Assessment of residual bio-efficacy and persistence of *Ipomoea cairica* plant extract against *Culex quinquefasciatus* Say mosquito

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Abstract. Specification on residual action of a possible alternative insecticide derived from plant materials is important to determine minimum interval time between applications and the environmental persistence of the biopesticides. The objective of this study is to evaluate crude acethonilic extract of *Ip. cairica* leaves for its residual and persistence effects against *Culex quinquefasciatus* larvae. Wild strain of *Cx. quinquefasciatus* larvae were used for the purpose of the study. Two test designs, replenishment of water and without replenishment of water were carried out. For the first design, a total of 10ml of test solution containing *Ip. cairica* extracts was replenished daily and replaced with 10ml of distilled water. For the second design, treatment water was maintained at 1500ml and only evaporated water was refilled. Larval mortality was recorded at 24 hours post-treatment after each introduction period and trials were terminated when mortality rate falls below 50%. Adult emergences from survived larvae were observed and number of survivals was recorded. For the non-replenishment design, mortality rate significantly reduced to below 50% after 28 days, meanwhile for replenishment of water declined significantly after 21 days (P < 0.05). There was no adult emergence observed up to seven days for non-replenishment and first two days for replenishment of water design. The short period of residual effectiveness of crude acethonilic extract of *Ip. cairica* leaves with high percentage of larval mortality on the first few days, endorses fewer concerns of having excess residues in the environment which may carry the risk of insecticide resistance and environmental pollution.

INTRODUCTION

Mosquitoes are well known as medically important vectors. *Culex quinquefasciatus* is one of the most abundant tropical house mosquito (Abu Hassan & Che Salmah, 1990) and urban vector of nocturnally periodic *Wuchereria bancrofti* (Cobbold) that causes lymphatic filariasis in Asia, Africa, the West Indies, South America, and Micronesia (Moses et al., 2009). More than 1.3 billion people in 72 countries worldwide are threatened by lymphatic filariasis and over 120 million people are currently infected, with about 40 million disfigured and incapacitated by this disease (WHO, 2012). Bancroftian filariasis is endemic in our neighboring countries, Indonesia, Bangladesh (Omar et al., 2001) and Thailand, particularly in rural, hilly, mostly forested areas of western regions along the Thai-Myanmar borders (Pothikasikorn et al., 2008). Study by Vythilingam et al. (2005) reported that laboratory strain of the Malaysian *Cx. quinquefasciatus* mosquitoes were susceptible to *W. bancrofti* where 33% that
fed on *W. bancrofti* patient were infective after 12-14 days. Therefore, with the influx of immigrants and movement across the international borders, there is a high possibility for an established urban transmission of *W. bancrofti* in Malaysia due to the presence of host and vector in abundance (Omar *et al*., 2001; Vythilingam *et al*., 2005).

*Culex quinquefasciatus* breeds in a wide variety of stagnant water habitats, including water barrels, wells, tanks, privies and canals near houses, provided the water has been sufficiently polluted (Moses *et al*., 2009). They prefer moderately aerated, polluted water which is rich in decaying organic matter for oviposition (Blackwell *et al*., 1993). This factor may increase chances of *Cx. quinquefasciatus* becoming a disease vector due to the presence of such sites usually near human habitations. Distribution of *Cx. quinquefasciatus* is increasing with urbanization and human activity and many rural pockets that were relatively free from this mosquito are increasingly colonized (Chavasse *et al*., 1995). Current trend for vector control has moved towards the use of substances of plant origin as they are rich in bioactive chemicals. This is due to concerns on the indiscriminate use of synthetic chemical insecticides that have resulted in physiological resistance problems in target insects, environmental pollution which pose a threat to human health and other organisms in the environment (Rahuman *et al*., 2009; Govindarajan *et al*., 2011).

Many studies on plant extracts on their potential for mosquito control as larvicidal, ovicidal, repellent and growth and reproduction inhibitors has been reported (Sivagnaname & Kalyanasundaram, 2004; Remia & Logaswamy, 2010; Govindarajan *et al*., 2011). Plants can produce many powerful chemicals which are used to defend themselves against herbivores and disease organisms (Freeman & Beattie, 2008). Plant extracts with insecticidal properties are possible alternatives to synthetic insecticide since it is safer, biodegradable and environmental friendly (Rahuman *et al*., 2009). Nevertheless, there is scarce information available on residual efficacy of the plant extracts identified with vector control potential. Specification on the effects of possible alternative insecticide derived from plant materials on its residual action is important to evaluate its toxicity and environmental persistence to minimize the impact on non-target organisms. Knowing the duration of the residual period of insecticide is crucial for vector control, since it indicates the minimum interval between applications to maintain persistence of the insecticide towards the targeted vector (Roseli *et al*., 2007).

*Ipomea cairica* from family Convolvulaceae is known by its characteristics as a twining herb, a climber, or even shrubs, and distributed in tropical and subtropical regions (Rong *et al*., 2008). This plant is commonly known as “Railway creeper” and it is a fencing ornamental plant found in most domestic and peri-domestic areas in Malaysia. The crude extracts of *Ip. cairica* leaves were reported to have remarkable larvicidal properties and high toxicity which causes 100% mortality in the larvae of *Cx tritaeniorhynchus* (100 ppm), *Aedes aegypti* (120 ppm), *Anopheles stephensi* (120 ppm) and *Cx. quinquefasciatus* (170 ppm) (Thomas *et al*., 2004; Rajkumar & Jebanesan 2007).

However, in the extent of our literature review, there have been no documented reports on residual efficacy of the *Ip. cairica* crude plant extract on any pest or insect vectors. In this study, our aim is to evaluate crude acethonilic extract of *Ip. cairica* Linn leaves (Family: Convolvulaceae) for its residual and persistence against *Cx. quinquefasciatus* larvae.

**MATERIALS AND METHODS**

**Mosquito colonies**

Wild strain of *Cx. quinquefasciatus* larvae were used for the purpose of the study. Larvae were collected from drains containing stagnant water in Bagan Dalam area, Penang, Malaysia (5°24'00"N, 100°23'00"E). Collected larvae were kept in enamel trays
containing dechlorinated tap water and reared to obtain F1 generation. F1 generation larvae were used in order to have standard size and age of late third instar larvae for this study. Larvae were reared and maintained at 100 larvae in 2L of dechlorinated tap water per tray. The larvae were fed daily with 0.5 mg fine powder mixture of dog biscuit, beef liver, yeast and milk powder at ratio of 2:1:1:1 by weight. Pupae were transferred into plastic containers, maintained in a mosquito cage and allowed to emerge. Adult mosquitoes were fed on 10% sucrose solution and periodically blood-fed on laboratory mice for egg production. The mosquito colonies were maintained under laboratory condition at a temperature of (28±2ºC) and (80±10%) relative humidity (RH).

**Plant extraction and dose preparation**

*Ipomea cairica* leaves were collected from a residential area in Relau, Penang, Malaysia (5º25'00"N 100º19'00"E) and were identified by Botanical Department of Universiti Sains Malaysia. Leaves were air dried for 1 to 2 weeks at room temperature and powdered mechanically using a stainless steel electric blender. A total of 120 g of the leaf powder were extracted with approximately 2 L of acetone as solvent in Soxhlet apparatus until complete extraction was achieved. Excess solvent in the crude extract was removed using rotary evaporator vacuum machine. Stock solution of 10,000 ppm was prepared by dissolving one gram of crude extract in 100 ml of acetone solvent. For the residual evaluation, 300 ppm of acetone extract of *Ip. cairica* leaves in 1500 ml of distilled water was prepared as test solution. Preliminary larvicidal bioassay showed that the particular concentration was capable of producing 100% mortality of *Cx. quinquefasciatus* larvae after 1 day of treatment (Thiagaletchumi et al., unpublished), therefore the residual effectiveness of the extract in long term use were analyzed in this study.

**Test designs**

Glass jars measuring 22.7 cm high, 8.7 cm surface diameter, 13.3 cm base diameter and 3 L volume were used. Twenty five late third and early fourth instar of *Cx. quinquefasciatus* larvae were introduced into the test jars for both replenishment and non-replenishment methods under laboratory conditions. Untreated jar with 1500 ml of distilled water was used as control. Larval mortality was recorded at 24 hours post-treatment after each introduction period on day 1, followed by day 3, day 5, week 1 until the efficacy dropped below 50%. New batch of *Cx. quinquefasciatus* larvae were introduced each treatment days. Larvae that survived the treatment were transferred into container that contained seasoned water with larval food. Adult emergence from survived larvae was observed and number of survival was recorded. Experiment was replicated 3 times and the trial was terminated when the mortality rate fell below 50%. Tests were carried out under laboratory conditions at 28±2ºC and 80±10 % relative humidity (RH).

**Replenishment and non-replenishment**

Two types of treatment were tested; (1) replenishment of water, and (2) non-replenishment of water. For non-replenishment method, distilled water was maintained at 1500 ml. Distilled water was only refilled up to the water mark level for all the jars to compensate for water loss due to evaporation. While, a total of 10 ml of test solution containing *I. cairica* extracts was replaced daily and topped up with 10 ml of distilled water in replenishment method. The daily replenishment of water is to simulate daily usage of water (Lee & Zairi, 2005). The replenishment of water imitates the natural environment such as drainage ditches, culverts and farm animal waste lagoons. The non-replenishment represents the condition such as lakes and artificial breeding sites.

**Statistical analysis**

The data collected for *Cx. quinquefasciatus* larval mortality and adult emergence were subjected to Analysis of Variance for randomized block design to test for significant effects among the treatments and post-treatment days. The level of significance for the statistical analyses was set at P<0.05. Analysis was carried out using the SPSS program for Windows version 20.0. Data were tested for normality using Kolomogorov-
Smirnov test and were log-transformed (y+1) prior to analysis to satisfy the requirement of normality for ANOVA.

RESULTS

Our study revealed that mortality of treated Cx. quinquefasciatus larvae was significantly influenced by the different treatment designs (df=7, MS=1.02, P< 0.05; Table 1) and length of post treatment time (df=1, MS=0.55, P< 0.05; Table 1). The results showed more than 50% reduction in Cx. quinquefasciatus larvae in treated containers up to three weeks post-treatment. Both non-replenishment and replenishment test design showed 100% mortality of the mosquito larvae on day 1 of the treatment with reduced efficacy over time. The larval mortality rates were then observed to differ between the treatments tested from day 3 onwards (Fig. 1). For the non-replenishment design, mortality rate significantly dropped below 50% after 28 days, meanwhile for replenishment of water the percentage mortality declined significantly after 21 days to less than 50% (P< 0.05; Fig. 1). However, throughout the study period, non-replenishment test design showed more effective results compared to replenishment method.

Cx. quinquefasciatus adult emergences from larvae treated with acethonilic extract of Ip. cairica was greatly affected both by the treatment designs (df=7, MS=6.99, P< 0.05; Table 1) and the duration of post treatment (df=1, MS=1.62, P< 0.05; Table 2). Even though a few Cx. quinquefasciatus larvae remained alive after the treatments, no adult emergence was observed up to seven days for non-replenishment and only first two days for replenishment water design (Fig. 2). Adult emergence was significantly controlled until day 14 non-replenishment design (P<0.05; Fig. 2). Meanwhile, in replenishment of water, the number of adult emergence was found higher than in non-replenishment with below than 13% after one week, despite, the number of larval mortality that were found almost similar in both treatments earlier. In general, the percentage of adult emergence was significantly controlled up to 7 days in replenishment water design and up to 14 days in non-replenishment water design (P<0.05, Fig. 2).

DISCUSSION

Our study had revealed that the effectiveness of acethonilic extract of Ip. cairica leaves last for up to 21 days with more than 50% reduction of Cx. quinquefasciatus larvae in treated containers. The established length of residual effectiveness of the extract indicates that the length of application time was effective under average weather conditions and could be useful to determine the accurate timing for reapplications in natural environment. Precise application of insecticide is important to prevent emergence of insecticide resistance in future. It is well known that overzealous use of chemical insecticides have caused resistance in target pest. Organophosphorus insecticide resistance has been reported in all major Culex vectors (Hemingway & Karunaratne, 1998), and pyrethroid Table 1. Effect of Ipomea cairica leaves extract on Cx. quinquefasciatus larval mortality based on post-treatment days and treatment designs (replenishment and non-replenishment) using ANOVA test. Significant values are in bold

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F-ratio</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-treatment (Days)</td>
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<td>47.40</td>
<td>0.00</td>
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<td>Treatment</td>
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<td>0.55</td>
<td>25.47</td>
<td>0.00</td>
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<tr>
<td>Error</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td></td>
<td></td>
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</table>

df-degree of freedom; MS-mean square
Table 2. Results of ANOVA on *Cq. quinquefasciatus* adult emergence from larvae treated with acethonilic extract of *I. cairica* leaves based on effects of post-treatment days and treatment designs (replenishment and non-replenishment). Significant values are in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F-ratio</th>
<th>Sig</th>
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<tr>
<td>Post-treatment (Days)</td>
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<tr>
<td>Treatment</td>
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</tr>
<tr>
<td>Error</td>
<td>39</td>
<td>0.10</td>
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<tr>
<td>Total</td>
<td>48</td>
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df-degree of freedom; MS-mean square

Figure 1. Mean percentage mortality of *Cq. quinquefasciatus* larvae after treated with acethonilic extract of *I. cairica* leaves using, (A) Replenishment, (B) non-replenishment test designs.

*Mean % mortality followed by the same letters are not significantly different (P>0.05, Tukey HSD).*

*Larval mortality in untreated jar is less than 10% and adult emergence is more than 90%.*
resistance had occurred in *Cx. quinquefasciatus* (Bencheikh *et al*., 1998; Chandre *et al*., 1998). The first generation of *Cx. quinquefasciatus* field strain was reported to develop 96.0 and 6.3 fold resistance to malathion and permethrin which are principle insecticides used in fogging operations in Malaysia, respectively (Nazni *et al*., 1998).

Non-replenishment water design gave longer residual effects in controlling *Cx. quinquefasciatus* larvae compared to those in replenishment design. It is possible that *Ip. cairica* extract particles that remained intact was higher in container without replenishment of water. This may have influenced the mortality rate of *Cx. quinquefasciatus* due to more contact with the extract particles. Meanwhile, the mortality rate observed in container with replenishment of water, suggested that even
if the extract was susceptible to wash off, enough residues still remained to kill more than 50% of the larvae for up to three weeks. In spite of that, *Cx. quinquefasciatus* larvae require to have contact with the remaining plant particles in treated container before it took effect.

Few chemical compounds has been extracted and isolated from *Ip. cairica* namely lignans, arctigenin, matairesinol and trachelogenin (Fuss, 2003; Kayser et al., 2003; Lin et al., 2008; Meira et al., 2012) trans-2, 3-dibenzylbutyrolactone, vanillic acid, p-hydroxybenzoic acid, methoxybenzoic acid, methylparaben stearic acid, palmitic acid, olenic acid, friedelinol, and a mixture of β-sitosterol and stigmasterol (Lin et al., 2008). Lignans together with other allelochemicals may play a role in synergizing antifeedant or insecticidal properties (Rios et al., 2002), suggesting better explanation of the larval mortality observed in the treated larvae in our study. The acethonilic extract of *Ip. cairica* may act as antifeedant through antibiosis mode of action as it causes *Cx. quinquefasciatus* larvae to subsequently cease feeding and eventually led to death. The larvae in the treated containers of both test designs were also observed to curl up, restless and show blackening of abdomen which is sign of neurotoxicity which may have caused by ingestion of the plant extract tested. Several studies indicated that the bioactivity of crude plant extract on insects may comprise of both toxic and antifeedant effects. *Bifora radians* was depicted as an antifeedant and exhibited toxic effects when ingested by obliquebanded leafroller, *Choristoneura rosaceana* larvae (Gokce et al., 2009). Crude methanolic extract of *Trichilia americana* was suggested to express both antifeedant and toxic activities against the larvae of *Spodoptera litura* (Wheeler & Isman, 2001). Azadirachtin is reported to be both a toxicant and antifeedant (Akhtar & Isman, 2004). Therefore, it can be suggested that acethonilic extract of *Ip. cairica* also exhibits both antifeedant and toxic bioactivity against *Cx. quinquefasciatus* larvae.

Mortality rate of treated *Cx. quinquefasciatus* larvae was found to decrease gradually with the increase of post treatment time. This indicated reduced residual activity of the plant extract with respect to time. The gradual decrease in residual efficacy of the plant extract might be due to degradation of active compounds. The plant extract tested was observed to form precipitate and settled at the bottom of the containers of both water designs after a few days. The precipitate formed may be breakdown product of the active compounds of plant extract as it decomposed. With regards to the decomposition action, the distribution and availability of the active compounds were reduced causing less contact with the *Culex* larvae. Hence, the mortality rate reduced gradually with higher adult emergence observed after 14 days.

Few other studies have reported the extent of toxicity of plant extract and essential oils against other insect pests. Commercial extract of neem (NeemAzal-T) has been proven to protect crop plants from damage of cabbage pest *Mamestra brassicae* for at least three weeks after application in a semi-field experiment (Seljåsen & Meadow, 2006). Whereas, Vatandoost & Vaziri (2004) concluded that Neemarin, a commercial neem extract product, at the recommended concentrations of 1 and 2 L/hectare in the field trials had significantly reduced the frequency of *An. stephensi* and *Cx. quinquefasciatus* larvae and the estimated residual effect was 7 days. While, toxicity of crude aqueous seed extracts of *Annona squamosa* against insidious flower bug, *Orius insidiosus* decreased significantly after 7 days the insidious over bug was exposed to the extract residues under laboratory conditions. The respective study also claimed that the toxicity of the extracts tested decreased with increasing residue time (Leatemia & Isman, 2004). UDA-245, essential oil extract from *Chenopodium ambrosioides*, a North American herbaceous plant was found to be persistent in the environment only for a few hours, the short residual activity indicated by mortality levels diminishing significantly when the two spotted spider mite, *Tetranychus urticae* Koch was introduced on treated foliage 1 h after application of an emulsifiable
concentrate UDA-245 in the test conducted under laboratory conditions (Chiasson et al., 2004).

Concisely, the result obtained in this study has proven that Ip. cairica extract which act as botanical insecticides are easily biodegradable and disappears in time. Biopesticides from plant origins are used against several insect species especially disease-transmitting vectors, based on the fact that compounds from plant origins are safer in usage, without phytotoxic properties and also leave no harmful residues in the environment (Alkofahi et al., 1989, Senthil Nathan et al., 2004, 2005a) as compared to synthetic chemical pesticides which are more expensive, hazardous to handle, leave toxic residues in food product and are not easily biodegradable (Rotimi et al., 2011). For example, DDT is biodegraded slowly, persists for a long time in the environment, and accumulates in the food chain and in the tissues of living organisms (Vladimir et al., 2012). The levels of selected organochlorine and organophosphate pesticides found in the Selangor River in Malaysia exceeded standard water quality levels and the wetland ecosystem was threatened by the occurrence of these residual pesticides (Kok et al., 2007).

Our study proved that the residual effectiveness of the acethonilic extract of Ip.cairica leaves to be far less persistent compared to conventional chemical insecticides. The short period of residual effectiveness of the plant extract tested endorsed fewer concerns of having excess residues in the environment which may risk environmental pollution, unlike conventional synthetic insecticides. However, the period of persistence given by plant extract is enough to eliminate the Cx. quinquefasciatus population especially within the first week after application. Furthermore, this plant product seems to eliminate the Culex population and also its progenies after only one application. Re-application needs to be done after some time due to the bio-degradable nature of this extract. Nevertheless, the efficacy of Ip.cairica plant extract under field conditions should be further assessed in order to develop and commercialize the plant extract into botanical derived insecticide as an environmentally friendly alternative in vector control programs.

In general, the studied plant extract have short effective residual period with good control on Culex mosquito population. Therefore, it does not accumulate in the environment and development of insect resistance will not arise as quickly as synthetic insecticides. Further studies on development and processing of the tested plant extract are required to establish it as a biopesticide. As acknowledged, phytochemicals are safer alternatives with less adverse effects and often active against a limited number of species including specific target insects.

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