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Proposal of a mosquito control plan of Smir-Restinga region (north-west of Morocco)

Mariam El Joubari^{1,2*}, Anass Louah³ and Oumnia Himmi⁴

Abstract

Background The present study conducted on mosquitoes in the region of Restinga Smir, located in the north-west of Morocco, attempts to provide a scientific platform for an appropriate mosquito control plan for this tourism spot. The aim of this plan is to perfect the methods of the means of mosquito control for an effective fight against mosquito toes in this area, known for their strong nuisance (bites) and their ability to transmit several deadly diseases.

Results Monitoring of mosquito species in the Smir marshes, especially those of public health interest, shows that they are distributed differently in the permanent and temporary environments. The establishment and analysis of their development cycles shows that their periods of activity in the different stations of the study area overlap in several cases, especially in the permanent stations. The study of the resistance of these species to the organophosphate insecticides commonly used for mosquito control in the region allowed to conclude the doses of insecticides (LC 90) that could be used for this mosquito control programme.

Conclusions The proposed mosquito control programme gives an estimate of the timing of larval treatments and the insecticides that may be used. This plan was concluded based on the results obtained from the study of life cycles, spatial and temporal distribution of mosquitoes in the Smir area and their resistance to the organophosphate insecticides most used by public health services.

Keywords Mosquitoes, Smir-Restinga, North-west of Morocco, Mosquito control, Organophosphate insecticides

Background

Larval control, particularly against disease-carrying mosquitoes, is widely used worldwide. World Health Organization (WHO) reports from its national malaria control programmes that 27 malaria-endemic countries around the world are conducting larviciding operations in some

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malaria transmission sites. Sixteen countries report using chemical treatments for this purpose and 13 others report using biological control (WHO, 2005a, 2005b, 2021, 2022).

The north of Morocco and in particular the Smir area is an area in full development. However, among the causes that hinder its socio-economic and particularly tourist expansion are the nuisances caused by mosquitoes. In addition to the risk that these haematophagous insects present as vectors of disease, their great aggressiveness, especially during the hot season, is well known.

The present work completes our previous studies about the geographical distribution of mosquitoes in the Smir region, their ecological preferences and the study of the resistance of species of interest for public health, namely *Anopheles labranchiae*, *Culex pipiens*, *Ochlerotatus detritus* and *Ochlerotatus caspius*, to the most commonly used insecticides in Morocco (El Joubari et al.,).



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The establishment of the life cycles of these mosquitoes allowed the determination of the periods of their maximum density by specifying the development periods of their larval generations. These data will allow us to propose an adequate scientific plan for mosquito control in this wetland. This control plan is essentially based on larval control. This control method is effective because the larvae are better controllable and a relatively large proportion of the adulticide products sprayed is lost to the environment (Bedos et al., 2002), more than 40% according to Marliere (2001). From an environmental and health perspective, the consequences of these products are particularly severe in terms of water contamination (surface and groundwater) (Brunet et al., 2013).

The insecticides proposed to be used for the present demoustication plan, whose doses and efficacy are tested, are the organophosphates most used in mosquito control in Morocco: Temephos, Malathion, Fenthion, Fenitrothion and chlorpyrifos (Faraj et al., 2010). These compounds are chemically similar, very little persistent in the environment and rapidly degrade in humid and warm climate (WHO, 1986, 2009). ANSES (National Agency for Food, Environmental and Occupational Health and Safety) states that these substances are interesting for pest control and have a medium toxicity/ecotoxicity profile (ANSES, 2011).

Methods

Study area

The Smir region is a SBEI (Site of Biological and Ecological Interest) due to its great biological and ecological biodiversity (AEFCS, 1996). It is located in the northwest of Morocco, on the eastern side of the peninsula of Tangier, near the town of Mdi'q and halfway between the cities of Tétouan and Sebta (Fig. 1). This Mediterranean wetland includes a lagoon, marshes, a wadi and the Smir dam. Its surface area is about 175 hectares and its climate is Mediterranean with an average annual rainfall of about 927.95 mm and an average annual temperature of 18.4 °C. Several types of hydrological inputs feed the Smir wetland: marine waters, sewage, groundwater and the flow of the Wadi Smir. It should be noted that this hydrology has been greatly modified by the various development projects that have been taking place in the area in recent years. (Ater et al., 1997, Stitou, 2002, Dakki et al., 2005, El Fellah, 2005, Ennabili et al., 2005, LWBA, 2012, El Joubari et al., 2014, 2015a).



Fig. 1 Location of the study area

On the ground

The present study was carried out by prospecting 20 potential mosquito breeding sites (from station 1 to station 20) in the Smir marshes was carried out every two weeks during the spring–summer periods of 2010 and 2011. The sampling of mosquito larvae was conducted using the « Dipping method» (Coffinet et al., 2009). A dip net with a mesh size of 0.1 mm, a diameter of 25 cm and a length of 50 cm was used.

In the laboratory

A binocular magnifying (Olympus), an optical microscope (Optech) and the mosquito identification keys of Himmi & al. 1995 and Tachet & al. 2006 were the tools for studying and identifying of collected mosquitoes larvae.

The susceptibility of mosquito larvae to the four insecticides studied (Temephos, Malathion, Fenthion, Fenitrothion and chlorpyrifos) was being carried out following the standard protocols of WHO: larvicidal bioassays test (Cup test) (WHO, 2005a, 2005b). This test consists of introducing 20 larvae of the 3rd and 4th stage (L3 and L4) in plastic beakers containing 1 ml of insecticide at different concentrations and 99 ml of distilled water. 5 to 8 concentrations are used for each test, in order to obtain, as a minimum, a mortality of less than 50% for two concentrations and a concentration causing 100% mortality of the mosquito larvae, where possible. The amount of insecticide is replaced by ethanol for the control test. Results are read after 24 h.

Data processing

The statistical treatment of the results of the tests of sensitivity of mosquitoes to insecticides studied was carried out using the log-probit analysis software (Win DL version 2.0) (Giner et al., 1999) for the determination of the lethal concentration (LC 90).

Results

Entomological prospecting and investigation

The monitoring of the 20 selected potential mosquito breeding sites in the region of Smir allowed us to collect a total of 25,940 mosquito larvae of which four species are harmful and of great interest for public health. These species represent the major part of the mosquitoes in the region of Smir with a percentage of 72.3% of the total mosquitoes captured. These species are: *Culex pipiens* (Linnaeus, 1758), *Anopheles labranchiae* ((Meigen, 1818), *Ochlerotatus detritus* (Haliday, 1833) and *Ochlerotatus caspius* (Pallas, 1771). These species represent, respectively, 40.24% (10,438 larvae), 14.79% (3836 larvae), 10.86% (2817 larvae) and 6.41% (1662 larvae) of the total culicidian fauna collected. They can cohabit in the same sites (Table 1), and therefore, their activity periods overlap in the majority of the stations studied.

Studies of the life cycles of mosquito species for the determination of timing of larval control interventions

The regular monitoring of the mosquito species studied at the different stations in the study area has also made it possible to establish their life cycles and specify their maximum activity periods. This will be useful in determining the appropriate periods for larval control interventions, which are summarised in Table 2.

Insecticide doses that can be used for antilarval interventions

According to the sensitivity tests of the species *A. labranchiae*, *C. pipiens*, *O. detritus* and *O. caspius* towards the five insecticides: Temephos, Malathion, Fenthion, Fenitrothion and chlorpyrifos, Table 3 represents LC 90 values of insecticides obtained from populations of mosquito larvae tested in the Smir region.

Proposed mosquito control plan for the Smir marshes

The combination of the data collected from the study of the distribution of culicidian species in the Smir marshes, their life cycles and their resistance to the insecticides tested has made it possible to propose a programme for the control of mosquitoes in this region (Table 4). It determines the antilarval control periods according to the mosquito species, the type(s) of insecticide(s) likely to be used (s) and the dose for each of these insecticides in mg/l.

When several species cohabit the same environment, the dose of insecticide that can be used for larval control treatments is the higher dose obtained (LC 90 higher). Similarly, overlapping control periods are extended over the longest period of coexistence of species in the environment.

Discussion

Studies of the life cycles of mosquito species for the determination of timing of larval control interventions

The determination of the larval control periods is relatively difficult to achieve because the precise setting of treatment dates is not possible, since it is based on monitoring results. During the cold season of the year, monitoring of the roosts every 15 days would be sufficient and would guarantee the treatment of the larvae. In contrast, during the warm season, the life cycles of mosquito species are shorter with eggs reaching the pupal stage in less

Type of environment	Stations	Percentage of species abundance					
		Culex pipiens (%)	Anopheles labranchiae (%)	Ochlerotatus detritus (%)	Ochlerotatus caspius (%)		
Permanent	Station 1	26.6	0	33.8	27.9		
	Station 2	63.4	11.4	0	5.4		
	Station 4	0	32.6	0	0		
	Station 6	31.9	12	0	0		
	Station 7	36.8	6.8	0	0		
	Station 8	0	0	49.4	39.7		
	Station 9	25.2	0	39.2	29.7		
	Station 10	59.8	2.4	15.3	6		
	Station 12	72.6	3	0	0		
	Station 13	0	17.3	0	0		
	Station 14	53	0	0	0		
	Station 15	29.6	21.6	0	0		
	Station 16	58.3	20.6	0	0		
Temporary	Station 3	28.4	17.8	0	0		
	Station 5	23.6	16.1	0	0		
	Station 11	71.8	0	18.8	0		
	Station 17	0	12.9	0	0		
	Station 18	0	25.5	0	0		
	Station 19	0.5	31.3	0	0		
	Station 20	100	0	0	0		

Table 1 Distribution of studied Culicidae species in the Smir marshes as a percentage of abundance

Table 2 Control periods of mosquito species in Smir Marshes

Type of environment	Stations	Control period		
		From	То	
Temporary	Station 3	End of February Mid of September	End of June Mid-December	
	Stations 5, 11, 19 and 20	End of February Mid of September	End of June Mid-December	
	Stations17 and 18	Mid of March Mid of October	End of June End of November	
Permanent	Station 4	Mid of March	End of November	
	Station 8	End of February	End of November	
	Station 13	Mid of March	End of November	
	Stations 1, 2, 6, 7, 9, 10, 12, 14, 15 and 16	Mid of February	End of December	

than 10 days, and therefore, a weekly visit of the breeding sites is mandatory. Furthermore, the mosquito species studied do not develop in the same way in permanent waters as in temporary ones.

The monitoring of the four targeted Culicidae species has enabled us to obtain the following results:

Culex pipiens

In Morocco, *C. pipiens* plays a major role in the severe nuisance experienced by most urban areas (Faraj et al.,

2006). This mosquito has been strongly suspected in West Nile virus transmission and Rift Valley fever in the Maghreb region (Amraoui, 2012, Amraoui et al., 2012). Several authors confirm that the role of this species in the transmission of outbreaks of West Nile virus that affected Morocco in 1996 and 2003 is strongly suspected (Murgue et al., 2001, EL Harrack et al., 1997, Schuffenecker et al., 2003, Faraj et al., 2006, El Ouali Lalami 2014).

In the permanent environment of the study region, the species is present continuously throughout the year with

Table 3 LC 90 values of insecticides obtained from populations of mosquito larvae tested in the Smir region

Species	Insecticides (mg/l)	LC 90 values of insecticide (mg/l)					
		Temephos	Malathion	Fenthion	Fenitrothion	Chlorpyrifos	
Culex pipiens	LC 90 of tested populations	0.0243	0.0585	0.0547	0.0026	0.0254	
	WHO *	**	0.035	**	**	**	
Anopheles labranchiae	LC 90 of tested populations	0.0168	0.0129	0.0073	0.0049	0.0139	
	WHO	0.125	3.125	0.05	0.125	0.025	
Ochlerotatus detritus	LC 90 of tested populations	0.0215	0.0498	0.0109	0.0201	0.0146	
	WHO	**	**	**	**	**	
Ochlerotatus caspius	LC 90 of tested populations	0.0378	0.0494	0.0109	0.0019	0.0220	
	WHO	**	**	**	**	**	

LC 90: The dose of insecticide resulting in the death of 90 per cent of the populations tested

* The diagnostic doses recommended by WHO

** Data not found

Table 4 Mosquito control plan for the Smir area

Type of environment	Station	Species	Control periods according to species		Insecticide (s)	Insecticide
			From	То		doses (mg/l)
Permanent	St 1	C. pipiens O. detritus O. caspius	Mid of February	End of December	Fenitrothion	0.0201
	St 2	C. pipiens A. labranchiae O. caspius	Mid of February	End of December	Fenitrothion	0.0201
	St 4	A. labranchiae	Mid of March	End of November	Temephos Malathion Fenthion Fenitrothion Chlorpyrifos	0.0168 0.0129 0.0073 0.0049 0.0139
	St 6	C. pipiens A. labranchiae	Mid of February	End of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0049
	St 7	C. pipiens A. labranchiae	Mid of February	End of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0049
	St 8	O. detritus O. caspius	End of February	End of November	Fenitrothion	0.0201
	St 9	C. pipiens O. detritus O. caspius	Mid of February	End of December	Fenitrothion	0.0201
	St 10	C. pipiens A. labranchiae O. detritus O. caspius	Mid of February	End of December	Fenitrothion	0.0201
	St 12	C. pipiens A. labranchiae	Mid of February	End of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0049
	St 13	A. labranchiae	Mid of March	End of November	Temephos Malathion Fenthion Fenitrothion Chlorpyrifos	0.0168 0.0129 0.0073 0.0049 0.0139
	St 14	C. pipiens	Mid of February	End of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0026
	St 15	C. pipiens A. labranchiae	Mid of February	End of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0049

Table 4 (continued)

Type of environment	Station	Species	Control periods according to species		Insecticide (s)	Insecticide
			From	То		doses (mg/l)
	St 16	C. pipiens A. labranchiae	Mid of February	End of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0049
Temporary	St 3	C. pipiens A. labranchiae	Mid of February Mid of September	End of June Mid of December	Malathion Fenthion Fenitrothion	00.0585 0.0547 0.0049
	St 5	C. pipiens A. labranchiae	End of February Mid of September	End of June Mid of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0049
	St 11	C. pipiens O. detritus	End of February Mid of September	End of June Mid of December	Fenitrothion	0.0201
	St 17	A. labranchiae	Mid of March Mid of October	End of June End of November	Temephos Malathion Fenthion Fenitrothion Chlorpyrifos	0.0168 0.0129 0.0073 0.0049 0.0139
	St 18	A. labranchiae	Mid of March Mid of October	End of June End of November	Temephos Malathion Fenthion Fenitrothion Chlorpyrifos	0.0168 0.0129 0.0073 0.0049 0.0139
	St 19	C. pipiens A. labranchiae	End of February Mid of September	End of June Mid of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0049
	St 20	C. pipiens	Mid of February Mid of September	End of June Mid of December	Malathion Fenthion Fenitrothion	0.0585 0.0547 0.0026

the exception of January and the first half of February when the species cannot withstand the winter cold. The treatment of *C. pipiens* permanent breeding sites should be carried out regularly: from mid-February (as soon as the first instar larvae appear) to the end of December.

Culex pipiens is present in the temporary stations from the end of February until the drying of the environment (around the end of June), and from around mid-September until mid-December. The treatment in this case should be spread over two periods: from the end of February until the end of June and from mid-September until mid-December following the appearance of L1 larvae.

Anopheles labranchiae

It is the mosquito species of radical importance to Moroccan human health. Apart from being a nuisance, *Anopheles labranchiae* is the major malaria vector in Morocco. This has been confirmed by numerous studies (Guy 1976, Louah 1995, Faraj et al., 2004, 2008, 2009, Himmi, 2007, El Ouali Lalami et al., 2010, Trari et al., 2011, Laboudi et al., 2012, 2014a, 2014b, El Joubari et al., 2015b).

In a permanent environment of Smir marshes, A. *labranchiae* is present from mid-March to the end of

November. Increases in the density of this species are noted mainly in the middle of spring and at the beginning of summer (May and June). This high abundance continues in the months of July and August.

Anopheles labranchiae is collected in the temporary environment from mid-March to the end of June (drying of the environment). It reappears around mid-October in this type of stations and disappears from the end of November.

Because of the specificity of this species, its monitoring would be meticulous especially with its great dispersion in the study area. The control of *A. labranchiae* is expected to take place from mid-March to the end of November as soon as L1 larvae appear in the permanent environments. The treatment of the temporary roosts must therefore be carried out in two periods: from mid-March to the end of June and from mid-October to the end of November as soon as the L1 larvae appear.

Ochlerotatus detritus and Ochlerotatus caspius

These two species of the genus *Ochlerotatus* are not declared disease vectors in Morocco, but their nuisance is extremely important for the majority of coastal towns in Morocco (Himmi, 2007, El Joubari et al., 2015b).

Ochlerotatus detritus occupies the permanent environment from the end of February to the end of November with 2 peaks of abundance: the first is in summer, marked in the period from the end of June to the beginning of July, and the second is in autumn, noted after the first autumnal rainfall (early October). The control of this species in the permanent stations would take place from the end of February to the end of November as soon as the first instar larvae (L1) appear.

Ochlerotatus detritus is present in temporary biotope from the beginning of April until the end of June (drying of the station). The species repopulates the area in early October and disappears towards the end of November. The treatment of the breeding sites of this species should therefore be spread over two periods: from the beginning of April to the end of June and from the early of October to the end of November as soon as the L1 larvae appear.

Ochlerotatus caspius is present continuously in the permanent environment of the study area from the beginning of April until the end of November. As with the previous species, both summer and fall peaks are also noted for *O. caspius.*

The treatment of the breeding sites of this species is therefore carried out from the beginning of April until the end of November since the appearance of the L1 larvae.

Table 3 summarizes the appropriate control periods for the Culicidae species studied in the temporary and permanent waters of the Smir marshes.

Insecticide doses that can be used for antilarval interventions

Culex pipiens

This is the most studied species among those presented in our work. For temephos, we found an LC 90 of 0.0243 mg/l. A similar result was reported by Sinegre (1984), who gave an LC 90 of 0.036 mg/l. On the other hand, Faraj et al., (2002) cite a higher LC 90 of 0.1 mg/l in the Mohammadia region of Morocco. In Tunisia, Chadli et al., (1986) report lower LC 90 values ranging from 0.0009 to 0.0166 mg/l.

The lethal concentration 90 of chlorpyrifos towards *Culex pipiens* found by the present study is 0.0254 mg/l. This result is consistent with those of Faraj et al., (2002), who reported an LC 90 of 0.02 mg/l in central Morocco, and Chadli et al., (1986), who found LC 90 s of between 0.0018 mg/l and 0.035 mg/l in Tunisia. However, the highest values are found by Ben Cheikh et al., (1995) still in Tunisia, who give a value of 0.27 mg/l, and in France, by Sinegre (1984), who finds that LC 90 of *C. pipiens* in relation to chlorpyrifos is 0.2 mg/l. Lower LC 90 values were reported in the south of France by Raymond et al.,

(1985), ranging from 0.012 to 0.02 mg/l, the upper limit of which is close to the value found in the present study.

For fenitrothion, the LC 90 found does not exceed 0.0026 mg/l. This value seems very low compared with other studies. Faraj et al., (2002) reported an LC 90 of 0.046 mg/l in the Skhirat-Temara region of Morocco. Sinegre et al., (1977) reported a higher LC 90 of 0.065 mg/l in the south of France.

For fenthion, the LC 90 found was 0.0547 mg/l. Unlike fenitrothion, this value is much higher than that found by Sinegre et al., (1977), who reported an LC 90 of 0.033 mg/l in southern France. In Tunisia, Chadli et al., (1986) report LC 90 values varying between 0.026 mg/l and 0.35 mg/l.

The LC 90 of *C. pipiens* in relation to malathion is 0.0585 mg/l. This value also seems very low compared with that cited by Faraj et al., (2002) in the Skhirat-Temara and Rabat regions of Morocco, which is 3.6 mg/l. Similarly, Sinegre et al., (1977) found a similarly high LC 90 in Midi France, equal to 0.1 mg/l.

Anopheles labranchiae

The LC 90 of chlorpyrifos for this species is 0.0139 mg/l. This value is very high compared with the range found by Krida et al., (1998) in several regions of Tunisia in 1997, where LC 90 values varied between 0.00437 mg/l and 0.00518 mg/l.

For temephos, Faraj et al., (2010) report LC 90 values of between 0.0362 mg/l and 0.1058 mg/l, while we found a lower value of 0.0168 mg/l. Larhbali et al., (2010) found in the province of Khémissat (Morocco) an LC 90 comparable to that of the present study, which was 0.015 mg/l.

The comparison of the LC 90 obtained for the five insecticides studied with the WHO recommended doses for the same insecticides shows that the latter are higher, and this can be explained by the fact that the tested populations of this species have not undergone excessive treatments by these organophosphate insecticides.

Ochlerotatus detritus and Ochlerotatus caspius

For these two species, our results are relatifly comparable with those obtained in France where Sinegre (1984) found, by testing the sensitivity of these two species to temephos and fenitrothion, that the LC 90 of fenitrothion is between 0.0014 mg/l and 0.016 mg/l. These results seem very similar to those of the present study where the LC 90 for fenitrothion is 0.0201 mg/l and 0.0019 mg/l for *O. detritus* and *O. caspius*, respectively. However, the same author states lower LC 90 values for temephos vary between 0.002 mg/l and 0.02 mg/l, whereas the values obtained by the present work are 0,0215 for *O. detritus* and 0.0378 mg/l for *O. caspius*.

The LC 90 obtained for chlorpyrifos in relation to *Ochlerotatus detritus* was 0.0146 mg/l, while that for temephos was 0.0215 mg/l. In Tunisia, Chadli et al., 1986 reported lower LC 90 values ranging from 0.0005 to 0.002 mg/l for chlorpyrifos and from 0.0009 to 0.0055 mg/l for temephos.

Conclusions

The results obtained during the study of the life cycles, the spatial and temporal distribution of mosquitoes in the Smir region and their resistance towards five organophosphate insecticides, served as the basis for the present proposal of a mosquito control plan for this wetland. This programme gives an estimate of the timing of larval treatments and the insecticides that may be used.

Depending on the species, monitoring of mosquitoes in Smir marshes generally takes place around the beginning of spring (end of February—early of March) until the temporary breeding sites dry out (around the end of June). It generally continues as soon as the sites are repopulated following autumn rainfall. In the permanent stations of the Smir area, the mosquito species develop continuously and their permanent monitoring particularly during the periods of their maximum abundance (spring–summer or summer–autumn depending on the species) would be of great interest for the success of the mosquito control programme.

The evaluation of the effectiveness of the larval treatments starts within 48 h after the treatment. Depending on the persistence of the insecticides used, the resumption of treatment is carried out as soon as the observation of a possible increase in the larval density of the species. This step is essential for monitoring the appearance of resistance to larvicide products used.

Abbreviations

FAO	Food and Agriculture Organization
WHO	World Health Organization
ANSES	National Agency for Food, Environmental and Occupational Health and Safety
LWBA	Loukkos Water Basin Agency

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Author contributions

OH co-supervises the work and reads, corrects and approves the final manuscript. AL supervises the work and reads, corrects and approves the final manuscript.

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Competing interests

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