

INFLUENCE OF IGR TREATMENT ON OVIPOSITION OF THREE SPECIES OF VECTOR MOSQUITOS AT SUBLETHAL CONCENTRATIONS

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Abstract. Sublethal effect of hexaflumuron, an insect growth regulator (IGR), on the oviposition of three species of vector mosquitos. *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* was studied. Significant reduction in oviposition was observed in the females of the above three species derived from fourth instar larvae and pupae exposed to sublethal (EI_5 and EI_{50}) doses. The reduction in egg laying is proportional to the dose of exposure and was found to be about twice higher in females of three species exposed to EI_{50} dose than those exposed to EI_5 dose. Among the three species exposed at larval and pupal stages, *Ae. aegypti* showed maximum reduction in egg laying (29.3-46.6%). Blood feeding was also reduced in females exposed to EI_{50} dose at larval stage and a positive correlation was demonstrated between the quantity of blood meal taken and the proportion of eggs laid. Significant reduction in the quantum of blood ingested by the treated females may be responsible for the reduced egg laying.

INTRODUCTION

Oviposition is an important event in the life cycle of mosquito species and its occurrence is under physiological and genetic control. Vector mosquitos usually require the taking of at least one blood meal for pathogen acquisition. Disease transmission generally requires the completion of at least one oviposition cycle before pathogen transfer can occur with a subsequent blood meal. Interplay of many chemical (Verma, 1986), physical (McCrae, 1984) and physiological (Shroyer and Sanders, 1979) factors affect the general aspects of mosquito oviposition in nature. Insect control agents including insect growth regulators (IGRs) have been found to alter the biology and behavior of insects (Brushwein, 1980, Armstong and Bonner, 1985).

Increasing attention has been paid to IGRs with greater specificity and lesser hazards than conventional insecticides for controlling insects of medical (Pawar *et al* 1995), veterinary (Matsumura, 1979) and agricultural (Hejazi and Granett, 1986) importance. Besides suppressing the adult emergence of target insects, IGRs have also been reported to have influenced several biological and reproductive parameters of insects at sublethal doses (Vasuki, 1992; Mazomenos *et al*, 1997, Parkman and Frank, 1998). Hence, it is necessary to examine the possible effect of IGRs on the essential biological processes such as oviposition. In the present study, an attempt has been made to find out the sublethal effects of an insect growth regulator, hexaflumuron, on ovi-

position and the relationship between egg laying and feeding of three species of vector mosquitos.

MATERIALS AND METHODS

Hexaflumuron (OMS 3031, XRD 473), chemically known as N-({[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy) phenyl]amino}carbonyl)-2,6-difluorobenzamide, was received gratis as 5% EC formulation from Dow Chemical Company, USA, through WHO, Geneva. Pupae and fourth instar larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* were obtained from the insectaries maintained at Vector Control Research Center.

Effect on egg laying

Fourth instar larvae and pupae of three species mentioned above were exposed to their respective sublethal (EI_5 and EI_{50}) doses (Table 1) (Vasuki, 1992). Two doses, a non selecting dose (EI_5) which gave nil mortality or minimum effect and a selecting dose (EI_{50}) that inhibited about 50% emergence in exposed mosquito immatures were chosen. The adults that survived the exposure of hexaflumuron at fourth instar stage (at EI_5 and EI_{50} doses) and pupal stage (at 0.005 and 0.05 mg/l) were separated sexwise during emergence cohabited at the ratio of 1:1 for three days and fed on restrained chick or rabbit. Twenty-five gravid females were allowed to oviposit individually. The number of oviposited females

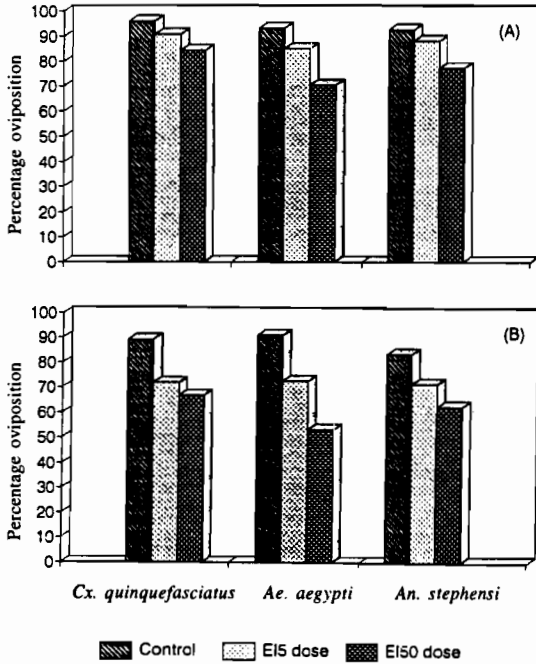


Fig 1—Percentage oviposition in the females of the three species of vector mosquitos exposed to sublethal concentrations at (A) fourth instar and (B) pupal stage.

were noted. The experiment was repeated ten times with appropriate control (untreated) at $27 \pm 2^\circ\text{C}$ and 70-80% RH. Observations on the ovipositional behavior and other abnormalities were also recorded. Percentage oviposition in the treated and untreated groups were statistically analysed by ANOVA variance test (Sokhal and Rohlf, 1981).

Relationship between egg production and feeding

The amount of blood meal taken by the females exposed to sublethal dose (EI_{50}) at fourth instar level was quantified by weighing 10 females before and after bloodmeal from a restrained chicken/rabbit and was repeated ten times. After weighing, the females were maintained separately and allowed to oviposit individually on appropriate oviposition medium. Untreated (Control) cohorts were maintained simultaneously in the same way as described for the treated cohorts. Quantum of blood intake per female and average number of eggs laid per female were calculated. A linear regression of the number of eggs laid per female to the quantity of blood meal taken/female was followed. The significance of the correlation (*r*-value) was tested by Students' *t* test.

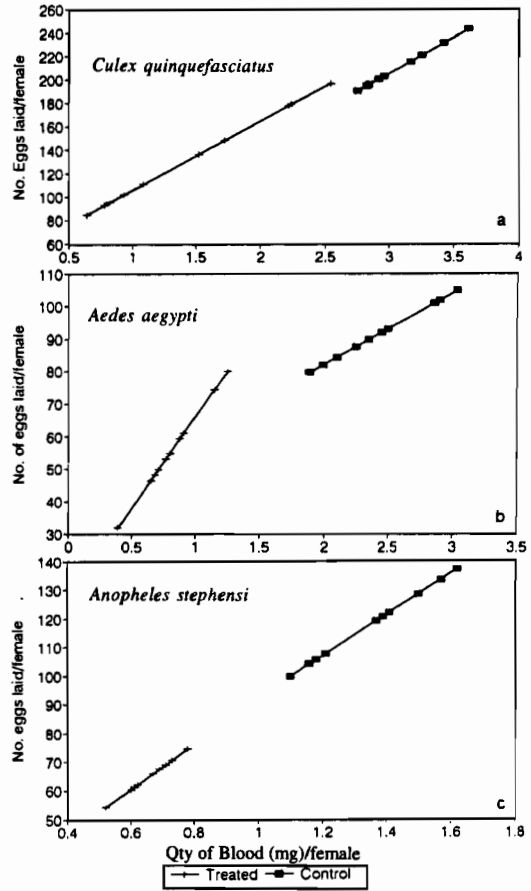


Fig 2—Relationship between egg production and feeding in (a) *Cx. quinquefasciatus* (b) *Ae. aegypti* and (c) *An. stephensi* when treated (at EI_{50} dose) females and untreated (control) females were compared.

RESULTS AND DISCUSSION

Effect on egg laying

Percentage oviposition of the three species, *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* emerged from treated fourth instar larvae at EI_5 and EI_{50} was found to be significantly lesser when compared to that of the respective control cohorts. On comparison, significant ($p < 0.05$) negative responses of 15.6%, 29.3% and 22.4% were seen in the ovipositing females of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* respectively when exposed as fourth instar to EI_{50} dose. The females of the treated group at higher concentration (EI_{50} dose) showed about 2 times higher negative response in the three test species when compared to that at lower concentration (Fig 1 A).

Table 1
Sublethal concentrations of fourth instar larvae
and pupae of the three species.

Species	Fourth instar larvae	
	EL ₅₀ (mg/l)*	EL ₉₀ (mg/l)*
<i>Cx. quinquefasciatus</i>	4.010x10 ⁻⁴	3.317x10 ⁻³
<i>Ae. aegypti</i>	6.273x10 ⁻³	3.242x10 ⁻³
<i>Ae. stephensi</i>	3.430x10 ⁻³	1.510x10 ⁻²

* -0.005 mg/l and 0.05 mg/l respectively for the pupae of three species (Vasuki, 1992).

Females of the group exposed as pupae to 0.005 and 0.05 mg/l exhibited significant reduction in egg laying in all the three species. Though marked decrease in the rate of oviposition was noticed in treated groups of the three species at 0.05 mg/l, *Ae. aegypti* showed the maximum of 46.6% (Fig 1 B).

On dissection, the females that had not oviposited (even after 48 hours) were found to retain fully developed eggs and also eggs at various stages of maturation. Few proportion of females was found dead during the course of oviposition with partly oviposited and partly retained eggs, which were scored as oviposited ones.

These results are in consistency with the observation made by Fujita and Kurihara (1984) on the reduced ovipositional activity of *Culex pipiens* exposed to methoprene. However, the present result is contrary to the earlier reports on *Rhodnius prolixus* (Ruegg and Davey, 1979) and *Ctenocephalides felis* (Olsen, 1985), where the treatment of IGR methoprene did not affect their oviposition or behavior.

Relationship between egg laying and feeding

A positive correlation of the number of eggs laid with the amount of blood meal taken was observed in treated (*Cx. quinquefasciatus* $r = 0.979$; *Ae. aegypti*, $r = 0.0865$; *An. stephensi*, $r = 0.973$) and untreated (*Cx. quinquefasciatus*, $r = 0.937$; *Ae. aegypti*, $r = 0.903$; *An. stephensi*, $r = 0.979$) females of the three species. However, a significant ($p < 0.05$) reduction in egg production was evident in the treated females of *Cx. quinquefasciatus* ($F = 6.14$), *Ae. aegypti* ($F = 4.43$) and *An. stephensi* ($F = 4.97$) than in controls. Thus, reduction in egg production could be an indirect indication of reduced feeding which was noticed to be significant ($p < 0.05$) in *Cx. quinquefasciatus* ($F = 6.30$), *Ae. aegypti* ($F = 3.39$) and *An. stephensi* ($F = 5.91$) when compared to controls (Fig 2 a-c). This clearly indicates that hexaflumuron at sublethal dosages

reduced the rate of oviposition due to insufficient intake of blood meal.

The results conclusively indicate that the oviposition responses of the females of the three species were adversely affected by the IGR treatment during fourth instar or pupal stage, particularly at higher sublethal concentrations. The influence of hexaflumuron on the oviposition behavior was more on *Ae. aegypti* than on other two species. The reason for the failure or inhibition in oviposition could be due to the interference of the treated IGR compound on the hormonal regulation in triggering the oviposition behavior, since oviposition and its regulation are under humoral and genetic control (Knowden and Blackmer, 1987). IGRs will play important role in the suppression of vector population and disease transmission by affecting the oviposition of vector mosquitos.

ACKNOWLEDGEMENTS

The author is thankful to the Director, Dr PK Das, Vector Control Research Centre, Pondicherry, for encouragement. The technical assistance of Mr K Sathianathan and Mr V Selvaraj is gratefully acknowledged. She is also thankful to WHO, Geneva, for the gratis supply of hexaflumuron, OMS 3031.

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