

1.5 Surveillance of pyrethroid resistance in important malaria vectors of western Rajasthan and studies on genetic and biochemical mechanisms of pyrethroid resistance in *An. stephensi* -

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OBJECTIVES

1. Periodic monitoring of insecticide susceptibility of *An. stephensi* and *An. culicifacies* to synthetic pyrethroids in the areas of their use to assess the development of insecticide resistance
2. Development of pyrethroid resistant strain of *An. stephensi* in the laboratory and investigation of the inheritance pattern of pyrethroid
3. Elucidation of the biochemical mechanism of pyrethroid resistance in *An stephensi* using laboratory selected strain and field collected resistant strains

PROGRESS

Three desert districts i.e. Barmer, Bikaner and Jaisalmer, where synthetic pyrethroids have already been introduced as indoor residual spray under national control programme, have been selected for the study. From each district ten villages, having different spray histories of last three years, were selected for the study.

Vector prevalence studies on *An. stephensi* and *An. culicifacies* have been carried-out in the selected villages using standard methods of entomological surveys to collect information on important aspects of vector bionomics such as adult mosquito density and breeding habitats, in post-monsoon, winter and pre-monsoon seasons. The adult collection of vector species has been done using WHO method, by sucking tubes and torch light.

The insecticide susceptibility status of adults of *An. stephensi* and *An. culicifacies* was determined against synthetic pyrethroids, DDT and Malathion and of larvae against temephos. For determination of current susceptibility status of field collected and laboratory reared individuals of both the species, WHO Standard Test Procedures were followed and diagnostic doses of test compounds were used. The status of resistance, if any, was recorded using WHO criteria:

Susceptible	: Mortality \geq 98.0%
Resistant	: Mortality 80.0 - <98.0%
Intermediate Resistant	: Mortality <80.0%

For the susceptibility status of larvae, the WHO larval test method was used to determine the susceptibility status of *An. stephensi* and *An. culicifacies* larvae using diagnostic dose of temephos, as temephos application is being undertaken in the desert areas for the control of malaria vectors under national control programme, as per WHO criteria.

Selection studies to establish a synthetic pyrethroid resistant strain of *An. stephensi* has been initiated. *An. stephensi* strain, collected from Kakku village of Bikaner district, has been selected for the purpose, as village Kakku, since last 3 years has no history of any insecticide spray and the strain of the village is susceptible to synthetic pyrethroids.

The base line data of the strain has been determined and the LT_{50} and LT_{90} values have been calculated using log-probit analysis. The selection studies using LT_{80} (selection pressure) value is in progress to develop a synthetic pyrethroid resistant strain which will be used for the biochemical and genetic studies next year.

Species Prevalence:

Season-wise prevalence of two important malaria vector species *viz.*, *An. stephensi* and *An. culicifacies* has been studied in 30 villages, 10 villages from each study district *viz.* Barmer, Bikaner and Jaisalmer. The district-wise details on the vector prevalence are as below:

Barmer district:

The field studies in this district have been carried-out during three seasons, i.e. post-monsoon, winter and pre-monsoon (Table 1). In post-monsoon, the survey was conducted in 10 villages, namely Kalyanpura, Jasol, Thob, Baitu, Rani, Gunga, Goliya, Parlu, Kawas and Nimbla. The study villages have insecticide spray history of only DDT since last three years. Villages like Kalyanpura, Jasol, Baitu, Goliya and Parlu have regular DDT spray, whereas, other villages have only two years DDT spray history i.e. during 2011 and 2012. *An. stephensi* was recorded from all the study villages during post-monsoon season, whereas, *An. culicifacies* was reported only from Thob, Gunga, Golia, Kawas and Nimbla. The per man hour (PMH) density of *An. stephensi* was recorded highest in village Gunga (PMH: 49.1) and minimum (PMH: 1.0) in village Golia (Table 1). *An. culicifacies* was also recorded maximum (PMH: 181.1) in village Gunga, but the minimum (PMH: 2.0) in village Golia.

In pre-monsoon season the entomological survey was conducted only in four villages *viz.*, Kalyanpura, Jasol, Thob and Parlu and among surveyed villages, in Jasol and Parlu villages no malaria vector species was recorded during this season, however, in village

Kalyanpur both *An. stephensi* (PMH: 7.3) and *An. culicifacies* (PMH: 3.3) were recorded. In winter, only Goliya village was surveyed and in this village no malaria vector species was recorded during this season (Table 1).

Table 1. Village-wise details of malaria vector species collected in Barmer district in different seasons

Name of Village	Insecticide Spray History			Season of Survey	Anopheles Species Collected	PMHD*
	2010	2011	2012			
Kalyan pura	DDT	DDT	DDT	Post-monsoon	<i>An. stephensi</i>	3.1
				Pre- monsoon	<i>An. stephensi</i> <i>An. culicifacies</i>	7.3 3.3
Jasol	DDT	DDT	DDT	Post- monsoon	<i>An. stephensi</i>	10.8
				Pre- Monsoon	Nil	0.0
Thob	No Spray	DDT	DDT	Post- monsoon	<i>An. stephensi</i> <i>An. culicifacies</i>	11.0 3.5
				Pre- monsoon	<i>An. stephensi</i>	1.3
Baitu	DDT	DDT	DDT	Post- monsoon	<i>An. stephensi</i>	3.6
Rani	No Spray	DDT	DDT	Post- monsoon	<i>An. stephensi</i>	2.3
Gunga	No Spray	DDT	DDT	Post- monsoon	<i>An. stephensi</i>	49.1
					<i>An. culicifacies</i>	181.1
Goliya	DDT	DDT	DDT	Post- monsoon	<i>An. stephensi</i> <i>An. culicifacies</i>	1.0 2.0
				Winter	Nil	-
Parlu	DDT	DDT	DDT	Post- monsoon	<i>An. stephensi</i>	1.2
				Pre- Monsoon	Nil	-
Kawas	No Spray	DDT	DDT	Post- monsoon	<i>An. stephensi</i>	12.9
					<i>An. culicifacies</i>	9.0
Nimbla	No Spray	DDT	DDT	Post- monsoon	<i>An. stephensi</i>	3.3
					<i>An. culicifacies</i>	7.7

*PMHD - Per Man Hour Density

The breeding of *An. stephensi* in Barmer district was detected in all the study villages in the habitats like indoor cemented ground tanks, indoor plastic, cemented and clay containers, village ponds and ditches, whereas of *An. culicifacies* was found breeding only in the village ponds of two villages i.e. Thob & Gunga. The detection of breeding of both *An. stephensi* and *An. culicifacies* species in village ponds, indicate that this habitat is being shared by both the malaria vectors species in the study area.

Bikaner district:

The field studies in this district have been carried-out during two seasons, i.e. post-monsoon and pre-monsoon (Table 2). In post-monsoon, the survey was conducted in 8 villages,

namely Khara, Shobhasar, Kodamdesar, Jagdewala Naal, Uttamdesar, Bhanipura and Kakku. The study villages have insecticide spray history of DDT and alpha-cypermethrin since last three years. Three villages namely Khara, Shobhasar and Jagdewala have the spray history of alpha-cypermethrin and DDT during 2011 and 2012 respectively, whereas, villages like Uttamdesar and Diyatra have DDT-alpha-cypermethrin-DDT spray history during last three years. Naal is a village where DDT has been sprayed only in 2012, and Kakku has not been sprayed since last two years.

An. stephensi was recorded from all the study villages during post-monsoon season, whereas, *An. culicifacies* only from three villages i.e. Shobhasar, Kodamdesar and Jagdewala. The per man hour (PMH) density of *An. stephensi* was recorded highest in village Shobhasar (PMH: 9.3) and minimum (PMH: 0.6%) in village Uttamdesar (Table 2). *An. culicifacies* was also recorded maximum (PMH: 6.0) in village Shobhasar, but the minimum (PMH: 4.0) in village Kodamdesar.

In pre-monsoon season the entomological survey was conducted only in six villages viz., Khara, Jagdewala, Naal, Bhanipura, Diyatra and Napasar. Diyatra and Napasar have been surveyed only in pre-monsoon season. In this season the highest density of *An. stephensi* was recorded from Naal village (PMH: 11.1) and lowest (PMH: 0.7) in Napasar village (Table 2). In this season, *An. culicifacies* was recorded only from three villages with highest density (PMH: 5.2) from village Bhanipura.

The breeding of both *An. stephensi* and *An. culicifacies* was detected in the study villages of this district. *An. stephensi* breeding was reported from all ten villages selected for the study, however, *An. culicifacies* was found breeding in only in two villages i.e. Kodamdesar and Jagdewala. The breeding of *An. stephensi* was found in indoor cemented containers/tanks, ground cemented open tanks, cattle tanks, village ponds and ditches, whereas, *An. culicifacies*, like Barmer district, only in village ponds. The village ponds were found shared by both *An. stephensi* and *An. culicifacies* species for breeding. In all study villages only larval forms were found in the breeding sites, whereas, in two villages, Diyatra and Napasar, besides larval forms, pupae were also detected.

Jaisalmer district:

The field studies in this district have been carried-out during two seasons, i.e. post-monsoon and pre-monsoon (Table 3). In post-monsoon, the survey was conducted in 5 villages, namely Awaya, Satyaya, Chinnu, Gomath and Indra Nagar. The study villages have insecticide spray history of DDT and alpha-cypermethrin since last three years. Two villages namely Satyaya and Chinnu have DDT spray history during 2010 and 2011 and no spray in 2012, whereas, other two villages, Gomath and Indra Nagar have spray history of alpha-cypermethrin and DDT during 2011 and 2012 respectively and no spray during

2010. *An. stephensi* was recorded from all the study villages during post-monsoon season, whereas, *An. culicifacies* only from four villages (Table 5). The per man hour (PMH) density of *An. stephensi* was recorded highest in village Chinnu (PMH: 12.6) and minimum (PMH: 2.3) in village Satyaya (Table 3). *An. culicifacies* was also recorded maximum (PMH: 29.8) in village Chinnu, but the minimum (PMH: 0.8) in village Gomath.

Table 2. Village-wise details of malaria vector species collected in Bikaner district in different seasons

Name of Village	Insecticide Spray History			Season of Survey	Anopheles Species Collected	PMHD*
	2010	2011	2012			
Khara	No Spray	Alpha-cypermethrin	DDT	Post-monsoon	<i>An. stephensi</i>	4.4
				Pre-monsoon	<i>An. stephensi</i>	8.4
Shobhasar	No Spray	Alpha-cypermethrin	DDT	Post-monsoon	<i>An. stephensi</i>	9.3
					<i>An. culicifacies</i>	6.0
Kodamdesar	DDT	No Spray	DDT	Post-monsoon	<i>An. stephensi</i>	1.6
					<i>An. culicifacies</i>	4.0
Jagdewala	No Spray	Alpha-cypermethrin	DDT	Post-monsoon	<i>An. stephensi</i>	2.0
					<i>An. culicifacies</i>	4.4
				Pre-monsoon	<i>An. stephensi</i>	2.2
					<i>An. culicifacies</i>	2.2
Naal	No Spray	No Spray	DDT	Post-monsoon	<i>An. stephensi</i>	3.2
				Pre-monsoon	<i>An. stephensi</i>	11.1
Uttamdesar	DDT	Alpha-cypermethrin	DDT	Post-monsoon	<i>An. stephensi</i>	0.6
Bhanipura	No Spray	Alpha-cypermethrin	No Spray	Post-monsoon	<i>An. stephensi</i>	0.8
				Pre-monsoon	<i>An. stephensi</i>	4.4
					<i>An. culicifacies</i>	5.2
Kakku	DDT	No Spray	No Spray	Post-monsoon	<i>An. stephensi</i>	6.0
Diyatra	DDT	Alpha-cypermethrin	DDT	Pre-monsoon	<i>An. stephensi</i>	1.8
					<i>An. culicifacies</i>	2.2
Napasar	DDT	DDT	No Spray	Pre-Monsoon	<i>An. stephensi</i>	0.7

*PMHD - Per Man Hour Density

In pre-monsoon season the entomological survey was conducted in nine villages viz., Awaya, Chinnu, Gomath, Indra Nagar, Tejpala, Sultana, Raimala, Hamira and Thaat (Table 3). Five newly added villages also have the insecticide spray history of DDT and alpha-cypermethrin. Three villages out of five had only DDT spray during 2012 and no spray in 2010 & 2011, whereas, village Thaat had DDT-alpha-cypermethrin-DDT spray history. In this season of *An. stephensi* was recorded from all the study villages with highest density (PMH: 72.0) in Tejpala village and lowest (PMH: 1.3) in Gomath village (Table 3), however, *An. culicifacies* was recorded only from five villages with highest density (PMH: 48.4) in village Tejpala only and lowest (PMH: 5.4) in village Hamira. The density of *An. culicifacies* was recorded comparatively high during this season. The density of both *An. stephensi* and *An. culicifacies* was recorded low in the villages where alpha-cypermethrin has been replaced by DDT in the following year.

The breeding of both *An. stephensi* and *An. culicifacies* was detected in the study villages of this district. *An. stephensi* breeding was reported from all ten villages selected for the study, however, *An. culicifacies* was found breeding in only in four villages. *An. stephensi* was found breeding in indoor underground (UG) & cemented tanks, metallic outdoor tanks, open ground tanks, coolers, ground cemented cattle tanks and ditches near canal, whereas, *An. culicifacies* in open ground tanks, cattle tanks and ditches near canal, which were also found sharing by *An. stephensi*. All aquatic stages i.e. eggs, larvae and pupae were recorded in the villages.

Current susceptibility status of malaria vector species:

In Barmer district, the current susceptibility status of *An. stephensi*, was determined against DDT, malathion and permethrin (Table 4). In village Gunga, *An. culicifacies* was found resistant to DDT, however, susceptible to both malathion and permethrin. In Kalyanpura village, *An. stephensi* was found resistant to DDT, intermediate resistant to malathion and susceptible to permethrin, whereas, in village Kawas and Baitu against DDT, the species was found intermediate resistant and resistant respectively, but in village Baitu against malathion exhibited intermediate resistance.

In Bikaner district, the current susceptibility status of *An. stephensi* was detected against DDT, malathion and cyfluthrin (Table 4). In village Khara, the species was found resistant to both DDT and malathion. Similarly in Naal village too this species exhibited resistance to DDT and malathion, however, against cyfluthrin the species was found totally susceptible. In village Kakku the species was found resistant to DDT and intermediate resistant to malathion.

In Jaisalmer district, the studies on the current susceptibility status revealed that *An. stephensi* in Raimala village is susceptible to DDT and permethrin; in Satyaya, susceptible to DDT and malathion; in Tejpala, susceptible to permethrin, intermediate resistant to malathion and resistant to DDT, and in village Thaat susceptible to permethrin and intermediate resistant to DDT and malathion. To DDT *An. culicifacies* exhibited resistance in Awaya village, intermediate resistant in Tejpala, and found susceptible in village Raimala. Against malathion and permethrin, *An. culicifacies* was found susceptible in village Awaya and village Tejpala, respectively.

Table 3. Village-wise details of malaria vector species collected in Jaisalmer district in different seasons

Name of Village	Insecticide Spray History			Season of Survey	<i>Anopheles</i> species collected	PMHD*
	2010	2011	2012			
Awaya	DDT	DDT	DDT	Post- monsoon	<i>An. stephensi</i>	2.8
					<i>An. culicifacies</i>	4.3
				Pre- monsoon	<i>An. stephensi</i>	11.7
					<i>An. culicifacies</i>	36.1
Satyaya	DDT	DDT	No Spray	Post- monsoon	<i>An. stephensi</i>	2.3
					<i>An. culicifacies</i>	2.9
Chinnu	DDT	DDT	No Spray	Post- monsoon	<i>An. stephensi</i>	12.6
					<i>An. culicifacies</i>	29.8
				Pre- monsoon	<i>An. stephensi</i>	9.1
					<i>An. culicifacies</i>	40.9
Gomath	No Spray	Alpha-Cypermethrin	DDT	Post- monsoon	<i>An. stephensi</i>	6.2
					<i>An. culicifacies</i>	0.8
				Pre- monsoon	<i>An. stephensi</i>	1.3
Indira Nagar	No Spray	Alpha-Cypermethrin	DDT	Post- monsoon	<i>An. stephensi</i>	10.2
				Pre- monsoon	<i>An. stephensi</i>	6.7
Tejpala	No Spray	No Spray	DDT	Pre- monsoon	<i>An. stephensi</i>	72.0
					<i>An. culicifacies</i>	48.4
Sultana	No Spray	No Spray	DDT	Pre -monsoon	<i>An. stephensi</i>	29.3
					<i>An. culicifacies</i>	7.3
Raimala	No Spray	DDT	DDT	Pre- monsoon	<i>An. stephensi</i>	11.1
					<i>An. culicifacies</i>	45.4
Hamira	No Spray	No Spray	DDT	Pre-monsoon	<i>An. stephensi</i>	8.9
					<i>An. culicifacies</i>	5.4
Thaat	DDT	Alpha-cypermethrin	DDT	Pre- monsoon	<i>An. stephensi</i>	2.7

*PMHD - Per Man Hour Density

Current susceptibility status of *An. stephensi* determined against temephos larvicide revealed that this species is susceptible to this larvicide in all the study districts, however, *An. culicifacies* in village Raimala of Jaisalmer district exhibited intermediate resistance against this larvicide, which needs further verification.

Table 4. Susceptibility status of malaria vector specie collected from study villages against different insecticides

District	Village	Species	Insecticide & diagnostic Dose	Exposure Time (in Hrs)	Percent Mortality	Susceptibility Status*
Bikaner	Khara	<i>An. stephensi</i>	DDT 4%	1.0	50.0	R
			Malathion 5%	1.0	20.0	R
	Kakku	<i>An. stephensi</i>	DDT 4%	1.0	80.0	R
			Malathion 5%	1.0	92.0	I R
			Permethrin 0.75 %	1.0	100.0	S
	Naal	<i>An. stephensi</i>	DDT 4%	1.0	12.5	R
			Malathion 5%	1.0	08.3	R
			Cyfluthrin 0.15 %	1.0	100.0	S
	Barmar	Gunga	<i>An. culicifacies</i>	DDT 4%	1.0	50.0
Malathion 5%				1.0	100.0	S
Permethrin 0.75 %				1.0	100.0	S
Kalyanpur		<i>An. stephensi</i>	DDT 4%	1.0	72.0	R
			Malathion 5%	1.0	88.0	IR
			Permethrin 0.75 %	1.0	100.0	S
Kawas		<i>An. stephensi</i>	DDT 4%	1.0	91.0	IR
Baitu		<i>An. stephensi</i>	DDT 4%	1.0	59.7	R
			Malathion 5%	1.0	96.7	IR
Jaisalmer	Raimala	<i>An. culicifacies</i>	DDT 4%	1.0	100.0	S
		<i>An. stephensi</i>	DDT 4%	1.0	100.0	S
			Permethrin 0.75 %	1.0	100.0	S
	Satyaya	<i>An. stephensi</i>	DDT 4%	1.0	100.0	S
			Malathion 5%	1.0	100.0	S
	Tejpala	<i>An. culicifacies</i>	DDT 4%	1.0	95.0	IR
			Permethrin 0.75 %	1.0	100.0	S
		<i>An. stephensi</i>	DDT 4%	1.0	68.0	R
			Malathion 5%	1.0	88.0	IR
	Thaat	<i>An. stephensi</i>	Permethrin 0.75 %	1.0	100.0	S
			DDT 4%	1.0	92.0	IR
			Malathion 5%	1.0	88.0	IR
	Awaya	<i>An. culicifacies</i>	DDT 4%	1.0	50.0	R
			Malathion 5%	1.0	100.0	S

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

Laboratory selection studies to establish a synthetic pyrethroid resistant strain:

Laboratory selection studies to establish a permethrin resistant strain of *An. stephensi* has been initiated. *An. stephensi* strain, collected from Kakku village of Bikaner district, has been selected for the purpose, as village Kakku since last 3 years has no history of any insecticide spray and the strain of the village is susceptible to permethrin (Table 4). The base line data of the strain has been determined and the LT_{50} and LT_{90} values have been calculated using log-probit analysis (Table 5).

Table 5. Determination of lethal time at 50 and 90 per cent mortality levels of *An. stephensi* of Kakku village against permethrin

Name of Insecticide	Diagnostic Dose (%)	Time of exposure (minutes)	Mortality %	LT_{50} and LT_{90}
Control	0.0	25	0.0	$LT_{50} = 10.5$ $LT_{90} = 20.6$ Df = 2 $\chi^2 = 5.98$
Permethrin	0.75	05	40.0	
Permethrin	0.75	10	68.0	
Permethrin	0.75	25	96.0	

The selection studies using LT_{80} (selection pressure) values are in progress to develop a permethrin resistant strain which will be used for the biochemical and genetic studies next year.