

Pirimiphos-methyl Resistance of *Culex pipiens* Populations (Diptera: Culicidae) in Central Tunisia: Status and Mechanisms Involved

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Abstract: In Tunisia, *Culex pipiens* plays a role in the high annoyance experienced by most urban cities, suburban and rural areas. Resistance to Pirimiphos-methyl was analyzed in five populations of *Culex pipiens* collected in central Tunisia. Bioassays were performed over different larvae samples. All samples were resistant to Pirimiphos-methyl. The study of the mechanisms involved in this resistance showed the involvement of two known targets in resistance to organophosphates: insensitive acetylcholinesterase and overproduced esterases. Results were discussed in comparison with previous studies. Our results will help to propose alternatives control strategies to limit the development of resistance on the ground.

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1. Introduction:

Stagnant wastewater collections, generated and maintained by human water use practices, are the main providers of mosquitoes. The larvae of *Culex pipiens* are particularly well adapted and developed massively in sump water (Subra, 1973). In Tunisia, *Culex pipiens* is the main source of nuisance in urban areas. This high nuisance is favored by the rapid and sometimes anarchic growth of cities with inadequate sanitation and environmental hygiene measures, the resistance of *Culex pipiens* to insecticides, inadequate public and private human and financial resources of vector control (Doannio, 1994).

The control of *Culex pipiens* is practiced mainly at the larval level. It is based, on the one hand, on methods of physical control and environmental management and on the other hand, on chemical control. Unfortunately, the latter quickly ran into problems of resistance to most commonly used insecticides (Chandref et al., 1998; Magnin et al., 1988; Weill et al., 2001; 2002; 2003; Ben Cheikh et al., 2008; 2009). Another problem is that the use of insecticides can have an impact on the ecological system, limiting their use (Hassal et al., 1990; Hougard et al., 1993) and explaining the growing interest in finding new agents vector control.

Pirimiphos-methyl is organophosphorus (OP) used in a wide range of pesticidal applications, including public health. It reacts by inhibition of acetylcholinesterase. The objective of this study which carried out in 2003-2004, was to test the susceptibility of

Culex pipiens populations to Pirimiphos-methyl in central Tunisia.

2. Materials and Methods

Mosquitoes: Five natural populations of *Culex pipiens* were taken as larvae and nymphs in the central Tunisia (Table 1, Figure 1). The S-Lab was a sensitive strain without any chemical resistance used as a reference (Georghiou et al., 1966). The identification of esterases enzymes on the starch gel was determined by referring to two strains (SA2 and SA5) whose enzymes are known (A2-B2 and A5-B5, respectively) (Berticat et al., 2002).

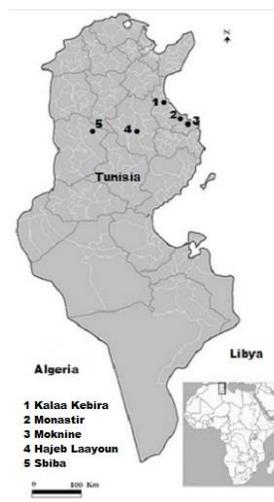


Figure 1. Geographic origin of Tunisian populations

Table 1: *Geographic origin of Tunisian populations, breeding site characteristics, and insecticide control*

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

C: Chlorpyrifos; T: Temephos; Pm: Pirimiphos methyl; F: Fenitrothion; P: Permethrin; D: Deltamethrin

***Culex pipiens* Laboratory Rearing:** The larvae are reared in plastic containers filled to a maximum of 2/3 tap water and covered with mosquito nets to avoid contamination by foreign laying. They are fed on rabbit croquette. The nymphs are transferred into pots containing water and placed in cubic cages where the emergence of the adults takes place 3 to 4 days later. Feeding the latter is done with sugar diluted in water. One week to 10 days after emergence, females need a meal of blood needed to carry their eggs to maturity and feed. The eggs obtained are regularly collected and reared as previously described.

Used Insecticides: The organophosphates Pirimiphos-methyl (91%o; American Cyanamid, Princeton, NJ) and the carbamate propoxur (997o; Mobay) were the two used insecticides for bioassays. Two synergists were used to help detect detoxification enzymes involved in resistance: S, S, S tributyl phosphorothioate (DEF), an esterase inhibitor, and piperonyl butoxide (Pb), an inhibitor of mixed function oxidases.

Bioassay Test for Mosquito Larvae and Data Analysis: Assays were performed as described by Raymond et al. (1986). The mortality data as a function of the dose were analyzed according to a log/probit program of Raymond et al. (1993).

Esterase's Detection: Authors undertook an electrophoretic study of the starch gel (Pasteur et al., 1987) in order to detect different esterases involved in Pirimiphos-methyl resistance.

3. Results and Discussion:

The linearity of the dose-mortality response was rejected for all samples, opposite to that of S-Lab which was accepted. All the studied samples were resistant to Pirimiphos-methyl. The RR_{50} ranged from 7 in sample # 1 to 115 in sample # 5 (Table 2). All samples were resistant

to Pirimiphos-methyl despite only two localities were treated by this OP insecticide. The agricultural pest control could explain the apparition of this high level of resistance.

The resistance of sample # 4 to Pirimiphos-methyl (OP insecticide) could be explained partly by the overproduced of EST (and/or GST) ($P < 0.05$, Table 2) because the RR_{50} remained high in the presence of the DEF ($RR_{50} > 1$, $p < 0.05$). In the same way, the CYP450 was involved partly in the Pirimiphos-methyl resistance (Table 2) because RR_{50} remained high in the presence of Pb ($RR_{50} > 1$, $p < 0.05$). However, esterases were detected in all studied samples. A1 was not detected in any studied sample. The highest frequencies of A2-B2 esterases (0.31) were recorded in samples # 5. A4-B4 and/or A5-B5 were detected in sample # 1, 2 and 3 with medium frequency (23 to 28%). A12 were detected in sample # 1, 3 and 4. C1 were detected in all samples except sample # 2 and 4. Bioassays realized on field samples again the propoxur indicated that rate of mortality ranged from 11% in samples # 3 to 79% in sample # 4 which had the highest resistance to Pirimiphos-methyl. Authors showed a significant correlation between the mortality due to propoxur and the LC_{50} of Pirimiphos-methyl ($P < 0.05$) indicated an insensitive acetylcholinesterase. In effect, this insecticide belongs to the chemical group of organophosphates which inhibit acetylcholinesterase, an enzyme involved in the regulation of nerve impulses (Aldridge, 1950).

The association between overproduced esterases and the insensitive target is very clear in our study. The only exception was for esterase A1 that was not detected in any of the studied populations. The limited number of used samples in an electrophoretic study of a starch gel may be at the origin of this absence. Our results are in agreement with those found by Raymond et al. (1989) who showed that the association of detoxification with an insensitive target is additive. Several other studies have shown this close association between the two targets at *Culex pipiens* of Tunisia but also of other countries (Ben Cheikh & Pasteur 1993; Ben Cheikh et al., 1995; 1998; 2008; 2009; Pasteur et al., 1999; Chevillon et al., 1999; Ben Cheikh et al., 1998; Raymond et al., 1998; Weill et al., 2002; 2003; Liu & Yue, 2000; Weill et al., 2001).

Other studies have suggested, contrary to what has cited above, the non-additive action of the two targets in *Culex pipiens* selected for several generations to temephos (OP insecticide) from Tunisia (Tabbabi et al., 2016; 2017). These authors did not detect any overproduced esterases and they associated the resistance to different used insecticides (organophosphorus and pyrethroid) for the second target (insensitive acetylcholinesterase), which seems to have the most part or even the totality of resistance recorded. It should be noted that previous study showed that all detoxification enzymes did not involve in the enormous resistance of

Culex pipiens to chlorpyrifos (OP insecticide) recorded in Tunisia and the mechanism involved remain to be elucidated (Ben Cheikh et al., 1998).

and pyrethroid insecticides in *Culex pipiens* (Diptera: Culicidae) from Tunisia (North Africa). *J. Med. Entomol*, 35(3), 251-260.

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

Population	<i>Pirimiphos methyl</i>			<i>Pirimiphos methyl</i> +DEF					<i>Pirimiphos methyl</i> +Pb				
	LC ₅₀ in µg/l	Slope	RR ₅₀	LC ₅₀ in µg/l	Slope	RR ₅₀	SR ₅₀	RSR	LC ₅₀ in µg/l	Slope	RR ₅₀	SR ₅₀	RSR
	(a)	± SE	(a)	(a)	± SE	(a)	(a)		(a)	± SE	(a)	(a)	
Slab	2.9 (2.5-3.4)	2.34 ± 0.18	-	0.30 (0.16-0.56)	1.7 ± 0.42	-	9.79 (6.16-15.5)	-	0.40 (0.31-0.55)	1.47 ± 0.18	-	7.2 (5.7-9.1)	-
1-kalaa Kebira	20 (14-29)	2.52 ± 0.34	7.0 (4.7-10.4)	5.7 (3.9-8.3)	1.96* ± 0.3	19.3 (11.9-31.4)	3.56 (2.33-5.43)	0.36	18 (10-30)	1.54 ± 0.25	45.1 (31.2-65.4)	1.1 (0.72-1.7)	0.16
2-Monastir	20 (15-27)	2.76* ± 0.31	6.9 (4.8-10.0)	7.3 (4.3-12)	2.05 ± 0.37	24.7 (14.2-43.0)	2.76 (1.70-4.48)	0.28	24 (16-34)	1.83* ± 0.22	59.7 (44.1-80.8)	0.84 (0.58-1.2)	0.12
3-Moknine	40 (31-53)	2.44* ± 0.31	13.9 (10.1-19.1)	9.2 (8.1-10)	2.08 ± 0.13	30.9 (20.3-47.1)	4.41 (3.37-5.77)	0.44	31 (23-41)	2.52 ± 0.35	77.1 (55.8-106)	1.3 (0.92-1.8)	0.18
4-Hajeb laayoun	117 (81-168)	2.57** ± 0.39	40.2 (26.5-61.0)	4.7 (4.0-5.3)	2.2 ± 0.2	15.7 (10.2-24.4)	24.9 (16.9-36.8)	2.6	4.7 (1.3-17)	1.87* ± 0.68	11.8 (6.6-20.9)	24.7 (12.8-47.4)	3.4
5-Sbiba	336 (70-1590)	4.11** ± 3.93	115 (9.9-1340)	42 (24-72)	2.3 ± 0.43	141 (77.7-258)	7.98 (0.76-83.5)	0.81	31 (22-42)	2.63 ± 0.34	77.3 (54.5-109)	10.8 (1.06-110)	1.5

(a), 95% CI; * The log dose-probit mortality response is parallel to that of S-Lab; ** Parallelism test positive 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).

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Conflicts of Interest:

Authors declared no conflicts of interest.

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