

# Current insecticide resistance status in *Culex quinquefasciatus* Say mosquito populations from couffo department and its implications for lymphatic filariasis control in south-western Republic of Benin, West Africa

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## ABSTRACT

**Background:** *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.

**Objectives:** The current study was aimed to investigate on the current insecticide resistance status in *Culex quinquefasciatus* Say mosquito populations from Couffo department and its implications for lymphatic filariasis control in south-western Republic of Benin, West Africa.

**Material and Methods:** Larvae and pupae of *Culex quinquefasciatus* populations were collected from the breeding sites in Couffo department from March to July 2024 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 and fenitrothion insecticides. There is possible resistance to bendiocarb and for that, further studies are needed to detect the real resistance status to this product. 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:** *Culex quinquefasciatus*, resistance, insecticide, lymphatic filariasis, Republic of Benin

## 1. INTRODUCTION

*Culex quinquefasciatus* Say, a member of the *Culex pipiens* group, is a medically important mosquito and major pest species with a worldwide distribution (1). *Culex quinquefasciatus* is known to be a major vector of filariasis (1), St. Louis encephalitis virus SLEV (2), West Nile virus WNV (3), and Rift Valley Fever virus RVFV (4,5). It is considered to be an opportunistic feeder, and while host choice is regionally variable, it feeds on many species of birds, mammals, and occasionally reptiles and amphibians (6,7). In North America, the human blood index (HBI) of *Cx. quinquefasciatus* varies from 1% (8) to 50% (9). In studies conducted in Kenya, HBIs ranged from 12-88% for indoor-collected mosquitoes and 3-23% for mosquitoes collected outdoors (10,11).

Resistance to insecticides in different mosquito species is a huge challenge that has continued to threaten vector control of mosquito-borne diseases (12). It is a major problem due to the rate at which insecticide resistance to recommended insecticides develop and spread in all countries in sub-Saharan Africa (13). Resistance in *Culex* spp. in particular has been reported in several west African countries (14,15). If insecticide resistance is not properly managed, it could lead to the failure of vector control efforts in the prevention, control and elimination of mosquito-borne diseases (16). 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.

Very few researches were published on insecticide resistance status in *Culex quinquefasciatus* mosquito populations from Couffo 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 *Culex quinquefasciatus* Say mosquito populations from Couffo department and its implications for lymphatic filariasis control in South-western Republic of Benin, West Africa.

## 2. MATERIAL AND METHODS

### Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in the six districts of this department (Figure 1). The choice of the study site took 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. These factors have a direct impact on the development of insecticide resistance in the local mosquito vectors. We took these factors into account to investigate on the current insecticide resistance status in *Culex quinquefasciatus* Say mosquito populations from Couffo department and its implications for lymphatic filariasis control in south-western Republic of Benin. Couffo 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 Republic of Benin showing the six districts surveyed in couffo department

### Mosquito sampling

*Culex quinquefasciatus* populations were collected from March to July 2024 during the great rainy season in Couffo department selected in the south-western of the country. Larvae and pupae were collected in this department 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. *Anopheles gambiae* sensu lato 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 (17). In this study, the insecticides tested were dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%), fenitrothion (1%) and bendiocarb (0.1%). We 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 used DDT to assess cross resistance with pyrethroids. Bendiocarb is the insecticide used for indoor residual spraying in the northern part of Benin. We used fenitrothion, an organophosphate to assess cross resistance with carbamate.

An aspirator was used to introduce 20 to 25 unfed female mosquitoes aged 2–5 days 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 one-hour exposure, mosquitoes were transferred back into holding tubes and provided with cotton wool moistened with a 10% honey solution. The number of mosquitoes “knocked down” at 60 minutes and mortalities at 24 hours were recorded following the WHO protocol (17).

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 laboratory LaRPET at 25+/-2°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 (18) 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.

## 3. RESULTS

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

The resistance ratios (RR50) of the wild populations of *Culex quinquefasciatus* from Couffo with regard to *Anopheles gambiae s.l.* Kisumu susceptible reference strain were 2.414, 1.670 and 1.820 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 1.921, 1.475 and 1.774 respectively for DDT, permethrin and deltamethrin. These results showed that the populations of *Culex quinquefasciatus* from Couffo were resistant to these products (Table 1).

**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	33.211	-	44.119	-
	Permethrin	37.103	-	50.251	-
	Deltamethrin	47.133	-	49.123	-
	Fenitrothion	Nd	-	Nd	-
	Bendiocarb	Nd	-	Nd	-
Couffo	DDT	80.184	2.414	84.763	1.921
	Permethrin	61.985	1.670	74.123	1.475
	Deltamethrin	85.797	1.820	87.179	1.774
	Fenitrothion	Nd	-	Nd	-
	Bendiocarb	Nd	-	Nd	-

Nd = No determined

- = Knock down (Kd) is not characteristic of Organophosphates and Carbamates

**Mortality of *Culex quinquefasciatus* populations from Couffo department after one hour exposure to WHO impregnated papers with dichlorodiphenyltrichloroethane (DDT) (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 hours mortality recording showed that female *Anopheles 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 hours mortality recording required by WHO (Table 2).

Regarding field collected female *Culex quinquefasciatus* populations from Couffo department, they were resistant to dichlorodiphenyltrichloroethane (DDT) (4%), permethrin (0.75%), deltamethrin (0.05%) and fenitrothion (1%) with the mortality rates of 12%, 23%, 37%, and 77% respectively. The mortality rate recorded with bendiocarb (0.1%) is 92% and indicated possible resistance to this product (Table 2).

**Table 2:** Determination of resistance status in *Culex quinquefasciatus* populations from Couffo 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
Couffo	DDT	100	12	R
	Permethrin	100	23	R
	Deltamethrin	100	37	R
	Fenitrothion	100	77	R
	Bendiocarb	100	92	r

S= susceptible

R= Resistant

r = possible resistance

#### 4. DISCUSSION

*Culex quinquefasciatus*, an arboviral and filarial vector, is present in high numbers throughout sub-Saharan Africa, and insecticide-resistant populations have been reported worldwide.

In the current study, the field collected female *Culex quinquefasciatus* populations from Couffo department, were resistant to dichlorodiphenyltrichloroethane (DDT), permethrin, deltamethrin and fenitrothion. Further studies are needed to detect the different mechanisms of resistance involved in *Culex quinquefasciatus* resistance to these products in this area. Our study corroborated with many reports of insecticide-resistant *Culex quinquefasciatus* in Africa. Resistance to pyrethroids has been documented in Tanzania (19), Benin (20), and Côte d'Ivoire (21), while organophosphate and carbamate resistance, whether through upregulated esterase or insensitive acetylcholinesterase, has been reported in Benin (22), Burkina Faso (23), and Côte d'Ivoire (24). Additionally, DDT resistance has been recorded in Benin (22). Another study carried out by Norris and Norris (25) had studied the insecticide resistance in *Culex quinquefasciatus* mosquitoes after the introduction of insecticide-treated bed nets in Macha, Zambia. In fact, in order to determine the insecticide resistance status of *Culex quinquefasciatus* in Macha, Zambia, adult mosquitoes reared from eggs collected from oviposition traps were tested by bioassay. High levels of resistance to DDT, pyrethroids (permethrin and deltamethrin), malathion (an insecticide of same class as fenitrothion), and deltamethrin-treated net material were detected, and molecular assays revealed that the knockdown resistance (*kdr*) allele was frequent in the *Culex quinquefasciatus* population, with 7.0% homozygous for the *kdr* L1014 allele and 38.5% heterozygous (0.263 *kdr* frequency). The *kdr* frequency was significantly higher in mosquitoes that had successfully fed on human hosts, and screening archived specimens revealed that *kdr* was present at lower frequency prior to the introduction of ITNs, indicating that ITNs might be a selective force in this population. Additionally, metabolic detoxification enzyme

activity assays showed upregulated glutathione S-transferases,  $\alpha$ -esterases, and  $\beta$ -esterases. Continued monitoring and assessment of the *Culex quinquefasciatus* population is necessary to determine levels of resistance.

In the current study, the mortality rate recorded with bendiocarb indicated possible resistance to this product. So, the resistance status to bendiocarb in *Culex quinquefasciatus* from Couffo department need to be monitored carefully in this area of the country.

### 5. 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.

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