

Short Communication



# Larvicidal Susceptibility Studies of a few Strains of Aedes Vectors of Bengaluru, Karnataka, India

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# ABSTRACT

*Objective:* To determine the larvicidal activity of four insecticides against different strains of two dengue vectors-*Aedes aegypti* and *Aedes albopictus* of Bengaluru. The study thus aims to determine the variation of resistance/ susceptibility levels in Aedes mosquitoes collected from different localities of Bengaluru against four insecticides viz. chlorpyrifos, temephos, cypermethrin and lambda-cyhalothrin.

*Methods:* Eighteen strains of *Ae. aegypti* and three strains of *Ae. albopictus* were collected from different parts of Bengaluru. They were maintained and reared in the laboratory for a few generations. Susceptibility studies were carried out on freshly collected third instar larvae according to the standard procedure of World Health Organisation.

*Results: Ae. aegypti* is tolerant to WHO recommended diagnostic dose of temephos. A moderate level of tolerance was found in HSR strain of *Ae. aegypti* to lambda-cyhalothrin ( $RR_{90}$ =38.8). *Ae. aegypti* proves to be more tolerant than *Ae. albopictus*, to all the larvicides tested. The synthetic pyrethroids used-cypermethrin and lambda-cyhalothrin were more effective in their larvicidal activity than the organophosphates studied-chlorpyrifos and temephos.

*Conclusion:* The present findings form baseline data on the susceptibility status of two arboviral vectors to different larvicides. This study would help in better vector control measures and effective source management in Bengaluru.

**Keywords:** Dengue Prevention, Insecticides, Mosquito, Resistance, Vector Control

# Introduction

Dengue virus and chikungunya virus are mosquito-borne viruses of medical concern in most tropical regions,<sup>1</sup> the transmission of which is effected through female *Aedes aegypti* Linnaeus 1762 and *Aedes albopictus* Skuse 1894.<sup>2,3</sup> Occupancy of artificial containers, human facilitated transport, desiccation-resistant eggs and associations

with human habitats have enabled these two Aedes species to become cosmopolitan.<sup>4</sup> In many developing tropical countries, dengue and chikungunya infections are significant causes of morbidity, mortality as well as adverse economic effects. One of the preventable viral diseases, dengue, in its 2006 Indian epidemic alone, had a record of 12,317 documented cases<sup>5</sup> and caused an economic burden of approximately US \$27.4 million.<sup>6</sup> The control of

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mosquito larvae worldwide depend primarily on continued applications of organophosphates and insect growth regulators, the repeated use of which has fostered several environmental and health concerns, including disruption of natural biological control systems, outbreak of other insect species, widespread development of resistance and undesirable effects on non-target organisms.<sup>7</sup> However, among the vector control practices in India, chemical control is given more priority than are others.<sup>8</sup> But, resistance to insecticides has appeared in major insect vectors from every genus, and this is expected to directly and profoundly affect the re-emergence of vector-borne diseases.<sup>9</sup>

Karnataka is among the 18 dengue endemic states/ union territories of India.<sup>5</sup> In the 2006 epidemic of chikungunya, Karnataka was one of the first states of India to be affected.<sup>10</sup> Bengaluru, the capital city of Karnataka state, witnesses regular outbreaks of these two arboviral fevers, and as per the November 2012 report by Bruhat Bengaluru Mahanagara Palike (BBMP), which is the municipal body of the city, there were a total of 976 reported dengue cases and 28 chikungunya cases in that year.<sup>11</sup> Most of the areas of Bengaluru are experiencing a surge of construction activity, creating ideal conditions for mosquito breeding; moreover the migration of construction workers from places where dengue are endemic, further adds to the problem.<sup>12</sup>

Previously, studies of susceptibility/ resistance status of different geographical strains of *Ae. aegypti* to malathion, carbofuran, alphamethrin, fenthion, temephos, propoxur, lambda-cyhalothrin, deltamethrin, and botanical extracts like Neem, *Centella asiatica, Eucalyptus globosus* have been conducted in our laboratory.<sup>12-15</sup> In addition to these, evaluation of susceptibility/ resistant status of Anopheline and Culicine mosquitoes of the river Cauvery basin, Karnataka to adulticides and larvicides was completed.<sup>16</sup>

The present study reports the susceptibility status of 18 strains of *Ae. aegypti* and 3 strains of *Ae. albopictus* of Bengaluru to chlorpyrifos and temephos (organophosphates), which are recommended by WHO to control mosquito larvae<sup>17</sup> and cypermethrin and lambdacyhalothrin (synthetic pyrethroids) approved by Ministry of Agriculture, Government of India for house-hold use against insects.<sup>18</sup> The results derived would help in determining the most effective insecticide that could be employed in controlling the dengue and chikungunya vectors and thus the diseases' prevalence in the area.

## **Materials and Methods**

A total of 18 strains of *Ae. aegypti* and 3 strains of *Ae. albopictus* were tested for their susceptibility status for four insecticides.

#### Spatial boundary of the study

Bengaluru, which is the capital city of Karnataka, also titled

the 'Silicon Valley of India' is selected as the study area. The area is situated at 12.79°N, 77.56°E, with an altitude of 920m, covering an area of 741 km<sup>2</sup>. Bengaluru is divided into eight zones which is further sub-divided, totally comprising 198 wards. The city municipality is responsible for mosquito control in the region and though measures are taken to control vector population, the region continues to report cases of dengue and chikungunya.

#### **Collection of mosquitoes**

Larvae, pupae and adults were freshly collected from the field from different areas of Bengaluru (Figure 1). *Ae. aegypti* larvae were in most cases, collected from water sumps and temporary reserves of water present in construction sites and nurseries and also from the stored water for domestic use in large drums, frequented in and outside the dwellings of people of low economic background. *Ae. albopictus* was collected from outdoor temporary sources of water like discarded tyres, coconut shells and garden tanks. A total of 21 strains were collected from different localities, of which 18 were of *Ae. aegypti* and 3 were of *Ae. albopictus* (Table 1). The collected mosquitoes were reared and maintained in the laboratory according to the method of Shetty,<sup>19</sup> with temperature maintained at 26±1°C and a relative humidity of 70-80%.

#### Insecticides tested

Two organophosphate insecticides – Chlorpyrifos (O,Odiethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate) (94.2%TC) (Tata Rallis, Mumbai), Temephos (O,O, O',O'-Tetramethyl O,O'-thiodi-p-phenylene bis (phosphorothioate)) (50% TC) (Greater Bangalore Municipal Corporation) and two synthetic pyrethroids - Cypermethrin (RS)-I-cyano-3-phenoxybenzyl (1RS)-cistrans-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane-1-carboxylate) (93% TC) (Greater Bangalore Municipal Corporation), Lambda cyhalothrin (A reaction product comprising equal amounts of S- $\alpha$ -cyano-3-phenoxybenyl (Z)-(1R,3R) 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2, 2-dimethyl-cyclopropanecarboxylate and R-α-cyano-3phenoxybenyl (Z)-(1S, 3S)3-(2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2, 2-dimethyl-cyclopropanecarboxylate) (10% TC) (Greater Bangalore Municipal Corporation) were used in testing larval susceptibility tests.

#### Larvicidal bioassay

Larvicidal susceptibility tests were conducted in accordance with the guidelines of WHO.<sup>20</sup> The two species of Aedes – *Ae. aegypti* and *Ae. albopictus* were the experimental species, on which technical grade formulations of chlorpyrifos, temephos, cypermethrin and lambda-cyhalothrin were tested. Working standard concentrations of each insecticide were prepared using denatured alcohol as solvent. The denaturation of absolute alcohol was achieved by adding 2% butanone (methyl ethyl ketone). Tests were conducted by adding batches of 25 late third instar larvae each in 300 ml wide-mouthed disposable bowls containing 249 ml of dechlorinated tap water and 1 ml of the designated insecticide of required concentrations. Each test concentration of each insecticide that was tested had 4 replicates. For the control, 1 ml of denatured alcohol was added instead of insecticide stock solution, to 249 ml of dechlorinated tap water. To prevent mortality due to lack of nutrition, yeast granules were added to all bowls, as larval diet. After twenty four hours, the number of living, dead and moribund larvae was recorded and percentage mortalities, with suitable corrections (when necessary), were calculated according to the guidelines of WHO.<sup>20,21</sup> The tests were carried out at a lab environment conducive for healthy sustenance of larvae under regular maintenance conditions.

#### Statistical analysis

The dosage mortality data were subjected to probit analysis<sup>22</sup>

to calculate  $LC_{50}$  and  $LC_{90}$  values for each insecticide. For each concentration, percentage mortality is calculated.

percentage mortality = 
$$\frac{\text{number of dead larvae}}{\text{number of larvae introduced}} X 100$$

Percentage mortality was corrected using Abott's formula<sup>23</sup> in case the percentage mortality in control happened to be 20% or less. The resistance ratio was calculated as per the formula described by Boike et al.<sup>24</sup>

The data were analysed using SPSS 20.0 version software for Windows.

#### Results

Larval susceptibility studies of a total of 21 strains of Aedes vectors were studied and presented. Regression equation, coefficient of correlation, resistance ratios and chi-square values corresponding to tests with different insecticides are presented in Tables 2-5.

Table I.Strains of Ae. aegypti and Ae. albopictus collected from different areas
of Bengaluru

Species	Strain No.	Strains	Strain abbreviation	Life stage collected	
	1	J. P. Nagar	JPN	larvae	
	2	Nagarbhavi	NAG	larvae	
	3	Koramangala	KOR	larvae	
	4	Jnanabharathi Campus	JBC	larvae	
	5	Marathahalli	MRT	larvae	
	6	Banaswadi	BNW	larvae	
	7	R. T. Nagar	RTN	Adults/ larvae	
	8	Nagasandra	NAS	larvae	
Aedes aegypti	9	H. S. R. Layout	HSR	larvae	
	10	Uttarahalli	UTH	larvae	
	11	Gottigere	GOT	larvae	
	12	Banashankari	BSK	larvae	
	13	Malleshwaram	MAL	larvae	
	14	Jeevanbhima Nagar	JBN	larvae	
	15	Yelahanka	YEL	larvae	
	16	K. R. Puram	KRP	larvae	
	17	Bagalakunte	BGL	larvae	
	18	Wilson Garden	WLG	adults	
	а	Kodigehalli	KDH	larvae	
Aedes albopictus	b	Jnanabharathi Campus	JBC	larvae	
	с	Nagavara	NGV	larvae	

_		LC <sub>50</sub>	LC <sub>90</sub>			χ²	df	Resistance Ratio		
Spe	Strain	(mg/l)	(mg/l)		r			RR <sub>50</sub>	<b>RR</b> <sub>90</sub>	
	JPN	0.1354	0.2761	y=4.138x+0.316	0.983	0.0273	5	2.6972	1.1999	
	NAG	0.0939	0.2301	y=3.291x+1.795	0.977	0.0410	5	1.8705	1.0000	
	KOR	0.0852	0.3550	y=2.066x+3.077	0.974	0.0225	5	1.6972	1.5428	
	JBC	0.1076	0.3459	y=2.523x+2.396	0.992	0.0092	5	1.6972	1.5033	
	MRT	0.1531	0.4025	y=3.052x+1.382	0.974	0.249	8	3.0498	1.7492	
	BNW	0.0912	0.4634	y=1.953x+3.025	0.958	0.0542	6	1.8167	2.0139	
	RTN	0.1633	0.5457	y=2.445x+2.033	0.904	0.0994	5	3.2530	2.3716	
	NAS	0.1569	0.4767	y=2.652x+1.829	0.951	0.189	6	3.1255	2.0717	
Ac commi	HSR	0.0917	0.4634	y=1.818x+3.250	0.923	0.1683	5	1.8267	2.0139	
Ae. aegypti	UTH	0.0765	0.3768	y=1.848x+3.367	0.974	0.0758	8	1.5239	1.6375	
	GOT	0.0948	0.497	y=1.779x+3.262	0.935	0.0542	5	1.8884	2.1599	
	BSK	0.0848	0.2318	y=2.954x+2.247	0.829	0.3027	5	1.6892	1.0074	
	MAL	0.1497	0.9061	y=1.637x+3.076	0.877	0.9956	11	2.9821	3.9379	
	JBN	0.0850	0.3475	y=2.092x+3.055	0.952	0.3223	9	1.6932	1.5102	
	YEL	0.1044	0.2868	y=2.919x+2.025	0.976	0.0645	6	2.0797	1.2464	
	KRP	0.207	1.241	y= 1.648x+2.829	0.989	0.0083	4	4.1235	5.3933	
	BGL	0.0926	0.5176	y=1.715x+3.340	0.984	0.0481	6	1.8446	2.2495	
	WLG	0.0502	0.2382	y=1.893x+3.673	0.981	0.0728	7	1.0000	1.0352	
	KDH	0.0010	0.0236	y=3.461x+1.524	0.944	1.225	6	1.0000	2.4300	
Ae. albopictus	JBC	0.0058	0.0139	y=3.377x+2.408	0.939	0.050	5	5.8000	1.4300	
	NGV	0.0045	0.0097	y=3.862x+2.460	0.906	0.1050	5	4.5000	1.0000	

Table 2.Susceptibility studies of Ae. aegypti and Ae. albopictus to chlorpyrifos

r - correlation coefficient

#### Table 3.Susceptibility studies of Ae. aegypti and Ae. albopictus to temephos

- Enc	Strain	LC <sub>50</sub>	LC <sub>90</sub>	Regression		~2	df	<b>Resistance Ratio</b>	
Spe	Strain	(mg/l)	(mg/l)	equation	r	χ²		<b>RR</b> <sub>50</sub>	<b>RR</b> <sub>90</sub>
	JPN	0.0109	0.0575	y=1.775x+3.156	0.947	0.1692	7	1.2674	3.0423
	NAG	0.0113	0.0250	y=3.696x+1.104	0.979	0.0294	4	1.3140	1.3228
	KOR	0.0127	0.0755	y=1.654x+3.173	0.932	0.1491	4	1.4767	3.9947
	JBC	0.0139	0.0304	y=3.782x+0.670	0.976	0.8695	4	1.6163	1.6085
	MRT	0.0177	0.0358	y=4.181x-0.128	0.979	0.9002	5	2.0581	1.8942
Ae. aegypti	BNW	0.0159	0.0502	y=2.565x+1.916	0.980	0.0254	5	1.8488	2.6561
	RTN	0.0091	0.0267	y=2.747x+2.358	0.920	0.0747	4	1.0581	1.4127
	NAS	0.0093	0.0263	y=2.827x+2.265	0.966	0.0530	4	1.0814	1.3915
	HSR	0.0128	0.0293	y=3.564x+1.048	0.984	0.0220	5	1.4884	1.5503
	UTH	0.0142	0.0372	y=3.061x+1.469	0.998	0.0031	4	1.6512	1.9683
	GOT	0.0393	0.1104	y=2.856x+0.445	0.969	0.0657	4	4.5698	5.8413

	BSK	0.0107	0.0189	y=5.243x-0.421	0.931	0.1897	4	1.2442	1.0000
	MAL	0.0100	0.0197	y=4.370x+0.616	0.981	0.0304	5	1.1628	1.0423
	JBN	0.0117	0.0326	y=2.885x+1.912	0.977	0.0521	6	1.3605	1.7249
	YEL	0.0271	0.0406	y=7.259x-5.406	0.994	0.0203	5	3.1512	2.1481
	KRP	0.0086	0.0295	y=2.396x+2.755	0.974	0.0358	4	1.0000	1.5608
	BGL	0.0216	0.1179	y=1.739x+2.677	0.935	0.0491	4	2.5116	6.2381
	WLG	0.0489	0.2517	y=1.801x+1.955	0.964	0.0593	5	5.6860	13.3175
_	KDH	0.0034	0.0076	y=3.755x+2.968	0.880	0.2612	6	1.1724	1.0410
Ae. albopictus	JBC	0.0029	0.0073	y=3.281x+3.455	0.963	0.0459	6	1.0000	1.0000
unoopicius	NGV	0.0058	0.0124	y=3.846x+2.070	0.975	0.0380	7	2.0000	1.698

r - correlation coefficient

#### Table 4.Susceptibility studies of Ae. aegypti and Ae. albopictus to cypermethrin

Spe	Churcha	LC <sub>50</sub>	LC <sub>90</sub>	Regression		?	df	Resistance Ratio		
	Strain	(mg'̃/l)	(mg/l)	equation	r	χ²		RR <sub>50</sub>	<b>RR</b> <sub>90</sub>	
	JPN	0.00032	0.00059	y=4.821x+2.554	0.881	0.6141	7	1.1034	1.0000	
	NAG	0.00049	0.00096	y=4.419x+1.943	0.941	0.0944	4	1.6897	1.6271	
	KOR	0.00029	0.00063	y= 3.770x+3.27	0.941	0.1604	6	1.0000	1.0678	
	JBC	0.00031	0.00114	y=2.252x+3.899	0.921	0.0616	6	1.0690	1.9322	
	MRT	0.00143	0.01558	y=1.235x+3.572	0.907	0.0776	5	4.9310	26.4068	
	BNW	0.00059	0.00090	y=6.948x-0.366	0.975	0.1265	8	2.0345	1.5254	
	RTN	0.00041	0.00186	y=1.489x+4.387	0.820	0.2732	7	1.4138	3.1525	
	NAS	0.00125	0.00465	y=2.248x+2.531	0.991	0.0062	6	4.3103	7.8814	
Ae.	HSR	0.00079	0.00186	y=2.576x+2.681	0.829	0.2672	5	2.7241	3.1525	
aegypti	UTH	0.00059	0.00119	y=4.179x+1.781	0.909	0.1958	5	2.0345	2.0169	
	GOT	0.00044	0.00067	y=7.063x+0.447	0.991	0.0216	7	1.5172	1.1356	
	BSK	0.00078	0.00186	y=3.390x+1.972	0.963	0.4032	5	2.6897	3.1525	
	MAL	0.0005	0.00187	y=2.248x+3.419	0.940	1.6000	6	1.7241	3.1695	
	JBN	0.00034	0.00071	y=4.033x+2.857	0.948	0.1490	6	1.1724	1.2034	
	YEL	0.00053	0.00113	y=3.881x+2.184	0.991	0.0152	5	1.8276	1.9153	
	KRP	0.00031	0.00065	y=3.980x+3.032	0.969	0.0919	5	1.0690	1.1017	
	BGL	0.00055	0.00091	y=5.912x+0.608	0.974	0.0951	6	1.8966	1.5424	
	WLG	0.00054	0.00108	y=4.314x+1.806	0.955	0.0497	5	1.8621	1.8305	
	KDH	0.00004	0.00010	y=3.531x+2.692	0.895	0.1202	7	1.3333	2.5000	
Ae. albopictus	JBC	0.00006	0.00012	y=4.660x+1.454	0.989	0.0228	4	2.0000	3.0000	
	NGV	0.00003	0.00004	y=5.029x+2.92	0.992	4.9905	5	1.0000	1.0000	

r - correlation coefficient

Chlorpyrifos, for *Ae. aegypti*, had a mean  $LC_{_{90}}$  value of 0.4556±0.059 mg/l and the different strains did not vary much in the susceptibility status (Table 2). KRP strain, however, showed a moderate tolerance (RR<sub>90</sub>=5.3933). But the mean  $LC_{_{90}}$  value for *Ae. albopictus* was much lower, at 0.01573±0.004 mg/l. The  $LC_{_{90}}$  values for *Ae. aegypti* for

the insecticide temephos varied from 0.0189 - 0.2517 mg/ I (Table3), with WLG strain showing highest tolerance with an  $RR_{_{90}}$  value of 13.3175.

An  $RR_{90}$  value of 26.41 was observed in *Ae. aegypti* strain – MRT, to cypermethrin (Table 4), making it highly tolerant

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to the insecticide. The other strains did not vary much in their susceptibility to the above said pyrethroid. To lambda-cyhalothrin, HSR strain of *Ae. aegypti* showed high resistance ( $RR_{90}$ =38.837) (Table 5). Lambda-cyhalothrin is more toxic than cypermethrin, as inferred by comparing their LC<sub>90</sub> value.

Ae. albopictus strains showed a homogenous response to bioassays, as shown by similar susceptibility rates. Differential response to insecticides was observed between species, with a general trend of Ae. aegypti being more resistant to all insecticides than Ae. albopictus. The susceptibility of various strains of Aedes exhibits a linear proportionality between log dose and probit mortality.

- Eno	Strain	train LC (mg/l) LC <sub>90</sub>	Regression		a.2	df	Resistance Ratio		
Spe	Strain	LC <sub>50</sub> (mg/l)	(mg/l)	equation	r	χ²	ar	RR <sub>50</sub>	RR <sub>90</sub>
	JPN	0.000058	0.00009	y=6.514x+0.003	0.979	0.0502	4	1.3182	1.0465
	NAG	0.000177	0.000565	y=2.540x+1.828	0.968	0.0844	6	4.0227	6.5698
	KOR	0.00005	0.000092	y=5.309x+1.140	0.984	0.0179	4	1.1364	1.0698
	JBC	0.000336	0.00267	y=1.421x+2.830	0.982	0.0113	5	7.6364	31.046
	MRT	0.000129	0.000482	y=2.245x+2.501	0.922	0.0532	4	2.9318	5.6047
	BNW	0.000082	0.00023	y=2.788x+2.440	0.935	0.1188	6	1.8636	2.6744
	RTN	0.000254	0.00104	y=2.082x+2.072	0.994	0.0041	4	5.7727	12.093
	NAS	0.000046	0.00015	y=2.517x+3.315	0.982	0.0126	5	1.0455	1.7442
Ao comunti	HSR	0.000393	0.00334	y=1.376x+2.806	0.96	0.0210	5	8.9318	38.837
Ae. aegypti	UTH	0.000069	0.000157	y=3.620x+1.948	0.937	0.1230	5	1.5682	1.8256
	GOT	0.000076	0.00027	y=2.316x+2.948	0.972	0.0185	5	1.7273	3.1395
	BSK	0.000044	0.000108	y=3.293x+2.870	0.968	2.1333	4	1.0000	1.2558
	MAL	0.000091	0.000228	y=3.203x+1.928	0.993	0.0083	5	2.0682	2.6512
	JBN	0.000044	0.000086	y=4.452x+2.100	0.981	0.0317	5	1.0227	1.0000
	YEL	0.000114	0.000480	y=2.051x+2.831	0.966	0.0340	6	2.5909	5.5814
	KRP	0.000045	0.000092	y=4.081x+2.345	0.995	0.0071	5	1.0000	1.0698
	BGL	0.000062	0.000135	y=3.826x+1.944	0.953	2.2157	4	1.4091	1.5698
	WLG	0.000254	0.000892	y=2.346x+1.704	0.970	0.0321	4	5.7727	10.372
	KDH	0.000006	0.000039	y=1.747x+3.584	0.913	0.0548	5	1.0000	1.0000
Ae. albopictus	JBC	0.000010	0.000059	y=1.739x+3.198	0.960	0.0646	6	1.6666	1.5128
anopicius	NGV	0.000009	0.000040	y=2.068x+2.946	0.857	0.1.562	5	1.5000	1.0250

Table 5 Suscentib	ility studies of Δe	$ae \sigma v b t i and \Delta e$	albobictus to	lambda-cyhalothrin
lable J.Jusceptib	incy scuttes of Ac.	. uegypti anu Ae.	uibopictus to	lambua-cynaiotin in

r - correlation coefficient

## Discussion

*Ae. aegypti* seems to be the more prevalent Aedes mosquito in Bengaluru. In this study, the Aedes species were randomly sampled, and of the 21 strains of Aedine mosquitoes collected, only three were of the species *Ae. albopictus*. According to a report from Delhi, of the three species of Aedes mosquitoes sampled there, only 9.52% were of *Ae. albopictus*,<sup>25</sup> which is similarly reflected in our sampling pattern too.

The susceptibility status of different strains of *Ae. aegypti* and *Ae. albopictus* of Bengaluru city, Karnataka to different insecticides- viz. chlorpyrifos, temephos, cypermethrin and

lambda-cyhalothrin was determined. Though cypermethrin and lambda-cyhalothrin are generally used as adulticides, studies have shown that they have effective larvicidal efficacy.<sup>26,27</sup> The response to an insecticide in this study was dependent on the class of insecticide that it belonged to. The synthetic pyrethroids were found to be more effective in their larvicidal activity when compared to organophosphates, with very low mean  $LC_{90}$  values (0.002124 ±0.0008 mg/ l for cypermethrin and 6.17x10<sup>-4</sup>±0.0002 mg/ l for lambda-cyhalothrin).

Studies on responses of *Ae. aegypti* and *Ae. albopictus* to different insecticides were conducted worldwide, because

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of their role of being effective arboviral vector. <sup>1,28-32</sup> Studies conducted in Central Africa on both these arboviral vectors reported that both the species are susceptible to temephos.<sup>1</sup> A case study conducted in Assam to determine susceptibility status to larvicides and adulticides reported susceptibility of both the Aedes species to temephos at its diagnostic dose.<sup>33</sup> However, an earlier study done in BBMP area, Bengaluru recommends a higher diagnostic dose of temephos to be used in the study area, because the strains of Ae. aegypti as well as Anopheles stephensi and Culex guinguefasciatus were tolerant to the WHO recommended diagnostic dose.<sup>12</sup> The present study concurs to the earlier studies, with respect to temephos, with most of the strains of Ae. aegypti showing tolerance to the WHO diagnostic dose of 0.02mg/ I. However, all the Ae. albopictus strains tested were susceptible to the recommended dosage of temephos.

The diagnostic dose of cypermethrin is 0.0125 mg/l,<sup>34</sup> and an LC<sub>90</sub> value of 0.0079 mg/l was reported for an *Ae. albopictus* strain,<sup>26</sup> but the current results show that the Bengaluru strains of *Ae. aegypti* are susceptible to the insecticide with an LC<sub>90</sub> values ranging from  $0.00059 \cdot 0.01558 \text{ mg/l}$ , and *Ae. albopictus* with LC<sub>90</sub> values of  $0.04 \cdot 0.12 \times 10.^3$  Thus these values deviate from the earlier published results for susceptibility to cypermethrin. According to a report in 2011,<sup>27</sup> 0.0002 mg/l dosage of lambda-cyhalothrin as larvicide, achieved larval mortality of 90% in a strain of *Culex quinquefasciatus*. Our study reports a dosage of 0.000039-0.000059 mg/l to achieve the same in *Ae. albopictus*. To *Ae. albopictus* a LC<sub>90</sub> value of 0.0069 mg/l of chlorpyrifos has been reported,<sup>26</sup> whereas the strains we studied are tolerant to those LC<sub>90</sub> levels.

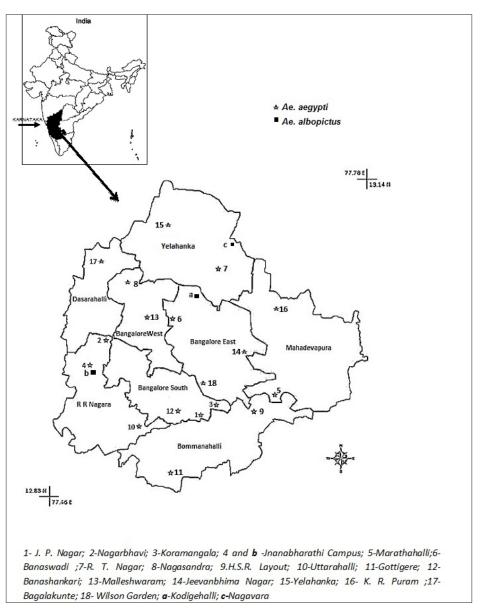


Figure 1.Map of Bengaluru, showing mosquito collection sites

Moderate to high level of tolerance/ resistance to insecticides has been observed in our study. Resistance to different insecticides are conferred to mosquitoes by various mechanisms. Resistance to chlorpyrifos has been linked to an increased expression of esterases.<sup>31,35,36</sup> Glutathione-s-Transferases (GST) levels elevation in chlorpyrifos-resistant *Anopheles stephensi* has also been reported.<sup>37</sup> At the cytogenetic level, chromosomal polymorphisms are also proved to be related to insecticide resistance; chromosomal inversions were discovered and described in ten insecticide-resistant strains derived from natural populations of *Anopheles stephensi*.<sup>38</sup> Knowledge of resistance status in a given area and the mechanisms involved to render resistance would help in vector-monitoring and effective control.

Exposure to insecticides, along with other environmental factors, can alter the genetic structure of vector mosquito species and most of the population gets wiped out. But the ones which survive are likely to pass on the survivability, and have altered physiology.<sup>39</sup> Alteration in life-history traits like fecundity, egg hatchability, sex ratio was observed in Anopheles stephensi strains exposed to sublethal doses of temephos and propoxur.<sup>40</sup> Thus, ineffective vector control programmes, in a long run, would worsen the public-health scenario. To have an understanding as to the efficacy of different insecticides in relation to environmental factors would in a long run help in better control and prevention of arboviral fevers that these Aedine species spread. WHO promotes Integrated Vector Management (IVM), which considers five key elements in management process to control dengue vectors. The guidelines stress the importance of methods based on knowledge of factors influencing local vector biology.<sup>41</sup> Thus this study, would help in framing a strategy to control dengue vectors in Bengaluru, taking into consideration the insecticidal susceptibility status, and efficacy of different insecticides.

# Conclusion

This is the first comparative study of the susceptibility status to various insecticides between two Aedes arboviral vectors in Bengaluru city. Both these vectors occupy overlapping habitats, and are susceptible to infections by arboviruses, and this preliminary study could have direct relevance in dengue prevention programmes, target intervention, reducing/ preventing outbreaks and spread of dengue and chikungunya. Since synthetic pyrethroids studied (cypermethrin and lambda-cyhalothrin) were found to be more effective than chlorpyrifos and temephos (organophosphates) in controlling different Aedes strains of Bengaluru, it is safe to recommend their use in the area for effective control of Aedine vectors.

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# Conflict of Interest: None

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