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Adult emergence inhibition and adulticidal activity of leaf crude extracts against Japanese encephalitis vector, *Culex tritaeniorhynchus*

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Abstract Mosquitoes have developed resistance to various synthetic insecticides, making its control increasingly difficult. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. The aim of this study was to evaluate the adult emergence inhibition (EI) and adulticidal activity of the leaf hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *Aegle marmelos* (Linn.) Correa ex Roxb, *Andrographis lineata* Wallich ex Nees., *Andrographis paniculata* (Burm.f.) Wall. ex Nees., *Cocculus hirsutus* L. Diels, *Eclipta prostrata* L. and *Tagetes erecta* L. were tested against Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). All plant extracts showed moderate EI and adulticidal activity effects after 24 h of exposure at 1,000 ppm; however, the highest EI activity was found in leaf methanol extracts of *A. marmelos*, *A. paniculata*, *T. erecta*, and chloroform extract of *E. prostrata* (EI_{50} = 141.94, 214.17, 166.43, and 184.58 ppm; EI_{90} = 590.26, 882.34, 532.00 and 571.81 ppm); and the effective adulticidal activity was observed in acetone extract of *A. marmelos*, hexane of *A. lineata*, ethyl acetate of *A. paniculata*, methanol extracts of *C. hirsutus*, *E. prostrata*, and *T. erecta* (LD_{50} = 139.05, 251.24, 205.06, 222.10, 166.73, and 232.74 ppm; LD_{90} = 426.19, 837.09, 813.59, 794.42, 579.43 and 807.41 ppm), respectively against *C. tritaeniorhynchus*. These results suggest that the leaf methanol extract of *C. hirsutus*, *E. prostrata*, and *T. erecta* have the

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potential to be used as an ideal eco-friendly approach for the control of the *C. tritaeniorhynchus*. Therefore, this study provides first report on the mosquito EI and adulticidal activity of plant extracts against a vector.

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1. Introduction

Japanese encephalitis (JE) outbreaks occur frequently in 14 Asian countries with about 3,060 million people at the risk of infection (Sabesan, 2003). JE is a major public health concern due to its high epidemic potential, high case fatality, and neuro-psychiatric sequel among survivors. The annual incidence and mortality estimates for JE are 30,000–50,000 and 10,000, respectively, (Solomon, 2004). Approximately 2 billion people live in countries where JE presents a significant risk to humans and animals, particularly in China and India, with at least 700 million potentially susceptible children (Gould et al., 2008) and it is highly endemic in few districts of Tamil Nadu, Southern India (Ravi et al., 1989; Reuben and Gajanana, 1997). *C. tritaeniorhynchus* is a primary vector of Japanese encephalitis virus, with a distribution throughout Southeast and South Asia. The larval habitat is mainly rice fields and shallow marshes. Mosquito control represents an important strategy for prevention of disease transmission and epidemic outbreaks. However, a high level of insecticide resistance has developed through chemical control of the vector and pests threatening the control strategies. To overcome these problems, it is necessary to search for alternative methods of vector control. The failure of chemical insecticides to control the insect and growing public concern for safe food and a healthy environment have catalyzed the search for more environmentally benign control methods for the management of the vectors. Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages (Liu et al., 2000).

The present study was carried out on *A. marmelos*, an Indian plant with religious and medicinal importance and the stem bark ethyl acetate extracts exhibited moderate insecticidal activity against *Phaedon cochleariae* and *Musca domestica* (Samarasekera et al., 2004). Zaridah et al. (2001) have reported that the aqueous extract from the dried leaves of *A. paniculata* showed the strongest activity against adult worms of *Brugia malayi*. Elango et al. (2010) reported that the hexane extracts of *A. marmelos* and *A. paniculata* served as a potential repellent, ovicidal, and oviposition deterrent against *C. tritaeniorhynchus*. *C. hirsutus* is a widely growing plant found in the plains of India in dry localities and the methanol extract showed effective oviposition repellency against *Anopheles subpictus* (Elango et al., 2009a). *E. prostrata*, a member of the Asteraceae family and commonly known as False Daisy and the ethyl acetate extract of *E. prostrata* and leaf hexane extract of *A. paniculata* have the potential to be used against the fourth-instar larvae of *A. subpictus* and *C. tritaeniorhynchus* (Elango et al., 2009b). Macêdo et al. (1997) have reported that the ethanol extract of *E. paniculata* and *Tagetes minuta* were significantly active against the larvae of *Aedes fluviatilis*.

The callus tissue of *T. erecta* which showed the presence of insecticidal pyrethrin mixture was screened against *Tribolium*

spp. Immediate ‘knock down’ effect was observed (Sarin, 2004), and the steam-distilled oils of *T. patula*, *T. erecta* and *T. minuta* were tested for larvicidal activity toward third instar *A. aegypti* and the results suggest a potential utilization of oil of *T. minuta* or its components for the control of *A. aegypti* and other species of mosquitoes (Green et al., 1991). The larvicidal potential of the essential oil of *T. patula* against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* was evaluated (Dharmagadda et al., 2005); the compounds, 4 thiophenes, 5-(but-3-ene-1-ynyl)-2,2'-bithiophene, 5-(but-3-ene-1-ynyl)-5'-methyl-2,2'-bithiophene, 2,2',5',2''-terthiophene, and 5-methyl-2,2',5',2''-terthiophene isolated from the floral extract of *T. minuta* were largely responsible for the toxicity exhibited against the adults of *A. aegypti* and *A. stephensi* (Perich et al., 1995).

The bioactive constituents of these plants could be either a single substance or a mixture of substances. The separation of the mixture is neither practical nor advantageous in the insect economic control strategies. The aim of this study was to investigate the activity of different solvent extracts of six plant species from Tamil Nadu, South India. As far as our literature survey could ascertain, no information was available on the adult emergence inhibition and adulticidal activities of the experimental plant species given here. Therefore, the present study was an attempt to assess the adult emergence inhibition and adulticidal activity leaf extracts against *C. tritaeniorhynchus*. This is the first report of the solvent extracts of selected plants.

2. Materials and methods

2.1. Collection of plants

The leaf of *A. marmelos* (Linn.) Correa ex Roxb (Rutaceae), *A. lineata* Wallich ex Nees. (Acanthaceae), *A. paniculata* (Burm.f.) Wall. ex Nees. (Acanthaceae), *C. hirsutus* (L.) Diels (Menispermaceae), *E. prostrata* L. (Asteraceae) and *T. erecta* L. (Compositae) were collected from the Tamil Nadu Medical Plant Farms and Herbal Medicine Corporation Limited, medicinal plant farm, Arumbakkam (13° 13' 4N, 79° 59' 7E Altitude 118 feet), Chennai, Tamil Nadu and the taxonomic identification was made by Dr. B. Annadurai, Department of Plant Biology and Biotechnology, C. Abdul Hakeem College, Melvisharam, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

2.2. Collection and rearing of mosquitoes

C. tritaeniorhynchus larvae were collected from a rice field and the stagnant water area of Melvisharam (12° 56' 23" N, 79° 14' 23" E) and identified in Zonal Entomological Research Centre, Vellore (12° 55' 48" N, 79° 7' 48" E), Tamil Nadu, to start the colony and the larvae were kept in plastic and enamel trays containing tap water. They were maintained, and all the experi-

ments were carried out, at $27 \pm 2^\circ\text{C}$ and 75–85% relative humidity under 14:10 light and dark cycles. Larvae were fed with a diet of brewer's yeast, dog biscuits, and algae collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary (45 cm × 45 cm × 40 cm) where adults emerged. Adults were maintained in glass cages and were continuously provided with 10% sucrose solution in a jar with a cotton wick. On day 5, the adults were given a blood meal from a pigeon placed in resting cages overnight for blood feeding by females. They were maintained and reared in the laboratory as per the method of Kamaraj et al. (2009).

2.3. Preparation of plant extracts

The dried leaf (800 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with hexane (1400 ml, Fine), chloroform (1400 ml, Fine), ethyl acetate (2200 ml, Qualigens), acetone (1200 ml, Qualigens), and methanol (2500 ml, Qualigens) in a soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22–26 mm Hg at 45°C and the residue obtained was stored at 4°C .

2.4. Adult emergence inhibition (EI) bioassay

The inhibition of adult emergence was evaluated by following the WHO standard procedure for testing insect growth regulators (WHO, 2005). Only 3rd instars larvae were used and followed the method of larvicidal activity. Because of the long duration of the test the larvae were fed with yeast at two-days intervals until the mortality counts were made. The yeast powder was prepared as stock suspension in water from which one or two drops were added per cup. One gram of crude extract was first dissolved in 100 mL of acetone (stock solution). From the stock solution, 15.625–1000 ppm were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. All the treated and control cups containing the pupae were kept separately in the net cage to prevent the successfully emerged adults from escaping into the environment. Mortality of the pupae was recorded at 24 h intervals. Observation was continued in treated and control cups (acetone, polysorbate 80 and de-chlorinated tap water) until the complete emergence of the adults. At the end of observation period, the impact was expressed as EI% based on the number of pupae that did not develop successfully into viable adults. In recording EI% for each concentration, moribund and pupae, as well as adult mosquitoes not completely separated from the pupal case, were considered as dead. The experiments stop when all the pupae in the controls have died or emerged as adults. The experimental media, in which 100% (EI) pupae occurs alone, were selected and dose response bioassay test done (Elimam et al., 2009b).

2.5. Adulticidal bioassay

C. tritaeniorhynchus mosquitoes were selected for the testing of adulticidal activities. The bioassay was performed by WHO (1981) method. Appropriate concentrations of the leaf plant solvent extracts were dissolved in 2.5 ml of acetone and applied on Whatman no. 1 filter papers (size $12 \times 15\text{ cm}^2$) as described by

Dua et al. (2008). Control papers were treated with acetone under similar conditions. The plant extracts were evaluated at seven concentrations 15.625, 31.25, 62.5, 125, 250, 500 and 1000 ppm to produce a range of mortality from 10% to 100% along with the control. Twenty mosquitoes (2–5 days old glucose fed, blood starved) were collected and gently transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1 h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept 24 h for the recovery period. A pad of cotton soaked with 10% glucose solution was placed on the mesh screen. Mortality of mosquitoes was determined at the end of 24 h recovery period. Percent mortality was corrected by using Abbott's formula (1925).

2.6. Dose response bioassay

From the stock solution, different concentrations ranging from 15.62 to 1000 ppm were prepared. Based on the preliminary screening results, crude different solvent leaf extracts prepared from *A. marmelos*, *A. lineata*, *A. paniculata*, *C. hirsutus*, *E. prostrata*, and *T. erecta* were subjected to dose response bioassay for percentage adult emergence inhibition and adulticidal activity were counted after 24 h of exposure, against the *C. tritaeniorhynchus*. The percentage adult mortality and EI% was reported from the average of five replicates.

2.7. Statistical analysis

Adult mortality counts were made after 24 h exposure. Bioassay test showing more than 10% control mortality was discarded and repeated. However, when control mortality ranged from 5% to 10%, the corrected mortality was calculated using Abbott's formula (Abbott, 1925). LD50, LD90 and the 90% confidence intervals (CI) of the lethal dosage of 50% and 90% calculated by a computerized log-probit analysis (Harvard Programming; Hg1, 2) was used to measure differences between test samples.

3. Results

The activity of crude plant extracts is often attributed to the complex mixture of active compounds. The preliminary screening is a good means of evaluation of the potential of plants adult emergence inhibition and adulticidal activity of crude plant extracts is often attributed to the different solvent extracts of six plants which are noted and presented in Table 1 and Fig 1. Among the crude extracts tested, the present results showed that emergence inhibition of leaf methanol extract of *A. marmelos*, *A. paniculata*, *T. erecta* and chloroform extract of *E. prostrata* ($EI_{50} = 141.94, 214.17, 166.43, \text{ and } 184.58\text{ ppm}$; $EI_{90} = 590.26, 882.34, 532.00 \text{ and } 571.81\text{ ppm}$), and the maximum adulticidal activity observed in acetone extract of *A. marmelos*, hexane extract of *A. lineata*, ethyl acetate extract of *A. paniculata*, methanol extract of *C. hirsutus*, *E. prostrata*, and *T. erecta* ($LD_{50} = 139.05, 251.24, 205.06, 222.10, 166.73, \text{ and } 232.74\text{ ppm}$; $LD_{90} = 426.19, 837.09, 813.59, 794.42, 579.43 \text{ and } 807.41\text{ ppm}$), respectively against *C. tritaeniorhynchus*. The chi-square values calculated during the analysis did not show any heterogeneity of the response. Table 2 and 3 and Fig. 2 and 3.

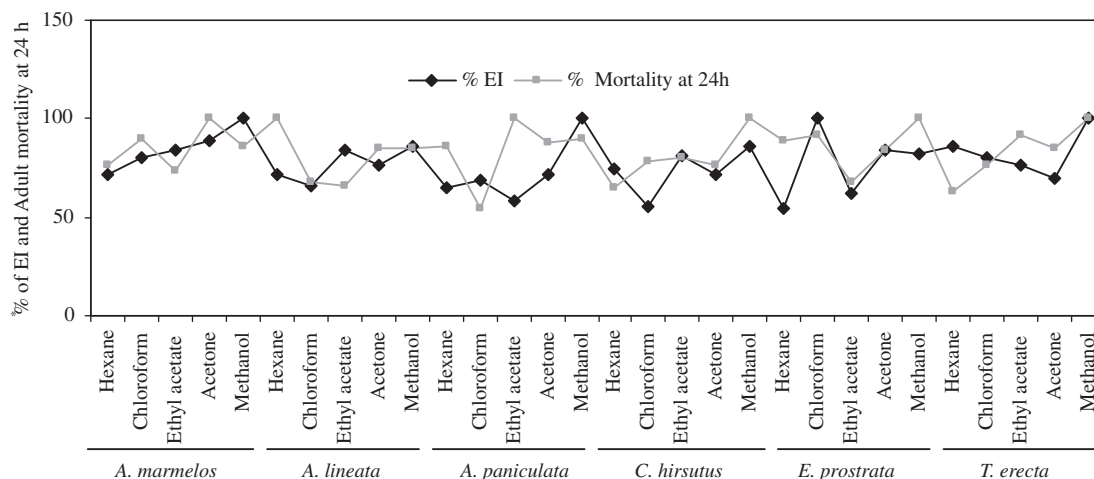


Figure 1 Adult emergence inhibition and adulticidal activity of different plant extracts against *C. tritaeniorhynchus* at 1000 ppm.

Table 1 Adult emergence inhibition and adulticidal activity of different plant extracts against *C. tritaeniorhynchus* at 1000 ppm.

Botanical name/Family (Herbarium numbers) Vernacular names	Solvents	*%EI	% mortality at 24 h
<i>Aegle marmelos</i> (Linn.) Correa ex Roxb/ Rutaceae (LE/ZB/007-05) Vilvam	Hexane	72 ± 2.21	76 ± 2.11
	Chloroform	80 ± 2.16	90 ± 1.60
	Ethylacetate	84 ± 1.72	74 ± 1.58
	Acetone	89 ± 1.68	100 ± 0.00
	Methanol	100 ± 0.00	86 ± 1.76
<i>Andrographis lineata</i> Wallich ex Nees./ Acanthaceae (ZD/AL/040-08) Siriyanangai	Hexane	72 ± 3.00	100 ± 0.00
	Chloroform	66 ± 1.42	68 ± 1.86
	Ethyl acetate	84 ± 1.38	66 ± 1.20
	Acetone	76 ± 0.00	85 ± 1.11
	Methanol	86 ± 1.65	85 ± 1.70
<i>Andrographis paniculata</i> (Burm.f.) Wall. ex Nees./Acanthaceae (ZD/AP/143-08) Periyanangai or Nilavembu	Hexane	65 ± 1.79	86 ± 1.62
	Chloroform	69 ± 1.98	54 ± 1.66
	Ethyl acetate	58 ± 1.84	100 ± 0.00
	Acetone	72 ± 1.00	88 ± 1.20
	Methanol	100 ± 0.00	90 ± 1.11
<i>Cocculus hirsutus</i> (L.) Diels/ Menispermaceae (ZD/CH/142-08) Vellakattukkodi	Hexane	75 ± 2.44	65 ± 1.40
	Chloroform	55 ± 1.41	78 ± 1.76
	Ethyl acetate	81 ± 0.00	80 ± 1.32
	Acetone	72 ± 1.82	76 ± 1.72
	Methanol	86 ± 1.71	100 ± 0.00
<i>Eclipta prostrata</i> L./Asteraceae (ZD/EP/ 114-08) Manjal Karisallankannai	Hexane	54 ± 2.14	89 ± 1.98
	Chloroform	100 ± 0.00	92 ± 1.68
	Ethyl acetate	62 ± 1.22	68 ± 1.86
	Acetone	84 ± 1.91	84 ± 1.76
	Methanol	82 ± 2.07	100 ± 0.00
<i>Tagetes erecta</i> L./Compositae(ZD/TE/ 156-08) Tulukkaccevvanti	Hexane	86 ± 1.48	63 ± 1.79
	Chloroform	80 ± 1.67	76 ± 1.75
	Ethyl acetate	76 ± 1.72	92 ± 1.68
	Acetone	70 ± 1.20	85 ± 1.62
	Methanol	100 ± 0.00	100 ± 0.00

EI = Emergence inhibition.

* Number of each replicates five, and mortality was recorded at 24 h recovery period.

4. Discussion

In this study it was observed that, six plant leaves extract of different solvents have tested adult emergence inhibition and adulticidal activity against *C. tritaeniorhynchus*. The biological

activity of the experimental plant extracts may be due to various compounds, including phenolics, terpenoides, flavonoids and alkaloids present in plants, of these compounds may jointly or independently contribute to produce adult emergence inhibition, and adulticidal effect against Japanese

Table 2 The adult emergence inhibition activity of leaves extract against *C. tritaeniorhynchus*.

Name of the plants	Solvents	EI ₅₀ ± SE (ppm)	UCL–LCL	EI ₉₀ ± SE (ppm)	UCL–LCL	Regression coefficient (slope)	χ ² (df = 4)
<i>A. marmelos</i>	Methanol	141.94 ± 9.64	160.84–123.04	590.26 ± 66.61	720.81–459.72	2.070	6.70
<i>A. paniculata</i>	Methanol	214.17 ± 14.04	241.69–186.65	882.34 ± 93.42	1005.6–639.22	2.305	18.96
<i>E. prostrata</i>	Chloroform	184.58 ± 10.79	205.73–163.42	571.81 ± 53.97	677.59–466.03	2.609	5.34
<i>T. erecta</i>	Methanol	166.43 ± 9.99	185.93–146.94	532.00 ± 51.38	632.69–431.30	2.539	4.76

UCL = upper confidence limit; LCL = lower confidence limit; χ² = Chi-square; df = degree of freedom.

Table 3 The adulticidal activity of leaves extracts against *C. tritaeniorhynchus*.

Name of the plants	Solvents	LD ₅₀ ± SE (ppm)	UCL–LCL	LD ₉₀ SE (ppm)	UCL–LCL	Regression coefficient (slope)	χ ² (df = 4)
<i>A. marmelos</i>	Acetone	139.05 ± 8.21	155.15–122.95	426.19 ± 40.25	505.25–347.29	2.634	2.89
<i>A. lineata</i>	Hexane	251.24 ± 15.23	281.67–221.82	837.09 ± 89.39	1012.30–661.88	2.452	16.72
<i>A. paniculata</i>	Ethyl acetate	205.06 ± 13.72	231.95–178.17	813.59 ± 95.94	1001.62–625.54	2.141	17.71
<i>C. hirsutus</i>	Methanol	222.10 ± 14.18	249.91–194.31	794.42 ± 87.12	965.17–623.67	2.315	16.67
<i>E. prostrata</i>	Methanol	166.73 ± 10.35	187.03–146.43	579.43 ± 58.59	694.28–146.43	2.633	6.57
<i>T. erecta</i>	Methanol	232.74 ± 14.60	261.36–204.22	807.41 ± 87.58	979.07–635.76	2.372	15.02

UCL = Upper confidence limit; LCL = lower confidence limit; χ² = Chi-square; df = degree of freedom. Mosquitoes was exposed for 1 h and mortality was recorded at 24 h recovery period.

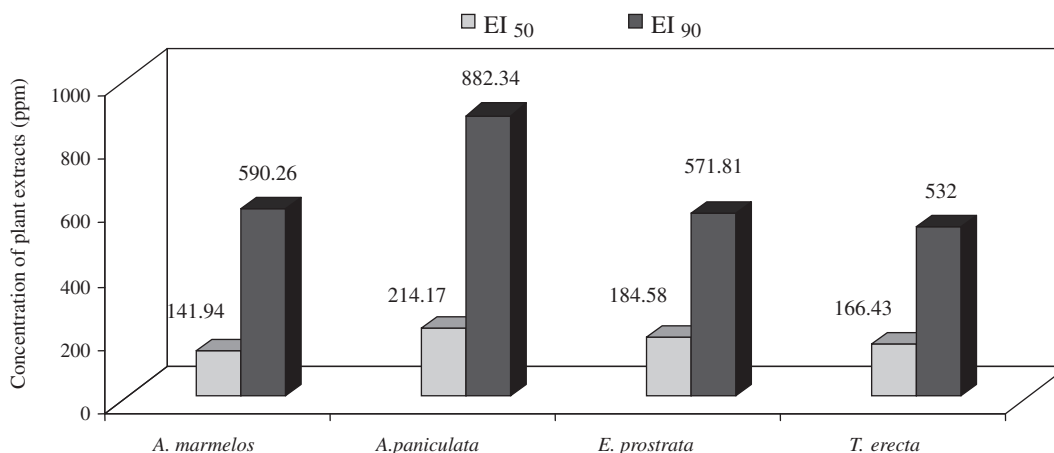


Figure 2 The adult emergence inhibition activity of leaves extract against *C. tritaeniorhynchus* expressed as EI₅₀ and EI₉₀.

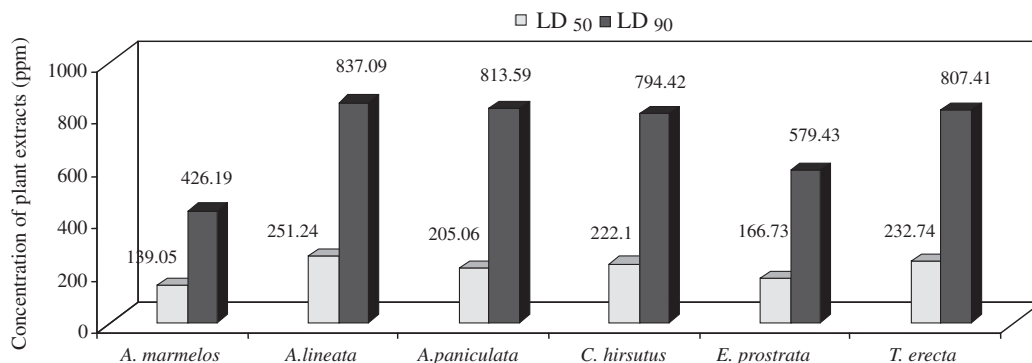


Figure 3 Adulticidal activity of different plant extracts against *C. tritaeniorhynchus* expressed as LD₅₀ and LD₉₀.

encephalitis vector, *C. tritaeniorhynchus*. Undoubtedly, plant-derived toxicants are a valuable source of potential insecticides. These and other naturally occurring insecticides play a prominent role in mosquito control programs in the future (Mordue and Blackwell, 1993). The results of this study may contribute to a great reduction in the application of synthetic insecticides, which in turn increase the opportunity for natural control of various medically important vectors by botanical chemicals. Since these are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control programme (Alkofahi et al., 1989), they could lead to the development of new classes of possible safer insect control agents. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Berenbaum, 1988; Murugan et al., 1996; Senthil Nathan et al., 2005).

Our results agree with some previous studies, such as the aqueous leaves extract of *Calotropis procera* reported that the 50% of adult emergence inhibition (EI₅₀) was shown at 277.90 and 183.65 ppm for *An. arabiensis* and *Cx. quinquefasciatus*, respectively, and the pupal stage was not affected till a concentration of 5000 ppm (Elimam et al., 2009b). Similar study was conducted by Elimam et al. (2009a) and they reported that aqueous extracts from leaves of *Ricinus communis* showed 50% of adult emergence inhibition (EI₅₀) was 374.97 and 1180.32 ppm against 3rd instar larvae of *Anopheles arabiensis* and *Cx. quinquefasciatus* and the extract showed oviposition deterrent effect against both species. The larvicidal, growth inhibitor and repellent actions of *Dalbergia sissoo* oil was evaluated against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* under laboratory conditions and observed no adult emergence was observed at 4 ml/m² (Ansari et al., 2000). Deformities produced in pupae further reduced adult emergence from 78% in the control to 75% at 6.25 ppm, 72% at 12.5 ppm, 70% at 25 and 50 ppm, and 66% at 100 ppm and the successful adult emergence from eggs treated was 78% in the control, which was reduced to 45%, 40%, 22%, 17%, and 10% at 6.25, 12.5, 25, 50, and 100 ppm, respectively was observed in methanol extract of *Azadirachta indica* on the development and growth index of *C. quinquefasciatus* (Sharma et al., 2009).

The effects of the tested extract, adult emergence and adulticidal activity of the mosquitoes are remarkably greater than those reported for other plant extracts in the literature. For example at the highest concentration, 50% inhibition of the emergence of the adult mosquitoes was observed by the use of the ethyl acetate fractions of *Calophyllum inophyllum* seed and leaf, *Solanum suratense* and *Samadera indica* leaf extracts and the petrol ether fraction of *Rhinocanthus nasutus* leaf extract on *C. quinquefasciatus*, *A. stephensi* and *A. aegypti* (Muthukrishnan and Puspalatha, 2001). Similarly 88% of the adult mortality was observed by the use of *Pelargonium citroasa* leaf extracts at 2% concentration against *A. stephensi* (Jeyabalan et al., 2003).

The present study results agree with Howard et al. (2009) who had reported that 50% inhibition of adult emergence (IE₅₀) of all larval instars obtained with <0.4 g of neem bark chippings of *A. indica* in 1 L of distilled water. The hexane extract obtained from leaves of *Eucalyptus citriodora* tested at

lowest concentration viz. 10 ppm, 73% larvae of *An. stephensi* failed to emerge as adult mosquito while in *Cx. quinquefasciatus* and *Ae. aegypti* only 10% and 6% larvae failed to emerge (Singh et al., 2007). Wiesman and Chapagain (2006) reported that the high total saponins compound isolated from methanol extracts of fruit mesocarp of *Balanites aegyptiaca* showed inhibition of 50% of the larval population from emerging adults (EC₅₀ at 0.0014% (w/v) against *A. aegypti*. The neem formulation, Neem Azal produced an overall mortality or inhibition of emergence of 90% (EI₉₀), when 3(rd) instar larvae were treated) at 0.046, 0.208 and 0.866 ppm in *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, respectively (Gunasekaran et al., 2009). The extracts of *Syzygium aromaticum* were less toxic to the larvae; however their influence on development was remarkable, causing complete inhibition of adult emergence at 200 and 600 ppm concentrations of the methanol and ether extracts, respectively against *Culex pipiens* (El Hag et al., 1999). Three limonoids, namely limonin, nomilin and obacunone, isolated from the seeds of *Citrus reticulata* showed that the EC₅₀ for inhibition of adult emergence were 6.31, 26.61 and 59.57 ppm for obacunone, nomilin and limonin, respectively on 4th instar larvae of mosquito *C. quinquefasciatus* (Jayaprakashia et al., 1997). Similar result was obtained in the root extract of *Valeriana jatamansi* which exhibited adulticidal activity of 90% lethal concentration against adult *An. stephensi*, *An. culicifacies*, *Ae. aegypti*, *Anopheles albopictus*, and *Cx. quinquefasciatus* and were 0.14, 0.16, 0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm², respectively (Dua et al., 2008). Nathan et al. (2005) considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity, *A. stephensi* and the larval mortality was dose-dependent with the highest dose of 1 ppm azadirachtin evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *A. stephensi*. Choochote et al. (2005) also reported that the in testing for adulticidal activity, the hexane-extracted *Curcuma aromatica* (LC₅₀: 1.60 microg/mg female) was found to be slightly more effective against female *A. aegypti* than volatile oil (LC₅₀: 2.86 microg/mg female). The highest adulticidal effect was established from *Piper sarmentosum*, followed by *Piper ribesoides* and *Piper longum*, with LD₅₀ values of 0.14, 0.15 and 0.26 microg/female, respectively (Choochote et al., 2006). In testing for adulticidal activity, the crude seed extract of celery, *Apium graveolens*, exhibited a slightly adulticidal potency on *A. aegypti* with LD₅₀ and LD₉₅ values of 6.6 and 66.4 mg/cm², respectively, (Choochote et al., 2004). The unsaponifiable portion and volatile oil of *Thymus capitatus* showed the highest adulticidal potency (LC₅₀ = 0.0070 and 0.0076 mg/cm², respectively against *Culex pipiens* (Mansour et al., 2000). This result is also comparable to earlier reports of Dua et al. (2010) who observed that the adulticidal activity of the essential oil of *Lantana camara* was evaluated against different mosquitoes species on 0.208 mg/cm² impregnated papers, and the KDT₅₀ and KDT₉₀ values of the essential oil were 20, 18, 15, 12 and 14 min and 35, 28, 25, 18 and 23 min against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluviatilis* and *An. stephensi* with their percent mortality of 93.3%, 95.2%, 100%, 100% and 100%, respectively. Also the result agrees with the finding of Halim (2008) who have reported the insecticidal activity of *Zingiber officinale* against the adult emergence of *A. pharoensis* 3rd stage was evaluated at the

concentrations of 100%, 70%, 50%, 25%, 5%, 2%, 1%, 0.9%, 0.7%, 0.5% and 0.3% showed 100% larval mortality rate and at 0.2% and 0.1% caused mortality of 66.7%, respectively.

All the toxins used in vector control pose some hazards to the user and also to the aquatic environment (Kreutzweiser, 1997). Hence this research is mainly focused on finding newer insecticides which will be more effective, biodegradable and also easily available at low cost. In our observation, acetone extract of *A. marmelos*, hexane, extracts of *A. lineata*, the methanol of *C. hirsutus*, *E. prostrata*, and *T. erecta* possessed higher EI and adulticidal activity than the other solvent extracts.

In conclusion, the present study clearly proved that the efficacy of plant leaves extracts of *A. lineata* and *T. erecta* can be suggested as an adult emergence inhibition and adulticidal activity against *C. tritaeniorhynchus* activity. The results reported that the efficacy for controlling mosquitoes and mortality properties of natural product extracts. Since it is considered environmentally safe, the isolation and purification of crude extract of leaf hexane of *A. lineata* and methanol of *T. erecta* are in progress.

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References

- Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Eco. Entomol.* 18, 265–266.
- Alkofahi, A., Rupperecht, 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.
- Ansari, M.A., Razdan, R.K., Tandon, M., Vasudevan, P., 2000. Larvicidal and repellent actions of *Dalbergia sissoo* Roxb (F. Leguminosae) oil against mosquitoes. *Bioresour. Technol.* 73, 207–211.
- Berenbaum, M.R., 1988. Allelochemicals in insect–microbe–plant interactions: agents provocateurs in the coevolutionary arms race. In: Barbosa, P., Lotourneau, D.K. (Eds.), *Novel Aspects of Insect–Plant Interactions*. Wiley, New York, pp. 97–123.
- Choochote, W., Tuetun, B., Kanjanapothi, D., Rattanachanpichai, E., Chaitong, U., Chaiwong, P., Jitpakdi, A., Tippawangkosol, P., Riyong, D., Pitasawat, B., 2004. Potential of crude seed extract of celery, *Apium graveolens* L., against the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). *J. Vector Ecol.* 29 (2), 340–346.
- Choochote, W., Chaiyasit, D., Kanjanapothi, D., Rattanachanpichai, E., Jitpakdi, A., Tuetun, B., Pitasawat, B., 2005. Chemical composition and anti-mosquito potential of rhizome extract and volatile oil derived from *Curcuma aromatica* against *Aedes aegypti* (Diptera: Culicidae). *J. Vector Ecol.* 30 (2), 302–309.
- Choochote, W., Chaitong, U., Kamsuk, K., Rattanachanpichai, E., Jitpakdi, A., Tippawangkosol, P., Chaiyasit, D., Champakaew, D., Tuetun, B., Pitasawat, B., 2006. Adulticidal activity against *Stegomyia aegypti* (diptera: culicidae) of three Piper spp. *Rev. Inst. Med. trop. S Paulo* 48 (1), 33–37.
- Dharmagadda, V.S., Naik, S.N., Mittal, P.K., Vasudevan, P., 2005. Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. *Bioresour. Technol.* 96 (11), 1235–1240.
- Dua, V.K., Alam, M.F., Pandey, A.C., Rai, S., Chopra, A.K., Kaul, V.K., 2008. Insecticidal activity of *Valeriana jatamansi* (Verbenaceae) against mosquitoes. *J. Am. Mosq. Control Assoc.* 24, 315–318.
- Dua, V.K., Pandey, A.C., Dash, A.P., 2010. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian J. Med. Res.* 131, 434–439.
- El Hag, E.A., El Nadi, A.H., Zaitoon, A.A., 1999. Toxic and Growth Retarding Effects of Three Plant Extracts on *Culex pipiens* Larvae (Diptera: Culicidae). *Phyther. Res.* 13, 388–392.
- Elango, G., Bagavan, A., Kamaraj, C., Zahir, A.A., Rahuman, A.A., 2009a. Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against *Anopheles subpictus* Grassi (Diptera: Culicidae). *Parasitol. Res.* 1051567, 1576.
- Elango, G., Rahuman, A.A., Bagavan, A., Kamaraj, C., Zahir, A.A., Venkatesan, C., 2009b. Laboratory study on larvicidal activity of indigenous plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Parasitol. Res.* 104 (6), 1381–1388.
- Elango, G., Rahuman, A.A., Kamaraj, C., Zahir, A.A., Bagavan, A., 2010. Studies on effects of indigenous plant extracts on filarial vector *Culex tritaeniorhynchus* Giles. *Parasitol. Res.* 107, 167–176.
- Elimam, A.M., Elmalik, K.H., Ali, F.S., 2009a. Efficacy of leaves extract of *Calotropis procera* Ait (Asclepiadaceae) in controlling *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes. *Saudi J. Biol. Sci.* 16, 95–100.
- Elimam, A.M., Elmalik, K.H., Ali, F.S., 2009b. Larvicidal, adult emergence inhibition and oviposition deterrent effects of foliage extract from *Ricinus communis* L Against *Anopheles arabiensis* and *Culex quinquefasciatus* in Sudan. *Trop. Biomed.* 26 (2), 130–139.
- Gould, E.A., Solomon, T., Mackenzie, J.S., 2008. Does antiviral therapy have a role in the control of Japanese encephalitis? *Antiviral Res.* 78 (1), 140–149.
- Green, M.M., Singer, J.M., Sutherland, D.J., Hibben, C.R., 1991. Larvicidal activity of *Tagetes minuta* (marigold) toward *Aedes aegypti*. *J. Am. Mosq. Control Assoc.* 7 (2), 282–286.
- Gunasekaran, K., Vijayakumar, T., Kalyanasundaram, M., 2009. Larvicidal & emergence inhibitory activities of NeemAzal T/S 1.2 per cent EC against vectors of malaria, filariasis & dengue. *Indian J. Med. Res.* 130 (2), 138–145.
- Halim, A.S.A., 2008. Efficacy of *Zingiber officinale* on third stage larvae and adult fecundity of *Musca domestica* and *Anopheles pharoensis*. *J. Egypt Soci. Parasitol.* 38 (2), 385–392.
- Howard, A.F., Adongo, E.A., Hassanali, A., Omlin, F.X., Wanjoya, A., Zhou, G., Vulule, J., 2009. Laboratory evaluation of the aqueous extract of *Azadirachta indica* (neem) wood chippings on *Anopheles gambiae* s.s. (Diptera: Culicidae) mosquitoes. *J. Med. Entomol.* 46 (1), 107–114.
- Jayaprakasha, G.K., Singh, R.P., Pereira, J., Sakariah, K.K., 1997. Limonoids from *Citrus reticulata* and their moult inhibiting activity in mosquito *Culex quinquefasciatus* larvae. *Phytochemistry* 44 (5), 843–846.
- Jeyabalan, D., Arul, N., Thangamathi, P., 2003. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. *Bioresour. Technol.* 89 (2), 185–189.
- Kamaraj, C., Bagavan, A., Rahuman, A.A., Zahir, A.A., Elango, G., Pandiyan, G., 2009. Larvicidal potential of medicinal plant extracts against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Parasitol. Res.* 104, 1163–1171.
- Kreutzweiser, D.P., 1997. Non-target effects of neem-based insecticides on aquatic Invertebrates. *Ecotoxicol. Environ. Safety* 36, 109–117.
- Liu, S.Q., Shi, J.J., Cao, H., Jia, F.B., Liu, X.Q., Shi, G.L., 2000. Survey of pesticidal component in plant. In *Entomology in*

- China in 21st Century, Proceedings of Conference of Chinese Entomological Society ed. Dianmo, Li Beijing, China: Science & Technique Press, pp. 1098-1104.
- Macêdo, M.E., Consoli, R.A., Grandi, T.S., dos Anjos, A.M., de Oliveira, A.B., Mendes, N.M., Queiróz, R.O., Zani, C.L., 1997. Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). Mem. Inst. Oswaldo. Cruz. 92 (4), 565–570.
- Mansour, S.A., Messeha, S.S., el-Gengaihi, S.E., 2000. Botanical biocides. 4 Mosquitocidal activity of certain *Thymus capitatus* constituents. J. Nat. Toxins 9 (1), 49–62.
- Mordue (Luntz), A.J., Blackwell, A., 1993. Azadirachtin an update. Insect. Physiol. 39, 903–924.
- Murugan, K., Babu, R., Jeyabalan, D., Senthil Kumar, N., Sivaramakrishnan, S., 1996. Antipupational effect of neem oil and neem seed kernel extract against mosquito larvae of *Anopheles stephensi* (Liston). J. Ent. Res. 20, 137–139.
- Muthukrishnan, J., Puspaltha, E., 2001. Effects of plant extracts on fecundity and fertility of mosquitoes. J. Appl. Entomol. 125, 31–35.
- Nathan, S.S., Kalaivani, K., Murugan, K., Chung, P.G., 2005. Effects of neem limonoids on malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Acta. Trop. 96, 47–55.
- Perich, M.J., Wells, C., Bertsch, W., Tredway, K.E., 1995. Isolation of the insecticidal components of *Tagetes minuta* (Compositae) against mosquito larvae and adults. J. Am. Mosq. Control Assoc. 11 (3), 307–310.
- Ravi, V., Vanajakshi, S., Gowda, A., Chandramuki, A., 1989. A laboratory diagnosis of Japanese encephalitis using monoclonal antibodies and correlation of findings with the outcome. J. Med. Virol. 29, 221–223.
- Reuben, R., Gajanana, A., 1997. Japanese encephalitis in India. Indian J. Pediatr. 64, 243–251.
- Sabesan, S., 2003. Forecasting mosquito abundance to prevent Japanese encephalitis. Curr. Sci. 84 (9), 1172–1173.
- Samarasekera, J.K., Khambay, B.P., Hemalal, K.P., 2004. A new insecticidal protolimonoid from *Aegle marmelos*. Nat. Prod. Res. 18 (2), 117–122.
- Sarin, R., 2004. Insecticidal activity of callus culture of *Tagetes erecta*. Fitoterapia 75 (1), 62–64.
- Senthil Nathan, S., Kalaivani, K., Murugan, K., Chung, P.G., 2005. The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenée), the rice leaffolder. Pest. Biochem. Physiol. 81, 113–122.
- Sharma, P., Mohan, L., Srivastava, C.N., 2009. Anti-juvenile activity of *Azadirachta indica* extract on the development and morphometry of filaria vector, *Culex quinquefasciatus* (Diptera: Culicidae) Say. Parasitol. Res. 105 (5), 1193–1203.
- Singh, R.K., Dhiman, R.C., Mittal, P.K., 2007. Studies on mosquito larvicidal properties of *Eucalyptus citriodora* Hook (family-Myrtaceae). J. commun. Dis. 39 (4), 233–236.
- Solomon, T., 2004. Flavivirus encephalitis. N. Engl. J. Med. 351, 370–378.
- WHO, 1981. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: diagnostic test. Geneva: WHO/VBC/81–807.
- WHO, 2005. Communicable disease tool kit, Sudan. World Health Organization, WHO/CDS/2005.26.
- Wiesman, Z., Chapagain, B.P., 2006. Larvicidal activity of saponin containing extracts and fractions of fruit mesocarp of *Balanites aegyptiaca*. Fitoterapia 77 (6), 420–424.
- Zaridah, M.Z., Idid, S.Z., Omar, A.W., Khozirah, S., 2001. In vitro antifilarial effects of three plant species against adult worms of subperiodic *Brugia malayi*. J. Ethnopharmacol. 78 (1), 79–84.