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ORIGINAL ARTICLE

Larvicidal and repellent effect of some *Tribulus terrestris* L., (Zygophyllaceae) extracts against the dengue fever mosquito, *Aedes aegypti* (Diptera: Culicidae)

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Abstract *Aedes aegypti* transmits etiologic agents of yellow fever and dengue. Vaccine for dengue virus is not available and vector control is essential to minimize dengue incidence. The larvicidal and repellent effect of the crude ethanol, acetone and petroleum ether extract leaves of *Tribulus terrestris*, against 3rd instar larvae and adults of mosquito, *Ae. aegypti* the vector of dengue fever was evaluated. The efficacy of petroleum ether extract seemed to be more effective with LC_{50} 64.6 ppm followed by acetone extract with LC_{50} 173.2 ppm and finally ethanolic extract with LC_{50} 376.4 ppm. Moreover, the acetone and petroleum ether extracts exerted a highly delayed toxic effect on the pupae and adults resulted from treated larvae, where the pupal mortality was 57.1% and 100% at concentrations 400 and 100 ppm, respectively. Also, the petroleum ether and acetone extracts showed reduction effects on adult emergence. The repellent action of the plant extracts tested was varied depending on the solvent used in extraction and the dose of the extract. The most effective plant extract that evoked 100% repellency or biting deterrence was petroleum ether extract at a dose of 1.5 mg/cm^2 compared with 100% repellency for commercial formulation, *N*,*N*-diethyl-3-methylbenzamide (DEET) at the same dose. Hence, these extracts can be used as an effective alternative to the existing synthetic pesticides for the control of *Ae. aegypti*.

1. Introduction

Insect-transmitted disease remains a major cause of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually (Taubes, 2000). Therefore, the control of mosquitoes is an important public health concern around the world. *Ae. aegypti* (Culicidae) occurs in Asia, Africa and Central and South America and transmits virus of

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Flavivirus genus, etiologic agents of human diseases like dengue and yellow fever (Roberts, 2002).

Yellow fever immunization programs have reduced the risk of outbreaks in some endemic countries and the disease still occurs in epidemic patterns only in some countries of Africa and Asia (World Health Organization, 2001). On the other hand, there is no vaccine for dengue fever; vector control is the only form to minimize the transmission of the virus. It can be caused by four serotypes of the DEN arbovirus and clinically can happen in asymptomatic forms, classic dengue fever, hemorrhagic dengue fever and other more severe forms. Worldwide, 2.5 billion of people are in risk to acquire the disease and 50 million are infected every year, characterizing a pandemia (World Health Organization, 2002). Dengue outbreaks in Geddah and Makkah in the Kingdom of Saudi Arabia (K.S.A.) in 1994 and re-emerging the second time in 2004, 2005, 2006, 2010 with cases of dengue registered by Ministry of Health reached 400, 291, 305, 500 and 710, respectively (http://www.aawsat.com).

Currently, most insecticides are non-selective and can be harmful to other organisms and to the environment. There is an urgent need to develop new materials for controlling mosquitoes in an environmentally safer way, using biodegradable and target-specific insecticides against them (Isman, 2006; Pavela,2007; Jawale et al., 2010). Bioactive organic compounds produced by plants can act as repellent, oviposition or food deterrents, growth inhibitors, and toxins (Ezeonu et al., 2001; Carlini and Grossi-de-Sá, 2002). Thus, crude plant extracts have been screened as natural and biodegradable forms to control pests and vectors of infectious diseases (Omena et al., 2007). Based on the foregoing, the present study aimed to scientifically evaluate the larvicidal and repellency or antifeedant activity of *Tribulus terrestris* leaf extracts against the larvae and adults of the mosquito vector, *Ae. aegypti*.

2. Materials and methods

2.1. Rearing of *Aedes aegypti*

The dengue fever mosquito, *Ae. aegypti* larvae were collected in December 2011 through dipping method from the natural sites located in a village called Sanba at a distance of 10 km from Jazan, Kingdom Saudi Arabia to establish the mosquito colony in our laboratory in the Department of Biology faculty of science, Jazan University. Larvae were then reared in 250 ml of glass beakers containing 200 ml of distilled water. 2% (w/v) of brewer's yeast solutions was routine wise supplied to them as a food source. Pupae were transferred to glass beakers (100 ml) containing 80 ml of distilled water and maintained in the standard mosquito cages ($30 \times 30 \times 30$ cm) for adult emergence. Adults were maintained by providing with cotton circles soaked with 10% honey solution. Two days after emergence, female mosquitoes were allowed to blood feed periodically from pigeon host. A few days after having a blood meal, gravid mosquitoes laid their eggs on the filter paper strips in the glass cups. The filter paper strips with eggs were brought to dry under laboratory condition for overnight and then kept in the same condition until use for larval hatching. Mosquito rearing and all experiments were conducted under laboratory conditions at 27 ± 2 °C, relative humidity 70 ± 10% and 12 h L:D phase.

2.2. Plant collection and extraction

Fresh leaves of *T. terrestris* (Family: Zygophyllaceae) were collected in the month of November 2011 from the Sabia city (Sabia Jazan desert road). The plant was identified by Dr. Wael Kassem, Assistant Professor of Plant Taxonomy, Biology Department, Faculty of Science, Jazan University, and by comparison with the published plant description in flora of Saudi Arabia (Migahid, 1987). A voucher specimen has been deposited in the herbarium of Biology Department, Faculty of Science, Jazan University. The leaves were washed and dried in the shade at room temperature (27–31 °C) for 7 days till they become brittle, then pulverized to powder in a hammer mill. The extraction was performed using 70% ethanol, acetone and petroleum ether solvents. One hundred grams of powder for each solvent separately was extracted three times with 300 ml of aqueous 70% ethanol, acetone and petroleum ether at room temperature. After 24 h, the supernatants were decanted, filtrated through a Whatman filter paper No. 5. and dried in a rotary evaporator at 40 °C for (2–3) hours to ethanol and (40–60) minutes to other solvents to obtain 14.7 g (ethanol), 6.3 g (acetone) and 2.2 g (petroleum ether) of a semi solid crude extract. The dry extracts were kept in a deep freezer (−4 °C) until used for experiments.

2.3. Larvicidal test

In order to study the toxicity of the concerned plant extracts, the tested material of the ethanolic extract was dissolved in 0.1 ml of 70% ethanol, while the tested material of acetone and petroleum extracts was dissolved in 2 drop of Tween. 80 as emulsifier to facility the dissolving of tested material in water. Different concentrations of each extract were prepared in order to detect mortalities. All tested materials were performed in 100 ml of dechlorinated tap water contained in 200 ml plastic cups. Then, third instar larvae were put immediately into plastic cups that contained different concentrations of extracts. At least three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions (27 ± 2 °C, RH 70 ± 10% and 12–12 light–dark regime). Control larvae received only 0.1 ml of 70% ethanol or 2 drop of Tween.80 in 100 ml water. Mortality was recorded daily and the dead larvae and pupae were removed until adult emergence.

2.4. Repellent/antifeedant test

Standard cages ($20 \times 20 \times 20$ cm) were used to test the repellent activity of plant extracts. Different weight from each extract was dissolved in 2 ml (70% ethanol or water + drop of Tween) in glass 4×4 cm to prepare different concentrations. One ml from each concentration was directly applied onto 5×6 cm of ventral surface of pigeon after removing feathers from the abdomen to evaluate the repellency against *Ae. aegypti*, compared with commercial repellent (Off!) 15% Deet (*N*,*N*-diethyl toulamide) (Johnson Wax Egypt) as a positive control. After 10 min of treatment, the treated pigeons were placed in the cages containing at least 20 *Ae. aegypti* starved females 5–7 d-old for 4 h. Control tests were carried out alongside with the treatments using ethanol or water. Each test was repeated thrice to get a mean value of repellent.