International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979, Impact Factor: RJIF 5.72
www.allsubjectjournal.com
Volume 4; Issue 7; July 2017; Page No. 327-330

Susceptibility of different geographical strains of *Culex pipiens* (diptera: culicidae) to temephos in grand Tunis area of Tunisia

[*1] Ahmed Tabbabi, [2] Jaber Daaboub, [3] Ali Lamari, [4] Raja Ben Cheikh, [5] Ben Jha Ibtissem, [6] Hassen Ben Cheikh

[1-6] Laboratory of Genetics, Faculty of Medicine of Monastir, Monastir University, Monastir, Tunisia
[2] Department of Hygiene and Environmental Protection, Ministry of Public Health, Bab Saadoun, Tunis, Tunisia

Abstract
Culex pipiens populations were collected in 5 localities of Grand Tunis area, Northeast Tunisia. Two samples (# 2, and 3) were susceptible to temephos insecticide. The resistant samples displayed RR_{50} ranged from 1 for the sample # 2 to 213 for the sample # 5. The resistance level was high for sample # 5 (>100-fold), and low, not exceeding 3-fold in the other resistant samples. The temephos resistance could be explained by the AChE 1 insensivity and the increased detoxification by the CYP450. In the samples # 1, and 4 (temephos RR_{50} <3-fold), the CYP450 were not involved in the temephos resistance and the insensitive AChE 1 could be the mechanism responsible for this resistance. The AChE 1 resistant phenotypes were present in the concerned samples with a frequency ranged from 0.18 to 0.46. For the sample which manifested high temephos resistance level (# 5), the insensitive AChE 1 seems play a role in the resistance, in addition to the major contribution of CYP450.

Keywords: *Culex pipiens*, temephos insecticide, resistance, ache 1, cyp450, grand tunis area

Introduction
Vector control was complicated by the emergence and spread of resistance to insecticides (David *et al*., 2013, El Ouali *et al*., 2014) [19, 9]. The first study on insect resistance to insecticides dates from 1914. In 2001, more than 540 species, including 198 of medical interest, showed resistance to at least one class of insecticide. Different methods using more specific insecticides, as well as rotations or mixtures of insecticides, make it possible to limit the selection pressure and therefore to limit the resistances. Resistance management, however, requires characterization. Considerable progress has been made in recent years in understanding their genetic mechanisms.

Various resistance mechanisms were selected in treated insect species, depending on the nature of the used insecticides and the control strategies. Two main mechanisms of resistance to insecticides have been found in treated populations of mosquitoes: the first, called metabolic resistance, corresponds to an increased detoxification of the insecticide by enzymes (monooxygenases, esterases, glutathione -S-transferases) which trap it and/or inactivate the insecticide before it reaches its target (Rooker *et al*., 1996; Hemingway *et al*., 1998, 2004; Karunaratne and Hemingway, 2001; Ranson *et al*., 2002) [24, 13, 14, 21]. In the case of resistance, these enzymes are often overproduced by a process of amplification of the genes that code them or by mutations of regulatory regions. Certain metabolic resistances are still imperfectly understood (genes involved, role of certain mutations, regulation...); the second mechanism corresponds to the mutation of the insecticides targets which modifies their conformation and their affinity for the insecticides (Devonshire *et al*., 1984; Oppenoorth, 1985; Pralavorio *et al*., 1992; Weill *et al*., 2003; Hemingway *et al*., 2004; Russel *et al*., 2004; Alout *et al*., 2007; Badawy *et al*., 2015) [8, 16, 20, 28 12, 25, 15, 2]. These resistances are generally better known (Knock down resistance or insensitive acetylcholinesterase and Gaba receptor mutations).

Studying and understanding these mechanisms makes it possible to improve the vector control, hence the objective of this study: Susceptibility of different geographical strains of *Culex pipiens* (Diptera: Culicidae) to temephos in Grand Tunis area of Tunisia.

Material and methods
Mosquito samples and strains
Culex pipiens populations were collected in 5 regions of Grand Tunis area, Northeast Tunisia (Figure 1, Table 1). Larvae were used for different bioassays and adults were stored in -80°C for esterases identification. We used three reference strains included S-Lab susceptible strain (Georghiou *et al*., 1966), and two strains homozygous for different over-produced esterases (SA2 and SA5, Berticat *et al*., 2002) [4].

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	Sidi Thabet	Ditch	Aug. 2004	Rare (C,P)	Yes
2	Sokra	Canal	June 2003	Very frequent (C, Pm, F, P, D)	Yes
3	Mannouba	River	June 2005	Occasional (P,D)	Yes
4	Ouardia	Ditch	Aug. 2005	Very frequent (C, F, P, D)	None
5	Ezzahra	Ditch	Nov 2005	Very frequent (C, F, P, D, T)	None

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

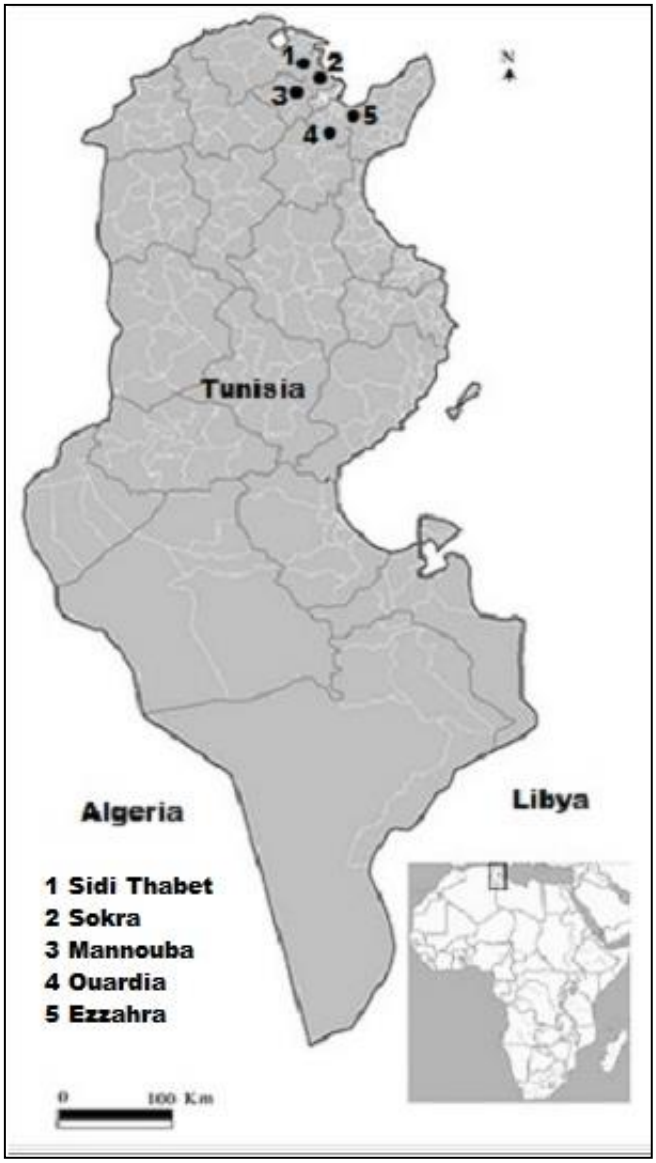

Fig 1: Geographic origin of Tunisian populations

Bioassays

Experimental protocol of Raymond *et al*. (1986) [22] was used for different bioassays using an organophosphorus (OP) insecticide, temephos (99.5% [AI]), and the carbamate propoxur (99.7%; Mobay). We used five doses and three replicates per dose. The effect of the two used synergists DEF (98% [AI], Chem Service, England), and the Pb (94% [AI], Laboratory Dr. Ehrenstorfer, Germany) was investigated to estimate the role of detoxification enzymes in the recorded resistance to chlorpyrifos insecticide. One dose (1mg/ml) was used for Propoxur insecticide to estimate the role of AChE 1. This concentration kills all susceptible mosquitoes and indicates a sensitive AChE 1. Data were analyzed using a log-probit program of Raymond *et al*. (1993) [23] based on Finney (1971) [10].

Esterases phenotypes

Ester locus was performed by starch gel electrophoresis using homogenates of adult thorax and abdomen as described by Pasteur *et al*. (1988) [19]. We compared electrophoretic mobility of esterases of collected populations and the laboratory reference strains to identify each enzyme.

Results

The linearity of the dose-mortality response was accepted ($p>0.05$) only for S-Lab and 2 among 5 field samples (# 2, and 3). Two samples (# 2, and 3) were susceptible (Table 2). The resistant samples displayed RR_{50} ranged from 1 for the sample # 2 to 213 for the sample # 5. The resistance level was high for sample # 5 (>100-fold), and low, not exceeding 3-fold in the other resistant samples.

The increased detoxification by the EST (and/or GST) was not involved in the temephos tolerance because the synergist effect was not significantly higher than that recorded in S-Lab in any tested sample (Table 1). With Pb, the resistance to temephos significantly increased in S-Lab (SR_{50}=0.56, $p<0.05$). The SR_{50} was significantly higher than that recorded in S-Lab in just 1 among 5 field samples (# 5). The addition of Pb to temephos bioassays completely suppressed the resistance in samples # 5 (RR_{50}= 8.5, $p>0.05$, RSR=25.0), indicating that the resistance mechanisms in this sample were inhibited by Pb. For samples # 1, and 4, which showed low resistance levels, not exceeding 3-fold, the addition of Pb to temephos bioassays did not decrease the resistance (95%CI of RR_{50} for temephos alone and temephos plus Pb include the common values), indicating that the resistance mechanisms inhibited by Pb were not involved in the resistance of these samples.

Mortality caused by propoxur ranged from 0% in samples # 5 which showed the highest resistance levels to studied temephos insecticide to 48% in sample # 2. The mortality due to propoxur was significantly correlated with the LC_{50} of temephos (Spearman rank correlation, (r) = -0.81 ($P<0.01$)).

We detected five esterases in studied field samples: A1, A2-B2, A4-B4 and/or A5-B5, B12, and C1. The frequencies of these esterases were ≥0.45 in all the collected samples.

Table 2: 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-Sidi thabet	2.2 (1.6-3.1)	1.85 ± 023	1.8 (1.3-2.3)	2.9 (2.1-5.8)	1.82 ± 0.40	9.0 (5.9-13.9)	0.76 (0.46-1.2)	0.19	3.2 (2.8-3.6)	2.0 * ± 0.14	1.4 (1.1-1.8)	0.70 (0.54-0.90)	1.2
2-Sokra	1.2 (0.83-1.7)	1.62 ± 0.21	1.0 (0.77-1.3)	-	-	-	-	-	-	-	-	-	-
3-Mannouba	1.5 (1.1-2.2)	1.99 ± 0.32	1.2 (0.95-1.6)	-	-	-	-	-	-	-	-	-	-

4-Ouardia	3.7 (2.1-6.5)	2.6 ± 0.43	2.9 (1.9-4.4)	1.2 (0.98-1.5)	1.44 ± 0.17	3.8 (2.8-5.1)	2.9 (1.9-4.6)	0.77	4.0 (3.1-5.5)	1.73 * ± 0.24	1.8 (1.3-2.4)	0.92 (0.58-1.4)	1.6
5-Ezzahra	265 (198-355)	6,8 ± 1,39	213 (126-361)	183 -	3.05 ± 1.72	567 (84.6-3808)	1.4 (0.20-10.2)	0.37	18 -	2.14 * ± 2.75	8.5 (0.86-84.2)	14.2 (1.35-148)	25.0

(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).

Discussion

Our study showed temephos resistance levels higher than those reported by previous studies. This recorded resistance could be explained by the prolonged use of OPs insecticides which caused cross-resistance with temephos insecticide (OP). The temephos resistance levels of the Tunisian *Culex pipiens* were low, not exceeding 10-fold (Ben Cheikh *et al*., 1998) [3]. The highest resistance level of this species in other areas of the world (200-fold) was signalled in Italy (Silvestrini *et al*., 1998) [26]. Less high resistance levels were recorded in other countries: 42-fold in Martinique (Yebakima *et al*., 2004) [30], 26-fold in Israel (Orshan *et al*., 2005) [17], 18-fold in Cote d'Ivoire (Chandre *et al*., 1998) [6] and 2.8-fold in Burkina Faso (Ouedraogo *et al*., 2005) [18].

The synergist study showed that the CYP450 have a major contribution in sample displaying high resistance levels (>100-fold) and were not involved in samples manifesting low resistance levels (<3 folds). These results agreed with those obtained on *Culex pipiens* by Ben Cheikh *et al*. (1998) [3] and Bisset *et al*. (2000) [5].

Our starch gel electrophoresis identified many esterases which could be involved in temephos resistance. These enzymes did not seem to have a significant role in the recorded resistance because of their presence in susceptible populations. Many previous studies reported the association between elevated esterase activity and temephos resistance (Vaughan *et al*., 1998; Wirth and Georghiou, 1999) [27, 29].

We also showed that the resistance to the studied temephos insecticide was correlated with the propoxur resistance. These results indicate that modifications of the target, AChE 1, can be involved in the temephos resistance. The selection of a modified AChE 1 less sensitive to OP and carbamate insecticides has been shown to be a common resistance mechanism, and was observed in numerous arthropod species (Alout *et al*., 2007) [1]. The resistance allele, *ace-1*^{R} is present worldwide and causes OP resistance in several mosquito species (Labbé *et al*., 2007) [15].

Acknowledgements

This work was kindly supported by the Ministry of Higher Education and Scientific Research of Tunisia by funds allocated to the Research Unit (Génétique 02/UR/08-03) and by DHMPE of the Minister of Public Health of Tunisia. We are very grateful to S. Ouanes, for technical assistance, A. Ben Haj Ayed and I. Mkada for help in bioassays, S. Saïdi, Tunisian hygienist technicians for help in mosquito collecting, and M. Nedhif and M. Rebhi for their kind interest and help.

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