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The effect of different extracts of Fattaka fruits (*Pergularia tomentosa* L.) on controlling the Dengue Fever vector (*Aedes aegypti*) larvae under laboratory conditions

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Abstract

This study was conducted as a pilot study to evaluate the toxicity of three different extracts (water, ethanol and acetone) from Fattaka fruits, *Pergularia tomentosa* L. (Asclepiadaceae), at five different concentrations against the fourth instar larvae of the Dengue Fever mosquito vector *Aedes aegypti* (Diptera: Culicidae). Results showed that the ethanolic extract caused a higher mortality of *A. aegypti* larvae compared with other solvents. In the ethanolic extract treatment, the percentage mortalities of the *A. aegypti* larvae ranged between 16.25% at the lowest concentration and 97.5% at the highest concentration after 24 hours. Also, toxicological parameters including LC_{50} and LC_{95}, toxicological index and Slope of the log-dose-probit lines showed that the ethanolic extract of this plant was more effective for controlling *A. aegypti*, showing LC_{50} of 0.06 ppm and LC_{95} of 2.37 ppm. This was not greatly different at 48 hours in which the mortalities of *A. aegypti* ranged between 32.5% and 98.75%, and the LC_{50} was 0.025 ppm and the LC_{95} was 1.668 ppm. Overall, this study suggests that more studies on the chemistry of *P. tomentosa* and its bioassays are required before confirming its use to biocontrol mosquitoes.

Keywords: *Pergularia tomentosa*, Fattaka, Dengue fever vector, Botanical pesticides, *Aedes aegypti* control.

1. Introduction

The Dengue Fever vector mosquito *Aedes aegypti* is the main vector of the viruses that cause Yellow Fever and Dengue Fever. Dengue Fever disease has seen a four-fold increase in incidence since 1970 and is a major public health problem threatening about 2.5 billion people worldwide, with 50–100 million new infections per year (Gubler, 1998; Remme *et al*., 2002). The World Health Organization (WHO) has reported that the global prevalence of Dengue Fever has increased dramatically, and this disease has become endemic in more than 100 countries in Africa, America, South-east Asia and the western Pacific (WHO, 2009). During the last two decades, the epidemic of dengue with sporadic incidence of dengue hemorrhagic fever (DHF) has occurred in the cities of Jeddah and Makkah, Kingdom of Saudi Arabia (KSA) (Gubler, 1997). This disease has a major impact on the health and economy of human populations (Alwafi *et al*., 2013).

The Dengue Fever vector *A. aegypti* is a robust mosquito species, suitable for mass-rearing. Controlling vectors and pest mosquitoes by using chemical pesticides has generated several problems including: safety risks for humans and domestic animals, insecticide resistance and other environmental concerns (Lacey, 1990). Generally, populations of larvae inhabiting ground pools are limited by natural enemies, whereas those encountered in containers are usually limited by resource availability. Such physical and biological features could have significant implications for the successful implementation of biocontrol programs (Washburn, 1995). In recent years, there is a growing interest for using botanical pesticides as an alternative to synthetic pesticides (Asiry, 2015). Many active chemicals can be derived from plants as secondary metabolic compounds such as alkaloids, terpenoids, cucurbitacin, glycosides, flavonoids and other compounds that have been used for controlling several insect pests including mosquito species (Asiry, 2015).

Pergularia tomentosa L. is a perennial plant about 50–60 cm high, reaching 1.0 m in good conditions and it is a poisonous plant (Babaamerab *et al*., 2013). This plant, known as Fattaka or Am Lebina in Saudi Arabia, belongs to the family of Asclepiadaceae and has a wide distribution, where it is commonly found in the Middle- East region including Saudi Arabia and Jordan. Also, it is known to be distributed in the Saharan and Sub-Saharan countries of North Africa including Algeria, Niger, and Egypt (Babaamerab *et al*., 2013).This plant is known to produce corrosive white latex that may severely harm the human skin. It has been shown that the crude alkaloids extract of *P. tomentosa* has a considerable larvicidal, antifeedant and weight loss effects against the fifth instar larvae of the migratory locust *Locusta migratoria*, suggesting this plant could be used as a natural product for locust larval control (Acheuk and Doumandji-Mitiche, 2013). However, no study has been conducted to test the effect of *P. tomentosa* on controlling mosquitos. The current study aimed at investigating the toxicity effect of different *P. tomentosa* fruit extracts against the 4th instar larvae of the Dengue Fever vector *A. aegypti* under laboratory conditions.

2. Materials and Methods

2.1. Preparation of *Pergularia tomentosa* extracts

The above-ground shoots of *P. tomentosa* were collected from the main campus of the University of Hail at Hail region which is located in the northern central part of Saudi Arabia. The collected plants were carefully isolated from combined impurities such as weeds, soil particles and other inessential matters. Fruits of the collected plants were washed, dried under room temperature, away from the direct sunlight, for four weeks. To prepare the extracts of *P. tomentosa*, dried fruits were powdered using an electric blender, and then 50 g of the powdered sample were taken and homogenized with 100 ml of the following solvents: water, ethanol, and acetone. The crude preparation was left for 24

hours in the shaker at room temperature, and then was centrifuged at 4000 rpm for 20 minutes. The supernatant containing the plant extract was then transferred to a 250 ml beaker and the extract was concentrated by evaporating the solvent at 60°C. The solid substance was weighed and dissolved in a known volume of distilled water to obtain the final concentrations of 5, 0.5, 0.05, 0.025 and 0.005 ppm.

2.2. Vectors rearing

Eggs of the Dengue Fever vector *A. aegypti* were obtained from the Laboratory of Public Health Pests, Al Amana, Jeddah Governorate, Saudi Arabia. Larvae were reared in plastic and enamel trays containing tap water in the Biological Control Laboratory at the University of Hail. Larvae were fed on a diet of aquarium fish foods that contained macro nutrients, trace elements and necessary vitamins. On a daily, 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 mosquito mortality

The experiment was carried out at 27 ± 2^0 C and 75–85% RH under a 14:10 L/D photo period. Larvicidal activities of the fruit extracts of *P. tomentosa* were determined by following the method of the WHO standard procedure (WHO, 2005). Twenty of the 4th instar larvae of *A. aegypti* were moved by means of dropper to the small test cups (250 ml), each containing 100 ml tap water to which five known concentrations were added. A completely randomized design in space was applied in which the tested cups were divided into 3 treatments and 5 concentrations. Five replicates of each concentration for each fruit extract of *P. tomentosa* were applied, resulting in the total of 75 tested cups (3 extracts × 5 concentrations × 5 replicates) used in this experiment. Larval mortality was assessed by observing and counting larvae that were dead in the tested cups after 24 hours and 48 hours. A control batch was also designed and included in this experiment.

2.4. Statistical data analyses

Larval mortality was subjected to statistical analysis using the Regression analysis tool included in the statistical analysis package SPSS® 14.0 for Windows. Concentrations were transferred to log (+3) to avoid the negative values (Finney, 1936). The percentage of larval mortality of *A. aegypti* was performed using the following formula:

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

To achieve the corresponding log-concentration probit lines, calculated percentage mortality of *A. aegypti* larvae was plotted with consistent concentrations on logarithmic probability paper. The regression lines were created to determine the lethal concentrations of 50% and 95% (LC_{50} and LC_{95}) on *A. aegypti* larvae. The probit and toxicity index (LC_{50} and LC_{95}) was calculated according to Abbot (1925), Finney (1936) and Busvine (1971). However, LC_{50} and LC_{95} were not calculated in the case of the aqueous extract because the *A. aegypti* larvae's mortality did not reach 20%.

3. Results

Data analysis showed the larvicidal potentiality of different fruit extracts of *P. tomentosa* on the mortality of the 4th instar larvae of *A. aegypti* after 24 and 48 hours. The percentage mortality (after 24 hours) of larvae of *A. aegypti* larvae calculated to be 65%, 97.5% and 12.5% when larvae were treated with the acetone, ethanol and aqueous extracts of *P. tomentosa* fruit, respectively, at the concentration of 5 ppm, showing the ethanol extract being the most effective in reducing the *A. aegypti* larvae after 24 hrs (Table. 1). Moreover, data revealed that the ethanol extract of *P. tomentosa* fruit was the most potent to control *A. aegypti* larvae when they were treated at lower concentration (0.005 ppm). This is very clear in Table (1) in which the percentages of larval mortality of *A. aegypti* ranged between 6.25% - 12.5% and between 5% - 18.75% when the *A. aegypti* larvae were treated with the aqueous extract for 24 hours and 48 hours, respectively.

Considerable mortalities were observed when the acetone extract was used against mosquito larvae. The mortality ranged between 11.25% at the lowest concentration (0.0005 ppm) to 65% at the highest concentration (5 ppm) at a submission period of 24 hours with LC_{50} of 2.62 ppm and LC_{95} of 17082.76 ppm. The case differed obviously at 48 hours in which the mortalities ranged between 22.5% - 82.5%; and the LC_{50} was 0.29 ppm and the LC_{95} was 549.54 ppm (Tables 1 & 2).

Concerning the ethanol extract, larval mortalities ranged between 16.25% at the lowest concentration to 97.5% at the highest concentration at 24 hours with LC_{50} of 0.06 ppm and LC_{95} of 2.37 ppm. The case was not greatly different at 48 hours as the mortalities ranged between 32.5% - 98.75%; and the LC_{50} was 0.025 ppm and the LC_{95} was 1.668 ppm (Tables 1 & 2).

The ethanolic extract of *P. tomentosa* showed an average of maximum toxicity (97.5 and 98.75%) in which this extract exhibited more effectiveness than the acetone and aqueous extracts. Slope measures of the toxicity lines of the aqueous, ethanolic and acetonic extracts of *P. tomentosa* fruit were 3.21 and 3.37, 3.74 and 3.13, and 3.77 and 3.53, respectively for 48 and 24 hours (Table 2 & Fig.1).

Table (1): The mean percentage mortality (%) of *Aedes aegypti* larvae treated with different concentrations of the aqueous, ethanol and acetone fruit extracts of *Pergularia tomentosa* fruits after 24 and 48 hours.

Concentration (ppm)	Acetone 24 hrs	Acetone 48 hrs	Ethanol 24 hrs	Ethanol 48 hrs	Water 24 hrs	Water 48 hrs
0.005	11.25	22.5	16.25	32.5	6.25	5
0.025	21.25	26.25	18.75	42.5	6.25	6.25
0.05	22.5	31.25	41.25	43.75	8.75	8.75
0.5	24	36.25	82.5	83.75	10	11.25
5	65	82.5	97.5	98.75	12.5	18.75
Control	0	0	0	0	0	0

Table (2): The transformed percentage mortality (%) and toxicological parameters of *Aedes aegypti* larvae treated with different concentrations of the aqueous, ethanol and acetone fruit extracts of *Pergularia tomentosa* fruits after 24 and 48 hours.

Concentration (ppm)	Log(conc.(+3))	Acetone 24 hrs	Acetone 48 hrs	Ethanol 24 hrs	Ethanol 48 hrs	Water 24 hrs	Water 48 hrs
0.005	0.699	3.77	4.26	4.01	4.56	3.45	3.36
0.025	1.000	4.19	4.36	4.12	4.82	3.45	3.45
0.05	1.699	4.26	4.5	4.77	4.85	3.66	3.66
0.5	2.699	4.29	4.64	5.95	5.99	3.72	3.77
5	3.699	5.39	5.95	7.05	7.33	3.82	4.12
Toxicological parameters							
R-square	-	0.801	0.8	0.993	0.936	0.924	0.972
slope	-	3.53	3.77	3.13	3.74	3.37	3.21
x-coefficient	-	0.43	0.5	1.04	0.9	0.13	0.24
LC_{50}	-	2.62	0.29	0.06	0.025	-	-
LC_{95}	-	17082.76	549.54	2.37	1.668	-	-

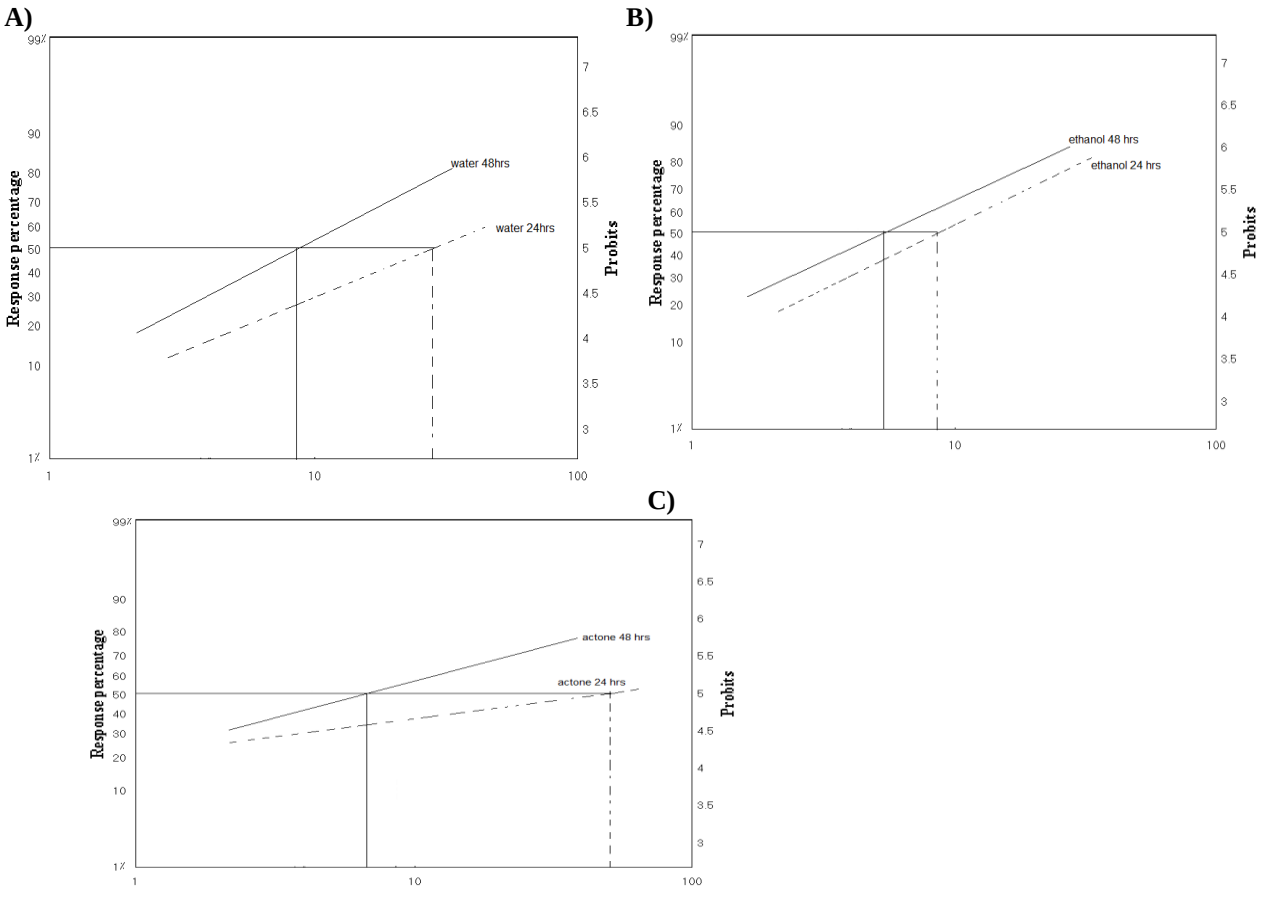

Figure (1): Log- Probity curves of the three fruit extracts of *Pergularia tomentosa* fruits: A) Water, B) Ethanol and C) Acetone that affected the mortality of *Aedes aegypti* larvae after 24 and 48 hours.

4. Discussion

The Dengue Fever vector *A. aegypti* is one of the most important mosquito species that are responsible for increasing the dengue fever in the western part of Saudi Arabia (Alshehri, 2013). There is a growing worry regarding the situation of Dengue Fever in Jeddah city as the number of the Dengue Fever cases are rapidly increased in the past few years (AL-Ghamdi *et al.*, 2009). This, in turn, has led to an increased application of synthetic pesticides to control the dengue fever vector. The extensive use of synthesized insecticides has adverse effects on humans and the environment (Marshall *et al.*, 2003; Pimentel, 2005; Palis *et al.*, 2006; Schou *et al.*, 2006; Winqvist *et al.*, 2011). Consequently, this study was conducted to evaluate the toxicity of different *P. tomentosa* fruit extracts, as an alternative biocontrol method to synthesized insecticides, against the 4th instar of the Dengue Fever vector *A. aegypti* under laboratory conditions for the first time.

The results showed a higher toxicity of the ethanloic extracts of *P. tomentosa* against the 4th instar larvae of the Dengue Fever vector *A. aegypti*, suggesting this plant is very poisonous to mosquito larvae. There are no other studies that have been carried out to examine the extracts of *P. tomentosa* against any species of mosquitoes in order to compare the current results with others. However, Acheuk and Doumandji-Mitiche (2013) reported the significant effect of alkaloids in *P. tomentosa* plant against the fifth instars larvae of the migratory locust *L. migratoria*, causing larvicidal, antifeedant and weight loss. This could explain in part the higher larval mortality of *A. aegypti* when they were treated with *P. tomentosa* fruit extracts in the current study.

This study suggests that more chemical and long-term studies on the bioassay of *P. tomentosa* extracts against mosquito species are needed to understand the underlining mechanism of how *P. tomentosa* extracts control the Dengue Fever vector *A. aegypti* larvae. This, in turn, could provide a decision for making a recommendation to use it as a sustainable biocontrol method.

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