New Esterases Amplification Involved in Organophosphate Resistance in *Culex Pipiens* Mosquitoes from Tunisia

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Abstract: In Tunisia, the mosquito *Culex pipiens* shows various organophosphate resistance alleles at Ester locus. Resistance to the organophosphate chlorpyrifos was investigated in one population of *Culex pipiens* collected in northwestern Tunisia. High resistance to chlorpyrifos was observed and new esterases were detected. These results must be considered in future mosquito control programs since detected esterases can lead to high resistance to several organophosphorus insecticides.

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Keywords: *Culex pipiens*, Tunisia, high resistance, chlorpyrifos, new esterases.

Short Report:

In most parts of Tunisia, mosquitoes have been subjected to organophosphate insecticide treatments since the mid-1960s, and resistance gene monitoring in the *Culex pipiens* complex (Diptera: Culicidae) started in only a few locations from the end of the 1980s (Ben Cheikh, 1999). The super-locus Ester is one of the two genome areas in the mosquito *Culex pipiens* involved in organophosphorus insecticide resistance (Lenomand et al, 1998; Bourget et al, 2004; Hanying et al, 2012). This super-locus is composed of two loci, Est-3 and Est-2, and both loci encode for detoxifying esterase. The resistance mechanism at Ester corresponds to an esterase over-production at one or both loci (Raymond et al, 1998; Bourget et al, 2004; Hanying et al, 2012). This study was conducted in order to assess the chlorpyrifos resistance status in Tunisia population of the *Culex pipiens* and to determine which resistance genes are associated with this resistance?

Culex pipiens were collected as larvae and pupae in the Governorate of Jandouba, northern Tunisia, in 2004. Resistance characteristics of larval population were determined by bioassays on fourth instar larvae, following the method described in Raymond & Marquine (1994). Reference strain used was S-LAB, insecticides susceptible strain without any known resistance genes (Georghiou et al, 1966). Chlorpyrifos insecticide (organophosphorus) was used in ethanol solutions. Mortality data were analysed by the log-probit program of Raymond (1993), based on Finney (1971). Esterase phenotypes were established by starch electrophoresis (TME 7.4 buffer system) as described by Pasteur et al. (1981, 1988) using homogenates of thorax and abdomen.

The linearity of dose–mortality responses was accepted ($P > 0.05$) for S-Lab and Jandouba population. RR at LC_{50} (RR_{50}) showed that the sample was resistant to chlorpyrifos. The RR_{50} reached a very high level with chlorpyrifos (RR_{50} = 8062). The addition of DEF (S,S,S-tributyl phosphorotrithioate) to chlorpyrifos bioassays did not decrease tolerance significantly ($P > 0.05$) in S-Lab and Jandouba sample. So, the increased detoxification by EST (and/or GST: Gluthations-S-Transferase) was not involved in chlorpyrifos resistance of this sample. However, Ben Cheikh et al, 2008 found an association between chlorpyrifos resistance and esterases.

A total of 20 mosquitoes were analyzed. Starch gel electrophoresis did not disclose any overproduced known esterase in the Jandouba samples. Two new patterns were observed (Figure 1). The first one (named New1 until further characterisation) displayed under esterase A1 and between A4/B4 and/or A5/B5. The second (New2) displayed under esterase A1. New1 and New2 are two new esterases and they are first detected in the present sample from 2004.

A new esterase, A13, characterised by the same electrophoretic migration as esterase A1 was identified in Tunisia by Ben Cheikh et al. (2009). New overproduced esterases detected could be responsible, at least partly, for the organophosphate resistance. In fact, theoretical studies showed that new alleles allow low rates of resistance compared to those already known (Raymond et al, 1989). These results must be considered in future mosquito control programs since the detected esterases can lead to high resistance to several organophosphorus insecticides.

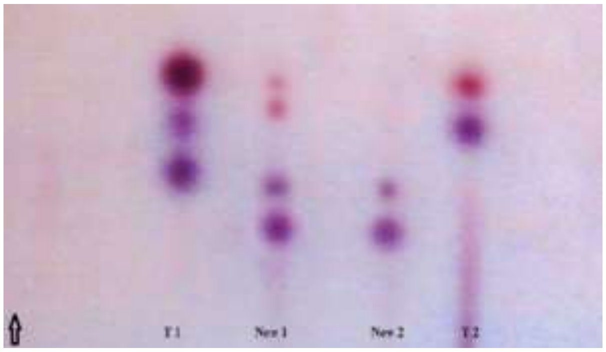

Figure 1. Activity esterases in single adults from the Jandouba sample analyzed on starch gel. The arrow indicates electrophoretic migration of the proteins. T1: A control mosquito displayed a phenotype with A2-B2/A4-B4 and/or A2-B2/A5-B5; T2: A control mosquito displayed a phenotype with A4-B4 and/or A5 -B5; New1 and New2: new esterases.

Declaration:

All authors declare they have no any conflicts of interests concerning on the report.

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