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# Insecticide susceptibility status of *Aedes aegypti* and *Anopheles stephensi* larvae against temephos in Delhi, India

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**Abstract**
Temephos is used as a larvicide in urban areas in India to control the population of mosquito vectors viz. *Anopheles stephensi* and *Aedes aegypti*. The susceptibility status of *Ae. aegypti* and *An. stephensi* to temophos in various zones of Municipal Corporation of Delhi was evaluated using the WHO method for determining larval susceptibility test kit. Results revealed that the larval mortality of *Ae. aegypti* collected from different localities ranged between 64.88% to 98.22%. The highest mortality was recorded from Sangam Vihar (98.22%) and lowest was recorded from Majnu ka tila (64.88%). *Ae. aegypti* larvae collected from Sangam Vihar locality was found fully susceptible to temephos, from two localities viz. Uttam Nagar and Pitampura of study area were tolerant to temephos, and from five localities viz. Majnu ka tila, Shastri Park, Mayur Vihar II, Tilak Bridge and Nagal Dewat showed development of resistance against temephos at diagnostic concentrations. However, larval populations of *An. stephensi* were fully susceptible to temephos in all the localities. The present study indicates the possible development of resistance against temephos in the larvae of *Ae. aegypti* in some areas in Delhi.

**Keywords:** *Anopheles stephensi*, *Aedes aegypti*, insecticide susceptibility status and temephos.

## 1. Introduction
*Aedes aegypti* (Linn) and *Anopheles stephensi* (Liston) are the two important urban mosquito vectors of dengue fever and malaria in India. *Ae. aegypti* has been found responsible for the outbreak of dengue fever (DF) and dengue hemorrhagic fever (DHF) in many urban areas of the country [1-2]. In India, there were 75454 dengue cases with 167 deaths as reported by National Vector Borne Disease Control Programme (NVBDCP) in 2013 [3]. *An. stephensi* is recognized primarily as an urban malaria vector in India. During 2012, a total of 82400 malaria cases in 19 states were reported under Urban Malaria Scheme (UMS) [3].
Both, *Ae. aegypti,* the dengue vector and *An. stephensi,* malaria vector, are prevalent in urban areas and co-breeds predominantly in over head tanks and storage containers having clean water. *Ae. aegypti* is widely distributed in different parts of the Delhi city and plays a key role in transmission of dengue fever [4]. To control urban mosquito vectors, mainly *An. stephensi*, anti larval methods involving the use of larvicides such as temephos, *Bacillus sphaericus* (BS) and *Bacillus thuringiensis var israelensis* (Bti) are commonly used under Urban Malaria Scheme (UMS). Although there is no specific control strategy for the control of dengue vectors, temephos, Bti and space spraying/ thermal fogging etc. as used under UMS are also now recommended for the control of *Aedes* mosquitoes. The temephos, an organophosphate compound is being used under the public health programme since 1980s.
One of the major problems for effective vector control is development of resistance to existing insecticides in the vectors. Recently, a survey of *Aedes* breeding habitats in different localities in Delhi revealed the presence of *Ae. aegypti* breeding in some habitats which were treated with temephos granules. It provides a clue that there may be resistance. Therefore, a study was carried out to determine the insecticide susceptibility of *Ae. aegypti* and *An. stephensi* larvae to temephos from different localities in Delhi. This paper reports the susceptibility status of *Ae. aegypti* and *An. stephensi* larvae to temephos in Delhi.

## 2. Methodology
### 2.1 Larval collection and rearing of mosquitoes for larval susceptibility tests
Over head tanks and desert coolers in 21 different localities under eight zones of Municipal

Corporation of Delhi (MCD) were surveyed for the presence of *Ae. aegypti* and *An. stephensi* breeding (Figure 1). *Ae. aegypti* breeding was observed from all the 21 localities, while *An. stephensi* larvae were collected from five localities only. To determine larval susceptibility, samples of *Ae. aegypti* were collected from one locality in each zone of MCD, but *An. stephensi* larvae could be collected only from five localities one each in five zones (Table 1). Collected mosquito larvae were separated, washed and kept under observation for 24 hrs to remove dead and unhealthy larvae. The healthy *Ae. aegypti* larvae of 8 localities, one locality in each zone were reared separately to F1 generation to determine the susceptibility against temephos. Similarly, *An. stephensi* larvae collected from five localities in five zones of MCD were reared separately to F1 generation to determine the susceptibility against temephos. The culture of both species from different localities was maintained separately.

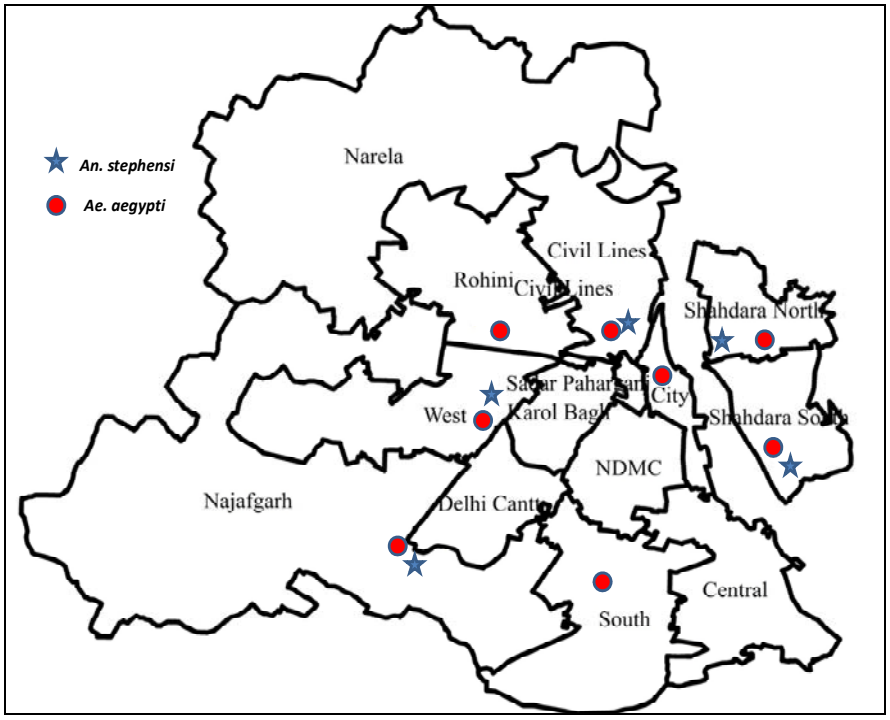


**Fig 1:** Map of Delhi showing study sites for mosquito larval collection in different MCD Zones

**2.2 Larval Bioassays**

Preliminarily larval bioassays were done on late III/early IV instar healthy larvae of *Ae. aegypti* and *An. stephensi* at WHO recommended diagnostic concentrations of temephos (0.02 mg/L for *Ae. aegypti* and 0.25 mg/L for *An. stephensi*) [5]. Experiments were performed by placing 25 larvae in a 500 ml capacity glass beaker containing 249 ml of water with 1 ml of temephos solution so as to get the final diagnostic concentrations. Three replicates for test and one for the control were used for larval bioassay at room temperature of 27±1 ºC and 70% humidity. *Ae. aegypti* larvae collected from all the localities showed less than 100% mortality at diagnostic concentrations of temephos while *An. stephensi* larvae from all the localities were fully susceptible. Further, larval bioassays experiments were done to determine the lethal concentrations of temephos for 50% ($LC_{50}$) and 90% ($LC_{90}$) mortality using dose-response mortalities bioassays against *Ae. aegypti* and *An. stephensi*.

A stock solution of 50 ppm dose was prepared by pipetting the appropriate standard temephos insecticide solution (50% EC) in ethanol. Stock solution was kept in a refrigerator until needed. Stock solution was appropriately diluted to make serial dilutions. From each of these serial dilutions 1 ml of the temephos solution was added to 249 ml of tap water taken in separate glass beakers to get final test concentrations. Each bioassay was done separately to determine the efficacy of temephos against late III/ early IV stages larvae of *An. stephensi* and *Ae. aegypti* by making various concentrations of temephos viz. 0.25 ppm, 0.125 ppm, 0.0625 ppm, 0.03125 ppm, 0.01562 ppm, 0.007812 ppm, 0.003906 ppm, 0.001953 ppm and 0.00097 ppm. Three replicates for each concentration and the control were used for larval bioassay as per WHO procedure [5]. Each of the larval bioassay was repeated thrice. 250 ml of each dilution was taken into a 500 ml glass beaker and 25 larvae of *An. stephensi* and *Ae. aegypti* were added separately. The experiments were conducted at room temperature of 27±1 ºC and 70% humidity. The larval mortality in each concentration and control was recorded after 24 hours of continuous exposure. The corrected mortality was determined using Abbott's formula whenever required [6]. The dose mortality data was analyzed by log-probit method of Finney [7] and lethal concentrations for 50% and 90% mortality were calculated by using the software SPSS (Statistical Product and Services Solutions) for windows.

**Table 1:** Table showing the different localities of MCD zones surveyed for *Aedes aegypti* and *Anopheles stephensi* larvae

| Name of MCD Zones | Localities surveyed for vector mosquito larvae | Place of *Ae. aegypti* mosquito larvae collection | Place of *An. stephensi* mosquito larvae collection |
|---|---|---|---|
| Civil Zone | Majnu ka tila, Rajpur Road, Sant Nagar, | Majnu ka tila | Majnu ka tila |
| Shahdara North Zone | Nand Nagari, Yamuna Vihar, Central Jail Mandoli, Shastri Park, Sonia Vihar, Dilshad Garden | Shastri Park | Shastri Park |
| West Zone | Raghuveer Nagar, Paschim Vihar, Nagloi, Uttam Nagar | Uttam Nagar | Uttam Nagar |
| South Zone | Sangam Vihar | Sangam Vihar | |
| Shahdara South Zone | Mayur Vihar II, | Mayur Vihar II | Mayur Vihar II |
| City Zone | Tilak Bridge, | Tilak Bridge | |
| Rohini Zone | Pitampura, Mangopuri, | Pitampura, | |
| Nazafgarh Zone | Vasant Kunj, Aaya Nagar, Nagal Dewat | Nagal Dewat | Nagal Dewat |

### 2.3 Abbotts Formula for Corrected % Mortality

$$\text{Corrected \% mortality} = \frac{\text{\% mortality (expt.) - \%mortality (cont.)}}{\text{100-\%mortality (cont)}} \times 100$$

## 3. Result

### 3.1 *Aedes aegypti*

Table 2 shows the susceptibility status of *Ae. aegypti* larvae collected from different localities against WHO suggested diagnostic concentration of temephos (0.02 ppm). The results of diagnostic dose tests revealed that larvae of *Ae. aegypti* collected from Sangam Vihar was susceptible to temephos, while from the two localities viz. Uttam Nagar and Pitampura of study area were tolerant to temephos, and from five localities viz. Majnu ka tila, Shastri Park, Mayur Vihar II, Tilak Bridge and Nagal Dewat showed development of resistance against temephos.

Table 3 shows the lethal concentrations of temephos for 50% ($LC_{50}$) and 90% ($LC_{90}$) mortality of *Ae. aegypti* larvae collected from different localities. The larvae from Sangam vihar locality were susceptible, while those from Shastri Park were least susceptible. The $LC_{50}$ and $LC_{90}$ value of *Ae. aegypti* larvae collected from Sangam Vihar locality were 0.07 and 0.10 ppm respectively and were significantly different from other localities.

**Table 2:** Insecticide susceptibility status of the larvae of *Aedes aegypti* against Temephos in various localities under different MCD zones in Delhi

| Name of localities | No. of mosquito larvae exposed | | No. of mosquito larvae dead | | Average mortality ( % ) | Susceptibility status |
|---|---|---|---|---|---|---|
| | Test | Control | Test | Control | | |
| Sangam vihar | 225 | 75 | 221 | 0 | 98.22 | S |
| Majnu ka tila | 225 | 75 | 146 | 1 | 64.88 | R |
| Shastri Park | 225 | 75 | 178 | 0 | 79.11 | R |
| Uttam Nagar | 225 | 75 | 214 | 0 | 95.11 | T |
| Mayur Vihar II | 225 | 75 | 151 | 0 | 67.11 | R |
| Tilak Bridge | 225 | 75 | 149 | 0 | 66.22 | R |
| Pitampura | 225 | 75 | 198 | 1 | 88.00 | T |
| Nagal Dewat | 225 | 75 | 169 | 0 | 75.11 | R |

WHO diagnostic concentration of (0.02 mg/L)

**Table 3:** Lethal concentrations of Temephos for 50% ($LC_{50}$) and 90% ($LC_{90}$) mortality of *Ae. aegypti* in various localities in Delhi

| Name of locality (MCD Zone) | $LC_{50}$ (Confidence limits) | $LC_{90}$ (Confidence limits) |
|---|---|---|
| Majnu ka tila | 0.015 (0.011-0.021) | 0.025 (0.018-0.037) |
| Shastri Park | 0.018 (0.012-0.026) | 0.032 (0.021-0.051) |
| Uttam Nagar | 0.015 (0.013-0.018) | 0.021 (0.018-0.025) |
| Mayur Vihar II | 0.016 (0.013-0.021) | 0.026 (0.021-0.034) |
| Pitampura, | 0.017 (0.013-0.021) | 0.025 (0.020-0.033) |
| Tilak Bridge | 0.017 (0.013-0.021) | 0.025 (0.020-0.033) |
| Sangam vihar | 0.007 (0.006-0.009) | 0.010 (0.008-0.012) |
| Nagal Dewat | 0.014 (0.011-0.014) | 0.019 (0.015-0.025) |

### 3.2 *Anopheles stephensi*

The diagnostic dose tests against *An. stephensi* larvae revealed that of larvae collected from all the five localities were 100% susceptible to temephos, but the dose-mortality susceptibility tests revealed some variations in the lethal concentrations for 50% and 90% mortality (Table 4). *An. stephensi* larvae collected from Uttam Nagar locality were least susceptible having $LC_{50}$ and $LC_{90}$ values of 0.015 and 0.022 ppm respectively, which were significantly different from the $LC_{50}$ and $LC_{90}$ values of the larvae collected from other localities.

**Table 4:** Lethal concentrations of Temephos for 50% ($LC_{50}$) and 90% ($LC_{90}$) mortality of *An. stephensi* in various localities in Delhi

| Name of locality | LC50 (Confidence limits) | LC90 (Confidence limits) |
|---|---|---|
| Majnu ka tila | 0.010 (0.008-0.012) | 0.013 (0.011-0.017) |
| Mayur Vihar II | 0.010 (0.008-0.012) | 0.013 (0.011-0.017) |
| Nagal Dewat | 0.008 (0.006-0.010) | 0.010 (0.008-0.014) |
| Shastri Park | 0.008 (0.006-0.011) | 0.012 (0.009-0.016) |
| Uttam Nagar | 0.015 (0.013-0.018) | 0.022 (0.018-0.027) |

## 4. Discussion

Studies have been undertaken by earlier investigators to assess the insecticide susceptibility against malaria and dengue vectors in different parts of the country [8-9]. The available report says that *Ae. aegypti* from India is still susceptible to temephos. Susceptibility of adult and immature stages of *Ae. aegypti* against different organophosphate and organochlorine was determined in Rajahmundry town of Andhra Pradesh. Larvae of *Ae. aegypti* were highly susceptible to temephos and fenthion [10]. Another susceptibility study has been done on adult and larvae of dengue vectors to DDT, malathion, deltamethrin and others insecticides including temephos and fenthion from different parts of Ranchi city of Jharkhand by Das *et al*, who reported highly susceptibility against temephos and fenthion in immature stages [11]. Similarly, Vasdev *et al*. determined susceptibility status of adult and immature stages of *Ae. aegypti* and reported that larvae of *Ae. aegypti* are still susceptible to temephos, fenthion and malathion from Assam [12]. However, the immature stages of *Ae. aegypti* mosquito has shown the tendency of developing induced resistance under the laboratory conditions to temephos [13]. But this is the first study from the country where tolerance/resistance against temephos is reported from field collected *Ae. aegypti* larvae in Delhi. Resistance in *Ae. aegypti* has however been reported from other tropical countries such as Malaysia, Brazil, Thailand, Cuba, Venezuela [14-17].

In urban areas, control of *An. stephensi* induced malaria is primarily dependent on antilarval methods and space spraying of insecticides. There are, however, some technical problems in the use of larvicides against *An. stephensi* in potable waters. Various studies undertaken from different parts of the country showed that *An. stephensi* is fully susceptible to temephos [18-19]. These reports are in conformity with the results of our study, which showed it as susceptible against temephos from Delhi. Although *An. stephensi* larvae collected from different localities in Delhi were susceptible to temephos significant difference in the susceptibility of larvae collected from Uttam Nagar locality indicates faster development of tolerance to temephos in this strain. Resistance to temephos in *An. stephensi* has, however been reported from Calcutta [20]. Development of resistance to microbial toxins of *Bacillus sphaericus* has also been reported recently in *An. stephensi* [21, 22]. These studies indicate the possibility of development of resistance in *An. stephensi* to all categories of insecticides that could be used for the control of *An. stephensi*. However, barring few reports, the data available on development of resistance against temephos in *An. stephensi* and *Ae. aegypti* from different parts of the country is very scanty, because the larval susceptibility monitoring has not been applied at the field level under the National Vector Borne Disease Control Programme.

## 5. Conclusion

The present study indicates the possible development of resistance against temephos in field collected *Ae. aegypti* larvae from Delhi, due to continuous use of temephos for over two decades as a part of the UMS. Under UMS, temephos-50% EC/granules has been used in these areas. Such development of resistance warrants a detailed evaluation of the efficacy of temephos against the larvae of *Ae. aegypti*. The purpose of the insecticide susceptibility test is to determine resistance in a mosquito population so that alternative control strategy can be implemented in time when the insecticide in question is no longer having the desired results. This raises the need for countrywide and regular surveys for monitoring the insecticide susceptibility status of major vectors, detecting resistance and assessing their implications on vector control activities. This study may help in reformulating control strategies including re-scheduling application of insecticide, or replacing the larvicide with other suitable compounds under the program.

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