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INFLUENCE OF SHORT TIME EXPOSURE TO AN INSECT GROWTH REGULATOR, HEXAFLUMURON, ON MORTALITY AND ADULT EMERGENCE OF VECTOR MOSQUITOES

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Hexaflumuron, an insect growth regulator (IGR), was found to greatly affect the development of immatures and emergence of adults of three species of vector mosquitoes, Culex quinquefasciatus, Aedes aegypti *and* Anopheles stephensi, *when larvae were subjected to short time exposure of ⩽1 h. This IGR could completely prevent adult emergence even at a minimum exposure time of 10 min at 0.001, 0.01 and 0.1 mg/l. On treatment, larval and pupal mortality as well as varying degrees of morphogenetic abnormalities were induced in immatures and adults of the three species. Four weeks of control achieved in a slow moving sullage canal breeding* Culex quinquefasciatus *indicates that this IGR can be of use in such breeding habitats.*

Key words: insect growth regulator – hexaflumuron – short time exposure – *Culex quinquefasciatus* – *Aedes aegypti* – *Anopheles stephensi*

Insect Growth Regulators (IGRs), the alternative insecticides for conventional ones, act through interference with growth and development rather than through direct toxicity (Staal, 1975). Benzoylphenylureas (BPUs), a group of IGRs, which are chitin synthesis inhibitors (Hajjar & Casida, 1978), however, are known for their toxic effects on egg (Miura et al., 1976), larva (Mulder & Gijiswijt, 1973) and pupa (De Loach et al., 1981) of insects. BPUs like hexaflumuron are largely stomach poisons (Leonard et al., 1987) controlling wide variety of insect pests either by disrupting larval development or preventing egg hatch presumably by interfering with cuticle deposition (Ishaaya & Cassida, 1974) and chitin synthesis (Post et al., 1974; Deul et al., 1978; Gijiswijt et al., 1979). The effects of BPUs are slower than those of most neurotoxic insecticides. Mortality occurs at the time of moulting, normally a few days after treatment. Therefore, the toxicity of BPUs is at least in part, related to the pesticide accumulating at the biochemical site of action (Neumann & Guyer, 1983). This toxicity is the result of uptake of chemical from the environment, uptake into the body and movement of the insecticide within the body to the active site (Hammock & Quistad, 1981). They are, therefore, considered as control agents with selectivity directed at disease bearing insects.

Hexaflumuron, a new acylurea, was found effective against *Heliothis* (Sbragia et al., 1983). Its efficacy on larvae and eggs of insect species like *Bemisia tabaci, Ostrinia nubilalis, Spodoptera littoralis, Carpophilus hemipterus* and *Liriomyza trifolii* has been compared with that of other benzoylphenylurea compounds (Ascher et al., 1986). The role of this IGR in controlling *Cx. quinquefasciatus, Cx. tarsalis, Cx. peus,* and *Ae. aegypti* has been established (Mulla & Darwazeh, 1988; Darriet, 1989; Mulla et al., 1989). Control potency of IGRs is usually assessed by exposing the immatures continuously or for 24 h to the treatment (WHO, 1981). But it is not known whether these compounds can exhibit similar effect when the immatures are exposed to the treatment for a limited period of time lesser than 1 h. Hence, an attempt was made to determine the influence of hexaflumuron on adult emergence when fourth instar larvae of three vector species were exposed to the compound for a minimum period of time (⩽1 h).

MATERIALS AND METHODS

Hexaflumuron (OMS 3031, XRD 473), chemically known as N-(((3,5-dichloro-4-

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(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. Immatures of *Cx. quinquefasciatus, Ae. aegypti* and *Anopheles stephensi* maintained at the Insectaries of Vector Control Research Centre, were used for this study. Twenty five fourth instar larvae of three species were exposed to varyin g concentrations of OMS 3031 for 10, 20, 30, 40, 50 and 60 min. The larvae were exposed to 250 ml of tap water with desired concentration of I GR (0.0001-0.1 mg/l) in a beaker of 500 ml capacity. Four replicates along with a control were maintained and repeated four times. The larvae were washed in running water after the required exposure time and then transferred to beakers with untreated tap water. Larval food was provided till pupation. Observations on the responses such as larval mortality, larval-pupal intermediate, pupal mortality, pupal-adult intermediate or incompletely emerged dead adults and normal adult emergence of treated larvae after limited exposure were recorded and expressed in percentage. Percentage inhibition in adult emergence at different exposure times and at various dosages were compared by Analysis of variance (ANOVA) after transforming the percentage to arcsine values to normalize the variance (Sokal & Rohlf, 1981).

TABLE I

Effect of short time exposure of hexaflumuron on the adult emergence of *Culex quinquefasciatus*

Exposure time (min)	Mortality and adult emergence in percentage[a] (mean ± SE) at different dosages									
	0.0001 mg/l					0.001 mg/l				
	I	II	III	IV	AE	I	II	III	IV	AE
10	7.3	0.0	3.0	0.0	89.6	15.3	16.0	50.0	17.3	1 3
	0.5	0.0	0.2	0.0	0.5	0.5	0.5	1.0	0.8	0.1
20	8 6	0.0	5.0	0.0	86.3	24.3	23.3	41.6	10.6	0.0
	0.4	0.0	0.2	0.0	0.4	0.5	0.7	1.0	0.5	0 0
30	15.6	0.0	6.6	0.0	77.6	42.3	25.0	23.6	9.0	0.0
	0.3	0.0	0.2	0.0	1.5	0.8	0.9	0.8	0.7	0.0
40	11.0	0.0	4.6	0.0	84.3	50.0	24.6	25.3	0.0	0.0
	0.3	0.0	0.2	0.0	0.4	0.9	0.8	0.8	0.0	0.0
50	16.6	0.0	7.6	0.0	75.6	53.3	31.3	15.3	0.0	0 0
	0.3	0.0	0.3	0.0	1.5	1.2	1.0	0.4	0 0	0.0
60	15.0	0.0	10.0	0.0	75.0	66.7	24.0	9.3	0.0	0 0
	0.6	0.0	0.4	0.0	0.8	0.9	0.7	0.3	0.0	0.0
Ctrl	5.0	0.0	2.3	0.0	92.6	3.0	0.0	2.0	0.0	95.0
	0.4	0.0	0.3	0.0	0.5	0.3	0.0	0.1	0.0	0.2
	0.01 mg/l					0.1 mg/l				
10	13.0	46.3	39.0	1.7	0.0	21.0	58.0	18.3	2.6	0.0
	0 3	1.1	1.0	0.2	0.0	0.6	1.4	1.0	0.2	0.0
20	15.6	47.3	34.6	2.3	0.0	18.3	68.6	12.6	0.3	0.0
	0.5	0.6	0.7	0.3	0.0	0.6	0.9	0.8	0 0	0 0
30	39.0	48.6	11.6	0.7	0.0	40.7	54.3	5.0	0 0	0 0
	1.0	0.8	0.4	0.1	0.0	1.8	1.4	0.3	0.0	0.0
40	48.3	44.6	6.3	0.7	0.0	61.7	35.0	3.3	0.0	0 0
	0.7	0.6	0.2	0.2	0.0	1.0	0.7	0.3	0.0	0 0
50	55.6	42.6	1.6	0.0	0.0	72.7	25.6	1.6	0.0	0.0
	1.0	1.0	0.1	0.0	0.0	1.3	1.3	0.3	0.0	0.0
60	82.0	17.0	1.0	0.0	0.0	87.0	13.0	0.0	0.0	0.0
	0.9	0.8	0.1	0.0	0.0	0.7	0.7	0.0	0.0	0.0
Ctrl	6.0	0.0	1.3	0.0	92.6	3.0	0.0	3.0	0.0	94.0
	0.2	0.0	0.1	0.0	0 2	0.3	0.0	0.3	0.0	0.3

a: n = 400.
AE: emerged adults.
Mortality at: I – larval stage; II – larval-pupal intermediate stage; III – pupal stage; IV – pupal-adult intermediate stage.

TABLE II

Effect of short time exposure of hexaflumuron on the adult emergence of *Aedes aegypti*

Exposure time (min)	Mortality and adult emergence in percentage[a] (mean ± SE) at different dosages									
	0.0001 mg/l					0.001 mg/l				
	I	II	III	IV	AE	I	II	III	IV	AE
10	9.3	2.0	5.0	0.0	83.6	15.3	55.6	14.3	9.3	5.3
	0.4	0.3	0.3	0.0	0.7	0.3	0.6	0.2	0.5	0.6
20	13.0	1.3	6.3	7.3	72.0	18.6	51.0	18.3	8.6	3.3
	0.4	0.1	0.2	1.6	1.6	0.4	0.6	0.4	0.6	0.3
30	18.3	0.6	7.0	2.3	71.6	29.6	50.0	12.6	7.6	0.0
	0.6	0.1	0.2	0.2	0.7	0.9	0.8	0.5	0.4	0.0
40	22.0	0.0	8.6	0.7	68.6	35.6	39.0	14.6	10.6	0.0
	0.6	0.0	0.4	0.2	0.9	0.9	0.7	0.7	0.4	0.0
50	29.0	2.3	3.3	2.0	63.3	49.0	26.0	17.3	7.6	0.0
	0.6	0.3	0.1	0.3	0.8	0.6	0.8	0.7	0.5	0.0
60	28.3	1.6	9.3	2.3	58.3	64.3	18.0	14.3	3.3	0.0
	0.8	0.2	0.4	0.2	1.0	0.8	0.6	0.5	0.3	0.0
Ctrl	4.6	1.3	4.3	0.0	89.6	3.0	0.0	0.0	0.0	96.6
	0.3	0.3	0.3	0.0	0.6	0.4	0.0	0.0	0.0	0.4
	0.01 mg/l					0.1 mg/l				
10	15.3	70.6	11.0	3.0	0.0	11.0	89.0	0.0	0.0	0.0
	0.3	0.7	0.4	0.4	0.0	0.3	0.3	0.0	0.0	0.0
20	31.6	46.0	17.0	5.3	0.0	22.3	77.6	0.0	0.0	0.0
	0.5	1.3	0.9	0.4	0.0	0.9	0.9	0.0	0.0	0.0
30	31.3	47.6	17.0	4.0	0.0	54.6	45.3	0.0	0.0	0.0
	0.6	0.8	0.7	0.3	0.0	0.7	0.7	0.0	0.0	0 0
40	35.3	50.0	10.6	4.0	0.0	76.6	23.3	0.0	0.0	0.0
	0.5	0.7	0.4	0.3	0.0	0.9	0.9	0 0	0.0	0.0
50	37.0	49.0	7.3	6.7	0.0	90.6	9.3	0.0	0.0	0 0
	0.4	0.8	0.7	1.0	0.0	0.6	0.6	0.0	0.0	0.0
60	52.6	40.3	6.3	0.7	0.0	100.0	0.0	0.0	0.0	0.0
	0.3	0.4	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0 0
Ctrl	4.3	0.0	1.0	0.0	94.6	2.0	0.0	1.6	0.0	96 3
	0.2	0.0	0.1	0.0	0.2	0.1	0.0	0.1	0 0	0 3

a: n = 400.
AE: emerged adults.
Mortality at: I – larval stage; II – larval-pupal intermediate stage; III – pupal stage; IV – pupal-adult intermediate stage.

A field trial was made to study the effect in natural habitat, i.e., a slow running shallow sullage canal breeding *Cx. quinquefasciatus*. The Uppar canal is the main sullage disposal facility in Pondicherry Town. The entire length of the canal (3 km) was surveyed for breeding of *Cx. quinquefasciatus*. Profuse breeding was observed in several pockets and a stretch of 225 m at the southern end of the canal situated at Attupatti was selected for the trial. The canal was 20 m wide at this section. A width of about 5 m from the margin on either sides of the canal was shallow with slow movement of sullage water and breeding was confined to this area. The surface area was 2250 m^2 and this was treated with 10% EC of hexaflumuron at an application rate of 200 gm/ha with a knapsack sprayer. Similar stretch of the canal was left untreated and maintained as control.

Assessment of pre-treatment density and percentage emergence was carried out for four days. Subsequent to treatment, immature density was assessed by dipper sampling on alternate days for the first week and at weekly interval thereafter for four weeks. Known number of live pupae and fourth instar larvae were brought to the laboratory and observed for adult emergence based on which emergence inhibition rate (EIR) was calculated (Amalraj & Velayudhan, 1989).

TABLE III

Effect of short time exposure of hexaflumuron on the adult emergence of *Anopheles stephensi*

Exposure time (min)	Mortality and adult emergence in percentage[a] (mean ± SE) at different dosages									
	0.0001 mg/l					0.001 mg/l				
	I	II	III	IV	AE	I	II	III	IV	AE
10	11.0	0.0	8.3	1.0	79.6	29.7	0.0	23 0	22.6	24.6
	0.2	0.0	0.3	0.2	0.5	0.9	0.0	1.1	0 8	0 8
20	17.3	0.0	12.0	0.0	70.6	14.7	0.0	25.6	21.6	38.0
	0.4	0.0	0.6	0.0	0.8	0 7	0.0	1 2	0.9	2.1
30	22.6	0.0	13.0	0.0	64.3	42.7	0.0	22.6	24.6	10.0
	0.5	0.0	0.5	0.0	0.5	1.2	0.0	0.4	1 0	0.7
40	27.6	0.0	16.3	0.0	56.0	48.0	0.0	15.3	27.6	9.0
	0.4	0.0	0.4	0.0	0.6	1.7	0.0	0 6	1.4	0.5
50	30.3	0.0	11.6	0.0	58.0	61.7	0.0	14.3	9.0	15.0
	0.7	0.0	0.5	0.0	0.7	0.8	0.0	0.6	0.5	0 9
60	35.6	2.3	11.6	0.0	50.3	87.7	1.0	3.6	5.6	2.0
	1.2	0.4	0.5	0.0	1.1	0.8	0.2	0.4	0.6	0.3
Ctrl	2.0	0.0	0.6	0.0	97.3	2.3	0.0	4.0	0.0	93.6
	0.2	0.0	0.1	0.0	0.2	0.2	0.0	0.4	0.0	0.4
	0.01 mg/l					0.1 mg/l				
10	21.0	0.0	21.6	28.6	28.6	18.3	0.0	19.6	45.6	16.3
	0.6	0.0	0.5	0.6	0.6	0.6	0.0	0.5	0.9	0.8
20	22.3	6.0	21.0	33.3	17.3	26.7	0.0	35.3	28.6	9.3
	0.6	1.4	0.6	1.2	0.8	0.9	0.0	1.1	1.4	1.2
30	24.3	0.0	30.6	29.0	16.0	52.3	0.3	24.6	22.6	0.0
	1.1	0.0	1.3	0.7	0.4	0.6	0.1	0.8	0.9	0.0
40	30.3	0.0	26.6	28.3	14.6	83.3	1.7	14.3	0.6	0.0
	1.0	0.0	1.2	1.1	0.5	0.4	0.2	0.4	0.1	0.0
50	45.0	3.3	20.0	26.0	5.6	89.7	2.0	8.3	0.0	0.0
	0.6	0.7	0.9	1.0	0.3	0.5	0.3	0.5	0.0	0.0
60	45.0	27.0	19.0	5.6	3.3	98.3	0.3	1.3	0.0	0.0
	1.2	1.7	0.7	0.5	0.2	0.2	0.1	0.1	0.0	0.0
Ctrl	1.6	0.0	1.3	0.0	97.0	4.0	0.0	1.3	0.0	94.6
	0.1	0.0	0.2	0.0	0.2	0.4	0.0	0.2	0.0	0 4

a: n = 400.
AE: emerged adults.
Mortality at: I – larval stage; II – larval-pupal intermediate stage; III – pupal stage; IV – pupal-adult intermediate stage.

RESULTS

The influence of short term exposure (10-60 min) on percentage adult emergence of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* was evident when fourth instar larvae were treated at varying dosages (Tables I-III). The percentage emergence inhibition obtained in *Cx. quinquefasciatus* was found to be significant ($F = 360.802$, $P = 0.000$) when larvae were exposed for short time (10-60 min) at varying dosages. While complete emergence inhibition was evident when larvae were exposed for 10-60 min at 0.001-0.1 mg/l, only about 75% inhibition in adult emergence was obtained at 0.0001 mg/l for the same exposure time. On analyzing the percentage of adult emergence, it was found that 98-100% emergence inhibition was obtained even at a minimum exposure time of 10 min at the dosages 0.001-0.1 mg/l ($F = 7.132$, $P = 0.000$) (Fig. 1).

Significant reduction in adult emergence was evident in *Ae. aegypti* when fourth instar larvae were exposed for 10-60 min at various concentrations ($F = 478.432$, $P = 0.000$). Maximum inhibition in adult emergence of 94.67-100% was obtained for an exposure time of 10 min at 0.001-0.1 mg/l ($F = 23.79$, $P = 0.000$) (Fig. 1).

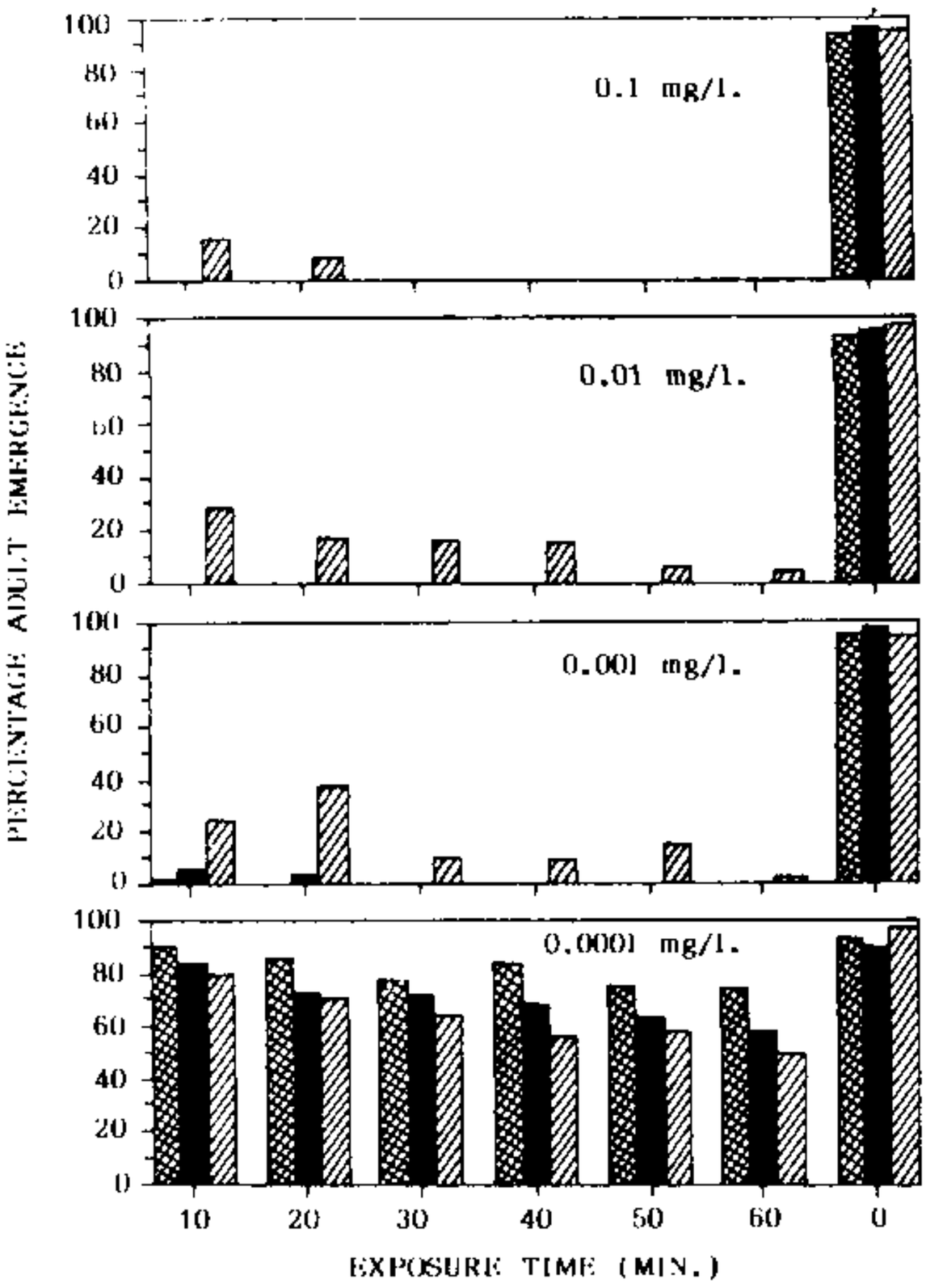

Fig. 1: percentage of adult emergence (n = 100) in *Culex quinquefasciatus* ▩ *Aedes aegypti* ■ and *Anopheles stephensi* ▨ at short time exposures to different concentrations of hexaflumuron.

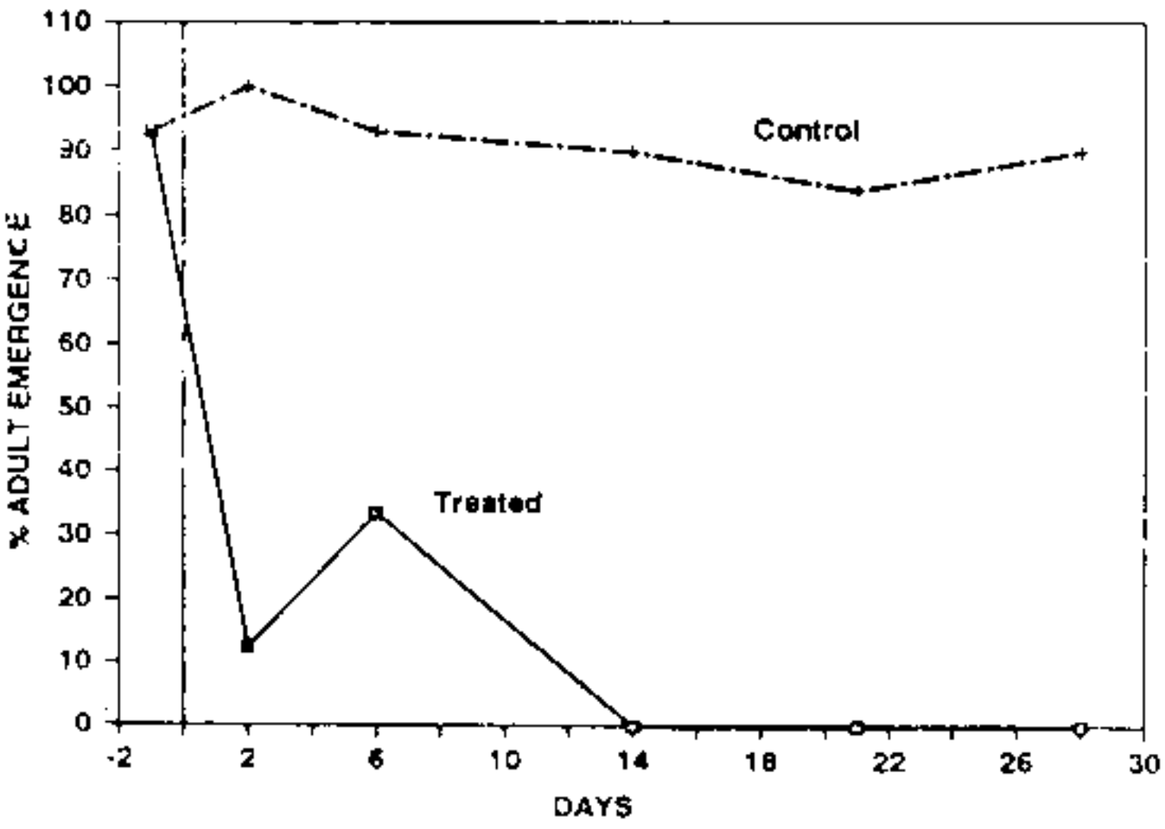

Fig. 2: efficacy of hexaflumuron against *Culex quinquefasciatus* in sullage canal.

The exposure time (10-60 min) considerably reduced (F = 554.88, P = 0.000) the adult emergence of *An. stephensi* from treated larvae at varying concentrations. However, the minimum exposure time of 10 min did not produce complete inhibition (100%) of adult emergence in fourth instar larvae at any of the treated dosages (Fig. 1).

Field evaluation of this compound in the slow moving sullage canal at the rate of 200 gm/ha showed high mortality of larvae subsequent to treatment up to three days and presence of pupae for a period of six days after which there was total absence of immatures for four weeks. When pupae were brought to laboratory and observed, adult emergence was found to be 12.5% and 33.5% on 2nd and 6th day respectively (Fig. 2), whereas in the control area adult emergence for the corresponding period ranged between 90-93%.

On treatment, hexaflumuron induced varying degrees of morphogenetic abnormalities which were visible in immatures and adults of all the three species tested. Such abnormalities resulted in mortality. Larval and pupal mortality also resulted due to direct kill by the toxic effect. Percentage larval mortality resulting from both was found to be higher at increasing exposure times with increasing concentration. Percentage pupal mortality and other abnormalities such as larval-pupal intermediate or pupal-adult intermediate were found to be more at higher concentrations.

DISCUSSION

From the observations and analysis it was established that effectiveness of IGR is dependent on exposure time as well as on the concentration. There was a significant time-concentration interaction observed in the adult emergence of the three vector species. At the application rates of 0.001, 0.01 and 0.1 mg/l, this IGR could completely prevent development of immatures and emergence of *Cx. quinquefasciatus* and *Ae. aegypti* adults, even at a minimum exposure time of 10 min.

This IGR induced notable abnormalities in larvae, pupae and adults of the three species of mosquitoes similar to those often induced by other IGRs (Arias & Mulla, 1975; Sharma et al., 1979; Kelada et al., 1980; Awad & Mulla, 1984). Most of the larvae showed extended body and characteristic dorsal splitting of thoracic cuticle after treatment (Fig. 3a). Many larvae were found attached to the previous moult and were observed moving rapidly from the water surface to the bottom of the beakers in an attempt to free themselves from larval cuticle. Even after repeated attempts the larvae died at prepupal stage (Sacher, 1971) with thoracic bulbous projection (Fig. 3b) or as larval-pupal intermediate with both larval and

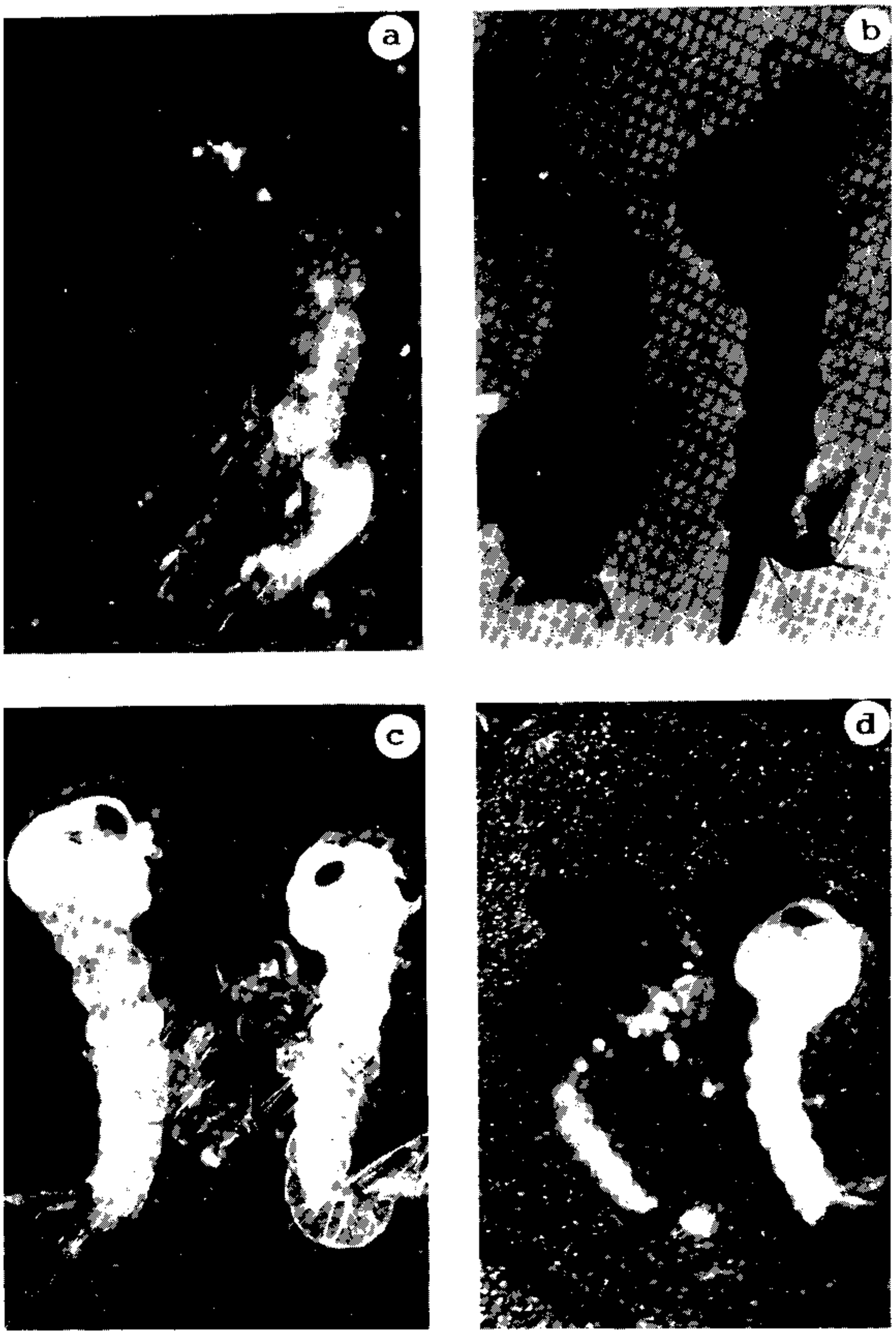

Fig. 3: *Culex quinquefasciatus* – a: dorsal splitting of thoracic cuticle. b: prepupal stage with thoracic bulbous projection. c: larval-pupal intermediates retaining larval cuticle. d: treated 'albino' (T) pupa and normal (C) pupa.

pupal characteristics. Such larval-pupal intermediate or prepupal stage larvae were immobile and dead shortly thereafter presumably from suffocation as reported earlier by Awad & Mulla (1984) in *Cx. quinquefasciatus* when treated with cyromazine.

Morphogenetic aberrations of other kinds were also noticed in pupae. Many pupae succeeded in splitting their exuviae and liberated themselves in part from the larval cuticle. Others retained their larval head capsule and were attached to the lower part of the abdominal cuticle (Fig. 3c). In few others the larval head capsule remained attached to the anterior portion of their cephalothorax while their entire body was free of the larval cuticle. Most of the pupae that were able to shed larval cuticle were white with soft body but without characteristic sclerotization of the pupal cu-

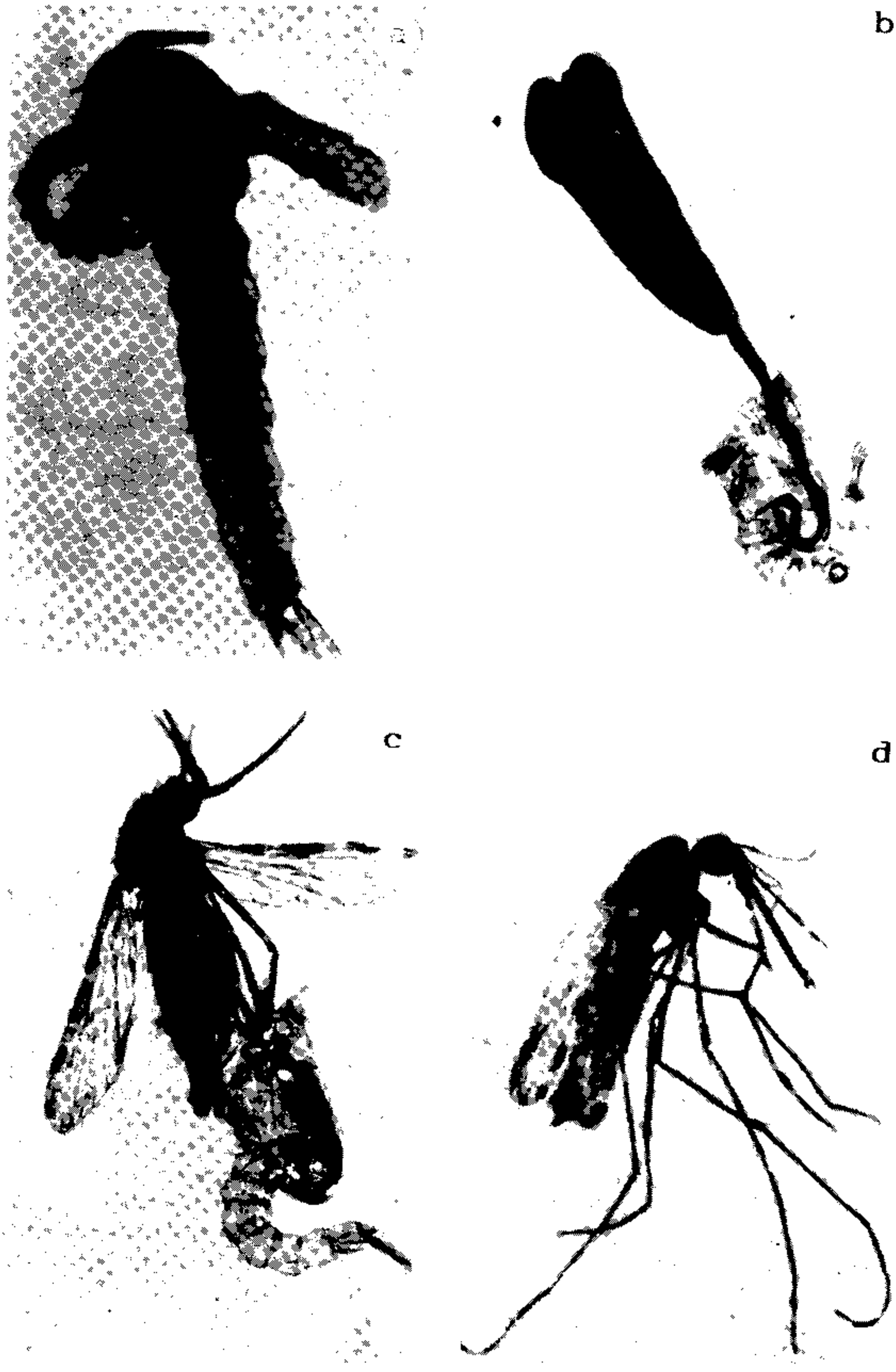

Fig. 4: *Anopheles stephensi* – a: pupal-adult intermediate with poorly developed adult characters. b: incompletely eclosed adult with legs and wings attached to the exuviae. c: incompletely emerged adult with legs attached to the exuviae. d: curved tarsi in the dead adult.

ticle. Such forms were earlier referred to as "albino" by Arias & Mulla (1975) (Fig. 3d). Most of the treated dead pupae were longer than normal pupae with distended body. Pupal-adult intermediates were found with poorly developed adult characters along with pupal structures (Fig. 4a). Many of the incompletely eclosed adults were attached to the exuviae by their legs or wings or both (Figs 4b, 4c). Many dead adults were also found with curved tarsi and crippled wings (Fig. 4d).

The actual mechanism involved in inducing morphogenetic abnormalities has not been elucidated for any benzoylphenylurea compound (Retnakaran, 1986). Information is restricted to studies with diflubenzuron which have shown that larvae ingesting this BPU

suffered from severe inhibition of chitin deposition in the cuticle and usually died attempting to moult (Retnakaran, 1982).

In field trial, four weeks control achieved in sullage canal indicates that this IGR can also be effectively used in the control of mosquitoes in flowing water habitats where the exposure time of the target population to the chemical would be minimized by various ecological and climatic conditions. Use of IGR in similar situation for the control of blackfly immatures has been demonstrated by McKague et al. (1978) against *Simulium verecundum* with Dimilin.

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