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Research Article

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Resistance of *Culex pipiens* (Diptera: Culicidae) to Chlorpyrifos Insecticide in Central Tunisia

Jaber Daaboub[1,2], Ahmed Tabbabi[1*], Raja Ben Cheikh[1], Ali Lamari[1], Ibtissem Ben Jha[1] and Hassen Ben Cheikh[1]

Abstract

Field populations collected as larvae in five localities of central Tunisia were used to study the resistance to chlorpyrifos insecticide. The resistance levels exceeded 10,000 folds in samples # 3 (East Center), and 5 (Western Center) not exceeding 5-fold in samples # 4 (East Center). Our result showed that detoxification by oxydases, EST and/or GST was responsible, at least in part, in resistance to chlorpyrifos in samples # 1, 3, and 5. Different esterases were detected in all studied samples. Except A2-B2 who recorded high frequencies ranged from 22% to 28%, all other detected esterases showed low percentages. Mortality caused by propoxur was very low in samples showed the highest resistance (# 3, and 5) and high in samples showed lowest resistant (# 1, 2, and 4) indicated an insensitive AChE 1. Our results of the resistance of *Culex pipiens* to chlorpyrifos in central Tunisia are consistent with those found in the literature. These data will help to better plan and program the vector control in Tunisia.

Keywords

Culex pipiens; Chlorpyrifos; Resistance; Detoxification enzymes; AChE 1; Central Tunisia

Introduction

The publication in 1962 of the book ''Silent Springs'' written by the biologist Rachel Carson, raised awareness of the problems related to pesticides on the environment. It was the first to denounce the harmful effects of these chemicals on non-target organisms such as birds. This work led to the banning of DDT in the United States in 1972. In addition to having an impact on non-target organisms, the intensive use of pesticides favors the emergence of resistance in pests. Misuse of these products can therefore cause their own inefficiencies on pests.

Today, the impact of phytosanitary products on the environment and living organisms is recognized and becomes a societal concern. A regulation has thus appeared to frame the use of these products. It is therefore necessary to understand the molecular and biochemical basis of resistance to improve insect pest control in the future. There are several types of mechanisms involved in resistance to insecticides. These mechanisms may be behavioral (different behavior of the insect in the presence of the insecticide), physiological (changes in the cuticle or changes in metabolism) or changes in the targets of the insecticide [1-5]. Ben Cheikh et al. [6,7] have reported that the Tunisian populations of *Culex pipiens pipiens* possess an AChE insensitive to propoxur inhibition and over-produced esterases known to be involved in OPs insecticides. These insecticides that have a role in the growth and development of the resistance are often used in the event of failure of other families of insecticides [8].

The current study was realized to evaluate the resistance of *Culex pipiens* of central Tunisia to chlorpyrifos insecticide. To contribute to the study of mechanisms involved in the observed resistances, we also investigated the effect of synergists, the S,S,S-tributyl-phosphorotrithioate (DEF) and the piperonly butoxide (Pb), on the resitance to chlorpyrifos insecticide. The cross-resistance between chlorpyrifos and propoxur, and the polymorphism of over-produced esterases were also investigated.

Material and Methods

Field populations collected as larvae in five localities of central Tunisia were used to study the resistance to chlorpyrifos insecticide. S-Lab was the susceptible strain used to do comparison with resistant strains. We used two resistant strains SA2 and SA5 selected for A2-B2 and A5-B5 esterases, respectively. Two insecticides were used for bioassays: chlorpyrifos (99.5% [AI]), brought from laboratory Dr Ehrenstorfer, Germany, and propoxur (99.9% [AI], Bayer AG, Leverkusen, Germany). The effect on chlorpyrifos resistance of 2 synergists, the DEF (98% [AI], Chem Service, England), and the Pb (94% [AI], Laboratory Dr Ehrenstorfer, Germany) was studied.

Bioassays were realized according to standard techniques used by Raymond et al. [9]. Mortality data were analyzed by using the log-probit program of Raymond [10], based on Finney [11]. We used starch gel electrophoresis to detect the presence of esterase's involved in resistance to chlorpyrifos [12,13]. Overproduced esterases from reference strains were run as controls: SA2 (A2-B2) and SA5 (A5-B5).

Results

As showed in Table 1, a large range of resistance to chlorpyrifos was recorded in studied samples (RR>1, $p<0.05$). The sample # 3 had the highest resistance to this insecticide reached 2747. The lowest resistance was recorded in sample # 4 (RR_{50}=4,5). The resistance levels exceeded 10,000 folds in samples # 3 and 5 not exceeding 5-fold in samples # 4. RR_{50} were 157 and 45, 9 for samples # 1, and 2, respectively.

Results of synergists tests were summarized in Table 1. These results showed that detoxification by EST and/or GST were responsible in resistance to chlorpyrifos in samples # 1, 3, and 5. The rate of resistance after addition of DEF remained high ($p<0.05$) that why we considered that detoxification enzymes had just a part of recorded resistance. Likewise, the effect of Pb on S-Lab and samples # 1, 3, and 5 is very clear in tale 1 showing the involvement of CYP450 in the recorded resistance. This implication was only partly because resistance to chlorpyrifos stay high after addition of the synergist (e.g., chlorpyrifos RR_{50}>1,000-fold in samples # 3). Different esterases were detected in all studied samples. Except A2-B2 who recorded high

***Corresponding author:** Tabbabi A, Laboratory of Genetics, Faculty of Medicine of Monastir, Monastir University, Monastir-5019, Tunisia, Tel: +216-97 085 424; E-mail: tabbabiahmed@gmail.com

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

Population	Chlorpyrifos			Chlorpyrifos +DEF					Chlorpyrifos +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	0.56 (0.53-0.58)	9.0 ± 1.04	-	0.17 (0.14-0.20)	2.85 ± 0.26	-	1.4 (1.08-1.8)	-	0.45 (0.17-1.3)	1.16 ± 0.43	-	0.53 (0.35-0.79)	-
1-kalaa Kebira	87 (52-148)	0.95 ± 0.1	157 (116-214)	32 (11-87)	0.76 ± 0.14	192 (128-288)	2.7 (1.8-3.8)	0.81	32 (14-71)	0.91 ± 0.15	73.5 (44.3-121)	2.6 (1.8-3.7)	2.1
2-Monastir	25 (16-37)	0.77 ± 0.06	45.9 (36.3-57.9)	11 (5.8-21)	0.88 ± 0.10	67.2 (47.6-94.9)	2.2(1.7-2.9)	0.68	63 (30-133)	0.99 ± 0.16	142 (85.3-239)	0.40 (0.28-0.55)	0.32
3-Moknine	1520 (951-2640)	0.43 ± 0.03	2747 (2227-3388)	404 (167-981)	0.67 ±0.09	2403 (1698-3400)	3.7 (2.9-4.8)	1.1	463 (101-2140)	0.65 ± 0.16	1040 (580-1864)	3.2 (2.2-4.7)	2.6
4-Hajeb laayoun	2.5 (1.1-5.6)	0.86 ± 0.16	4.5 (3.1-6.5)	1.2 (0.84-1.7)	1.09 ± 0.10	7.3 (5.6-9.5)	2.0 (1.4-2.8)	0.62	9.5 (4.5-20)	1.07 ± 0.18	21.4 (12.5-36.4)	0.26 (0.17-0.40)	0.21
5-Sbiba	1270 (705-2320)	1.3 ± 0.19	2288 (1541-3397)	210 (105-422)	0.93 ± 0.12	1249 (863-1806)	6.0 (4.1-8.8)	1.8	167 (135-206)	1.30 ± 0.07	375 (258-545)	7.5 (5.5-10.4)	6.1

(a): 95% CI; 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)

frequencies ranged from 22% to 28%, all other detoxification enzymes showed low percentages.

Mortality caused by propoxur explained logically the different rate of resistance recorded in studied samples. The rate of mortality was very low in samples showed the highest resistance (# 3, and 5) and high in samples showed lowest resistant (# 1, 2, and 4). We noticed a strong correlation between the mortality due to propoxur and the LC_{50} of chlorpyrifos (Spearman rank correlation, (r) = -0.90 (P<0.01)) indicated an insensitive AChE 1.

Discussion

Our results showed that the rate of resistance varied between 4,5 and 2747. The investigations of breeding sites characteristics showed the absence of insecticides control in the sample which had the lowest resistance and a frequent control in the sample which had the highest resistance to chlorpyrifos. Ben Cheikh et al. [7]. showed very important level of resistance to chlorpyrifos in many samples collected from Tunisia. Several previous studies showed similar results and confirmed a large variation in the tolerance to this insecticide [14-21].

Our result showed that detoxification by EST and/or GST was responsible partially in the recorded resistance to chlorpyrifos. Starch gel electrophoresis confirmed the synergist tests by detecting of several esterases in studied samples. The chlorpyrifos is an organophosphate insecticide and the strong correlation between overproduced esterase and resistance to different insecticides belonging to this family (OPs) was studied and confirmed by several authors [7,18,22-35]. Same authors showed the strong correlation between the insensitive AChE 1 and the resistance to OPs insecticides.

This implication of oxydases was partly involved in the recorded resistance to chlorpyrifos which confirmed previous studies on involvement of CYP450 in resistance to OPs insecticides [7,18,22,36,37].

Conclusion

Our results of the resistance of *Culex pipiens* to chlorpyrifos in central Tunisia are consistent with those found in the literature. These data will help to better plan and program the vector control in Tunisia.

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Author Affiliation

Top

[1]*Laboratory of Genetics, Faculty of Medicine of Monastir, Monastir University, Monastir-5019, Tunisia*
[2]*Department of Hygiene and Environmental Protection, Ministry of Public Health, Bab Saadoun, Tunis-1006, Tunisia*