Resistance status to deltamethrin pyrethroid of *Culex pipiens pipiens* (Diptera: Culicidae) collected from three districts of Tunisia

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Abstract

Objectives: The aim of the present study was to determine the susceptibility status of *Culex pipiens pipiens* populations against deltamehtrin insecticide.

Methods: Larvae of *Culex pipiens pipiens* were collected from three breeding places in Northern and Southern Tunisia between 2003 and 2005. Early third and late fourth instars were tested against deltamethrin pyrethroid insecticide. Cross-resistance with DDT resistance was evaluated in studied samples to estimate the role of target site insensitivity and two synergists including piperonyl butoxide (Pb) and S,S,S-tributyl phosphorotrithioate (DEF) were used to estimate the role of detoxification enzymes.

Results: Our results revealed that the level of deltamehtrin resistance ranged from 0.67 to 31.4. We also showed the non-involvement of kdr resistance in pyrethroid resistance and no cross-resistance with DDT resistance was detected in all studied populations including the most resistant one. Synergists study on the resistant population (sample # 1) showed the involvement of CYP450 in the recorded resistance to the deltamethrin insecticide.

Conclusion: The results obtained from this study should be considered in the current control programs to combat mosquitoes in Tunisia.

Keywords: *Culex pipiens pipiens*, deltamethrin resistance, kdr mutation, detoxification, Tunisia.

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Introduction

Culex mosquitoes are known as main vectors of lymphatic filariasis and several viral pathogens[1] including West Nile encephalitis which regularly strikes Tunisia, North Africa[2]. Mosquito-borne diseases continue to dramatically affect public health and to constrain economic development worldwide. Due to absence of vaccination available for some of the most devastating mosquito-borne diseases, mosquito control is considered as the better method of intervention[3]. Most mosquito control programs still mostly depend on chemical insecticides[4] including pyrethroids which are the most commonly used insecticides due to the relatively low mammalian toxicity and rapid knockdown effect on insects[5]. However, these gains are threatened by the rapid development and spread of insecticide resistance that would threaten the efficacy of control programs. Hence, it is important to prevent or delay the emergence and development of resistance to pyrethroids to improve vector control efforts. Knowledge of resistance status and understand its mechanisms would be of great importance.

Increased detoxification and target site insensitivity[6] are the two main resistance mechanisms of mosquitoes to pyrethroids. Three major gene families of detoxification

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enzymes are well documented[7] and have associated with pyrethroid resistance in mosquitoes[8-10]: cytochrome P450 monooxygenases (CYP450), carboxyl/choline esterases (CCEs) and glutathione-S-transferases (GSTs). The target sites of pyrethroids, known as knockdown resistance (kdr), encode voltage-gated sodium channels, and mutations in the sodium channel have been shown in several insect species[11] to reduce neuronal sensitivity to DDT and pyrethroids[12].

Previous studies reported low, moderate and high level of resistance to pyrethroids in *Culex* mosquitoes from Tunisia[4,13]. Here, we studied the resistance status of *Culex pipiens pipiens* to deltamethrin insecticide in Tunisia. Cross-resistance with DDT resistance was evaluated in studied samples to estimate the role of target site insensitivity and two synergists including piperonyl butoxide (Pb) and S,S,S-tributyl phosphorotrithioate (DEF) were used to estimate the role of detoxification enzymes.

Materials and methods

Larvae of *Culex pipiens pipiens* were collected from three breeding sites in Northern and Southern Tunisia between 2003 and 2005. 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). Late 3rd or early 4th instar larvae were identified morphologically[14] and tested for susceptibility to deltamethrin pyrethroid insecticide. The synergists tested to estimate metabolic resistance were piperonyl butoxide (Pb) and S,S,S-tributyl phosphorotrithioate (DEF). We evaluated the DDT resistance of studied samples to detect cross-resistance with pyrethroid resistances which have a common target site. Standard methods of Raymond et al[15] for testing mosquito larvae were essentially followed to performed bioassays. Bioassays were performed on field populations and/or F1 and F2 laboratory generations in order to finalize all necessary tests. Deltamethrin bioassays included 5 concentrations providing between 0 and 100% mortality and 5 replicates per concentration on sets of 20 late 3rd and early 4th instars in a total volume of 100 ml of water containing 1 ml of ethanol solution of the tested insecticide. The serial dilutions of each insecticide were performed to generate concentration-mortality curves. The effect on pyrethroid resistance of 2 synergists: the DEF (98%, Chem Service, England), and Pb (94%, Laboratory Dr Ehrenstorfer, Germany) , was studied by exposing larvae to a standard sub-lethal doses of 0.08 mg/l for DEF , and 2.5 mg/l for Pb , 4h before the addition of the insecticide[15]. Tests were cancelled if mortality exceeded 10% in control beakers. LC_{50}, LC_{95} and regression line were calculated by log probit program of Raymond[16], based on Finney[17].Values of LC_{50}, LC_{95}, confidence limits at 95% and slopes were computed. Susceptible strain was used to calculate the Resistance ratio at LC_{50} which is LC_{50} of field population/LC_{50} of sensitive strain and synergism ratio at LC_{50} which is LC_{50} in absence of synergist/LC_{50} in presence of synergist.

Results

In the present study, three field-populations of *Culex pipiens pipiens* were collected from different parts of Tunisia. The results of experiments have been shown in Table 1 that reveals the resistance of studied populations to deltamethrin insecticide which ranged from 0.67 to 31.4. Bioassays showed that the sample # 1 was resistant to used insecticide reaching 31.4.

Table 1: Deltamethrin resistance characteristics of Tunisian *Culex pipiens pipiens*

Population	Deltamethrin			Deltamethrin +DEF					Deltamethrin +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	0.18 (0.17–0.20)	3.53 ± 0.24	-	0.18 (0.07–0.47)	2.59 ± 1.07	-	1.02 (0.41–2.56)	-	0.02 (0.01–0.06)	1.20 ± 0.33	-	10.0 (6.27–16.1)	-
1- Sidi Hcine	5.7 (2.8–11)	1.36 ± 0.25	31.4 (21.1–46.6)	4.5 (3.1–6.5)	1.34± 0.12**	25.5 (10.4–62.1)	1.26 (0.84–1.89)	1.23	0.03 (0.02–0.05)	1.45 ± 0.21	1.74 (1.04–2.92)	181 (116–282)	18.0
2- El Fahs	0.12 (0.07–0.23)	2.01 ± 0.42	0.67 (0.41–1.08)	-	-	-	-	-	-	-	-	-	-
3- Jebeniana	0.15 (0.06–0.40)	1.23 ± 0.31	0.81 (0.53–1.24)	-	-	-	-	-	-	-	-	-	-

(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).
Note: the empty cells was due to the loss of some populations.

However, samples # 2 and 3 were susceptible and their resistance rations did not exceed 0.81. No cross-resistance between pyrtehoird and DDT insecticides (Table 2) was detected in all samples showing any correlation between both insecticides and indicated the non-involvment of kdr mutations since both insecticides target the voltage-gated sodium channel of insect. Indeed, the alone resistant population to deltamethrin showed low resistance ratio to DDT insecticide (1.95). Likewise, the two susceptible population recorded low resistance level to DDT not exceeding 4-folds. Bioassays synergists (Table 1) realized on the resistant population (sample # 1) showed that there was no significant effect of DEF synergist on the toxicity of deltamethrin insecticide in the studied sample, suggesting the non-involvement esterase (and/or GST) in the recorded resistance. Indeed, the SR50 was not significantly higher than that recorded in S-Lab in the studied sample. However, resistance ratio of sample # 1 was affected by Pb synergist showing the involvement of CYP450 in the recorded resistance (RSR>18).

Table 2: DDT resistance characteristics of Tunisian *Culex pipiens pipiens*

Population	LC_{50} in µg/l (a)	Slope ± SE	RR_{50} (a)
Slab	3,1 (2,7–3,4)	3,26 ± 0,26	-
1-Sidi Hcine	6.1 (4–9.3)	1,78 ± 0,23	1,95 (1,40–2,73)
2-El Fahs	14 (5,1–39)	1,29± 0,29	4,53 (2,83–7,26)
3-Jebeniana	6,7 (5–8,8)	1,54 ± 0,17	2,13 (1,67–2,72)

(a), 95% CI; RR_{50}, resistance ratio at LC_{50} (RR_{50}=LC_{50} of the population considered/LC_{50} of Slab).

Discussion

The present paper reported low and high resistance levels deltamethrin pyrethroids. Previous studies showed that some populations showed high resistance to permethrin pyrethroids (up to 5,000-fold) in Tunisia[4]. Nine years earlier, resistance ratio levels of 9092-folds and 453-folds of *Culex pipiens pipiens* from Tunisia was recorded to permethrin and deltamethrin, respectively[13]. Similar results were found in the most parts of the worldwide although low resistance ratios were also recorded to permethrin insecticide: <4-folds in Venezuella[18], 18.3-folds in California[19], 9.5 to 82-folds in Ivory Coast and 17 to 49-folds in Burkina Faso[20], 2500-folds in Saudi Arabia[21] and 2800-folds in Martinique[22]. In contrast, resistance to deltamethrin insecticide was lower than recorded in Tunisia: 9 to 38-folds in West Africa[20] and 12-folds in California[19].

Synergist assays indicated that CYP450 were involved as the resistance mechanism to deltamethrinin in the alone resistant *Culex pipiens pipiens* population tested. Daaboub et al[13] showed that permethrin and deltamethrin resistances recorded in *Culex pipiens pipiens* from Tunisia was almost completely suppressed by Pb and partially suppressed by DEF synergists, suggesting the major and the minor involvement of cytochrome P450 and esterases (and/or GSTs) in recorded resistance, respectively. Using the same synergist, Ben Cheikh et al[4] reported that esterases (and/or GSTs) were not involved in the resistance to permethrin pyrethroids in Tunisian populations of *Culex pipiens* although CYP450s played only a minor role. The involvement of detoxification enzymes in pyrethroid resistance was widely documented. Amin and Hemingway[21] reported the important contribution of oxidases in the high resistance to permethrin (2500-fold) of *Culex pipiens quinquefasciatus* from Saudi Arabia. According to McAbee et al[19], carboxylesterases and CYP450 played an important role in the resistance to permethrin pyrethroids of *Culex pipiens pipiens* from California. Synergistic and biochemical tests revealed that the resistance to permethrin pyrethroids (3750-fold) of *Culex pipiens quinquefasciatus* from West Africa was due in part to CYP450[20]. However, Bisset et al[18] showed that detoxification enzymes were not involved in resistance to permethrin and deltamethrin in *Culex pipiens quinquefasciatus* from Venezuela.

The present study reported a negative correlation between resistance to DDT and deltamethrin insecticides. Contrary, opposite observations have been observed in several mosquito species including *Aedes aegypti*[23], *Culex pipiens quinquefasciatus*[24], *Anopheles quadrimaculatus*[25], *Culex pipiens pipiens*[4], *Anopheles gambiae*[26] and *Aedes albopictus*[23]. It is important to note that the prolonged and intensive use of DDT against malaria vectors in these countries could be probably responsible for the cross-resistance resistance expressed by their common target site (kdr mutation). Indeed, previous studies reported that CNaVD modification was implicated, in addition to detoxification enzymes particularly CYP450, in permethrin pyrethroids resistance of *Culex pipiens quinquefasciatus*[20] mosquitoes, *Anopheles stephensi*[27] and *Culex pipiens pipiens*[19].

Raymond et al[28] have shown that the association of detoxification with an insensitive target is additive with a major role of target site. The absence of the important mechanism in the resistant studied sample suggests the intervention of other factors in the recorded resistance. In this context, we should note that detoxification enzymes may be insensitive to the effects of synergists which probably explain the absence of esterases in the studied sample.

Figure 1: Geographic origin of Tunisian populations.

Conclusion

The results obtained from this study revealed different levels of deltamehtrin resistance in *Culex pipiens pipiens* from Tunisia. Considering the ecological plasticity of this species and their role in the transmission of several diseases, further investigation are needed to well understand the resistance mechanisms of this species against insecticides using molecular and biochemical methods.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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