Oviposition deterring and oviciding potentials of *Ipomoea cairica* L. leaf extract against dengue vectors

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Abstract. Bioprospecting of plant-based insecticides for vector control has become an area of interest within the last two decades. Due to drawbacks of chemical insecticides, phytochemicals of plant origin with mosquito control potential are being utilized as alternative sources in integrated vector control. In this regard, the present study aimed to investigate oviposition deterring and oviciding potentials of *Ipomoea cairica* (L.) (Family: Convolvulaceae) crude leaf extract against dengue vectors, *Aedes aegypti* and *Aedes albopictus*. *Ipomoea cairica* is an indigenous plant that has demonstrated marked toxicity towards larvae of *Ae. aegypti* and *Ae. albopictus*. Leaves of *I. cairica* were extracted using Soxhlet apparatus with acetone as solvent. Oviposition deterrent activity and ovicidal assay was carried out in oviposition site choice tests with three different concentrations (50, 100, 450 ppm). Acetone extract of *I. cairica* leaf strongly inhibited oviposition with 100% repellence to *Ae. aegypti* at lower concentration of 100 ppm, while for *Ae. albopictus* was at 450 ppm. The oviposition activity index (OAI) values which ranged from -0.69 to -1.00 revealed that *I. cairica* demonstrated deterrent effect. In ovicidal assay, similar trend was observed whereby zero hatchability was recorded for *Ae. aegypti* and *Ae. albopictus* eggs at 100 and 450 ppm, respectively. It is noteworthy that *I. cairica* leaf extract had significantly elicited dual properties as oviposition deterrent and oviciding agent in both *Aedes* species. Reduction in egg number through oviposition deterring activity, reduction in hatching percentage and survival rates, suggested an additional hallmark of this plant to be integrated in *Aedes* mosquito control. *Ipomoea cairica* deserved to be considered as one of the potential alternative sources for the new development of novel plant based insecticides in future.

INTRODUCTION

Aedes aegyti and *Aedes albopictus* are two main vectors of dengue, a mosquito-borne infectious disease that constitutes on a growing global threat. Establishment of *Aedes* mosquitoes around the world via accidental transport in cargo (CDC, 2012) coupled with its ability to lay non-dessicating eggs has aided successful expansion across continents and dissemination of dengue viruses (Medlock *et al.*, 2006). Dengue is the most important arboviral disease affecting humans (Bhatt *et al.*, 2013) in terms of morbidity and global economic impact

(Gubler, 2012). The latest studies estimated 50 to 100 millions new infections in 100 nations worldwide, with over two millions severe cases resulting in 20 000 deaths annually (Beatty *et al.*, 2008; WHO, 2012). The continuous spread of dengue over the past five decades is due to population growth, unplanned urbanization, increase in air transport, virus evolution and deteriorating vector control (Focks *et al.*, 2000; Guzman, 2002; WHO, 2009; Gubler, 2011; Morens *et al.*, 2013).

Public health regulators have adopted substantial effort to combat dengue transmission in endemic areas by

suppressing mosquito vector populations through a combination of chemical, biological methods and management of breeding sites (WHO, 2009). Indeed, vector control will remain fundamental tool in any sustainable programme (Scott & Morrison, 2010). Adulticide and larvicide are the common strategies used in vector control. Due to the limited movement of mosquito immature stages compared with that of free-flying adult mosquitoes, control of the immature stages is more efficient and necessary (Elimam *et al.*, 2009). Thus, advances in vector control by identifying and targeting on the most productive larval habitat and breeding sites are very essential (Blaustein & Kotler, 1993; WHO, 1996, 2006).

Recent researches in vector control programmes focused more on plant derived insecticides (Cavalcanti *et al.*, 2004; Das *et al.*, 2007; Govindarajan *et al.*, 2011) as they are rich in bioactive compounds. Plant extracts, essential oils, secondary metabolites and lectins from several plant species have been proven to function as general toxicant, growth and reproductive inhibitor, insect repellent, larvicidal, ovicidal and ovipostion-deterrent against mosquito vectors (Sukumar *et al.*, 1991; Muthu-krishnan & Pushpalatha, 2001; Rajkumar & Jebanesan, 2007; Rahuman *et al.*, 2008; Govindarajan *et al.*, 2008; Khandagle, 2011).

The abundance of *Aedes* mosquitoes is typically assessed using ovitraps (Service, 1992; Reiskind & Zarrabi, 2012). Domestic *Ae. aegypti* and *Ae. albopictus* tend to have ubiquitous breeding sites in artificial containers and natural sites close to human habitations (Scott *et al.*, 2000; Gubler, 2002; Thavara *et al.*, 2004; Dieng *et al.*, 2010). Oviposition site preferences play important role in the planning of vector control programmes against *Aedes* mosquitoes (Yap *et al.*, 1995) as disease propagation is closely associated with dispersal for oviposition (Honorio *et al.*, 2003).

Oviposition is considered as one of the most crucial events. After sufficient blood meal is taken to initiate ovarian development and egg- maturation period is completed, the mosquito must locate suitable oviposition sites. Pathogen carrying vector mosquito need to complete at least one oviposition cycle before transferring pathogen to a host during subsequent blood meal (Bently & Day, 1989). Preventing oviposition and egg-hatching could disrupt mosquito life cycle and subsequently suppress population growth and pathogen dissemination. In this case, potential mosquito egg-laying site could be treated with oviposition deterrents and oviciding agents, as part of integrated vector management strategies.

Research on oviposition deterrents must be conducted to ward off female mosquitoes from breeding sites. Oviposition involves complex interaction between physical and chemical cues by the mosquitoes (Bently & Day, 1989). The choice of oviposition media is related to chemosensory cues (semiochemicals) which the gravid females can detect through specialized cuticular structures, sensilla and chemosensory neurons found in antennae, mouthparts, wing margins and legs (Bohbot & Vogt, 2005). Olfactory sensilla respond to airborne volatiles, while gustatory sensilla react to low-volatility chemicals (Bohbot & Vogt, 2005).

Ipomoea cairica (L.) (Family: Convolvulaceae) is a herbaceous perennial, which is widely distributed in tropical and subtropical regions (Kao, 1987; Liu *et al.*, 2011), and is commonly known as railway creeper or coastal morning glory (Rajkumar & Jebanesan, 2007). It is used in Brazilian folk medicine for the treatment of inflammations and rheumatism (Ferreira *et al.*, 2005) and drinks made from its crushed leaf is used to cure body rashes (Watt & Breyer-Brandwijk, 1962). In addition to its use in traditional medicine, the essential oil extracted from *I. cairica* has also shown larvicidal activity (Thomas *et al.*, 2004) and repellency (Rajkumar & Jebanesan, 2007) against several mosquito species. However, no information is available on the oviposition deterrent and ovicidal activities of *I. cairica* against *Ae. aegypti* and *Ae. albopictus*. Thus, this research aimed to determine oviposition deterrent and ovicidal activities of acetonic crude extract of *I. cairica* leaf against *Ae. aegypti* and *Ae. albopictus*.

MATERIALS AND METHODS

Ethics

This study was approved by Ethics Committee of School of Biological Sciences, Universiti Sains Malaysia.

Collection of plant materials

Fresh leaves of *I. cairica* were collected from Bayan Baru, Penang, Malaysia (5º25'N, 100º19'E) and verified by Botany Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.

Preparation of plant extract

Ipomoea cairica leaves were shade-dried under room temperature for 7-10 days. Dried materials were powdered mechanically by using commercial electrical stainless steel blender (Panasonic: MX-899TM). The finely ground leaves were subjected to extraction using acetone (2000 ml, Qualigens) in Soxhlet apparatus (Favorit, Malaysia). A total of 40 g ground leaves were placed in paper thimble (Favorit cellulose extraction thimbles: size 43 x 123 mm), together with some clean pebbles to ensure optimum solvent flow through the plant powders. The apparatus was set to the boiling point of 56ºC for acetone (Amer & Paxton, 1956). The apparatus was run for approximately three hours, until the solvent colour in the siphon side arm turned almost colourless. The procedure was repeated twice by replacing the leaf powder for each cycle.

The excess solvent from collected crude extract was evaporated under reduced pressure using rotary evaporator machine. The speed was set up to 100 rpm and the temperature of water bath was fixed to boiling point of acetone at 55ºC. The solvent from the concentrated crude extract were further removed by placing it in electrical oven at 37ºC for two days. Stock solution at 10,000 ppm was prepared by dissolving one gram of crude extract in 100 ml of acetone solvent. From the stock solution, 1000 ppm was prepared by dissolving 50 ml of the stock solution in 450 ml of distilled water and subsequent dilutions were made to prepare different concentrations.

Mosquito Culture

Eggs of *Ae. aegypti* were obtained by placing ovitraps in Bagan Dalam, Butterworth, Penang (5º24'N, 100º23'E), while *Ae. albopictus* was obtained from Durian Valley, Universiti Sains Malaysia, Penang, (5º26'N, 100º49'E) two weeks prior to the experiment. To start the colony, eggs were hatched in enamel trays containing dechlorinated tap water. Larvae were fed daily with 0.5 gm of fine powder made from dog biscuit, beef liver, yeast, and milk powder at the ratio of 2:1:1:1. Adult mosquitoes were provided with a mixture of sucrose solution and vitamin B-complex solutions. After five to six days of adult emergence, females were provided with blood meal from a restrained rat placed in resting cage overnight. Two days after blood feeding, a glass petri dish lined with moist filter paper was placed inside the cage as an oviposition substrate. Mosquito culture was maintained at (28±2)ºC, 70%–85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

Oviposition deterrent assay

Laboratory bioassay of oviposition deterrence was performed using the method by Sivagnaname *et al.* (2001) in multiple concentration tests with small alterations. A total of 20 gravid females of *Ae. albopictus* and *Ae. aegypti* were introduced into the test cages (30x30x30 cm). Experiment for both mosquito species were ran separately. Plastic containers (4.5x5.6 cm) containing 50 ml of distilled water were prepared with different concentration of extract (50, 100, 450 ppm) and a container with distilled water and 10% acetone was the control. These solution containers were placed in a square block in the cage within 15 cm between each other. The position of the containers was rotated clockwise everyday to nullify any effect of site preference. A bamboo chopstick of 6 cm was placed in each container (4x6 cm) to facilitate oviposition. The number of eggs laid in treated and control containers were counted under dissecting microscope and recorded daily for five consecutive days. Each set of experiment was replicated for three times.

Ovicidal assay

A total of 100 eggs of *Ae. aegypti* and *Ae. albopictus* were submerged separately in 100 ml of acetonic extract of *I. cairica* leaf test solutions. Four test solutions were prepared at 50, 100, 450 ppm and a control containing 100 ml of water and 10% acetone. After 5 h of submergence, eggs from each concentration were sieved through muslin cloth, rinsed in tap water and transferred individually to different plastic containers containing dechlorinated tap water for hatching assessment. The number of eggs hatched was determined by counting 1st instar larvae daily for 98 h of post-treatment. The experiment was replicated four times.

Statistical analysis

For oviposition-deterrence assay, the data were expressed as mean number of eggs laid. The percentage effective repellency (ER) (Xue *et al.*, 2001) for each concentration was calculated using the following formula:

$$ER\% = \frac{NC - NT}{NC} \times 100$$

Where ER= effective repellency, NC= number of eggs in control, and NT= number of eggs in treatment.

The oviposition activity index (OAI) (Kramer & Mulla, 1979; Elango *et al.*, 2010) was calculated by using the formula:

$$\frac{NT - NS}{NT + NS}$$

where NT denotes mean number of eggs in test solution and NS denotes total number of eggs in control solution.

Compounds are considered as oviposition attractants if the OAI value is +0.3 and above, while those with OAI value of -0.3 and below are considered as repellents (Kramer & Mulla, 1979).

In ovicidal assay, percentage hatchability of larvae for each concentration was calculated according to the following formula:

$$\% \text{ hatchability} = \frac{\text{Number of larvae hatched}}{\text{Total number of eggs}} \times 100$$

Data from all oviposition deterrent and ovicidal activity studies were tested for normality (Shapiro-Wilk) prior to analysis. Data for number of eggs were natural-log transformed (ln[y+1]) due to violations of homogeneity of variance (Blaustein *et al.*, 2005). One-way analysis of variance (ANOVA) was conducted on the number of oviposited eggs with concentrations of oviposition medium as factor for both *Aedes* species using SPSS software (version 20). An arc sine square root transformation was made before analyzing percentage data from egg hatchability in ovicidal study. Percentage hatchability were also analyzed using one-way ANOVA to determine significant treatment differences (Elango *et al.*, 2010), where the concentrations of *I. cairica* extract exposed to eggs was considered as factor. Results with P<0.05 were considered as statistically significant.

RESULTS

Oviposition deterrence assay

The acetone leaf extract strongly deterred oviposition by *Ae. aegypti* and *Ae. albopictus* gravid females at all the concentrations tested as they preferred to lay eggs in control medium compared to treated solutions (Table 1). The results clearly indicated that the oviposition deterrent activity was dose dependent for both species tested. Oviposition of *Ae. aegypti* was strongly deterred (100% effective repellency) with no oviposited eggs at lower concentration of 100 ppm, while *Ae. albopictus* was deterred at 450 ppm. There was also significant difference in the number of eggs laid in each concentration by *Ae. aegypti* (F=67.41, df=3, P<0.05) and *Ae. albopictus* (F=6.78, df=3, P<0.05). All the three concentrations tested showed strong deterrence activity for both species tested, with the lowest effective repellency (ER) of 81.72%. All the OAI values recorded for 50, 100 and 450 ppm for both species were negative values that ranged

Table 1. Oviposition deterrent activity of *Ipomoea cairica* leaf extract against *Ae. aegypti* and *Ae. albopictus*

	Concentration (ppm)	Mean no. of eggs ± SE		ER%	OAI
		Control	Treated		
Ae. aegypti	50	449.33±53.80a	8.00±8.00b	98.21	-0.97
	100		0.00±0.00b	100	-1.00
	450		0.00±0.00b	100	-1.00
Ae. albopictus	50	317.33±114.29a	58.00±17.10a	81.72	-0.69
	100		3.67±3.67b	98.84	-0.98
	450		0.00±0.00b	100	-1.00

*Mean ± SE values followed by different letters within the same row of each mosquito species are significantly different (one-way ANOVA followed by Tukey's test, P <0.05). *ppm* part per million, *ER* effective repellency, *OAI* oviposition activity index

Table 2. Hatching percentage of *Ae. aegypti* and *Ae. albopictus* eggs for ovicidal activities after exposure to *Ipomoea cairica* leaf extract

Concentration (ppm)	*Aedes aegypti*				*Aedes albopictus*			
	Percentage of egg hatching ± SE	df	F	P	Percentage of egg hatching ± SE	df	F	P
Control	85.50±2.40a	3	843.67	0.000	84.00±3.14a	3	507.62	0.000
50	7.00±1.58b				12.50±2.52b			
100	0.00±0.00c				2.00±2.16b			
450	0.00±0.00c				0.00±0.00c			

*Mean ± SE values followed by different letters within the same column of each mosquito species are significantly different (one-way ANOVA followed by Tukey's test, P <0.05)

from -0.69 to -1.00, which indicates strong repellency towards test solution.

Ovicidal assay

The percentage of eggs hatchability of *Ae. aegypti* and *Ae. albopictus* with acetonic crude extract of *I. cairica* leaf is presented in Table 2. There was no hatching from eggs to 1st instar larvae observed for both *Ae. aegypti* (F=843.67, df=3, P<0.05) and *Ae. albopictus* (F=507.62, df=3, P<0.05) at 450 ppm of *I. cairica* extract. This indicated that *I. cairica* extract had significantly elicited strong ovicidal activity in both *Aedes* species. Even at 100 ppm, no eggs of *Ae. aegypti* hatched and only a small percentage of eggs hatched for *Ae. albopictus* (Table 2).

DISCUSSION

In our study, it is evident that *I. cairica* leaf extract possessed excellent oviposition deterrent and ovicidal activity against *Ae. aeygpti* and *Ae. albopictus* under laboratory conditions. It is interesting to note that *I. cairica* produced dual properties as oviposition deterrent and ovicidal response, which were found to be concentration-dependent in both *Aedes* mosquito species. *Aedes aegypti* was found to be more susceptible towards this phytochemical insecticide property when compared to *Ae. albopictus*. Since dengue viruses involves transovarial transmission (Lee & Rohani, 2005), preventing egg laying and egg

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hatching might be one of the strategy in controlling spreading this disease.

Previous reported studies provide information on a wide variety of plant phytochemicals that showed dual properties as oviposition deterrent and oviciding agent against medically important mosquitoes (Tripathi *et al.*, 2004; Prajapati *et al.*, 2005; Govindarajan *et al.*, 2008; Chenniappan & Kadarkarai, 2008; Elango *et al.*, 2010; Phasomkusolsil & Soonwera, 2012; Cheah *et al.*, 2013). Oviposition site preference by gravid females is a fundamental aspect that determines individual fitness, species proliferation, population densities and dispersion in different geographical areas (Spencer *et al.*, 2002; Waliwitiya *et al.*, 2009). Plant phytochemicals can act as oviposition deterrents in which the deterrent compounds could be used to suppress egg laying activity in specific habitats.

The selection of an oviposition site involves visual, olfactory, and tactile responses from the gravid females (Bently & Day, 1989). In the presence of oviposition deterrents, gravid females seek and land upon a site, assess site quality, but lay few or no eggs before flying away (Waliwitiya *et al.*, 2009). *Ipomoea cairica* leaf extract deterred both *Aedes* mosquito species at higher concentrations tested. From our observation, *Aedes* mosquitoes were found to access the water surface, but fly away afterwards without demonstrating any egg laying position. El-gendy & Shaalan (2012) stated that oviposition deterrence may be due to degradation of various chemical compounds of the plant product in the oviposition medium which then produces secondary metabolites that act independently or synergistically to inhibit mosquitoes from laying eggs. Thus, it might be possible that secondary metabolites present in *I. cairica* extract are responsible for deterring oviposition by *Aedes* mosquitoes.

Our present findings on *I. cairica* crude plant extract showed excellent results with lower dosage and are comparable with other previously laboratory screened of essential oils and crude extracts. For example, Warikoo *et al.* (2011) reported that essential oil obtained from peppermint (*Mentha piperita*),

basil (*Ocimum basilicum*), rosemary oil (*Rosemarinus officinalis*), citronella oil (*Cymbopogon nardus*), and celery seed oil (*Apium graveolens*) showed 100% oviposition deterrence activity against *Ae. aegypti* when pure oils of 10 0000 ppm were used. Similar activity was observed in the present study, but at much lower doses (100 and 450 ppm). Also, *Imperata cylindrica* possessed 100% anti-oviposition activity at 1000 ppm against *Culex quinquefasciatus* (Mohsen *et al.*, 1995) and the concentration is 10 times higher than our present concentration (100 ppm). Comparatively, stem oil from burweed (*Achyranthes aspera*) produced 100% oviposition deterrence against *Ae. aegypti* at 100 ppm (0.1%) (Khandagle *et al.*, 2011).

The exposure of eggs to various concentrations of *I. cairica* extract caused failure in egg hatching. *Aedes* mosquitoes usually lay eggs on damp surfaces or above the waterline in containers. The eggs may have to survive for prolonged periods until favourable environmental conditions for hatching is present. During this period, the eggs might be exposed to dirt, organic materials, chlorine and other contaminants which may prevent egg hatching. The efficiency of the oviciding compound present in the plant extract to affect the embryo inside the egg shell depends on its efficient penetration, which in turn is influenced by exposure time (Grosscurt, 1977). In this study, exposure time was fixed for 5 h for all the replicates conducted. Extract effect on fresh eggs is more effective than that of older eggs because mosquito eggs become impervious once they harden (Shaalan *et al.*, 2005; Chenniappan & Kadarkarai, 2008). Thus, eggs of two days old were chosen for this study with exposure time of 5 h. Severe egg mortality was observed at concentration of 450 ppm for both *Aedes* species tested, leading to total failure of egg hatching. Similarly, when eggs were exposed directly to high concentration of extract, more penetration of the chemicals get inside the egg shell through chorion and suppressed further embryonic development (Broadbent & Pree, 1984). We speculated that the cytotoxicity of coumarines in *Ipomoea*

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cairica, which is one of the major constituents (Lima and Braz-Filho, 1997), may cause some unfavourable alterations on embryogenesis of *Ae. albopictus* and *Ae. aegypti* eggs and thus decrease their hatchability. However, further study is needed to validate cytotoxicity of coumarines.

The potential of phytochemicals as larvicide in mosquito control programmes has been known for a long time. However, reduction in egg number through oviposition deterring activity, reduction in hatching percentage and survival rates suggests additional advantages of phytochemicals present in acetonic extract of *I. cairica* leaf. Plant extracts that possess oviposition deterrent and ovicidal activities along with other mosquitocidal effects against *Ae. aegypti* and *Ae. albopictus* would spark an interest among entomologists, researchers and the government to develop an alternative to chemical insecticides and to be incorporated into integrated vector management programmes. *Ipomoea cairica* should be further assessed in semifield and field trials, studied for better understanding of active ingredients and related mode of actions responsible for mosquitocidal properties.

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