International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2018; 5(2): 35-38

Received: 07-01-2018
Accepted: 08-02-2018

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Larvicidal effect of *Artemisia annua* (Asterales: asteraceae) against the dengue fever mosquito vector *Aedes aegypti* (Diptera: Culicidae)

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Abstract
The larvicidal activity of four solvents leaf extracts from *Artemisia annua* (Asterales: Asteraceae), with four different concentrations, on controlling the Dengue Fever mosquito vector *Aedes aegypti* (Diptera, Culicidae) larvae was investigated under laboratory conditions. The solvents that used in the experiment are aqueous, chloroform, ethyl acetate and ethanol. Results showed that the ethanolic and aqueous extracts caused a higher mortality of *Ae. Aegypti*. The percentage mortality of the *Ae. Aegypti* larvae by using the ethanolic extract at 50000 ppm were 99%, 100% after 24 and 48 hours. In the same concentration, a hsigher toxicity of the aqueous extract has been shown against the *Ae. Aegypti* larvae showing percentage mortality of 72%, 84% and 93% after 24, 48 and 72 hours, respectively. The LC_{50} values of the aqueous and ethanolic extracts of *A. annua* for Aedes *aegypti*, were recorded as 1000.38, 45.25, and 120.37, 14.29 ppm after 24 and 72 hours, respectively. The results indicated that different solvent of leaf extracts of A. *annua* showed a concentration-dependent deterrent activity and had a strong mortalities effect when the ethanolic and aqueous extracts were used compared with other extracts. In conclusion, this study recommends that the crude extract of *A. annua* (ethanolic and aqueous) could be used as an alternative method in the dengue fever vector management programs.

Keywords: *Aedes aegypti*, the Dengue Fever Vector, *Artemisia annua*, aqueous extract, ethanolic extract, larvicidal activity

1. Introduction
Mosquitoes cause great threats to human health throughout their transmission of serious human diseases [1-2]. Malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection are example diseases that can be transformed to human by mosquitoes in almost tropical, subtropical countries and several regions in the world [1]. In tropical countries, these diseases can be significantly responsible to disease burden, death, poverty and social weakness [3]. In Saudi Arabia, Dengue Fever, which is transmitted by the Dengue Fever mosquito vector *Aedes aegypti* (Diptera, Culicidae), has a negative effect on human populations and the country's economy in the western parts of the country [4-5]. Worldwide, mosquito control is essential to improve the quality of the environment and public health in any society [6].

Classical mosquito control programs focus on larval stages to which synthetic insecticides solutions in oils as emulsions, wettable powder or dusts, have been applied in the breeding sites [7]. Indeed, this has not worked due to many factors such as human, technical, operational, ecological, and economic factors. In mosquito control programs, theuse of many synthetic insecticides is uncompleted. This is because many reasons include a lack of new insecticides, the cost of synthetic insecticides, concern the sustainability of the environment, negative effects on the human health and other non-target species as well as insecticide resistance on a large-scale [8]. This, in turn, has required to find a method that can be described as an environmentally safe for vector control.

Today, there is an increased attention for using botanical insecticides for controlling insect pests as an alternative to synthetic insecticides [9]. Plant extracts can offer a great promise as a source of phyto-chemicals with a potential effect as insecticides which can play an important role on controlling mosquitoes [10]. The medicinal herbal plant *Artemisia annua*, (Asterales: Asteraceae) also known as annual wormwood or sweet wormwood, grows naturally about

1000-1500 m above sea level and can be seen as annual weedy herb, usually single-stemmed, reaching up to 2 to 3 m in height in many countries including China, USA, Hungary, Bulgaria, Romania, Turkey, Argentina, Italy, France, Spain and India [11-12]. Traditionally, this herbal plant has been long used for many purposes including medicine and pest control. In the United Kingdom, the dried leaves of *A. annua* were used as a treatment for the great flea epidemic in the 18th century while the same plant materials were used to eradicate mosquitoes in China without any toxic effects on the environment [11]. There are many empirical studies showed the effect of *A. annua* on controlling mosquito species. For instance, [13] found that hexane extract of *A. annua* had a higher toxicity against *Anopheles sinensis* and *Aedes aegypti*. The effectiveness of *A. annua* as a larvicide and oviposition deterrent cause and its capability to reduce the egg-hatching rate in mosquitoes has led to suggest that the crude extract of *A. annua* is a potential control agent that can be used in the vector control programs [14]. Thus, this study aimed at investigating the toxicity effect of different *A. annua* leaf extracts against the 4th instar larvae of the Dengue Fever vector *Ae. Aegypti* under laboratory conditions.

2. Materials and Methods

2.1 Plant extracts

Foliages of *A. annua* were gathered from Al-Nafud Desert, which is placed in the northern part of Hail region, Saudi Arabia. Combined impurities such as weeds, soil particles and other inessential matters were carefully removed from the collected foliages of *A. annua*. After that, leaves of A. *annua* were separated, washed, dried under room temperature and kept away from the direct sunlight for three months. Then, an electric blender was used to powder the dried leaves of *A. annua*. Subsequently, 50 g of the powdered trial were obtained and homogenized with 100 ml of the following solvents: water, ethanol, chloroform and ethyl acetate. The shaker was used to mix the crude preparation for 24 hours at room temperature and then centrifuged at 4000 rpm for 20 minutes. This mixture was then relocated to a 250 ml beaker and the solvent was evaporated at 60 °C to concentrate the plant extract. To acquire the final concentrations of 50, 500, 5000 and 50000 ppm, the solid material of the plant extract was weighed and dissolved in a known amount of distilled water.

2.2 Mosquito cultures

Eggs of the Dengue Fever vector *Ae. Aegypti* were obtained from the Laboratory of Public Health Pests, Al Amana, Jeddah Governorate, Saudi Arabia. Plastic and enamel trays containing tap water were used to rear larvae of the Dengue Fever vector *Ae. Aegypti*. A diet of aquarium fish foods that contained macro nutrients, trace elements and necessary vitamins, was used to feed the larvae. Every day, the pupae were transferred to small bowls containing clean water. The bowls were placed in a cage (measuring 50×50×50 cm) covered with a fine mesh for adult emergence. From the day of emergence, adults were fed on 5% (W/V) sugar solution (as a carbohydrate source) absorbed on cotton in a small conical flask that was placed inside the cage, in addition to intervals of blood meals (on 1-week-old chicks).

2.3 Bioassay and the Dengue Fever mosquito vector mortality

Larvicidal activity of the leaf extracts of *A. annua* was determined by following the method of the WHO standard procedure [15]. In this case, means of dropper were used to move twenty five of the 4th instar larvae of *Ae. Aegypti* to the small test cups (250 ml), each containing 100 ml tap water to which four known concentrations were added. A completely randomized design in space was applied in which the tested cups were divided into 4 extract treatments and 4 concentrations. Each concentration was replicated four times. Also, a control treatment was designed and included in this experiment. The larval mortality was observed and counted after 24, 48 and 72 hours, respectively, of the exposure period.

2.4 Statistical data analyses

Larval mortality was subjected to statistical analysis using the Regression analysis tool included in the statistical package SPSS® 14.0 for Windows. The percentage of larval mortality of *Ae. Aegypti* was performed using the following formula:

$$\% \text{ of larvae mortality} = \frac{Mean\ larvae\ mortality}{Total\ of\ introduced\ larvae} \times 100$$

To determine the lethal concentrations of 50% (LC_{50}) on *Ae. Aegypti* larvae, the regression lines were created. Values of LC_{50} were calculated according to [16]. Data were corrected for control mortality using [17].

3. Results and Discussion

The mortality percentages of *Ae. Aegypti* larvae after 24, 48 and 72 hours are presented in table 1. In this table, ethanolic extract of *A. annua* was the most effective, where its concentration 50000 ppm, ethanolic and aqueous extracts caused 100% and 84% mortalities of the *Ae. Aegypti* after 48 hours, respectively. The ethanolic and aqueous extracts produced 100% and 93% mortalities of *Ae. Aegypti* showing their positive effect on controlling the *Ae. Aegypti* after 72 hours. The LC_{50} values and 95% confidence limits (LC) are given in table 2. All solvents of *A. annua* were toxic to *Ae. Aegypti* larvae in dose dependant manner, although the toxic action was relatively slow for chloroform and Ethyl acetate solvents and their efficacy varied (table1). The range of acute (48hr) and chronic (72 hr) LC_{50} values were 1000 and 45, 1919 and 58, 14432 and 105.9 and 120.4 and 14.3 ppm for aqueous, chloroform, ethyl acetate and ethanolic extracts, respectively (Table 2). Both ethanolic and aqueous extracts of *A. annua* had significantly lower LC_{50}s (48 and 72 hrs) than the chloroform and ethyl acetate extracts.

The findings of the present study indicate larvicidal activity in the four solvent extracts of *A. annua* against *Ae. Aegypti*. [18] Reported that 48% mortalities of the larval stages of *Xanthogaleruca luteola* when they were treated with a methanolic extract of *A. annua*. Comparing these results with that obtained in the current study, it can be shown that the ethanolic extract of *A. annua* has an excellent larvicidal effect against *Ae. Aegypti.*

The results of the present study illustrated a higher deterrence effect of *A. annua* extract. This finding is in agreement with the conclusion of [14] when they mentioned that the methanol

extract of *A. annua* could be considered as a strong deterrent effect which also affected the biochemical metabolism of the target pest. This study clearly showed that *A. annua* extract severely affects larval feeding behavior and mainly acts as an antifeedant. Certain compounds of plant origin on contact, such as monoterpinoids, may affect the nervous system [19]. By increasing concentration, deterrence index increases and a good dose-response can be achieved. Deterrence index in the highest concentration of ethanolic extract of *A. annua* reached 100% mortalities of the *Ae*. *aegypt* larvae and in the lowest concentration showed 69% mortality. In higher concentrations, the larvae might be leave a treated food may be due to a rapid deterrence evoked by chemical sensila on mouth parts or retracted pulses coming from stomodael nervous system after ingestion [20] or toxic effects after ingestion. However, [21] reported that toosendanin is not toxic. In addition, [13] reported moderate larvicidal effects of the hexane extract of *A. annua* against mosquitoes. In their experiment and 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 hexane extract of *A. annua* against mosquito species showed to be effective as a larvicide and oviposition deterrent agent as well as its ability to reduce the egg-hatching rate against mosquitoes. Moreover, [22] showed that Phytoproducts possess different bioactive components that can be used as general toxicants against various larval stages of mosquito's especially ethanolic extract of *Artemisia molinier*.

Table 1: Percentage mortality (%) of *Aedes aegypti* larvae treated with different concentrations of the four leaf extracts of *Artemisia annua* (aqueous, chloroform, ethyl acetate and ethanol) after 24, 48 and 72 hours.

	Aqueous			Chloroform			Ethyl acetate			Ethanol		
Conc. (ppm)	**(24h)**	**(48h)**	**(72h)**	**(24h)**	**(48h)**	**(72h)**	**(24h)**	**(48h)**	**(72h)**	**(24h)**	**(48h)**	**(72h)**
50	36	55	73	29	45	58	27	48	52	61	64	69
500	46	70	83	35	58	69	36	56	67	67	78	81
5000	55	71	85	39	70	75	49	58	80	76	92	98
50000	72	84	93	57	74	83	53	68	91	99	100	100
Control	1	2	6									

Table 2: Toxicological parameters of *Aedes aegypti* larvae treated with different concentrations of the four leaf extracts of *Artemisia annua* (aqueous, chloroform, ethyl acetate and ethanol) after 24, 48 and 72 hours.

	Aqueous			Chloroform			Ethyl acetate			Ethanol		
Toxicological parameters	**(24h)**	**(48h)**	**(72h)**	**(24h)**	**(48h)**	**(72h)**	**(24h)**	**(48h)**	**(72h)**	**(24h)**	**(48h)**	**(72h)**
R-square	0.8134	1.030	0.887	2.477	0.739	0.136	0.685	0.481	0.149	0.339	3.257	4.447
Slope (b)	0.30	0.28	0.26	0.29	0.27	0.24	0.24	0.62	0.42	1.45	0.62	0.72
LC_{50} (ppm)	1000.38	99.91	45.25	1919.12	120.66	58.50	14432.24	250.18	105.88	120.37	29.05	14.29
Probability(P)	0.666**	0.579	0.644	0.292	0.690	0.934	0.711	0.786	0.928	0.844	0.462	0.103

4. Conclusion

In conclusion, this study recommends that a crude extract of *A. annua* could be used as a alternative mean to synthetic pesticides in vector management program, especially in controlling the Dengue Fever vector *Ae. Aegypti*.

5. Acknowledgements

The author would like to thank Mr. Fahad Al- Zahrani for allowing me to conduct the experiment in the Laboratory of Public Health Pests facilities, Al Amana, Jeddah Governorate, Saudi Arabia.

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