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Biological effects of Chitin-synthesis inhibitor, Hexaflumuron compound on the desert locust, *Schistocerca gregaria* (Forskal)

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ABSTRACT

Feeding application of different concentrations of Consult on one day old and six day old of the 5th nymphal instar of the desert locust, *Schistocerca gregaria* (Forskal) showed mortal action on treated nymphs, failure in ecdysis to adults and prolongation in the 5th nymphal age and adults had malformed wings, colour changes of body and failure of completely getting rid the last nymphal exuvia.

Keywords: Chitin-synthesis inhibitor, Hexaflumuron, the desert locust, *Schistocerca gregaria*

INTRODUCTION

The desert locust, *Schistocerca gregaria* (Forskal) is one of the most economic pests causing severe damage to crops, which consider the main food for human and animals. So, it seemed necessary to develop an effective preventive control strategy depends upon the early warning to suppress this multiplication and prevent the outbreak of the mobile swarms by an effective control tool against its nymphal instars.

In the last few years, scientists directed their efforts towards the control of insects by the use of insect growth regulators to avoid the hazards of insecticides (Bakr *et al*. 1984 & 1989).

IGRs are diverse groups of chemical compounds that are highly active against immature stage of insects and have a good margin of safety to most non-target biota including invertebrates, fishes, birds and other wild life, they are also safe to man and domestic animals, they will play an important role in vector control programs in the future (Mulla, 1995 and Bakr *et al*. 2008).

The main types of insect growth regulators used commercially are juvenile hormone analogues and chitin synthesis inhibitors (Parrella and Murphy, 1998).

MATERIALS AND METHODS

1. Maintenance of the culture:

1.1. Origin of stock culture and rearing in the laboratory:

The stock colony of *Schistocerca gregaria* was maintained for several years at the Locust research Division, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. The insects were reared and handled under the following technique described by Abbassi *et al*. 2003 and Bakr *et al*. 2008.

Leaves of leguminous plant, *Medicago sativa* were daily placed as feeding material. The cages were incubated in a constant room temperature (32±2ºC) and (30-50% RH).

1.2. Experimental insects:

The experimental nymphs were segregated from the gregarious stock colony at the beginning of the first nymphal instar and held up in cages (30x30x30 cm) in diameter. The cages were a wooden farmed equipped with zinc bottom covered by thin layer of sand, glass covered sides and a wire-gauze top provided with a little door.

All cages were incubated at (32±2ºC) and (30-50% RH). Unconsumed food, dead locusts and faces were removed daily.

The whole cage was thoroughly washed and effectively sterilized with an antiseptic agent every (4-6 weeks) or when ever it becomes empty or at the end of any experiment.

2. Insecticides used:

Chitin-synthesis inhibitor, Hexaflumuron (10% EC):

Hexaflumuron (Consult), [N 3, 5-dichloro-4- (1,1,2,2-tetrafluro-ethoxy) (phenyl-amino) carbony l-2,6- difluroben- zamide].

3. Treatment of experimental insects:

Both sexes of nymphs of one-day old and 6-day old of the 5th nymphal instars of *S. gregaria* during synthesis and deposition of the newly adult cuticle (Taha & El-Gammal 1990 and Bakr *et al*. 2008) were treated by feeding technique with Consult as the following:

Leaves of *M. sativa* were dipped in 50, 75 and 100 ppm of Consult for two minutes. Then leaves were air dried before being offered to the nymphs for feeding on it. Three replicates of 20 nymphs were subjected to each of the treated leaves.

After feeding for 24 hours on the treated leaves, the alive nymphs were transferred onto untreated leaves and left to feed for 24 hours after that mortality counts or malformed individuals were recorded.

5. Calculations and data analysis:

Statistical analysis of results was statistically analyzed by analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS, 1988).

RESULTS

1. Biological effects:

1. 1. Effects of Consult on some biological aspects of *S. gregaria:*

Results in Table (1) and graphically illustrated in Figs (1&2) showed that the effects of Consult (chitin synthesis inhibitor) on the 5th nymphal instar of *S. gregaria* during one day old by feeding technique.

Table (1): Effect of Consult on some biological aspects of the desert locust, *Schistocerca gregaria* treated as 1-day old of the 5th nymphal instar.

Conc. (ppm)	Duration of Nymphal instar			Adult stage			%Total inhibition of adult emergence Mean ±SE
	% Nymphal mortality Mean ±SE	% Failure to ecdysis to adults Mean ±SE	Old of nymphal instar/day Mean ±SE	% Adult mortality Mean ±SE	% Malformed adult Mean ±SE	% Adult emergence Mean ±SE	
Control	0.0±0.0	0.0±0.0	8.0±0.5	0.0±0.0	0.0±0.0	100±0	0±0.0
50	0.0±0.0	0.0±0.0	8.76±0.1	0.0±0.0	100±0.0	0.0±0.0	100±0.0
75	8.33±0.33	0.0±0.0	9.36±0.1	8.33±0.33	83.34±0.30	0.0±0.0	100±0.0
100	11.01±1.0	8.33±0.33	10.16±0.15	8.33±0.3	72.33±0.33	0.0±0.0	100±0.0
F 0.05	380.84***	191.54***	37.82***	227.83***	117.01***	-	-
LSD	0.99	0.31	0.49	0.31	0.41	-	-

F: Measurement of distance between individual distributions
LSD: Least Significant Deference

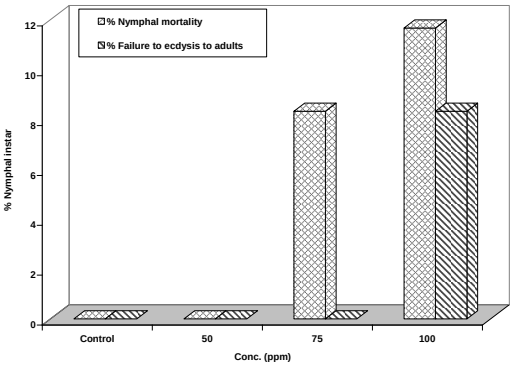


Fig. (1): Effect of Consult on some biological aspects of the desert locust nymphs treated as 1-day old of the 5th nymphal instar.

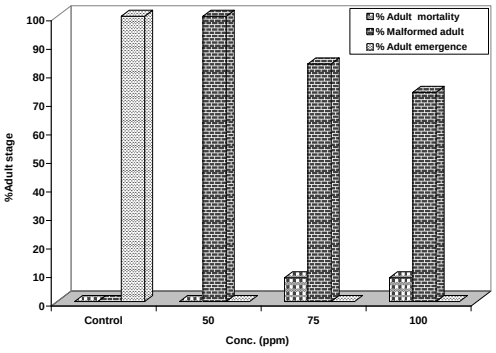


Fig. (2): Effect of Consult on some biological aspects of the desert locust adults treated as 1-day old of the 5th nymphal instar.

Data cleared that the percentages of nymphal mortality of the 5th nymphal instars of *S. gregaria* were 0.0, 8.33 and 11.01% after one day old

treatment with 50, 75 and 100 ppm of Consult, respectively comparing with control (0.0%), whereas, the percentages of adult mortality were 0.0, 8.33 and 8.33%, respectively comparing with control (0.0%). While, the percentage of adult emergency was 0.0% for all concentrations. On the other hand, the percentage of total inhibition adult emergence was 100% for all Consult concentrations comparing with control.

Also, the percentages of malformed adults reached 100, 83.34 and 72.33% after one day treatment of the 5th nymphal instar of *S. gregaria* when treated with 50, 75 and 100 ppm of Consult concentrations, respectively comparing with control. The percentages of nymphal instars failed to ecdysis to adult were 0.0, 0.0 and 8.33% when treated with Consult concentrations 50, 75 and 100 ppm, respectively (Table 1 & Figs 1&2).

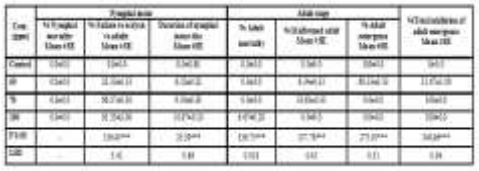
Statistical analysis in (Table 1) showed highly significant differences between the effect of Consult concentrations comparing with control in%nymphal mortality, %failure to ecdysis to adults,% adults mortality and %malformed adults after one day old of the 5th nymphs of *S. gregaria* treatments.

The obtained results in (Table 2 and Figs 3&4) showed that the effects of different concentrations of Consult on six day old of the 5th nymphal instars of *S. gregaria*. The percentage of nymphal mortality was 0.0% for all different Consult concentrations; while the percentages of adult mortality were 0.0, 0.0 and 6.67%, respectively, also % adult emergence were 68.33, 0.0 and 0.0% at 50, 75 and 100 ppm of Consult concentrations, respectively.comparing with control (0.0%). On the other hand, the percentages of total inhibition of adult emergence increased by 31.67, 100 and 100% with the increase of concentrations when comparing with control (0.0%).

Also, the results showed that the percentages of nymphs failed to ecdysis to adults when treated with different concentrations as follow: 23.33, 66.37 and 93.33% at 50, 75 and 100 ppm of Consult, respectively, while with the above mentioned concentrations the percentages of malformed adults reached 8.34, 33.63 and 0.0%, respectively.

Statistical analysis in (Table 2) showed highly significant differences between Consult concentrations comparing with control.

Table (2): Effect of Consult on some biological aspects of the desert locust, *Schistocerca gregaria* treated as 6-day old of the 5th nymphal instar.



F: Measurement of distance between individual distributions
LSD: Least Significant Deference

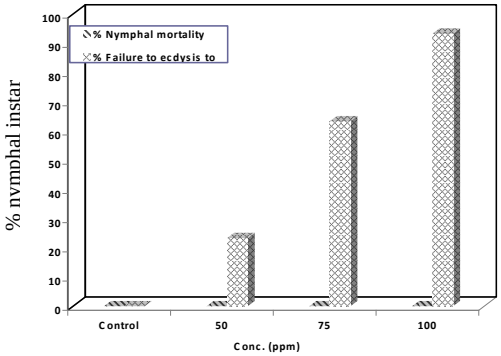


Fig. (3): Effect of Consult on some biological aspects of the desert locust nymphs treated as 6-day old of the 5th nymphal instar.

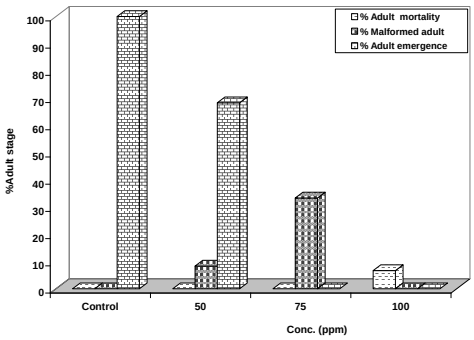


Fig. (4): Effect of Consult on some biological aspects of the desert locust adult treated as 6-day old of the 5th nymphal instar.

2.prolongation effect of Consult on *Schistocerca gregaria*:

Usually, the duration of the 5th nymphal instars of *S.gregaria* is about 8-day, but the application of Consult affects on this duration.

The duration of treated and untreated nymphs calculated according to Dembester equation.

Data in Tables (1&2) and Fig. (5) showed the effect of Consult concentrations on the duration of the 5th nymphal instars of *S. gregaria.* The experiments were carried out on one and six-day old of the 5th nymphal instar with different concentrations of Consult (50,75and 100 ppm). It was observed that, the duration of the treated nymphs was increased as result of treatment with Consult. The duration of treated nymphs during one day old reaching 8.76, 9.36 and 10.16 days after treatment with 50, 75 and 100 ppm, respectively, also the duration of treated nymphs during six day old were 8.32, 9.30 and 10.97 days with the same concentrations, respectively. It was concluded that the duration of treated nymphs had positive relationships with increasing Consult concentrations.

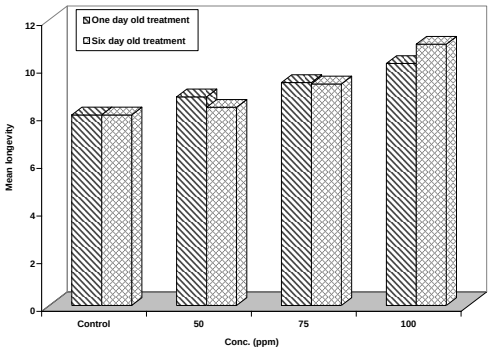


Fig. (5): Effect of Consult on the longevity of the 5th nymphal instar of *Schistocerca gregaria* treated as one day and six day old.

Statistical analysis in (Tables 1&2) shows highly significant differences between Consult concentrations comparing with control in old of nymphal instar/day after one and six day old of the 5th nymphs of *S. gregaria* treatments (F = 37.82 and 25.39 & LSD = 0.49 & 0.86).

1.3. Developmental events:

The treatment of nymphs of *S. gregaria* with tested compounds; Consult induced malformation in both nymphal instars and emerged adults as sown in Fig. (6). When the 5th nymphal instars of *S. gregaria* were treated some nymphs were unable to moult into adult stage and died without completing the moulting process. Different deformities were observed, some were able to split the old cuticle but unable to wriggle out of the old exuvia, some were able to complete the moulting process but the old cuticle connected with the resulting adults in different positions as legs or wings and some were able to complete the moulting process without any deformity in the resulting adults.

Treatment of the 5th nymphal instars of *S. gregaria* with different concentrations of Consult resulted in moulting disturbances which increased with the increase of Consult concentrations. The observation of resulted adults, indicated that, the most of adult emergence were unable to fly and sluggish in walking, jumping and climbing, also they have curled wings, grayish and buff solitary colour and absence of wing patches.

Fig. (6): shows that:
1: Normal adult.
2: Treated adult which treated as one day old of the 5th nymphal instar with Consult showed old exuvia connected to wings.
3: Treated adult which treated as one day old of the 5th nymphal instar with Consult showed twisted wings and absence of wings batches.
4: Treated adult, which treated as six day old of the 5th nymphal instar with Consult showed curled wings and buff colour of body.

DISCUSSION

1.Biological effects of Consult on *Schistocerca gregaria* by feeding technique:

The present investigation revealed that, the treatment with insect growth regulators has toxic effects on the desert locust, *Schistocerca gregaria.*

These effects depend on the concentration of the compounds and the age of the treated insects. In the present study, hexaflumuron (Consult) act as chitin synthesis inhibitor (CSI), was used against the 5th nymphal instar of *S. gregaria* during one day and six day old by feeding technique.

Chitin synthesis inhibitors act on insects by disturbing the deposition of chitin of the insect cuticle so that the moulting process is inhibited.

The present study showed that, the treatment with different concentrations against one day and six day old of the 5th nymphal instar of *S. gregaria* caused nymphal mortality and failure to ecdysis to adult increased with the increase of Consult concentrations. Also, the percentage of total inhibition of adult emergence reached to 100%.

More or less, the present results are agreed with those finding by several chitin synthesis inhibitors against the same acridide species, *S. gregaria* such as:diflubezuron, which interfered with the chitin synthesis during the nymphal ecdysis to the last instar causing some mortalities (Taha and El-Gammal, 1985), also diflubenzuron when injected to the 5th nymphs of *S. gregaria* was observed that, some treated instars were unable to moult and died without completing the moulting process, some were able to split the old cuticle but unable to wriggle out of the exuvia, some were able to complete moulting process but the resulting adults were deformed to varying degrees and some were able to moult without deformity in the resulting adults (Roa and Mehrotra, 1986), the greatest mortality was recorded during ecdysis of early the 4th nymphal instar to the 5th nymphal instar of *S. gregaria* when treated with chlorfluazuron (Abo El-Ela *et al.*, 1993b), also chlorfluazuron induced appreciable failure in ecdysis to adult stage when applicated on the last nymphal instar (El-Gammal *et al.*, 1993), Coppen and Jepson (1996a) when treated the 2nd nymphal instars of *S. gregaria* with diflubenzuron, hexaflumuron and teflubenzuron, they recorded mortality after all other treatments. Triflumuron caused different mortalities after 5 to 15 days of the barrier application in Mauritania (Wilps and Diop, 1997).

Also, the results are in agreement with some authors who applicated hexaflumuron on another insects such as (Haagsma and Rust, 2005), on western subterranean termite and (Marzban and Baniameri, 2005) on the diamondback moth and (Vahabzadeh *et al.*, 2007) on eastern subterranean termite.

2.Prolongation effects of consult on *Schistocerca gregaria*:

When applied the selected compounds, insect growth regulators, on the 5th nymphs of *S. gregaria* by using of the feeding technique, the age of the treated nymphs were affected when treated one and six day old of the 5th nymphal instars with different concentrations of Consult (hexaflumuron). The age of untreated nymphs was about 8-day, while the age of treated nymphs was prolonged by treatment with consult.

The prolongation, which happened in the age of the treated nymphs may be due to the effect of consult on the ecdysteriod and JH titers in haemolymph of the treated nymphs, this explanation was in agreement with (Key and Edney, 1936) who suggested that, the moults and the instar number in locusts may be under hormonal control.

Whereas the moulting hormone (ecdyson), which were produced from prothoracic gland, controls the moulting process and the duration of each nymphal instars of *Locusta.* That was recorded by implantation of an extra gland in the 4th instar shortened the mean duration of the instar from 8.11 to 6.25 days and implantation of an extra gland in the 5th instar reduced the duration. While extirpation of the gland from the 4th instar either prolonged it, or caused the hopper to remain longer than the normal without moulting; such individuals were called permanent larvae although they died within eighteen to twenty-two days, that was recorded by (Sir, 1966). Therefore, decreased levels of the ecdyson hormone resulted in prolongation of the nymphal duration.

Similar observations were also reported by Eid *et al.* (1982a), they noticed that, treatment with precocoene II and juvenile hormone III extended the duration of the 4th and 5th nymphal instars, whereas treatment with cycloheximide extended the duration of the 4th nymphal instar only. Also, the formation of a long-lasting additional instar, when exposed second-instar nymphs of *S. gregaria* to precocene II at 15 µg/cm^2 that was recorded by Salem *et al.* (1982b), but when the 4th nymphal instars was exposed to precocene II gave rise to precocious adults.

Other nymphs, which were treated by different concentration of Consult, died without moulting to the adult stage, this may be due to the reduction effect of consult on protein levels of haemolymph of the treated nymphs. This may be attributed to that; orthopterous insects have an unusually high requirement for protein, of which approximately 50% is deposited in the cuticle.

Also, the prolongation effect of diflubenzuron reached to 14 days when Azam and Seegh (1993) treated the 2nd nymphal instars of *S. gregaria* with diflubenzuron. Also the duration of the 2nd and 3rd instar of *S. gregaria* were significantly prolonged when they exposed to three benzoylphenyl urea's, diflubenzuron, hexaflumuron and teflubezuron that was observed by (Coppen and Jepson 1996a).

3. Developmental events:

Application of IGR generally disturbs hormone balance inside the insect body.

The present investigation revealed that the morphologenetic aberrations induced by the used of CSI (Consult) fed to the one day and six day old 5th nymphal instars of *S. gregaria* for 24 hours dietary period were concentrations dependent.

The application of Consult resulted in moulting disturbance and different morphological defects in treated adult locusts appeared as twisted wings, colour changes and the failure of get rid the last nymphal exuvia.

Several hypothesis have been introduced to explain the mode of action of Consult (CSI in general) on the morphologensis since Mulder and Gijswijt (1973) supported that, the ingestion of these compounds by insect larvae disturbed the endocuticular deposition during moulting process because they block the chitin synthesis. The blockage of chitin synthesis occurs due to disruption of function of connecting N-acetylg lucose amine moieties; to the chitin chain in spite of that the coupling of uridine disphosphate-N-acetyle-glucose amine (UDPAG), the ultimate chitin precursor, to chitin synthetase still proceeds (Post *et al.*, 1974).

Several lines of experiments argue explain direct interaction of these inhibitors with the chitin synthetase, instead, they may alter either vesicle transport or fusion, inhibit the translocation of chitin fibrils across the plasma membrane (Cohen, 2001), or

interfere with the hormonal regulation of chitin synthesis by influencing ecdysteroid production (Fournet *et al.*, 1995 and Bakr *et al*. 2008). However, the exact mode of action of this CSI on the adult transformation and exclusion is still puzzling (Merzendorfer and Zimoch, 2003).

The results of Consult treatments were in agreement with (Mariy *et al.*, 1981) who concluded that the effect of diflubenzuron on the development of nymphs and the reproductive potential of females. Also, 4 categories of morphological infestations due to diflubenzuron treatment was observed by Rao and Mehrotra (1986). While in (1987) Rao and Mehrotra injected diflubenzuron into 2 to 3 day old 5^{th} nymphal instars of *S. gregaria* and recorded, a significant reduction in the chitin content of the wings. Both diflubenzuron and teflubenzuron caused abortive moult on the 5^{th} nymphal instars of *S. gregaria* and most survivors developed twisted or misshape wings (Wakgari, 1997).

Also, IKI induced appreciable failure in ecdysis to adult stage when treated one-day old of the 5^{th} nymphal instars for one day (El-Gammal *et al.*, 1993).

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ARABIC SUMMARY

التأثيرات البيولوجية لمثبت النمو الحشري هكسافلوميرون على الجراد الصحراوي *شيستوسيركا جريجاريا*

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تعتبر آفة الجراد الصحراوي *شيستوسيركا جريجاريا* إحدي أهم الآفات الحشرية من الناحية الإقتصادية حيث أنها تدمر المحاصيل الزراعية التي تمثل الغذاء الأساسي للأنسان والحيوان.

لذلك أصبح ضروريا أن نمنع تكاثر وهجرة هذه الأسراب كي نتجنب هذا الضرر، ومن ثم فإن معظم الدراسات تتجه لتقييم التأثيرات المختلفة لبعض العناصر الفعالة من منظمات النمو الحشرية ضد حوريات الجراد الصحراوي عمر أول وسادس للحصول علي طريقة مكافحة جديدة وفعالة وتجنب المشكلات الناجمة عن استخدام المبيدات الكيميائية المضرة بالإنسان والبيئة لذلك وضعت هذه الدراسة الحالية لتقييم تأثير مركب من منظمات النمو الحشري (الكونصلت) على حوريات العمر الخامس من الجراد الصحراوي.

وتهدف هذه الدراسة الي تقييم التأثيرات البيولوجية وشوهدت النتائج في العمر الخامس للحورية والطور اليافع للحشرة.

ويمكن تلخيص أهم النتائج المتحصل عليها فيما يلي:

أولا: التأثيرات البيولوجية والتغيرات في السلوك:

أسفرت النتائج عن أن تغذية حوريات العمر الخامس أول يوم وسادس يوم للجراد الصحراوي علي أوراق البرسيم الحجازي المغمورة فى تركيزات مختلفة من الكونصلت (٥٠، ٧٥، ١٠٠ جزء من المليون)، كان له تأثيرات سلبية على الحوريات الحية حيث يؤدى الى موت بعض الحوريات قبل الانسلاخ الى الطور اليافع ولوحظ اطالة لعمر الحوريات التى عوملت بهذه المادة مع فشل نسبة

من الحوريات فى الانسلاخ للطور اليافع تزداد حتي تصل الى ١٠٠% بزيادة التركيزات كما أن الحشرات اليافعة من الحوريات المعاملة حدث لها تشوهات فى الاجنحة وفشل بعضهم فى التخلص التام من جليد الحوريات وكذلك تميزهم باللون الرمادى.