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Comparison Of Susceptibility To Lambdacyhalothrin In Female Adult Anopheles Gambiae Sensu Lato From Dogbo District With Their F1 Progeny Susceptibility In South-Western Benin, West Africa

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Abstract
Mosquito control programs are now threatened by the selection of mosquito populations resistant to the chemical insecticides. The current study was aimed to compare the susceptibility to lambdacyhalothrin in female adult Anopheles gambiae sensu lato from Dogbo district with their F1 progeny susceptibility in South-Western Benin, West Africa. Larvae and pupae of An. gambiae s.l. mosquitoes were collected from March to July 2023 during the rainy season in the locations of Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni and reared up for obtaining F1 progeny. Female adult Anopheles gambiae s.l. mosquitoes were also collected from window traps put on windows of rooms in Dogbo district surveyed. Female adult Anopheles gambiae species collected from window traps were morphologically identified using morphological keys and then transferred into mosquito cages for WHO bioassays performed with impregnated papers of lambdacyhalothrin (0.05%). WHO bioassays were also performed with F1 progeny.The physiological age of female adult Anopheles gambiae s.l. collected from window traps was determined through dissection using Detinova method. The results showed that female adult Anopheles gambiae s.l. from Dogbo district collected from window traps were more susceptible to lambdacyhalothrin than their F1 progeny. Changes in mosquito physiology occur with senescence. The resistance is a hereditary and dynamic phenomenon.

Keywords: *F1 progeny, Lambdacyhalothrin Resistance, Anopheles gambiae, window traps*

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I. Introduction

Globally, in 2023, the number of malaria cases was estimated at 263 million, with an incidence of 60.4 cases per 1000 population at risk. This is an increase of 11 million cases from the previous year and a rise in incidence from 58.6 cases per 1000 population at risk in 2022. The WHO African Region continues to carry the heaviest burden of the disease, accounting for an estimated 94% of malaria cases worldwide in 2023. The WHO Eastern Mediterranean Region has experienced a 57% increase in incidence since 2021, rising to 17.9 cases per 1000 population at risk in 2023 [1].

Globally, in 2023, the number of deaths was estimated at 597 000, with a mortality rate of 13.7 per 100 000. The number of malaria deaths and the mortality rate steadily decreased from 622 000 and 14.9 deaths per 100 000, respectively, in 2020. The WHO African Region continues to carry the heaviest burden of mortality, with 95% of estimated malaria deaths worldwide [1].

The ongoing spread of insecticide-resistant genes, such as the well-characterized *kdr* mutations [2-3] in populations of the major African malaria vectors, *An. gambiae*, can seriously jeopardize the efficacy of vector control programmes [4].The monitoring of insecticide resistance in malaria vectors is of prime importance

especially where control programmes are planned or already running, in order to assess potential selection effects of insecticidal compounds on vector populations, and to take appropriate measures such as switching to other classes of compounds. For this goal, the presence and frequency of the *kdr* mutations constitute a valuable and useful resistance marker for two main reasons.

Pyrethroid is the general term for a group of synthetic chemicals that are structurally modified from natural pyrethrins derived from Chrysanthemum flowers. Most of the pyrethroids are nontoxic to mammals as compared to the insecticide groups and possess high knockdown activity against insects. Nowadays, pyrethroids are emerging as the predominant insecticides for vector control. They are used in various formulations such as long-lasting insecticide-treated nets (LLIN) for the long-time prevention of mosquito bites in malaria endemic areas, indoor residual spray, and ultra-low volume (ULV) sprays for emergency control of dengue vectors. In fact, pyrethroids comprise 40% of the insecticides used annually on a global level for indoor residual spraying against malaria vectors and 100% of the WHO -recommended insecticides for the treatment of mosquito nets [5]. Pyrethroids are the only group of insecticides currently recommended for net treatment. The determination of insecticide susceptibility status of the target vectors will help monitor the insecticidal efficacy and possible development of resistance at early stages. So, the early detection of insecticide resistance development is the most important aspect that guides vector control programmes. The emergence of resistance in populations of *Anopheles gambiae* to common classes of insecticides used in public health has been reported in Benin [6-14]**.**

Beninese National Malaria Control Programme has frequently implemented large-scale and free distribution of long-lasting insecticidal nets (LLINs) throughout the entire country to increase coverage of LLINs. It is crucial that information on current status of *Anopheles gambiae s.l.* resistance to pyrethroid being investigated. This will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate resistance management strategies. In this study, we compare the susceptibility to lambdacyhalothrin in female adult *Anopheles gambiae s.l.* from Dogbo district with their F1 progeny susceptibility in South-Western Benin.

II. Materials 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 Dogbo district. 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 compare the susceptibility to lambdacyhalothrin in female adult *Anopheles gambiae s.l.* from Dogbo district with their F1 progeny susceptibility in South-Western 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.

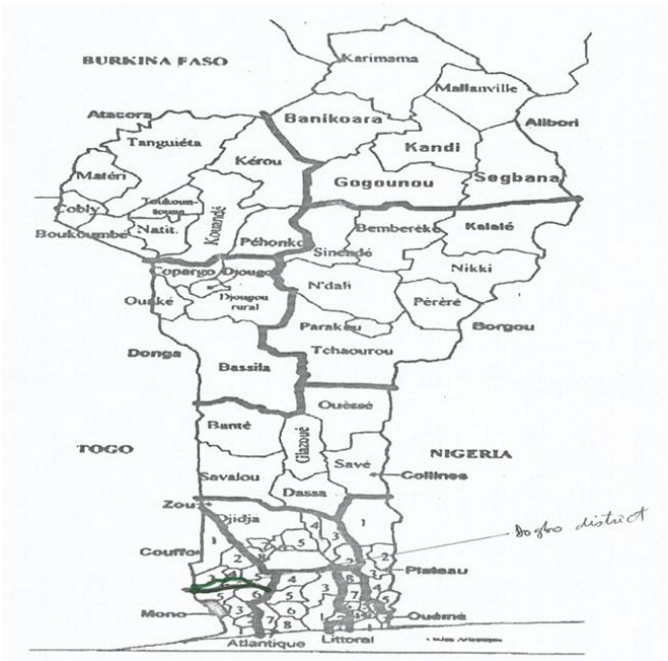

Figure 1: Map of Republic of Benin showing Dogbo District

Mosquito sampling

Anopheles gambiae sensu lato mosquitoes were collected from March to July 2023 during the rainy season in Dogbo district. Larvae and pupae were collected from breeding sites using the dipping method and kept in labeled bottles. The samples were reared to adults in the insectary of Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) in Department of Sciences and Agricultural Techniques located in Dogbo district. *Anopheles gambiae* s.l. mosquitoes were also collected from March to July 2023 during the rainy season in window traps put on windows of four rooms in each of locations surveyed in Dogbo district such as Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni. Between 6.00 a.m. to 7.00 a.m., aspirators were used to collecting mosquitoes from these window traps. They were then put in some plastic cups covered with small cutting untreated net on which was put cotton wool moistened with a 10% honey solution.

Obtaining of F1 progeny

After larvae and pupae were collected in locations of Dogbo district, they were reared up to adult emergence at insectary. Male and female adult mosquitoes aged 5-7 days old were used in the reproduction. After the female mosquitoes had been mated and given rabbit's blood meal, an ovipositor was put in mosquito cage containing these females. After the eggs were laid by these females, they were placed in some containers which contained water. Larvae of first stage were fed with yeast. They were then reared up to F1 progeny emergence for bioassays tests.

Mosquito species identification

After female Anophelines were collected from window traps, they were identified to species based on morphological characters using identification keys [15] and then transferred into mosquito cages for bioassays tests.

Testing insecticide susceptibility

WHO protocol

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 [16]. In this study, the insecticide tested was lambdacyhalothrin (0.05%). The choice of lambdacyhalothrin was justified by the recent use of pyrethroids on LLINs which were used by NMCP for implementation of large-scale and free distribution through the entire country to increase coverage.

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 [16].

An. gambiae Kisumu, a reference susceptible strain was used as a control for the bioassay tests. We used Kisumu more precisely to confirm the quality of WHO impregnated papers of lambdacyhalothrin.

All susceptibility tests were done following WHO protocol in Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) of the Department of Sciences and Agricultural Techniques located in Dogbo district at 25+/-2°C and 70 to 80% relative humidity.

Dissection of *Anopheles gambiae* mosquitoes

The physiological age of female adult *Anopheles gambiae* from window traps was determined through dissection using Detinova method [17].

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.

Analysis using Fisher's exact test and test of proportion was performed on the data sets gathered from the locations surveyed in Dogbo district to compare mortality rates obtained with F1 progeny to those of their parent female adult *Anopheles gambiae s.l.* mosquitoes.

III. Results

Susceptibility status to lambdacyhalothrin in *Anopheles gambiae s.l.* populations from Dogbo district

Kisumu strain (control) confirmed its susceptibility status as a reference strain. The 24 hours mortality recording shows that female *Anopheles gambiae* Kisumu which were exposed to WHO papers impregnated with lambdacyhalothrin (0.05%) were fully susceptible to this product. They were dead and none of them could fly after 24 h mortality recording required by WHO (Table 1 and Table 2).

Regarding F1 progeny *Anopheles gambiae s.l.* mosquitoes from Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni in Dogbo district, they were resistant to lambdacyhalothrin with the mortality rates of 43%, 25%, 39%, 51%, 37% and 29% respectively (Table 1).

Table 1: Mortality of F1 progeny *Anopheles gambiae s.l.* mosquitoes from Dogbo district after one hour exposure to WHO impregnated papers with lambdacyhalothrin (0.05%)

Locations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Ayomi	100	43	R
Dévé	100	25	R
Honton	100	39	R
Lokogohoué	100	51	R
Madjrè	100	37	R
Totchangni	100	29	R

Regarding field collected female adult *Anopheles gambiae s.l.* populations from Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni in Dogbo district, they were also resistant to lambdacyhalothrin with the mortality rates of 67%, 43%, 53%, 69%, 55% and 47% respectively (Table 2). But, these mortality rates were higher than those recorded with their F1 progeny.

Table 2: Mortality of female adult *Anopheles gambiae s.l.* mosquitoes from Dogbo district after one hour exposure to WHO impregnated papers with lambdacyhalothrin (0.05%)

Locations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Ayomi	100	67	R
Dévé	100	43	R
Honton	100	53	R
Lokogohoué	100	69	R
Madjrè	100	55	R
Totchangni	100	47	R

Dissection of *Anopheles gambiae* mosquitoes

The results obtained regarding the physiological age of female adult *Anophles gambiae* determined through dissection using Detinova method showed that almost all surviving *Anopheles gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes in the different locations surveyed were very few and ranged from Zero (0) to five (5) mosquitoes (Table 3).

Table 3: Determination of physiological ages with surviving *Anopheles gambiae* mosquitoes from WHO bioassays

					Physiological ages			
Locations		Number tested			Pare			Nullipare
Ayomi		49			5			44
Dévé		27			1			26
Honton		33			1			32
Lokogohoué		22			0			22
Madjrè		45			1			44
Totchangni		39			0			39

The advantages and disadvantages of using *Anopheles gambiae* F1 progeny were shown in table 4.

Table 4: Advantages and disadvantages of using F1 progeny

Vector sample	Advantages	Disadvantages
F1 progeny	Age of vectors can be kept constant between tests, allowing results from different times and places to be compared.	Requires better entomological facilities, which limits where the tests can be carried out. Environmental conditions will differ

	In areas with low mosquito density, can be used even if it is not possible to catch sufficient numbers of adult wild female mosquitoes.	from those within the insectary. Since many eggs may be derived from just a few adult females, the number of genomes sampled from the wild population is likely to be less than the number of insects tested.

IV. Discussion

Female adult *Anopheles gambiae s.l.* mosquitoes collected from window traps put on windows of rooms in Dogbo district surveyed were those from emergence of pupae of *Anopheles gambiae s.l.* populations collected from the breeding sites using the dipping method. So, even if in the current study, the F1 progeny were obtained from mosquitoes collected from the breeding sites using the dipping method and reared up to adult emergence, they (F1 progeny) also have the female adult *Anopheles gambiae s.l.* mosquitoes collected from window traps put on windows of rooms as parent.

The F1 progeny *Anopheles gambiae s.l.* mosquitoes from Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni in Dogbo district were resistant to lambdacyhalothrin. In similar way, field collected female adult *Anopheles gambiae s.l.* populations from window traps in the same locations in Dogbo district were also resistant to lambdacyhalothrin. The resistance of *Anopheles gambiae s.l.* populations from Dogbo district to lambdacyhalothrin may be explained by increased use of various insecticidal products (including pyrethroids) for crop protection. In fact, Akogbéto *et al.* [19] had shown that after pesticide treatments in agricultural settings, residues of insecticides get into mosquito breeding sites. These residues have lethal effects on larvae of some populations of mosquito whereas they exert a selective pressure on other populations, leading to a gradual tolerance of insecticide concentrations and to the emergence of resistant populations. The status of lambdacyhalothrin resistance was already studied by Aïzoun *et al.* [20] in *Anopheles gambiae* sensu lato populations from Kandi district in north of Benin.

The mortality rates recorded with female adult *Anopheles gambiae s.l.* mosquitoes collected from window traps put on windows of rooms in Dogbo district surveyed were higher than those obtained with their F1 progeny *Anopheles gambiae s.l.* mosquitoes obtained after reproduction. So, more the mosquito was old, more it was susceptible to lambdacyhalothrin. Otherwise, the young *Anopheles gambiae* mosquitoes were more resistant to lambdacyhalothrin than the old. According to Chouaibou *et al.* [21], changes in mosquito physiology that is not specifically associated with insecticides but that occurs with senescence such as an increase in the rate of cuticle permeability or a decrease in the rate of xenobiotic excretion, could also lead to an increase in susceptibility to insecticides.

The results obtained regarding the physiological age of female adult *Anopheles gambiae* determined through dissection using Detinova method showed that almost all surviving *Anopheles gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes in the different locations surveyed were very few and ranged from zero to five mosquitoes. That is good news for malaria vector control in Dogbo district and for Benin country.

Hakizimana *et al* [22] had studied the susceptibility of *Anopheles gambiae* to insecticides used for malaria vector control in Rwanda. In fact, the widespread emergence of resistance to pyrethroids is a major threat to the gains made in malaria control. To monitor the presence and possible emergence of resistance against a variety of insecticides used for malaria control in Rwanda, nationwide insecticide resistance surveys were conducted in 2011 and 2013. For that, larvae of *Anopheles gambiae sensu* lato mosquitoes were collected in 12 sentinel sites throughout Rwanda. Mortality results indicated a significant increase in resistance to lambdacyhalothrin from 2011 to 2013 in 83% of the sites.

As advantages of using F1 progeny, age of vectors can be kept constant between tests, allowing results from different times and places to be compared. Also, in areas with low mosquito density, can be used even if it is not possible to catch sufficient numbers of adult wild female mosquitoes. As disadvantages of using F1 progeny, Requires better entomological facilities, which limits where the tests can be carried out. In addition, environmental conditions will differ from those within the insectary. Also, since many eggs may be derived from just a few adult females, the number of genomes sampled from the wild population is likely to be less than the number of insects tested.

V. Conclusion

Monitoring resistance patterns over time is essential for developing effective vector control strategies to combat mosquito-borne diseases. Changes in mosquito physiology occur with senescence. The resistance is a hereditary and dynamic phenomenon.

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