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First investigation of deltamethrin pyrethroid susceptibility and resistance status of *Anopheles labranchiae* (Falleroni, 1926), potential malaria vector in Tunisia

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ABSTRACT

Objective: To evaluate the deltamethrin pyrethroid insecticides against *Anopheles labranchiae*, potential malaria vector in Tunisia.

Methods: Six field populations of *Anopheles labranchiae* mosquitoes were collected from six localities in Northern and Central Tunisia between October and November 2016. Different bioassays were performed to estimate the level of resistance in each collected population. Two synergists were used to estimate the involvement of detoxification enzymes in insecticide resistance.

Results: All studied strains were resistant and the RR_{50} ranged from 12.5 in sample #1 to 72.5 in sample #6. Synergist tests using piperonyl butoxide indicated the involvement of monoxygenases enzymes in the recorded resistance. In contrast, the increase of deltamethrin mortality was not significant in presence of S,S,sributyl phosphorothioate ($0.8 < SR < 1.2$), suggesting no role of esterases (and/or GST) in the resistance phenotype. The correlation recorded between mortality due to DDT and the LC_{50} of deltamethrin insecticide indicated an insensitive sodium channel affected by Kdr mutation (Spearman rank correlation, $r = -0.59$, $P < 0.01$).

Conclusions: These results should be considered in the current mosquitoes control programs in Tunisia. The use of pesticides and insecticides by both agricultural and public health departments in Tunisia should be more rational to reduce the development of resistance in populations. Different insecticide applications should be implemented alternately.

1. Introduction

Malaria was endemic in Tunisia before its elimination in 1980 due to the malaria eradication program. The incidence of 10 000 cases was recorded every year [1]. *Anopheles labranchiae (An. Labranchiae)* (Falleroni, 1926) was incriminated as the principal vector of autochthonous transmission malaria in a large part of the country, particularly in the northern and central governorates (Wernsdorfer W and Iyengar MO, unpublished data). In fact, several authors showed experimentally that *An. labranchiae* can successfully transmit *Plasmodium falciparum (P. falciparum)* [2,3]. Despite Italian populations were refractory to African strains of *P. falciparum* [4,5]. This species was also the main vector incriminated in autochthonous transmission of *Plasmodium vivax* (*P. vivax)* in Corsica, Greece, and Italy [6–8]. In Tunisia, Aoun *et al.* [9] mentioned recently an increase in imported cases of *P. vivax* highlighting a risk for the re-emergence of local foci in Tunisia. Furthermore, *An. labranchiae* was the main vector responsible for recent epidemic outbreaks in Morocco [10] due to *P. falciparum*, *Plasmodium malariae*, and *P. vivax*.

Tabbabi *et al.* [11,12] have retained *An. labranchiae* as the only member of *Anopheles maculipennis* complex in Tunisia and North Africa. These authors recently reported for the first time their spatial distribution and larval habitat diversity in Tunisia to identify areas that is at higher risk of malaria transmission. Due to the importance of public health and the long history of insecticide/larvicides resistance in *Anopheles* mosquitoes in Africa and other continents, it is essential to evaluate the resistance status of this species at regular intervals using WHO standard bioassay tests and to map areas of their levels of susceptibility/resistance. Therefore this study was aimed to determine for the first time the deltamethrin pyrethroid resistance status of *An. labranchiae* (Falleroni, 1926), potential

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malaria vector in Tunisia. Results could improve vector control implementation through targeted strategies.

2. Materials and methods

2.1. Mosquitoes and areas study

A sensitive strain of *An. labranchiae* was used as a standard reference. Mosquito larvae were collected from six breeding sites in October and November 2016. *An. labranchiae* larvaes were identified using the keys of Brunhes *et al.* [13]. The localities of the larval collection are cited in Tables.

2.2. Insecticides and synergists

Two insecticides and two synergists were used: the pyrethroid deltamethrin (95.7Vo, ICI Americas, Inc., Richmond, CA), and the organochloric DDT (99.9Vo; Mobay), S,S,sributyl phosphorothioate (DEF), an esterase inhibitor, and piperonyl butoxide (Pb), an inhibitor of mixed function oxidases.

2.3. Dose-response bioassay

Different bioassays were performed following the standard procedure of Raymond *et al.* [14] to estimate the level of resistance to deltamethrin insecticide in each collected population. Late third and early fourth instar larvae were used. At least three replicate groups of 20 larvae placed in 100 mL of water treated with serial dilutions of insecticide were performed in each bioassay. Ethanol replaced insecticide in control group was tested. The assay was repeated if the rate of mortality in the control group exceeded 10%. The mean lethal concentrations of temephos causing 50% and 95% mortality (LC_{50} and LC_{95}) of exposed larvae after 24 h of treatment, which were estimated through a probit analysis linear regression of Raymond [15], based on Finney [16]. The Mazzarri and Georghiou [17] criteria were followed to classify the resistance level of each population tested as follows: low [resistance ratio (RR) < 5], moderate ($5 \leq RR \leq 10$) or high (RR > 10).

3. Results

3.1. Deltamethrin resistance

The LC_{50} values demonstrated that the resistance to deltamethrin of the larvae of *An. labranchiae* collected from Northern and Central Tunisia was highest in Monastir, followed by Jendouba, Kairouan, Beja, Ariana and Ben Arous (Table 1). Sample #6 showed the highest resistance to deltamethrin insecticide with resistance ratio at LC_{50} (RR_{50}) of 72.5, followed by samples #5 and #4 with RR_{50} of 42.50 and 40.83, respectively. Sample #1 showed the lowest susceptibility to deltamethrin with RR_{50} of 12.5. The linearity of the dose-mortality response was accepted ($P < 0.05$) for all studied samples including reference strain ($P < 0.05$). Regression slope showed the homogeneity of tested phenotypes.

Table 1

Resistance to deltamethrin in *An. labranchiae* from Tunisia.

Population	LC_{50} (μg/L)		RR_{50}
	95% *CI*	Slope ± SE	
Sensitive strain	0.12 (0.05–0.17)	2.10 ± 0.32	–
1-Ben Arous	1.50 (0.50–2.20)	1.22 ± 0.17	12.50 (10.20–14.10)
2-Ariana	2.60 (1.90–3.10)	0.87 ± 0.14	21.66 (20.30–23.50)
3-Beja	2.90 (2.00–3.50)	0.89 ± 0.12	24.16 (23.40–25.70)
4-Jendouba	5.10 (4.10–6.40)	1.02 ± 0.16	42.50 (40.20–44.20)
5-Kairouan	4.90 (4.10–5.90)	1.42 ± 0.33	40.83 (39.20–42.30)
6-Monastir	8.70 (7.20–9.40)	0.91 ± 0.13	72.50 (69.20–75.10)

RR_{50}: resistance ratio at LC_{50} (RR_{50} = LC_{50} of the population considered/LC_{50} of Slab).

3.2. Synergism tests

In the presence of Pb, the toxicity of deltamethrin significantly increased in samples #5 and #6 (Table 3). The median-lethal doses of deltamethrin were about 40 and 7 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 h prior to treatment with insecticide, toxicity of deltamethrin was unchanged (Table 2) and the mixture did not show any synergistic interactions in *An. labranchiae* [0.8 < synergism ratio (SR) < 1.2].

3.3. Cross-resistance deltamethrin/DDT

Significant correlation was observed between mortality due to DDT and the LC_{50} of deltamethrin insecticide (Spearman rank correlation, $r = -0.59$, $P < 0.01$) indicating cross-resistance to these two insecticides. Sample #6 having the highest resistance to deltamethrin showed the lowest mortality to DDT (12% at 1 mg/L).

Table 2

Effect of DEF synergist on deltamethrin toxicity in *An. labranchiae* from Tunisia.

Population	LC_{50} (μg/L)		RR_{50}	SR_{50}	RSR
	95% *CI*	Slope ± SE			
Sensitive strain	0.15 (0.14–0.20)	1.23 ± 0.12	–	0.80 (0.28–1.20)	–
1-Ben Arous	1.20 (0.90–1.80)	0.85 ± 0.17	8.00 (7.40–9.20)	1.25 (1.00–1.90)	1.56
2-Ariana	2.40 (1.80–2.90)	0.89 ± 0.19	16.00 (15.20–16.90)	1.08 (0.69–1.61)	1.35
3-Beja	2.50 (2.00–2.90)	0.95 ± 0.17	16.66 (15.10–17.50)	1.16 (0.87–1.88)	1.45
4-Jendouba	4.10 (3.40–5.20)	0.89 ± 0.13	27.33 (25.80–29.20)	1.24 (1.00–2.20)	1.55
5-Kairouan	4.30 (3.90–4.80)	1.22 ± 0.41	28.66 (26.30–29.60)	1.13 (0.69–1.48)	1.42
6-Monastir	7.50 (6.90–8.30)	0.83 ± 0.09	50.00 (48.20–52.60)	1.16 (0.75–1.78)	1.45

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

Effect of Pb synergist on deltamethrin toxicity in *An. labranchiae* from Tunisia.

Population	LC_{50} (μg/L)		RR_{50}	SR_{50}	RSR
	95% *CI*	Slope ± SE			
Sensitive strain	0.15 (0.08–0.55)	1.04 ± 0.32	–	0.80 (0.41–0.99)	–
1-Ben Arous	0.90 (0.11–1.41)	0.75 ± 0.20	6.00 (5.40–6.80)	1.66 (1.40–2.30)	2.08
2-Ariana	2.10 (1.89–2.54)	1.52 ± 0.11	14.00 (12.20–15.30)	1.23 (0.92–2.20)	1.54
3-Beja	2.70 (1.10–3.20)	1.12 ± 0.12	18.00 (14.60–19.00)	1.07 (0.80–1.90)	1.34
4-Jendouba	4.90 (3.40–5.90)	0.87 ± 0.10	32.66 (30.50–34.20)	1.04 (0.80–1.50)	1.30
5-Kairouan	0.12 (0.01–0.45)	1.78 ± 0.14	0.80 (0.40–1.30)	40.83 (38.20–42.00)	51.03
6-Monastir	1.10 (0.70–1.80)	1.25 ± 0.12	7.33 (6.60–8.00)	7.90 (6.30–8.60)	9.89

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).

4. Discussion

The RR_{50} to deltamethrin insecticide differed from population to population. The lowest resistance rate was obtained in Monastir locality while the highest rate was obtained in Ben Arous locality. These results showed the existence of ancient mechanisms that become effective against deltamethrin pyrethroid insecticide and still effective to organochlorine insecticides in the populations such as DDT intensively used for malaria eradication in Tunisia [18]. This resistance can only be based on a genetic factor. Authors [19] concluded that the resistance due to the massif use of DDT may limit the effectiveness of insecticides having the same resistance mechanisms. In fact, mosquito's populations may develop cross-resistance even if the use of DDT insecticide has been interrupted since a long time. The results are in agreement with previous studies on DDT and other insecticides from the same groups. It is already known that malaria vectors such as *Anopheles albimanus*, *Anopheles stephensi* and *Anopheles gambiae* [20–23] have developed pyrethroid resistance following the use of DDT. In Tunisia, DDT was the main insecticide used for malaria eradication programs before 1980 [18] and recently other pesticides including pyrethroids for agricultural purposes. We concluded that *An. labranchiae* have developed this resistance after exposure to insecticides pressures. In fact, *Anopheles sacharovi* is a member of *Anopheles maculipennis* species complex developed resistance to DDT and lindane in 1959, although they were used in the 1950s and 1960s [24]. Hemingway *et al.* [25] reported the same results in *Anopheles sacharovi* populations in 1984 although malathion replaced DDT in malaria control. We should note that the sample size of this study was large enough to draw and definite the resistance status to deltamethrin of Tunisian populations. In contrast, several factors such as climate, season, year or period in which samples are collected and season-based population movements may probably influence the obtained results. In fact, bioassays applied in a single of the year are not sufficient to demonstrate the resistance or susceptibility of the populations, that's why molecular and biochemical studies on mechanisms of resistance are needed.

The correlation recorded between mortality due to DDT and the LC_{50} of deltamethrin insecticide indicating an insensitive sodium channel affected by Kdr mutation (common target site). This mutation was observed in many *Anopheles* mosquitoes [26–29] including *Anopheles sinensis* and *Anopheles peditaeniatus* [26]. The target site mutations that offer cross-resistance to DDT and pyrethroids (kdr) have also been observed in *Anopheles gambiae* [30–32]. In this study, the high resistance recorded in some samples led us to suggest that resistance may be caused by other mechanisms such as metabolic resistance. Indeed, Raymond *et al.* [33] have showed that the resistance caused by detoxification enzymes and insensitive target is additive. The restoration of deltamethrin susceptibility in presence of Pb in some studied samples of *An. labranchiae* suggests the involvement of monoxygenases enzymes in the recorded resistance. Elevated levels of P450 activity have been observed in pyrethroid-resistant malaria vectors in Africa, particularly in *Anopheles funestus* from southern Africa [34–36]. In contrast, the increase of deltamethrin mortality was not significant in presence of DEF, suggesting no role of esterases (and/or GST) in the resistance phenotype. It should be noted that resistance is not always affected by synergist's actions. Several authors showed that resistance to DDT was not due to detoxification enzymes in *Anopheles* and other species of mosquitoes including *Anopheles maculatus*, *Culex pipiens quinquefasciatus* and *Aedes aegypti* in Malaysia [37], *Anopheles albimanus* in Guatemala [38] and *Aedes aegypti* in Thailand [39].

The use of pesticides and insecticides by both agricultural and public health departments in Tunisia should be more rational to reduce the development of resistance in populations. Moreover, non persistent and fast-acting insecticides must be used in right doses and replace permanent insecticides for which *Anopheles* mosquitoes have developed extremely high levels of resistance in several localities of the country. Different insecticide applications should be implemented alternately.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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