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Comparison of susceptibility to deltamethrin in female adult *Anopheles gambiae s.l.* from Dogbo district with their F1 progeny susceptibility in South-Western Benin, West Africa

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Abstract

The current study was aimed to compare the susceptibility to deltamethrin in female adult *Anopheles gambiae s.l.* from Dogbo district with their F1 progeny susceptibility in South-Western Benin, West Africa. Larvae and pupae of *Anopheles gambiae s.l.* populations were collected from the breeding sites in Couffo department in 2020 and reared up for obtaining F1 progeny. Female adult *An. gambiae s.l.* mosquitoes were also collected from window traps put on windows of rooms in Dogbo district surveyed. *An. gambiae s.l.* mosquitoes were collected from March to July and August to November 2020 during the rainy season in the locations of Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni. Female adult *An. 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 deltamethrin (0.05%). WHO bioassays were also performed with F1 progeny. The physiological age of female adult *An. gambiae s.l.* collected from window traps was determined through dissection using Detinova method. The results showed that the mortality rates recorded with female adult *An. gambiae s.l.* mosquitoes collected from window traps were higher than those obtained with their F1 progeny *An. gambiae s.l.* mosquitoes obtained after reproduction of parent mosquitoes. The current study clearly shows that changes in mosquito physiology occur with senescence.

Keywords: F1 progeny; Resistance; *Anopheles gambiae*; Window traps; Deltamethrin; Benin

1. Introduction

National programmes reported that 135 million people representing 4% of the global population at risk were protected by IRS in 2012. The proportion of the population protected by IRS increased substantially in the African Region during 2006 and 2008, and the increased coverage was maintained during 2009 and 2011, at 10% and 12% of the population at risk. In 2012, a total of 58 million people, or 8% of the population at risk, were protected [1]. IRS involves the application of residual insecticides to the inner surfaces of dwellings targeting *Anopheles* mosquitoes that rest on walls after having taken a blood meal. IRS programmes can rapidly reduce local malaria incidence and mortality, provided that most houses and animal shelters in targeted communities are sprayed. WHO recommends the spraying ofat least 80% (and ideally 100%) of houses, structures and units in the targeted area inany round of spraying [2].

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The ongoing spread of insecticide-resistant genes, such as the well-characterized *kdr* mutations [3-4] in populations of the major African malaria vectors, *An. gambiae*, can seriously jeopardize the efficacy of vector control programmes [5].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.

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. Recently, the emergence of resistance in populations of *An. gambiae* to common classes of insecticides used in public health has been reported in Benin [6-15].

Beninese National Malaria Control Programme has recently implemented large-scale and free distribution oflong-lasting insecticidal nets (LLINs) throughout the entire country to increase coverage of LLINs. It is crucial that information on current status of *An. 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 deltamethrin in female adult *Anopheles gambiae s.l.* from Dogbo district with their F1 progeny susceptibility in South-Western Benin.

2. Material and methods

2.1. 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-westernBenin 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 deltamethrin 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.

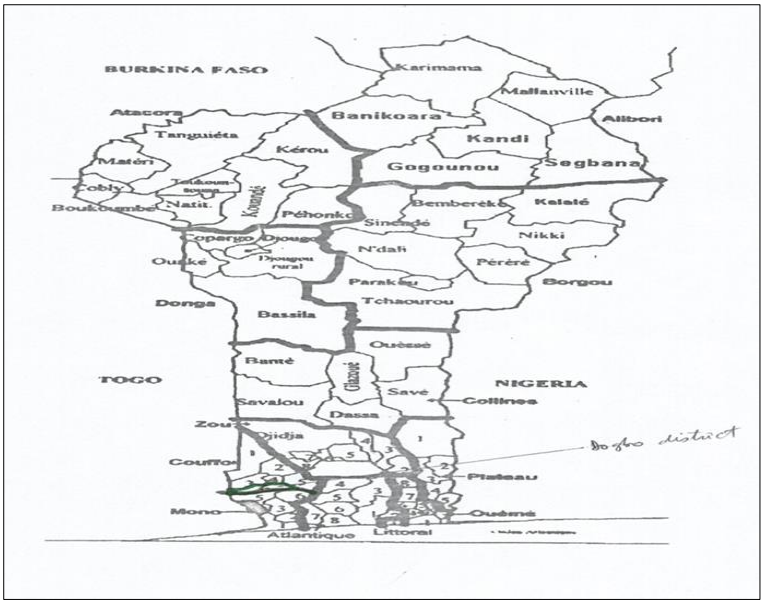

Figure 1a Map of Republic of Benin showing Dogbo District

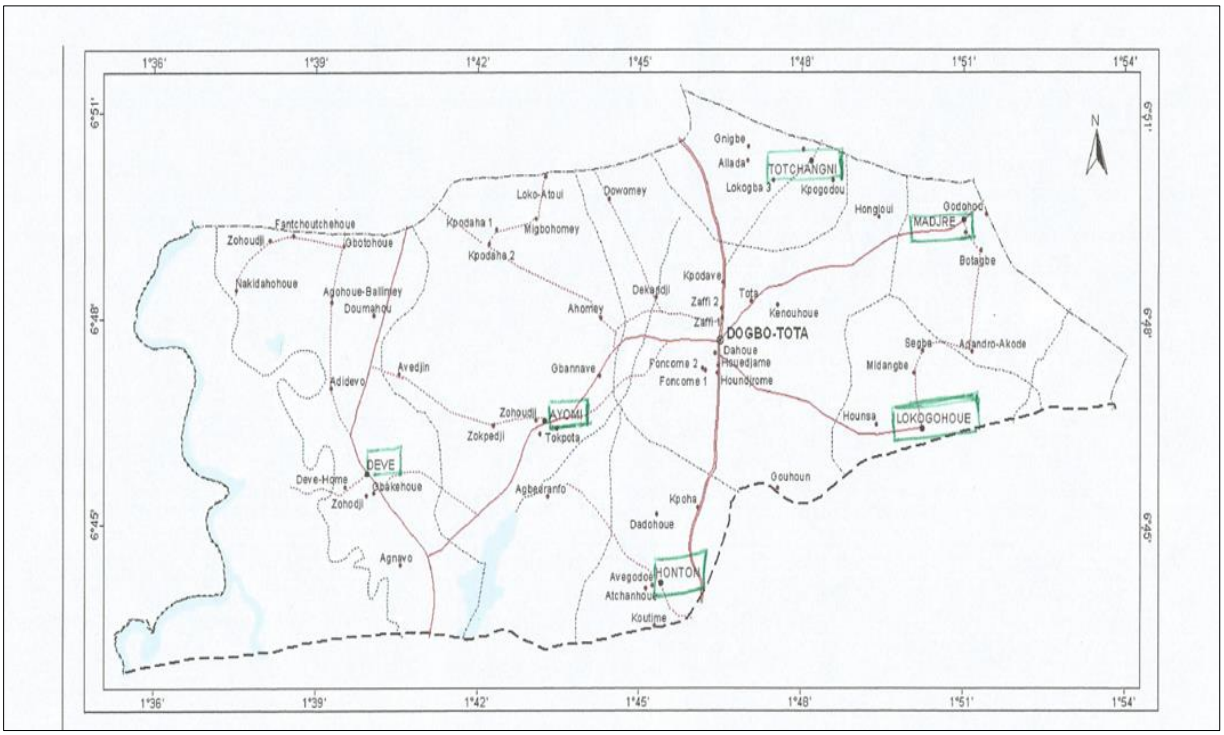

Figure 1b Map of Dogbo District showing the study area

2.2. Mosquito sampling

An. gambiae s.l. mosquitoes were collected from March to July and August to November 2020 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 the Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques located in Dogbo district.

Anopheles gambiae s.l. mosquitoes were also collected from March to July and August to November 2020 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.

2.3. 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.

2.4. Mosquito species identification

After female Anophelines were collected from window traps, they were identified to species based on morphological characters using identification keys [16] and then transferred into mosquito cages for bioassays tests.

2.5. Testing insecticide susceptibility

The principle of the WHO bioassay is to expose insectsto 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 insecticide tested was deltamethrin (0.05%). The choice of deltamethrin 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 fiveWHO 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].

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 deltamethrin.

All susceptibility tests were done following WHO protocol in Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques located in Dogbo district at 25+/-2°C and 70 to 80% relative humidity.

2.6. Dissection of *Anopheles gambiae* mosquitoes

The physiological age of female adult *An. gambiae* from window traps was determined through dissection using Detinova method [18].

2.7. Statistical analysis

The resistance status of mosquito samples was determined according to the WHO criteria [19] as follows:

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

Abbott s formula was not used in this study for the correction of mortality rates in test tubesbecause the mortality rates in control tube were less than 5% [20].

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.

3. Results

3.1. Susceptibility status to deltamethrin 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 deltamethrin (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).

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

Locations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Ayomi	100	53	R
Dévé	100	35	R
Honton	100	46	R
Lokogohoué	100	41	R
Madjrè	100	29	R
Totchangni	100	31	R

Regarding F1 progeny *An. gambiae s.l.* mosquitoes from Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni in Dogbo district, they were resistant to deltamethrin with the mortality rates of 53%, 35%,46%, 41%, 29% and 31% respectively (Table 1).

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 deltamethrin with the mortality rates of 77%, 83%, 63%, 79%, 75% and 67% respectively (Table 2).

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

Locations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Ayomi	100	77	R
Dévé	100	83	R
Honton	100	63	R
Lokogohoué	100	79	R
Madjrè	100	75	R
Totchangni	100	67	R

3.2. Dissection of *Anopheles gambiae* mosquitoes

The results obtained regarding the physiological age of female adult *An. gambiae* determined through dissection using Detinova method showed that almost all surviving *An. gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes in the different locations surveyed were very few and ranged from 00 to 07mosquitoes (Table 3).

Table 3 Determination of physiological ages with surviving *An. gambiae* mosquitoes from WHO bioassays

Physiological ages			
Locations	**Number tested**	**Pare**	**Nullipare**
Ayomi	23	03	20
Dévé	17	00	17
Honton	37	01	36
Lokogohoué	21	04	17
Madjrè	25	00	25
Totchangni	33	07	26

4. Discussion

Female adult *An. 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 *An. gambiae s.l.* mosquitoes collected from window traps put on windows of rooms as parent.

The F1 progeny *An. gambiae s.l.* mosquitoes from Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni in Dogbo district were resistant to deltamethrin. 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 deltamethrin. The resistance of *Anopheles gambiae s.l.* populations from Dogbo district to deltamethrin may be explained by increased use of various

insecticidal products (including pyrethroids) for crop protection. In fact, Akogbéto *et al.* [21] showed 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 deltamethrin resistance was already studied by Aïzoun *et al.* [9] in *Anopheles gambiae sensu lato* populations from Ouémé department in southern Benin. In fact, from 2008 to 2010, the National Malaria Control Program (NMCP) had undertaken a full coverage of IRS in no-flood zones in the Ouémé region, coupled with the distribution of LLINs in flood zones. IRS was not implemented in the flood zone because of the presence of water bodies, which could be at risk of contamination by insecticides. For that reason, Long-Lasting Insecticidal Nets (LLINs) (Permanet 2.0) were distributed to households, with particular attention to children under-five and pregnant women in October 2008 and May 2009. Permanet 2.0 is a LLIN impregnated with deltamethrin which is the insecticide tested in the current study.

The mortality rates recorded with female adult *An. 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 *An. gambiae s.l.* mosquitoes obtained after reproduction. According to Chouaibou *et al.*[22], 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 *An. gambiae* determined through dissection using Detinova method showed that almost all surviving *An. gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes in the different locations surveyed were very few and ranged from 00 to 07 mosquitoes. So, more the mosquito was old, more it was susceptible to deltamethrin. Otherwise, the young *An. gambiae* mosquitoes were more resistant to deltamethrin than the old.

The current study clearly showed that female adult *An. gambiae s.l.* mosquitoes collected from window traps put on windows of rooms in Dogbo district surveyed were resistant like their F1 progeny obtained from mosquitoes collected from the breeding sites using the dipping method. So the resistance is a hereditary phenomenon. However, the mortality rates recorded with female adult *An. gambiae s.l.* mosquitoes collected from window traps were higher than those obtained with their F1 progeny *An. gambiae s.l.* mosquitoes obtained after reproduction of parent mosquitoes.

5. Conclusion

The current study clearly shows that changes in mosquito physiology occur with senescence and the resistance is a hereditary phenomenon.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest regarding the publication of this paper.

Statement of ethical approval

The study follows proper ethical procedures.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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