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Mosquitocidal properties of *Basella rubra* and *Cleome viscosa* against *Aedes aegypti* (Linn.) (Diptera:Culicidae)

K. KRISHNAPPA, K. ELUMALAI*

Department of Zoology, Poompuhar College, Melaiyur, Nagapattinam District, Tamilnadu, India
*Unit of Entomotoxicity, Department of Advanced Zoology and Biotechnology, Govt. Arts College (Autonomous), Nandanam, Chennai, Tamilnadu, India

Abstract. – OBJECTIVE: To investigate the larvicidal and ovicidal activities of acetone, benzene, hexane and methanol leaf extracts of *Basella rubra* and *Cleome viscosa* against dengue vector, *Aedes aegypti*.

MATERIALS AND METHODS: Twenty five early third instar larvae of *Aedes aegypti* were exposed to various concentrations and were assayed in the laboratory by using the protocol of WHO 2005. The 24h LC_{50} values of the *Basella rubra* and *Cleome viscosa* leaf extracts were determined by probit analysis. The ovicidal activity was determined against *Aedes aegypti* to various concentrations ranging from 50-420 ppm under laboratory conditions. The hatch rates were assessed 48 h post treatment.

RESULTS: The LC_{50} value of acetone, benzene, hexane and methanol leaf extracts of *Basella rubra* were 72.63, 53.62, 122.64 and 63.28 ppm, respectively. *Cleome viscosa* shows the LC_{50} values of 126.12, 82.43, 179.26 and 123.34 ppm, respectively. Among two plant solvents tested, *Basella rubra* extracts were found to be most significant ovicidal activity 100% egg mortality (zero hatchability) observed at 300 ppm and 420 ppm for *Cleome viscosa*.

CONCLUSIONS: The crude extracts of *Basella rubra* and *Cleome viscosa* were an excellent potential for controlling *Aedes aegypti* mosquitoes.

Key Words:
Basella rubra, Cleome viscosa, Aedes aegypti, Larvicidal activity, Ovicidal activity.

Introduction

Mosquitoes are vectors of several diseases affecting humans and domestic animals worldwide. Mosquitoes are the major vectors for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis, etc., causing millions of deaths every year[1]. Mosquitoes cause substantial mortality and morbidity among people living in tropical and sub tropical zones[2]. *Aedes (Ae.) aegypti* is arguably one of most domestic mosquito vectors, feeding predominantly on man, mating and resting indoors and breeding in man-made containers in and around human habitations, especially in urban environments[3]. *Aedes aegypti* is highly anthropophilic and day-biter mosquitoes which reside in peridomestic habitats, serving as important vectors of arboviruses throughout the world[4]. The incidence of dengue has grown dramatically around the world in recent decades[5].

Basella rubra known as Malabar spinach is also known as cyclone spinach. It belongs to *Basellaceae* family. It is a climbing perennial plant. Spinach extracts have been demonstrated to exert numerous beneficial effects such as chemo and central nervous system protection, anticancer and antiaging function[6]. *Cleome viscosa* (Capparidaceae) is a weed of woodland, grassland, fallow land, fields, roadsides and wasteland, often found growing on sandy soils but sometimes on calcareous and rocky soils. The common names are tickweed, wild mustard and spider plant (English). A number of pharmacological properties of this plant have been reported[7]. The plant constitutes a rich source of bioactive compounds that are biodegradable into nontoxic products[8]. These botanical insecticides are believed to pose little threat to the environment or to human health and may provide a practical substitute for synthetic insecticides[9]. Several phytochemicals extracted from various botanical sources have been reported to have detrimental effects on mosquitoes[10]. Over the past 50 years, more than 2,000 plant species belonging to different families and genera have been reported to contain toxic principles, which are effective against insects[11]. The extensive and indiscriminate application of synthetic chemical insecti-

Corresponding Author: K. Elumalai, Ph.D.; e-mail: zoologyresearcher@gmail.com

cides leads to environmental and health concerns, widespread development of resistance by mosquitoes and unwarranted toxic or lethal effects on non-target organisms[12]. These well known drawbacks with synthetic insecticides shifted the mosquito control programme to use of eco-friendly, bio-degradable plant compounds with mosquitocidal property. A variety of secondary metabolites in the extracts obtained from different parts of a whole range of plants have been found to kill adult mosquitoes or reduce/inhibit feeding, egg laying, growth and development of mosquito larvae and pupae[13]. The phytochemicals derived from plant resources can act as larvicides, adulticides, repellent and ovipositional attractants, having deterrent activities in different researchers and may be alternative sources of mosquito larval control agents[14]. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal and ovicidal potential of the extracts from the medicinal plants against medically important species of chikungunya vector, *Aedes aegypti*.

Materials and Methods

Plant Material

Plant sampling was carried out during the growing season (March-April) of 2010 from different places of Koothur, Sirkali, Nagapattinam districts of the Tamilnadu, India. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder. At the time of collection, two pressed voucher Herbarium specimens were prepared per species and identified with the help of plant taxonomist, Department of Botany, Annamalai University, India.

Extraction Method

The dried leaf (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with acetone,benzene, hexane and methanol (500 ml, Ranchem (Ranboxy, Mumbai, Maharashtra, India), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22-26 mmHg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C.

Mosquito Rearing

Eggs of *Aedes aegypti* were collected from ICMR centre, Virudachalam. The eggs were then brought to the laboratory. The eggs were placed in enamel trays (30 × 24 × 5 cm) each containing 2 l of tap water and kept at room temperature (28 ± 2°C) with a photoperiod of 16:8 h (L:D) for larval hatching. The larvae of each mosquito species were maintained in separate trays under the same laboratory conditions and fed with a powdered feed containing a mixture of dog biscuit and baker's yeast (3:1 ratio). The trays with pupae of each mosquito species were maintained in separate mosquito cages at 26±2°C and relative humidity of 85±3% under a photoperiod of 16:8 h (L:D) for adult emergence. Cotton soaked in 10% aqueous sucrose solution in a Petri dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized young chick was placed for 3 h inside the cage in order to provide blood meal especially for female mosquitoes. A plastic tray (11 × 10 × 4 cm) filled with tap water with a lining of partially immersed filter paper was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched larvae/pupae of *Ae. aegypti* were used in all bioassays.

Larvicidal Activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO[15]. From the stock solution, five different test concentrations (*viz*., 50, 100, 150, 200, 250 and 300 ppm were prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of *Aedes aegypti*. The larvae of test species (25) were introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1 ml of emulsifier) and the required amount of plant extract was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC_{50} value was calculated by using probit analysis[16].

Ovicidal Activity

The method of Su and Mulla[17] was slightly modified and used to test the ovicidal activity. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of *Ae. aegypti* were counted individually with the help of

hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to dimethylsulfoxide (DMSO) in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) and *chi*-square value were calculated using the SPSS software package 12.0. Results with $p < 0.05$ were considered to be statically significant.

Results

The toxicity of different solvent crude extracts of *Basella rubra* and *Cleome viscosa* were tested against larvae of *Aedes aegypti.* The data were recorded and statistical data ranging LC_{50}, LC_{90}, LCL, UCL and *chi*-square value were calculated. The experiment conducted for evaluating larvicidal efficacy of leaf extracts of *Basella rubra* and *Cleome viscosa* revealed that *Basella rubra* exerted effective larvicidal properties than *Cleome viscosa* againt *Aedes aegypti*. The benzene extract of *Basella rubra* was more effective and the methanol extract was least effective and LC_{50} values ranging from 53.62 to 63.28 ppm. The leaf extracts of *Cleome viscosa* revealed the same results like *Basella rubra*: benzene extract exerted effective larvicidal efficacy and the methanolic extract was least effective with the LC_{50} values ranging from 82.43 to 123.34 ppm (Table I). The mean present hatchability of *Aedes aegypti* with *Basella rubra* and *Cleome viscosa* was shown in table II and III. The toxicity of leaf extracts was dependent on its concentration. Zero hatchability (100% mortality) was attained at the concentration of 300 ppm for *Basella rubra* and 420 ppm *Cleome viscosa*. In control experiments there was 100% hatchability (Tables II and III). The tested plant crude extracts have exerted promising larvicidal and ovicidal against selected mosquitoes.

Discussion

Our results showed that crude extracts of *Basella rubra* and *Cleome viscosa* have significant larvicidal and ovicidal activities against *Aedes aegypti* mosquitoes. The results are comparable with an earlier report by Govindarajan et al[18] who have reported that the crude extract of *Ervatamia coronaria* exerted zero hatchability (100% mortality) at 250, 200 and 150 ppm for *Culex. quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*, respectively. The crude extract of *Caesalpinia pulcherrima* zero hatchability (100% mortality) at 225 ppm for *Culex. quinquefasciatus, Aedes aegypti* and *Anopheles stephensi*, respectively. Kweka et al[19] reported that necessitated the search and development of environmentally safe, biodegradable, low-cost, and indigenous methods for vector

Table I. Larvicidal activity of *Basella rubra* and *Cleome viscosa* leaf extracts against freshly moulted (0-6h old) third instar larvae of *Aedes aegypti.*

Name of the plant	Solvent	LC_{50} (ppm)	LC_{90} (ppm)	95% confidence limit (ppm)		χ^2 value (df)*
				LCL	UCL	
Basella rubra	Hexane	122.64 ± 1.35	256.43 ± 1.66	88.28 ± 1.62	164.37 ± 1.64	24.25 (5)*
	Acetone	72.63 ± 1.22	137.28 ± 1.39	54.48 ± 1.34	84.12 ± 1.46	16.49 (5)*
	Benzene	53.62 ± 1.36	86.42 ± 1.84	44.38 ± 1.26	63.52 ± 1.23	9.18 (5)*
	Methanol	63.28 ± 0.64	112.34 ± 1.84	58.46 ± 1.66	72.62 ± 1.35	12.68 (5)*
Cleome viscosa	Hexane	179.26 ± 1.95	325.64 ± 1.47	138.33 ± 1.68	208.37 ± 1.55	13.47 (5)*
	Acetone	126.12 ± 1.33	224.16 ± 1.64	94.36 ± 1.62	169.35 ± 1.43	14.83 (5)*
	Benzene	82.43 ± 1.38	148.67 ± 1.66	63.28± 1.57	96.82 ± 1.53	18.92 (5)*
	Methanol	123.34 ± 1.47	216.18 ± 1.47	92.82 ± 1.23	161.83 ± 1.88	10.66 (5)*

Mortality of the larvae observed after 24h of exposure period. *Significant at $p < 0.05$ level. Value represents mean ± S.D. of five replications. LC_{50} = Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table II. Ovicidal activity of *Basella rubra* leaf extracts against *Aedes aegypti.*

Solvent	Percentage of egg hatchability, concentration (ppm)						
	Control	50	100	150	200	250	300
Hexane	100 ± 0.0	94.2 ± 2.3	75.6 ± 1.3	63.2 ± 2.7	43.6 ± 2.3	22.4 ± 2.3	NH
Acetone	100 ± 0.0	83.5 ± 1.4	69.6 ± 1.4	57.2 ± 1.3	38.4 ± 1.8	17.4 ± 1.8	NH
Benzene	100 ± 0.0	72.3 ± 1.5	54.4 ± 1.9	39.2 ± 1.7	18.3 ± 1.4	8.3 ± 1.4	NH
Methanol	100 ± 0.0	79.4 ± 1.8	62.8 ± 1.4	52.6 ± 1.1	21.4 ± 1.8	13.8 ± 1.9	NH

Values represent mean ± S.D. of five replications. Eggs in control groups were sprayed with no phytochemicals. NH: No hatchability (100% mortality).

control, which can be used without risk of harm to individuals and communities. They used the methanolic extracts of *Acalypha indica*, and *Achyranthes aspera* leaves against *Aedes aegypti*. Based on LC_{50} values for 4th instar *Aedes aegypti,* the combined extracts showed the strongest larvicidal activity (277 ppm). *Achyranthes aspera* and *Acalypha indica* extracts individually gave similar results (409 and 420 ppm, respectively). Respective LC_{50} values for pupae were 326 ppm, 456 ppm, and 467 ppm[20]. Govindarajan and Karuppannan[21] reported that the LC_{50} values of benzene, hexane, ethyl acetate, methanol and chloroform extract of *Eclipta alba* against early third instar larvae of *Aedes aegypti* were 151.38, 165.10, 154t.88, 127.64 and 146.28 ppm, respectively. Tren and Roberts[22] reported the efficacy shown by *Schinus terebinthifolia* for knockdown time and 100% mortality after 24 hours to adult mosquitoes from wild resistant population. Recently, the highest repellency was observed in *Zingiber officinale*; an higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150 and 180 min against *Culex. tritaeniorhynchus* and *Anopheles subpictus*, respectively[23]. Sivakumar et al[24] also reported the dodecanoic, hexadecanoic and tetradecanoic acid (isolated from conspecific eggs) against gravid female *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. Since there is no previous reports of literature available about the mosquitocidal activity of the selected plant *Basella rubra* and *Cleome viscosa* our investigations serve as first hand information. So, these phytochemicals could possibly utilized in Integrated Vector Control Programme after a detailed analysis of active principles.

Conclusions

The findings of the present study revealed that the leaf extracts of *Basella rubra* and *Cleome viscosa* possessed remarkable larvicidal and ovicidal activities against medically important species of chikungunya vector, *Aedes aegypti*.

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Conflict of Interest

None declared.

Table III. Ovicidal activity of *Cleome viscosa* leaf extracts against *Aedes aegypti.*

Solvent	Percentage of egg hatchability, concentration (ppm)						
	Control	70	140	210	280	350	420
Hexane	100 ± 0.0	95.5 ± 1.8	80.3 ± 1.2	68.4 ± 1.2	49.8 ± 1.5	26.8 ± 1.8	NH
Acetone	100 ± 0.0	91.2 ± 1.4	77.6 ± 1.8	62.2 ± 1.5	43.6 ± 1.2	22.4 ± 1.3	NH
Benzene	100 ± 0.0	82.3 ± 1.6	64.6 ± 1.4	48.2 ± 1.6	33.8 ± 1.7	12.3 ± 1.3	NH
Methanol	100 ± 0.0	88.8 ± 1.5	72.8 ± 1.3	54.8 ± 1.4	38.8 ± 1.2	17.8 ± 1.5	NH

Values represent mean ± S.D. of five replications. Eggs in control groups were sprayed with no phytochemicals. NH: No hatchability (100% mortality).

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