Heliyon 9 (2023) e12709

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

First comprehensive report of the resistance of *Culex quinquefasciatus* Say (Diptera: Culicidae) to commonly used insecticides in Riyadh, Saudi Arabia

Abdulwahab M. Hafez [*]

Pesticides and Environmental Toxicology Laboratory, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Keywords:
Southern house mosquito
Vector-borne diseases
Conventional insecticides
Field-evolved-resistance
Human health and environmental safety
Insect-vector-management

ABSTRACT

The mosquito *Culex quinquefasciatus* is a vector of various pathogens including West Nile virus, Saint Louis encephalitis virus, and Western equine encephalitis virus. Insecticides are the main tools for *Cx. quinquefasciatus* control, but this overreliance on chemical tools has led to the development of resistance to many insecticides in this important insect vector. The resistance of eight field populations of *Cx. quinquefasciatus* to 10 commonly used insecticides was evaluated. Based on the resistance ratios (RRs), the adults of *Cx. quinquefasciatus* field populations displayed susceptibility to the organophosphates (OPs) except Al-Masanie adults which exhibited low resistance to fenitrothion (RR_{50} = 3.62). Conversely, the mosquitoes exhibited susceptibility, low resistance, and moderate resistance to the pyrethroids alpha-cypermethrin (RR = 0.59–2.56), bifenthrin (RR = 0.59–2.19), deltamethrin (RR = 0.60–7.07), cypermethrin (RR = 0.60–2.66), and cyfluthrin (RR = 0.58–2.39). At the larval stage, *Cx. quinquefasciatus* field populations displayed susceptibility to low resistance to the OPs chlorpyrifos (RR = 0.03–1.75), malathion (RR = 0.19–3.42), fenitrothion (RR = 0.11–2.78), and pirimiphos-methyl (RR = 0.08–1.15). Although these results in *Cx. quinquefasciatus* field populations indicated that the OPs and pyrethroids maintained high efficacy in controlling this species in the geographical area of this study, these findings should be utilized wisely to avoid any potential negative effects on human health and environmental safety attributable to the application of these broad-spectrum conventional insecticides. However, these findings provide a solid basis for decision-making for *Cx. quinquefasciatus* integrated vector management programs.

1. Introduction

The climate of tropical and subtropical countries usually promotes the growth of insect vectors, which, especially when associated with improper insect vector management, threatens public health by fueling the spread of serious vector-borne diseases [1,2]. Mosquitoes in turn are among the most important vectors causing severe illness and death in humans and animals globally by transferring diseases such as malaria, dengue fever, West Nile fever, yellow fever, Rift Valley fever (RVF), Zika, Japanese encephalitis, and lymphatic filariasis [3,4].

Culex quinquefasciatus Say (Diptera: Culicidae) is an important vector of different vector-borne diseases including Japanese

* Corresponding author.
E-mail address: hafez@ksu.edu.sa.

https://doi.org/10.1016/j.heliyon.2022.e12709
Received 22 July 2022; Received in revised form 2 November 2022; Accepted 21 December 2022
Available online 29 December 2022

encephalitis, filariasis, Saint Louis encephalitis, West Nile fever, and Western equine encephalitis [3,5,6], in addition to the discomfort associated with the bite itself [7,8]. The aquatic habitats of *Cx. quinquefasciatus* larvae are diverse and include temporary standing rainwater, mangrove swamps, edges of rivers and streams, and freshwater/saltwater marshes [9–10].

Although various practices including chemical, biological, and cultural management strategies have been used to control *Cx. quinquefasciatus* [11], chemical approaches are the main strategies for controlling this insect vector to prevent transmission of the associated diseases [12,13]. For decades, organophosphates (OPs) and pyrethroids have been the most commonly used insecticides globally to control mosquitoes, and they can be administered using different modalities including indoor residual sprays, space spraying, and mosquito coils [11]. In Riyadh province, Saudi Arabia, mosquito control programs are conducted on a seasonal basis (spring: March to May, fall: September to November) because of its severe desert climate, and in most cases, insecticides are applied once monthly [14].

However, this overreliance has led to the development of resistance to commonly used insecticides including malathion, chlorpyrifos, dimethoate, permethrin, and deltamethrin in *Cx. quinquefasciatus* [15]. This resistance caused difficulties in insect vector control, resulting in the resurgence of many diseases that threaten human health, including lymphatic filariasis, RVF, and West Nile fever [16]. In addition, insecticide overuse to overcome resistance has increased the economic cost of insect vector chemical control and resulted in negative consequences on environmental safety [17–18]. The escalated problem of resistance and its negative consequences necessitate the adoption of integrated vector management (IVM) approaches to maintain the ability to control insect vectors, including *Cx. quinquefasciatus*, and ensure human and environmental safety.

Insecticide resistance monitoring is a baseline for novel and effective strategies to control insect vectors, including *Cx. quinquefasciatus* [8,19]. However, poor understanding of the resistance status of insect vectors can lead to a failure to control their spread. This failure in control programs could require increases of the application of insecticides, the applied field rate, or both. This strategy to overcome the failure of control programs will definitely increase the risks to human health and environmental safety, especially when using broad-spectrum conventional insecticides, including OPs and pyrethroids, with histories of negative impacts on both human health and environmental safety [20–21]. These broad-spectrum insecticides pose a major risk to humans especially children and pregnant women [22]. Where the acute and chronic exposure to OPs and pyrethroids can cause deaths or adverse health effects such as neurological symptoms, respiratory diseases, neurodevelopmental syndrome, hormonal and reproductive disturbance, chronic diseases, and cancer risks [23–24]. This risk has necessitated periodic and comprehensive monitoring of insecticide resistance in insect vectors, including *Cx. quinquefasciatus*. In Saudi Arabia, despite the availability of many different insecticides for *Cx. quinquefasciatus* control, information on the resistance status of this serious disease vector is limited. Therefore, this study evaluated the resistance of *Cx. quinquefasciatus* to 10 commonly used OPs and pyrethroids in different locations in Riyadh, Saudi Arabia. The findings of this study will help control *Cx. quinquefasciatus* in an appropriate and effective manner.

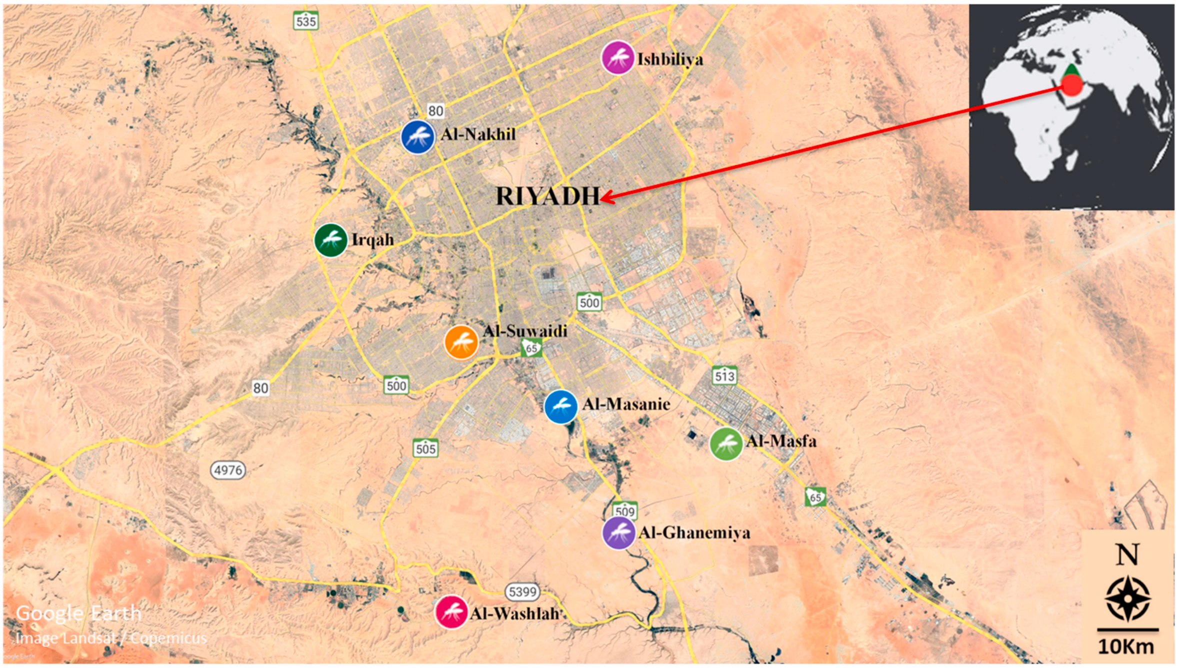

Figure 1. Collection locations of *Culex quinquefasciatus* field populations in Riyadh city.

2. Materials and methods

2.1. *Cx. quinquefasciatus* collection and rearing

Different stages of *Cx. quinquefasciatus* larvae were collected (approximately 200) from eight locations in Riyadh, Saudi Arabia (Figure 1, Table 1). Prior to use in this study, all collected populations were identified at King Saud University Museum of Arthropods (Riyadh, Kingdom of Saudi Arabia). Collected larvae were separately transported to the laboratory in 30 × 30 cm^2 plastic containers, fed cattle food ad libitum, maintained until pupation, then transferred emerging adults to 40 × 40 cm^2 cages and provided 10% sugar solution in soaked wicks as food. For blood feeding in females, cotton wicks saturated with defibrinated bovine blood obtained from the authorized Educational and Research Farm, Department of Animal Production, College of Food & Agriculture Sciences, King Saud University were placed in petri dishes and provided to mosquitoes in the adult cages. Following the blood meal, water containers (10 × 7 cm^2) were placed in the cages for egg laying and obtaining uniform F1 populations. Then transferred the collected egg rafts to the aforementioned larval containers. Some of the emerging larvae were used in the larval bioassays, and the others were allowed to develop until pupation to obtain adults for adult bioassays. The susceptible reference strain was obtained in 1990 from Alexandria University (Alexandria, Egypt) and maintained subsequently with no exposure to any chemicals.

2.2. Tested insecticides

Full information about the 10 tested OPs and pyrethroids is listed in Table 2.

2.3. Adult bioassays

Following the feeding bioassay of Shah et al. [8]; with some modifications, the toxicities of the tested insecticides were determined in *Cx. quinquefasciatus* adults (5 days old). Using serial dilution in deionized water, 100 mL 10% sugar stock solution of each tested insecticide was prepared. Prior to the main bioassays, 0 and 100% mortalities were determined in each population using five concentrations of each tested insecticide. For each bioassay, 150 mixed-sex adults were assigned to receive one of five concentrations of each tested insecticide, and three replicates were used for each concentration (10 adults per replicate). The adults were starved for 2 h and then provided cotton wicks saturated with 10% sugar solution containing an insecticide for feeding. Each bioassay was repeated three times. Meanwhile, 10% sugar solution with no insecticide was used as the control (3 replicates of 10 adults each). Mortality was determined after 24 h, and any adult that did not move was considered deceased.

2.4. Larval bioassays

Following the World Health Organization protocol [25], a larval bioassay was conducted using *Cx. quinquefasciatus* third instar larvae. Prior to the main bioassays, 0 and 100% mortalities were determined in each population using five concentrations of each tested insecticide. Each bioassay was repeated four times with fresh stock solution each time [26]. Ten larvae were placed in a plastic cup containing 400 mL of each tested concentration. Four replicates (10 larvae each) were used for each of the five/six concentrations, giving 200–240 larvae for each bioassay/insecticide/population. Deionized water was used as the control (4 replicates of 10 larvae each). Mortality was determined after 24 h, and any larva that did not move was considered deceased.

2.5. Laboratory conditions

Cx. quinquefasciatus rearing and bioassays were performed under temperature = 27 °C ± 2 °C, humidity = 65% ± 5%, and a photoperiod light:dark = 12-h:12-h.

Table 1
Culex quinquefasciatus field populations collected in Riyadh city.*

Location[1]	Nature of the collection location	Coordinates	Date of collection
Ishbiliya	Adjacent to a residential district park	24.802°N, 46.803°E	January 2020
Al-Suwaidi	Adjacent to a residential district park	24.590°N, 46.676°E	January 2020
Al-Ghanemiya	Adjacent to a small farm	24.482°N, 46.798°E	January 2020
Al-Masfa	Adjacent to livestock barns	24.471°N, 46.861°E	January 2020
Al-Masanie	Adjacent to a small farm	24.558°N, 46.743°E	January 2020
Al-Nakhil	Adjacent to a residential district park	24.737°N, 46.620°E	January 2020
Al-Washlah	Adjacent to private resorts	24.409°N, 46.660°E	January 2020
Irqah	Adjacent to a water filling and distribution station	24.677°N, 46.575°E	January 2020

* Average temperature, 14.7 °C (maximum, 22.2 °C; minimum, 8.9 °C); average humidity, 36.4% (maximum, 50%; minimum, 23%).
[1] Approximately 200 larvae were collected at each site.

Table 2
List of tested insecticides in the toxicity bioassay of *Culex quinquefasciatus*.

Active ingredient	[1]Form.	Trade name	Manufacturer	[2]IRAC number	Mode of action
50% fenitrothion	500 EC	Fentox	Pioneers Chemicals Factory Co., Saudi Arabia	2A	Acetylcholinesterase inhibitor
48% chlorpyrifos	48 EC	Chlorfet	Masani Chemicals, Jordan		
57% malathion	570 EC	Delthion	Saudi Delta Company, Saudi Arabia		
60% diazinon	60 EC	Diazinon	APCO, Saudi Arabia		
50% pirimiphos-methyl	500 EC	Actikil	Astrachem, Saudi Arabia		
10% cypermethrin	10 EC	Montothrin	Montajat Agrochemicals, Saudi Arabia	3A	Sodium channel modulator
7.9% bifenthrin	8SC	Biflex	FMC, Belgium		
2.5% deltamethrin	25SC	K-Othrine	Bayer Crop Sciences, France		
5% cyfluthrin	050 EW	Solfac	Bayer Crop Sciences, Germany		
10% alpha-cypermethrin	100 EC	Alphaquest	Astrachem, Saudi Arabia		

[1] Formulation.
[2] Insecticide Resistance Action Committee, EC = emulsifiable concentrate, SC = soluble concentrate, EW = emulsion in water.

2.6. Data analyses

Bioassay data were analyzed by probit analysis using POLO Plus software [27] to calculate the median lethal concentration (LC_{50}), 95% fiducial limit (FL), standard error (SE), and chi-square (χ^2). If required, the mortality of each bioassay was corrected by that of the control using Abbott's formula [28]. The field populations were considered significantly different from the susceptible strain when their 95% FLs did not overlap [29]. The resistance ratio (RR) was calculated by dividing the LC_{50} of each field population by that of the susceptible strain. In the results, RR < 5 indicated low resistance, RR = 5–10 indicated moderate resistance, and RR > 10 indicated high resistance [25,30].

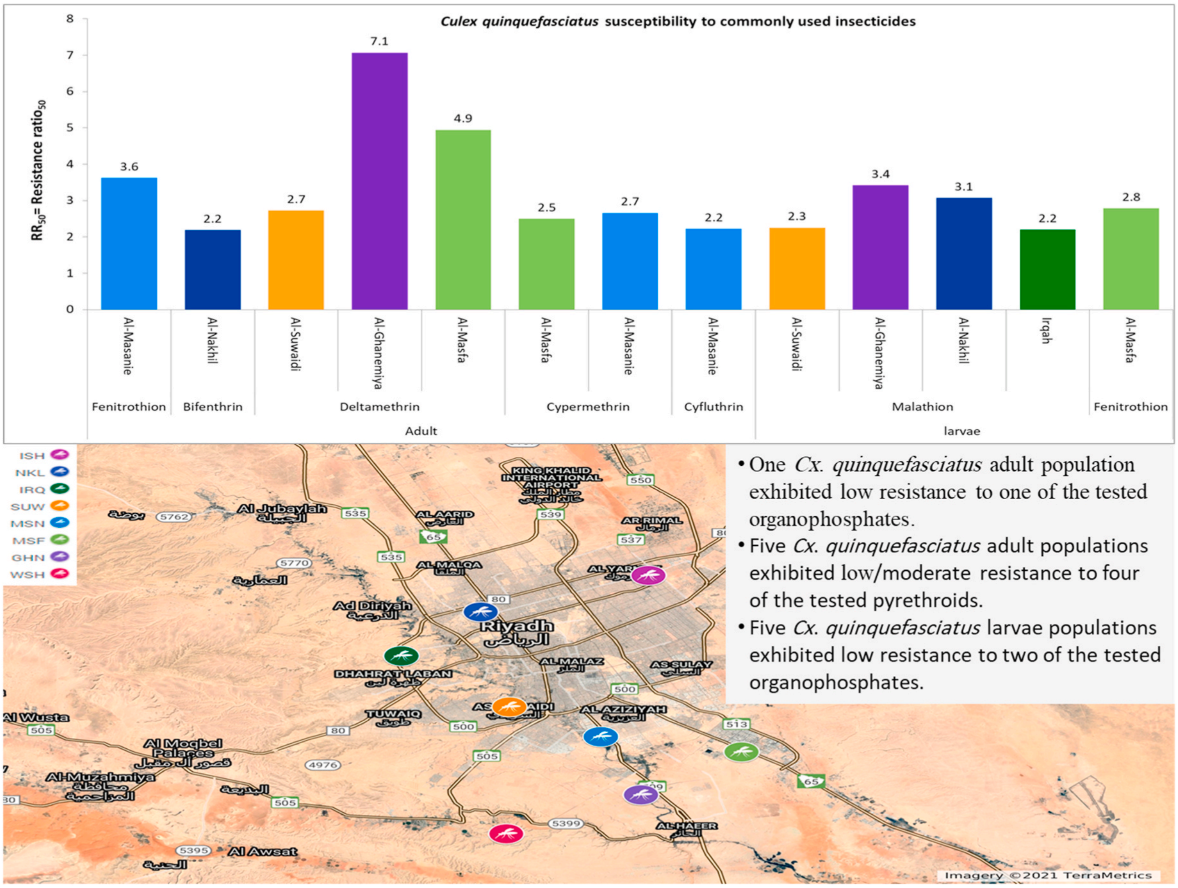

Figure 2. Graphical abstract.

3. Results

3.1. OP toxicities against *Cx. quinquefasciatus*

Based on the LC_{50s} of the tested OPs, no significant differences were detected regarding their toxic effects between the field population adults and susceptible strain adults. However, significantly higher susceptibility was detected in the adults of some field populations in comparison to the susceptible strain adults as follows: chlorpyrifos (RR_{50} = 0.23), malathion (RR_{50} = 0.44), and pirimiphos-methyl (RR_{50} = 0.38) in Al-Ghanemiya; fenitrothion in Al-Suwaidi and Al-Masfa (RR_{50} = 0.09 and 0.43, respectively); and diazinon in Al-Washlah (RR_{50} = 0.11). The only low resistance case was recorded to fenitrothion (RR_{50} = 3.62) in the adults of the Al-Masanie field population (Table 3, Figure 2).

Based on the LC_{50s} of the tested OPs, significantly greater susceptibility was detected in the larvae of some field populations in comparison to the susceptible strain as follows: chlorpyrifos, malathion, fenitrothion, and pirimiphos-methyl in Ishbiliya (RR_{50} = 0.08, 0.19, 0.33, and 0.19, respectively); chlorpyrifos and fenitrothion in Al-Suwaidi (RR_{50} = 0.03 and 0.44, respectively); fenitrothion and pirimiphos-methyl in Al-Ghanemiya (RR_{50} = 0.11 and 0.35, respectively); chlorpyrifos and malathion in Al-Masfa (RR_{50} = 0.17 and 0.56, respectively); chlorpyrifos and pirimiphos-methyl in Al-Masanie (RR_{50} = 0.25 and 0.19, respectively); pirimiphos-methyl in Al-Washlah (RR_{50} = 0.50); and chlorpyrifos and pirimiphos-methyl in Irqah (RR_{50} = 0.17 and 0.08, respectively). Meanwhile, significant

Table 3
The susceptibility of *Culex quinquefasciatus* adults to organophosphates.

Insecticide	Population	LC_{50} (95% FL), μg/mL	LC_{90} (95% FL), μg/mL	Slope ± SE	χ^2	RR_{50}	RR_{90}
Chlorpyrifos	Susceptible	0.044 (0.029–0.062)	0.316 (0.183–0.923)	1.50 ± 0.28	2.37	1.00	1.00
	Ishbiliya	0.024 (0.013–0.035)	0.112 (0.075–0.227)	1.91 ± 0.39	1.28	0.55	0.35
	Al-Suwaidi	0.024 (0.010–0.038)	0.212 (0.121–0.722)	1.35 ± 0.31	5.06	0.55	0.67
	Al-Ghanemiya	0.010 (0.005–0.015)	0.056 (0.038–0.117)	1.75 ± 0.36	0.39	0.23	0.18
	Al-Masfa	0.031 (0.009–0.053)	0.119 (0.068–0.563)	2.18 ± 0.42	3.12	0.70	0.38
	Al-Masanie	0.033 (0.017–0.049)	0.351 (0.183–1.545)	1.24 ± 0.27	1.44	0.75	1.11
	Al-Nakhil	0.031 (0.021–0.042)	0.165 (0.110–0.345)	1.77 ± 0.21	1.18	0.70	0.52
	Al-Washlah	0.035 (0.014–0.058)	0.494 (0.220–4.284)	1.11 ± 0.28	0.09	0.80	1.56
	Irqah	0.033 (0.022–0.045)	0.197 (0.126–0.450)	1.66 ± 0.30	1.39	0.75	0.62
Malathion	Susceptible	0.066 (0.048–0.092)	0.395 (0.232–1.067)	1.65 ± 0.28	0.64	1.00	1.00
	Ishbiliya	0.089 (0.049–0.137)	0.467 (0.263–1.988)	1.78 ± 0.45	1.79	1.35	1.18
	Al-Suwaidi	0.035 (0.013–0.061)	0.591 (0.242–8.041)	1.05 ± 0.31	0.83	0.53	1.50
	Al-Ghanemiya	0.029 (0.018–0.039)	0.169 (0.110–0.378)	1.66 ± 0.30	1.01	0.44	0.43
	Al-Masfa	0.095 (0.065–0.162)	0.997 (0.425–7.092)	1.26 ± 0.27	0.03	1.44	2.52
	Al-Masanie	0.066 (0.047–0.096)	0.482 (0.262–1.618)	1.49 ± 0.27	0.72	1.00	1.22
	Al-Nakhil	0.060 (0.040–0.090)	0.581 (0.284–2.790)	1.30 ± 0.27	0.88	0.91	1.47
	Al-Washlah	0.095 (0.051–0.199)	1.462 (0.485–40.197)	1.08 ± 0.29	0.97	1.44	3.70
	Irqah	0. 049 (0.031–0. 072)	0.496 (0.247–2.308)	1.28 ± 0.27	0.22	0. 74	1.26
Fenitrothion	Susceptible	0.058 (0.041–0.082)	0.399 (0.226–1.205)	1.54 ± 0.28	2.36	1.00	1.00
	Ishbiliya	0.036 (0.015–0.061)	0.167 (0.088–1.299)	1.91 ± 0.31	3.98	0.62	0.42
	Al-Suwaidi	0.005 (0.000–0.012)	0.049 (0.027–0.135)	1.25 ± 0.42	1.15	0.09	0.12
	Al-Ghanemiya	0.053 (0.016–0.121)	3.897 (0.646–102480.469)	0.68 ± 0.25	0.22	0.91	9.77
	Al-Masfa	0.025 (0.018–0.032)	0.084 (0.062–0.138)	2.46 ± 0.41	0.72	0.43	0.21
	Al-Masanie	0.210 (0.150–0.404)*	0.647 (0.310–22.398)	2.62 ± 0.51	3.63	3.62	1.62
	Al-Nakhil	0.031 (0.008–0.058)	0.121 (0.063–1.561)	2.17 ± 0.33	6.08	0.53	0.30
	Al-Washlah	0.043 (0.023–0.065)	0.340 (0.186–1.245)	1.42 ± 0.30	0.46	0.74	0.85
	Irqah	0.048 (0.035–0.064)	0.254 (0.162–0.564)	1.77 ± 0.29	1. 51	0.83	0.64
Pirimiphos-methyl	Susceptible	0.039 (0.026–0.053)	0.228 (0.144–0.526)	1.65 ± 0.29	6.07	1.00	1.00
	Ishbiliya	0.082 (0.040–0.148)	0.829 (0.348–9.971)	1.27 ± 0.34	0.75	2.10	3.64
	Al-Suwaidi	0.036 (0.024–0.048)	0.137 (0.096–0.247)	2.21 ± 0.38	2.02	0.92	0.60
	Al-Ghanemiya	0.015 (0.010–0.020)	0.071 (0.049–0.138)	1.91 ± 0.35	5.88	0.38	0.31
	Al-Masfa	0.027 (0.014–0.040)	0.133 (0.088–0.287)	1.86 ± 0.38	2.68	0.69	0.58
	Al-Masanie	0.037 (0.019–0.058)	0.551 (0.245–4.403)	1.10 ± 0.26	1.09	0.95	2.42
	Al-Nakhil	0.046 (0.032–0.063)	0.287 (0.174–0.734)	1.61 ± 0.28	1.85	1.18	1.26
	Al-Washlah	0.023 (0.015–0.031)	0.075 (0.055–0.126)	2.52 ± 0.47	1.75	0.59	0.33
	Irqah	0.030 (0.020–0.041)	0.158 (0.106–0.324)	1.77 ± 0.31	4.99	0.77	0.69
Diazinon	Susceptible	0.046 (0.031–0.063)	0.314 (0.184–0.886)	1.53 ± 0.28	2.27	1.00	1.00
	Ishbiliya	0.031 (0.020–0.044)	0.144 (0.091–0.354)	1.92 ± 0.38	0.45	0.67	0.46
	Al-Suwaidi	0.024 (0.010–0.038)	0.212 (0.121–0.717)	1.35 ± 0.31	5.06	0.52	0.68
	Al-Ghanemiya	0.033 (0.013–0.057)	0.141 (0.076–1.104)	2.04 ± 0.33	4.41	0.72	0.45
	Al-Masfa	0.030 (0.021–0.040)	0.140 (0.096–0.267)	1.92 ± 0.32	2.42	0.65	0.45
	Al-Masanie	0.028 (0.018–0.041)	0.272 (0.132–1.482)	1.29 ± 0.28	1.03	0.61	0.87
	Al-Nakhil	0.043 (0.014–0.086)	0.287 (0.124–13.072)	1.55 ± 0.28	4.25	0.93	0.91
	Al-Washlah	0.005 (0.003–0.007)	0.036 (0.021–0.105)	1.45 ± 0.28	0.95	0.11	0.11
	Irqah	0.024 (0.015–0.032)	0.107 (0.075–0.195)	1.97 ± 0.35	5.35	0.52	0.34

LC = lethal concentration, FL = fiducial limit, SE = standard error, χ^2 = chi-square, RR = resistance ratio (LC of the insecticide in the field population/LC of the insecticide in the susceptible strain).
* The field population was significantly more resistant to the insecticide than the susceptible strain.

low levels of resistance were recorded in the larvae of other field populations in comparison to the susceptible strain. Specifically, significant low levels of resistance were recorded against malathion in the larvae of Al-Suwaidi, Al-Ghanemiya, Al-Nakhil, and Irqah (RR_{50} = 2.25, 3.42, 3.07, and 2.20, respectively) and against fenitrothion in the larvae of Al-Masfa (RR_{50} = 2.78; Table 4, Figure 2). The LC_{50} ranges for the tested OPs in the larvae of eight field populations were 0.0003–0.021 μg/mL for chlorpyrifos, 0.011–0.202 μg/mL for malathion, 0.001–0.025 μg/mL for fenitrothion, and 0.002–0.030 μg/mL for pirimiphos-methyl. The RR_{50} ranges for the tested OPs in the larvae of eight field populations were chlorpyrifos = 0.03–1.75, malathion = 0.19–3.42, fenitrothion = 0.11–2.78, and pirimiphos-methyl = 0.08–1.15 (Table 4).

3.2. *Pyrethroid toxicities against Cx. quinquefasciatus*

Based on the LC_{50s} of the tested pyrethroids, no significant differences were detected regarding their toxicities between the field population adults and susceptible strain adults, excluding deltamethrin in Al-Suwaidi, deltamethrin in Al-Ghanemiya, deltamethrin and cypermethrin in Al-Masfa, cypermethrin and cyfluthrin in Al-Masanie, and bifenthrin in Al-Nakhil (Table 5, Figure 2). In comparison to the susceptible strain, no significant differences in the susceptibility to alpha-cypermethrin were detected in the adults of all field populations with an RR_{50} range of 0.59–2.56. For bifenthrin, Al-Nakhil adults displayed the only low resistance case with RR_{50} of 2.19, whereas no significant differences in susceptibility were detected in the adults of the remaining field populations in comparison to the susceptible strain (RR_{50} = 0.59–1.66). For deltamethrin, the adult of Al-Ghanemiya displayed moderate resistance (RR_{50} = 7.07), and those of Al-Suwaidi and Al-Masfa exhibited low resistance (RR_{50} = 2.72 and 4.94, respectively). Meanwhile, no significant differences in susceptibility were detected in the adults of the other field populations (RR_{50} = 0.60–1.54) in comparison to those of the susceptible strain. For cypermethrin, the adults of Al-Masfa and Al-Masanie exhibited low resistance (RR_{50} = 2.50 and 2.66, respectively), whereas no significant differences in susceptibility were detected in the adults of the remaining field populations (RR_{50} = 0.60–1.97) in comparison to the susceptible strain. For cyfluthrin, Al-Masanie adults displayed the only low resistance case with RR_{50} of 2.22, whereas no significant differences in susceptibility were detected in the adults of the other field populations, in

Table 4
The susceptibility of third instar *Culex quinquefasciatus* larvae to organophosphates.

Insecticide	Population	LC_{50} (95% FL), μg/mL	LC_{90} (95% FL), μg/mL	Slope ± SE	χ^2	RR_{50}	RR_{90}
Chlorpyrifos	Susceptible	0.012 (0.005–0.027)	2.410 (0.316–3430.572)	0.55 ± 0.16	1.32	1.00	1.00
	Ishbiliya	0.001 (0.000–0.004)	0.053 (0.027–0.468)	0.72 ± 0.26	0.37	0.08	0.02
	Al-Suwaidi	0.0003 (0.0002–0.0005)	0.003 (0.002–0.006)	1.39 ± 0.25	3.55	0.03	0.001
	Al-Ghanemiya	0.007 (0.003–0.011)	0.110 (0.051–0.843)	1.07 ± 0.26	1.31	0.58	0.05
	Al-Masfa	0.002 (0.001–0.003)	0.019 (0.011–0.055)	1.28 ± 0.21	1.81	0.17	0.01
	Al-Masanie	0.003 (0.002–0.004)	0.012 (0.008–0.020)	2.03 ± 0.34	1.51	0.25	0.005
	Al-Nakhil	0.021 (0.013–0.041)	0.398 (0.128–10.180)	1.00 ± 0.26	1.81	1.75	0.17
	Al-Washlah	0.009 (0.007–0.012)	0.037 (0.026–0.065)	2.09 ± 0.32	1.50	0.75	0.02
	Irqah	0.002 (0.001–0.003)	0.022 (0.009–0.175)	1.13 ± 0.26	0.30	0.17	0.01
Malathion	Susceptible	0.059 (0.046–0.077)	0.324 (0.215–0.602)	1.73 ± 0.20	7.17	1.00	1.00
	Ishbiliya	0.011 (0.002–0.021)	0.892 (0.230–146.839)	0.67 ± 0.20	0.46	0.19	2.75
	Al-Suwaidi	0.133 (0.092–0.229)*	1.143 (0.521–5.133)	1.41 ± 0.24	0.70	2.25	3.53
	Al-Ghanemiya	0.202 (0.145–0.354)*	0.964 (0.495–3.860)	1.89 ± 0.36	0.99	3.42	2.98
	Al-Masfa	0.033 (0.025–0.043)	0.164 (0.111–0.298)	1.84 ± 0.24	2.79	0.56	0.51
	Al-Masanie	0.045 (0.033–0.058)	0.199 (0.135–0.379)	1.98 ± 0.30	4.82	0.76	0.61
	Al-Nakhil	0.181 (0.088–2.880)*	12.799 (1.350–6084033.5)*	0.69 ± 0.25	2.18	3.07	39.50
	Al-Washlah	0.079 (0.057–0.114)	0.508 (0.282–1.570)	1.59 ± 0.28	1.21	1.34	1.57
	Irqah	0.130 (0.080–0.183)*	1.279 (0.739–3.708)*	1.29 ± 0.23	0.38	2.20	3.95
Fenitrothion	Susceptible	0.009 (0.007–0.011)	0.043 (0.030–0.073)	1.90 ± 0.27	7.14	1.00	1.00
	Ishbiliya	0.003 (0.001–0.005)	0.034 (0.022–0.089)	1.14 ± 0.25	2.09	0.33	0.79
	Al-Suwaidi	0.004 (0.002–0.005)	0.009 (0.007–0.014)	3.42 ± 0.81	0.93	0.44	0.21
	Al-Ghanemiya	0.001 (0.000–0.003)	0.013 (0.008–0.030)	1.32 ± 0.40	2.17	0.11	0.30
	Al-Masfa	0.025 (0.018–0.038)*	0.187 (0.096–0.620)*	1.46 ± 0.23	4.10	2.78	4.35
	Al-Masanie	0.007 (0.006–0.008)	0.020 (0.015–0.029)	2.80 ± 0.35	1.39	0.78	0.47
	Al-Nakhil	0.012 (0.003–0.025)	0.874 (0.151–17593.656)*	0.68 ± 0.25	0.30	1.33	20.33
	Al-Washlah	0.015 (0.011–0.021)	0.099 (0.059–0.254)	1.57 ± 0.26	0.05	1.67	2.30
	Irqah	0.008 (0.005–0.012)	0.079 (0.043–0.285)	1.32 ± 0.27	1.95	0.89	1.84
Pirimiphos-methyl	Susceptible	0.026 (0.017–0.047)	0.527 (0.189–4.177)	0.98 ± 0.17	3.80	1.00	1.00
	Ishbiliya	0.005 (0.004–0.006)	0.013 (0.011–0.019)	2.98 ± 0.46	3.90	0.19	0.02
	Al-Suwaidi	0.021 (0.017–0.026)	0.058 (0.043–0.091)	2.88 ± 0.39	1.71	0.81	0.11
	Al-Ghanemiya	0.009 (0.006–0.013)	0.105 (0.055–0.353)	1.21 ± 0.20	2.09	0.35	0.20
	Al-Masfa	0.014 (0.010–0.017)	0.053 (0.037–0.095)	2.17 ± 0.31	2.14	0.54	0.10
	Al-Masanie	0.005 (0.003–0.006)	0.024 (0.017–0.043)	1.83 ± 0.26	0.72	0.19	0.05
	Al-Nakhil	0.030 (0.025–0.036)	0.060 (0.048–0.087)	4.18 ± 0.64	1.57	1.15	0.11
	Al-Washlah	0.013 (0.011–0.016)	0.039 (0.030–0.057)	2.73 ± 0.32	1.74	0.50	0.07
	Irqah	0.002 (0.001–0.003)	0.008 (0.006–0.014)	2.32 ± 0.44	2.14	0.08	0.02

LC = lethal concentration, FL = fiducial limit, SE = standard error, χ^2 = chi-squared, RR = resistance ratio (LC of the insecticide in the field population/LC of the insecticide in the susceptible strain).

* The field population was significantly more resistant to the insecticide than the susceptible strain.

comparison to the susceptible strain (RR_{50} = 0.58–2.39; Table 5).

4. Discussion

Globally, insect vector management relies mainly on insecticides, which has necessitated periodic and comprehensive monitoring of resistance to commonly used insecticides in insect vectors, including *Cx. quinquefasciatus* [31–32]. OPs and pyrethroids, which inhibit acetylcholinesterase and modulate sodium channels, respectively [33], are the most commonly used insecticides for controlling insect vectors, especially mosquitoes, in countries including Saudi Arabia [31,34–36]. Therefore, resistance to commonly used insecticides in Saudi Arabia has been documented in *Cx. pipiens* and *Aedes aegypti* (L.) [34,37,38].

The findings of this study revealed low levels of resistance to commonly used OPs in *Cx. quinquefasciatus* field populations. However, other studies have documented field-evolved resistance to commonly used OPs in *Cx. quinquefasciatus* [2,8,19,39], *Cx. pipiens* [34,40], *Ae. albopictus* [41,42], and *Ae. aegypti* [43].

Concerning pyrethroids, the findings of this study indicated susceptibility, low resistance, or moderate resistance to commonly used pyrethroids including deltamethrin, cypermethrin, alpha-cypermethrin, cyfluthrin, and bifenthrin in *Cx. quinquefasciatus* field populations. Globally, resistance to commonly used pyrethroids has been well documented in different insect vectors, including *Cx. quinquefasciatus* [8,36,44], *Ae. aegypti* and *Ae. albopictus* [41,45,46–49], *Cx pipiens* [40], *Anopheles gambiae* Giles [50,51], and *An.*

Table 5
The susceptibility of *Culex quinquefasciatus* adults to pyrethroids.

Insecticide	Population	LC_{50} (95% FL), μg/mL	LC_{90} (95% FL), μg/mL	Slope ± SE	χ^2	RR_{50}	RR_{90}
Alpha-cypermethrin	Susceptible	6.11 (4.33–8.29)	35.09 (21.81–83.75)	1.69 ± 0.29	1.69	1.00	1.00
	Ishbiliya	4.28 (2.97–5.66)	20.01 (13.70–38.23)	1.91 ± 0.32	7.58	0.70	0.57
	Al-Suwaidi	11.56 (6.13–33.72)	511.41 (97.15–573310.00)*	0.78 ± 0.25	0.62	1.89	14.57
	Al-Ghanemiya	7.70 (4.64–12.55)	115.97 (46.06–1250.58)	1.09 ± 0.26	0.20	1.26	3.30
	Al-Masfa	15.63 (8.17–82.61)	888.02 (127.81–12967000.00)*	0.73 ± 0.25	0.48	2.56	25.31
	Al-Masanie	3.60 (0.66–6.74)	170.93 (45.878–52897.00)	0.76 ± 0.25	2.61	0.59	4.87
	Al-Nakhil	11.39 (7.69–18.99)	120.50 (51.84–842.58)	1.25 ± 0.27	0.31	1.86	3.43
	Al-Washlah	7.68 (4.45–12.97)	137.01 (49.91–2229.10)	1.02 ± 0.26	0.05	1.26	3.90
	Irqah	12.17 (7.54–24.95)	225.97 (70.834–6292.10)	1.01 ± 0.26	0.13	1.99	6.44
Bifenthrin	Susceptible	22.79 (15.53–31.42)	148.20 (88.97–391.04)	1.58 ± 0.28	1.60	1.00	1.00
	Ishbiliya	21.07 (11.69–32.11)	270.60 (125.44–1748.10)	1.16 ± 0.26	1.20	0.92	1.83
	Al-Suwaidi	27.35 (16.38–42.92)	371.40 (158.71–3064.50)	1.13 ± 0.26	1.31	1.20	2.51
	Al-Ghanemiya	13.41 (2.77–24.56)	519.40 (158.40–55403.00)	0.81 ± 0.25	0.26	0.59	3.50
	Al-Masfa	35.82 (16.04–69.77)	540.92 (192.56–13841.00)	1.09 ± 0.31	0.13	1.57	3.65
	Al-Masanie	25.74 (15.66–39.04)	301.57 (140.48–1792.89)	1.20 ± 0.26	0.10	1.13	2.04
	Al-Nakhil	49.85 (34.56–81.15)*	443.50 (204.02–2427.26)	1.35 ± 0.27	1.34	2.19	2.99
	Al-Washlah	32.04 (14.60–70.21)	1459.40 (308.83–1069900)	0.77 ± 0.25	0.17	1.41	9.85
	Irqah	37.92 (25.72–58.87)	365.86 (171.75–1933.09)	1.30 ± 0.27	0.11	1.66	2.47
Deltamethrin	Susceptible	10.87 (5.91–17.17)	169.94 (70.54–1698.50)	1.07 ± 0.26	0.87	1.00	1.00
	Ishbiliya	7.14 (2.34–12.19)	184.58 (66.46–5146.63)	0.91 ± 0.26	0.69	0.66	1.09
	Al-Suwaidi	29.57 (17.61–81.12)*	705.16 (176.45–61693)	0.93 ± 0.26	0.85	2.72	4.15
	Al-Ghanemiya	76.82 (33.97–2542.64)*	3968.30 (406.58–609720000)	0.75 ± 0.26	0.42	7.07	23.35
	Al-Masfa	53.73 (30.06–229.10)*	1054.30 (241.08–110600)	0.99 ± 0.27	1.29	4.94	6.20
	Al-Masanie	6.56 (2.84–10.32)	90.58 (44.29–536.96)	1.12 ± 0.27	3.35	0.60	0.53
	Al-Nakhil	10.53 (7.40–14.22)	59.30 (37.48–136.06)	1.71 ± 0.29	1.04	0.97	0.35
	Al-Washlah	16.78 (7.40–45.71)	1056.90 (175.45–9767800)	0.71 ± 0.25	0.09	1.54	6.22
	Irqah	15.52 (9.55–25.75)	230.87 (90.16–2621.00)	1.09 ± 0.26	1.61	1.43	1.36
Cypermethrin	Susceptible	4.64 (2.89–6.53)	34.61 (20.34–99.72)	1.47 ± 0.28	1.37	1.00	1.00
	Ishbiliya	3.83 (1.09–6.84)	17.99 (9.336–231.16)	1.91 ± 0.33	4.62	0.83	0.52
	Al-Suwaidi	5.82 (4.19–7.74)	29.50 (19.28–62.00)	1.82 ± 0.30	5.95	1.25	0.85
	Al-Ghanemiya	9.13 (5.31–17.04)	195.47 (61.54–6288.30)	0.96 ± 0.25	0.44	1.97	5.65
	Al-Masfa	11.58 (7.26–22.35)*	195.63 (65.57–4005.60)	1.04 ± 0.26	0.98	2.50	5.65
	Al-Masanie	12.33 (8.64–19.65)*	102.10 (48.63–501.24)	1.40 ± 0.28	2.72	2.66	2.95
	Al-Nakhil	2.78 (0.41–5.06)	16.56 (8.52–252.14)	1.65 ± 0.32	3.76	0.60	0.48
	Al-Washlah	7.10 (2.37–17.01)	533.91 (86.25–15644071)	0.68 ± 0.25	0.25	1.53	15.43
	Irqah	7.07 (4.10–11.52)	114.09 (44.51–1388.00)	1.06 ± 0.26	0.71	1.52	3.30
Cyfluthrin	Susceptible	2.57 (1.45–3.86)	30.31 (14.65–166.76)	1.20 ± 0.26	0.89	1.00	1.00
	Ishbiliya	1.93 (1.05–2.83)	17.56 (9.90–59.09)	1.34 ± 0.28	7.69	0.75	0.58
	Al-Suwaidi	3.49 (2.94–5.11)	31.71 (16.23–131.96)	1.34 ± 0.27	0.52	1.36	1.05
	Al-Ghanemiya	3.36 (1.05–7.74)	252.48 (41.62–6604754)	0.68 ± 0.25	0.22	1.31	8.33
	Al-Masfa	4.95 (3.30–8.00)	54.24 (23.75–364.88)	1.23 ± 0.26	0.05	1.93	1.79
	Al-Masanie	5.70 (4.19–8.23)*	33.82 (19.06–100.46)	1.66 ± 0.29	0.80	2.22	1.12
	Al-Nakhil	1.48 (0.83–2.10)	9.06 (5.91–20.34)	1.63 ± 0.31	2.57	0.58	0.30
	Al-Washlah	6.13 (2.86–34.15)	546.25 (63.92–505362240)	0.66 ± 0.25	0.05	2.39	18.02
	Irqah	3.28 (2.17–4.72)	27.58 (14.74–101.38)	1.39 ± 0.27	1.10	1.28	0.91

LC = lethal concentration, FL = fiducial limit, SE = standard error, χ^2 = chi-square, RR = resistance ratio (LC of the insecticide in the field population/LC of the insecticide in the susceptible strain).

* The field population was significantly more resistant to the insecticide than the susceptible strain.

stephensi Liston [52].

The possible causes of resistance to commonly used OPs and pyrethroids in these insect vectors include increased metabolic enzyme activities [41,53,54] and target site insensitivity caused by knockdown resistance (kdr) mutations (L1014F, F1534C, and I1532T in voltage-gated sodium channels; V1016I and F1534C in the 11S6 domain) for pyrethroids and the G119S mutation in the acetylcholinesterase 1 gene for OPs [36,46,55–57]. Previously, multiple insecticide resistance mechanisms were reported in *Cx. quinquefasciatus* [35,36,58–60]. For example, the metabolic enzymes including mixed-function oxidase, esterases, and glutathione S-transferase played an important role in different cases of resistance to commonly used insecticides in *Cx. quinquefasciatus* populations [58–60]. In addition, mutations in the acetylcholinesterase and voltage-gated sodium channel genes have been identified in *Cx. quinquefasciatus* populations with resistance to OPs and pyrethroids, respectively [35,36]. In the current study, the absence or low levels of resistance in the tested *Cx. quinquefasciatus* populations did not necessitate the action of resistance mechanism. However, we are currently conducting further studies on resistance in *Cx. quinquefasciatus* in consideration of cross-resistance patterns, metabolic mechanisms, and target-site resistance mutations to deeply explore the resistance phenomena in *Cx. quinquefasciatus*.

In the current study, *Cx. quinquefasciatus* field populations were collected from the Riyadh region, which has a severe desert climate that naturally controls insect vectors including *Cx. quinquefasciatus*. Due to this severe climate, the application of OPs and pyrethroids is on a seasonal basis (in March–May and September–November) with one application per month in most cases [14]. This possible low exposure might explain the documented susceptibility and low level of resistance to commonly used OPs and pyrethroids in the field populations of *Cx. quinquefasciatus*. However, the moderate field-evolved resistance documented in some *Cx. quinquefasciatus* field populations to the pyrethroid deltamethrin highlights the need for careful pyrethroid use in this geographical region and periodic and comprehensive monitoring of resistance to detect, at an early stage, any field-evolved resistance in *Cx. quinquefasciatus* to commonly used insecticides.

In addition to the documented susceptibility/low level of resistance in the field populations of *Cx. quinquefasciatus* to old classes of insecticides (OPs and pyrethroids) in this study, Hafez and Abbas [61] documented significant higher levels of resistance to new classes of insecticides in the same field populations of *Cx. quinquefasciatus*. These unexpected findings of both studies could be explained by the circumstances of the collection locations. *Cx. quinquefasciatus* field populations were collected from locations adjacent to parks and farms, in which the use of old classes of insecticides was restricted or prevented over the last two decades due to the pesticide laws and regulations which also permitted their replacement by new classes of insecticides in plant protection programs [11]. This may have increased the selection pressure against these new classes of insecticides in *Cx. quinquefasciatus* populations attributable to accidental exposure over the last two decades.

In conclusion, the documented susceptibility and low level of resistance to commonly used OPs and pyrethroids in *Cx. quinquefasciatus* suggests that they remain effective for vector control in Riyadh, Saudi Arabia. However, *Cx. quinquefasciatus* should be controlled via a strategic IVM program including periodic monitoring of resistance, the use of insecticides with different modes of actions in a rotational manner, and both cultural and biological control practices. This strategic IVM program could maintain the efficacy of insecticides by preventing or delaying the development of resistance and ensuring the safety of humans and the environment by minimizing the overreliance on chemical control.

Finally, the findings of this study provide a solid basis for decision-making for *Cx. quinquefasciatus* integrated vector management programs. However, it is extremely important to wisely utilize the findings of this study. Although the tested OP and pyrethroid insecticides were found to be effective chemicals for controlling *Cx. quinquefasciatus*, these old chemicals should not be the first options in *Cx. quinquefasciatus* control programs, and agents with histories of negative impact on human health and environmental safety (e.g., chlorpyrifos) should be completely avoided. In addition, the findings of this study are limited to the geographical area of analysis, and any generalization to other regions should be avoided. A wider geographical monitoring of the resistance status in *Cx. quinquefasciatus* is necessary to better understand the emergence of resistance to insecticides.

Ethical approval

No ethical approval was required to conduct this study on the unregulated invertebrate animal *Cx. quinquefasciatus*.

Declarations

Author contribution statement

Abdulwahab M. Hafez: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Dr. Abdulwahab Hafez was supported by the Deputyship for Research & Innovation, "Ministry of Education" in Saudi Arabia through the project number IFKSUDR-H212.

Data availability statement

Data associated with this study has been deposited at https://doi.org/10.21203/rs.3.rs-907877/v1.

Declaration of interest's statement

The author declares no competing interests.

Additional information

No additional information is available for this paper.

References

[1] E.I. Khater, M.M. Sowilem, M.F. Sallam, A.M. Alahmed, Ecology and habitat characterization of mosquitoes in Saudi Arabia, Trop. Biomed. 30 (2013) 409–427.
[2] P. Rai, M. Bharati, A. Subba, D. Saha, Insecticide resistance mapping in the vector of lymphatic filariasis, *Culex quinquefasciatus* Say from northern region of West Bengal, India, PLoS One 14 (2019), e0217706.
[3] WHO, Global programme to eliminate lymphatic filariasis: progress report on mass drug administration, Wkly. Epidemiol. Rec. 86 (2011) 377–387, 2010.
[4] S. Eybpoosh, M. Fazlalipour, V. Baniasadi, M.H. Pouriayevali, F. Sadeghi, A. Ahmadi Vasmehjani, M.H. Karbalaie Niya, R. Hewson, M. Salehi-Vaziri, Epidemiology of West Nile Virus in the Eastern Mediterranean region: A systematic review, PLoS Negl. Trop. Dis. 13 (2019), e0007081.
[5] M.S. Godsey Jr., R. Nasci, H.M. Savage, S. Aspen, R. King, A.M. Powers, K. Burkhalter, L. Colton, D. Charnetzky, S. Lasater, West Nile virus–infected mosquitoes, Louisiana, 2002, Emerging Infect. Dis. 11 (2005) 1399.
[6] N. Nitatpattana, C. Apiwathnasorn, P. Barbazan, S. Leemingsawat, S. Yoksan, J. Gonzalez, First isolation of Japanese encephalitis from *Culex quinquefasciatus* in Thailand, Southeast Asian J. Trop. Med. Public Health 36 (2005) 875.
[7] L.B. Amorim, E. Helvecio, C.M.F. de Oliveira, C.F.J. Ayres, Susceptibility status of *Culex quinquefasciatus* (Diptera: Culicidae) populations to the chemical insecticide temephos in Pernambuco, Brazil, Pest Manag. Sci. 69 (2013) 1307–1314.
[8] R.M. Shah, M. Alam, D. Ahmad, M. Waqas, Q. Ali, M. Binyameen, S.A. Shad, Toxicity of 25 synthetic insecticides to the field population of *Culex quinquefasciatus* Say, Parasitol. Res. 115 (2016) 4345–4351.
[9] T. Su, J.P. Webb, R.P. Meyer, M.S. Mulla, Spatial and temporal distribution of mosquitoes in underground storm drain systems in Orange County, California, J. Vector Ecol. 28 (2003) 79–89.
[10] R.M. Shah, Q. Ali, M. Alam, S.A. Shad, S. Majeed, M. Riaz, M. Binyameen, Larval habitat substrates could affect the biology and vectorial capacity of *Culex quinquefasciatus* (Diptera: Culicidae), J. Med. Entomol. 54 (2017) 638–645.
[11] G. Benelli, C.L. Jeffries, T. Walker, Biological control of mosquito vectors: past, present, and future, Insects 7 (2016) 52.
[12] L. McCarroll, J. Hemingway, Can insecticide resistance status affect parasite transmission in mosquitoes? Insect Biochem. Mol. Biol. 32 (2002) 1345–1351.
[13] H. Liu, E.W. Cupp, K.M. Micher, A. Guo, N. Liu, Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefaciatus*, J. Med. Entomol. 41 (2004) 408–413.
[14] S.A. Riyadh Municipality, Riyadh Municipality, Riyadh Province, Saudi Arabia, 2022. https://www.alriyadh.gov.sa/en/Pages/Revamp3.aspx. (Accessed 23 March 2019). Accessed.
[15] Q. Xu, H. Liu, L. Zhang, N. Liu, Resistance in the mosquito, *Culex quinquefasciatus*, and possible mechanisms for resistance, Pest Manag. Sci. 61 (2005) 1096–1102.
[16] Y. Fang, E. Tambo, J.-B. Xue, Y. Zhang, X.-N. Zhou, E.I. Khater, Detection of DENV-2 and insect-specific flaviviruses in mosquitoes collected from Jeddah, Saudi Arabia, Front. Cell. Infect. Microbiol. 11 (2021) 52.
[17] A. Kasai, T.I. Hayashi, H. Ohnishi, K. Suzuki, D. Hayasaka, K. Goka, Fipronil application on rice paddy fields reduces densities of common skimmer and scarlet skimmer, Sci. Rep. 6 (2016) 1–10.
[18] M.A. Soares, L.C. Passos, M.R. Campos, L.J. Collares, N. Desneux, G.A. Carvalho, Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*, J. Pest Sci. 92 (2019) 1447–1456.
[19] V. Shetty, D. Sanil, N.J. Shetty, Insecticide susceptibility status in three medically important species of mosquitoes, *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*, from Bruhat Bengaluru Mahanagara Palike, Karnataka, India, Pest Manag. Sci. 69 (2013) 257–267.
[20] P.R. Salameh, I. Baldi, P. Brochard, C. Raherison, B. Abi Saleh, R. Salamon, Respiratory symptoms in children and exposure to pesticides, Eur. Respir. J. 22 (2003) 507–512.
[21] H. van den Berg, R. Velayudhan, R.S. Yadav, Management of insecticides for use in disease vector control: Lessons from six countries in Asia and the Middle East, PLoS Negl. Trop. Dis. 15 (2021), e0009358.
[22] S.R.S. Simaremare, C.-C. Hung, C.-J. Hsieh, L.-M. Yiin, Relationship between organophosphate and pyrethroid insecticides in blood and their metabolites in urine: a pilot study, Int. J. Env. Res. Public Health 17 (2020) 34.
[23] A. Pilkington, D. Buchanan, G.A. Jamal, R. Gillham, S. Hansen, M. Kidd, J.F. Hurley, C.A. Soutar, An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers, Occup. Environ. Med. 58 (2001) 702–710.
[24] K.R. Fluegge, M. Nishioka, J.R. Wilkins III, Effects of simultaneous prenatal exposures to organophosphate and synthetic pyrethroid insecticides on infant neurodevelopment at three months of age, Journal of Environmental Toxiology and Public Health 1 (2016) 60–73.
[25] WHO, Monitoring and managing insecticide resistance in *Aedes* mosquito populations: interim guidance for entomologists, 2016.
[26] J.L. Robertson, N. Savin, H.K. Preisler, R.M. Russell, Bioassays with arthropods, CRC press, 2007.
[27] S. LeOra, Poloplus, a user's guide to probit or logit analysis, LeOra Software, Berkeley, CA, 2003.
[28] W. Abbott, A method of computing the effectiveness of an insecticide, J. Econ. Entomol. 18 (1925) 265–267.
[29] J.T. Litchfield, F. Wilcoxon, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther. 96 (1949) 99–113.
[30] M.B. Mazzarri, G.P. Georghiou, Characterization of resistance to organophosphate, carbamate, and pyrethroid insecticides in field populations of *Aedes aegypti* from Venezuela, Journal of the American Mosquito Control Association-Mosquito News 11 (1995) 315–322.
[31] N. Abbas, S.A. Shad, M. Ismail, Resistance to conventional and new insecticides in house flies (Diptera: Muscidae) from poultry facilities in Punjab, Pakistan, J. Econ. Entomol. 108 (2015) 826–833.
[32] N. Abbas, A.M. Hafez, Resistance to insect growth regulators and age-stage, two-sex life table in *Musca domestica* from different dairy facilities, PloS One 16 (2021), e0248693.
[33] IRAC, IRAC: Mode of action classification scheme, 2020, pp. 1–26. Version 9.4, www.irac-online.org-documents-moa-classification.
[34] A.S. Al-Sarar, Insecticide resistance of *Culex pipiens* (L.) populations (Diptera: Culicidae) from Riyadh city, Saudi Arabia: Status and overcome, Saudi J. Biol. Sci. 17 (2010) 95–100.
[35] R.P. Lopes, J.B.P. Lima, A.J. Martins, Insecticide resistance in *Culex quinquefasciatus* Say, 1823 in Brazil: a review, Parasit. Vectors 12 (2019) 1–12.
[36] R. Maestre-Serrano, J. Lara-Cobos, D. Gomez-Camargo, G. Ponce-Garcia, P. Pareja-Loaiza, A.E. Flores, Susceptibility to pyrethroids and the first report of L1014F kdr mutation in *Culex quinquefasciatus* (Diptera: Culicidae) in Colombia, J. Med. Entomol. 57 (2020) 1830–1834.
[37] A. Alsheikh, W. Mohammed, E. Noureldin, O. Daffalla, K. Shrwani, Y. Hobani, F.A. Alsheikh, M.H. Alzahrani, A.A. Binsaeed, Resistance status of *Aedes aegypti* to insecticides in the Jazan Region of Saudi Arabia, Biosci, Biotech Res Asia 13 (2016) 155–162.
[38] O. Dafalla, A. Alsheikh, W. Mohammed, K. Shrwani, F. Alsheikh, Y. Hobani, E. Noureldin, Knockdown resistance mutations contributing to pyrethroid resistance in *Aedes aegypti* population, Saudi Arabia, East. Mediterr. Health Journal 25 (2019) 905–913.

[39] K.B. Campos, A.J. Martins, C. de Melo Rodovalho, D.F. Bellinato, L. dos Santos Dias, M.d.L. da Graça Macoris, M.T.M. Andrighetti, J.B.P. Lima, M.T. Obara, Assessment of the susceptibility status of *Aedes aegypti* (Diptera: Culicidae) populations to pyriproxyfen and malathion in a nation-wide monitoring of insecticide resistance performed in Brazil from 2017 to 2018, Parasit. Vectors 13 (2020) 1–18.
[40] J.G. Scott, M.H. Yoshimizu, S. Kasai, Pyrethroid resistance in *Culex pipiens* mosquitoes, Pestic. Biochem. Physiol. 120 (2015) 68–76.
[41] H.A.A. Khan, Resistance to insecticides and synergism by enzyme inhibitors in *Aedes albopictus* from Punjab, Pakistan, Sci. Rep. 10 (2020) 1–8.
[42] N.M.R. Elia-Amira, C.D. Chen, V.L. Low, K.W. Lau, A. Haziqah-Rashid, Z.H. Amelia-Yap, H.L. Lee, M. Sofian-Azirun, Adulticide resistance status of *Aedes albopictus* (Diptera: Culicidae) in Sabah, Malaysia: A statewide assessment, J. Med. Entomol. 56 (2019) 1715–1725.
[43] H.M. Al-Amin, F.T. Johora, S.R. Irish, M.R.H. Hossainey, L. Vizcaino, K.K. Paul, W.A. Khan, R. Haque, M.S. Alam, A. Lenhart, Insecticide resistance status of *Aedes aegypti* in Bangladesh, Parasit. Vectors 13 (2020) 1–15.
[44] C. Delannay, D. Goindin, K. Kellaou, C. Ramdini, J. Gustave, A. Vega-Rúa, Multiple insecticide resistance in *Culex quinquefasciatus* populations from Guadeloupe (French West Indies) and associated mechanisms, PloS One 13 (2018), e0199615.
[45] H.A.A. Khan, W. Akram, Resistance status to deltamethrin, permethrin, and temephos along with preliminary resistance mechanism in *Aedes aegypti* (Diptera: Culicidae) from Punjab, Pakistan, J. Med. Entomol. 56 (2019) 1304–1311.
[46] H.A. Aponte, R.P. Penilla, F. Dzul-Manzanilla, A. Che-Mendoza, A.D. López, F. Solis, P. Manrique-Saide, H. Ranson, A. Lenhart, P.J. McCall, The pyrethroid resistance status and mechanisms in *Aedes aegypti* from the Guerrero state, Mexico, Pestic. Biochem. Physiol. 107 (2013) 226–234.
[47] L.B. Smith, S. Kasai, J.G. Scott, Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases, Pestic. Biochem. Physiol. 133 (2016) 1–12.
[48] A.S. Estep, N.D. Sanscrainte, C.M. Waits, J.E. Louton, J.J. Becnel, Resistance status and resistance mechanisms in a strain of *Aedes aegypti* (Diptera: Culicidae) from Puerto Rico, J. Med. Entomol. 54 (2017) 1643–1648.
[49] S. Demok, N. Endersby-Harshman, R. Vinit, L. Timinao, L.J. Robinson, M. Susapu, L. Makita, M. Laman, A. Hoffmann, S. Karl, Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Papua New Guinea, Parasit. Vectors 12 (2019) 1–8.
[50] N. Protopopoff, J. Matowo, R. Malima, R. Kavishe, R. Kaaya, A. Wright, P.A. West, I. Kleinschmidt, W. Kisinza, F.W. Mosha, High level of resistance in the mosquito *Anopheles gambiae* to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania, Malar. J. 12 (2013) 149.
[51] O. Chukwuekezie, E. Nwosu, U. Nwangwu, F. Dogunro, C. Onwude, N. Agashi, E. Ezihe, C. Anioke, S. Anokwu, E. Eloy, Resistance status of *Anopheles gambiae* (sl) to four commonly used insecticides for malaria vector control in South-East Nigeria, Parasit. Vectors 13 (2020) 1–10.
[52] H.A.A. Khan, W. Akram, S. Lee, Resistance to selected pyrethroid insecticides in the malaria mosquito, *Anopheles stephensi* (Diptera: Culicidae), from Punjab, Pakistan, J. Med. Entomol. 55 (2018) 735–738.
[53] O. Komagata, S. Kasai, T. Tomita, Overexpression of cytochrome P450 genes in pyrethroid-resistant *Culex quinquefasciatus*, Insect Biochem. Mol. Biol. 40 (2010) 146–152.
[54] F. Yang, S. Schildhauer, S.A. Billeter, M. Hardstone Yoshimizu, R. Payne, M.J. Pakingan, M.E. Metzger, K.A. Liebman, R. Hu, V. Kramer, Insecticide resistance status of *Aedes aegypti* (Diptera: Culicidae) in California by biochemical assays, J. Med. Entomol. 57 (2020) 1176–1183.
[55] S. Balaska, E.A. Fotakis, I. Kioulos, L. Grigoraki, S. Mpellou, A. Chaskopoulou, J. Vontas, Bioassay and molecular monitoring of insecticide resistance status in *Aedes albopictus* populations from Greece, to support evidence-based vector control, Parasit. Vectors 13 (2020) 1–13.
[56] M.H. Yoshimizu, K. Padgett, V. Kramer, Surveillance of a kdr Resistance Mutation in *Culex pipiens* (Diptera: Culicidae) and *Culex quinquefasciatus* in California, J. Med. Entomol. 57 (2020) 645–648.
[57] Y. Fang, E. Tambo, J.B. Xue, Y. Zhang, X.N. Zhou, E.I.M. Khater, Molecular analysis of targeted insecticide resistance gene mutations in field-caught mosquitos of medical importance from Saudi Arabia, J. Med. Entomol. 58 (2021) 1839–1848.
[58] V. Corbel, R. N'guessan, C. Brengues, F. Chandre, L. Djogbenou, T. Martin, M. Akogbeto, J.-M. Hougard, M. Rowland, Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa, Acta Trop 101 (2007) 207–216.
[59] N. Liu, T. Li, W.R. Reid, T. Yang, L. Zhang, Multiple cytochrome P450 genes: their constitutive overexpression and permethrin induction in insecticide resistant mosquitoes, *Culex quinquefasciatus*, PloS One 6 (2011), e23403.
[60] V.L. Low, C.D. Chen, H.L. Lee, T.K. Tan, C.F. Chen, C.S. Leong, Y.A.L. Lim, P.E. Lim, Y. Norma-Rashid, M. Sofian-Azirun, Enzymatic characterization of insecticide resistance mechanisms in field populations of Malaysian *Culex quinquefasciatus* Say (Diptera: Culicidae), PloS One 8 (2013), e79928.
[61] A.M. Hafez, N. Abbas, Insecticide resistance to insect growth regulators, avermectins, spinosyns and diamides in *Culex quinquefasciatus* in Saudi Arabia, Parasit. Vectors 14 (2021) 1–9.