(13): 5922–5526.

Ranson, H., Jensen, B., Vulule, J.M., Wang, X., Hemingway, J., Collins, F.H., 2000. 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.

WHO., 2011. World Malaria Report. World Health Organization: Geneva.