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Histopathological, biological and biochemical effects of lufenuron on *Schistocerca gregaria* (Orthoptera: Acrididae)

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Abstract:

Locusts are considered a dangerous insect attacking almost the important economic crops. One of these injurious insects is the desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae), which has a great economic importance in Egypt. Newly moulted second, third, fourth and fifth instar nymphs of the desert locust, *S. gregaria* was sprayed with the chitin synthesis inhibitors cymax, lufenuron in a recommended dose 40ml/100L. water to assignment the percentages of mortality, malformation and prolongation in life period also to evaluate histological changes in testis compared with the normal histological structure of control testis and determine total protein. Mortality percentages were 26.67, 23.33, 16.67, 10 and 10% for second, third, fourth, fifth nymphal instars and adult insects respectively, but were 40, 46.67, 36.67 and 36.67% through moulting. Also, malformation percentages were 30, 23.33, 20 and 23.33% in second third fourth fifth nymphal instars respectively. The life period of the treated nymphs reached at 7.33, 8.33, 8.67 and 10.33 days to second, third, fourth and fifth nymphal instar, respectively, but normally the duration of life to untreated nymphs were 5.67, 6.33, 7 and 7.67 days, respectively. The treatment induced variable histological changes in the testis. Effects on the testis were manifested by cytoplasmic vacuolation. Also, the treatment caused many ultrastructural changes in male germ cells including scattered vacuoles, degeneration, ad-electron mitochondria and many vacant areas. The levels of haemolymph protein decreased after 5,10 and 15 days when the males were treated for one day old and reached 61.25, 40.53and 26.59 mg/100 ml, respectively as compared with 79.86, 75.42 and 82.03 mg/100ml of untreated males, respectively. Those changes may lead to infertile males.

Introduction

Throughout the world are spreading locusts and grasshoppers widely. Locusts occur great damage in Egypt to many economic crops as, clover, maize, sugarcane, cotton, leguminous and cereals, one of these insects is the desert locust, *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) (Soliman, 2019). The desert locust *S. gregaria* is invading many countries in Africa and Asia (Hamadah

et al., 2013). Locust and grasshoppers have ability to eat their own weight (2-3g) of invaded plants every day (Alomenu, 1998). Chemical insecticides are the common method to locusts control with using Ultra Low Volume application method. Protection plants from locusts infestation needs using fast acting pesticides, like Organophosporous and Pyrethroids. The widespread use of traditional insecticides has caused serious environmental impacts. To avoid these risks, proceed has been made through last decades to develop new pesticides. This trend has led to find Insect Growth Regulators (IGRs) (Abdel-Kerim and Shebl, 2002). IGRs are different groups of chemical compounds which are highly effective against immature stage of insects, also they have a well border of safety to generality non-target organisms such as invertebrate also, to domestic animals and human, they will play an essential role in control programs in the future (Mulla, 1995). The major types of IGRs which used commercially are Juvenile hormone analogues and chitin synthesis inhibitors (Parrella and Murphy,1998). IGRs can be divided according to the mode of action as: chitin synthesis inhibitors and substances that intervene with insect hormone action (i.e. JH analogues, and ecdysteroids) (Tunaz, and Uygun, 2004). The lufenuron (Match) is chitin synthesis inhibitor that overlaps with chitin synthesis (Oberlander and Silhacek, 1998). As little attention has been given to the effect of IGR, such as lufenuron on desert locust. The present study is undertaken to appear the effect of this IGR on the second, third, fourth and fifth nymphal instars mortality and malformed percentages and to determine the total proteins and examine the histological effects in the male testis.

Materials and methods

1. Experimental Insect:

Experimental Insect was desert locust *S. gregaria* which took from the culture of department of locust and grasshopper, agricultural research center. The insects were reared according to Hassanein (1965). Fresh clover leaves, *Trifolium alexandrinum* were presented during the period of study.

2. Insect growth regulator:

Cymax 5% EC (Lufenuron) It was obtained from Shoura Chemicals Company, Km 28, Cairo- Alexandria desert road.

3. Treatment of the desert locust with chitin synthesis inhibitor:

Newly moulted adult and 2^{nd} , 3^{rd} , 4^{th} , 5^{th} instar nymphs and fresh clover, *Trifolium alexandrinum* was sprayed with chitin synthesis inhibitor (CSI), Lufenuron which was prepared as recommended concentration 40ml/100L. water. and leaved even feed on treated clover leaves for 24hrs. The control insects were fed on untreated clover leaves and kept under the same conditions. Each treatment was divided to three replicates (10 nymphs/rep.). Surviving nymphs were kept at 32 + 2 °C to follow up its moulting to the next instar.

4. Histological preparations:

Electron microscope**:** Immature adult males treated with Lufenuron and leaved even became mature males were dissected in insect saline solution. For transmission electron microscopy (TEM) and examined with JEOL JEM-1400 Electron microscope Kv 120.

5. Determination of the total proteins

Samples collection and preparation: Adult were taken for experiments where, the biochemical effects of Lufenuron on haemolymph components were evaluated. The experiments were carried out by treatment of 1-day old under laboratory conditions (Robert *et al.*, 2002). Samples of the haemolymph were taken

at different intervals of 5, 10, and 15days after treatment. Total proteins were determined by the method of Bradford (1976).

Results and discussion

1.Mortality and malformed percentages of *Schistocerca gregaria* after treated with lufenuron:

Table (1) and Figure (1) showed the effectiveness of chitin synthesis inhibitor (CSI), lufenuron on the 2nd, 3rd, 4th and 5th nymphal instar and adult of *S. gregaria* during one-day old by spraying technique. Data cleared that the percentages of nymphal mortality were 26.67, 23.33, 16.67 and 10% after treatment with recommended concentration of Lufenuron, respectively, comparing with control (0.0%). When the 2nd, 3rd, 4th and 5th nymphal instar of *S. gregaria* were treated, some nymphs were unable to moult into next stage and died without completing the moulting process where mortality percentages through moulting were 40, 46.67, 36.67 and 36.67%, respectively. Different deformities were observed, some were able to split the old cuticle but unable to complete the moulting process but the old cuticle connected with the resulting adults in different positions as legs or wings malformation percentages were 30, 23.33, 20 and 23.33% and some were able to complete the moulting process without any deformity in the resulting adults. Most of adult emerged were unable to fly and sluggish in walking, jumping and climbing. All adults, which resulted of treatment, showed the following morphological changes, hypertrophied and twisted wings, absence of the most wing patches, also they have curled wings. It was noticed that, the duration of the treated nymphs was prolonged as result of usage lufenuron. This duration reached to 7.33, 8.33, 8.67 and 10.33 days to second, third, fourth and fifth nymphal instar, respectively, but normally the duration of untreated nymphs was 5.67, 6.33, 7 and 7.67 days to second, third, fourth and fifth nymphal instar, respectively.

Table (1): Mortality percentages and mlaformes of Lufenuron on nymphal instars and adult of the desert locust.

Effect / Treat.	Mortality percentage	mortality percentage through moulting	Duration of untreated instars/day	Duration of treated instars/day	Malformed percentage	emergence percentage	Total percentage
2nd instar	26.67	40	5.67±0.38	7.33±1.45	30	3.33	100
3rd instar	23.33	46.67	6.33±0.26	8.33±0.88	23.33	6.67	100
4th instar	16.67	36.67	7	8.67±0.33	20	26.66	100
5th instar	10	36.67	7.67±0.26	10.33±0.67	23.33	30	100
Adult	10	0	0	0	0	90	100

Means +/- Standard Error

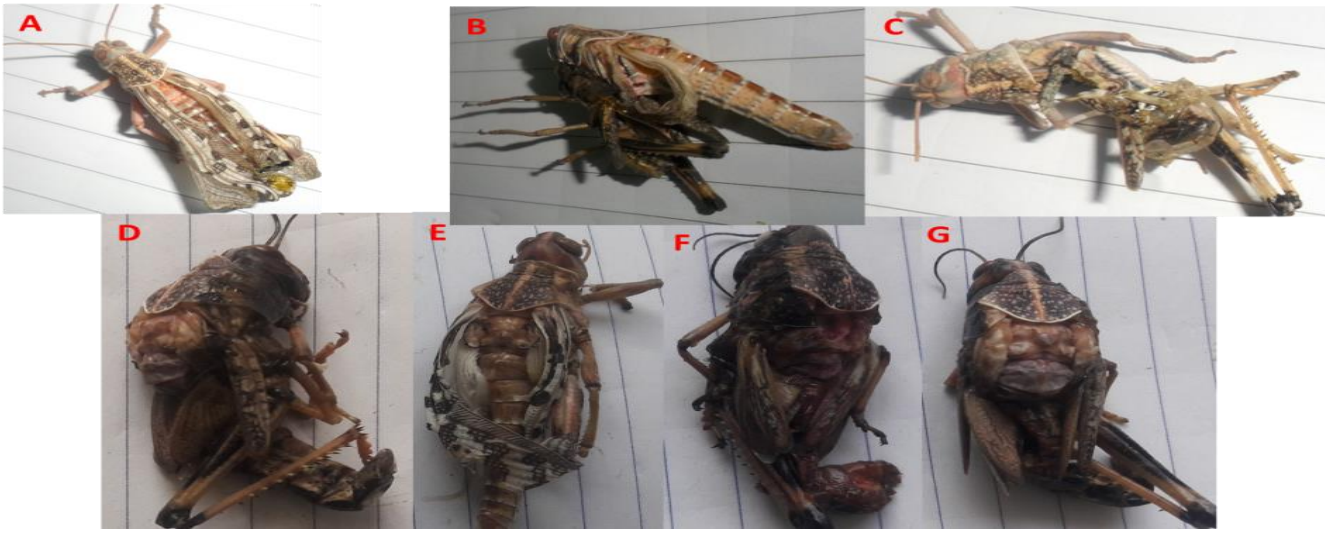

Figure (1): Malformes of Lufenuron on nymphal instars of the desert locust *Schistocerca gregaria* A. Curled wings – E. Twisted wings – B and C. Old cuticle connected with the result adults in different positions D., F. and G. Nymphs were unable to moult into adult stage and died without completing the moulting process.

Lufenuron may be considered selective and safe compound to target insects only. This study is a step in understanding histological, biological and biochemical effects of the Chitin synthesis inhibitor, Lufenuron on the desert locust *S. gregaria*. Usage chitin synthesis inhibitor CSI, lufenuron (Cymax 5% EC) on the desert locust in a recommended dose was used through the spray on the newly moulted adult and nymphal instars second, third, fourth and fifth. The lethal action of Lufenuron was detected for either the nymphal instars or adult stage. The present results agree with Taha and El-Gammal (1985) found that diflubenzuron (DFB) caused some mortalities to *S. gregaria* during the ecdysis to the last instar. Also, Tiwari (2000) appeared that chlorfluazuron affected the survival potential. When treatment of the second nymphal instar with diflubenzuron caused various mortality percentages after 14 days (Azam and Al-Seegh, 1993). The CSI, IKI-7899 caused high mortality during the fourth and fifth instars (Abdel-Magid, 1993). After treatment insects with 100 µg/insect Imidazole compound caused 80% mortality (Kumari *et al*., 2001). The present study showed different degrees of the mortal potential of (Cymax) depending on nymphal instar and adult. Probably, mortalities percentages ascribed to inhibition of chitin synthesis or to indirect effects on hormones levels (Hajjar and Casida, 1978). Also, the insect death by Lufenuron may be causing as a result to some attribution in the newly formed cuticle (Fytizus and Mourikis, 1979). Or that insects were unable to moult into next stage. These suggestions can be showed for the killing power of Lufenuron as recorded in the present study on *S. gregaria.*

2. Histological studies on testis of the desert locust *Schistocerca gregaria* :

Histological examination of sections of testes of *S. gregaria* treated with lufenuron showed some alterations in primary spermatogonium. Many spermatocyte nuclei show pyknosis where the chromatin contracts into a dense, deeply stained irregular mass and Spermatids are reduced in number showing either degeneration or malformation, also spermatocytes showed degeneration (Figures 2 and 3).

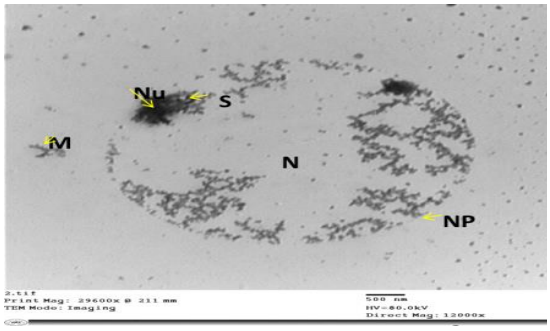

Figure (3): Photomicrograph of a longitudinal section of the testis of treated *Schistocerca gregaria* showing definitive primary spermatogonium. Nucleus (n) with irregular outline; nucleolus (nu) Apical cell cytoplasm

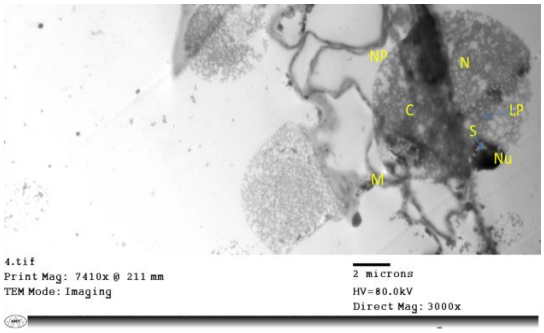

Figure (2): Photomicrograph of a longitudinal section of the testis of control *Schistocerca gregaria* showing definitive primary spermatogonium. Nucleus (n) with irregular outline; nucleolus (nu): apical cell cytoplasm

The male reproductive system of *S. gregaria* in this study showed like that in chrotogonus trachypterus trachypterus (Wagan, 1977), *P. pictus* (Wagan and Pitafi, 1990) and the histology of *S. gregaria* testis agrees with conformable structures of the acridids *Locusta migratoria* L. (Chapman, 1985) and *Poekilocerus pictus* (Fabr.) (Orthoptera: Acrididae) (Saxena and Aditya, 1969). The present study on the desert locust *S. gregaria* treated with lufenuron demonstrated histopathological changes of treated males. Those changes because of the impact of lufenuron appear as degeneration of spermatogenic stages and cytoplasmic vacuolation. These results agree with Saxena and Aditya (1969) who showed on *P. pictus* after treatment with apholat and with Shayin and Usharani (1996) who used cypermethrin and sodium selenite on *P. pictus*. Also, with *L. migratoria* treated with hempa (Güven, 1989); on *Earias insulana* (Boisd.) (Lepidoptera:Noctui dae) treated with pyriproxyfen (Hussein *et al*., 1993); These vacuolation reflects the primary morphological response to cell injury in several ways. This is due to the detrimental or toxic impacts of treatment on the cell membrane; this induces significant disruptions both structurally and functionally within its permeability system. This can contribute to an increased impregnation of water into the cells if it accumulates enough in the cell, these intracellular waters create transparent cytoplasmic vacuoles suggesting the presence of generally referred to as hydropic degeneration pathological symptoms (Sakr *et al*., 2000).

Application of pyriproxyfen to *S. gregaria* caused cytoplasmic vacuolation in the testis which was known to be the primary stage of cell deterioration. Among other studies Cellular vacuolation was identified as associated with fatty degeneration that occurs under the influence of a deferent of chemical and physical factors (Saleh, 1996). These findings are close to those reported in apholate-treated grasshopper *P. pictus* (Saxena and Aditya, 1969), cypermethrin and sodium selenite (Shayin and Usharani, 1996); hempa-treated *L. migratoria* (Güven, 1989) and in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) treated with extracts of some botany (Assar *et al*., 2001).

Many authors documented the reduction and damaging effect of these chemosterilant on the number and structure spermatids. Hussein *et al*. (1993**)** revealed in this respect decrease in the number of spermatids produced

in the testis of pyriproxyfen-treated *E. insulana*. They have also noted that the testicular follicles inhabit only early stages of spermatogenesis. Also noted reduction and degradation of different stages of spermatogonic clumping in *Eyprepocnemis plorans plorans* (Charpentier) (Orthoptera: Acrididae) which have been treated with lead nitrate. Similarly, Assar *et al*. (2001) noted depletion of various spermatogenesis stages in *T. castaneum* treated with some extracts of the botany.

This study showed that lufenuron was responsible for significant histopathological changes. When the male of *S. gregaria* treated with lufenuron has identified cellular degeneration and vacuolation. It can be concluded that lufenuron is successful at the histological level which leads to the prevention of normal sperm formation of ending with distorted sperm that cannot fertilize the ovum. Hence, Lufenuron may cause the male fertility reduction of *S. gregaria*. There are conflicting views regarding lufenuron′ role in testis production and the spermatogenesis process in insects. Spermatogenesis occurs in many species of insect in the larval or pupal stage when the hormone from the prothoracic glands may induce the process (Engelmann, 1970) possibly under a low titre of lufenuron. Foster (1967) has stressed that spermatogenesis doesn't seem to be under the influence of the corpus allatum, Metwally (1979) studies on *Spodoptera littoralis* (Boisd.) (Lepidoptera:Noctuidae), Yagi and Kuramochi (1976) on *S. littoralis* and Leviatan and Friedlander (1979) on *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) have showed, however, that corpus allatum hormone or its analogs can alter or inhibit the spermatogenesis process or can produce defective sperms. Thus, two mechanisms tend to clarify lufenuron′s mode of action on *S. gregaria* testis. The first of these is a clear influence on testis. The lack of spermatide and sperm indicates a separation mechanism has been compromised. This seems to point to an impact overloading caused by Lufenuron. Lufenuron′s this morphogenetic action. The second mechanism that may account for the pathological condition is that the corpora allata interferes with natural hormone production. Since differentiation regulation is believed to be in the corpora allata (Wigglesworth, 1973), it could be argued that one of the modes of action may be the corpora allata′s reduction in the production of hormones.

3. Effect of lufenuron on the total protein of the desert locust *Schistocerca gregaria*:

Data in Table (2) and showed that, the levels of haemolymph protein decreased after 5,10 and 15 days. After 5,10 and 15 days the haemolymph protein levels in male reached 61.2533, 40.5267 and 26.59mg/100ml respectively as compared with 79.86, 75.42333and 82.02667 mg/100ml of untreated males (F = 327.9 and LSD = 5.45).

Table (2): Effect of lufenuron on the total protein of the desert locust *Schistocerca gregaria*:

Days after application	Lufenuron			mg Protein \100ml haemolymph (Mean±S.E)	Control			mg Protein \100ml haemolymph (Mean±S.E))
	Rep.1	Rep.2	Rep.3	mean	Rep.1	Rep.2	Rep.3	Mean
5th days	60.56	63.35	59.85	61.2533+/1.068a	80.54	77.91	81.13	79.86+/0.99b
10th days	40.4	41.8	39.38	40.5267+/-0.7a	77.3	75.64	73.33	75.42333+/1.15b
15th days	29.9	22.27	27.6	26.59+/-2.26a	83.16	82.2	80.72	82.02667+/0.71b

[b] significant; [a] non-significant

Means, within row, bearing different subscripts are significantly different (p<0.05%)

In this study lufenuron appeared reduction in protein level in treated insects of *S. gregaria* as a compare with untreated insect. Those changes in total protein may be due to the toxic action of CSI, this tested compound changes the expression process of protein. Specific biological processes occur as a result of protein (Bakr *et al*., 2010). The total protein concentration of treated insects decreased during the study period after 5,10 and 15days after treatment than in untreated insects. The decline in protein content gradually increased until reached minimum value after 15th day of application.

Some of this protein contents use in the synthesis structural proteins and enzymes and may be storage compounds. Part of them is used as an energy source or in the developing larva is used in the synthesis of nonprotein components. haemolymph protein is considered importance for the understanding many different physiological processes that are very important for the reproduction in insects, such as, maturation, egg production, development and ecdysis therefore, quantitative assay of protein is important. Changes in proteins are great during stages of development and tissues differentiation such as, during metamorphosis (Alrubeai and Gorell, 1982) and sexual maturity of the reproductive organs (Engelmann, 1970). Normally, in the case of normal male, total protein declined due to the tissues differentiation of reproductive organs and spermatogenesis process in testes. In addition to, the result indicated that Lufenuron after 15th days of treatment caused decrease of total protein in adults of *S. gregaria* less than the control insects. Those results were likewise demonstrated by Lusis (1963) who detailed that, the neurosecretion from the standards intercerebralis of the brain influenced proteins synthesis in the fat body. In this manner, lacking incitement of neurosecretory activity would prompt protein insufficiency and decreased the measure of the protein, which required for oocytes development, and consumed from the haemolymph. Comparative results were accounted in ladybird beetles, Slogget and Lorenz (2008) observed that protein content decays pitifully over development. The results concur with Steel and Hall (1985), they reported that protein levels of the treated nymphs were not exactly in the control individuals, when treated the 5th nymphal instar of *S. gregaria* by benzoyl-phenyl urea (S-71624). likewise, the protein levels were decreased in the haemolymph of the treated females and males of the fifth nymphal instar of *S. gregaria* was found by Badawy and El-Gammal (2000), who treated the fifth male and female nymphal instar by (S-71624). The deccreae of the concentrations of

protein, ecdysteroids and juvenile hormone in the haemolymph of the fourth and fifth nymphal instars of *L. migratoria* were also noted by Baehr *et al*. (1979) when examined the impact of ecdysteroids and juvenile hormone, on protein levels in the haemolymph of the fifth nymphal instar.

It can be concluded that those changes which occurred as result to use Lufenuron may lead to infertile males. Lufenuron also caused similar damaging effects to those caused by many chemical insecticides and chemosterilants which have many hazardous effects on livestock and household including mammals. This suggests that the applying this IGR on a wide scale safely, especially among illiterate farmers.

References

Abdel-Kerim, A. I. and Shebl, D. E. (2002). Biological parameters for evaluating the effect of insect growth regulators against grasshopper *Eyprepocnemis plorans plorans* (Charp.) and cotton leaf worm, *Spodoptera littoralis* (Boisd.). Egyptian Journal of Agricultural Research, 80(2): 571 – 581.

Abdel-Magid, A. D. (1993). Effects of some insect growth regulators on the developmental stages of *Schistocerca gregaria*. Unpublished M.Sc. Thesis, Zagazig University.

Alomenu, H. S. (1998): Current trend in African migratory locust plague prevention. Outlook on Agriculture, 14: 165-173.

Alrubeai, H. F. and Gorell, T. A. (1982): Electrophoretic analysis of testicular protein components in developing *Tenebrio molitor*. Insect Biochemistry, 12 (2): 171-175.

Assar, A. A.; Emara, T. E. and Abou El-Mahase, M. M. (2001): Histopathological effects of some botanical extracts on the body wall, midgut and gonads of the rust-red flour beetle, *Tribolium castaneum* (Coleopter: Tenebrionidae). J. Egypt. Ger. Soc. Zool. 35E:17-30 (11th) International Conference, Faculty of Science (Aswan) South Valley University.

Azam, K. M. and Al-Seegh, A. A. A. (1993): Effect of diflubenzuron on second instar nymphs of desert locust *Schistocerca gregaria* (Forsk.). Journal of Research Andhra Pradesh Agricultural University, 21: 48-50.

Badawy, N. S. and El-Gammal, A. M. (2000): The biochemical effects of the antichitin synthesis compound (S-71624) in the haemolymph of the fifth nymphal instar of *Schistocerca gregaria* (Forskal). Egyptian Journal of Applied Science, 15(9): 279-290.

Baehr, J. C.; Prochereon, P.; Papillion M. and Dry, F. (1979): Haemolymph levels of juvenile hormone, ecdysteroids and protein during the last two larval instars of *Locusta migratoria*. Journal of Insect Physiology, 25: 415-421.

Bakr, R. F.; Helmy, N.; Namwr, G. A.; El Ibrahim S. and Helmy, O. M. (2010): Changes in protein content of *Culex pipiens* mosquito treated with two agriculture waste extracts. Egyptian Academic Journal of Biological Sciences, 3(1): 95-103.

Bradford, M. (1976): Rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein dye

binding. Annual Biochemistry, 72: 248-254.

Chapman, R. F. (1985): Coordination of digestion F. A. Kerkut, L. I. Gilbert (Eds.), Comprehensiv e Insect Physiology, Biochemistry and Pharmacology, Vol. 4, Pergamon Press, Oxford , 213-240.

Engelmann, F. (1970): The physiology of insect reproduction. Department of Zoology, California Univ., Los Angeles, Oxfod, Engl. Pergamon,pp. 307 ..

Foster, W. (1967): Hormone-mediated nutritional control of sexual behaviour in male dung flies. Science, 158: 1596–1597.

Fytizus, E. and Mourikis, P. A. (1979): Some aspects of the action of a juvenoid on the growth of *Heliothis armigera* (Hbn.) (Lepidoptera: Noctuidae), body weight, feeding and utilization of food. Zeitschrift für Angewandte Entomologie, 88: 542-547.

Güven, T. (1989): Hexamethyl phosphortriamide (HEMPA) kemosterilantının *Locusta migratoria* (Orthoptera: Acrididae) spermatositlerinin ince yapısıana etkileri. Doğa Türk Biyoloji Dergisi, 13(2): 72-87.

Hajjar, N. P. and Casida, J. E. (1978). Insecticidal benzoylpyridlyureas: Structure – activity relationships as chitin synthesis inhibitors. Science, 200: 1499-1500.

Hamadah, Kh. Sh.; Ghoneim, K. S.; El-Hela, A. A.; Amer, S. M. and Mohammad, A. A. (2013): Disturbed Survival, Growth and Development of the Desert Locust *Schistocerca gregaria* by different extracts of *Azadirachta indica* (Meliaceae) and *Nigella sativa* (Ranunculaceae). Egyptian Academic Journal Biological Science, 6(2): 1 -21.

Hassanein, M. S. (1965): Laboratory and outdoor cultures and breeding of locusts and grasshoppers. FAO Publications, 5/31901, pp.10.

Hussein, N. M.; Enan, R. A. and Mohamed, M. I. (1993): Effects of the juvenoid pyriproxyfen on gonad development in male, *Earias insulana* Boisd. (Lepidoptera: Noctuidae). Al-Azhar Journal of Agricultural Research, 17: 129-140.

Kumari, A.; Kumar, U. and Agawal, H. C. (2001): Inhibitory effects of 1-benzyl-5[(c)-2,6-dimethyl-1,5-heptadienyl] imidazale (KK-42) on desert locust, *Schistocerca gregaria* (Forsk.). Indian Journal of Experimental Biology, 39: 811-813.

Leviatan R. and Friedländer M. (1979): The eupyrene-apyrene dichotomous spermatogenesis of Lepidoptera. Developmental Biology, 68(2): 515-524.

Lusis, O. (1963). The histology and histochemistry of development and resorption in the terminal oocytes of the desert locust, *Schistocerca gregaria*. Quarterly Journal of Microscopical Science, 104: 57-68.

Metwally, S. M. I.; Abd El-Rahim, W. A. and Dakroury, F. (1979): Effect of certain physical and chemical characteristics of some cotton genotypes on the biological aspects of the cotton leaf worm *Spodoptera littoralis* (Boisd.); in Egypt. Tanta Journal of

Agricultural Research, 5(2): 243-248.

Mulla, M. S. (1995): The future of insect growth regulators in vector control. Journal of American Mosquito Control Associate, 11(2): 269-273.

Oberlander, H. and Silhacek, D. L. (1998): New perspectives on the mode of action of benzoylphenyl urea insecticides. In: Ishaaya I, Degheele D, Editors. Insecticides with Novel Modes of Action, 92-105.

Parrella, M. P. and Murphy, B. C. (1998): Insect growth regulators. Growers Talks, 62(2): 86-89.

Robert, M. O.; Andrena, K.; Goettel, M. S.; Jacques, B. and Micheal, J. B. (2002): Attenuation of fungal infection in thermoregulating *Locusta migratoria* is accompanied by changes in haemolymph proteins. Journal of Invertebrate Pathology, 81: 19-24.

Sakr, S. A.; AL-Rawi, M. M. and Saleh, A. T. (2000): Changes in carbohydrates and total proteins of rat ovarian tissue intoxicated with a carbamate insecticide. Oxford Research Forum Journal, I (2): 25-30.

Saleh, A. T. (1996): Effect of the organophosphate insecticide, Sumithion (fenitrothion) on the liver of Albino rats. Saudi Journal of Biological Science, 6 (2): 179-1 87.

Saxena, S. C. and Aditya, V. (1969): Histopathology and Histochemistry of Insects Treated with Chemosterilants. II. On the Studies of DNA in Active Testes of *Poicelocerus Pictus* (Orthoptera: Acrididae) Treated with Apholate. Cytologia (Tokyo), 34(3): 405-407.

Shayin, S. and Usharani (1994): Histopathological effects of sodium selenite and its co-exposure with cypermethrin on the testis of adult *Poekilocercus pictus* (Orthoptera: Acrididae). Journal of Environmental Biology, 17(1): 11-15.

Sloggett, J. and Lorenz, W. M. (2008): Egg composition and reproductive investment in aphidophagous ladybird beetles (Coccinellidae: Coccinellini): egg development and interspecific variation. Physiological Entomology, 33: 200-208.

Soliman, M. M. M.; Mohanna, K. M.; Abdel-Fattah, T. T. A.; Moustafa, O. R. M. and El-Sheikh, W. E. A. (2019): Efficiency of Agroecosystem Compounds against the desert locust *Schistocerca gregaria* (Forskal) and the African migratory locust *Locusta migratoria migratorioides* (Reiche and Fairmaire)". Scientific Journal of Agricultural Science, 1(1): 30-37.

Steel, J. E. and Hall, S. (1985): Trehalose synthesis and glycogenolysis as sites of action for the corpora cardiacum in *Periplaneta americana*. Insect Biochemistry, 15: 529-536.

Taha, G. Z. and El-Gammal, A. M. (1985): Laboratory evaluation of diflubenzuron against the fourth instar nymphs of *Schistocerca gregaria* (Forskal). First International Conference of Applied Science, Zagazig University, IV: 269-278.

Tiwari, L. D. (2000). Toxicity of chlorfluazuron to fifth instar

hoppers of *Schistocerca gregaria*. Indian Journal of Entomology, 62(2): 211-213.

Tunaz, H. and Uygun, N. (2004): Insect growth regulators for insect pest control. Turkish Journal of Agriculture and Forestry, 28: 337-387.

Wagan, M. S. (1977): A comparative study of male reproductive organs of Orthoptera of Sind (Pakistan). Pakistan Journal of Zoology, 9(1): 35-40.

Wagan, M. S. and Pitafi, K. D. (1990): The anatomy and histology of male reproductive organs of *Poekilocercus pictus* (Fabricius) (Pyrgomorphidae: Acrididae: Orthoptera). Pakistan Journal of Zoology, 22(2): 117-121.

Wigglesworth, V. B. (1973): The significance of "apolysis" in the moulting of insects. Physiological Entomology, 47: 115–209.

Yagi, S. and Kuramochi, K. (1976): The role of juvenile hormone in larval duration and spermatogenesis in relation to phase variation in the tobacco cutworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). Applied Entomology and Zoology, 11: 133–138.