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Sensitivity of the Mosquito *Culex pipiens* Diptera: Culicidae towards Temephos in Southern Central Morocco

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mosquito species are responsible for the transmission of many parasitic diseases and their control leads to the phenomenon of resistance to insecticides including temephos, mostly used by hygienic services. This article reports the results of studies conducted using WHO sensitivity tests on larvae local populations of *Culex pipiens* collected in three lodging in the city of Marrakesh (Southern-Central, Morocco), towards temephos. Five concentrations of insecticide in addition to a control, were used to determine the DC50 and DC 90 of *Culex pipiens* species towards temephos. Sensitivity tests were carried out at the entomology unit and monitoring of insect sensitivity towards insecticides installed at the Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene (LRDEHM), Marrakesh, under the Regional Directorate of Health of Marrakesh-Safi. The bioassay results affirmed the presence of resistance in *Culex pipiens* larvae towards temephos. This species has also developed similar and comparable resistance levels in the three lodgings studied, with resistance rates recorded varying between 15.2 and 128.5. This study results can be used as database for the control of mosquito resistance to insecticides at local and national level.

Keywords: Culex pipiens, Resistance, Temephos, Marrakesh, Southern-Central Morocco.

1. INTRODUCTION

Culicidae are responsible for the transmission of pathogens that they can inoculate during their blood meal. They therefore represent a real public health problem. Among these mosquitoes, some are a source of nuisance difficult to bear. This is relevant for *Culex pipiens* Linné, 1758, very widespread throughout the world, especially in tropical and temperate zones [1]. Monitoring the sensitivity of culicidae to insecticides is essential step to develop guidelines for of any vector control program required to carry out a control plan in time, allowing to counter the situation when the insecticide used is no longer effective. In Morocco, the species *C.pipiens* was strongly suspected as being the most probable vector in the transmission of the West Nile virus epidemics which affected Morocco in 1996 [2,3] and in 2003 [4]. At the authors' knowledge, with the exception of the work of Bouallam et al. [5]; Faraj et al. [6]; Faraj et al. [7]; Larhbali et al [8]; El Joubari et al. [9]; El Ouali Lalami et al. [10] and Bkhache et al. [11], no other study has been published on the sensitivity of *C. pipiens* to temephos in Morocco. This work, carried out for the first time in south-central Morocco, aimed to determine the resistance levels of the *C. pipiens* species to temephos, considered as the insecticide usually used in larval control in the study area.

2. MATERIALS AND METHODS

2.1 Study Location

This study was carried out between 2011 and 2013 during the period of mosquito activity in three stations in the region of Marrakech located in south-central Morocco (Fig. 1): Station 1: rural commune Souihla; Station 2: Douar François (rural commune of Saada) and Station 3: Targa (urban commune of Marrakech).

2.2 Strain to Test

The larvae of *C. pipiens* collected at the stations studied were kept in the laboratory in rectangular plastic trays (50 x 30 x 5 cm) filled with breeding water at an average temperature of 21.3 °C ± 2°C, and 70 to 80% humidity. The test conditions are summarized in Table 1.

The mosquito larvae identification was done using for Moroccan culicids [12], and that of African culicids published by the Institute of Research for Development [13].

2.3 Larval Sensitivity Testing

The sensitivity of *C. pipiens* was studied according to "the experimental protocol recommended and standardized by the WHO [14] modified.

Five concentrations of insecticide were prepared from the WHO kit of a stock solution of temephos at 156.2 mg/l, plus a control, with 5 replicates per concentration.

The LD50 and LD90, concentrations corresponding to 50% and 90% mortality, were determined graphically, by the linear relationship between the decimal logarithm of the insecticide concentrations (on the abscissa) and the mortality percentages transformed into probit values (on the ordinate), on logarithmic gausso papers.

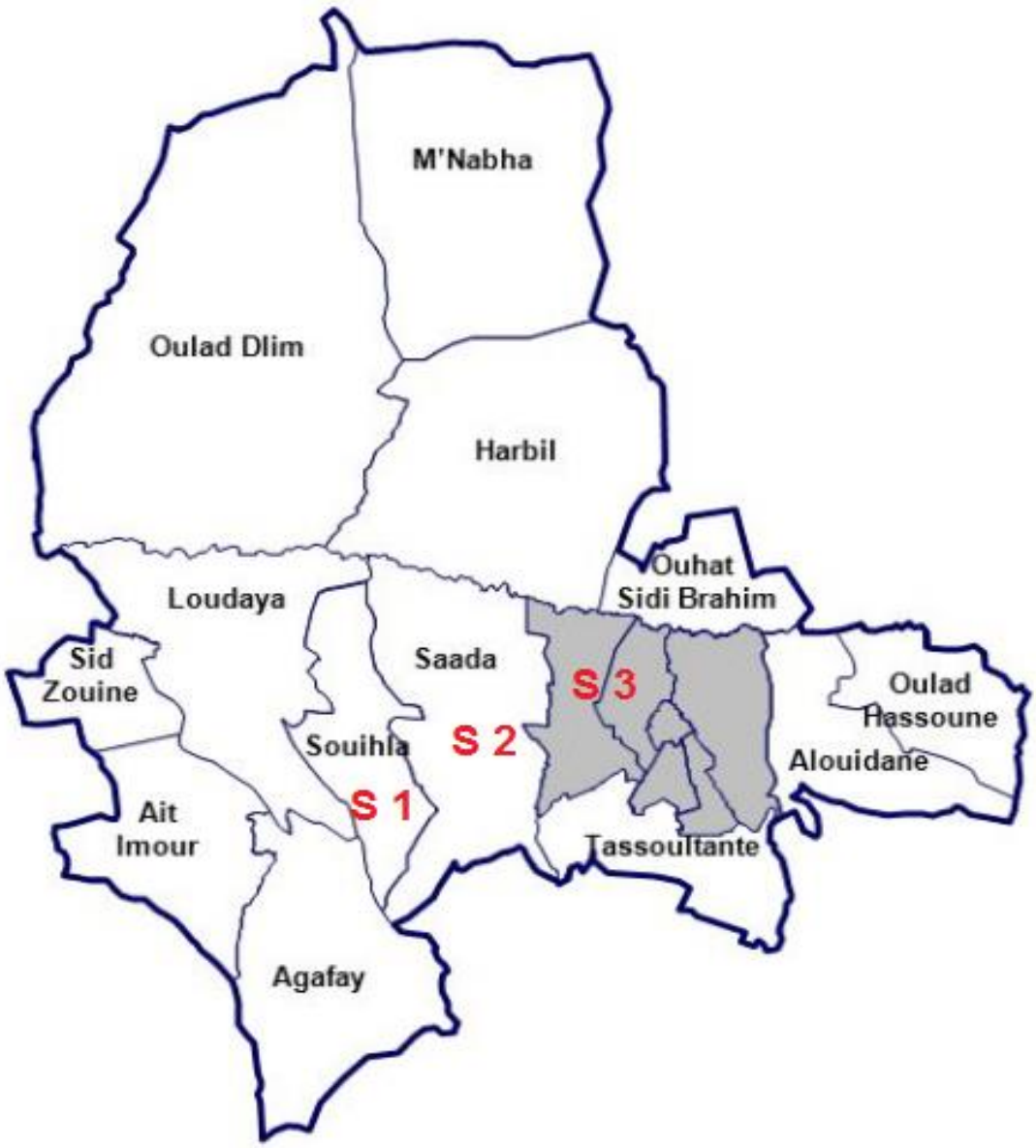

Fig. 1. Location of the area and sites of study

Table 1. The conditions of larval tests

	Cx. pipiens **(Station 1)**	***Cx. pipiens*** **(Station 2)**	***Cx. pipiens*** **(Station 3)**
Larval stage (L) and age (Day)	L3 age 4 days	L3 age 4 days	L3 age 4 days
Temperature (°C)	20.2	26.7	24
Processing method	direct contact	solution contact	solution contact
Number of insects tested in the control sample	100	100	100

The differentiation between susceptible and resistant strains is based on the resistance rate. The latter was calculated by referring to the LD 90 of the *C. pipiens* S-Lab strain (0.0007 mg/l).

3. RESULTS

The results of the sensitivity tests carried out at the 3 study stations are summarized in Tables 2, 3 and 4.

Table 2. Sensitivity to Temephos of larvae of *C. pipiens* in the station 1 (south-central Morocco)**)**

Gites prospected	Dose (mg/L)	Population tested	Mortality	Corrected mortality (%)	LD50 (mg/L)	LD90 (mg/L)	Resistance rate	Chi2 calculated (χ^2)
Station1:	0.0025	100	26	0.2449	0.0052	0.0214	30.5	2.260
C. Rural	0.0050	100	46	0.4490				
commune	0.0125	100	82	0.8163				
Souihla	0.0250	100	95	0.9490				
	0.0500	100	97	0.9694				

Remarks number of dead insects in the control sample: 2 / Percentage of natural mortality in the control sample: 2%

Table 3. Sensitivity of *C. pipiens* larvae to temephos in the station 2 (Douar François)**)**

Gites prospected	Dose (mg/L)	Population tested	Mortality	Corrected mortality (%)	LD50 (mg/L)	LD90 (mg/L)	Resistance rate	Chi^2 calculated (χ^2)
Station 2:	0.0025	100	11	0.0720	0.01397	0.0899	128.5	8.788
Saada	0.0030	100	26	0.2284				
	0.0070	100	27	0.2389				
	0.0125	100	53	0.5099				
	0.0250	100	59	0.6780				

Remarks number of dead insects in the control sample: 4 / Percentage of natural mortality in the control sample: 4%

Table 4. Sensitivity to temephos of *C. pipiens* larvae in the station 3 (Targa)

Gites prospected	Dose (mg/L)	Population tested	Mortality	Corrected mortality (%)	LD50 (mg/L)	LD90 (mg/L)	Resistance rate	Chi2 calculated (χ^2)
Station 3	0.0025	101	13	0.1287	0.00464	0.0107	15.2	10.696
Targa: Urban	0.0050	100	34	0.3400				
community	0.0125	101	66	0.6535				
	0.0250	100	96	0.9600				
	0.0500	100	100	1.0000				

Remarks number of dead insects in the control sample: 0 / Percentage of natural mortality in the control sample: 0%

4. DISCUSSION

The mosquito resistance levels were calculated with reference to a *C. pipiens* strain. The differentiation between susceptible and resistant strains is based on the resistance rate. The latter was calculated by referring to the LD 90 of the *.x. pipiens* S-Lab strain (0.0007 mg/l). It was considered as a reference strain sensitive to organophosphates given that the LD50 and LD90 values corresponding to this strain are comparable to those established on reference strains in other regions of the world [6,10,15, 16,17, 18]. Based on the study results (Tables 2, 3 and 4), the biotests revealed the sensitivity to temephos, of the strain *C. pipiens* from "Station 3", i.e. LD50 = 0.0107 mg/l (equation of weighted regression lines: Y = 1.58500 X + 2.93985). Furthermore, by comparing the sensitivity of different populations of *C. pipiens* sampled from the 3 investigated stations in the study, it was found that this species had developed varying levels of resistance depending on the sampling sites. The highest resistance rates were recorded in station 2, while *C. pipiens* resistance was still moderate, but significant, in station 1, and the lowest levels were found in station 3 (urban area). In fact, *C. pipiens* considered as a ubiquitous mosquito well adapted to different biotopes and environments. It grows in both urban and rural environments, in polluted and clean water. In several regions, it is active throughout the year, reaching maximum development during the hot seasons [19].

Thus, the current study findings can be explained by the fact that temephos insecticide is widely used in the fight against mosquito larvae in stations 1 and 2. Chavasse and Yap 1997 [20] confirmed that prolonged and long-term use of an organophosphate results in the appearance of cross-resistance to other organophosphates, and sometimes to other products of the carbamate family. Indeed, Sinegre et al. [16] managed to establish an obvious correlation between the degrees of mosquito resistance and the frequency of treatments with a given product. Sensitivity to temephos appears identical between the two strains sampled from stations 1 and 2, with 0.0052 mg/l, and a calculated resistance rate of 30.5 for the "Station 1" strain, versus 0.01397 mg/l and a calculated resistance rate of 128.5 for the "Station 2" strain (weighted regression lines: Y = 2.60915 X + 6.61374 and Y =3.1 X + 5.8). Similar results regarding the high resistance of *C. pipiens* to temephos have been reported by several studies in Morocco [7, 8, 11].

In central Morocco, El-Akhal et al. [17] reported that *C.. pipiens* develop significant resistance to temephos (LD50 = 0.0081 mg/l and LD90 = 0.0305 mg/l). Furthermore, Faraj et al. [7] indicated that larval populations of *C. pipiens* are resistant to temephos in the Moroccan prefectures of Mohammedia, Rabat and Skhirat-Témara, with respective resistance rates of 143, 20 and 30. Similarly, El Joubari et al. [9] found that the populations of C.. pipiens tested are resistant to temephos (LD50 = 0.0056 mg/l and LD90 = 0.0243 mg/l) in North-West Morocco. Similar findings to the present study have been documented, by other authors, such as Paul et al. [21] in New York (United States), and Larhbali et al. [8] in Khemisset (Morocco), confirming the resistance of *C. pipiens* to temephos. In another hand, a study on the valorization, as a bioinsecticide, of two essential oils of *Citrus sinensis* and *Citrus aurantium* cultivated in the center of Morocco, showed that the essential oil of *Citrus aurantium* has an interesting larvicidal activity against *Culex pipiens* as compared to the essential oil of *Citrus sinensis*, with respective LC50 and LC90 (139.48; 212.04 ppm) and (280.82; 516ppm as reported by El-Akhal et al. [22]). Similar results have been reported by Belaqziz et al. [23]) on the insecticidal activity against *C.pipiens* compared to the essential oils of *Thymus broussonettii* and *Thymus maroccanus*.

5. CONCLUSION

This study investigated on the sensitivity of the mosquito *Cx. pipiens* to temephos, one of the most used insecticides for vector control in Morocco. findings showed that:

Populations of the species *Cx. pipiens* tested, collected at stations 1 (rural area) and 2 (urban area), are resistant to témephos with respective rates of 128.5 and 30.5);

Populations of the species *Cx. pipiens* tested, collected at the station, are sensitive to temephos with 15.2.

Mosquito resistance to temephos was significantly higher in rural stations as compared to urban stations, which can be related to treatment frequency.

Regular monitoring of the sensitivity of target mosquito populations and the resistance

mechanisms possibly involved are essential and should be an integrated in any vector control program in the study area.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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