

Laboratory evaluation of the insect growth regulator dudim and the plant extract neem oil against *Culex pipiens* mosquitoes with reference to its side effects on aquatic nontarget organisms

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ABSTRACT

The biological effects of the insect growth regulator (IGR), Dudim and the plant extract Neem oil on the developmental stages of *Culex pipiens*, the dominant mosquito species in Jeddah, Saudi Arabia, were evaluated. The records showed that the response of *Cx. pipiens* to the test compounds depends on the differential mode of action of the compounds and its effective concentrations. According to IC₅₀ values (concentration which to inhibit the emergence of 50% of mosquito adults emergence), the IGR Dudim (0.0003 ppm) proved to be more effective against *Cx. pipiens* than the Neem oil (70 ppm). On the other hands, treatment with IC₉₀ values (concentration which to inhibit the emergence of 90% of mosquito adults emergence) of Dudim (0.0032 ppm) and Neem oil (140 ppm) did not exhibit any market effect on nontarget organisms (naiads Odonata, aquatic hemipterans, aquatic coleopterans) associated with mosquito breeding sites.

Key words: Insect growth regulator, *Culex pipiens*, neem oil, aquatic organisms.

INTRODUCTION

With increasing problems of environmental pollution by chemical insecticides and development of resistance in many species of mosquito vectors, intensive work was currently paid to the use of non-conventional insecticides as insect growth regulator (IGR) and plant extracts for mosquito control in different parts of the world (Saleh and wright, 1989; Chowdhury *et al.*, 2007; Seng *et al.*, 2008).

The present work was planned in part to evaluate the biological effects of two non-conventional insecticides, (IGR), Dudim and the plant extract Neem oil, against *Cx. pipiens*, the

dominant mosquito species in Jeddah, Saudi Arabia. Additional test were also conducted to study the side effects of the test compounds on some nontarget organisms associated with mosquito habitats.

MATERIAL AND METHODS

Mosquito strain

Tests were performed on a field strain of *Cx. pipiens* raised from wild larvae, collected from Jeddah governorate, Saudi Arabia, and had been maintained in the laboratory under controlled conditions of 27± 1 °C and 70 ± 5% R.H, with a 14 : 10 (L :D) photoperiod.

Compounds tested

Two non-conventional insecticides were used

1. The insect growth regulator Dudim (4G%); 1-(4-chlorophenyl)-3-(2,6-difluorbenzoyl)-urea, kindly supplied by Dr. KH. Al. Ghamdi, Fac. of science, King Abdulaziz Univ.
2. The plant extract Neem oil (*Azadirachtain indica*) kindly supplied by Dr. M. A. Khan, Dept. of Zoology, Saifia Science College Bhopal, India. The stock solution of the plant extract was prepared by adding 1 ml of it to 99 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.

Test experiments

The Larval susceptibility test was conducted according to the method of WHO (1981). Treatments were carried out by exposing early 4th instar larvae of *Cx. pipiens* to various concentrations of the tested compounds in groups of glass beakers containing 100ml of tap water. Five replicates of 20 Larvae each per concentration, and so for control trials were set up. The Larvae were given the usual larval food during the experiments. Mortalities of larvae and pupae were recorded daily. The percentage of inhibition of adult formation was recorded and compared with those of the controls under the same conditions of tests (Abbott, 1925). Log concentration – probability regression lines were drawn for the tested compounds and statistical parameters were also calculated using the method of Litchfield and Wilcoxon (1949).

Additional tests were also conducted to study the side effects of the IGR Dudim and the plant extract Neem oil on some aquatic nontarget organisms associated with mosquito breeding habitats. The test organisms were collected from a pond water known to be mosquito breeding sites. The nontarget organisms were Odonata naiads, aquatic bugs (Order: Hemiptera) and aquatic beetles (Order: Coleoptera).

The concentrations corresponding to IC₉₀ values (concentration which to inhibit the emergence of 90% of mosquito adults emergence) were derived from the toxicity lines of Dudim and Neem oil. These concentrations were prepared and chosen for treating the aquatic organisms. Treatments were carried out by exposing the individuals to the test concentrations in 500 ml glass beakers with 100 ml of tap water. Three replicates of 4 individuals each per concentration, and so for control were set up. Mortalities were recorded after 24hr post-treatment.

RESULTS AND DISCUSSION

Percentage mortalities of *Cx. pipiens* larvae and inhibition of adult emergence following treatments with the IGR Dudim and the plant extract Neem oil are shown in table 1. In general, 2-30% and 11-65% larval mortalities were obtained when 4th instar larvae of *Cx. pipiens* were treated with the effective concentrations of the above compounds, respectively. This means that the test compounds did not appear to give high percentages of mortality against larval stages. In other words, the results thus may confirm the unsuitability of larval mortality

Table 1: The percentage inhibition of adult emergence following treatment of *Cx. pipiens* mosquito larvae with the IGR Dudim and the plant extract Neem oil

Compound	Effective concentrations (ppm)	larval ^a mortality (%)	Inhibition of adult emergence ^b (%)	Statistical parameters ^c		
				IC ₅₀	IC ₉₀	Slop
DudimControl	0.0001- 0.005	2 - 304	22.5 – 94.67	0.0003	0.0032	1.54
Neem oil	40 - 150	11 - 65	23.4 – 92.5	70	140	3.6
Control		3	6			

a: Five replicates, 20 larvae each

b: Corrected by Abbotts formula (Abbott, 1925)

c: Litchfield and Wilcoxon (1949)

records as a measurement for evaluating the efficacy of such compounds (Saleh, 1985).

The biological effects of the present compounds were often manifested by the formation of a type of larval – pupal intermediate. Moreover, most pupae were died before the adult emerged. Some adults emerged incompletely or left their tarsi attached in the pupal exuvia (Al-sharook *et al.*, 1991). Therefore, the biological effects of Dudim and Neem oil were expressed as the percentage of larvae that do not develop into successfully emerging adults or the inhibition of adult emergence (WHO, 2005).

Generally, the effective concentrations of Dudim and Neem oil in respect were 0.0001-0.005 ppm and 40-150 ppm. The corresponding percentages of inhibition of adult emergence were 22.5-94.6% and 23.4 -92.5%, respectively. According to IC_{50} values (concentration which to inhibit the emergence of 50% of mosquito adults emergence), the IGR Dudim (0.0003 ppm) proved

to be more effective against *Cx. pipiens* mosquitoes than the plant extract Neem oil (70 ppm). It can be concluded that the response of *Cx. pipiens* to the test compounds depends entirely on the differential mode of action of these compounds and its effective concentrations. Studies in this respect were carried out by other investigators using the IGR cyromazine against *Cx. pipiens* and *Aedes egypticus* (Saleh and Wright, 1989); the IGR methoprene against *Ae. nigromaculis* (Cornel *et al.*, 2000) and the plant extract *Solanum villosum* against *Cx. quinquefasciatus* (Chowdhury *et al.*, 2007). However, consideration must be taken to study the effect of environmental conditions on the effectiveness of such compounds when applied for field control measures.

On the other hand, the side effect of the IGR Dudim and the plant extract Neem oil on three nontarget organisms viz. Odonata naiads, aquatic bugs (Order: Hemiptera) and aquatic beetles (Order: Coleoptera) was evaluated in the laboratory. In general, the records indicated that treatment with

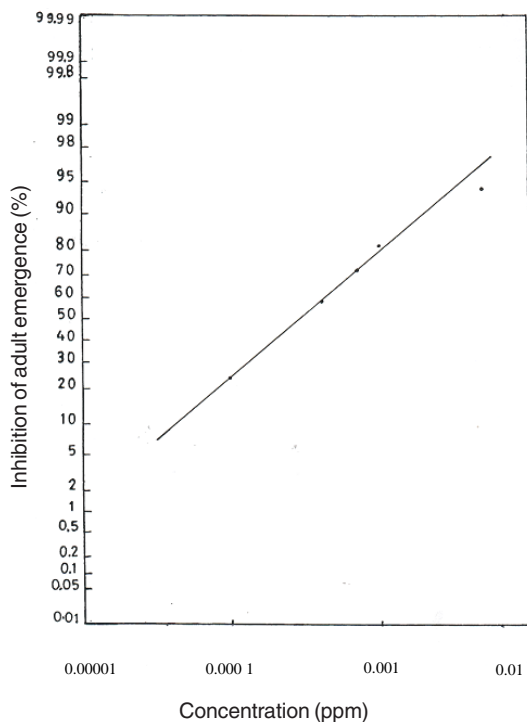


Fig. 1 : The relation between concentrations of Dudim and the percentage of inhibition of adult emergence after treatment of *Cx. pipiens* larvae

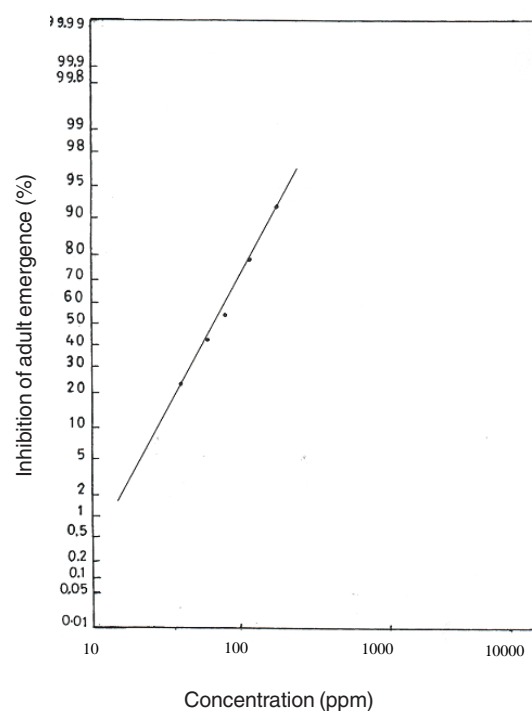


Fig. 2 : The relation between concentrations of Neem oil and the percentage of inhibition of adult emergence after treatment of *Cx. pipiens* larvae

the concentrations corresponding to IC₉₀ values of Dudim (0.0032ppm, Fig.1) and Neem oil (70ppm, Fig.2) did not exhibit any marked effect on the test nontarget organisms. Similar findings have been obtained by Miura and Takahashi (1975) who found that treatment with the IGR TH6040, at rates used for mosquito control, did not have any adverse effects against aquatic beetles and spiders associated with mosquito larvae in the pond water. Gunasekaran *et al.* (2004) reported that treatment with the sub-lethal doses of the bioinsecticide Teknar HP-D did not affect on *Gambusia* fish and water bugs of *Notonecta* sp. and *Diplonychus indicus*.

Moreover, treatments with the IGR pyriproxyfen (sumilarv) against mosquito larvae did not have any impact on aquatic hemipterans (Vythilingam *et al.*, 2005).

Generally, it can be concluded that the present IGR Dudim and the plant extract Neem oil seem to have a good margin of safety to aquatic natural enemies prevailing in mosquito breeding habitats. Therefore, the use of such non-conventional compounds in mosquito control programs offers certain advantages over some currently used chemical insecticides

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