First Comprehensive Report of Conventional Insecticide Resistance Status of the Mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) in Saudi Arabia

Abdulwahab M. Hafez (hafez@ksu.edu.sa)
King Saud University

Research

Keywords: southern house mosquito, field-evolved-resistance, pyrethroids, organophosphates, insect vector management

Posted Date: September 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-907877/v1

Abstract

Background

The mosquito *Culex quinquefasciatus* Say is a vector of various fatal diseases including West Nile fever, filariasis, and Japanese encephalitis. The major approach in controlling *Cx. quinquefasciatus* to prevent associated disease transmission has focused on insecticides. However, because of overreliance on such measures, *Cx. quinquefasciatus* has developed resistance to these insecticides including organophosphates and pyrethroids.

Methods

We evaluated 10 commonly used conventional insecticides (five OPs and five pyrethroids) for toxicity/resistance in *Cx. quinquefasciatus* in adults and larvae in eight populations collected from the environs around Riyadh, Saudi Arabia.

Results

The LC_{50} values for the tested insecticides in adults did not differ significantly from those of a susceptible strain, except for bifenthrin, deltamethrin, cypermethrin, cyfluthrin, and fenitrothion against populations from Al-Nakhil, Al-Suwaidi, Al-Ghanemiya, Al-Masfa, and Al-Masanie regions. All *Cx. quinquefasciatus* adult populations exhibited susceptibility/low resistance to the tested organophosphates with resistance ratios of 0.23–0.80 for chlorpyrifos, 0.44–1.97 for malathion, 0.09–3.62 for fenitrothion, 0.05–2.10 for pirimiphos-methyl, and 0.11–0.93 for diazinon. The *Cx. quinquefasciatus* adult populations exhibited susceptibility/moderate resistance to the tested pyrethroids with resistance ratios of 0.59–2.56 for alpha-cypermethrin, 0.59–2.19 for bifenthrin, 0.60–7.07 for deltamethrin, 0.60–2.66 for cypermethrin, and 0.58–2.39 for cyfluthrin. In *Cx. quinquefasciatus* larva populations, susceptibility/low resistance to the tested organophosphates was observed with resistance ratios of 0.03–1.75 for chlorpyrifos, 0.19–3.42 for malathion, 0.11–2.78 for fenitrothion, and 0.08–1.15 for pirimiphos-methyl.

Conclusions

The susceptibility/low resistance of *Cx. quinquefasciatus* adults to OP and pyrethroid insecticides suggests that they still have good potency against the adults of this species in Riyadh, Saudi Arabia. These results provide a baseline for decision-making in integrated vector management programs for *Cx. quinquefasciatus*.

Background

Climatic conditions that promote the growth of insect vectors in tropical and subtropical countries, alongside improper management, result in vector-borne diseases impacting public health [1, 2]. Among such vectors, mosquitoes transfer diseases to humans and animals, including dengue fever, yellow fever, malaria, Rift Valley fever (RVF), West Nile fever, lymphatic filariasis, Japanese encephalitis, and Zika virus, collectively leading to severe illness and death on a global scale [3, 4]. The mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) is a significant insect vector that transmits fatal diseases including West Nile fever, filariasis, and Japanese encephalitis [3, 5, 6] and also causes discomfort by biting its hosts [7, 8]. Poor hygiene in human settlements, livestock farms, and agricultural lands provides ideal larval habitats for this mosquito, the larvae thriving in various aquatic habitats including mangrove swamps, fresh or salt water marshes, stream and river edges, and temporary standing rainwater [9–11].

Various cultural management practices along with the use of chemical and biological agents have been used to manage insect vectors, including *Cx. quinquefasciatus* [12]. The major approach in controlling *Cx. quinquefasciatus* to prevent associated disease transmission has focused on insecticides [13, 14]. For example, organophosphate (OP) and pyrethroid insecticides are used for mosquito and vector control globally, via space spraying, mosquito coils, and indoor residual sprays [12]. However, because of overreliance on such measures, *Cx. quinquefasciatus* has developed resistance to OPs and pyrethroids, including chlorpyrifos, malathion, dimethoate, deltamethrin, and permethrin [15]. This resistance threatens human welfare in terms of vector management, and many fatal diseases such as West Nile fever, lymphatic filariasis, and RVF have resurged because of difficulties in vector control [16]. Furthermore, the over application of insecticides has increased the preventive costs of chemical control and resulted in the destruction of natural control agents [17–19]. These factors now necessitate the instigation of integrated vector management programs against *Cx. quinquefasciatus* to ensure continued efficacy.

Insecticide resistance monitoring is crucial for designing novel control strategies against insect vectors, including *Cx. quinquefasciatus* [7, 20]. Despite the availability of many different conventional insecticides against such vectors in Saudi Arabia, information on the resistance status of *Cx. quinquefasciatus* is limited. We evaluated the toxicity and resistance levels of 10 conventional insecticides (OP and pyrethroid) against *Cx. quinquefasciatus* from different areas in Riyadh, Saudi Arabia. Baseline susceptibility data from this study will direct appropriate and effective insecticide strategies for controlling *Cx. quinquefasciatus*.

Methods

Culex quinquefasciatus **populations**

Approximately 200 *Cx. quinquefasciatus* larvae at mixed stages were collected from standing water and plastic containers placed at the following eight locations in Riyadh, Saudi Arabia: Ishbiliya (24.802°N, 46.803°E), Al-Suwaidi (24.590°N, 46.676°E), Al-Ghanemiya (24.482°N, 46.798°E), Al-Masfa (24.471°N, 46.861°E), Al-Masanie (24.558°N, 46.743°E), Al-Nakhil (24.737°N, 46.620°E), Al-Washlah (24.409°N, 46.660°E), and Irqah (24.677°N, 46.575°E). Larvae were collected, maintained separately, placed in separate plastic containers (30 × 30 cm), and moved to the laboratory where they were provided with cattle food *ad libitum* until pupation. Emerging adults were transferred to cages (40 × 40 cm), and cotton wicks soaked in 10% sugar solution were used for food. These wicks were moistened every 2 days and replaced when dirty. Following a blood meal, water containers (10 × 7 cm) were placed in cages to promote fecundity and generate uniform F1 populations. Eggs were transferred into plastic containers and emerging larvae were provided with cattle food *ad libitum* until pupation, and emerging adults were used for bioassays. Populations were maintained separately at 27°C ± 2°C, 65% ± 5% humidity, and a 12:12 h (light:dark) photoperiod in the laboratory. The susceptible reference strain was originally obtained from the High Institute of Public Health, Alexandria University, Egypt, in 1990 and had been maintained since then with no exposure to any kind of chemicals.

Insecticides

Ten formulated commercial-grade, commonly used OP and pyrethroid class insecticides were utilized for bioassays (Table 1).

Adult bioassays

Insecticide toxicity against 5-day-old *Cx. quinquefasciatus* adults was determined using a feeding bioassay according to the methods of Shah et al. [7], with some modifications. For each insecticide and population, five concentrations causing mortality, from > 0%–<100%, were prepared in 10% sugar solutions via the serial dilution method. Stock solutions (100 mL) were made for each insecticide and from these, and 50 mL solutions were poured into glass beakers along with 50 mL deionized water. Mixed-sex adults were placed in plastic jars (500 mL) and starved for 2 h. Thereafter, cotton wicks maintained in a saturated state with insecticide dilutions were provided for feeding. Insecticide dilution bioassays were performed three times, with 10 adults per replicate, with a total of 150 adults used for each bioassay. For control treatments, 30 adults comprising three replicates (10 adults per replicate) were fed 10% sugar solution without insecticide. All bioassays were conducted under the aforementioned laboratory conditions. Mortality data were recorded after 24 h; adults that did not move were considered dead.

Larva bioassays

Bioassay of OP insecticides against third instar *Cx. quinquefasciatus* larvae was performed according to the protocol formulated by the World Health Organization [21]. Five to six concentrations causing mortality between > 0% and < 100% were prepared in tap water by serial dilutions from a stock solution (1,000 mL) for each insecticide. Fresh stock solution was prepared for each replication, and assays were performed at different times to ensure true replication [22]. The third instar larvae were held in plastic cups containing 400 mL of the test solution. Insecticide dilution bioassays were performed four times, with 10 larvae per replicate, and a total of 200–240 larvae were used for each bioassay. In the control, 40 larvae with four replicates (10 larvae per replicate) were used. All bioassays were conducted under the aforementioned laboratory conditions. Mortality data were recorded after 24 h.

Data analyses

To determine median lethal concentration (LC_{50}) and 95% fiducial limit (FL), standard error (SE), and chi-squared (χ^2), bioassay data were analyzed using POLO Plus software [23]. In each bioassay, mortality rates were corrected using mortality rates from control treatments following Abbot's formula Abbott [24], if required. The LC_{50} value for a field population was considered significantly different when its 95% FL did not overlap with those of the susceptible strain [25]. Resistance ratios (RRs) were calculated as (LC_{50} for the field population/LC_{50} for the susceptible strain). The RRs were classified as RR = < 5 (susceptibility/low resistance), RR = 5–10 (moderate resistance), and RR = > 10 (high resistance) [21, 26].

Results

Cx. quinquefasciatus **resistance to organophosphates**

OP insecticide toxicities against *Cx. quinquefasciatus* adult field populations were not significantly different from that of the susceptible strain (overlapped 95% FL). However, some of the adult field populations showed significantly higher susceptibility when compared with the susceptible strain (nonoverlapped 95% FL), viz.: Al-Ghanemiya to chlorpyrifos (RR = 0.23), malathion (RR = 0.44), and pirimiphos-methyl (RR = 0.38); Al-Suwaidi and Al-Masfa to fenitrothion (RR = 0.09 and 0.43, respectively); Al-Washlah to diazinon (RR = 0.11); and Irqah to fenitrothion and pirimiphos-methyl (RR = 0.14 and 0.05, respectively). The adults of the Al-Masanie field population provided the only case of significant resistance (low level) against fenitrothion (RR = 3.62) (nonoverlapped 95% FL). Generally, all of the *Cx. quinquefasciatus* adult populations exhibited susceptibility/low resistance to chlorpyrifos (RR = 0.23–0.80), malathion (RR = 0.44–1.97), fenitrothion (RR = 0.09–3.62), pirimiphos-methyl (RR = 0.05–2.10), and diazinon (RR = 0.11–0.93) when compared with the susceptible strain (Table 2).

OP insecticide toxicities against *Cx. quinquefasciatus* larva field populations were not significantly different from that of the susceptible strain (overlapped 95% FL). However, some of the larva field populations showed significantly higher susceptibility when compared with the susceptible strain (nonoverlapped 95% FL), viz.: Ishbiliya, Al-Suwaidi, Al-Masfa, Al-Masanie, and Irqah to chlorpyrifos (RR = 0.08, 0.03, 0.17, 0.25, and 0.17, respectively); Ishbiliya and Al-Masfa to malathion (RR = 0.19 and 0.56, respectively); Ishbiliya, Al-Suwaidi, and Al-Ghanemiya to fenitrothion (RR = 0.33, 0.44, and 0.11, respectively); and Ishbiliya, Al-Ghanemiya, Al-Masanie, Al-Washlah, and Irqah to pirimiphos-methyl (RR = 0.19, 0.35, 0.19, 0.50, and 0.08, respectively). Other larva field populations showed significant low levels of resistance when compared with the susceptible strain (nonoverlapped 95% FL), viz.: Al-Suwaidi, Al-Ghanemiya, Al-Nakhil, and Irqah to malathion (RR = 2.25, 3.42, 3.07, and 2.20, respectively) and Al-Masfa to fenitrothion (RR = 2.78). The LC_{50} values for chlorpyrifos, malathion, fenitrothion, and pirimiphos-methyl ranged 0.0003–0.021 μg/mL, 0.011–0.202 μg/mL, 0.001–0.025 μg/mL, and 0.002–0.030 μg/mL, respectively, against the eight larva populations tested. RR values ranged from 0.03 to 1.75 for chlorpyrifos, 0.19 to 3.42 for malathion, 0.11 to 2.78 for fenitrothion, and 0.08 to 1.15 for pirimiphos-methyl (Table 3).

Cx. quinquefasciatus **resistance to pyrethroids**

Pyrethroid insecticide toxicities against *Cx. quinquefasciatus* adult field populations were not significantly different from that of the susceptible strain (overlapped 95% FL), except for Al-Nakhil to bifenthrin; Al-Suwaidi, Al-Ghanemiya, and Al-Masfa to deltamethrin; Al-Masfa and Al-Masanie to cypermethrin; and Al-Masanie to cyfluthrin (nonoverlapped 95% FL). The *Cx. quinquefasciatus* adult field populations from Ishbiliya (RR = 0.70), Al-Suwaidi (RR = 1.89), Al-Ghanemiya (RR = 1.26), Al-Masfa (RR = 2.56), Al-Masanie (RR = 0.59), Al-Nakhil (RR = 1.86), Al-Washlah (RR = 1.26), and Irqah (RR = 1.99) did not significantly differ in susceptibility to alpha-cypermethrin from the susceptible strain. For bifenthrin, the RRs of all tested *Cx. quinquefasciatus* adult field populations ranged from 0.59 to 2.19, and the only case of significant resistance (low level) was found in the Al-Nakhil adult field population (RR = 2.19). The adult field populations from Al-Ghanemiya (RR = 7.07) showed moderate resistance, and those from Al-Suwaidi (RR = 2.72) and Al-Masfa (RR = 4.94) showed low resistance to deltamethrin, whereas the remaining populations did not significantly differ from the susceptible strain. For cypermethrin, the adult field populations from Al-Masfa (RR = 2.50) and Al-Masanie (RR = 2.66) showed low resistance, whereas the remaining populations did not significantly differ from the susceptible strain. For cyfluthrin, the RRs of all tested *Cx. quinquefasciatus* adult field populations ranged from 0.58 to 2.39, and the only case of significant resistance (low level) was found in the Al-Masanie adult field population (RR = 2.22) (Table 4).

Discussion

Globally, insecticides are a part of major management solutions against various insect vectors; thus, determining susceptibility to commonly used insecticides is crucial for the selection of appropriate and effective treatment [27, 28]. OPs, including chlorpyrifos, malathion, fenitrothion, pirimiphos-methyl, and diazinon, are acetylcholinesterase inhibitors, whereas pyrethroids, including alpha-cypermethrin, cypermethrin, deltamethrin, bifenthrin, and cyfluthrin, are sodium channel modulators [29]. These conventional insecticides are the most commonly used adulticides for controlling mosquitoes and other medically important insect pests globally, including in Saudi Arabia [27, 30–32]. Therefore, conventional insecticide resistance has arisen and been reported in *Cx. pipiens* and *Aedes* in Saudi Arabia [30, 33, 34].

In the present study, susceptibility/low resistance to chlorpyrifos, malathion, fenitrothion, pirimiphos-methyl, and diazinon was observed in *Cx. quinquefasciatus* populations from the study regions. Field-evolved resistance to OPs has been well documented in *Cx. quinquefasciatus* [1, 7, 20, 35], *Cx. pipiens* [30, 36], *Aedes albopictus* [37, 38], and *Ae. aegypti* [39].

In this study, susceptibility/low to moderate resistance to alpha-cypermethrin, cypermethrin, deltamethrin, bifenthrin, and cyfluthrin was observed in *Cx. quinquefasciatus* populations from the study regions. Resistance to pyrethroid insecticides has been observed for many insect vectors, including *Cx.*

quinquefasciatus [7, 32, 40], *Ae. aegypti* and *Ae. albopictus* [28, 37, 41–44], *Cx pipiens* [36], *Anopheles gambiae* Giles [45, 46], and *An. stephensi* Liston [47]. The OP and pyrethroid resistance in these insect vectors may be due to increased metabolic enzyme activities [37, 48, 49] and target site insensitivity due to Kdr mutations, L1014F, F1534C, and I1532T on VGSC, V1016I, F1534C on 11S6 domain, and G119S on the acetylcholinesterase 1 gene [32, 43, 50–52].

In the Riyadh region, one that experiences a severe desert climate, OPs and pyrethroids are applied once a month during the periods March–May and September–November to control different household insect vectors, e.g., mosquitoes, in human dwellings. This low level of exposure may explain the detected susceptibility/low resistance to OPs and pyrethroids in the *Cx. quinquefasciatus* field populations. However, it appears that resistance to deltamethrin (pyrethroid) is increasing in the Al-Ghanemiya and Al-Masfa populations, where moderate RRs of 7.07 and 4.94, respectively, were observed. Thus, deltamethrin should be used carefully and correctly at these locations to prolong efficacy.

Interestingly, besides this finding of susceptibility/low resistance in *Cx. quinquefasciatus* populations to conventional insecticides, Hafez and Abbas (Unpublished data) found significant and higher levels of resistance in the same field populations toward new classes of insecticides, including insect growth regulators. Since the field populations in this study were collected from locations adjacent to farms or parks, their findings could be due to pesticide laws and regulations that have restricted or prevented the use of many conventional insecticides over the last two decades but permitted their replacement by new insecticides in the plant protection programs [12]. This may have led to accidental exposure to the new insecticides, giving rise to a selection pressure in these *Cx. quinquefasciatus* populations over the last two decades, and this could be the reason for these unexpected findings.

In conclusion, the susceptibility/low resistance of *Cx. quinquefasciatus* adults to OP and pyrethroid insecticides suggests that they still have good potency against the adults of this species in Riyadh, Saudi Arabia. A strategic program must be instituted to control *Cx. quinquefasciatus* to sustain the efficacy of other OP and pyrethroid insecticides before resistance develops. Periodic monitoring must be continued to detect any increase in resistance to these conventional insecticides. Additionally, cultural practices, such as the removal of larval habitats and the use of biological control agents [12], must be used to manage larvae of *Cx. quinquefasciatus* to minimize overreliance on chemical insecticides. Our findings serve as a reference point for future monitoring efforts of *Cx. quinquefasciatus* insecticide susceptibility.

Declarations

There are no interests to declare.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

The author declares that he has no competing interests.

Funding

This work was funded by the Deanship of Scientific Research at King Saud University through Research Group number G-1441-480.

Author contribution

AH designed the experiment. AH performed the experiment. AH analyzed the data and wrote the manuscript. AH reviewed and edited the final manuscript. The author read and approved the manuscript.

Acknowledgments

The author extends his appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group number G-1441-480. The author thanks the Deanship of Scientific Research and Researchers Support and Services Unit (RSSU) at King Saud University for their technical support. The author would like to thank Dr. Naeem Abbas from the Pesticides and Environmental Toxicology Laboratory for his great support at all stages of this work. The author would like also to thank the researchers and technicians from Pesticides and Environmental Toxicology Laboratory: Mohammed Ali Albaqiyah and Safwat Gamal Hassan Sabra for their help in collecting and maintaining the mosquito field populations in the laboratory and for other laboratory work.

Author details

Abdulwahab M. Hafez (+966566444577, hafez@ksu.edu.sa)

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.

References

1. Rai P, Bharati M, Subba A, Saha D. Insecticide resistance mapping in the vector of lymphatic filariasis, *Culex quinquefasciatus* Say from northern region of West Bengal, India. PloS One. 2019;14:e0217706.

2. Khater EI, Sowilem MM, Sallam MF, Alahmed AM. Ecology and habitat characterization of mosquitoes in Saudi Arabia. Trop Biomed. 2013;30:409–27.
3. WHO. Global programme to eliminate lymphatic filariasis: progress report on mass drug administration, 2010. Wkly Epidemiol Rec. 2011;86:377–87.
4. Eybpoosh S, Fazlalipour M, Baniasadi V, Pouriayevali MH, Sadeghi F, Ahmadi Vasmehjani A, Karbalaie Niya MH, Hewson R, Salehi-Vaziri M.. Epidemiology of West Nile Virus in the Eastern Mediterranean region: A systematic review. PLoS Negl Trop Dis. 2019;13:e0007081.
5. Godsey MS Jr, Nasci R, Savage HM, Aspen S, King R, Powers AM, Burkhalter K, Colton L, Charnetzky D. Lasater S. West Nile virus–infected mosquitoes, Louisiana, 2002. Emerging Infect Dis. 2005;11:1399.
6. Nitatpattana N, Apiwathnasorn C, Barbazan P, Leemingsawat S, Yoksan S, Gonzalez J.. First isolation of Japanese encephalitis from *Culex quinquefasciatus* in Thailand. Southeast Asian J Trop Med Public Health. 2005;36:875.
7. Shah RM, Alam M, Ahmad D, Waqas M, Ali Q, Binyameen M, Shad SA. Toxicity of 25 synthetic insecticides to the field population of *Culex quinquefasciatus* Say. Parasitol Res. 2016;115:4345–51.
8. Amorim LB, Helvecio E, de Oliveira CMF, Ayres CFJ. Susceptibility status of *Culex quinquefasciatus* (Diptera: Culicidae) populations to the chemical insecticide temephos in Pernambuco, Brazil. Pest Manag Sci. 2013;69:1307–14.
9. Su T, Webb JP, Meyer RP, Mulla MS. Spatial and temporal distribution of mosquitoes in underground storm drain systems in Orange County, California. J Vector Ecol. 2003;28:79–89.
10. Shah RM, Ali Q, Alam M, Shad SA, Majeed S, Riaz M, Binyameen M. Larval habitat substrates could affect the biology and vectorial capacity of *Culex quinquefasciatus* (Diptera: Culicidae). J Med Entomol. 2017;54:638–45.
11. Joseph AO, Adepeju S-OI. Omosalewa OB. Distribution, abundance and diversity of mosquitoes in Akure, Ondo State, Nigeria. Journal of Parasitology Vector Biology. 2013;5:132–6.
12. Benelli G, Jeffries CL. Walker T. Biological control of mosquito vectors: past, present, and future. Insects. 2016;7:52.
13. Liu H, Cupp EW, Micher KM, Guo A, Liu N. Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefaciatus*. J Med Entomol. 2004;41:408–13.
14. McCarroll L, Hemingway J.. Can insecticide resistance status affect parasite transmission in mosquitoes? Insect Biochem Mol Biol. 2002;32:1345–51.
15. Xu Q, Liu H, Zhang L, Liu N.. Resistance in the mosquito, *Culex quinquefasciatus*, and possible mechanisms for resistance. Pest Manag Sci. 2005;61:1096–102.
16. Fang Y, Tambo E, Xue J-B, Zhang Y, Zhou X-N. Khater EI. Detection of DENV-2 and insect-specific flaviviruses in mosquitoes collected from Jeddah, Saudi Arabia. Frontiers in cellular and infection microbiology. 2021;11:52.

17. Soares MA, Passos LC, Campos MR, Collares LJ, Desneux N, Carvalho GA. Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*. J Pest Sci. 2019;92:1447–56.
18. Kasai A, Hayashi TI, Ohnishi H, Suzuki K, Hayasaka D. Goka K. Fipronil application on rice paddy fields reduces densities of common skimmer and scarlet skimmer. Scientific Reports. 2016;6:1–10.
19. Jinguji H, Ohtsu K, Ueda T, Goka K. Effects of short-term, sublethal fipronil and its metabolite on dragonfly feeding activity. PloS one. 2018;13:e0200299.
20. Shetty V, Sanil D, Shetty NJ. 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. 2013;69:257–67.
21. WHO. Monitoring and managing insecticide resistance in *Aedes* mosquito populations: interim guidance for entomologists. 2016.
22. Robertson JL, Savin N, Preisler HK, Russell RM. Bioassays with arthropods, CRC press 2007.
23. LeOra S. Poloplus, a user's guide to probit or logit analysis. Berkeley: LeOra Software; 2003.
24. Abbott W. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925;18:265–7.
25. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther. 1949;96:99–113.
26. Mazzarri MB, Georghiou GP. 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. 1995;11:315–22.
27. Abbas N, Shad SA, Ismail M. Resistance to conventional and new insecticides in house flies (Diptera: Muscidae) from poultry facilities in Punjab, Pakistan. J Econ Entomol. 2015;108:826–33.
28. Khan HAA, Akram W. Resistance status to deltamethrin, permethrin, and temephos along with preliminary resistance mechanism in *Aedes aegypti* (Diptera: Culicidae) from Punjab, Pakistan. J Med Entomol. 2019;56:1304–11.
29. IRAC. IRAC: Mode of action classification scheme, Version 9.4,.www.irac-online.org › documents › moa-classification. 2020:pp, 1–26.
30. Al-Sarar AS. Insecticide resistance of *Culex pipiens* (L.) populations (Diptera: Culicidae) from Riyadh city, Saudi Arabia: Status and overcome. Saudi J Biol Sci. 2010;17:95–100.
31. Lopes RP, Lima JBP, Martins AJ. Insecticide resistance in *Culex quinquefasciatus* Say, 1823 in Brazil: a review. Parasit Vectors. 2019;12:1–12.
32. Maestre-Serrano R, Lara-Cobos J, Gomez-Camargo D, Ponce-Garcia G, Pareja-Loaiza P, Flores AE. Susceptibility to pyrethroids and the first report of L1014F kdr mutation in *Culex quinquefasciatus* (Diptera: Culicidae) in Colombia. J Med Entomol. 2020;57:1830–4.
33. Dafalla O, Alsheikh A, Mohammed W, Shrwani K, Alsheikh F, Hobani Y, Noureldin E. Knockdown resistance mutations contributing to pyrethroid resistance in *Aedes aegypti* population, Saudi Arabia.

East Mediterr Health Journal. 2019;25:905–13.

34. Alsheikh A, Mohammed W, Noureldin E, Daffalla O, Shrwani K, Hobani Y, Alsheikh FA, Alzahrani MH, Binsaeed AA. Resistance status of *Aedes aegypti* to insecticides in the Jazan Region of Saudi Arabia. Biosci, Biotech Res Asia. 2016;13:155–162.
35. Campos KB, Martins AJ, de Melo Rodovalho C, Bellinato DF, dos Santos Dias L, da Graça Macoris MdL, Andrighetti MTM, Lima JBP, Obara MT. 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. 2020;13:1–18.
36. Scott JG, Yoshimizu MH, Kasai S.. Pyrethroid resistance in *Culex pipiens* mosquitoes. Pestic Biochem Physiol. 2015;120:68–76.
37. Khan HAA. Resistance to insecticides and synergism by enzyme inhibitors in *Aedes albopictus* from Punjab. Pakistan Scientific Reports. 2020;10:1–8.
38. Elia-Amira NMR, Chen CD, Low VL, Lau KW, Haziqah-Rashid A, Amelia-Yap ZH, Lee HL. Sofian-Azirun M. Adulticide resistance status of *Aedes albopictus* (Diptera: Culicidae) in Sabah, Malaysia: A statewide assessment. J Med Entomol. 2019;56:1715–25.
39. Al-Amin HM, Johora FT, Irish SR, Hossainey MRH, Vizcaino L, Paul KK, Khan WA, Haque R, Alam MS. Lenhart A. Insecticide resistance status of *Aedes aegypti* in Bangladesh. Parasit Vectors. 2020;13:1–15.
40. Delannay C, Goindin D, Kellaou K, Ramdini C, Gustave J, Vega-Rúa. A. Multiple insecticide resistance in *Culex quinquefasciatus* populations from Guadeloupe (French West Indies) and associated mechanisms. PloS One. 2018;13:e0199615.
41. Smith LB, Kasai S, Scott JG. Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases. Pestic Biochem Physiol. 2016;133:1–12.
42. Estep AS, Sanscrainte ND, Waits CM, Louton JE, Becnel JJ. Resistance status and resistance mechanisms in a strain of *Aedes aegypti* (Diptera: Culicidae) from Puerto Rico. J Med Entomol. 2017;54:1643–8.
43. Aponte HA, Penilla RP, Dzul-Manzanilla F, Che-Mendoza A, López AD, Solis F, Manrique-Saide P, Ranson H, Lenhart A. McCall PJ. The pyrethroid resistance status and mechanisms in *Aedes aegypti* from the Guerrero state, Mexico. Pestic Biochem Physiol. 2013;107:226–34.
44. Demok S, Endersby-Harshman N, Vinit R, Timinao L, Robinson LJ, Susapu M, Makita L, Laman M, Hoffmann A. Karl S. Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Papua New Guinea. Parasit Vectors. 2019;12:1–8.
45. Protopopoff N, Matowo J, Malima R, Kavishe R, Kaaya R, Wright A, West PA, Kleinschmidt I, Kisinza W. Mosha FW. High level of resistance in the mosquito *Anopheles gambiae* to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania. Malaria Journal. 2013;12:149.
46. Chukwuekezie O, Nwosu E, Nwangwu U, Dogunro F, Onwude C, Agashi N, Ezihe E, Anioke C, Anokwu S. Eloy E. Resistance status of *Anopheles gambiae* (sl) to four commonly used insecticides for malaria vector control in South-East Nigeria. Parasit Vectors. 2020;13:1–10.

47. Khan HAA, Akram W, Lee S. Resistance to selected pyrethroid insecticides in the malaria mosquito, *Anopheles stephensi* (Diptera: Culicidae), from Punjab, Pakistan. J Med Entomol. 2018;55:735–8.
48. Yang F, Schildhauer S, Billeter SA, Hardstone Yoshimizu M, Payne R, Pakingan MJ, Metzger ME, Liebman KA, Hu R. Kramer V. Insecticide resistance status of *Aedes aegypti* (Diptera: Culicidae) in California by biochemical assays. J Med Entomol. 2020;57:1176–83.
49. Komagata O, Kasai S, Tomita T.. Overexpression of cytochrome P450 genes in pyrethroid-resistant *Culex quinquefasciatus*. Insect Biochem Mol Biol. 2010;40:146–52.
50. Balaska S, Fotakis EA, Kioulos I, Grigoraki L, Mpellou S, Chaskopoulou A, Vontas J. Bioassay and molecular monitoring of insecticide resistance status in *Aedes albopictus* populations from Greece, to support evidence-based vector control. Parasit Vectors. 2020;13:1–13.
51. Yoshimizu MH, Padgett K, Kramer V. Surveillance of a kdr Resistance Mutation in *Culex pipiens* (Diptera: Culicidae) and *Culex quinquefasciatus* in California. J Med Entomol. 2020;57:645–8.
52. Fang Y, Tambo E, Xue JB, Zhang Y, Zhou XN, Khater EIM. Molecular analysis of targeted insecticide resistance gene mutations in field-caught mosquitos of medical importance from Saudi Arabia. J Med Entomol. 2021;58:1839–48.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.jpg