

Effect of Four Indigenous Medicinal Plants on Dengue Fever, Vector *Aedes aegypti* from Jeddah, Saudi Arabia

Abbas M. Al-Azab^{1*}, Ahmed A. Zaituon², Khalid M. Al-Ghamdi³ and Fahd M. Abd Al-Galil^{4,5}

¹Department of Biological Sciences, Faculty of Sciences, Sana'a University, Yemen.

²Department of Arid Land Agriculture, Collage of Meteorology, Environment and Arid Land Agriculture - King Abdulaziz University, Saudi Arabia,

³Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Saudi Arabia,

⁴Department of Biological Sciences, Faculty of Sciences, University of Bisha, Bisha 61922, P. O. Box 551- Saudi Arabia and

⁵Department of Biological Sciences, Faculty of Sciences, Tamar University- Yemen.

ABSTRACT

Aedes aegypti is the vector mosquito of dengue fever, which is an endemic disease in Saudi Arabia, Jeddah. The use of conventional insecticides in water sources introduces risks to people and/or the environment. In this study, mosquitocidal activity and adult development inhibition were investigated using four medicinal plants (*Rhazya stricta*, *Lantana camara*, *R. chalepensis*, and *Punica granatum*) against *Ae. aegypti* under laboratory conditions. A laboratory bioassay was conducted to assess larvicidal activity of leaves and peels crude acetonetic extract of *L. camara*, *Ruta chalepensis*, *Rh. stricta* and *P. granatum*. Under controlled laboratory conditions, late 3rd or early 4th instar larvae were exposed to various concentrations – ranging from 150ppm to 1000 ppm – of the extracts to obtain the median lethal concentration (LC50) values for each plant extract tested. Mortalities were observed to increase with the increase in concentrations. Acetone extract of *Rh. Stricta* revealed high activity against *Ae. aegypti* compared to the rest of the extracts. The larval mortality rates of mosquito larvae ranged from 25 to 97% for *Rh. stricta*, 23 to 94% for *L. camara*, 17 to 96% for *R. chalepensis*, and 10 to 72% for *P. granatum*. In terms of effects on adult emergence, the inhibition percentage of the mosquito ranged from 26 to 100% for *Rh. stricta*, 13 to 100% for *L. camara*, 10 to 100% for *R. chalepensis*, and 22 to 92% for *P. granatum*. The evaluated medicinal plants seemed to be a better alternative to synthetic insecticides for controlling *Ae. aegypti*.

KEY WORDS: *AEDES AEGYPTI*, BIOASSAY, DENGUE FEVER, *LANTANA CAMARA*, MEDICINAL PLANTS.

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*Corresponding Author: abbasazab2000@gmail.com

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INTRODUCTION

Dengue fever is an arthropod-borne viral disease found in many tropical and subtropical countries throughout the world (Gubler, 2018; Hosseini et al., 2018). *Aedes aegypti* (L) is presently considered the main vector of dengue, yellow fever, Zika, and chikungunya viruses in many parts of the world (Kraemer et al., 2015; Mayer et al., 2017). For many decades, synthetic pesticides have been employed to successfully control the infection cycle. However, the development of insect resistance to synthetic pesticides has also been documented globally, (Smith et al., 2016, Goindin et al., 2017; David et al., 2018). High operational costs and environmental pollution have created the need for developing alternative approaches for controlling vector-borne disease (Ohia and Ana, 2015). In early historical time, humans used plant extracts and concentrated compounds to control certain pests and disease-causing insects (Pavela, 2016). Overall, the search for such compounds has been directed extensively at the plant kingdom, (Veer and Gopalakrishnan, 2016.; Ninan et al., 2017 Lakshmi et al., 2018).

Medicinal plants are common sources of bioactive compounds, especially in tropical and subtropical countries (Van Wyk and Wink, 2017). The use of phytochemicals is one such strategy that may be suitable for mosquito control. The first, commonly used plant extract against adult mosquitoes was the flower extract of *Chrysanthemum cinerariaefolium* in South Africa and India, (Maheswaran and Ignacimuthu 2012 and Govindarajan, 2016) Many authors across the world started large screening activities for using extracts of medicinal and herbaceous plants to control mosquitoes. Extracts and oils from leaves, flowers, and roots of plants were found to display mosquito larvicidal activity (Chansang et al., 2018; Pavela et al., 2018).

Several researches from all over the world have documented the toxic effect of plant extracts such as *L. camara* and *Rh. stricta* and those of essential oils in controlling *Ae. aegypti* L mosquito (Ghosh et al., 2012; Hari and Mathew, 2018; Kalita et al., 2012; Kumar and Maneemegalai, 2008; Mappau and Ganing, 2018; Muangmoon et al., 2018; Ved et al., 2018). *Lantana camara* Linn. (Verbenaceae) is a hardy, evergreen, straggling shrub with a characteristic odor; it has antibacterial, antifungal, and insecticidal properties, and its oil provides protection from *Aedes* mosquitoes (Mossa et al., 1987). *R. chalepensis* L. (*Rutaceae*), commonly known as “ruda” is a perennial herb, which is widely distributed in the Mediterranean region and the Kingdom of Saudi Arabia area (Verma, 2018). The toxic effect of the *R. chalepensis* extract against insects, particularly mosquitoes, have been reported previously, (Morsy et al., 1998, Al-Myah et al., 2011; Madkour et al., 2012). It is an ancient medicinal plant still used in the traditional medicine of many countries as recently reported by Bedini et al., (2018).

There is a scarcity of literature regarding the insecticidal potential of *P. granatum*. Its reported larvicidal activity was observed against the instar larvae of *Chrysomya albiceps* decades ago in Egypt (Sameeh et al., 2010). Moreover, its larvicidal activity was also reported recently against the larvae of *Anopheles pharoensis* (Eidi et al., 2005). To the best of the author's knowledge, this is the first study to address the larvicidal properties of *P. granatum* and *Rh. stricta* against *Ae. aegypti*. In this study, mosquito larvicidal activity and adult development inhibition were investigated using four medicinal plants (*Rh. stricta*, *L. camara*, *R. chalepensis*, and *P. granatum*) against the dengue fever vector *Ae. aegypti* under laboratory conditions.

MATERIALS AND METHODS

Mosquito strain source and rearing: *Ae. aegypti* (Diptera; Culicidae) was obtained from the mosquito laboratory at Al-Amara, Jeddah, Saudi Arabia. The *Ae. aegypti* (L) larvae were reared under laboratory conditions for over 12 generations using diet media fish food. Larvae from the culture were used for the different treatments described in this study. The stock colony was maintained at room temperature ($27\pm 1^\circ\text{C}$) and relative humidity of $70\pm 5\%$ with a 14:10 (LD) photoperiod throughout the study. The adults were fed on a 10% sugar solution.

Plant collection and extraction: The medicinal plants *L. camara*, *R. chalepensis*, *Rh. stricta*, and *P. granatum* were collected from the campus of King Abdulaziz University, Jeddah and were identified by an experienced botanist at the department of Botany. Fresh leaves from the plants were washed and shade-dried at room temperature for one week. They were then prepared to extract the effective ingredients according to the published standard method (WHO, 2005). Forty to sixty gm of leaf tissues were finely ground and loaded into a 250 ml glass stoppered Soxhlet apparatus. Absolute Acetone (200 ml) was added to the glass and the extraction was performed for 6 hours. The rotary evaporator was used to concentrate the extracts to turn them into a semi-dry material. The extracted components were kept at -10°C until use for testing against selected insect stages.

Bioassay of plant extract against *Ae. aegypti* (L): The larvicidal bioassay followed the WHO standard protocols (Finney, 1972). The stock solution of each plant extract was prepared by adding 1 ml of re suspended extracts to 99 ml of distilled water containing 0.3% dimethyl sulphoxide (DMSO). From this stock solution, concentrations of 150, 250, 500, 800, and 1000 ppm were prepared in distilled water. Larval treatments were carried out by exposing the late 3rd or early 4th instar larvae continuously to various concentrations of the plant extracts. Tests were performed in groups of 250

ml glass beakers containing 100 ml tap water. Four replicates of 25 larvae for each concentration and control trial were assembled. The larvae were given the usual larval food during these experiments. After 24 hours of exposure time, the percent of mortality was recorded. The cumulative mortalities of larvae and pupae were recorded daily for the tested plants. Live pupae were transferred to untreated water in new beakers for further observation. X Actelic 50% EC (Primiphosmethyle) was tested as a positive control, while acetone was the negative control.

Statistical Analysis: This study used a completely randomized design (CRD) in a factorial experiment. The collected data were statistically analyzed using analysis of variance (ANOVA) tools, and the means were compared by LSD at $p \leq 0.05$ using the SAS software program [SAS Institute (2006) version 9.3]. IC50 and IC95 were calculated according to the Probit analysis program (Finney 1972, Abbott, 1925). The 95% confidence intervals, values, the χ^2 goodness of

fit test, and regression equations were estimated by a computerized log-probit analysis. The mortalities were corrected for control mortality using Abbott's formula (Tisgratog et al., 2016).

RESULTS AND DISCUSSION

The screening of herbal bioactive insecticides is a rapidly emerging research field that aims to find potential mosquito control agents with advantages such as low costs, ease of administration, and risk-free properties that can overcome the resistance to synthetic insecticides, and safety to the environment (Remia and Logaswamy, 2010). In the present study, crude leaves / peel extracts of *Rh. stricta*, *L. camara*, *R. chalepensis* and *P. granatum* showed larvicidal activity against *Ae. aegypti* when the larvae were exposed to concentrations of the plant extract ranging from 150 to 1000 ppm for 24 hours. The detailed results of the larvicidal activity of, *Ae. aegypti* larvae are presented in Tables 1 and 2 and depicted graphically in Figs. 1–4.

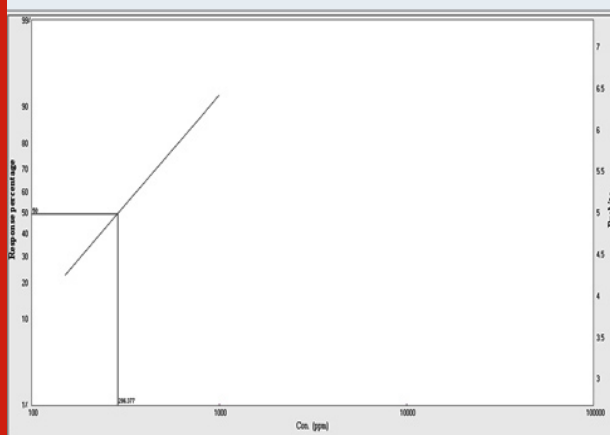
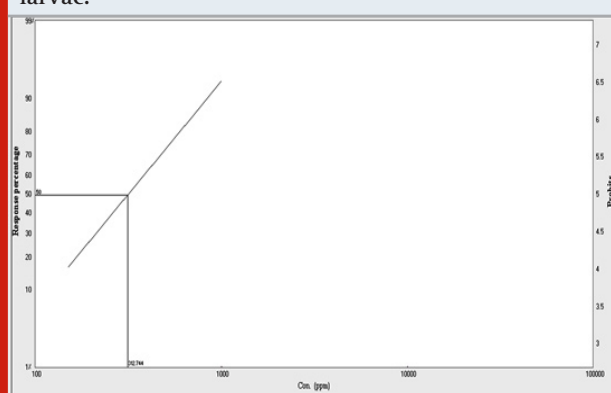
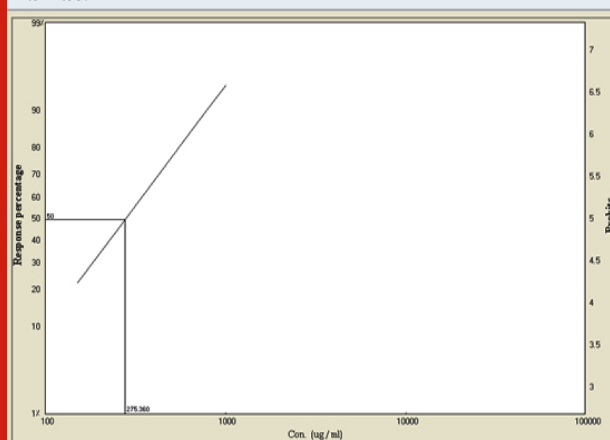
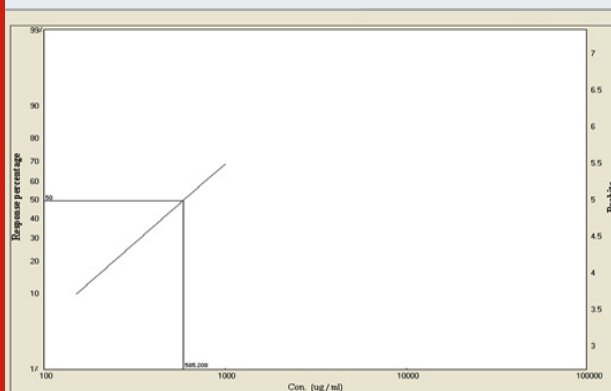
Table 1. Toxicity effect of four plant extracts against different stages of *Ae. aegypti*.

Plant extract	Conc. (ppm)	Larval Mortality (%) ^A	Pupation %	Adult emergence	
				Total	Inhibition (%) ^B
<i>Rh. stricta</i>	150	25	75	67	26
	250	42	58	50	44
	500	77	23	17	80
	800	90	10	10	89
	1000	97	3	0	100
Control	8	92	90	10	
<i>L. camara</i>	150	23	77	69	27
	250	44	66	38	60
	500	74	26	23	76
	800	86	14	7	93
	1000	94	6	0	100
Control	3	97	95	5	
<i>R. chalepensis</i>	150	17	83	77	33
	250	39	61	40	60
	500	72	28	21	79
	800	87	13	8	92
	1000	96	4	0	100
Control	2	98	96	4	
<i>P. granatum</i>	150	10	90	72	22
	250	23	77	51	47
	500	41	59	36	61
	800	60	40	20	78
	1000	72	28	7	92
Control		5	95	92	8
Positive control	2	100			

A Four replicates of 25 larvae each B Corrected with Abbott's formula [35]

Table 2. Statistical parameters of plant extract against *Ae. aegypti*.

Statistical parameters	<i>Rh. stricta</i>	<i>L. camara</i>	<i>R. chalepensis</i>	<i>P. granatum</i>
LC ₅₀ (ppm)	275	286	312	585
Inhibition of (%) adult development	48	68	75	71
95% (*F. L)	233–298	251–320	280–345	550–696
LC95 (ppm)	729	1203	1095	2050
95% (*F. L)	648–842	990–1560	931–1149	1628–2873
Slope	2.8	2.63	3.02	2.43
Calculated (Chi) ²	1.26	0.87	1.3	1.04
Tabulated (Chi) ²	7.8	7.8	7.8	7.8
R ²	0.96	0.90	0.92	0.98

Figure 1: The relationship between concentrations of *L. camara* extract and mortality percentage of *Ae. aegypti* larvae.Figure 3: The relationship between concentrations of *R. chalepensis* extract and mortality percentage of *Ae. aegypti* larvae.Figure 2: The relationship between concentrations of *Rh. stricta* extract and mortality percentage of *Ae. aegypti* larvae.Figure 4: The relationship between concentrations of *P. granatum* extract and mortality percentage of *Ae. aegypti* larvae.

The mortality rates of the larvae exposed to various concentrations of the extracts were 25–97% (*Rh. stricta*), 23–94% (*L. camara*), 17–96% (*R. chalepensis*), and 10–72% (*P. granatum*).

The acetone extract of *Rh. stricta* showed a significantly high larvicidal activity against *Ae. aegypti* compared with the other three extracts. The LC₅₀/ LC₉₅ for the four plant values were 275/729, 286/1203, 312/1095 and 585/2050 ppm for *Rh. stricta*, *L. camara*, *R. chalepensis*, and *P. granatum* respectively.

The findings of the present study are comparable to several earlier studies, (Remia and Logaswamy, 2010, Ghosh et al., 2012; Mappau and Ganing, 2018). Indeed, Ghosh and associates reported that the LC50 value of the *Rh. stricta* plant extract against mosquitoes was 251 ppm, while Remia and Logaswamy reported that the highest mortality of acetone extracts of *L. camara* leaves against *Ae. aegypti* larvae with LC50 value was 230.7 ppm (Ghosh et al., 2012; Remia and Logaswamy, 2010). However, a higher value for LC50 (468.5 ppm) was reported by Indian scholars (Jayaraman et al., 2015). On the other hand, a higher mortality rate (85% at 150 ppm conc.) of *Ae. aegypti* 4th instar larva was reported in a recent study (Hemalatha et al., 2015).

Currently, the larvicidal mechanisms of phytochemicals are poorly understood. Recently, it has been reported that some alkaloids inhibit in vitro activity of glutathione S-transferase (GST) and acetylcholinesterase (AChE) of *Lymantria dispar* and the octopaminergic system / receptor in *Ae. aegypti* (Wang et al., 2019; Zou et al., 2017). However, subsequent studies revealed that protein carbonylation mediates an oxidative damage to the proteins involved in the energy metabolism of *Ae. aegypti* larvae (Rodríguez-Cavallo et al., 2018). The presence of certain phytochemicals (alkaloids, steroids, and phenolics) in plant extracts might be the reason for their larvicidal activity (Lakshmi et al., 2018). Unfortunately, scientific literature is unavailable regarding the efficacy of *R. chalepensis* and *P. granatum* against *Ae. aegypti*. However, their effects against other species of mosquitoes have been tested. For instance, *P. granatum* has been evaluated for its larvicidal activities against *Culex gelidus* and *Culex quinquefasciatus* mosquitoes. Methanol extracts showed absolute mortality of the larvae at 500 µg/ml after 24 hours (Kamaraj and Rahuman, 2010). Strikingly, a recent Libyan study found the acetone extract of *R. chalepensis*' aerial parts to demonstrate powerful larvicidal activity (LC50 at 1.08 ppm) against *Culex pipiens* (El-Bokl, 2016).

The cumulative mortalities during larval development to pupae and adults were recorded for the evaluation of the tested plants. The inhibition percentage of adult emergence were 26–100%, 27–100%, 33–100%, and 22–92% for *Rh. stricta*, *L. camara*, *R. chalepensis*, and *P. granatum* respectively (Table 1). Furthermore, mortality rates were observed at both the pupal stage and incomplete adult emergence. This could be due to morphological aberrations leading to failure of emergence from the exuviae of the pupal stage (Yu et al., 2015). The *R. chalepensis* extract exhibited the highest inhibition of adult emergence (75%) at LC50 (275 ppm), whereas the *Rh. stricta* extract showed the lowest activity (48%) at LC50 value (585 ppm) (Table 2). As shown in Table 2, when compared with earlier studies, these results revealed that the experimental plant extracts were effective in

controlling *Ae. aegypti* and showed promising results (Ghosh et al., 2012; Morsy et al., 1998). The activity of plant extracts is often attributed to the complex mixture of active compounds (Kumar et al., 2012). The high efficacy of *Rh. stricta* and *L. camara* extracts may be due to the presence of chemical components including furanonaphthaquinones regioisomers and camaric acid as reported previously (Kamaraj et al., 2011). However, the biological effect of *R. chalepensis* against mosquitoes might be attributed to its high content of biologically active alkaloids (Sousa and Costa, 2012).

CONCLUSION

The acetone extract of *Rh. stricta*, *L. camara*, *R. chalepensis*, and *P. granatum* could comprise other approaches to be utilized for controlling the dengue vector mosquito, *Ae. aegypti*. These results could encourage the search for new active natural compounds offering a suitable alternative to insecticides synthesized from other medicinal plants; these alternatives would be safer and easier to handle than synthetic insecticides.

Authors' Contributions: A Al-Azab performed the experiments, analyzed the data, and wrote and revised the manuscript. Both A Zaituon and K Al-Ghamdi conceptualized the research idea designed the study, coordinated the work and wrote and revised the manuscript. F. Abd Al Galil assisted in collecting the literature search, read and revised the final manuscript. All authors read and approved the final manuscript for publication

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