Hereditary Genetics

Tabbabi et al., Hereditary Genet 2017, 6:1
DOI: 10.4172/2161-1041.1000176

Research Article OMICS International

Study Confirming Resistance to Fenitrothion of *Culex pipiens* (Diptera: Culicidae) from Central Tunisia

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Abstract

Five natural populations of *Culex pipiens* were taken as larvae in the central Tunisia to evaluate their resistance level of fenitrothion. Our study showed that all samples were resistant to fenitrothion at LC50. The RR50 ranged from 9.2 in sample # 2 to 59.2 in sample # 5. Starch electrophoresis detected the overproduced esterases in all studied samples. The most frequent esterase A2B2 was detected in samples # 5 with a frequency of 31%. Three other esterases were detected in samples # 1, 2, 3, and 4: A4-B4 and/or A5-B5, A12, and C1. Synergists showed that the involvement of CYTP450 in the resistance to fenitrothion (OP) is not neglected. Cross-resistance of fenitrothion and propoxur was detected indicate the involvement of target site alteration (AChE1) in fenitrothion resistance. It should be noted that study of the polymorphism of AChE 1 will be of great importance.

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

Introduction

In the absence of vaccine and therapeutic treatment, the control of mosquitoes by the use of chemical insecticides remains the preferred weapon. Unfortunately, the intensive and repeated use of the same insecticides (especially pyrethroids and organophosphates) for more than forty years has led to the selection and diffusion of resistance on a global scale [1-4]. The management of these resistances becomes problematic because very few new insecticides are developed for public health. Indeed, almost all the insecticides used against mosquitoes come from the agricultural market, because of the lack of investment by the agrochemical companies in a public health market considered too narrow and not very profitable.

Insecticide resistance is now seen by the World Health Organization (WHO), as a major obstacle to the control of mosquito-borne diseases. Resistance is likely to contribute to the re-emergence of arboviruses because of the inability to maintain effective control of mosquito populations.

The objectives of this work are multiple: to identify areas where insecticide resistance may challenge the control of mosquito vectors and to provide recommendations to the government to improve resistance management and to encourage the deployment of alternative control methods. We tested the fenitrothion insecticide which is an organophosphorous compounds largely used in *Culex pipiens* control.

Materials and Methods

Mosquitoes

We used eihgt colonies of *Culex pipiens* in this study: Five natural populations were taken as larvae in the central Tunisia (Table 1 and Figure 1), a sensitive strain called S-Lab to do comparisons with resistant strains, and two resistant strains SA2 and SA5 characterized by the presence of A2B2 and A5B5, respectively. The two last strains were used as reference in starch electrophoresis to identify overproduced esterases of collected populations.

Code	Locality	Breeding sites	Date of collection	Mosquito control (used insecticides)	Agricultural pest control
1	Kalaa kebira	River	July. 2003	Occasional (F, Pm, P, D)	None
2	Monastir	Ditch	Aug. 2003	Rare (C,F)	Yes
3	Moknine	Water pond	Aug. 2003	Very frequent (C)	Yes
4	Hajeb Laayoun	River	July. 2004	None	Yes
5	Sbiba	River	Sept. 2004	Rare (Pm, P)	Yes

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.

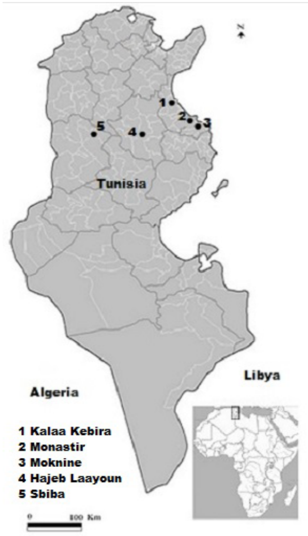

Figure 1: Geographic origin of Tunisian population.

Rearing of *Culex pipiens* in the laboratory

Larvae were directly transferred to the laboratory and putted in plastic basins containing water and rabbit crop which serves as food.

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Received May 04, 2017; **Accepted** May 10, 2017; **Published** May 15, 2017

Citation: Tabbabi A, Daaboub J, Laamari A, Cheikh RB, Cheikh HB, et al. (2017) Study Confirming Resistance to Fenitrothion of *Culex pipiens* (Diptera: Culicidae) from Central Tunisia. Hereditary Genet 6: 176. doi:10.4172/2161-1041.1000176

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-kalaa Kebira	51 (26-98)	1.52 ± 0.3	15.2 (7.18-32.3)	17 (8.5-36)	1.07 ± 0.19	13.3 (8.8-19.9)	2.8 (1.8-4.5)	1.1	14 (12-17)	1.6 ± 0.11	5.0 (2.0-12.4)	3.5 (2.4-5.1)	3.0
2-Monastir	30 (25-37)	1.27 ± 0.08	9.2 (4.8-17.4)	21 (12-35)	1.04 ± 0.12	16.1 (11.5-22.5)	1.4 (1.1-1.8)	0.57	24 (19-30)	1.23* ± 0.08	8.3 (3.2-21.0)	1.2 (1.1-1.5)	1.1
3-Moknine	115 (45-291)	0.93 ± 0.18	34.4 (16.8-70.4)	38 (15-97)	0.81 ± 0.15	28.8 (19.4-42.9)	2.9 (1.9-4.6)	1.2	105 (45-249)	0.63 ± 0.08	36.4 (14.7-90.3)	1.0 (0.74-1.6)	0.94
4-Hajeb laayoun	103 (63-169)	1.56 ± 0.25	30.9 (15.2-62.5)	17 (9.6-31)	1.32 ± 0.29	13.1 (8.8-19.5)	5.9 (3.9-8.9)	2.3	12 (9.1-17)	1.76 ± 0.26	4.2 (1.3-13.0)	8.4 (5.6-12.8)	7.3
5-Sbiba	198 (103-377)	2.54 ± 0.64	59.2 (24.3-144)	247 (111-548)	1.82 ± 0.43	185 (101-337)	0.80 (0.36-1.7)	0.32	61 (46-81)	1.89 ± 0.19	21.3 (8.5-53.6)	3.2 (1.7-5.8)	2.8

(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.

Adults were transferred to cages and both sexes were fed on sugar water. Only females then blood fed on birds to be able to lay.

Chemical insecticides

Two insecticides were used for bioassays: the organophosphates fenitrothion (98.5% [AI]), brought from laboratory Dr Ehrenstorfer, Germany) and the carbamate propoxur (99.9% [AI], Bayer AG, Leverkusen, Germany).

Bioassays and data analysis

Third and fourth instar larvae were used to do bioassays according standard protocol of the World Health Organization (WHO). [5]. Brieftly, five insecticide concentrations were used for each assay and five replicates for each concentration. Lethal concentrations (LCs) and all related data were calculated via probit analysis [6]. The sensitive strain S-Lab was used as reference to do analysis.

Mechanisms involved in the resistance to fenitrothion

We used two synergists to identify the role of esterases, GST, and CYTP450 in the recorded resistance. The only exception compared to previous assays was to add 0.5 ml of the maximum sub-lethal concentration of an esterase inhibitor, S,S,S-tributylphosphorotrithioate, (0.5 µg/ml) to each cup with 0.5 ml of insecticide and piperonyl butoxide (pb), an inhibitor of mixed function oxidases. The addition should be done 4 hours before the start of the bioassays. Esterase phenotypes were established by starch electrophoresis (TME 7.4 buffer system) as described by Pasteur et al. [7,8] using adults specimens.

Results

Fenitrothion resistance

The S-Lab was the only strain with accepted linearity of the dose-mortality response (p<0.05). As indicate in Table 2, all samples were resistant to fenitrothion at LC50. The RR50 ranged from 9.2 in sample # 2 to 59.2 in sample # 5.

The tolerance to fenitrothion insecticide decreased in S-Lab (SR50=2.5, p<0.05) and 4 among 5 field samples when DEF were added to bioassays (Table 2) despite SR of all samples were not significantly higher than that recorded in S-Lab. That's why no detoxification role in resistance has been given to EST (and/or GST). The fenitrothion resistance in S-Lab did not change after addition of PB synergist (SR50 = 1.16, p<0.05). As showed in Table 2, only the SR50 of sample # 4 was significantly higher than that recorded in S-Lab. That's why detoxification caused by CYTP450 played a minor role in recorded resistance in sample # 4 (RR50=4.2, p<0.05, RSR=7.3).

Cross-resistance fenitrothion/Propoxur

Mortality caused by propoxur were 72%, 41%, 11%, 79% and 12% for samples # 1, 2, 3, 4 and 5, respectively. A strong correlation were recorded between mortality due to propoxur and LC50 of fenitrothion [Spearman rank correlation, (r) = -0.69 (P<0.01)].

Esterase's activities

Starch electrophoresis detected the overproduced esterases in all studied samples. The most frequent esterase A2B2 was detected in samples # 5 with a frequency of 31%. Three other esterases were detected in samples # 1, 2, 3, and 4: A4-B4 and/or A5-B5, A12 and C1.

Discussion

The status of fenitrothion resistance in *Culex pipiens* was studied in central Tunisia to have data on levels of resistance of this species to this insecticide. Our study showed different levels of resistance to fenitrothion in the five population of *Culex pipiens* collected in central Tunisia despite almost all the insecticides used against mosquitoes come from the agricultural market (Table 1). Cross-resistance of public health and agriculture insecticides could explain the recorded resistance in all studied populations. It should be noted that fenitrothion resistance levels recorded in other areas of the wolrd is lower than recorded in Tunisia [9,10].

Our synergist study showed the non-involvement of EST (and/or GST) in fenitrothion resistance, although starch electrophorsis showed several overproduced esterases in all studied samples. These enzymes are probably involved in recorded resistance. Indeed, the action of the synergist employed in the toxicological tests (DEF) does not always result in the inhibition of esterases and GSTs. Many previous studies confirmed the association between resistance to OPs insecticides and overproduced esterases and/or GST [11-15]. Similar results have been reported in Malaysian *Culex quinquefasciatus* [16] and Malaysian *Aedes* aegypti [17].

The involvement of CYTP450 in the resistance to fenitrothion (OP) *could explain its involvement in the resistance to chlorpyrifos (OP) on Culex pipiens f*rom Tunisia [11]. However, previous studies showed the strong correlation between oxydases and pyrethroid resistance in

Malaysian *Culex quinquefasciatus* [18], *Aedes albopictus* [19,20], *and Aedes aegypti* [21].

A strong correlation was recorded between mortality due to propoxur and LC50 of fenitrothion (OP) indicating the involvement of AChE 1 in the recorded resistance. Similar studies were recorded by Labbé et al. [22] in several mosquito species.

The involvement of both metabolic mechanisms and target site alteration in multiple insecticide resistance has been reported in *Culex quinquefasciatus* from many parts of the world [23-25]. Furthermore, reduced insecticide penetration has been recorded in *Culex quinquefasciatus* [26].

Conclusion

In conclusion, it would be interesting to develop a spatial analysis using geographic information systems (GIS) to correlate the presence of different insecticides, treatments and tolerance of populations to insecticides throughout the country.

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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