Int. J. Adv. Res. Biol. Sci. 2(10): (2015): 195–202

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

Larvicidal, ovicidal and pupicidal activity of *Celosia argentea* Linn. (Amaranthaceae) against the malarial vector, *Anopheles stephensi* Liston (Culicidae : Diptera)

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Abstract

Mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. Phytochemicals may serve as suitable alternative biocontrol techniques in the near future. Although several plants have been reported for mosquitocidal activity, the information pertaining to pesticidal activity in general and mosquitocidal activity in particular about *Celosia argentea* (Amaranthaceae) has not been reported so far. Crude extracts of mature leaves of *C. argentea* was assayed for larvicidal activities against *Anopheles stephensi* Liston. (Diptera: Culicidae), the vector of human malarial pathogen. A significant variation in various bases was noted with respect to concentration. Results pertaining to the experiment clearly revealed that the methanol extract showed significant larvicidal, ovicidal and pupicidal activity against the *An. stephensi.* Larvicidal activity of methanol extracts of *C. Trifolia* showed a maximum mortality in 250ppm concentration (96.0 ± 2.4%). Furthermore, the LC_{50} was found to be 141.25 (LCL=11.68 and UCL=175.87) and the LC_{90} value was recorded to be 260.01ppm (LCL=218.21 and UCL=343.03). The Ovicidal activity of methanol extract was assessed by assessing the egg hatchability. The highest concentration of both solvent extracts exhibited 100% ovicidal activity. Similarly, pupae exposed to different concentrations of methanol extract were found dead with 58.10% adult emergence when it was treated with 25 ppm concentration. Similarly, 19.58 ± 2.62 (n=30; 65.26%); 23.64 ± 1.65 (78.80) and 23.38 ± 2.83 (77.93) pupal mortality were recorded from the experimental pupae treated with 50, 75 and 100ppm concentration of extracts. Three fractions have been tested for their larvicidal activity of which the Fraction 3 showed the LC_{50} and LC_{90} values of 23.23 and 40.39 ppm. With regard to the ovicidal effect fraction 3 showed highest ovicidal activities than the other two factions. Furthermore, there were no hatchability was recorded above 50ppm (100% egg mortality) in the experimental group. Statistically significant pupicidal activity was recorded from 75ppm concentration. It is apparent that, fraction 3 possesses a novel and active principle which could be responsible for those biological activities. *Celosia argentea* offers promise as a potential phytopesticidal agent against *An. stephensi* which can be effectively used in the National Malaria Eradicating Program (NMEP).

Keywords: *Celosia argentea*, Crude extract, Fractions, Larvicidal activity, Ovicidal activity, Pupicidal activity.

Introduction

Bacillary dysentery and enteric fevers continue to be Ecologically, mosquitoes are important components of aquatic and terrestrial food chains as they serve as food for a number of animals, such as fish and birds. With respect to the human well-being, mosquitoes are of great economic impact because their bites are annoying and may cause skin allergies, and they are vectors for a number of diseases, such as malaria, yellow fever, dengue, filariasis, and certain types of encephalitis such as West Nile Fever (Srvice, 1993; Nasci and Miller 1996). *Anopheles stephensi* (Liston) is the primary vector of malaria in India and other West Asian countries, and improved methods of control are urgently needed (Burfield and Reekie, 2005). Insect vectors, especially mosquitoes are responsible for spreading serious human diseases like malaria (Halstead, 2000). The distribution and

abundance of these diseases are strongly influenced by the presence of humans and by the level of poverty of the population (Mendonca *et al* ., 2005). Malaria is by far the most important insect transmitted disease [6], remaining a major health problem in many parts of the world and is responsible for high childhood mortality and morbidity in Africa and Asia (Kleinshmidt *et al* ., 2000; Pates and Curtis, 2005; Senthil Nathan *et al* ., 2005). *Anopheles stephensi* have, therefore, become a challenging problem to public health worldwide, and it has a serious social and economical impact, especially in tropical and subtropical countries (Borovsky, 2003, Spielman, 2003; Bossche and Coetzer, 2008).

An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. It has also provoked undesirable effects, including toxicity to non-target organisms, and fostered environmental and human health concerns (Lee *et al* ., 2001). Thus, the effort towards mosquito control continues to be an important strategy in preventing the mosquito-borne diseases (Billingsley *et al* ., 2008). 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. In India, there are various plants known for their insecticidal property and are popular as pesticides. Plant derived compounds (phytopesticides) in general have been recognized as an important natural resource of insecticides (Gbolade *et al* ., 2000). Several phytochemicals have been reported to exhibit detrimental effects on mosquitoes (Kuo *et al* ., 2007; Ghosh *et al* ., 2008; Rahuman *et al* ., 2009).

Materials and Methods

Plant sampling was carried out during the growing season (March– April) of 2014 from different places of Dharapuram, of the Tamilnadu. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder. The dried leaf (100g) was powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with chloroform, ethyl acetate, acetone and methanol (500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22–26 mmHg at 45°C by 'Rota-vapour' and the residue obtained was stored at 4°C.

Eggs of *Anopheles stephensi* were collected from ICMR centre, Virudachalam. The egg rafts 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 a 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 *Anopheles stephensi* were used in all bioassays.

Bioassay

Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005). From the stock solution, five different test concentrations (*viz*., 50, 100, 150, 200 and 250 ppm were prepared and they were tested against the freshly moulted (0 – 6 hrs) third instar larvae of *An. stephensi*. The larvae of test species (25) were introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1ml of emulsifier) and the required amount of plant extract were 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 percentage of mortality was calculated by using Abbott's formula (1925). The LC_{50}, LC_{90}, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), chi-square values and the degrees of freedom were calculated by using Probit analysis with Statistical Package for Social Sciences (SPSS) 17.0 Version in MS-Excel, 2007.

Ovicidal activity

The method of Su and Mulla *(*1998*)* 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 *An. stephensi* were counted individually with the help of a hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to 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 by the following formula.

$$\%\text{Ovicidal Activity} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs treated}} \text{ X } 100$$

Pupicidal assay

Batches of ten pupae were introduced into 500 ml of the test medium containing a particular concentration of the crude extract in a plastic cup in five replications. In control, the same number of pupae was maintained at 500 ml of dechlorinated water containing appropriate volume of DMSO. All containers were maintained at room temperature (28±2°C) with naturally prevailing photoperiod (12: 12h / L: D) in the laboratory. Any pupa was considered to be dead if did not move when prodded repeatedly with a soft brush. Mortality of each pupa was recorded over 24 of exposure to the extract.

Lethal concentration (LC_{50}) represents the concentration of the test material that caused 50% mortality of the test (target and non target) organisms within the specified period of exposure, and it was determined by exposing various developmental stages of the mosquitoes to different concentrations of the extract. Based on the mortality of the test organisms recorded in these bioassays, LC_{50} was calculated along with their fiducial limits at 95% confidence level by probit analysis using the SPSS software package.

The effective plant extract was used for further analysis to identify the number of compounds responsible for their effectiveness. The extract was run on pre coated Thin Layer Chromatography (TLC) sheets. The solvent mixture consisting of hexane : methanol 1:9 ratio. Then the maximum number of fractions (3 fractions) was obtained with the same solvent system in Column Chromatography.

Results

The larvicidal activity of *C. argentea* ethyl acetate and methanol extracts were tested against fourth instar larvae of *Anopheles stephensi*. Data pertaining to the results clearly revealed that minimum larval mortality was observed in the ethyl acetate extract of *C. argentina* with 31.2 ± 0.6 at 50 ppm concentration and the maximum mortality was observed from the same extract with 96.0 ± 2.4%. Furthermore, the LC_{50} and LC_{90} value for ethyl acetate extract was found to be 121.79 and 231.98ppm. Similarly larvae exposed to 50 ppm concentration of methanol extract showed less susceptibility whereas, experimental larvae exposed to 250ppm concentration showed more susceptibility to the same extract. Furthermore, the LC_{50} was found to be 141.25 (LCL=11.68 and UCL=175.87) and the LC_{90} value was recorded to be 260.01ppm (LCL=218.21 and UCL=343.03). The recorded data were found statistically significant (Table 1; DMRT, $p<0.05$). The ovicidal activity of ethyl acetate and methanol extract was assessed by assessing the egg hatchability. It was noted that 100% hatchability was noted from the control groups, which means 0% ovicidal activity. The highest concentration of both solvent extracts exhibited 100% ovicidal activity as it was evident from the table 2. Further, as the concentration increased the mortality of the eggs were also increased with decreased hatching percentage. The data obtained in the experiments were statistically significant over the control. Effect of ethyl acetate and methanol crude extract of the *Celosia argentea* tested on the pupae of *Anopheles stephensi*, data obtained from the experiment are presented in table 3.

In the above results it is evident that methanol extract of *C. argentea* exhibited strong activity against the mosquito species. Hence, it was fractioned using TLC with varying solvent systems and finally three fractions were obtained from hexane: methanol (9:.1). Further, the three fractions were checked for their bioefficacy against the selected mosquito species.

Three fractions have been tested for their larvicidal activity against the larvae of *Anopheles stephensi*, and the results are shown in table 5. It is apparent that, fraction 3 possesses a novel and active principle which could be responsible for those biological activities. Hence, a detailed spectral analysis is to be made to identify the compound (s).

Table 1. Larvicidal activity of *Celosia argentea* at different concentration tested against freshly moulted (0-6h old) 4th instar larvae of *Anopheles stephensi*

Concentration	Mortality* (%)	LC_{50} (ppm)	95% Confidence Limits (ppm)		LC_{90} (ppm)	95% Confidence Limits (ppm)		Degrees of freedom	χ^2 value
			LCL	UCL		LCL	UCL		
Ethyl acetate extract									
Control	1.6 ± 0.6 [a]								
50	31.2 ± 0.6 [b]								
100	42.8 ± 1.6 [c]								
150	56.4 ± 1.8 [d]	121.79	88.91	154.01	231.98	191.28	316.65	5	17.152
200	78.2 ± 1.6 [e]								
250	96.0 ± 2.4 [f]								
Methanol extract									
Control	1.4 ± 0.8 [a]								
50	21.2 ± 1.6 [b]								
100	35.6 ± 0.6 [c]								
150	49.4 ± 1.6 [d]	144.25	115.68	175.87	260.01	218.21	343.30	5	13.707
200	64.6 ± 1.6 [e]								
250	92.2 ± 1.2 [f]								

The value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 24h of the exposure period. 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; Values in a column with a different superscript alphabet are significantly different at $P < 0.05$ level DMRT Test.

Table 2. Ovicidal activity (% egg hatchability) of *Celosia argentea* crude extract on eggs (0-6h old) of *Anopheles stephensi*.

Concentrations tested	Ethyl acetate extract	Methanol extract
Control	100.00±0.00 [e] (0.00)	
50 ppm	87.64±1.82 [d] (12.36)	81.34±1.62 [d] (18.66)
100 ppm	63.63±1.64 [c] (36.37)	52.25±1.83 [c] (47.75)
150 ppm	48.42±128 [b] (51.58)	35.36±1.44 [b] (63.65)
200 ppm	0.00±0.00 [a] (100.00)	0.00±0.00 [a] (100.00)

Values represent mean ± S.D. of five replications. Different alphabets in the column are statistically significant at p<0.05level DMRT Test. Eggs in the control groups were sprayed with no phytochemicals. Parentheses hold ovicidal activity.

Table 3. The pupicidal activity of ethyl acetate and methanol extract of *Celosia argentea* at different concentrations tested against the pupae of *Anopheles stephensi.*

Concentration (ppm)	n*	Mortality**		Adult emergence	
		Pupal mortality	% mortality	Adult	% emergence
Ethyl acetate extract					
50	30	13.62 ± 1.56[b]	45.40	16.38 ± 1.34[d]	54.60
100	30	17.43 ± 1.38[c]	58.10	12.57 ± 1.65[c]	41.90
150	30	20.33 ± 1.69[d]	67.76	9.67 ± 0.87[b]	32.23
200	30	25.48 ± 2.33[e]	84.93	4.52 ± 1.23[a]	15.06
Control	30	3.45 ± 0.98[a]	11.50	26.55 ± 2.33[e]	88.50
Methanol extract					
50	30	12.57 ±1.25[b]	41.90	17.43 ± 1.22[d]	58.10
100	30	18.36 ± 1.38[c]	61.20	11.64 ± 1.36[c]	38.8
150	30	21.28 ± 1.29[d]	70.93	8.72 ± 1.45[b]	29.06
200	30	27.33 ± 1.36[e]	91.10	2.67 ± 0.32[a]	8.9
Control	30	1.83 ± 1.87[a]	6.10	28.17 ± 1.37[e]	93.9

The value represents mean ± S.D. of five replications.* Number of pupae subjected to the experiment. **Mortality of the pupae observed after 7 days of exposure period). Values in the column with a different superscript alphabet are significantly different at $P < 0.05$ level DMRT Test).

Table 4. The larvicidal activity of different fractions of *Celosia argentea* tested against freshly moulted (0-6h old) 4th instar larvae of selected mosquito species.

Fractions tested	LC50 (ppm)	95% Confidence limit(ppm)		LC90	95% Confidence limit(ppm)		df	Chi-square value
		LCL	UCL		LCL	UCL		
				Anopheles stephensi				
Fraction 1	30.50	25.81	35.72	31.59	44.55	64.22	4	10.841
Fraction 2	39.45	37.08	42.20	62.71	57.98	60.06	4	7.189
Fraction 3	23.23	18.41	27.91	40.39	34.58	50.69	4	13.465

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 5. The ovicidal activity (% egg hatchability) of *Celosia argentea* fractions on eggs of *Anopheles stephensi*

Fractions tested	Concentrations tested	
	50ppm	100ppm
Control	98.84.00±0.00	
Fraction 1	46.32±2.36	12.38±1.22
Fraction 2	48.66±2.44	14.72±1.42
Fraction 3	18.08±1.34	0.00±0.00

Values represent mean ± S.D. of five replications. Different alphabets in the column are statistically significant at p<0.05level DMRT Test. Eggs in the control groups were sprayed with no phytochemicals.

Table 6. Pupicidal activity of *Cayratia trifolia* fractions tested against the pupae *Anopheles stephensi*.

Concentration (ppm)	n*	Pupal Mortality**	Adult emergence
Fraction1			
25	30	6.54±0.82[b]	23.46±1.36
50	30	10.66±1.29[c]	19.34 ± 168[c]
75	30	26.18± 1.56[d]	3.82 ± 1.23[b]
Control	30	1.24 ±0.26[a]	28.76 ± 1.33[d]
Fraction 2			
25	30	8.64± 1.33[b]	21.36 ± 1.84[c]
50	30	19.28± 1.16[c]	10.72 ± 1.82[b]
75	30	23.22 ± 0.00[d]	6.78 ± 1.44[a]
Control	30	1.46 ±0.26[a]	28.54 ± 2.36[d]
Fraction 3			
25	30	12.45± 1.23[b]	17.55 ± 1.36[c]
50	30	24.62± 1.64[c]	5.38 ± 1.23[b]
75	30	30.00 ± 0.00[d]	0.00 ± 0.00[a]
Control	30	1.33 ±0.26[a]	28.67 ± 2.33[d]

The value represents mean ± S.D. of five replications.* Number of pupae subjected to the experiment. **Mortality of the pupae observed after 7 days of exposure period). Values in the column with a different superscript alphabet are significantly different at $P < 0.05$ level DMRT Test).

Discussion

With increasing legislative restrictions being implemented concerning the use of pesticides, safe, but efficient alternatives and application techniques must be developed to allow the least-toxic but more efficient means of integrated vector control, especially during emergency situations (Chavasse and Yap, 1997; Anonymous, 2007). The methanolic extracts of the few plants exhibited larvicidal activity against *C. quinquefasciatus* (Venkatachalam and Jebanesan, 2001). In the case of ovicidal activity, exposure to freshly laid eggs was more effective than to the older eggs. It has been shown that the age of the embryos at the time of treatment played a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin to *C. quinquefasciatus* (Miura *et al* ., 1976). Rajkumar and Jebanesan (2002) reported that increase in the concentration of leaf extract of *Solanum aerianthum* induced the oviposition attractant activity in *C. quinquefasciatus*. Exposure of *A. stephensi* larvae to sub-lethal doses of neem extracts in the laboratory prolonged larval development, reduced pupal weight, high oviposition deterrence and high mortality (Su and Mulla, 1998).

Recently Mathivanan *et al* . (2010) reported that the methanol extract of *Ervatamia coronaria* showed promising larvicidal and ovicidal activity against *An. stephensi*. The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for management of *An. stephensi*. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future (Wandscheer *et al* ., 2004). Although the botanical insecticides are the lesser of many hazards in terms of general pesticide toxicities, they are toxins nonetheless. All toxins used in pest control pose some hazards to the user and also to the aquatic environment (Wandscheer *et al* ., 2004). Mullai and Jebanesan (2007) have reported that ethyl acetate, petroleum ether and methanol leaf extracts of *Citrullus colocynthis* and *Cucurbita maxima* showed LC_{50} values of 47.58, 66.92 and 118.74 ppm and 75.91, 117.73 and 171.64 ppm, respectively, against *Cx. quinquefasciatus* larvae.

Rahuman *et al* (2008) have reported that the LC_{50} value of petroleum ether extracts of *Jatropha curcas, Pedilanthus tithymaloides, Phyllanthus amarus, Euphorbia hirta,* and *Euphorbia tirucalli* were 8.79, 55.26, 90.92, 272.36 and 4.25 ppm, respectively, against *Ae. aegypti* and 11.34, 76.61, 113.40, 424.94 and 5.52 ppm, respectively, against

Cx. quinquefasciatus. Karunamoorthi *et al* (2008) reported that the petroleum ether extracts of the leaves of *V. negundo* were evaluated for larvicidal activity against larval stages of *Cx. tritaeniorhynchus* in the laboratory with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/l, respectively. The methanol leaf extracts of *V. negundo, V. trifolia, V. peduncularis and V. altissima* possessed varying levels of larvicidal activity on *Cx. quinquefasciatus* and *An. stephensi* and found with LC_{50} value of 212.57, 41.41, 76.28 and 128.04 ppm, respectively *(*Pushpalatha and Muthukrishnan, 1995*)*. The peel methanol extract of *Citrus sinensis* and the leaf and flower ethyl acetate extracts of *Ocimum canum* were tested against the larvae of *An. stephensi* (LC_{50} = 95.74, 101.53, 28.96, LC_{90} = 303.20, 492.43 and 168.05 ppm), respectively *(*Kamaraj and Rahuman, 2008*)*.

This study reveals that the *C. argentea* has remarkable mosquitocidal properties against *An. stephensi* mosquitoes. Since there is no previous record of literature available about the mosquitocidal activity of the selected plant *C. argentea* these present investigations serve as first hand information. The finding of the present investigation revealed that the leaf extract of *C. argentea* possessed remarkable larvicidal, ovicidal activity and pupicidal activity against the malarial vector *An. stephensi*. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to The Principal, Professor and Head, Department of Advanced Zoology and Biotechnology, Govt. Arts College (Autonomous), Nandanam, Chennai-600035, Tamilnadu, India for the facilities provided and their encouragement.

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