J Vector Borne Dis 52, June 2015, pp. 142–146

Effectiveness and feasibility of methanol extracted latex of *Calotropis procera* as larvicide against dengue vectors of western Rajasthan, India

Manju Singhi, Anil Purohit & Sushmita Chattopadhyay

Desert Medicine Research Centre (ICMR), Jodhpur, India

ABSTRACT

Background & objectives: Identification of novel effective larvicide from natural resources is essential to combat developing resistances, environmental concerns, residue problems and high cost of synthetic insecticides. Results of earlier laboratory findings have shown that *Calotropis procera* extracts showed larvicidal, ovicidal and refractory properties towards ovipositioning of dengue vectors; further, latex extracted with methanol was found to be more effective compared to crude latex. For testing efficacy and feasibility of extracted latex in field, the present study was undertaken in different settings of Jodhpur City, India against dengue vectors.

Methods: Study areas were selected based on surveillance design for the control of dengue vectors. During the study period domestic and peri-domestic breeding containers were treated with methanol extracted latex and mortality was observed after 24 h as per WHO guidelines. Latex was manually collected from internodes of *Calotropis procera* and extracted using methanol (AR) grade.

Results: Methanol extracted latex of *C. procera* was found effective and feasible larvicide against dengue vectors in the field conditions. Cement tanks, clay pots and coolers (breeding sites) were observed as key containers for the control of dengue transmission.

Interpretation & conclusion: Today environmental safety is considered to be very important. Herbal composition prepared by the extraction of latex of *C. procera* can be used as an alternative approach for the control of dengue vectors. This will reduce the dependence on expensive products and stimulate local efforts to enhance the public involvement.

Key words *Calotropis procera*; dengue vectors; larvicide; methanol extracted latex

INTRODUCTION

Dengue fever [associated with or without dengue haemorrhagic fever (DHF)] is the most important public health problem in tropical and sub-tropical regions of the world including India[1-2]. Recent estimates of WHO indicate that about 50–100 million infections, including 0.5 million DHF cases and 24,000 deaths (mostly among children) occur every year throughout the world due to dengue. In India, >19 states have been affected by dengue and DHF including Rajasthan[3]. *Aedes aegypti* (L.) (Diptera: Culicidae) is the principal vector transmitting dengue which breeds in water storage containers, such as jars, drums, tanks, coolers, cement tanks and clay pots available in and around houses[4]. In the absence of any specific antiviral therapy or vaccines, the virus transmission by the vector can be effectively controlled by targeting immatures of vector and thus eliminating the disease before it is transmitted. During the immature stages, mosquitoes are relatively immobile remain more concentrated than they are in the adult stage[5].

Development of resistance in *Ae. aegypti* towards temephos (extensively used insecticide in past) has been reported in several studies[6-9]. Consequently, to minimise the dependency on chemical based insecticides, more efforts are required towards development of alternative methods for controlling vector mosquitoes. In this regard, development of bioinsecticide, has received much attention as they are considered to be efficient, safe to environment and biodegradable compared to synthetic insecticides. Additionally, unlike commercial insecticides that are based on single active component, herbal origin insecticide comprises of number of secondary metabolites which act on both behavioural and physiological processes, therefore ruling out the chances of development of resistance[10]. The variation in the effectiveness of phytochemical compounds on target mosquito species *viz-à-viz* plant species depends on their geographical origin, plant part used, age of plant, extraction methodology and the polarity of the solvent[11] .

Calotropis procera R. Br. (Asclepiadaceae) is a plant widely distributed in Asia including arid and semi-arid parts of Rajasthan[12]. The latex in the green parts of the plant is produced and accumulated as a defence strategy against organisms such as virus, fungi, and insects[13]. The latex of *C. procera* possess schizonticidal activity[14] and has been reported to control mosquito population. Our laboratory studies have shown latex of *C. procera* as larvicidal and refractory towards ovipositioning and ovicidal against dengue vectors[15-16] and further methanol extract was found to be more effective than other solvents tested[17].

The present study is a translational research in which the efficacy of methanol extracted latex of *C. procera* has been tested in positive breeding containers at different socioeconomic settings of dengue endemic areas of western Rajasthan against dengue vectors.

MATERIAL & METHODS

Selection of study settings

The study areas were selected as per the dengue surveillance design[18] for testing efficacy and feasibility of application of extracted latex against dengue vectors. The city has two distinct regions, an inner ward city with clustered houses and outer city with cosmopolitan population. Common socioeconomic characteristics of population were extrapolated over municipal city wards and the areas with common characteristics were grouped to select representative study settings as follows:

High socio-economic inside city (HSIC): It includes households having high socioeconomic status inside the city characterized by presence of plastic buckets, clay pots, cement tanks, few underground and overhead tanks as water storage containers.

High socioeconomic outside city (HSOC): It includes households having high socioeconomic status outside the main city characterized by properly built large water storage tanks as overhead, underground tanks, coolers and clay pots.

Low socioeconomic inside city (LSIC): It includes households having low socioeconomic status inside the city characterized by more number of water filled containers and unorganized water storage system which often remains neglected for longer period (resulting into persistent breeding) such as clay pots, *matkas*, plastic containers, cement tanks, metallic vessels, very few overhead tanks and coolers.

Low socioeconomic outside city (LSOC): It includes households having low socioeconomic status outside the city characterized by more number of clay pots, plastic drums, cement tanks with very few overhead tanks and coolers.

Collection and extraction of latex

Latex was collected from naturally grown *C. procera* plant, in and around Desert Medicine Research Centre campus in three seasons, *i.e.* winter, summer and post-rainy. The latex was manually collected from internodes of plant directly into disposable bottles. The freshly collected latex was immediately subjected to extraction using methanol (100% AR). After one hour the mixture was filtered through Whatman filter paper Grade 1. The clear filtrate was collected in petri-dishes and left for air drying in a cool and dry place at room temperature till a dried layer of extract is left on the plates. The dried extract was crushed into powder form and used as larvicide by formulating 1 g dry powder in 1000 ml distilled water to attain 1000 ppm stock solution.

Dose optimization

Dose optimisation studies were carried out in DMRC campus as per WHO guidelines[19]. The *Ae. aegypti* larvae used in the experiments were collected from their natural breeding sites of the selected settings. The III instar larvae in batches of 20 were used for each experiment for each concentration of larvicide. The experiments were carried out in five replicates along with controls for each dose ranging from 10–120 ppm. Mortality was observed after 24 h. The LC_{50} and LC_{90} values along with their 95% fiducial limits, regression equation, chi-square and test of significance were determined by log-probit regression analysis[20].

Study area, pre-application surveillance of breeding and identification of key containers

The field study was carried out from January to December 2011 in the selected socioeconomic settings of Jodhpur City (26°17' 12"N, 73°1' 47"E), an arid region of Rajasthan. During the study period, a total 600 households from selected settings were surveyed in three seasons for the presence of *Aedes* breeding in domestic and peri-domestic containers. All the positive breeding containers were marked for the testing efficacy. Consent was obtained from residents of the household before carrying out interventions and the study has the approval of Ethical Committee of the Centre.

Field application study

All the marked positive breeding containers in different settings were applied with 100 ppm concentration of extracted latex according to the volume of water and mortality was observed after revisiting the area after 24 h.

Feasibility study

To know the cost effectiveness and average yield of latex from *C. procera* plant, the volume of latex collected and amount extracted were measured for calculation. Latex was collected from 40 twigs/plant for 50 days in the morning (0900–1000 hrs) and after extraction of this volume; dry powder was measured for each collection during two seasons, *i.e.* summer and post-rainy. During the study, awareness training was given to local inhabitants about the transmission, prevention and control of disease.

RESULTS

The results of dose optimization for the toxicity of extracted latex of *C. procera* against *Ae. aegypti* larvae exposed from four settings are presented in Table 1. The LC_{50} value for setting I was 36.80, setting II was 28.347, setting III was 29.55, and setting IV was 29.75 mg/l. The LC_{90} values were 66.13 for setting I, 61.72 for setting II, 56.58 for setting III, and 62.73 mg/l for setting IV. This clearly showed that latex was found effective at low concentration against all the larvae irrespective of area.

A dose of 100 ppm extracted latex was selected as field application dose to ensure the mortality in all types of water storage breeding containers irrespective of study settings. During the study period mainly cement tanks, clay pots, metallic vessels, underground tanks, plastic drums, *matkas* and coolers were observed as breeding containers (Table 2). In total, 305 different breeding containers were treated with optimized dose. All 305 treated containers showed 100% larval mortality after exposure of 24 h (Table 3). Main key breeding containers observed were cement tanks, clay pots and coolers.

Results of feasibility study have shown that in average 24.5 and 38.2 ml of latex was obtained in summer and post-rainy season respectively, from 40 twigs/plant which on extraction yielded 1.53 and 2.52 g of dry latex. The community accepted its application in breeding containers.

Table 2. Details of positive breeding containers of different settings

Type of containers	No. of containers in four settings				Total containers	Positive containers
	I	II	III	IV		
Cement	166	140	119	68	493	110
Clay	389	403	239	172	1203	70
Plastic	316	290	235	167	1008	39
Metallic	111	136	56	36	339	9
Underground	26	56	81	145	308	22
Overhead	75	108	114	150	447	0
Coolers	95	58	59	70	282	55
Total	1178	1191	903	808	4080	305

Table 3. Percent larval mortality of treated positive breeding containers

Season	Settings	Total No. of containers	Positive containers	Treated containers	% Larval mortality
Winter	I	205	12	12	100
	II	212	19	19	100
	III	209	4	4	100
	IV	149	8	8	100
Summer	I	623	43	43	100
	II	651	44	44	100
	III	399	12	12	100
	IV	410	10	10	100
Post-rainy	I	350	62	62	100
	II	328	53	53	100
	III	295	24	24	100
	IV	249	14	14	100
Total		4080	305	305	100

Table 1. Dose optimization

Settings	LC_{50} (Confidence limits)	LC_{90} (Confidence limits)	Regression equation
I	LC_{50}=36.009 (33.46546–41.3345	LC_{90}=66.13 (62.77884–70.11434)	$y = -13.48+17.48x$, $x^2 = 9.270$; df = 10; $p = 0.507$
II	LC_{50}=28.347 (25.21400–31.5730)	LC_{90}=61.72 (8.16187–65.99694)	$y = -10.15+15.89x$, $x^2 = 3.160$; df = 10; $p = 0.997$
III	LC_{50}=29.50 (27.07–31.93)	LC_{90}=56.58 (53.42–60.39)	$y = -11.67+15.87x$, $x^2 = 4.91$; df = 10; $p = 0.89$
IV	LC_{50}=29.75 (26.70–32.49)	LC_{90}=62.73 (59.21–66.93)	$y = -10.74+16.28x$, $x^2 = 4.43$; df = 10; $p = 0.92$

I— High socioeconomic inside city; II—High socioeconomic outside city; III—Low socioeconomic inside city; IV—Low socioeconomic outside city; df— Degree of freedom.

DISCUSSION

The results of the present study revealed that methanol extracted latex of *C. procera* was found effective and its application is feasible in different mosquito breeding containers of different socioeconomic settings against dengue vectors. The latex of *C. procera* has shown larvicidal efficacy against *Ae. aegypti, An. stephensi* and *Cx. quiquefasciatus*[21-22]. Shahi *et al*[23] reported high efficacy of extracted latex of *C. procera* against *Cx. quinquefasciatus* and *An. stephensi*. Laboratory studies of Bansal *et al*[24] showed that methanol extracts of seed, leaf and flower of *C. procera* are more effective as compared to aqueous extract against three important vectors of arid Rajasthan. Giridhar *et al*[21] showed larvicidal efficacy of aqueous extract of *C. procera* to control mosquito population in the laboratory and its effectivity in stagnant and polluted water in comparison to pyrethrum insecticide.

The therapeutic use of latex was also reported to be safe at low doses[25]. Laboratory findings of larvicidal potential of *C. procera* had not been so far attempted to translate into field for the public health use. The only field testing of plant derived larvicide was conducted by Vatandoost *et al*[26] reporting effectiveness of neemarin from *Azadirachta indica* against *Anopheles stephensi* and *Culex quinquefasciatus*. The bioactivity of phytochemicals against mosquito larvae varies significantly depending on plant species, plant part used and the solvent used for extraction. Fernando *et al*[27] have demonstrated limitations of using temephos (short residual activity and variability) in the field, due to which there exists a wide gap between expected and actual duration of its effect, leading to partial larval control. Water storing habits depend on cultural practices, socioeconomic status, improper supply and availability of water. These factors altogether affect dengue transmission dynamics.

In western Rajasthan, socioeconomic status of people was found to be closely associated with water management and storage practices which influenced vector breeding. The prevalence of vector breeding was also associated with the seasonality as maximum vector density was observed in rainy and post-rainy season (August–September) followed by summer (April–July) and winter (January–March), in arid parts of Rajasthan. Botanical derivatives may be the future of mosquito control. Studies on *C. procera* extracts showed larvicidal, repellent and ovideterrent properties against mosquitoes and can be used as good alternative to synthetic insecticides. Further, studies are indicated for identificaion of active principles and large-scale field trials to assess the efficacy in field conditions.

ACKNOWLEDGEMENTS

The authors are grateful to Dr V.M. Katoch, the then Secretary, Department of Health Research and Director General, ICMR, New Delhi for awarding translational research funds and Director, DMRC, Jodhpur for providing necessary research facilities for the completion of the study.

REFERENCES

1. *Dengue guidelines for diagnosis, treatment, prevention and control*. Geneva, Switzerland: World Health Organization 2009; p. 147.
2. *Report of the scientific working group on dengue*. Geneva, Switzerland: World Health organization 2006.
3. Sharma SN, Raina VK, Kumar A. Dengue/DHF: An emerging disease in India. *J Commun Dis* 2000; *32*(3): 175–9.
4. Harrington LC, Scott TW, Lerdthusnee K, Coleman RC, Costero A, *et al*. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *Am J Trop Med Hyg* 2005; *72:* 209–20.
5. Rutledge CR, Clarke F, Curtis A, Sackett S. Larval mosquito control. *Tech. Bull Florida Mosq Control Assoc* 2003; *4:* 16–9.
6. *Vector resistance to pesticides*. Fifteenth report of the WHO Expert Committee on Vector Biology and Control. *WHO Tech Rep Ser* 1992; *818:* 22.
7. Mulla MS. Laboratory and field evaluation of insect growth regulators against mosquitoes. *Proc Pap Calif Mosq Control Assoc* 1974; *42:* 175–6.
8. Lima JB, Da-Cunha MP, Da Silva RC, Galarado AK, Soares Soda S, Braga IA, *et al*. Resistance of *Aedes aegypti* to organophosphates in several municipalities in the State of Rio de Janeiro and Espírito Santo, Brazil. *Am J Trop Med Hyg* 2003; *68*(3): 329–33.
9. Braga IA, Lima JB, Soares Sda S, Valle D. *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe, and Alagoas, Brazil. *Mem Inst Oswaldo Cruz* 2004; *99*(2): 199–203.
10. Shaalan EA, Canyon D, Younes MW, Abdel-Wahab H, Mansour AH. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int* 2005; *31*(8): 1149–66.
11. Sukumar K, Perich MJ, Boober. Botanical derivatives in mosquito control: A review. *J Am Mosq Control Assoc* 1991; *7:* 210–37.
12. Bhandari MM. *Flora of the Indian desert*, Rev edn. Jodhpur, India: MPS Repros 1990; p. 1–435.
13. Larhsini M, Bousaid M, Lazrek HB, Jana M, Amarouch H. Evaluation of antifungal and molluscicidal properties of extracts of *Calotropis procera*. *Fitoterapia* 1997; *68:* 371–3.
14. Sharma JD, Sharma P. *In-vitro* schizonticidal screening of *Calotropis procera*. *Fitoterapia* 2000; *71:* 77–9.
15. Singhi M, Joshi V, Sharma RC, Sharma Keerti. Ovipositioning behaviour of *Aedes aegypti* in different concentrations of latex of *Calotropis procera*: Studies on refractory behaviour and relative preference trend across gonotrophic cycles. *Dengue Bull* 2004; *28:* 184–9.
16. Singhi M, Joshi V, Sharma RC, Adha Sandeep, Dixit AK. Larvi-

cidal efficacy of *Calotropis procera* against vectors of dengue, malaria and lymphatic filariasis in arid zone of Rajasthan. *Annals Arid Zone* 2005; *44*(2): 185–9.

17. Annual Report 2005–06; Jodhpur: Desert Medicine Research Centre 2006; p. 24–8.
18. Joshi V, Sharma RC, Adha S, Sharma Y, Sharma K, Singh H, *et al*. Importance of socioeconomic status and tree holes in distribution of *Aedes aegypti* in a dengue endemic town Jodhpur, India. *J Med Entomol* 2006; *43*(2): 330–6.
19. *Guidelines for laboratory and field testing of mosquito larvicides*. Geneva: World Health Organization 2005. WHO/CDS/WHOPES/GCDPP/2005.13.
20. Finney DJ. Probit analysis, III edn. New York: Cambridge University Press 1971; p. 1–333.
21. Giridhar G, Deval K, Mittal PK, Vasudevan P. Mosquito control by *Calotropis procera* latex. *Pesticides* 1984; *18:* 26–9.
22. Singh RK, Mittal PK and Dhiman RC. Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family: Asclepiadaceae) against mosquito larvae. *J Commun Dis* 2005*; 37:* 109–13.
23. Shahi M, Hanafi-Bojd M, Iranshahi H, Vatandoost H, Hanafi-Bojd MY. Larvicidal efficacy of latex and extract of *Calotropis procera* against *Culex quinquefasciatus* and *Anopheles stephensi*. *J Vector Born Dis* 2010; *47:* 185–8.
24. Bansal SK, Singh KV, Sharma Sapna, Sherwani MRK. Laboratory observations on the larvicidal efficacy of three plant species against mosquito vectors of malaria, dengue fever/dengue hemorrhagic fever and lymphatic filariasis in the semi-arid desert . *J Environ Biol* 2012; *33:* 617–21.
25. Singhal VA, Kumar VL. Effect of aqueous suspension of dried latex of *Calotropis procera* on hepato renal functions in rat. *J Ethnopharmacol* 2009; *122*(1): 172–4.
26. Vatandoost H, Oshaghi MA, Abaie MR, Shahi M, Yaaghoobi F, Baghali M, *et al*. Bionomics of *Anopheles stephensi* Liston in the malarious area of Hormozgan province, southern Iran 2000. *Acta Trop* 2006; *97:* 196–203.
27. Fernando M, Manuel O, Diego Weinberg, Maria A. Trinelli, Ricardo E.Gurtler. Water use practices limit effectiveness of a temephosbased *Aedes aegypti* larval control programme in northern Argentina. *PLoS Negl Trop Dis* 2011; *5*(3): e991.

Correspondence to: Dr Manju Singhi, Scientist 'C', Desert Medicine Research Centre, Jodhpur–342 005, Rajasthan, India.
E-mail: manju@dmrcjodhpur.org

Received: 22 January 2014 *Accepted in revised form:* 23 March 2015