1.3 Field efficacy trials of extracted latex of *Calotropis procera* for its public use as bio-larvicide against dengue vectors-

Principal Investigator: *Dr. Manju Singhi, Scientist 'C'*
Co-Investigators: *Dr.Vinod Joshi, Scientist'F'* and *Mr. Anil Purohit, Research Assistant*
Commencement: January, 2011 **Duration:** Two year **Status:** Completed
Funding agency: Indian Council of Medical Research - Translational Research Project (Extrmural)

AIMS

Present work involves testing of efficacy and feasibility of small scale application of chemically extracted latex of *Calotropis procera* in +ve breeding habitats of different socio-economic areas of Jodhpur city for the control of dengue vectors.

OBJECTIVES

1. Studies on comparative efficacy of extracted latex against dengue vectors
2. Efficacy of latex in clear and polluted water against dengue vectors
3. Feasibility studies

RATIONALE

The present study is a translational research programme wherein the basic research conducted by us has been attempted to translate into public health by testing the efficacy and feasibility of a herbal composition for the control of dengue vectors. We have reported '*A herbal composition*' as new bio larvicide useful for the control of dengue vectors from latex of *Calotropis procera*. Since dengue fever associated with Dengue Hemorrhagic Fever (DHF) is endemic in many state of India and we do not have any specific chemotherapy or vaccine yet developed against dengue and also report of development of resistance by *Ae. aegypti*, there is a need to apply a safe and easily available plant product to control breeding of dengue vectors.

PROGRESS

Our earlier work report of translational research has shown that extracted latex as bio larvicide is effective in field conditions in all + breeding containers of different socio-economic areas of Jodhpur. During the reported year a study has been undertaken to know the comparative efficacy of latex collected from different parts of Arid Rajasthan and neighboring state. To accomplish this objective latex has been collected from C. *procera* grown in the field of Jaisalmer, Bikaner, and Ganganagar and near Sabarmati Lake of Gujarat. The collected latex has been extracted by methanol as per the protocol. A field survey has been made in selected areas and marked +ve breeding containers have been applied the extracted latex collected

from Jaisalmer, Bikaner, Sri- Ganganagar and Sabarmati areas of Gujarat. The result has shown that latex collected from any part of arid Rajasthan has shown same efficacy in field conditions against dengue vectors (Tables 1-5).

Since mosquito vectors also breed in polluted water, Experiments have been undertaken to compare efficacy of latex in clear and polluted water for its optimum use in control of mosquito (Table 1). The polluted water such as biological, industrial, textile and sewerage water has been collected from different places of Salawas area and susceptibility tests have been undertaken. The experiments have been undertaken in polluted water with the application of crude latex, methanol extracted latex and acetonitrile extracted latex. 100 ppm concentration of extracted latex was applied to each polluted water containing larvae of mosquito along with control and mortality was observed after 24hr.

The results have shown that efficacy of extracted latex in polluted water was found low as compared to clean water and methanol and acetonitrile extracts (Tables 1-3) have shown comparable efficacy while latex in water has shown very low efficacy as shown in tables.

Frequency of latex application:

Series of experiments have been undertaken to study frequency of latex application in + breeding container. 20 third instar larvae were put into clay containers (5 replicates) with separate control containing measured quantity of water and after acclimatization for 3 hrs larvae have been treated by optimized dose. Containers were monitored in 24 hrs to record mortality. After 24 hours all the dead larvae were removed from the water using glass droppers and a new batch of 20 third instar larvae were added. This batch was kept in the same water having extracted latex up to 5 days (Table 4). Larval mortality was recorded in every 24 hr period in treated and control. Results have shown that latex is effective up to 24 hours causing 100 % mortality .After 24 hours latex has shown growth inhibitory effect.

Average yield of latex /plant of *C. procera* (Table 5) has been calculated by measuring the volume of latex collected and amount of extract obtained by the extraction. Latex has been collected from 40 twigs/plant for 50 days in the morning from 9-10 AM and measured the quantity of latex collection in summer as well as in post rainy season. The collected latex from each plant was extracted with methanol and developed into herbal composition and this was measured in grams for each collection as shown in table-5.

The result has shown that volume of latex produced by the plant is more in post rainy season as compared to summer (Table 5). This difference in latex volume can be attributed to the fact that in summers the plant growing in arid areas is under stress due to high temperature and unavailability of sufficient water causing the latex to be more concentrated.

Table 1. Comparative efficacy of methanol extracted latex in clear and polluted water

Type of water	Dose applied (100 ppm)	Replicates of larvae exposed															% mortality
		t_1			t_2			t_3			t_4			t_5			
		D	L	P	D	L	P	D	L	P	D	L	P	D	L	P	
Biological	Control	0	20	0	0	20	0	1	19	0	0	20	0	1	19	0	2
	treated	16	3	1	17	3	0	17	3	0	19	1	0	19	1	0	88
Industrial	Control	1	19	0	1	19	0	1	19	0	0	20	0	0	20	0	3
	treated	17	3	0	17	3	0	18	2	0	17	3	0	18	2	0	87
Sewerage	Control	1	19	0	1	19	0	2	18	0	1	19	0	0	20	0	5
	treated	6	14	0	7	13	0	7	13	0	7	13	0	6	14	0	34
Clear	Control	0	20	0	0	20	0	0	20	0	0	20	0	0	20	0	0
	treated	20	0	0	20	0	0	20	0	0	20	0	0	20	0	0	100
Textile	Control	1	19	0	11	9	0	20	0	0	20	0	0	20	0	0	2
	treated	16	4	0	17	3	0	16	4	0	16	4	0	15	5	0	80

D- No. of dead larvae., L- No. of live larvae., P-pupae emerged

Table 2. Comparative efficacy of Acetonitrile extracted latex in clear and polluted water

Type of water	Dose applied (100 ppm)	Replicates of larvae exposed															% mortality
		t_1			t_2			t_3			t_4			t_5			
		D	L	P	D	L	P	D	L	P	D	L	P	D	L	P	
Biological	Control	0	20	0	1	19	0	1	19	0	0	20	0	0	20	0	2
	treated	17	2	1	18	2	0	18	2	0	19	1	0	19	1	0	92
Industrial	Control	1	19	0	1	19	0	0	20	0	0	20	0	0	20	0	2
	treated	18	2	0	18	2	0	18	2	0	17	3	0	18	2	0	91
Sewerage	Control	1	19	0	1	19	0	1	19	0	1	19	0	0	20	0	4
	treated	8	12	0	9	11	0	7	13	0	7	13	0	8	14	0	40
Clear	Control	0	20	0	0	20	0	0	20	0	0	20	0	0	20	0	0
	treated	20	0	0	20	0	0	20	0	0	20	0	0	20	0	0	100
Textile	Control	1	19	0	1	19	0	1	19	0	2	18	0	0	20	0	5
	treated	18	2	0	17	3	0	18	2	0	16	4	0	17	3	0	86

D- No. of dead larvae., L- No. of live larvae., P-pupae emerged

Table 3. Comparative efficacy of crude latex in clear and polluted water

Type of water	Dose applied (100 ppm)	Replicates of larvae exposed															% mortality
		t_1			t_2			t_3			t_4			t_5			
		D	L	P	D	L	P	D	L	P	D	L	P	D	L	P	
Biological	Control	1	19	0	1	19	0	1	19	0	0	20	0	0	20	0	3
	treated	5	15	0	5	15	0	2	18	0	3	17	0	5	15	0	20
Industrial	Control	1	19	0	1	19	0	0	20	0	0	20	0	0	20	0	2
	treated	4	16	0	3	17	0	3	17	0	3	17	0	4	16	0	17
Sewerage	Control	1	19	0	1	19	0	1	19	0	0	20	0	0	20	0	3
	treated	1	18	1	3	17	0	1	17	2	2	17	1	1	18	1	8
Clear	Control	0	20	0	0	20	0	0	20	0	0	20	0	0	20	0	0
	treated	5	15	0	4	16	0	4	16	0	5	15	0	5	15	0	23
Textile	Control	1	19	0	1	19	0	1	19	0	0	20	0	0	20	0	3
	treated	1	18	1	1	19	0	2	18	0	2	18	0	5	15	0	11

D- No. of dead larvae., L- No. of live larvae., P-pupae emerged

Table 4. Average yield of latex obtained in summer and rainy season collection

Time (hrs)	Replicates											
	Control						Treated					
	R1	R2	R3	R4	R5	Total	R1	R2	R3	R4	R5	Total
24	20	20	20	20	20	100	0	0	0	0	0	0
%mortality	0	0	0	0	0	0 %	100	100	100	100	100	100%
48	20	20	20	20	20	100	17	17	20	17	19	97
%mortality	0	0	0	0	0	0%	5	5	0	0	0	3%
72	20	20	20	20	20	100	19	19	20	19	20	98
%mortality	0	0	0	0	0	0%	5	5	0	5	0	3%
96	20	20	20	20	20	100	19	19	20	20	20	98
%mortality	0	0	0	0	0	0%	5	5	0	0	0	2%
120	20	20	20	20	20	100	19	19	20	20	20	98
%mortality	0	0	0	0	0	0%	5	5	0	0	0	2%

Table 5. Average yield of latex collected during summer and rainy season

Season	collection /plant	Average latex obtained (ml)	Average amount of extract (g)	Extract(g)/ 100ml latex
Summer	40	24.5	1.53	6.24
Rainy	40	38.2	2.52	6.60

IMPORTANT FINDINGS

- Developed herbal composition has been found effective in all natural breeding containers of different socio-economic areas of Jodhpur.
- No major difference was observed in the feasibility studies of application of latex in the study settings.
- Cement tanks, coolers and clay pots were observed to be key breeding containers in the study areas.
- The efficacy of latex against mosquito vectors is more in clear water as compared to polluted water.
- Application of larvicide in key containers during interphase of the disease will reduce larval breeding responsible for disease occurrence.

INFERENCES

- Developed herbal composition against dengue vectors can be used as an effective bio-larvicide which along with larval control would also eliminate virus foci possibly transmitted through vertical transmission.