Evaluation of resistance to temephos insecticide in *Culex pipiens pipiens* larvae collected from three districts of Tunisia

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Abstract

Background: Mosquitoes are considered as the main groups of arthropods that cause nuisance and public health problems.

Objectives: Evaluation of resistance to temephos insecticide in *Culex pipiens pipiens* larvae collected from three districts of Tunisia.

Methods: Late third and early fourth instars larvae of *Culex pipiens pipiens* were collected in three localities of Northern and Southern Tunisia. Field collected populations were tested against temephos insecticide and compared to bioassays of a susceptible reference strain. The cross-resistance between temephos and propoxur, and the polymorphism of over-produced esterases and AChE 1 were investigated.

Results: Studied populations exhibited tolerance to temephos with low and high levels of resistance. The resistance ratio (RR_{50}) values of temephos ranged from 1.34 to 114. Synergists and starch electrophoresis showed that the metabolic resistances were involved in the recorded resistance. Likewise, the resistant target site (acetyl cholinesterase: AChE 1) was responsible for the recorded resistance to temephos compound in *Culex pipiens pipiens*.

Conclusion: The low and high resistance recorded to temephos insecticides is particularly interesting, because it leaves a range of tools useable by vector control services. However, further studies are needed to determine its spread and anticipate vector control failure where these insecticides are used.

Keywords: *Culex pipiens pipiens*, temephos resistance, Tunisia.

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Introduction

Mosquitoes including *Culex pipiens* are responsible for several serious diseases and able to transmit diseases agents infecting over 600 million people per year[1,2]. *Culex pipiens pipiens* are known by their ecologic plasticity which explains their wide geographical distribution (temperate and tropical regions). This species may be the cause of strong nuisance and can transmit several parasitosis such as the Japanese Encephalitis, the West Nile fever[3-6], the Rift Valley virus and certain filariasis[7-11]. *Culex pipiens* mosquito has been strongly suspected as the most likely vector in the transmission of West Nile virus outbreaks that have affected Tunisia[12-15] in 1997, 2003, 2007, 2010, 2011 and 2012. This situation forces us to investigate one of the major obstacles to vectors disease control, the mos-

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quito resistance to insecticides since the use of chemical insecticides for mosquito control remains the most widespread.

In general, resistance to organophosphates including temephos insecticides can be metabolic or due to target site modifications. For target-site resistance, several authors have been identified and reported a relatively small number of highly conserved point mutations in the gene encoding the acetylcholinesterase (AChE) enzyme of many species[16,17]. For metabolic resistance, other studies on a range of insect species identified and reported genomic changes which lead to gene amplification, over expression and/or modification of genes encoding members of the glutathione S-transferases (GSTs), cytochrome P450 (CYTP450) and carboxylesterases (CEs)[18]. Unfortunately, the massive use of insecticides during the malaria eradication program between 1967 and 1978 led to the development of strong resistance worldwide in *Culex pipiens* from Tunisia[19-22]. The aim of the present study was to evaluate the status of temephos resistance in larval populations of *Culex pipiens pipiens* (Diptera: Culicidae) in Tunisia and estimate the involved mechanisms using different synergists. The cross-resistance between temephos and propoxur, and the polymorphism of over-produced esterases and AChE 1 were also investigated.

Methods

Standard methods of Raymond et al.[23] were performed on larvae populations of *Culex pipiens pipiens* mosquitoes collected in three breeding sites in northern and Southern Tunisia (Figure 1). Identification of collected populations was done morphologically using the key of Brunhes et al[24]. Collected larvae were transported to the laboratory and directly transferred into plastic trays containing distilled water with rabbit croquette which served as food under standard insectary conditions (25 ± 1°C and 70 ± 5% RH). Bioassays were performed on both early third and late fourth instars. However, young larvae were reared until advanced instars. Field collected strains were tested against temephos insecticide and compared to bioassays of a susceptible reference strain. Five concentrations of temephos (100, 10, 1, 0,1, 0,01 ppm) providing between 0 and 100% mortality were used in a total volume of 100 ml of water containing 1 ml of ethanol solution of the tested insecticide. The tests were replicated five times per concentration. In each replicate, 20 early third and late fourth instar larvae were released. After a period of 24 hours, larval mortality was recorded. The Mazzarri and Georghiou[25] criteria were followed to classify the resistance level of each population tested as follows: low (RR<5), moderate (5≤RR≤10) or high (RR>10). We assessed also the effect of the synergists piperonyl-butoxide (Pb) on metabolic insecticide resistance. Esterase's activities were characterized on homogenates of adult thorax and abdomen according to the method of Pasteur et al.[26]. Propoxur bioassays which included one dose (1mg/l) was done to estimate the common mechanism of resistance to temephos insecticide: acetylcholinesterase resistance. According the method described by Bourguet et al.[27], AChE1 polymorphism was analyzed to comparing AChE1 activity of homogenates of adult heads in the absence or presence of propoxur. Three phenotypes including ace-1R, ace-1S alleles, and duplicated haplotype were separated using this enzyme bioassay. The obtained results were analyzed by using the log probit program of Raymond[28], based on Finney[29] to obtain LC_{50}, LC_{95} and regression line.

Figure 1: Geographic origin of Tunisian populations

Results

The resistance to temephos insecticide in field populations of *Culex pipiens pipiens* are presented in Table 1. The resistance ratio (LC_{50}) values of temephos ranged from 1.34 to 114 and showed their low and high levels according Mazzarri et al[25] criteria.

Table 1: Temephos resistance characteristics of Tunisian *Culexpipienspipiens*

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	-	3,84 (2,89–5,09)	-	2,2 (1,7–2,8)	1,94 ±0,28	-	0,56 (0,44–0,72)	-
1- Sidi Hcine	142 (65,5–308)	2,41 ± 0,75**	114 (60,2–216)	-	-	-	-	-	7,3 (3–17)	3,44 ± 2,48**	3,32 (1,12–9,84)	19,4 (6,00–63,0)	34,33
2- El Fahs	3,1 (2,1–4,4)	1,69 ± 0,31	2,55 (1,93–3,37)	-	-	-	-	-	-	-	-	-	-
3-Dgach	1,6 (0,98–2,8)	2,05 ± 0,35	1,34 (0,87–2,05)	-	-	-	-	-	-	-	-	-	-

(a), 95% CI; ** Parallelism test positif but without probability.
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).

The addition of Pb synergist in sample # 1 increased the toxicity to used insecticide (RSR>30), indicating that the resistance mechanisms inhibited by synergist (CYTP450) were involved in the resistance of the studied sample. Starch electrophoresis was realized to verify the involvement of detoxification (esterase) in the recorded resistance because it was not possible to do all synergists bioassays mainly DEF synergist. Two esterases were detected in the most resistant sample with high frequency of A4-B4 (45%). Three esterases including A4-B4, B12 and C1 were revealed in sample # 2 with low frequencies. Low frequencies of two esterases including A4-B4 and C1 were reported in sample # 3 which showed the lowest level of resistance to temephos and propoxur.

Cross-resistance temephos/propoxur was detected using bioassays and the mortality due to propoxur was significantly correlated with the LC_{50} of temephos insecticide indicating the resistance of the common target site: AChE 1. The propoxur mortality was low in the most resistant population (32%). The high moralities, 38% and 99%, were associated to the susceptibility of samples # 2 and 3, respectively. The polymorphism of AChE 1 showed that the highest frequencies of acetylcholinesterase 1 (AChE 1) resistant phenotypes was recorded in the most resistant population (sample # 1) with a percentage of 74% including an important percentage of duplicate phenotype [RS]. However, the highest frequencies of acetylcholinesterase 1 (AChE 1) susceptible phenotypes was reported in the two susceptible populations (samples # 2 and 3) with a percentages of 80 and 75%, respectively.

Discussion

Unfortunately, the massive use of insecticides during the malaria eradication program between 1967 and 1978 has led to the development of strong resistance worldwide in *Culex pipiens* from Tunisia[19-22]. Furthermore, organophosphates actually remain one of the major tools for culicinae control including *Culex pipiens*. It is clear that closer collaboration between resistance experts in agriculture and public health is needed. Indeed and despite the absence of agricultural control in the studied areas, we can consider that the migration including passive transportation of mosquitoes and gene flow play major roles in the dispersion of resistance genes between distant populations[30]. Except the previous and ancient studies of Ben Cheikh and his collaborators[19-22,31], there are no previous published studies about the resistance status of Tunisian *Culex pipiens pipiens* populations to chemical insecticides. Therefore, these populations of *Culex pipiens pipiens* need to be monitored for insecticide resistance in this area. Previous findings[19-22,31] showed that organophosphate and carbamate resistance was widespread in Tunisian populations of *Culex pipiens*. Ben Cheikh et al[19] showed low resistance ratio levels of *Culex pipiens pipiens* to temephos insecticide, not exceeding 10-fold. However, high resistance level of this species to temephos was recorded in Tunisia and reached 400-fold[31].

The use of studied insecticides in public health explained the recorded resistance of *Culex pipiens pipiens*. Carbamate and organophosphate resistance was strongly correlated with the presence of an insensitive acetylcholinesterase which results in reduced sensitivity to inhibition of the enzyme. This target site resistance has been found and documented in many vectors including *Culex pipiens* and other mosquitoes[19,32-34]. In Benin, a cross-resistance between organophosphatess (fenitrothion) and carbamates (bendiocarb and propoxur) in *Anopheles gambiae* was reported with the presence of insensitive acetylcholinesterase[35]. The polymorphism of AChE 1 showed that the highest frequencies of acetylcholinesterase 1 (AChE 1) resistant phenotypes was recorded in the most resistant population with an important percentage of duplicate phenotype [RS]. This could be probably due to the fitness costs associated to different alleles. Indeed, the overall fitness advantage of the duplicated haplotype may result from a much lower fitness cost[36].

Esterases, CYTP450 and glutathione-S-transferases are the three detoxification enzymes known to confer resistance to insecticides in mosquitoes vectors for all major classes of insecticides currently used for vector control, including organochlorine, organophosphates, carbamates, and pyrethroids. In the present study, synergist bioassays and biochemical characterization showed that esterases and CYTP450 were involved in the recorded resistance. Our results are in agreement with previous studies on the role of the CYTP450, esterases and the glutathione-S-transferases in the organophosphate including temephos resistance[19,37,38]. However, authors showed that AChE 1 identified above is known to be a dominant feature in resistance insects[39].

Conclusion

In the present study, both resistant target site and detoxification enzymes were identified and therefore confer the recorded resistance to temephos and propoxur compounds in *Culex pipiens pipiens*. The selection pressure for resistance could have risen mainly from the use of these insecticides in agriculture, as well as in public health. Given the increase of resistance to temephos insecticide in *Culex pipiens pipiens* compared to previous studies, there is an urgent need for field and laboratory monitoring of insecticide resistance.

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Conflict of interest

The authors declare that they have no conflict of interest.

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