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Pyrethroid resistance in *Aedes aegypti* populations in southern Benin, West Africa

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Abstract
Aedes aegypti the main vector of dengue fever is present year round in several cities of the Republic of Benin. It is therefore unfortunate that the international focus is on Anopheles control and not so much on *Ae. Aegypti*, a rather more resilient mosquito to many insecticides that deserves attention. In this study, we assessed the resistance status of *Ae. Aegypti* to organochlorine, pyrethroids and carbamates.
We collected *Ae. Aegypti* mosquitoes in Dandji from June to August 2017. Bioassay tests were performed to assess the susceptibility of dengue vectors to various agricultural insecticides where females mosquitoes aged 2-5 days old were exposed to five insecticide impregnated papers (permethrin at 0.75%; deltamethrin at 0.05%; DDT at 4%, lambdacyhalothrin (0.05%) and bendiocarb at 0.1%) following WHOPES guidelines.
Results from this study showed that *Ae. Aegypti* developed a strong resistance to DDT (10% of mortality), and moderate resistance to pyrethroids (35% with permethrin, 60% with deltamethrin and 72% with lambdacyhalothrin). However, the same populations were fully susceptible to bendiocarb
These findings show for the first time in Benin the resistance of *Ae. Aegypti* populations to organochlorines and pyrethroids.
Measures must be taken by Benin Health authorities for a continuous monitoring of the susceptibility of *Ae. Aegypti* across the country. These measures will enable the selection of effective insecticides for arbovirus control in Benin in case of a dengue fever outbreak.

Keywords: *Aedes aegypti*, insecticides, resistance, Dandji, Benin

Introduction

With more than 50 million cases reported to the World Health Organization (WHO) each year, Dengue Virus (DENV) is now regarded as the world's most important mosquito-borne virus (mobovirus) [1]. Its global prevalence has grown dramatically in recent decades [1-2]. DENV infection is caused by one of four DENV serotypes (DENV 1, DENV 2, DENV 3, and DENV 4) and its circulation has been reported in 34 countries in Africa[3] but also in travellers returning from many African countries like Benin [4]; Cote d'Ivoire and Tanzania [5].
Widely distributed *Aedes* spp. mosquitoes are the primary vectors of dengue [2]. The virus is transmitted to humans through the bites of infected female mosquitoes. After virus incubation for 4-10 days, an infected mosquito is capable of transmitting the virus for the rest of its life [6]. Symptoms of mobovirus infection in humans can vary extensively and therefore, mobovirus infections are often misdiagnosed. In a time of changing environmental, ecological and socio-economic conditions, moboviruses surveillance is an important forecasting tool for emerging and re-emerging arboviruses. In Africa, the knowledge about DENV epidemiology and its impact on public health is poor. Because of a low awareness of health care providers and lack of diagnostic testing and systematic surveillance, the epidemiology and effect of dengue in Africa has not been [3, 7].
Recently, outbreaks of dengue have been reported in many African countries. In fact, in 2009, 696 suspected cases were recorded in Senegal with 196 confirmed [8]. In Cape Verde, an estimated 210,000 clinical cases were documented of which 174 fitted the WHO definition of DHF/DSS and six were fatal [8]. This re-emergence of dengue fever has been observed also in Mauritius [9], in Cameroon [10] and in Senegal [8].

As there is still no vaccine or specific treatment for DENV, vector control remains the cornerstone of prevention and outbreak control. Conventional control strategies rely on the reduction of larval sources by eradicating water-holding containers that serve as larval habitats, and by using larvicides (e.g. temephos and *Bacillus thuringiensis var israeliensis* [Bti]) in natural and/or domestic breeding sites [11]. However, for the past 10 years, insecticides of the organophosphate (OP) and pyrethroid (PY) classes have been utilized intensively in many parts of the world against *Ae. Aegypti*. This situation had contributed to the selection of resistant *Ae. Aegypti* populations to insecticides reported worldwide [12-13].
In Benin, despite the use of insecticides for crops control and in public health against mosquitoes [14-15], there is a lack of information on the phenotypic resistance of *Ae. Aegypti* to the four classes of insecticides used in public health. Giving a recent report by Yadouleton *et al*. [16] showing the presence throughout the year of *Ae. Aedes* in Benin, it is unfortunate that the international focus is on Anopheles control and not so much on *Ae. Aegypti*, a rather more resilient mosquito to many insecticides that deserves attention.
The present study was designed to assess the resistance status of *Ae. Aegypti* to organochlorine, pyrethroids and carbamates in southern Benin.

Materials and Methods

Study Area

The study was carried out in Dandji, an urban area located in Cotonou, in southern Benin. This part of southern Benin (figure 1) is characterized by two rainy seasons (March- July and October-November) and two dry seasons (December-March and August-September). The annual mean rainfall is 1,500 mm in July, relative humidity (RH) of 70% ± 5 and a minimum/maximum and the temperature ranging from 23 to 32 °C. The choice of the study area is based on the weak level of urbanization. Additionally, lots of second hand tires from Europe and Asia, which are good breeding sites for *Ae. Aegypti*, are stored and sold in this area.

Fig 1: Map of Benin showing the study site

Mosquito Collections

Ae. Aegypti immature stages (larvae/pupae) were sampled from domestic water sources (e.g. jars, tanks), peridomestic (e.g. tires) and natural sources (e.g. tree holes) during the dry and rainy season. Larvae or pupae collected at the study site were stored in plastic boxes and brought to the "Centre de Recherche Entomologique de Cotonou" (CREC) insectary for bioassay tests.

Insecticide Susceptibility Test

Aedes aegypti bioassays were performed with non-blood-fed females according to the standard WHO guidelines [17]. Tests were performed on 2-5 day-old unfed females. Batches of 20-25 females were exposed to the insecticide-impregnated papers: deltamethrin (0.05%); permethrin (0.75%); DDT (4%); lambdacyhalothrin (0.05%) and bendiocarb (0.1%). The number of knockdown mosquitoes was recorded every 10 min during exposure. The mortality rate was recorded after 24 h. Tests with untreated papers were systematically run as control. When control mortality was between 5 and 20%, mortality rate in tested samples was corrected using Abbott formula [18]. We used the *Ae. Aegypti* SBE strain originating from Benin which is free of any detectable resistance mechanisms [19].

Data Interpretation

Percentage knockdown and percentage mortality to the five insecticides for the mosquitoes from the study locality were determined. The resistance/susceptibility status of *Ae. Aegypti* populations were evaluated using WHO method [20]. Mortality rates <80% at 24 h post exposure indicated resistance, >97% indicated susceptibility and mortality rates between 80 and 97% indicated that resistance is suspected. The knockdown times for 50% (KdT_{50}) and 95% (KdT_{95}) of tested mosquitoes were calculated using a log-probit software [21].

Results
Resistance to Insecticides
A total of 1,000 females of *Ae. Aegypti* collected from the study site of Dandji were exposed to impregnated papers with discriminating doses of permethrin (0.75%); deltamethrin (0.05%); DDT (4%); lambdacyhalothrin (0.05%) and bendiocarb (0.1%). The knockdown times (KdT50, KdT95) of *Ae. Aegypti* populations for permethrin, deltamethrin, DDT, and ambdacyhalothrin were significantly longer than the one from the susceptible strain SBE (p<0.05) (Table 1). However, the knockdown times (KdT50, KdT95) for bendiocarb were not significantly different from susceptible strain SBE (p>0.05) (Table 1).

Table 1: Knockdown times (KdT_{50} and KdT_{95}) of *Ae. Aegypti* populations from the study site after exposure to DDT 4%; permethrin 0.75%; Lambda-cyhalothrin 0.05% and bendiocarb 0.1%

	Insecticide	N	kdT50 [Cl95] (min)	kdT95 [Cl95] (min)
Wild population of *Ae. Aegypti* from Dandji	DDT	100	65.1 [57.5-73.4]	152.1 [118.4-228.1]
	Permethrin	100	32.4 [25.8-30.2]	60.1 [50.1-78.6]
	Deltamethrin	100	30.3 [25.4-38.9]	72.6 [50.2-90.5]
	Lambda-cyhalothrin	100	38.1 [29.4-36.5]	65.1 [57.5-86.5]
Susceptible strain (SBE)	Bendiocarb	100	13.2 [9.4-18.2]	25.5 [20.2-37.1]
	DDT	100	25.7 [24.3-27.0]	40.7 [38.0- 44.5]
	Permethrin	100	15.1 [13.6-20.1]	56.5 [43.2-77.2]
	Deltamethrin	100	13.2 [9.4-18.2]	25.5 [20.2-37.1]
	Lambda-cyhalothrin	100	10.9 [9.7-12.0]	18.1 [16.0-21. 6]
	Bendiocarb	100	11.0 [8.7-17.6]	23.5 [18.4-35.8]

Moreover, mortality rates recorded with DDT (10% of mortality), permethrin (35%), deltamethrin (60%) and lamdacyhalothrin (72%) were significantly higher compared to the control strain respectively with the insecticides cited above (Figure 2).

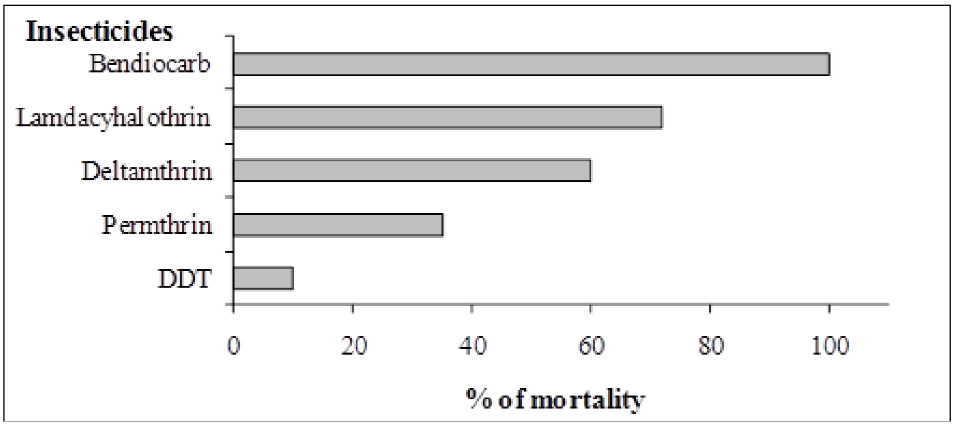

Fig 2: Moratlity of *Ae. Aegypti* populations in Dandji after exposure to DDT 4%, permethrin 0.75%, Lambda-cyhalothrin 0.05% and bendiocarb 0.1%

Discussion
Our findings document for the first time the occurrence of pyrethroids and organochlorine resistance in *Ae. Aegypti* populations in southern Benin. The results of our investigation in Dandji showed that many people use insecticide bombs and coils which active ingredients are pyrethroids inside their households to fight against mosquitoes bites. The repetitive use of these products may have contributed to the selection of insecticide resistance in *Ae. Aegypti* populations in Dandji. This situation can explain the WHO biossay results on pyrethroids resistance in *Ae. Aegypti* where the mosquito developed resistance to 0.75% permethrin (mortality rate = 35%), 0.05% deltamethrin (mortality rate = 60%) and 0.05% lambdacyhalothrin (mortality rate = 72%). Pyrethroids resistance in *Ae. Aegypti* dated long time ago and are worldwide [22-23]. This resistance to PY can be explained by the presence of agricultural activities in our larval site collection. In fact, such activities in urban areas directly led to an improper use of insecticides to control vegetable pests, thus exerting a huge selection pressure on mosquito larval populations [24].

Despite the explanation of the resistance of *Ae. Aegypti* to PY, the source of the selection of the resistance seem to be unclear. In fact, in Benin, there is no program specifically targeting arbovirus vectors particularly *Ae. Aegypti*. It is possible that insecticides used to control other insects of medical or agricultural interest exert indirect selection pressure on *Ae. Aegypti* mosquito species.

Conclusion
These findings show for the first time in Benin the resistance of *Ae. Aegypti* populations to organochlorines and pyrethroids. Measures must be taken by Benin Health authorities for continuous monitoring of the susceptibility of *Ae. Aegypti* across the country. Such measures will enable the selection of effective insecticides for arbovirus control in

Benin in case of a dengue fever outbreak.

Competing Interests

The authors declare that they have no competing interests.

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