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

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Abstract

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 *etal*., 2016), with high morbidity and mortality, particularly in tropical regions (Koudou *et al.,* 2005 ; Ferraguti *etal*., 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).

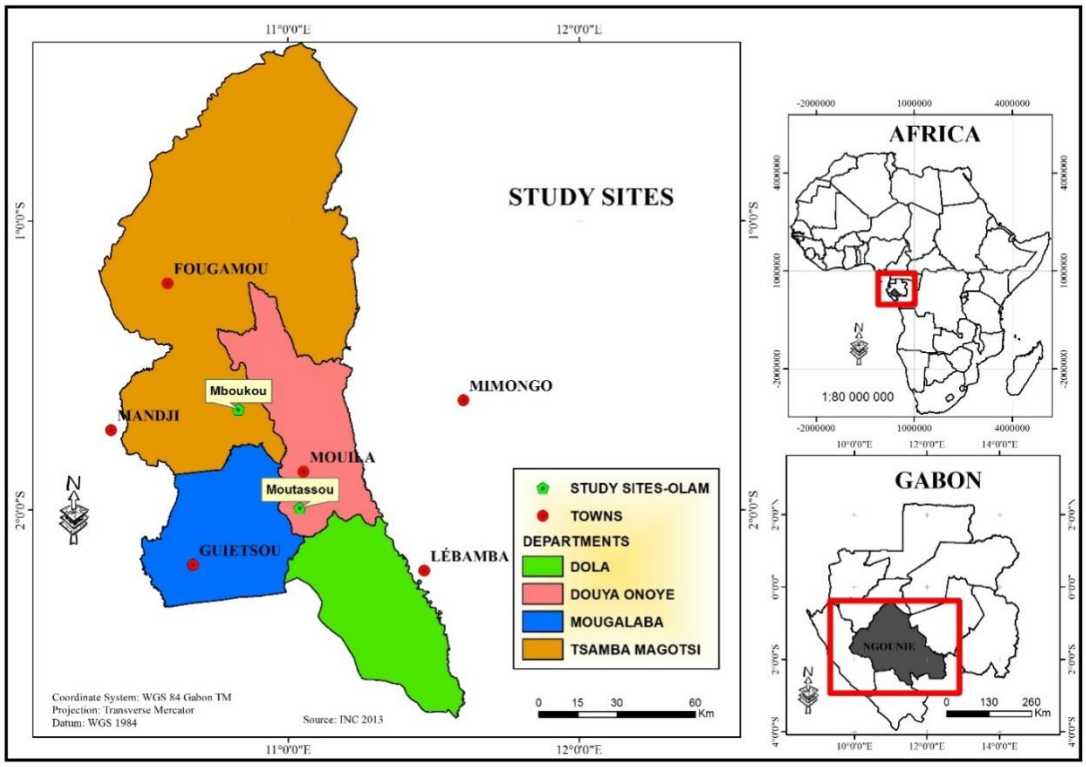

Figure 1:Geographical location of the study sites

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 Moulandoufouala, 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 gambiaes.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 Foumou 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) andby 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

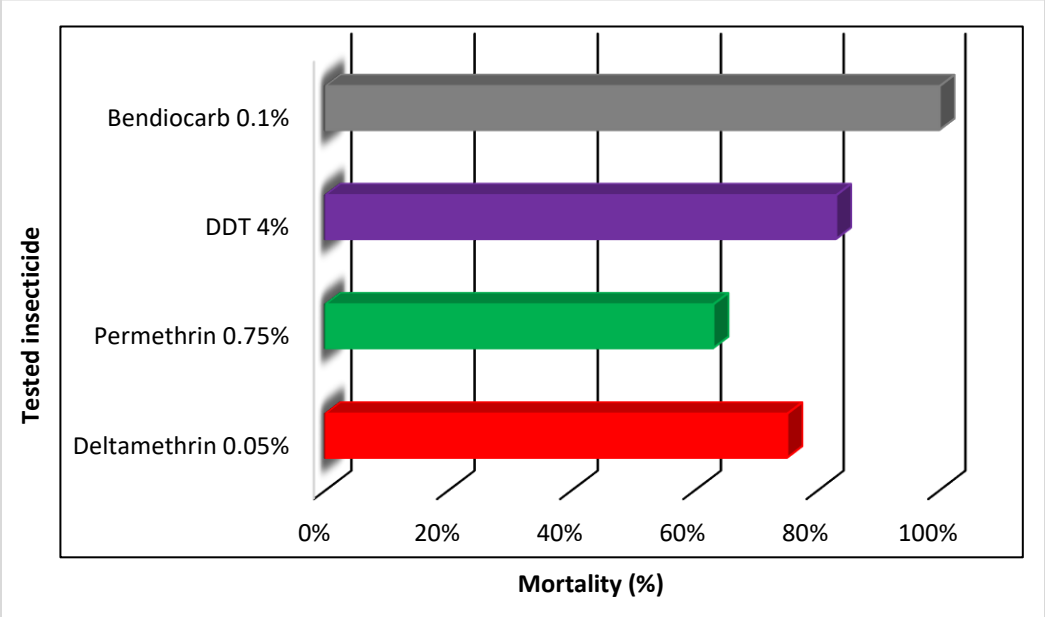

Figure 2: Mortality rate of adult* Culex sp. *from study sites, 24 h after exposure to deltamethrin, permethrin, DDT and bendiocarb.

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

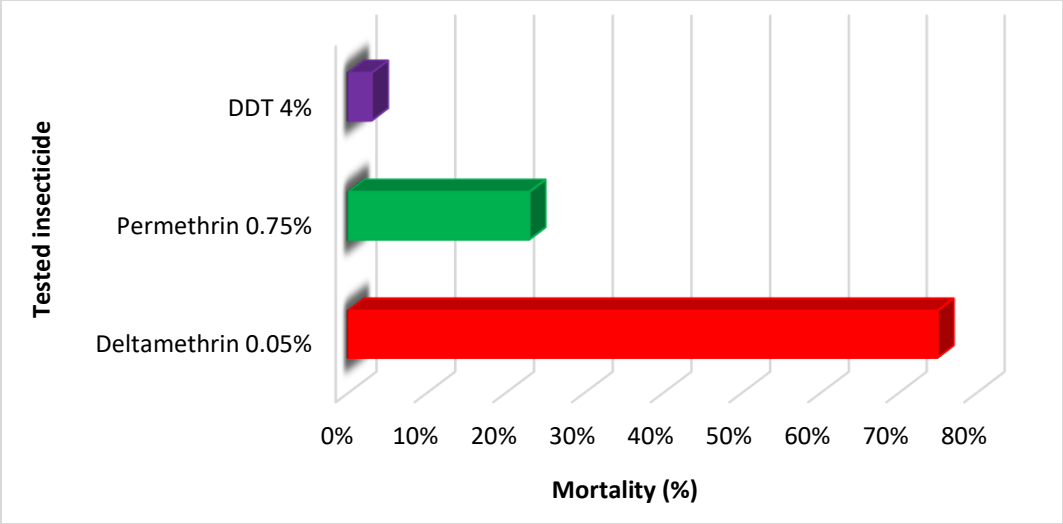

Figure 3a: Mortality rates of adult* Anopheles gambiae s.l. *from the study sites, 24 h after exposure to deltamethrin, permethrin and DDT

***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. *Anophelesgambiae s.l.* populations had higher levels of resistance to permethrin and DDT compared than *Culex sp*. This difference was not significant with deltamethrin.

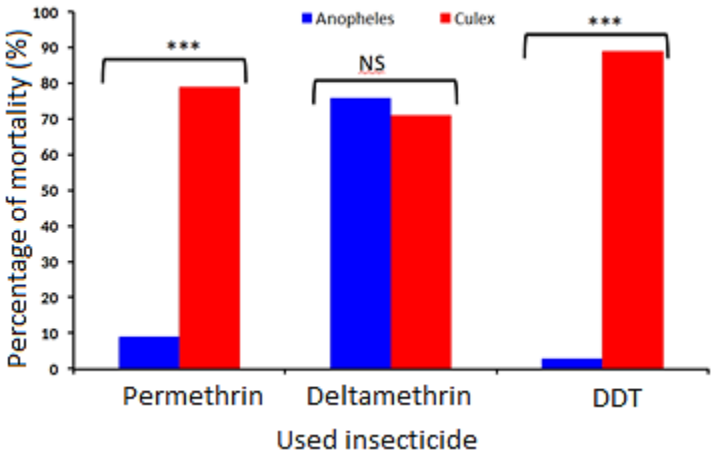

Figure 3b: Comparison of* Anopheles gambiae s.l. *susceptibility to that of* Culex sp. *from the study sites of Mouila, 24 h after exposure to DDT, deltamethrin and permethrin.

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.

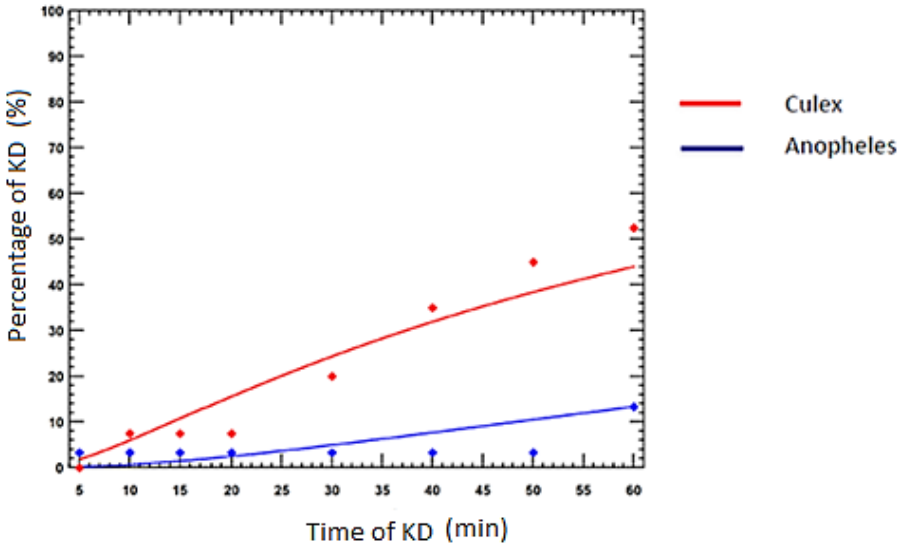

Figure 4 : Change in percentage of knocked down mosquitoes (*Anopheles gambiae s.l.*and* Culex sp.*) for 60 minutes after exposure to DDT

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.*

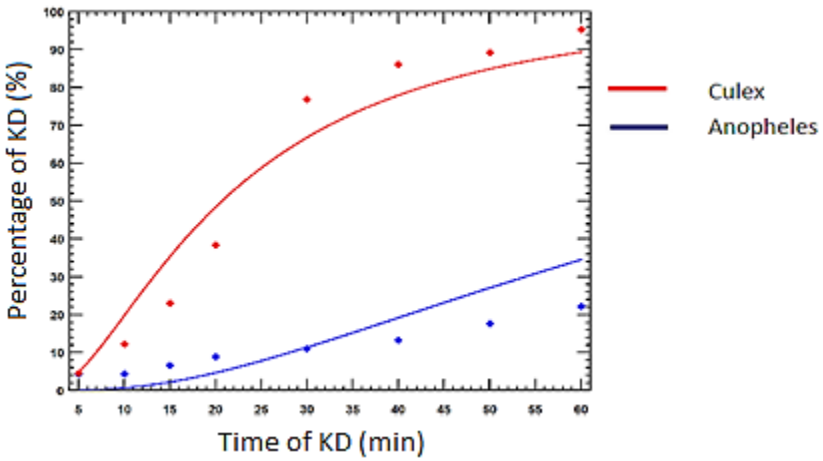

Figure 5: Change in percentage of knocked down mosquitoes (*Anopheles gambiae s.l.*and* Culex sp.*) for 60 minutes after permethrin exposure

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.*).

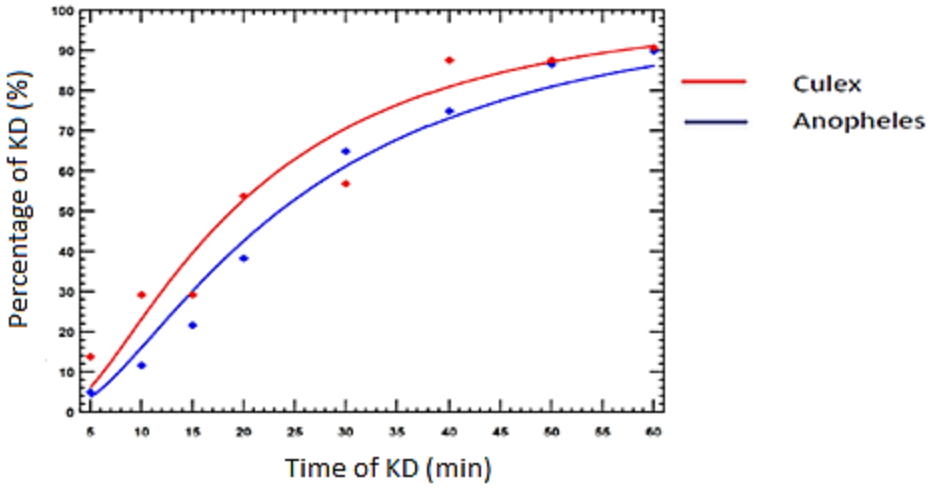

Figure 6: Change in percentage of knocked-down mosquitoes (Anopheles gambiae s.l. and Culex sp.) for 60 minutes after deltamethrin exposure

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. gambiaes.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 *Anophelesgambiae 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 (KdT50 and KdT95) 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
Anopheles gambiae s.l.	ND		ND		ND		ND		23.6	(21.3-26.1)	81.3	(67.5-104)
Culex 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 (A*nopheles*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 arbovirosis 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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