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Research Article

Physiological and reproductive fitness cost in *Aedes aegypti* on exposure to toxic xenobiotics in New Delhi, India

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Abstract

Aedes aegypti, is a well-known vector of dengue, Chikungunya and Zika at the global level. Primary use of pyrethroids as control interventions has caused the development of a considerable level of immunity in *Ae. aegypti*. The current study assessed the efficacy of a pyrethroid, α-cypermethrin on the survival and various life parameters of *Ae. aegypti*. The larvicidal studies with α-cypermethrin revealed the respective LC_{50} and LC_{90} values as 0.26526 mg/L and 0.60211 mg/L. The impact of LC_{50} level was assessed on the growth and life attributes; such as gonotrophic cycle, egg development, hatchability, development and survival of immature stages, adult longevity, reproduction rate and generation time; of fourth instar of susceptible (S) and α-cypermethrin -exposed population (E). The exposed population showed diminished fitness as compared to the susceptible population. The individual female fecundity in susceptible population was recorded as 79.6 with 61.6% hatchability rate as compared to the 28 eggs/female and 25% hatchability in the exposed population. The mean egg hatch time in S strain increased by 2-fold in E strain. The proportion of immature survival observed in S strain was 0.88 for fourth instar to pupa (P/I), 0.94 for pupa to adult (A/P) and an overall 0.83 for fourth larva to adult (A/I), which respectively reduced to 0.32, 0.86 and 0.27 in E strain of *Ae. aegypti*. Likewise, the net reproductive rate, birth rate and death rate were significantly ($p < 0.05$) higher in S than in E strain. This study demonstrates the negative impact of α-cypermethrin on the physiological and reproductive fitness of *Ae. aegypti*.

Keywords: *Aedes*, α-cypermethrin, Larvicidal bioassay, Life table, Pyrethroids

INTRODUCTION

Aedes aegypti is the predominant disease vector responsible for the transmission of several diseases of human concern. The continuous increase in these diseases has made the management of *Ae. aegypti* indispensable to improve the quality of the environment and public health (Benelli *et al.,* 2016). Due to lack of successful medication and vaccine against these diseases, prime mitigation approach is the disruption of disease transmission either by killing different developmental stages of a mosquito or by preventing adult bites using chemical or natural repellents (Achee *et al.,* 2019).

In India, Ministry of Family and Health Welfare recorded 99,913 cases of dengue and 220 fatalities in 2015, which rose to 1,57,315 cases and 166 deaths in 2019 (NVBDCP, 2020a). In addition, the outbreak of Chikungunya across India registered 81,914 cases in 2019, highest since last fifteen years (NVBDCP, 2020b). These diseases are distributed in almost all the Indian states and Union Territories; majorly in the Gujarat, Maharashtra, Rajasthan, Karnataka, Kerala, Tamil Nadu, Telangana, Uttarakhand and Uttar Pradesh (NVBDCP, 2020a).

Chemical-based interventions are being practised since decades for mosquito management. Several organochlorines, organophosphates and carbamates have been formulated and utilized for field and domestic use. Nevertheless, rapid resistance development in mosquitoes and adverse environmental impacts caused by their frequent use led to the employment of relatively safer pyrethroid compounds. Pyrethroids, the synthetic analogues of pyrethrins isolated from the flowers of *Chrysanthemum*, are labelled as safe by World Health Organization (WHO) and are frequently used as indoor residual sprays (IRS) and in bed-nets as a vector-based intercession scheme (Raghavendra *et al*., 2010). However, like other xenobiotics, the extensive use of pyrethroids has also resulted in harmful effects on aquatic organisms and the development of resistance to pyrethroids (Kumar *et al*., 2009).

Alpha-cypermethrin is a recommended pyrethroid by World Health Organization for IRS (WHO, 2009). Different formulations of the compound have been tested against mosquitoes by WHO in the field conditions (WHO, 1998). In Darwin city, Australia, α-cypermethrin could prevent mosquito larval colonization of water-containing receptacles efficiently (Pettit *et al.,* 2010). In Pondicherry (now Puducherry), India, the WP (Wettable powder) of α-cypermethrin when sprayed indoors @100 mg a.i./m^2, significantly reduced density of *Anopheles subpictus* and *Culex quinquefasciatus* with residual efficacy of 18–27 weeks on different surfaces (Amalraj *et al.,* 1987).

Most of the studies with α-cypermethrin have been carried out against adult mosquitoes (Dong, 2007; Rinkevich *et al*., 2013). With several reports regarding development of pyrethroid resistance in mosquitoes, more systematic and sophisticated insecticide resistance monitoring in the field populations of mosquito is vital for the success of mosquito control programs. A better understanding of the factors contributing to the mechanism governing resistance development can help to formulate the strategies for mosquito management. Hence, the current study was held to assess the impact of alpha-cypermethrin on the survival and life parameters of *Ae. aegypti* larvae. In addition, the variations in the life-table characteristics of *Ae. aegypti* in susceptible (S) and α-cypermethrin exposed (E) population was investigated to understand the population dynamics of this important arboviral vector better, the dynamics of dengue transmission and control under local and regional conditions.

MATERIALS AND METHODS

Establishment of *Aedes aegypti* culture

Culture of dengue fever mosquitoes, *Ae. aegypti* pure line was procured from ICGEB (International Centre for Genetic Engineering and Biotechnology), New Delhi, India. The colony of *Ae. aegypti* were maintained in an insect rearing room at 28± 1 °C, 80 ± 5% RH, 14 h/10 L photo-regime (Kumar *et al*., 2002; Samal and Kumar, 2018). Adults were fed upon sugary juices of water-soaked raisins. Occasional blood meals were given to female mosquitoes for egg maturation by keeping albino rats in the cage, reared for the purpose. The eggs were collected on the moist Whatman filter paper strips in an ovitrap. Eggs were hatched into the enamel trays filled with 1.5-2.0 L of dechlorinated water and the larvae were reared on a diet of dog biscuits and yeast in a ratio of 3:1 (Warikoo *et al*., 2012). The pupae were collected on a regular basis and kept in cages for adult emergence.

Preparation of insecticidal solutions

Alpha-cypermethrin was procured from Sigma-Aldrich, India. The 10 mg of insecticide was diluted in 10 mL ethanol (eMerck) to form desired concentration of 1 mg/mL and was stored at 4 °C.

Larvicidal bioassay

The efficacy of α-cypermethrin as larvicide against *Ae. aegypti* was investigated by adopting WHO protocol (2016). The graded series of the concentration was prepared. A total of 25 early fourth instar larvae of *Ae. aegypti* taken in 199 mL of dechlorinated water were exposed to 1 mL of a particular concentration of the alpha-cypermethrin (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg/L) for 24 h. Three replicates of each dilution were run simultaneously. The dead and moribund larvae were confirmed by gently touching with a glass rod. Control was run in the same manner by substituting insecticidal solution with absolute ethanol.

Data analysis

The assay was carried out again in case of more than 20% pupal mortality in control, while the test results with 5-20% larval mortality in the control were corrected by Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{\%Test mortality - \%Control mortality X 100}}{\text{100 - \%Control}} \qquad \text{……….Eq. 1}$$

The corrected data was analyzed statistically using software program SPSS 19.0 and the lethal values; LC_{30}, LC_{50}, LC_{70} and LC_{90}; regression coefficient, 95% fiducial limits and chi-square values were computed.

Investigations on physiological and reproductive fitness in *Aedes aegypti* population

The efficacy of α-cypermethrin was studied on the life table attributes of early fourth instar of *Ae. aegypti* at median lethal (LC_{50}) dosages. The alterations recorded were compared with the parameters observed in a susceptible laboratory-bred population (S).

A total of 200 early fourth instar of the susceptible population (S) were exposed to α-cypermethrin for 24 h and marked as exposed population (E). One mL of α-cypermethrin at LC_{50} level was added to 110 mL of dechlorinated water in 250 mL beaker. The solution was stirred with a glass rod to ensure the homogeneity of solution. Subsequently, 89 mL of dechlorinated water with 200 early fourth instar of 'S' population of *Ae. aegypti* was transferred to the 250 mL beaker. Another set of 200 larvae was exposed to ethanol simultaneously and taken as control. After 24 h, the larvae survived in each set were strained carefully and washed thoroughly. The larvae were reared and their development to adult and to next generation was studied. Various life table attributes and growth parameters were recorded on a daily basis till the emergence of next filial.

Toxicity and morphological alterations

The prospective use of α-cypermethrin as effective toxicant at the larval stage was assessed. The number of dead larvae was scored and examined for any morphological changes.

Delayed toxicity and adult emergence

The pupae developed and the adults emerged from the larvae exposed to LC_{50} level of α-cypermethrin were scored. The delayed toxicity effects of the alpha-cypermethrin was estimated on the pupae and adults, if any.

Fecundity and reproductive fitness of surviving adults

The adult females that emerged from the exposure were provided with blood meal after 3 days of emergence. The fecundity was scored by counting the number of eggs laid by the female *Ae. aegypti*, whereas the reproductive fitness was calculated by emergence of the next generation from the collected eggs.

Growth regulatory effects

The larvae hatched from the eggs laid were developed till adults. The larval duration of each instar and pupal duration were recorded.

Life table and survivorship

Based on the collected data, life table and survivorship curves were prepared using the following formulae (Sowilem *et al*., 2013).

A. Egg development

H_{50}= Median hatching time (time taken for 50% hatching of eggs): The H_{50} was computed by fitting a regression equation; P=a + b.In(x); where 'P' is the cumulative proportion of eggs hatched on each day (x) transformed to probits, 'a' is the intercept and 'b' is the slope/ regression coefficient.

B. Development of immature stages (Survivorship parameters): Various survivorship parameters calculated include; fourth instar to pupa (P/I); pupa to adult stage (A/P) and total survivorship from early fourth instar to adult stage (A/I).

Where, 'I' is the number of early fourth instar at the start of the experiment; 'P' is the number of pupae and 'A' denoted the number of adult emerged.

C. Adult parameters: The adult attributes calculated were duration of first gonotrophic cycle; Oviposition by female adults (Fecundity) and sex ratio (Number of Males or Females emerged/Total adults emerged).

D. Life table and survivorship attributes: Adult longevity at emergence (e_x) was obtained from the following series of calculations:

- L_x= [l_x + l(x+1)]/2); it denotes the number of mosquitoes survived between the days x and x+1; where l_x is the proportion of alive adults at the beginning of day x, and l(x+1) is the proportion of alive adults at the beginning of the next day (x+1).
- $l_x = y_x/y_0$; where y_x is number of mosquitoes that were alive on the day x and y_0 is the initial number of mosquitoes in the population
- T_x = summation of L_x (x to w); T_x is the total number of survivors beyond age x; where w is the day when the last individual died.
- $e_x = T_x/l_x$; where e_x is the adult life expectancy, i.e., the mean number of days remaining for the survivors at age x.

Net Reproductive rate R_0 = Mean number of offspring produced by single female from a cohort during the course of it lifespan

- R^0 = {(H/UH)/T}*100
- H= number of hatched eggs (viable)
- UH=number of unhatched eggs (non-viable)
- T= Total number of eggs laid

Intrinsic rate of increase (r_m): average of number of adults alive on day x and x+1

Mean Generation time (G): the average time between two consecutive generations in the lineage of a population

Birth rate (B): number of birth per 1000 individual

Death rate (D): number of death per 1000 individual

RESULTS

Larval susceptibility to α-cypermethrin

The results demonstrated the considerable larvicidal efficacy of α-cypermethrin against early fourth instars of dengue vector resulting in respective LC_{50} and LC_{90} values of 0.26526 mg/L and 0.60211 mg/L after 24 h of exposure. It was also observed that the treatments resulted in complete mortality without any pupa or adult emergence (Table 1; Fig. 1). The Larval mortality was dose-dependent, increasing with the enhanced dosage of toxicant used.

Table 1. Larvicidal activity (mg/L) of α- cypermethrin against early fourth instar of *Aedes aegypti*.

Lethal concentrations		**95% Fiducial limits**		**Slope ± SE**	**χ^2 (df)**	***p*-value**
		Lower	**Upper**			
LC_{30} (mg/L)	0.09684	0.07125	0.13163	3.566 ± 0.0681	26.496 (3)	0.0001
LC_{50} (mg/L)	0.26526	0.19516	0.36054			
LC_{70} (mg/L)	0.43368	0.31908	0.58946			
LC_{90} (mg/L)	0.60211	0.44299	0.81837			

LC_{30} - Lethal Concentration that kills 30% of the exposed larvae, LC_{50} - Lethal Concentration that kills 50% of the exposed larvae, LC_{70} - Lethal Concentration that kills 70% of the exposed larvae, LC_{90} - Lethal Concentration that kills 90% of the exposed larvae, S.E. = Standard Error, χ^2 = Chi-square, df = degree of freedom.

Table 2. Comparative life table attributes of *Aedes aegypti* under control and α- cypermethrin exposed conditions.

Life Attributes	**Control condition (Mean ± SEM)**	**Exposure to LC_{50} of α-cypermethrin (Mean ± SEM)**
Egg Development		
Incubation period in days	3.384 ± 0.058 a	3.852 ± 0.099 b
Median time to egg hatch (H_{50}) in days	1.696 ± 0.078 a	3.296 ± 1.023 b
Hatchability %	61.639 ± 4.507 a	36.774 ± 7.650 b
Development of Immature Stages		
Fourth larvae to pupae (P/l)	0.885 ± 0.007 a	0.320 ± 0.009 b
Pupae to Adult (A/P)	0.943 ± 0.012 a	0.859 ± 0.008 b
Fourth larvae to Adult (A/l)	0.835 ± 0.009 a	0.275 ± 0.005 b
Adult Parameters		
Gonotrophic cycle (in days)	11 ± 1.025 a	7 ± 2.851 b
Female Fecundity	79.625 ± 3.589 a	50.833 ± 6.235 b
Sex ratio of emerged adults (M/F)	3.595 ± 0.988 a	1.115 ± 0.265 b
Female survival (%)	86.486 ± 6.359 a	46.154 ± 8.254 b
Male survival (%)	95 ± 11.256 a	65.517 ± 9.645 b
Life table and Survivorship		
Adult Mean Longevity (e_x)	2.041 ± 0.287 a	1.980 ± 0.325 a
Net Reproductive rate (R_0)	23.278 ± 9.018 a	-26.451 ± 11.383 b
Intrinsic rate of increase (r_m)	5.702 ± 0.965 a	-7.784 ± 3.458 b
Mean generation time (G)	25.605 ± 9.325 a	26.465 ± 7.256 a
Birth Rate (B)	1.347 ± 0.654 a	0.202 ± 0.006 b
Death Rate (D)	1.201 ± 0.230 a	0.408 ± 0.008 b
r_m/B	4.233 ± 1.259 a	-38.534 ± 9.564 b
B/D	1.215 ± 0.068 a	0.495 ± 0.071 b

*SEM= Standard error of mean; Figures in each row followed by different letters are significantly different ($p < 0.05$), one-way ANOVA followed by Tukey's all pair wise multiple comparison test

Evaluation of life table attributes of *Aedes aegypti*

The fecundity of *Ae. aegypti* female of control population was recorded as 79.6 eggs with an overall 61.6% egg hatch. On the other hand, larval exposure to α-cypermethrin reduced oviposition by 28 eggs/female and 25% egg hatch. The mean time needed for eggs to hatch into first instars under controlled condition was 1.7 days which increased to 3.3 days in case of α-cypermethrin-exposed larvae (Table 2).

The immature survivorship was recorded as 0.88 for fourth instar larva to pupa (P/l), 0.94 for pupa to adult (A/P) and an overall 0.83 for fourth larva to adult (A/l), whereas larval exposure to α- cypermethrin reduced the respective survivorship parameters to 0.32, 0.86 and 0.27 (Table 2, Fig. 2). Likewise, other attributes; the net reproductive rate, birth rate and death rate were significantly ($p < 0.05$) higher in controlled conditions than the exposed one. The birth and death rate; calculated

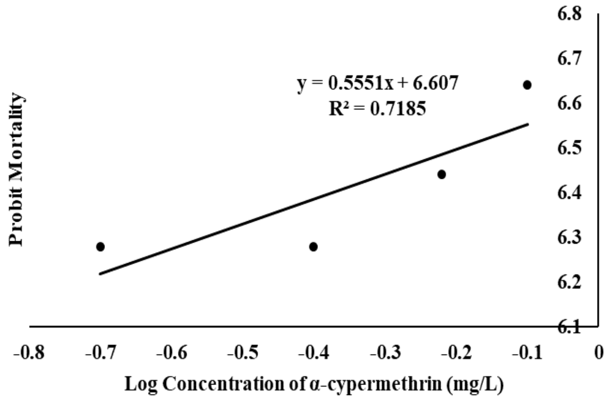

Fig. 1. *Dosage-mortality regression line on larvicidal bioassay with α-cypermethrin against larvae of Aedes aegypti.*

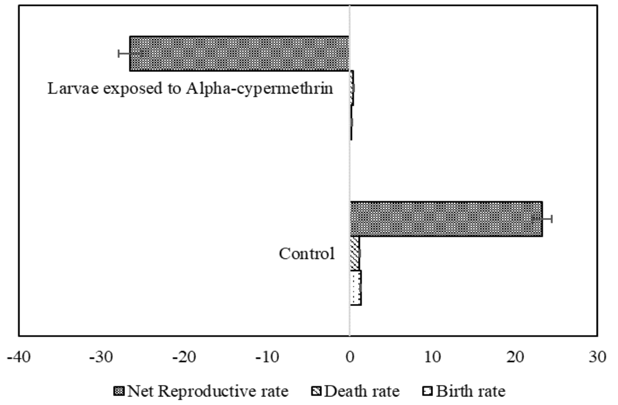

Fig. 3. *Decline in the birth, death and net reproductive rate in α- cypermethrin exposed population of Aedes aegypti.*

as 1.347 and 1.201 in control conditions; diminished to 0.202 and 0.408 in α-cypermethrin-exposed environment (Table 2). The calculated r_m/B and B/D ratios were (-)38.534 ± 9.564 and 0.495 ± 0.071, respectively for exposed larvae. These results indicate that the growth potential of this exposed colony is relatively lower in comparison to the susceptible strain.

The life expectancy (e_x) decreased to 1.980 ± 0.325 (E) from 2.041 ± 0.287 (S) with increasing age till death. The average sex ratio of emerged adults in experimental population (1.115) was insignificantly different from the 1:1 ratio. Our investigations also revealed that *Aedes* population under alpha-cypermethrin exposed condition showed higher number of deaths than births, indicating that just one-time exposure of the insecticide can disrupt the developmental cycle of successive generation. Consequently, the exposed population exhibited negative net reproductive rate and intrinsic rate of increase unlike in the control (Fig. 3). The net reproductive rate (R_0) in adults emerged from α- cypermethrin exposed larvae was (-) 26.451 ± 11.383, which was significantly different ($p < 0.05$) from the R_0 of susceptible strain (23.278 ± 9.018). Likewise, the mean intrinsic growth rate (r_m) obtained as (-) 7.784 ± 3.458 in 'E' population was significantly different from that of susceptible strain ($p < 0.05$). However, the mean generation time (G) computed in both S and E was not significantly ($p > 0.05$) different from each other.

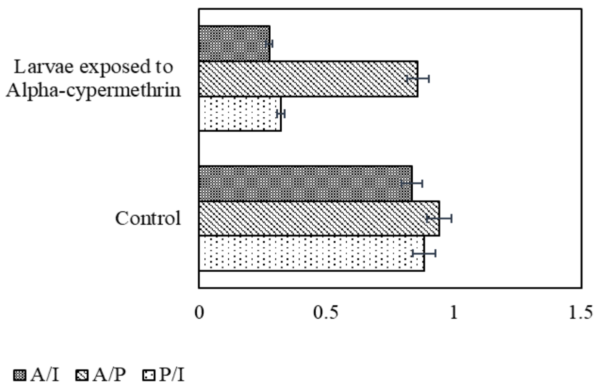

Fig. 2. *Decline in generation time in α- cypermethrin exposed population of Aedes aegypti; A/I = total survival from early fourth instar to adult; A/P = pupa to adult; P/I = fourth instar to pupa.*

DISCUSSION

The data on the effects of α-cypermethrin on the survival and, growth and development parameters of laboratory-bred strain (S) and α-cypermethrin-exposed population (E) of *Ae. aegypti* revealed a series of increasing deleterious effects on both the physiological and reproductive fitness of exposed population of *Ae. aegypti* with consequent reduction of general fitness.

Present studies demonstrated larvicidal efficacy of α-cypermethrin against *Ae. aegypti.* Alpha-cypermethrin has been investigated against different species of *Aedes, Culex* and *Anopheles* in various parts of the world. It was found efficient to prevent colonization of water-containing receptacles by larvae of mosquito species (Pettit *et al.,* 2010). In India, indoor spraying of α-cypermethrin WP @100 mg a.i./m^2 significantly reduced density of *An. subpictus* and *Cx. quinquefasciatus* and showed residual efficacy of 18–27 weeks on different surfaces (Amalraj *et al.,* 1987).

Utilisation of pyrethroids, nevertheless has induced resistance in mosquitoes making their management difficult (Samal and Kumar, 2020). Hence, comparative investigations of life parameters of susceptible and pyrethroid-exposed population of *Ae. aegypti* could help to strategize the control interventions. The mosquito larvae exhibit higher susceptibility to insecticide stress, predation, and even destruction of their habitat; in comparison to the adults. As increase in longevity enhances the number and frequency of blood meals and fecundity, survival is considered a major and important component of mosquito fitness (Charlwood, 2004).

The reports suggest that In the absence of insecticide applications, resistant alleles can cost energy, growth and physiological fitness under reduced insecticide applications when compared to their susceptible counterparts (Alvarez-Gonzalez *et al*., 2017). Insecticide resistance-based fitness cost in *Cx. quinquefasciatus* has been found either due to the laboratory selection or conducting backcrosses with laboratory strains to produce lineages differing only in the resistance traits (Berticat *et al*., 2008; Melo-Santos *et al*., 2010; Brito *et al*., 2013; Jaramillo *et al*., 2014; Alvarez-Gonzalez *et al*., 2017). This approach perhaps measures variations in the insecticide resistance-based fitness parameters of mosquitoes more accurately than the genetic differences. However, loss of genetic variability due to intense inbreeding may not reflect the resistance features in the field (Kliot and Ghanim, 2012).

Investigations regarding fitness cost, or life history, are generally performed through comparisons of biological parameters, such as developmental kinetics, fecundity, or even growing rates, under controlled laboratory conditions (Foster *et al*., 2003; Ffrench-Constant and Bass, 2017). The present study demonstrated the several life-traits of susceptible and α- cypermethrin exposed population of *Ae. aegypti*. The exposed strains showed decreased fecundity, egg hatch and adult emergence. The malformation in the development pattern and diminished reproductive fitness observed in the exposed strain of *Ae. aegypti* indicated fitness cost induced by α- cypermethrin stress. Rigby *et al.* (2020) observed increase in the development duration, reduced adult emergence and a shorter average lifespan in pyrethroid -resistant *Ae. aegypti* in comparison to the susceptible strain. Similar delay in the larval development, decreased longevity and a reduced fecundity in a field population; strongly resistant to temephos (RR_{95} > 200) was reported by Diniz *et al*. (2015). On the contrary, field populations of *Ae. aegypti*, in Brazil, exhibiting temephos RR_{95} in the range of 7.4 to 19.2 showed lower reproductive fitness than susceptible population with reduction in size of blood meal, oviposition index, and fecundity (Belinato *et al*., 2012).

Mebrahtu *et al*. (1997) recorded lower rate of insemination and lower fecundity in pyrethroid-resistant *Ae. aegypti* females than the susceptible females. The reduced fitness in resistant strains may be attributed to the diversion of energy resources from the fecundity and oviposition to elevated production of detoxifying enzymes providing them survival advantage under insecticide selection pressure (Rivero *et al*., 2010; Kliot and Ghanim, 2012).

Present investigation also reported a significantly prolonged development phase in exposed larvae (E) compared to the susceptible generation (S). Likewise, prolonged development period in a pyrethroid/DDT-resistant strain of *Ae. aegypti* was observed in Thailand (Saingamsook *et al.,* 2019). They also observed shorter wing length, diminished egg hatch and viability and reduced lifespan in the resistant population. According to Berticat *et al*. (2004), a prolonged larval phase in the natural environment would expose them more to the risk of predation, reduced breeding sites and xenobiotics stress representing an adaptive disadvantage and consequently, reduced number of generations.

Conclusion

Impact of insecticide selections on the fitness costs of mosquitoes can be useful while evaluating specific traits under laboratory conditions. However, these results may not correspond under the environmental conditions where the insect is under multifarious stress. Nevertheless, reductions in adult longevity can not only affect virus transmission substantially but also decrease gonotrophic cycles and fecundity resulting in reduced vector abundance. The study provides baseline information for better understanding of population dynamics and designing appropriate strategies for mosquito management.

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Conflict of interest

The authors declare that they have no conflict of interest.

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