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ORIGINAL PAPER

Larvicidal and ovicidal properties of leaf and seed extracts of *Delonix elata* (L.) Gamble (Family: Fabaceae) against malaria (*Anopheles stephensi* Liston) and dengue (*Aedes aegypti* Linn.) (Diptera: Culicidae) vector mosquitoes

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Abstract Mosquito-borne diseases with an economic impact create loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates. Mosquito control is facing a threat because of the emergence of resistance to synthetic insecticides. Extracts from plants may be alternative sources of mosquito control agents because they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use to control mosquitoes. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. 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 crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts from the medicinal plant *Delonix elata* against the medically important mosquito vectors, *Anopheles stephensi* and *Aedes aegypti* (Diptera: Culicidae). The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in methanol extract of leaf of *D. elata* against the larvae of *A. stephensi* and *A. aegypti* with the LC_{50} and LC_{90} values being 93.59 and 111.83, and 163.69 and 202.77 ppm, respectively. Compared to leaf extracts, seeds have low potency against two mosquitoes with the LC_{50} and LC_{90} values being 115.28 and 139.04, and 225.07 and 273.03 ppm, respectively. The mean percent hatchability of the eggs was observed after 48 h post-treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. All the five solvent extracts showed moderate ovicidal activity; however, the methanol extract showed the highest ovicidal activity. One hundred percent mortality was observed at 300 ppm for leaf methanol extract and 500 ppm for seed methanol extract of *D. elata* against *A. stephensi* and *A. aegypti*, respectively. These results suggest that the leaf and seed extracts have the potential to be used as an ideal eco-friendly approach for the control of mosquitoes. This is the first report on the mosquito larvicidal and ovicidal activities of the reported *D. elata* plant.

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Introduction

Mosquitoes being vector for many tropical and subtropical diseases are the most important single group of insect well-known for their public health importance. Despite progress in vaccine development, no effective and acceptable multivalent vaccines are currently available against mosquito-borne diseases. The approach to combat these diseases largely relies on interruption of the disease transmission cycle by either destruction of the aquatic stages or by killing the adult mosquitoes using chemical insecticides. The drastic effects of chemical insecticide-based intervention measures for the control of disease vectors have received wide public apprehension and have caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans, and other non-target organisms. To alleviate these problems, major emphasis has been on the use of natural plant-based products as larvicides which can provide an alternate to synthetic insecticides. Plants are rich sources of bioactive compounds that

can be used to develop environmentally safe vector and pest-managing agents. A number of plants and microbes have been reported as selective with little or no harmful effect on non-target organisms and the environment (Govindarajan and Sivakumar 2011; Govindarajan et al. 2008a). Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal, and adulticidal properties. Many synthetic insecticides and naturally occurring chemical cues have been shown to influence mosquito oviposition (Geetha et al. 2003). A few insecticides in common use have also exhibited high-deterrent activity, causing negative ovipositional response (Moore 1977). The effects of fruit and senescent leaf extracts of *Melia azedarach* (Coria et al. 2008) and the piperitenone oxide isolated from essential oil of *Mentha spicata* (Tripathi et al. 2004) were investigated for their larvicidal, ovicidal, and oviposition deterrence effects against *Anopheles stephensi* and *Aedes aegypti*. The active components dymalol, nymania-3, and triterpenes isolated from the extract of *Dysoxylum malabaricum* act as an oviposition repellent and/or deterrent to *A. stephensi* (Hisham et al. 2001). The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth, and reproductive inhibitors, repellents, and oviposition deterrent.

A. aegypti L., a vector of dengue that carries the arbovirus responsible for these diseases, is widely distributed in the tropical and subtropical zones. In Maharashtra, dengue fever has spread to 209 villages in the state infecting 31,000 peoples. There have been reports of large-scale outbreak of this virus in southern India. At least 80,000 people in Gulbarga, Tumkur, Bidar, Raichur, Bellary, Chitradurga, Davanagere, Kolar, and Bijapur districts in Karnataka state, Andhra Pradesh, and Tamil Nadu are known to have been affected since December 2005 (Ravi 2006). However, recent reports of large-scale outbreaks of fever caused by Chikungunya virus infection in several parts of southern India have confirmed the reemergence of this virus (WHO 2006). Malaria is one of the serious scourges inflicted upon humanity. It causes human mortality and morbidity along with great financial loss. In general, transmission of malaria occurs between 64° N and 32° S of the Earth in more than 100 countries throughout the Africa, Asia, and Latin America along with certain Caribbean and Pacific islands where there are favorable conditions for completion of life cycle of malaria parasite (Zarchi et al. 2006). Among 53 anopheline species present in India, nine are vectors of malaria. *A. stephensi* is responsible for transmission of malaria in urban regions of India (Rahman et al. 1989). In India, malaria is still the most important cause of morbidity and mortality with approximately two to three million new cases arising every year (Sharma et al. 2009). Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings (Mathew et al. 2009). It is known that larvicides and ovicides play a vital role in controlling mosquitoes in their breeding sites. Mosquito control has been mainly affected by use of conventional insecticides, but these have caused their own problems, such as adverse effects on the environment and the encouragement of pesticide resistance in some mosquitoes (Su and Mulla 1998). These problems stimulated a search for safer and effective alternative bioactive ovicidal and larvicidal material. Although various biocontrol measures are in vogue, their effective control of eggs and larval mosquitoes has not been hitherto highlighted.

The common mosquito larvicides, nowadays, include an organophosphate temephos, methoprene, phytochemicals, soil bacterium, *Bacillus thuringiensis israelensis*, and *Bacillus sphaericus*. However, the high amount of chemical larvicides could lead to long-term residual effect to the environment and chronic effects on non-target organisms. The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. It can act as larvicides, insect growth regulators, repellents, and ovipositional attractants, having deterrent activities observed by different researchers (Amer and Mehlhorn 2006a, b; Govindarajan et al. 2011a). Govindarajan et al. (2008b) reported that methanolic leaf extract of *Cassia fistula* was tested for larvicidal and ovicidal activity against *Culex quinquefasciatus* and *A. stephensi*. The leaf extract of *Acalypha indica* with different solvents viz., benzene, chloroform, ethyl acetate, and methanol was tested for larvicidal, ovicidal activity, and oviposition attractancy against *A. stephensi* (Govindarajan et al. 2008c). The leaf extract of *C. fistula* with different solvents viz., methanol, benzene, and acetone was studied for the larvicidal, ovicidal, and repellent activity against *A. aegypti* (Govindarajan 2009). Samidurai et al. (2009) observed that the leaf extracts of *Pemphis acidula* were evaluated for larvicidal, ovicidal, and repellent activities against *C. quinquefasciatus* and *A. aegypti*. The acetone, chloroform, ethyl acetate, hexane, and methanol leaf extracts of *A. indica*, *Achyranthes aspera*, *Leucas aspera*, *Morinda tinctoria*, and *Ocimum sanctum* were studied against the early fourth-instar larvae of *A. aegypti* and *C. quinquefasciatus* (Bagavan et al. 2008). Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, *Citrullus colocynthis*, *Coccinia indica*, *Cucumis sativus*, *Momordica charantia*, and *Trichosanthes anguina*, was tested against the early fourth-instar larvae of *A. aegypti* L. and *C. quinquefasciatus* (Rahuman et al. 2008).

Govindarajan and Karuppannan (2011) investigate the larvicidal and ovicidal activities of benzene, hexane, ethyl acetate, methanol, and chloroform leaf extract of *Eclipta alba* against dengue vector, *A. aegypti*. The larvicidal and ovicidal efficacy of different extracts of *Cardiospermum*

halicacabum L. against *C. quinquefasciatus* and *A. aegypti* (Govindarajan 2011a)—the larvicidal and ovicidal activity of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of the leaf of three plants, *E. alba*, *C. halicacabum*, and *Andrographis paniculata*—were tested against the early third-instar larvae of *A. stephensi* (Govindarajan 2011b). Muthukrishnan and Puspalatha (2001) evaluated the larvicidal activity of extracts from *Calophyllum inophyllum* (Clusiaceae), *Rhinacanthus nasutus* (Acanthaceae), *Solanum suratense* (Solanaceae), *Samadera indica* (Simaroubaceae), and *Myriophyllum spicatum* (Haloragaceae) against *A. stephensi*. Several indigenous plants viz., *Ocimum basilicum*, *Ocimum santum*, *Azadirachta indica*, *Lantana camera*, *Vitex negundo*, and *Cleome viscosa* were studied for their larvicidal action on the field which collected fourth-instar larva of *C. quinquefasciatus* (Kalyanasundaram and Dos 1985). Murugan and Jeyabalan (1999) reported that *L. aspera*, *O. santum*, *A. indica*, *Allium sativum*, and *Curcuma longa* had a strong larvicidal, anti-emergence, adult repellency, and antireproductive activity against *A. stephensi*.

Elango et al. (2009) have reported that the leaf acetone, chloroform, ethyl acetate, hexane, and methanol extracts of *Aegle marmelos*, *Andrographis lineata*, *A. paniculata*, *Cocculus hirsutus*, *Eclipta prostrate*, and *Tagetes erecta* were tested against fourth-instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus*. The ethanol extract of *Curcuma aeruginosa*, *Curcuma aromatica*, and *Curcuma xanthorrhiza* was tested for repellent activity against *Aedes togoi*, *Armigeres subalbatus*, *C. quinquefasciatus*, and *C. tritaeniorhynchus* (Pitasawat et al. 2003). Murugan et al. (2003) studied the interactive effect of botanicals (neem, pongamia) and *L. aspera* and *B. sphaericus* against the larvae of *C. quinquefasciatus*. Vahitha et al. (2002) studied the larvicidal efficacy of *Pavonia zeylanica* L. and *Acacia ferruginea* against *C. quinquefasciatus* Say. Shigeo et al. (2004) reported the larvicidal effect of neem against *A. aegypti* and chironomid larvae. Mullai et al. (2008) have reported that the leaf extract of *Citrullus vulgaris* with different solvents, viz., benzene, petroleum ether, ethyl acetate, and methanol, was tested for larvicidal, ovicidal, repellent, and insect growth regulatory activities against *A. stephensi*. Ovicidal effects of the seed extract of *Atriplex canescens* were reported against *C. quinquefasciatus* (Ouda et al. 1998) and the larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogon citratus*, *Cinnamomum zeylanicum*, *Rosmarinus officinalis*, and *Zingiber officinale* against *C. tritaeniorhynchus* and *A. subpictus* (Govindarajan 2011c). Su and Mulla (1998) reported the ovicidal activity of the neem product azadirachtin against the mosquitoes *Culex tarsalis* and *C. quinquefasciatus*.

Delonix elata (Syn. *Poinciana elata*) commonly known as white gold mohur (Fabaceae) is used by folklore for joint pains and in flatulence. In Indochina, the bark is considered as febrifuge and antiperiodic. The leaf and bark in the form of paste are used by local people to reduce inflammation and pain. It has been used in traditional Indian medicine for the treatment of rheumatism and stomach disorders (Thirugnanam 2003), and its leaves are used in the treatment of bronchitis and pneumonia in infants. Leaf extracts of *D. elata* are reported for strong anti-inflammatory activity (Sethuraman and Sulochana 1986). As far as our literature survey could ascertain, no information was available on the ovicidal and larvicidal activities of the experimental plant species given here against *A. stephensi* and *A. aegypti*. Therefore, the aim of this study was to investigate the mosquito ovicidal and larvicidal activities of the different solvent extracts of *D. elata* plant species from Tamil Nadu, India. This is the first report on the mosquito larvicidal and ovicidal activity of the solvent extracts of selected plant.

Materials and methods

Collection of plants

Fully developed leaves and seeds of the *D. elata* (Fig. 1) were collected from Thanjavur District (between 9°50′ and 11°25′ of the north latitude and 78°45′ and 70°25′ of the east longitude), Tamil Nadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the Herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University.

Extraction

The leaves and seeds were washed with tap water, shade-dried, and finely ground. The finely ground plant leaf powder (1.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with five different solvents, namely hexane, benzene, chloroform, ethyl acetate, and methanol, individually (Vogel 1978). The solvents from the extracts were removed using a rotary

Fig. 1 leaves and seeds of *D. elata*

vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in ethanol. From this stock solution, different concentrations were prepared, and these solutions were used for larvicidal and ovicidal bioassays.

Test organisms

The mosquitoes *A. stephensi* and *A. aegypti* were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week-old chick for blood meal. Mosquitoes were held at 28±2°C, 70–85% relative humidity, with a photoperiod of 14:10-h light/dark cycle.

Larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by World Health Organization (2005). Batches of 25 third-instar larvae were transferred to a small disposable test cups, each containing 200 ml of water. The appropriate volume of dilution was added to 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration. Four replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. To this, 1 ml of ethanol was added. The LC_{50} value was calculated after 24 h by probit analysis (Finney 1971).

Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla (1998) was performed. The eggs of *A. stephensi* and *A. aegypti* were collected from vector control laboratory, Annamalai University. The leaf and seed extracts were diluted in the ethanol to achieve various concentrations ranging from 75 to 600 ppm. Eggs of these mosquitoes species (100) were exposed to each concentration of leaf and seed extracts. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post-treatment by the following formula.

$$\%\ \text{of mortality} = \frac{\text{No of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

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 values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

Result

The results of the larvicidal activity of crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of leaf and seed of *D. elata* against the larvae of two important vector mosquitoes viz., *A. stephensi* and *A. aegypti* are presented in Tables 1, 2, 3, and 4 and Figs. 2 and 3. The experiments conducted for evaluating larvicidal efficacy of leaf and seed extracts of *D. elata* revealed that leaf extract exerted effective larvicidal activity. Among the extracts tested, the highest larvicidal activity was observed in leaf methanol extract of *D. elata* against *A. stephensi* than *A. aegypti* with the LC_{50} and LC_{90} values being 93.59 and 111.83, and 163.69 and 202.77 ppm, respectively. Compared to leaf extracts, seeds have low potency against two mosquitoes. The LC_{50} and LC_{90} values were 115.28 and 139.04, and 225.07 and 273.03 ppm, respectively. The chi-square values are significant at $p<0.05$ level. The chi-square values in the bioassays indicated probably the heterogeneity of the test population. The 95% confidence limits (LC_{50} (LCL–UCL)) and (LC_{90} (LCL–UCL)) were also calculated. The mean percent of egg hatchability of *A. stephensi* and *A. aegypti* were tested with five different solvents at different concentrations of *D. elata* leaves and seed extracts, and the results are listed in Tables 5 and 6. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Among the extracts tested for ovicidal activity against *A. stephensi* and *A. aegypti*, the leaf and seed methanol extract of *D. elata* exerted 100% mortality (zero hatchability) at 300 and 500 ppm, respectively. The leaf extract of *D. elata* was found to be most effective than seed against larvae and eggs of two vector mosquitoes. Control eggs showed the 100% hatchability.

Discussion

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. Phytoextracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties. Our result showed that the crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of leaf and seed of *D. elata* have significant larvicidal and ovicidal properties against two important

Table 1 Larvicidal activity of different solvent leaf extracts of *D. elata* against *A. stephensi*

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2
Methanol	Control	0.0±0.0	93.59 (73.81–112.87)	163.69 (139.74–206.61)	13.828*
	40	23.5±1.2			
	80	44.6±1.8			
	120	63.2±1.6			
	160	85.1±0.8			
	200	99.7±2.0			
Ethyl acetate	Control	0.0±0.0	112.13 (83.86–138.96)	201.49 (169.00–263.61)	16.578*
	50	26.7±1.0			
	100	48.1±1.2			
	150	66.5±1.6			
	200	84.8±1.8			
	250	99.9±2.0			
Chloroform	Control	0.0±0.0	118.26 (91.79–144.07)	207.58 (175.86–266.55)	15.259*
	50	23.4±1.4			
	100	44.6±2.0			
	150	62.7±1.6			
	200	83.1±2.2			
	250	99.8±0.8			
Benzene	Control	0.0±0.0	126.03 (100.27–151.82)	216.19 (184.23–275.26)	14.767*
	50	19.5±1.6			
	100	40.6±1.8			
	150	59.8±2.0			
	200	78.7±1.6			
	250	99.6±1.0			
Hexane	Control	0.0±0.0	137.23 (102.67–170.27)	245.71 (205.77–323.12)	17.040*
	60	25.6±1.2			
	120	48.2±1.4			
	180	63.9±1.8			
	240	84.0±0.8			
	300	99.8±2.0			

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 chi-square

*$P<0.05$

vector mosquitoes viz., *A. stephensi* and *A. aegypti.* This result is also comparable to earlier reports of Govindarajan (2009) reporting that the leaf extract of *C. fistula* with different solvents viz., methanol, benzene, and acetone were studied for the larvicidal, ovicidal, and repellent activity against *A. aegypti*. The 24-h LC_{50} concentration of the extract against *A. aegypti* was observed at 10.69, 18.27, and 23.95 mg/l, respectively. The culture filtrates of five different soil fungi tested larvicidal activity against larvae of *C. quinquefasciatus*. The LC_{50} values of *Aspergillus flavus*, *Aspergillus parasiticus*, *Penicillium falicum*, *Fusarium vasinfectum*, and *Trichoderma viride* were 38.34, 40.39, 44.97, 50.03, and 54.16 mg/l, respectively (Govindarajan et al. 2005). Among the five different fungi, the culture filtrates of *A. flavus* were found to be more toxic than the other four species of fungi against *C. quinquefasciatus*. The testing for larvicidal activity of secondary metabolites of fungal isolates, 95 isolates of them were larvicidal positive against *A. aegypti*. Further, on screening the 95 isolates, four isolates of *Aspergillus* (F91, F42, F52, F27), two isolates of *Penicillium* (F189, F28), and one isolates of each *Trichoderma* (F102), *Fusarium* (F65), and *Paecilomyces* (F74) were found to be active against mosquito larvae and the LC_{50} values of the metabolites ranged from 16.09 to 59.38 ml/ml. Among them, an isolates of *Aspergillus* (F91) exerted a very low LC_{50} value of 16.09 ml/ml (Govindarajan et al. 2006a).

The secondary metabolites of *Streptomyces* spp. were examined for oviposition attractancy of the mosquito, *C. quinquefasciatus*. The highest effective attractancy of 98.84% was observed at 100 μl/ml of the culture filtrate of *Streptomyces aureofaciens*. The lowest effective attractancy of 65.18% was observed at 25 μl/ml (Govindarajan et al.

Table 2 Larvicidal activity of different solvent seed extracts of *D. elata* against *A. stephensi*

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2
Methanol	Control	0.0±0.0	115.28 (69.49–154.36)	225.07 (180.50–324.34)	23.323*
	60	38.7±1.8			
	120	57.3±1.6			
	180	73.4±2.0			
	240	88.9±1.2			
	300	99.6±0.8			
Ethyl acetate	Control	0.0±0.0	150.02 (99.94–199.18)	292.14 (233.24–439.36)	22.809*
	60	32.1±1.2			
	120	45.2±0.8			
	180	58.7±1.0			
	240	71.8±1.8			
	300	93.4±1.6			
Chloroform	Control	0.0±0.0	162.26 (117.71–209.38)	310.43 (251.35–449.43)	18.930*
	60	27.8±1.2			
	120	42.7±1.8			
	180	54.6±1.6			
	240	68.1±2.0			
	300	89.9±0.8			
Benzene	Control	0.0±0.0	172.92 (132.57–218.18)	326.41 (267.34–458.24)	15.825*
	60	25.2±1.2			
	120	39.4±1.6			
	180	51.3±1.8			
	240	65.8±2.0			
	300	86.2±1.4			
Hexane	Control	0.0±0.0	188.45 (154.06–229.56)	344.82 (288.23–459.86)	11.876*
	60	20.4±1.0			
	120	34.9±2.2			
	180	46.7±1.6			
	240	62.1±1.8			
	300	81.9±1.4			

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 chi-square

*$P<0.05$

2006b). Pushpanathan et al. (2006a) reported the larvicidal efficacy on three essential oils. The LC_{50} values of the three essential oils of *C. citratus*, *Mentha arvensis*, and *Pelargonium graveolens* were 144.54, 162.02, and 190.47 ppm. Pushpanathan et al. (2006b) stated that essential oils from *C. citratus* were evaluated for larvicidal, ovicidal, and repellent activities against the filarial mosquito *C. quinquefasciatus*. The LC_{50} values calculated for the second, third, and fourth larval instar were 144.54±2.3, 165.70±1.2, and 184.18± 0.8 ppm, respectively; 100% ovicidal activity was observed at 300 ppm; skin repellent test at 1.0, 2.5, and 5.0 mg/cm^2 concentration of *C. citratus* gave 100% protection up to 3.00, 4.00, and 5.00 h, respectively; the total percentage of protection of this essential oil was 49.64% at 1.0 mg/cm^2, 62.19% at 2.5 mg/cm^2, and 74.03% at 5.0 mg/cm^2 for 12 h. The larvicidal activity of four isolates of *Streptomyces*(A14, A21, A49, A63) and each one isolate of *Micromonospora* (A32) and *Actinoplanes* (A52) were found to be more active against the larvae of *A. aegypti*. The LC_{50} values of the metabolites ranged from 15.83 to 68.06 µl/ml. Among the six isolates, an isolate of *Streptomyces* (A21) exerted a very low LC_{50} value of 15.83 µl/ml (Govindarajan et al. 2007). Methanolic leaf extract of *C. fistula* was found to be more lethal to the larvae of *A. stephensi* than *C. quinquefasciatus* with LC_{50} values of 17.97 and 20.57 mg/l, respectively (Govindarajan et al. 2008b); the essential oil from *P. graveolens* was evaluated three different concentrations 1.0, 2.5, and 5.0 mg/cm^2 exerted 100% protection up to 3.0, 4.0, and 5.30 h, respectively. The total percentage of protection of *P. graveolens* was 49.64% at 1.0 mg/cm^2, 62.19% at 2.5 mg/cm^2, and 74.03% at 5.0 mg/cm^2 for 10 h (Pushpanathan et al. 2008a). In the larvicidal efficacy of the leaf extract of *C. colocynthis* with four different solvents, the

Table 3 Larvicidal activity of different solvent leaf extracts of *D. elata* against *A. aegypti*

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2
Methanol	Control	0.0±0.0	111.83 (81.00–140.96)	202.77 (168.11–272.68)	18.736*
	50	28.2±1.6			
	100	49.2±1.8			
	150	63.4±1.4			
	200	85.7±1.2			
	250	99.8±0.8			
Ethyl acetate	Control	0.0±0.0	118.44 (91.65–144.44)	211.18 (178.82–271.13)	14.855*
	50	24.1±0.8			
	100	46.3±1.2			
	150	61.8±2.0			
	200	82.5±1.6			
	250	98.8±1.8			
Chloroform	Control	0.0±0.0	138.14 (102.38–172.39)	247.53 (206.42–328.99)	17.924*
	60	25.8±1.8			
	120	47.6±0.8			
	180	63.4±1.2			
	240	83.2±1.6			
	300	99.9±1.4			
Benzene	Control	0.0±0.0	142.56 (110.12–174.22)	250.67 (211.85–323.72)	15.687*
	60	23.5±1.6			
	120	44.8±1.4			
	180	61.6±1.8			
	240	82.9±2.0			
	300	99.7±0.8			
Hexane	Control	0.0±0.0	150.98 (123.18–178.81)	256.28 (221.13–317.71)	12.633*
	60	18.5±1.4			
	120	40.6±1.2			
	180	58.7±0.8			
	240	81.2±2.0			
	300	99.5±1.6			

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 chi-square

*$P<0.05$

maximum efficacy was observed in ethyl acetate. The LC_{50} values of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* were 37.02±1.04, 47.58±1.43, and 55.71±0.97 ppm, respectively (Pushpanathan et al. 2008b). The larvicidal property of extracellular secondary metabolites of 64 actinomycetes isolated from soil samples was tested against early third instar of *A. stephensi*. The 23 isolates of *Streptomyces* (A14, A21, A49, A63) and each one isolate of *Micromonospora* (A32) and *Actinoplanes* (A52) were found to be highly active against mosquito larvae with ranging from 9 to 60 ppm (Govindarajan et al. 2008d); four isolates of *Aspergillus* (F91, F42, F52, F27), two isolate of *Penicillium* (F189, F28), and one isolate of each *Trichoderma* (F102), *Fusarium* (F65), and *Paecilomyces* (F74) were found to be active against *A. stephensi* mosquito larvae, and the LC_{50} values of the metabolites ranged from 11.58 to 48.67 ppm (Govindarajan et al. 2008e).

Larvicidal activity of *O. basilicum*, *Thymus vulgaris*, *C. citratus*, *M. arvensis*, and *P. graveolens* essential oils was tested against the late third instar of mosquito, *C. quinquefasciatus*; the LC_{50} values of *O. basilicum*, *T. vulgaris*, *C. citratus*, *M. arvensis*, and *P. graveolens* were 29.98, 30.31, 165.70, 178.04, and 226.52 ppm, respectively (Pushpanathan et al. 2008c). Essential oils from *Z. officinalis* were evaluated for larvicidal and repellent activity against the filarial mosquito *C. quinquefasciatus*; the LC_{50} value was 50.78 ppm; and skin repellent test at 1.0, 2.0, 3.0, and 4.0 mg/cm^2 concentration of *Z. officinalis* gave 100% protection up to 15, 30, 60, and 120 min (Pushpanathan et al. 2008d). The leaf extract of *A. indica* with different solvents viz., benzene, chloroform, ethyl acetate, and methanol were tested for larvicidal, ovicidal activity, and oviposition attractancy against *A. stephensi*; the LC_{50} values are

Table 4 Larvicidal activity of different solvent seed extracts of *D. elata* against *A. aegypti*

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL—UCL)	χ^2
Methanol	Control	0.0±0.0	139.04 (87.38–183.55)	273.03 (220.97–384.57)	21.338*
	70	36.8±1.6			
	140	56.7±1.8			
	210	72.9±2.0			
	280	86.7±1.2			
	350	98.4±1.0			
Ethyl acetate	Control	0.0±0.0	164.73 (110.05–215.78)	319.83 (258.24–461.78)	21.538*
	70	31.4±1.8			
	140	49.8±1.4			
	210	62.6±1.2			
	280	76.1±0.8			
	350	95.3±1.6			
Chloroform	Control	0.0±0.0	185.33 (131.00–241.75)	352.70 (284.05–519.24)	20.974*
	70	27.6±2.0			
	140	45.1±1.2			
	210	53.6±1.6			
	280	69.3±1.8			
	350	92.2±0.8			
Benzene	Control	0.0±0.0	206.32 (164.57–253.35)	380.29 (317.02–510.23)	13.450*
	70	22.3±1.0			
	140	36.9±2.2			
	210	51.7±1.2			
	280	64.5±1.4			
	350	86.3±1.6			
Hexane	Control	0.0±0.0	221.09 (185.12–263.31)	398.40 (338.43–511.48)	10.062*
	70	19.6±1.8			
	140	32.2±0.8			
	210	46.4±1.2			
	280	63.5±1.4			
	350	82.0±1.6			

SD standard deviation, *LCL* lower confidence limits , *UCL* upper confidence limits, χ^2 chi-square

*$P<0.05$

19.25, 27.76, 23.26, and 15.03 ppm, respectively. The highest effective attractancy of 90.09%, 94.20%, 85.43%, and 95.75% was observed at 100 ppm concentration viz., benzene, chloroform, ethyl acetate, and methanol, respectively; the lowest effective attractancy of 47.17%, 61.94%, 49.28%, and 68.12% were observed at 25 ppm concentration viz., benzene, chloroform, ethyl acetate, and methanol, respectively (Govindarajan et al. 2008c). The LC_{50} values of methanol, benzene, and acetone extract of *P. acidula* against *C. quinquefasciatus* and *A. aegypti* were 10.81, 41.07, and 53.22 and 22.10, 43.99, and 57.66 ppm, respectively. One hundred percent ovicidal activities were observed at 350 and 450 ppm for *C. quinquefasciatus* and *A. aegypti* mosquitoes, respectively; 1.0, 2.5, and 5.0 mg/cm^2 concentrations of *P. acidula* gave 10% protection up to 2.30, 4.00, and 6.45 and 2.45, 4.30, and 7.0 h, respectively (Samidurai et al. 2009). The lethal concentration (LC_{50}) values of *Ficus benghalensis* against early second, third, and fourth larvae of *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* were 41.43, 58.21, and 74.32; 56.54, 70.29, and 80.85; and 60.44, 76.41, and 89.55 ppm, respectively (Govindarajan and Angelina 2010).

Mathivanan et al. (2010) determine the LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ervatamia coronaria* on *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* larvae in 24 h were 72.41, 65.67, and 62.08 and 136.55, 127.24, and 120.86 mg/l, respectively; Govindarajan (2010a) evaluate the larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging between 38 to 48 mg/L; the crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *A. stephensi* for 180 min followed by *A. aegypti* (150 min)

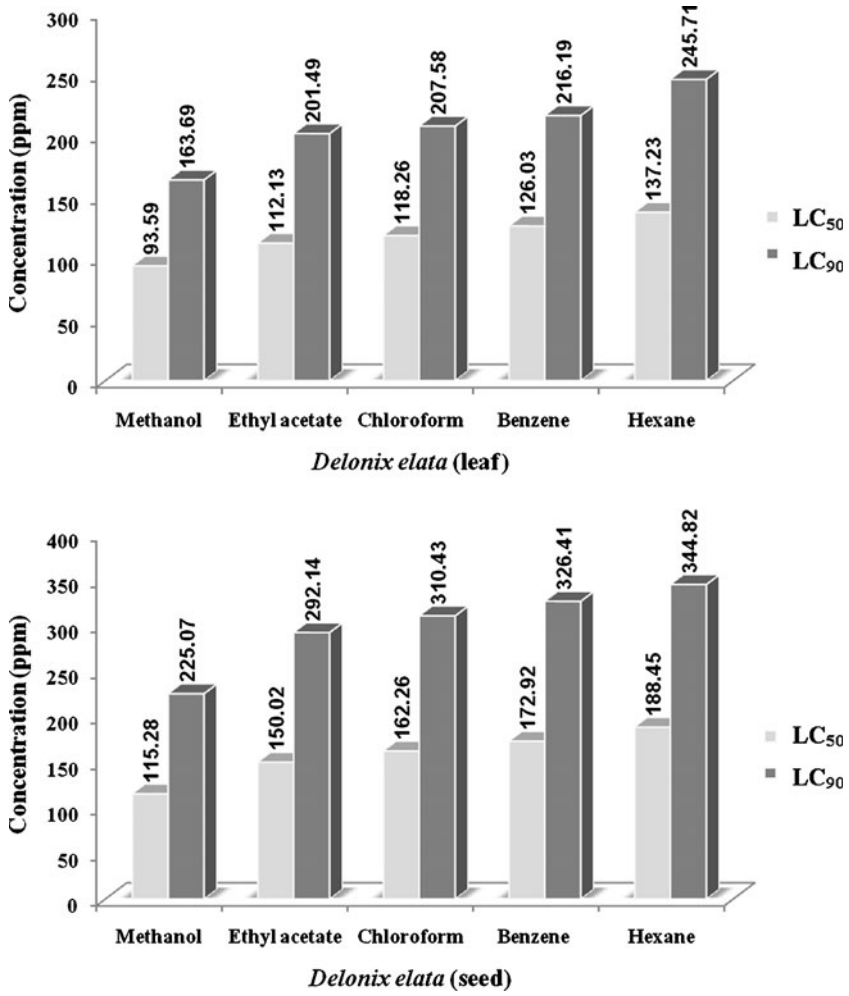

Fig. 2 Larvicidal activity of different solvent leaf and seed extracts of *D. elata* against *A. stephensi*

and *C. quinquefasciatus* (120 min). The essential oil from the leaves of *Clausena anisata* exhibited significant larvicidal activity, with 24 h LC_{50} values of 140.96, 130.19, and 119.59 ppm, respectively (Govindarajan 2010b). The larvicidal activity of *S. acuta* was evaluated against third-instar larvae of *A. subpictus* and *C. tritaeniorhynchus*. The leaf extract and active compound cryptolepine showed negligible mortality against early third-instar larvae of *A. subpictus* and *C. tritaeniorhynchus*; the 24 h LC_{50} value was observed at 38.68 and 50.81, and 9.98 and 12.69 mg/l for crude leaf extract and active compound cryptolepine, respectively (Niraimathi et al. 2010). The LC_{50} values of benzene, hexane, ethyl acetate, methanol, and chloroform extract of *E. alba* against early third-instar larvae of *A. aegypti* were 151.38, 165.10, 154.88, 127.64, and 146.28 ppm, respectively (Govindarajan and Karuppannan 2011), and for the larvicidal efficacy of benzene, hexane, ethyl acetate, methanol, and chloroform leaf extract of *C. halicacabum* against *C. quinquefasciatus* and *A. aegypti*, the LC_{50} values were 174.24, 193.31, 183.36, 150.44, and 154.95 and 182.51, 200.02, 192.31, 156.80, and 164.54 ppm, respectively (Govindarajan 2011a).

The larvicidal and ovicidal activity of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of the leaf of three plants, *E. alba*, *C. halicacabum*, and *A. paniculata* against *A. stephensi*, the highest larval mortality was found in methanol extract of *A. paniculata*, *E. alba*, and *C. halicacabum* against the larvae of *A. stephensi* (LC_{50}= 79.68, 112.56, and 133.01 ppm; LC_{90}=154.66, 220.68, and 270.72 ppm), respectively (Govindarajan 2011b); the highest larvicidal activity was observed in the essential oil from *Z. officinale* against *C. tritaeniorhynchus* and *A. subpictus* with the LC_{50} and LC_{90} values as 98.83 and 57.98, and 186.55 and 104.23 ppm, respectively (Govindarajan 2011c). The larvicidal activity of crude benzene and ethyl acetate extracts of leaf of *E. coronaria* and *Caesalpinia pulcherrima* against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. The highest larval mortality was found in benzene extract of *E. coronaria* against the larvae of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* with the LC_{50} and LC_{90} values being 79.08, 89.59, and 96.15 and 150.47, 166.04, and 174.10 ppm, respectively (Govindarajan et al. 2011b). The crude extract of *E. coronaria* and *C. pulcherrima* exerted zero hatchability

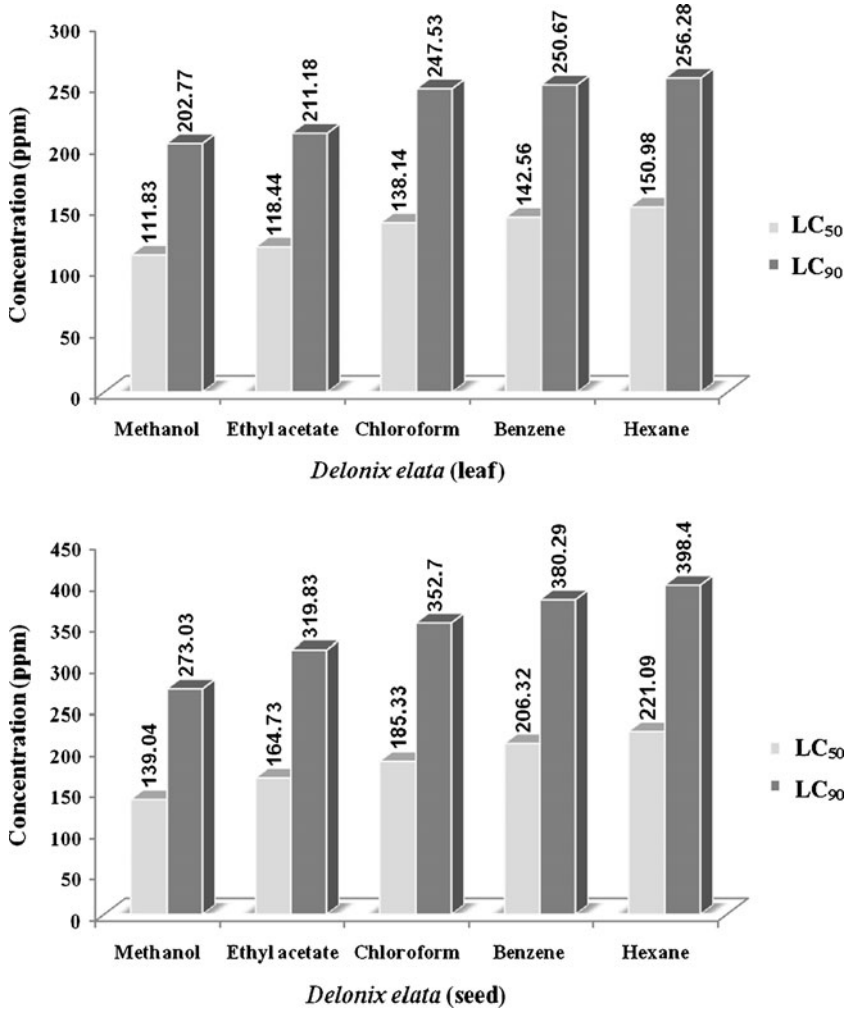

Fig. 3 Larvicidal activity of different solvent leaf and seed extracts of *D. elata* against *A. aegypti*

(100% mortality) at 250, 200, and 150 and 375, 300, and 225 ppm for *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi*, respectively. The methanol extract of *E. coronaria* found to be more repellent than *C. pulcherrima* (Govindarajan et al. 2011c). The benzene, hexane, ethyl acetate, methanol, and chloroform leaf extract of *A. paniculata* was found to be more effective against *C. quinquefasciatus* than *A. aegypti*. The LC_{50} values were 112.19, 137.48, 118.67, 102.05, and 91.20 and 119.58, 146.34, 124.24, 110.12, and 99.54 ppm, respectively (Govindarajan 2011d); the LC_{50} and LC_{90} values methanol extract of *F. benghalensis* against early third instar of *C. tritaeniorhynchus* and *A. subpictus* were 100.88 and 159.76, and 56.66 and 85.84 ppm, respectively (Govindarajan et al. 2011d).

Earlier authors reported that the effect of water extract of citrus seed extract showed LC_{50} values of 135,319.40 and 127,411.88 ppm against the larvae of *A. aegypti* and *C. quinquefasciatus* (Sumroiphon et al. 2006). Dua et al. (2006) have reported that the mean median lethal concentration values of the aqueous extract from the roots of *Hibiscus abelmoschus* against the larvae of *Anopheles culicifacies*, *A. stephensi*, and *C. quinquefasciatus* were 52.3, 52.6, and 43.8 ppm, respectively. The aqueous extract of *R. nasutus* showed LC_{50} values of 5,124 and 9,681 mg/l against *C. quinquefasciatus* and *A. aegypti*, respectively (Chansang et al. 2005). A preliminary screening of crude acetone extract of *Cuscuta hyalina* was conducted against the laboratory-reared preadult stages of common house mosquito *C. quinquefasciatus* (Say) (Diptera: Culicidae). Twenty-four-hour LC_{50} of third- and fourth-instar larvae and pupae were 303, 306.44, and 97.66 ppm, respectively (Mehra and Hiradhar 2002). Sharma et al. (2005) reported that the acetone extract of *Nerium indicum* and *Thuja orientelis* have been studied with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against third-instar larvae of *A. stephensi* and *C. quinquefasciatus*, respectively. A crude extract of seeds of *Millettia dura* showed high activity (LC_{50}=3.5 μg ml^{-1} at 24 h) against second-instar larvae of *A. aegypti* (Yenesew et al. 2003).

Mullai and Jebanesan (2007) have reported that the methanol leaf extracts of *C. colocynthis* and *Cucurbita maxima* showed that the LC_{50} values were 117.73 and 171.64 ppm,

Table 5 Ovicidal activity of *D. elata* plant leaf extracts against *A. stephensi* and *A. aegypti*

Mosquito	Name of the solvent	Percentage of egg hatch ability						
		Concentration (ppm)						
		Control	75	150	225	300	375	450
Anopheles stephensi	Hexane	100±0.0	67.8±1.4	53.6±1.6	45.9±1.4	34.1±1.4	20.4±1.4	NH
	Benzene	100±0.0	62.4±1.0	50.7±1.8	41.8±1.7	29.6±1.8	17.4±1.7	NH
	Chloroform	100±0.0	55.7±2.1	47.3±2.1	35.1±2.0	24.7±1.1	NH	NH
	Ethyl acetate	100±0.0	49.1±1.6	40.8±1.7	26.9±1.1	NH	NH	NH
	Methanol	100±0.0	46.6±1.9	35.1±1.8	21.3±1.6	NH	NH	NH
Aedes aegypti	Hexane	100±0.0	70.1±2.1	59.1±1.4	48.4±1.2	37.1±1.4	23.7±1.1	NH
	Benzene	100±0.0	67.2±1.0	56.2±1.2	45.1±1.0	33.8±1.9	19.9±2.0	NH
	Chloroform	100±0.0	61.8±1.6	51.4±1.6	39.2±0.9	27.3±1.2	NH	NH
	Ethyl acetate	100±0.0	55.4±1.1	46.6±1.3	33.7±2.1	19.4±1.7	NH	NH
	Methanol	100±0.0	49.9±1.5	39.7±0.9	24.5±1.2	NH	NH	NH

NH no hatch ability

respectively, against *C. quinquefasciatus* larvae. Larvicidal efficacies of methanol extracts of *M. charantia*, *T. anguina*, *Luffa acutangula*, *Benincasa cerifera*, and *C. vulgaris* tested with LC_{50} values were 465.85, 567.81, 839.81, 1,189.30, and 1,636.04 ppm, respectively, against the late third larval age group of *C. quinquefasciatus* (Prabakar and Jebanesan 2004). The methanol leaf extracts of *V. negundo*, *Vitex trifolia*, *Vitex peduncularis*, and *Vitex altissima* were used for larvicidal assay with LC_{50} values of 212.57, 41.41, 76.28, and 128.04 ppm, respectively, against the early fourth-instar larvae of *C. quinquefasciatus* (Kannathasan et al. 2007). The methanol extracts of *Euphorbia tirucalli* latex and stem bark were evaluated for larvicidal activity against laboratory-reared larvae of *C. quinquefasciatus* with LC_{50} values of 177.14 and 513.387 mg/L, respectively (Yadav et al. 2002a, b). Compared with earlier reports, our results revealed that the experimental plant extracts were effective to control *A. stephensi* and *A. aegypti.* From these results, it was concluded that the plant *D. elata* exhibits larvicidal and ovicidal activity against two important vector mosquitoes. 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 insecticides from other medicinal plants.

Table 6 Ovicidal activity of *D. elata* seed extracts against *A. stephensi* and *A. aegypti*

Mosquito	Name of the solvent	Percentage of egg hatch ability						
		Concentration (ppm)						
		Control	100	200	300	400	500	600
Anopheles stephensi	Hexane	100±0.0	81.2±1.4	69.8±1.4	53.7±1.5	40.2±2.1	28.5±1.9	NH
	Benzene	100±0.0	76.2±1.8	62.4±1.7	48.6±1.7	36.2±1.9	25.4±1.1	NH
	Chloroform	100±0.0	70.4±1.6	57.8±1.6	43.1±1.2	31.9±1.3	20.3±2.0	NH
	Ethyl acetate	100±0.0	65.3±1.4	52.4±1.8	39.5±0.9	25.6±0.9	NH	NH
	Methanol	100±0.0	59.3±1.3	47.6±1.6	32.4±2.0	20.1±1.4	NH	NH
Aedes aegypti	Hexane	100±0.0	85.1±1.9	72.6±1.9	58.4±1.4	44.6±1.2	31.4±1.4	NH
	Benzene	100±0.0	79.9±2.0	65.5±0.9	51.1±1.9	39.2±0.9	27.6±1.3	NH
	Chloroform	100±0.0	73.6±1.7	60.8±2.0	47.6±1.7	34.2±2.1	22.9±0.9	NH
	Ethyl acetate	100±0.0	68.8±1.3	56.7±2.1	42.3±1.1	29.9±1.6	17.9±1.7	NH
	Methanol	100±0.0	63.1±0.9	51.9±1.0	37.1±2.0	22.8±1.0	NH	NH

NH no hatch ability

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