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Susceptibility status of dengue vector *Aedes aegypti* (L.) against various larvicides and insecticides in Udaipur district of Southern Rajasthan, India

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Abstract
Dengue fever is a developing disease in India, responsible for causing significant morbidity and mortality in most of tropical and sub-tropical countries of the world including in Rajasthan (India). Many insecticides have been used to control of vectors, in recent days; unfortunately, vector control programs are facing operational challenges because of the emergence and development of insecticide resistance in dengue vectors, especially *Aedes aegypti* (L.). The insecticide susceptibility status of *Aedes aegypti* (L.) larval populations and Mosquito adults of *Aedes aegypti* (L.) was ascertained, using World Health Organization standard diagnostic concentrations and test procedures. Larval samples of *Aedes aegypti* (L.) were susceptible to all two larvicides, including Temephos and *Bacillus thuringiensis var. israelensis* Bti (WP). Whereas adults of *Aedes aegypti* (L.) were resistant to DDT, with a mortality of <80%, but fully susceptible to Deltamethrin at the given diagnostic concentrations. *Aedes aegypti* (L.) response to pyrethroids (Malathion) with a mortality of 97% for 24 hours and 89% knock down in 1 hour. The present study revealed that the use of temephos and *Bacillus thuringiensis var. israelensis* Bti (WP) larvicides for larval control, Malathion and Deltamethrin insecticides to adult's control, is best.

Keywords: dengue, insecticides, susceptible, organophosphorous, pyrethroids

1. Introduction

Dengue fever is an aedes mosquito-borne human viral pathogen caused by the genus Flavivirus of family Flaviviridae. There are main four known virus serotypes (DEN 1, DEN 2, DEN 3, and DEN 4) [4, 7, 21]. Dengue virus is spreaded and transmitted in humans by two species of aedes mosquitoes named as, *Aedes aegypti* (L.) and *Aedes albopictus*. *Aedes aegypti* (L.) and *Aedes albopictus* both vectors are reported prevalent in the North-east India, because this part offers best conditions for explosion of these vectors and the spread of mosquito borne disease [3]. Although infection with one serotype of dengue virus confers lifetime immunity against re-infection by the same serotype of dengue virus, but there is no evidence of cross immunity [10]. These days, there has been dramatic increase in dengue cases globally and approximately half of the world's population is estimated to be living at danger of the disease. World Health Organization (WHO, 2017) currently stated that the incidence of dengue has been increased thirty times more over the last 50 years, as 50–100 million cases of Dengue infections occur worldwide every year, putting almost half of the world's population at risk [22].

In India dengue is prevalent and endemic in major and metropolitan cities. However, with increased population migration and urbanization, the dengue disease is supposedly spreading to other metropolitan cities or areas that were up till now free from dengue disease [1, 3, 20]. According to National Vector Borne Disease Control Programme (NVBDCP, 2020), about 1, 36,422 dengue cases with 132 deaths were in 2019, in India. According to the data on the website of National Vector Borne Disease Control Programme (NVBDCP, 2013) and publications by National Institute of Virology (NIV) dengue has been endemic in sixteen states since the beginning: Andhra Pradesh, Chandigarh, Delhi, Goa, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Panduchery, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal [12, 23].

Rajasthan is an arid region geographically still dengue cases and patients are seen in a regular manner. Dengue is observed in Rajasthan because of the common practices of the people living here. People here use to store the rainy water for the drinking and also for other purposes as here is scarcity of drinking water. So to get water without difficulty they have built the practices to collect drinking water in the pots, underground cemented takas and underground takas which are the good place for the mosquitoes to breed [27].

In Rajasthan, in 2009 there were 1389 dengue cases with eighteen deaths, in 2010, 1823 dengue cases with nine deaths, in 2011, 1072 dengue cases with four deaths, in 2012, 1295 dengue cases with ten deaths, in 2013, 4413 dengue cases with ten deaths, in 2014, 1243 dengue cases with seven deaths and in 2015, 2376 dengue cases with five deaths but recently 12664 suffered from dengue with ten deaths were observed. At present out of the total population of the state one percent of the population is suffering with dengue and dengue is the major reason of the deaths 11,12.

The use of chemicals or insecticides to control insects or arthropods possibly dates back to standard Greece and Rome. In the twentieth century, after the insecticidal prospective of DDT, other insecticidal organophosphorous insecticides- folithion, Malathion and temephos were developed and used to control vectors [8]. Pyrethroids (PYs) named as, cyphenothrin, deltamethrin, lambda-cyhalothrin

and permethrin, organophosphates (OPs) named as, fenitrothion, malathion and pirimiphos-methyl are the insecticides, recommended by WHO for space spraying [9]. The fenitrothion was applied in space spraying for dengue and malaria mosquitoes control since 1970s [6, 15, 17].

In recent days, emergence and development of insecticide resistance in dengue vectors, especially in *Aedes aegypti* (L.) are main barriers vector control programs. Resistance to organophosphates (OPs) and pyrethroids (PYRs) has been reported since the 1980s and 1990s respectively [16]. All *Aedes aegypti* (L.) populations have become resistant to temephos, deltamethrin and other insecticides also at many places but mostly susceptible to *Bacillus thuringiensis israelensis* (Bti). This resistance has recently been shown to be negatively impacting on the efficacy of vector control interventions taken together, so it is necessary to calculate susceptibility status of vectors against insecticides before applications of insecticides [18].

In our present study, we calculated and determined susceptibility status of dengue vector named as *Aedes aegypti* (L.) against larvicides namely, Temephos and *Bacillus thuringiensis var. israelensis* Bti (WP) and insecticides namely as, DDT, Malathion and Deltamethrin. The susceptibility status data against these insecticides have not been recorded before in Udaipur district of Southern Rajasthan, so our study will be one of the pioneer studies to control dengue vectors, for vector control programs of NVBDCP and others also. Further study of insecticides susceptibility against dengue vectors is very important for effective control and control of disease spread.

2. Materials and Methods

Insecticide susceptibility status of larval populations of *Aedes aegypti* (L.) mosquito

The insecticide susceptibility status of larval populations of *Aedes aegypti* (L.) mosquito was measured using WHO standard diagnostic dosages and test procedures [25]. Late second or early third instar larvae of *Aedes aegypti* (L.) were separated from the colony and used for the test. Thirty larvae were put in 150 ml container containing 100 ml of water with the required or recommended dose of Larvicides (Temephos- 0.02ml/100ml and Bacillus thuringiensis var. israelensis Bti (WP)-2.5gm/100ml). Four replicates of test and one replicate of control were run in parallel for each experiment. Mortality was made after 24 hours exposure period and reading were noted for percent larval mortality calculation. From the obtained result percent larval mortality (for LC_{50} and LC_{90}) were calculated. Actual (Corrected mortality) mortality of mosquitoes was checked and calculated using Abbtots formula by Probit analysis [5]. Each experiment was repeated three times. All experiment was performed under controlled laboratory conditions 27±2 ºC, 70-80 percent.

Insecticide susceptibility status of adult mosquitoes of *Aedes aegypti* (L.)

The insecticide susceptibility status of adult mosquitoes of *Aedes aegypti* (L.) was measured using WHO standard diagnostic dosages and test procedures [24]. 20-25 unfed, 2–3-days old female mosquitoes, which were emerged from field-collected larval populations, were introduced into holding tubes for 1 hour for accumulation and then transferred into the exposure tube and placed vertically for 1 hour to the test insecticides, namely organochlorine (4 % DDT), organophosphorous (5% Malathion), and synthetic pyrethroids (0.05% Deltamethrin). Knocked-down and dead mosquitoes were noted after this time (1 hour.) before being transferred into the holding tubes. A mosquito was recorded as knockdown if it was lying on surface and was unable to maintain flight after a gentle tap. Two controls were tested as described above, with a batch of 20-25 unfed females per replicate against each insecticide. Mortality was recorded 24 hours after exposure. Four replicates of test and two replicates of control were at a time and each test was repeated three times. All experiments were completed in controlled temperature- 27 ± 2°C and humidity-70–80 percent. Ten per cent sugar-soaked cotton was provided for feeding to the females during the 24-h holding period.

Percent mortality

$$= \frac{\text{Number of Dead Larvae}}{\text{Number of Tested Larvae}} \times 100$$

Corrected mortality

$$= \frac{\text{Mortality in treated- Mortality in Control}}{\text{100- Mortality in Control}} \times 100$$

Statistical analysis

Data from larval bioassays were subject to probit analysis, using SPSS software version 22 for determining the 50% and 90% lethal concentrations (LC50 and LC90), with 95% confidence intervals.

3. Results

Insecticide susceptibility status of larval populations of *Aedes aegypti* (L.) mosquito

Table 1: Statistical analysis on susceptibility test results of mosquito larvae of *Aedes aegypti* (L.) to various larvicides in Udaipur district of Southern Rajasthan

Larvicide (Diagnostic Concentration)	Mosquito species	Number of larvae assayed	Number of Dead larvae after 24 hours	Regression equation	Pearson X^2 goodness of fit (df)	LC50 (95% confidence Interval)	LC90 (95% confidence Interval)
Temephos (0.02ml/100ml)	*Aedes aegypti*(L.)	150	114	Y=-1.834+178.28x	0.626 (6)	0.010(0.009 to 0.012)	0.017(0.016 to 0.020)
Bacillus thuringiensis var. israelensis Bti (WP) (2.5gm/100ml)	*Aedes aegypti*(L.)	150	115	Y= -1.834+0.169x	0.302 (6)	1.084(0.697 to 1.411)	1.842(1.515 to 2.222)

Larval samples of *Aedes aegypti* (L.) were susceptible to all two larvicides, including Temephos (0.02ml/100ml) and *Bacillus thuringiensis var. israelensis* Bti (WP) (2.5gm/100ml), at lower dosages or given diagnostic concentrations. For larvicide assay of larval population of *Aedes aegypti* (L.), the LC50 to LC90 values for Temephos 0.010ml/100ml to 0.017ml/100ml and for *Bacillus thuringiensis var. israelensis* Bti (WP) 1.084gm/100ml to 1.842gm/100ml, respectively (Table 1).

Insecticide susceptibility status of adult mosquitoes of *Aedes aegypti* (L.)

Table 2: Insecticide susceptibility status of *Aedes aegypti* (L.), dengue mosquito vector species in Udaipur district of Southern Rajasthan

Insecticide	Discriminating dose (%)	Exposure time (hours)	Number of Mosquitoes exposed (Number of replicates)	Number (%) of Mosquitoes Knockdown in 60 minutes	Number (%) of Mosquitoes dead in post 24 hrs of exposure	Susceptibility status
DDT	4.0	1	87 (4)	1 (1)	30 (34)	R
Malathion	5.0	1	96 (4)	86 (89)	93 (97)	VR
Deltamethrin	0.05	1	93 (4)	90 (97)	93 (100)	S

R: resistant (mortality <80%); VR: verification required (mortality 81–97%); S: susceptible (mortality 98–100%). Mortality in control replicates was <5%.

The results of the susceptibility test of *Aedes aegypti* (L.) females to various insecticides are presented in Table 2. *Aedes aegypti* (L.) were resistant to DDT (4%), with a mortality of <80%, but fully susceptible to Deltamethrin (0.05%) at the given diagnostic concentrations. *Aedes aegypti* (L.) response to pyrethroids (Malathion- 5%)) with a mortality of 97% for 24 hours and 89% knock down in 1 hour. Thus, the susceptibility status was borderline (verification required) according to the given WHO criterion.

Table 3: Determination of Knockdown Time of *Aedes aegypti* (L.) against Insecticides (DDT, Malathion & Deltamethrin) in Udaipur district of Southern Rajasthan

Insecticide	Discriminating dose (%)	KT50 (minutes) with fiducial limits	KT90 (minutes) with fiducial limits	X^2 Value (df)
DDT (Organochlorine)	4.0	93.79 (72.70- 114.88)	112.51 (90.74-165.33)	0.626(10)
Malathion (Organophosphorous)	5.0	37.54 (21.46-54.68)	57.93 (45.16-118.69)	6.65(3)
Deltamethrin (Synthetic pyrethroids)	0.05	35.02 (30.67-39.42)	51.65 (46.25-60.32)	1.24(3)

KT50& KT90= Time required to Knock down 50 & 90 individuals

The Knockdown effect of insecticides (DDT, Malathion & Deltamethrin) was performed against adults of *Aedes aegypti* (L.), dengue vector in Udaipur district of Southern Rajasthan, using WHO test kit, to study the knockdown time responses of these insecticides. The data was subjected to log probit analysis for the calculation of KT50 and KT90 values (Table 3), which respectively reflects the time requires for 50 and 90 percent Knockdown of the exposed population. The knockdown time of DDT, Malathion and Deltamethrin at KT50 and KT90 levels were determined as 93.79 & 112.51, 37.54 & 57.93 and 35.02 & 51.65 minutes respectively, which indicates that Deltamethrin and Malathion have more knockdown effect than DDT, as these take less time (KT50-35.02 & KT90-51.65 minutes and KT50-37.54 & KT90-57.93minutes) to knockdown effect comparison to DDT (KT50-93.79 & KT90-112.51 minutes).

4. Discussion

The results emphasized that the larvae of dengue vector (*Aedes aegypti* (L.)) in Udaipur district were susceptible to both larvicides, viz. Temephos (0.02ml/100ml) and *Bacillus thuringiensis var. israelensis* Bti (WP) – 2.5gm/100ml) as 95.83 to 96.67% mortality was found within 24 hours of treatment. In adult susceptibility tests, studies revealed that dengue vector mosquitoes were resistant to DDT with a mortality of 34%, and are susceptible to Deltamethrin with 100% motality. But in case of Malathion mortality was as 97% means vectors were slightly resistant against Malathion, so verification is required. Resistance to DDT may be due to excessive or prolonged use of inadequate dosages of DDT in Indoor Residual Spray for mosquito control since 1958. There is however no report available on the development of resistance in *Aedes* mosquitoes to insecticides from Udaipur district of Southern Rajasthan.

Similarly, Singh *et. al*, (2011) studied that the larvae of both *Aedes* species were susceptible to all the three larvicides, viz. temephos (0.02 mg/L), malathion (1.0 mg/L) and fenthion (0.05mg/L) as 96.53 to 100% mortality was found within 24 hours of treatment. In adult susceptibility tests, they revealed that both species *Aedes* aegypti (L.) and *Aedes albopictus* were resistant to DDT and susceptible to other insecticides (Malathion, lambda-cyhalothrin, deltamethrin, permethrin and cyfluthrin) but some areas in case of malathion both species were slightly resistant (Verification required) [19].

Dev *et.al* (2014) reported that *Aedes aegypti* (L.) *and Aedes albopictus* were susceptible to all three larvicides, including temephos (0.02 mg/L), malathion (1.0 mg/L) and fenthion (0.05 mg/L). They also studied that *Aedes aegypti* (L.) and *Aedes albopictus* were resistant to DDT (4%), with a mortality of <80%, but susceptible to malathion (5%). *Aedes aegypti* (L.) was observed to be resistant to deltamethrin (0.05%) as well as permethrin (0.75%) [2].

Paeporn *et al*, (2004) studied larval populations of both species were susceptible to all three larvicides, malathion (1.0 mg/L), temephos (0.02 mg/L) and fenthion (0.05 mg/L). In case of adults they found that populations of *Aedes aegypti* (L.) *and Aedes albopictus* were resistant to both deltamethrin and permethin resistance in Thailand [13, 14].

5. Conclusions

The present study revealed that in Udaipur district of Southern Rajasthan *Aedes aegypti* (L.) larvae were susceptible to both larvicides named as Temephos and *Bacillus thuringiensis var. israelensis* Bti (WP).While adults *Aedes aegypti* (L.) were resistant to DDT but still susceptibility to Malathion and Deltamethrin. In view of the presence susceptibility for temephos and *Bacillus thuringiensis var. israelensis* Bti (WP), and resistance for DDT in the dengue vector (*Aedes aegypti* (L.)) the use Temephos and *Bacillus thuringiensis var. israelensis* Bti (WP) larvicides for larvae control and Malathion or

Deltamethrin insecticides to adults control, can be a good option for their effective control. The study also revealed that there is an urgent need to district-wise mapping in whole Southern Rajasthan, on susceptibility status of dengue vectors to the insecticides being used by the NVBDCP in the vector control programme.

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