Tropical Biomedicine 31(3): 466–476 (2014)

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

Maniam Thiagaletchumi[1], Wan Fatma Zuharah[1,2*], Rattanam Ahbi Rami[1], Nik Fadzly[1,3], Hamady Dieng[1], Abu Hassan Ahmad[1] and Sazaly AbuBakar[4]
[1]School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia
[2]Vector Control Research Unit, Universiti Sains Malaysia, Penang 11800, Malaysia
[3]Centre of Marine & Coastal Studies (CEMACS), Universtiti Sains Malaysia, Penang 11800, Malaysia
[4]Department of Medical Microbiology, University of Malaya, Kuala Lumpur 50603, Malaysia
[*]Corresponding author: email: wfatma@usm.my
Received 23 August 2013; received in revised form 10 October 2013; accepted 25 December 2013

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 *Ipomoea 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 prescence 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). Vector control has proven highly efficient in preventing disease transmission in major vector-borne diseases (Townson *et al.*, 2005). 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 declorinated 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.

Culex 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

Source	df	MS	F-ratio	Sig
Post-treatment (Days)	7	1.02	47.40	**0.00**
Treatment	1	0.55	25.47	**0.00**
Error	39	0.02		
Total	48			

df-degree of freedom; MS-mean square

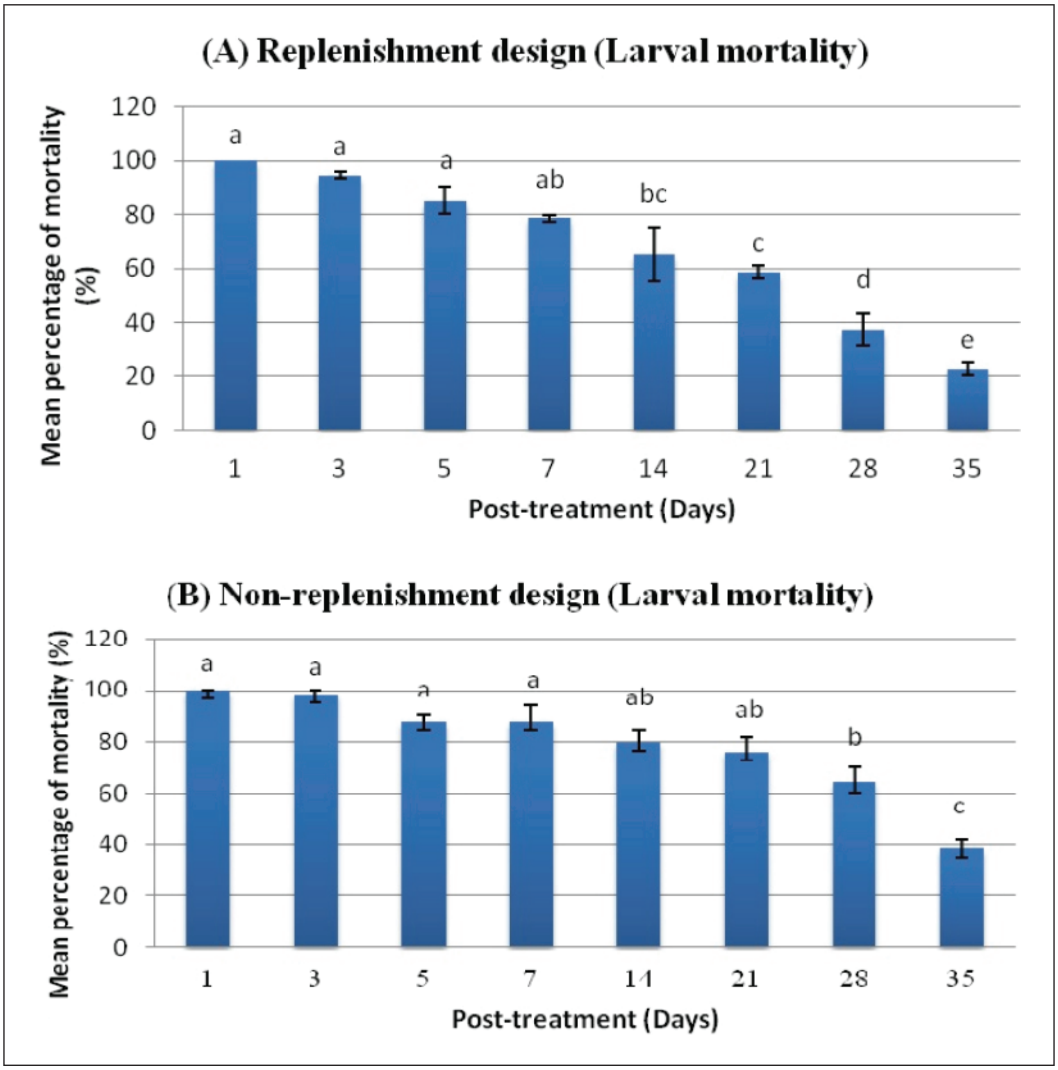

Figure 1. Mean percentage mortality of *Cx. quinquefasciatus* larvae after treated with acethonilic extract of *I.cairica* leaves using, (A) Replenishment, (B) non-replenishment test designs
*Mean % mortality followed by the same letters are not significantly different (P>0.05, Tukey HSD).
*Larval mortality in untreated jar is less than 10% and adult emergence is more than 90%.

Table 2. Results of ANOVA on *Cx. quinquefasciatus* adult emergence from larvae treated with *Ipomea cairica* leaves extract based on effects of post-treatment days and treatment designs (replenishment and non-replenishment). Significant values are in bold

Source	df	MS	F-ratio	Sig
Post-treatment (Days)	7	6.99	71.75	**0.00**
Treatment	1	1.62	16.58	**0.00**
Error	39	0.10		
Total	48			

df-degree of freedom; MS-mean square

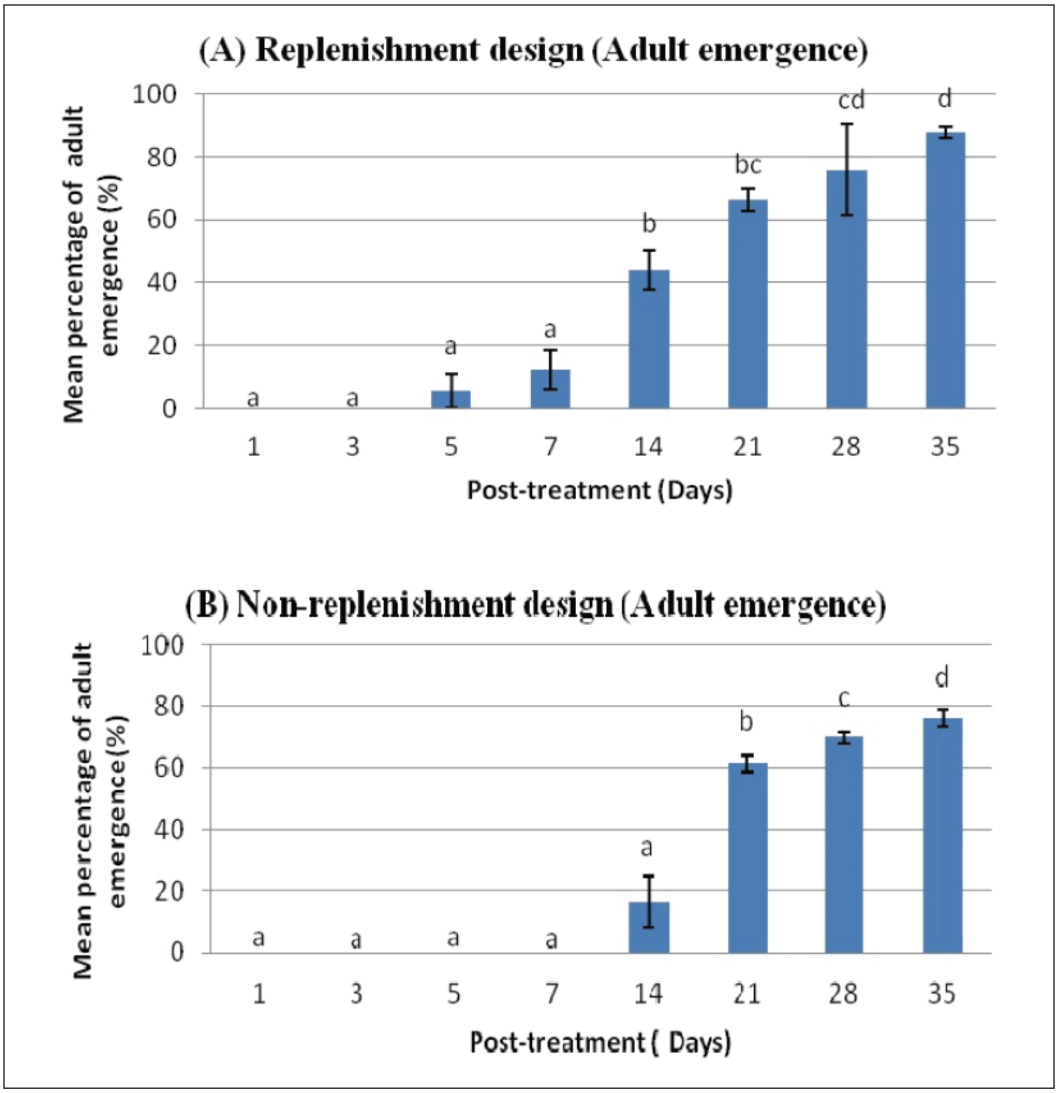

Figure 2. Mean percentage of *Cx. quinquefasciatus* adult emergences from larvae treated with acethonilic extract of *Ipomea cairica* leaves using two treatment designs; (A) replenishment, (B) non-replenishment

*Mean value followed by the same letters within the same column 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, methoxybenzoicacid 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). Azaridachtin 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 signicantly after 7 days the insidious ower 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. Bio-pesticides 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.

Acknowledgements. The authors are grateful to the staff of the Vector Control Unit and School of Biological Sciences, Universiti Sains Malaysia for assisting in sample collections and their continuous support during the course of the experiment. This project was partially funded by Long Term Research Grant (LRGS) for Infectious Diseases, 2011-2014 Ministry of Higher Education Malaysia and Universiti Sains Malaysia (304/PBIOLOGI/650577/U112) and Short Term Grant USM (304/PBIOLOGI/6312026).

REFERENCES

Abu Hassan, A. & Che Salmah, M.R. (1990). A checklist of the mosquitoes of Malaysia. *Journal of Bioscience* **1**: 29-41.

Alkofahi, A., Rupprecht, J.K., Anderson, J.E., Mclaughlin, J.L., Mikolajczak, K.L., Scott, B.A. (1989). Search for new pesticides from higher plants. In: Arnason, J.T., Philogene, B.J.R., Morand, P. (Eds.), Insecticides of Plant Origin. American Chemical Society, Washington, DC, pp. 25-43.

Akhtar, Y. & Isman, M. B. (2004). Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. *Journal of Applied Entomology* **128**(1): 32-38.

Bencheikh, H., Ben Ali Haouas, Z., Marquine, M. & Pasteur, N. (1998). Resistance to organophosphorus and pyrethroid insecticides in *Culex pipiens* (Diptera: Culicidae) from Tunisia. *Journal of Medical Entomology* **35**: 251-260.

Blackwell, A., Mordue, L.A.J., Hansson, B.S., Wadhams, L.J. & Pickett, J.A. (1993). A behavioral and electrophysiological study of oviposition cues for *Culex quinquefasciatus*. *Physiological Entomology* **18**: 343-348.

Chandre, F., Darriet, F., Darder, M., Cuany, A., Doannio, J.M.C., Pasteur, N. & Guillet, P. (1998). Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Medical and Veterinary Entomology* **12(**4): 359-366.

Chavasse, D.C., Lines, J.D., Ichimori, K. & Marijani, J. (1995). Mosquito control in Dar es Salaam. I. Assessment of *Culex quinquefasciatus* breeding sites prior to intervention. *Medical and Veterinary Entomology* **9**: 141-146.

Chiasson, H., Bostanian, N.J. & Vincent, C. (2004). Acaricidal properties of a Chenopodium-based botanical. *Journal of Economic Entomology* **97**(4): 1373-1377.

Freeman, B.C. & Beattie, G.A. (2008). An Overview of Plant Defenses against Pathogens and Herbivores. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2008-0226-01. Available at http://www.apsnet.org/edcenter/intropp/topics/Pages/OverviewOfPlantDiseases.aspx

Fuss, E. (2003). Lignans in plant cell and organ cultures: an overview. *Phytochemistry Reviews* **2**(3): 307-320.

Gokce, A., Stelinski, L.L., Whalon, M.E. & Gut, L.J. (2009). Toxicity and antifeedant activity of selected plant extracts against larval obliquebanded leafroller, *Choristoneura rosaceana* (Harris). *The Open Entomology Journal* **3**: 30-36.

Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K. & Anandan, A. (2011). Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine* 43-48.

Hamdan, H., Sofian-Azirun, M., Nazni, W.A. & Lee, H.L. (2005). Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae against malathion, permethrin and temephos. *Tropical Biomedicine* **22**(1): 45-52.

Hemingway, J. & Karunaratne, S.H.P.P. (1998). Mosquito carboxylesterases: a review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Medical and Veterinary Entomology* **12**: 1-12.

Kayser, O., Masihi, K.N. & Kiderlen, A.F. (2003). Natural products and synthetic compounds as immunomodulators. *Expert review of anti-infective therapy* **1**(2): 319-335.

Kok, H.L., Benjamin, T. & Ali, M. (2007). Contamination levels of selected organochlorine and organophosphate pesticides in the Selangor River, Malaysia between 2002 and 2003. *Chemosphere* **66**(6): 1153-1159.

Leatemia, J. & Isman, B. (2004). Toxicity and antifeedant activity of crude seed extracts of *Annona squamosa* (Annonaceae) against lepidopteran pests and natural enemies. *International Journal of Tropical Insect Science* **24**(1): 150-158.

Lee, Y.W. & Zairi, J. (2005). Laboratory evaluation of *Bacillus thuringiensis* H-14 against *Aedes aegypti*. *Tropical Biomedicine* **22**(1): 5-10.

Lin, R.J., Chen, C.Y. & Lo, W.L. (2008). Cytotoxic activity of Ipomoea cairica. *Natural Product Research* **22**(9): 747-753.

Meira, M., Silva, E.P.D., David, J.M. & David, J.P. (2012). Review of the genus Ipomoea: traditional uses, chemistry and biological activities. *Revista Brasileira de Farmacognosia* **22**(3): 682-713.

Moses, J.B., Erling, M.P., Graham, B.W. & Edwin, M. (2009). Role of Vector Control in the Global Program to Eliminate Lymphatic Filariasis. *Annual Review of Entomology* **54**: 469-487.

Nazni, W.A., Lee, H.L. & Sa'diyah, I. (1998). Rate of resistance development in wild *Culex quinquefasciatus* (Say) selected by malathion and permethrin. Available at: http://imsear.hellis.org/handle/123456789/31009

Omar, A.W., Sulaiman, O., Yusof, S., Ismail, G., Fatmah, M.S., Rahmah, N. & Khairul, A. A. (2001). Epidemiological screening of lymphatic filariasis among immigrants using dipstick colloidal dye immuno-assay. *The Malaysian Journal of Medical Sciences: MJMS* **8**(2): 19.

Pothikasikorn, J., Bangs, M.J., Boonplueang, R. & Chareonviriyaphap, T. (2008). Susceptibility of various mosquitoes of Thailand to nocturnal subperiodic *Wuchereria bancrofti*. *Journal of Vector Ecology* **33**(2): 313-320.

Rahuman, A.A., Bagavan, A., Kamaraj, C., Saravanan, E., Zahir, A.A. & Elango, G. (2009). Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitology Research* **104**(6): 1365-1372.

Rajkumar, S. & Jebanesan, A. (2007). Repellent activity of selected plant essential oils against the malarial fever mosquito *Anopheles stephensi*. *Tropical Biomedicine* **24**(2): 71-75

Remia, K.M. & Logaswamy, S. (2010). Larvicidal efficacy of leaf extract of two botanicals against the mosquito vector *Aedes aegypti* (Diptera: Culicidae) *Indian Journal of Natural Products and Resources* **1**(2): 208-212.

Ríos, J.L., Giner, R.M. & Prieto, J.M. (2002). New findings on the bioactivity of lignans. *Studies in Natural Products Chemistry* **26**: 183-292.

Rong, J.L., Chung, Y.C. & Wen, L.L. (2008). Cytotoxic activity of *Ipomoea cairica*. *Natural Product Research* **22**(9): 747-753.

Roseli, L.C.S., Arnaldo, S.F., Antonio, E.F.A., Durval, B.R.V. & Marinete, M.P. (2007). Evaluation of the residual effect of pyrethroids on Anopheles in the Brazilian Amazon. *Revista Saúde Pública* **41**(2).

Rotimi, O.A., Chris, O.A., Olusola, O.O., Joshua, R. & Josiah, A.O. (2011). Bioefficacy of Extracts of some Indigenous Nigerian Plants on the developmental stages of mosquito (*Anopheles gambiae*). *Jordan Journal of Biological Sciences* **4**(4): 237-242

Seljåsen, R. & Meadow, R. (2006). Effects of neem on oviposition and egg and larval development of< i> Mamestra brassicae </i> L: Dose response, residual activity, repellent effect and systemic activity in cabbage plants. *Crop Protection* **25**(4): 338-345.

Senthil, N.S., Chung, P.G. & Murugan, K. (2004). Effect of botanicals and bacterial toxin on the gut enzyme of *Cnaphalocrocis medinalis*. *Phytoparasitica* **32**: 433-443.

Senthil, N.S., Kalaivani, K., Murugan, K. & Chung, P.G. (2005a). The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guene´e), the rice leaf folder. *Pesticide Biochemistry and Physiology* **81**: 113-122.

Sivagnaname, N. & Kalyanasundaram, M. (2004). Laboratory Evaluation of Methanolic Extract of *Atlantia monophylla* (Family: Rutaceae) against Immature Stages of Mosquitoes and Non-target Organisms. *Memorias do Institute of Oswaldo Cruz, Rio de Janeiro* **99**(1): 115-118.

Thomas, T.G., Rao, S. & Lal, S. (2004). Mosquito larvicidal properties of essential oil of an indigenous plant, *Ipomoea cairica* Linn. *Japanese Journal of Infectious Disease* **57**: 176-177.

Townson, H., Nathan, M.B., Zaim, M., Guillet, P., Manga, L., Bos, R. & Kindhauser, M. (2005). Exploiting the potential of vector control for disease prevention. *Bulletin of World Health Organisation* **83**(12): 942-947. Available at: http://www.scielosp.org/scielo.php?script=sci_arttext&pid=S0042-96862005001200017&lng=en. http://dx.doi.org/10.1590/S0042-96862005001200017.

Vatandoost, H. & Vaziri, V.M. (2004). Larvicidal activity of a neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran. *Eastern Mediterranean Health Journal* **10**(4-5): 573-581.

Vladimir, T., Valery, R. & Lorenzo, T. (2012). Dichlorodiphenyltrichloroethane (DDT): Ubiquity, Persistence, and Risks. *Environmental Health Perspectives* **110**(2): 125-128.

Vythilingam, I., Huat, T.C. & Ahmad, N.W. (2005). Research Note Transmission potential of *Wuchereria bancrofti* by *Culex quinquefasciatus* in urban areas of Malaysia. *Tropical Biomedicine* **22**(1): 83-85.

Wheeler, D.A. & Isman, M.B. (2001). Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of Spodoptera litura. *Entomologia Experimentalis et Applicata* **98**(1): 9-16.

WHO 2012. Available at: http://www.who.int/mediacentre/factsheets/fs102/en/