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Physico-chemical Parameters in Breeding Sites of *Culex Quinquefasciatus* Conferring Larvicide Tolerance in Dogbo District in South-Western Republic of Benin, West Africa

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Abstract Mosquito larva tolerance to larvicides is widely spread across Africa region and there is a need to search for contributing factors. This study aims to establish the correlation between the presence of physico-chemical parameters in breeding sites of *Culex quinquefasciatus* and the larva tolerance to larvicides. The study was carried out in November 2021 during the small rainy season to collect information about the visual characteristics of *Culex quinquefasciatus* breeding sites present in Dogbo district. Then, the measurements of physico-chemical parameters in breeding sites were done in laboratory. The results showed that different *Culex quinquefasciatus* breeding sites were found in this study. They were: the sump and the ditch. Both were semipermanent stagnant and polluted water. The relationships between physico-chemical parameters and mosquito larva tolerance to larvicides were evaluated. The breeding sites with higher physico-chemical parameters such as: hydrogen potential and total dissolve solids were considered polluted and conferring larvicide tolerance to *Culex quinquefasciatus*. However, there was a negative correlation between some parameters such as salinity and nitrites and *Culex quinquefasciatus* larva tolerance to larvicides. The breeding sites of *Culex quinquefasciatus* were influenced by physico-chemical parameters such as temperature, hydrogen potential, conductivity and total dissolve solids of the water body. There is a relationship between the presence of physico-chemical parameters in *Culex quinquefasciatus* breeding sites and the tolerance of these mosquito larvae to larvicides.

Keywords Physico-chemical Parameters, *Culex Quinquefasciatus*, Larval Tolerance, Benin

Introduction

Culex species and particularly mosquitoes of the *Culex pipiens* complex, are considered vectors of several diseases such as Lymphatic filariasis, West Nile Virus, Japanese Encephalitis, Saint Louis Encephalitis, Dengue and Rift Valley Fever with some being fatal in the absence of treatment and others causing lifelong disabilities and impairment [1]. In addition to their role as vectors, *Culex* species are also responsible for a high nuisance problem [2-3]. Indicatively *Culex* mosquitoes exhibit high biting rates exceeding 100 bites/person/night [2;4]. The *Culex pipiens* complex member *Culex quinquefasciatus* is a prominent vector species which feeds on both humans and animals [5-6], increasing its implication in pathogen transmission to both host groups.

Despite a growing interest in the promotion of integrated vector control strategies co-targeting different vector species, control efforts and relative entomological, epidemiological and insecticide resistance studies primarily focus on anophelines resulting in important knowledge gaps regarding *Culex* species and their control.

There is an accelerated and disorganized process of urbanization in the last decades, especially in the tropical, low income countries. Additionally, in well-developed regions, the density of the mosquitoes may be positively correlated with seasonal high temperatures [7-8]. In addition, very recently, Aïzoun and Assongba [9] showed the temephos tolerance in larvae of mosquitoes, vectors of malaria and in *Culex quinquefasciatus* in Dogbo district in south-western of Benin republic. Therefore, there is a need to search for the physico-chemical parameters of the breeding sites that confer tolerance in mosquito vectors.

Very few researches were published on the measurements of physico-chemical parameters in breeding sites of *Culex quinquefasciatus* conferring larvicide tolerance to this mosquito vector in Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to measure the physico-chemical parameters in breeding sites of *Culex quinquefasciatus* conferring larvicide tolerance to this mosquito vector in Dogbo district in South-western Republic of Benin.

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.1). The southern borders of this district are Lokossa and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km^2 and belongs to geographic region of ADJA. 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. We took these factors into account to measure the physico-chemical parameters of breeding sites of *Culex quinquefasciatus* conferring larvicide tolerance in Dogbo district in Department of Couffo in South-western Republic of 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.

Visual characteristics of breeding sites

The study was carried out in November 2021 during the small rainy season to collect information about the visual characteristics of *Culex quinquefasciatus* breeding sites present in Dogbo district. Photos of breeding sites were taken during our survey.

Obtaining of the water of breeding sites of *Culex quinquefasciatus*

Water was taken from breeding sites using the dipping method [10] and kept in labeled bottles. Then, it was carried out to the Laboratory of Hygiene-Sanitation Ecotoxicology Environment Health (HECOTES) of the Interfaculty Center of Training and Research in Environment for Sustainable Development (CIFRED) for the measurements of the physico-chemical characteristics.

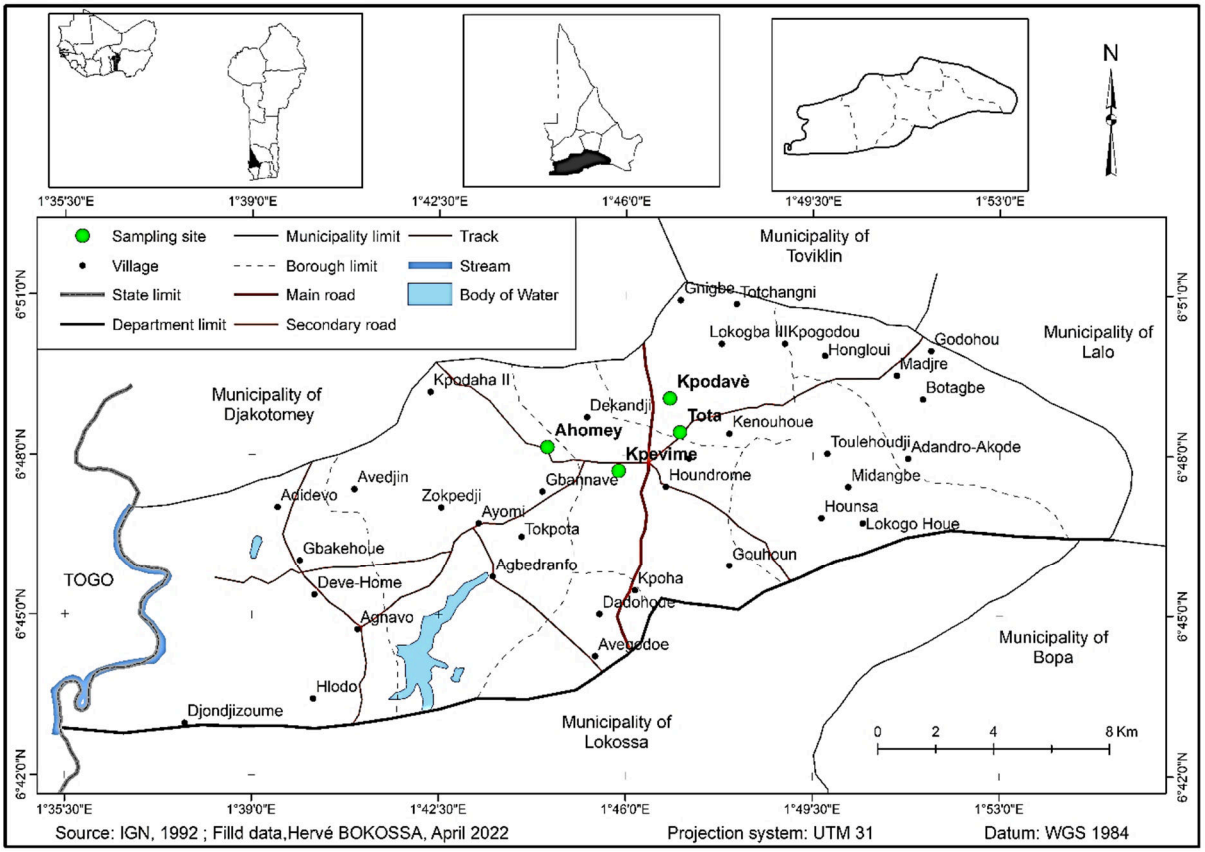

Figure 1: Map of Dogbo District showing sampling points of Culex quinquefasciatus breeding sites in Republic of Benin

Measurements of physico-chemical parameters in breeding sites of *Culex quinquefasciatus*

The measurements of physico-chemical parameters in breeding sites were done. Parameters measured were temperature, hydrogen potential, conductivity, total dissolve solids, salinity and nitrites. Temperature, pH, TDS and salinity were determined by electrochemical method by using HI 991001, pH meter, HACH session 5, conductivity meter while nitrites contents were measured using spectrophotometer DR 6000. These parameters were measured in distilled water as control and compared to those measured in water taken from breeding sites surveyed.

Statistical analysis

The physico-chemical parameters were tested with univariate analysis. *P* values <0.05 were considered significant. All these analyses were carried using the software R.

Results and Discussion

Characteristics of the different breeding sites of *Culex quinquefasciatus*

The different *Culex quinquefasciatus* breeding sites found during our survey where water of breeding sites was taken for measurements of physico-chemical parameters are: the sump and the ditch (Fig. 2 and Fig. 3).

Figure 2: A sump as breeding site of Culex quinquefasciatus larvae surveyed in KPEVIME location in Dogbo district

Figure 3: A ditch as breeding site of Culex quinquefasciatus larvae surveyed in KPODAVE location in Dogbo district

Measurements of physico-chemical parameters in breeding site waters.

The relationships between physico-chemical parameters and mosquito larva tolerance to larvicides were evaluated. The analysis of table 1 showed that all the values of hydrogen potential obtained were superiors to 7 (the mean was 7.48) except that of breeding site water taken near of a muck-heap in TOTA location. But, this value was slightly inferior to 7. The same remark was made with the values of total dissolve solids which were very superior to that of the distilled water (control). Regarding the values of temperature in the different breeding site waters, they were nearly uniform in all breeding site waters and ranged from 27.1 to 27.6°C (the mean was 27.35) comparatively to that of the distilled water (control) which was 25°C. All the values of conductivity were very high (the mean was 476.67) ($P < 0.05$) whereas all the values of salinity and nitrites recorded in the current study were very low. The mean was 0.225 for salinity and 1.5 for nitrites.

Table 1: Recording of the values of physico-chemical parameters

Parameters	Unities	Symbols	Used Methods	Obtained values				
				1	2	3	4	Distilled water (Control)
Temperature	°C	T	Electrometric	27.6	27.3	27.4	27.1	25
Hydrogen Potential	-	pH	Potentiometric	8.4	7.14	7.60	6.79	6.5
Conductivity	µS/cm	C	Electrometric	304	369	77.7	1156	nd
Total Dissolve Solids	mg/L	TDS	Electrometric	146.3	177.9	36.7	570	0.7
Salinitty	%	-	Electrometric	0.1	0.2	0.0	0.6	0.0
Nitrites	mg /L	NO_2^-	By diazotation	0	0	4	2	0.1

nd = no determined; 1 = *Culex quinquefasciatus* breeding site water taken under a bridge in AHOMEY location ; 2 = *Culex quinquefasciatus* breeding site water taken in a ditch in KPODAVE location ; 3 = *Culex quinquefasciatus* breeding site water taken in a sump in KPEVIME location ; 4 = *Culex quinquefasciatus* breeding site water taken near of a muck-heap in TOTA location ; 5 = Distilled water

Different *Culex quinquefasciatus* breeding sites were found during our survey where water of breeding sites was taken for measurements of physico-chemical parameters. They were: the sump and the ditch. Both were semipermanent stagnant and polluted water. In fact, *Culex quinquefasciatus* mosquitoes are predominant in most cities across Sub-Saharan Africa and they are of major epidemiological significance as vectors of important diseases like West Nile Virus and filariasis. This vector displays a variety of breeding habitats including swamps, drains, pit latrin and permanent or semipermanent stagnant water bodies full of organic matters [11-12], commonly found within and around African cities. Notably, the rapid unplanned urbanization of major cities in Africa, has favoured the installation of *Culex quinquefasciatus* within the urban environment [2;13]. Although *Culex* spp. females preferentially lay their eggs in collections of water, either stagnant or gentle flow, rich in organic matter, *Cx. quinquefasciatus* is very opportunistic so that any permanent or temporary collection of water may serve as a potential breading site for their larvae [14-15]. Therefore, vector control planning has to focus on breeding site elimination or treatment by improving the basic sanitary infrastructure of water supply and waste destination, as well as activities to promote community engagement within an environmental agenda. As *Culex* mosquitoes are usually found in sympatry with anophelines in sub-Saharan Africa, it is possible that local *Culex* populations have also received high insecticide selection pressures [15-16], driving the development of resistance in important vector species.

The environmental chemical compounds and various environmental xenobiotics present in high levels in some breeding sites contributed to the development of larvicide tolerance in mosquito species. But, some agrochemicals other than pesticides, such as fertilizers also play an important role. The multiple insecticide resistance mechanisms observed in *Cx. quinquefasciatus* populations by Corbel *et al.* [17] in Benin suggest high selective pressure taking place in urban environment. In fact, in the urban area of Cotonou (Ladji and Asecna) in southern Benin, the *Culex*

quinquefasciatus exhibited high *kdr* frequency and elevated levels of esterases and GST activity (5 to 7 fold higher than in SLAB) with high frequencies of resistance to permethrin, DDT and carbosulfan.

In addition to insecticides, xenobiotics could also induce high resistance in this mosquito species due to its preference for organically polluted habitats at the larval stage [18]. Several xenobiotics, such as, heavy metals, petroleum products, regularly influence species biological responses and probably affect vectors susceptibility to insecticides.

In the current study, the analysis of the results obtained regarding the measurements of physico-chemical parameters present in breeding sites of *Culex quinquefasciatus* showed that all the values of hydrogen potential obtained were superiors to 7 except that of breeding site water taken near of a muck-heap in TOTA location. But, this value was slightly inferior to 7. The same remark was made with the values of total dissolve solids which were very superior to that of the distilled water (control). The breeding sites with higher physico-chemical parameters were considered polluted. Similar results were reported by Emidi *et al.* [19]. In their study, these authors had shown that mosquito can breed suitably in a water body whose pH is above 7. In the current study, regarding the values of temperature in the different breeding site waters, they were nearly uniform in all the breeding site waters comparatively to that of the distilled water (control). A moderately high temperature is said to be suitable to facilitate the growth and development of mosquito larva. In the current study, the values of conductivity of the different breeding sites were higher. So, *culex quinquefasciatus* could tolerate high concentrations of this parameter. All the values of salinity and nitrites recorded in the current study were very low. So, there was a negative correlation between these parameters and *culex quinquefasciatus* larvae tolerance to larvicides.

Conclusion

The current study clearly described the *Culex quinquefasciatus* mosquito larva breeding sites including their visual description but also the knowledge of physico-chemical parameters which could guarantee a better classification of breeding sites and therefore contributed to their management, treatment or destroying. The breeding characteristics were influenced by physico-chemical parameters such as temperature, hydrogen potential, conductivity, total dissolve solids of the water body. There is a relationship between the presence of **physico-chemical** parameters in *Culex quinquefasciatus* breeding sites and the tolerance of these mosquito larvae to larvicides.

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Conflicts of interest

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

Contributions from authors

Each author contributed equitably

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