

ESTABLISHMENT OF THE CORRELATION BETWEEN RESISTANCE LEVEL TO PERMETHRIN AND PHYSIOLOGICAL AGE OF AN. GAMBIAE SENSU LATO POPULATIONS FROM DOGBO DISTRICT IN SOUTH-WESTERN BENIN, WEST AFRICA

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ABSTRACT

Background: Monitoring of insecticide resistance is a necessary element of any medium-scale or large-scale deployment of an insecticidal intervention. **Objective:** The current study was aimed to establish the correlation between the resistance level to permethrin and physiological age of *An. gambiae* sensu lato populations from Dogbo district in South-Western Benin, West Africa. **Methodology:** Female *An. gambiae* s.l. mosquitoes were 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 *An. gambiae* species were morphologically identified using morphological keys and then transferred into mosquito cages for WHO bioassays performed with impregnated papers of permethrin (0.75%). The physiological age of adult female *An. gambiae* was determined through dissection using Detinova method. **Results:** The results showed that more *An. gambiae* mosquitoes were old, more they were susceptible to permethrin. Otherwise, the young *An. gambiae* mosquitoes were more resistant to permethrin than the old. There is a correlation between resistance level to permethrin and physiological age of *An. gambiae* sensu lato populations from Dogbo district in South-Western Benin. **Conclusion:** The current study clearly shows that there is a correlation between resistance level to permethrin and physiological age of *An. gambiae* sensu lato populations from Dogbo district in South-Western Benin.

KEYWORDS: Resistance, *Anopheles gambiae*, physiological age, permethrin, Benin.**INTRODUCTION**

Malaria poses a serious obstacle to development in sub-Saharan Africa.^[1] To control transmission, the National Malaria Control Program (NMCP) of Benin relies on an integrated approach to control the intense transmission, characteristic of many programs in this region, through vector-control, early diagnosis and treatment plus prophylaxis during pregnancy.

In sub-Saharan Africa, insecticide treated nets (ITNs) and indoor residual insecticide spraying (IRS) are the cornerstones of malaria vector control.^[2] These vector control methods aim to reduce morbidity and mortality caused by malaria. ITNs and IRS have each been shown to be highly effective methods of malaria vector control in their own right. A recent review of the evidence of cost and consequences of large-scale vector control for malaria concluded that both ITNs and IRS are highly

cost effective vector control strategies.^[3] ITNs have been the mainstay of vector control in many countries in which the disease is endemic and where infrastructure limits or precludes the implementation of IRS.^[4] Unfortunately the resistance of *An. gambiae* to insecticides used for malaria vector control has occurred. This resistance has been associated with all insecticidal compounds used for insect vectors of human disease, including African malaria vectors.^[5] The ongoing spread of insecticide-resistant genes, such as the well-characterized kdr mutations^[6-7] in populations of the major African malaria vectors, *An. gambiae* and its sibling species *An. arabiensis*^[8-11], can seriously jeopardize the efficacy of vector control programs.^[12]

In the recent decade, many countries across sub-Saharan Africa are rapidly increasing insecticide-treated nets (ITNs) coverage through several strategies including, social marketing^[13,14], free distribution to target

groups^[15,16] and more recently, free universal population based distribution campaigns target the entire population at risk.^[15,17] If the results are below what is expected, this is due to several factors. In addition to the extension of resistance to pyrethroids in malaria vectors which represents a serious obstacle to the implementation and use of LLINs in Africa^[18], significant problems remain, endangering the sustainability goals and achievements. Indeed, in recent years, the National Malaria Control Program (NMCP) in African countries has invested heavily in the acquisition and distribution of LLINs for malaria control. However, monitoring the use of these materials is often overlooked.

In Benin as across Africa, malaria control relies heavily on vector control through the use of insecticide-treated nets (ITN) and indoor residual spraying (IRS). In West Africa, the main mechanism involved in pyrethroid-resistance in *Anopheles gambiae* is caused by target site insensitivity through a knockdown resistance (*kdr*)-like mutation caused by a single point mutation (Leu-Phe) in the para-sodium channel gene.^[8] Malaria vector resistance to insecticides in Benin is conferred by two main mechanisms: (1) alterations at site of action in the sodium channel, viz the *kdr* mutations and (2) an increase of detoxification and/or metabolism through high levels of multi-function oxidases (MFOs), non-specific esterases (NSEs).^[18-22]

Beninese National Malaria Control Programme has recently 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 *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 establish the correlation between resistance level to permethrin and physiological age of *An. gambiae sensu lato* populations from Dogbo district in South-Western Benin, West Africa.

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 (Fig.1a and Fig.1b). 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 establish the correlation between resistance level to permethrin and physiological age of *An. gambiae sensu lato* populations from Dogbo district. 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.



Fig. 1a: Map of Republic of Benin showing Dogbo District.

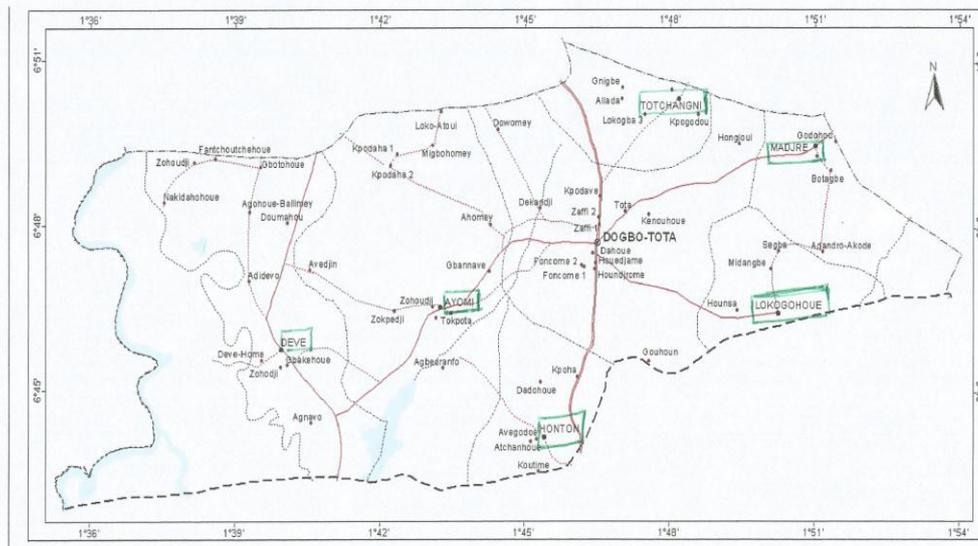


Fig.1b: Map of Dogbo District showing the study area.

Mosquito sampling

Anopheles 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 in window traps put on windows of four rooms in each of locations surveyed in Dogbo district. 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.

Mosquito species identification

Female Anophelines were identified to species based on morphological characters using identification keys^[23] 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.^[24] In this study, the insecticide tested was permethrin (0.75%). The choice of permethrin was justified by the recent use of pyrethroids on LLINs which were used by National Malaria Control Programme (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.^[24]

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 permethrin.

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 27°C +/- 2°C and 75% +/- 10% relative humidity.

Dissection of *Anopheles gambiae* mosquitoes

At the end of WHO bioassays, the physiological age of alive adult female *An. gambiae* was determined through dissection using Detinova method.^[25]

Statistical analysis

The resistance status of mosquito samples was determined according to the WHO criteria^[26] 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 tubes because the mortality rates in control tube were less than 5%.^[27]

RESULTS

Susceptibility status to permethrin 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 impregnated papers with permethrin (0.75%) 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).

Regarding field collected female *Anopheles gambiae s.l.* populations from Ayomi, Dévé, Honton, Lokoghoué, Madjrè and Totchangni in Dogbo district, they were resistant to permethrin with the mortality rates of 41%, 36%, 27%, 39%, 28% and 31% respectively (Table 1).

Table 1: Mortality of *An. gambiae s.l.* populations from Dogbo district after one hour exposure to WHO impregnated papers with permethrin (0.75%).

Locations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Ayomi	100	41	R
Dévé	100	36	R
Honton	100	27	R
Lokoghoué	100	39	R
Madjrè	100	28	R
Totchangni	100	31	R

Physiological age determination of alive *Anopheles gambiae* mosquitoes

The results obtained regarding the physiological age of survivor female adult *An. gambiae* determined through dissection using Detinova method showed that almost all

alive *An. gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes observed in the different locations surveyed were very few and ranged from 00 to 03 mosquitoes (Table 2).

Table 2: Physiological age determination of survivor *An. gambiae* mosquitoes from WHO bioassays.

Locations	Number tested	Physiological ages	
		Pare	Nullipare
Ayomi	34	00	34
Dévé	39	02	37
Honton	48	01	47
Lokoghoué	36	00	36
Madjrè	47	03	44
Totchangni	44	02	42

DISCUSSION

The control of vector borne diseases uses different methods depending on physiological, behavioural and ecological features of the vector. Knowledge regarding the level and mechanisms of resistance occurring in a vector population is very important for integrated vector control, in order to decide which control method is effective, efficient, and will not encourage further resistance.^[28] Resistance management strategies are mainly based on the rational use of the compounds already available, especially in public health because the number of insecticides is very limited. Resistance to these insecticide families is now widespread in the main malaria vectors *Anopheles gambiae s.l.* from Benin. The emergence and spread of *kdr* resistance among *Anopheles gambiae* should burden the large scale programmes of impregnated net distribution that are promoted all over African countries.

Field collected female *Anopheles gambiae s.l.* populations from Dogbo district, were resistant to permethrin. The resistance of *Anopheles gambiae s.l.* populations from Dogbo district to permethrin may be explained by increased use of various insecticidal products (including pyrethroids) for crop protection. In fact, Akogbéto *et al.*^[29], 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. According to Zaim *et al.*^[30], pyrethroids have unique modes of action such as fast knockdown and excito-repellent effects. The status of permethrin resistance was already studied by Aizoun *et al.*^[22] in *Anopheles gambiae sensu lato* populations from Agbalilamè in Sèmè district in Ouémé department in southern Benin. It is worth mentioning that the locality of Agbalilamè is crossed by the Nokoue Lake streams,

which sweep and converge several environmental pollutants and pesticide residues from the neighbouring peri-urban cities and farms to the coastal locality of Agbalilamè. It is also possible that several ranges of xenobiotics present in these water bodies around Agbalilamè might have also contributed to the selection of this resistance in *Anopheles gambiae*.

The results obtained regarding the physiological age of adult female *An. gambiae* determined through dissection using Detinova method showed that almost all alive *An. gambiae* mosquitoes from bioassays dissected were nullipares. The number of pare mosquitoes in the different locations surveyed were very few and ranged from 01 to 03 mosquitoes. Nullipare mosquitoes were younger than pares. More the mosquito was old, more it was susceptible to permethrin. Otherwise, the young *An. gambiae* mosquitoes were more resistant to permethrin than the old.

CONCLUSION

The current study clearly shows that there is a correlation between resistance level to permethrin and physiological age of *An. gambiae sensu lato* populations from Dogbo district in South-Western Benin.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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