

## Susceptibility of Mosquito Vectors to Insecticides: A Focus on Malaria and Arbovirus Transmission in Mouila, Gabon

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### **Abstract**

#### **Keywords:**

*Susceptibility, Anopheles, Culex, insecticides, Mouila, Gabon*

**Objective:** To assess the susceptibility status of the culicid fauna in Mouila and its environs (southwest Gabon).

**Methods:** This study was carried out in August 2017 and focused on insecticides recommended for public health that contain DDT (4%), deltamethrin (0.05%), permethrin (0.75%) and bendiocarb (0.1%). The *Anopheles* and *Culex* larvae were collected from different breeding sites of the oil palm plantations and neighboring villages, in the Mouila area. All larvae were transported to the laboratory and reared to the adult stage. The susceptibility tests were performed on the emerging adult mosquitoes using unfed adult females aged 2 to 4 days old. These mosquitoes were exposed to insecticides according to WHO standard protocol.

**Results:** Mosquitoes showed a susceptibility to carbamates (bendiocarb; mortality=100%) and a resistance to organochlorines (DDT; mortality=89%) and pyrethroids (deltamethrin and permethrin; mortalities=71% and 79%). Moreover, the susceptibility tests carried out on *Anopheles* mosquitoes revealed a resistance to pyrethroids (deltamethrin and permethrin) and organochlorines (DDT), with the respective mortality rates of 76%, 9 % and 7%. Populations of both *Culex* and *Anopheles* adult mosquitoes were resistant to the tested insecticides that contain DDT and pyrethroids. The resistance level of *Anopheles* mosquitoes to permethrin and DDT were very high compared with those of *Culex* mosquitoes.

**Conclusion:** The results of these analyses will allow a better understanding of the resistance mechanisms developed by these insect vectors in the Mouila region. Molecular analyses of the resistant specimens are in process to determine the genes involved in the resistance to these insecticides

### **Introduction**

Mosquitoes are responsible for transmission of numerous pathogenic organisms and transmit many vector-borne diseases such as malaria, chikungunya, yellow fever, filariasis, encephalitis, Rift valley fever, dengue, Zika virus and West Nile virus infections (Koua, 1994 ; WHO, 2004 ; Bkhache, 2016 ; Labbé *et al.*, 2017). These illnesses constitute an important global public health issue (Johnston *et al.*, 2014 ; Ferraguti *et al.*, 2016), with high morbidity and mortality, particularly in tropical regions (Koudou *et al.*, 2005 ; Ferraguti *et al.*, 2016 ; Labbé *et al.*, 2017). The prevention of these diseases is partly based on the control of the insect vectors populations (N'Guessan *et al.*, 2007 ; Djogbenou, 2009; Akono *et al.*, 2017). Vector populations are effectively controlled by removing potential breeding sites from the environment, introducing predators, and the use of long-lasting insecticide-treated bed-nets (LLINs) and indoor residual spraying (IRS) (Carnevale *et al.*, 2009). By contrast, vector control using chemical insecticides can be ineffective, particularly if mosquito vectors develop resistance (Faye *et al.*, 1991 ; Kant *et al.*, 2013 ; El Ouali *et al.*, 2014 ; Labbé *et al.*, 2017). Indeed, according to several authors (Gariou & Mouchet, 1961 ; Curtis, 2001 ; Hemingway *et al.*, 2002 ; Tia *et al.*, 2017), the ignorance of the resistance status of mosquito populations to insecticides can compromise the success of vector control interventions. Consequently, it is essential to know the susceptibility level of target vector populations to different insecticides (Manirakiza *et al.*, 2003 ; Konan *et al.*, 2011 ; Toto *et al.*, 2011), before starting vector control programs like indoor residual sprayings, which can be expensive and difficult to implement (Tia *et al.*, 2006 ; Toto *et al.*, 2011).

In Gabon, infections transmitted by mosquitoes constitute an important source of disease. Indeed, in 2010, about 45% of children and 71% of pregnant women were hospitalized because of serious forms of malaria (PNLP, 2010). To combat vector-borne diseases, Gabon adopted a vector control strategy focused on the distribution of long-lasting insecticide-treated mosquito bed-nets and indoor residual spraying. However, the effectiveness of these interventions relies on proper knowledge of vector resistance to the insecticides that are used. Nevertheless, in this tropical Africa country, mosquito susceptibility to insecticides remains poorly characterized (Pinto *et al.*, 2006, Mourou *et al.*, 2010). The existing studies of insecticide resistance in Gabon concern only the cities of Libreville and Port-Gentil (Pinto *et al.*, 2006, Mourou *et al.*, 2010). For other cities and regions, this information is almost non-existent. In this study, we investigate the susceptibility of two important mosquito vectors to three insecticides in the oil palm exploitation sites in Mouila and its surroundings.

## Materials and methods

### Study sites

This study was conducted in August 2017 in the oil palm exploitation sites in Mouila (southwest Gabon), specifically at sites in Mboukou and Moutassou, as well as the neighbouring villages (Figure 1).

The Mboukou site is the palm plantations zone called « lot 1 ». It is located in the department of Tsamba-Magotsi about 35 kilometers from the city of Mouila. It is bounded in the east by the Ngounié River and the villages of Saint-Martin and Migabe, then in the west by Douya, Doubou, Mboukou and Rembo villages (Ecosphère, 2011; PNLP, 2017). This industrial site covers nearly 35 300 hectares and is geographically located between 1°39'06" South and 10°49'42.6" East (Burton *et al.*, 2016; PNLP, 2017).

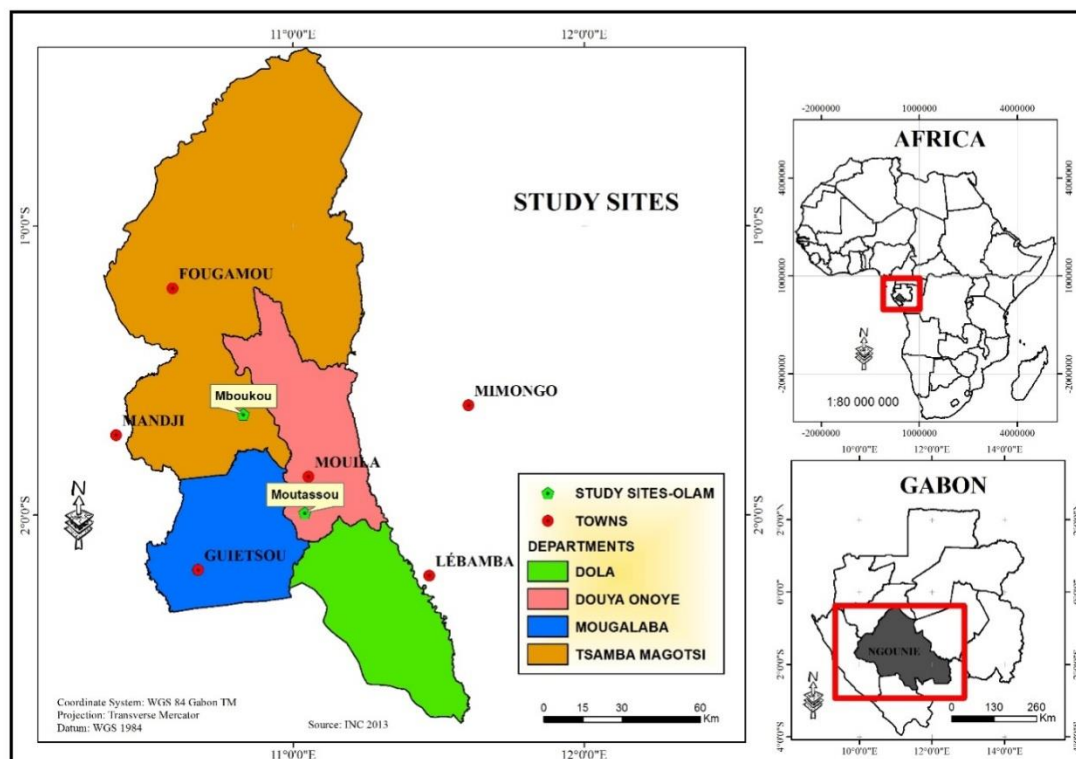


Figure 1: Geographical location of the study sites

The Moutassou site constitutes another oil palm plantation zone called "lot 3". This agro-industrial site covers 23 780 hectares and is located in the department of Douya-Onoye about 13 kilometers from the town of Mouila (Ecosphère, 2014; PNLP, 2017). This site is geographically located between 1°59'33.8" S and 11°02'25.2" E (PNLP, 2017). It is bounded in the north by the town of Mouila, in the east by the Mouladoufouala, Mbengui and Mbadi villages and to the West by the Moutassou, Koumbanou, Ikolo-Ikolo and Digabosse villages (Ecosphère, 2014). It is

an ecosystem dominated by the savannah-forest mosaics with savannahs occupying about 75% of the exploitation permit (Ecosphere 2014; PNLP 2017).

### **Collection and rearing of mosquito larvae**

*Anopheles gambiae* s.l. and *Culex* sp. larvae were collected from nine sampling stations selected from the study area. The surveyed villages were Mboukou village and Moutambe Sane Fomou village. In addition, the surveyed sampling stations were Doubou camp, Ngounie camp, PK19, Mboukou camp, Moutassou camp and Mavassa camp. Sampling stations were chosen based on their ecological profile and the structuring of their landscape. The collection of immature stages was performed using the "dipping" method (Service, 1989; Coffinet *et al.*, 2009; Bakwo-Fils *et al.*, 2010; Talipouo *et al.*, 2017) and standard dippers (300 mL). The larvae were put in trays and transported in coolers to the laboratory (Akono *et al.*, 2010). In the laboratory, the captured larvae were selected, counted and put in the rearing-trays containing the water from the larval habitat and covered with the tulle from a mosquito net. These larvae were then fed every two days with aquarium fish feed. Each day, new pupae were separated from the larvae and transferred to the rearing cages until the emergence as adults. The adults were fed on 8% sugar solution soaked in cotton wool before being subjected to the susceptibility test.

### **Insecticide susceptibility assays**

Susceptibility tests were performed on *Anopheles* and *Culex* mosquitoes from Mboukou and Moutassou sites and surrounding areas. All *Anopheles* and *Culex* obtained within 30 km radius of the study sites were grouped together for susceptibility tests because of the small numbers of adult mosquitoes obtained during this study (dry season). Thus, the tests were conducted on two-to four-day-old non-blood fed female adult mosquitoes from different larval breeding sites of study sites and reared to adult stage in the laboratory. We used the standard WHO susceptibility test protocol and mortality rates calculated after 24 h (WHO, 1998; WHO, 2013; Viana-Medeiros *et al.*, 2017). A susceptible strain of *An. gambiae* (Kisumu) was used as reference strain for the bioassays (Djogbenou *et al.*, 2011). Insecticide-impregnated test papers with the WHO diagnostic dosages were supplied by the Universiti Sains Malaysia, Penang. Test papers were impregnated with pyrethroids (0.05% deltamethrin and 0.75% permethrin), carbamates (0.1% bendiocarb) and organochlorines (4.0% DDT). For each insecticide, five tubes were prepared plus a tube for control. Twenty-five randomly selected female mosquitoes were used at 26–29 °C and 74–82% relative humidity. The knockdown effect of insecticides on the mosquitoes were observed firstly for every 5 min the first 20 min and then every 10 min till the total time was an hour to obtain the knockdown effect (KD). Thereafter, mosquitoes were observed for 24 h with a piece of cotton soaked with sugar solution (8%) on the grille of the cork to feed the mosquitoes. The percentage of female mosquitoes that died after the 24 h was recorded as the mortality rate for each insecticide followed WHO standards.

All *Anopheles* and *Culex* mosquitoes (dead and alive) from each site were conditioned and stored in 1.5 ml Eppendorf tubes containing silicagel. We used only *Anopheles* samples for molecular identification (Fanello *et al.*, 2002; Djogbenou *et al.*, 2011). Specific identification of *Anopheles* were done with identification key (Baldacchino & Paupy, 2010) and by PCR using the technique of Fanello *et al.* (2002).

### **Data analysis**

The susceptibility level of the tested mosquitoes was evaluated using mortality observation in accordance with WHO's interpretation criteria (WHO, 2013). The tests were validated with mortality rates in mosquito control trials of less than 5%. When mortality rates in the control tubes were between 5% and 20%, rates were corrected by applying the Abbott's formula (Abbott, 1925).

The knock-down times or the necessary time so that 50% ( $KdT_{50}$ ) and 95% ( $KdT_{95}$ ) of mosquitoes are knocked down after one hour of contact with an insecticide, were estimated from the regression line of knock-down effect or with the Probit analysis software (Djogbenou *et al.*, 2011; Akono *et al.*, 2017). The comparison tests of Fisher's Least Significant Difference were performed at the 5% threshold using SYSTAT 5.0 software.

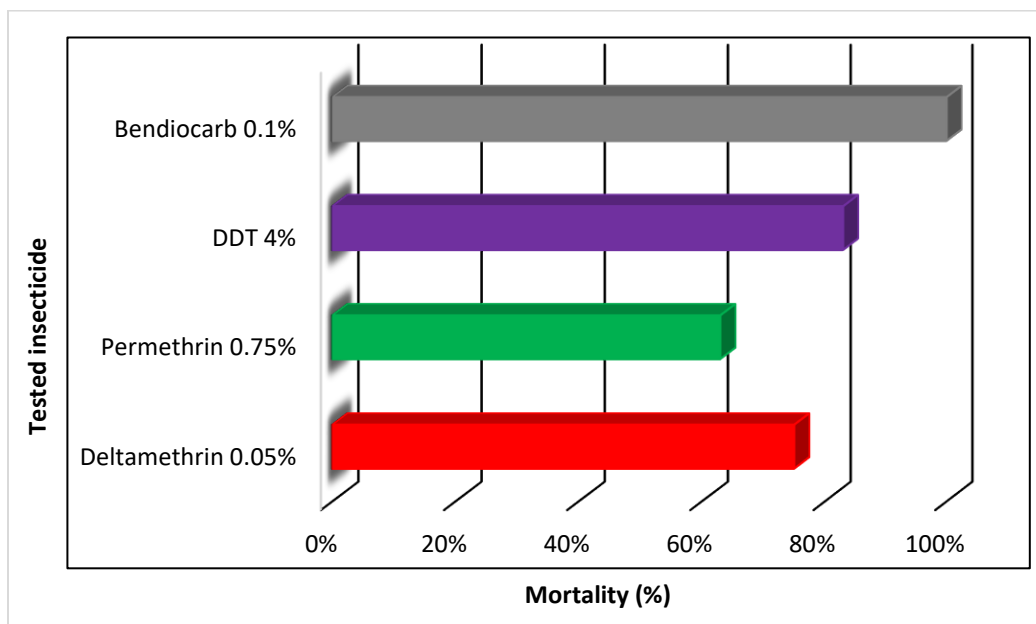
## **Results**

### **Susceptibility of *Culex* sp. mosquitoes to DDT, permethrin, deltamethrin and bendiocarb**

The analysis of Table 1 and Figure 2 shows a clear susceptibility of *Culex* sp. mosquitoes to bendiocarb (carbamates), with 100% mortality rate. However, they were resistant to insecticides of the pyrethroids (deltamethrin and permethrin) and organochlorines classes (DDT).

**Table 1. Results of *Culex* sp. mosquitoes susceptibility test in the study sites**

Origin	Insecticide used	Control mortality	Corrected mortality	Results
F0	Deltamethrin 0.05%	6%	75%	Resistant
F0	Permethrin 0.75%	6%	63%	Resistant
F0	DDT 4%	5%	85%	Resistant
F0	Bendiocarb 0.1%	0%	100%	Susceptible

**Figure 2: Mortality rate of adult *Culex* sp. from study sites, 24 h after exposure to deltamethrin, permethrin, DDT and bendiocarb.****Susceptibility of adult *Anopheles gambiae* s.l. to DDT, permethrin and deltamethrin**

The results of the susceptibility test (Table 2 and Figure 3a) show that *Anophelesgambiae* s.l. obtained from the larvae collected from the larval habitats of the study sites were resistant to the tested insecticides.

**Table 2. Results of the susceptibility tests of adult *Anopheles gambiae* s.l from the study sites**

Origin	Testedinsecticide	Controlmortality	Correctedmortality	Results
F0	Deltamethrin 0.05%	13%	75%	Resistant
F0	Permethrin 0.75%	13%	24%	Resistant
F0	DDT 4%	7%	3%	Resistant

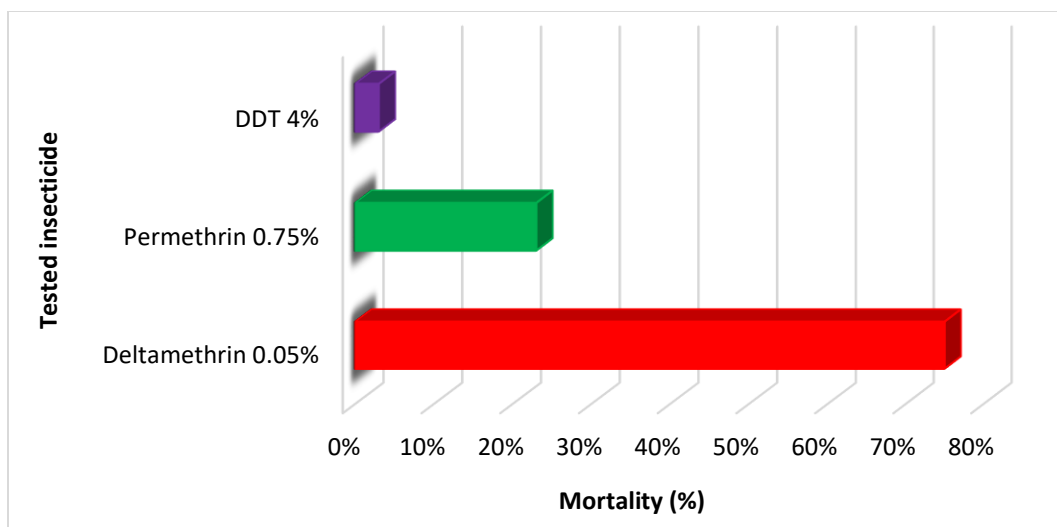


Figure 3a: Mortality rates of adult *Anopheles gambiae* s.l. from the study sites, 24 h after exposure to deltamethrin, permethrin and DDT

#### Comparison of the insecticide susceptibility level of adult *Anopheles gambiae* s.l. with adult *Culex* sp

Figure 3b shows the susceptibility of adult *Anopheles gambiae* s.l. compared with adult *Culex* sp. obtained from larvae collected from Mouila, 24 h after exposure to DDT, deltamethrin and permethrin. The results obtained show that populations of these both groups of mosquitoes were resistant to the tested insecticides according to WHO interpretation criteria. *Anopheles gambiae* s.l. populations had higher levels of resistance to permethrin and DDT compared than *Culex* sp. This difference was not significant with deltamethrin.

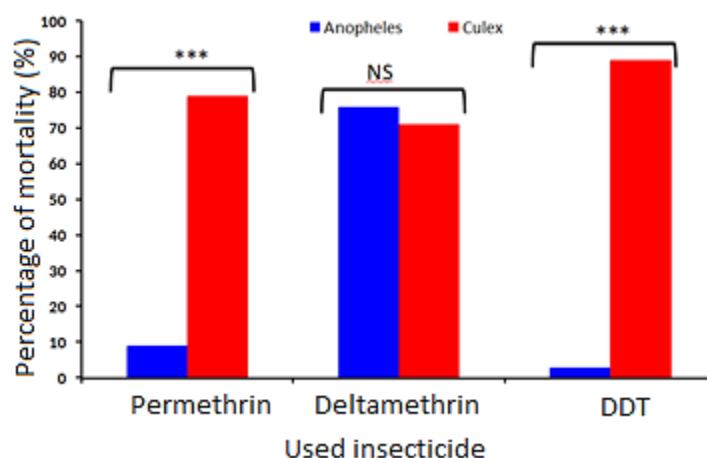


Figure 3b: Comparison of *Anopheles gambiae* s.l. susceptibility to that of *Culex* sp. from the study sites of Mouila, 24 h after exposure to DDT, deltamethrin and permethrin.

#### Change in Knockdown (KD) effect of mosquitoes (*Anopheles gambiae* s.l. and *Culex* sp.) after DDT exposure

Figure 4 summarizes the rate of mosquito knockdown with respect to the time of exposure to DDT insecticide. These results show that although KD was low for both mosquito groups, it was relatively higher in *Culex* sp. This was related to the low KD effect of DDT.

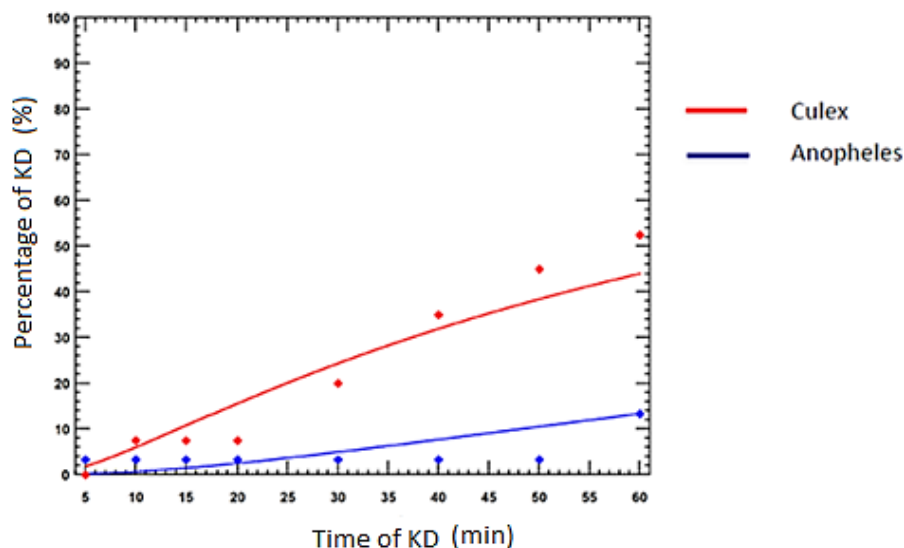


Figure 4 : Change in percentage of knocked down mosquitoes (*Anopheles gambiae* s.l. and *Culex* sp.) for 60 minutes after exposure to DDT

#### Change in Knockdown (KD) effect of mosquitoes after permethrin exposure

The change in percentage of knocked down mosquitoes with respect to time of permethrin exposure is represented in Figure 5. These results show that KD remained higher in *Culex* sp. after permethrin exposure while remaining relatively low in *Anopheles gambiae* s.l.

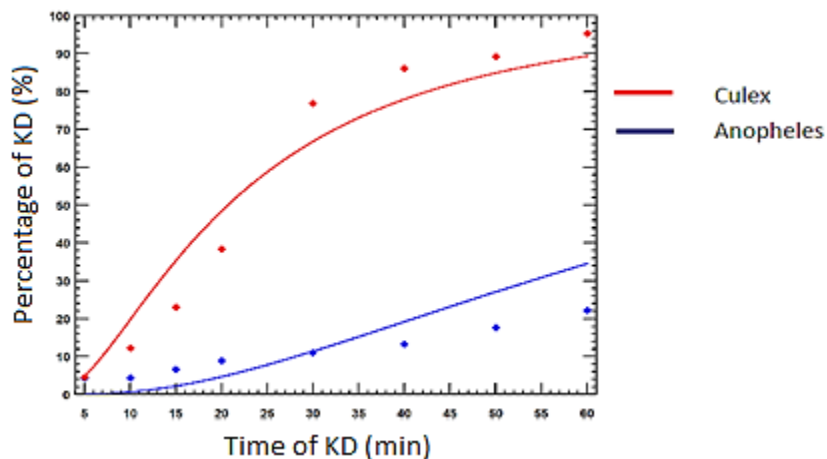


Figure 5: Change in percentage of knocked down mosquitoes (*Anopheles gambiae* s.l. and *Culex* sp.) for 60 minutes after permethrin exposure

#### Change in Knockdown effect of mosquitoes after deltamethrin exposure

The curves of Figure 6 reflect the changing rate of mosquito knockdown over time after exposure to 0.05% deltamethrin. The effects were very similar for each group, with each curve showing a similar shape. The level of KD resistance to deltamethrin was almost the same for both groups of mosquitoes (*Anopheles gambiae* s.l. and *Culex* sp.).

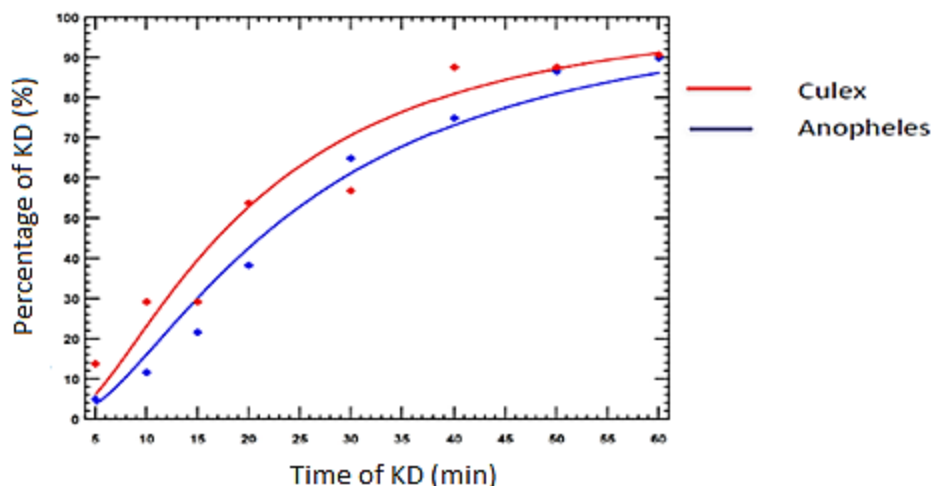


Figure 6: Change in percentage of knocked-down mosquitoes (*Anopheles gambiae* s.l. and *Culex* sp.) for 60 minutes after deltamethrin exposure

#### Evolution of $KdT_{50}$ and $KdT_{95}$ according to mosquito genera

In the presence of these insecticides,  $KdT_{95}$  remained higher than 62 minutes for *Culex* sp. and less than 82 minutes for *An. gambiae* s.l. females. Indeed, the highest  $KdT_{95}$  were observed with DDT followed by deltamethrin and permethrin (Table 3, Figures 4, 5 & 6). In addition, for *Culex* sp.,  $KdT_{50}$  remained below 60 minutes in the presence of 4% DDT (59.2 minutes) (Figure 4), 0.75% permethrin (21.2 minutes) and 0.05% deltamethrin (18.5 minutes). In tested *An. gambiae* s.l.  $KdT_{50}$  remained below 60 minutes only in the presence of deltamethrin (23.6 minutes, Table 3).  $KdT_{50}$  for permethrin and DDT could not be estimated for *Anopheles gambiae* s.l. because of the low number of mosquitoes knocked down after 60 minutes of exposure (less than 10%).

Table 3. Time of Knock-down ( $KdT_{50}$  and  $KdT_{95}$ ) with DDT, permethrin and deltamethrin of *Anopheles gambiae* s.l. and *Culex* sp. populations for study sites in Mouila

Taxa	DDT 4%				Permethrin 0.75%				Deltamethrin 0.05%			
	$KdT_{50}$ (min)	95% CI	$KdT_{95}$ (min)	95% CI	$KdT_{50}$ (min)	95% CI	$KdT_{95}$ (min)	95% CI	$KdT_{50}$ (min)	95% CI	$KdT_{95}$ (min)	95% CI
<i>Anopheles gambiae</i> s.l.	ND		ND		ND		ND		23.6	(21.3-26.1)	81.3	(67.5-104)
<i>Culex</i> sp.	59.2	(48.5 - 80.6)	286.6	(172.6 - 708.7)	21.2	(18.2 - 24.5)	62.3	(49.7 - 87.3)	18.5	(14.1-23.3)	90.8	(60.7-188.3)

ND = When the knock-down effect is less than 10% after 60 minutes of exposure; CI= Interval of confidence at 95%;  $KdT$ = Knock-down Time.

#### Specific identification of the tested anopheles mosquitoes

The molecular identification of mosquitoes in the *Anopheles* genus showed that all the tested specimens were *An. gambiae* s.s. (85/85= 100%). No mosquitoes belonging to *An. coluzzii* or *An. arabiensis* species were found among the tested specimens.

#### Discussion

The study of the culicid fauna susceptibility in the Mouila region revealed that *Anopheles gambiae* s.s and *Culex* populations developed resistance to DDT (4%), deltamethrin (0.05%) and permethrin (0.75%). The resistance to



carbamates and pyrethroids is not surprising, since several studies have reported others cases in sub-saharan Africa (Faye *et al.*, 1991; Chandre *et al.*, 1999; Awolola *et al.*, 2005; Djogbenou *et al.*, 2007; N'guessan *et al.*, 2007; Djouaka *et al.*, 2008; Corbel *et al.*, 2004; Dabiré *et al.*, 2008; Betson *et al.*, 2009; Djogbenou *et al.*, 2011; Toto *et al.*, 2011). According to Akogbéto *et al.* (2005), some populations of *An. gambiae* lay their eggs in larval habitats contaminated by the insecticide residues. This is explained by excessive agricultural insecticide use (Koudou *et al.*, 2005). In Gambia, DDT residues were found in the soil samples (Manirakiza *et al.*, 2003). It is therefore possible that the use of DDT and pyrethroids in the past has encouraged selection for resistance in *Anopheles gambiae* populations over time. Therefore, this may explain the significant decrease of susceptibility observed during this first evaluation of insecticide susceptibility at Mouila in 2017. This resistance manifested by adult stages (F0) from immature stages that are permanently vulnerable to selection pressures of the resistance, due to the high use of many insecticides in the oil palm plantations and workers' camps.

The results of Fisher's tests indicated that the mosquitoes of the *Anopheles* genus seem to present a higher resistance to Permethrin 0.75% and DDT 4% compared to those of the *Culex* genus, which, although resistant to these insecticides, have higher mortality rates. These results corroborate those obtained by Fofana *et al.* (2010), which showed a slight susceptibility of *Culex* species to permethrin 0.75%. However, this difference of susceptibility between *Anopheles gambiae* s.l. and *Culex* sp. is not significant for deltamethrin 0.05%.

The knockdown (KD) rates recorded in our study are relatively higher for deltamethrin than DDT or permethrin. The reduction of this KD effect in comparison with other insecticides leads us to suspect the existence of resistance genes of kdr type for DDT and pyrethroids. These results are similar to those obtained by Mourou *et al.* (2010), who found resistance genes Kdr-w and Kdr-e in mosquitoes from the cities of Libreville and Port-Gentil. In addition, the rate of knockdown for DDT (4%) was low for both genera of mosquitoes (*Anopheles* and *Culex*). This is in accordance with the resistance level of *Anopheles gambiae* s.l. and *Culex* sp. to DDT in our study zone. These results are also consistent with those observed by Toto *et al.* (2011) in Angola and Fofana *et al.* (2010) in Ivory Coast. These authors showed that mosquitoes of *Culex* and *Anopheles* genera are resistant to DDT. The resistance of mosquitoes in the Mouila region to pyrethroids and organochlorines insecticide classes may have a negative impact on control efforts against these vectors (Namountougou *et al.*, 2012).

The molecular analyzes performed on the tested mosquitoes revealed the exclusive presence of *An. gambiae* s.s. and the absence of specimens of other malaria vector species in Gabon (*An. coluzzii* and *An. arabiensis*) in the samples. This result could be related to the low number of breeding sites identified during this study. This could also be related to the period of larval habitats surveys and test realization.

## Conclusion

The present work revealed the current resistance status of the culicid fauna of the Mouila region to insecticides. Our study indicated that adult mosquitoes (*Anopheles* and *Culex*) are resistant to permethrin 0.75%, deltamethrin 0.05% and DDT 4%. Moreover, all *Anopheles* identified by molecular biology tools belong only to *Anopheles gambiae* s.s. species. These results show the need to take into account the insecticide susceptibility of vectors in this study zone when planning the vector control strategy. The data from this study can be used as a basis for the monitoring program of malaria and arbovirolosis vectors susceptibility at the local level. The resistance of mosquito populations to insecticides used for the public health may be impede insect control efforts that use insecticide-treated mosquito nets. Consequently, it is essential to implement a monitoring system of mosquito populations and their susceptibility to insecticides to understand the evolution of resistance mechanisms in these mosquitoes.

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## References

1. Koua Kouakou H, Mise en évidence de l'activité larvicide de *Persea americana* Miller, 1768 (*Lauraceae*) sur *Anopheles gambiae* Gilles, 1902 (*Diptera, Culicidae*) un moustique d'importance médicale. Thèse de doctorat 3<sup>ème</sup> cycle, Université d'Abidjan, Abidjan, Côte d'Ivoire, pp. 1-133, 1994.
2. WHO. Global Strategic Framework for Integrated Vector Management. World Health Organization, Geneva, 2004.
3. Bkhache M, Tmimi FZ, Charafeddine O, Faraj C, Failloux AB, Sari M. First report of L1014F-kdr mutation in *Culex pipiens* complex from Morocco. *Parasites & Vectors*, vol. 9, pp. 644-654, 2016.
4. Labbé P, David JP, Alout H, Milesi P, Djogbenou L, Pasteur N, Weill M. Evolution of resistance to insecticide in disease vectors. *Genetics and Evolution of Infectious Diseases*, pp. 313-339, 2017.
5. Johnston E, Weinstein P, Slaney D, Flies AS, Fricker S, Williams C. Mosquito communities with trap height and urban-rural gradient in Adelaide, South Australia: implications for disease vector surveillance. *Journal of Vector Ecology*, vol. 39 (1), pp. 48-55, 2014.
6. Ferraguti M, De la Puente JM, Roiz D, Ruiz S, Soriguer R, Figuerola J. Effects of landscape anthropization on mosquito community composition and abundance. *Scientific Reports*, vol. 6, 29002; doi: 10.1038/srep29002, pp. 1-9, 2016.
7. Koudou BG, Yao T, Doumbia M, Nsanzabana C, Cissé G, Girardin O, Dao D, N'goran EK, Vounatsou P, Bordmann G, Keiser J, Tanner M, Utzinger J. Malaria transmission dynamics in Central Côte d'Ivoire: the influence of changing patterns of irrigated rice agriculture. *Medical Veterinary Entomology*, vol. 18, pp. 1-11, 2005.
8. N'Guessan R, Corbel V, Akogbéto M, Rowland M. Reduced efficacy of insecticide treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerging Infectious Diseases*, vol. 13, pp. 199-206, 2007.
9. Djogbenou L. Lutte antivectorielle contre le paludisme et résistance des vecteurs aux insecticides en Afrique. *Médecine Tropicale*, vol. 69 (2), pp. 160-164, 2009.
10. Akono Ntonga P, Tonga C, Mbida Mbida JA, Ndo C, Awono Ambene P, Lehman LG, Ngaha R, Ngo Hondt OE, Peka Nsangou MF, Tedongmo Lopedji N. Transmission du paludisme et sensibilité aux insecticides de la faune culicidienne agressive (Deido, Douala, Cameroun). *Médecine et Santé Tropicales*, vol. 27 (1), pp. 82-89, 2017.
11. Carnevale P, Robert V, Manguin S, Corbel V, Fontenille D, Garros C, Rogier C. Les Anophèles : Biologie, transmission du Plasmodium et lutte antivectorielle. IRD éditions, Collections Didactiques, pp. 1-402, 2009.
12. Faye O, Gaye O, Diallo S. Evaluation of the sensitivity of *Anopheles gambiae* to fenitrothion, malathion and DDT in Senegal. *Dakar Medical*, vol. 36, pp. 170-177, 1991.
13. Kant R, Haq S, Srivastava HC, Sharma VP. Review of the bioenvironmental methods for malaria control with special reference to the use of larvivorous fishes and composite fish culture in central Gujarat, India. *Journal of Vector Borne Disease*, vol. 50, pp. 1-12, 2013.
14. El Ouali Lalami A, El-Akhal F, El Amri N, Maniar S, Faraj C. Etat de la résistance du moustique *Culex pipiens* vis-à-vis du téméphos au centre du Maroc. *Bulletin de la Société de Pathologie Exotique*, vol. 107, pp. 194-198, 2014. DOI 10.1007/s13149-014-0361-x.
15. Gariou J, Mouchet J. Apparition d'une souche d'*Anopheles gambiae* résistante à la dieldrine dans la zone de la campagne antipaludique du Sud-Cameroun. *Bulletin de la Société de Pathologie Exotique Filiales*, vol. 54, pp. 870-875, 1961.
16. Curtis CF. Insecticide resistance and mosquito-borne disease. *Lancet*, vol. 357(9257) : 656, 2001.
17. Hemingway J, Field L, Vontas J. An overview of insecticide resistance. *Science*, vol. 298 (5591): 96-107, 2002.
18. Tia E, Chouaibou M, Gbalégba CNG, Boby AMO, Koné M, Kadjo A. Distribution des espèces et de la fréquence du gène Kdr chez les populations d'*Anopheles gambiae* s.s. et d'*Anopheles coluzzii* dans cinq

- sites agricoles de la Côte d'Ivoire. Bulletin de la Société de Pathologie Exotique, vol. 110, pp. 130-134, 2017.
19. Manirakiza P, Akinbamijo O, Covaci A, Pitonzo R, Schepens P. Assessment of organochlorine pesticide residues in West Africa city farms: Banjul and Dakar case study. Archives of Environmental Contamination and Toxicology, vol. 44, pp. 171-179, 2003.
  20. Konan KG, Koné AB, Konan YL, Fofana D, Konan KL, Diallo A, Ziogba JC, Touré M, Kouassi KP, Doannio JMC. Résistance d'*Anopheles gambiae* s.l. aux pyréthrinoides et au DDT à Tiassalékro, village de riziculture irriguée en zone sud forestière de Côte-d'Ivoire. Bulletin de la Société de Pathologie Exotique, vol. 104, pp. 303-306, 2011. DOI 10.1007/s13149-011-0176-y.
  21. Toto J. C., Besnard P., Le Mire J., Almeida D. S. I., Dos Santos M. A., Fortes F., Foumane V., Simard F., Awono-Ambene H. P. & Carnevale P. Premiers tests OMS d'évaluation de la sensibilité aux insecticides chez *Anopheles gambiae* et *Culex quinquefasciatus* à Lobito, Angola. Bulletin de la Société de Pathologie Exotique, 104:307-312, 2011. DOI 10.1007/s13149-010-0125-1.
  22. Tia E, Akogbéto M, Koffi A, Toure M, Adja AM, Moussa K, Yao T, Carvenale P, Chandre F. Situation de la résistance d'*Anopheles gambiae* s.s. (Diptera : Culicidae) aux pyréthrinoides et au DDT dans cinq écosystèmes agricoles de Côte d'Ivoire. Bulletin de la Société de Pathologie Exotique, vol. 99 (4), pp. 278–282, 2006. [http://www.pathexo.fr/documents/articles-bull/T99-4-2877-5p.pdf].
  23. PNLP. Statistique épidémiologique du Gabon, Rapport d'activités. Programme National de Lutte contre le Paludisme, Gabon, pp. 1-30, 2010.
  24. Pinto J, Lynd A, Elissa N, Donnelly MJ, Costa C, Gentile G, Caccone A, Do Rosario VE. Co-occurrence of East and West kdr mutations suggests high levels of resistance to pyrethroid insecticides in *Anopheles gambiae* from Libreville, Gabon. Medical and Veterinary Entomology, vol. 20, pp. 27-32, 2006.
  25. Mourou JR, Coffinet T, Jarjaval F, Pradines B, Amalvict R, Rogier C, Kombila M, Pagès F. Malaria transmission and insecticide resistance of *Anopheles gambiae* in Libreville and Port-Gentil, Gabon. Malaria Journal, vol. 9 (32), pp. 1-8, 2010.
  26. Ecosphère. Etude d'Impact Environnemental relative à l'implantation d'une palmeraie dans la zone de Mouila (Lot 1). Rapport d'EIES, Olam Palm Gabon, pp. 1-300, 2011.
  27. PNLP. Etude entomologique en prélude à la Pulvérisation Intra-Domiciliaire dans les base-vies et bureaux d'Olam Palm Gabon à Mouila, Rapport d'étude. Programme National de Lutte contre le Paludisme, Gabon, pp. 1-79, 2017.
  28. Burton MEH, Poulsen JR, Lee ME, Medjibe VP, Stewart CG, Venkataraman A, White LJT. Reducing Carbon Emissions from Forest Conversion for Oil Palm Agriculture in Gabon. Conservation Letters, vol. 0 (0), pp. 1-11, 2016.
  29. Ecosphère. Pré-évaluation de la zone du projet d'aménagement d'une palmeraie à Mouila Lot 3. Rapport de mission, Olam Palm Gabon, pp. 1-30, 2014.
  30. Service MW. Irrigation/boom or bane? In: "Service M.W. Demography and vector-borne diseases". CRC Press (eds) Boca Raton, pp. 283–301, 1989.
  31. Coffinet T, Rogier C, Pages F. Evaluation de l'agressivité des anophèles et du risque de transmission du paludisme : méthodes utilisées dans les Armées françaises. Medecine Tropicale, vol. 69, pp. 109-122, 2009.
  32. Bakwo-Fils EM, Ntonga Akono P, Belong P, Messi J. Impact des aménagements piscicoles sur le pullulement culicidien à Yaoundé, Cameroun. Entomologie Faunistique-Faunistic Entomology, vol. 62 (3), pp. 109-114, 2010.
  33. Talipouo A, Ntonga Akono P, Tagne D, Mbida Mbida A, Etang J, Tchoffo Fobasso R, Ekoko W, Binyang J, Dongmo A. Comparative study of *Culicidae* biodiversity of Manoka island and Youpwe mainland area, Littoral, Cameroon. International Journal of Biosciences, vol. 10 (4), pp. 9-18, 2017.
  34. Akono Ntonga P, Bakwo EM, Belong P, Kekeunou S, Foko G, Messi J. Abondance et diversité de la faune culicidienne à Yaoundé, Cameroun. Entomologie Faunistique-Faunistic Entomology, vol. 62 (3), pp. 115-124, 2010.
  35. WHO. Tests Procedures for Insecticide Resistance Monitoring in Malaria Vectors, Bio efficacy and Persistence of Insecticides on Treated Surfaces. Report of the WHO Informal Consultation, WHO/MAL/98. Geneva. World Health Organization, pp. 1-43, 1998.
  36. WHO. Test procedures for Insecticide Resistance Monitoring in malaria vector mosquitoes, Report of the WHO, WHO Library Cataloguing-in-Publication Data, Geneva. World Health Organization, pp. 1-40, 2013.

37. Viana-Medeiros PF, Bellinato DF, Martins AJ, Valle D. Insecticide resistance, associated mechanisms and fitness aspects in two Brazilian *Stegomyia aegypti* (*Aedes aegypti*) populations. Medical and Veterinary Entomology, pp. 1-11, 2017.
38. Djogbenou L, Pasteur N, Akogbeto M, Weill M, Chandre F. Insecticide resistance in the *Anopheles gambiae* complex in Benin: a nationwide survey. Medical and Veterinary Entomology, vol. 25, pp. 256–267, 2011.
39. Fanello C, Petrarca V, Della Torre A. Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR/RFLP. Medical and Veterinary Entomology, vol. 16, pp. 461-464, 2002.
40. Baldacchino F, Paupy C. Clé de détermination des *Culicidae* présents en Afrique Centrale et au Gabon, pp. 1-110.
41. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, vol. 18, pp. 265-267, 1925.
42. Chandre F, Darrier F, Manga L, Akogbeto M, Faye O, Mouchet J, Guillet P. Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. Bulletin of World Health Organization, vol. 77, pp. 230-234, 1999.
43. Awolola TS, Oyewole IO, Amajoh CN, Idowu ET, Ajayi MB, Oduola A, Manafa OU, Ibrahim K, Koekemoer LL, Coetzee M. Distribution of the molecular forms of *Anopheles gambiae* and pyrethroid knock down resistance gene in Nigeria. Acta Tropica, vol. 95, pp. 204-209, 2005.
44. Djogbenou L, Weill M, Hougard JM, Raymond M, Akogbeto M, Chandre F. Characterization of Insensitive Acetylcholinesterase (Ace-1R) in *Anopheles gambiae* (Diptera: Culicidae): Resistance Levels and Dominance. Entomological Society of America, vol. 44 (5), pp. 805-810, 2007.
45. Djouaka RF, Bakare AA, Coulibaly ON, Akogbeto MC, Ranson H, Hemingway J, Strode C. Expression of the cytochrome P450s, CYP6P3 and CYP6M2 are significantly elevated in multiple pyrethroid resistant populations of *Anopheles gambiae* s.s. from Southern Benin and Nigeria. BMC Genomics, vol. 9, pp. 538-548, 2008.
46. Corbel V, Raymond M, Chandre F, Darriet F, Hougard JM. Efficacy of insecticide mixtures against larvae of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) resistant to pyrethroids and carbamates. Pest Management Science, vol. 60, pp. 375-380, 2004.
47. Dabiré KR, Diabaté A, Agostinho F, Alves F, Manga L, Faye O, Baldet T. Distribution of the members of *Anopheles gambiae* and pyrethroid knock-down resistance gene (kdr) in Guinea-Bissau, West Africa. Bulletin de la Société de Pathologie Exotique, vol. 101, pp. 119-123, 2008.
48. Betson M, Jawara M, Awolola TS. Status of insecticide susceptibility in *Anopheles gambiae* s.l. from malaria surveillance sites in the Gambia. Malaria Journal, vol. 8, pp. 187-193, 2009.
49. Akogbeto M, Djouaka R, Noukpo H. Use of agricultural insecticides in Benin. Bulletin de la Société de Pathologie Exotique, vol. 98, pp. 400-405, 2005.
50. Fofana D, Konan KL, Djohan V, Konan YL, Koné AB, Doannio JMC, N’Goran KE. Diversité spécifique et nuisance Culicidienne dans les villages de N’gatty et d’Allaba en milieu côtier lagunaire de Côte d’Ivoire. Bulletin de la Société de Pathologie Exotique, vol. 3, pp. 333-339, 2010.
51. Namountougou M, Simard F, Baldet T, Diabaté A, Ouédraogo JB, Martin T, Dabiré RK. Multiple insecticide resistance in *Anopheles gambiae* s.l. populations from Burkina Faso, West Africa. PLOS ONE, vol. 7, Issue 11, pp. 1-10, 2012.