

COMPARATIVE SUSCEPTIBILITY TO LAMBDCYHALOTHRIN OF TWO *Aedes aegypti* POPULATIONS FROM COUFFO DEPARTMENT AND ITS IMPLICATIONS FOR DENGUE VECTOR CONTROL IN SOUTH-WESTERN BENIN

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ABSTRACT

Aedes aegypti is a vector of viruses that negatively impact human health. The understanding of the factors which influence vector susceptibility to insecticide is important. The current study aimed to compare susceptibility to lambdacyhalothrin of two *Aedes aegypti* populations from Couffo department in South-western Benin, regarding mosquito sex, physiological status, and mosquito age. Larvae and pupae of *Aedes aegypti* mosquitoes were collected from April to July 2024 during the great rainy season in the breeding sites in Couffo department. The WHO susceptibility tests were conducted on unfed male and female mosquitoes aged 2-5 days old. WHO susceptibility tests were also conducted on unfed, blood fed and gravid female mosquitoes aged 2-5 days old. These susceptibility tests were also conducted on unfed, blood fed and gravid female mosquitoes aged 20 days old. The results showed that *Aedes aegypti* male adult were not more susceptible than the females. There was no increasing in vector susceptibility to lambdacyhalothrin after females had taken their blood meals. There also was no increasing in vector susceptibility to lambdacyhalothrin after females possess eggs in their abdomen. The results also showed that more mosquitoes were old more they were susceptible to lambdacyhalothrin. Many factors influence vector susceptibility to insecticide. Among these factors, there are mosquito sex, physiological status and mosquito age. Therefore, it is useful to respect the WHO criteria in the assessment of insecticide susceptibility tests in human disease vectors including the dengue vectors.

KEYWORDS: Mosquito sex, Physiological status, Mosquito age, Susceptibility, lambdacyhalothrin.

1. INTRODUCTION

Mosquitoes have become a major public health concern due to the blood feeding females of different species transmitting malaria causing protozoa, elephantiasis causing filarial worms, and dengue fever, yellow fever, Rift valley fever, West Nile fever causing viruses.^[1]

Historically, *Aedes aegypti* has been an efficient vector for various arboviruses due to its preference for human blood and its behavior of biting multiple hosts in a single feeding.^[2] In contrast, *Aedes albopictus* has a diurnal biting activity, feeds on a wider variety of mammals, and prefers colder habitats, making it less efficient as a vector. Furthermore, *Aedes aegypti* prefers indoor habitats, reproducing in water containers close to human dwellings. Its life cycle lasts from 8 to 15 days, with an adult phase of four to six weeks. This species is most active at dawn and dusk but can bite at different times of the day depending on the availability of blood sources.^[3]

Aedes aegypti, originally from Africa, and *Aedes albopictus*, from Asia, have become significantly globalized. The adaptation of *Aedes aegypti* to synanthropic environments has facilitated their proliferation, exacerbated by urbanization and increased international travel and trade, making them effective vectors of arboviruses.^[4,5]

Additionally, climate change has contributed to their expansion, allowing these mosquitoes to colonize new geographic areas.^[6]

The prevention and control of *Aedes aegypti* are crucial for public health, especially in tropical and subtropical regions. Various control methods, including chemical, biological, and environmental management, target different stages of the mosquito's life cycle and are often applied simultaneously. *Aedes aegypti* is a vector of viruses that negatively impact human health. Insecticide resistance complicates mosquito control efforts, but understanding the mechanisms of resistance can help to improve management practices.

Very few researches were published on Comparative susceptibility to lambda-cyhalothrin of *Aedes aegypti* populations from Couffo department in South-western Benin, regarding mosquito sex, physiological status and mosquito age. Therefore, there is a need to carry out new researches for this purpose.

The aim of this study was to compare susceptibility to lambda-cyhalothrin of two *Aedes aegypti* populations from Couffo department and its implications for dengue vector control in South-western Republic of Benin, West Africa.

2. MATERIALS 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-western Republic of Benin and the study was carried out in Dogbo and Lalo districts (**Figure 1**). The choice of the study sites took into account the economic activities of populations, their usual protection practices against mosquito bites, the Long-Lasting Insecticidal Nets, Permanets and OlysetNets distribution frequently by National Malaria Control Program in these localities and peasant practices to control farming pests. These factors have a direct impact on the development of insecticide resistance in the local mosquito vectors. Couffo has a climate with four seasons, two rainy seasons (March-July and August-November) and two dry seasons (November-March and July-August). The temperature ranged from 25 to 32°C with the annual mean rainfall, which is between 900 and 1100 mm.

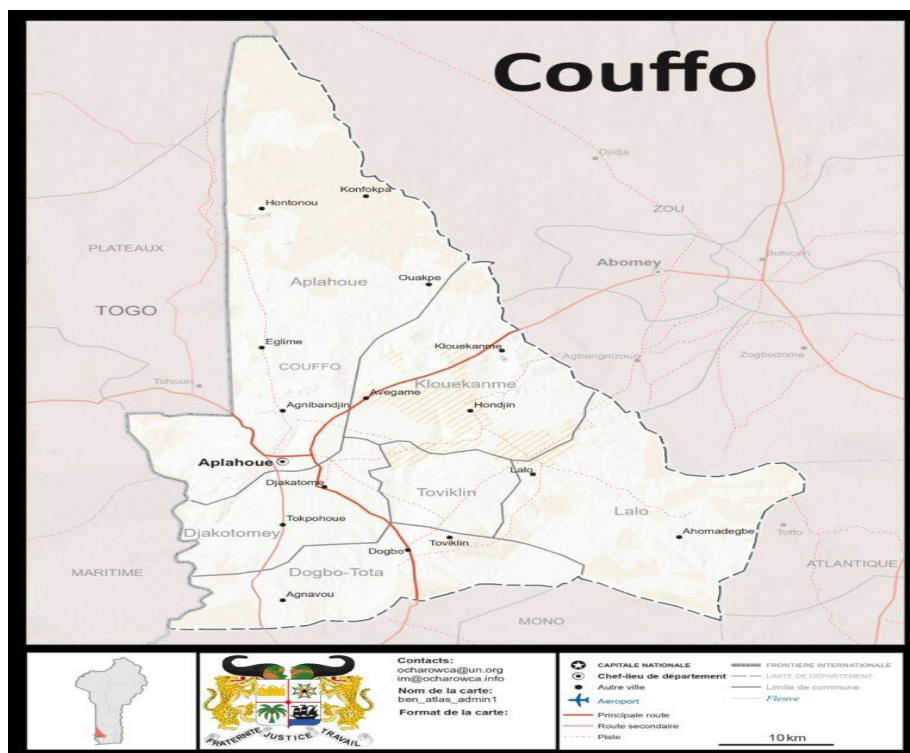


Figure 1: Map of the study area showing Dogbo and Lalo districts.

2.2. Mosquito sample collection

Aedes aegypti populations were collected during the great rainy season from April to July 2024 across Dogbo and Lalo districts selected in South-western Benin. Larvae and pupae were collected in breeding sites using the dipping method. They were then kept in separated labeled bottles related to each locality. The samples were reared up to adult emergence at insectary of Department of Sciences and Agricultural Techniques located in Dogbo district in Normal High School of Technical Teaching (ENSET) of Lokossa. *Anopheles gambiae* Kisumu, a reference susceptible strain was used as a control for the bioassay tests. Susceptibility tests were carried out following World Health Organization (WHO) protocol. All susceptibility tests were conducted in the laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) at 25+/-2°C and 70% to 80% relative humidity.

2.3. Obtaining of blood fed and gravid mosquitoes

After larvae and pupae *Aedes aegypti* mosquitoes were collected in Dogbo and Lalo districts, they were reared up to adult emergence at insectary. The female adult mosquitoes had been mated and given rabbit's blood meal to have blood fed mosquitoes. Then, male and female adult mosquitoes aged 5-7 days old were used in the reproduction to have gravid mosquitoes.

2.4. Obtaining of old female mosquitoes

After larvae and pupae of *Aedes aegypti* mosquitoes were collected in Dogbo and Lalo districts, they were reared up to adult emergence at insectary. Adult mosquitoes were provided with cotton wool moistened with a 10% honey solution until they were 19 days old. On Day 20, they were separated in two batches. The first batch was fed with rabbit's blood meal and susceptibility tests were assessed the same day on blood fed old female mosquitoes. On this same day (Day 20), the second batch containing unfed old female mosquitoes was also used in the assessment of the susceptibility tests.

2.5. Testing insecticide susceptibility

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.^[7] In this study, the insecticide tested was lambda-cyhalothrin (0.05%). We used lambda-cyhalothrin, an insecticide of the same family as permethrin (pyrethroids) which is insecticide used on Long-Lasting Insecticidal Nets, OlysetNets distributed frequently by National Malaria Control Program.

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.^[7]

2.6. Statistical analysis

Stata 12 was used to analysis the data sets gathered from the two districts surveyed to compare for the tested insecticide, the mortality rates of *Anopheles gambiae* populations obtained regarding mosquito sex, physiological status and mosquito age. Data are presented with 95% confidence limits.

3. RESULTS

3.1. Comparison of mosquito susceptibility regarding their sex

The analysis of Table 1 showed that both sexes of *Anopheles gambiae* Kisumu populations were fully susceptible to lambda-cyhalothrin when they were unfed and aged 2-5 days old. Regarding *Aedes aegypti* populations from Dogbo and Lalo, females were more susceptible than males when they were unfed and aged 2-5 days old ($P < 0.05$) (**Table 1**).

Table 1: Comparative susceptibility of *Aedes aegypti* populations aged 2-5 days old regarding their sex.

Populations	Mosquito sex	Physiological status	Number tested	Mortality (%)
Kisumu	Male	Unfed	100	100
	Female	Unfed	100	100
Dogbo	Male	Unfed	100	25
	Female	Unfed	100	53
Lalo	Male	Unfed	100	33
	Female	Unfed	100	61

3.2. Comparison of *Aedes aegypti* mosquito susceptibility regarding their physiological status

The analysis of Table 2 showed on the one hand that female *Anopheles gambiae* Kisumu populations were fully susceptible to lambda-cyhalothrin when they were unfed, blood fed, gravid and aged 2-5 days old. The analysis of Table 2 showed on the second hand that the mortality rates of blood fed *Aedes aegypti* female populations from Dogbo and Lalo aged 2-5 days old were lower than those obtained when these females were unfed ($P < 0.05$). Regarding the mortality rates of gravid females from Dogbo and Lalo, they were lower and significantly different from those obtained when these females were unfed ($P < 0.05$) (**Table 2**).

Table 2: Comparative susceptibility of *Aedes aegypti* populations aged 2-5 days old regarding their physiological status.

Populations	Mosquito sex	Physiological status	Number tested	Mortality (%)
Kisumu	Female	Unfed	100	100
	Female	Blood fed	100	100
	Female	Gravid	100	100
Dangbo	Female	Unfed	100	53
	Female	Blood fed	100	32
	Female	Gravid	100	23
Lalo	Female	Unfed	100	61
	Female	Blood fed	100	43
	Female	Gravid	100	31

3.3. Comparison of mosquito susceptibility regarding their age

The analysis of Table 3 showed on the one hand that female *Anopheles gambiae* Kisumu populations were fully susceptible to lambda-cyhalothrin when they were unfed and aged 2-5 days old and when they were unfed and aged 20 days old. The analysis of Table 3 showed on the second hand that female *Anopheles gambiae* Kisumu populations were fully susceptible to lambda-cyhalothrin when they were blood fed aged 2-5 days old and when they were blood fed and aged 20 days old. The analysis of this table showed that the mortality rates of blood fed and aged 20 days old *Aedes aegypti* female populations from Dogbo and Lalo were higher than those obtained when these populations were blood fed and aged 2-5 days old. A similar pattern was observed in *Aedes aegypti* female populations from Dogbo and Lalo when they were unfed and aged 20 days old comparatively to when they were unfed and aged 2-5 days old (Table 3).

Table 3: Comparative susceptibility of *Aedes aegypti* populations regarding their age.

Populations	Mosquito sex	Physiological status	Mosquito age	Number tested	Mortality (%)
Kisumu	Female	Unfed	2-5 days old	100	100
	Female	Unfed	20 days old	100	100
	Female	Blood fed	2-5 days old	100	100
	Female	Blood fed	20 days old	100	100
Dogbo	Female	Unfed	2-5 days old	100	53
	Female	Unfed	20 days old	100	73
Lalo	Female	Unfed	2-5 days old	100	61
	Female	Unfed	20 days old	100	74
Dogbo	Female	Blood fed	2-5 days old	100	60
	Female	Blood fed	20 days old	100	71
Lalo	Female	Blood fed	2-5 days old	100	55
	Female	Blood fed	20 days old	100	69

4. DISCUSSION

In the current study, *Aedes aegypti* female populations from Dogbo and Lalo were more susceptible than males when they were unfed and aged 2-5 days old. Although males are usually smaller and more fragile than females,^[8] they were not more susceptible than the females. However, it was not recommended to assess susceptibility tests with males because that tends to have higher control mortalities.^[8,9]

In the current study, the mortality rates of blood fed *Aedes aegypti* female populations from Dogbo and Lalo aged 2-5 days old were lower than those obtained when these females were unfed. This result showed that there was no increasing in vector susceptibility to lambda-cyhalothrin after females had taken their blood meals. A similar pattern was observed in *Anopheles funestus*.^[10] In addition, the mortality rates of gravid females from Dogbo and Lalo were

lower and significantly different from those obtained when these females were unfed. This result showed that there was no increasing in vector susceptibility to lambda-cyhalothrin after females possess eggs in their abdomen.

In the current study, the mortality rates of blood fed and aged 20 days old *Aedes aegypti* female populations from Dogbo and Lalo were higher than those obtained when these populations were blood fed and aged 2-5 days old. A similar pattern was observed in *Aedes aegypti* female populations from Dogbo and Lalo when they were unfed and aged 20 days old comparatively to when they were unfed and aged 2-5 days old. These results showed that more mosquitoes were old more they were susceptible to insecticides. According to Chouaibou *et al.*,^[11] 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. A similar pattern was also observed in *Anopheles gambiae* Giles from Zanzibar by Lines and Nassor who showed that the mortality rate rose with age when mosquitoes were old.^[12] Another similar pattern was also observed in *Anopheles funestus* FUMOZ-R from Southern Mozambique.^[13]

A research carried out by Rault *et al.*^[14] had studied the association of age, sex, and pyrethroid resistance status on survival and cytochrome P450 gene expression in *Aedes aegypti* (L.). In fact, their study examined different factors that could influence the interpretation of toxicity bioassays and gene expression studies in *Aedes aegypti*, including sex and age, in the context of resistance to pyrethroids. Bioassays using a pyrethroid-resistant strain, Puerto Rico (PR), and a pyrethroid-susceptible strain, Rockefeller (Rock), of *Aedes aegypti* were conducted with females and males of three age groups to determine differences in mortality induced by deltamethrin. Overall, strain was the only factor with a significant effect on the LD₅₀. Enzyme assays showed that cytochrome P450 monooxygenase activity in PR was constitutively higher than in Rock, and that pretreatment with the cytochrome P450 inhibitor piperonyl butoxide (PBO) followed by a topical application of deltamethrin (LD₂₅) significantly increased mortality in both strains. Evaluation of the expression levels of seven CYP9J genes previously reported to be involved in pyrethroid resistance revealed that CYP9J10, CYP9J19, and CYP9J28 were more highly expressed in PR than in Rock at all ages of females and males, indicating that they may be essential for resistance. The expression of CYP9J24, CYP9J26, CYP9J27, and CYP9J32 was higher in PR males compared to other groups, including PR females. Significant differences in expression between sexes and strains were also observed as a result of age.

5. CONCLUSION

Many factors influence vector susceptibility to insecticide. Among these factors, there are mosquito sex, physiological status and mosquito age. Therefore, it is useful to respect the WHO criteria in the assessment of insecticide susceptibility tests in human disease vectors including the dengue vectors.

Ethical statement

Not applicable

Conflict of Interest

The authors declare that they have no known competing financial interests.

Consent for publication

Not applicable.

Availability of supporting data

The data is made available on request from the authors.

Funding

Not applicable

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