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Insecticide resistance in *Culex quinquefasciatus,* a vector of lymphatic filariasis at Djougou, north-western of Benin: Public health concern

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Abstract
To have an idea about the insecticide resistance profiles of *Culex quinquefasciatus* in one endemic district of Lymphatic Filariasis (LF) in Benin, susceptibility status of this mosquito in two urban areas (Founga and Zountori) and two rural areas (Nima and Bougou 2) to insecticides 0.75% permethrin, 0.05% deltamethrin, 4% DDT and 1% of bendiocarb was determined using the standard WHO susceptibility tests. Moreover, mutations in the acetylcholinesterase (*ace-1*) and knock down resistance (kdr) were screened from surviving mosquitoes using PCR methods. Results from the bioassays revealed that *Cx. quinquefasciatus* developed resistance to DDT (2% and 5% as average of mortality in urban and peri-urban areas, respectively), permethrin (14% and 22% in urban and peri-urban areas, respectively), deltamethrin (35% regardless of the areas of collection), but still susceptible to bendiocarb. Moreover, the kdr mutation was present in all populations of *Cx. quinquefasciatus* with frequency ranging from 0.89 to 0.9 and 0.86 to 0.87 in urban and rural areas, respectively. A low frequency (0.22 - 0.25 and 0.20 - 0.23 respectively in urban and rural areas) of *ace-1* mutation was also found in the mosquitoes collected.
These findings showed that *Cx. quinquefasciatus* populations of Djougou have developed resistance to organochlorine, pyrethroids with a high level of kdr mutation. The low level of *ace-1* mutation suggested that insecticides belonging to carbamate and organophosphate families could be used to control *Cx. quinquefasciatus* in this district, however, regular surveillance needs to be conducted on the susceptibility of *this mosquito* to the insecticides of these families.

Keywords: insecticides, resistance, *Culex quinquefasciatus,* Djougou, Benin

Introduction

Culex quinquefasciatus largely distributed in subtropical and tropical areas is one of the vector of filarial parasite *Wuchereria bancrofti*, [1-6]. This mosquito is a major biting nuisance and lives close to people [7]. It's responsible for many diseases (Rift Valley fever virus, West Nile virus etc;) particularly in the weak level of urbanization in many cities in Africa [8-12].

The district of Djougou located in northern Benin with low level of urbanization is characterized by the lack of storm-water drainage systems with the absence of hygiene service, thus offering favorable conditions for larvae and adults of *Cx. quinquefasciatus* and therefore could contribute to the emergence of lymphatic filariasis (LF).

Despite the use of mass drug administration against LF in this district, the new strategy of the national vector control programme is based on the use of insecticides for indoor residual spraying (IRS**)** and insecticide-treated nets (ITNs) with synthetic pyrethroids to control *Cx. quinquefasciatus*. However, many reports showed that the massive use of different families of insecticide both in agriculture for pests control and in public health for vector control seemed to be one of the key of insecticide resistance in *Cx. Quinquefasciatus* **[13]**. Resistance of this mosquito has been reported in many countries [14-17] wheras in Benin, there is no data available on the resistance profiles of *Cx. quinquefasciatus* to different families of insecticide in the north of the country.

It therefore crucial to know the insecticide resistance profiles of this mosquito in this district in case of LF outbreaks.

Materials and Methods

Study area

The study was conducted at Djougou, a district located in the north of Benin characterize by a sub-equatorial type climate with one rainy season (June - November) and one dry season (December-May) with temperature ranges between 22 and 40°C. The choice of this district is based on the weak level of urbanization where the lack of sanitation is the most striking

Mosquito collections

Cx. quinquefasciatus larvae were collected during the rainy season in the district of Djougou (1°23 E, 10°18 N) in two urban areas (Founga and Zountori) and two rural areas (Nima and Bougou 2) (Figure 1). The samples were collected from polluted drain. Larvae and pupae were collected from polluted water and brought to insectary to be reared for adults susceptibility tests.

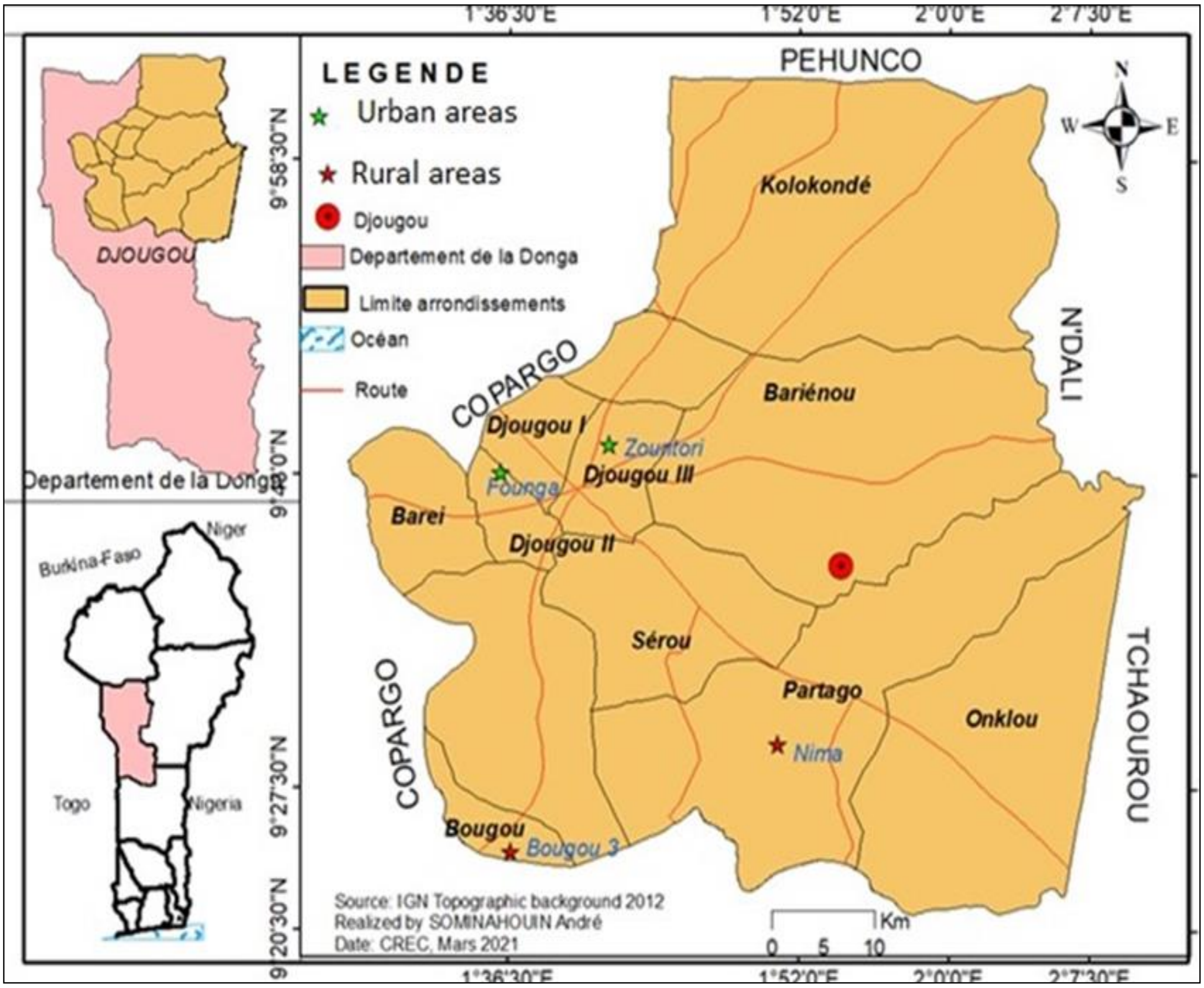

Fig 1: Map of Djougou district showing the localities where Culex larvae were collected

Insecticide susceptibility test

Adults females (150) mosquitoes aged 2–5 days from each study site were subjected to the susceptibility tests using insecticide- impregnated papers (bendiocarb (0.1%), permethrin (0.75%), deltamethrin (0.05%), DDT (4%) base on WHO testing protocol [18]. Twenty-five mosquitoes were tested per insecticide and every ten minutes, the number of knock down mosquitoes was recorded. After 1h exposing to insecticide- impregnated papers, they were transferred into new tubes with untreated white filter papers for 24h recovery period. They were fed with 10% honey solution during the 24h recovery period. Mortality rate was recorded after 24h and dead and living mosquitoes were stored at -20 °C for further analysis.

All results were compared with the susceptible reference strain SLAB.

Detection of L1014F and G119S mutations

The presence of Kdr L1014F was investigated on 400 of mosquitoes (100 from each site) using two separate PCRs [19]. The G119S a*ce-1* mutation in *Cx. quinquefasciatus* was also searched from the same populations using the PCR-RFLP test [20].

Data Interpretation

Mortality rates were calculated using Abbott's formula [21]. Base on the WHO criteria, all mortality less than 80%, indicate resistance, while those greater than 98% indicate susceptibility [18]. Mortality between 80%-98%, suggests the possibility of resistance that needs to be verified [18]. The Genepop software (version 3.3) was used to estimate the frequency of *ace-1* and the *kdr* mutation resistance [22]. The Knock Down (KD) data was subjected to Probit analysis using statistical software to compute the KDT50 and KDT90 (time taken to knock down 50 and 90 % of the exposed mosquitoes) and their 95 % confidence intervals.

Results

Phenotypic resistance in *Cx. quinquefasciatus*

Overall, a total of 2,400.0 adults from larvae collection, 600 S-LAB of *Cx. quinquefasciatus* were exposed to four different insecticides (DDT, Permethrin, deltamethrin and bendiocarb). Results from the bioassays revealed that *Cx. quinquefasciatus* developed resistance to DDT (2% and 5% as average of mortality in urban and peri-urban areas, respectively), permethrin (14% and 22% in urban and peri-urban areas, respectively), deltatmethrin (35% regardless of the areas of collection), but still susceptible to bendiocarb (100% of mortality) (Table 1).

Moreover, Table1 showed also that KDT_{50} and KDT_{90} values of DDT, permethrin of *Cx quinquefasciatus* from urban and peri urban areas was 3 to 6 times higher than those exposed to bendiocarb.

The Slab *Cx. quinquefasciatus* was susceptible (100%) to all insecticide-impregnated papers listed above.

Table 1: Detection of Knockdown time (KDT50 and KDT95) and the susceptibility status of *Cx quinquefasciatus* populations from the study sites

Sites	Ecological zone	Insecticide	Number of tested *Cx. quinquefasciatus*	kdT50 [Cl95] (min)	kdT95 [Cl95] (min)	% Mortality	Resistance status
Urban area	Founga	DDT	150	368.80	1140.58	4	R
		Permethrin	150	141.70	321.48	12	R
		Deltamethrin	150	209.37	589.78	36	R
		Bendiocarb	150	53.12	99.16	100	S
	Zountori	DDT	150	-------	-----	0	R
		Permethrin	150	148.54	288.26	16	R
		Deltamethrin	150	331.82	1089.21	34	R
		Bendiocarb	150	52.80	99.56	100	S
Peri urban area	Nima	DDT	150	218.34	465.22	6	R
		Permethrin	150	218.75	485.59	24	R
		Deltamethrin	150	285.12	812.39	36	R
		Bendiocarb	150	58.31	101.12	100	S
	Bougou 2	DDT	150	389.10	1089.45	4	R
		Permethrin	150	136.41	198.51	20	R
		Deltamethrin	150	367.15	1079.65	34	R
		Bendiocarb	150	58.72	102.17	100	S
Cx. SLAB	Susceptible reference strain	DDT	150	48.12	98.1	100	S
		Permethrin	150	57.43	91.72	100	S
		Deltamethrin	150	39.12	78.39	100	S
		Bendiocarb	150	49.48	98.54	100	S

Detection of genes of resistance

Resistance allele frequency at the *kdr* and *ace.1 of Cx. quinquefasciatus* populations from the study sites is showing in Table 2. Regardless of the areas of collection, the kdr mutation was present in all populations of *Cx. quinquefasciatus* with frequency ranging from 0.89 to 0.9 and 0.86 to 0.87 in urban and rural areas, respectively. The *ace-1* mutation was also found in low frequency (0.22 to 0.25 and 0.20 to 0.23 in urban and rural areas, respectively.

Table 2: *Kdr* and *Ace1R* mutations detected in *Cx. quinquefasciatus* populations from the study sites

		Mutation *Kdr*				Mutation *Ace-1*			
Study Sites	**Localities**	**SS**	**RS**	**RR**	**F(R)**	**SS**	**RS**	**RR**	**F(R)**
Urban areas	Founga	3	9	90	0.9	70	08	22	0.22
	Zountori	3	8	89	0.89	70	05	25	0.25
Rural areas	Nima	4	9	87	0.87	68	12	20	0.20
	Bougou 2	5	9	86	0.86	63	14	23	0.23

Discussion

Results from this study showed the susceptibility profiles of *Cx. quinquefasciatus* to different families of insecticide in urban and peri urban areas in the district of Djougou, Northern Benin. Overall, *Cx. quinquefasciatus* developed resistance to organochlorine (DDT), pyrethroids (permethrin, deltamethrin, lambdacyhalothrin) but susceptible to carbamate (bendiocarb). Regardless of the areas of *Cx. quinquefasciatus* larvae collection, the resistance is strong with DDT (mortality less than 6%) and pyrethroids (mortality less than 6%) permethrin and deltamethrin with mortality rates less than 30%), but susceptible with bendiocarb based on the WHO criteria for characterizing insecticide resistance/susceptibility.

Indeed, several reports on the susceptibility profiles of *Cx. quinquefasciatus* have shown that this mosquito developed a high level of resistance to [23]. The district of Djougou characterized by subsistence agriculture and cotton production, where many families of insecticides particularly pyrethroids, are used for crops protection could be one of the factors that contribute to the resistance of *Cx. quinquefasciatus*. These insecticides widely used to control agricultural pests in Africa can pose additional selection pressure on mosquitoes when insecticide contaminated ground water permeates their larval habitats [24]. During the treatments for crops protection, insecticide residues are washed into mosquito breeding sites which exerting selection pressure on mosquito larval populations and could explain the high level of resistance in *Cx. quinquefasciatus* [25]. This hypothesis could explain the pyrethroids and DDT resistance in *Cx. quinquefasciatus* observed in the district of Djougou. Similar results were reported in Cameroon [25-26]. The kdr mutation is reported to be closely associated with DDT and pyrethroids resistance It has been reported that the kdr mutation was associated with PYR and DDT [27]. The *Cx. quinquefasciatus* resistance to DDT and pyrethroids in this district could explain the high level of kdr frequency resistance in mosquitoes We found that the diagnostic values of the ace-1 and kdr mutations were different: susceptibility was high for the kd and could explain the widespread of kdr in Djougou peri urban and urban areas. The low frequency of *ace-1* mutation showed that the kdr mutation seems to be the important mechanism of *Cx. quinquefasciatus* resistance found.

Conclusion

The emergence of pyrethroid resistance couple with the high level of kdr mutation in *Cx quinquefasciatus* in the district of Djougou has become a serious concern for Lymphatic filariasis control. The low level of Ace-1 mutation seems to be a hope to control this mosquito which is the most important LF. However, regular surveillance need to be carried out on the susceptibility of *Cx. quiquefasciatus* from this district to carbamate and organophosphate.

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