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Evaluation of some plant extracts of neem *Azadirachta indica* A. Juss and *Nerium oleander* Linn. against mosquito larvae of *Aedes aegypti*

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ABSTRACT

The biological effects of neem seed kernel extracts of *Azadirachta indica* and flower extracts of *Nerium oleander* against mosquito larvae of *Aedes aegypti* have been evaluated. The results indicated that the test extracts did not appear to give high percentages of mortality against larval stages of. *A. aegypti.* However, taking IC_{50} values (concentration which to inhibit the emergence of 50% of mosquito adults survived from larval treatments) into consideration, the acetonic extract of neem seeds (28 ppm) proved to be more effective against *A. aegypti* than the ethanolic extract (37 ppm) by about 1.3 times. On the other hand, the records indicated that the ethanolic extract of *N. oleaner* white flowers proved to be the most effective extract against *A. aegypti* , followed by the acetonic white flower extract and the aqueous red flower extract while the crude extract of white flowers was the least effective one. This was highly pronounced on the basis of IC_{50} values which were 50 , 68 , 110 and 140ppm, respectively. Variations in the susceptibility levels of *A. aegypti* mosquito larvae may probably due to differences in the levels of toxicity among the active ingredients of plant extracts and the type of solvents used.

Key words: *Aedes aegypti*, susceptibility status, mosquito larvae, plant extracts.

INTRODUCTION

Drawbacks associated with widespread use of chemical insecticides to control mosquitoes have not only resulted in the development of resistance in many species of mosquito vectors, but have also caused environmental pollution. Therefore, more attention has been recently paid to the use of non – conventional insecticides such as insect growth regulators, bioinsecticides and plant extracts for mosquito control in different part of the world (Saleh and Wright, 1989; Batra *et al*., 1998; Moore *et al*., 2003; Bai *et al*., 2007, Siddigui *et al*., 2009).

The present study was planned to evaluate the biological activity of neem seed kernel extracts of *Azadirachta indica* and flower extracts of *Nerium oleander* against mosquito larvae of *Aedes aegypti,* the primary vector of dengue fever in Jeddah governorate, Saudi Arabia.

MATERIAL AND METHODS

Mosquito strain

A field strain of *Aedes aegypti* (L.) larvae was collected from Jeddah governorate, Saudi Arabia, and had been maintained at a room temperature of 27 ± 1 °C and 70 ± 5% R.H., with

14 : 10 (L:D) photoperiod throughout this study. The larvae were reared until pupation and adult emergence took place for maintaining the stock culture.

Preparation of plant extracts

***Azadirachta indica* extracts**

Ripe fruits of *Azadirachta indica* A. Juss were collected from neem trees grown in the garden of Faculty of Science – King Abdulaziz University – Jeddah – Saudi Arabia.

Fruits were manually de – pulped and kernel endocarps were thoroughly washed with distilled water, air dried and triturated in a commercial blender at maximal speed for 5 min. in successive intermittent cycles thus avoiding heating of sample. To prepare the acetonic and ethanolic extracts of neem seed kernel powder, 40 g of powder was added to 200 ml of each solvent at room temperature for 24h in the dark using sealed Erlenmeyer flasks that had been flushed with nitrogen and kept under agitation in a rotator shaker. Extracts were filtered through Whatman no. 3 paper and the residue twice re – extracted with warmed (50 °C) ethanolic. The solvent of the combined extracts was removed in a rotator evaporator and most of the residual moisture eliminated in a vacuum centrifuge (Savant). The extracts were then lyophilized (Freeze Dryer 4.5; Labconco). Stock powders resulted from 40 g neem extracts were 4.1 and 2.9 g acetonic and ethanolic extracts, respectively.

***Nerium oleander* extracts**

Fresh *N. oleander* white flowers (800g), collected from the plants grown in the garden of king Abdulaziz University were extrated in methylene chloride methanolic (1:1) to obtain the organic extract. The extract was filtered, evaporated under vacuum and a yellowiash precipitate was obtained. It was separated to a liquid fraction and a solid one. The liquid fraction (WF1) was mixed with 250 celiet and fractionated by elution with solvents of increasing polarity as follows : hexane, chloroform, chloroform methanol (1:1) and methanol. The solid fraction was dissolved in hot acetonic and filtered yielding the acetonic soluble and acetonic insoluble fractions. The acetonic soluble fraction was cooled at 5 °C and a precipitate was formed. The solution was then filtered yielding the filtrate and precipitate the filtrate was subjected to column chromatography fractionation on silica gel. The column was eluted with cyclohexane acetonic (7:3), then increasing the polarity to (6:4), 100% acetone and finally with methanolic water (1:1). Column fractions were then analyzed by TLC on silica gel whatman, developed with cyclohexane (8:2) and methylene chloride (9:1). The plates were sprayed with anizaldehyde sulfuric acid reagent. Similar fractions were considered yielding 18 fractions and tested on brine shrimp. Also the mortality of precipitate red flower, supernatant red flower, aqueous extract of red flower and aqueous extract of white flower was performed as previously described.(el sawi etal 1999) .

Bioassay tests

The stock solution of each tested plant extract was prepared by adding 0.2 gm (or 0.2 ml) of it to 10 ml of distilled water containing 0.5% triton X- 100 as an emulsifier to ensure complete solubility of the extract in water. Series of concentrations were prepared in distilled water.

The standard WHO larval susceptibility test method (WHO, 1981) was used. Treatments were carried out by exposing 3rd instar larvae of *A. aegypti* to various concentrations of the tested plant extracts in groups of glass beakers of 20 larvae each per concentrations, and so for control trials were set up. The larvae were given the usual larval food during these experiments. Larval mortalities were recorded daily. Live pupae were transferred to untreated water in new beakers for further observation. Partially emerged adults or these found completely emerged but unable to leave the water surface were recorded and scored as dead. Therefore the biological effect of the test plant extract was expressed as the percentage of larvae that do not develop into successfully emerging adults, or the inhibition of adult emergence (WHO, 2005).

The inhibition of adult emergence – concentration – probability line (IC – p line) was drawn for each extract using the method of Litchfield and Wilcoxon (1949). Statistical parameters of the IC – p lines were also calculated. The criterion used to evaluate the biological effects of these plant

extracts was the median inhibitory concentration of adult formation (IC_{50}).

RESULTS AND DISCUSSION

Susceptibility levels of *A.aegypti* larvae following treatments with different concentrations of neem seed kernel extracts of *Azadirachta indica* and flower extracts of *Nerium oleander* are shown in Table (1) and illustrated by figure (1) .

In general, 3 – 14% and 9 – 43% larval mortalities were obtained when the 3rd instar larvae of *A.aegypti* were treated with the effective concentrations of acetonic (15 – 60ppm) and ethanolic (20 – 80ppm) extracts of *A. indica*. This means that the above extracts did not appear to give high percentages of mortality against larval stages of *A.aegypti.* The same trend was obtained with white and red flower extracts of *N. oleander* against the present larvae. The effective concentrations of the acetonic, ethanolic and crude extracts of white flowers gave in respect 9 – 41% , 2 – 6% and 9 – 18% larval mortality while the aqueous extract of red flowers induced 5 – 23% larval mortality (Table 1).

Therefore, in the present study, cumulative mortalities during larval development to pupae and adults have been taken as a criterion for the evaluation of the tested extracts as they have more juvenilizing effects than toxic mode of action (WHO, 2005).

Generally, larval treatment with the effective concentrations of acetonic and ethanolic extracts of *A. indica* neem seeds caused 18 – 94% and 19 – 89% inhibition of adult emergence, respectively. Taking IC_{50} values (concentration which to inhibit the emergence of 50% of adults) into consideration, the acetonic extract (28ppm) proved to be more effective against *A.aegypti* than the ethanolic extract (37ppm) by about 1.3 times. Laboratory and field studies in this respect were carried out by several authors to evaluate the biological effects of neem plant extracts (Leaves, seeds, kernels, coat of seeds) against a wide spectrum of mosquito species. Most of these extracts have been reported to exhibit mosquito larvicidal activities (Batra *et al*, 1998; Moore *et al*., 2003; Okumu *et al*., 2007; Howard *et al*., 2009; Siddigui *et al*. , 2009).

The effective concentrations of acetonic, ethanolic, and crude extracts of *N. oleander* white flowers as well as the aqueous extract of *N. oleander* red flowers were in respect 3-150 ppm, 25 -130 ppm 50 – 350 ppm and 50 – 250 ppm. The corresponding percentages of inhibition of adult emergence were 15 – 89% , 17 – 91% , 16 – 88% and 17 – 92% , respectively. Their IC_{50} values were 68, 50, 140 and 110 ppm (Table 1). The records indicated that the ethanolic extract of white flowers proved to be the most effective extract against *A.aegypti* followed by the acetonic white flower extract and the aqueous red flower extract while the crude extract of white flowers was the least

Table 1: The biological effects of neem seed kernel extracts of *A. indica* and flower extracts of *N. oleander* on the developmental stages of *A. aegypti*

Plant extracts	Effective concentrations (ppm)	Larval *a* mortality (%)	Pupae Produced (%)	Adult emergence		IC_{50} (ppm)
				Total	Inhibition	
Acetonic neem extract	15-60	3-14	97-86	82 - 6	18-94	28
Ethanolic neem extract	20-80	9-43	91-57	81-11	19-89	37
Acetonic extract of *N. oleander* white flowers	30-150	9-41	91-59	80-10	15-89 [b]	68
Aqueous extract of *N. oleander* red flowers	50-250	5-23	95-77	83-8	17-92	110
Ethanolic extract of *N. oleander* white flowers	25-13	12-6	88-94	83-9	17-91	50

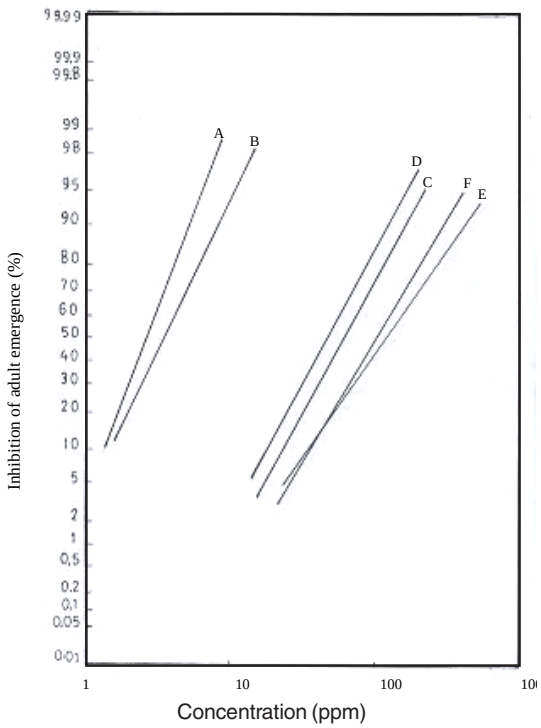

Fig. 1: The effect of larval treatment with the acetonic (A) and ethanolic (B) extracts of *A. indica* and the acetonic (C) , ethanolic (D) and crude (E) extracts of *N.oleander* white flowers and the aqueous extract (F) of *N.oleander* red flowers on *Ae. aegypti* adults survived from these treatments.

effective one. This was highly pronounced on the basis of IC_{50} values (Fig. 1) which were 50, 68, 110 and 14 ppm, respectively. In other words, results thus indicate that the ethanolic extract of *N. oleander* white flowers is about 1.4, 2.2 and 2.8 times as effective as the above extracts, respectively. Generally, it can be concluded that the response of 3rd instar larvae of *A.aegypti* depends entirely on the type of solvents used and the effective concentrations. The fluctuations in the percentage inhibition of adult emergence obtained for the different concentrations of the test plant extracts against the present mosquito strain support this conclusion (Pushpalatha and Muthukrishnan, 1995; Sharma *et al*., 2005). However, long term follow – up studies are needed to evaluate the possible delayed effects of such plant extracts on some biological and behavioral aspects of mosquito vectors.

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