

## 1.4 Current status of susceptibility of *Aedes aegypti* and *Anopheles stephensi* against larvicides/insecticides being used in National Programme in Rajasthan -

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**Commencement:** October, 2010

**Duration:** Two Year

**Status:** Completed

**Funding :** Desert Medicine Research Centre (Intramural)

### OBJECTIVES

1. To determine the current status of susceptibility of *Ae. aegypti* and *An. stephensi* in rural areas, with particular reference to arid situations, and urban areas of Rajasthan against conventional insecticides being used under national control programme
2. Determination of biochemical mechanisms involved in the development of insecticide resistance, if any

### PROGRESS

The studies were carried-out in 9 districts of Rajasthan state viz., Ajmer, Alwar, Barmer, Bikaner, Jaipur, Jaisalmer, Jodhpur, Pali and Udaipur and in all the districts only urban localities were considered during the report period. During the field investigations, the collection of larval forms of *Ae. aegypti* species in different habitats potential for its breeding was made.

Susceptibility tests were carried out with the larvae of *Ae. aegypti*, collected from the field or reared in the insectary under controlled conditions of temperature (28–2°C) and humidity (75–5%). Third or early fourth instar larvae were tested as per standard WHO methods for the determination of the baseline data on their susceptibility status using diagnostic doses of temephos. Four to five replicates of each observation were made and the data was subjected to log probit regression analysis for the determination of lethal concentrations at 50 and 90 percent levels. WHO criteria was followed for the determination of the susceptibility status of individual species.

The susceptibility status of *Ae. aegypti* larvae, collected from urban areas of Alwar, Barmer, Bikaner, Kota, Udaipur, Jaipur, Jodhpur, Pali and Udaipur was determined against technical grade of temephos (Table 1). The results of the experiments revealed that the species has developed resistance to temephos in Alwar, Bikaner, and Udaipur, and intermediate resistant in Jodhpur, Kota, Jaipur and Pali districts. In Ajmer and Barmer districts *Ae. aegypti* was found susceptible.

**Table 1. Susceptibility status of *Ae. aegypti* against Temephos (Technical)**

Study Districts	Diagnostic Dose (ppm)	No. exposed	No. Dead	Mortality %	Susceptibility status*
Ajmer	0.02	225	221	98.2	S
Alwar	0.02	200	152	76.0	R
Barmer	0.02	125	125	100.0	S
Bikaner	0.02	100	23	23.0	R
Jaipur	0.02	175	170	97.4	IR
Jodhpur	0.02	350	341	97.4	IR
Pali	0.02	100	96	96.0	IR
Kota	0.02	200	187	93.5	IR
Udaipur	0.02	100	52	52.0	R

\*S- Susceptible, R- Resistant, IR- Intermediate Resistant

The lethal concentrations of temephos were also determined at 50 and 90 percent levels against *Ae. aegypti* in seven districts and it was found that at 50 percent level, temephos was maximum effective in Jodhpur ( $LC_{50} = 0.0040$ ), followed by Alwar ( $LC_{50} = 0.0044$ ), Jaisalmer ( $LC_{50} = 0.0060$ ), Ajmer ( $LC_{50} = 0.0063$ ), Jaipur ( $LC_{50} = 0.0071$ ), Udaipur ( $LC_{50} = 0.0169$ ) and Bikaner ( $LC_{50} = 0.0271$  mg/l). The Chi-square values revealed that heterogeneity of the response of *Ae. aegypti* populations in Ajmer, Jaipur, Alwar, Bikaner and Jodhpur cities were found statistically significant, However, at 90 per cent level it exhibited maximum efficacy ( $LC_{90} = 0.0144$ ) in Alwar and minimum ( $LC_{90} = 0.2979$ ) in Udaipur (Table 2).

The enzymatic assays were conducted on the resistant *Ae. aegypti* showed that the percent reduction in Acetyl cholinesterase enzyme (AChE) was significantly higher ( $60.21 \pm 17.12\%$ ) in DDT resistant individuals in comparison to susceptible ones ( $10.29 \pm 0.40$ ). The values of  $\alpha$  and  $\beta$  esterase activities ( $\alpha = 0.008 \pm 0.008$  od/min/mg protein;  $\beta = 0.059 \pm 0.015$  od/min/mg protein) in DDT resistant individuals than susceptible mosquitoes ( $\alpha = 0.0049 \pm 0.00007$  od/min/mg protein;  $\beta = 0.006 \pm 0.0004$  od/min/mg protein) were recorded higher. The above observations indicate the involvement of AChE and esterases in the development of DDT resistance in *Ae. aegypti*.

**Table 2. Determination of lethal concentrations of Temephos (Technical) at 50 and 90 percent levels against *Ae. aegypti***

Study Districts	Regression Equation	LC <sub>50</sub> with Fiducial limits (ppm)	LC <sub>90</sub> with Fiducial limits (ppm)	Chi square (df)
Ajmer	Y = -6.101 + 8.710x	0.0063	0.0167	87.80* (4)
Alwar	Y = -5.055 + 10.14x	0.0044	0.0144	42.67* (4)
Bikaner	Y = -7.814 + 7.417x	0.0271	0.0525	35.31* (4)
Jaipur	Y = -7.086 + 7.361x	0.0071	0.1385	51.83* (2)
Jaisalmer	Y = 0.6087 + 1.778x	0.0060	0.018	1.17 (4)
Jodhpur	Y = 0.663 + 10.57x	0.0040	0.0860	26.12* (4)
Udaipur	Y = 2.371 + 3.311x	0.0169	0.2979	1.903 (4)

\*Heterogeneity of the response was found statistically significant

**Table 3. Biochemical assays of *Ae. aegypti* against DDT and Malathion**

Insecticide	Status (No. exposed)	Enzyme activity (value in od/min/mg protein)		
		Ach E (%)± SE(σ)	α Esterase ± SE(σ)	β Esterase ± SE(σ)
DDT (4.0%)	R (n=39)	60.21±17.12	0.008±0.008	0.059±0.015
	S (n=16)	10.29±0.40	0.00490.00007	0.006±0.0004
Malathion (5.0%)	R (n=22)	21.48±3.99	0.075±0.012	0.059±0.010
	S (n=33)	18.77±11.26	0.0008±0.008	0.014±0.010

However, against Malathion resistance, though the experiments exhibited the involvement of Acetyl cholinesterase enzyme (AchE) but the difference of percent inhibition in resistant and susceptible individuals was not very high (Table 3). The higher values of α and β esterase activities (α = 0.075±0.012 od/min/mg protein; β = 0.059±0.01 od/min/mg protein) in DDT resistant individuals than susceptible ones (α = 0.0008±0.008 od/min/mg protein; β = 0.014±0.010 od/min/mg protein) showed involvement of esterases.