Research Article

Comparative Susceptibility to Deltamethrin of two *Culex Quinquefasciatus* Say Populations from Mono Department in South-Western Benin, regarding Mosquito Sex, Physiological Status and Mosquito Age

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ABSTRACT

Background: *Culex quinquefasciatus* is a vector of viruses that negatively impact human health. The understanding of the factors which influence vector susceptibility to insecticide is important.

Objective: The current study was aimed to compare susceptibility to deltamethrin of two populations of *Culex quinquefasciatus* Say mosquitoes from Mono department in South-western Benin, regarding mosquito sex, physiological status and mosquito age.

Materials & Methods: Larvae and pupae of *Culex quinquefasciatus* mosquitoes were collected from April to July 2024 during the great rainy season in the breeding sites in Mono 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.

Results: The results showed that *Culex quinquefasciatus* male adult were not more susceptible than the females. There was no increasing in vector susceptibility to deltamethrin after females had taken their blood meals. There was no increasing in vector susceptibility to deltamethrin after females possess eggs in their abdomen. These results also showed that more mosquitoes were old more they were susceptible to deltamethrin.

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 vectors of human diseases.

Keywords: Mosquito sex, Physiological status, Mosquito age, Susceptibility, Deltamethrin.

INTRODUCTION

Mosquitoes can transmit more life-threatening diseases such as malaria, dengue, chikungunya, zika, west Nile, yellow fever, encephalitis and filariasis and make life at risk millions of people particularly in subtropical/tropical world[1-2].

The control of mosquito in integrated pest management has been focused on alteration of disease transmission by eliminating the mosquitoes, preventing human contact with the mosquitoes or killing the larvae at their breeding site, though the most effective control measures are those targeted at the larval stages due to their confined habitats[3].

Chemical control is a key strategy for combating arbovirus disease as it addresses both immature stages (eggs, larvae, pupae) and the adult mosquitoes. Personal protection through the use of domestic and commercial insecticides, as well as synthetic repellents, can also have a significant entomological and epidemiological impact[4]. However, the indiscriminate use of chemicals has led to numerous environmental issues, such as the pollution of water, air, and soil, and the development of insect resistance.

To prevent occurrence of mosquito-borne diseases and promote public health, it is necessary to control their population. The use of organochlorine and organophosphorus insecticides is in first line of fighting against mosquitoes, but in recent years, due to high cost of these insecticides, their stability in environment, increase in resistance to insecticides, harmful effects on human health and other non-target populations, they have been used of them is limited[5]. These reasons have prompted researchers to look for alternative methods, especially use of natural substances to fight and reduce the mosquito population, which is compatible with environment.

Very few researches were published on comparative susceptibility to insecticides of *Culex quinquefasciatus* populations from Mono 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 deltamethrin of two *Culex quinquefasciatus* populations from Mono department in South-western Benin, regarding mosquito sex, physiological status and mosquito age.

MATERIALS AND METHODS

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Mono. Mono department is located in the south-western Republic of Benin and the study was carried out in Lokossa and Comè 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. Mono 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 et1100 mm.

Figure 1: Map of Republic of Benin showing the two districts surveyed in Mono department

Mosquito sample collection

Culex quinquefasciatus mosquitoes were collected during the great rainy season from April to July 2024 across Lokossa and Comè 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.

Obtaining of blood fed and gravid mosquitoes

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

Obtaining of old female mosquitoes

After larvae and pupae of *Culex quinquefasciatus* mosquitoes were collected in Lokossa and Comè 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.

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[6]. In this study, the insecticide tested was deltamethrin (0.05%). The choice of deltamethrin was justified by its use on Long-Lasting Insecticidal Nets, Permanets which is 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[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.

RESULTS

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 deltamethrin when they were unfed and aged 2-5 days old. Regarding *Culex quinquefasciatus* Lokossa and Comè populations, females were more susceptible than males when they were unfed and aged 2-5 days old (P <0.05) (Table 1).

Comparison of *Culex quinquefasciatus* 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 deltamethrin 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 *Culex quinquefasciatus* female populations from Lokossa and Comè 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 Lokossa and Comè, they were lower and significantly different from those obtained when these females were unfed (P <0.05) (Table 2).

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 deltamethrin 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 deltamethrin 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 *Culex quinquefasciatus* female populations from Lokossa and Comè were higher than those obtained when these populations were blood fed and aged 2-5 days old. A similar pattern was observed in *Culex quinquefasciatus* female populations from Lokossa and Comè when they were unfed and aged 20 days old comparatively to when they were unfed and aged 2-5 days old.

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

Populations Mosquito sex	Physiological status	Number tested	Mortality (%)
Kisumu Male	Unfed	100	100
(Control) Female	Unfed	100	100
Lokossa Male	Unfed	100	15
Female	Unfed	100	36
Comè Male	Unfed	100	13
Female	Unfed	100	44

Table 2: *Culex quinquefasciatus* mosquito susceptibility regarding their physiological status

Populations Mosquito sex	Physiological status	Number tested	Mortality (%)
Kisumu Female	Unfed	100	100
(Control) Female	Blood fed	100	100
Female	Gravid	100	100
Lokossa Female	Unfed	100	36
Female	Blood fed	100	17
Female	Gravid	100	22
Comè Female	Unfed	100	44
Female	Blood fed	100	21
Female	Gravid	100	27

Table 3: Mosquito susceptibility regarding their age

Populations	Mosquito sex	Physiological status	Mosquito age	Number tested	Mortality (%)
Kisumu	Female	Unfed	2-5 days old	100	100
(Control)	Female	Unfed	20 days old	100	100
	Female	Blood fed	2-5 days old	100	100
	Female	Blood fed	20 days old	100	100
Lokossa	Female	Unfed	2-5 days old	100	36
	Female	Unfed	20 days old	100	51
Comè	Female	Unfed	2-5 days old	100	44
	Female	Unfed	20 days old	100	63
Lokossa	Female	Blood fed	2-5 days old	100	52
	Female	Blood fed	20 days old	100	61
Comè	Female	Blood fed	2-5 days old	100	33
	Female	Blood fed	20 days old	100	49

DISCUSSION

In the current study, females *Culex quinquefasciatus* populations from Lokossa and Comè 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[7], 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[7-8].

In the current study, the mortality rates of blood fed *Culex quinquefasciatus* female populations from Lokossa and Comè 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 deltamethrin after females had taken their blood meals. A similar pattern was observed in *Anopheles funestus*[9]. In addition, the mortality rates of gravid females from Lokossa and Comè 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 deltamethrin after females possess eggs in their abdomen.

In the current study, the mortality rates of blood fed and aged 20 days old *Culex quinquefasciatus* female populations from Lokossa and Comè were higher than those obtained when these populations were blood fed and aged 2-5 days old. A similar pattern was observed in *Culex quinquefasciatus* female populations from Lokossa and Comè 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*[10], 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[11]. Another similar pattern was also observed in *Anopheles funestus* FUMOZ-R from Southern Mozambique[12].

A research carried out by Tsecouras *et al*[13] had studied the effect of sex and age on survival of adult *Culex tarsalis* from a susceptible laboratory strain exposed to permethrin in the CDC bottle bioassay. Permethrin is an insecticide of same class as deltamethrin used in the current study. In addition, *Culex* tarsalis is a specie of same genus as *Culex quinquefasciatus* Say mosquitoes. In their study, they investigated the effect of mosquito sex and age on the survival and resistance determination of adult *Culex tarsalis* exposed to permethrin, a pyrethroid commonly used for mosquito control, using the Centers for Disease Control and Prevention (CDC) bottle bioassay method. A permethrin-susceptible strain of *Culex tarsalis* (Bakersfield strain) was used in this study. Survival was compared for young adult females (2-5 days old) relative to older adult females (7-10 days old) and separately for female and male mosquitoes of the same age (2-5 days old). Mortality was slightly higher for males than females during the first observation period (0-5 min) following permethrin exposure, and higher for older females relative to younger females from 5 to 10 min following permethrin exposure, with no differences in mortality by either sex or age for observation periods during the remainder of the diagnostic period. When evaluated over the full diagnostic period, survival varied with mosquito age but not sex. However, all mosquitoes, regardless of sex or age, died within the 30-min diagnostic period for this species, confirming their permethrin susceptibility per the CDC bottle bioassay. This research

contributes valuable insight into the potential impact of sex and age on mosquito susceptibility to insecticides in the context of insecticide resistance determination.

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 vectors of human diseases.

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