

## Susceptibility of Larval Stage of Mosquito *Culex pipiens* against Chlorpyrifos Insecticide in Southern Tunisia

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### Abstract

Three populations of mosquito *Culex pipiens* were used to study the susceptibility of larval stage against organophosphate chlorpyrifos and carbamate propoxur insecticides in Southern Tunisia. All samples were resistant to chlorpyrifos (RR>1, p<0.05) and the tolerance to this insecticide was varied between 1.8 and 1318. The sample # 3 was the most susceptible and disclosed a little difference when compared to S-Lab (RR50=1.9). The highest resistance levels (>1,000-fold) was recorded in samples # 2 (South East). The level of resistance was lower, not exceeding 2-fold in samples # 3 (Far South). Total esterase activity of field populations detected one or several esterases in all the studied samples, with the exception of the sample # 3 despite the addition of DEF showed that the increased detoxification by EST and/or GST was not responsible for chlorpyrifos resistance in these samples. Oxidative metabolism accounted for only a small part of the observed resistances because chlorpyrifos resistance ratios remained high in the presence of the Pb. The mortality due to propoxur was significantly correlated with the LC50 of chlorpyrifos and indicated an insensitive AChE in samples # 2 which showed the highest resistance levels to chlorpyrifos insecticide (1%). Results were discussed in relation to resistance mechanisms and *Culex pipiens* control.

**Keywords:** *Culex pipiens*; Chlorpyrifos; Propoxur resistance; Resistance mechanisms; Detoxification enzymes; Insensitive AChE 1; Southern Tunisia

### Introduction

The vector control must be rational and sustainable, based on surveillance of vectors and pathogens, and seeking to minimize adverse effects. The sustainability of the effectiveness of active substances depends on preventive strategy of developing the resistance by alternating the used substances. When the resistance to an active substance becomes observable in a vector, it is that it has already reached an irremediable level which will rapidly lead to the operational inefficiency of this substance. Knowledge of resistance levels is basic information to be acquired for all vector / family of insecticides in all territories. Moreover, the monitoring of the evolution of the resistances must be carried out.

Knowledge of the mechanisms of action of insecticidal active substances for understanding the efficacy of the product, to promote the development of new substances through a better understanding of (the) target (s), but also to better manage risks of resistance developed by insects and limiting adverse effects on non-target organisms and the environment.

To be effective, an insecticide should contact the insect to be transported to its target to exert its toxic activity. All the mechanisms that prevent or modify the effect of the insecticide in the body can lead to the development of resistance. In fact, insecticide resistance corresponds to a genetic event that leads to a decreased sensitivity of insects to a given insecticide. There are four main types of resistance: enzymatic detoxification (metabolic resistance) [1-3]; the change in the quality of the insecticide target [3-7]. Altering the amount of target; and reducing the penetration of the insecticide into the insect as a result of a modification of the structure of the cuticle. It is a mechanism which causes only low levels of resistance.

Ben Cheikh et al. [8] showed that resistance to chlorpyrifos of *Culex pipiens* collected from Northern Tunisia was very important, reaching the highest level >10,000-folds recorded worldwide. The aim of this paper was to study the susceptibility of larval stage of mosquito *Culex pipiens* against chlorpyrifos insecticide in Southern Tunisia.

### Materials and Methods

#### Mosquito strains

Six strains were used to study the susceptibility of larval stage of mosquito *Culex pipiens* against chlorpyrifos insecticide in Southern Tunisia: three field populations collected from three localities in Southern Tunisia (Figure 1), a sensitive strain called S-Lab used as reference strain, and two strains resistant to chlorpyrifos called SA2 and SA5 characterized by overproduced esterases A2-B2 and A5-B5, respectively.

#### Insecticides and synergists

Assays were performed using ethanol solutions of organophosphate chlorpyrifos (99.5% [AI]), and carbamate propoxur (99.9% [AI], Bayer AG, Leverkusen, Germany). The effect on chlorpyrifos resistance of 2 synergists, the DEF (98% [AI], Chem Service, England), and the Pb (94% [AI], Laboratory Dr Ehrenstorfer, Germany), was studied. The study of AChE 1 activity was performed by propoxur bioassays.

#### Bioassay procedures and data analysis

Bioassay tests utilized standard methods [9]. There is no difference between insecticides and synergism tests. The only exception was to add 0.5 ml of the desired concentration of synergist to each cup, 4 hours after putting the concentration of insecticide. Data were subjected to probit analysis [10] using a BASIC program [11].

#### Esterase assay protocol

Total esterase activity of field populations was determined according to the method of Pasteur et al. [12,13].

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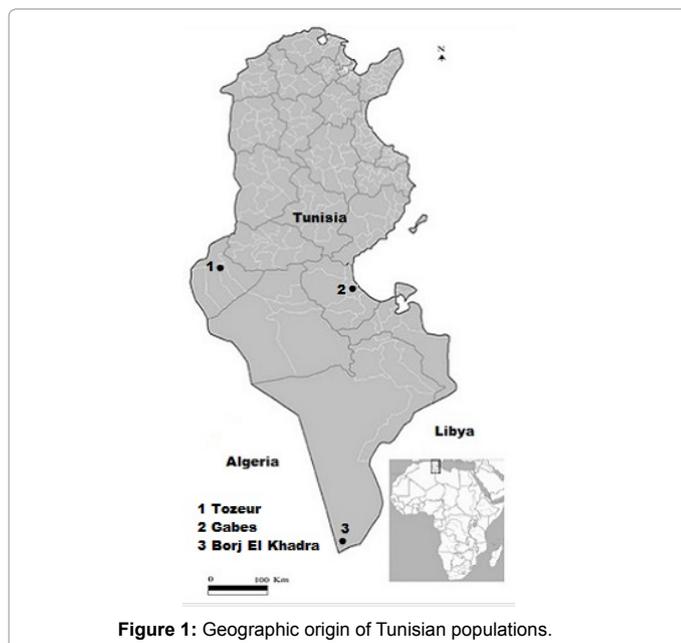
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## Results

### Chlorpyrifos resistance

The linearity of the dose–mortality response is accepted ( $p > 0.05$ ) only for S-Lab and samples # 1. All samples were resistant to chlorpyrifos ( $RR > 1$ ,  $p < 0.05$ ) and the tolerance to this insecticide was varied between 1.8 and 1318 (Table 1). The sample # 3 was the most susceptible and disclosed a little difference when compared to S-Lab ( $RR_{50} = 1.9$ ). The highest resistance levels (>1,000-fold) was recorded in samples # 2 (South East). The level of resistance was lower, not exceeding 2-fold in samples # 3 (Far South). At  $LC_{95}$ , all the samples showed a more important resistance to chlorpyrifos.  $RR_{95}$  was 4 in sample # 3. The resistance levels exceeded 10,000 folds in sample # 1 (South East).

The addition of DEF decreased significantly the tolerance to chlorpyrifos in S-Lab ( $SR_{50} = 1.41$ ,  $p < 0.05$ ) (Table 1). So, the increased detoxification by EST and/or GST was not responsible for chlorpyrifos resistance in these samples. The addition of Pb to chlorpyrifos bioassays significantly increased the tolerance of S-Lab ( $SR = 0.53$ ,  $p < 0.05$ ) and decreased the resistance of samples # 1 (Table 1). The recorded SR in this sample was significantly higher than that observed in S-Lab. However, oxidative metabolism accounted for only a small part of the observed resistances because chlorpyrifos resistance ratios remained high in the presence of the Pb.



### Cross-resistance to Chlorpyrifos/Propoxur

Mortality caused by propoxur was 41% in samples # 1 and 1% in samples # 2 which showed the highest resistance levels to chlorpyrifos insecticide. 100% was the percentage recorded in sample # 3 which showed the lowest resistance levels to studied insecticide. The mortality due to propoxur was significantly correlated with the  $LC_{50}$  of chlorpyrifos, (Spearman rank correlation,  $r = -0.90$  ( $P < 0.01$ )) and indicated an insensitive AChE.

### Overproduced esterases

One or several esterases were detected in all the studied samples, with the exception of the sample # 3.

### Discussion

The present study showed that two *Culex pipiens* field samples were resistant to chlorpyrifos and one was sensitive compared to the reference strain S-Lab. This result may be due to massif mosquito control using chemical insecticides in east and west south, and to occasional treatment by just permethrin in the far South. Many previous studies showed that resistance of *Culex pipiens* to chlorpyrifos varied between high and low [8,14-21].

The highest resistance levels (>1,000-fold) was recorded in samples # 2 (South East). A strong resistance of Tunisian *Culex pipiens* population (Gara) to OP chlorpyrifos (410 000-folds) was recorded by Pasteur et al. [22] This resistance was not related to *Ace-1R* allele because they had the same propoxur resistance of the reference strain SR which had the same *Ace-1R* allele [23], but differed highly in resistance to Chlorpyrifos. Carboxylesterases or cytochrome P450 oxidases were not involved in this recorded resistance. Authors lost the strain but suggested that this resistance unique in the world by its scope was due to a single major gene (or a group of linked genes), distinct from *Ace-1*, but epistatic with and/or genetically linked to it. Later, Alout et al. [24] confirmed this hypothesis on *Culex pipiens* collected worldwide supposing a particular combination of *Ace-1* and another gene linked to the sex factor and *Ace-2*.

Our and previous study confirmed the presence of stronger esterase activity in the most resistant chlorpyrifos samples (samples # 1 and 2) [8,18,25-39]. The esterase activity is very variable between individuals of the same sample, as evidenced by the results observed on single individuals after starch gel electrophoresis of the extracts, but it is clear that the proportion of individuals with high esterase activity is more important in samples where resistance is high. This heterogeneity of esterase activity may reflect a mixture of individuals with different levels of resistance. It would therefore be important in the future to

Population	Chlorpyrifos			Chlorpyrifos +DEF					Chlorpyrifos +Pb				
	LC <sub>50</sub> in µg/l (a)	Slope ± SE	RR <sub>50</sub> (a)	LC <sub>50</sub> in µg/l (a)	Slope ± SE	RR <sub>50</sub> (a)	SR <sub>50</sub> (a)	RSR	LC <sub>50</sub> in µg/l (a)	Slope ± SE	RR <sub>50</sub> (a)	SR <sub>50</sub> (a)	RSR
Slab	0.56 (0.53-0.58)	9.0 ± 1.04	-	0.17 (0.14-0.20)	2.85 ± 0.26	-	1.4 (1.08-1.8)	-	0.45 (0.17-1.3)	1.16 ± 0.43	-	0.53 (0.35-0.79)	-
1-Tozeur	69 (56-87)	1.38 ± 0.11	126 (100-158)	23 (5.9-95)	0.86 ± 0.24	141 (81.5- 245)	2.9 (1.8- 4.7)	0.89	4.1 (1.6-7.4)	0.84 ± 0.14	9.3 (6.0-14.5)	16.7 (12.5-22.2)	13.4
2-Gabès	732 (380-1420)	1.28 ± 0.24	1318 (868-2003)	1090 (579-2050)	2.25 ± 0.48	6490 (3528-11938)	0.67 (0.38-1.1)	0.20	649 (313-1350)	0.77 ± 0.11	1457 (904-2348)	1.1 (0.76-1.6)	0.90
3- Bordj El Khadra	1.0 (0.68-1.6)	3.01 ± 0.66	1.8 (1.08-3.3)	-	-	-	-	-	-	-	-	-	-

(a), 95% CI.

RR<sub>50</sub>, resistance ratio at LC<sub>50</sub> ( $RR_{50} = LC_{50}$  of the population considered/LC<sub>50</sub> of Slab); SR<sub>50</sub>, synergism ratio (LC<sub>50</sub> observed in absence of synergist/LC<sub>50</sub> observed in presence of synergist). RR and SR considered significant ( $P < 0.05$ ) if their 95% CI did not include the value 1.

RSR, relative synergism ratio (RR for insecticide alone/RR for insecticide plus synergist).

**Table 1:** Chlorpyrifos resistance characteristics of Tunisian *Culex pipiens* in presence and absence of synergists DEF and Pb.

determine precisely the nature of the link between the resistance rate and the esterase activity it contains each individual, since the analysis of this activity is likely to provide the vector control staff with valuable insight into the level of resistance to organophosphorus insecticides existing or likely to develop in each population.

Our study showed that oxidative metabolism accounted for only a small part of the resistance to chlorpyrifos. Similar results were previously obtained in Tunisia and in other countries of the world on several insects [8,18,37,40].

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