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Susceptibility of field-collected mosquitoes (*Culex pipiens*) in Northern Tunisia to temephos, an organophosphate insecticide

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ABSTRACT

In order to understand the resistance of *Culex pipiens* to temephos and provide parameters for management programs, we evaluated the susceptibility levels to temephos of individuals collected in five localities of Northern Tunisia. Our results showed that sample # 2 was susceptible. The resistant samples displayed RR_{50} ranged from 1.3 in sample # 5 to 440 in sample # 4. Mortality caused by propoxur ranged from 0% in sample # 4, which showed the highest resistance levels to studied temephos insecticide and indicated an important contribution of AChE 1, to 68% in sample # 5. Starch gel electrophoresis identified many esterases in studied samples with an important frequency (85%) in the sample # 4. This sample showed the highest resistance to temephos with a major contribution of CYP450, esterases, and AChE 1. Both detoxification mechanisms and target site alteration were involved in the resistance to temephos as reported in our study. This is not a new phenomenon in mosquitoes, in which multiple insecticide resistance mechanisms has been reported worldwide.

Key Words: *Culex pipiens*, temephos resistance, detoxification mechanisms, target site alteration, Northern Tunisia.

Introduction

In Tunisia, *Culex pipiens* is very spread. This mosquito is strongly fought, especially by the use of insecticides because of the nuisance that it causes and its transmission of West Nile Virus [1-4]. For years, the organophosphates (OPs) and synthetic pyrethroids have been widely used in the mosquito control programs. Currently, in addition to pyrethroid insecticides (permethrin and deltamethnin), many organophosphates (OPs) including the temephos insecticide were largely used in *Culex pipiens* control [5,6].

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It's effective as larvicide for mosquitoes, it is inexpensive and it has low toxicity to mammals and, for this reason, it's widely used in mosquito control efforts [7]. In order to understand the resistance of *Culex pipiens* to temephos and provide parameters for management programs, we evaluated the susceptibility levels of individuals collected between 2002 and 2005 in five localities of Northern Tunisia.

Material and Methods

Mosquito strains: Eight strains were used for bioassays and biochemical study. Five field populations collected from Northern Tunisia. Three long established laboratory reference strains: S-Lab a susceptible strain was used for comparisons, SA2 and SA5 characterized by overproduced esterases A2-B2 and A5-B5, respectively were used to identify detected esterases in field populations [8].

Insecticides and synergists: The organophosphate temephos (95.5% [AI]), and the carbamate propoxur (99.9% [AI], Bayer AG, Leverkusen, Germany) were

used to test the susceptibility of different strains. S,S,Sributyl phosphorothioate (DEF), and piperonyl butoxide (PB) were used as synergists to detect different enzymes involved in the recorded resistance.

Bioassay: Bioassay tests utilized standard methods of Raymond et al. [9]. Data were subjected to probit analysis [10] using a BASIC program [11].

Esterases phenotypes: We determined esterase activity in individual mosquitoes of field populations according to the method of Pasteur et al. [12,13].

Results

Our results showed the susceptibility of sample # 2 with RR50 of 0.72 (Table 1). The resistant samples displayed RR50 ranged from 1.3 in sample # 5 to 440 in sample # 4. The synergist (DEF) effect was significantly higher than that recorded in S-Lab only in sample # 1 (Table 1). This indicates that the increased detoxification by the EST (and/or GST) was involved in the temephos tolerance only for this sample. The addition of Pb to temephos bioassays in sample # 5 did not decrease the resistance, considerably decreased the tolerance in samples # 3 (RR50=2.3, $p<0.05$, RSR=29.9) and 4 (RR50=21.4, $p<0.05$, RSR=20.6), and completely suppressed the resistance in samples # 1 (RR50= 0.23, $p<0.05$, RSR=6.6). Hence this mechanism was involved in the recorded resistance with different rates of contribution. Mortality caused by propoxur ranged from 0% in sample # 4, which showed the highest resistance levels to studied temephos insecticide and indicated an important contribution of AChE 1, to 68% in sample # 5. Starch gel electrophoresis identified many esterases in studied samples with an important frequency (85%) in the sample # 4 despite the increased detoxification by the EST (and/or GST) was not detected by synergists tests. This sample showed the highest resistance to temephos with a major contribution of CYP450, esterases, and AChE 1.

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-Ousja	1.9 (0.68-2.9)	1.3 ± 0.28	1.5 (1.05-2.2)	0.51 (0.44-0.60)	1.71 ± 0.11	1.6 (1.2-2.1)	11.7 (8.2-16.7)	0.96	0.51 (5.5-9.1)	1.72 ± 0.21	0.23 (0.16-0.32)	11.9 (7.2-19.4)	6.6
2-Krib	0.90 (0.77-1.0)	2.6 ** ± 0.3	0.72 (0.58-0.89)	-	-	-	-	-	-	-	-	-	-
3-Belli	86 (39-188)	2.24 * ± 0.66	69.4 (43.5-110)	-	-	-	-	-	5.1 (4.5-5.6)	2.87 ± 0.21	2.3 (1.8-2.9)	16.9 (10.9-26.1)	29.9
4-Tazarka	547 (208-1500)	3.77 ± 1.43	440 (171-1132)	321 (291-353)	7.63 ± 1.17	992 (666-1476)	1.7 (0.48-5.9)	0.44	47 (25-86)	5.09 ** ± 2.48	21.4 (7.0-65.6)	11.6 (2.7-48.4)	20.6
5-Sidi khalifa	1.7 (1.4-1.9)	2.27 * ± 0.2	1.3 (1.2-1.6)	0.57 (0.12-2.4)	2.51 ± 1.02	1.7 (0.60-5.0)	3.0 (1.2-7.3)	0.78	2.7 (2.1-3.4)	2.17 ± 0.28	1.2 (0.91-1.6)	0.62 (0.48-0.82)	1.1

(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).

Discussion

Between 1990 and 1996, Ben Cheikh et al. [5] reported the resistance to temephos insecticide (OP) on *Culex pipiens* collected from Tunisia. In their report, the most resistant population showed that resistance to temephos was uniformly low and reached 10-folds. Our results reported more high resistance reached 400-folds in one among five studied populations. Previous studies carried out in other countries showed that this level ranged from 200 to 2.8-folds [14-19].The results found in our study can be explained by the massif use of temephos and other insecticides in the control of mosquito larvae in these areas. They are part of the products used in the context of the fight against larval by DHMPE of the Minister of Public Health of Tunisia. According to Faraj et al. [20], resistance levels in *Culex pipiens* larvae, if not due to intensive previous use, can only be explained by acquisition of cross-resistance. Indeed, Sinègre et al. [21] found resistance to other organophosphorus compounds in *Culex pipiens* treated with chlorpyriphos. Chavasse and Yap. [22] confirmed also that the prolonged use of an organophosphorus insecticide always leads to the appearance of cross-resistance to other organophosphates.Synergist tests and starch gel electrophoresis showed the partial involvement of esterases in the recorded resistance to temephos. Kao et al. [23], Yan and Sudderuddin [24], and Chen et al. [25] found similar finding, in which a strong correlation was reported between EST activity and temephos resistance in *Musca domestica* and *Aedes aegypti*. On the other hand, this resistance was not associated with esterases enzymes in larvae and adults of *Aedes Albopictus* and *Aedes aegypti* [26].

The oxidases activity was involved in the recorded resistance with different rates of contribution. Nazni et al. [27] found the same results and confirmed that *Aedes Aegypti* larvae resistance to temephos could be due to the presence of oxidases activity. However, Paeporn et al. [28] reported that these enzymes are not playing any role in temephos resistance.

Mortality caused by propoxur indicated an important contribution of AChE 1 in resistant samples. This result was confirmed by many previous studies [5,29,30]. However, insensitive AChE did not play a clear role in temephos resistance as reported by Macoris et al. [31] and Saelim et al. [32].

Conclusion

Both detoxification mechanisms and target site alteration were involved in the resistance to temephos as reported in our study. This is not a new phenomenon in mosquitoes, in which multiple insecticide resistance mechanisms has been reported worldwide [33,34].

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