



# Sensitivity of *Culex quinquefasciatus* to Insecticides Used in Brazzaville (Republic of Congo)

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**How to cite this paper:** Nianga Bikouta, G.O.T., Bitsindou, P., Belle Mbou Okassa, M., Dibangou, V. and Lenga, A. (2025) Sensitivity of *Culex quinquefasciatus* to Insecticides Used in Brazzaville (Republic of Congo). *Open Access Library Journal*, 12: e13921. <https://doi.org/10.4236/oalib.1113921>

**Received:** July 7, 2025

**Accepted:** September 20, 2025

**Published:** September 23, 2025

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## Abstract

*Culex quinquefasciatus* is a major source of culicidian nuisance in the city of Brazzaville. To fight against this nuisance, the populations use different means of control, in particular the insecticide sprays sold on the market. This study was carried out in August and October 2016, and in January 2017. The objective was to assess the sensitivity of *Culex quinquefasciatus* to insecticides used in public health in order to adopt effective control strategies against this mosquito. *Culex quinquefasciatus* larvae bred to obtain adult mosquitoes were collected from different waste water collections in the city. Susceptibility tests were carried out according to the WHO protocol (1998) with *Culex* females aged 2 to 5 days. The insecticides used were DDT 4%, permethrin 0.75%, deltamethrin 0.05%, carbosulfan 0.6%, bendiocarb 0.1%, fenitrothion 1%, lambdacyhalothrin 0.05%, malathion 5% and cyfluthrin 0.15%. Susceptibility tests showed high levels of resistance to all insecticides used. *Culex quinquefasciatus* being resistant to these insecticides, the identification of the mechanisms that support this resistance is necessary to put in place an effective strategic plan.

## Subject Areas

Entomology

## Keywords

*Culex quinquefasciatus*, Wastewater, Insecticides, Resistance, Congo

## 1. Introduction

*Culex quinquefasciatus* belongs to the *Culex pipiens* Linnaeus complex. It is one of the subspecies of this complex found in Africa [1] [2]. This species is the vector of several diseases such as *Japanese encephalitis*, *West Nile infection*, *Wuchereria bancrofti lymphatic filariasis*, *Rift Valley fever* and *Ross River Fever* [3]-[7]. In addition to being a vector of these diseases, this species is a culicidal nuisance in urban environments. It thrives in various water collectors such as septic tanks, open sumps and blocked sewage drains [8]-[10].

Brazzaville, like other large African cities, has a very inefficient wastewater collection system [11]. Throughout the city, we regularly see clogged wastewater collectors, stagnant wastewater and open cesspools. These various water collectors are veritable breeding grounds for the proliferation of *Culex quinquefasciatus* in Brazzaville. This proliferation means that the rate of aggressiveness attributed to *Culex quinquefasciatus* in Moundali, Poto-poto and Ouenze, Brazzaville's densely populated neighborhoods, can reach 500 bites/man/night [12] [13]. To protect themselves from the bites of *Culex quinquefasciatus*, populations use various means of protection, such as mosquito nets, smoke coils and insecticide sprays. The aim of this study was to assess the susceptibility of *Culex quinquefasciatus* to insecticides belonging to four families (organochlorines, organophosphates, pyrethroids and carbamates) used in public health, with a view to adopting effective control strategies against this species in Brazzaville.

## 2. Material and Methods

### 2.1. Study Site

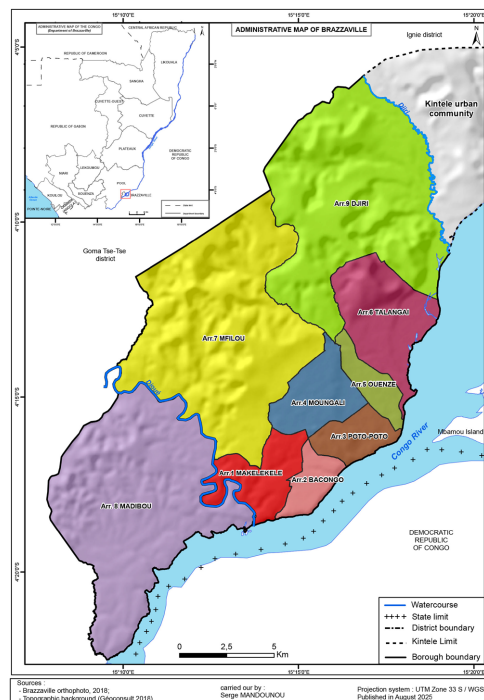


Figure 1. Map of brazzaville (source: geoconsult 2018).

The study was conducted in Brazzaville, the political capital of the Republic of Congo. The city is located on the right bank of the river of the same name. It covers an area of 100 km<sup>2</sup> and its geographical coordinates are 4°15'6"S and 15°15'11"E. **Figure 1** represents the city of Brazzaville and the sites where the data were collected [14] [15]. The city is subdivided into 9 districts, with an equatorial climate characterized by a dry season from June to September and a rainy season from October to May. It is watered by numerous tributaries from the Plateaux Batékés that flow into the Congo River, and its vegetation, and that of the surrounding area, is made up of forests and savannahs [16]. The population of Brazzaville is estimated at 1,408,150 [17].

## 2.2. Larvae Collection

The adult mosquitoes used in this study were derived from larvae and nymphs collected in the city of Brazzaville. These larvae and nymphs were collected in clogged wastewater drainage pipes in August and October 2016 and January 2017. Field-collected larvae and pupae were reared to obtain females of the same age for susceptibility testing. The larvae were kept in the insectarium at a temperature of 27°C ± 2°C and a relative humidity of 70% ± 10%. They were fed Comipex® powdered fish feed. The adults, which emerged from the larvae, were placed in the rearing cages and fed with 10% sugar water.

## 2.3. Insecticide Sensibility Tests

Susceptibility tests were performed on fasting females aged 2 to 5 days, according to the WHO protocol [18]. Tests were carried out under an ambient temperature of 25°C ± 2°C and a relative humidity of 70% ± 10% RH. For each insecticide, four replicates of 25 females were exposed to insecticide-impregnated papers. And one replicate of 25 females was exposed to non-insecticide-impregnated paper. Tests were carried out with eight insecticides: deltamethrin 0.05%, cyfluthrin 0.15%, permethrin 0.75%, lambdacyhathrin 0.05%, DDT 4%, bendiocarb 0.1%, fenitrothion 1% and malathion 5%.

During the 60-minute insecticide exposure period, the knock-down effect was recorded every 5 minutes. At the end of this period, females were transferred to observation tubes. Mortality was then assessed 24 hours after exposure to the insecticide [19].

## 2.4. Validity Criteria for Sensitivity Tests

Population susceptibility has been classified according to WHO criteria, along with the associated tests. The knock-down effect of the insecticide was assessed during the 1-hour exposure period, and its lethal effect at 24 hours post-exposure. Adult mortality rates were corrected using Abbott's formula [20] if mortality was greater than 5%, but less than 20% in control tubes. Population status was defined by mortality rate: resistance was confirmed if mortality was 98% [19].

## 2.5. Statistical Data Processing

In this study, a two-factor analysis of variance (ANOVA) was performed, using SPSS software version 26.0 [21], to evaluate the efficacy of 7 types of insecticide in the natural environment. Kd times were determined using the Log-time Probit model from the Dose Effect Function package on XLSTAT 2020 software. *Culex* sensitivity was assessed by quantifying mortality rates to the various insecticides using Fisher's exact statistical test (when the number of individuals tested was less than 100), Pearson Chi2 test (when the number of individuals tested was 100) and Student Newman-Keuls test. A probability value of p less than or equal to 0.05 was considered significant.

## 3. Results

The results of the sensitivity tests showed that the *Cx. quinquefasciatus* populations tested had high levels of resistance to the insecticides used (Table 1). Kd times and mortality rates varied according to insecticide, with significant differences of 50% and 95% recorded ( $P < 0.001$ ).

Kd 50% times ranged from 34 to 2000 minutes, and Kd 95% times from 66 to 3800 minutes (Table 1). The lowest Kd 50% time (34 min) was recorded with bendiocarb 0.1% at almost half the insecticide exposure time. And the highest (2000 min) with fenitrothion 0.1% beyond the duration of exposure to this insecticide.

The lowest Kd 95% time (66 min) was recorded with bendiocarb 0.1% 6 minutes after the insecticide exposure time. And the highest (3800 min) with fenitrothion 0.1% beyond the duration of exposure to this insecticide.

**Table 1.** Presentation of KD50% and KD95% times for the different insecticides of *Cx. quinquefasciatus* used in Brazzaville in August, October 2016 and January 2017.

Chemical families	Insecticides	Month and Year	N	KD <sub>50%</sub> (min)	KD <sub>95%</sub> (min)
organochlorines	DDT 4%	August 2016	100	500 ± 1.03 d	950 ± 0.13 g
	cylfluthrin (0.15%)	August 2016	100	375 ± 1.81 c	713 ± 0.22 ef
pyrethroids	deltamethrin (0.05%)	August 2016	100	103 ± 0.99 ab	197 ± 1.09 b
	permethrin (0.75%)	August 2016	100	286 ± 0.05 bc	542 ± 1.01 d
	lamdacyhathrin (0.05%)	August 2016	100	250 ± 1.34 b	475 ± 0.46 d
carbamates	bendiocarb (0.1%)	October 2016	100	34 ± 2.01 a	66 ± 0.96 a
organophosphates	fenitrothion (1%)	January 2017	100	2000 ± 0.4 h	3800 ± 2.07 j
	malathion (5%)	August 2016	100	80 ± 2.36 a	154 ± 1.22 b

KdT<sub>50</sub> and KdT<sub>95</sub>: 50% and 95% knockdown times (in minutes); 95% CI: 95% confidence interval.

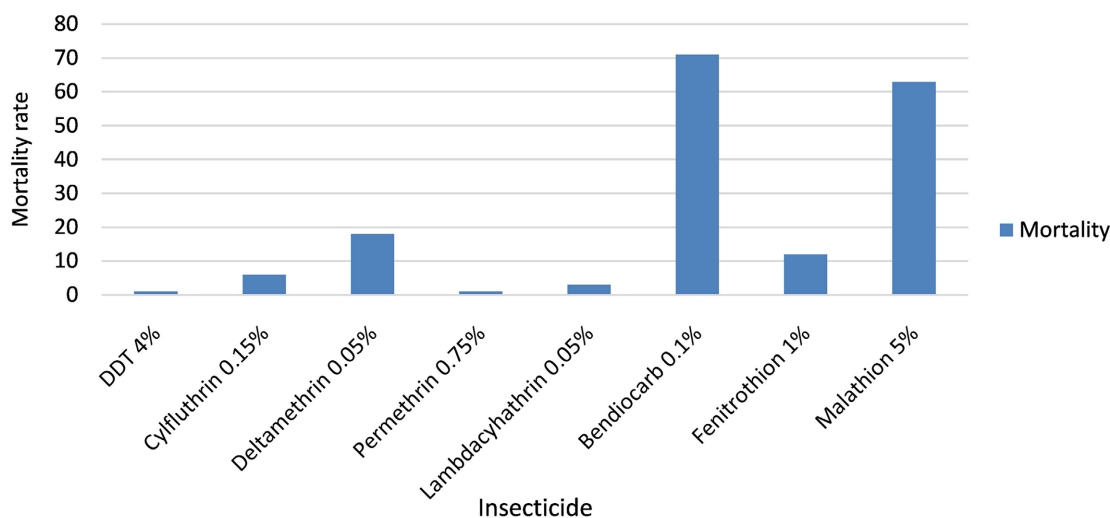
Mortality rates at 24 h ranged from 1% to 71% (from DDT 4% to bendiocarb 0.1%). Analysis of mortality rates for the different insecticides showed significant

differences ( $P < 0.05$ ) in mortality rate and insecticide used (Table 2 and Figure 2). The lowest mortality rates were recorded with DDT 4% and permethrin 0.75%. The highest mortality rate was recorded with bendiocarb (0.1%). Analysis of variance showed no significant difference in mortality rates between the insecticides cyfluthrin (0.15%), permethrin (0.75%) and bendiocarb (0.1%) ( $P < 0.05$ ).

**Table 2.** Presentation of mortality rates of *Cx. quinquefasciatus* 24 hours after exposure to insecticides used in Brazzaville in August, October 2016 and January 2017.

Chemical families	Insecticides	Month and Year	N	Mortality %	Results
organochlorines	DDT 4%	August 2016	100	1 ± 0.02 a	R
	cyfluthrin (0.15%)	August 2016	100	6 ± 0.4 a	R
pyrethroids	deltamethrin (0.05%)	August 2016	100	18 ± 0.11 b	R
	permethrin (0.75%)	August 2016	100	1 ± 0.2 a	R
	lamdacyhathrin (0.05%)	August 2016	100	3 ± 0.09 a	R
carbamates	bendiocarb (0.1%)	October 2016	100	71 ± 0.41 f	R
organophosphates	fenitrothion (1%)	January 2017	100	12 ± 0.07 b	R
	malathion (5%)	August 2016	100	63 ± 0.66 ef	R

Mortality: Figures with different letters in the column are significantly different at the  $P < 0.05$  threshold according to the Student Newman-Keuls test. R: Resistant.



**Figure 2.** Mortality rate of *Cx. quinquefasciatus* to the different insecticides used in Brazzaville in August, October 2016 and January 2017.

#### 4. Discussion

The present study reports the level of sensitivity of *Cx. quinquefasciatus* to the insecticides tested. According to WHO standards, *Cx. quinquefasciatus* showed variability in its sensitivity to insecticides, depending on the type used. Very high resistance was observed to DDT, permethrin, deltamethrin, cyfluthrin, deltamethrin and lamdacyhathrin, with mortality rates below 13%. And moderate re-

sistance to bendiocarb and malathion, with mortality rates above 50% (71% and 63% respectively).

Similar results have been obtained in other countries where resistance of *Cx. quinquefasciatus* to several insecticides has been demonstrated [22]-[30].

This resistance has been attributed to 5 main factors. Firstly, the adaptation of *Cx. quinquefasciatus* to its living environment, which is extremely polluted in the city. In fact, its habitat is wastewater, characterized by a multi-faceted pollution of all kinds of physical or chemical matter (e.g. dissolved detergents, insecticide residues, organic matter). In the context of this pollution, a study carried out in the city of Constantine, Algeria, showed that mosquitoes of the *Culex* genus develop best in breeding grounds rich in organic matter and ammonium [31]. The development of these species in these habitats may be supported by biochemical or genetic mechanisms that enable them to live in these environments. These mechanisms may also enable resistance to xenobiotics, including insecticides, in order to resist insecticides. These larvae were collected in clogged drains, during both seasons; in the dry season (in August) and in the rainy season (in October 2016 and January 2017). This type of larval site had no impact on larval density during these two seasons. Resistance mechanisms were not identified, and it is therefore essential to identify these mechanisms, as it is possible that there is a relationship between the type of larval nest and the development of resistance.

The use of insecticide impregnated mosquito nets can be mentioned as a second factor. In 2019, for example, 3,325,355 mosquito nets impregnated with permethrin (pyrethroids) were distributed nationwide. The massive use of these impregnated nets has exerted selection pressure on culicid populations. Resistant populations were selected to the detriment of susceptible populations. This may have led to an increase in the frequency of pyrethroid resistance. This hypothesis has been supported by other authors, who have demonstrated that the massive use of impregnated mosquito nets was a determining factor in the emergence of pyrethroid resistance [26] [32]. As pyrethroids and DDT have the same mode of action, the resistance observed with DDT may be cross-resistance [9] [18] [23] [29] [33].

Finally, the largest quantity of insecticides used worldwide is for agricultural purposes. For example, in 2007, 404,000 tonnes of insecticides were applied worldwide. The quantity of insecticide used for agricultural purposes represented 98.6%. To combat crop pests, horticulturalists use pesticides which they mix with water for irrigation. When these waters or rainwater run-off are used to water plants, the pesticides mix with and contaminate the water in the larval breeding grounds [34]. This contamination can exert a constant and regular selection pressure on the larval *Culex* populations living in these breeding sites. This situation may explain the emergence of insecticide resistance in *Cx. Quinquefasciatus*. The molecules used in vector control are very often the same as those used against crop pests. In addition, other xenobiotics such as herbicides, nematocides and fungicides are used in agriculture. These can modulate the detoxification system of *Cx.*

*quinquefasciatus* and thus increase its tolerance to insecticides.

The use of insecticides by private individuals, in the form of aerosol sprays, coils and wafers, to control pests (flies, Culicidae, cockroaches, spiders, etc.) could also have a significant impact. Culicidae are nocturnal and bite mainly in the evening, so their contact with insecticides is frequent. The consequence is a probable selection of populations to the detriment of susceptible populations. This makes these populations resistant to insecticides.

## 5. Conclusion

The results of this study showed that *Culex quinquefasciatus* is resistant to all the insecticides tested. The populations studied showed high resistance to DDT, permethrin, deltamethrin, cyfluthrin, deltamethrin and lamdacyathrin. On the other hand, resistance to malathion and bendiocarb was considered moderate. This resistance was essentially attributed to the use of insecticide-impregnated mosquito nets. Indeed, these represent a major pillar of vector control, mainly targeting malaria vectors. This situation is likely to slow down the effectiveness of these nets, as people lose interest in using them, believing them to be ineffective. As *Cx. quinquefasciatus* is an opportunistic species, it thrives in man-made habitats such as permanent or temporary water collections. As a result, the sanitation of living environments and the observance of hygiene rules by the community are essential in the fight against *Cx. quinquefasciatus*.

## Conflicts of Interest

The authors declare no conflicts of interest.

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