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ORIGINAL PAPER

Larvicidal, oviposition, and ovicidal effects of *Artemisia annua* (Asterales: Asteraceae) against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract This study focuses on the larvicidal, oviposition, and ovicidal effects of a crude extract of *Artemisia annua* against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*. Dried cells of *Artemisia annua* from cell suspension cultures were extracted using hexane. The extract showed moderate larvicidal effects against mosquitoes. At 24-h post treatment, the LC_{50} values for *Anopheles sinensis*, *Aedes aegypti*, and *Culex quinquefasciatus* were recorded as 244.55, 276.14, and 374.99 ppm, respectively. The percentage mortality of larvae was directly proportional to the tested concentration. *Anopheles sinensis* was found to be the most susceptible species, whereas *Culex quinquefasciatus* was the most tolerant to the *Artemisia annua* extract. The results indicated that the *Artemisia annua* extract showed concentration-dependent oviposition deterrent activity and had a strong deterrent effect. At 500 ppm, the percentage effective repellency was more than 85 % compared with the control group for all the species, with oviposition activity index values of −0.94, −0.95, and −0.78 for *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*, respectively. In the ovicidal assay, the percentage hatchability of eggs after treatment with 500 ppm of *Artemisia annua* extract was significantly lower than the control, with values of 48.84±4.08, 38.42±3.67, and 79.35±2.09 % for *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*, respectively. *Artemisia annua* was found to be more effective against *Aedes aegypti* and *Anopheles sinensis* compared with *Culex quinquefasciatus*. This study indicated that crude extract of *A. annua* could be a potential alternative for use in vector management programs.

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Introduction

Mosquitoes have long been known for their importance as vectors of disease (Cheng et al. 2004; Wang et al. 2011). In addition to being a nuisance, they threaten public health and cause allergic responses in humans. Despite their small size, mosquitoes are of economic and medical importance. The genera *Aedes*, *Anopheles*, and *Culex* are important vectors of mosquito-borne diseases worldwide. Mosquito-borne diseases cause economic loss and are commonly found in tropical rather than temperate region (Adanan et al. 2005). *Aedes aegypti*, the primary carrier of the dengue virus, which is predominant in tropical regions, also transmits yellow fever in Africa and South America (Govindarajan et al. 2011; Kumar et al. 2011). Cases of dengue fever and dengue hemorrhagic fever have increased every year and resulted in high number of deaths in Malaysia (Lee and Zairi 2005). On the other hand, malaria remains one of the most important diseases worldwide and one of the world's biggest killers, with 350–500 million of cases occurring annually that are transmitted by *Anopheles sp*. mosquitoes (Hemingway and Bates 2003). Lymphatic filariasis and Japanese encephalitis, which are transmitted by *Culex sp*. mosquitoes, cause millions of deaths every year, especially in India and Africa (Pavela 2008; Govindarajan et al. 2011).

Vector control programs using chemical and synthetic insecticides have long been utilized to prevent the transmission of these diseases. However, use of these chemicals resulted in numerous problems, such as insecticide resistance, environmental pollution, and adverse effects on

humans and other organisms (Georghiou and Taylor 1977; Hemingway and Ranson 2000; Nauen 2007). As a result, research on alternative control methods, such as microbial and botanical insecticides, has been carried out over the last few years. Botanical insecticides are environmentally friendly, readily available, and relatively safe for non-target pests (Markouk et al. 2000; Isman 2006; Jbilou et al. 2008; Pavela 2008; Shekari et al. 2008; Cheng et al. 2009; Govindarajan et al. 2012a). To date, no studies have shown resistance to botanical-based insecticides among vector pests due to the limited use of botanical agents in vector control programs (Shaalan et al. 2005).

Artemisia annua from the family Asteraceae has been used to treat fever since ancient times (Tawfiq et al. 1989; Paniego and Giulietti 1994; Balint 2001). Artemisinin, a compound found in this plant, is effective against *Plasmodium falciparum*, the parasite that causes malaria tropica (Tawfiq et al. 1989; Paniego and Giulietti 1994; Bhakuni et al. 2001). Thus, *Artemisia annua* is a potential antimalarial plant that is used in the treatment of malaria in endemic regions, such as in Africa. Moreover, *Artemisia annua* can be used to treat cancer and act as antibacterial, antifeedant, and anti-inflammatory agent (Bhakuni et al. 2001; Baldi and Dixit 2008). Several studies have been performed indicating the effectiveness of *Artemisia annua* against insect pests, such as mosquitoes (Sharma et al. 2006), elm leaf beetles (Shekari et al. 2008), stored-product beetles (Tripathi et al. 2000), and lesser mulberry pyralids (Roya et al. 2010).

Larvicide and adulticide can be effective strategies in mosquito control. Due to the limited area of mosquito larvae movement compared with that of free-flying adult mosquitoes, the control of larvae is more effective than the control of adults (Lee and Zairi 2005; Amer and Mehlhorn 2006; Rajkumar and Jebanesan 2009; Waliwitiya et al. 2009). Oviposition is one of the important processes in the life cycle of a mosquito. Mosquitoes can lay their eggs directly on the water surface or on moist areas above the water level. Oviposition could be influenced by a variety of environmental factors, such as water temperature, salinity, and level of pollution. Female mosquitoes need to feed on vertebrate blood to obtain essential protein for egg maturation before laying eggs, and this is the process by which pathogens are transferred to humans.

Because *Artemisia annua* can be used to effectively treat malaria parasites, it is important to study the potential of this plant as a mosquito insecticide. This research aimed to (1) investigate the effects of an *Artemisia annua* extract on mosquitoes at the larval stage through larval bioassays, (2) examine the effects of an *Artemisia annua* extract on the oviposition activity, and (3) determine the ovicidal activity of an *Artemisia annua* extract against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*.

Materials and methods

Cell suspension culture of *Artemisia annua*

Artemisia annua originated from Vietnam. A callus culture of *Artemisia annua* was established in glass-culture vessels containing Murashige and Skoog (MS) medium (1962) supplemented with 30 g/L sucrose and 8 g/L agar to solidify the medium. No agar was added to medium used for cell suspension cultures. The medium was fixed at pH 5.7–5.8. Flasks filled with medium were autoclaved at 121 °C for 11 min to prevent contamination. Cell suspension cultures were initiated by introducing 2.0±0.3 g of green, soft callus into a 250-ml Erlenmeyer flask containing 50 ml of MS medium (1962) supplemented with 30 g/L sucrose. The flasks were shaken at 110 rpm and placed under light at a constant temperature of 25±2 °C. The cell cultures were subcultured every 2 weeks to maintain the clone. Excess cells that remained after sub-culturing were filtered using a Buchner funnel, placed in a petri dish and air dried at room temperature before being used in the extraction process.

Extraction

Dried cells of *Artemisia annua* were ground into a powder using a mortar and pestle. Dried *Artemisia annua* powder (10 g) was extracted using 60 ml of hexane (Labchem, Petaling Jaya, Malaysia) and allowed to sit for 24 h at room temperature prior to removal of the supernatant. The process was repeated three times, and the supernatant was filtered through Whatman No. 1 filter paper to separate the powder from the hexane. The extract was placed in the fume hood until the solvent was completely evaporated, and a dark green residue was obtained. A 7 % stock solution was prepared by dissolving the residue in acetone. Different concentrations of the *Artemisia annua* crude extract were prepared by serial dilution using acetone.

Mosquito culture

All mosquito species used in the study were laboratory strains obtained from the Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia. Mosquito eggs were submerged in an enamel tray filled with chlorine-free water. Larvae were reared under laboratory conditions at a temperature of 27±2 °C and relative humidity of 70±5 %. The larvae were fed on a fine mixture of dog biscuits, beef liver, milk powder, and yeast powder at a ratio of 2:1:1:1. The water was changed every day to avoid scum formation. Pupae were collected and transferred into a plastic container filled with clean water. The plastic container was placed in a cage (30×30×30 cm) covered with a mosquito net for adult emergence. Adult mosquitoes were provided with a sucrose

solution mixed with vitamin B solution soaked in cotton. On the fifth to sixth day post-emergence of adult mosquitoes, female mosquitoes were fed on a constrained mouse. Due to the different biting behavior, the mouse was placed in the cage during the day for *Aedes aegypti*, and during the night for *Anopheles sinensis* and *Culex quinquefasciatus*. For oviposition, moist filter papers were fitted onto Petri dishes for adult *Aedes aegypti* females to lay their eggs. Petri dishes filled with clean water were prepared as oviposition sites for *Anopheles sinensis* and *Culex quinquefasciatus*.

Larval bioassays

Late third and early fourth instar larvae were used in the larval bioassays. Larval bioassays were conducted in accordance with the WHO standard method (WHO 2005). Ten larvae were transferred into a paper cup filled with 99 ml of chlorine-free water. For *Aedes aegypti* and *Anopheles sinensis*, 1 ml of extract was added to the water to obtain concentrations of 100, 200, 300, 400, 500, and 600 ppm. For *Culex quinquefasciatus*, the tested concentrations were 200, 300, 400, 500, 600, and 700 ppm. Acetone (1 ml) was added to the controls. Larval mortality was recorded at 24- and 48-h post treatment. The mosquito larvae were considered dead if they were unable to move after gentle touching with a needle or glass rod. Moribund larvae were unable to rise to the surface when the water was disturbed (WHO 2005). Larvae were provided with food after 24 h. Ten replicates with a total of 100 larvae were performed simultaneously for each of the tested concentrations.

Oviposition deterrent assay

Petri dishes filled with 50 ml of tap water were prepared as oviposition sites. For *Aedes aegypti*, moist filter papers were fitted onto the petri dishes for oviposition. For *Anopheles sinensis* and *Culex quinquefasciatus*, clean water was provided for oviposition. *Artemisia annua* extract was added to the dishes to obtain test solutions of 50, 200, 300, and 500 ppm. Acetone (1 ml) was added to the control dish. One treated dish and one control dish were placed in the opposite corners of the cage (30×30×30 cm) containing 15 blood-fed female mosquitoes. The positions of the dishes were rotated between the different replicates to abolish any effect of position on oviposition. Three replicates were performed for each concentration. All experiments were conducted at a room temperature of 27±2 °C and relative humidity of 70±5 %. Eggs were collected daily until no eggs were laid for at least 48 h. The eggs were counted under a dissecting microscope.

Ovicidal assay

One blood-fed female was transferred into a paper cup and allowed to lay eggs. For *Aedes aegypti*, the bottom of the cup was lined with filter paper on wet cotton (provided as an oviposition site), whereas clean water was provided for *Anopheles sinensis* and *Culex quinquefasciatus*. After 2 days, the eggs were collected and counted under a dissecting microscope. The eggs were submerged in four different concentrations of *Artemisia annua* extract (50, 200, 300, and 500 ppm). Acetone (1 ml) was added to the control. After 5 h of exposure, the eggs from each replicate were transferred to a different plastic container filled with chlorine-free water for hatching assessment. The hatched larvae were collected and counted daily until no larvae were hatching for at least 48 h. Only fully hatched larvae were counted. Five replicates were performed for each concentration.

Statistical analysis

Control bioassay tests with more than 20 % mortality were discarded and repeated. If 5–20 % mortality was observed in a control group, the observed mortality was corrected using Abbott's formula (Abbott 1925):

$$\text{Observed mortality} = \frac{\text{Test mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

The data from larval bioassays were analyzed using a computerized log-probit analysis. The 50 and 95 % lethal concentrations (LC_{50} and LC_{95}) at 24- and 48-h post treatment were obtained using SPSS 16.0. The 95 % confidence limits (LCL–UCL) were also calculated.

In the oviposition deterrent assay, the percent effective repellency (ER) for each concentration was calculated using the following formula:

$$\%\text{ER} = \frac{NC - NT}{NC} \times 100$$

where *NC* is the number of eggs in the control group and *NT* is the number of eggs in the treated group.

The oviposition activity index (OAI) for each concentration was calculated using the following formula:

$$\text{OAI} = [(\text{NT} - \text{NC})/(\text{NT} + \text{NC})]$$

where *NC* is the number of eggs in the control group and *NT* is the number of eggs in the treated group.

In the ovicidal assay, percentage hatchability of larvae was calculated using the following formula:

$$\%\text{hatchability} = \frac{\text{Number of larvae hatched}}{\text{Total number of eggs in each replicate}} \times 100$$

Comparison data were analyzed using independent *t* test and one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) using SPSS (2007) 16.0 at $P < 0.05$.

Results

The results indicated that the percentage mortality of larvae was directly proportional to the concentration of *Artemisia annua* extract at 24- and 48-h post treatment. The LC_{50} values at 24-h post treatment were 244.55 ppm (186.36–300.93), 276.14 ppm (212.09–343.22), and 374.99 ppm (348.90–400.87) for *Anopheles sinensis*, *Aedes aegypti*, and *Culex quinquefasciatus*, respectively (Table 1). When the incubation time was extended to 48 h, the LC_{50} values were 187.10 ppm (128.29–238.26), 213.98 ppm (149.56–272.22), and 304.55 ppm (252.75–348.39) for *Anopheles sinensis*, *Aedes aegypti*, and *Culex quinquefasciatus*, respectively (Table 2). The LC_{50} values decreased with increasing exposure time. According to the results, *Culex quinquefasciatus* was significantly more tolerant to *Artemisia annua* extract compared with *Anopheles sinensis* and *Aedes aegypti*.

On the other hand, the oviposition deterrent activity was dependent on the concentration of *Artemisia annua* extract. At all concentrations, there was a significant difference between control and treated groups with respect to the number of eggs laid for all three species with the exception of 50 ppm in *Culex quinquefasciatus* (Table 3). The extract had strong deterrent effects as its concentration increased. At 500 ppm, the percentage of effective repellency was more than 85 % for all three species, with OAI values of −0.94, −0.95, and −0.78 for *Aedes aegypti*, *Anopheles sinensis*, and *C. quinquefasciatus*, respectively. Elango et al. (2010) reported that compounds are considered as oviposition attractants if the OAI is +0.3 and above, whereas those with an OAI of −0.3 and below are considered as oviposition repellents. The lowest percentage of effective repellency was 19.08±7.18 % at 50 ppm of *Artemisia annua* extract in *Culex quinquefasciatus*, whereas the highest was achieved at 500 ppm of *Artemisia annua* extract in *Anopheles sinensis* with 97.28±1.40 %.

In the ovicidal assay, 500 ppm of *Artemisia annua* extract was found to have a severe ovicidal effect. The hatchability percentages in the 500 ppm group were 48.84±4.08, 38.42±3.67, and 79.35±2.09 % for *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*, respectively (Table 4). From these results, we observed that *C. quinquefasciatus* eggs were more tolerant to *Artemisia annua* extract. *Artemisia annua* extract at 500 ppm significantly

Table 1 Larvicidal activity of *Artemisia annua* against *Aedes aegypti*, *Anopheles sinensis* and *Culex quinquefasciatus* at 24-h post treatment

	Concentration (ppm)	Percentage mortality (mean ± SE)	LC_{50} in ppm (LCL–UCL)	LC_{95} in ppm (LCL–UCL)	Slope	*df*	χ^2
Aedes aegypti	100	10±2.1	276.14 (212.09–343.22)	886.97 (621.63–1,928.76)	3.2±0.3	4	14.05
	200	35±3.1					
	300	48±1.3					
	400	62±3.3					
	500	78±2.5					
	600	96±1.6					
Anopheles sinensis	100	13±2.6	244.54 (186.36–300.93)	751.28 (546.83–1,436.92)	3.4±0.3	4	13.64
	200	37±2.6					
	300	58±2.9					
	400	69±2.3					
	500	84±2.7					
	600	99±1.0					
Culex quinquefasciatus	200	17±3.7	374.99 (348.90–400.87)	1018.3 (880.92–1,240.85)	3.8±0.3	4	6.3
	300	39±3.8					
	400	47±3.0					
	500	63±3.3					
	600	79±3.2					
	700	90±3.2					

ppm part per million, *LC* lethal concentration, *LCL* lower confidence limit, *UCL* upper confidence limit, *df* degree of freedom; χ^2 chi-square value

Table 2 Larvicidal activity of *Artemisia annua* against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus* at 48-h post treatment

	Concentration (ppm)	Percentage mortality (mean ± SE)	LC_{50} in ppm (LCL–UCL)	LC_{95} in ppm (LCL–UCL)	Slope	*df*	χ^2
Aedes aegypti	100	18±3.9	213.98 (149.56–272.22)	704.95 (497.49–1,528.62)	3.2±0.2	4	16.07
	200	48±4.2					
	300	59±4.6					
	400	72±4.2					
	500	95±1.7					
	600	100±0					
Anopheles sinensis	100	22±3.3	187.1 (128.29–238.26)	555.39 (405.58–1,083.96)	3.5±0.3	4	17.04
	200	53±2.1					
	300	66±2.7					
	400	83±2.1					
	500	98±1.3					
	600	100±0					
Culex quinquefasciatus	200	25±2.2	304.55 (252.75–348.39)	718.93 (588.01–1,036.42)	4.4±0.4	4	9.18
	300	49±2.3					
	400	63±2.6					
	500	78±2.5					
	600	92±2.9					
	700	99±1.0					

ppm part per million, *LC* lethal concentration, *LCL* lower confidence limit, *UCL* upper confidence limit, *df* degree of freedom; χ^2 chi-square value

reduced the hatchability rate compared with the other concentrations and the control group in *Aedes aegypti* (F=19.871; df=4, 20; P <0.05), *Anopheles sinensis* (F=44.495; df=4, 20; P <0.05), and *Culex quinquefasciatus* (F=23.976; df=4, 20; P <0.05).

Discussions

Several studies have reported that mosquito control should be carried out at the larval stage due to the limited living habitat of mosquito larvae (Amer and Mehlhorn 2006;

Table 3 Oviposition deterrent activity of *Artemisia annua* against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*

Mosquito species	Concentration (ppm)	Number of eggs (mean ± SE)		% ER (mean ± SE)	OAI
		Control	Treated		
Aedes aegypti	50	1,100.67±81.49 a	725.67±36.10 b	33.40±5.81 a	−0.21
	200	1,050.67±88.52 a	592.33±37.02 b	43.38±1.66 a	−0.28
	300	1,296.33±29.17 a	456±23.07 b	64.79±1.94 b	−0.48
	500	1,752.67±68.18 a	52.67±30.31 b	97.12±1.61 c	−0.94
Anopheles sinensis	50	477.33±13.54 a	352.33±10.84 b	26.13±2.22 a	−0.15
	200	633.67±14.71 a	167.33±40.54 b	73.85±5.67 b	−0.58
	300	733.33±24.63 a	83.33±10.48 b	88.52±1.80 bc	−0.80
	500	765.67±22.30 a	20.33±10.65 b	97.28±1.41 c	−0.95
Culex quinquefasciatus	50	877±49.69 a	705±45.74 a	19.08±7.18 a	−0.11
	200	1,162.67±66.50 a	678.33±31.40 b	41.57±1.29 b	−0.26
	300	1,266.33±10.17 a	479.33±23.68 b	62.17±1.62 c	−0.45
	500	1,769.67±73.32 a	218±27.02 b	87.68±1.44 d	−0.78

In the "Number of eggs" column, (mean ± SE) values followed by different letters within the same row are significantly different (independent *t* test, P <0.05). In the "% ER" column, (mean ± SE) values followed by different letters within the same column for each species are significantly different (one-way ANOVA followed by Tukey's HSD, P <0.05)

ER effective repellency, *OAI* oviposition activity index

Table 4 Percentage hatchability of *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus* eggs after treatment with different concentrations of *Artemisia annua* extract

Concentration	Percentage hatchability (mean ± SE)		
(ppm)	*Aedes aegypti*	*Anopheles sinensis*	*Culex quinquefasciatus*
Control	89.60±2.31 a	84.85±2.04 a	96.30±1.26 a
50	86.25±2.13 a	80.16±3.74 a	95.06±1.02 a
200	83.50±4.63 a	77.91±0.79 ab	92.10±1.17 a
300	75.62±4.48 a	67.30±2.62 b	94.93±1.35 a
500	48.84±4.08 b	38.42±3.67 c	79.35±2.09 b

Mean ± SE values followed by different letters within the same column are significantly different (one-way ANOVA followed by Tukey's HSD, $P<0.05$)

Waliwitiya et al. 2009). The larvicidal activity of *Artemisia annua* extract against three species of mosquitoes was investigated in this study. The LC_{50} and LC_{95} values had an inverse relationship with time such that the values decreased after 48 h of exposure to the *Artemisia annua* extract. Different species of mosquitoes may have different sensitivities to insecticides (Amer and Mehlhorn 2006). In this study, the larval stage of *Culex quinquefasciatus* was found to be least susceptible to the *Artemisia annua* extracts, followed by *Aedes aegypti*, and *Anopheles sinensis*. The current results were similar to those of Govindarajan et al. (2011), who revealed that LC_{50} and LC_{90} values were lowest in *Anopheles stephensi*, higher in *Aedes aegypti*, and highest in *Culex quinquefasciatus* after treatment with benzene extract of *Ervatamia coronaria*. However, different results were obtained by Shaalan et al. (2005), who stated that *Aedes sp*. larvae are generally less susceptible to insecticides and botanical extracts compared with *Culex sp*. larvae. Khandagle et al. (2011) also reported that *Zingiber officinalis* was a more effective larvicide against *Culex quinquefasciatus* compared with *Aedes aegypti*, with LC_{50} values of 154 and 197 ppm, respectively. Govindarajan et al. (2012b) found that hexane extract of *Delonix elata* was less effective compared with methanol, ethyl acetate, chloroform, and benzene extracts in terms of their larvicidal and ovicidal effects against *Anopheles stephensi* and *Aedes aegypti*. Moreover, the solvent used for extraction may affect the toxicity against the vector because different organic solvents show different polarity gradients in dissolving toxic components (Karmegam et al. 1997). Therefore, the solvent used for extraction must be carefully considered before determining the potential of insecticides of plant origin.

Because dengue fever and dengue hemorrhagic fever can be transmitted transovarially, knowledge regarding the selection of oviposition site by mosquitoes is very crucial in combating this disease. Moreover, the mosquito population could be reduced by preventing their oviposition (Rajkumar and Jebanesan 2009). Therefore, oviposition deterrent activity was investigated in our study. Yap et al. (1996) showed that the number of eggs produced decreased with increasing age in *Aedes aegypti* and *Culex quinquefasciatus*. Thus, mosquitoes of the same age were used in this study to avoid inaccurate results. Adanan et al. (2005) indicated that *C. quinquefasciatus* produced more eggs compared with *Aedes aegypti* under laboratory conditions. Similar results were obtained in this study. Previous studies showed that the essential oil of *Z. officinalis* had promising larvicidal activity and oviposition deterrence activity, whereas the essential oil of *Achyranthes aspera* showed significant oviposition deterrence activity rather than larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* (Khandagle et al. 2011). In the present study, *Artemisia annua* extracts were found to perform effectively as oviposition deterrent agent rather than larvicides. The oviposition deterrent and skin repellent activity of *Solanum trilobatum* leaf extracts against *Anopheles stephensi* could be due to the compounds that exist in plants, such as phenolics, terpenoids, and alkaloids (Rajkumar and Jebanesan 2005). Artemisinin, which is found in *Artemisia annua*, is a sesquiterpene lactone with an endoperoxide bridge. The insecticidal properties of this compound should be investigated in the future.

Mosquito eggs can become impervious once they harden; therefore, freshly laid eggs were used in the ovicidal assay (Kuppusamy and Murugan 2008). The hatchability rate was inversely proportional to the concentration of *Artemisia annua* extract in this study (Table 4). Govindarajan et al. (2011) reported that the ovicidal activity of *E. coronaria* extract and *Caesalpinia pulcherrima* extract against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* showed similar results. The current results are also comparable to the effects of ethanolic extract of *Andrographis paniculate* on *Anopheles stephensi*, where increasing concentrations of plant extracts decreased the hatchability rate (Kuppusamy and Murugan 2008). Another study reported that zero hatchability was observed in *Anopheles stephensi* and *Aedes aegypti* exposed to 300 ppm of *D. elata* leaf methanol extract and 500 ppm of *D. elata* seed methanol extract, respectively (Govindarajan et al. 2012b). Al-Doghairi et al. (2004) reported that 1,000 ppm of *Solenostemma argel* methanolic extract reduced egg hatching in *Culex pipiens* by 33.7 %. It is possible that *Artemisia annua* extract may be a

more effective ovicide than methanolic extract of *S. argel*. Indeed, at a concentration of 500 ppm, *Artemisia annua* extract reduced egg hatching by 45.5, 54.7, and 17.6 % in *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*, respectively.

In conclusion, hexane extract of *Artemisia annua* was found to be more effective against *Anopheles sinensis* and *Aedes aegypti* compared with *Culex quinquefasciatus*. Its effectiveness as a larvicide and oviposition deterrent agent as well as its ability to reduce the egg-hatching rate in mosquitoes indicate that crude extract of *Artemisia annua* is a potential control agent that can be used in vector control programs. However, the mode of action of this extract and formulations for improving its larvicidal potency must be investigated to ensure better outcomes.

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