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ORIGINAL ARTICLE

EVALUATION OF SUBLETHAL EFFECTS OF *Ipomoea cairica* LINN. EXTRACT ON LIFE HISTORY TRAITS OF DENGUE VECTORS

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SUMMARY

Plant derived insecticides have considerable potential for mosquito control because these products are safer than conventional insecticides. This study aimed to investigate sublethal activities of *Ipomoea carica* or railway creeper crude acethonilic extract against life history trait of dengue vectors, *Aedes albopictus* and *Aedes aegypti*. The late third instar larvae of *Ae. albopictus* and *Ae. aegypti* were exposed to a sublethal dose at LC_{50} and larvae that survived were further cultured. Overall, *Ipomea cairica* crude extracts affected the whole life history of both *Aedes* species. The study demonstrated significantly lower egg production (fecundity) and eggs hatchability (fertility) in *Ae. albopictus*. The sublethal dose of crude extracts reduced significantly the width of larval head capsule and the wing length of both sexes in both *Aedes* species. The significance of sublethal effects of *I. cairica* against *Aedes* mosquitoes was an additional hallmark to demonstrate further activity of this plant despite its direct toxicity to the larvae. The reduced reproductive capacity as well as morphological and physiological anomalies are some of the effects that make *I. cairica* a potential candidate to be used as a new plant-based insecticide to control dengue vectors.

KEYWORDS: *Aedes*; Control; Mosquito; Plant extract; Sublethal effects.

INTRODUCTION

Dengue fever has become a pandemic threat[1] and a major public health challenge to the health officials and policy makers in nearly all the tropical areas of the world[2]. Frequent outbreaks[3], hospitalisations[4] and the increasing number of people at risk, over 40% of the world's population[1], are reminders of the constant menace of this disease. Efforts to reverse the trend to a higher incidence of mosquito-borne outbreaks have mainly being based on the use of synthetic insecticides, particularly organophosphates and pyrethroids[5]. Dinetro-o-cresol was first used as a synthetic insecticide in 1892, and since then more potential insecticides have been discovered[6]. Although insecticide-based strategies have sometimes been successful[7,8], the monolithic reliance on these two classes of products has led to adverse effects. Their widespread misuse have caused the development of mosquitoes[9] resistance, with the main vector, *Aedes aegypti*, ranking eighth in the list of species with the highest reported number of resistant cases worldwide[10]. The fact that these two classes of insecticides have identical modes of action has generated a cross-resistance problem[11,12]. These problems have hampered the effectiveness of current control measures. Despite the efforts to test other substances, only a few insecticides have become available[13]. These circumstances have stimulated the search for new sustainable control strategies, such as the use of botanical insecticides[14,15].

Plants produce a broad range of bioactive chemical compounds[16]. Several plants have been screened due to their insecticidal properties and crude extracts of many plant species have shown promising mosquitocidal activities[17,18]. Some phytochemical insecticides are even more effective than synthetic ones[19]. *Aedes aegypti* in Thailand was found to be susceptible to the essential oil of the plants *Carum carvi, Curcuma zedoria, Piper longum, Apium graveolens* and *Illicium verum* even though these mosquitos had already shown resistance to the permethrin insecticide[20]. Besides being generally target-specific[21,22], plant-derived insecticides decompose quickly[23], and they are not stored in organisms[24], and in ecosystems[25]. Due to a poor penetrance in ecosystems, large animals are not affected by plant-derived insecticides[26]. They contain no halogen molecules and are bioactive at lower concentrations[27]. Reductions in progeny size and longevity, delayed development and repellency to adults parasitoids[28] as well as the development of resistance due to overuse[29], are characteristics that have been attributed to botanical insecticides. These characteristics have raised some arguments on the need to reduce the amount of botanical insecticides applied[30]. Phototoxic activities, the geographical source of plant, the use of solvents during extraction are factors that could also affect the effectiveness of plant-derived insecticides on mosquitoes[17]. The formulation of spot treatment applications are recommended rather than broadcasting over large areas, as this represents a serious menace for non-target invertebrates

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and biological control agents. This strategy is also important for dengue control perspectives due to an increased interest of integrating botanical and microbial pesticides to control dengue mosquitoes.

One way to avoid pesticide residue hazards and the associated disruption of natural enemies would be to combat the evolution of resistance by reducing the amount of insecticide applied. The use of sublethal doses can help minimizing the effects on the physiology and behaviour of individuals that have been exposed to a pesticide[31]. Exposure to sublethal doses of botanical insecticides has been proven to disrupt the insect nervous system and cause hormonal changes, which alter both the behaviour and the physiological processes[32]. In *Ae. aegypti*, exposure to sublethal doses of botanical insecticides disrupts the development, the larval eclosion and the reproduction outcome[33]. Two key fitness parameters for all the insects[34,35], the body size and reproduction have always been ignored by researchers as main sublethal effects of botanicals extracts, including dengue mosquitoes[36,37]. Applied in the field, this efficiency may be offset by many environmental factors such as temperature and rain[38]. It seems likely, therefore, that many insect pests targeted by the application of lethal insecticide doses in nature, end up with sublethal doses. This situation is likely to be more prevalent in countries like Malaysia where the rain falls all year round.

Ipomoea cairica Linn. (Family Convolvulaceae) is an herbaceous perennial plant distributed worldwide[39]. This plant was chosen in this study due to the fact that it is considered as unwanted invasive weeds in nature, and it is widely used in fencing of domestic and peri-domestic areas. The study on sublethal effects of this particular plant against *Aedes* mosquitos has been little or has not been investigated. Therefore, this study was conducted to verify whether sublethal doses of *I. cairica* acethonilic extracts on parent generation would modify the fecundity, fertility, and the development in the life history traits of progenies of *Ae. albopictus* and *Ae. aegypti.* Both of the *Aedes* species tested are main vectors of dengue and dengue haemorrhagic fever in Malaysia.

MATERIALS AND METHODS

Collection of plant samples

Samples of *Ipomoea cairica* (Linn.) (Convolvulaceae) were collected from Bayan Baru, Penang, Malaysia (5°25'N, 100°19'E) by using the random sampling method and the taxonomic authentication was made by the Botany Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia with the identification number HERB No 9251.

Mosquitoes colony

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, University of 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 until reached early 3rd instar larvae to be used in sublethal studies. 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. Mosquito cultures were maintained at (28 ± 2 ºC), with 70%-85% relative humidity (RH), and a period of 14 h of light and 10 h of darkness.

Preparation of plant extracts

Ipomoea cairica leaves were air-dried for 7-10 days in the shadow. Dried materials were powdered mechanically by using a commercial electrical stainless steel blender (Panasonic: MX-899TM). The finely ground leaves were extracted using the Soxhlet apparatus (Favorit, Malaysia) with acetone solvent (2000 mL, Qualigens). A total of 40 g of 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 powder. The apparatus was set to the boiling point of 56 ºC for acetone[40]. The apparatus ran for three hours, until the solvent colour in the siphon side arm became almost clear. The procedure was repeated twice by replacing the 40 g of leaves powder.

The excess of solvents from collected crude extracts was removed by using a rotary evaporator machine. The speed was set up to 100 rpm and the temperature of the water bath was fixed to the boiling point of acetone at 56 ºC. The solvent from the concentrated crude extract was further removed by placing it in an electrical oven at 37 ºC for a week. The yield of the crude residue from plants varied according to the solvents used. The collected crude extract was weighed and stored in petri dishes at 4 ºC until use.

Dose preparation

A stock solution at 10,000 ppm was prepared by dissolving one gram of crude extracts in 100 ml of acetone solvent. From the stock solution, 1,000 ppm was prepared by dissolving 50 mL of the stock solution in 450 mL of distilled water and subsequent dilutions were made as needed. The crude extract obtained and the stock solutions were stored in a refrigerator until use[41].

The preliminary bioassay tests revealed that crude acetone extracts of *I. cairica* leaves were more toxic than the methanol extract from other parts of the plant[42]. Hence this fraction of acethonilic leaves extract was selected to study the sublethal effects on two species of *Aedes* mosquitoes: *Ae. albopictus* and *Ae. aegypti*. The sublethal dose (LC_{50}) which caused 50% of larval mortality was used in this study. In this case, we assumed that 50% of the parent mosquitoes would stay alive and continue to have the first generation progeny. The LC_{50} value for *Ae. albopictus* was 105.59 ppm, while for *Ae. aegypti* it was 101.94 ppm. All of the solutions mentioned above were prepared by using serial dilutions from the stock solution. Mosquito larvae that were treated with LC_{50} concentrations were considered as treated groups. The control group was exposed only to the distilled water with addition of 1 mL of acetone and used for comparisons. Acetone was added to the control experiment to ensure that the control group would be identical in every aspect of the test solution since we have predicted that a little amount of solvent would still be present in the crude extract.

Method of exposure of third instar larvae to sublethal doses of the crude extract

Six batches of 25 third instar larvae of *Ae. albopictus* and *Ae. aegypti* exposed to sublethal concentrations (LC_{50}) of the leaves crude extract were tested separately following the WHO procedures for standard bioassay tests[35]. Experiments were carried out at 28 ± 2 ºC and 70%-

85% of relative humidity (RH). Twenty-four hours post-treatment, larvae that survived were counted, isolated and rinsed with seasoned water and reared in enamel trays until pupal emergence[32]. Larvae in both treated and control groups were fed with a mixture of dog biscuit, beef liver, yeast, and milk powder in ratio of 2:1:1:1. Adults were allowed to emerge in emergence cages (45 × 45 × 40 cm) and were provided with cotton wick soaked with 10% sucrose. Subsequent studies on life traits were conducted on the next generation.

Egg production (fecundity)

After five days of the adults emergence, both treated and control mosquitoes, 30 males and 30 females from each species, *Ae. albopictus* and *Ae. aegypti* were transferred separately into oviposition cages using a manual aspirator. Females and males mosquitoes were allowed to mate. Females were then provided with blood meal from restrained rats placed in resting cages during the day (for 12 hours) and they were subsequently supplied with 10% sucrose solutions in a tube with cotton wick for two days. Two days after the blood meal, 30 engorged females were transferred into individual paper cups covered with mesh net. A moist of no.1 Whatman filter paper cut in cone shape was used as the oviposition site. The eggs laid by the mosquitoes were counted daily for a maximum period of seven days under a dissecting microscope (×20).

Egg hatchability (fertility)

To investigate the hatchability of both *Aedes* mosquito species, 10 paper cones with eggs were randomly selected. Paper cones were allowed to dry for 24 hours before hatching in enamel culture trays containing seasoned water. Eggs that hatched and turned into first instar larvae were counted.

Egg length

For the measurement of the eggs length, paper cones containing eggs from the fecundity study were used. Three paper cones from both control and treated groups of each *Aedes* sp were selected. The length of 10 randomly selected eggs from each paper cone was measured using a stereomicroscope (Olympus EX41), at the Electron Microscopy Unit, School of Biological Sciences, University of Sains Malaysia (100×) (Fig. 1) and the data were recorded.

Fig. 1 - Measurement of the egg length. The egg length from the anterior to the posterior end was measured using a stereomicroscope (100×)

Fig. 2 - Measurement of the width of the head capsule. The width of the head capsule across the dorsal surface at the base of the antennae was measured using a stereomicroscope (100×).

Width of larval head capsule

The eggs obtained from the fecundity study were hatched and reared until they turned into third instar larvae. Thirty larvae from the treated and the control groups of each mosquito species were selected for the measurement of the width of the larval head capsule. Larvae were immobilised using 60% alcohol. The width of the head capsule across the dorsal surface at the base of the antennae was measured using a stereomicroscope (100×) (Fig. 2).

Adult wing length

The measurement of the adult mosquito wing length which estimates the body size of aedes mosquitoes was used in this study[61]. After the adult emergence (from the fertility studies), 30 females and 30 males of both species (*Ae. albopictus* and *Ae. aegypti*) and tested groups (treated and control) were randomly aspirated and maintained in separate cages. Then, all the individuals were anesthetized with 60% alcohol and the right wing of each individual was detached from the thorax and the length from the axial vein to the outmost extreme of the R1 vein[50] was measured using a stereomicroscope (100×) (Fig. 3).

Data analysis

The effective reduction percentage (ER%) for the fecundity was calculated as follows[29]:

ER% = [NC-NT/NC] × 100

NC- number of eggs in the control group

NT- number of eggs in the treated group

The percentage of hatchability (fertility) was calculated as follows[32]:

% hatchability = (number of larvae/ number of eggs) × 100

Fig. 3 - Measurement of the wing length. The wing length was measured from the axial vein to the outmost extreme of the R1 vein of an adult mosquito under a stereomicroscope (100×)

Data from all the experiments of the sublethal effect studies were tested for normality (Shapiro-Wilk) prior to analysis. All the data were natural-log transformed (ln[y+1]) due to violations of the homogeneity of variance[11]. The transformed data were analysed using independent T-test by means of the SPSS software (version 20) to determine the significant differences at $p < 0.05$ between treated and untreated groups (control). An Arc sine transformation was performed before the analysis of the percentage of data from the fertility study.

RESULTS

Our data suggested that acethonilic leaves extract of *I. cairica* had a significantly higher impact on the reduction of fecundity and fertility of *Ae. Albopictus*, while in *Ae. aegypti* a lower impact was observed in the first generation. Our results indicated that *I. cairica* acethonilic extract can reduce the populations of *Ae. albopictus* in parent generation and subsequently reduce the next generation population. Exposure to a sublethal dose (LC_{50}) of *I. cairica* crude extract of third instar larvae of *Ae. albopictus* and *Ae. aegypti* decreased their fecundity by 34.68% and 16.61% compared to the respective controls. Significant reduction on the number of eggs laid by female mosquitoes was only observed in *Ae. albopictus* mosquitoes (t = 3.12, df = 58, p = 0.030; Table 1).

Fertility of *Ae. albopictus* was measured by the percentage of hatched eggs, and it was significantly lower in treated groups compared to controls, a 49.06% of reduction (t = -4.857, df = 3.833, p = 0.009; Table 2), whereas in *Ae. aegypti*, the eggs hatchability was reduced only by 18.55% a not significant reduction in comparison to the control group (t = -2.592, df = 2.969, p = 0.082; Table 2). The statistical significance obtained has clearly depicted that eggs become less fertile or less productive after the parental mosquitoes exposure to the sublethal dose of *I. cairica*.

However, we found that the length of treated eggs for both species was significantly different from control groups ($p < 0.05$), as well as the decreased hatchability. The sublethal dose of crude extracts reduced the egg length in *Ae. aegypti* by 0.02 mm followed by *Ae. albopictus* (0.01 mm) in comparison to controls.

Table 1

Sublethal effects of acetone extract of *Ipomea cairica* leaf (LC50 dose) on fecundity, fertility, size of egg length and width of head capsule for *Aedes albopictus*

Parameter	Treated	Control
Fecundity		
No. of eggs	50.2 ± 6.33a	76.6±6.80b
ER (%)	34.68	
Fertility		
No. of eggs	17.18±3.50a	66.24±11.19b
ER (%)	49.06	
Egg length (mm)	0.59±0.01a	0.60±0.01a
Width of head capsule (mm)	0.82±0.01a	0.85±0.01b

ER: Effective reduction

Table 2

Effects of sublethal dose (LC50) of acethonilic *Ipomea cairica* leaves crude extracts on fecundity, fertility, size of egg length and width of head capsule on *Aedes aegypti* mosquitoes

Parameter	Treated	Control
Fecundity		
No. of eggs	56.57±4.23 a	67.83±4.81 a
ER (%)	16.61	
Fertility		
No. of eggs	17.03±4.72 a	35.58±4.56 a
ER (%)	18.55	
Egg length (mm)	0.57±0.00 a	0.59±0.01 a
Width of head capsule (mm)	0.84±0.01 a	0.86±0.01 b

ER: Effective reduction

Even though the size of eggs was not affected by the sublethal dose, the size of the head capsule in third instar larvae was significantly reduced in both *Ae. albopictus* (t = -3.560, df = 56.645, p = 0.001) and *Ae. aegypti* (t = -2.505, df = 57.972, p = 0.015). Significantly narrower head capsules were found in both species with reductions of 0.03 mm for *Ae. albopictus* and 0.02 mm for *Ae. aegypti*, resulting in smaller larvae in comparison to the control group.

In relation to the smaller size of head capsule, the emerged female and male adult mosquitoes had shorter wing length which indicates smaller body sizes of the adult mosquitoes. As shown in Table 3, the mean wing length of both treated female and male mosquitoes was significantly reduced compared to controls for both mosquito species, *Ae. albopictus* (Female: t = -5.109, df = 46.39, p = 0.000; Male: t = -2.113, df = 57.70, p = 0.039) and *Ae. aegypti* (Female: t = -6.526, df = 58, p = 0.000; Male: t = -2.519, df = 57.50, p = 0.015).

DISCUSSION

The present study has shown that exposure of *Aedes* mosquitoes to sublethal doses of *Ipomoea cairica* acethonilic extracts has affected the life history traits of these mosquitoes under laboratory conditions. *Ae. albopictus* was found to be the most affected species regarding their

Table 3
Sublethal effects of acetone extract of *Ipomea cairica* leaf on adult wing length (mm) of *Aedes albopictus* and *Aedes aegypti*.

Species	Female		Male	
	Treated	Control	Treated	Control
Aedes albopictus	2.30±0.05 a	2.61±0.03 b	2.04±0.03 a	2.14±0.03 b
Aedes aegypti	2.24±0.06 a	2.67±0.04 b	2.09±0.19 a	2.21±0.19 b

progeny after exposure to lethal concentrations (LC_{50}) of acethonilic *I. cairica* extracts. The acethonilic extract of *I. cairica* has been found to be more effective to cause mortality in both *Aedes* species compared to the methanolic extract[42]. However, *Ae. aegypti* fecundity and fertility were less influenced by the *I. cairica* acethonilic extract.

The decrement in fecundity was observed in *Ae. albopictus* females, indicating that the sublethal dose of *I. cairica* leaf extract has induced reproductive disadvantages in the exposed group. A similar result has been previously reported following exposure of *Culex quinquefasciatus*, *Anopheles stephensi,* and *Ae. aegypti* larvae to other plant extracts such as *Calophyllum inophyllum*, *Solanum suratense*, *Samadera indica* and *Rhinocanthus nasutus* extracts that caused a significant reduction in fecundity[43]. The methanolic extract of seed and leaf of chinaberry tree, *Melia azedarach*[44], methanolic extract of Indian white cedar, *Dysoxyllum malabaricum*[45], limonoids of neem, *Azadirachta indica*[44] at various concentrations have remarkably decreased the fecundity of *An. stephensi.* Pyrethrin derived from *Tanacetum cinerariaefolium* which is known to be a highly effective insecticide against both *Aedes* species can cause a reduction of eggs production in emerging adults after exposure[46]. In addition, the naturally-derived insecticide spinosad has significantly decreased the fecundity in exposed females compared to unexposed females of *Ae. aegypti*[47].

Similarly, several available studies have reported a fecundity and reproductive potential reduction in mosquito species after exposure to a sublethal dose of chemical insecticides. Temephos and propoxur have induced a marginal fecundity reduction in *An. stephensi*[32] and the exposure of *Ae. aegypti* females to higher concentrations of temephos, malathion and alpamenthtin has shown decreasing numbers of eggs production[48,49]. Also, a decreased fecundity upon exposure to entomopathogenic fungi, *Leptolegnia chapmanii*[50] and bacterial agents, *Bacillus thuringiensis* H-14[51] has been observed in *Ae. aegypti.*

The fecundity reduction in the present study led us to speculate that the plant extract used has a certain mode of action to induce reproductive disadvantages in the *Aedes* treated group. The insects that were fed on plant secondary metabolites have shown toxic effects that in turn have affected the insects' physiology in different ways and at various receptor sites[15]. It has been reported that *I. cairica* contains alkaloids[52] which act as plant phytochemicals[53] and natural mosquito larvicides[54]. The mode of action of alkaloids on insect vectors varies according to the molecules structure, but they are mainly reported to act on acetylcholinestrase (AChE) and sodium channels, which are the most important physical disruption in insects[15]. The insects' neuro-hormone system plays a key role in regulating a complex behavioural and physiological mechanisms related to their reproduction. Exposure to insecticide toxins cause coordination disruption resulting in a diminished reproductive capability[55,56].

Regarding the eggs hatchability, the present study has shown that the eggs produced after exposure of parent mosquitoes to a sublethal dose of *I. cairica* leaf extracts were less fertile. It has been reported that temephos and propoxur-treated females produced less viable eggs which may be due to the direct toxic effect of the insecticide that remained un-metabolized in the body of the parent and may have been transmitted to the eggs[32]. A decrease in the production of a viable offspring is a common end result of sublethal poisoning[55]. Besides lignans, *I. cairica* was reported to contain coumarins as one of its major constituents[57], so it might be possible that the cytotoxicity of coumarines caused some unfavourable alterations on the embryonic development of *Ae. albopictus* eggs and thus decreased their hatchability. Similarly, coumarines from *Mammea siamensis* were believed to cause abnormalities in the development of reproductive organs, have direct effect on the eggs and affect some fundamental aspects related to the embryonic development[58]. Similar findings were reported in fertility studies of *Stegomyia albopicta*, in which the percentage of egg hatching was reduced after sublethal exposure of females to boric acid sugar bait[59]. The treatment of *Cx. quinquefasciatus* and *Ae aegypti*, with 50% EC_{50} of *Calophyllum inophyllum* and *Rhinocanthus nasutus* leaf extract has reduced the fertility from 94.9 to 30.2 % and 82.2 to 37.7% respectively, when compared to controls[43]. Also, adults of *An. stephensi* exposed as larvae to sublethal concentrations of alkaloids isolated from *Annona squamosa* seeds were found to produce eggs with a 27.7% reduced hatchability in comparison to controls and the plant extract has disrupted some growth stimulating activities in the tested mosquitoes[60].

The present study showed a slight but not significant reduction of the egg length when the parent larval stage was exposed to a sublethal concentration of *I. cairica* plant extract in both mosquito species. However, few studies have reported the positive sublethal effects of phytochemicals on mosquito egg length. Females of *Cx. quinquefasciatus* laid shorter eggs than the control group following exposure to a sublethal dose of malathion and methoprene[61]. In contrast, our study suggested that the eggs length were insignificantly reduced in size when a sublethal effect of the extract was used. Regarding the reproductive disadvantages caused by the plant extract, the sublethal dose did not affect the size or the length of the produced eggs. It is possible that the sublethal dose effect was more pronounced on the quantity and quality of the produced eggs. A similar finding was reported with respect to the turnip moth (*Agrotis segetum*). Females treated with the dithiocarbamate fungicide mancozeb showed a reduced fecundity but there were no significant differences in egg length[62].

The results from our study have also revealed that third instar larvae of both *Aedes* species which emerged from eggs of treated parents had significantly narrower width of head capsules in comparison to the control group. The measurement of the larval head capsule is an important indicator of insect larvae developmental stages[63]. So far, there are no published reports regarding sublethal effects of plant extracts on the width of mosquito larval head capsules. As mentioned previously, the egg length was not significantly reduced, but we have observed a significant decrease in the larval head capsule. Thus, it might be possible that properties of phytochemicals from plant extracts have produced morphological abnormalities in various developmental stages of *Aedes* mosquitoes. In this case, narrower head capsules indicated abnormal growth and development among the treated *Aedes* larvae compared to the control group. Herbicides such as atrizin and glyphosate were reported to decrease *Ae. albopictus* vectorial capacity in term of sex ratio distortion, adults emergence rate and body size[6]. Abnormalities were also observed at pupal stages of *Ae. albopictus* exposured to LC50 dose of *Citrus* contains *R-(+)-limonene* with larvae that failed to turn into adults and died in the pupa form which became elongated, showing the emergence only of the head and thorax from the puparium, with legs still attached to the pupal case or crumpled wing in adults[64]. A sublethal dose did not only alter the morphology, but also the genetic structure of *Ae. albopictus*. Exposure to cadmium, inorganic mercury and methylmercury metals can change the morphology of *Ae. albopictus'* cell line, affecting the development of filopodia[65].

Following the reduction in width of larval head capsules, the wing lengths of both males and females from the first generation of *Ae. albopictus* and *Ae. aegypti* were found to be significantly reduced after treatment with *I. cairica* leaf extract. A similar finding was reported regarding the wing length of males and females of *Ae. aegypti* which emerged from larvae treated with microalgal chloorophytes, *Scenedesmus quadricaula* and *Chlorococcum* sp.[66], and entomopathogenic fungus, *Leptolegnia chapmanii*[50]. However, a contrasting finding has also been reported in females of *Ae. aegypti* that survived the treatment with spinosad at $LC_{50,}$ resulting in progenies with significantly longer wings compared to the control group[47].

Reduction in wing length is considered as one of the morphological abnormalities caused by plant phytochemicals in insects. Wing length is a reliable parameter used as an indicator of body size in mosquitoes compared to their weight[30,67]. It has been reported that the body size positively correlates with the fecundity of mosquitoes, which means larger females tend to lay more eggs compared to smaller ones[68,69]. In addition, shorter wing length may lead to a reduction of flight capabilities. Here, we postulate that the survival of *Aedes* mosquito treated with phytochemicals tend to be lower in time. Mosquitoes with shorter wings are unable to a long-distance migration and the host-seeking ability of blood feeding will be reduced as well. Smaller females tend to die before reaching hosts due to a lack of substantial energy[67]. Blood meals are essential for ovarian maturation and eggs production and therefore, we can predict a decrease in mosquito size leading to a reduced egg production. An observation of reciprocity in body size and ovarioles production in *Ae. aegypti*[70] seems to support this statement. In addition, a decreased blood feeding activity reduces the chances of female mosquitoes in acquiring and transmitting the dengue virus[71]. Hence, the reduction in wing length of male and female mosquitoes of *Ae. albopictus* and *Ae. aegypti* after exposure of parents to a sublethal dose of *I. cairica* leaf extract is a collateral advantage for the control of these mosquitoes species and the associated disease.

While most of the literature information resides on the larvicidal, ovicidal, adulticidal and repellency effects of phytochemicals on mosquitoes species, the present study is the first attempt to use a sublethal dose of *I. cairica* plant crude extract on the reproductive responses (fecundity), eggs hatchability (fertility), egg length, width of larval head capsules and wing length of *Aedes* mosquitoes.

CONCLUSIONS

The crude extract of *Ipomoea cairica* leaf has not only caused a high mortality rate in larvae of dengue vectors but has also caused sublethal effects on the offsprings of treated mosquitoes in terms of biological and morphological aspects. The reproductive potential (fecundity and fertility), width of larval head capsule and wing length were significantly reduced, especially in *Ae. albopictus*, indicating that this plant extract could be incorporated to integrated pest management aedes control programs.

COMPETING INTERESTS

The author (s) declare that they have no competing interests.

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CO-AUTHOR CONTRIBUTIONS

Designed the experiment: WFZ, AR, NF, HD. Carried out the experiments: WFZ, AR, TM. Analyzed the data: WFZ, AR, NF. All the authors read and approved the final version of manuscript.

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