Hereditary Genetics

Tabbabi et al., Hereditary Genet 2017, 6:2
DOI: 10.4172/2161-1041.1000177

Research Article Open Access

Study on Resistance of *Culex pipiens* (Diptera: Culicidae) Populations to Fenitrothion in Northern Tunisia

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Abstract

Four populations of *Culex pipiens* were collected as larvae in Northern Tunisia to evaluate their resistance status against two insecticides: fenitrothion and propoxur. At LC50, the sample # 1 was susceptible, whereas all the other samples were resistant. The RR50 ranged from 1.08 in sample # 1 to 550 in sample # 3. The A2-B2, A4-B4 (and/or A5-B5), B12 and C1 esterases were found in collected samples and the frequencies ranged from 0.02 to 0.42. Propoxur caused a mortality of 0% in samples # 3 which showed the highest resistance levels to fenitrothion insecticide and 87% in sample # 1 which was susceptible hence the involvement of AChE 1 in the recorded resistance. Our results are essential for the development of such strategies of vector control.

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

Introduction

Formerly, insect populations resistant to pesticides were controlled either by increasing the quantity of product used, either by applying new active ingredients. Both strategies are now over. The use of increasing amounts of insecticides is a danger to the environment and is very costly; moreover, the discovery and development of new insecticides is clearly decreasing. There are thus few alternatives to control insects such as *Culex pipiens* which are resistant to insecticides, whether organophosphates, carbamates or pyrethroids [1-10]. Fenitrothion is one of the most popular organophosphorus (OP) insecticides used worldwide, which inhibits arthropod [11].

All of these considerations prompt urgent action, based on the development of appropriate strategies for the use of pesticides. Data inherent to insecticides, their toxicity and their interactions with arthropod action sites, to the knowledge of biochemical resistance mechanisms, are essential for the development of such strategies. It is in this context that this document is inscribed. We reported in this paper a study on fenitrothion resistance of *Culex pipiens* populations collected in four breeding sites of Northern Tunisia (Figure 1 and Table 1).

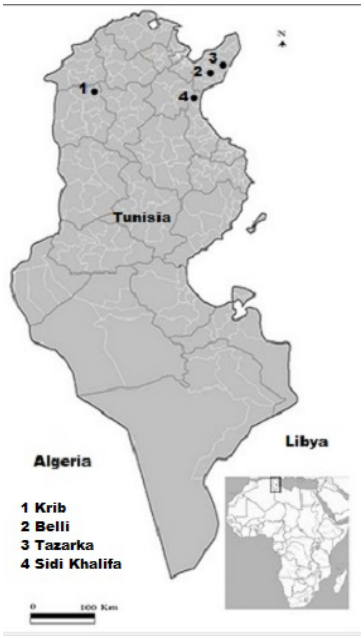

Figure 1: Geographic origin of Tunisian populations.

Materials and Methods

Mosquito strains

Four populations of *Culex pipiens* were collected as larvae and pupae in Northern Tunisia between August 2003 and October 2005. Collected samples were reared in the laboratory for further bioassays. Three strains were used as references: S-Lab was a sensitive strain, SA2, and SA5 were resistant strains with A2-B2 and A5-B5 esterases, respectively.

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. We used two synergists in order to detect detoxification enzymes involved in resistance: S, S, S {ributyl phosphorothioate (DEF), an esterase inhibitor, and piperonyl butoxide (pb), an inhibitor of mixed function oxidases.

Bioassay procedures and data analysis

Bioassays were realized on late third and early fourth instar larvae according to procedures of WHO [12]. Results were analysed for the median lethal concentration (LD50) and LD95 by probit analysis using a Basic program [13].

Esterase's detection

Esterase phenotypes were established by starch electrophoresis (TME 7.4 buffer system) as described by Pasteur et al. [14,15] using homogenates of thorax and abdomen.

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Received May 04, 2017; **Accepted** May 10, 2017; **Published** May 19, 2017

Citation: Tabbabi A, Daaboub J, Ben Cheikh R, Feriani M, Boubaker C, et al. (2017) Study on Resistance of *Culex pipiens* (Diptera: Culicidae) Populations to Fenitrothion in Northern Tunisia. Hereditary Genet 6: 177. doi:10.4172/2161-1041.1000177

Code	Locality	Breeding site	Date of collection	Mosquito control (used insecticides)	Agricultural pest control
1	Krib	River	0ct. 2005	Occasional (P)	Yes
2	Belli	River	Aug. 2003	Rare (C,D)	Yes
3	Tazarka	River	May 2005	Very frequent (C, T, Pm, F, P, D)	Yes
4	Sidi khalifa	Water pond	July 2004	None	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)	-
Krib	3.6 (1.9-6.6)	2.36 ± 0.52	1.08 (0.47-2.4)	-	-	-	-	-	-	-	-	-	-
Belli	8.7 (5.0-14)	1.13 ± 0.15	2.6 (1.3-5.1)	7.5 (3.2 - 17)	0.94 ± 0.19	5.6 (3.7-8.4)	1.1 (0.78-1.7)	0.46	14 (9.8-20)	1.58* ± 0.2	4.9 (1.9-12.5)	0.61 (0.43-0.84)	0.52
Tazarka	1840 (1710-1980)	6.38 ± 0.61	550 (241-1250)	1990 (1790-2220)	3.52 ± 0.25	1497 (1140-1965)	0.92 (0.71-1.1)	0.37	1040 (953-1130)	4.55 ± 0.35	360 (119-1087)	1.7 (1 .3-2.3)	1.5
Sidi khalifa	126 (74-215)	1.43 ± 0.21	37.7 (18.6-76.5)	26 (8.0-51)	0.71 ± 0.17	19.8 (14.3-27.4)	4.7 (3.1-7.2)	1.9	37 (26-54)	1.56* ± 0.24	13.0 (4.2-40.3)	3.3 (2.2-5.0)	2.9
(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).													

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

Results

Fenitrothion resistance

The linearity of the dose-mortality response was accepted (p<0.05) for S-Lab and field samples # 3. At LC50, the sample # 1 was susceptible, whereas all the other samples were resistant (Table 2). The RR50 ranged from 1.08 in sample # 1 to 550 in sample # 3. High resistance levels were manifested by sample # 3 (>500 folds). The samples # 1 and 2 showed low resistance levels, not exceeding 10-fold. At LC95, RR95>100 in samples # 3 and 4.

The addition of DEF to fenitrothion bioassays decreased significantly the tolerance in S-Lab (SR50=2.5, p<0.05) and sample # 4 (Table 2). The SR was not significantly higher than that recorded in S-Lab in all samples. These results indicate that the increased detoxification by the EST (and/or GST) did not play any role in the resistance. The Pb had not a significant effect on the fenitrothion resistance in S-Lab (SR50 = 1.16, p<0.05). The resistance decreased significantly in sample # 4, but the SR50 was not significantly higher than that recorded in S-Lab in any samples (Table 2). These mechanisms did not account any portion of the fenitrothion resistance for all samples.

Cross-resistance of fenitrothion/propoxur

Propoxur caused a mortality of 0% in samples # 3 which showed the highest resistance levels to fenitrothion insecticide. The highest percentage of mortality was recorded in sample # 1 (87%) which showed a susceptibility to fenitrothion. Mortalities due to propoxur were 39% and 68% in resistant samples # 2 and 4, respectively. A strong correlation were found between mortality due to propoxur and the LC50 of fenitrothion (Spearman rank correlation, (r) = 0.69 (P<0.01)).

Esterase's activities

The A2-B2, A4-B4 (and/or A5-B5), B12, and C1 esterases were found in collected samples and the frequencies ranged from 0.02 to 0.42. The A1 esterase was not detected in any used sample.

Discussion

Our study on resistance of *Culex pipiens* to fenitrothion showed high levels compared to other studies on different mosquitoes in the world [16-18]. In addition to the treatments carried out in vector control, arthropod vectors are also subjected, depending on their ecology, to the insecticidal pressure resulting from agriculture or domestic uses, thus accelerating the appearance of the phenomenon and the spread of resistant alleles in the vector populations, resulting in a loss of effectiveness of the treatments.

Fenitrothion is one of the most popular organophosphorus insecticides used worldwide [11]. Intensive insecticide applications often result in accelerated biodegradation of the insecticide in the environment [19-21]. Many studies confirmed drastic increase of fenitrothion-degrading Pseudomonas, Flavobacterium, and Burkholderia in agricultural field soils [22-24].

Our synergist study showed that the increased detoxification by EST (and/or GST) and oxydases were not involved in the recorded resistance. Starch electrophoresis detected many esterases in all studied samples. This confirms the hypothesis that somes esterases, GSTs, and cytochrome P450 enzymes may be insensitive to the action of DEF and Pb. The involvement of EST and the GST in the OPs resistance was confirmed by many previous studies [2,3,25-32]. Our study is in agreement with previous publication on correlation between cytochrome P450 enzymes and resistance to pyrethroids [33].

We also showed that the resistance to the studied OP was correlated with the propoxur resistance hence the involvement of AChE 1, where mutations changed the sensitivity of AChE, in the recorded resistance. Our results were in agreement with many previous studies that have shown the role offered by the resistant allele, Ace-1, in many areas of the world [34-37].

Acknowledgements

This work was kindly supported by the Ministry of Higher Education and

Scientific Research of Tunisia by funds allocated to the Research Unit (Génétique 02/UR/08-03) and by DHMPE of the Minister of Public Health of Tunisia. We are very grateful to S. Ouanes, for technical assistance, A. Ben Haj Ayed and I. Mkada for help in bioassays, S. Saïdi, Tunisian hygienist technicians for help in mosquito collecting, and M. Nedhif and M. Rebhi for their kind interest and help.

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