

## Evaluation of Chlorpyrifos Resistance and Biochemical Mechanisms of *Culex pipiens* in Five Localities of Grand Tunis Area, Northeast Tunisia

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### Abstract

Five *Culex pipiens* samples were collected at preimaginal stages from breeding sites in 5 localities of Grand Tunis area, Northeast Tunisia, between March 2002 and November 2005. Larvae were used for bioassays using ethanol solutions of chlorpyrifos and propoxur insecticides. All samples were resistant to chlorpyrifos (RR>1, p<0.05). The highest resistance levels (>1,000-fold) were recorded in samples # 2, 4, and 5. Our synergist study showed that the increased detoxification by EST (and/or GST) had only a minor role in the chlorpyrifos resistance, although several overproduced esterases, known to be involved in the OPs resistance, were detected in all the resistant field samples. The mortality due to propoxur was significantly correlated with the LC<sub>50</sub> of chlorpyrifos and indicated an insensitive AChE.

**Keywords:** *Culex pipiens*; Chlorpyrifos; Resistance; Esterases; Insensitive AChE I; Grand Tunis area

### Introduction

Overall, surveillance activities of potential mosquito vectors of disease are very limited in Tunisia in terms of both spatial and temporal coverage and effectiveness. The problem is essentially linked to the lack of human and material resources mobilized for the activity. The hygienist technicians responsible for the activities of the entomological units are involved, at the same time, in other activities and the means at their disposal are very limited.

The current mosquito surveillance system is far from being able to predict epidemics of vector-borne diseases, evidenced by the occurrence of the several outbreak of West Nile Virus in 2003, 2007, 2010, 2011 and 2012 [1-4] despite the surveillance system putted in place after the first epidemic of 1997.

The other problem relating to potential vectors of diseases, which should be mentioned, concerns the development by mosquitoes of strong resistance to insecticides. The results of the study of insecticide resistance in populations of *Culex pipiens*, the most frequent and abundant mosquito in Tunisia, captured in different parts of the country showed their high level of resistance to chlorpyrifos [5-8]. This strong resistance concerns mainly Grand Tunis area of northern Tunisia.

The primary mechanism of toxicity of organophosphorus pesticides, such as chlorpyrifos, is cholinesterase inhibition (ChE). Inhibition of the enzyme acetylcholinesterase (AChE) results in an accumulation of acetylcholine (ACh) at choline receptors, resulting in continuous nerve stimulation [9]. The current study was realized to study the status of tolerance of *Culex pipiens* to chlorpyrifos insecticide (OP) in five localities of Grand Tunis Area of Tunisia.

### Materials and methods

#### Study area

Tunis, Ariana, Manouba and Ben Arous are the four states of Grand Tunis area, Northeast Tunisia.

#### Mosquitoes

Five *Culex pipiens* samples were collected at preimaginal stages from breeding sites in 5 localities between March 2002 and November 2005 (Table 1). Larvae were used for bioassays and pupae were reared to imago under laboratory conditions. Two to three days after their

emergence, some adults from each collection were stored in liquid nitrogen for biochemical investigations. Reference strains included S-Lab, an insecticide-susceptible strain without any known resistance genes [10], and two OPs resistant strains: SA2, a resistant strain homozygous for Ester<sup>2</sup>, displaying overproduced esterases A2-B2, and SA5, a resistant strain homozygous for Ester<sup>5</sup>, displaying overproduced esterases A5-B5 [11].

#### Bioassays

Assays were performed as described by Raymond et al. [12], using ethanol solutions of chlorpyrifos (99.5% [AI]), brought from laboratory Dr Ehrenstorfer, Germany, and 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 by exposing larvae to a standard sublethal doses of 0.08 mg/liter for DEF, and 2.5 mg/liter for Pb, 4 h before the addition of the insecticide.

#### Over-produced esterases

Esterases of high activity were characterized on homogenates of adult thorax and abdomen by studying esterase activity in the presence of  $\alpha$ - and  $\beta$ -naphthyl acetate after protein separation by starch-gel electrophoresis (TME 7,4 buffer system) as described by Pasteur et al. [13] and were identified by comparing their electrophoretic mobility to that of known over-produced esterases.

#### Data analysis

Larval mortality was recorded after 24-h exposures, and data were analyzed using a log-probit program of Raymond et al. [14] based on Finney. [15].

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Populations	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-Sidi thabet	90 (49-166)	0.79 ± 0.08	163 (121-221)	846 (220-3230)	0.99 ± 0.23	5032 (2769-9145)	0.10 (0.06-0.17)	0.03	241 (87-531)	0.97 ± 0.17	481 (276-838)	0.42 (0.29-0.61)	0.33
2-Sokra	1230 (665-2270)	1,71 ± 0.39	2219 (1349-3652)	1460 (1120-1900)	1.75 ± 0.16	8715 (6398-11870)	0.84 (0.53-1.3)	0.25	305 (232-381)	1.39 ± 0.14	684 (466-1005)	4.0 (2.5-6.4)	3.2
3-Mannouba	35 (19-66)	0.99 ± 0.14	64.6 (45.8-91.0)	14 (5.5-37)	0.74 ± 0.12	85.9 (58.4-126)	2.4 (1.7-3.5)	0.75	21 (13-35)	0.84 ± 0.08	48.8 (32.8-72.7)	1.6 (1.2-2.2)	1.3
4-Ouardia	682 (470-1030)	0,65 ± 0,05	1229 (989-1527)	129 (95-177)	0.74 ± 0.06	771 (615-967)	5.2 (4.5-6.0)	1.6	1860 (1020-4580)	0.81 ± 0.13	4183 (2716-6441)	0.36 (0.28-0.47)	0.29
5-Ezzahra	4950 (3830-6850)	1.67 ± 0.19	8929 (6773-11773)	4740 (3690-6430)	1.43 ± 0.22	28199 (21728-36598)	1.04 (0.80-1.3)	0.31	6340 (2910-55400)	0.95 ± 0.33	14228 (8361-24209)	0.78 (0.50-1.2)	0.62

(a), 95% CI. RR50, resistance ratio at LC50 (RR50=LC50 of the population considered/LC50 of Slab); SR50, synergism ratio (LC50 observed in absence of synergist/LC50 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.

## Results

### Chlorpyrifos Resistance in presence and absence of synergists DEF and Pb

All samples were resistant to chlorpyrifos (RR>1, p<0.05) (Table 1). The highest resistance levels (>1,000-fold) were recorded in samples # 2, 4, and 5. At LC<sub>95</sub>, the resistance levels exceeded 10,000 folds in samples # 1, 2, 4, and 5.

The addition of DEF decreased significantly the resistance (SR50>1, p<0.05) of sample # 4 where the SR was significantly higher than that recorded in S-Lab (Table 1). So, the increased detoxification by EST and/or GST was responsible, at least in part, for chlorpyrifos resistance in this sample. The addition of Pb to chlorpyrifos bioassays significantly decreased the resistance of samples 2 and 3 (Table 1). The recorded SR in these samples 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 significant high in the presence of the Pb.

### Cross-resistance Chlorpyrifos/Propoxur

The mortality due to propoxur varied from one sample to another. It was 0% in the most resistant strain and 21% in the most sensitive strain indicated an insensitive AChE. The mortality due to propoxur was significantly correlated with the LC<sub>50</sub> of chlorpyrifos (Spearman rank correlation, (r)=-0.90 (P<0.01)).

### Overproduced esterases

C1 A1, A2-B2, A4-B4 and/or A5-B5, and B12 are the five esterases detected in studied samples. It should be noted that frequency of each enzyme was not correlated with the LC<sub>50</sub> of chlorpyrifos. For example, the sample # 3 had 47% of A4-B4 and/or A5-B5 despite its lowest resistance to chlorpyrifos.

## Discussion

The present study showed that all the studied Tunisian *Culex pipiens* field samples were resistant to chlorpyrifos. The resistance levels were very high in some samples (RR<sub>50</sub>>1,000). Similar chlorpyrifos resistance levels of *Culex pipiens* were previously reported in Tunisia [6]. These authors showed that resistance to chlorpyrifos in populations of *Culex pipiens* collected from Tunisia was very important, reaching the highest

level >10,000-folds recorded worldwide. The resistance of *Culex pipiens* populations collected in Grand Tunis area may be associated with the use of chlorpyrifos and other insecticides at different intensities and frequencies of application. The highest chlorpyrifos resistance level of *Culex pipiens* reported in other areas of the world was of 800-fold [16]. The resistance levels to chlorpyrifos in *Culex pipiens* from other regions are lower: 700-fold in Israel [17], 186-fold in Italy [18], 123-fold in Martinique [19], 34-fold in Venezuela [20], 30-fold in Cote d'Ivoire [21], 14-fold in China [22] and 4-fold in Burkina Faso [23]. Other previous studies revealed that pressure using high concentrations of chlorpyrifos may induce resistance [24]. This may explain the difference in resistance to chlorpyrifos in regions under selection pressure [25].

The increased detoxification by EST and/or GST was responsible, at least in part, for chlorpyrifos resistance in just one among 5 samples despite several esterases were detected in all resistant samples. So these enzymes were not involved in recorded resistance. Our results are in agreement with previous studies on the role of the EST and the GST in the OPs resistance [6, 26] and the resistance levels conferred by the overproduced esterases, A2-B2, A4-B4, A5-B5 [27], C1 [6] and B12 [28]. In contrast, several previous studies showed significant elevation in the activity of esterases implicated in the resistance to OP insecticides [20,29-35]. Likewise, several studies reported the implication of GST in OPs resistance in mosquitoes including *Culex* and *Anopheles* [36-40]. Our result mentioned the minor role of CYP450 in the recorded chlorpyrifos resistance. Similar results were reported in many insects, including mosquitoes of many countries of the world [6, 20, 26, 41].

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