Egypt. Acad. J. biolog. Sci., 1 ( 1) 13 - 21 (2008) ISSN: 1687 – 8809
E. mail. egyptianacademic@yahoo.com
Received: 10/11/2007

# Effect of Cascade, *Oriza sativa* bran extract and Karate on fine structure of the ovary of *Schistocerca gregaria.*

**Mohamed A Hussein[1].; Laila S. Hamouda[1]; Reda F. A. Bakr[1]; Hassan H.A.[2];and Elsokary Z.F.[2]**
1-Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt.
2-Plant Protec. Res. Instit., Agric. Res. Center, Giza, Egypt

## ABSTRACT

The ultrastructure changes in the ovarian follicle of normal adult females and those developed from treated 5th nymphal instar of *Schistocerca gregaria* with $LC_{50}$ of anti-chitin compound (cascade), plant extract (*Oriza sativa* bran extract) and synthetic pyrethroid (Karate) were examined.

In the present study electron micrographs of follicle cells of treated adult females with previous compounds showed a degeneration of ovarioles and oocytes .There is also evidence of mitochondrial disintegration . It is also noticed that vacuoles were increased and enlarged in follicle cells and yolk bodies were cracked mostly in two halves.

**Key words:** *Schistocerca gregaria* – Orthoptera - IGRs - plant extracts- ovaries - ultrastructure - histopathological aberrations.

## INTRODUCTION

The desert Locust *S. gregaria* is potentially the most dangerous of the locust pests because of the ability of swarms to fly rapidly across great distances.The control strategy for controlling the nymphal instars of desert locust depends upon some insecticidal agents such as insect growth regulators, plant extracts as well as synthetic pyrethroids. The previous evaluation of these new compounds can prohibit metamorphosis and block embryonic development (Taha and El-Gammal, 1990 ; El-Gammal, 1992 and El-Gammal *et al.,* 1994).

*Schistocerca. gregaria* had panoistic ovary, which is composed of number of ovarioles (Weber,1954 and Martoja,1964). Each ovariole was surrounded by basal lamina and consists of follicle cells and oocytes. The ultrastructure and function of follicles in locust ovary were described by ( Kimber, 1980 and Davies & King 1972).

The aim of the present study is to examine the ultastructural changes occurred in the ovarioles of the female *S. gregaria* developed from the treatment of 5th nymphal instars with sublethal concentration ($LC_{50}$) of anti-chitin compound (cascade), plant extract (*Oriza sativa* bran extract) and synthetic pyrethroid (Karate).

## MATERIALS AND METHODS

### Origin of population:

The stock colony of *Schistocerca gregaria* was maintained for several years at the locust Research Division, plant protection Research Institute, Agricultural Research Center, Dokki, Cairo. The insects were reared and handled according to the method described by Abbassi *et al.* (2003).

**Tested compounds:**

a) Chitin synthesis inhibitor: Cascade 10% E.C (Flufenoxuron)
b) Plant extract: Bran of rice (*Oriza sativa*)
C) Synthetic pyrethroid : karate 20% E.C (Lambdacyhalothrin)

The 5th nymphal instar of *S. gregaria* was fed on Leaves of *Medicago sativa* that dipped in 30 ppm for cascade, in [$5.5x10^3$ ppm] for rice bran extract and in 98 ppm in case of karate for three minutes. After feeding for 24 hours on the treated leaves, the alive nymphs were transferred onto untreated leaves until the adult females emergence.

**Electron microscopy studies:**

Adult females of *S. gregaria* were prepared for electron microscopy. The insects were killed by twisting the head to break the "neck" membrane. The posterior tip of the abdomen was cut off and the head, with the gut attached, was removed. Ovary cleaned from the surrounding fat body and then it was dissected in ice-cold (0-5 °C) karnovsky fixative, pH 7.3 (Karnovsky, 1965). The tissues were transferred to fresh ice-cold fixative for 1hr. After washing for 30 min in 0.1M sodium cacodylate buffer, pH 7.3, the tissues were post-fixed for further 1h in 1% osmium tetroxide in 0.1M sodium cacodylate buffer, pH 7.3 at 4.°C (Brissan *et al.,* 1996).

The samples were then dehydrated at room temperature via a graded series of ethanol solutions to propylene oxide prior to embedding in Araldite epoxy resin. Semithin sections were cut from these blocks stained with toluidine blue and examined by the light microscope (Spnrr, 1969).

The obtained ultrathin sections obtained from selected blocks were mounted on copper grids stained with uranyl acetate and lead citrate and then viewed in a Jeol transmission electron microscope (12000 $EX_2$) at the central laboratory, Faculty of Science, Ain Shams University.

## RESULTS

**Structure of ovarian follicle of normal adult**

The ovarian follicle was examined by transmission electron microscopy. The follicular epithelium of vitellogenic follicles is composed a single layer of more or less columnar to cuboidal cells without nuclei. Electron micrograph of follicle cells showed abundant of rough endoplasmic reticulum and Golgi bodies, oval shape mitochondria in the apical cytoplasm of follicle cell (Fig.1).

Follicle cell nuclei are large and occupied most of the cell volume throughout vitellogenesis. The nuclei are also irregular in shape and contain numerous cytoplasmic intrusions. Also, channels exist between follicle cells suggest that, the channel walls consist of only part of the total width of the follicle cell coated pits occur on the follicle cell membranes along these intercellular channels and the channels are filled with a flocculent material having the same appearance as that filling the spaces between the epithelium and the
oocyte (Figs. 2 & 3).

In follicle cells which containing mature oocytes are characterized by having an abundance of large yolk bodies each of which is surrounded by a granulated rim (Fig. 4).

**Ovarian follicle of treated adult with cascade**

The histopathological examination of the ovarioles of adult female *S. gregaria* showed different deterioration effects on organelles after the treatment with cascade at ($LC_{50}$).

Ovarian follicles have degeneration of cell components (Fig. 5). Their cytoplasm and organells were lyzed and vacuolized. There is disorganization and aggregation of mitochondria with malformation of their border (Fig. 6).

In oocytes, the yolk bodies are surrounded by relatively clear rings, some yolk bodies showed cracks and fissures dividing the yolk bodies into 2 halves, while others showed normal appearance. Large lipid droplets of different sizes were also presented in the oocyte (Fig. 7).

**Ovarian follicle of treated adult with rice bran extract**

Electron micrograph of ovarioles showed degeneration of cell components of the follicular epithelial cytoplasm, aggregation of mitochondria and the cytoplasm was vacuolized.

The nucleus contains abnormal and dark chromatin clumps and have irregular chromatin pattern (Fig. 8).

Oocytes were also showed the most obvious signs of damage and the yolk bodies are surrounded by relatively clear rings with marginal and central pale colour (Fig. 9).

**Ovarian follicle of treated adult with karate.**

Ultrastructure of ovaryof treated adults of *S. gregaria* ovarioles with karate, showed the ovarian follicle has abnormal appearance in which the cytoplasm is severely degenerated and vacuolized. Mitochondria disorganized, aggregated and showed differentiation deformity of their outline and disintegration of their crista (Fig. 10).

Oocytes showed the presence of numerous rough endoplasmic reticulum and the yolk bodies have spherical shape and granulated with different sizes (Fig. 11).

## DISCUSSION

*Schistocerca gregaria* as well as other orthopterous insects has panoistic ovarioles enclosed in nucleated ovarian sheath. Each ovariole consists of: terminal filament, attaching the ovariole to the fat body and inner body wall. Followed by a germarium which contained undifferentiated germ cells and vitellarium which consists of number of follicles or egg chambers and contains oocytes in different developmental stages (Engelmann, 1970).

There are four distinct periods during oocyte development in *S.gregaria* were recorded by Tobe and Pratt (1975): an early growth period, previtellogenic period, vitellogenic period when vitellogenin is deposited, and finally chorionation, which proceedes ovulation.

Follicle cell cytoplasm in early stages exhibited an organizational polarity with respect to the conspicuous organelles. The apical cytoplasm is rich with ribosomes and mitochondria, while rough endoplasmic reticulum (RER) and Golgi complexes occupy the basal and basolateral regions of the cells. During the early stages, when polarity exists, the RER and Golgi are active as inferred from the large number of small vesicles associated with the Golgi stacks. The polarity begins to disappear during late stage reflects a shift to chorion protein synthesis (John and James, 1989).

Junctional complexes between follicle and between epithelium and oocyte are present in insect ovarian follicles, but spatial and temporal organization of junctional complexes varies among insect species.

Morphological data suggested that desmosomal and septate junctions between follicle cells are regarded to the baseolateral and apical membranes, while the apicolateral membranes are free of junctional complexes.

During yolk deposition, the junctional complex presumably help to maintain intercellular communication and the structural integrant of the epithelium, but the fat of junction complexes at the end of yolk deposition varying among species (John and James, 1989) and (Ferenz, 1993).

Treatment of newly emerged 5$^{th}$ nymphs with cascade, rice bran extract and karate, each at $LC_{50}$ produced disturbance in protein synthesis of the ovary, which reflect an inhibition of ovary maturation. Electron micrographs in the present study showed a degeneration of ovarioles and occytes, disintegrated mitochondria, vacuoles were increased moreover enlarged and yolk bodies were cracked mostly in two halves.

Histopathological changes in treated ovaries of different locust species with insecticidal agents could be recorded (Lim and Lee, 1982). They treated the grosshopper, *Oxya japonica* with diflubenzuron and found a retard ovarian development, causing an increase in the receptor tyrosine kinase present on the adjacent follicle cells. This localized signal from the oocyte to the follicle cells leading to dorsal follicle cell delimiting and orienting the future dorsoventral axis of the embryo.

Similarly, Polivanova and Triseleva (1989) feeding the nymphs of *Locusta migratoria* in the laboratory on the *Ageratum houstonianum* extract, and they found completely suppressed oocyte development. Also, Shalom *et al.* (1993) treated the 5$^{th}$ nymphal instar of *Locusta migratoria* with azadirachtin and methoprene, and they found a limited vitellogenic oocytes development, which still remained incomplete. Similarly, Ghazawi *et al.* (2007) found that, topically treated of female nymphs of *Heteracris littoralis* (orthoptera: acrididae) with serial concentrations of Azadirachtin, showed revealed disintegration and destruction in follicle cells and mitochondria.

## REFERENCES

Abbassi K.; Zineb A. and Ghaout, S. (2003): Biological effects of alkaloids extracted from: Three plants of *Moroccan avid* areas on the desert locust. J. Physiol. Entomol., 28: 232-236.

Brissan, A.; Gharibian, S.; Agen, R.; Leclerc, D.F. and Breuil, C. (1996): Localization and characterization of the melanin granules produced by the sap-staining fungus *Ophiostoma piceae*. Material and Organismen, 30: 23-32.

Davies, P. and King, P.E. (1972): The ultrastructure of oösorption in *Locusta migratoria migratorioides*. J. Cell and Tissue Res.135(2) : 275-286.

EL-Gammal, A.M. (1992): Evaluation of diflubenzuron against the 4$^{th}$ instar nymph of *Schistocerca gregaria*. Egypt. J. Agric. Res. 70(3): 835-843.

El-Gammal, A.M.; Gadulla, A.I.; Amer, M.S.; Metwally, M.A. (1994): Evaluation of the anti-moulting action of diflubenzuron on *Schistocerca gregaria* Egypt. J. Agric. Res., 72(1): 147-153.

Engelmann, F. (1970): The physiology of insect reproduction. Department of zoology, Univ of California, Los Angleles, Engel. Pergamon: 307 pp.

Ferenz, H.J. (1993): Yolk protein accumulation in *Locusta migratoria* oocytes. J. Insect Morphol. Entomol., 22 (2-4): 295-314.

Ghazawi, A.N.; El-Shranoubi, E.D.; El-Shazly, M.M. and Abdel Rahman, A. (2007): Effects of Azadirachtin on mortality and reproductive system of the grasshopper, *Heteracris littoralis* Rambo. (Orthoptera): Acrididae). J. Orthoptera Res. 16(1): 57-65.

John, C.D. and James, T.B. (1989): Ovarian follicle development during vitellogenesic in the house cricket *Achreta domesticus*. J. Morphology. 200: 185-198.

Karnovsky, M.J. (1965): The localization of cholinesterase activity in rat cardiac muscle by electron microscopy. J. Cell. Biol. 23, 217-232.

Kimber, SJ (1980): The secretion of the eggshell of *Schistocerca gregaria*: ultrastructure of the follicle cells during the termination of vitellogenesis and eggshell secretion J. Cell Science,46 (1): 455-477.

Lim,S.J.and Lee,S.S.(1982): Toxicity of diflubenzuron to the grasshopper, *Oxya japonica*: Effects on reproduction. Entomologica, Experimetnalis. Et Applicata,31 (2): 154-168.

Martoja, R. (1964): Untype particulier d'appareil genital femelle chez les insects: les ovarioles adenomorphes du coleopteran, *Steraspis speciosa.* Bull. Soc. Zool. Fr. 89: 614-41.

Polivanova, E.N. and Triseleva, T.A. (1989): Suppression of development of *Locusta migratoria* during nymphal feeding on plants containing precocences and prospects for using these plants in biological control. Doklady. Biolo. Sci. 306 (1-6): 289-299.

Shalom, U.; Pener, M.P. and Allebaum, S. (1993): Corpus allatum activity and the effects of juvenile hormone on oocyte development in azadirachtin-induced averaged nymphs of *Locusta migratoria.* Inver. Rep. and Develop., 23(1): 1-9.

Spnrr, A.R. (1969): A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure. Res., 26: 31-43.

Taha, G.Z. and El-Cammal, A.M. (1990): Morphogenetic effects of non-terpenoid juvenile hormone analogue, S,31183 on metamorphosis of last lymphal instar of *Schistocerca gregaria*. Egypt. J. Appl. Sci., 5: 75-81.

Tobe, S.S. and Pratt, G.E. (1975): Corpus allatum activity *in vitro* during ovarian maturation in the desert locust, *Schitocerca gregaria*. J. Exp. Biol, 62: 611-627.

Weber, H. (1954): Grundriss der Insekt-enkunde. Gustav Fischer Verlag Stuttgart, 65(2): 29-37.

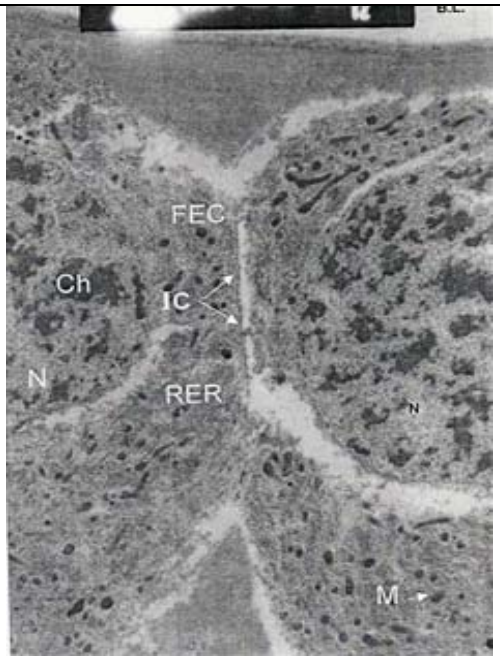


Fig.(1)**:**Electron micrograph of the follicular epithelial cells (FEC) of untreated female *Schistocerca gregaria* showing: intercellular channel (IC), rough endoplasmic reticulum (RER), oval shape mitochondria (M) and nucleus (N) containing normal chromatin (Ch). (X:3000)

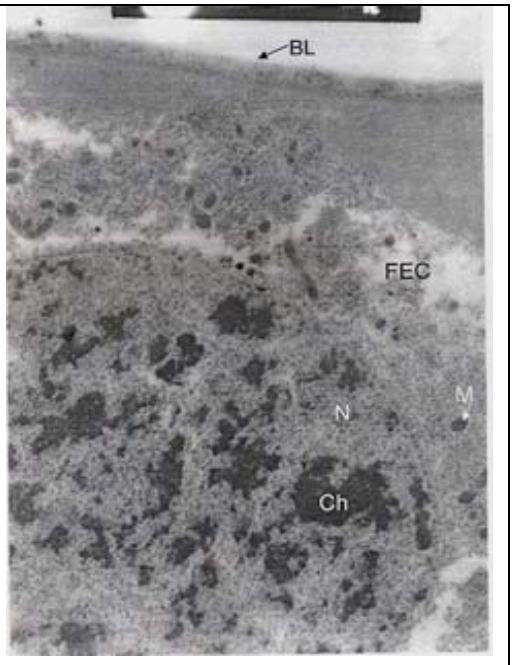


Fig. (2): Higher magnification of follicular epithelial cells (FEC) of untreated female *Schistocerca gregaria* showing basal lamina (BL) nucleus (N) with normal chromatin (Ch). (X: 5000)

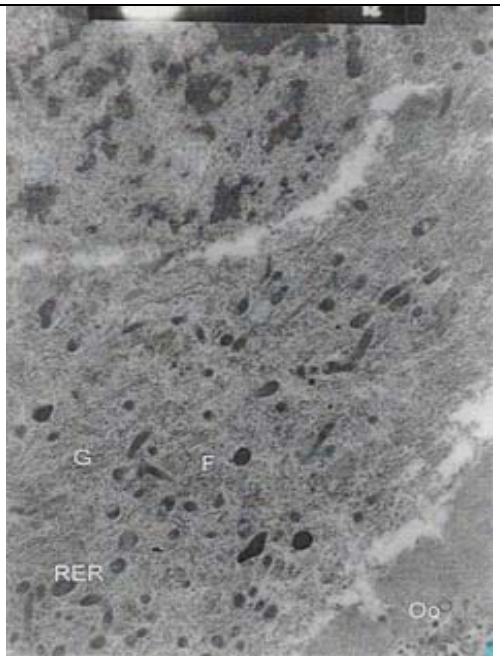


Fig. (3): Higher magnification of follicular cell(f), showing: high concentrations of rough endoplasmic reticulum (RER), Golgi bodies (G) and oocyte (Oo). (X: 5000)

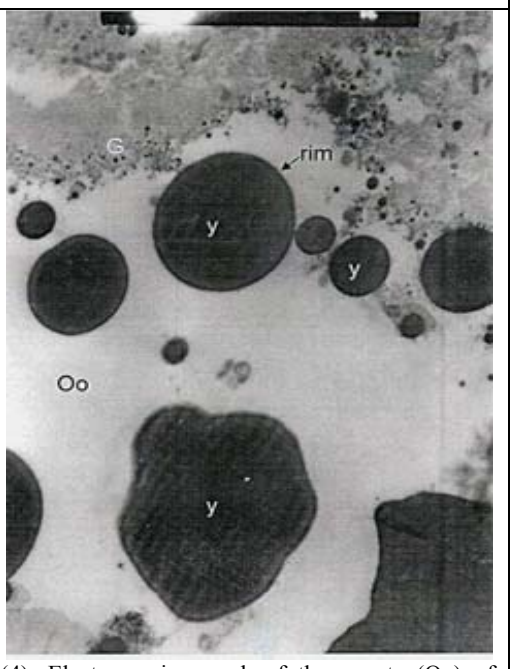


Fig. (4): Electron micrograph of the oocyte (Oo) of untreated female *Schistocerca gregaria* showing: yolk bodies (y) with granulated rim (rim). (X: 3000)

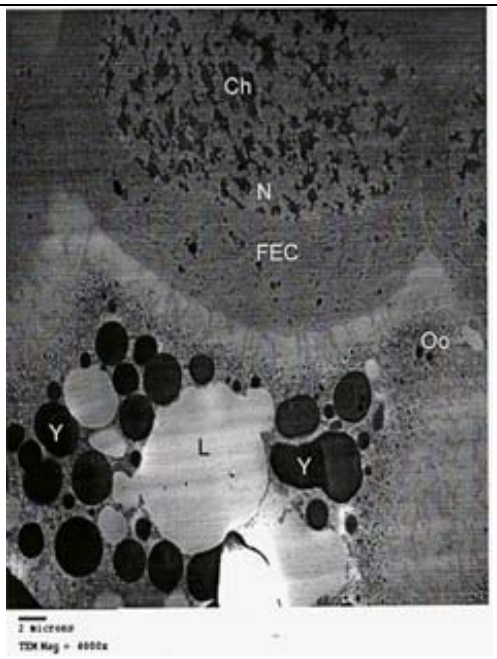


Fig. (5): Electron micrograph of the follicle cell of adult female *Schistocerca gregaria* showing: disorganization of follicular epithelial cell (FEC) nucleus (N) with disintegrated chromatin material (Ch), oocyte (Oo) with large lipid droplets (L) and cracks yolk bodies (y) (X: 4000)

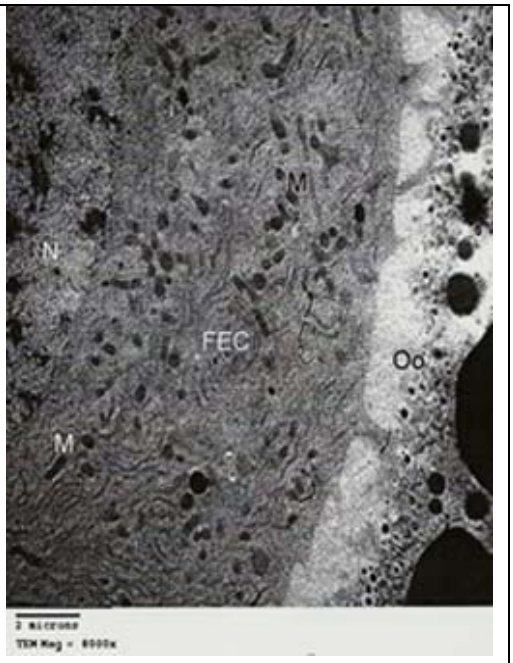


Fig. (6): Higher magnification of follicle cell of treated adult female *Schistocerca gregaria* with cascade at ($LC_{50}$) showing aggregation of malformed mitochondria (M) (X: 8000)

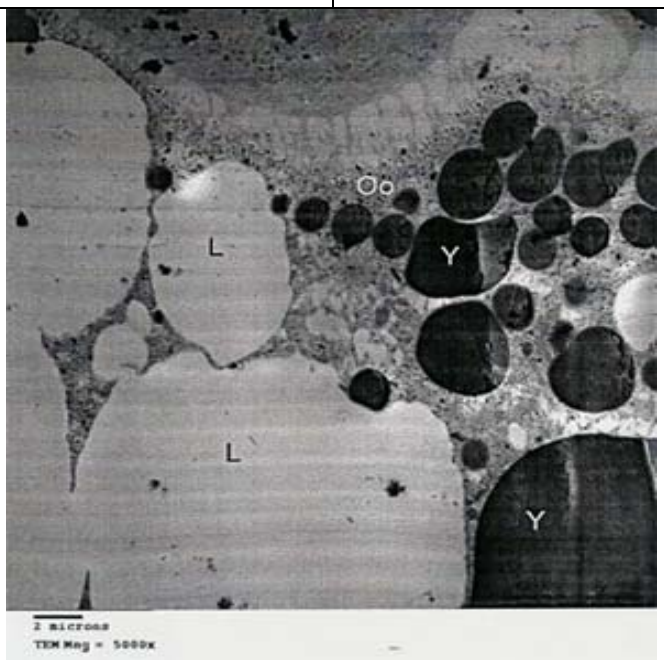


Fig. (7): Higher magnification of treated adult *Schistocerca gregaria* oocyte with cascade at $LC_{50}$, showing cracking of yolk bodies (y), large and numerous lipid droplets (L) with different sizes. (X: 5000)

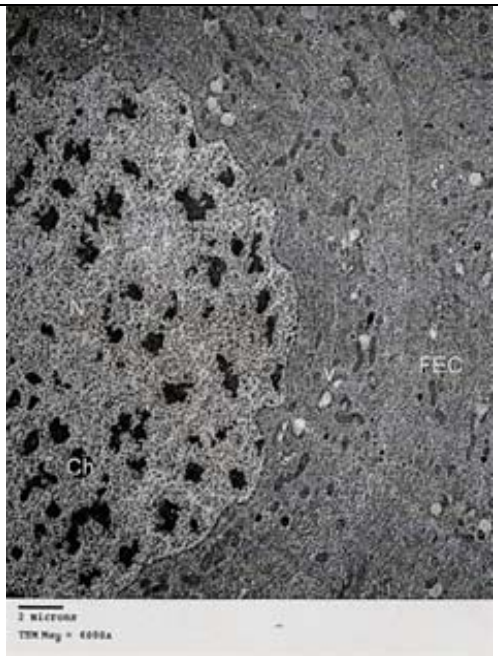


Fig. (8): Electron micrograph of follicle cell of treated adult female of *Schistocerca gregaria* with bran of *Oriza sativa* extract at ($LC_{50}$), showing degeneration and vaculization of follicular epithelial cell cytoplasm (FEC) and irregular shape of nucleus (N) with dark and abnormal chromatin materials (Ch) (X: 6000)

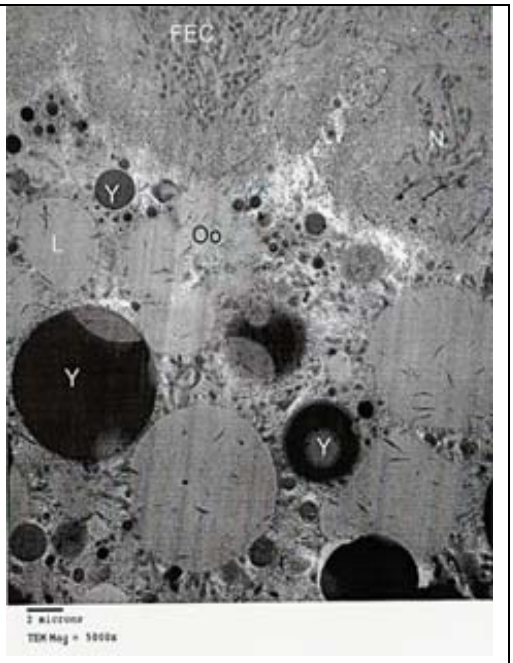


Fig. (9): Electron micrograph of the oocyte (Oo) of treated adult female of *Schistocerca gregaria* with *Oriza sativa* bran extract, showing yolk bodies (y) surrounded by relatively clear rings with marginal and central pale colour. (X: 5000)

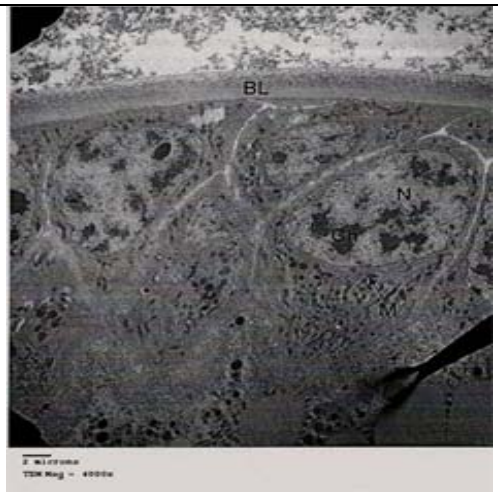


Fig. (10): Electron micrograph of the follicle cell of adult female *Schistocerca gregaria* from treatment with karate showing aggregation of mitochondria (M) and nucleus (N) with dark and abnormal chromatin (ch). (X: 6000)

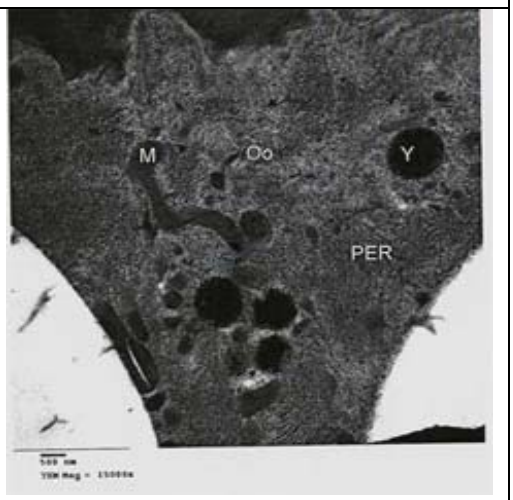


Fig. (11): Electron micrograph of treated oocyte (Oo) of adult female *Schistocerca gregaria* with karate, showing rough endoplasmic reticulum (RER), malformed mitochondria (M) and spherical and granulated yolk bodies (y) (X: 15000)

**ARABIC SUMMERY**

**تأثير الكاسكيد و مستخلص نخالة الأرز و الكارات على التغيرات التركيبية الدقيقة لأنابيب البيض فى الجراد الصحراوى .**

**أ.د. محمد عادل حسين[1] ،أ. د. ليلى سيد حموده[1], ا.د. رضا فضيل على بكر[1]،.د. هبه حسن عبد الوهاب[2]، زينب فتحى عبد الحميد السكرى [2].**

**1- قسم علم الحشرات – كلية العلوم – جامعة عين شمس – القاهرة- مصر**

**2- معهد وقاية النباتات - الدقى- مصر**

تم فحص التغيرات التركيبية الدقيقة لأنابيب البيض فى الاناث البالغة و التى نتجت من المعاملة فى العمر الخامس بالتركيز القاتل للنصف لكل من مثبط الكيتين و المستخلص النباتى و الكارات ( البيروثرويد المخلق ) .

أظهرت الدراسة تحلل فى مكونات الخلايا الطلائية لأكياس البيض و كذلك تحلل فى مكونات الميتوكندريا . كما لوحظ زيادة الفجوات فى أنابيب البيض و حدوث إستطالة فى أحجامها مع حدوث تشقق للأجسام المكونة للمح.

تبين هذه الدراسة أهمية المركبات المستخدمة و التى لها تأثير سلبى على تكوين البيض مما يؤدى فى النهايه إلى تناقص أعداد الجراد بالقدر الكافى لتفادى خطورته على المزروعات .