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Effects of synergist ethacrynic acid (ETAA or EA) on dichlorodiphenyltrichloroethane (DDT) tolerance in *Anopheles gambiae s.l.* larvae from mono department in south-western Benin, West Africa

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Abstract

The current study was aimed to study the effects of synergist ethacrynic acid (ETAA or EA) on dichlorodiphenyltrichloroethane (DDT) tolerance in *Anopheles gambiae s.l.* larvae from mono department in south-western Benin, West Africa. Larvae and pupae were collected from March to July and August to November 2018 during the rainy season in the locations of Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa. Larval bioassays were performed on these collected *Anopheles gambiae s.l.* larvae using dichlorodiphenyltrichloroethane (DDT) as larvicide and ethacrynic acid (ETAA or EA) as enzyme inhibitor or synergist. The results showed that glutathione S-transferases (GSTs) may play a role in *An. gambiae s.l.* larvae from Athiemè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa resistance to DDT.

Keywords: DDT, ethacrynic acid, tolerance, malaria vectors, Benin

Introduction

Resistance to the insecticides used is a major operational concern in vector control efforts. Because monitoring efforts have been limited, the current spectrum of the insecticide resistance problem is not known in malaria endemic regions, particularly in Africa (Coleman *et al.*, 2006). Resistance to DDT was widespread in the early 1970s because of its intensive use in public health and agriculture (Metcalf, 1973) and emerged after about 11 years of application (Magesa *et al*., 1991). Although DDT has been used in limited quantities for disease vector control during the past 3 decades, there have been recent reports of resistance in malaria vectors from African countries (Coetzee *et al.*, 2006; Hargreaves *et al*., 2003 ; Ranson *et al*., 2000 ; Tia *et al*., 2006).

An elaborate three phase detoxification system is used by all animal species including *An. gambiae* to defend themselves against the toxic effects of these environmental xenobiotic substances. The three phase system metabolize the toxic substances into a less harmful one and excrete them out of the cell (Xu *et al.*, 2005). Among these detoxification phases, the phase I detoxification mechanism is the most elaborate; employing activities of enzymes belonging to the cytochrome P450 family. In phase II, the by-products of phase I reaction are further detoxified by means of enzymes belonging to the Glutathione S-Transferase and α & β-esterases families (Misra *et al.*, 2011). When organisms are exposed to environmental toxicants, a transcriptional response is activated which leads to upregulation of the genes involved in the detoxification machinery. This is called induction. Induction of detoxification enzymes in response to xenobiotic exposure has received greater attention in higher animals, because of its important implication in drug metabolism and discovery. Studies on induction of detoxification enzymes in insect vectors have tended to focus more on adaptation; how a particular strain of insect has adapted to a particular environment which could then select it for insecticide resistance (Perry *et al.*, 2011). However, evidence have emerged that insects like other higher animals have the ability to regulate the transcription of their detoxification genes in response to environmental xenobiotics.

Several previous studies (Mwangagngi *et al*., 2010; Animut *et al*., 2012; Imbahale *et al*., 2011; Mala *et al*., 2011) have established the impact of several breeding sites ecogeographical, topographical, agricultural, and other environmental indices on *Anopheles* larval diversity, abundance, and dynamics, as well as breeding sites productivity. Also, induction of detoxification enzymes by various environmental xenobiotics in many species of insects has been well documented (David *et al.*, 2013).

Very few researches were published on organochlorine tolerance in *Anopheles gambiae s.l.* larvae from mono department in south-western Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to explore the detoxification enzymes mechanisms conferring organochlorine tolerance in *Anopheles gambiae s.l.* larvae in Benin.

Materials and Methods

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Mono. Mono department is located in the south-western Benin and the study was carried out more precisely in the locations of Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa. The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. These factors have an impact on resistance development in the local vector mosquitoes. We took them into account to determine the effects of synergist Ethacrynic acid (ETAA or EA) on dichlorodiphenyltrichloroethane (DDT) tolerance in *Anopheles gambiae s.l.* larvae from this department. Mono has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

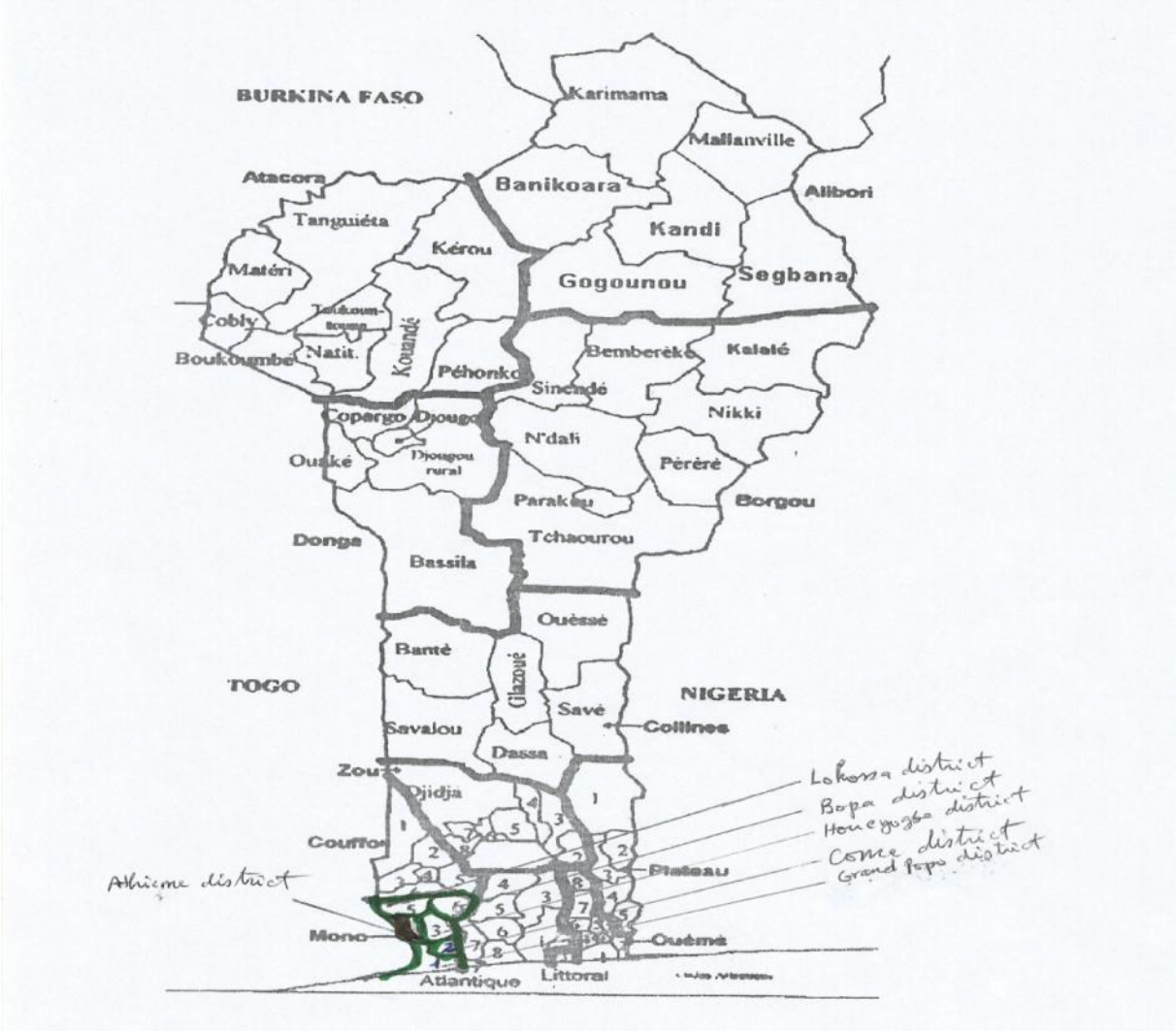

Figure 1: Map of districts of mono department surveyed

Mosquito sampling

An. gambiae s.l. larvae were collected from March to July and August to November 2018 during the rainy season in the locations of Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa selected in south-western Benin. Larvae and pupae were collected in these localities within both padding and town 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 gutters). Once, larvae and pupae collected, they were then kept in labeled bottles related to the localities surveyed. Otherwise, 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 under insectary conditions of 25+/-2°C and 70 to 80% relative humidity at Department of Sciences and Agricultural Techniques located in Dogbo district in south-western Benin. *An. gambiae* Kisumu larvae, a reference susceptible strain was used as a control for the larval bioassays. All larval bioassays were conducted in the Laboratory of Applied Entomology and Vector Control of the Department of Sciences and Agricultural Techniques at 25+/-2°C and 70 to 80% relative humidity.

Preparation of stock solutions or suspensions and test concentrations

Stock solutions and serial dilutions were prepared following the protocol described in WHO guidelines (WHO, 2005). The volume of stock solution was 20 ml of 1%, obtained by weighing 200 mg of dichlorodiphenyltrichloroethane (DDT) and adding 20 ml solvent to it. It was kept in a screw-cap vial, with aluminium foil over the mouth of the vial.

Then, it was shacked vigorously to dissolve or disperse the dichlorodiphenyltrichloroethane in the solvent. The stock solution was then serially diluted (ten-fold) in ethanol (2 ml solution to 18 ml solvent). Test concentrations were then obtained by adding 0.1–1.0 ml (100–1000 µl) of the appropriate dilution to 100 ml or 200 ml distilled water.

Bioassays

Initially, the mosquito larvae were exposed to a wide range of test concentrations of dichlorodiphenyltrichloroethane and a control to find out the activity range of the larvicide under test. After determining the mortality of larvae in this wide range of concentrations, a narrower range (of 4-5 concentrations, yielding between 10% and 95% mortality in 24h or 48h) was used to determine LC50 and LC90 values (WHO, 2005).

Batches of 25 third or fourth instar larvae were transferred by means of strainers, screen loops or droppers to small disposable test cups or vessels, each containing 100-200 ml of water. Small, unhealthy or damaged larvae were removed and replaced. The depth of the water in the cups or vessels was remained between 5 cm and 10 cm; deeper levels may cause undue mortality.

The appropriate volume of dilution was added to 100 ml or 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration. Four replicates were set up for each concentration and an equal number of controls were set up simultaneously with tap water, to which 1 ml alcohol was added. Each test was run three times on different days. For long exposures, larval food was added to each test cup, particularly if high mortality was noted in control. The test containers were held at 25-28°C and preferably a photoperiod of 12h light followed by 12h dark (12 L: 12 D).

After 24 h exposure, larval mortality was recorded. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed. The results were recorded on the result form, where the LC50 and LC90 values, and slope and heterogeneity analysis were also noted. The form was accommodated three separate tests of six concentrations of dichlorodiphenyltrichloroethane, each of four replicates (WHO, 2005).

Biochemical assays using synergist

The presence of metabolic-based resistance mechanisms was investigated by exposing larvae to enzyme inhibitor prior to bioassays with DDT. For that, as all tested samples showed high tolerance to DDT in *Anopheles gambiae s.l.* larvae from Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa, they were exposed to the effects of synergist: Ethacrynic acid (ETAA or EA) (80 µg per test cup), which inhibits glutathione S-transferases activity. The test allowed us to compare the obtained percentage of dead larvae before the addition of the synergist to that obtained after the addition of the synergist.

Data analysis

Data from all replicates were pooled for analysis. LC50 and LC90 values were calculated from a log dosage-probit mortality regression line using computer software programs. Bioassays were repeated at least three times, using new solutions or suspensions and different batches of larvae each time. Standard deviation or confidence intervals of the means of LC50 values were calculated and recorded on a form. A test series was valid if the relative standard deviation (or coefficient of variation) was less than 25% or if confidence limits of LC50 overlap (significant level at $P < 0.05$). Abbott's formula was not used in this study for the correction of mortality rates in test cups because the mortality rates in all controls was always less than 5% (Abbott, 1987). To appreciate the effects of synergist ETAA on *Anopheles gambiae s.l.* larvae tolerance to DDT, we used a Kruskal-Wallis test. LC50 and LC90 values were estimated using SPSS version 16.0 (SPSS Inc., Chicago, IL). The significance level was set at 5%.

Results and Discussion

The analysis of table 1 showed that all tested larvae or strains from mono department were highly resistant to DDT (see Resistance ratios RR50 and RR95). After the addition of synergist ETAA in test cups, the LC50 values obtained with larvae from Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa which were 99 µg per liter, 91.6 µg per liter, 91.4 µg per liter, 91.8 µg per liter, 91.3 µg per liter and 99.1 µg per liter respectively were lower than those obtained with DDT

alone which were 990 µg per liter, 988.9 µg per liter, 989 µg per liter, 990.1 µg per liter, 988.8 µg per liter and 988.7 µg per liter respectively (Table 2). The Synergism Ratios (SR50) (before addition of ETAA/after addition of ETAA) were 10.00, 10.79, 10.82, 10.78, 10.83 and 09.97 respectively (Table 2).

In the same way, the LC95 values obtained with larvae from Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa which were 390.1 µg per liter, 388.9 µg per liter, 389.9 µg per liter, 390.3 µg per liter, 390.4 µg per liter and 390.2 µg per liter respectively were lower than those obtained with DDT alone which were 4210 µg per liter, 4208 µg per liter, 4207 µg per liter, 4206 µg per liter, 4204 µg per liter and 4205 µg per liter respectively (Table 3). The Synergism Ratios (SR95) (before addition of ETAA/after addition of ETAA) were 10.79, 10.82, 10.78, 10.77, 10.76 and 10.77 respectively (Table 3). These results showed that glutathione S-transferases (GSTs) may play a role in *An. gambiae s.l.* larvae from Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa resistance to DDT.

Table-1: Determination of Lethal Concentrations LC50 and LC95

Strain	LC50 (mg/l)	LC95 (mg/l)	RR50	RR95
Kisumu	0.0176	0.0553	_	_
Athiémè	0.9900	4.210	56.25	76.13
Grand Popo	0.9889	4.208	56.18	76.09
Comè	0.9890	4.207	56.19	76.07
Lokossa	0.9901	4.206	56.25	76.05
Houéyogbé	0.9888	4.204	56.18	76.02
Bopa	0.9887	4.205	56.17	76.03

Table-2: Determination of Lethal Concentrations LC50 of *An. gambiae s.l.* larvae to DDT with and without ETAA and Synergism ration SR50

	Without ETAA		With ETAA		
Strain	Number tested	LC50 (mg/l)	Number tested	LC50 (mg/l)	Synergism Ratio (SR50)
Athiémè	25	0.9900	25	0.0990	10.00
Grand Popo	25	0.9889	25	0.0916	10.79
Comè	25	0.9890	25	0.0914	10.82
Lokossa	25	0.9901	25	0.0918	10.78
Houéyogbé	25	0.9888	25	0.0913	10.83
Bopa	25	0.9887	25	0.0991	09.97

Table-3: Determination of Lethal Concentrations LC95 of *An. gambiae s.l.* larvae to DDT with and without ETAA and Synergism ration SR95

	Without ETAA		With ETAA		
Strain	Number tested	LC95 (mg/l)	Number tested	LC95 (mg/l)	Synergism Ratio (SR95)
Athiémè	25	4.210	25	0.3901	10.79
Grand Popo	25	4.208	25	0.3889	10.82
Comè	25	4.207	25	0.3899	10.78
Lokossa	25	4.206	25	0.3903	10.77
Houéyogbé	25	4.204	25	0.3904	10.76
Bopa	25	4.205	25	0.3902	10.77

All tested larvae from Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa in mono department in south-western Benin were highly resistant to DDT. According to Akogbeto and Yakoubou (1999), the emergence of DDT resistance recorded in *An. gambiae* from meridian regions was related to two phenomena: the massive use of DDT and dieldrin for house-spraying applications in southern villages from 1953 to 1960 during WHO programmes of malaria eradication and the massive use of organochlorine in agricultural settings during the 1950s (OMS, 1976).

The synergist assay with ETAA, an inhibitor of Glutathione S-transferases (GSTs), indicated that this enzyme family plays a role in this DDT resistance observed in larvae from Athiémè, Grand Popo, Comè, Lokossa, Houéyogbé and Bopa. The use of synergist ETAA to overcome DDT resistance in these *An. gambiae s.l.* larvae showed that this synergist has partially inhibited Glutathione S-transferases (GSTs) activity and therefore slightly improved DDT effectiveness in these larvae. Previous studies conducted in others departments in the country on adults also showed the involvement of GSTs in *An. gambiae s.l.* populations from Suru-léré and Akron resistance to DDT (Aïzoun *et al.*, 2014a) and in *An. gambiae s.l.* populations from Ladji resistance to DDT (Aïzoun *et al.*, 2014b). But GSTs may play no role in *An. gambiae s.l.* populations from Parakou and Bohicon resistance to DDT (Aïzoun *et al.*, 2014c). They may also play no role in *An. gambiae s.l.* populations from Sèkandji resistance to DDT (Aïzoun *et al.*, 2014b). 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.

Conclusion

Glutathione S-transferases played a role in *Anopheles gambiae s.l.* larvae tolerance to DDT in mono department in south-western Benin. However, further studies using a microarray approach followed by quantitative real-time RT-PCR validation are need to identify detoxification genes putatively involved in metabolic resistance. This will improve the implementation and management of future control programs against this important malaria vector particularly in Benin and in Africa in general.

References

1. Abbott, W.S. (1987): A method of computing the effectiveness of an insecticide**.** *J. Am. Mosq. Cont. Assoc*, 3(2): 302 303.
2: Aïzoun, N., Azondekon, R., Aïkpon, R., Anagonou, R., Gnanguenon, V., and Akogbéto, M. (2014a): Dynamics of insecticide resistance and exploring biochemical mechanisms involved in pyrethroids and dichlorodiphenyltrichloroethane (DDT) cross-resistance in *Anopheles gambiae s.l.* populations from Benin, West Africa. *J. Cell. Anim. Biol*, 8(3): 41-50.
3: Aïzoun, N., Aïkpon, R., Gnanguenon, V., Azondekon, R., Oké-Agbo, F., Padonou, G.G., Akogbéto, M. (2014b): Dynamics of insecticide resistance and effect of synergists piperonyl butoxide (PBO), S.S.S-tributylphosphoro-trithioate (DEF) and ethacrynic acid (ETAA or

EA) on permethrin, deltamethrin and dichlorodiphenyltrichloroethane (DDT) resistance in two *Anopheles gambiae s.l.* populations from southern Benin, West Africa. *J. Parasitol. Vector. Biol*, 6(1):1-10.

4: Aïzoun, N., Azondekon, R., and Akogbéto, M. (2014c): 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. *Int. J. Curr. Microbiol. App. Sci*, 3(9): 392-403.

5: 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.

6: Animut, A., Gebre-micheal, T., Balkew, M., Lindtjorn, B. (2012): Abundance and dynamics of Anopheline larvae in a highland malarious area of south-central Ethiopia. Parasit. Vectors, 5:117.

7: Coetzee, M., Van, Wyk, P., Booman, M., Koekemoer, L.L., Hunt, R.H. (2006): Insecticide resistance in malaria vector mosquitoes in a gold mining town in Ghana and implications for malaria control. *Bull. Soc. Pathol. Exot*, 99*:* 400–403.

8: Coleman, M., Sharp, B., Seocharan, I., Hemingway, J. (2006): Developing an evidence-based decision support system for rational insecticide choice in the control of African malaria vectors. *J. Med. Entomol*, 43*:* 663–668.

9: David, J.P., Ismail, A.M., Chander-Proust, A., Paine, M.J. (2013): Role of cytochrome P450s in insecticide resistance: Impact on the control of mosquito-borne diseases and use of insecticides on earth. *Phil Trans R Soc B*, 368:20120429.

10: Hargreaves, K., Hunt, R.H., Brooke, B.D., Mthembu, J., Weeto, M.M., Awolola, T.S., Coetzee, M. (2003): *Anopheles arabiensis* and *An. quadiannulatus* resistance to DDT in South Africa. *Med. Vet*. *Entomol*, 17: 417–422.

11: Imbahale, S.S., Paajimans, K.P., Mukabana, W.R., Van Lammeren, R., Githeko, A.K., Takken, W. (2011): A longitudinal study on Anopheles mosquito larval abundance in distinct geographical and environmental settings in western Kenya. *Malar. J*, 10:81.

12: Magesa, S.M., Wilkes, T.J., Mnzava, A.E., Njunwa, K.J., Myamba, J., Kivuyo, M.D., Hill, N., Lines, J.D., Curtis, C.F. (1991): Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2. Effects on the malaria vector population. *Acta. Trop*, 49*:* 97–108.

13: Mala, A.O., Irungu, L.W., Shililu, J.I., Muturi, E.J., Mbogo, C.C., Njagi, J.K., Githure, J.I. (2011): Dry season ecology of *Anopgeles gambiae* complex mosquitoes at larval habitats in two traditionally semi-arid villages in Baringo, Kenya. *Parasit. Vectors*, 4:25.

14: Martin, S.H., Ottea, J.A., Leonard, B.R., Graves, J.B., Burris, E., Micinski, S., 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.

15: Metcalf, R.L. (1973): A century of DDT. *J. Agric. Food Chem*, 21: 511–519.

16: Misra, J.R., Horner, M.A., Lam, G., Hummel, C.S. (2011): Transcriptional regulation of xenobiotic detoxification in Drosophila. Genes & Development, 25:1796-1806.

17: Mwangagngi, J.M., Shililu, J., Muturi, E.J., Muriu, S., Jacob, B., Kabiru, E.W., Mbogo, C.M., Githure, J., Novak, R.J. (2010): Anopheles larval abundance and diversity in three rice agrovillage complexes mwea irrigation scheme, central Kenya. *Malar. J*, 9:228.

18: OMS (1976): Résistance des vecteurs et des réservoirs de maladies aux pesticides: 22 ème rapport du comité OMS d'experts des insecticides. Cah. Tech. Afro, 585: 30-31.

19: Perry, T., Batterham, P., Daborn, P.J. (2011): The biology of insecticidal activity and resistance. *Insect Biochem. Mol. Biol*, 41:411-422.

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

21: Tia, E., Akogbeto, M., Koffi, A., Toure, M., Adja, A.M., Moussa, K., Yao, T., Carnevale, P., Chandre, E. (2006): Pyrethroid and DTT resistance of *Anopheles gambiae* s.s. (*Diptera: Culicidae*) in five agricultural ecosystems from Cote-d'Ivoire [in French]. *Bull. Soc. Pathol. Exot*, 99*:* 278–282.

22: WHO (2005): "Guidelines for laboratory and field testing of mosquito larvicides," World Health Organization Communicable Disease Control, Prevention and Eradication WHO Pesticide Evaluation Scheme. WHO/CDS/WHOPES/GCDPP/2005.13.

23: Xu, Q., Li, C.Y., Kong, A.N. (2005): Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch. Pharm. Res*, 28(3):249-68.

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