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Temephos Resistance in Three Populations of *Culex pipiens* Collected from Three Districts of Southern Tunisia and Its Significance for the Resistance Mechanism

Jaber Daaboub[1,2], Ahmed Tabbabi[1*], Ali Lamari[1], Mohamed Feriani[1], Chokri Boubaker[1] and Hassen Ben Cheikh[1]

Abstract

Objectives: The aim of this study was to investigate the resistance to temephos (OP) in three populations of Culex pipiens collected from three districts of Southern Tunisia and its significance for the resistance mechanism.

Methods: Resistance to temephos insecticide was studied on *Culex pipiens* mosquitoes collected in three localities of Southern Tunisia. Larvae were used for different bioassays and adults were stored in -80°C for esterases identification.

Results: Bioassays revealed the susceptibility of sample # 3. The weakest resistance was recorded in sample # 3 (RR50 = 0.68) and the strongest resistance for the sample # 2 (RR50 = 3.6). At LC95, two samples (# 1, and 3) were susceptible. The use of synergists showed the non-involvement of resistance mechanisms inhibited by DEF and Pb. Our investigation reported that temephos resistance could be explained by the two most common mechanisms of resistance to OP (overproduced esterases and AChE 1 mutation).

Conclusion: Both detoxification mechanisms and target site alteration were involved in the resistance to temephos as reported in our study. These results are very important for the implementation and development of vector control strategies.

Keywords

Culex pipiens; Temephos resistance; Overproduced esterases; AChE 1 mutation; Southern Tunisia

Introduction

The use of insecticides around the world to control mosquitoes is regularly disputed, in particular because of the increasing resistance of these insects to products. This adaptation of mosquitoes threatens the prevention of epidemics in the absence of an alternative to insecticides. It is important to understand that the mosquito does not mutate to resist insecticides! Numerous mutations preexist in the immense populations of mosquitoes. When insecticides are present in the environment, mosquitoes that have mutations favorable to their survival reproduce and pass them on to their offspring, while sensitive mosquitoes die. Mutations that give mosquitoes the ability to resist organophosphates (OPs) are not spawned, but are selected by the environment. More simply, the frequency of mosquitoes carrying these mutations increases in a toxic environment.

Only three loci are responsible for major resistances, *Est-2*, *Est-3* and *ace-1* [1-4]. *Est-2* and *Est-3* form a super locus (designated by *Ester*) as they are very close in the genome; these genes encode esterases which trap or metabolise insecticides before they can inhibit acetylcholinesterase synapses. In the case of resistance, these esterases are produced in excess by a process of amplification of the number of copies of the genes which encode them in the genome or an increase in their expression [5-9]. Some resistance alleles have up to 50 copies of *Ester*, while the sensitive allele contains only one copy [10]. The ace-1 gene codes for the target of OPs insecticides, acetylcholinesterase 1 (AChE 1). In the case of resistance, this target is mutated, which reduces its affinity for OPs [7,11-13].

Temephos, belongs to the family of organophosphate (OP) insecticides, used in the fight against immature mosquitoes vectors due to its cost-effectiveness and community acceptance [14]. Recent study showed their effectiveness as a larvicide for mosquito control [15]. Many countries used this insecticide in mosquito control. However, its massive use had led to the development of resistance in different countries including Tunisia [16,17].

The aim of this study was to investigate the resistance to temephos (OP) in three populations of *Culex pipiens* collected from three districts of Southern Tunisia and its significance for the resistance mechanism.

Material and Methods

Strains

Between 2002 and 2005, *Culex pipiens* were sampled in three localities of Southern Tunisia and used for different bioassays tests. S-Lab [18] was used as a reference population for bioassays. SA2 and SA5 [19], were used as reference population for biochemical tests.

Insecticides and used synergists

Assays were performed as described by Raymond et al. [20], using ethanol solutions of temephos (95.5% [AI]), 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.

Bioassay tests for mosquito larvae and data analysis

Bioassays were done on larvae preferably on early fourth stage. Each bioassay test included five different temephos concentrations. Tests were carried out in triplicate with five repetitions of controls without insecticide. The mortality results were read after 24h hours of exposure and were analyzed by using the log-probit program of Raymond [21], based on Finney [22].

Starch gel electrophoresis

From each collection, we used sample of adult mosquitoes to study the elevated esterases according to the method of Pasteur [23].

***Corresponding author:** Ahmed Tabbabi, Laboratory of Genetics, Faculty of Medicine of Monastir, Monastir University, 5019, Monastir, Tunisia, Tel: +00216 97 085 424; E-mail: tabbabiahmed@gmail.com

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Table 1: Temephos resistance characteristics of Tunisian *Culex pipiens* in presence and absence of synergists DEF and Pb.

Population	Temephos			Temephos +DEF					Temephos +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	1.2 (1.1-1.4)	2.34 ± 0.22	-	0.32 (0.28-0.36)	4.99 ± 0.69	-	3.8 (2.8-5.0)	-	2.2 (1.7-2.8)	1.94 ± 0.28	-	0.56 (0.44-0.72)	-
1-Tozeur	2.3 (1.4-3.9)	2.73 * ± 0.63	1.9 (1.2-3.0)	-	-	-	-	-	-	-	-	-	-
2-Gabès	4.6 (3.8-5.4)	2.35 * ± 0.24	3.6 (2.9-4.5)	2.3 (1.5-3.4)	3.46 ± 0.53	7.2 (4.0-12.8)	1.9 (1.1-3.4)	0.51	6.3 (4.2-9.5)	3.51 ± 1.09	2.9 (1.6-5.1)	0.72 (0.40-1.2)	1.3
3- Bordj El Khadra	0.86 (0.62-1.1)	5.07 ± 1.5	0.68 (0.40-1.1)	-	-	-	-	-	-	-	-	-	-

(a), 95% CI; * The log dose-probit mortality response 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 used two references strains (SA2 and SA5) to identify detected esterases.

Results

Analysis of resistance levels

Linearity of the dose-mortality response was accepted (p>0.05) only for S-Lab and rejected for all samples except # 2. Bioassays revealed the susceptibility of sample # 3 (Table 1). The weakest resistance was recorded in sample # 3 (RR_{50} = 0.68) and the strongest resistance for the sample # 2 (RR_{50} = 3.6). At LC_{95}, 2 samples (# 1, and 3) were susceptibles. The use of synergists showed the non-involvement of resistance mechanisms inhibited by DEF and Pb (Table 1).

Analysis of resistance mechanisms and genes

We investigated the two most common mechanisms of resistance to OP (overproduced esterases and AChE1 mutation) on field collected populations. The over-produced esterases encoded by the *Est-2*, and *Est-3* loci were present in sample # 1 with frequency of 0.56 and 0.81 in sample # 2. The study of cross-resistance temephos/propoxur showed a strong correlation. In fact the mortality due to propxur was 1% for the sample having the strongest resistance to temephos (sample # 2) and 100% for the sample having the lowest resistance (sample # 3) indicated an insensitive AChE.

Discussion

Previous studies of Ben Cheikh et al. [16] showed that the temephos resistance levels of the Tunisian *Culex pipiens* were low (LC_{50} ranges of 0.0021–0.015lmg/l). This is in agreement with our results showing a low rate of resistance to this insecticide in Southern Tunisia. The resistance of *Culex pipiens* population collected in South east (sample # 3) may be associated with the use of temephos and other insecticides at different intensities and frequencies of application. In contrast, the susceptibility recorded in far South (sample # 3) could be explained by the absence of vector control in this locality because of lack of human population. Other studies reported LC50 of 0.01 mg/l in this species from India [24]. This value was 0.0000473 mg/l for *Culex quinquefasciatus* collected in the same country [25]. Recently, Ben Cheikh et al. [17] showed the contribution of overproduced esterases in the recorded resistance to the temephos insecticide which confirms our starch gel electrophoresis but not synergists results. The involvement of esterases enzymes system in resistance to OPs, carbamates [26,27] and pyrethroids [28] has been showed in mosquitoes and other insects.

It should be noted that despite many studies reported the temephos resistance in mosquitoes, the involved mechanism are not well characterized. Our investigation reported that temephos resistance could be explained by the AChE 1 insensivity and the increased detoxification by esterases enzymes. This result are in agreement with previous studies showing mutations on the acetylcholinesterase (*ace-1*) gene have been associated with OP resistance [29-31]. Previous studies on *Culex* populations showed that mutation in the acetylcholinesterase (*ace-1*) gene leading to G119S substitution is responsible for insensitivity to OP and carbamate insecticides [11].

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Author Affiliations

Top

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

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