Temephos Resistance and Esterase Activity in Selected Laboratory Population of *Culex Pipiens* from Tunisia

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Abstract: Selection for resistance to temephos (an organophosphorus insecticide) was performed by exposing larvae of the field-collection population of *Culex pipiens* to increasing doses of temephos for four generations. Mosquito larval specimens were captured in Northwestern Tunisia and identified using morphological keys. Two insecticides including temephos and propoxur were used evaluating the susceptibility status of the selected population. Bioassays results showed high resistance to temephos and no esterase activity. A new esterase was first detected in one sample among 60 tested. This result will help to elucidate resistance mechanisms, which is essential for the planning of future insecticide resistance management programs.

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1. Introduction:

The mosquito *Culex pipiens*, common in temperate and tropical countries, is subjected to insecticide control in many places. Its distribution is wide so that this species approximately present in all continent of the world (Mitchell et al., 1980; Vinogradova, 2000; Smith and Fonseca, 2004; Savage et al., 2007; Mullen, 2009; Strickman and Fonseca, 2012). In Tunisia, *Culex pipiens* is the most abundant mosquito species, particularly in urban areas (Daaboub et al., 2008; Ben Cheikh et al., 2009; Wasfi et al., 2016) and is generally controlled by conventional insecticides such as organophosphorus, carbamate and pyrethroids. Temephos is an organophosphate insecticide currently used primarily as a mosquito larvicide. In Tunisia, *Culex pipiens* has a low resistance to temephos comparing to other insecticides (Ben Cheikh & Pasteur, 1993). There are three loci involved in organophosphate resistance: Est-2, Est-3 and Ace-1. Est-2 and Est-3 coding for esterase detoxification. Ace-1 coding for the acetylcholinesterase AChE 1 which is present in the synaptic cleft of the acetylcholine neurons and catalyzes the degradation of the neurotransmitter (Bourguet et al., 1997; Lenormand et al., 1998; Weill et al., 2003; 2004). The present study was performed for evaluating the susceptibility status of *Culex pipiens* population after selection pressures with temephos and identifies mechanisms that may cause of resistance.

2. Materials and Methods:

2.1. Mosquito strains:

A resistant strain of *Culex pipiens*, collected as larvae from Tunisia, was obtained after four generations of selection pressure to temephos. We used the insecticide susceptible laboratory strain "S-Lab" from the University of Montpellier, France, as the reference strain.

2.2. Insecticides:

Two technical grade insecticides were used for selection and bioassay: the organophosphates temephos (9l%o; American Cyanamid, Princeton, NJ), and the carbamate propoxur (997o; Mobay).

2.3. Bioassay procedures and data analysis:

Bioassays were performed on larvae after four generations of selection pressures to assess the susceptibility of *Culex pipiens* to temephos (Raymond et al., 1986). Mortality data were analyzed by using the log-probit program of Raymond (1993), based on Finney (1971). By the method of Finney, the lethal mortality for 50% and 95% (LT50 and LT95) values, their 95% confidence interval and Probit regression line parameters were determined for both strains (S-Lab and selected population).

2.4. Esterase's detection:

Biochemical assays were carried out to detect the presence of esterase's involved in resistance (Pasteur et al., 1981; 1988). Esterases were identified in single individual homogenates analyzed by starch electrophoresis using TME 7.4 buffer systems and revealed according to Pasteur et al. (1988). Overproduced esterases from reference strains were run as controls: T1 (A2-B2) and T2 (A4-B4 and/or A5-B5).

3. Results and Discussion:

The linearity of concentration-mortality curves was rejected ($P<0.05$) for the selected strain and the value

slope was 0.87. Heterogeneity tended to increase (18.40) because there was an increase in the heterogeneity of the population as selection pressure favored an increased frequency of resistant alleles (Brown & Pal, 1971). The RR95 reached a very high level with temephos (RR95 = 780.14). Resistance temephos was not affected by DEF (S, S, S-tributyl phosphorotrithioate). Although the direct association of temephos resistance and esterases has not been demonstrated, considerable circumstantial evidence supports this association (Raymond et al., 1998, Ben Cheikh et al., 2008). Bioassays showed propoxur resistance of selected strain and revealed an insensitive acetylcholinesterase.

Interestingly, we have detected for the first time a new esterase in the temephos selected strain. The new esterase displayed between A4-B4 and/or A5-B5 (Figure 1). The new overproduced esterase could be responsible, at least in part, in the recorded resistance. However, several studies of the interactions between resistance genes suggested that any new gene only offered a low resistance rate compared to genes that have already existed (Cui et al., 2006; 2007). Within the near future one or several of the existing alleles will probably be eliminated (Pasteur et al, 1981; Villani et al, 1986; Severeni et al., 1993; Chevillon et al, 1995; Brown and Brogdon, 1987; French-Constant and Roush, 1990; Qiao and Raymond, 1995).

Bioassays results showed high resistance to temephos and no esterase activity. The situation of our study can be explained by rare mutation alleles generating esterase alleles. Indeed, several studies on mosquitoes without overproduced esterases from different regions revealed a very important polymorphism (Raymond et al., 1996). Detecting only the B2 allele, despite the importance of the neutral polymorphism of the esterase B region in non-resistant individuals, led the authors to interpret this as the result of mosquito migration and not the result of mutations. Moreover, the analysis of the gene encoding the esterases A in the resistant and sensitive individuals showed that these sequences varied enormously between sensitive individuals. On the other hand, these sequences were perfectly identical between resistant mosquitoes from the five continents (Guillemaud et al., 1996).

Deleting constraints are probably responsible for this low number of mutations. In *Myzus persicae*, individuals who show high expression of overproduced esterases suffer from high mortality compared to susceptible individuals during the winter. In fact, the cost of resistance depends on the level of overproduction of esterases. The insensitive acetylcholinesterase may explain the appearance of a high resistance to temephos insecticides after a few generations of selections.

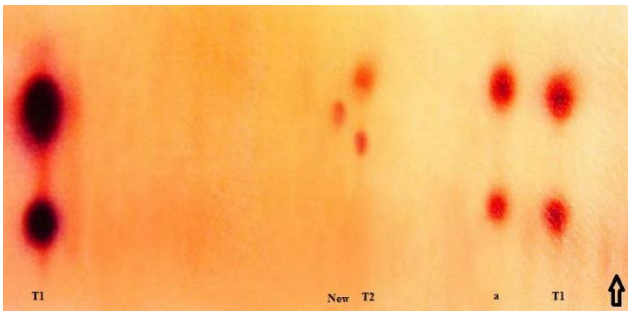

Figure 1. The activity of esterases detected in selected laboratory population of *Culex pipiens* by starch gel electrophoresis. The arrow indicates electrophoretic migration of the proteins. T1: A control mosquitoes displayed a phenotype with A2-B2; T2: A control mosquito displayed a phenotype with A4-B4 and/or A5 - B5; a: resistant strain; New: new esterase.

4. Conclusion:

Highly resistance level and new esterase were detected in selected population. Authors suggest biochemical and molecular investigations, to detect resistance mechanisms in the selected population for the further decision of vector control.

Conflicts of Interest:

Authors declared no conflicts of interest.

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