

# COMMUNICABLE DISEASES

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

**Principal Investigator:** Dr. Karam V. Singh, Scientist 'F'

**Co- Principal Investigator:** Dr S. K. Bansal, Scientist 'F' and Dr. Himmat Singh, Technical Assistant

**Commencement:** June 1, 2012      **Duration:** Two Years      **Status:** Ongoing

**Funding Agency:** Vector Science Forum, ICMR (Extramural)

### 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

The studies were initiated in June 2012 in three desert districts i.e. Barmer, Bikaner and Jaisalmer, where synthetic pyrethroids have already been introduced as indoor residual spray under national control programme. From each district ten villages, having different spray histories of last three years, were selected for the study. Entomological surveys was conducted in each village for the collection of adult and larval forms of anopheline vector species from human- and cattle- sheds for presence adult form and domestic and peridomestic containers, tankas in each households, community tanks, ponds, ditches, 'Digis' (open cement tanks for collection water for village supply) etc for the presence of larval forms. The insecticide susceptibility status of the collected individuals was determined as per WHO method. The progress report of the work carried-out during the report period is as under:

#### Vector Species Prevalence

To study the season-wise prevalence of important malaria vectors of desert parts of Rajasthan, during the report period, the entomological surveys were conducted in 30 villages, 10 villages from each study district *viz.* Barmer, Bikaner and Jaisalmer during post-monsoon season. The district-wise details on the vector prevalence is as below:

**Barmer district:** The field studies in this district have been carried-out only during post-monsoon and pre-monsoon season in all 10 study villages (Table 1). In 07 study villages both *An. stephensi* and

*An. culicifacies* were collected in only one village i.e. Kalyanpur only *An. stephensi* was recorded, however in Baitu and Goliya villages no adult anophelines were collected (Table 1). The per man hour density (PMHD) of *An. stephensi* was recorded highest in village Nimbla (PMHD: 11.3) and minimum (PMHD: 0.5) in village Parlu (Table 1). *An. culicifacies* was also recorded maximum (PMHD: 16.6) in village Gunga, but the minimum (PMHD: 0.4) in village Thob.

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* only in the village ponds and ditches of three villages i.e. Jasol, Rani and Nimbla. The common breeding of both *An. stephensi* and *An. culicifacies* was observed in village ponds and ditches.

**Table 1: Village-wise details of malaria vector species collected in Barmer district in monsoon season.**

S. No.	Name of Village	Anopheline Species Collected	No. of Mosquitoes collected	PMHD*
1.	Kalyanpura	<i>An. stephensi</i>	02	0.8
2.	Jasol	<i>An. stephensi</i>	13	4.7
		<i>An. culicifacies</i>	08	2.9
3.	Thob	<i>An. stephensi</i>	03	1.1
		<i>An. culicifacies</i>	01	0.4
4.	Baitu	Nil	-	-
5.	Rani	<i>An. stephensi</i>	04	1.1
		<i>An. culicifacies</i>	07	1.9
6.	Gunga	<i>An. stephensi</i>	08	1.6
		<i>An. culicifacies</i>	83	16.6
7.	Goliya	Nil		-
8.	Parlu	<i>An. stephensi</i>	02	0.5
		<i>An. culicifacies</i>	02	0.5
9.	Kawas	<i>An. stephensi</i>	13	1.5
		<i>An. culicifacies</i>	02	0.5
10.	Nimbla	<i>An. stephensi</i>	31	11.3
		<i>An. culicifacies</i>	12	4.4

\*PMHD – Per Man Hour Density

**Bikaner district:** The field studies in this district too have been carried-out during post-monsoon and pre-monsoon in all 10 study villages namely Khara, Shobhasar, Kodamdesar, Jagdewala Naal, Uttamdesar, Bhanipura, Kakku, Diyatra and Napasar (Table 2). *An. stephensi* was recorded from all the study villages during post-monsoon season, whereas, *An. culicifacies* only from nine villages except Khara. The per man hour density (PMHD) of *An. stephensi* was recorded highest in village Kakku (PMHD: 5.9) and minimum (PMHD: 0.3%) in village Khara and Bhanipura (Table 2). *An. culicifacies* was recorded maximum (PMHD: 8.0) in village Shobhasar, but the minimum (PMHD: 0.2) in village Naal.

**Table 2: Village-wise details of malaria vector species collected in Bikaner district in monsoon season.**

S. No.	Name of Village	Anopheline Species Collected	No. of Mosquitoes collected	PMHD*
1.	Khara	<i>An. stephensi</i>	01	0.3
2.	Shobhasar	<i>An. stephensi</i>	19	5.1
		<i>An. culicifacies</i>	30	8.0
3.	Kodamdesar	<i>An. stephensi</i>	15	3.1
		<i>An. culicifacies</i>	04	0.8
4.	Jagdewala	<i>An. stephensi</i>	03	0.9
		<i>An. culicifacies</i>	01	0.3
5.	Naal	<i>An. stephensi</i>	08	1.5
		<i>An. culicifacies</i>	01	0.2
6.	Uttamdesar	<i>An. stephensi</i>	02	0.4
		<i>An. culicifacies</i>	06	1.3
7.	Bhanipura	<i>An. stephensi</i>	01	0.3
		<i>An. culicifacies</i>	03	0.9
8.	Kakku	<i>An. stephensi</i>	37	5.9
		<i>An. culicifacies</i>	03	0.5
9.	Diyatra	<i>An. stephensi</i>	10	3.0
		<i>An. culicifacies</i>	13	3.9
10.	Napasar	<i>An. stephensi</i>	10	2.8
		<i>An. culicifacies</i>	01	0.3

\*PMHD – Per Man Hour Density

In Bikaner district *An. stephensi* breeding was reported from all ten study villages, however, *An. culicifacies* was found breeding only in five villages i.e. Shobhasar, Kodamdesar, Uttamdesar, Jagdewala and Diyatra. The breeding of *An. stephensi* was found in indoor cemented containers/tanks, ground cemented open tanks, cattle tanks, village ponds and bird pots, whereas, *An. culicifacies* was found only in village ponds and community water tanks. The village ponds and community water tanks were found shared by both *An. stephensi* and *An. culicifacies* species for breeding.

**Jaisalmer district:** The field studies in this district too have been carried-out during post-monsoon in all 10 study villages (Table 3). Both *An. stephensi* and *An. culicifacies* were recorded from nine study villages except Gomath village. The per man hour density (PMHD) of *An. stephensi* was recorded highest in village Tejpala (PMHD: 8.9) and minimum (PMHD: 0.3) in villages Chinnu and Indira Nagar. *An. culicifacies* was recorded maximum (PMHD: 60.0) in village Raimala, but the minimum (PMHD: 0.3) in village Indira Nagar (Table 3).

**Table 3: Village-wise details of malaria vector species collected in Jaisalmer district in monsoon season.**

S. No.	Name of Village	Anopheline species collected	No. of mosquitoes	PMHD*
1.	Awaya	<i>An. stephensi</i>	08	1.5
		<i>An. culicifacies</i>	33	6.3
2.	Satyaya	<i>An. stephensi</i>	18	3.8
		<i>An. culicifacies</i>	03	0.7
3.	Chinnu	<i>An. stephensi</i>	01	0.3
		<i>An. culicifacies</i>	13	4.0
4.	Gomath	Nil	-	-
5.	Indira Nagar	<i>An. stephensi</i>	01	0.3
		<i>An. stephensi</i>	01	0.3
6.	Tejpala	<i>An. stephensi</i>	38	8.9
		<i>An. culicifacies</i>	211	49.6
7.	Sultana	<i>An. stephensi</i>	17	3.9
		<i>An. culicifacies</i>	98	22.5
8.	Raimala	<i>An. stephensi</i>	10	2.0
		<i>An. culicifacies</i>	300	60.0
9.	Hamira	<i>An. stephensi</i>	02	0.7
		<i>An. culicifacies</i>	01	0.4
10.	Thaat	<i>An. stephensi</i>	04	0.9
		<i>An. culicifacies</i>	06	1.4

\*PMHD – Per Man Hour Density

In Jaisalmer district *An. stephensi* breeding was reported from all ten study villages, however, *An. culicifacies* was found breeding in only in five villages viz., Awaya, Satyaya, Tejpala, Sultana and Raimala. *An. stephensi* was found breeding in indoor underground (UG) and cemented tanks, open ground tanks, coolers, ground cemented cattle tanks, whereas, *An. culicifacies* in village pond and cattle tanks, which were also found sharing by *An. stephensi*.

#### Current susceptibility status of malaria vector species:

The current susceptibility status of *An. stephensi*, in Barmer district, was determined against DDT, malathion, alpha-cypermethrin, cyfluthrin and permethrin (Table 4). *An. stephensi* in village Kalyanpura was found susceptible to cyfluthrin (Mortality: 100.0%), intermediate resistant to DDT (Mortality: 92.0%) and resistant to malathion (Mortality: 72.0%); in village Thob, resistant to DDT and intermediate resistant to malathion (Mortality: 90.0%) and cyfluthrin (Mortality: 96.0%); in village Kawas susceptible to permethrin (Mortality: 100.0%); in village Baitu intermediate resistant to cypermethrin (Mortality: 96.0%), and in village Gunga susceptible to alpha-cypermethrin (Mortality: 100.0%). The experiments of *An. stephensi* against alpha-cypermethrin in village Baitu and Bhanipura and cyfluthrin in village Thob need repetition with adequate no. of individuals for verification of the results.

In Bikaner district, the current susceptibility status of *An. stephensi* was detected against DDT, malathion and permethrin (Table 4). *An. stephensi* in village Napasar was found susceptible to

permethrin (Mortality: 100.0%) and intermediate resistant to DDT (Mortality: 95.6%); in village Kakku susceptible to permethrin (Mortality: 100.0%); in village Diyatra, intermediate resistant to DDT (Mortality: 88.0%) and susceptible to malathion (Mortality: 100.0%); in village Naal against DDT intermediate resistant (Mortality: 88.0%), and in village Bhanipura intermediate resistant to alpha-cypermethrin (Mortality: 88.0%). The experiments of *An. stephensi* against alpha-cypermethrin in village Bhanipura need repetition with adequate no. of individuals for verification of the results.

In Jaisalmer district studies on the current susceptibility status were carried-out with both *An. stephensi* and *An. culicifacies* against DDT, malathion cyfluthrin and permethrin. *An. stephensi* in village Hameera was found susceptible to permethrin (Mortality: 100.0%), intermediate resistant to malathion (Mortality: 92.0%) and resistant to DDT (Mortality: 80.0%); in village Satyaya, susceptible to both cyfluthrin and permethrin (Mortality: 100.0%) and intermediate resistant to malathion (Mortality: 93.3%) and DDT (Mortality: 84.4%); in village Tejpala, intermediate resistant to cyfluthrin (Mortality: 96.0%), malathion (Mortality: 96.0%) and DDT (Mortality: 96.0%); in village Thaat, susceptible to permethrin (Mortality: 100.0%), intermediate resistant to DDT (Mortality: 86.0%) and resistant to malathion (Mortality: 68.0%), and in village Gomath susceptible to permethrin (Mortality: 100.0%), intermediate resistant to malathion (Mortality: 88.0%) and resistant to DDT (Mortality: 76.0%). The experiments of *An. stephensi* against cyfluthrin in village Tejpala village need repetition with adequate no. of individuals for verification of the results (Table 4).

#### **Laboratory selection studies to establish a synthetic pyrethroid resistant strain:**

Laboratory selection studies to establish a permethrin resistant strain of *An. stephensi* are continued. The strain collected from Kakku village of Bikaner district, has been selected for the purpose, as village Kakku since last 2 years has no history of any insecticide spray and the strain of the village was susceptible to permethrin. The selection studies using LT80 (selection pressure) value are in progress to develop a permethrin resistant strain which will be used for the biochemical and genetic studies.

**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)	% Mortality	Susceptibility Status*	
Bikaner	Napasar	<i>An. stephensi</i>	DDT 4.0%	1.0	95.6	IR	
			Malathion 5.0%	1.0	95.6	IR	
			Permethrin 0.75 %	1.0	100.0	S	
	Kakku	<i>An. stephensi</i>	Permethrin 0.75 %	1.0	100.0	S	
	Naal	<i>An. stephensi</i>	DDT 4.0%	1.0	88.0	IR	
	Diyatra	<i>An. stephensi</i>	DDT 4.0%	1.0	88.0	IR	
			Malathion 5.0%	1.0	100.0	S	
Bhanipura	<i>An. stephensi</i>	$\alpha$ - cypermethrin 0.05%	1.0	88.0	IR		
Barmer	Baitu	<i>An. stephensi</i>	$\alpha$ - cypermethrin 0.05%	1.0	96.0	IR	
	Kalyan-pur	<i>An. stephensi</i>	DDT 4.0%	1.0	92.0	IR	
			Malathion 5.0%	1.0	72.0	R	
			Cyfluthrin 0.15%	1.0	100.0	S	
	Kawas	<i>An. stephensi</i>	Permethrin 0.75 %	1.0	100.0	S	
	Thob	<i>An. stephensi</i>	DDT 4.0%	1.0	73.3	R	
			Malathion 5.0%	1.0	90.0	IR	
			Cyfluthrin 0.15%	1.0	90.0	IR	
	Gunga	<i>An. stephensi</i>	$\alpha$ - cypermethrin 0.05%	1.0	100.0	S	
	Jaisalmer	Hameera	<i>An. stephensi</i>	DDT 4.0%	1.0	80.0	R
Malathion 5.0%				1.0	92.0	IR	
Permethrin 0.75 %				1.0	100.0	S	
Satyaya		<i>An. stephensi</i>	DDT 4.0%	1.0	84.4	IR	
			Malathion 5.0%	1.0	93.3	IR	
			Permethrin 0.75 %	1.0	100.0	S	
			Cyfluthrin 0.15%	1.0	100.0	S	
Tejpala		<i>An. culicifacies</i>	Cyfluthrin 0.15%	1.0	100.0	S	
			<i>An. stephensi</i>	DDT 4.0%	1.0	96.0	IR
				Malathion 5.0%	1.0	96.0	IR
				Cyfluthrin 0.15%	1.0	96.0	IR
Thaat		<i>An. stephensi</i>	DDT 4.0%	1.0	86.0	IR	
			Malathion 5.0%	1.0	68.0	R	
			Permethrin 0.75 %	1.0	100.0	S	
Gomath		<i>An. stephensi</i>	DDT 4.0%	1.0	76.0	R	
			Malathion 5.0%	1.0	88.0	IR	
			Permethrin 0.75 %	1.0	100.0	S	
Chinnu	<i>An. culicifacies</i>	DDT 4.0%	1.0	100.0	S		
		Malathion 5.0%	1.0	100.0	S		

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

## 8. EXPECTED OUTCOME:

The findings would reveal whether the populations of *An. stephensi* and *An. culicifacies* select for resistance to synthetic pyrethroids or not in the field. The knowledge of the genetic and biochemical mechanisms of resistance would be useful in resistance management and control of *An. stephensi*.