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Research Article

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Pirimiphos-methyl Resistance in *Culex pipiens* (Diptera: Culicidae) in Southern Tunisia

Tabbabi Ahmed[1*], Daaboub Jaber[1,2], Laamari Ali[1], Ben Cheikh Raja[1] and Ben Cheikh Hassen[1]

Abstract

Pirimiphos-methyl is an organophosphorus insecticide having a fast action with less toxicity for humans and environment. The aim of this study was to *investigate* the *pirimiphos-methyl resistance* of three populations of *Culex pipiens* in southern Tunisia. Three *Culex pipiens* samples were collected in 3 localities (South east, south west, and extreme south) of southern Tunisia between March 2002 and October 2005. The *susceptibility* of the third population (extreme south) of *Culex pipiens* to *pirimiphos-methyl was not considered* due to control-level mortality. Both other studied samples were resistant to pirimiphos methyl and to propoxur. A significantly correlation was observed between the mortality due to propoxur and the LC_{50} of pirimiphos methyl ($P<0.01$) showed an insensitive acetylcholinesterase (AChE). The characterization of Esterase phenotypes confirmed the previous studies which showed the major implication of esterases in the resistance to organophosphorus insecticides. Our results can *improve* current control and mosquito surveillance *strategies*.

Keywords

Culex pipiens; Pirimiphos-methyl; Resistance; Insensitive AChE; Esterases; Southern Tunisia

Introduction

Culex pipiens is showing locally invasive or interfering with everyday life, tourism and economic activities [1-3]. Many control methods have been tested. Many of these actions have had unintended negative or collateral consequences, and none has shown lasting results. This mosquito is favored by the fact that it is rapidly reproduced in large numbers and is highly adaptive. In particular, it has demonstrated the ability to adapt to many insecticides, which are sometimes only temporarily or partially effective [4-9], while affecting non-target species that were precisely predators of the mosquito.

The majority of synthetic insecticides are compounds that inactivate a vital target of the nervous system, resulting in death of the insect. Unfortunately, the populations of treated insects evolve very rapidly towards resistance [10,11]. In order to combat resistance, the use of insecticidal molecules directed against another target may prove effective for a more or less short time depending on the frequency of the occurrence of new adaptive mutations.

***Corresponding author:** Tabbabi A, Laboratory of Genetics, Faculty of Medicine of Monastir, Monastir University, Monastir- 5019, Tunisia, Tel: +216 97 085 424 ; E-mail: tabbabiahmed@gmail.com

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After the massive use of DDT (organochlorine), resistance was very quickly selected in the 1960s. Two other families of insecticides were then used: organophosphates (OP) and carbamates (CX). These molecules inhibit AChE, the enzyme responsible for the hydrolysis of acetylcholine in cholinergic synapses. This inhibition prolongs the nerve impulse, which leads rapidly to the death of the insect by tetany. In the mosquito *Culex pipiens*, two main mechanisms of resistance to these insecticides have been demonstrated: The first corresponds to an increased detoxification of OPs by carboxylesterases that trap or degrade them [12]. In the case of resistance, these esterases are overproduced by a process of amplification or overexpression of the genes that encode them [13,14]. The second mechanism corresponds to a modification of the target, AChE, which reduces its affinity for insecticides and leads to its insensitivity [15,16].

Pirimiphos-methyl is an organophosphorus insecticide used in a wide range of vectors control and is being considered by WHO for addition to potable water in containers as a mosquito larvicide treatment. Recently, results have shown that pyrimiphos-methyl alone has little effect on larvae of *Aedes aegypti* whereas its efficacy on adults is particularly good [17]. The aim of this study is to investigate the pirimiphos-methyl resistance of three populations of *Culex pipiens* in southern Tunisia.

Materials and Methods

Mosquitoes

Three *Culex pipiens* samples were collected at preimaginal stages from breeding sites in 3 localities of southern Tunisia between March 2002 and October 2005 (Table 1 and Figure 1). Comparisons of different bioassays were made with a reference strain called S-Lab [18]. SA2 and SA5 are two resistant strains homozygous for Ester2 and Ester5, respectively [19]. They were used for correct identification of detected esterases.

Bioassays and data analysis

Assays were realized according to Raymond et al. [20], using ethanol solutions of pirimiphos methyl (99% [AI]), brought from laboratory Dr Ehrenstorfer, Germany, and propoxur (99.9% [AI], Bayer AG, Leverkusen , Germany). The effect on OPs resistance of 2 synergists, the DEF (98% [AI], Chem Service, England), and the Pb (94% [AI], Laboratory Dr Ehrenstorfer, Germany) , was studied. Data were analysed by probit analysis [21] using a BASIC program [22].

Over-produced esterases

Esterases were characterized on homogenates of adult thorax and abdomen using the method of Pasteur et al. (1981,1988) [23,24].

Results and Discussion

S-Lab was the only strain that has linear dose-mortality response. With the exception of susceptibility to propoxur, the susceptibility of the third population (sample # 3) of *Culex pipiens* to pirimiphos-methyl was not considered due to control-level mortality. Both other studied samples were resistant to pirimiphos methyl. This result can be explained by the frequent control of these areas against this mosquito (frequent monitoring by the pirimiphos methyl, Table 1)

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

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

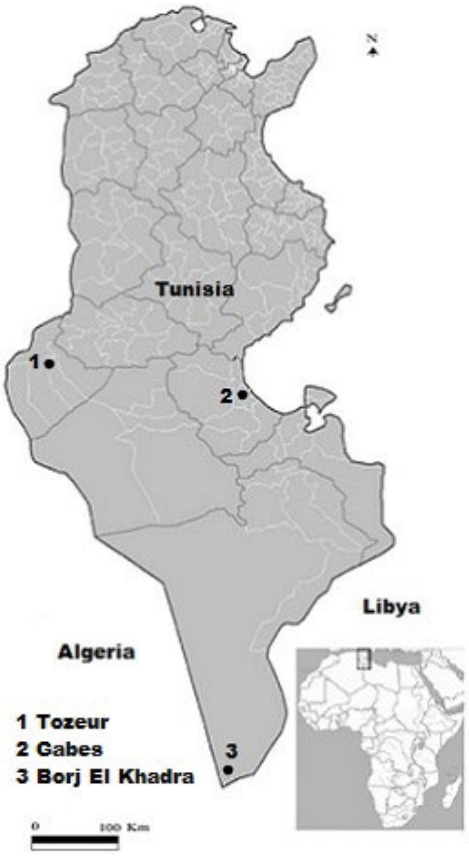

Figure 1: Geographic origin of Tunisian populations.

because of the alerted by inhabitants about the mosquito bites. The RR_{50} was 6.9 in sample # 1 and 27 in sample # 2 (Table 2).

The tolerance to pirimiphos methyl in S-Lab and the two field samples decreased significantly with the addition of the DEF (SR_{50}>1, p<0.05) (Table 2). No synergistic action was recorded by the DEF in samples # 1 and 2. Therefore, esterases (and/or GST) had no effect on the pirimiphos methyl resistance in both samples. These results confirm those found by Tabbabi et al. [9] which showed the noninvolvement of esterases (and/or GST) in the resistance of *Culex pipiens* strain subjected to temephos (organophosphorus) selection pressure. The tolerance to pirimiphos-methyl decreased significantly with the addition of the Pb in S-Lab (SR_{50}=7.24, p<0.05) and in both samples, and the SR was not significantly higher than that recorded in S-Lab (Table 2). Therefore, the CYP450 had no effect on the pirimiphos methyl resistance in both populations. Other previous studies on *Culex pipiens* from Tunisia [4], *Culex quinquefasciatus* from Venezuela [25] and USA [26], and *Musca domestica* [27] showed the minor role of oxydases in resistance to chlorpyrifos insecticide (organophosphorus).

Mortality caused by propoxur was 1% in samples # 2, 46% in samples # 1 and 100% in sample # 3. A significantly correlation was observed between the mortality due to propoxur and the LC_{50} of pirimiphos methyl (P<0.01) showed an insensitive AChE. These results are in agreement with previous studies [9,28,29] which showed the major role of insensitive AChE in resistance to OPs insecticides. It was not possible to carry out toxicological tests on population # 3 because of control-level mortality but as it was even possible to keep samples and to study its AChE and esterases activities. The latter were very sensitive to propoxur. This can be explained by the absence of control actions in this region (occasionally by the permethrin).

With the exception of the sample # 3, all the studied samples showed the presence of one or more esterases in their electrophoretic profiles. The frequency of 0.03 and 0.56 were those of A4-B4 (and / or A5-B5) esterases found in samples # 1 and 2, respectively. The frequency of 0.03 was those of The B12 esterases observed in samples # 2. The frequency of C1 esterases was 0.28 in sample # 1. The A2B2 esterases were observed just in sample # 2 with a frequency of

Table 2: Pirimiphosmethyl resistance characteristics of Tunisian *Culex pipiens* in presence and absence of synergists DEF and Pb.

Population	Pirimiphosmethyl			Pirimiphosmethyl +DEF					Pirimiphosmethyl +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
Slab	2.9 (2.5-3.4)	2.34 ± 0.18	-	0.30 (0.16-0.56)	1.7 ± 0.42	-	9.79 (6.16-15.5)	-	0.40 (0.31-0.55)	1.47 ± 0.18	-	7.2 (5.7-9.1)	-
1-Tozeur	20 (12-31)	2.49* ± 0.31	6.9 (4.4-10.7)	1.6 (0.39-6.5)	1.05 ± 0.3	5.42 (2.91-10.1)	12.4 (6.70-23.2)	1.3	9.0 (5.0-13)	1.01 ± 0.22	22.3 (17.2-29.1)	2.2 (1.3-3.7)	0.31
2-Gabès	78 (31-200)	1.84 ± 0.66	27.0 (14.3-50.7)	33 (30-36)	3.65 ± 0.29	113 (71.7-178)	0.59 (0.39-0.91)	0.23	54 (34-85)	3.9 ± 1.2	135 (71.0-258)	0.36 (0.17-0.77)	0.20
3-Borj El Khadra	-	-	-	-	-	-	-	-	-	-	-	-	-

(a): 95% CI

*The log dose-probit mortality response is parallel to that of S-Lab

**Parallelism test positive but without probability;

RR_{50}: resistance ratio at LC_{50} (RR_{50}=LC_{50} of the population considered / LC_{50} of Slab)

SR_{50}: synergism ratio (LC_{50} observed in absence of synergist / LC_{50} 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)

0.11. Our results confirms the previous studies which showed the major implication of esterases in the resistance to OPs insecticides [4,25,27,30-36].

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Author Affiliation

Top

[1]*Laboratory of Genetics, Faculty of Medicine of Monastir, Monastir University, Monastir-5019, Tunisia*

[2]*Department of Hygiene and Environmental Protection, Ministry of Public Health, Bab Saadoun, Tunis-1006, Tunisia*