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Research Article

MOSQUITOCIDAL ACTIVITY OF *PLUMBAGO ZEYLANICA* LINN. (PLUMBAGINACEAE) METHANOL EXTRACT AGAINST HUMAN VECTOR MOSQUITOES

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ABSTRACT

Mosquitoes transmit serious human diseases, causing millions of deaths every year and the development of resistance to chemical insecticides resulting in rebounding vectorial capacity. Plants may be alternative sources of mosquito control agents. The present study assessed the larvicidal and oviposition - deterrence activities of methanol extract of *Plumbago zeylanica* against the fourth instar larvae of vector mosquitoes. Larvicidal activities of $LC_{50}$was studied in the range of 20 to 320 ppm in the laboratory bioassays against early 4th instar larvae of selected mosquitoes. The mortality data were subjected to probit analysis to determine the lethal concentrations (LC_{50} and LC_{90}) to kill 50 and 90 per cent of the treated larvae of the respective species. Oviposition attractancy were calculated for oviposition active index and oviposition attractancy concentration which were calculated by the standard procedure. The experiments showed significant activity and larvicidal activity were tested after 24 h of exposure. The LC_{50} andLC_{90} value of *Plumbago zeylanica* methanol extract against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* were 47.24, 46.52, 48.28 and 94.00, 92.28 and 98.05ppm respectively. The oviposition attractancy concentration of (OAC_{50}) *Plumbago zeylanica* methanol extract against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* were 55.508, 33.348 and 60.438 respectively. Our data suggest that the leaf methanol extract of *Plumbago zeylanica* have the potential to be used as an ecofriendly approach for the control of selected vector mosquitoes.

INTRODUCTION

Insect-borne diseases remain to this day a major source of illness and death worldwide (Pavela, 2009). Mosquitoes alone transmit such diseases in more than 700 million people annually worldwide (Aregawi *et al.,* 2008). They are the vectors for the transmission of malaria, dengue fever, chikungunya, yellow fever, lymphatic filariasis, Japanese encephalitis, etc., causing millions of deaths every year (Rahuman *et al.,* 2008). Mosquitoes are one of the most medically significant vectors, and they transmit parasites and pathogens, which continue to have a devastating impact on human beings and other animals (Krishnappa and Elumalai, 2013). Several mosquito species belonging to genera *Anopheles, Aedes* and *Culex* are the vectors for the pathogens of various diseases and contribute significantly to poverty and social debility in tropical countries (Jiang *et al.,* 2009). *An. stephensi* is the primary vector of malaria in India and other West Asian countries (Mittal and Subbarao, 2003). Larvae of the *Anopheles* species are generally found in distinctly different habitat and are nocturnal, crepuscular in nature and also transmit the filarial worm causing filariasis (Dean, 2001). *Ae. aegypti* the yellow fever mosquito spreads dengue fever, chikungunya and yellow fever, viruses and other diseases. It is a vector for transmitting several tropical fevers and only the female bites for blood which she needs to mature her eggs (Hahn *et al.,* 2001). *Cx. quinquefasciatus* is the predominant house-reaching mosquito in many tropical countries. It is an important vector of filariasis and breeds in polluted waters. Lymphatic filariasis is probably the fastest spreading insect-

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borne disease of man in the tropics, affecting about 146 million people (Krishnappa and Elumalai, 2012). One of the approaches for controlling mosquitoes borne diseases is the interruption of disease transmission either by killing, preventing mosquito bite by using repellents or by causing larval mortality in a large scale at the breeding centres of the vector. The control of mosquito larvae worldwide depends on continued application of organophosphates and insect growth regulators (Rahuman *et al.,* 2009). These problems have highlighted the need for new strategies for mosquito larvae control. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and has led to mosquito resistance to these insecticides. Furthermore, Environmental and health influences of synthetic insecticides have stimulated the search for new more efficient and safer insecticides derived from plants (Brogdon and McAllister, 1998).

The search for new strategies with natural products to control destructive insects and vectors of diseases is desirable due to the prevalence of vector resistance to synthetic insecticides, problem of toxicity, non-biodegradable effects, and effects on non-target organisms (Jantan *et al.,* 2005). Extracts from plants may be alternative sources of mosquito control agents since they constitute rich sources of bioactive compounds that are biodegradable into non-toxic products and potentially stable (Amer and Melhorn, 2006). Further, phytochemicals are advantageous due to their ecosafety, target specificity, higher acceptability, and suitability for rural areas. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, while others interfere with growth or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant (Markouk *et al.,* 2001). In this regard, the chemicals derived from plants have been projected as weapons in future mosquito control programs. According to Krishnappa and Elumalai (2015), more than 1,000 plant species in India are found to possess insecticidal properties, 384 contain antifeedants, 297 contain repellents, and 27 contain attractants and possess growth inhibitors. These botanical materials can be used as an alternative to chemical pesticides. This will be very helpful in minimizing the undesirable side effects of synthetic pesticides. The present experiment was, undertaken to study the mosquitocidal of *Plumbago zeylanica* against selected vector mosqitoes.

MATERIALS AND METHODS

Collection and extraction of plant material

Fresh leaves of Indian medicinal plant, *Plumbago zeylanica* Linn. (Plumbaginaceae) was collected from Salem District, Tamilnadu, India. Then washed and shade dried. After shade drying, the leaves were powdered by using electronic blender. 100 grams of the dry powder were extracted in 500 ml of chloroform, ethyl acetate and methanol using soxhlet apparatus and the crude extracts were individually condensed with a rotary evaporator for 30 min at 45^0C and crude extract were stored in refrigerator for further studies. The extracts obtained were dissolved in the corresponding pure solvent until a 10% (w/v) stock solution was obtained.

Rearing of Vector Mosquitoes

The vector mosquitoes such as *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L.) were chosen as the candidate species in the present investigation. Mosquito colony were maintained at insectary (54cm x 45cm x 40cm) at 27 ± 2^0C and 80 ± 2 % Relative humidity with a photoperiod of 12:10 hours light and dark cycles. The egg strips were obtained from Malaria Research Center (MRC), Chennai to start the colony. The strips were immersed in dechlorinated tap water for hatching. To obtain the larvae of equal developmental stage, eggs were introduced by adding a stimulant such as ascorbic acid (100 mg/L) to water. This has tended the eclosion process. The emerged larvae were maintained in Petri dishes (10.5cm diameter) with dechlorinated tap water. Larvae were fed with a diet of yeast and dog biscuits in the ratio of 3: 1. The first instar larvae developed into pupae in about 7 - 10 days through four stages. The pupae were separated by using a glass dropper into glass Petri dishes and were kept in mosquito net cages (40 cm x 45 cm x 40 cm) for emergence. The newly emerged mosquitoes were provided with 5 % glucose solution soaked in cotton wool, which was placed inside the mosquito net cage for nourishment (Verma and Rahman, 1986). After three days of emergence, adults were given a blood meal of pigeon. Glass Petri dishes of 50 ml of tap water lined with filter paper was kept inside the cage for oviposition. The eggs thus obtained were immersed in larval trays containing dechlorinated tap water for hatching.

Larvicidal activity

Larvicidal activity of the extract was determined by following the standard procedure WHO (2005). Initially, mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the aqueous extract of plant under test. After determining the mortality of larvae in this wide range of concentrations, a narrow range of 20, 40, 80, 160 and 320ppm concentrations were used to determine the lethal concentration of 50% (LC_{50}) and the lethal concentration of 90% (LC_{90}) values. DMSO (emulsifier) in water served as a control. The larvae of these mosquito species (25 nos.) were introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1ml of emulsifier) and the required amount of plant extract was added. Five replicates were setup for each test concentration. In each replicate 25 larvae were used, with five replicate of control. The experiment was performed under laboratory conditions at 25-28 ± 2^oC. If the control mortality is between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott (1925) formula. The LC_{50}, LC_{90}, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), chi-square values and the degrees of freedom were calculated by using Probit analysis with Statistical Package for Social Sciences (SPSS) 17.0 Version in MS-Excel, 2007.

Oviposition - deterrence assay

The oviposition deterrence effect and the number of eggs deposited in the presence of different solvent extracts of experimental plants, a multiple concentration test was carried out. For bioassay test, 20 males and 20 females were separated in the pupal stage and were introduced into screen cages

(45×45×40 cm) in a room at 27±2ºC and 75-85% relative humidity with a photoperiod of 14:10 h light and dark cycles. The sex of individual pupae can be determined by looking at the ninth segment of the abdomen and by the size of the pupae. The ninth segment on male mosquitoes is more prominent during the pupal stage, while the female pupa is usually larger in size than the male (male, 1.28-1.60mm and female, 2.11-2.43mm). The pupae were allowed to emerge into adults in the test cages. Adults were provided continuously with 10% sucrose solution in a plastic cup with a cotton wick. They were blood fed (from pigeon) on day five after emergence. In the multiple concentration test, five cups, each containing 100 ml distilled water with a 9- cm piece of white filter paper for oviposition as well as solvent extracts at a concentration of 20, 40, 80, 160 and 320 ppm were placed in each cage. A sixth cup without extract served as a control. The control was set up with acetone, water and polysorbate 80. The positions of the plastic cups were alternated between the different replicates so as to nullify any effect of position on oviposition. Five replicates for each concentration were run with cages placed side by side for each bioassay. After 24 h, the number of eggs laid in treated and control cups were counted under a stereomicroscope. The percent effective repellency for each concentration was calculated using the following formula.

ER% = x 100 ER% = [(*Nc* - *Nt*)/*Nc*] x 100

Where ER=effective repellency, NC=number of eggs in control, and NT=number of eggs in treatment (Rajkumar and Jebanesan, 2009). The oviposition experiments were expressed as mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula.

OAI = NT - NS/ NT + NS

Where NT = total number of eggs in the test solution and NS = total number of eggs in the control solution.

Oviposition active index of +0.3 and above are considered as attractants, while those with - 0.3 and below are considered as repellents (Kramer and Mulla, 1979). Positive values indicate that more eggs were deposited in the test cups than in the control cups and that the test solutions were attractive. Conversely, negative values indicate that more eggs were deposited in the control cups than in the test cups and that the test solutions were a deterrent.

RESULTS

Larvicidal activity of the P. zeylanica methanol extract against A. aegypti

The efficacy of different concentrations of the extract *viz*. 20, 40, 80,160 and 320 ppm on the larvicidal activity against *A. aegypti* is furnished in Table 1. Results clearly indicates that the highest of 93.22 % larval mortality was observed at 320 ppm concentration of plant extract, whereas, the lowest mortality of 22.22 % was recorded at the 20 ppm concentration. The larval mortality of 44.48, 64.46 and 83.78 % were observed at 40, 80 and 160 ppm concentrations respectively. The total mortality of 1.34 % was observed in methanol served as a control. As the concentration of the plant extract increases the total mortality of *A. aegypti* was also found to be increased. The LC_{50} and LC_{90} values of the *P. zeylanica* methanol extract were 47.24 and 94.00 ppm respectively. The 95 % confidence limit of LCL and UCL were 40.21 and 51.33 ppm respectively. The Chi-square value was 1.024 which indicates significant larvicidal activity at 0.05 % level. The dead larvae were collected from the experimental cups for microscopic studies.

Table 1 Larvicidal activity of the *Plumbago zeylanica* methanol plant extract against *Aedes aegypti*

Concentrations (ppm)	Mortality	LC_{50} (ppm)	95 % Fiducial Limit		LC_{90} (ppm)	95 % Fiducial Limit		X^2
			LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)	
20	22.22 ± 0.02^{b}							
40	48.48 ± 1.18^{c}							
80	64.46 ± 1.34^{d}	47.24	40.21	51.33	94.00	86.24	103.50	1.024
160	83.78 ± 1.82^{e}							
320	93.22 ± 1.24^{f}							
Control	1.34 ± 0.68^{a}							

Each value represents Mean ± Standard deviation of six replicates. Mean followed by different superscripts denotes significant at 5 % level (DMRT Test).

Investigations on the cuticular sculpturing and ornamentation of the treated and control larvae depicted similarity in the structure of head capsule including antennae, compound eyes, clypeal, frontal, sutural, transutural, lateral and ventral hairs; simple and branched hairs of prothorax, mesothorax and metathorax; simple and branched hairs of nine abdominal segments: anal papillae; saddle and ventral brush. The respiratory siphon of control larvae showed normal intact cuticle but a shrunken cuticle has been found in treated larvae.

Larvicidal activity of the P. zeylanica methanol extract against A. stephensi

The consequences of different concentrations of the *plant* extract *viz*. 20, 40, 80, 160 and 320 ppm on the larvicidal activity against *A. stephensi* were depicted in table 2. The results revealed that the highest larval mortality of 93.46 % was observed at 320 ppm concentration, whereas, the lowest mortality of 21.00 % was noted at 20 ppm concentration. The mortality of 48.52, 66.36 and 82.28 % were observed at 40, 80 and 160 ppm concentration respectively. In the control, the total mortality of 1.44 % was observed. The results clearly indicated that the larvicidal activity of plant extract was directly related with the concentration of the extract. The 24 h LC_{50} and LC_{90} values of the plant extract were 46.52 and 92.28 ppm respectively. The 95 % lower confidence limits (LCL) and upper confidence limit (UCL) were 40.64 and 52.48 ppm respectively. The Chi-square value was 1.127 and it indicated that the larvicidal activity was significant at 0.05 % level (Table 2).

Table 2 Larvicidal activity of the *Plumbago zeylanica* methanol plant extract against *Anopheles stephensi*

Concentrations (ppm)	Mortality	LC_{50} (ppm)	95 % Fiducial Limit		LC_{90} (ppm)	95 % Fiducial Limit		X^2
			LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)	
20	21.00 ± 0.06^{b}							
40	48.52 ± 1.62^{c}							
80	66.36 ± 1.22^{d}	46.52	40.64	52.48	92.28	82.38	108.22	1.127
160	82.28 ± 1.64^{e}							
320	93.46 ± 1.22^{f}							
Control	1.44 ± 0.80^{a}							

Each value represents Mean ± Standard deviation of six replicates. Mean followed by different superscripts denotes significant at 5 % level (DMRT Test).

The microscopic observations showed normal intact in both treated and control larvae but the respiratory siphon of treated

larvae was partially affected and showed shrunken cuticle. The respiratory siphon was cone shaped in control larvae whereas in treated larva the siphon was partially disappeared. Examination of alimentary system and brainstem of both treated and control larvae showed similar arrangement.

Larvicidal activity of the P. zeylanica methanol extract against C. quinquefasciatus

The larvicidal effect and different plant extract concentration of 20, 40, 80 160 and 320 ppm on the larvicidal activity against *C. quinquefasciatus* are presented in table 3.

Table 3 Larvicidal activity of the *Plumbago zeylanica* methanol plant extract against *Culex quinquefasciatus*

Concentrations (ppm)	Mortality	LC_{50} (ppm)	95 % Fiducial Limit		LC_{90} (ppm)	95 % Fiducial Limit		X^2
			LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)	
20	18.82 ± 1.24^{b}							
40	49.38 ± 1.46^{c}							
80	63.60 ± 1.24^{d}							
160	79.68 ± 1.82^{e}	48.28	40.36	53.74	98.05	84.48	108.64	2.211
320	92.22 ± 1.62^{f}							
Control	1.26 ± 0.80^{a}							

Each value represents Mean ± Standard deviation of six replicates. Mean followed by different superscripts denotes significant at 5 % level (DMRT Test).

The percentage age of larval mortality was found to be increased of 92.22 % at 320 ppm concentration of the plant extract and least larval mortality 18.82% were recorded at 20 ppm concentration. The mortality of 49.38, 63.60 and 79.68 % were observed at 40, 80 and 160 ppm concentration respectively. The total mortality of 1.26 % was recorded in methanol, which served as a control. The increase in the concentration of the plant extract was found to increase the total mortality of *C. quinquefasciatus*. The LC_{50} and LC_{90} values of plant extract 48.28 and 98.05 ppm respectively. The 95 % of LCL and UCL were 40.36 and 53.74 ppm respectively. The Chi-square value was 2.211, which indicated that the larvicidal activity was significant at 0.05 % level. The dead larvae were removed from the plant extract treated cups for microscopic studies. Investigations on the cuticular sculpturing and ornamentation of the treated and control larvae revealed the similarity in the structure of head capsule including antennae, compound eyes, clypeal, frontal, sutural, transutural, lateral and ventral hairs; simple and branched hairs of prothorax, mesothorax and metathorax; simple and branched hairs of nine abdominal segments; anal papillae; saddle and ventral brush. The respiratory siphon of control larvae showed normal intact cuticle but in treated larvae shrunken cuticle has been found.

Oviposition activity of the P. zeylanica methanol extract against A. aegypti

The results of dose - effect relationship studies of the oviposition activity in the laboratory are given in the table 4. The treated with different concentration of the plant extracts such as 20, 40, 80, 160 and 320 ppm concentration. No egg strips were obtained 320 ppm and 160 ppm concentration, whereas, control acquired 9.30 % and 16.76 respectively. 80 ppm concentration resulted with the minimum number of 2.44 % egg strips with 12.00 % in control. 5.82 % egg strips were noticed at 40 ppm concentration whereas, the control showed oviposition of 10.28 %.

Table 4 Effect of the *Plumbago zeylanica* methanol plant extract on oviposition attractancy of *Aedes aegypti*

Concentration (ppm)	Number of Egg Strips		Effective Attractancy (%)	OAC_{50}	OAI
	Treated	Control			
320	0.0^{a}	9.30 ± 0.52^{ab}	100.00		1.0540
160	0.0^{a}	16.76 ± 1.46^{d}	100.00		0.5216
80	2.44 ± 0.24^{b}	12.00 ± 0.58^{c}	75.80	55.508	0.2225
40	5.82 ± 0.72^{c}	10.28 ± 0.56^{b}	43.28		0.0283
20	6.86 ± 0.80^{d}	8.00 ± 0.42^{a}	8.56		0.0

Each value represents Mean ± Standard deviation of six replicates. Mean followed by different superscripts denotes significant at 5 % level (DMRT Test). OAI = Oviposition Active Index. OAC_{50}= 50 % Oviposition Attractancy Concentration.

Minimum of 6.86% egg strips was observed at 20 ppm concentration whereas the control received 8.00 % egg strips. 100.00% was observed at 320 and 160 ppm concentration the lowest effective attractancy and the highest effective attractancy of 8.56% was noticed at20 ppm concentration. The effective repellency of 75.80 and 43.28 % was observed at 80 and 40 ppm concentration respectively. The 50 % oviposition repellency concentration was noted at 55.508 ppm. The lowest oviposition active index of 0.0 was noted at 20ppm concentration. The oviposition active index was observed to be decreased at the decreasing concentration of the plant extract. The results disclosed that the oviposition repellency of the plant extract significantly enhanced by decreasing the concentration.

Oviposition Activity of the P. zeylanica methanol extract against A. stephensi

Methanol extract of *P. zeylanica* was tested for its oviposition repellent activity with different concentrations *viz*. 20, 40, 80, 160 and 320 ppm. Gravid mosquitoes exposed to 320 ppm and 160 ppm concentration were deterred by the extract significant to a greater extent whereas control received 12.28and 19.46 % respective concentrations. The effective repellency of 84.30 and 72.21 % was observed at 80 and 40 ppm concentration respectively. The 50% oviposition repellency concentration was noted at 33.348 ppm. The lowest oviposition active index of 0.1264 was noted at 20 ppm concentration, descending order of the oviposition active index was observed as the concentration of the plant extract decreases (Table 5).

Table 5 Effect of the *Plumbago zeylanica* methanol plant extract on oviposition attractancy of *Anopheles stephensi*

Concentration (ppm)	Number of Egg Strips		Effective Attractancy (%)	OAC_{50}	OAI
	Treated	Control			
320	0.0^{a}	12.28 ± 0.48^{bc}	100.00		1.00
160	0.0^{a}	19.46 ± 1.62^{d}	100.00		1.00
80	2.25 ± 0.80^{b}	14.28 ± 0.43^{c}	84.30	33.348	0.7211
40	2.80 ± 0.25^{c}	12.33 ± 0.64^{b}	72.21		0.5870
20	6.82 ± 0.86^{d}	6.82 ± 0.82^{a}	23.84		0.1264

Each value represents Mean ± Standard deviation of six replicates. Mean followed by different superscripts denotes significant at 5 % level (DMRT Test). OAI = Oviposition Active Index. OAC_{50}= 50 % Oviposition Attractancy Concentration.

Oviposition Activity of the P. zeylanica methanol extract against C. quinquefasciatus

The dose - effect relationship studies for the oviposition deterrent activity against the gravid mosquitoes of *C.*

quinquefasciatus are shown in the Table 6. They were treated with different concentrations of the plant extracts (20, 40, 80, 160, and 320 ppm). No egg rafts were found at 320 and 160 ppm concentration whereas control received 12.46 and 15.58 % respectively. The maximum effective repellency of 100.0 % was observed at 320 ppm and 160 ppm concentration and the minimum repellency of 19.24 % was observed at 20 ppm concentration. The 50% oviposition active concentration was noted at 60.438 ppm. The lowest oviposition active index of 0.1040 was noted as 20 ppm concentration.

Table 6 Effect of the *Plumbago zeylanica* methanol plant extract on oviposition attractancy of *Culex quinquefasciatus*

Concentration (ppm)	Number of Egg Strips		Effective Attractancy (%)	OAC_{50}	OAI
	Treated	Control			
320	0.0^{a}	12.46 ± 0.64^{c}	100.00		1.00
160	0.0^{a}	15.58 ± 1.77^{d}	100.00		1.00
80	3.32 ± 0.54^{b}	12.36 ± 0.60^{bc}	72.56	60.438	0.5290
40	5.22 ± 0.60^{c}	8.24 ± 0.68^{a}	24.80		0.1468
20	7.28 ± 0.62^{d}	9.94 ± 0.82^{b}	19.24		0.1040

Each value represents Mean ± Standard deviation of six replicates. Mean followed by different superscripts denotes significant at 5 % level (DMRT Test). OAI= Oviposition Active Index. OAC_{50} = 50 % Oviposition Attractancy Concentration.

DISCUSSION

The widespread use of synthetic organic insecticides during the last five decades has resulted in environmental hazards and development of resistance in the majority of vector species. This has necessitated the search and development of environmentally safe, biodegradable, low-cost, and indigenous methods for vector control which can be used with minimum care by individuals and communities in specific situations (Mittal and Subbarao, 2003). Mosquito-borne diseases are one of the most serious public health problems in developing countries. It could be controlled by preventing mosquito bites using repellents, larvicides, and eliminating the mosquitoes. In this regard, a number of plant extracts have been reported to have mosquitocidal or repellent activities, but very few plant products have shown practical utility for mosquito control (Sukumar *et al.,* 1991; Kalyanasundaram and Das, 1985; Kalyanasundaram and Babu, 1982). Our results showed that the crude methanol extract of *Plumbago zeylanica* have significant larvicidal and oviposition-deterrent properties against the three important vector mosquito species tested. Similarly, Prathibha *et al.,* (2011) have reported the larvicidal efficacy of *E. ridleyi* against *C. quinquefasciatus*. Our results are also comparable to that of Vahitha *et al.,* (2002) who have tested *Pavonia zeylanica* and *Acacia ferruginea* for their larval efficacy against *C. quinquefasciatus*. Recent studies on the larval and pupal mortality of *Anopheles stephensi* after the treatment of methanol extract of *Clerodendron inerme* leaf extract showed 22% mortality at I instar larvae as a result of treatment at 20 ppm; in contrast, it was increased to 81% at 100 ppm of *C. inerme* leaf extract of larval and pupal mortality of *A. stephensi* (I-IV instars) after the treatment of methanol extract of *Acanthus ilicifolius* at different concentrations (20-100 ppm). A 23% mortality was noted at I instar larvae by the treatment of *A. ilicifolius* at 20 ppm, whereas it was increased to 89% at 100 ppm of *A. ilicifolius* leaf extract treatment (Kovendan and Murugan, 2011).

The isolated compound saponin from ethyl acetate extract of *Achyranthes aspera* was effective against the larvae of *A. aegypti* and *Culex quinquefasciatus* with LC_{50} value of 18.20 and 27.24 ppm, respectively (Bagavan *et al.,* 2008). The neem formulation, Neem Azal, produced an overall mortality or inhibition of emergence of 90% (EI_{90}, when third-instar larvae were treated) at 0.046, 0.208, and 0.866 ppm in *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti*, respectively (Gunasekaran *et al.,* 2009). Fraction A1 of ethanol from *Sterculia guttata* seed extract was found to be most promising; its LC_{50} was 21.552 and 35.520 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively (Katade *et al.,* 2006a,b). With *A. barbadensis* the larvicidal activity increases with increase in the exposure period from 24 to 48 h with decrease in LC_{50} values from 15.31 to 11.01 ppm (carbon tetrachloride extract), 25.97 to 16.60 ppm (petroleum ether extract) and 144.44 to 108.38 ppm (methanol extract). Similar trend was also observed in case of *Cannabis sativa* with LC_{50} values 88.51 to 68.69 ppm (carbon tetrachloride extract), 294.42 to 73.32 ppm (petroleum ether extract) and 160.78 to 71.71 ppm (methanol extract) on increase in the exposure period. Further, Barnard and Rui De (2004) observed the repellent activity of *A. vera* against *A. albopictus* and *Culex nigripalpus*. The leaf extract of *Acalypha alnifolia* with different solvents - hexane, chloroform, ethyl acetate, acetone, and methanol - were tested for larvicidal activity against three important mosquitoes such as malarial vector, *A. stephensi*, dengue vector, *A. aegypti* and *Bancroftian filariasis* vector, *C. quinquefasciatus* and highest larval and pupal mortality were found in the leaf extract of methanol *Carica papaya* against the first to fourth instar larvae and pupae of values LC_{50} = 51.76, 61.87, 74.07, 82.18 and 440.65 ppm, respectively (Kovendan *et al.,* 2012a,b). However, in our case, early detection of resistance against *B. sphaericus* could be better for the management of resistance development. Experts of resistance management have been involved in resistance detection with generations of mosquitoes. Moreover, the detection of resistance in an early stage could be a better approach to control mosquitoes. Laboratory- and field-collected *C. quinquefasciatus* exposed to *B. sphaericus* strain 2362 for 35 generations in the laboratory showed a level of resistance 43- and 12-fold than that of potential generation, respectively (Rodeharoen and Mulla, 1991). *B. sphaericus*, a spore-forming, entamopathogenic bacterium, has been shown to possess potent larvicidal activity against several species of mosquito larvae (Davidson, 1983; Yousten and Wallis, 1987). *B. sphaericus* showed a good control over *A. stephensi* which may be due to the presence of Bin and mosquitocidal toxins (Mtxs). As a consequence of the specific toxicity to mosquito larvae of Bin and Mtxs produced during the sporulation and vegetative stages, respectively, some toxic strains have been widely used for many years as bio-pesticides in the field of mosquito control programs (Bei *et al.,* 2006).

Furthermore, the same hexane extract of *L. acidissima* showed 100% oviposition deterrent activity at all the tested concentrations (62.5-500 ppm) against *Cx. quinquefasciatus* and *Ae. aegypti* adult females. Previously, some investigators reported the oviposition deterrent effect of plant extracts against vector mosquitoes. Coria *et al.,* (2008) reported 100% oviposition deterrent effect obtained with *Melia azedarach* L. leaf extract at 1 g/L concentration

against *Ae. aegypti*. Autran *et al*., (2009) recorded the oviposition deterrent effect of essential oil obtained from leaves, inflorescence, and stem of *Piper marginatum*. Their results showed that essential oil of leaves and stems of *P. marginatum* exhibited oviposition deterrent effect on *Ae. aegypti* females at 50 ppm and 100 ppm concentration and that the number of eggs laid was significantly lower (<50%) compared to control. Similarly, Prajapati *et al.,* (2005) reported that the bark oil of *Cinnamomum zeylanicum* reduced the oviposition of *Ae. aegypti* to 50% at 33.5 ppm concentration. Rajkumar and Jebanesan, (2009) have reported the oviposition deterrence effects of the extracts of *Cassia obtusifolia* with repellency at higher concentration (400 mg/L). Mehra and Hiradhar, (2002) revealed that the crude acetone extract of *Cuscuta hyalina* was an effective oviposition deterrent against *C. quinquefasciatus* at a concentration of 80 ppm. This result clearly reveals that the *Plumbago zeylanica* leaf methanol extract have bio-control agent for vector mosquitoes, which could be served as a potential larvicidal agents against importance vector mosquitoes. Therefore, the present strategy should be promoted in vector control programme. The mode of action and larvicidal and oviposition efficiency of the *Plumbago zeylanica* extract under the field conditions should be scrutinized and determined. Besides, further investigation regarding the effect on non-target organism is extremely important and imperative in the near future.

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