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Research Article

Assessment of Lambda-cyhalothrin Toxicity on the Developmental and Reproductive Fitness of Dengue Vector, *Aedes albopictus* (Skuse) and the Associated Changes in the Activity of Insecticide Detoxifying Enzymes

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I N F O

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A B S T R A C T

Introduction: The emergence of resistance development in mosquitoes is a huge challenge for successful vector control programmes. Insecticides used in vector control mainly target the eradication of the vector population but often neglect their residual and long-time effect on the surviving population.

Methods: In this study, *Aedes albopictus* were selected with sublethal doses of lambda-cyhalothrin and its effects on their life parameters and major detoxifying enzymes were analysed.

Results: In the twelfth generation of the resistant population, there was a 101-fold increase in resistance ratio values of LC_{50}. In addition, larval development time prolonged for 2 days and hatchability and fecundity were reduced by 52% and 93%, respectively when compared to the control group. Moreover, longevity was shortened by 9.43 days in females and 2.18 days in males. Significant changes in the activity of major detoxifying enzymes were observed where monooxygenase level was highest which indicates its role in lambda-cyhalothrin degradation leading to resistance, followed by both β-esterase and GST.

Conclusion: The findings of the current study can be helpful in integrated mosquito management (IMM), where based on their life history traits, the vulnerable stage of vectors can be targeted for effective resistance management.

Keywords: *Aedes albopictus*, Synthetic Pyrethroid, Lethal Concentration, Life-traits, Detoxifying Enzymes

Introduction

Aedes albopictus, also known as "the Asian tiger mosquito", is an established vector in most parts of the world and has the most widespread distribution.[1] It is a dominant outdoor breeding species capable of spreading dengue, chikungunya, and Zika virus.[2-5] Being an important disease-causing agent, it also creates irritation and biting nuisance affecting the daily life of humans.[6] Factors like climate change and unplanned urbanisation have led to frequent exposure of humans to this mosquito.[7,8] Moreover, human practices and transportation networks are the important factors that enhance the spread of these disease-transmitting mosquitoes in different environments.[9] Due to the absence of effective medication against these diseases, the preventive method is either the destruction of the larval habitats or preventing the adult bite by using chemical or natural repellents.[10] The use of synthetic pyrethroid, lambda-cyhalothrin, is recommended in the public health sector for the treatment of mosquito nets, indoor residual spraying, and space spraying as per WHO specifications.[11] Over the years, control and management of vector and insect pests have become a difficult task due to the emergence of insecticide resistance led due to the inappropriate use of insecticides.[12,13] Often resistance studies in vectors depend on field surveys where they already are exposed to different insecticides, thus the effect of specific insecticide selection pressure is less known.[14] In addition, the spreading of the resistant population in the environment is of prime concern as they are less affected by novel vector control strategies due to the development of cross-resistance.[15]

In the process of dealing with the toxic effect of insecticide, vectors often modify their behavioural, physiological, and molecular tactics which seem to be very costly when compared to their susceptible counterpart.[16] A knowledge of the biotic parameters of pesticide-resistant vectors is very important for vector control, but little has been done in this field. A better understanding of the vector's upcoming population size, development, and survival after attaining resistance is crucial information for the development of cost-effective and usable vector control policies. Thus, the current study assessed the toxic effect of lambda-cyhalothrin on the developmental and reproductive parameters of *Ae. albopictus* . Data from current studies may be helpful in enhancing the vector control strategies which would further help in better control of the resistant mosquitoes.

Method

Ethics Approval

The use of rats for blood feeding was approved by the Institutional Animal Ethics Committee (IAEC) of the Department of Zoology, University of North Bengal (Regn no. 840/GO/Re/S/04/CPCSEA and approval no. IAEC/NBU/2019/19).

Mosquito Strain Used

The wild population of *Ae. albopictus* was collected from Siliguri (26^0 42' 36.18" N, 88^0 21' 31.9248" E), West Bengal, India. This study site was selected as any insecticide had not been applied here for a long period of time. The samples were brought to the laboratory and identified using the standard identification catalogue by Tyagi et al.[17] The sampling was done during April to September 2021.

Laboratory Rearing

Pupae were separated from the collected sample and kept inside the cage for the emergence of adults. Cotton balls soaked with 10% sucrose solution were kept for adult feeding. The remaining larvae were kept in enamel trays, containing tap water and provided with ground fish feed as a source of food. Three days old females were starved for 24 h followed by a blood meal. Ovitrap was placed for egg laying. Regular monitoring was done for egg collection. Thereafter, the eggs laid were transferred into water-filled enamel trays for hatching until the development of the pupae. This population was referred to as F1 (Filial 1) generation. After the emergence of adults, the above process was repeated for successive generations to maintain the laboratory colony.

Larval Bioassay

The larval bioassay was conducted by adopting WHO protocol with some modifications.[18] Prior to exposing the larvae to lambda-cyhalothrin, the resistance level and lethal concentration were evaluated through dose-response bioassay. The graded series of the concentration was prepared to start with the lowest concentration and 20 late 3rd instar larvae taken in 100 ml water were exposed to a range of concentrations of lambda-cyhalothrin. Mortality was counted and the larvae that survived after 24 h of exposure were further shifted to a clean enamel tray with fresh water. Control was set in the same manner but without the insecticide. Lethal concentration (LC) was calculated using the probit analysis and the resistance ratio (RR) values were calculated by dividing the LC_{50} values of treated generation by the LC_{50} value of the control population. If RR value was < 5, the population was considered susceptible, if the value ranged from 5 to 10 mosquitoes, then it was considered moderate resistance, and when the RR value was >10, the population was considered highly resistant.

Pyrethroid Selection

The collected immature stages were exposed to two different sublethal concentrations (LC_{20} and LC_{50}) of lambda-cyhalothrin. LC_{20} and LC_{50} are abbreviations used for the concentration of insecticide which kills 20% and 50% of

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the exposed population. After exposure for 24 h, they were shifted to an enamel tray containing fresh tap water. Individuals that survived were further raised into adults for obtaining the next generation. The treated and control group were maintained in identical environment side by side.

Life History Traits

Hatchability Rate: Eggs obtained from both treated and control groups were kept in separate enamel trays/ beakers containing tap water. Hatchability was recorded every 24 h (Figure 1a).

Feeding Rate: For observing the feeding rate, adult female mosquitoes from selected and control populations were starved for 24 h and then provided with blood meal. Blood-fed females were counted and isolated in a cage provided with a 10% sucrose solution.

Fecundity: Blood-fed females were randomly selected and placed in mosquito cages with 10% sucrose solution and ovipositing bowl. After every 24 h, the number of eggs laid was counted (Figure 1b).

Larval Development Time: At least 150-200 eggs were counted and placed in an enamel tray (11" x 12" cm) containing 2 L water and were covered with a net to avoid oviposition by other females. Changes in larval development were recorded every 24 h in both selected (F12) and control populations (Figure 1c).

Longevity: 150 freshly emerged males and females (three replicates) were kept in separate cages and provided with sucrose solution (Figure 1d). The base of the cage was covered with a white cloth to count the dead mosquitoes.

Sex Ratio: A total of 150 pupae from both populations were separated and kept inside mosquito cages (Figure 1d). The sex ratio (female/male) was calculated once the adult emerged from the pupa.

Detoxifying Enzyme Activity: Biochemical experiments were carried out using the protocol recommended by WHO with a few modifications.[19] Thirty non-blood-fed mosquitoes were homogenised in 200 µl of ice-cold sodium phosphate buffer (0.1 mMol). The homogenate was subjected to centrifugation at 13000 rpm for 10 min. The protein concentration of individual homogenates was determined following the standard protocol in order to analyse the specific activities of enzymes.[20] For monooxygenase, a total of 200 µl of working solution (0.01 g 3,3-5,5-tetramethyl-benzidine in 5 ml methanol + 15 ml sodium acetate buffer) was added to 20 µl of mosquito homogenate, and 25 µl 3% hydrogen peroxide (H_2O_2) was added afterwards. After 2 h of incubation at room temperature, the plate was read at 650 nm (Figure 2a). For esterase activity, 20 µl of mosquito homogenate was mixed with 200 µl of working solution (1 ml of 30 mM α-naphthyl/ β-naphthyl acetate in acetone solution in 99 ml of 0.02M phosphate buffer). The mixture was incubated at room temperature for 15 min and 50 µl of Fast Blue BB Salt (FBBS) solution was added to it. The plate was read at 570 nm (Figure 2b). For Glutathione-S-transferase (GSTs), 200 µl of working solution (350 µl of 63 mM 1-chloro-2,4-dinitrobenzene (cDNB) + 7 ml of 10 mM reduced glutathione) was added to 20 µl of mosquito supernatant. The absorbance was read at 340 nm together with control where homogenate was absent (Figure 2c).

Statistical Analysis: Values of lethal concentration (LC) were determined by probit analysis using IBM SPSS version 21. Enzyme activities and levels were compared between the treated and control group using the Analysis of Variance (ANOVA) test. The comparison of different life traits between groups was performed using one-way ANOVA which was followed by Tukey's test. Descriptive statistics were used for hatchability and fertility tests.

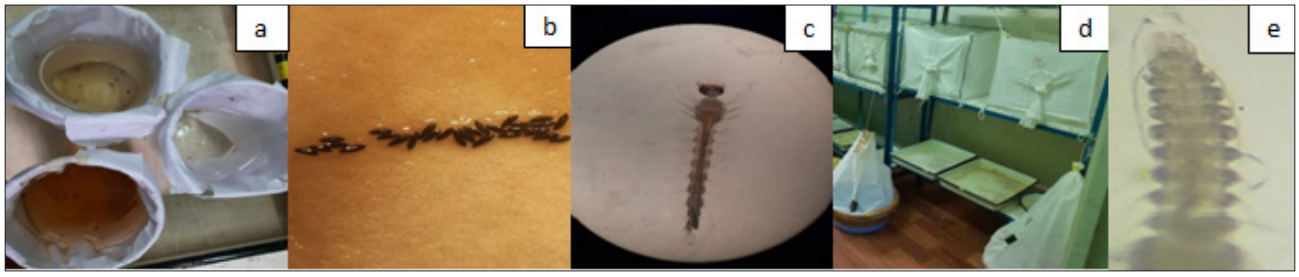

Figure 1. Experimental Set-up for Observing Key Life-traits of *Ae. albopictus* (a) Ovitrap kept for Recording Egg Hatchability (b) Eggs obtained from Individual Gravid Females for Fecundity Study (c) Larva kept for identifying their Developmental Stage and Time (d) Cage Set-up for observing Longevity and Sex Ratio of the Mosquito (e) Unhatched Eggs of Highly Resistant Mosquito exposed to Bleaching and Decalcifying Liquid

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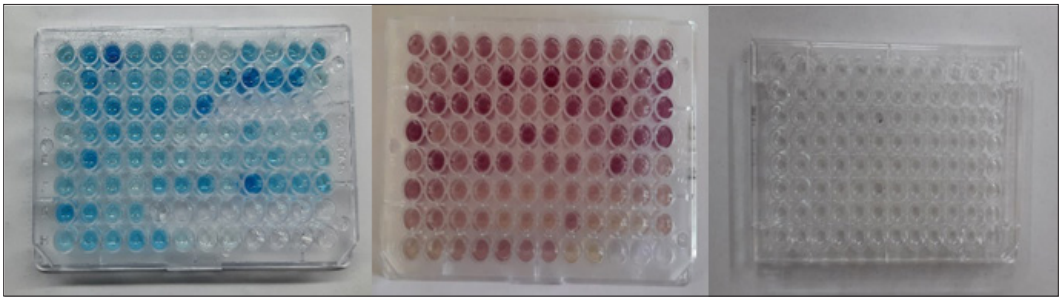

(a) (b) (c)

Figure 2.Microplate prepared for Measuring Activities of Detoxifying Enzymes (a) Cytochrome P450 (b) β-esterases (c) GST

Table 1.Status of Lambda-cyhalothrin Resistance Development in Different Generations of *Ae. albopictus*

Population	LC_{50} mg/L (95% CI)	RR	Status
Control group (n = 200)	0.007	-	-
Insecticide-treated group (n = 200)			
F1	0.011	1.6	S
F3	0.024	3.42	S
F4	0.033	4.7	S
F5	0.059	8.42	MR
F8	0.262	37.77	HR
F12	0.719	102.7	HR

Table 2.Reproductive Parameters in Control and Insecticide-treated Mosquitoes

Parameters	Control Strain (n = 150)	Treated Strain (LC_{20}) (n = 150)	Treated Strain (LC_{50}) (n = 150)	p Value
	Rates (%)	Rates (%)	Rates (%)	
Blood feeding	35.067	34.514	38.479	0.895
Fecundity	135.464[a]	125.421[a]	42.349[b]	0.0001
Hatchability	94.32[a]	82.97[a]	42.69[b]	0.0004

The same superscript letters in each row indicate no significant difference (p > 0.05); n: sample size.

Results

In this study, *Ae. albopictus* was selected for 12 generations. Table 1 shows the LC_{50} values of lambda-cyhalothrin treated and control population. With the succeeding generation, there was a gradual increase in LC_{50} values from 0.011 mg/L to 0.719 mg/L i.e., a 101 times increase in resistance ratio values from F1 to F12 generations.

Life History Traits

Hatchability: It is clear from Table 2 that exposure to lambda-cyhalothrin sublethal dose of LC_{20} had no significant difference observed but at LC_{50} dose, the hatchability decreased significantly by 52% compared to a control group ($p < 0.0004$). Upon exposure of unhatched eggs to a bleaching agent, a fully developed larva was found (Figure 1e).

Feeding Rate: There was no significant difference in feeding rate between treated and control populations (Table 2).

Fecundity: Significant reduction (93%) in fecundity was observed in *Ae. albopictus* population exposed to LC_{50} as compared to the control population. However, there was no significant difference between LC_{20} treated population and the control group.

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Larval Development Time: Comparison between the treated and control population was performed until pupae were formed. As it is clear from Table 3, LC_{50}-treated larvae took a significantly longer time to develop into pupae (appx. 9 days) and LC_{20}-treated larvae took 8 days in comparison with the control population (7 days).

Longevity: In the treated group, the life span of the female adults decreased significantly by 7.67 days in LC_{20} dose and by 9.43 days in LC_{50} dose. Moreover, male longevity also decreased significantly by 2.18 days with respect to the control population.

Sex Ratio: As shown in Table 3, sex ratio of the mosquito population in the control group was found to be 1.07 while that of the populations exposed at LC_{20} and LC_{50} were 1.05 and 0.82, respectively.

Detoxifying Enzyme Activity

It was found that the mean activity of monooxygenase significantly increased by 2.4-fold in LC_{20} treated group and by 2.6-fold in LC_{50} group ($F(2,69) = 4.630$, $p = 0.013$), respectively, in comparison to the control group (Figure 3a). For carboxylesterase activity (β-esterase), LC_{20} treated group exhibited a 1.8-fold increase and LC_{50} treated group exhibited a 2.6-fold increase ($p < 0.043$), respectively (Figure 3b). Likewise, for GST activity, a 2.4-fold increase in LC_{20} ($p < 0.002$) and a 3.3-fold increase in LC_{50} treated population ($p < 0.001$) were observed (Figure 3c).

Discussion

Insecticides used in vector control mainly target eradicating the vector population but often neglect their residual and long-time effect on the surviving population. Thus, the current study was undertaken to develop the lambda-cyhalothrin resistant population and to observe the associated changes in the life trait of mosquitos with special emphasis on the role of major detoxifying enzymes behind the resistance development. The data obtained showed that after continuous exposure to the sublethal dose of lambda-cyhalothrin, there was an increase in the resistance ratio value of LC_{50} by 101-fold where moderate resistance (MR) was developed at F5 generation (RR = 5-10), and in F12, high resistance (HR) was noted with RR > 10 (Table 1). According to the present study, it took approximately 60 days to obtain a resistant population i.e., at F5 generation (Table 1), after continuous exposure to lambda-cyhalothrin. Therefore, it is better to avoid continuous use of lambda-cyhalothrin for a longer period of time and proper surveillance should be done before applying the insecticide mainly in the area where insecticide resistance has been reported. A similar increase in resistance ratio values was reported in *Ae. aegypti* population that was resistant to deltamethrin (118-fold), fipronil (76.75-fold), and imidacloprid (372-fold).[21] Another study on *Culex pipiens pallens* revealed that the RR value increased by 617 times in the deltamethrin-resistant population as compared to the unselected population.[22]

Table 3.Developmental Parameters between Control and Insecticide-treated Mosquitoes

Parameters	Control Strain (n = 150)	Treated Strain (LC_{20}) (n = 150)	Treated Strain (LC_{50}) (n = 150)	p Value
	Means ± SE	Means ± SE	Means ± SE	
LDT	7.333 ± 0.256[a]	8.26 ± 0.427[a]	8.90 ± 0.323[b]	0.006
Longevity female	13.33 ± 0.303[a]	5.66 ± 0.718[b]	3.90 ± 0.319[c]	0.000
Longevity male	5.33 ± 0.186[a]	5.43 ± 0.598[a]	3.15 ± 0.276[b]	0.000
Sex ratio	1.07 ± 0.094	1.05 ± 0.224	0.82 ± 0.223	0.614

The same superscript letters in each row indicate no significant difference ($p > 0.05$); n: sample size; SE: Standard Error; LDT: Larval Development Time.

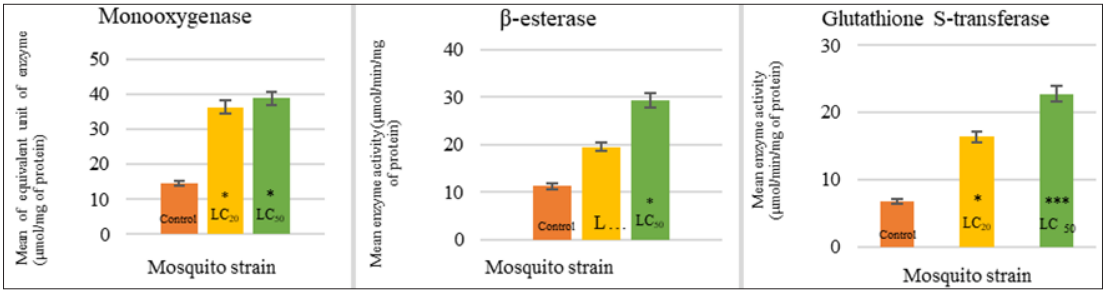

Figure 3.Effect of Lambda-cyhalothrin Exposure on the Activities of Major Detoxifying Enzymes in *Ae. albopictus* (a) Monooxygenase (b) β-esterase (c) Glutathione-S-transferase activity in the control and treated group (Lc_{20} and LC_{50})

* indicates $p < 0.05$; *** indicates $p < 0.001$

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Most studies report the fitness disadvantage of resistant populations on continuous exposure to insecticides.[23,24] However, studies on the developmental and reproductive parameters of *Aedes albopictus* are very few although it is a potential vector of dengue and chikungunya. According to our study, the length of larval development time was longer in LC_{50} and LC_{20} treated groups in comparison to the control group. A similar result was obtained in deltamethrin selected *An. gambiae* in western Kenya where larval development time in resistant populations was extended by 2 days.[25] Moreover, temephos-resistant *Ae. aegypti* in Brazil had an additional 5-7 days larval stage.[26] In contrast to this, one study showed that the resistant strain of *Ae. aegypti* in Thailand had no differences in the larval development time.[27]As juvenile hormone and ecdysone are important hormones that regulate growth in mosquitoes, prolonged larval duration might occur due to the disturbance in their synthesis and secretion or alteration of the regulatory mechanism involved.[28] Therefore, delayed development in our result may occur due to the sublethal effect of lambda-cyhalothrin on the synthesis of these hormones. The prolonged larval stage is of concern as it extends the life cycle of the vector, which means it may be found apart from its peak season which further may increase the risk of disease transmission throughout the year.

The effect of insecticide exposure on the feeding rate of *Ae. albopictus* is less known as there are fewer data available to corroborate our study. In this study, the comparison of feeding rates between the treated and control groups did not show any significant difference, indicating no effect of insecticide resistance on feeding rate. In contrast to this, it was observed in one study that the blood feeding rate was high in the control population, unlike their resistant counterpart.[29]

Furthermore, in the present study, reduced fecundity in *Ae. albopictus* was observed which was similar to the study where temephos-resistant *Ae. aegypti* also had decreased fecundity.[30] Another study conducted in New Delhi (India) had similar findings where α-cypermethrin exposed *Ae. aegypti* showed decreased fecundity in the resistant population (28 eggs/female) as compared to the susceptible population (79.6 eggs/female).[31] This reduction in fecundity may occur due to the toxic effects exerted by lambda-cyhalothrin on the growth of egg follicles.[32] Moreover, the feeding rate is not affected yet the reduction in fecundity of the resistant population is observed. It may occur due to a compromise in ovarian development or synthesis of vitellin as the influence of lambda-cyhalothrin.

Hatchability in LC_{20} treated group had no significant difference but exposure to LC_{50} reduced it by 52%. The use of bleaching agent[33] on unhatched eggs gives a clear indication that the unhatched eggs had fully developed larvae (Figure 1e). So, the reason behind it may be the thick calcification of the exochorion of eggs which reduced the hatchability. Otherwise, the eggs may have gone into dormant condition for some duration to escape from the stressful (insecticide pressure) environment. Further study is yet to be done for detailed information. As reported in another study, a similar reduction in the hatchability of *An. Stephensi* was found when exposed to a sublethal dose of temephos and propoxur.[34]

In the case of longevity, the treated female had a significantly shorter life span of 7 days (LC_{20}) and 9 days (LC_{50}), whereas, in the case of males, it was reduced by 2 days (LC_{50}) (Table 3). As longevity is regarded as one of the important criteria due to its effect on the vectorial capacity, shorter longevity in vectors is a fitness disadvantage. Contrary to our findings, a longer life span in resistant females of *An. funestus* was observed more than the susceptible counterpart.[35] Moreover, it was also reported that lambda-cyhalothrin resistant strain of *Ceratitis capitata* (Wiedemann) commonly known as the Mediterranean fruit fly had increased longevity in both females and males by 8 days.[36]

No obvious difference was observed in the sex ratio of the treated and the control population which is alike the study in deltamethrin-resistant *Culex pipienspallens*[22] and *An. coluzzii* population of Cameroon.[29]

Numerous studies have associated the activity of major detoxifying enzymes with the development of resistance to pyrethroids.[37,38] Thus, upon selection with lambda-cyhalothrin, a significant increase in monooxygenase, carboxylesterase (β-esterase), and GST activity was observed, indicating their vital role in pyrethroid resistance development, where monooxygenase level was found to be highest followed by both β-esterase and GST. As the resistance ratio (RR) value increases with generation, the activity of detoxifying enzymes also increases which indicates the significant role of metabolic detoxification behind the resistance development of lambda-cyhalothrin. Moreover, other factors/ mechanisms responsible for pyrethroid resistance development should be studied for a clear conclusion.

More recent reports have shown a similar involvement of monooxygenase, β-esterases, and GSTs in pyrethroid resistance mosquitoes [14] On contrary, a different result was obtained where an elevated level of GST was found in deltamethrin-resistant *Ae. aegypti*.[21]

Conclusion

In the current study, the laboratory selection of *Ae. albopictus* over generations diminished its fitness parameters like fecundity, hatchability, longevity, and prolonged immature duration. This type of study helps in the better understanding of the changes in population

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dynamics of resistant vectors which is crucial information and should be taken into account while designing vector control programmes. Furthermore, findings of the current study can also be used to select the vulnerable stage of vectors based on their life history traits, which then can be targeted for effective control of the resistant population. Overall, as the fitness components of the resistant population have already been hampered, eventually decreasing their population, continuous use of insecticides should be avoided mostly in the areas where resistance is reported and insecticides with a different mode of action should be implemented. Besides, for efficient resistance management, proper vector population surveillance should be done prior to the implementation of other alternatives.

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Authors' Contributions

Conceptualisation, supervision and validation: DS; investigation, methodology and data curation: NL; Writing - original draft preparation: NL; Writing - review and editing: DS and NL.

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References

1. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, Hendrickx G, Schaffner F, Elyazar IR, Teng HJ, Brady OJ, Messina JP, Pigott DM, Scott TW, Smith DL, Wint GR, Golding N, Hay SI. The global distribution of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Elife. 2015;4:e08347. [PubMed] [Google Scholar]
2. Lee HL, Rohani A. Transovarial transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus* in relation to dengue outbreak in an urban area in Malaysia. Dengue Bull. 2005;29:106-9. [Google Scholar]
3. Chompoosri J, Thavara U, Tawatsin A, Boonserm R, Phumee A, Sangkitporn S, Siriyasatien P. Vertical transmission of Indian Ocean Lineage of chikungunya virus in *Aedes aegypti* and *Aedes albopictus* mosquitoes. Parasit Vectors. 2016;9(1):227. [PubMed] [Google Scholar]
4. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. *Aedes (Stegomyia) albopictus* (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis. 2013;7(8):e2348. [PubMed] [Google Scholar]
5. Reegan AD, Ceasar SA, Paulraj MG, Ignacimuthu S, Al-Dhabi NA. Current status of genome editing in vector mosquitoes: a review. Biosci Trends. 2017;10(6):424-32. [PubMed] [Google Scholar]
6. Halasa YA, Shepard DS, Fonseca DM, Farajollahi A, Healy S, Gaugler R, Bartlett-Healy K, Strickman DA, Clark GG. Quantifying the impact of mosquitoes on quality of life and enjoyment of yard and porch activities in New Jersey. PloS One. 2014;9(3):e89221. [PubMed] [Google Scholar]
7. Monaghan AJ, Sampson KM, Steinhoff DF, Ernst KC, Ebi KL, Jones B, Hayden MH. The potential impacts of 21st century climatic and population changes on human exposure to the virus vector mosquito *Aedes aegypti*. Clim Change. 2018;146(3):487-500. [PubMed] [Google Scholar]
8. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. Climate change and vector-borne diseases: a regional analysis. Bull World Health Organ. 2000;78(9):1136-47. [PubMed] [Google Scholar]
9. Bennett KL, Gómez Martínez C, Almanza A, Rovira JR, McMillan WO, Enriquez V, Barraza E, Diaz M, Sanchez-Galan JE, Whiteman A, Gittens RA, Loaiza JR. High infestation of invasive *Aedes* mosquitoes in used tires along the local transport network of Panama. Parasit Vectors. 2019;12(1):264. [PubMed] [Google Scholar]
10. Achee NL, Grieco JP, Vatandoost H, Seixas G, Pinto J, Ching-Ng L, Martins AJ, Juntarajumnong W, Corbel V, Gouagna C, David JP, Logan JG, Orsborne J, Marois E, Devine GJ, Vontas J. Alternative strategies for mosquito-borne arbovirus control. PLoS Negl Trop Dis. 2019;13(1):e0006822. [PubMed] [Google Scholar]
11. World Health Organization [Internet]. WHO Specifications and evaluations for public health pesticides; 2015 [cited 2022 Feb 12]. Available from: https://extranet.who.int/pqweb/sites/default/files/vcp-documents/WHOVC-SP_Lambda-cyhalothrin_2015.pdf
12. Bellinato DF, Viana-Medeiros PF, Araújo SC, Martins AJ, Lima JB, Valle D. Resistance status to the insecticides temephos, deltamethrin, and diflubenzuron in Brazilian *Aedes aegypti* populations. Biomed Res Int. 2016;2016:8603263. [PubMed] [Google Scholar]
13. Benelli G, Jeffries CL, Walker T. Biological control of mosquito vectors: past, present, and future. Insects. 2016;7(4):52. [PubMed] [Google Scholar]
14. Machani MG, Ochomo E, Zhong D, Zhou G, Wang X, Githeko AK, Yan G, Afrane YA. Phenotypic, genotypic and biochemical changes during pyrethroid resistance selection in *Anopheles gambiae* mosquitoes. Sci Rep. 2020;10(1):19063. [PubMed] [Google Scholar]
15. Ranson H, Lissenden N. Insecticide resistance in African *Anopheles* mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 2016;32(3):187-96. [PubMed] [Google

Scholar]
16. Kliot A, Ghanim M. Fitness costs associated with insecticide resistance. Pest Manag Sci. 2012;68(11):1431-7. [PubMed] [Google Scholar]
17. Tyagi BK, Munirathinam A, Venkatesh A. A catalogue of Indian mosquitoes. Int J Mosq Res. 2015;2(2):50-97. [Google Scholar]
18. World Health Organization [Internet]. Monitoring and managing insecticide resistance in Aedes mosquito populations; 2016 [cited 2022 Jan 27]. Available from: https://apps.who.int/iris/bitstream/handle/10665/204588/WHO_ZIKV_VC_16.1_eng.pdf [Google Scholar]
19. World Health Organization [Internet]. Techniques to detect insecticide resistance mechanisms (field and laboratory manual); 1998 [cited 2022 Jan 27]. Available from: http://apps.who.int/iris/bitstream/10665/83780/1/WHO_CDS_CPC_MAL_98.6.pdf [Google Scholar]
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75. [PubMed] [Google Scholar]
21. Sumra MW, Freed S, Shah MS, Nazar MZ, Hussain S, Naeem A. Biochemical characterization of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) resistance to deltamethrin, fipronil, and imidacloprid. Environ Monit Assess. 2021;193(10):665. [PubMed] [Google Scholar]
22. Li X, Ma L, Sun L, Zhu C. Biotic characteristics in the deltamethrin-susceptible and resistant strains of *Culex pipiens pallens* (Diptera: Culicidae) in China. Appl Entomol Zool. 2002;37(2):305-8. [Google Scholar]
23. Rahim J, Ahmad AH, Maimusa AH. Effects of temephos resistance on life history traits of *Aedes albopictus* (Skuse) (Diptera: Culicidae), a vector of arboviruses. Rev Bras Entomol. 2017;61:312-7. [Google Scholar]
24. Martins AJ, Ribeiro CD, Bellinato DF, Peixoto AA, Valle D, Lima JB. Effect of insecticide resistance on development, longevity and reproduction of field or laboratory selected *Aedes aegypti* populations. PloS One. 2012;7(3):e31889. [PubMed] [Google Scholar]
25. Osoro JK, Machani MG, Ochomo E, Wanjala C, Omukunda E, Munga S, Githeko AK, Yan G, Afrane YA. Insecticide resistance exerts significant fitness costs in immature stages of *Anopheles gambiae* in western Kenya. Malar J. 2021;20(1):259. [PubMed] [Google Scholar]
26. Diniz DF, de Melo-Santos MA, de Mendonça Santos EM, Beserra EB, Helvecio E, de Carvalho-Leandro D, dos Santos BS, de Menezes Lima VL, Ayres CF. Fitness cost in field and laboratory *Aedes aegypti* populations associated with resistance to the insecticide temephos. Parasit Vectors. 2015;8(1):662. [PubMed] [Google Scholar]
27. Plernsub S, Stenhouse SA, Tippawangkosol P, Lumjuan N, Yanola J, Choochote W, Somboon P. Relative developmental and reproductive fitness associated with F1534C homozygous knockdown resistant gene in *Aedes aegypti* from Thailand. Trop Biomed. 2013;30(4):621-30. [PubMed] [Google Scholar]
28. Venkatesan R, Ravindran J, Eapen A, William J. Insecticidal and growth regulating activity of crude leaf extracts of *Cassia occidentalis* L. (Caesalpinaceae) against the urban malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Asian Pac J Trop Dis. 2014;4:S578-82. [Google Scholar]
29. Nkahe DL, Kopya E, Djiappi-Tchamen B, Toussile W, Sonhafouo-Chiana N, Kekeunou S, Mimpfoundi R, Awono-Ambene P, Wondji CS, Antonio-Nkondjio C. Fitness cost of insecticide resistance on the life-traits of *Anopheles coluzzii* population from the city of Yaoundé, Cameroon. Wellcome Open Res. 2020;5:171. [PubMed]
30. Belinato TA, Martins AJ, Valle D. Fitness evaluation of two Brazilian *Aedes aegypti* field populations with distinct levels of resistance to the organophosphate temephos. Mem Inst Oswaldo Cruz. 2012;107(7):916-22. [PubMed] [Google Scholar]
31. Gupta A, Samal RR, Kumar S. Physiological and reproductive fitness cost in *Aedes aegypti* on exposure to toxic xenobiotics in New Delhi, India. J Appl Nat Sci. 2021;13(1):71-8. [Google Scholar]
32. Kumar S, Pillai MK. Correlation between the reproductive potential and the pyrethroid resistance in an Indian strain of filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Bull Entomol Res. 2011;101(1):25-31. [PubMed] [Google Scholar]
33. Trpiš M. A new bleaching and decalcifying method for general use in zoology. Can J Zool. 1970;48(4):892-3. [Google Scholar]
34. Sanil D, Shetty NJ. The effect of sublethal exposure to temephos and propoxur on reproductive fitness and its influence on circadian rhythms of pupation and adult emergence in *Anopheles stephensi* Liston—a malaria vector. Parasitol Res. 2012;111(1):423-32. [PubMed] [Google Scholar]
35. Okoye PN, Brooke BD, Hunt RH, Coetzee M. Relative developmental and reproductive fitness associated with pyrethroid resistance in the major southern African malaria vector, *Anopheles funestus*. Bull Entomol Res. 2007;97(6):599-605. [PubMed] [Google Scholar]
36. Guillem-Amat A, López-Errasquín E, Sánchez L, González-Guzmán M, Ortego F. Inheritance, fitness cost, and management of lambda-cyhalothrin resistance in a laboratory-selected strain of *Ceratitis capitata* (Wiedemann). Insects. 2020;11(9):551. [PubMed] [Google Scholar]
37. David JP, Ismail HM, Chandor-Proust A, Paine MJ. Role

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of cytochrome P450s in insecticide resistance: impact on the control of mosquito-borne diseases and use of insecticides on Earth. Philos Trans R Soc Lond B Biol Sci. 2013;368(1612):20120429. [PubMed] [Google Scholar]

38. Bharati M, Rai P, Saha D. Insecticide resistance in *Aedes albopictus* Skuse from sub-Himalayan districts of West Bengal, India. Acta Trop. 2019;192:104-11. [PubMed] [Google Scholar]

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