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Impacts of agricultural practices on pyrethroid resistance in *Culex pipiens pipiens*, an important vector of human diseases, from Tunisia

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Abstract. Agricultural pesticides may play a profound role in selection of resistance in field populations of mosquito vectors. The aim of the present study was to examine the relationship between agricultural pesticide use and development of resistance to insecticides in *Culex pipiens pipiens* from Tunisia. Entomological surveys were conducted in three various districts from Tunisia differ in insect control in agriculture and in public health. A reference locality without any chemical activities was used to do different comparisons. Our results revealed that the level of permethrin resistance ranged from 40.9 to 7438. Practically no susceptible populations were found and resistance to permethrin was important, but significantly higher in site submitted to both agricultural and public health applications. However, resistance ratio has been decreased 7000 folds in site not submitted to agricultural pests. These observations expressed an important influence of agricultural applications on permethrin resistance and need an urgent coordination between the integrated vector control program and the Ministry of Agriculture to reduce the development of resistance in populations. The recorded resistance was slightly associated with DDT suggest the involvement of their common mechanism (target site). Synergist's tests indicated that different enzymes played an important role in the detoxification of this insecticide.

INTRODUCTION

Vector-borne diseases are major contributors to the global disease burden and represent a major burden in terms of economy and development worldwide (WHO, 2011). Vector control using insecticides applications constitutes an important element in the current global strategies for the control of major vector-borne diseases (McCarroll *et al.*, 2002; Najera *et al.*, 2001). Pyrethroids are currently the only class of insecticides approved for controlling mosquitoes worldwide including *Culex pipiens* which was the main vector of West Nile virus that affected Tunisia in 1997, 2003, 2007, 2010, 2011 and 2012 (Triki *et al.*, 2001; Hachfi *et al.*, 2010; Bouatef *et al.*, 2012; Riabi *et al.*, 2014) because of their rapid effects on mosquitoes at low dosages. However the overuse and misuse of insecticides have led to the emergence of resistance, which undermines the potentiality of vector control (Curtia *et al.*, 1998; Enayati *et al.*, 2006). Resistance is defined by the World Health Organisation as "the development of an ability in a strain of some organism to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species" (WHO, 1957). Pyrethroid insecticides have been widely used for decades in Tunisia and highly resistant populations of *Culex pipiens* have been reported in several Tunisian provinces (Daaboub *et al.*, 2008). The mechanisms of resistance in mosquitoes have attracted a great deal of attention from researchers, because they elucidate pathways of resistance development and help those designing novel strategies to prevent or minimize the spread and evolution of resistance. Diverse resistance mechanisms have already been identified in several insect species including mosquitoes (Pasteur *et al.*, 1996; Brengues *et al.*, 2003). There are two major mechanisms of pyrethroid resistance in insects including increases in the rate of metabolic detoxification of the insecticide and changes in target site sensitivity which caused the knockdown resistance (kdr) mutation (Daaboub *et al.*, 2008).

It is known that pyrethroid efficacy is now threatened by the rise of resistance in target populations. However, studies pointed out the possible role of other factors including the use of agricultural pest and mosquitoes control activities in the selection of inherited resistance mechanisms or in the higher tolerance of mosquitoes to pyrethroids. Other studies showed that anthropogenic pollutants may be an important origin of pyrethroids resistance in mosquitoes. These factors may affect the distribution of disease vectors and subsequently the transmission and incidence of human pathogens (Gould & Higgs, 2009; Peterson *et al.*, 2005). A better understanding of factors affecting mosquito response to pyrethroid may provide unforeseen perspectives for controlling mosquito populations and developing innovative insecticide resistance management strategies. In this context, the present work aims at revealing the impact of agricultural pesticides and/or mosquitoes control activities on mosquito response to pyrethroids in three field populations of *Culex pipiens pipiens* collected along three various districts from Tunisia differ in insect control in agriculture and in public health. For each population, different mechanisms leading to resistance to pyrethroids were identified and discussed.

MATERIALS AND METHODS

Larvae of *Culex pipiens pipiens* were collected from three field populations of *Culex pipiens pipiens* along three various districts from Tunisia (Figure 1) differ in insect control in agriculture and in public health. A reference locality without any chemical activities was used as reference to do different comparisons. The characteristics of study areas including insecticides usage is given in Table 1. Data were collected according to both ministries of health and agriculture and during individual interviews with the collection sites residents. Late 3rd or early 4th instar larvae were identified morphologically according to the standardized key for the mosquitoes of Mediterranean Africa (Brunhes *et al.*,

Figure 1. Geographic origin of Tunisian populations.

Table 1. Geographic origin of Tunisian populations, breeding site characteristics, and agricultural pest and mosquitoes control activities

Code	Locality	Breeding site	Date of collection	Mosquito control (used insecticides)	Agricultural pest control
1	Tazarka	River	May 2005	Both Organophosphates and pyrethroids (C, T, Pm, F, P, D)	Yes
2	Gabes	Drain	June 2005	Both Organophosphates and pyrethroids (C, Pm, P, D)	None
3	Sidi khalifa	Water pond	July 2004	None	None

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

1999) and used for different bioassays. In this paper, we applied DDT bioassays to elucidate the resistance mechanisms in permethrin-resistant and DDT/permethrin cross-resistant populations. Resistance to permethrin insecticide was evaluate under standard insectary conditions (25 ± 1°C and 70 ± 5% RH) in late third and early fourth instar larvae from studied populations according to standard methods of Raymond *et al.* (1986). Serial dilutions of each insecticide were performed to generate concentration-mortality curves. According to the methods of Raymond *et al.* (1986), five concentrations of used insecticide (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. After a period of 24 hours, larval mortality was recorded. Two synergists including the DEF (98%, Chem Service, England), and Pb (94%, Laboratory Dr Ehrenstorfer, Germany) were associated with some bioassays to estimate the role of detoxification enzymes in permethrin resistance. The synergists effect 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 to estimate the role of detoxification enzymes. Tests were cancelled if mortality exceeded 10% in control beakers. Resistance ratios (RR_{50} and RR_{95}) for the various field populations were calculated by comparison with lethal concentrations (LC_{50} and LC_{95}) obtained for the S-Lab strain. LC_{50}, LC_{95} and regression line were calculated using log probit program of Raymond (1993), based on Finney (1971). This program tests the linearity of a dose-mortality response, computes the different lethal doses (LCs) and their confidence interval (CI) at the chosen probability (here P=95%). It also compares pairs of dose-mortality responses. When mortality responses are linear, it determines whether they are parallel and estimates the difference by computing a ratio with its 95% confidence interval (95% CI). For mortality responses which are not linear or/and not parallel, the program analyses the difference at each LC by computing a ratio and its 95% CI. In this study, the ratios computed were: the resistance ratio (RR) comparing each sample to the reference strain (S-Lab), and the synergism ratio (SR) comparing mortality data observed in presence of the insecticide alone to mortality data observed in presence of the insecticide plus the synergist in each sample. RR and SR are considered significant ($P<0.05$) when their 95% CI does not include the value 1. To test whether a synergist was more efficient in the field population than in the S-Lab, relative synergism ratios (RSR) were determined. The RSR is equal to the RR for insecticide alone divided by the RR for insecticide plus synergist. A RSR>1 indicates that the synergist has a stronger effect in the field population than in the S-Lab, that is, that the detoxifying mechanism synergized is enhanced in the field population; a RSR<1 shows that the two samples compared are not different as far as the mechanism inhibited by the synergist is concerned (Poirié *et al.*, 1992).

RESULTS

Three field populations of *Culex pipiens pipiens* were collected along three various districts from Tunisia differ in insect control in agriculture and in public health. A reference locality without any chemical activities was used to do different comparisons. As shown in Table 2, the resistance to permethrin insecticide ranged from 40.9 to 7438. The highest resistance ratio of the median lethal dose (RR50) was recorded in the field-population submitted to both agricultural and public health departments (sample 1). However, resistance ratio has been decreased 7000 folds in sample # 2 not submitted to agricultural pests (Table 2). These observations expressed an important influence of agricultural applications on permethrin resistance. In this context, it is important to noted that anthropogenic pollutants may be probably the origin of the moderate level of resistance to the tested insecticide in sample 3 (Table 2).

In the presence of Pb, the toxicity of permethrin significantly increased in samples 1, 2 and 3 (Table 2). The median-lethal doses of permethrin were about 7000, 200 and 2 times lower than that obtained without synergists, respectively. This indicates that cytochrome- P450 monooxygenases played an important role in the detoxification of this insecticide. Applying DEF 4 hours prior to treatment with insecticide, toxicity of permethrin was unchanged (Table 2) and the mixture did not show any synergistic interactions in sample 2 (synergism ratio (SR) < 0.55). However, the toxicity of permethrin increased in samples 1 and 3 and the median-lethal doses of permethrin were about 3.5 and 2 times lower than that obtained without synergists, respectively. These finding showed the involvement of esterases (and/or GST) in the recorded resistance.

A positive correlation was revealed between permethrin and DDT insecticides (Table 3). These finding indicated the involvement of their common target site (voltage-gated sodium channel) in the recorded resistance conferring cross-resistance to both DDT and pyrethroids.

DISUCSSION

Agriculture has been an essential industry for nearly all major economies in the world including Tunisia. Pesticides have been routinely used by both small-scale and marginal farmers and these applications causes the selection of resistance in mosquitoes, threatening effectiveness and sustainability of vector control programs (Chouaibou *et al.*, 2008; Diabate *et al.*, 2002; Yadouleton *et al.*, 2009, 2011). Indeed, as most insecticides used in agriculture are of the same chemical classes and share the same targets and modes of action as those used for vector control, they have the potential to select for resistance in mosquitoes (Khambay & Jewess, 2010). On the other hand, several chemical products including pyrethroid insecticides are widely used for mosquitoes control activities and contribute to the selection of resistance in mosquitoes (Daaboub *et al.*, 2017). 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 (Ben Cheikh *et al.*, 1998). Furthermore, pyrethroid insectcides remain actually one of the major tools for culicinae control including *Culex pipiens* which was the main vector of West Nile virus that affected Tunisia in 1997, 2003, 2007, 2010, 2011 and 2012 (Triki *et al.*, 2001; Hachfi *et al.*, 2010; Bouatef *et al.*, 2012; Riabi *et al.*, 2014). Previous studies carried out in Africa showed that the intensive use of agricultural pesticides is the origin of the recorded resistance in *Anopheles* mosquitoes. However, control vector activities may contribute to a moderate level of resistance (Chandre *et al.*, 1999; Chouaibou *et al.*, 2008; Diabate *et al.*, 2002; Ranson *et al.*, 2009; Yadouleton *et al.*, 2011).

It is important to note that many anthropogenic pollutants including chemicals

Table 2. Permethrin resistance characteristics of Tunisian *Culex pipiens pipiens* in presence and absence of synergists DEF and Pb

Population	*Permethrin*			*Permethrin +DEF*					*Permethrin+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	0.41 (0.38–0.44)	4.7 ± 0.55	–	0.41 (0.26–0.71)	1.22 ± 0.25	–	0.99 (0.73–1.3)	–	0.13 (0.10–0.19)	1.83 ± 0.26	–	3.1 (2.3–4.2)	–
1-Tazarka	3050 (1430–6740)	1.07 ± 0.16	7438 (5015–11,032)	890 (284–2790)	0.79 ± 0.16	2150 (1327–3484)	3.4 (2.1–5.3)	3.5	12 (11–14)	2.97 ± 0.19	98.4 (73.5–131)	236 (165–336)	75.6
2-Gabes	45 (36–55)	1.29 ± 0.08	110 (90.6–135)	82 (54–126)	1.18 ± 0.12	199 (138–286)	0.55 (0.43–0.69)	0.55	0.78 (0.13–4.4)	0.83 ± 0.23	5.9 (3.1–11.2)	58.6 (34.3–100)	18.6
3-Sidi khalifa	16 (12–22)	3.43 ± 0.5	40.9 (25.3–66.3)	8.5 (5.8–12)	1.43 ± 0.18	20.6 (14.8–28.6)	1.9 (1.2–3.1)	2.0	0.19 (0.12–0.26)	1.82 ± 0.31	1.4 (0.96–2.0)	90.1 (51.0–158)	2.9

(a), 95% CI.
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).

Table 3. 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-Tazarka	206 (96–443)	1.04 ± 0.18	65.7 (45.5–94.9)
2-Gabes	13 (8–22)	1.6 ± 0.24	4.2 (3.0–6.0)
2-Sidi Khalifa	5 (3.8–6.4)	2.38 ± 0.34	1.5 (1.1–2.1)

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

commonly used in agriculture also include fertilizers, herbicides, fungicides and various adjuvants may put indirect pressure of the resistance of mosquitoes to pyrethroids in the reference locality. Previous studies showed that *Aedes albopictus* larvae developed an important tolerance to carbaryl after exposition to fungicides (Suwanchaichinda & Brattsten, 2001). Similarly, *Aedes aegypti* larvae exposed to different herbicides led to a significant increase of their resistance to permethrin together with the induction of multiple detoxification genes (Riaz *et al.*, 2009). The cross-resistance between insecticides and some pollutants was well investigated. However, the quantification of these chemicals in relation to their contribution to insecticide resistance of field mosquito populations, where pollutants are represented as complex heterogeneous mixtures remains challenging. In this context, further investigations are needed to elucidate the mechanism of resistance using molecular and biochemical methods and therefore knowing whether insecticide resistance mutations involve each chemical pollutant. Understanding the mode of action of both anthropogenic and natural pollutants in contact with mosquitoes will represent a significant step forward for predicting the dynamics of resistance in diverse environments.

In the present study, both target-site insensitivity and metabolic resistance were involved in the recorded resistance to permethrin of all studied samples. Previous studies carried out on *Aedes aegypti* showed a positive correlation between resistance levels and the expression of particular detoxification genes with agriculture activities (Marcombe *et al.*, 2012). These results should be considered in the current mosquitoes control programs in Tunisia and the combination of insecticides and synergists will be of great importance to reduce the development of resistance.

CONCLUSIONS

Development of insecticide resistance management strategies involves better understanding of the environmental factors that may lead to the resistance. Among those factors, the use of chemical products by both public health and agricultural department's are likely to have a significant pressure selection of pyrethroid resistance but rarely studied in detail. In addition to field studies, laboratory and field experiments are needed to valid the impact of agricultural practices on mosquito resistance and to estimate the selection pressure of each chemical product.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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