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Research Article

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Evaluation of Fenitrothion against *Culex pipiens* (Diptera: Culicidae) Larvae in Grand Tunis Area of Tunisia

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Abstract

Resistance toward 2 insecticides (fenitrothion and propoxur) was analyzed in 5 samples of *Culex pipiens* populations collected from various localities of Grand Tunis area, Northeast Tunisia. All studied samples were resistant to fenitrothion and propoxur insecticides. The study of esterase's activities showed the existence of five overproduced esterases: C1, A1, A2-B2, A4-B4 (and/or A5-B5) and B12. One or several esterases were detected in the studied samples. We also showed that the resistance to fenitrothion was correlated with the propoxur resistance indicating that modifications of the target, the AChE 1, can be involved in the fenitrothion resistance. These results should be considered in the current mosquitoes control programs in Tunisia.

Keywords

Culex pipiens; Grand Tunis area; Fenitrothion; Propoxur; Resistance; Esterases; AChE; Control programs

Introduction

The control of mosquito vectors of human diseases is a global health issue. Their ability to resist to insecticide treatments threatens the prevention of epidemics. This systematic use of insecticides to control mosquito larvae has led to the occurrence of resistance phenomena, which considerably reduces the effectiveness of the treatments [1]. Between 1948 and 1990, the number of insect resistance cases increased steadily: 14 species in 1948, 224 in 1969 and more than 500 in 1990 [2]. In several species of mosquitoes, including *Culex pipiens*, these resistances have already been demonstrated for conventional insecticides [3,4]. Resistance is studied at several biological levels, using molecular, genetic, cellular, metabolic and ecological techniques among others [5-12]. The detection and monitoring of the resistance developed by natural mosquito populations will be essential to better manage them in the field as long as alternatives to insecticides are not put in place.

The organophosphate insecticides (OPs) and synthetic pyrethroids have been used for a long time to fight against mosquitoes. Fenitrothion belongs to the chemical group of organophosphates which inhibit acetylcholinesterase, an enzyme involved in the regulation of nerve impulses [13]. This insecticide has moderate toxicity to mammals. The aim of this paper was to evaluate the resistance level of fenitrothion against *Culex pipiens* larvae in Grand Tunis Area of Tunisia.

Materials and Methods

Mosquitoes

Five populations of *Culex pipiens* were collected from natural habitats of Grand Tunis area, Northeast Tunisia, between June 2003 and November 2005 (Table 1 and Figure 1). A susceptible strain named S-Lab was used as reference. Two other strains characterized by the presence of esterases A2B2 (SA2 strain) and A5B5 (SA5 strain) were used as positive control in starch electrophoresis.

Chemical insecticides

Assays were performed using two insecticides: fenitrothion (98.5% [AI], brought from laboratory Dr Ehrenstorfer, Germany), and propoxur (99.9% [AI], Bayer AG, Leverkusen , Germany), organophosphates and carbamates compounds, respectively.

Bioassays test and data analysis

Twenty third and/or fourth instar larvaes were used for bioassays. We used five different fenitrothion and propoxur concentrations which affect a mortality of 0% to 100%. Bioassays carried out in triplicates except control where we made five repetitions without insecticide. Reading of the larval mortality was carried out after 24 hours of exposure. Mortality data were analyzed by using the log-probit program of Raymond [14], based on Finney [15].

Synergism tests and esterase's detection

Synergism tests consists to perform the same previous bioassays with the addition of 0.5 ml of the maximum sub-lethal concentration of an esterase inhibitor DEF, S,S,S-tributyl phosphorotrithioate (0.5 μg/ml) to each cup. The same for piperonyl butoxide (PB), an inhibitor of mixed function oxidases. The two synergists were added 4 hours before the start of bioassays. Esterase phenotypes were established by starch electrophoresis (TME 7.4 buffer system) as described by Pasteur et al. [16,17] using adults specimens.

Results

Fenitrothion resistance

S-Lab and 4 field samples (# 1, 3, 4, and 5) showed a linear dose-mortality responses ($p<0.05$). As indicated in Table 2, all studied samples were resistant at LC_{50}. The RR_{50} varied from 14.5 in sample # 3 to 887 and sample # 5. Agricultural pest control and used other OPs insecticides were probably responsible for the resistance of populations collected from sites not subject to fenitrothion control.

The study of the effect of DEF on fenitrothion resistance showed the minor role played by the EST (and/or GST) in sample # 4. In fact, the SR was higher than that found in S-Lab and $RR_{50}>1$ in the presence of DEF (Table 2). The effect of Pb on fenitrothion resistance showed the same situation in sample # 5. P450 cytochrome mediated monooxygenases inhibited by Pb account for only a part of the fenitrothion resistance for sample # 5 ($RR_{50}=112$, $p<0.05$, RSR=7.9).

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

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, F, 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

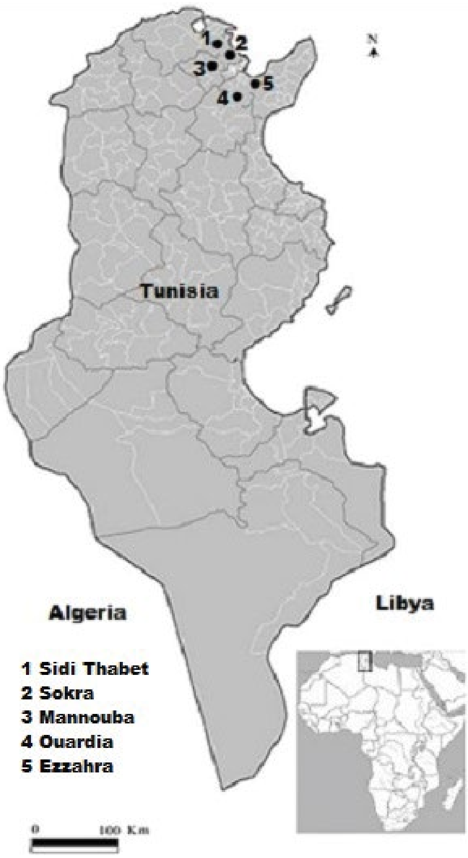

Figure 1: Geographic origin of Tunisian populations.

Cross-resistance fenitrothion/propoxur

The different percentages of mortalities caused by propoxur 46%, 21%, 48%, 18%, and 0% were recorded in the resistant samples # 1, 2, 3, 4, and 5, respectively. All recorded percentages were correlated with the resistance rates of fenitrothion (P<0.01).

Esterase's activities

The study of esterase's activities showed the existence of five esterases: C1, A1, A2-B2, A4-B4 (and/or A5-B5) and B12. One or several esterases were detected in the studied samples with different percentages ranged from 0.02 to 0.47. The involvement of these enzymes in the recorded resistance confirmed partially the previous results of the synergist used in bioassays.

Discussion

Several authors studied and evaluated the resistance rate of many mosquitos' species in the world like Rong et al. [18] who carried out the level of fenitrothion resistance and other insecticides on *An. gambia* from Benin. Authors in this study showed the high level of resistance to fenitrothion of *Culex pipiens* populations collected from Tunisia. This level is higher than existed in other countries of the world: It was 11.2-fold in *Cx. pipiens quinquefasciatus* from Brazil [19], and 4.1-fold in *Aedes aegypti* from Cuba [20]. The fenitrothion resistance levels of *Culex pipiens* were low in Senegal [21,22].

The study of the effect of DEF on fenitrothion resistance showed the minor role played by the EST (and/or GST) in one among five used samples. These results were not in agreement with those found by starch electrophoresis where five esterases were detected to be involved in the resistance of all field samples. Several studies confirmed the correlation between OPs insecticides and overproduced esterases and GST in *Culex pipiens* and other insect species [8,10,23-25]. CYP450 inhibited by Pb account for only a part of the fenitrothion resistance for one among 5 tested samples. These results were in agreement with those found by Ben Cheikh et al. [8] obtained on *Culex pipiens* from Tunisia resistant to chlorpyrifos (OP).

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

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-Sidi thabet	239 (195-296)	1.39 ± 0.12	71.5 (36.5-140)	124 (79-196)	1.45 ± 0.2	93.7 (65.0-134)	1.9 (1.4-2.5)	0.76	178 (173-237)	2.46 ± 0.29	61.7 (24.0-158)	1.3 (0.98-1.8)	1.2
2-Sokra	291 (5.2-139)	3.77 ± 1.37	85.0 (10.8-701)	668 (478-946)	4.08 ± 0.88	501 (319-787)	0.43 (0.04-4.2)	0.17	257 (221-288)	4.36 ± 0.45	89.0 (31.2-254)	1.1 (0.15-8.1)	0.95
3-Mannouba	48 (37-61)	1.27 ± 0.11	14.5 (7.2-29.1)	23 (15-34)	0.99 ± 0.1	17.4 (13.2-23.1)	2.0 (1.6– 2.6)	0.83	22 (16-31)	1.62* ± 0.23	7.81 (2.5-24.0)	2.1 (1.6-2.8)	1.8
4-Ouardia	308 (233-418)	0.87 ± 0.06	91.9 (47.8-176)	20 (8.1-52)	0.63 ± 0.11	15.5 (10.9-21.9)	14.8 (11.4-19.3)	5.9	257 (117-565)	1.94 ± 0.41	89.2 (28.9-275)	1.1 (0.73-1.9)	1.03
5-Ezzahra	2970 (2740-3210)	8.74 ± 1.08	887 (370-2124)	1430 (685-3050)	3.31 ± 0.85	1075 (539-2143)	2.0 (0.83-5.1)	0.82	325 (162-537)	1.8 ± 0.5	112 (28.0-451)	9.1 (5.6-14.8)	7.9

(a): 95% CI; * The log dose-probit mortality responses is parallel to that of S-Lab;

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)

We showed also a strong correlation between fenitrothion resistances of all resistant samples with the propoxur resistance. The AChE 1 is probably involved in the recorded resistance. The resistance allele, *Ace-1R,* is present worldwide and causes OPs resistance in several mosquito species [3,9,26].

Our field work presents a certain weakness due to a lack of physicochemical characterization of the mosquito breeding sites. A hypothesis about a possible relationship between chemical disturbances of water and the potential for resistance of larvae to insecticides would be of great importance.

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