



RESEARCH ARTICLE

CURRENT INSECTICIDE RESISTANCE STATUS IN *Culex quinquefasciatus* SAY MOSQUITO POPULATIONS FROM MONO DEPARTMENT IN SOUTH-WESTERN REPUBLIC OF BENIN, WEST AFRICA

Lègba Nazaire Aïzoun^{1*}, Mindégnisèa Habib Tamègnon¹, Seindé Espérance Mèdoatinsa², Chèpo Daniel Chougourou³

¹Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET), Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, Cotonou, Benin.

²Unit of Experimental and Clinical Biology, Laboratory of Medical and Pharmaceutical Biotechnology Research, National High School of Applied Biosciences and Biotechnology (ENSBBBA) of Dassa-Zoumè, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, Benin.

³Department of Environment Genius, Polytechnic School of Abomey-Calavi (EPAC), University of Abomey-Calavi (UAC), Cotonou, Benin.

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*Address for Correspondence:

Dr. Nazaire Aïzoun, Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET), Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, Cotonou, Benin. Tel: +229 0195317939 ; E-mail: aizoun.nazaire@yahoo.fr

Abstract

Background and aims: *Culex quinquefasciatus*, an important vector in the transmission of human diseases has developed resistance to commonly used classes of insecticides in populations worldwide in public health. The current study was aimed to investigate on the current insecticide resistance status in *Culex quinquefasciatus* Say mosquito populations from Mono department in South-western Republic of Benin, West Africa.

Methods: Larvae and pupae of *Culex quinquefasciatus* populations were collected from the breeding sites in Mono department in 2023 during the great rainy season. WHO susceptibility tests were conducted on unfed female mosquitoes aged 2-5 days old. WHO bioassays were performed with impregnated papers of dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%).

Results: The results showed *Culex quinquefasciatus* mosquito populations were resistant to DDT, permethrin, deltamethrin insecticides. There is possible resistance to fenitrothion and bendiocarb. For that, further studies are needed to detect the real resistance status to these products. There is cross-resistance to both pyrethroid and organochlorine insecticides.

Conclusion: The physical barrier of Long Lasting Insecticidal Nets (LLINs) which are regularly distributed free by Beninese National Malaria Control Program throughout the entire country to increase coverage of LLINs is still important despite the insecticide resistance observed.

Keywords: Benin, *Culex quinquefasciatus*, Insecticide resistance, lymphatic filariasis.

INTRODUCTION

Undoubtedly, mosquitoes are the most important dipterans accountable for the spread of mosquito-borne diseases like malaria, dengue and filarial fevers, and so on. Among these diseases, malaria in Africa is caused by *Anopheles gambiae* (Giles, 1902) while filariasis is caused by *C. quinquefasciatus* (Say, 1823) with Nigeria having highest global records of morbidity and mortality of 25 % and 24 % malaria, and 14.3 % and low than 10 % filarial disease¹. *Culex* mosquitoes particularly *C. quinquefasciatus* are important arboviral and filariasis vectors. More than 17% of the global

burden of infectious threat is caused by vector-borne diseases (VBDs), which increase the levels of morbidity and mortality in tropical and subtropical areas².

C. quinquefasciatus is considered to be a major nuisance mosquito in many urban and semi-urban tropical settings, and its distribution is likely to extend further globally. Vector control is an important element in the prevention, control and elimination of major vector-borne diseases². *Culex* mosquitoes are responsible for discomfort to the population. Thus, innovative and effective vector control tools are needed to sustain the fight against resistant and nuisance

populations of *C. quinquefasciatus*. Even when considering human behaviour, the combination of screening and eave tubes has the potential to reduce mosquito entry and kill mosquitoes³.

Mechanistically focal *kdr* resistance, identified as a single nucleotide polymorphism (SNP) resulting in an amino acid change from leucine to phenylalanine at position 1014 (1014L to 1014F or occasionally 1014S), has been described in numerous populations, but published work indicates that enzymatic resistance often accounts for a large proportion of the observed IR phenotype in *C. quinquefasciatus* and that these same enzymes are able to degrade other classes of insecticides resulting in cross-resistance⁴⁻⁶. The use of insecticides with new modes of action, or those with multiple classes of insecticides in locations where there is broad resistance in adult mosquito populations will help support resistance management and aid in the optimization of sustainable integrated vector control strategies. Multiple insecticides formulations are now being effectively used in long lasting insecticidal nets to control resistant malaria vectors⁷.

Chemical control approaches based on insecticide use remain the standard strategy for both public health responses to disease outbreaks and general indoor vector pest control⁸. The use of chemical insecticides is one of the best way for controlling medically important vectors like mosquitoes that transmit various diseases. These insecticides are very effective, but it has a lot of problems such water and environmental pollution, effects on non target insects and development of insecticides resistance. When insecticide resistance in mosquitoes is not properly managed, it could lead to the failure of vector control efforts in the prevention, control and elimination of mosquito-borne diseases⁹. Likewise, the development and spread of insecticide resistance in *Culex* species could be a huge challenge if *Culex*-borne diseases re-emerge in areas where elimination had previously been achieved or is introduced into communities where mass drug administration (MDA) has not yet covered. Notably, insecticide resistance in *Culex* species could also impact malaria elimination programmes if communities perceive reduced efficacy of IRS and ITN/LLINs due to insecticide resistance in *Culex* spp. and the usage rate of these tools decline¹⁰.

Very few researches were published on insecticide resistance status in *C. quinquefasciatus* mosquito populations from Mono department. Therefore, there is a need to carry out new researches for this purpose.

The aim of this study was to investigate on the current insecticide resistance status in *C. quinquefasciatus* mosquito populations from Mono department in South-western Republic of Benin, West Africa.

MATERIALS AND METHODS

Study area

The study area is the department of Mono in Benin Republic. The study was carried out in the six districts of this department in south-western Benin. We have taken into account the economic activities of populations, their usual protection practices against

mosquito bites, the Long-Lasting Insecticidal Nets, Permanets and OlysetNets distribution frequently by National Malaria Control Program in these localities and peasant practices to control farming pests in the choice of the study site. The development of insecticide resistance in the local mosquito vectors depends of these factors. We took these factors into account to investigate on the current insecticide resistance status in *C. quinquefasciatus* Say mosquito populations from Mono department in South-western Republic of Benin. Four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August) characterize Mono department. The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

Mosquito sampling

We have collected in 2023 during the great rainy season in Mono department larvae and pupae 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 gutters). Then, they were kept in separated labeled bottles related to each district surveyed. Otherwise, larvae collected from multiple breeding sites were pooled together related to each district surveyed and 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 Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) of the Department of Sciences and Agricultural Techniques located in Dogbo district at Normal High School of Technical Teaching (ENSET) of Lokossa. *A. gambiae* Kisumu, a reference susceptible strain was used as a control for the bioassay tests.

Testing insecticide susceptibility

The principle of the WHO bioassay is to expose insects to a given dose of insecticide for a given time to assess susceptibility or resistance. The standard WHO discriminating dosages are twice the experimentally derived 100% lethal concentration (LC100 value) of a reference susceptible strain according to the tests procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces (1998). In this study, we have tested dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%). We have used permethrin and deltamethrin, insecticides of the same family as pyrethroids which are used on Long-Lasting Insecticidal Nets, OlysetNets and Permanets distributed frequently by National Malaria Control Program. We have used DDT to assess cross resistance with pyrethroids. Bendiocarb is the insecticide used for indoor residual spraying in the northern part of Benin. Fenitrothion was used in present study as an organo-phosphate to assess cross resistance with carbamate.

A batch of 20 to 25 unfed female mosquitoes aged 2–5 days were introduced into five WHO holding tubes (four tests and one control) that contained untreated

papers. They were then gently blown into the exposure tubes containing the insecticide impregnated papers. After 1 hr exposure, mosquitoes were transferred back into holding tubes and provided with cotton wool moistened with a 10% honey solution. Then, we have recorded the number of mosquitoes “knocked down” at 60 minutes and mortalities at 24 hrs following the WHO protocol (1998).

We have done the susceptibility tests on unfed females mosquitoes aged 2-5 days old reared from larval and pupal collections. All susceptibility tests were conducted in 2023 in the laboratory LaRPET at $25\pm 2^\circ\text{C}$ and 70 to 80% relative humidity.

Statistical analysis and data interpretation

The resistance status of mosquito samples was determined according to the WHO criteria (2017, seconde edition) as follows:

- Mortality rates between 98-100% indicate full susceptibility
- Mortality rates between 90-97% indicate possible resistance
- Mortality rates <90%, the population is considered resistant to the tested insecticides.

Analysis using ANOVA test and test of proportion was performed on the data sets gathered from the localities surveyed in Mono department to compare each of the tested insecticides and assess the resistance status of *C. quinquefasciatus* populations using WHO method.

RESULTS

Determination of Knock down time (Kdt) and resistance ratio (RR)

The resistance ratios (RR50) of the wild populations of *C. quinquefasciatus* from Mono with regard to *A. gambiae* Kisumu susceptible reference strain were 1.809, 1.470 and 1.793 respectively for DDT, permethrin and deltamethrin. These resistance ratios (RR50) were all higher than 1 (Table 1). The same remark was made with the resistance ratios (RR95). They were 1.860, 1.279 and 1.646 respectively for DDT, permethrin and deltamethrin. These results showed that the populations of *C. quinquefasciatus* from Mono were resistant to these products (Table 1). Mortality of *C. quinquefasciatus* populations from Mono department after 1 hr exposure to WHO impregnated papers with dichlorodiphenyl-trichloroethane (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%). Kisumu strain (control) confirmed its susceptibility status as a reference strain. The 24 hrs mortality recording showed that female *A. gambiae* Kisumu which were exposed to WHO papers impregnated with dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%) were fully susceptible to these products. They were dead and none of them could fly after 24 hrs mortality recording required by WHO (Table 2). Regarding field collected female *C. quinquefasciatus* populations from Mono department; they were resistant to dichloro-diphenyltrichloroethane (DDT) (4%), permethrin (0.75%) and deltamethrin (0.05%) with the mortality rates of 14%, 25% and 49% respectively. The mortality rates recorded with fenitrothion (1%) and bendiocarb (0.1%) were 91% and 94% respectively and indicated possible resistance to these products (Table 2).

Table 1: Determination of Knock down times 50 (Kdt50), Knock down times 95 (Kdt95), resistance ratios 50 (RR50) and resistance ratios 95 (RR95).

Populations	Insecticides	Kdt50 (min)	RR50	Kdt95 (min)	RR95
Kisumu (Control)	DDT	43.201	-	49.119	-
	Permethrin	58.352	-	70.189	-
	Deltamethrin	36.793	-	45.698	-
	Fenitrothion	Nd	-	Nd	-
	Bendiocarb	Nd	-	Nd	-
Mono	DDT	78.184	1.809	91.389	1.860
	Permethrin	85.797	1.470	89.777	1.279
	Deltamethrin	65.985	1.793	75.245	1.646
	Fenitrothion	Nd	-	Nd	-
	Bendiocarb	Nd	-	Nd	-

Table 2: Determination of resistance status in *C. quinquefasciatus* populations from Mono to DDT, permethrin, deltamethrin, fenitrothion and bendiocarb.

Populations	Insecticides	Number tested	% Mortality	Resistance status
Kisumu (Control)	DDT	100	100	S
	Permethrin	100	100	S
	Deltamethrin	100	100	S
	Fenitrothion	100	100	S
	Bendiocarb	100	100	S
Mono	DDT	100	14	R
	Permethrin	100	25	R
	Deltamethrin	100	49	R
	Fenitrothion	100	91	r
	Bendiocarb	100	94	r

DISCUSSION

In the current study, the field collected female *C. quinquefasciatus* populations from Mono department, were resistant to dichlorodiphenyltrichloroethane (DDT), permethrin and deltamethrin. Further studies are needed to detect the different mechanisms of resistance involved in *C. quinquefasciatus* resistance to these products in this area. Current study corroborated with that by Modak *et al.*¹¹, who had shown that *C. quinquefasciatus* populations from sub-Himalayan Region of West Bengal in India are highly resistant against synthetic pyrethroids and possess L1014F *kdr* mutation. Current study also corroborated with that by Danladi *et al.*¹², who had evaluated the resistance of *Culex* mosquitoes in Zagaina, Gombe State in Nigeria, to commonly used insecticides, providing insights for managing insecticide resistance in disease vector control. Their results showed that high resistance was observed across all tested insecticides, with mortality rates of 15% for DDT, 7% for Permethrin, 65% for Deltamethrin,

Unlu *et al.*¹³, had detected resistance to two pyrethroid insecticides in all tested locations (permethrin: 27 locations, deltamethrin: 28 locations) in Miami-Dade. The 1014F allele was widely distributed throughout all 17 locations sampled; however, 29.4% of these locations lacked 1014F homozygotes even though phenotypic pyrethroid resistance was present. Organophosphate resistance was more variable; 20.7% of the locations tested were susceptible to malathion, and 33.3% of the populations were susceptible to naled. Current study also corroborated with that by Poumachu *et al.*¹⁴, who had studied the trends in insecticide resistance in natural populations of *C. quinquefasciatus* and its impact on mosquito fitness in Dschang in West Cameroon. Their results showed that *C. quinquefasciatus* were resistant to pyrethroids such as permethrin and deltamethrin but full susceptible to carbamates such as propoxur and organochlorites such as malathion. The resistance intensity of the *C. quinquefasciatus* population was moderated when the population was exposed to deltamethrin at 10 \times . Pre-exposure to PBO led to the restoration of full susceptibility to both deltamethrin and permethrin. Compared with that of the control group, female fecundity rates, egg hatchability, and pupation rates were significantly lower in the insecticide exposed groups. Larval development time and adults emergence rates were comparable between insecticide-exposed groups and the control. Insecticide-exposed adults lived longer than control adults did. The findings of these authors suggest that the mechanisms selected for pyrethroid resistance are associated with negative impacts on different life-trait parameters and support the hypothesis that insecticide resistance is related to a high fitness cost. Mosquitoes also sometimes resist to insecticides by increasing the production of metabolic enzymes for detoxification or reducing the degree of sympatry between target sites and insecticide molecules. Vereecken *et al.*¹⁵, had studied the phenotypic insecticide resistance status of the *C. pipiens* complex. In addition, a study carried

out by Omotayo *et al.*¹⁶, also showed high pyrethroid-resistance intensity in *C. quinquefasciatus* populations from Jigawa, North-West, Nigeria.

Current study also corroborated with that by Talipouo *et al.*⁹, In fact, these authors had assessed the insecticide resistance status of *C. quinquefasciatus* populations from four districts of Yaoundé (Cameroon). All *C. quinquefasciatus* populations except one displayed high resistance to bendiocarb and malathion while high resistance intensity against both permethrin and deltamethrin was recorded. Molecular analyses revealed high frequencies of the ACE-1 G119S mutation (ranging from 0 to 33%) and *kdr* L1014F allele (ranging from 55 to 74%) in all *C. quinquefasciatus* populations. Significant over-expression was detected for cytochrome P450s genes *CYP6AA7* and *CYP6Z10*, as well as for *Esterase A* and *Esterase B* genes. The total cuticular hydrocarbon content, a proxy of cuticular resistance, was significantly increased (compared to the S-lab strain) in one population. Their study confirms strong insecticide resistance mediated by different mechanisms in *C. quinquefasciatus* populations from the city of Yaoundé. These authors think that the expansion of insecticide resistance in *Culex* populations can affect the effectiveness of current vector control measures and stress the need for the implementation of integrated vector control strategies in urban settings.

In the current study, the mortality rates recorded with fenitrothion and bendiocarb indicated possible resistance to these products. So, the resistance status to fenitrothion and bendiocarb in *C. quinquefasciatus* from Mono department need to be monitored carefully in this area of the country.

Limitations of the study

Only one department on twelve departments of Republic of Benin concerned the current study. More departments and more insecticides will be taken into account with different mosquito's species in future studies. The mechanisms selected for insecticide resistance will be investigated too.

CONCLUSION

There is cross-resistance to both pyrethroid and organochlorine insecticides. However, the physical barrier of Long Lasting Insecticidal Nets (LLINs) which are frequently distributed free by Beninese National Malaria Control Program throughout the entire country to increase coverage of LLINs is still important despite the insecticide resistance observed. Only the torn nets cannot protect people from vector bites. Monitoring resistance patterns over time is essential for developing effective vector control strategies to combat mosquito-borne diseases.

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AUTHOR'S CONTRIBUTIONS

Aïzoun LN: investigation, formal analysis and writing original draft. **Tamègnon MH:** validation, methodology, writing, critical review. **Mèdoatinsa SE:** **Chougourou CD:** supervision, review and editing. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

There are no conflicts of interest in regard to this project.

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