

MORPHOMETRIC AND MORPHOGENETIC ABERRATIONS INDUCED BY THE
IGR'S CHLORFLUAZURON (IKI) AND TWO FORMULATIONS OF
TRIFLUMURON IN *SCHISTOCERCA GREGARIA* FORSK.

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INTRODUCTION

In recent years, the development of insect growth regulators has received much attention as a potential measure for selective control of many insects. The use of IGRs during the sensitive periods of locust development may cause many morphogenetic abnormalities, as well as death of the treated insects, thus providing an excellent tool for locust control and suppressing the outbreak of mobile swarms.

The chitin synthesis inhibitors, Triflumuron (Bay Sir 8514) and/or Chlorfluazuron (IKI) have been reported to induce morphogenetic abnormalities in many insect species as *Tribolium confusum* (Ishaeya, *et al.*, 1981) and *Musca domestica* (Weaver and Kondo, 1987 and Bakr *et al.*, 1991). In the present study, the morphogenetic as well as the morphometric abnormalities which may be induced by these compounds in *Schistocerca gregaria* were investigated.

MATERIALS AND METHODS

Experimental Insect

The test insect, *Schistocerca gregaria* Forsk, was reared and handled according to the method described by Salem *et al.*, (1983) and Pener *et al.*, (1989), with some modifications. Fresh clover in winter and the leaves of *Sesbania aegyptiaca* in summer were used for feeding insects.

Experimental nymphs were isolated from the stock colony at the beginning of the first instar and reared in groups of 100 hoppers per cage (30X30X30 cm) in a walk-in insectary at the Faculty of Science, Benha University. An electric bulb (150 Watt) adjusted to a photoperiod of 12 hours was placed in each cage to provide additional heat to maintain an ambient temperature of $32 \pm 2^\circ\text{C}$.

Insect Growth Regulators :

Triflumuron (Bay Sir 8514, 6.5% EC) was kindly provided by Prof. Hammed, M.S., Department of Entomology, Faculty of Science, Ain Shams University.

Chlorfluazuron (IKI, 5% EC) and Triflumuron (Sir 8514, 6.5% FC) were kindly provided by Prof. Zidan, H., Sumitomo Co., Zamalek, Cairo.

Application Technique :

The LD50 of each compound was dissolved in 4 μl of solvent (IKI and Bay Sir 8514 were dissolved in acetone whereas Sir 8514 was dissolved in water), and applied topically on the dorsal surface of newly moulted 4th instar nymphs. Each treatment was replicated three times of ten individuals each. The untreated check groups received 4 μl of solvent only. The treated and untreated insects were incubated at constant conditions of $32 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ R.H.

Morphometric Measurements :

Fifth instar nymphs and adults which survived treatment with the IGRs were killed and various measurements were taken in order to quantify the morphological changes in the treated insects. Measurements of a group of untreated nymphs and adults were also taken as check. The measurements taken were Elytron (E), Femur (F) and the maximum width of the head (C). The phase ratio E/F and F/C were calculated in order to demonstrate the main deviation towards the solitarious phase according to the technique of Stower (1959).

Morphogenetic Abnormalities :

The morphological deformities, precocious adults and permanent nymphs were assessed. All treated and untreated locusts were examined daily for nymphal and imaginal colour changes according to the phase theory of locusts (Uvarov, 1928) in order to detect any abnormalities in metamorphosis process.

RESULTS AND DISCUSSION

Morphometric Measurements :

The present study revealed that, the LD50 of the used IGRs significantly ($P < 0.001$) reduced elytron length (E) of the resulting 5th instar nymphs and adults of the treated group. The hind femur (F) measurements were relatively close to the normal dimensions and showed no overall pattern of change. The head width (C value) was significantly decreased ($P < 0.001$) in 5th instar nymphs treated with Bay Sir 8514 and Sir 8514. A significant decrease ($P < 0.01$ and 0.001) was found in C values of adults descended from nymphs treated with Bay Sir 8514 and Sir 8514, respectively. The E/F ratio was significantly decreased ($P < 0.001$) in 5th instar nymphs of the treated groups. Also a significant decrease ($P < 0.05$, 0.01 and 0.01) was found in the E/F ratio of adults resulted from the groups treated with Bay Sir 8514, Sir 8514 and IKI, respectively. The F/C ratio was significantly increased ($P < 0.001$) in 5th instar nymphs and adults in all treated groups (Table I).

Implantation of Corpora allata in crowded nymphs of *Locusta migratoria* by Joly (1952) resulted in a similar decrease in E/F ratio. The significant increase of F/C ratio observed during the present study was due to a decrease in C value, as the F value did not change significantly. Similar results were found by Staal (1961) after the implantation of extera corpora allata in the 2nd nymphal instar of *L. migratoria*. This change in morphometric measurements indicate a shifting towards the solitary characteristics, and is probably due to the high titre of the JH occurring in treated insects. This shifting towards the solitary characteristics was also observed by Eid *et al.*, (1985) and Philips and Loughton (1979), using Cycloheximide against *S. gregaria* and *L. migratoria*.

Morphogenetic Abnormalities :

The present investigation revealed that, the morphogenetic aberrations induced in *S. gregaria* by the used IGRs were dose dependent. The morphogenetic aberrations increased with the increase of doses. However, at higher doses, most aberrations occurred during ecdysis of 4th instar. The highest doses of Sir 8514 and IKI produced a complete inhibition of adult emergence, (Table II).

S. gregaria exhibited various morphological abnormalities (Fig. 1) in response to the three IGRs used. Most treated insects were partially exuviated and dead within the exuviae. Some were completely exuviated but still attached to exuviae by legs or wings or mouth parts. Still many of the deformed 5th instar nymphs developed cuticular bulges. Permanent 5th instar in IKI treated

TABLE (I)

Morphometric Measurements of 5th Instar Nymphs and Adults of *S. gregaria* Resulted from Treated 4th Instar Nymphs with BAY SIR 8514¹, SIR 8514² and IKI³ (all measurements in mm.).

Dimensions	Normal 5th Instar Nym.	Treated 5th Instar Nymphs		
		Bay Sir 8514	Sir 8514	IKI
Elytron (E) ± S.E.	11.43±0.31	08.33±0.39	07.53±0.25	08.40±0.50
Hind femur (F) ± S.E.	17.41±0.26	17.11±0.86	17.83±0.92	17.02±0.39
Head width (C) ± S.E.	07.24±0.15	06.24±0.12	06.00±0.17	06.95±0.17
E/F Ratio ± S.E.	00.66±0.01	00.49±0.02	00.42±0.03	00.49±0.03
F/C Ratio ± S.E.	02.41±0.01	02.74±0.01	02.97±0.01	02.45±0.02
Sample size	15	10	10	10

(TABLE I Cont.)

Dimensions	Normal Adults	Treated Adults		
		Bay Sir 8514	Sir 8514	IKI
Elytron (E) ± S.E.	53.63±0.40	50.53±0.40	49.73±0.23	49.77±0.34
Hind femur (F) ± S.E.	24.46±0.28	24.16±0.28	24.05±0.23	24.76±0.50
Head width (C) ± S.E.	08.17±0.17	07.19±0.29	07.10±0.12	07.30±0.17
E/F Ratio ± S.E.	02.19±0.03	02.09±0.05	02.06±0.01	02.01±0.04
F/C Ratio ± S.E.	02.99±0.02	03.36±0.03	03.39±0.03	03.38±0.05
Sample size	15	9	8	7

* Significant ($P < 0.05$),** Highly significant ($P < 0.01$),*** Very highly significant ($P < 0.001$).

(1) 0.75 ug/nymph.

(2) 0.085 ug/nymph.

(3) 0.55 ug/nymph.

TABLE (II)

Morphogenetic aberrations induced by, Bay Sir 8514, IKI and Sir 8514 in *S. gregaria*.

IGR	Doses ug/nymph	Dead in 4th instar	Failure in 4th ecdyses	Normal 5th instar	Deformed 5th instar	Permanent 5th instar	Failure in adult emer.	Nym-adult intermediate	Precocious		Deformed	
									adult	adult	adult	adult
	0.480	10.00	16.67	66.66	6.67	0.0	06.67	0.00	0.00	0.00	59.99	0.00
BAY	0.560	16.67	13.33	59.99	10.10	0.0	06.67	0.00	0.00	0.00	49.90	3.33
SIR	0.640	13.33	20.00	43.34	23.33	0.0	06.67	3.33	0.00	0.00	30.01	3.33
8514	0.840	10.00	26.67	30.00	33.33	0.0	03.33	0.00	3.33	3.33	20.01	3.33
	0.920	16.67	43.33	16.67	23.33	0.0	06.67	0.00	0.00	0.00	6.67	3.33
	0.200	00.00	16.67	80.00	03.33	0.0	06.67	0.00	0.00	0.00	73.33	0.00
	0.300	00.0	31.11	46.69	20.00	2.2	06.67	0.00	0.00	0.00	40.02	0.00
IKI	0.400	00.00	23.33	50.00	26.67	0.0	23.33	0.00	0.00	0.00	26.67	0.00
	0.600	00.00	50.00	30.00	20.00	0.0	20.00	0.00	0.00	0.00	6.67	3.33
	0.800	00.00	76.67	06.66	16.67	0.0	06.66	0.00	0.00	0.00	0.00	0.00
	0.026	10.00	10.00	66.67	13.33	0.0	10.00	0.00	0.00	0.00	56.67	0.00
	0.052	03.33	36.67	46.67	13.33	0.0	06.67	0.00	0.00	0.00	40.0	0.00
SIR	0.078	06.67	43.33	36.67	13.33	0.0	10.00	0.00	0.00	0.00	26.67	0.00
8514	0.104	00.00	56.67	16.66	26.67	0.0	03.33	0.00	0.00	0.00	13.33	0.00
	0.130	13.33	66.67	06.67	13.33	0.0	06.67	0.00	0.00	0.00	0.00	0.00

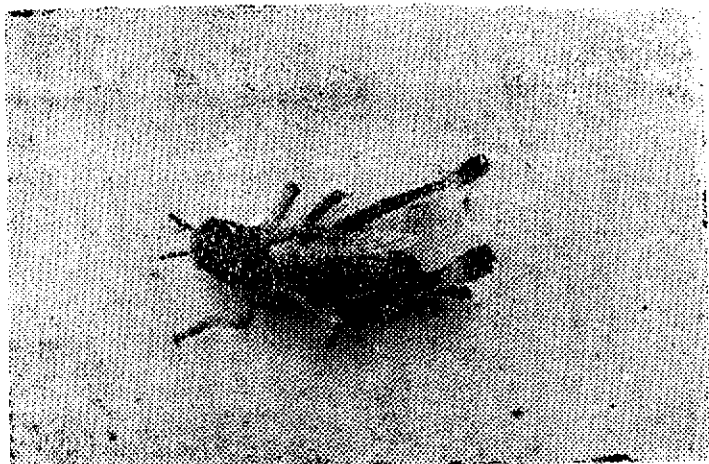
N.B. : 30, 4th instar nymphs were used in each treatment

nymphal-adult intermediates & precocious adults in Bay Sir 8514 treated insects were also observed.

Failure in ecdysis may be due to interference with chitin synthesis, inability to retract the mouth parts and/or failure in general contraction of the treated insects due to muscle weakness. Similar findings have been observed by Ghoneim (1988) who observed that 50 and 40 $\mu\text{g}/\text{nymph}$ of Cycloheximide applied topically to 0-24 h old 4th instar nymphs of *S. gregaria* resulted in Morphogenetic abnormalities of *S. gregaria* topically treated in fourth nymphal instar with the IGRs, Chlorfluazuron and Triflumuron.



A. Partially exuviated fifth instar nymph with deformed wings glued to the body and the gut extruded from the thorax.



B. Abnormal small fifth instar nymph with reduced compressed abdomen, crushed thorax and bent tibiae.



C. Permanent fifth instar nymph, with a deep dark pattern, slightly deformed wings, broken antennae, invisible eye stripes and indistinguishable POP.



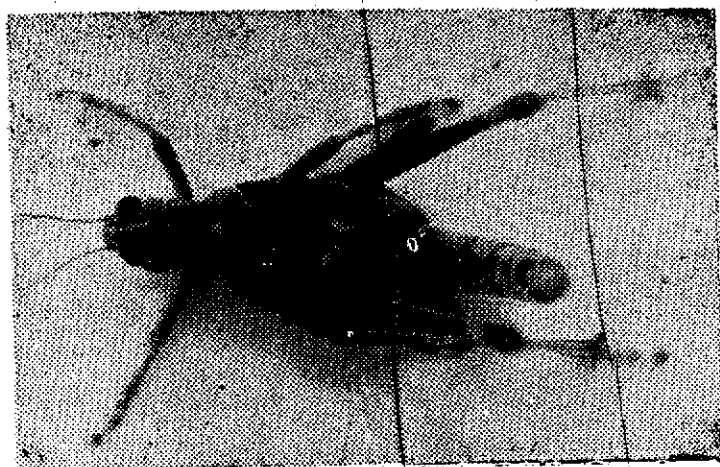
D. Precocious adult, completely free from the nymphal exuvia except for mouth parts and curled antennae. The size is small and the wings are short.

deformed 5th instar nymphs which failed to shed their exuviae completely and died. Similar effect was also observed by Farag and Varjas (1983) using Fluoromevalonate (FMeV, ZR-351b) against *Hyphantria cunea*.

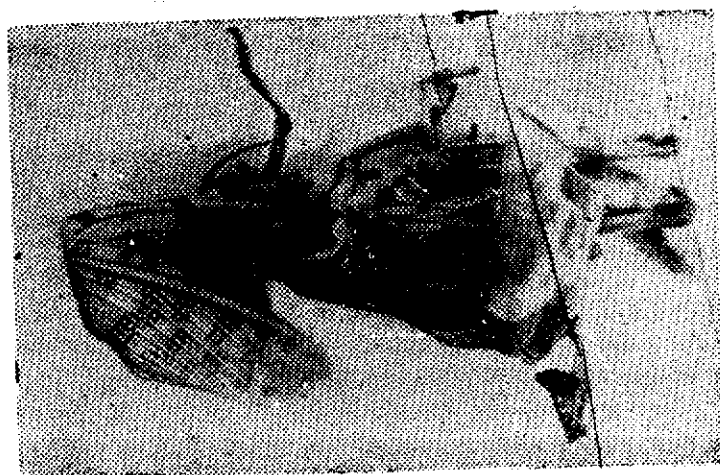
Bakr *et al.*, (1991) mentioned that, the total pool of free amino acids (FAA) increased as a result of protein synthesis inhibition by treatment with

Bay Sir 8514, it may be suggested that, the variations in quality and quantity of the free amino acid contents may interfere in the transcript of DNA during the process of protein synthesis which may be the reason for the observed abnormalities in fully ecdysed insects.

Wing deformations in treated insects were also observed by other authors, as Salem *et al.*, (1985), who used Precocene II and Cycloheximide against 4th nymphal instar of *S. gregaria*. De Kort and Koopmanschap (1991) after treatment of *L. migratoria* with Pyriproxyfen, observed that, a single injection as low as 2 μg induced malformation of the wings.



E. Nymphal-adult intermediate with enlarged wing buds and large pronotum.



F. Deformed adult with twisted abdomen and crumpled wings.

Cuticular bulges might be due to localized thinning of the cuticle which is produced into the balloon shaped bulges under the internal pressure of the gut extruding out from the thorax. This postulation is supported by Soltani (1983) who stated that, Diflubenzuron may induce a reduction in the thickness of post ecdysial cuticle. Similar observations have been described by Ghoneim (1988) who treated 4th nymphal instar of *S. gregaria* with Cycloheximide at a dose of 30 $\mu\text{g}/\text{nymph}$. The author found 40% failure in penultimate ecdysis, producing 5th instar nymphs with a very weak and thin cuticle extruding out the gut from the thorax causing death. Similar findings were also recorded by other authors in other insect species such as *Musca domestica* using Bay Sir 8514 & Altosid (Bakr *et al.*, 1991) and Bay Sir 8514. (Weaver & Begley, 1982).

Permanent 5th instar nymphs may be due to a depression in esterases activity in the early 5th instar nymphs, which in turn would result in prolonged high titre of JH in the haemolymph. The formation of permanent nymphs in *S. gregaria* following IGRs treatment was also observed by El-Gammal and Taha (1984) using Dimilin against 4th instar nymphs, Salem *et al.*, (1985) and Ghoneim (1988) using Cycloheximide.

Nymphal-adult intermediates may be attributed to undernutrition, muscle fatigue and/or hypoxia. Nymphal-adult intermediates were also recorded by Miall (1980) using Precocene II against 4th nymphal instar of *L. migratoria*. Bakr *et al.* (1991) used Bay Sir 8514 against *M. domestica* larvae and obtained larval-pupal intermediates.

Precocious adults may be produced as a result of the inhibitory effect of Bay Sir 8514 on Protein synthesis, especially JH-binding protein which protects JH from degeneration by general esterases (Hammock, *et al.*, 1975 and Sandburge, *et al.*, 1975).

SUMMARY

Early 4th nymphal instar of *Schistocerca gregaria* was topically treated with different concentrations of Chlorfluazuron (IKI, EC) and two different formulations of Triflumuron (Bay Sir 8514 EC and Sir 8514 FC). The LD50 of the used IGRs significantly reduced elytron (E) and head width (C) of the resulting 5th instar nymph and adult. The hind femur measurements (F) were relatively close to the normal dimensions. The E/F ratio was significantly decreased and the F/C ratio was increased. These results indicate a shifting towards the solitary phase.

The induced morphogenetic aberrations were dose dependent, however,

at higher doses, most aberrations occurred during the ecdysis of 4th instar. Most treated insects were partially exuviated and died within exuviae, some were completely released from exuviae but exhibited some deformations in legs, wings or mouth parts. Permanent 5th instar in IKI treated nymphs and nymphal adult intermediates and precocious adults in Bay Sir 8514 — treated insects were also observed.

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