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Susceptibility status of *Aedes aegypti* larvae against temephos 50% EC in South Zone, Delhi, India

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Abstract
Introduction: Dengue, a major re-emerging mosquito-borne viral disease, continues to pose a serious public health threat in India, including Delhi. With no effective vaccine or antiviral treatment available, vector control particularly the use of insecticides like Temephos remains the primary prevention strategy. However, repeated use of the same insecticides has led to growing concerns about resistance in *Aedes aegypti* mosquitoes.
Method: A door-to-door entomological survey conducted in South Zone, Delhi during July 2025 to assess the susceptibility of *Aedes aegypti* larvae against Temephos using WHO protocols. Larvae collected from various household containers were exposed to four concentrations of Temephos, with mortality recorded after 24 hours. Probit analysis revealed reduced susceptibility in field populations, highlighting the need for regular monitoring and alternative control strategies.
Results: The bioassay results show that *Aedes aegypti* larvae from South Zone, Delhi exhibited varying levels of resistance against Temephos. At lower concentrations (0.002-0.004 mg/L), mortality remained below the WHO threshold, confirming resistance, while higher doses (0.008 mg/L) showed possible resistance. Complete susceptibility was observed only at 0.012 mg/L, with LC50 and LC90 values calculated as 0.0022 mg/L and 0.0058 mg/L, respectively.
Conclusion: This study from eight selected localities of South Zone, Delhi reveals reduced susceptibility of *Aedes aegypti* larvae against Temephos, with resistance detected at lower concentrations and susceptibility restored only at higher doses. Continuous use of Temephos under the Urban Malaria Scheme (UMS) for over two decades appears to be driving this trend. The findings emphasize the need for routine resistance monitoring, rotational use of alternative larvicides, and stronger Integrated Vector Management (IVM) practices. Regular surveillance and timely policy adjustments are essential to sustain the effectiveness of dengue control programs and protect public health.

Key words: *Aedes aegypti*, larval susceptibility, resistance, larvicide, percent mortality

Introduction
Dengue is recognized as one of the most serious re-emerging viral diseases spread by *Aedes* mosquito species. Each year, close to 390 million new infections are estimated to occur worldwide [1]. The illness is caused by the dengue virus (DENV), which has four serotypes (DENV-1 to DENV-4), and it is considered one of the most important arboviral diseases in tropical and subtropical parts of the world [2]. Over the past five decades, the number of dengue cases has steadily increased [3]. Still, a large portion of those infected do not show clear symptoms or remain only mildly ill [4]. Because of this, the number of officially reported cases is often much lower than the actual infections in the community.
The spread of dengue has expanded rapidly and today it is endemic in 129 countries across Africa, the Americas, the Eastern Mediterranean, South-East Asia, and the Western Pacific regions identified by the World Health Organization (WHO). South-East Asia Region, except the Democratic People's Republic of Korea, are considered endemic for dengue [5]. Within India, every state and Union Territory, apart from Ladakh and Lakshadweep, has reported both cases and deaths due to this disease [6]. Each year, hundreds of thousands of people develop

severe forms of dengue, and about 20,000 deaths are recorded. The disease also contributes significantly to the loss of health, with around 264 disability-adjusted life years (DALYs) lost per million population [7].

Aedes aegypti is mainly a domestic and peri-domestic breeder, preferring to lay eggs in clean water found in man-made containers. On the other hand, *Aedes albopictus* is more commonly associated with natural breeding sites such as tree holes, though it is also reported from peri-domestic containers and discarded tyres for breeding [8]. Since there is no effective vaccine available for treatment and prevention of dengue control [9]. The most successful of these strategies is thought to be source reduction, which involves covering or removing water storage containers to stop breeding. When this is not possible, pesticides are used in accordance with WHO protocol [10].

Over the years, various groups of insecticides have been widely used in NCVBDC in India. These include organochlorines such as Dichloro- diphenyl trichloroethane (DDT), organophosphates like malathion and, pyrethroids. While organochlorines are largely banned for agricultural purposes by the Environmental Protection Agency (EPA) because of their high toxicity to non-target organisms, they continue to be used in public health campaigns [11]. Other control tools employed under the Urban Malaria Scheme which also help in reducing *Aedes* populations include Temephos and space spraying or thermal fogging.

Temephos, an organophosphate larvicide, has been a key component of public health programs since the 1980s. Research has shown that *Aedes* larvae are susceptible to Temephos at a concentration of 0.02 mg/L. Similarly, adult mosquitoes remain sensitive to malathion, though some tolerance has been reported. When applied at WHO-recommended dosages, these insecticides remain cost-effective and useful in controlling dengue and dengue haemorrhagic fever (DHF) [12-13].

A major challenge, however, is insecticide resistance. Resistance tends to develop when the same chemical is used repeatedly in a given mosquito population. In some cases, resistance has even been recorded against insecticides that are now excluded from public health programs [14-16]. Such resistance is a growing concern as it can reduce the effectiveness of mosquito control strategies, particularly in urban environments where outbreaks are common.

Delhi, one of India's largest metropolitan cities has faced repeated dengue outbreaks in recent years [17]. There is favourable habitat for *Aedes aegypti* in and around National Capital Region (NCR), which may be affecting the susceptibility of local mosquito populations to insecticides.

The present larval susceptibility study that was conducted in July 2025, was designed to evaluate the susceptibility status i.e. resistant, possible resistant and susceptible of *Aedes aegypti* populations in selected localities of South Zone, Delhi against Temephos 50% EC, which is widely used in public health programme. No current baseline data or studies are available for susceptibility status against dengue vectors in selected localities of south zone, Delhi and these findings will provide baseline data for future planning and preparation of vector control strategies by the programme.

Material & methods

2.1 Study Area: A door-to-door entomological survey was carried out in eight selected localities of the South Zone, Delhi, with informed consent from household owners. Larval sampling was performed using flashlights along with dipping and pipetting techniques, following WHO guidelines. The study sites were chosen on the basis of reported dengue cases in previous years and socio-economic diversity of the society including two government institutions i.e. Indira Gandhi National Open University, Maidan Garhi, and the Indian Institute of Technology Delhi, Hauz Khas, two high-income residential areas (Asian Games Village Complex, Siri Fort, and Vasant Kunj), two urban villages (Munirka and Saidulajab), and two slum settlements (Bengali Camp, Masoodpur, and Parvatiya Camp, Sector-4, R.K. Puram) [Fig. 1]. Mapping of localities was done with the help of QGIS and DIVA GIS.

The larval susceptibility bioassay was designed to evaluate the susceptibility status of *Ae. aegypti* populations from eight localities within the South Zone of Delhi against Temephos 50% EC as per the World Health Organization (WHO) guidelines [18].

Immature stages of *Aedes* larvae were collected from various domestic and peri-domestic water-holding containers, including plastic containers, earthen pots, flower pots, coolers, metal containers, discarded tyres, cement cisterns, water tanks and others. Only late third-instar larvae were selected to ensure uniformity in age and developmental stage, as larval size and physiological status influence susceptibility results [19]. The study was conducted at the School of Life Sciences, Indira Gandhi National Open University, Maidan Garhi, Delhi, India.

2.2 Preparation of Stock and Test Solutions

Temephos 50% EC (commercial name Paraguard) was used as per the WHO standard protocol. A stock solution of 250 mg/L was prepared by adding 0.5 mL (500 µL) of the formulation to 1 litre of distilled water. Four test concentrations were prepared from this stock solution i.e. 0.002 mg/L, 0.004 mg/L, 0.008 mg/L, and 0.012 mg/L. For each concentration, the required volume of stock solution was added to 250 mL of distilled water in glass beakers i.e. 2µL, 4 L, 8 µL, and 12 µL respectively (Table 1).

2.3 Larval Bioassay

For each concentration, four replicates were set up with 25 larvae each, totalling 100 larvae per concentration along with 25 larvae in control i.e. normal water. The total number of larvae used across all treatments and controls was 500. A total of 500 larvae were utilized in all tests including control.

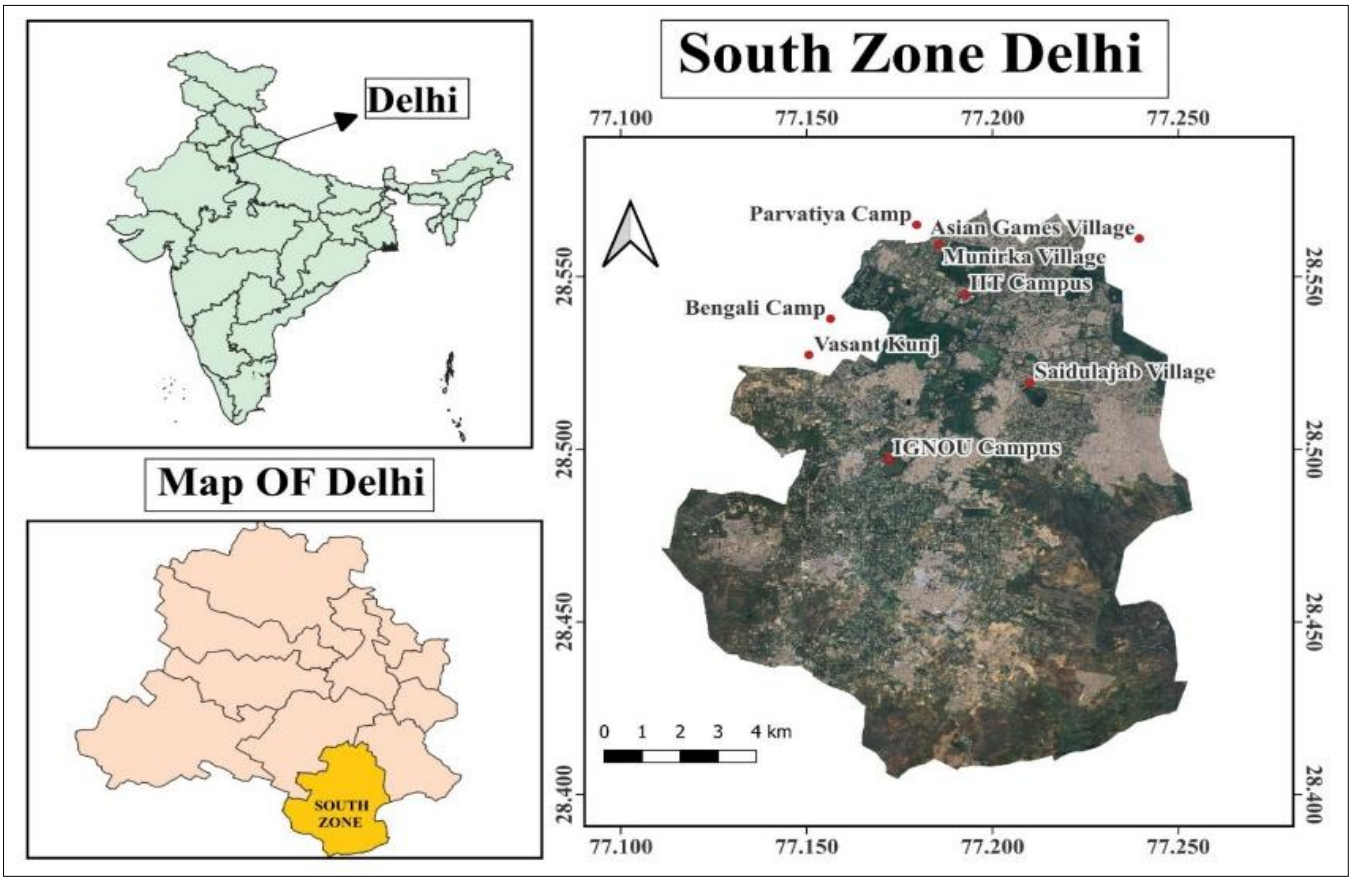

Fig 1: Map showing the study sites in eight selected localities of South Zone, Delhi for collection of *Aedes* larvae.

Table 1: Preparation of different concentrations of Temephos 50% EC used for dose-mortality response analysis.

Test Dose	Final Concentration (mg/L)	Temephos Required in 250 mL (mg)	Microliters (µL) of Stock A to Add
Test 1	0.002	0.0005	2 µL
Test 2	0.004	0.001	4 µL
Test 3	0.008	0.002	8 µL
Test 4	0.012	0.003	12 µL
Control	0.000	0.000	0 µL

The tests were conducted in laboratory at 25-27°C under ambient light conditions. Larvae were not fed during 24-hour exposure period. Mortality was recorded after 24 hours of exposure by gently prodding larvae with a fine brush; lack of movement was recorded as mortality.

Data Analysis

Mortality data were first corrected using Abbott's formula when control mortality ranged from 5-20% [20]. The formula used for correction is shown below:

$$\% \text{ Mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Observed mortality in control}} \times 100$$

In this case, control mortality was 1%, so no correction was needed. Resistance classification followed WHO criteria: >98% mortality = susceptible, 90-97% = possible resistance, and <90% = resistant. Probit analysis software was used to determine LC_{50} and LC_{90} values. Mortality percentages were plotted against log-transformed concentrations, and lethal concentration values were derived by interpolation.

Results: Table 2 & Figure 2 depict, the susceptibility status of *Aedes aegypti* larvae collected from selected localities of South Zone, Delhi as per WHO standard protocol. Mortality rates varied substantially across concentrations. At 0.002 mg/L, mortality was 41%, indicating confirmed resistance. At 0.004 mg/L, mortality increased to 84% but still was within the resistance range. At 0.008 mg/L, mortality reached 91%, suggesting possible resistance, and at 0.012 mg/L, complete mortality (100%) was achieved, classifying the population as susceptible at this highest dose.

Table-2: Dose -Mortality response of 3rd instar larvae of *Ae. aegypti* against Temephos 50% EC.

Sl.No	Concentrations (mg/L)	No of Larvae Exposed								Total test mortality	% of mortality	Interpretation
		Replicate 1		Replicate 2		Replicate 3		Replicate 4				
		Exposed	Dead	Exposed	Dead	Exposed	Dead	Exposed	Dead			
1.	0.002ppm	25	12	25	10	25	11	25	08	41	41%	Resistant
2.	0.004 ppm	25	22	25	20	25	19	25	23	84	84%	Resistant
3.	0.008ppm	25	21	25	23	25	24	25	23	91	91%	Possible Resistance
4.	0.012ppm	25	25	25	25	25	25	25	25	100	100%	Susceptible
5.	Control	25	0	25	0	25	0	25	01	01	1%	Susceptible

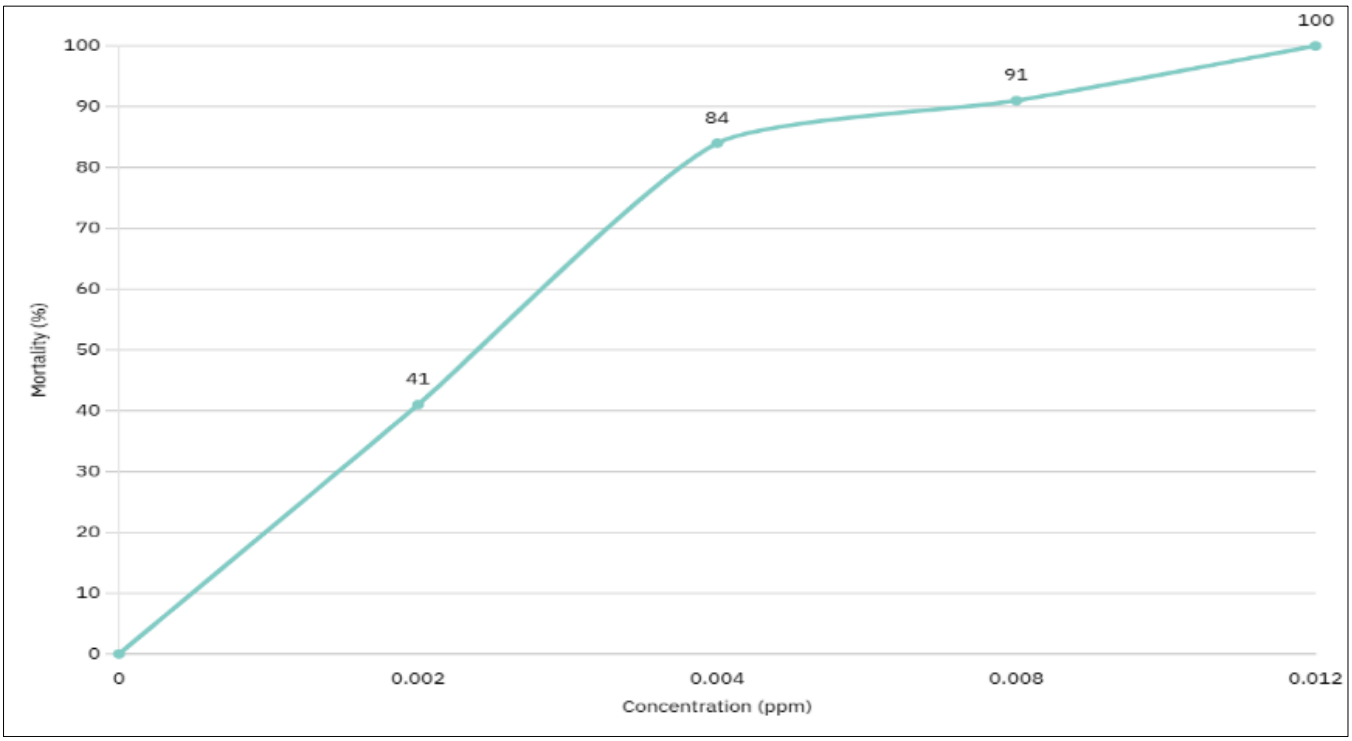

Fig 2: Probit regression curve showing observed and predicted mortality rates for different concentrations of Temephos 50% EC.

The lethal concentrations of Temephos required to cause 50% (LC_{50}) and 90% (LC_{90}) mortality of *Ae. aegypti* larvae collected from selected localities of South Zone, Delhi, were determined through probit analysis. In the larvicidal toxicity effects of Temephos at various concentrations against the dengue vector, *Ae. aegypti*, shows the highest mortality rate with LC_{50} and LC_{90} values corresponding to 0.0022 and 0.0058 mg/L respectively (Table 3). The larval mortality rate of *Ae. aegypti* increased with the increase in concentration of Temephos.

Table 3: Lethal Concentration of Temephos for 50% (LC_{50}) and 90% (LC_{90}) mortality of *Ae. aegypti* in study areas.

LC_{50} (ppm)	LC_{90} (ppm)	95% confidence limit		Chi square χ^2	Sig. P-value
		LCL LC50 range	UCL LC90 range		
0.0022	0.0058	0.0000035-0.003944	0.003-63.27	8.337	0.015

Control - nil mortality; within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT; LCL - lower confidence limit, UCL - upper confidence limit, *P<0.05level

Discussion

Earlier studies indicate that, *Ae. aegypti* population is susceptible against Temephos [21-22]. *Ae. aegypti* larvae were found highly sensitive against Temephos and Fenthion in Rajahmundry, Andhra Pradesh [23]. Similar findings were reported by Das *et al.* in Ranchi, Jharkhand, where both adult and larval stages of dengue vectors were evaluated against DDT, Malathion, Deltamethrin, and other insecticides, including Temephos and Fenthion [24]. Vasdev *et al.* also documented that, *Ae. aegypti* populations from Assam remained susceptible to Temephos, Fenthion, and Malathion [25]. However, laboratory-based studies have demonstrated that immature stages of *Ae. aegypti* can develop induced resistance to Temephos under controlled conditions [26]. Notably, resistance in *Ae. aegypti* against Temephos has already been reported from tropical countries, including Malaysia, Brazil,

Thailand, Cuba, and Venezuela [27-30].

The LC_{50} was calculated at 0.0022 mg/L, and the LC_{90} at 0.0058 mg/L. These values are consistent with resistance patterns documented in other metropolitan cities of Chennai, Kolkata, and Delhi, where prolonged use of Temephos in public health programs has been linked to reduced susceptibility [31]. The gradual increase in mortality with concentration reflects partial resistance, where some proportion of the population remains unaffected at doses near the diagnostic threshold.

The findings parallel reports from Brazil, Malaysia, and Cuba, where similar resistance profiles have emerged following sustained temephos usage [32 & 33]. Mechanistically, resistance in *Ae. aegypti* to organophosphates like Temephos is often associated with elevated esterase activity or mutations in target enzymes, reducing binding efficiency [34].

Conclusion

The susceptibility status data from eight surveyed localities in South Zone, Delhi, indicate a clear reduction in the susceptibility of *Ae. aegypti* populations against Temephos. Resistance was observed at lower concentrations (0.002 mg/L), and susceptibility was restored only at the highest tested dose (0.012 mg/L). The calculated LC_{50} and LC_{90} values emphasize the need to revisit the operational doses currently being used in Vector control programme. These results strongly highlight the importance of continuous monitoring of resistance and the need for proactive management strategies.

Routine bioassays should be made an integral part of vector control programs to identify early changes in susceptibility levels before they compromise control measures. Adopting a rotational use of larvicides with different modes of action, such as *Bacillus thuringiensis israelensis* (Bti) and insect growth regulators (IGRs), can reduce the risk of resistance development. In addition, Integrated Vector Management (IVM) approaches combining environmental management, biological methods, and the careful use of chemical insecticides should be prioritized. Sustained success also requires strong community participation through awareness campaigns, source reduction practices, and active collaboration between health authorities and local residents.

Globally, resistance to Temephos in *Ae. aegypti* has become an increasing concern, and the present findings from Delhi reflect this wider trend. If left unaddressed, resistance will likely intensify, making dengue control efforts less effective. The study also suggests that decades of continuous Temephos use under the Urban Malaria Scheme (UMS), where 50% EC or granular formulations have been routinely applied, may have contributed to this emerging resistance. Such evidence makes it necessary to carefully re-evaluate the continued effectiveness of Temephos in Delhi's vector control program.

The primary objective of insecticide susceptibility testing is to detect resistance in mosquito populations so that timely adjustments can be made whether by rescheduling applications, rotating insecticides, or replacing ineffective compounds with alternative larvicides. Therefore, regular nationwide surveillance programs are urgently needed to track susceptibility patterns in major mosquito vectors and to assess their implications for control strategies.

The outcomes of this study can play an important role in reformulating dengue control approaches in India. They provide a basis for considering modifications in the application schedule of Temephos or replacing it with safer and more effective alternatives. By using data-driven policies, strengthening resistance monitoring, and promoting integrated approaches, public health programs can maintain the effectiveness of vector control tools and continue to protect communities from dengue and related diseases.

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There is no source of funding for the study.

Conflict of interest

There is no conflict of interest.

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