

Innovative Biocontrolling Method of Dengue Fever Vector, *Aedes aegypti* (Diptera: Culicidae)

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Abstract

The control of mosquitoes is an important public health concern around the world. The activity of petroleum ether extracts of *Dodonaea viscosa*, *Lantana camara* and *Ruta chalephensis* have been investigated towards larval development of *Aedes aegypti*. The plant extracts exhibit variable biological activity against *Ae. aegypti*. The greatest effect was observed with *Dodonaea viscosa* and *Lantana camara*. They showed acute LC₅₀ (2 days) of 126.2, 136.9 and chronic LC₅₀ (10 days) of 64.6 and 68.5 ppm, respectively. Larvae suffered chronic toxicities leading to 97.8% mortality using *Dodonaea viscosa* and 95.6% using *Lantana camara*. Egg hatchability was significantly reduced in *Dodonaea viscosa* and *Lantana camara* concentrations. All extract concentrations of *Dodonaea viscosa* and *Lantana camara* as well as higher extract concentration of *Ruta chalephensis* caused significant high hindrance of subsequent larval development and consequently reduced both pupation and adult emergence. Drastic retardation of development was shown using *Dodonaea viscosa* extracts (17.3, 7.4%) and *Lantana camara* extracts (19.3, 9.0%) from larval stage managed to pupal and adult stages, when reared in very low concentration (100 ppm) of the extracts. *Ruta chalephensis* was only more effective at higher concentrations. Hence, application of these extracts to *Ae. aegypti* mosquito breeding sites can be used as an innovative valuable alternative to the existing synthetic pesticides for the control of *Ae. aegypti*.

Keywords: petroleum ether extraction, toxicity, *Aedes aegypti*, *Dodonaea viscosa*, *Lantana camara* and *Ruta chalephensis*

1. Introduction

Insect transmitted diseases remain a major source of illness and death worldwide. Mosquitoes are still the world's number one vectors of human and animal diseases. These diseases including malaria, filariasis, yellow and dengue fever and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries (Jang, Kim, Ahn, & Lee, 2002; Rajkumar & Jebanesan, 2005). *Aedes aegypti*, the main carrier for viruses that cause dengue and dengue hemorrhagic and yellow fevers, is found majorly in the tropics and subtropics (Langat et al., 2012). There is no effective vaccine against dengue, and thus the only way of significantly lowering the incidence of this disease is through mosquito control (Malavige, Fernando, Fernando, & Seneviratne, 2004). The target of mosquito control is to prevent proliferation of mosquito borne diseases and to improve quality of environment and public health. Mosquitoes are conspicuous nuisance pests as well, even after massive efforts of eradication or control (El-Maghraby, Nawwar, Bakr, Helmy, & Kamel, 2012). The extensive use of chemical pesticides or insecticides resulted in inducing resistance by insect pests besides, residue contamination of human food, mammalian toxicity and environmental pollution (Domingues, Agra, Monaghan, Soares, & Nogueira, 2010). One alternative approach is the use of insecticides from natural origin, especially plant-derived products to resolve these problems (Elhag, Harraz, Zaitoun, & Salama, 1996). Several studies have emphasized the importance of research and development of herbal substances for controlling mosquitoes (Shaalan, Canyon, Younes, Abdel-Wahab, 2012). Sukumar, Perich, and Boobar (1991) listed and

discussed results of 344 plant species that only exhibited mosquitocidal activity. The phytochemicals derived plant recourses can act as larvicides, insect growth regulators, repellent and ovipositional attractant (Das, Baruah, Talukadar, & Das, 2003; Venkatachalam & Jebanesan, 2001). Extracts of onion, garlic, eucalyptus and tobacco are reported to control many plant pathogenic fungi and insects. The neem biopesticides is usually used for all biological materials and organisms, which can be formulated for use as pesticides for the control of pests (Praveena, Venkatasubbu, & Jegadeesan, 2012). Kumar, Dhamodaran, Nilani, and Balakrishnan (2012) found that the hole extracts of *Tephrosia purpurea* (L.) has larvicidal activity against the larvae of *Culex quinquefasciatus*. The three plants of *Dodonaea viscosa*, *Lantana camara* and *Ruta chalepensis* are available in Saudi Arabia and used in folk medicine (Migahid, 1978). Mogahed and Gesraha (2005) found that the extract of *Dodonaea viscosa* significantly reduced the infestation of cotton plant by many insect pests. Also, *Lantana camara* and *Ruta chalapensis* were tested for their efficacy against parasitic bee mite, *Varroa destructor* and toxic effect against the mite (Zaitoun & Madkour, 2012).

Therefore, the main objective of the present study was to investigate the effect of petroleum ether extracts from three plant species: *Dodonaea viscosa*, *Lantana camara* and *Ruta chalapensis* on egg hatchability and larval development in *Aedes aegypti* mosquitoes.

2. Materials and Methods

2.1 Insects

The mosquito *Ae. Aegypti*, larvae was collected from the natural sites located in Jeddah city, Saudi Arabia. Colonies were maintained in the laboratory. Mosquitoes were held at 27 ± 1 °C, $70 \pm 5\%$ RH, and photo regime of 14:10 h (light: dark). Adults were provided with a 10% sucrose solution as food source. Female mosquitoes were allowed to blood feed periodically from pigeon host. Larvae were reared in dechlorinated water under the same laboratory conditions and were fed with fish food. The experiments were carried out at the Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia.

2.2 Plant Collection and Extractions

Test materials, *Dodonaea viscosa*, *Lantana camara* and *Ruta chalapensis* were collected from different parts of Saudi Arabia, and were authenticated by a specialist from Faculty of Pharmacy, King Abdulaziz University, Saudi Arabia. Plant leaves were air dried in the laboratory, ground in a mortar and extracted with petroleum ether. One hundred grams of powder for each solvent separately were extracted three times with 300 ml of petroleum ether at room temperature. After 24 h, the supernatants were decanted, filtrated through filter paper and dried in a rotary evaporator to obtain a semi solid crude extract according to Chitra, Janardhan, Kameswara, and Nagaiah (1993). The dry extracts were kept in refrigerator until using for experiments.

2.3 Test Procedure

Stock solution of the three plant petroleum ether extracts were prepared by dissolving the dry extract in warm distilled H₂O (0.5 g/100 mL H₂O). Two drops of Tween. 80 as emulsifier were used to facilitate the dissolving of tested material in water. Different concentration of 100, 200, 300, 400 and 500 ppm were prepared from the stock solution. About 30 freshly laid eggs or 30 second instars larvae were transferred from the culture into plastic cups (8-cm diameter, 10 cm deep), each containing 30 ml of the desired concentration. Treatments were carried out in triplicate and control larvae received only 2 drop of Tween. 80 in distilled water. Larvae were fed *ad libitum* and kept under laboratory conditions. Larval mortalities were counted at 2, 4 and 10 days after treatment. Percentage of successful pupation and adult emergence were determined by monitoring on daily basis until all adults in the control have emerged.

Data were analyzed using maximum likelihood procedures and the effectiveness was expressed as LC₅₀ values according to Finney (1971). Data were corrected for control mortality (Abbot, 1925). Egg hatchability data were analyzed by an analysis of variance. If significant differences ($p < 0.05$) occurred, means were separated by Duncan's multiple range test.

3. Results and Discussion

Data given in table (1) indicated the biological activity of petroleum ether extracts of *Dodonaea viscosa*, *Lantana camara* and *Ruta chalepensis* against the 2nd instar larvae of *Ae. aegypti*. The mortality percentages of *Ae. aegypti* larvae, their LC₅₀ values and 95% confidence limits at 2, 4, 10 days after treatment are shown in Table 1, 2. Data shows that 97.8 and 95.6% mortality of larvae reached after 10 days of exposure to 500 ppm of *D. viscosa* and *L.camara* extracts, respectively. However, the lowest *D. viscosa* concentration (100 ppm) caused 47.8% mortality after 2 days of treatment. *Ruta chalepensis* petroleum ether extract caused the lowest mortalities, whereas highest concentration (500 ppm) caused 84.4% mortality after 10 days of treatment compared to 3.3%

for the controls. Data of Table 2 showed significant differences. LC_{50} for second instars larvae were (126.18 and 64.56), (136.89 and 68.49) and (173.66 and 75.43) for *Dodonaea viscosa*, *Lantana camara* and *Ruta chalepensis*, respectively after 2 and 10 days from exposure of larvae to plant extracts. *Dodonaea viscosa* and *Lantana camara* were significantly more toxic at all exposure times than *Ruta chalepensis*.

Table 1. Effect of plant extract on the mortality percentages of larvae of *Aedes aegypti*

Plant Extract	Conc. (ppm)	% Mortality after		
		2 nd day	4 th day	10 th day
<i>Dodonaea viscosa</i>	100	47.8	52.2	66.7
	200	56.7	67.8	77.8
	300	70.0	73.3	84.4
	400	81.1	85.6	92.2
	500	90.0	94.4	97.8
	control	00.0	00.0	03.33
<i>Lantana camara</i>	100	45.6	51.1	65.6
	200	54.7	68.9	74.4
	300	67.8	71.1	81.1
	400	80.0	83.4	91.1
	500	85.6	93.3	95.6
	Control	00.0	00.0	06.7
<i>Ruta chalepensis</i>	100	41.1	45.6	60.0
	200	46.7	62.2	66.7
	300	61.1	67.8	76.7
	400	71.1	75.8	81.3
	500	75.6	81.1	84.4
	Control	00.0	00.0	03.0

Table 2. LC_{50} values and 95% confidence limits of *Aedes aegypti* larvae in media containing petroleum ether plant extracts

Plant material	Assay time (days)	Slope	LC_{50} (95%CL)
<i>Dodonaea viscosa</i>	2	1.64	126.18 (96.55-164.68)
	4	1.83	104.25 (78.57-138.13)
	10	1.78	64.56 (41.84-99.30)
<i>Lantana camara</i>	2	1.66	136.89 (106.94-175.63)
	4	1.76	105.31 (78.70-140.69)
	10	1.62	68.49 (41.08-104.04)
<i>Ruta chalepensis</i>	2	1.37	173.66 (136.08-221.39)
	4	1.40	123.40 (89.67-169.52)
	10	1.19	75.43 (45.55-142.42)

Egg hatchability was significantly lower ($p < 0.05$) in all extracts than control (Table 3). At 100 ppm concentration, *Dodonaea viscosa* and *Lantana camara* had the most severe effect on egg hatching rate which were reduced by about 35% and 33.5% compared with 26% for *Ruta chalepensis*. At their highest concentration (500 ppm), the tree plant extract reduced egg hatchability percentages by about 90%, 87% and 69% for *Dodonaea*

viscose, *Lantana camara* and *Ruta chalepensis* respectively. In fact, about 90% of the emerging larvae died within the first 2 days in the *Dodonaea viscose* extract at this concentration.

Table 3. Egg hatchability percentages of *Aedes aegypti* in media containing plant extracts

Plant extract	Concentration(ppm)	Mean% Egg Hatchability*
<i>Dodonaea viscosa</i>	100	65.0 cd
	200	52.6 e
	300	45.7 f
	400	22.5 i
	500	10.3 j
<i>Lantana camara</i>	100	66.5 c
	200	54.9 e
	300	47.2 f
	400	26.7 i
	500	13.0 j
<i>Ruta chalepensis</i>	100	73.7 b
	200	61.3 d
	300	53.9 e
	400	40.6 g
	500	31.2 h
	Control	97.5 a

*Means followed by the same letter are not significantly different at 5% level, Duncan's multiple tests.

On the other hand all plant extracts had an evidence inhibitory effect even at their lowest concentrations (100 ppm), where the successful pupation were only 17.3, 19.3 and 39.3 for *Dodonaea viscose*, *Lantana camara* and *Ruta chalepensis*, respectively. Complete suppression of adult emerging was evident in the 500 ppm concentration of *Dodonaea viscose* and *Lantana camara*. The adult emerging percentages from the 100 ppm treatments were 7.4, 9.0 and 14.9 for *Dodonaea viscose*, *Lantana camara* and *Ruta chalepensis*, respectively (Table 4).

Table 4. Successful pupation and adult emergence of *Aedes aegypti* larvae reared in media containing plant extracts

Plant extract	Conc. (ppm)	% Successful pupation or adult emergence	
		Pupation	Adult emergence
<i>Dodonaea viscosa</i>	100	17.3	7.4
	200	12.2	3.7
	300	6.1	2.2
	400	1.1	0.0
	500	0.0	0.0
<i>Lantana camara</i>	100	19.3	9.0
	200	15.7	7.1
	300	8.4	5.3
	400	5.1	2.2
	500	0.0	0.0
<i>Ruta chalepensis</i>	100	39.3	14.9
	200	23.9	10.3
	300	14.3	7.5
	400	8.7	3.7
	500	2.1	0.0
	Control	96.7	93.2

Considerable biological activity related to toxicity and hindrance of growth and development of the larvae of *Ae. aegypti* has been observed with the petroleum ether extracts of the three plant materials investigated. Of the three plant extracts, *Dodonaea viscosa* and *Lantana camara* were found to cause higher rate of mortality compared with *Ruta chalepensis*. Thus, the extract of *Dodonaea viscosa* caused toxicity at 300 ppm concentration leading up to 70% larval mortality over a period of 48 hours, and 84.4% mortality after exposure for 10 days. Praveena, Venkatasubbu and Jegadeesan (2012) found that *Dodonaea viscosa* extract has antifeedant activity against spotted bollworm. Also, Zaitoun and Madkour (2012) found that *L. camara* extract was very effective against *Varroa destructor*. Emam, Swelam, and Megally (2009) found that furocoumarin and quinolone alkaloid isolated from *Ruta chalepensis*, showed larvicidal and antifeedant activity against the cotton pest, *Spodoptera littoralis* larvae.

5. Conclusions

The results obtained in this study demonstrated the importance, the toxic, growth and development-retarding influence of the extracted plant materials, especially *Dodonaea viscosa* and *Lantana camara* on *Ae. aegypti* mosquitoes. It is anticipated that such effects may be observable when these materials are applied in natural larval breeding habitats in rural as well as in urban localities. Moreover, application of these materials is not likely to leave harmful residues in the environment since they are naturally occurring among the local flora.

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