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Pirimiphos-Methyl Resistance Status of Field Populations of *Culex pipiens* (Diptera: Culicidae) From Grand Tunis Area, Northeast Tunisia

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Abstract

Mosquito species are responsible for the transmission of many parasitic diseases and their control leads to the phenomenon of resistance to insecticides. Five *Culex pipiens* samples were collected from various localities of Grand Tunis area, Northeast Tunisia, between June 2003 and November 2005. All the studied samples were resistant to pirimiphos methyl (organophosphorus insecticide). Our results showed the implication of insensitive acetylcholinesterase and overproduced esterases in the résistance of the Tunisian populations of *Culex pipiens*. Results were discussed in relation to resistance mechanism.

Keywords: *Culex pipiens*; Resistance; Pirimiphos-methyl; Insensitive acetylcholinesterase; Overproduced esterases; Northeast Tunisia

Introduction

In addition to their nuisance, mosquitoes cause vector-borne diseases [1-3] and their impact on human health is very considerable [4]. Worldwide, mosquito species such as *Culex* are responsible for the transmission of parasitic diseases such as filariasis, yellow fever and West Nile virus [5,6]. Mosquito control by insecticides is very effective on culicidae mosquitoes, but has several disadvantages. In addition to a detrimental effect on aquatic life, they can be the cause of various environmental problems [5], in particular the phenomenon of insect resistance to insecticides [7-12].

Due to the problem of resistance of vectors to chemical insecticides, several alternative insecticides have been developed to evaluate their efficiencies. Pirimiphos-methyl is an organophosphorus (OP) insecticide having a fast action with less toxicity for humans and environment. The use of this insecticide against mosquito adults has already been reported [13-15]. The low remanence of pyrimiphos-methyl, used as adulticide, is due to its high vapor pressure which diffuses it rapidly into the ambient atmosphere [16,17]. It should be mentioned that according to our knowledge, no published studies have been done on resistance of *Culex pipiens* to pirimiphos methyl in Tunisia.

The aim of this study was to determine the pirimiphos-methyl resistance status of field populations of *Culex pipiens* (Diptera: Culicidae) from Grand Tunis Area, Northeast Tunisia.

Materials and Methods

Study area

Grand Tunis is the name for the greatest metropolitan area in Tunisia, which assembles four of the following states: Tunis, Ariana, Manouba and Ben Arous.

Mosquitoes

Five *Culex pipiens* samples were collected at preimaginal stages from breeding sites in 5 localities between June 2003 and November 2005 (Table 1 and Figure 1). Reference strains included S-Lab, an insecticide-susceptible strain without any known resistance genes [18]. SA2 and SA5 were used as references of resistant strains with A2-B2 and A5B5, respectively.

Bioassays

Assays were performed as described by Raymond et al. [19], 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.

Over-produced esterases

Esterases were characterized on homogenates of adult thorax and abdomen according to the method of Pasteur et al. [20,21].

Data analysis

Data were subjected to probit analysis [22] using a BASIC program [19].

Results and Discussion

The linearity of the dose-mortality response was rejected for all samples, with the exception of S-Lab and 3 field samples (#2, 3, and 5). All the studied samples, collected between June 2003 and November

Code	Locality	Breeding sites	Date of collection	Mosquito control (used insecticides)	Agricultural pest control
1	Sidi Thabet	Ditch	Aug, 2004	Rare (C, P)	Yes
2	Sokra	Canal	June, 2003	Very frequent (C, Pm, tF, P, D)	Yes
3	Mannouba	River	June, 2005	Occasional (P, D)	Yes
4	Ouardia	Ditch	Aug, 2005	Very frequent (C, F, P, D)	None
5	Ezzahra	Ditch	Nov, 2005	Very frequent (C, F, P, D, T)	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.

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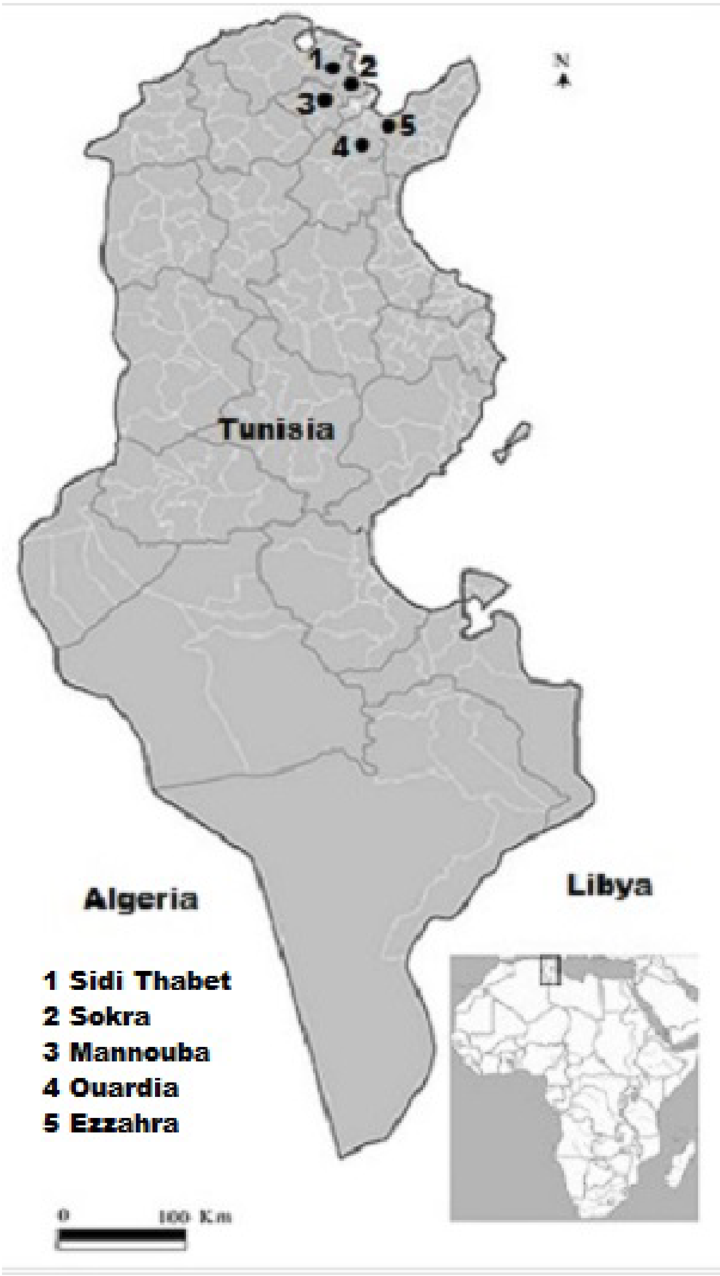

Figure 1: Geographic origin of Tunisian populations.

2005, were resistant to pirimiphos methyl. The RR50 ranged from 12.6 in sample #3 to 231 in sample #5 (Table 2). This can be explained by the massive use of this insecticide in the control against these insects but the survey carried out is not in agreement with the results found: only one locality by five prospected was the object of control by pirimiphos-methyl.

The DEF decreased significantly the tolerance to pirimiphos methyl in S-Lab and the five field samples (SR50>1, p<0.05) (Table 2). The DEF had a synergistic effect significantly higher than that recorded in S-Lab (RSR>1) only in samples #4. Therefore, the increased detoxification by EST (and/or GST) was involved in the pirimiphos methyl resistance only in this sample. This mechanism accounts for only a part of this resistance because the RR50 remained significant in the presence of the DEF (RR50>1, p<0.05). The addition of Pb decreased significantly the tolerance to pirimiphos-methyl in S-Lab (SR50=7.24, p<0.05) and in samples #2 and #5, but the SR was significantly higher than that recorded in S-Lab only in sample #5 (Table 2). Therefore, the increased detoxification by the CYP450 was involved in the pirimiphos methyl resistance only in this population. This mechanism explains only a part of this resistance because RR50 remained significant in the presence of Pb (RR50>1, p<0.05). Our synergist study showed that the increased detoxification by EST (and/or GST) and CYP450 had only a minor role in the pirimiphos methyl resistance, results already confirmed by previous studies [23,24]. It should be noted that esterases, GSTs and cytochrome P450 enzymes are not always sensitive to used synergists.

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-Sidi Thabet	67 (29-155)	1.16 ± 0.25	23.2 (15.2-35.5)	24 (16-35)	2.15 ± 0.32	82.2 (49.5-136)	2.77 (1.72-4.45)	0.28	43 (37-49)	2.34 ± 0.21	107 (85.5-133)	1.5 (1.04-2.3)	0.22
2-Sokra	135 (102-184)	1.17 ± 0.12	46.2 (37.4-57.1)	60 (32-110)	5.37** ± 3.46	201 (59.1-688)	2.24 (0.63-7.96)	0.22	37 (19-72)	2.52 ± 0.64	94.1 (61.2-144)	3.5 (2.1-5.7)	0.49
3-Mannouba	37 (31-43)	1.57 ± 0.13	12.6 (10.4-15.3)	6.9 (4.7-10)	1.21* ± 0.16	16.7 (12.1-23.0)	5.33 (4.22-6.72)	0.75	32 (15-66)	2.2 ± 0.7	80.3 (43.1-149)	1.1 (0.62-2.0)	0.16
4-Ouardia	38 (27-52)	2.52 ± 0.43	13.1 (9.02-19.0)	1.3 (0.33-2.9)	0.34 ± 0.08	4.5 (2.8-7.0)	28.5 (20.0-40.6)	2.9	23 (15-41)	1.07* ± 0.15	58.5 (44.4-77.2)	1.6 (1.09-2.4)	0.22
5-Ezzahra	676 (656-689)	19.2 ± 2.36	231 (172-311)	376 (258-789)	1.33* ± 0.32	1262 (726-2195)	1.79 (1.24-2.59)	0.18	27 (18-38)	2.8 ± 0.71	67.6 (44.2-103)	24.8 (15.6-39.3)	3.4

(a) 95% CI; * The log dose-probit mortality response is parallel to that of S-Lab; ** Parallelism test positif but without probability.
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: Pirimiphosmethyl resistance characteristics of Tunisian *Culex pipiens* in presence and absence of synergists DEF and Pb.

Mortality caused by propoxur were 0% in sample #5 which showed the highest resistance levels to studied pirimiphos-methyl insecticide, 18% in sample #4, 21% in sample #2, 46% in sample #1 and 48% in sample #3. The mortality due to propoxur was significantly correlated with the LC50 of pirimiphos methyl (P<0.05) indicates an acetylcholinesterase insensitive. Five esterases were observed in studied field samples. The esterase C1 encoded by the Est-1 locus and four esterases encoded by the *Ester* super locus: A1, A2-B2, A4-B4 (and/or A5-B5, which has the same electrophoretic mobility) and B12. One or several esterases were detected in all the studied samples. The A1 esterases were observed only in samples #3 and #5, with a low phenotypic frequency of 0.03. The C1 esterases were observed only in samples #1 and #5, with a low phenotypic frequency of 0.06 and 0.11, respectively. The other esetrases were detected almost in all collected samples with frequency which vaired between 0.03 and 0.48. Esterases play an important role in the resistance of several insects in the world [25-31]. Our results confirms the previous results of Ben Cheikh et al. [28] which reported the existence of a correlation between the frequency of individuals possessing the Ace-1R allele and those overproduced esterases A and B among the Tunisian populations of *Culex pipiens*. However, other studies are not in agreement with these results and suggested that overproduction of esterases and modifications of AChE are not correlated [11,12].

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