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Research Article

Resistance of *Culex pipiens* (Diptera: Culicidae) to Organophosphate Insecticides in Centeral Morocco

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ABSTRACT
In this work, carried out for the first time in the center of Morocco, the activity of four insecticides was studied in 2010 on the larvae of the stages three and four of *Culex pipiens*, which are known to be the responsible of nuisance and/or transmission of some parasitic illnesses especially during spring and summer. Six concentrations were used: 0.00125, 0.0025, 0.005, 0.0125, 0.025, 0.0625 mg/l and the negative control (0 mg/l). The lethal concentrations (LC_{50} and LC_{90}) were determined according to the WHO protocol. LC_{50} of the four insecticides (Malathion, Fenthion, Temephos and Fenitrothion) were respectively 0.0066, 0.0081, 0.0053, 0.0069 mg/l and they were respectively 0.0228, 0.0188, 0.0305, 0.0314 mg/l for LC_{90}.The population of *Culex pipiens* studied presents an important resistance for Temephos and Fenitrothion of 13.26 and 12.07 respectively. However, a beginning of resistance for Malathion was observed with the rate of 0.456, and 0.343 for Fenthion. A regular survey of the sensitivity of the dominant population imposes itself in the density and the mechanisms of the possible resistance implied. The results of this study can be used as database for the management of mosquito resistance to insecticides at local and national level.

Keywords: *Culex pipiens*, Larvae, Insecticides, Organophosphate, Resistance, Central Morocco.

INTRODUCTION

Mosquitoes are vectors of diseases agents infecting over 600 million people per year[1,2] and are responsible for serious human and animal diseases. Those from *Culex pipiens* complex have a wide geographical distribution and are very abundant in temperate and tropical regions. *Culex pipiens* (Diptera: Culicidae) is one of the mosquito responsible for the transmission of certain parasitosis such as the Japanese Encephalitis, the West Nile fever[3-6] the Rift Valley virus[7-10] and certain filariasis[11-15]. This species is also responsible for the strong nuisance being experienced in the majority of the urban and rural areas in the world. The *Culex pipiens* has been strongly incriminated as the most likely vector in the transmission of epidemics (human and equine) of West Nile virus that have affected Morocco, in 1996[16,17], 2003[18,19] and 2010[20,21]. The research work conducted about the Culicidae, in the region of Fez, in the North Center of Morocco, showed that the *Culex* species existed during the whole year[22]. Thus, they could constitute a menace to the population of the region of Fez having particularity the association of a number of risk factors for including: a semi-arid bioclimate and the presence of foreign citizens in this region from Africa, Asia and Europe by many diseases transmitted by *Culex*[23,24]. The vector control by means of insecticides faces the problem of resistance organophosphates and pyrethroids, including the *Culex pipiens* complex[25-32]. Now, each of the four chemical families of insecticides, organophosphates, organochlorines, carbamates and pyrethroids, which are available in the world have been the subject of resistance, they are larvicides or adulticides[33,34]. The observation is true both in Morocco and France as a country in the European continent. Indeed, in the south of France, the *Culex pipiens* mosquito was found resistant to organophosphates and Bacillus sphaericus[35,36]. However, published studies on the sensitivity of mosquito larvae Culex towards insecticides are very limited in Morocco. Indeed, with the exception of the work[37-39], and recently those of El Joubari et al., (2015)[40] no further study has been published on insecticide susceptibility of mosquito larvae of *Culex pipiens* complex. Furthermore, in central Morocco the work on insecticide susceptibility of mosquito species (larvae or adults) are absent except one

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Table 1: Effect larvicide of four insecticides (Temephos, Fenthion, Malathion and Fenitrothion) on the larvas of *Culex pipiens* after 24 hours exhibition.

Insecticides	CL_{50} mg /l (Ll-Ul)*	CL_{90} mg /l (Ll-Ul)*	Chi 2 calculated($\chi 2$)
Malathion	0.0066 (0.0039-0.0091)	0.0228 (0.0171-0.0340)	4.08966
Fenthion	0.0081 (0.0060-0.0100)	0.0188 (0.0153-0.0255)	4.99115
Temephos	0.0053 (0.0022-0.0087)	0.0305 (0.0205-0.0497)	2.350028
Fenitrothion	0.0069 (0.0055-0.0086)	0.0314 (0.0232-0.0476)	2.87204

*Ll-Ul: Lower limit- Upper limit

preliminary study on the sensitivity of *Culex pipiens* towards solely insecticide Temephos which was carried out during the period from years 2007 to 2010 and highlighted the presence of resistance in this strain varying from 12.17 to 14.34[41].

Thus, in the framework of the national program for monitoring the sensitivity of mosquitoes to insecticides and also the research conducted in our laboratory on mosquito resistance to insecticides Culicidae, the sensitivity of larval population of *Culex pipiens* local was studied towards four insecticides (Malathion, Fenthion, Temephos and Fenitrothion). This work, carried out for the first time in the central region of Morocco, was conducted for verifying and confirming the sensitivity or resistance of *Culex pipiens* in Temephos insecticide the most used locally in the anti larval fight and studied the effect of this mosquito larvicide towards other insecticides in order to identify those which could be used as a substituent in case of resistance.

MATERIALS AND METHODS

Characteristics of the site studied

The collection of larvae of *Culex pipiens* was performed in a breeding site located in the urban area of the city of Fez, called Moulay Driss. It is considered as a permanent source site of 500 m² of water and recognized as an ideal habitat for Culex. This site is located at the proximity of uncontrolled waste discharge of all kinds. The characteristics of Lambert of the studied site are as follow: 395 m, 34°04'00.76' latitude and 4°59'28.96' longitude.

Identification of larvae

The morphological identification of larvae appealed to the Moroccan identification key of Culicidae[42] and the identification software of mosquitoes of the Mediterranean Africa[43].

Insecticides tested

The insecticides used to realize the larval tests are organophosphates: Malathion, Fenthion, Temephos and Fenitrothion. We prepared a range of six dilutions from the kit WHO corresponding stock solutions (1.25 and 6.25 mg/l).

Larval susceptibility test

The larval tests were carried out according to the WHO protocol[2]. For each dilution, a series of three beakers was used. In each beaker, we poured 99 ml of distilled water, and then we filed 20 larvae of stages three and four pre-sorted with a pipettor pear. According to the previously selected dilution ranges, we added by increasing dose and X ml beaker of a partial dilution using a micropipette P 1000. Finally, we added 1-X ml of ethanol to supplement 100 ml in each beaker. In the tumblers containing the control larvae, we added solely 1 ml of ethanol. After a contact time of 24 h with the insecticide tested, we counted the dead and living larvae. During those 24 hours, the larvae were not fed. The tumblers were placed in the Unit of Entomology at RDLEEHF, under standard conditions (25 ± 2 ° C and humidity of 70-80%). The protocol of larval test is based on the trial method of the three-fold, that is, three tests under the same experimental conditions. The results of the sensitivity tests are expressed in the percentage of the larval mortality versus the concentrations of insecticides used. If the percentage of mortality in controls exceeds 5%, the larvae exposed to the insecticide must be corrected using the formula of the law of independent probabilities[44].

% Mortality corrected = [(% Mortality observed -% Mortality control) / (100 -% Mortality Control)] × 100.

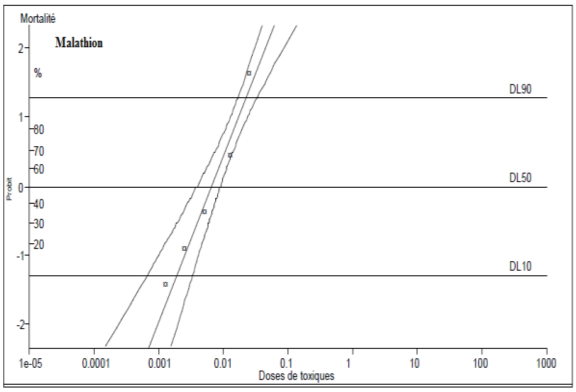
If the mortality in control exceeds 20%, the test is invalid and should be repeated.

Statistical Analysis

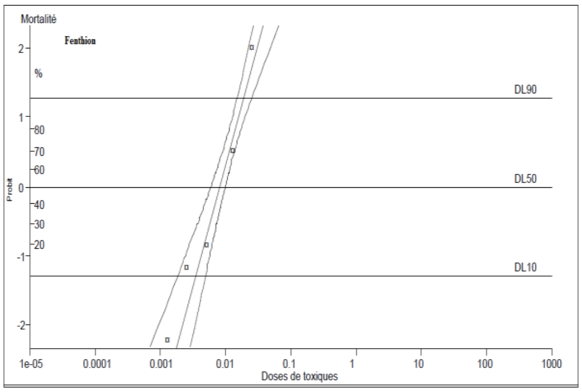
For inputting and processing data, we used the analysis software Log- probit (WinDL version 2.0)[45] developed by CIRAD-CA / MABIS.

RESULTS AND DISCUSSION

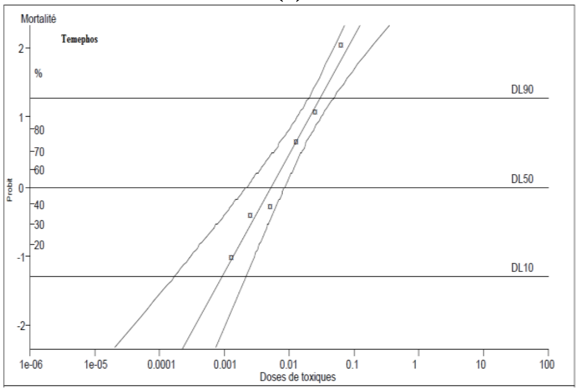
After 24 h of contact, the insecticides studied proved being more efficient at least against the *Culex pipiens* strain (Table 1). Indeed, Temephos present towards the larvae of *Culex pipiens* a lower LC_{50} value of 0.0053mg/l and an LC_{90} of 0.0305mg/l (the right side of equation weighted regression Fenthion: Y = 7.33453 + 3.51138X). For other insecticides, Malathion, Fenitrothion and Fenthion recorded LC_{50} and LC_{90} equal to 0.0066 and 0.0228 mg/l (equation of the weighted regression of Malathion: Y = 5.21035 + 2.39374X); and 0.0069 and 0.0314 mg/l (the right equation of weighted regression Fenitrothion is Y = 4.23014+1.96229 X) and 0.0081 and 0.0188 mg/l (the right side of equation weighted regression Temephos was Y = 3.84133+1.68901X) (Figure 1a-d). Schaefer and Wilder in (1970)[46] reported that the operational failures of an organophosphorus



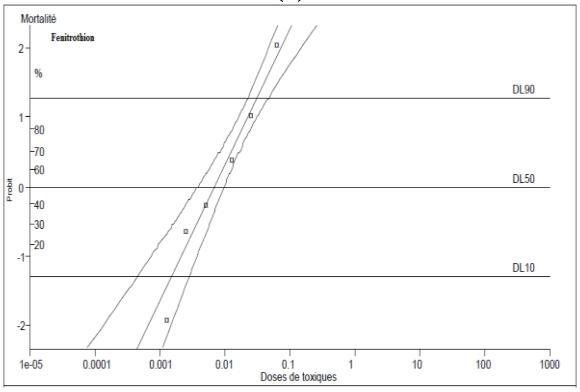
(a)



(b)



(c)



(d)

Figure 1a-d: Curves of resistance to the Malathion (a), Fenthion (b), Temephos (c) and Fenitrothion (d) observed in larvae of *Culex pipiens.*

(Chlorpyrifos) started to turn out to be in a strain when the rate of resistance exceeds 5; whereas Sinègre et al. (1976)[47] found that a strain may contain resistant individuals when its LC_{90} exceed 5 times LC_{90} the base. In our study, the rate of resistance calculated for the four insecticides (Malathion, Fenthion, Temephos and Fenitrothion) are respectively of the following order 0.456; 0.343; 13.26 and 12.07 (Table 2).

All of these results therefore confirm the presence of resistance in *Culex pipiens* to various insecticides tested, including Temephos, and are consistent with those seen in northern Morocco in 2002 by Faraj et al. (2002)[38] who found that the larvae of *Culex pipiens* are developing transient resistance levels as prospected towards certain organophosphorus (Temephos, Chlorpyrifos, Malathion, Fenitrothion, Pirimiphos-methyl) areas. These results mostly confirm the data found by El Ouali Lalami et al. (2014)[41] and El Joubari et al. (2015)[40] particularly related to the resistance of *Culex pipiens* in Temephos. An early resistance of Fenthion and Malathion was also observed. This resistance recorded by the larvae of *Culex pipiens* to different insecticides tested might be explained by the fact that in this area of the city, a very Temephos used in the fight against the insecticide mosquito larvae. Indeed, according to the health services, the site of the Edge of My Driss close to an uncontrolled site has been the subject of treatment by organophosphates which includes the Temephos. High rates of resistance to this species, which has acquired these organophosphates including Temephos, are therefore, not surprising in the measure in which this product is used by health services. Our results are consistent with those reported in the literature. Indeed, it was reported in Morocco by Bouallam et al. (1998)[37]; Faraj et al. (2002)[38]; El Ouali Lalami et al. (2014)[41] and El Joubari et al. (2015)[40], and in Tunisia by Chadli et al. (1986)[48] and very recently in China by Dong et al. (2013)[49], that a rate of resistance was acquired in urban larval population of the mosquito *Culex pipiens*. Otherwise, by comparing the sensitivity of *Culex pipiens* towards the four insecticides covered in this study, it was found that this species has developed variable levels of resistance used as insecticides. The highest rates were recorded for Fenitrothion (12.07) and the Temephos (13.26); whereas the weakest were for Malathion (0.456) and Fenthion (0.343) (Table 2). According to the works of Chavasse and Yop in (1997)[50]; Sinègre et al. (1977)[51] and Faraj et al. (2002)[38], the resistance levels observed in larvae of *Culex pipiens* towards insecticides are due to operations of interior treatment or cross resistance. The same author reported that the use of organophosphorus for a long duration, always leads to the occurrence of cross-resistance to other organophosphate insecticides. *Culex pipiens* has therefore become resistant to Temephos in the site of the Edge the My Driss and equally to other insecticides tested. Even if the resistance levels and particularly those recorded towards the Temephos are substantially stable over time, the values

Table 2: Resistance rates, coefficient of slope, and susceptibility of the mosquito species tested with the four insecticides studied.

Species	Insecticides	Temephos	Malathion	Fenthion	Fenitrothion
	CL90	0.0305	0.0228	0.0188	0.0314
C. pipiens	CL 90 SS*	0.0023(1)	0.05(2)	0.0547(3)	0.0026(3)
	Resistance Rate	13.26	0.456	0.343	12.07

* SS : Sensitive Reference Strain(1) (El Ouali Lalami et al. 2014)[41] (2) (Faraj et al. 2002)[38] (3) (El Joubari et al. 2015)[40]

recorded by other insecticides show that their use in control operations are likely to be compromised in the near future, which would impose resort to other larvicides or other control methods. Thus, the use of other alternative products such as the bio-insecticide based on essential oils, or aqueous extracts of aromatic plants[52-54], or on entomopathogenic bacteria[37], or in alternation with other insecticides; might be a secondary method the fight against the mosquito *Culex pipiens* to quickly explore. According to Faraj et al. (2002)[38], the prolonged, uncontrolled and irrational employment of insecticides in the fight against mosquito larvae may create other defense mechanisms in *Culex pipiens*, to extend its spectrum of resistance and later make an inefficient use of organophosphates. Based on our results and the work conducted by El Ouali Lalami et al. (2014)[41] and El Joubari et al. (2015)[40], the *Culex pipiens* have developed variable resistance levels depending on the site in the city of Fez. Regular monitoring of the sensitivity of the dominant population density and resistance mechanisms which may be involved is essential and becomes mandatory in order to detect without delay the onset of resistance. This better rationalizes the use of insecticides available and equally better manages the evolution of resistance.

It would be also useful to recommend studies related to the mechanisms of resistance for determining the presence or absence of this mutation by biochemical tests and/or complementary molecular. The mechanisms of resistance of *Culex pipiens* and in particular OP insecticides are known and have been studied throughout toxicological enzymatic and molecular aspects[55-57]. In our case and in our study area, we are intentioned to analyze for the first time the factors already known for resistance.

CONCLUSION

This study on the sensitivity of the *Culex pipiens* insecticides in central Morocco has shown that the insecticides studied (Malathion, Fenthion, Temephos and Fenitrothion) have proved being more efficient at least against the *Culex pipiens* strain. Resistance rates found for the four insecticides (Malathion, Fenthion, Temephos and Fenitrothion) are respectively 0.456; 0.343; 13.26 and 12.07. These results on the study of the sensitivity of the *Culex pipiens* to insecticides in central Morocco are consistent with those in the literature, including those found in the cities prospected in Morocco which confirm the resistance of *Culex pipiens* to Temephos, and advise for the first time the start of resistance towards other organophosphates. These data will help to better plan and program the vector control at the level in site of the city of Fez and recommend monitoring and resistance management as a part of any program of vector control.

CONFLICT OF INTEREST

None

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