Investigation of Pirimiphos-methyl Resistance Status of *Culex pipiens* (Diptera: Culicidae) Populations in Northern Tunisia

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Abstract: This article reports Pirimiphos-methyl resistance status of *Culex pipiens* populations harvested in four breeding sites in Northern Tunisia. Our results showed the resistance of all samples to Pirimiphos-methyl. The RR_{50} ranged from 3.3 1 to 62.1. The sample collected from Tazarka had the highest resistance among all studied populations. This status could be explained by its frequency of mortality caused by propoxur (0%) and also by its frequency of detected esterases (85%). Authors confirmed the implications of insensitive acetylcholinesterase and esterases enzymes in the resistance of *Culex pipiens* populations to Pirimiphos-methyl. It is the first investigation of Pirimiphos-methyl resistance status of *Culex pipiens* populations from Tunisia and it is very important for the implementation and development of vector control strategies.

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1. Introduction:

The main approach is reducing the risk of infection by mosquito-borne diseases is to significantly reduce populations of disease-carrying mosquitoes. Landscape modification programs such as stream containment have reduced breeding sites and led to the eradication of malaria in the 1950s (Serandour et al., 2007). However, these approaches alone are not enough, and the most effective and widely used method of controlling mosquitoes in the world today is the use of chemical insecticides (Davidson, 1964; Mukhopadhyay et al., 1993; Ben Cheikh et al., 1998; Bisset et al., 1999; Martinez - Torres et al., 1999; Weill et al., 2001; 2002; 2003; Corbel et al., 2007; Tantely et al., 2010; Toma et al., 2011; Jones et al., 2012; Pocquet et al., 2013). The insecticides can be used as larvicide or as adulticides depending on the target species and the local context in terms of the topography of the breeding sites, legislation, and available means. The larviciding approach is generally preferred when breeding sites are easily identifiable and reachable while the use of adulticides is used when breeding sites are too diffuse in space and time.

The development of chemical insecticides began after the Second World War with the discovery of the insecticidal properties of DDT (dichlorodiphenyltrichloroethane) by Paul Hermann Müller in 1939. From the family of organochlorines, this first-generation insecticide has served many purposes by reducing or even eradicating malaria in some countries. However, its intensive and repeated use has led to the appearance of numerous cases of resistance limiting its effectiveness (Hemingway et al., 2002). In addition, its high bioaccumulation capacity, environmental persistence, and toxicity in mammals have led to its ban in many countries (Brown, 1986). Subsequently, advances in the chemical industry and the growth of intensive agriculture led to the development of the second generation of insecticides, represented by three major families: organophosphates (OP), carbamates and synthetic pyrethroids.

In Tunisia, information on the susceptibility or resistance to Pirimiphos-methyl insecticide (OP) of mosquitoes (larvae and adults) which are vectors of diseases or pests are non-existent. It should also be pointed out that Pirimiphos-methyl has been effective in many countries of South-East Asia in cold or hot spraying against arbovirus vectors. This article reports the results of the studies carried out between 2003 and 2005 using the WHO susceptibility tests on larvae of local populations of *Culex pipiens* harvested in four breeding sites in the Northern Tunisia.

2. Materials and Methods

Mosquitoes: Four field populations of *Culex pipiens* was taken as larvae and nymphs in the Northern Tunisia (Figure 1, Table 1). The S-Lab, sensitive strain, was used as a reference (Georghiou et al., 1966). Two strains (SA2 and SA5) with known esterases (A2-B2 and A5-B5

respectively) (Berticat et al., 2002) were used to be able to identify the detected esterases in field populations.

Figure 1. Geographic origin of Tunisian populations

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

Code	Locality	Breeding Site	Date of Collection	Mosquito control (used Insecticides)	Agricultural Pest Control
1	Krib	River	0ct. 2005	Occasional (P)	Yes
2	Belli	River	Aug. 2003	Rare (C, D)	Yes
3	Tazarka	River	May 2005	Very frequent (C, T, Pm, F, P, D)	Yes
4	Sidi Khalifa	Water pond	July 2004	None	None

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

Used Insecticides: The organophosphates Pirimiphos-methyl (9l%o; American Cyanamid, Princeton, NJ) and the carbamate propoxur (997o; Mobay) were used for different assays. S, S, S tributyl phosphorothioate (DEF), an esterase inhibitor, and piperonyl butoxide (Pb), an inhibitor of mixed function oxidases are the two synergists used to detect the presence or absence of detoxification enzymes involved in resistance.

Bioassay Test for Mosquito Larvae and Data Analysis: Bioassay tests utilized standard methods (Raymond et al, 1986). The results of different used tests were analyzed using a log/probit program of Raymond et al. (1993).

Esterase's Detection: An electrophoretic study of the starch gel was realized to detect different esterases involved in resistance of field populations to Pirimiphos-methyl (Pasteur et al., 1987).

3. Results and Discussion:

The linearity of the dose-mortality response was rejected for all samples. The linearity was accepted in S-Lab strain because of its homogeneity of sensitive characters. Our results showed the resistance of the four collected samples to Pirimiphos-methyl. The RR_{50} ranged from 3.3 in sample # 1 to 62.1 in sample # 3 (Table 2). It seems that the Pirimiphos-methyl resistance levels of the Tunisian *Culex pipiens* were higher than those signaled in other areas of the world (Bisset et al., 1999; Rodriguez et al., 2001). In the laboratory, susceptibility to Pirimiphos-methyl of wild populations of *Aedes Aegypti* and *Aedes Albopictus* of Singapore was compared to susceptible reference strains belonging to these two species. The results showed that *Aedes Aegypti* and *Aedes Albopictus* had not developed a mechanism of resistance to this compound despite its use for more than nine years in LAV programs (Ping et al., 2001).

The increased detoxification by EST (and/or GST) and the CYP450 were not involved in the Pirimiphos-methyl resistance in all samples. In effect, the two synergists did not decrease the tolerance to Pirimiphos-methyl in any collected populations. In contrast, the DEF and the Pb decreased significantly the tolerance to this insecticide in s-Lab strains ($p<0.05$) (Table 2). It should be noted that cytochrome P450, esterases and/or GSTs enzymes may be insensitive to the action of the two used synergists (DEF and Pb). The detection of esterases by electrophoretic starch gel in all studied samples (results presented below) confirmed this hypothesis.

The sample # 3 had the highest resistance among all studied populations. This status could be explained by its frequency of mortality caused by propoxur (0%) and also by its frequency of detected esterases (85%). Many esterases were detected in these samples with different frequencies (A2-B2, A4B4 and/or A5B5, B12, and C1). Mortality caused by propoxur was 39% in samples # 2, 68% in samples # 4, and 87% in sample # 1. The mortality caused by propoxur indicated an insensitive acetylcholinesterase significantly correlated with the LC_{50} of Pirimiphos-methyl ($P<0.05$). Bourguet et al. (1996) showed the existence of two loci AChE in *Culex pipiens*, *Ace-1* is involved in resistance to organophosphates (Scott, 1990; Feyereisen, 1995; Taylor and Feyereisen, 1996, Weill et al., 2001; 2002; 2003) and, *Ace-2* whose role is unknown.

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

Population	*Pirimiphos methyl*			*Pirimiphos methyl +DEF*					*Pirimiphos methyl +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	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-Krib	9.6 (4.0-23)	2.23** ± 0.59	3.3 (1.8-5.9)	-	-	-	-	-	-	-	-	-	-
2-Belli	11 (8.8-16)	2.46* ± 0.33	4.1 (2.8-5.8)	7.5 (5.9-9.4)	2.41 ± 0.26	25.2 (16.0-39.6)	1.59 (1.12-2.26)	0.16	27 (22-34)	2.64 ± 0.29	69.4 (51.5-93.4)	0.42 (0.29-0.61)	0.06
3-Tazarka	181 (166-196)	3.93 ± 0.22	62.1 (50.5-76.4)	122 (110-136)	4.48 ± 0.37	411 (251-673)	1.48 (1.15-1.90)	0.15	184 (113-299)	4.68 ± 1.03	457 (256-816)	0.98 (0.56-1.7)	0.13
4-Sidi khalifa	33 (21-52)	1.48 ± 0.25	11.4 (8.3-15.7)	29 -	2.64 ± 0.90	97.9 (36.7-260)	1.14 (0.44-2.98)	0.12	15 (10-20)	2.11 ± 0.34	39.6 (28.2-55.5)	2.0 (1.3-3.2)	0.29

(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).

The A2-B2 esterases were revealed in samples # 2, 3, and 4 with a frequency of 0.03, 0.15 and 0.06, respectively. The A4-B4 (and/or A5-B5) esterases were present in all samples with a frequency of 0.03, 0.42, 0.50, and 0.19, respectively. The B12 esterases were observed in # 2, 3, and 4 with a frequency of 0.19, 0.02, and 0.14, respectively. The C1 esterases were found in # 2, 3, and 4 with a frequency of 0.11, 0.04, and 0.03, respectively. The A1 esterase was not detected in any used sample It should be noted that the implication of esterases in the resistance to OPs was confirmed by several authors (Guillemaud et al., 1996; Guillemaud et al., 1997; Chevillon et al., 1999; Ben Cheikh et al., 1998; Raymond et al., 1998; Liu et al., 2000; Weill et al., 2001).

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Conflicts of Interest:

Authors declared no conflicts of interest.

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