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Evaluation of Fenitrothion Resistance and Biochemical Mechanism in three Populations of *Culex pipiens* (Diptera: Culicidae) from Southern Tunisia

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Abstract

Evaluation of fenitrothion resistance was realized in three populations of *Culex pipiens* collected in Southern Tunisia between March 2002 and October 2005. It was not possible to considered bioassays tests to fenitrothion in sample # 3 due to their control-level mortality. The RR50 were 27.1 in sample # 1 and 179 in sample # 2. All the studied samples showed the presence of one or more esterases in their electrophoretic profiles except the sample # 3 which was sensitive to propoxur. The addition of Pb to fenitrothion bioassays indicated the involvement of CYTP450 in the recorded resistance. This result could be explained by the massive use of the permethrin in the control against these insects in southern Tunisia. We also showed that the resistance to fenitrothion was correlated with the propoxur resistance. These results indicate that modifications of the target, AChE1, can be involved in the recorded resistance.

Keywords: *Culex pipiens;* Resistance; Fenitrothion; Propoxur; Esterases; CYP450; AChE1; Southern Tunisia

Introduction

The resistance of pathogen vectors that cause human or animal diseases to insecticides affects both the economy and public health globally: it requires increasing the amount of used insecticides and developing new molecules or formulations (thus raising costs). It makes the available products and vector control strategies inefficient, thus leading to an increased prevalence of the pathogens and diseases they transmit [1-3].

The resistance of a target species can be defined as an inheritance reduction of susceptibility to an insecticide [2]. At the fundamental level, this is an adaptation to the new environment selected by the pressure exerted by one or more insecticides, according to a natural selection process. Resistant individuals carry one or more gene mutations (known as resistance alleles) encoding proteins that interact with the insecticide. Thus, the mutated proteins prevent the insecticide from reaching its target, for example by degrading it, or by modifying this target allowing the insects carrying these mutations to survive doses of insecticide normally lethal [4-11].

For years, the organophosphates (OPs) and synthetic pyrethroids have been widely used in the mosquito control programs. Currently, in addition to pyrethroid insecticides (permethrin and deltamethnin), many OPs including fenitrothion insecticide were largely used in *Culex pipiens* control.

This study reported the resistance of *Culex pipiens* to fenitrothion (OP). The aim is to present the main mechanisms of resistance to fenitrothion as well as the current situation in terms of resistance to this insecticide.

Materials and Methods

Mosquito strains

Six strains of mosquitoes were used in this study: three field populations of *Culex pipiens* collected in Southern Tunisia between March 2002 and October 2005 (Table 1 and Figure 1), S-Lab reference used as sensitive strain, SA2 and SA5 with overproduced esterases A2-B2 and A5-B5, respectively.

Insecticides and synergists

Two insecticides were used for bioassays: the organophosphate fenitrothion (98.5% [AI]), brought from laboratory Dr Ehrenstorfer, Germany) and the carbamate propoxur (99.9% [AI], Bayer AG, Leverkusen, Germany). Two synergists were used to help detect detoxification enzymes involved in resistance: S,S,S tributyl phosphorothioate (DEF), an esterase inhibitor, and piperonyl butoxide (PB), an inhibitor of mixed function oxidases.

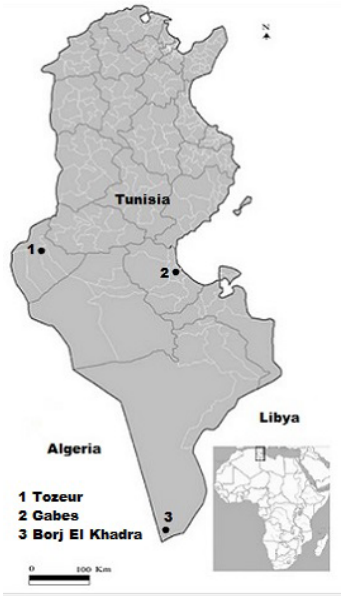

Figure 1: Geographic origin of Tunisian populations.

Bioassay procedures

We used standard methods to do bioassays tests [12,13]. Data

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Code	Locality	Breeding site	Date of collection	Mosquito control (used insecticides)	Agricultural pest control
1	Tozeur	Ditch	Oct. 2005	Frequent (C, Pm, F, P, D)	None
2	Gabes	Drain	June 2005	Frequent (C, Pm, P, D)	None
3	Borj El-Khadra	Water pond	Mar 2002	Occasional (P)	None
C: Chlorpyrifos ; T: Temephos ; Pm: Pirimiphos methyl ; F: Fenitrithion ; P: Permethrin ; D: Deltamethrin					

Table 1: Geographic origin of Tunisian populations, breeding site characteristics and insecticide control.

Population	Fenitrothion			Fenitrothion +DEF					Fenitrothion +Pb				
	LC_{50} in µg/l (a)	Slope ± SE	RR_{50} (a)	LC_{50} in µg/l (a)	Slope ± SE	RR_{50} (a)	SR_{50} (a)	RSR	LC_{50} in µg/l (a)	Slope ± SE	RR_{50} (a)	SR_{50} (a)	RSR
S-Lab	3.3 (1.7-6.3)	3.19 ± 0.94	-	1.3 (1.0-1.6)	2.43 ± 0.26	-	2.5 (1.2-5.2)	-	2.8 (0.18-44)	1.44 ± 0.93	-	1.1 (0.34-3.9)	-
1-Tozeur	91 (47-179)	1.31 ± 0.17	27.1 (12.9-56.8)	10 (5.0-21)	1.01 ± 0.14	7.7 (5.3-11.2)	8.8 (5.9-12.9)	3.5	3.6 (0.61-21)	1.31 ± 0.45	1.2 (0.35-4.6)	24.6 (10.8-56.0)	21.3
2-Gabès	601 (488-738)	3.4 ± 0.59	179 (89.5-359)	601 (488-738)	3.40 ± 0.59	451 (310-654)	1.0 (0.69-1.4)	0.40	367 (238-564)	5.06 ± 1.76	127 (36.2-446)	1.6 (0.78-3.4)	1.4
(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 2: Fenitrothion resistance characteristics of Tunisian *Culex pipiens* in presence and absence of synergists DEF and Pb.

were analyzed by probit analysis [14] using a BASIC program [15]. We calculated the resistance ratios at the median lethal concentration (LC50) and LC95 by comparing the lethal concentration values of field populations and S-Lab strain. Synergism tests were similar to the bioassay tests except that 0.5 ml of the desired concentration of synergist was added four hours before adding the concentration of insecticide to each cup.

Esterase assay

Total esterase activity in individual, frozen adult mosquitoes from field populations was determined by starch electrophoresis according to the method of Pasteur et al. [16,17].

Results

Fenitrothion resistance

The linearity of the dose-mortality response was accepted ($p<0.05$) just for S-Lab. It was not possible to considered bioassays tests to fenitrothion in sample # 3 due to their control-level mortality. The RR50 were 27.1 in sample # 1 and 179 in sample # 2.

The additions of DEF to fenitrothion bioassays indicate that the increased detoxification by the EST (and/or GST) played only a minor role in the resistance in samples # 1. In fact, The SR was significantly higher than that recorded in S-Lab in samples # 1 which showed RR50>1 in the presence of DEF. The tolerance was not decreased after addition of DEF in sample # 2 (Table 2). The Pb had not a significant effect on the fenitrothion resistance in S-Lab (SR50 = 1.16, $p<0.05$). The SR50 was significantly higher than that recorded in S-Lab only in samples # 1 (Table 2). The addition of Pb to fenitrothion bioassays eliminated completely the resistance of sample # 1 (RR50=1.2, $p>0.05$, RSR=21.3), indicating that the resistance mechanisms in this sample were inhibited by Pb.

Cross-resistance of fenirtothion/propoxur

Mortality caused by propoxur ranged from 1% in samples # 2 which has the highest resistance rate to fenitrothion to 100% in samples # 3 which was sensitive. Significant correlation was recorded between mortality due to propoxur and the LC50 of fenitrothion (Spearman rank correlation, (r) = -0.69 ($P<0.01$)). This was expressed by a percentage of 41% in samples # 1 which had a medium rate of resistance.

Esterase's activities

All the studied samples showed the presence of one or more esterases in their electrophoretic profiles except the sample # 3 which was sensitive: A4-B4 (and / or A5-B5) which had the highest frequency (59%), C1 (28%), A2-B2 (11%), and B12 (3%).

Discussion

Its lack of selectivity towards non-target wildlife combined with a probable risk of toxicity to humans has prevented its use on a larger scale. High resistance levels were demonstrated by our two studied samples. Moderate resistance has been detected in Brazil in some wild populations of *Aedes aegypti* by Macoris et al. Similarly, resistances have been found on *Aedes aegypti* against OPs, including fenitrothion in many Caribbean islands [18]. Hidayati et al. [19] showed that level of resistance to fenitrothion could be the result of repetitive exposure with malathion which is the same insecticide class. Similar results were recorded by Corena et al. [20] who reported a cross-resistance to temephos and three other OPs insecticides (fenitrothion, fenthion and malathion).

Observation of insecticide resistance in a vector population is not necessarily associated with treatment failures. Indeed, the resistance must be widely distributed among the target population in order to have a visible operational impact. In addition, some insecticides have, in addition to their lethal action, a repellent action. For permethrin, for example, the major effect of which is repulsion, resistance can have only a limited effect on the overall effectiveness of the intervention. However, it is essential to detect as soon as possible the appearance of resistance, in order to allow operators to adapt their control strategy.

Our study detected many esterases probably involved in the recorded resistance to fenitrothion (OP). The involvement of these enzymes in the OPs resistance was confirmed on *Culex pipiens* and other insects species by many previous studies [5,21-23]. Esterase's activities showed the implication of many esterases in resistance of studied populations. However, many previous studies observed elevated esterase levels, but found no correlation with resistance [24-29].

The addition of Pb to fenitrothion bioassays indicated the involvement of CYP450 in the recorded resistance. This result could be explained by the massive use of the permethrine in the control

against these insects (Table 1). Our study is in agreement with previous publication on correlation between cytochrome P450 enzymes and resistance to pyrethroids [30]. Multidisciplinary insect resistance studies are needed to determine how resistance to an insecticide may develop after exposure to another family of pesticides, and whether complementarities exist between these mechanisms of detoxification.

We also showed that the resistance to fenitrothion was correlated with the propoxur resistance. These results indicate that modifications of the target can be involved in the recorded resistance. The involvement of AChE1 in OPs insecticides was confirmed in several mosquito species [31].

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