THE ANTI-JUVENILE HORMONE ACTION OF CYCLOHEXIMIDE (RNA AND PROTEIN SYNTHESIS INHIBITOR) IN SOME NYMPHAL INSTARS OF, *SCHISTOCERCA GREGARIA* (FORSKAL).

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Abstract

Topical application of RNA and protein synthesis inhibitor, cycloheximide (3;2 (3,5-Dimethyl-1-2-oxocyclohexyl) -2-hydroxy ethyl glutarimide) to 0-24 H-old fourth instar female nymph, *Schistocerca gregaria* (Forskal) revealed that, biological activities are dose dependent. This treatment induced three types of responses: the high doses resulted in different percentages of the permanent fourth instar nymphs, the low doses produced precocious adults, meanwhile the lower doses, especially 30 µg/insect, inhibited the metamorphosis completely. C16-JH extra cycloheximide therapy restored the normal development and metamorphosis of the resulting fifth instar nymphs, which confirm that the last two types of response are related to juvenile hormone (JH) deficiency as a result of anti-JH action of cycloheximide.

On the other hand, the lowest doses of this compound (20, and 10 µg /insect) had produced the solitary green colour in the resulting fifth instar nymphs, that considered as an indicator for the high level of JH in their haemolymph. Owing to these obtained results agreed with the dual effect of anti-JH (precocene II), cycloheximide acts as anti-JH in *S. gregaria*.

INTRODUCTION

Ferkovich *et al* (1977) stated that cycloheximide inhibited JH- binding protein in the tissue culture of *Plodia interpunctella* fat body. So, general esterase's could degenerate Juvenile hormone (JH) causing a deficiency in its level. Injection of cycloheximide into fourth instar nymphs of *Locusta migratoria* by Phillips and Loughton (1979) inhibited JH esterase activities resulting in a solitary green colour fifth instar nymphs in response to the high level of JH; Szibbo and Tobe (1981) on *Diploptera punctata* found a partial destruction of the corpus allatum (CA) causing a reduction in the titre of JH which was followed by a compensatory hormone biosynthesis by the remaining cells resulting in a high level of JH.

Induction of precocious metamorphosis had been obtained by Unnithan and Nair (1979) on *Oncopeltus fasciatus*; El-Gammal *et al* (1986) on *S. gregaria* as a result of JH deficiency in response to anti-JH effects of precocenes. Morevere, Cassier and Delmore-Joulie (1976) found that, the presence of ecdysone alone during the last two days of the fourth instar nymphs and the early days of the fifth instar, *S. gregaria* favour gregarious features, while the presence of ecdysone and JH together resulted in solitarious development. Simonds *et al* (1994) stated that, ecdysteroid was affected in favour of JH in the azadirachtin treated nymphs of *L. migratoria.* Also, Mohamed and El-Gammal (2002) found that, the injection of azadirachtin into one day old 4th instar nymphs, *S. gregaria* prolonged the duration of the resulting 5th instar.

The present study aimed to provide an evidence for the unexpected anti-JH effects of cycloheximide.

MATERIALS AND METHODS

1.Insects: First instar nymphs were selected from the stock colony of *Schistocerca gregaria* (Forskal) which maintained under the crowed conditions of Hunter-Jones (1961) at Locust Res. Section, Plant Proc. Res. Inst., Agric. Res. Center. These nymphs were captured in cages (30 x 30 x 30 cm in diameters) and incubated at 32 C ± 2 in a constant room temperature adjusted photophase of 12 h and 65% ± 5 RH. Fresh leaves of the plant *Sesbania aegyptiaca* were daily provided as a feeding material.

1. **Cycloheximide administration:** Different concentrations of cycloheximide (3, 2 (3, 5-dimethyl – 2 oxocyclohexl)-2-hydroxy-ethyl-glutarimide) were dissolved in acetone and applied topically to 0-24 h old fourth instar nymphs. Groups of 20 4th instar nymphs were treated between the first and the second abdominal sternum with 480, 200, 100, 50, 40, 30, 20, and 10 µg/nymph. The untreated control nymphs were received 4 µl acetone. The treated and untreated insects were incubated as previously mentioned.

2. **C16-JH replacement cycloheximide therapy**

It was observed from the primary trials that, 30 µg cycloheximide per nymph prevented metamorphosis completely. Therefore, 40 newly moulted fourth instar nymphs (less than 24 h post-ecdysis) were treated with a dose of 30 µg cycloheximide mide. Twenty nymphs from this group were treated again with a dose of 40 µg C16-

JH. This dose was selected as it generally restored the normal growth and development. Another group of 12 newly moulted fourth instar nymphs were treated with a dose of 40 µg C16-JH per nymph. The hormone was applied topically to the ventral abdominal tips with 4 µl acetone.

Morphogenetic observation: The morphological deformities, precocious adults and permanent nymphs were measured. Experimental nymphs were examined daily for nymphal and imaginal colour changes. The nymphal duration's were calculated by Dembestars equation (1957).

RESULTS AND DISCUSSION

Table (1) shows that, treatment with the higher doses of cycloheximide (480, 200 and 100 µg/insect) resulted in 72.50, 70.0 and 60.0% permanent fourth instar nymphs for each dose, respectively. These nymphs seemed to be weak and inactive with a deep dark pattern (Fig. 1 A) and stay alive for more than 30 days.

The second treatment with the high doses, 50 and 40 µg, of cycloheximide per fourth instar nymph induced 6.7 and 36.67% precocious adult for each dose, respectively (Table 1). The precocious adults were miniature in size and wings, having the same pink colour of the normal gregarious adults (Fig. 1 B).

these two doses, Moreovere, resulted in 46.7 and 56.66% of deformed fifth instar nymphs with a patch of the adult cuticle at the site of cycloheximide application. These nymphs failed to shed their exuvia completely and died.

Also, Table (1) illustrates, that the dose of 30 µg cycloheximide per fourth instar nymph prevented metamorphosis completely. Among these amorphed individuals, 10% were permanent nymphs and 40% failed in penultimate ecdysis producing fifth instar nymphs with a very weak and thin cuticle getting out the gut from the thorax and abdomen, causing death. The rest (50%) of the resulting 5^{th} instar failed to reach the adult stage because they could not shed off their exuvia inspite of the occurrence of the adult cuticle. This phenomenone may be correlated to the action of cycloheximide as RNA and protein synthesis inhibitor during synthesis and deposition of cuticular protein. This explanation was introduced by Phillips and Loughton (1979) who stated that cycloheximide caused almost complete inhibition of protein synthesis in *L. migratoria*.

Table (1) indicates that, the low doses 20 and 10 µg from cycloheximide per fourth instar nymph resulted in 30 and 50% fifth instar nymphs with solitary green colour for each dose, respectively (Fig.2).This feature in agreement with the results of Phillips and Loughton (1979) in *L. migratoria.* They claimed that the injection of cycloheximide into the fourth instar nymphs caused the development of the next fifth instar with a solitary green colour in response to the producing high level of JH. They revealed that this high level of JH occurred by depressing of JH esterase activities by cycloheximide (RNA and protein synthesis inhibitor).

On the other hand, the main feature of this study in Table (1) is the high percentages of precocious adults obtained by the high doses of cycloheximide

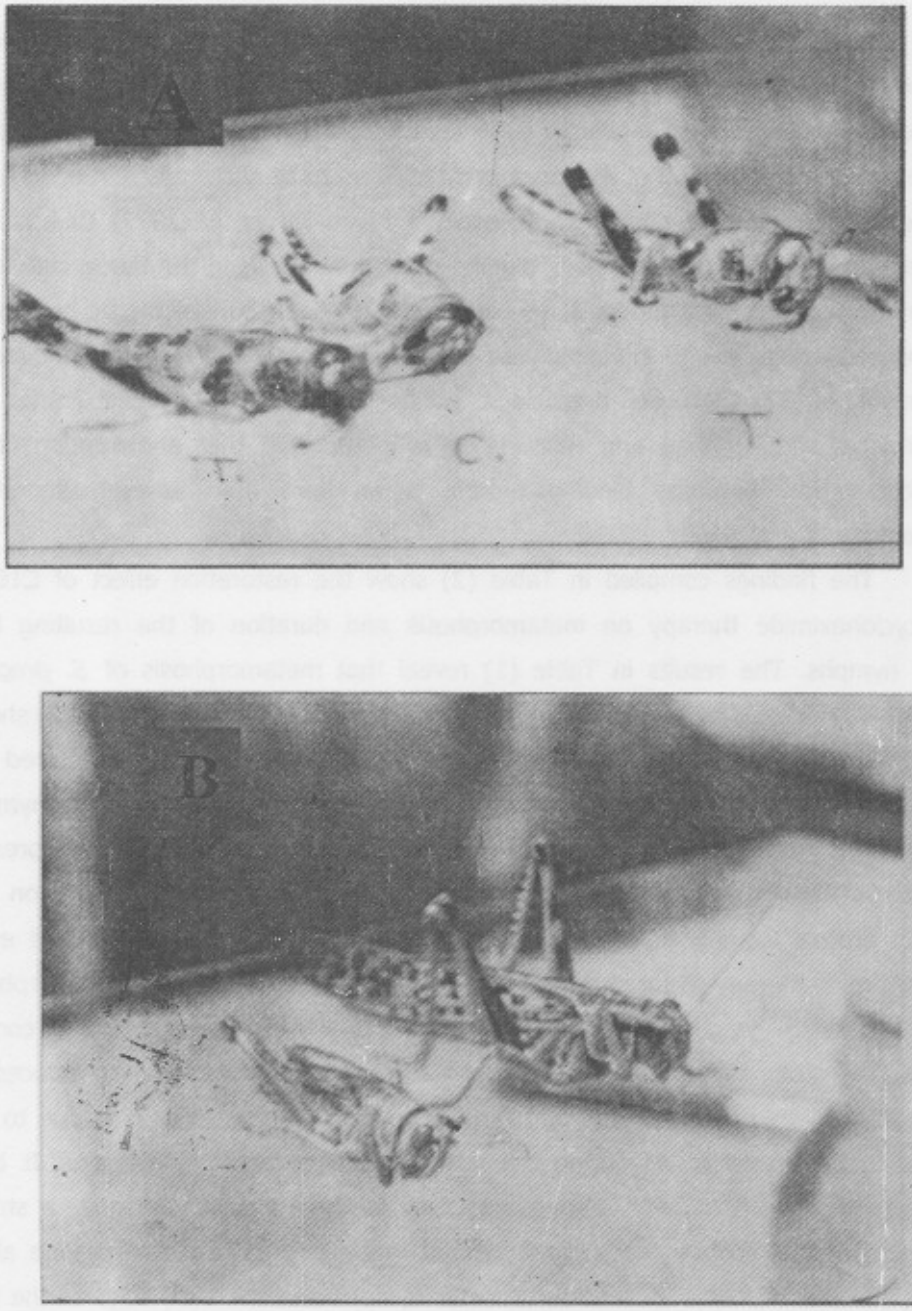

Fig 1. Indicates the resulting permanent 4th instar nymphs (A - T) compared with the untreated control one (A – C) and the precoucsious adults (B) after cycloheximide application to 4th instart, S. gregaria.

(Fig. 1 B). it may be produced as a result of the inhibitory effects of this compound on RNA and protein synthesis, especially JH-binding protein which protects JH from degradation. Hammock *et al* (1975), suggested that, the main function of JH-binding protein in the haemolymph of *Manduca sexta* appears to be the protection of JH from degradation by general esterases. In addition Ferkovich *et al* (1977) found that, cycloheximide inhibited the fat body binding protein synthesis in the tissue culture of *Plodia interpunctella*. Oberlander and Silkhacek (1976) deduced that the fat body of *P. interpunctella* degrades JH in vitro unless *M. sexta* binding protein has been added to the medium. Thus, these precocious adults obtained by cycloheximide are conformable with Unithan and Nair (1979) who reported that a disruption of or competition for, hormone binding protein is an item from several theoretical mechanisms of anti-JH.

The findings compiled in Table (2) show the restoration effect of C16-JH extracycloheximide therapy on metamorphosis and duration of the resulting fifth instar nymphs. The results in Table (1) reveal that metamorphosis of *S. gregaria* nymphs was blocked by a dose of 30 µg cycloheximide/nymph. While Table (2) shows that, treatment with 40 µg C16-JH nymph; extra 30 µg cycloheximide restored the normal duration and metamorphosis producing 50% of the perfect fifth instar nymphs and their corresponding adults (Table 2). These results supported the present hypothesis about the anti-JH effects of cycloheximide which acts directly on JH-binding protein leading to deficiency in JH level. The application of C16-JH extra cycloheximide therapy restored the normal duration, development and metamorphosis (Table 2) of *S. gregaria* in comparison with C16-JH, cycloheximide, and control results. The prolongation in the duration of the resulting fifth instar which induced by the extra C16-JH therapy or the administration of JH alone (Table 2) is due to the producing high level of JH during the last nymphal instar of *S. gregaria*. It is in concerte with the finding of Cassier and Delmore-Joulie (1976) who gave a strong evidence for this solitary feature, they found that the presence of ecdysone alone during the last two days of the fourth instar nymphs and the early days of the fifth instar, *S. gregaria* favour gregarious features, while the presence of ecdysone and JH together resulted in a solitarious development. Simonds *et al* (1994) stated that ecdysteroid was affected in favour of JH in the azadirachtin treated nymphs of *L. migratoria*. Also, Mohamed and El-Gammal (2002) found that, the injection of azadirachtin into one day old 4^{th} instar nymphs, *S. gregaria* prolonged the duration of the resulting 5^{th} instar.

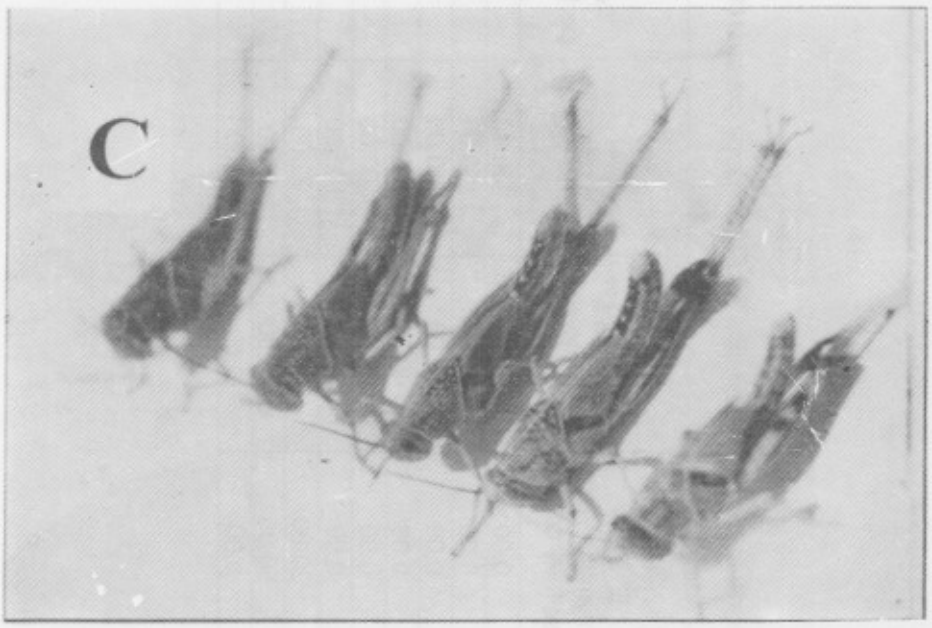

Fig 2. The produced solitary green color in resulting 5th instar nymphs, S. gregaria from the treated 4th instar nymphs with cycloheximide.

Table 1. The anti- Juvenile hormone effects of cycloheximide in the fourth instar female nymphs of *Schistocerca gregaria* (Forskal).

No. of insects used	Dose µg/nymph	(%) Permanent 4th instar nymphs	(%) Precocious adults obtained	(%) Resulting 5th instar nymphs with solitary colour	(%) Deformed 5th instar nymphs	(%) Final mortality	(%) Resulting perfect adults
40	480	72.50	0.00	0.00	0.00	100.00	0.00
40	200	70.00	0.00	0.00	0.00	100.00	0.00
40	100	60.00	0.00	0.00	10.00	100.00	0.00
30	50	6.70	6.70	0.00	46.79	100.00	0.00
30	40	6.70	36.67	0.00	56.66	100.00	0.00
40	30	10.00	0.00	0.00	50.00	100.00	0.00
30	20	0.00	0.00	20.00	50.00	70.00	30.00
40	10	5.00	10.00	50.00	35.00	15.00	50.00
30	Acetone	0.00	0.00	0.00	0.00	0.00	100.00

Table 2. Effects of C16 JH and C16 JH extra cycloheximide therapy on duration and metamorphosis of the female nymphal instars of *Schistocerca gregaria* Forskal.

Treatments	No. of nymphs used	Doses in µg	Fourth instar nymphs			Fifth instar nymphs		Total late nymphal periods (in days)	(%) Resulting adults
			Duration in days	(%) Failure in ecdysis	(%) Permanent nymphs	Duration in days	(%) Failure in the last ecdysis		
Cycloheximide	40	30 µg/nymph	5.88	40.00	10.00	8.80	50.00	14.68	0.00
C16-JH	24	40 µg/nymph	5.50	8.33	0.00	10.86	0.00	16.36	91.67
Cycloheximide + C16-JH	40	30±40 µg/nymph	6.17	40.00	10.00	8.80	0.00	14.97	50.00
Untreated Control	40	Acetone	4.55	0.00	0.00	7.00	0.00	11.95	100.00

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التأثيرات المضادة لهرمون الحداثة لمركب السيكلوهكساميد (مضاد تخليق الحمض النووى RNA والبروتين) على طور الحورية للجراد الصحراوى (شيستو سيركا جريجاريا فورسكال)

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أوضحت المعاملة الموضعية بمركب السيكلوهكساميد (مضاد تخليق الحمض النووى **RNA** والبروتيـــن) ضـــد حوريات العمر الرابع للجراد الصحراوى أن التأثيرات البيولوجية لهذا المركب يعتمد على الجرعة المستخدمة منه ٠ وأدت هذه المعاملة إلى ظهور ثلاثة تأثيرات واضحة حيث أدت الجرعات العالية منه إلى ظهور حوريات دائمة للعمر الرابع أما الجرعات المنخفضة أدت إلى ظهور الحشـــرات الكاملـــة قـــبل أوانهـــا من العمر الرابع مباشرة والجرعات الأكثر إنخفاضاً خاصة ٣٠ ميكروجرام / حورية أوقفت التطور إلى الحشرة الكاملة كلية ٠

وعند المعاملـــة التعويضية للحشرات المعاملة بهرمون الحداثة الثالث (وهـــــــو C) 16- JH أعـــادت حالة النمو الطبيعى لحوريات العمر الرابع وإختفت التأثيرات البيولوجيـــة السابقة وظهر العمر الحورى الخامس بصورة طبيعية ، مما يؤكد أن هذه التأثيرات الغير طبيعية كانت نتيجة لتأثير مركب السيكلوهكساميد على هرمون الحداثة بدم الحشرات المعاملـــة ٠

ومـــن جهـــة أخـــرى قـــد أدت الجـــرعات الأقـــل إنخفاضاً من هذا المركب (٢٠ ، ١٠ ميكروجرام/ حورية) إلى ظهور اللون الأخضر المميز للحوريات الإنفرادية فى الحوريات التجمعية المعاملة ، مما يشير إلى زيادة هرمون الحداثة فى دم حوريات العمر الخامس الناتجـــة، مما يتوافق مع الفعل المزدوج لهذا المركب ضد حوريات الجراد الصحراوى ٠