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Susceptibility status of *Aedes aegypti* (Diptera: Culicidae) to insecticides in Rajkot Urban Region, India

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Abstract
Background: Dengue is a significant global health concern, with India experiencing a marked increase in the number of cases. The primary vector, *Aedes aegypti*, has developed resistance to insecticides, undermining the efficacy of control programs.
Methods: This study evaluated the susceptibility of *Aedes aegypti* populations in Rajkot, India to commonly used larvicides and adulticides. Bioassays were conducted using field-collected larvae against Abate (Temephos) and Baytex 1000 (Fenthion), and adult mosquitoes were tested for Deltamethrin and Malathion. Commercial fumigants containing Cyfluthrin and Transfluthrin were also evaluated.
Results: The results showed that the mosquito population had significant resistance to the recommended field dosages of both organophosphate larvicides, Abate and Baytex 1000. Adult mosquitoes showed high susceptibility to deltamethrin (100% mortality) but high resistance to malathion (61% mortality). Commercial fumigants were largely ineffective, with a mortality rate of 65 %after 4 h.
Conclusion: The study concluded that the *Aedes aegypti* population in urban Rajkot has developed resistance to key organophosphate larvicides and malathion. These findings underscore the need to continuously monitor insecticide resistance and regularly change the insecticides used. This will help in the effective and sustainable control of pests.

Keywords: *Aedes aegypti*, insecticide susceptibility, resistance, larvicide, percentage mortality

Introduction

Dengue is a viral disease transmitted through the bite of an Aedes mosquito carrying the virus and represents a major health concern globally, impacting over 128 countries. According to the World Health Organization (WHO), there are an estimated 390 million cases of dengue annually. The incidence of this disease has increased by more than 30-fold globally over the last five decades, with epidemiological data from India showing a progressive increase in reported cases. For example, 1,24,493 cases were reported in 2018, which is expected to increase to 2,33,251 cases by 2022. Despite this increase, the case fatality rate (CFR) has declined substantially from 3.3% in 1996 to 0.1% since 2019, which has been consistently maintained (NCVBDC 2023).

The dengue virus (DENV) is a member of the Flaviviridae family and comprises four distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4 (WHO, 2022). These serotypes are primarily transmitted by Aedes mosquitoes and are responsible for a range of clinical presentations, from mild dengue fever to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Kariyawasam *et al*., 2023; Park *et al*., 2022) [8, 13]. The increasing number of dengue cases, exceeding 400 million annually, highlights the critical demand for effective vaccines and treatments (Kariyawasam *et al*., 2023; Pandey *et al*., 2008) [8, 12]. Disease transmission is a multifaceted process influenced by the presence of viruses, vectors, and vulnerable hosts. Additionally, it is worsened by environmental and societal factors, including high population density, unplanned urban growth, insufficient water supply, inadequate waste management and shifting climatic conditions. For instance, studies in Pakistan have shown a strong positive correlation between high rainfall, humidity, and temperature and increased dengue incidence (Association of Dengue Case Load and Environmental Factors in Four Potentially Disease Risk Areas of Pakistan, 2023) [2].

Aedes aegypti is consistently known as the principal dengue vector in India, with high infestation rates and a significant role in disease transmission across various regions (Reegan *et al*., 2018; Ramliana *et al*., 2024) [15, 17]. Although *Aedes albopictus* is recognized as a secondary vector, its prevalence is generally lower than that of *A. aegypti* in most areas (Sarkar *et al*., 2024) [19]. Urban areas provide ideal habitats for mosquito breeding because of their high population density and inadequate waste management, creating hotspots for *Aedes aegypti* and increasing the risk of diseases such as Dengue and Chikungunya, also transmit other arboviruses, including Chikungunya and Zika (Evans *et al*., 2022; Sushma *et al*., 2025) [6, 22]. *A. aegypti* breeds primarily in artificial containers, such as discarded containers and tires, which are less common in rural environments (Zahouli *et al*., 2017; Tauil, 2001) [27]. this is closely associated with human habitation and thriving year-round in urban areas (Dalpadado *et al*., 2022; Tandon & Ray, 2000) [5, 23]. In contrast, *A. albopictus* prefers natural water collections, such as tree holes and bamboo stumps, and is more prevalent in outdoor environments, indicating an adaptation to less disturbed habitats (Ray & Tandon, 1999; Dalpadado *et al*., 2022) [5, 16].

In the absence of a promising vaccine or effective antiviral agent for dengue, vector control strategies are crucial for managing disease transmission (WHO, 2025). These measures include source reduction, which involves removing or covering the water storage containers (Bhatt *et al*., 2013; Lim *et al*., 2025) [3, 10]. When source reduction is not feasible, insecticides approved by the national control programs are used. Insecticides have historically been the

cornerstone of vector-control strategies. However, the extensive use of chemicals, such as DDT and malathion, has led to significant resistance among mosquito populations in India, complicating disease management efforts (Shroff *et al*., 2020) [20]. Despite the ban on many organochlorines owing to their toxicity, DDT is still used for public health purposes, particularly in malaria control, under specific conditions. This heavy reliance on chemical insecticides has resulted in widespread resistance patterns, highlighting the urgent need to re-evaluate the current strategies (Shroff *et al*., 2020) [20]. India is increasingly promoting alternatives such as Long-Lasting Insecticidal Nets (LLINs) and biopesticides to reduce its dependence on DDT (Kumari & Swamy, 2024) [9].

Regular monitoring of insecticide resistance is crucial for maintaining the efficacy and flexibility of vector control methods (Raghavendra *et al*., 2017) [14]. Although there is no specific control strategy for dengue vectors, various methods and insecticides used in urban malaria schemes have been applied to control Aedes mosquitoes. These include temephos, *Bacillus thuringiensis* (Bti), Insect Growth Regulators (IGRs), and space spraying or thermal fogging (Sushma *et al*., 2025) [22]. Temephos, an organophosphate compound, has been used in public health programmes since the 1980s. Although studies have shown that Aedes larvae are susceptible to temephos (0.02 mg/L) and that adult mosquitoes are generally susceptible to malathion, the emergence of resistance in certain populations underscores the critical need for ongoing susceptibility testing (Sivan *et al*., 2015; Boyer *et al*., 2022) [4, 21]. Insecticide resistance, driven by the overuse and misuse of chemical agents, poses a serious threat to the efficacy of vector control programs and can lead to persistent mosquito populations and an increased risk of disease outbreaks. Understanding the resistance status of local mosquito populations is a prerequisite for developing suitable and effective vector control programs (Haidy *et al*., 2025). Therefore, this study aimed to evaluate the susceptibility of *Aedes aegypti* to insecticides in an urban region of Rajkot, India.

Materials and Methods

Larvae of the 3rd and 4th instar stages and adults were randomly collected from different locations in Rajkot City. Larvae were separated according to their stages, and pupae were reared into adults for identification using a standard identification key. and were used as the materials. Baytex 1000 (Fenthion 82.5% EC), Abate (Temephos 50% EC) larvicides, malethion 5%, deltamethrin 0.05%, and a commercially used insecticide containing cyfluthrin (0.025%, transfluthrin 0.04%) were selected for the test.

1. Bioassay of Larvae (The Eillot Larval Test)

Stock solutions of Baytex 1000 (Fenthion 82.5% EC) and Abate (Temephos 50% EC) were prepared in absolute alcohol at concentrations of 1, 0.1, 0.01, and 0.001%. A range of working concentrations (200, 100, 50, 10, 1, 0.1, and 0.01 mg/L) were subsequently prepared from these stock solutions.

Transparent plastic polymer cups, each with a capacity exceeding 100 ml, were used to conduct the bioassay. The experimental setup included three distinct groups: exposure set, cups containing larvicide concentrations for larval exposure; recovery set, cups containing water without larvicide for post-exposure observation; and control set, cups with untreated water to serve as a baseline.

A standardized diet of crushed biscuits and yeast powder was provided to the larvae in all sets. One-milliliter graduated pipettes with micropipette fillers were used to prepare the solutions, with one pipette designated for each solution. Larval transfer was conducted using small pieces of cotton mesh cloth, with a separate piece used for each larvicidal concentration.

1.1 Procedure

Each cup of the recovery set contained twenty-five larvae. Each cup in the exposure set received a pre-mixed larvicide dilution. After that, the larvae were moved from the recovery set to the exposure set.

Each concentration was given to larvae for one hour. Each batch of larvae was moved to the recovery set after exposure, and the initial observation was noted. A second observation was made after the larvae had been left undisturbed for five hours. After a day, a third observation was noted. Levels of susceptibility, tolerance, and resistance were determined by calculating mortality as a percentage. Plotting the collected data on a logarithmic scale allowed for the estimation of LC_{50} and LC_{98} values.

1.2 Bioassay of Adult

The experiment was conducted using a standard WHO (1995) susceptibility test kit provided by the Malaria Department of the Rajkot Municipal Corporation. The kit includes an instrument with two clear, detachable plastic tubes: a holding tube and an exposure tube, separated by a sliding plastic plate. One side of the plate contained a hole, whereas the other side did not contain a hole. The test used pre-prepared paper with insecticide solutions of malathion (5 %) and deltamethrin (0.05 %).

Twenty-five field-collected, blood-fed, and taxonomically identified mosquitoes were introduced into a holding tube. Throughout all stages of the experiment, mosquitoes were provided with a 10% glucose solution via cotton soaked in the solution. Filter paper impregnated with the selected insecticide solution was placed inside the exposure tube. Mosquitoes were transferred from the holding tube to the exposure tube by sliding the intermediate partition separating the two tubes. A control set, without insecticide-impregnated paper, was run simultaneously. The experiment was conducted with four replicates under identical conditions.

Mosquitoes were exposed to the insecticide-treated paper in the exposure tube for a period of 1 hour. After exposure, the mosquitoes were transferred back to the holding tube, and the first mortality observation was recorded. A second observation was recorded after 24 hours.

Mortality was calculated as a percentage, and the mean mortality was determined using the following formula:

$$\textit{Average mortality} = \frac{\text{Total mortality}}{\text{Total number of replications}}$$

2. Bioassay Test For Fumigant Effect Of Commercially Used Insecticides On Adult Mosquitoes

An adult mosquito bioassay was conducted in a rectangular metallic cage measuring 4 × 15 × 20 cm. The study used commercially available insecticide spray solutions containing cyfluthrin 0.025% and transfluthrin 0.04% for the tests.

2.1 Procedure

Number of mosquitoes per cage: Twenty-five blood-fed mosquitoes of the target species were introduced into each test cage. Subsequently, the cage was placed in a designated area for the insecticide efficacy trials.

Positions of cages: The cages were suspended in a 10 × 10 ft room. The cages were suspended at two levels, 50 cm from the walls. The other cage was suspended in the middle of the room for comparison.

Time of exposure: The insecticide was sprayed in the room, and the cages containing the mosquitoes were exposed for 4 h. At the end of the exposure period, the mosquitoes were transferred to clean cages and provided with 10% glucose solution on cotton wool for 24 h.

Reading of mortality: Mortality was measured by counting the number of dead and knocked down mosquitoes at the end of the initial exposure period and after 24 h. The final results were calculated as a percentage of the total number of captured mosquitoes.

Results

1. Larvicidal bioassay

Aedes aegypti larvae were exposed to varying concentrations of abate (Temephos 50% EC) and Baytex 1000 (Fenthion 82.5% EC). Achieving optimal larval mortality was inversely proportional to the exposure duration, indicating that a longer exposure period required a lower larvicide concentration. Conversely, a higher dose was required to achieve maximum larval mortality within a shorter time frame. A critical finding was the development of resistance in mosquito larvae to field-recommended dosages of both larvicides.

1.1 *Aedes aegypti* - Abate (Temephos 50% EC)

This was observed in the case of *Ae. aegypti* (Table.2) after exposure for 1 h, and an average concentration of more than 200 mg/l of average concentration is required to kill 100% of the larvae of this species in 1 h. The average tolerance of *Ae. aegypti* was 51-200 mg/l and larvae of this species showed an average resistance of 0.01-50 mg/l concentrations (Table.1).

Table 1: Dose response of *Ae. aegypti* larval mosquito species against *Abate (Temephos 50% EC)*

***Ae. aegypti* larval against Abate (Temephos 50% EC)**			
Time Duration In Hours	**Level in mg/l**		
	Susceptibility	**Tolerance**	**Resistance**
1	>200	51-200	0.01-50
5	146-200	0.1-145	0.01-0.09
24	5-200	0.04-4.9	0.01-0.03

The Lc_{50} and Lc_{98} values for *Ae. aegypti* were 0.019 and >200 mg/l, respectively (Table. 2), respectively. Five hours after exposure, the average susceptibility level was 146-200 mg/l, the average tolerance level was 0.1-145 mg/l and the average resistance level was 0.01-0.09 mg/l (Table. 1). Lc_{50} and Lc_{98} values after five hours of exposure to *Ae. aegypti* were 0.007 mg/L and 146 mg/l, respectively (Table.2). Twenty-four hours after exposure, the average susceptibility level was 5-200 mg/l, the average tolerance level was 0.04-4.9 mg/l and the average resistance level was 0.01-0.03 mg/l (Table. 1). The Lc_{50} and Lc_{98} values were 0.004 and 6 mg/l, respectively (Table. 2).

Table 2: Larvicidal action of Abate and Baytex 1000 on *Ae. aegypti* mosquito species

Con. (stock)/100ml	**Mortality (%)**					
	Larvicidal action of Abate (Temephos 50% EC			**Larvicidal action of Baytex 1000 (Fenthion 82.5% EC)**		
	1h	5hrs	24hrs	1h	5hrs	24hrs
Control 0(0ml)	0	0	0	0	0	0
200(2ml)	90	100	100	85	100	100
100(1ml)	85	95	100	83	98	100
50(0.5ml)	80	93	100	73	85	100
10(0.1ml)	78	87	100	70	83	98
1(0.1ml)	73	83	95	63	78	93
0.1(0.1ml)	68	80	90	57	70	87
0.0375*(0.1ml)	50	68	80	48	65	77
0.01(0.1ml)	48	60	78	45	63	76
Lc_{50}	0.019	0.007	0.004	0.072	0.006	0.004
Lc_{98}	>200	68	6	>200	100	10

* Dosage levels recommended by larvicide producers.

1.2 *Aedes aegypti* - Baytex 1000 (Fenthion 82.5% EC)

This was observed in the case of *Ae. aegypti* (Table. 2) After exposure for 1 h, an average concentration of more than 200 mg/l of the average concentration is required to kill 100% of the larvae of this species in 1 h. The average tolerance of *Ae. aegypti* was 80-200 mg/l and the larvae of this species showed an average resistance of 0.01-97.9 mg/l concentrations (Table. 3). The Lc_{50} and Lc_{98} values for *Ae. aegypti* were 0.072 mg/L and >200 mg/l (Table.2), respectively Five hours after exposure, the average susceptibility level was 100-200 mg/l, the average tolerance level was 3.0-99.9 mg/l and the average resistance level was 0.01-2.9 mg/l (Table.3). Lc_{50} and Lc_{98} values after five hours of exposure to *Ae. aegypti* were 0.006 mg/L and 100 mg/l, respectively (Table.2). Twenty-four hours after exposure, the average susceptibility level was 10-200 mg/l, the average tolerance level was 0.08-9.9 mg/l and the average resistance level was 0.01-0.07 mg/l (Table. 3). The Lc_{50} and Lc_{98} values were 0.004 and 10 mg/l, respectively (Table.2).

Table 3: Dose response of *Ae. aegypti* larval mosquito species against Baytex 1000 (Fenthion 82.5% EC)

***Ae. aegypti* larval against Baytex 1000 (Fenthion 82.5% EC)**			
Time Duration In Hours	**Level in mg/l**		
	Susceptibility	Tolerance	Resistance
1	>200	80-200	0.01-97.9
5	100-200	3-99.9	0.01-2.9
24	10-200	0.08-9.9	0.01-0.07

2. Adult bioassay Test

Field-collected mosquitoes *Ae. aegypti* when exposed to the selected insecticides, deltamethrin 0.05% and malathion 5%, the following results were obtained. *Ae. aegypti* mosquitoes were more sensitive to deltamethrin 0.05% than to 5% malathion

Ae. aegypti exhibited tolerance 1 hour post-exposure, with a mortality rate of 97% against 0.05% deltamethrin. Twenty-four hours after exposure, *Ae. aegypti* was found at the susceptible level, with 100% mortality against deltamethrin 0.05%. *Aegypti* was resistant to malathion at a given dosage. The resistance level of *Ae. aegypti* with 42% mortality after 1 h of exposure to malathion 5%. Twenty-four hours after exposure, *Ae. aegypti* showed a similar level of resistance, with 61% mortality against malathion 5% (Table. 4).

Table 4: Dose response of selected adult *Ae. aegypti* against insecticides after 1 & 24 hr

adult *Ae. aegypti* against insecticides			
Deltmethrin 0.05%		Malathion 5%	
1 hr	24 hr	1 hr	24 hr
97 T	100 S	42 R	61 R

*S=Susceptible, T=Tolerent, R=Resistant

3. Effectiveness of Commercially used insecticide

Caged *Ae. aegypti* When exposed to a room area treated with a fumigant insecticide containing cyfluthrin (0.025%) and transfluthrin (0.04%), the following results were recorded: The average mortality 4 h after exposure to *Ae. aegypti* in all three cages were 63, 67%, and 65%, respectively. Twenty-four hours after exposure, the average resistance recorded in Cage I was 80%, and the average tolerances in Cages II and III were 87% and 83%, respectively.

Table 5: Effect of commercially used insecticides on *Ae. Aegypt*

Insecticide containing (Cyfluthrin 0.025%, Transfluthrin 0.04%) on *Ae. Aegypti*			
Time Duration In Hours	**Cage Numbers**		
	I	**II**	**III**
	Mortality %		
4	63 R	67 R	65 R
24	80 R	87 T	83 T

*S=Susceptible, T=Tolerent, R=Resistant

Discussion

In the present study, different chemical larvicides and insecticides showed different effects on mosquitoes. *Aedes aegypti* larvae and adults exhibited different reactions to certain larvicides and insecticides. These differences in response encompass susceptibility, tolerance, and resistance to stress. The selected vector mosquito species, *Ae. aegypti* are developing resistance to field-applied larvicides and insecticides in the Rajkot region. Insecticide resistance and cross-resistance among mosquito species, particularly vectors, are well-known problems that hamper sustainable and cost-effective vector control (Ansari, 2004) [1].

Larvicidal efficacy was assessed 24 h post-treatment, with optimal efficacy observed at lower concentration. However, achieving a high mortality rate in a short timeframe necessitates a higher dosage, which poses environmental hazards to non-target organisms. The study revealed that *Aedes aegypti* larvae developed resistance to the recommended field application doses of Abate (Temephos 50% EC) and Baytex 1000 (Fenthion 82.5% EC), indicating a serious issue of insecticide resistance in the study area. Furthermore, a comparative analysis showed that Abate was more effective than Baytex 1000 in Rajkot City, suggesting that it may provide superior results for field-based larvicide applications.

Insecticide applications in Rajkot City are primarily conducted through fuming. In this study, exposure of field-collected *Aedes aegypti* to deltamethrin 0.05% and malathion 5% demonstrated that deltamethrin was the most effective insecticide. Compared with the findings of Vyas (2008) [24] on *Anopheles stephensi* and *Culex quinquefasciatus*, this study revealed that the sensitivity of Anopheles and Aedes to the selected insecticides was greater than that of Culex. This variation is attributed to resting behavior, as Anopheles and Aedes primarily rest indoors, whereas *Culex* rests in both indoor and outdoor environments. Higher exposure of Culex to insecticide residues has led to the development of resistance and increased tolerance capacity, thereby reducing their susceptibility to insecticides compared to *that of Anopheles stephensi* and *Aedes aegypti*.

Commercially available insecticides that provide a fumigant effect and contain cyfluthrin (0.025%) or transfluthrin (0.04%) have shown poor efficacy. However, the effectiveness of this insecticide on vector mosquitoes in Rajkot is poor. mosquito species *Ae. aegypti* were resistant after 4 h and tolerant after 24 h of exposure to the given dose. The present study compared two species of Vyas (2008) [24], which were studied as *Culex. Quinquefasciatus* was more resistant than *Anopheles spp. stephensi* and *Ae.*

Aegypti. It can be mentioned that the capacity of developing resistance in *Cx. quinquefasciatus* was higher than that of other selected vector mosquito species.

Conclusion

The present study revealed that the *Aedes aegypti* population in Rajkot has become resistant to common insecticides to varying extents. In particular, larval populations were resistant to the suggested field dosages of Baytex 1000 (Fenthion 82.5% EC) and Abate (Temephos 50% EC), which require higher and potentially dangerous concentrations for efficient control. Adult mosquitoes, a promising substitute for adulticidal control, demonstrated notable resistance to malathion but remained extremely vulnerable to deltamethrin. Furthermore, commercially available pyrethroid-based fumigants, including transfluthrin and cyfluthrin, were ineffective. For urban mosquito vector control programs to remain effective over the long term, these findings highlight the vital necessity of ongoing monitoring of insecticide resistance and deliberate rotation of insecticide classes.

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Author Contributions: SMV conducted the experiments, collected the data, and performed the primary review of the manuscript.

SKC contributed to manuscript review, data analysis, and equipment operation.

DRP Drafted the manuscript.

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