Hindawi
Psyche: A Journal of Entomology
Volume 2022, Article ID 5576317, 7 pages
https://doi.org/10.1155/2022/5576317

Research Article

Insecticide Resistance in the Disease Vector *Culex pipiens* in Morocco: Intensity, Mechanisms, and Contribution in Insecticide Resistance Management

Souhail Aboulfadl,[1,2] **Fouad Mellouki**,[1] **Btissam Ameur**,[3] **and Chafika Faraj**[2]

[1]*Hassan II University of Casablanca, Faculty of Sciences and Technologies Mohammedia, Microbiology, Hygiene and Bioactive Molecules Team, BP28806, Casablanca, Morocco*
[2]*National Institute of Hygiene, Medical Entomology Laboratory of Rabat, BP769, Rabat, Morocco*
[3]*Epidemiology and Disease Control Directorate, Anti-Vector Control Service, BP335, Rabat, Morocco*

Correspondence should be addressed to Chafika Faraj; chafikaf@gmail.com

Received 15 March 2021; Revised 10 February 2022; Accepted 4 April 2022; Published 28 April 2022

Academic Editor: Cleber Galvão

West Nile (WN) virus has been detected in Morocco in 1996, 2003, and 2010. One WN human case and 94 equine cases with 42 deaths were reported in the first outbreak. The second and the last epizootics were in Kénitra and Mohammedia regions, respectively. *Culex pipiens* mosquitoes were strongly suspected as WN vectors during these epizootics in Morocco. To help propose the first insecticide resistance management strategy in Morocco, we provide baseline data on the resistance status of *C. pipiens* mosquitoes to insecticides most used in public health in Morocco. We investigated the resistance intensity of *C. pipiens* and the role of monooxygenases in pyrethroids resistance using the synergist piperonyl butoxide (PBO) in three prefectures of central Morocco: Mohammedia, Benslimane, and Skhirate. Bioassays were carried out using WHO (World Health Organization) diagnostic test kits for adult mosquitoes, with DDT 4%; deltamethrin 0.05%, 0.25%, and 0.5%; bendiocarb 0.1%, 0.5%, and 1%; and malathion 5%. The synergist PBO 4% was used to check for the involvement of detoxification enzymes in pyrethroids resistant populations. Mosquito populations tested displayed high resistance intensity to pyrethroids and carbamates in Mohammedia and moderate resistance intensity in Skhirate. In Benslimane, the resistance intensity of *C. pipiens* was high to carbamate and moderate to pyrethroids. The pre-exposure to PBO restored full or partial susceptibility to pyrethroids in the different regions. According to our finding, Mohammedia and Benslimane are at risk level 3 of control failure, while Skhirate is at risk level 2 of it. The employment of IRM is therefore imperative in all study sites.

1. Introduction

Culex pipiens mosquitoes are the most widely distributed and abundant species, and their nuisance have serious negative impacts on the standard of living in many Moroccan urban communities. Moreover, this species is the principal vector of WNV (West Nile virus) in Morocco [1]. *C. pipiens* is highly suspected in the WN virus transmission during epizootics that had affected Morocco in 1996, 2003, and 2010 [1–3]. Until now, there are no highly effective vaccines for all mosquito-borne infections other than yellow fever and Japanese encephalitis [4]. For this reason, considerable efforts in mosquito control have been performed since the 1980s to decrease the nuisance generated by *C. pipiens* and prevent the WNV transmission in Morocco [5].

Chemical insecticides usage was the main strategy in the vector control national program using organophosphates and pyrethroids against both larvae and adult mosquitoes, respectively. According to Moroccan public health services, mazout diesel, chlorpyriphos, malathion, and then temephos were used in larval control through time. On the other side, DDT, permethrin, cypermethrin, tetrametrin, and deltamethrin were used in adult control. The state of susceptibility to insecticides of this mosquito is the object of regular surveillance by the Ministry of Health since 2003, but the published data remain scarce [2]. Most of the published studies, mainly conducted on larvae, showed significant resistance to insecticides used in the majority of urban areas: Rabat, Sale, Skhirate, and Mohammedia to organophosphates (OP) [6]; Khemisset to organochlorines (OC) [7]; Fes to OP [8]; Smir to OP [9]; Casablanca, Tanger, and Marrakech to pyrethroids (PYR) [10]; and Mohammedia, Benslimane, Skhirate, and Sale to OP [5]. However, the continuous chemical treatment of resistant mosquitoes can make the use of insecticides ineffective compromising the strategy of mosquito control.

In order to better manage *C. pipiens* resistance in Morocco, it is essential to study the resistance intensity and the mechanisms involved in this species. In other words, the operational impact of a given resistance might not be achieved yet what makes the resistance intensity (RI) investigation so important [11]. The objective of our study was first to assess the current susceptibility status of *C. pipiens* adult mosquitoes to DDT, deltamethrin, bendiocarb, and malathion and second to know the resistance intensity as well as the mechanisms involved in those populations from Mohammedia, Skhirate, and Benslimane in Northwestern Morocco.

The results of our study can be used, as a guide to the choice of mosquito control and resistance management strategies. However, it is important to collaborate with public hygiene services to ensure the transfer of our basic research to field operations in order to assess and optimize the vector strategy proposed. To our knowledge, this is the first study that gives basic data on resistance intensity and metabolic mechanism and has practical implications for resistance management in adult *C. pipiens* from Morocco.

2. Materials and Methods

2.1. Study Area. This study was conducted in three urban districts: Ouled Hamimoun (33.673748, −7.445547), Lahjar (33.849538, −7.018181), and El-Jazeera (33.780420, −7.235220) from three coastal cities, respectively: Mohammedia, Skhirate, and Benslimane, Northwestern Morocco (Figure 1). We chose Mohammedia for this study because it was the subject of a West Nile virus epizootic in 2010 transmitted by *C. pipiens*. Benslimane and Skhirate, as for them, are selected because they have recently experienced a very strong proliferation of *C. pipiens* due to the growth of urbanization in these regions. Ouled Hamimoun site from the Mohammedia region is an open drain very close to settlements and industrial companies. However, the Lahjar site from Skhirate and the El-Jazeera site from Benslimane are swamps too close to settlements and food companies, respectively. All sites are receiving a daily charge of waste that forms breeding sites very rich in organic matters and therefore suitable for *C. pipiens* proliferation. Although the three breeding sites are located in coastal cities, the geography of the regions affects the risk of a possible epidemic or epizootic. For example, the Mohammedia site exists in a semiurban wetland complex that represents a refuge for migratory birds, the main reservoir of the West Nile virus. The risk of a vector-borne disease in this region remains very high. On the other hand, Benslimane and Skhirate sites are inside an urban complex. The absence of migratory birds in these regions decreases the risk above, but it stills a nuisance for the inhabitants.

2.2. Sampling. *C. pipiens* larvae and pupae were collected by dipping and netting methods using a ladle and a vacuum landing net with a mesh size of 0.1 mm and kept in the water sampled from the larval habitat at $27 \pm 2^{\circ}$C until adult stages [12]. We morphologically identified the tested mosquitoes using the Culicidae of Mediterranean Africa Identification Software (CulAfMe) [13].

2.3. WHO Susceptibility Assays. We tested adult susceptibility using WHO discriminating dosages (DD) of four insecticides belonging to four chemical classes: organochlorine (DDT4%), pyrethroid (deltamethrin 0.05%), carbamate (bendiocarb 0.1%), and organophosphate (malathion 5%). We determined the resistance intensity by using 5x and 10x DD of deltamethrin (0.25% and 0.5%) and bendiocarb (0.5% and 1%). All bioassays were conducted following WHO protocols [11]. Four batches of 25 *C. pipiens* unfed females, aged 2–5 days, were exposed for 4 h to DDT insecticide-treated papers, 2 h to bendiocarb, and 1 h to deltamethrin and malathion. We recorded the knocked down number during the exposure time for DDT every 30 min and for deltamethrin every 10 min. After the exposure period, we transferred all mosquitoes to observation tubes with a 10% sugar solution supplement, and the mortality rate was recorded after 24 h. Batches exposed to oil-treated papers were run in parallel and used as control. Knockdown time (KdT_{50} and KdT_{90} indicating 50% and 90% of knocked down tested populations, respectively) were calculated using WinDL32 software [14].

2.4. Synergist-Insecticide Bioassay. In order to assess the involvement of detoxifying enzymes, mainly monooxygenases in producing resistant phenotypes to pyrethroids, the synergist-insecticide bioassay was carried out using PBO 4%. Unfed females, aged 2–5 days, were pre-exposed to 4% PBO-impregnated papers for 1 h before they were exposed to insecticide-impregnated papers (deltamethrin 0.05%). Control mosquitoes were exposed to oil-impregnated papers for 1 h. Mortality was recorded after 24 h. Three replicates were performed for each bioassay.

2.5. Data Analysis. The WHO criteria were adopted for distinguishing between resistance/susceptibility status and resistance intensity of the tested mosquito populations [11]. When more than 98% mortality at DD was observed, the population was considered "susceptible"; when less than 90% was observed, the population was considered "resistant." Mortality of 98–100% at the 5x DD indicates low resistance intensity. When mortality <98% but ≥98% at the 10x DD, resistance intensity is moderate, and when mortality <98% at the 10x DD, resistance intensity is high. In

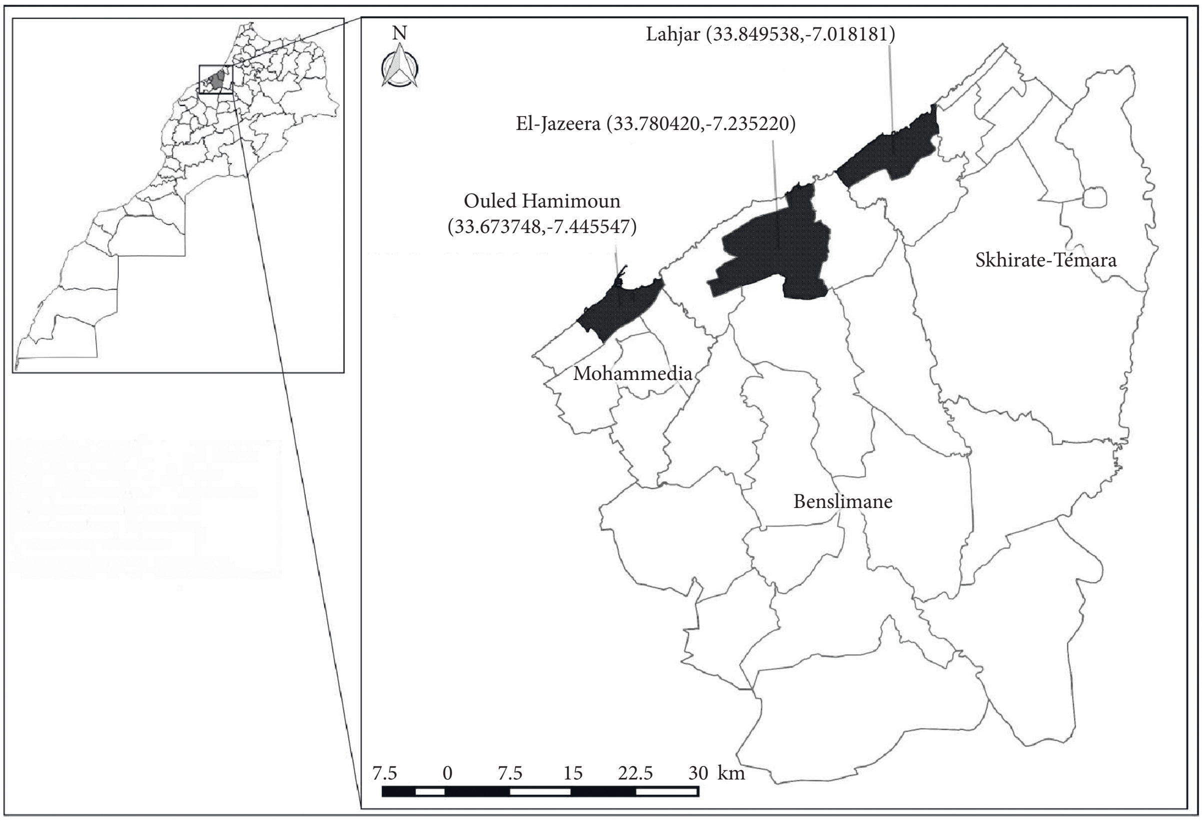

Figure 1: Map of Mohammedia, Benslimane, and Skhirate cities showing the study sites.

regard to the population pre-treated by PBO 4%, when mean mortality was ≥98% in the "PBO followed by insecticide" samples, this implies that a monooxygenase-based resistance mechanism fully accounts for the expression of the resistant phenotype in the tested population. When mean mortality in the "PBO followed by insecticide" samples is superior to mean mortality in the "insecticide only" samples but <98%, the phenotype resistance observed is partially due to a monooxygenase-based resistance mechanism, and other resistance mechanisms are likely to be present in the tested population. When mean mortality in the "PBO followed by insecticide" samples is ≤= mean mortality in the "insecticide only" samples, the resistance phenotype detected is not based on monooxgenase-mediated detoxification.

3. Results

Results showed the presence of resistance from the three *C. pipiens* populations to the four chemical classes: organochlorates (DDT), pyrethroids (deltamethrin), carbamates (bendiocarb), and organophosphates (malathion; Table 1). The highest knockdown time (KdT) was observed in the population exposed to DDT from Benslimane with $KdT_{50} = 1{,}771$, and the lowest KdT was observed in the population exposed to deltamethrin from Mohammedia with $KdT_{50} = 12$. These values agree with the resistance state obtained in both populations. Mortality, as for it, is very high in the Skhirate population exposed to deltamethrin with %Mr = 85 and is very low in the Benslimane population exposed to DDT with %Mr = 2.

The mortality obtained of adult *C. pipiens* from Mohammedia, Benslimane, and Skhirate after being exposed to deltamethrin ×10 was 97%, 99%, and 99%, respectively, and to bendiocarb ×10 was 94%, 92%, and 98%, respectively. On the other side, KdT_{50} decreased in all three populations after being exposed first to deltamethrin ×5 and then to deltamethrin ×10. The resistance intensity of *C. pipiens* from Mohammedia was high to both deltamethrin and bendiocarb, whereas, in Benslimane, it was high to bendiocarb and moderate to deltamethrin. In Skhirate, it was moderate to both deltamethrin and bendiocarb (Table 2).

The pre-exposure to piperonyl butoxide (PBO) completely eliminated the phenotypic resistance to deltamethrin observed in adult *C. pipiens* from Mohammedia and Skhirate. The populations pre-exposed to the synergist showed mortality of 99 and 100, respectively. However, the resistance to deltamethrin in the population of Benslimane has been partially removed after a pre-exposure to PBO with a mortality of 87% (Table 3).

4. Discussion

In our work, we give basic data on the resistance status of *C. pipiens* adult populations from three regions in central Morocco. Our results showed that culicidian populations of adult *C. pipiens* from Mohammedia, Benslimane, and Skhirate have a resistant phenotype towards DDT 4% (OC), deltamethrin 0.05% (PYR), bendiocarb 0.1% (CX), and malathion 5% (OP) with dissimilar intensities. The

Table 1: Susceptibility of *Culex pipiens* to DDT, deltamethrin, bendiocarb, and malathion in Mohammedia, Benslimane, and Skhirate.

Insecticide	DDT 4%					Deltamethrin 0.05%					Bendiocarb 0.1%			Malathion 5%		
Study sites	*N*	KdT_{50} [CI]	KdT_{90} [CI]	%Mr [SE]	*S*	*N*	KdT_{50} [CI]	KdT_{90} [CI]	%Mr [SE]	*S*	*N*	%Mr [SE]	*S*	*N*	%Mr [SE]	*S*
Mohammedia	91	166 [45–262]	816 [424–1,700]	74 [0.9]	R	100	12 [0.9–19]	30 [19–183]	75 [0.5]	R	100	74 [1.1]	R	89	82 [0.9]	R
Benslimane	100	1,771 [1,200–1,886]	6,978 [5,661–10,523]	2 [0.2]	R	100	50 [24–65]	122 [80–211]	44 [0.7]	R	94	32 [0.5]	R	103	70 [2]	R
Skhirate	101	112 [98–123]	266 [238–311]	84 [0.7]	R	99	23 [21–25]	49 [44–55]	85 [0.7]	R	101	72 [0.5]	R	98	83 [1.2]	R

N: number of mosquitoes, KdT_{50}: time of which 50% of the tested population suffers a knockdown, KdT_{90}: time of which 90% of the tested population suffers a knockdown, %Mr: mortality percentage, S: status, and R: resistant.

Table 2: Resistance intensity of adult *Culex pipiens* to deltamethrin and bendiocarb in Mohammedia, Benslimane, and Skhirate.

Insecticide	Deltamethrin 0.25%				Deltamethrin 0.5%					Bendiocarb 0.5%		Bendiocarb 1%		
Study sites	*N*	KdT_{50} [CI]	KdT_{90} [CI]	%Mr [SE]	*N*	KdT_{50} [CI]	KdT_{90} [CI]	%Mr [SE]	RI	*N*	%Mr [SE]	*N*	%Mr [SE]	RI
Mohammedia	100	13 [11–14]	23 [21–26]	93 [0.7]	103	5 [1–11]	69 [42–138]	97 [0.7]	HR	94	96 [0.9]	102	94 [0.6]	HR
Benslimane	98	25 [23–27]	44 [41–49]	89 [0.9]	101	25 [4–169]	86 [42–172]	99 [0.3]	MR	99	87 [1.3]	102	92 [1]	HR
Skhirate	100	9 [7–10]	21 [19–24]	84 [0.6]	101	6 [4–7.5]	21 [10–42]	99 [0.3]	MR	102	96 [0.2]	107	98 [1.1]	MR

N: number of mosquitoes, KdT_{50}: time of which 50% of the tested population suffers a knockdown, KdT_{90}: time of which 90% of the tested population suffers a knockdown, %Mr: mortality percentage, RI: resistance intensity, HR: high resistance, and MR: moderate resistance.

Table 3: Mortality rates of *Culex pipiens* with deltamethrin alone and deltamethrin + PBO in Mohammedia, Benslimane, and Skhirate, 24 hours after exposure.

Insecticide	PBO only				PBO + deltamethrin 0.05%				Deltamethrin 0.05% only				Solvent control			
Study sites	*N*	KdT_{50}	KdT_{90}	% Mr	*N*	KdT_{50} [CI]	KdT_{90} [CI]	%Mr [SE]	*N*	KdT_{50} [CI]	KdT_{90} [CI]	%Mr [SE]	*N*	KdT_{50}	KdT_{90}	% Mr
Mohammedia	75	—	—	0	75	18 [16–19]	27 [25–29]	99 [0.2]	76	23 [7.5–30]	37 [28–104]	83 [0.8]	75	—	—	0
Benslimane	75	—	—	0	71	11 [0.02–24]	25 [1.1–39]	87 [1.3]	69	19 [13–24]	43 [33–68]	59 [1.3]	76	—	—	0
Skhirate	77	—	—	0	78	11 [2–21]	24 [3–34]	100 [0]	77	18 [6–26]	46 [34–55]	88 [0.7]	75	—	—	0

N: number of mosquitoes, KdT_{50}: time of which 50% of the tested population suffers a knockdown, KdT_{90}: time of which 90% of the tested population suffers a knockdown, and %Mr: mortality percentage.

resistance intensity was high in Mohammedia and moderate in Skhirate to both deltamethrin and bendiocarb, while, for Benslimane, it was moderate to deltamethrin and high to bendiocarb. The current insecticides used by public health services in all study sites are temephos and deltamethrin to control mosquito larvae and adults, respectively. However, the insecticide quantities and the treatment frequencies made in vector control might affect the resistance as well as its intensity. The average quantity of an organophosphate, for example, "Abate[R]" (temephos), over the past five years in prefectures of Mohammedia and Skhirate was 120 L on 100 ha of mosquito breeding sites and 47 L on 13 ha, respectively [5]. Despite the rate of 3.6 of temephos quantities used in Skhirate being 2.4 higher than the rate of 1.2 of temephos quantities used in Mohammedia, the resistance intensity of adult *C. pipiens* to CX and PYR in Mohammedia is still higher than Skhirate. This could be explained by the treatment frequency of each mosquito habitat. The Ouled Hamimoun (Mohammedia) breeding site is a permanent open drain located close to human habitats and is therefore regularly treated [5], contrary to the Lahjar (Skhirate) breeding site that is a temporary swamp and only treated depending on the presence of the water. El-Jazeera (Benslimane) breeding site is a permanent swamp located outside the city and is regularly treated by only temephos-based products. Larval and adult control is maintained in both Ouled Hamimoun and Lahjar breeding sites, whereas, at El-Jazeera, only larvae are treated. The different intensities of adult resistance to the three neurotoxic insecticides indicate that the chemicals present in the environment could not only induce direct adult resistance but also interact with other molecules from the environment and thus develop cross-resistance processes [15]. Our results agree with those obtained by Sinegre et al. [16] who have established an observable correlation between the level of resistance and the frequency of treatments.

Moreover, we demonstrated that the pyrethroids resistance within mosquitoes from Mohammedia and Skhirate is completely metabolic and contributed to full involvement of P450 monooxygenases in the resistant populations. However, the pre-exposure to PBO in *C. pipiens* from Benslimane revealed that the pyrethroids resistance is partly metabolic and caused by limited involvement of P450 monooxygenases. The action of other resistance mechanisms in this population could also be in play.

Our finding investigates for the first time the resistance intensity and the mechanism involved in the disease vector *C. pipiens* from Mohammedia, Benslimane, and Skhirate. According to our results, we prove the necessity of the implementation of insecticide resistance management (IRM) in all study sites in order to maintain the effectiveness of chemical insecticides used in vector control in these regions. In other words, Mohammedia and Benslimane regions are at level 3 of control failure, and IRM should be implemented immediately with unrelated insecticides in these areas following the flow chart to support the decision-making of IRM strategy by Dusfour et al. [17]. Contrariwise, the Skhirate region is at level 2 of control failure, and IRM should be implemented without the need for unrelated insecticides at this level. Environment management, biological control, use of biocides, insecticide rotation, mosaic, and mixture or combination are all strategies for IRM. However, the use of these strategies must be appropriate to the Culicidean population resistance mechanism. Rotating insecticides with different modes of action, for example, will be unnecessary and will not reduce the resistant mosquitoes' adaptive value in a case of metabolic cross-resistance [17]. The susceptibility state and resistance intensity of the same mosquito species could vary from one breeding site to another within the same prefecture and from one city to another within the same country. In this context, it is important for implementing an effective IRM to have precise, detailed, and deep knowledge of the Culicidean populations (susceptibility state, resistance intensity, mechanisms involved, etc.), their habitats (geographical data of the breeding sites, sites type, areas type, etc.), and their vector control strategies. According to our results, the best IRM strategy that could be applied in Mohammedia and Skhirate is the use of a combination of pyrethroids and the synergist PBO in adult control because of their resistance mechanism that is completely metabolic due to cytochromes P450 monooxygenases, whereas, in Benslimane, the combination of PYR and PBO synergist could be a good strategy but not sufficient for IRM. This is due to the limited involvement of P450 monooxygenases in metabolic resistance. For this reason, other complementary strategies must be implemented in this region as rotating insecticides with different target sites, biological control, or environment management.

Definitely, the biosphere, which largely overlaps with what is now called the "environment," is profoundly transformed (growing demography and/or population densification, the evolution of farming and breeding practices, draining of wetlands, hydro-agricultural developments, deforestation, reforestation, and climate change) making it very difficult to define constants [18]. These environmental factors have a crucial impact on the vector system and possibly on mosquito resistance, so our understanding of the interaction environment x mosquitoes could optimize IRM. The urbanization degree, for example, has a significant impact on mosquito resistance. A study showed that the occurrence of insecticide resistance is strongly related to increasing human urbanization [19]. Indeed, the resistant populations are highly resistant in most urbanized areas and low resistant in natural areas [20]. Moreover, Grossman et al. [21] highlighted the importance of gene $\times$ environment interaction in re-establishing mosquito susceptibility. They found that the *Aedes aegypti* resistant population was no longer phenotypically resistant at high density (93% were knocked down when exposed to permethrin with a decrease in C1534 kdr allele frequency from 0.98 ± 0.04 to 0.69 ± 0.04 in only one generation of selection). According to the authors, this gain of susceptibility is due to the selective force induced by density-dependent intraspecific competition. This environment influence leads us to take into account the local dimension, which is that of populations and not of species [18].

Furthermore, mosquito resistance is mainly due to two mechanisms: metabolic resistance and target site modification. In metabolic resistance, several classes of enzymes are involved in the detoxification of chemical insecticides, the major ones being cytochrome P450 oxidases (monooxygenases), esterases, and glutathione-S-transferases. Cyt P450 oxidases play the main role in resistance to pyrethroids, while esterases are largely responsible for resistance to organophosphates and carbamates [22]. Monooxygenases are also responsible for activating organophosphorus molecules, that is, the OP that are able to quickly penetrate the insect's cuticle, are applied in the form of thionates, and monooxygenases convert the thionate analogues to highly toxic oxon analogues inside the mosquito [23]. Metabolic resistance to pyrethroids is due to the overexpression of one or more P450s or also to mutations that transform the P450 protein [24]. However, there are more than 100 P450s in mosquitoes' genomes, the thing that makes a determination of the specific P450(s) liable for the resistance a real challenge [25]. Molecular tools can help choose the best alternative insecticide, by determining which resistant alleles are responsible for certain cross-resistance mechanisms [26]. However, other resistance mechanisms such as metabolic resistance have not been sufficiently studied, and therefore, alleles that induce this type of mechanisms are rarely explored. Diagnostic tests for such resistance mechanisms are currently failing, which is mainly due to the lack of validated DNA markers [17].

Our finding shows that metabolic resistance is involved completely in the resistant population from Mohammedia and Skhirate and partly in the resistant population from Benslimane. Although these results help in the decision-making of the insecticide resistance management, complementary studies are needed to know which cytochrome P450 genes are responsible for the observed resistance. Thus, understanding what kind of insecticide and the precise P450 behind its metabolism is important to delineate the potential strengths and liabilities of insecticide compounds [27].

5. Conclusion

The results of this study showed the presence of resistance with variable intensities in the three *C. pipiens* populations to chemical insecticides used for mosquito control in three coastal cities: Mohammedia, Benslimane, and Skhirate. According to our finding, the implementation of insecticide resistance management in all study sites is imperative in order to avoid a critical resistance state in *C. pipiens* populations. In Mohammedia and Skhirate where resistance is proved completely metabolic, the IRM strategies such as insecticide rotation, mosaic, and mixture or combination will be unnecessary and will not reduce the proportion of resistant mosquitoes against the tested insecticides. However, the possible strategies that could be implemented in these areas are biological control, physical treatment, or combining PBO with the insecticide, whereas, in Benslimane, the resistance mechanism is partly metabolic and needs further studies to know which IRM strategy is ideal to implement. Insecticide resistance aspects in mosquitoes could be affected by several factors, but still decreasing insecticide pressure is the best strategy to apply in IRM. Thus, it is important to collaborate with public hygiene services to ensure the transfer of our basic research to field operations in order to assess and optimize the vector strategy proposed.

Data Availability

No data were used to support this study.

Ethical Approval

No specific authorization is required for activities in the field that do not concern endangered or protected species. The National Institute of Hygiene in Morocco is a public health and scientific research institution under the supervision of the Ministry of Health. In this context, it participates in vector control activities that allow it to operate without specific authorization to access breeding sites and mosquito collections.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

Graphical abstract: Intensive use of chemical insecticides in vector control in Morocco leads, through time, to a mosquito resistance to all chemical insecticide classes. Mosquito populations tested displayed high resistance intensity to pyrethroids and carbamates in Mohammedia and moderate resistance intensity in Skhirate. In Benslimane, the resistance intensity of *C. pipiens* was high to carbamates and moderate to pyrethroids. The pre-exposure to PBO reveals that the tested Culicidian populations in Mohammedia and Skhirate have a completely metabolic resistance mechanism, as for Benslimane, the mosquitoes' populations have a partly metabolic resistance mechanism. Finally, Mohammedia and Benslimane are at risk level 3 of control failure while Skhirate is at risk level 2 of it. The employment of IRM is therefore imperative in all study sites. (*Supplementary Materials*)

References

[1] F. Amraoui, G. Krida, A. Bouattour et al., "*Culex pipiens*, an experimental efficient vector of West Nile and rift valley fever viruses in the maghreb region," *PLoS One*, vol. 7, no. 5, Article ID e36757, 2012.

[2] A. B. Failloux, A. Bouattour, C. Faraj et al., "Surveillance of arthropod-borne viruses and their vectors in the mediterranean and black sea regions within the MediLabSecure Network," *Current Tropical Medicine Reports*, vol. 4, no. 1, pp. 27–39, 2017.

[3] C. Faraj, M. Elkohli, and M. Lyagoubi, "The gonotrophic cycle of *Culex pipiens* (Diptera: Culicidae) potential vector of West Nile virus in Morocco: evaluation of its duration in laboratory," *Bulletin de la Société de Pathologie Exotique*, vol. 99, p. 2, 2006.

[4] N. M. Ferguson, "Challenges and opportunities in controlling mosquito-borne infections," *Nature*, vol. 559, no. 7715, pp. 490–497, 2018.

[5] S. Aboulfadl, F. Mellouki, B. Aouinty, and C. Faraj, "Susceptibility status of *Culex pipiens* larvae (Diptera: Culicidae) to the main insecticides used in larval control in the regions of Rabat and Casablanca in Morocco," *International Journal of Pest Management*, vol. 66, pp. 1–7, 2020.

[6] C. Faraj, M. El Kohli, M. El Rhazi, M. Laqraa, and M. Lyagoubi, "Niveau actuel de la résistance du moustique *Culex pipiens* aux insecticides au Maroc," *Science Letter*, vol. 4, no. 1, p. 4, 2002.

[7] Y. Larhbali, D. Belghyti, Y. El Guamri et al., "Sensibilité de deux moustiques culicidés (*Anopheles labranchiae* et *Culex pipiens*) aux insecticides," *Bulletin de la Societe de pharmacie de Bordeaux*, vol. 149, pp. 33–42, 2010.

[8] A. El Ouali Lalami, F. El Akhal, N. El Amri, S. Maniar, and C. Faraj, "State resistance of the mosquito *Culex pipiens* towards temephos central Morocco," *Bulletin de la Société de Pathologie Exotique*, vol. 107, pp. 194–198, 2014.

[9] M. El Joubari, C. Faraj, A. Louah, and O. Himmi, "Sensibilité des moustiques *Anopheles labranchiae, Culex pipiens, Ochlerotatus detritus* et *Ochlerotatus caspius* de la région de Smir (Nord-Ouest du Maroc) aux organophosphorés utilisés en santé publique," *Environnement, Risques & Santé*, vol. 14, pp. 72–79, 2015.

[10] M. Bkhache, FZ. Tmimi, O. Charafeddine, C. Faraj, AB. Failloux, and M. Sarih, "First report of L1014F-kdr mutation in *Culex pipiens* complex from Morocco," *Parasites & Vectors*, vol. 9, no. 1, p. 644, 2016.

[11] World Health Organization, *Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes*, World Health Organization, Geneva, Switzerland, 2016.

[12] European Centre for Disease Prevention and Control; European Food Safety Authority, *Field Sampling Methods for Mosquitoes, Sandflies, Biting Midges and Ticks—VectorNet Project 2014–2018*, ECDC and EFSA, Stockholm, Sweden, 2018.

[13] J. Brunhes, A. Rhaim, B. Geoffroy, G. Angel, and J. P. Hervy, *The Mosquitoes of the Mediterranean Africa*, Collection Didactiques, IRD Editions, Montpellier, France, 1999.

[14] M. Giner, C. Vassal, Z. Kouaik, F. Chiroleu, and J. M. Vassal, *Win DL [CD-ROM]*, CIRAD, Paris, France, 1999.

[15] Y. Jin-Clark, M. J. Lydy, and K. Yan Zhu, "Effects of atrazine and cyanazine on chlorpyrifos toxicity in *Chironomus tentans* (Diptera: chironomidae)," *Environmental Toxicology & Chemistry*, vol. 21, no. 3, pp. 598–603, 2002.

[16] G. Sinegre, J. Jullien, and B. Gaven, "Acquisition progressive de la résistance au chlorpyriphos chez les larves de *Culex pipiens* (L.) dans le midi de la France [Progressive acquisition of resistance to chlorpyriphos in *Culex pipiens* (L.) larvae in the south of France]," *Parasitologia*, vol. 19, pp. 1-2, 1977.

[17] I. Dusfour, J. Vontas, J. P. David et al., "Management of insecticide resistance in the major *Aedes vectors* of arboviruses: advances and challenges," *PLoS Neglected Tropical Diseases*, vol. 13, no. 10, Article ID e0007615, 2019.

[18] V. Robert, "Introduction à l'entomologie médicale et vétérinaire," in *Entomologie médicale et vétérinaire*, G. Duvallet, D. Fontenille, and V. Robert, Eds., pp. 37–59, Quae, Marseille, France, 2017.

[19] T. B. T. Satoto, H. Satrisno, L. Lazuardi et al., "Insecticide resistance in *Aedes aegypti*: an impact from human urbanization?" *PLoS One*, vol. 14, no. 6, Article ID e0218079, 2019.

[20] A. Tabbabi, J. Daaboub, R. B. Cheikh et al., "The potential role of urbanization in the resistance to organophosphate insecticide in *Culex pipiens pipiens* from Tunisia," *African Health Sciences*, vol. 19, no. 1, pp. 1368–1375, 2019.

[21] M. K. Grossman, V. Uc-Puc, A. E. Flores, P. C. Manrique-Saide, and G. M. Vazquez-Prokopec, "Larval density mediates knockdown resistance to pyrethroid insecticides in adult *Aedes aegypti*," *Parasites & Vectors*, vol. 11, no. 1, p. 282, 2018.

[22] A. Farajollahi, D. M. Fonseca, L. D. Kramer, and A. Marm Kilpatrick, ""Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology," *Infection, Genetics and Evolution*, vol. 11, no. 7, pp. 1577–1585, 2011.

[23] S. H. P. P. Karunaratne, "Insecticide resistance in insects," *Ceylon Journal of Science*, vol. 25, pp. 72–99, 1998.

[24] J. G. Scott, "Cytochromes P450 and insecticide resistance," *Insect Biochemistry and Molecular Biology*, vol. 29, no. 9, pp. 757–777, 1999.

[25] J. G. Scott, M. H. Yoshimizu, and S. Kasai, "Pyrethroid resistance in *Culex pipiens* mosquitoes," *Pesticide Biochemistry and Physiology*, vol. 120, pp. 68–76, 2015.

[26] C. V. Edi, L. Djogbenou, A. M. Jenkins et al., "CYP6 P450 enzymes and ACE-1 duplication produce extreme and multiple insecticide resistance in the malaria mosquito *Anopheles gambiae*," *PLoS Genetics*, vol. 10, no. 3, Article ID e1004236, 2014.

[27] J. P. David, H. M. Ismail, A. Chandor Proust, and M. J. I. Paine, "Role of cytochrome P450s in insecticide resistance: impact on the control of mosquito-borne diseases and use of insecticides on Earth," *Philosophical Transactions of the Royal Society B*, vol. 368, no. 1612, Article ID 20120429, 2013.