Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading doi: 10.1016/S2222-1808(14)60676-9

Bioefficacy of *Anamirta cocculus* Linn. (Menispermaceae) seed extracts against dengue vector, *Aedes aegypti* Linn. (Diptera: Culicidae)

Umer Qadir*

Entomology and Microbiology Research Unit, Department of Zoology, Annamalai University, Annamalainagar-608002, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 21 Apr 2014
Received in revised form 7 May 2014
Accepted 25 Jul 2014
Available online 18 Aug 2014

Keywords:
Anamirta cocculus
Aedes aegypti
Larvicidal
Adulticidal
Mosquitocidal
Insecticidal
Dengue
Biocontrol

ABSTRACT

Objective: To evaluate the larvicidal and adulticidal activity of various extracts of *Anamirta cocculus* (*A. cocculus*) (Linn.) seeds against dengue vector *Aedes aegypti* (*Ae. aegypti*).

Methods: The endosperms were removed from the seeds of *A. cocculus* and were extracted with petroleum ether, benzene, chloroform, acetone and aqueous solvents by using Soxhlet apparatus for organic solvents and cold extraction procedure for aqueous solvent. Extracts were evaluated further for the determination of 24 h LC_{50}, LC_{90} and LC_{99} values by probit analysis using SPSS 16 package.

Results: All the tested seed extracts of *A. cocculus* showed larvicidal and adulticidal effects after 24 h of exposure against *Ae. aegypti*. Petroleum ether extract was the most potential extract with LC_{50}, LC_{90}, LC_{99} values of 56.731, 87.908, 127.474 and 140.161, 178.285, 214.713 mg/L, respectively against larvae and adults of *Ae. aegypti*. The second effective mosquitocidal extract was aqueous extract with LC_{50} values of 57.726 mg/L and 141.930 mg/L against larvae and adult mosquitoes respectively followed by benzene, chloroform and acetone extracts.

Conclusions: Present study reveals that *A. cocculus* has considerable potentiality for dengue vector control. This potency could be exploited for the development of safer and effective botanical mosquitocidal/insecticidal tool for the management of *Ae. aegypti*.

1. Introduction

Mosquitoes are serious threat to public health through which several dreadful diseases are transmitted to both animals and human beings[1]. They are the primary vectors for many acute, chronic and fatal diseases such as dengue, malaria, Japanese encephalitis, yellow fever and filariasis which are transmitted by three genera of mosquitoes, namely, *Anopheles*, *Culex* and *Aedes*. Mosquitoes are known to transmit diseases to more than 700 million people every year. Insect-transmitted diseases are the major sources of infections, discomforts and deaths worldwide. There are over 3000 mosquito species belonging to 34 genera in the World. Of these, only about 300 species are dreadful and can transmit human and animal diseases. These diseases devastate Indian and World economy every year[2]. Mosquitoes are also becoming increasingly resistant to traditional chemical pesticides and there is growing concern about the potential health and environmental risks surrounding these products. Environmental protection agencies have banned or placed severe restrictions on the use of many pesticides which were formerly used in mosquito control programmes and there are now fewer mosquitocides available than there have been for the last 20 years[3]. *Aedes aegypti* (*Ae. aegypti*) alone transmits dengue hemorrhagic fever and chikungunya disease to human beings[4]. Dengue infestation has spread throughout the world in recent decades and is prominent especially in underdeveloped countries. World Health Organization has estimated 50 million dengue infections worldwide each year and about 2.5 billion people (two fifths of the world's population) are at risk of dengue[5]. *Ae. aegypti*, a vector of dengue that carries the arbovirus responsible for this disease, is widely distributed in the tropical and subtropical zones. The symptoms of dengue are severe pain in the joints and muscles, skin eruptions and fever. However, dengue fever is rarely fatal. In recent years dengue fever continues to afflict millions of people and cause thousands

*Corresponding author: Umer Qadir, Entomology and Microbiology Research Unit, Department of Zoology, Annamalai University, Annamalainagar-608002, Tamil Nadu, India.
Tel: +91 7418808600
E-mail: umerqadir85@gmail.com

of deaths annually. The vector of dengue infection (female *Ae. aegypti*) breed, profusely in rainwater storage containers like cisterns, barrels, pots and other contaminant water bodies. Dengue outbreaks are often associated with urban areas due to irregular potable water supply. The female *Ae. aegypti* preferably lays eggs in artificial collections of water. The hatched larvae undergo growth and metamorphosis and attain adult stage. Identification of *Ae. aegypti* larvae, pupae and adults by their morphologic features immediately after collection is of considerable value in recognizing vector prevalence[6]. The only way to prevent dengue virus transmission is to combat the disease-carrying mosquito which is done by chemical and botanical insecticides. Due to environmental concern of use of synthetic insecticides for vector control and exist widespread insecticide resistance, lack of alternative, cost-effective and safe insecticides, interest on possible use of environment friendly natural products such as extracts of plant or plant parts has tremendously increased for vector control[7]. Therefore the use of botanicals/phytochemicals is a kind of strategy and alternative to synthetic insecticides or along with other insecticides under integrated vector control programs that may be suitable for mosquito control. Phytochemicals are advantageous due to their eco-safety, target-specificity, no drug resistant, reduced number of applications, higher acceptability and suitability for rural areas. Thus, an attempt to develop novel materials as mosquitocide is the need of hour. Biologically active plant materials have attracted considerable interests in mosquito control programs in the recent times. Many studies on plant extracts and their active constituent compounds against mosquitoes have been conducted around the world[8]. There are 346 species of plants which has been tested against mosquitoes for testing mosquitocidal activities such as growth inhibition, ovipositional detterrency and repellency of adults mosquitoes.

Anamirta cocculus (*A. cocculus*) (Linn.) is a wild woody climber belonging to the family Menispermaceae. It is widely distributed throughout India as well as South-East Asia. The seeds of *A. cocculus* are commonly known as Indian fish berry or cocculus indicus (pharmacology) and are being exploited by humans for several purposes including hunting, fishing, traditional remedy to cure many disorders and as an insecticidal agent[9,10]. The seeds are also utilized in eradicating the unwanted wild fishes from aquaculture ponds[11,12]. Recently its fruits have been reported to possess antiepileptic, wound healing and anti-inflammatory activities[13,14]. Picrotoxin (cocculin) is the major reported pharmacological component of the seed and is composed of poisonous picrotoxinin and the bitter non-poisonous picrotin[10,15,16]. The wide range of active phytochemicals has been detected in *A. cocculus* which may render insecticidal and mosquitocidal activity to this plant[12]. As a first report of mosquitocidal activity of the selected plant, efforts have been made in the present study to demonstrate the adulticidal and larvicidal activity of *A. cocculus* (Linn.) seed extracts *viz.*, petroleum ether, benzene, chloroform, acetone and aqueous extracts against dengue vector mosquito *Ae. aegypti* in the laboratory.

2. Materials and methods

2.1. Plant material

Fruits of *A. cocculus* were collected from the forests of Vadattupara (10°17′ N, 76°69′ E), Kerala State, India. They were brought to the laboratory of Department of Zoology, Annamalai University in air tight polythene bags where they were air dried completely. The endosperms were then collected by breaking the shells of the seeds.

2.2. Preparation of plant extracts

The air dried seeds of *A. cocculus* were broken with a hammer and the endosperms were separated from the shells of the seeds by a sterilized clean needle. The endosperms were ascetically powdered with the help of an electric grinder. Each of the powdered endosperms (300 g) were packed in the thimble of the Soxhlet apparatus and were extracted separately by using 1 000 mL of each polar and non-polar solvents *viz.*, petroleum ether, benzene, chloroform and acetone. In order to prepare the aqueous extract of the seeds of *A. cocculus*, the powdered endosperms (100 g) were kept in 100 mL triple distilled water (1:1) overnight at room temperature with continuous shaking. The liquid portion was then filtered and used for further processes. Cold extraction with aqueous solvent was carried out to protect the degradation of ingredients from excess temperature required for Soxhlet extraction with triple distilled water (high boiling point of 100 °C). Each extracts of organic and aqueous solvents were then concentrated using rotary vacuum evaporator and the concentrated extracts were preserved in Borosil vials for further assays.

2.3. Rearing of Ae. aegypti

Ae. aegypti were reared in the insect rearing Laboratory of Department of Zoology, Annamalai University. Immature mosquitoes (early third instar larvae) were obtained from the laboratory colonized mosquitoes of F1 generation. Larvae were fed once a day initially and twice during the later stages of development with a diet of finely ground Brewer's yeast and dog biscuits (3:1)[17]. Adult mosquitoes were treated with 10% sucrose solution and one-week-old chick for blood meal. Mosquitoes were held at (27±2) °C, 75%-85% relative humidity, with a photo period of 13-hour light and 11-hour dark.

2.4. Bioassay for larvicidal activity

For evaluation of larvicidal bioassay of *A. cocculus* against *Ae. aegypti*, the standard protocol of World Health Organization (1981) was used in the present study with minor modifications[18]. In this study the third instar larvae of *Ae. aegypti* were exposed to appropriate test concentrations (15, 30, 45, 60, 75 and 90 mg/L) of petroleum ether, benzene, chloroform, acetone and aqueous extracts of seeds of *A. cocculus* in 100 mL of tap water taken in a series of glass beakers (capacity of 250 mL). Small, unhealthy or damaged larvae were removed. Each experiment was performed in three replicates with a final total of 75 larvae for each concentration. Each batch of replicates contained one plain control (without extract). The number of dead larvae at the end of 24 h was recorded in the data record form. During the treatment with extracts no food was offered to larvae. Moribund larvae were counted and added to dead larvae for calculating mortality percentage. Initially the mosquito larvae were exposed to a wide range of test concentrations. After determining the mortality of larvae in this wide range of concentrations a narrow range of 6 concentrations yielding significant mortality in 24 h were used to determine lethal concentration that killed 50%, 90% and 99% larval population (LC_{50}, LC_{90} and LC_{99}).

2.5. Bioassay for adulticidal activity

Ae. aegypti adulticidal bioassay was also performed according to World Health Organization protocol with minor modifications[18]. Appropriate concentrations (90, 110, 130, 150, 170 and 190 mg/L) of the petroleum ether, benzene, chloroform, acetone and aqueous extracts were prepared by dissolving the solid extract in 2.5 mL of acetone and soaked on Whatman No. 1 filter papers (size 11 cm×14 cm) separately, and then were dried according to the method of Dua *et al*[19]. Control filter papers were treated with the respective solvents (without the extract). A group of adult *Ae. aegypti* (25/group) of 4 to 5 days old under blood starved and being fed with glucose were cautiously transferred into plastic holding tubes separately and kept 1 h for acclimatization inside the tube environment. Then the adult mosquitoes were exposed to the treated filter papers for 1 h. They were transferred back to the holding tube and kept there for 24 h for recovery. Mosquitoes were provided with 10% glucose solution soaked in cotton ball as food. Control experiment was performed similarly. The test was repeated four times. Adulticidal efficacy of the seed extracts of *A. cocculus* was expressed in terms of mortality rate of the *Ae. aegypti* mosquitoes after 24 h of recovery period.

2.6. Statistical analysis

The percentage of corrected mortality was calculated following Rawani *et al*[20]. From the mortality data each value of LC_{50}, LC_{90} and LC_{99} was estimated according to Finney's method by probit analysis along with the slope values by using SPSS (16 version) computer software[21]. *Chi*-square test was used to check the heterogeneity of the data for the LC_{50} values in the respective larvicidal and adulticidal modes of experiments. Results with $P<0.05$ were considered as statistical significance and the 95% fiducial level of upper and lower confidence limits for each of the median lethal concentrations was also calculated.

3. Results

From the present observation, the percentage mortality of *Ae. aegypti* by petroleum ether, benzene, chloroform, acetone and aqueous extracts of *A. cocculus* seeds were on an increasing order with the increase in concentration (Figures 1 and 2). Petroleum ether extract showed highest percentage mortality of (97.33±1.96) at 90 mg/L concentration against *Ae. aegypti* larvae while aqueous extract showed the second highest mortality percentage followed by benzene extract, chloroform extract and acetone extract. Petroleum ether extract showed 100% mortality of adult mosquitoes at 190 mg/L, demonstrating that petroleum ether extract is the most potential extract to kill disease spreading vectors like *Ae. aegypti*.

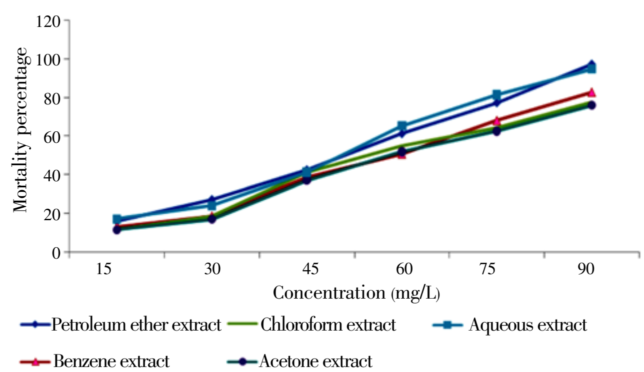

Figure 1. Dose response relationship for various seed extracts of *A. cocculus* applied for 24 h on the third instar larvae of *Ae. aegypti*.

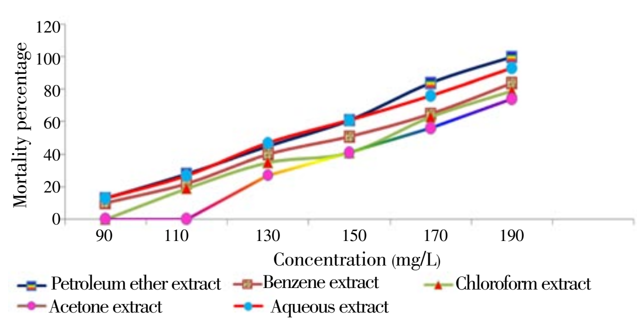

Figure 2. Dose response relationship for various seed extracts of *A. cocculus* applied for 24 h on adult mosquito of *Ae. aegypti*.

The larvae were more sensitive towards the extracts than the adults. Results of 24 h larvicidal bioassay are presented in Tables 1, 2 and 3. It shows that in the case of larvae of *Ae. aegypti*, petroleum ether extract was more active than other tested extracts. LC_{50} value of petroleum ether extract (56.731 mg/L) against larvae of *Ae. aegeypti* was also lower than other extracts. Similarly the LC_{90} and LC_{99} values of petroleum ether extract (87.908 and 127.474 mg/L) against larvae of *Ae. aegypti* were lower than the other extracts.

Table 1

At the end of 24 h, LC_{50} values of *A. cocculus* different extracts against *Ae. aegypti* at larval and adult modes with their 95% confidence limits, calculated χ^2 values and slopes for each. mg/L.

Treatment mode	Solvent extract	LC_{50}	Lower limit	Upper limit	χ^2	Slope
Larval treatment	Petroleum ether	56.731	48.204	62.207	5.825*	1.416
	Benzene	61.168	54.197	68.753	1.051*	1.628
	Chloroform	61.290	52.214	69.050	1.039*	1.832
	Acetone	64.165	55.591	71.892	0.612*	1.775
	Aqueous	57.726	48.509	60.892	0.964*	1.425
Adult treatment	Petroleum ether	140.161	28.522	158.748	10.646*	1.194
	Benzene	148.303	132.476	158.445	2.499*	1.356
	Chloroform	152.251	89.800	181.923	10.148*	1.239
	Acetone	160.992	124.411	192.850	13.036*	1.256
	Aqueous	141.930	127.284	148.879	3.106*	1.290

*: Significant at P<0.05.

Table 2

At the end of 24 h, LC_{90} values of *A. cocculus* different extracts against *Ae. aegypti* at larval and adult modes with their 95% confidence limits, calculated χ^2 values and slopes for each. mg/L.

Treatment mode	Solvent extract	LC_{90}	Lower limit	Upper limit	χ^2	Slope
Larval treatment	Petroleum ether	87.908	80.846	104.764	5.825*	1.560
	Benzene	116.331	100.146	152.793	1.051*	1.957
	Chloroform	133.503	110.175	191.985	1.039*	2.415
	Acetone	134.154	111.020	192.115	0.612*	2.279
	Aqueous	88.742	80.165	101.960	0.964*	1.578
Adult treatment	Petroleum ether	178.285	159.536	530.070	10.646*	1.224
	Benzene	219.600	203.740	251.414	2.499*	1.462
	Chloroform	221.766	185.482	393.997	10.148*	1.289
	Acetone	216.004	182.284	338.825	13.036*	1.311
	Aqueous	194.555	184.266	211.200	3.106*	1.360

*: Significant at P<0.05.

Tables 1, 2 and 3 also expresses the results of adulticidal activities of the different extracts of *A. cocculus* seeds against *Ae. aegypti* after 24 h of exposure. Similar to larval stage, mortality rates of adult mosquitoes also increased in a dose dependent pattern which is signified by *Chi*-square and slope values in each case. The lowest value of adult LC_{50} was shown by petroleum ether extract (140.161 mg/L), hence it is more effective than others to kill and eradicate adult dengue mosquitoes; whereas for aqueous extract, the LC_{50} value is 141.930 mg/L, which is very close to the previous LC_{50} value of petroleum ether extract. Similarly LC_{90} (178.285 mg/L) and LC_{99} (214.713 mg/L) values indicate petroleum ether extract as the most potent in all the extracts against adult *Ae. aegypti*. It is clear from these results that LC_{90} or LC_{99} concentration of *A. cocculus* extracts can be used to completely eradicate dengue vectors from the surroundings.

Table 3

At the end of 24 h, LC_{99} values of *A. cocculus* different extracts against *Ae. aegypti* at larval and adult modes with their 95% confidence limits, calculated χ^2 values and slopes for each. mg/L.

Treatment mode	Solvent extract	LC_{99}	Lower limit	Upper limit	χ^2	Slope
Larval treatment	Petroleum ether	127.474	107.382	183.897	5.825*	1.909
	Benzene	193.887	148.833	325.213	1.051*	2.798
	Chloroform	251.844	179.131	499.604	1.039*	3.984
	Acetone	244.756	175.897	474.921	0.612*	3.615
	Aqueous	127.804	108.122	173.328	0.964*	1.941
Adult treatment	Petroleum ether	214.713	183.752	500.651	10.646*	1.297
	Benzene	302.426	261.019	406.131	2.499*	1.716
	Chloroform	301.338	234.091	458.865	10.148*	1.465
	Acetone	274.495	219.356	608.634	13.036*	1.439
	Aqueous	254.183	229.299	305.162	3.106*	1.525

*: Significant at P<0.05.

A corresponding decrease in the toxicity was noticed (petroleum ether> aqueous> benzene> chloroform> acetone extracts) as evident by the increased LC_{50}, LC_{90} and LC_{99} values, which were statistically significant (P<0.05) along with relevant slopes. The mortality rates were found to increase with the concentration of the extracts. Therefore the higher the concentration, the lower the LC_{50} in both larval and adult modes of exposure. The calculated χ^2 values were less than the corresponding table values. Further, on extrapolation of percentage of mortality, near 100% mortality of the larvae as well as adult *Ae. aegypti* could be predicted at the calculated 24 h LC_{90} (Table 2) of the seed extracts of *A. cocculus*. Therefore the calculated 24 h LC_{90} and much stronger LC_{99} may be considered as the absolute acute concentration causing 100% mortality in the corresponding exposure periods.

4. Discussion

Dengue fever is a dangerous mosquito-borne illness in major global public health concern especially in underdeveloped countries. It is endemic to tropical and subtropical countries, especially in the urban and suburban areas where public hygiene is being given little or no attention[22]. But in the case of advanced countries various steps have been successfully taken to curb vector borne infestations. Nowadays mosquito control is being strengthened in many areas, but there are still many challenges, including an increasing mosquito resistance to insecticides and lack of alternative, cost-effective, and safe insecticides. Bioactive plant extracts or isolated phyto-constituents from the plants could be used as an alternative to the currently used synthetic insecticides. The biological activity of plant extracts might be due to various compounds, including phenolics, saponins, volatile oils, terpenoids, and alkaloids present in plants[23]. Advance of using complete mixture may act synergistically and may show greater overall bioactivity compared to the individual constituents[24,25]. These natural insecticides may play a more prominent role on mosquito control programs in the future[26]. Nowadays, the control of mosquitoes is focused on principally larval stages with the plant extracts. The advantage of targeting mosquitoes at the larval stage is that they can not escape from their breeding sites and also reduce the overall pesticide use to control adult mosquitoes by aerial application of insecticides.

Our preliminary screening for larvicidal properties of various solvent extracts of *A. cocculus* seeds revealed high larvicidal potency with low lethal concentrations (LC_{50}=56.731 mg/L of petroleum ether extract) against the third instar larvae of *Ae. aegypti*. Similarly adulticidal activity in the present investigation of *A. cocculus* seed extracts is quite satisfactory with low lethal adulticidal concentration (LC_{50}=140.161 mg/L) shown by petroleum ether extract. Rather than the whole seeds, endosperms were used for the present study because most of the reported cytotoxic phytochemicals are present in the endosperms[12]. The shells of the seeds contained mainly the nontoxic menispermine and paramenispermine[10]. The highest mosquitocidal activity of petroleum ether extract against *Ae. aegypti* may be related to the presence of alkaloids in it. The second and third highest mosquitocidal activity respectively shown by the aqueous and benzene extracts may also be due to the presence of alkaloids in them. The possible mosquitocidal mechanism of alkaloids is generally due to cytotoxic nature of these phytochemicals and damage of protein kinase which is involved in signal transduction and development process among most cells and tissues of mosquitoes[27,28]. The presence of saponins in chloroform extract of *A. cocculus* seeds may make substance freely soluble in both organic solvents and water and work by interacting with cuticle membrane of larvae ultimately and then disarranging the membrane, which is the probable reason for the larval death[29]. Many studies reported that saponins showed 100% mortality against mosquito larvae[30]. They may interact with the adult mosquito membranes in the same way and can cause their mortality as well. Hence, the presence of these compounds in *A. cocculus* supports the observed insecticidal activities. The lowest insecticidal activity shown by acetone extract may be due to the absence of alkaloids, phenolic compounds and saponins and the presence of volatile oils; volatile oils may affect the cuticle of mosquitoes[31,32]. The mode of action of volatile oils is presumed to be interference with the neuromodulator octopamine or gaba-gated chloride channels through fumigant activity[33-36]. However, the exact mode of action is still unclear[31]. Glycosides present in the acetone extract may also contribute to some insecticidal activity because glycosides are also reported to possess mosquitocidal property[37].

The results of present study are comparable with earlier reports. Imam *et al*. reported the larvicidal activity of petroleum ether and ethyl alcohol extracts of *A. calamus* applied for 24 h on the third and forth instar larvae of *Ae. aegypti* and concluded that petroleum ether extract possessed most mosquitocidal activity[38]. Larval mortality was detected by Shivakumar *et al.*[39], in acetone extract of *Eleusine indica* (LC_{50} 90.89, LC_{90} 217.21 and LC_{99} 441.88 mg/L) and *Mangifera indica* acetone extract (LC_{50} 173.21, LC_{90} 289.86 and LC_{99} 441.04 mg/L) against the forth instar larvae of *Ae. aegypti*. The leaf extract of *Amelanchier alnifolia* (*A. alnifolia*) with different solvents like hexane, chloroform, ethyl acetate, acetone and methanol were tested for larvicidal activity against malaria vector. The different extracts of *A. alnifolia* against early fourth instar larvae of *Anopheles stephensi* had values of LC_{50} (197.37, 178.75, 164.34, 149.90 and 125.73 mg/L) and LC_{90} (477.60, 459.21, 435.07, 416.20, and 395.50 mg/L), respectively. The results of the leaf extract of *A. alnifolia* thus possess promising larvicidal activity against the mosquito vector, *Anopheles stephensi*[40].

The present observation has involved the promising mosquitocidal property of *A. cocculus* and therefore the present attempt is part of devising active bioinsecticide from *A. cocculus* to control dengue infestation. The present study indicates the potential of *A. cocculus* to eradicate the dengue caused by *Ae. aegypti* vector at larval as well as adult stages. This observation is significant in view of the reports that a large quantity of the chemical insecticides reach non target species creating environmental concerns[41], decrease biodiversity and soil quality[42], deplete the habitat for birds and can threaten endangered species[41]. On the basis of these harmful effects of chemical insecticides, there is a need to find and develop such pesticides that would ensure environmental safety and high rate of degradation. By the application of bioinsecticides, which are inherently less noxious than the chemical mosquitocides but effective at small quantities and often decompose quickly, we can avoid many of the ecotoxicological problems caused by the chemical insecticides. The present study reveals the ability of the seeds of *A. cocculus* to serve as the raw material for an effective bioinsecticide.

The mortality caused by the *A. cocculus* seed extracts at larval and adult stages points to the significant relationship between percentage of mortality and concentration of the extracts. Various factors such as accelerated penetration of active moieties, nature of the slope as well as concentration of the active biological molecules in the extracts *etc*., might influence the mortality rate of *Ae. aegypti*. According to Tiwari *et al.*[43], such mediating variables can act separately or conjointly. The increased concentration of the extracts might facilitate penetration of active moieties into the body of larvae/adults and thereby causing an increase in mortality at higher concentration. The significant χ^2 values indicate that mortality rates in the present study are not significantly heterogeneous and variables such as individual resistance do not significantly affect the LC_{50} values since they lie within 95% confidence limit. The steepness of the slope also indicates a sharp increase in the mortality rate with a small increase in the exposure concentration.

The possibility of using *A. cocculus* seed extract as a mosquitocide against dengue caused by *Ae. aegypti* vector has great significance as the expected result is seen at lower exposure concentration and largely avoids the pollution problem caused by chemical insecticides. Its application as a bioinsecticide may not alter ecological balance and may not greatly affect the ecological significance of food chains and food webs. The findings of the present investigation revealed that the seed extracts of *A. cocculus* possess remarkable larvicidal and adulticidal activity against the dreadful vector *Ae. aegypti*. Therefore these results should encourage further studies on the identification of the active principles involved and their mode of action. Field trials are also needed to recommend *A. cocculus* as an anti-mosquito product to combat and protect from mosquitoes in a control program.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The author is thankful to Department of Zoology, Annamalai University and Department of Entomology, Faculty of Agriculture, Annamalai University for providing necessary facilities. Author acknowledges the immense help from the scholars whose articles are cited and included in references of this manuscript. The author is also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

References

[1] Govindarajan M, Sivakumar R. Adulticidal properties of *Cardiospermum halicacabum* plant extracts against three important vector mosquitoes. *Eur Rev Med Pharmacol Sci* 2012; **16**(Suppl 3): S95-S104.

[2] Jaswanth A, Ramanathan P, Ruckmani K. Evaluation of mosquitocidal activity of *Annona squamosa* leaves against filarial vector mosquito, *Culex quinquefasciatus* Say. *Indian J Exp Biol* 2002; **40**: 363-365.

[3] Carvalho AF, Melo VM, Creveiro AA, Machado MI, Bantim MB, Rabelo EF. Larvicidal activity of the essential oil from *Lippia sidoides* Cham against *Aedes aegypti* linn. *Mem Inst Oswaldo Cruz* 2003; **98**: 569-571.

[4] Sivakumar R, Jebanesan A, Govindarajan M, Rajasekar P. Larvicidal and repellent activity of tetradecanoic acid against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say.) (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(9): 706-710.

[5] World Health Organization. Dengue/dengue haemorrhagic fever. Geneva: World Health Organization. [Online] Available from: http://www.who.int/csr/disease/dengue/en/ [Accessed on 20 Apr 2014]

[6] Andrew J, Bar A. Morphology and morphometry of *Aedes aegypti* adult mosquito. *Annu Rev Res Biol* 2013; **3**(1): 52-69.

[7] Saxena A, Saxena G, Singh M, Neeraj PK. Study on larvicidal potential of flavonoid extracted from *Ageratum conyzoides* (Asteraceae) for controlling *Culex quinquefaciatus* (Culicidae) Diptera. *Sci Secure J Biotechnol* 2013; **2**(3): 75-82.

[8] Maheswaran R, Ignacimuthu S. A novel herbal formulation against dengue vector mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Parasitol Res* 2012; **110**: 1801-1813.

[9] Jothivel N, Paul VI. Evaluation of the acute toxicity of the seeds of *Anamirta cocculus* (Linn.) and its piscicidal effect on three species of freshwater fish. *Internet J Toxicol* 2008; **5**: 1-11.

[10] Satya V, Paridhavi M. Ethno-botanical, phytochemical and pharmacological review of *Anamirta cocculus* (Linn.) Wight and arn. *Int J Rev Life Sci* 2012; **2**: 1-6.

[11] Jothivel N, Paul VI. Exploitation of acute toxicity of the seeds of *Anamirta cocculus* (Linn.) as a potential aquaculture management tool to eradicate unwanted fish fauna. *Asian Fish Sci* 2008; **21**:

457-467.

[12] Qadir U, Muzaffar U. Exploitation of piscicidal potential of *Anamirta cocculus* (Linn.) seed extracts for the eradication of predatory wild fish *Mystus vittatus* (Bloch.). *Int J Sci Res* 2014; **3**(4): 453-454.

[13] Satya V, Paridhavi M. Evaluation of anti-inflammatory activity of *Anamirta cocculus* Linn. fruit. *Int J Res Pharm Sci* 2013; **4**(1): 30-35.

[14] Satya V, Paridhavi M. Wound healing activity of fruits of *Anamirta cocculus* in rats. *Int J Pharm Pharm Sci* 2013; **5**: 280-282.

[15] Johnston GA. Advantages of an antagonist: bicuculline and other GABA antagonists. *Br J Pharmacol* 2013; **169**: 328-336.

[16] Lalith JG, Wannigamma P, Macleod JK. Triterpenoids from *Anamirta cocculus*. *Phytochemistry* 1993; **34**(4): 1111-1116.

[17] Asahina S. Food material and feeding procedures for mosquito larvae. *Bull World Health Organ* 1964; **31**: 465-466.

[18] World Health Organization. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Geneva: World Health Organization; 1981. [Online] Available from: http://apps.who.int/iris/handle/10665/69615?locale=en [Accessed on 1st September, 2013]

[19] Dua VK, Alam MF, Pandey AC, Rai S, Chopra AK, Kaul VK, et al. Insecticidal activity of *Valeriana jatamansi* (Valerianaceae) against mosquitoes. *J Am Mosq Control Assoc* 2008; **24**(2): 315-318.

[20] Rawani A, Ghosh A, Laskar S, Chandra G. Aliphatic amide from seeds of *Carica papaya* as mosquito larvicide, pupicide, adulticide, repellent and smoke toxicant. *J Mosq Res* 2012; doi: 10.5376/jmr.2012.02.0002.

[21] Finney DJ. *Probit analysis*. 3rd ed. London: Cambridge University Press; 1971, p. 1-333.

[22] Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: a continuing global threat. *Nat Rev Microbiol* 2010; **8**(Suppl 12): S7-S16.

[23] Rafael L, Teresinha N, Moritz JC, Maria IG, Eduardo MD, Tania SF. Evaluation of antimicrobial and antiplatelet aggregation effect of *Solidago chilensis* eyen. *Int J Green Pharm* 2009; **3**: 35-39.

[24] Huang EC, Zhao Y, Chen G, Baek SJ, McEntee MF, Minkin S, et al. Zyflamend, a polyherbal mixture, down regulates class I and class II histone deacetylases and increases p21 levels in castrate-resistant prostate cancer cells. *BMC Complement Altern Med* 2014; doi:10.1186/1472-6882-14-68.

[25] Chen W, Isman MB, Chiu SF. Antifeedant and growth inhibitory effects of the limonoid toosendanin and *Media toosendan* extracts on the variegated cutworm *Peridroma saucia* (Lep., Noctuidae). *J Appl Entomol* 1995; **119**: 367-370.

[26] Al-Mehmadi RM, Al-Khalaf AA. Larvicidal and histological effects of *Melia azedarach* extract on *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *J King Saud Univ Sci* 2010; **22**: 77-85.

[27] Goel G, Makkar HP, Francis G, Becker K. Phorbol esters: structure, biological activity, and toxicity in animals. *Int J Toxicol* 2007; **26**: 279-288.

[28] Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res* 2012; **135**(5): 581-598.

[29] Bagavan A, Kamaraj C, Rahuman AA, Elango G, Zahir AA, Pandiyan G. Evaluation of larvicidal and nymphicidal potential of plant extracts against *Anopheles subpictus* Grassi, *Culex tritaeniorhynchus* Giles and *Aphis gossypii* Glover. *Parasitol Res* 2009; **104**: 1109-1117.

[30] Ojha K, Pattabhiramaiah M. Evaluation of phytochemicals, larvicidal activity of *Jatropha curcas* seed oil against *Aedes Aegypti*. *Int J Appl Res Stud* 2013; **2**(12): 1-12.

[31] Ebadollahi A. Iranian plant essential oils as sources of natural insecticide agents. *Int J Biol Chem* 2011; **5**: 266-290.

[32] Tong F, Bloomquist JR. Plant essential oils affect the toxicities of carbaryl and permethrin against *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 2013; **50**(4): 826-832.

[33] Douiri LF, Boughdad A, Alaoui MH, Moumni M. Biological activity of *Rosmarinus officinalis* essential oils against *Callosobruchus maculatus*, (Coleoptera, Bruchinae). *J Biol Agric Healthc* 2014; **4**(2): 5-14.

[34] Chaubey MK. Combinatorial action of essential oils towards pulse beetle *Callosobruchus chinensis* Fabricius (Coleoptera: Bruchidae). *Int J Agric Res* 2011; **6**(6): 511-516.

[35] Bairwa R, Sodha RS, Rajawat BS. *Trachyspermum ammi*. *Pharmacogn Rev* 2012; **6**(11): 56-60.

[36] Mann RS, Tiwari S, Smoot JM, Rouseff RL, Stelinski LL. Repellency and toxicity of plant-based essential oils and their constituents against *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *J Appl Entomol* 2012; **136**: 87-96.

[37] Amin E, El-Hawary SS, Fathy MM, Mohammed R, Ali Z, Tabanca N, et al. Triterpenoidal saponins: bioactive secondary metabolites from *Zygophyllum coccineum*. *Planta Med* 2011; **77**: 488-491.

[38] Imam H, Zarnigar, Sofi G. Mosquito larvicidal efficay of *Acorus calamus* extracts against *Aedes aegypti* L. larvae. *Asian Pac J Trop Dis* 2014; **4**(Suppl 1): S181-S185.

[39] Shivakumar MS, Srinivasan R, Natarajan D. Larvicidal potential of some Indian medicinal plant extracts against *Aedes aegypti* (L.). *Asian J Pharm Clin Res* 2013; **6**(3): 77-80.

[40] Kovendan K, Murugan K, Vincent S. Evaluation of larvicidal activity of *Acalypha alnifolia* Klein ex Willd. (Euphorbiaceae) leaf extract against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and *Bancroftian filariasis* vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2012; **110**: 571-581.

[41] Miller GT. *Sustaining the earth*. 6th ed. California: Thompson Learning Inc. Pacific Grove; 2004, p. 211-216.

[42] Upadhyay M. Use of inorganic chemical and its impact on human health: a case study of farmers in India. *Asian J Pharm Res* 2014; **4**(2): 68-73.

[43] Tiwari SP, Singh P, Singh A. Toxicity of *Euphorbia tirucalli* plant against freshwater target and non-target organisms. *Pak J Biol Sci* 2003; **6**: 1423-1429.