Histopathological and Ultrastructure Changes in the Embryonic Development of *Schistocerca Gregaria* (Forskal) (Orthoptera): Acrididae Induced by Lufenuron (CSI) and Rice Bran Extract (Waste Product)

Original Article

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ABSTRACT

Background: Waste product compound of rice bran and lufenuron are considered safe compounds to the human and to the environment. They can be used to control *Schistocerca gregaria.*

Objective: The aim of the present study is to examine the ultrastructural changes and histopathological alteration in the Schistocerca gregaria embryogenesis induced by selected waste product compound of rice bran (Oryza sativa) and a chitin synthesis inhibitor (lufenuron).

Materials and Methods: Histological and ultrastructure study of normal and affected eggs of Schistocerca gregaria were conducted to demonstrate the effects of lufenuron and rice bran extract on the embryogenesis. Cleavages started about 5 hrs post oviposition (pop) and continue to divide until formation of cellular blastodrem by 1 day pop. By 2 days pop germ band is formed, differentiated into ectoderm and mesoderm. At 3 days pop segmentation of germ band into mouthpart and three thoracic segments occur. Antenna was observed at 3 days pop. Fore and midgut were detected by 5 days pop. Hindgut was also observed by 5 days pop. By 4 days pop, eyes and brain appeared. Brain appeared as two ganglionic masses separated by oesophagus, which by 5 days pop appears as 2 large interconnect cerebral lobes enwrapped by neurilemma. Histological section of affected eggs showed great effects on brain, alimentary canals and compound eyes.

Results: Ultrastructure study of newly deposited eggs showed that, the chorion consists of several easily distinguishable layers and in 30-hour-old eggs, cleavage nuclei of different shapes could be observed. The nuclei of the blastoderm cell have spindle shape and have condensed chromatin, which attaches to nuclear membrane. Electron micrograph of lufenuron-affected eggs revealed abnormal chorion and cleavage nuclei. In rice bran affected eggs, disintegrated blastoderm that failed to arranged and sever malformed nuclei were seen. Vaculation and lysis of cell components leaving cavities within the ooplasm were detected in both treatments.

Conclusion: The tested compounds induced serious changes to the embryos of Schistocerca gregaria as revealed by the histological and ultrastructure studies.

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Key Words: Embryogenesis; histological and ultrastructure study; lufenuron (chitin synthesis inhibitors); oryza sativa bran extract (waste product); schistocerca gregaria.

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INTRODUCTION

The desert locust, Schistocerca gregaria is one of the most economic pests causing severe damage to crops, which are considered the main food for a human and animals. The desert locust is potentially the most dangerous of the locust pests because of its polyphagous behavior, the ability of its swarms to fly rapidly across great distances and it has two to five generations per year[1]. This species in its gregarious phase can cause up to 100 % of crop loss[2]. 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 mobile swarms by effective control tool against its nymphal instar[3].

Chemical insecticides have been widely developed and are extensively used for the control of this pest because of their effectiveness and easy application and storage. However, the widespread use of these chemicals resulted in inducing resistance by insect pests, contamination of human food, mammalian toxicity, reducing beneficial non-target biota and environmental pollution[4].

To overcome these problems, it is necessary to seek safe, convenient, environmental, and low-cost alternatives agents for pest control, among these agents are the insect growth regulators (IGRs) and botanical extracts, which act as potential acute or chronic insecticides. Over one thousand plant species contain bioactive substances, many of these contain phyto ecdysones, phytojuvenoids and anti-

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juvenile hormones, which act as IGRS[5,6,7]. CSIs (Chitin synthesis inhibitors) are a group of IGRs interfering with chitin biosynthesis in insects and thus prevent moulting, or produce an imperfect cuticle. These compounds have a good lethal effect on the desert locust[8,9,10]. Numerous studies have proven ovicidal activity of lufenuron (CSI) on pests at the recommended dose[11,12,13].

Ultrastructure of embryogenesis in order orthoptera was studied; concentrated on early embryogenesis and the eggshell[14]. illustrated the stages of the grasshopper embryo by light micrographs. Insect eggs are characterized by egg shell or chorion. The insect egg shell is commonly composed of 3 layers; the vitelline membrane, the endochorion and the exochorion (passing from the oocyte outwards). The endochorion and exochorion together are known as the chorion. The chorion secreted by follicular epithelium which provides mechanical protection to developing embryo and an inner vitelline envelope[15]. In Orthoptera Acrididae[16], observed a mucous sheath, referred to epichorion is also present externally to chorion. Ultrastructure of egg shell was studied in different insect species[17]; detected 3 layers of the chorion of screw worm Chochliomyia hominivorax; but in cat flea it is made up of 4 layers[18].

The aim of the present study is to examine the ultrastructural changes and histopathological alteration in the Schistocerca gregaria embryogenesis induced by selected waste product compound of rice bran (Oryza sativa) and a chitin synthesis inhibitor (lufenuron).

MATERIALS AND METHODS

1- Rearing of the insect

The stock colony of Schistocerca gregaria was maintained at the Locust Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. The insects were reared and handled according to the technique described by[19].

2- Tested compounds

A Chitin Synthesis Inhibitor Lufenuron (10%) and waste product Oryza sativa (Rice bran) were used. Rice bran was extracted according to the method described by[20]. The resulted crude extract yield from 100 gm of O. sativa bran was weighted before storage at 4^{o}C in screw capped vials, until use.

3- Bioassays studies

3.1. Treatment of experimental insects

One-day old females of the 5th nymphal instars of S. gregaria were treated by feeding technique with serial appropriate concentrations of Lufenuron (10%) and rice bran extract (Oryza sativa).

3.2. Evaluation of tested compounds

Eggs from normal female and affected eggs of adult females resulted from 5th nymphal instars treated with LC50 of lufenuron (10%) and rice bran extract, were separated from the sand particles with a fine brush, placed on a wet cotton pad in petri dishes and incubated at 32 ºC to follow their hatchability and to study the effect of the tested compounds on the embryonic development. Eggs from treatment with LC50 of lufenuron (10%) and rice bran extract (Oryza sativa) were processed for histological examination. Selected stages from normal and affected eggs were investigated by light and electron microscopy.

4- Histological studies

Eggs from five females were selected fore studding the embryonic development. Eeggs were prepared for microscopic examination according to[21]. Eggs were dechorionted by immersion for 1 min in 6% sodium hypochlorite followed by three rinses in distilled water. Eggs were then placed in aqueous bouin solution for 7 days and dehydrated in consecutive baths of 30, 50, 70, 80, 95 and 100 ethanol. These steps is followed by Clearing twice in xylene,embedding in paraffin wax and sectioning 5-6 µm. thick sections using a rotary microtome, were placed on slides and stainedusing Harris' hematoxylin stain.

5 - Ultrastructural study

For transmission electron microscopy (TEM), eggs were dechorionted by immersion for 1 min in 6% sodium hypochlorite followed by three rinses in distilled water. Embryos were then immersed in 3% glutaraldehyde then transferred to initial fixative. Embryos were fixed overnight and given 3 brief rinses in sucrose – cacodylate buffer (pH 7.2); Post fixed 1h in 1% osmium tetroxide in 0.1M cacodylate buffer, rinsed 3 times in distilled water, and dehydrated through a graded series of ethanol. Then specimens were infiltrated with spur's epoxy resin in a graded series of absolute alcohol-spur's resin mixtures, and then embedded in freshly prepared spur's resin at 70°C for 72hrs. Embedded embryo were sectioned with glass knives on a reichet OM- 2 ultra- microtome, collected on form-coated copper grids, stained with uranyl acetate counterstained with lead citrate, and examined with Seo100 cx TEM at 75 KV.

RESULTS

1-Susceptibility of one-day old females of the 5th nymphs of Schistocerca gregaria to Lufenuron (CSI) and Rise bran extract (waste product)

The insecticidal activity of Lufenuron and Oryza sativa bran extract against one-day old females of the 5th nymphs of Schistocerca gregaria was graphically illustrated in (Figure 1). Data showed that, lethal effect of LC50 value after treatment with Lufenuron was more effective than Oryza sativa extract. From the propit dose response curve, the LC50 of Lufenuron accounted for 95 ppm while that of Oryza sativa bran extract was 14.3×10^3 ppm.

2-Studies of embryonic development

Histological sections and electron micrograph of eggs were examined as early as (0-5 hrs) after oviposition, to

describe the structure and cleavage of the zygote. The major stages "namely" blastoderm formation, gastrulation, segmentation and organogenesis were studied.

2.1- Histological studies

- Cleavage and blastoderm formation

The oocyte has intact homogeneous chorionic layer around it. The chorion is differentiated into two layers (the endochorion and exochorion). The endochorion forms an intact homogeneous sheath around the oocyte, beneath it vitelline membrane enclosed the oocyte. The exochorion constitutes a compound layer. The oocyte is characterized by having an abundance yolk at ooplasm and also vacuoles (Figure 2). Cleavage started 5 hours post oviposition. In newly oviposit eggs, cleavage nuclei increased in number (Figure 2). These nuclei migrate posteriorly away from the center to become distributed in the yolk (Figure 3). At the same time, dark granules begin to appear in the periplasm at the posterior pole of the egg forming pole cells (Figure 4). Some of cleavage nuclei migrate back into yolk as secondary vitellophages (Figure 4). The vitellophages increase in number at this stage by mitotic division. By 1 day post oviposition (pop), nearly all the cleavage nuclei have reached the periphery under vitelline membrane, and then the cell furrows growing inwards from vitelline membrane to form cell wall of blastoderm cells. (Figure 3). Mitotic division and nucleus migration continued until the blastoderm formation by 1 day post oviposition (pop). Blastoderm is formed from one layer of cells arranged in a complete ring enclosing the yolk and surrounded by cytoplasmic membrane (Figure 3).

- Gastrulation

In 2 days old eggs, the blastoderm thickens forming the germ band (Figure 5). The cells of the blastoderm that do not take part in the germ band formation become flattened, to form the serosa (Figure 6).About 2 days (pop) ,the embryonic envelopes begin to appear with differentiation of the inner layer (Figure 5), this is brought by inward folding of the lateral borders of the germ band. These folds including the tail fold increase in width, the edges approaching each until they meet. The outer surface composed of the pavement cells (the serosa), the cover over of the germ band, the amnion (Figuree 5,6) By 2 days (pop) the embryonic envelopes ,amnion and serosa are completed. The germ band at this stage is formed of multilayer strips of cells that are differentiated into 2 layers of ectodermal and mesodermal cells (Figure 5).

- Segmentation and organogenesis

About 3 days (pop), inner layer in the head and thorax is divided into segments corresponding to the labrum, mandibles and maxillae and three thoracic segments (Figure 7). Antennae originate from the lateral margin of the blastocephalon as 2 ectodermal buds (Figure 7).The foregut develops as stomodeal invagination near the cephalic end of the embryo (Figure 7) .The hindgut develops as proctodeal invagination in a manner similar to the stomodeal invagination but at caudal end of the body, about 7 hours after stomodeal appearance .

By 5 days (pop), the crop posterior part of esophagus dilates to form a thick hard conical structure, the proventriculus (Figure 8). The proventriculus is formed as a result of the projection of the posterior end of the esophagus into anterior region of the midgut. The proventriculus consists of 3 layers, an inner and outer layers which are formed by the esophagus folding back and medial layer which is derived from midgut cells.

The anterior midgut gives rise to 2 ribbons of cells on the ventrolateral sides directed posteriorly between the mesoderm and the yolk .Similarly, a second pair of cell strips arise from the posterior midgut directed posteriorly. When the proctodaeam reaches its final position, these cords are directed ventrally and anteriorly fusing with anterior midgut cords (Figure 8).

By the time this process is completed, the midgut now is a closed tube completely surrounding the yolk mass (Figure 8). A prominent inner layer of yolk cells is present in the midgut (Figures 8 and 9). The ectodermal cells of terminal unit of the embryo elongate and migrate inwardly to form a deep cavity. Mesodermal cells in the posterior part of the segment generate a thin layer around this pocket. The invaginating cells undergo a series of cell division at the rim of the invagination and cells at the posterior end from the posterior midgut, its base differentiate into the proctodaeum (Figure 10).

By 5 days (pop) trachea appears as an ectodermal invagination (Figure 9). By this time, the heart is formed from cardio blasts differentiated as two strings of single large rounded cells lying on the dorsolateral borders and extending through thorax and abdomen (Figure 9). By 5 days pop, brain appears as 2 large interconnect cerebral lobes which should be enwrapped by a neurilemma (Figure 9).

By 4 days (pop), on the dorsolateral edge of the head lobes. Eye discs appeared as a thickened masses of which the peripheral nuclei are aligned in a single row but in the rest of the mass they exhibit no regular arrangement(Figure 11). By 4 days (pop) brain appeared as two ganglionic masses separated by an oesophagus. The dorsal ganglion is the superaoesophageal ganglion. The latter starts to develop early as 2 large masses proliferating bilaterally from the ectodermal cell in the posterior region of the blastocephalon. It exhibits a trilobed mass. The anterior most is the deutocerebrum appearing as an elongated structure. The median lobe is tritocerebrum, the smallest of 3 units. The largest lobe (the protocerebrum) located posteriorly (Figure 11). The ventral ganglion is a subesophageal ganglion which is made of neural cell aggregates of the gnathal regions (Figure 11).

2.2- Histopathological studies

Affected eggs were processed for histopathological studies. In both treatments, the most affected organs during the embryonic development stages were brain and the alimentary canals. Eggs of 2 days old resulted from lufenuron treatment showed signs of deterioration. Warping of egg chorion was detected. Serosa become thinner and detached. Lysis of ooplasm is greatly increased (Figure 12). In eggs from lufenuron treatment , the development of some embryos were blocked at germ band formation. At 5 days old eggs, the structure of chorion has abnormal appearance, ruptured is most clear (Figure 13). Lysis of mid gut yolk granules was detected, degeneration of epithelial cells at both midgut and hindgut were observed (Figure 13). The crop structure appeared atrophied in the size. The brain become compressed and did not differentiate into known basic structure (Figure 14). Histopathological investigations of the affected eggs, produced of females, which resulted from rice bran treatment showed that, the development of some embryos were blocked at cleavage stage (Figure 15). Eggs had an abnormal shape. Sign of deterioration was detected, wide spread cytolysis was observed, lysis of yolk granules and disintegration of egg content (Figure 15). At eggs of 5 days old affected with rice bran extract, midgut epithelial cells seem to be degenerated (Figures 16 and 17). Yolk granules were deteriorated and dispersed (Figures 16 and 17). In some embryos, compound eyes showed severe shrinkage and were asymmetrical (Figure 17). The brain loses its morphic nature, appeared compressed and lysed (Figure 17).

3- Ultrastructure studies

For detail investigations of successive stages of embryonic development. Ultrastructure and histopathological changes in the eggs were examined.

Normal eggs

Electron micrograph of newly deposited eggs showed that, the chorion consists of several easily distinguiable layers (Figure 18). The inner one is endochorion and the outer exochorion (Figures 18 and 19). The vitelline membrane is the inner most chorionic layer (Figure 18). The vitelline membrane is scalloped layer (Figure 18). The endochorion consists mainly of tubules linked by strands of flocculent material and separated by large spaces (Figure 19). The outer layer of endochorion is denser and contains fewer tubules (Figures 18 and 19). The exochorion is composed of web of fibrillars substance and islands of endochorion material are present in this web adjacent to endochorion (Figures 18 and 19) .A layer of curly fibers the extra chorion covers the outer layer of laid egg. (Figures 18 and 19). Air layers are present at the end of chorion tubules (Figure 20). Vitelline membrane appear to encircle the egg ooplasm (Figure 21).

According to electron micrographs of 30 hrs old eggs, cleavage nuclei of different shapes could be observed (Figure 22). The nuclei are round to oval in shape contain clumped chromatin, The cytoplasm is rich with mitochondria, ribosomes, microtubules and dense vesicles (Figure 22).The large numbers of cleavage nuclei form a syncytium Figure 22). The nuclei are heterochromatic (Figure 23). Nuclei form syncytium, whereas there is no cell boundaries, embedded in continuous matrix of granulated cytoplasm (Figures 24,25 and 26). The microtubules run parallel to long axis along the lateral sides of the cell forming a stripe which make palisade for primary vitellophage (Figures 24,25 and 26). The nuclei are more or less round, heterochromatic and are surrounded by distinct double membrane (Figures 24 and 25). Nuclei are oval or ellipsoid and have condensed chromatin which attaches to the nuclear membrane. The cytoplasm is granulated, contains few dense vesicles, microtubules, mitochondria, ribosomes, cisternae of endoplasmic reticulum (Figures 25 and 26). The nuclei of syncytium are spindle in shape and have condensed chromatin, which tend to adhere to nuclear membrane. The pole cell is also lying in between the primary vitellophage (Figure 27).

After a set of mitotic division, some cleavage nuclei remain adjacent to the yolk granules as secondary vitellophages. Other cleavage nuclei migrated to the periphery of egg and form a single layer of epithelial cells called the blastoderm (Figures 28,29 and 30). Blastoderm cells arranged in a linear manner adjacent to stripe formed of microtubules (Figures 28,29 and 30). The nuclei of the blastoderm cell have spindle shape and have condensed chromatin which attaches to nuclear membrane (Figures 28,29 and 30)

- Lufenuron affected egg

Electron micrographs of 5 hrs old eggs affected of treatment with LC50 of lufenuron showed that signs of deterioration, whereas chorion exhibit abnormal appearance. The chorion thickness is greatly reduced and become lighter in color than normal. The structural component of extra chorion, exochorion and endochorion are completely lysis. The chorion involved the vitelline membrane (the most inner layer of it) has irregularities of the outlines (Figure 31). Some cleavage nuclei have abnormal appearance and short stripe of microtubules is obvious (Figure 31). Electron micrograph of 30 hrs old eggs showed gross disintegration of cell organelles leaving wide empty space or cell debris, vacuolated cytoplasm. Irregular shape of cleavage nuclei, which have poor chromatin and very thin nuclear membrane (Figures 32 and 33). The yolk granules spherical texture are change to become pear in shape. Yolk bodies are surrounded by relatively clear ring, others have a clear rim (Figure 32).

- Rice bran extract affected egg

Electron micrographs of eggs affected of treatment with LC50 of rice bran extract showed signs of deterioration. Thirty hrs old affected eggs have vaculation and lysis of cell components, leaving cavities within the ooplasm. Blastoderm is disintegrated and failed to arrange

(Figure 34). The nuclei have severe malformation, show irregularity of the out lines and suffer chromatolysis, where chromatin is very poor and dispersed. Vacuolated cytoplasm are also observed, include peculiar vesicle like body filled with remnants of degeneration, also involving yolk and other debris (Figure 35).

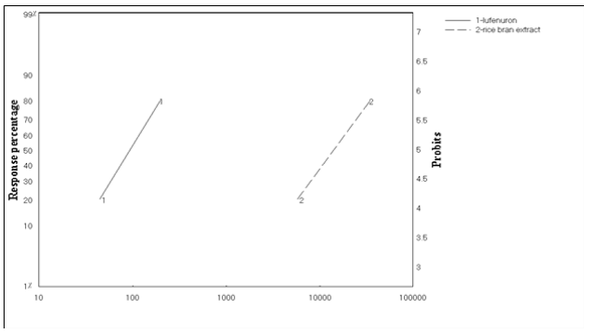

Fig. 1: Regression lines of lufenuron and rice bran extract on the one-day old of the 5th nymphs of Schistocerca gregaria females.

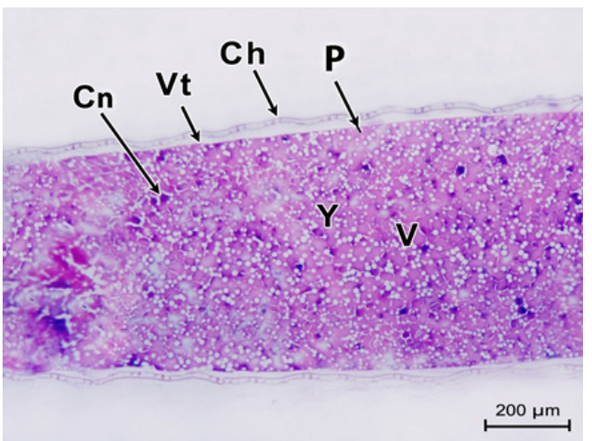

Fig. 2: Photograph of longitudinal section of 0 day old egg of Schistocerca gregaria, showing chorion (Ch), vitelline membrane (Vt), yolk (Y), vacuoles (V), cleavage nuclei (Cn) and periplasm (P).

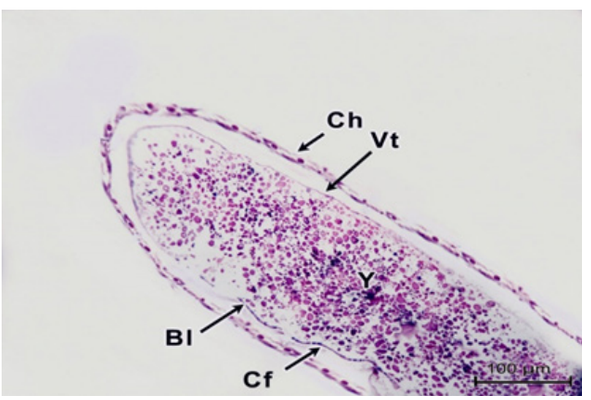

Fig. 3: Photograph of longitudinal section of 1 day old egg of Schistocerca gregaria showing, chorion (Ch), vitelline membrane (Vt), yolk (Y), blastoderm (Bl) and cell furrows (Cf).

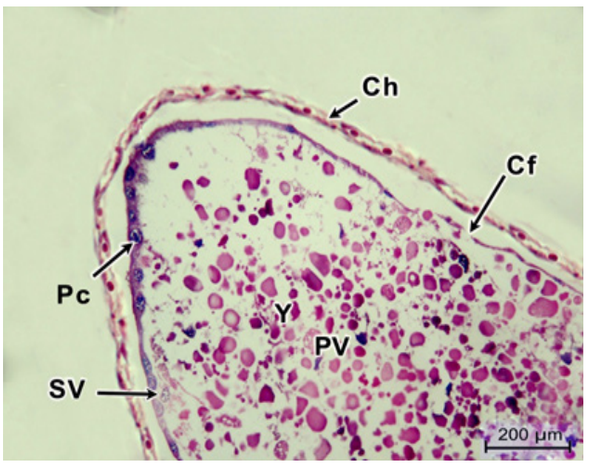

Fig. 4: Photograph of longitudinal section of 1 day old egg of Schistocerca gregaria showing, chorion (Ch), cell furrows (Cf), yolk (Y), primary vitellophages (PV), secondary vitellophages (SV), and pole cells(Pc)

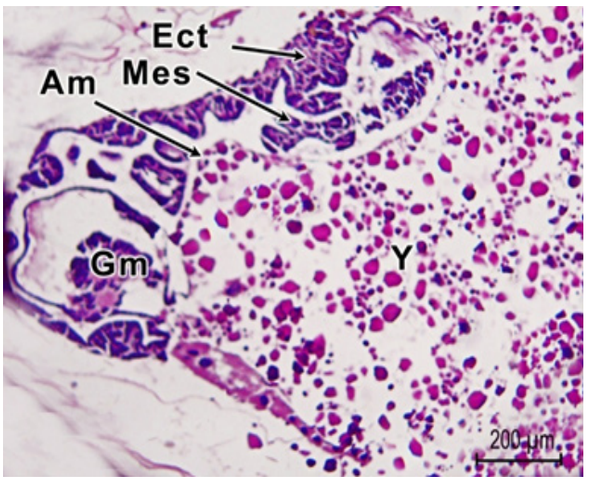

Fig. 5: Photograph of Longitudinal section of 2 days old egg of Schistocerca gregaria showing, yolk (Y), germ band (Gm), amnion (Am), mesoderm (Mes) and ectoderm (Ect)

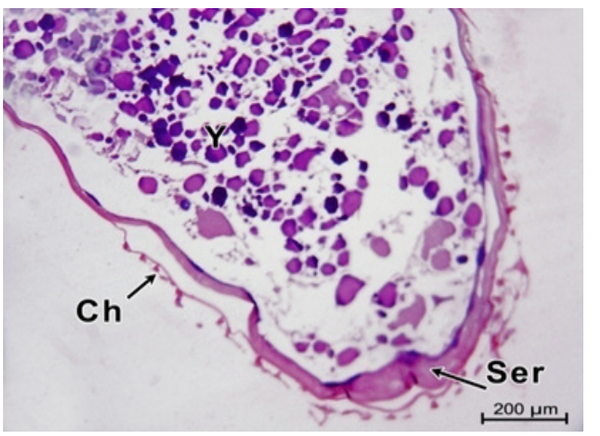

Fig. 6: Photograph of longitudinal section of 2 days old egg of Schistocerca gregaria, showing chorion (Ch) , yolk (Y) and serosa (Ser)

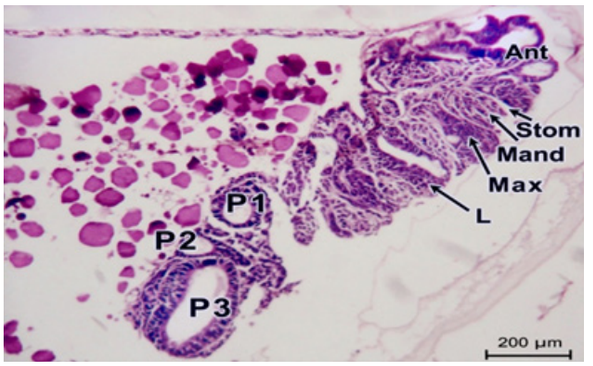

Fig. 7: Photograph of longitudinal section of 3 days old egg Schistocerca gregaria showing, antenna (Ant), stomodaeum (Stom), mandibular segment (Mand), maxillary segment (Max), labial segment (L), first thoracic segment (P1), second thoracic segment (P2) and third thoracic segment (P3)

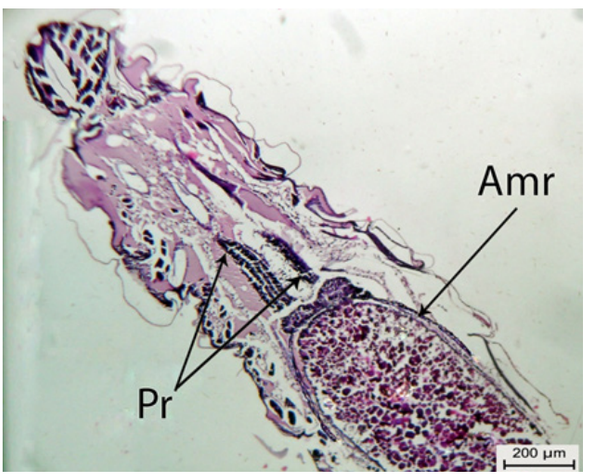

Fig. 8: Photograph of longitudinal section 5 days old egg of Schistocerca gregaria, showing anterior midgut rudiment (Amr), and proventriculus (Pr)

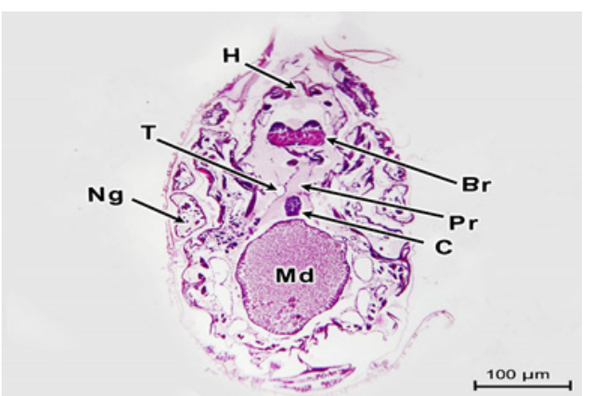

Fig. 9: Photograph of sagittal section 5 days old egg of Schistocerca gregaria ,showing midgut (Md) , crop (C), proventriculus (Pr), brain (Br), nerve ganglion (Ng), heart (H) and trachea (T)

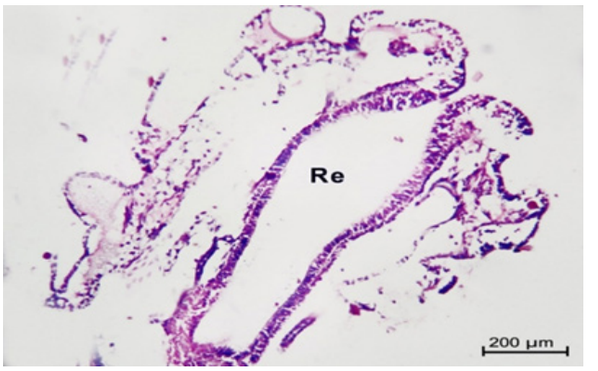

Fig. 10: Photograph of longitudinal section of 5 days old egg of Schistocerca gregaria, showing rectum (Re)

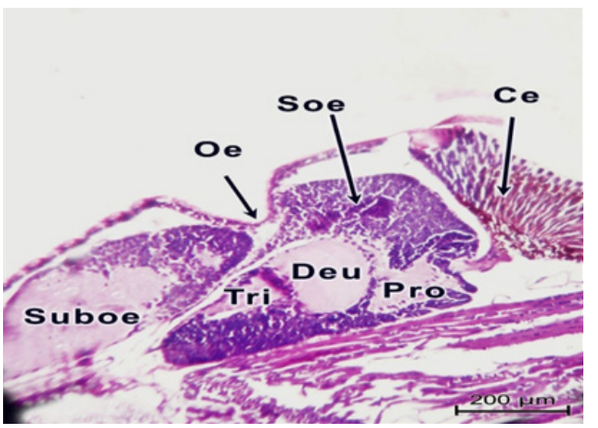

Fig. 11: Photograph of longitudinal section of 4 days old egg of Schistocerca gregaria, showing tritocerebrum (Tri),deutocerebrum (Deu) ,protocerebrum (Pro), compound eye (Ce), suboesaphageal ganglion (Suboe), oesophagus (Oe) and supraoesaphageal ganglion (Soe)

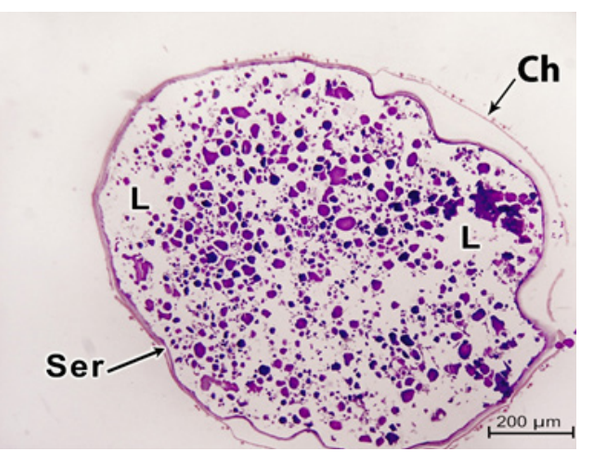

Fig. 12: Photograph of sagittal section of 2 days old egg of Schistocerca gregaria affected with LC50 lufenuron treatment showing, detached chorion (Ch) & serosa (Ser) and lysis of germ band (L)

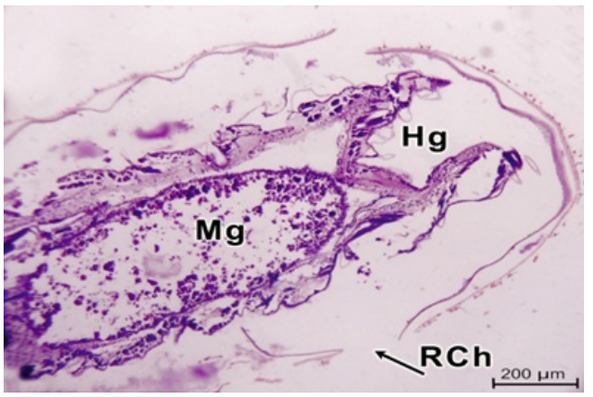

Fig. 13: Photograph of longitudinal section of 5 days old egg of Schistocerca gregaria affected with LC50 lufenuron treatment showing ruptured chorion (RCh), lysis of midgut yolk (Mg), and distortion of hindgut (Hg)

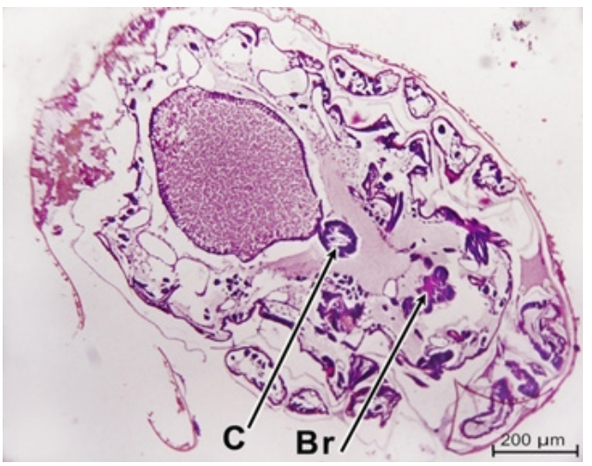

Fig. 14: Photograph of sagittal section of 5 days old egg of Schistocerca gregaria affected with LC50 lufenuron treatment showing abnormal shape of crop (C) and compressed brain (Br)

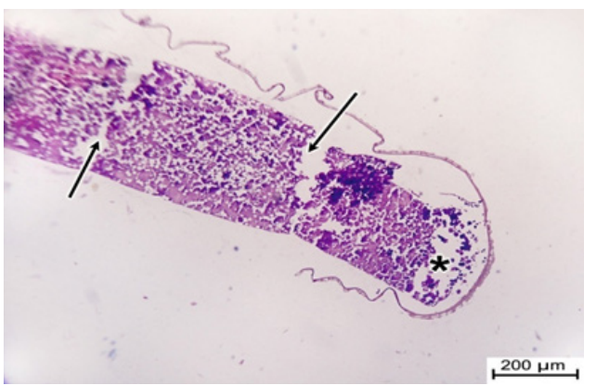

Fig. 15: Photograph of longitudinal section of newly oviposited egg of Schistocerca gregaria affected with LC50 rice bran extract treatment showing ruptured and disintegration of the egg content (indicated by stars and arrows)

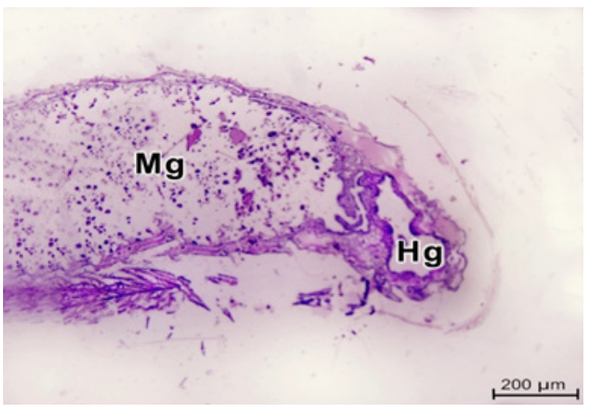

Fig. 16: Photograph of longitudinal section of 5 days old egg of Schistocerca gregaria affected with LC50 rice bran extract treatment showing distortion & lysis of midgut (Mg) and hindgut (Hg)

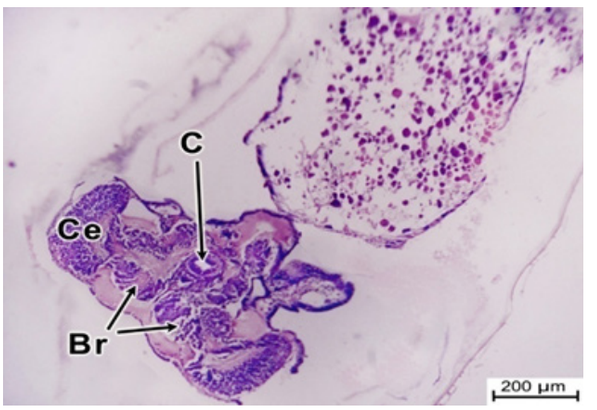

Fig. 17: Photograph of longitudinal section of 5 days old egg of Schistocerca gregaria affected with LC50 rice bran extract treatment showing crop (C), shrinkage compound eye (Ce) and compressed & lysis brain (Br)

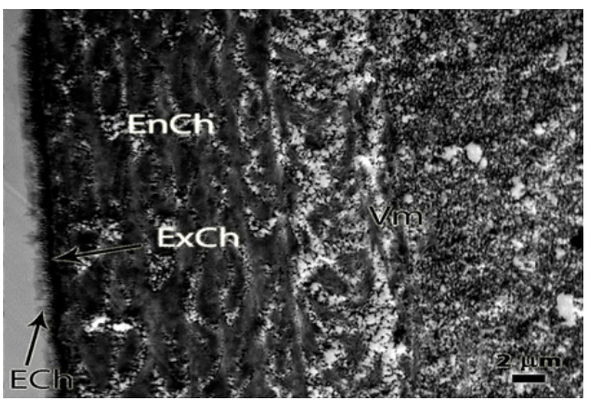

Fig. 18: Electron micrograph of 5 hrs old egg of Schistocerca gregaria showing, extrachorion(ECh), exochorion (ExCh), endochorion (EnCh) and vitelline membrane (Vm)

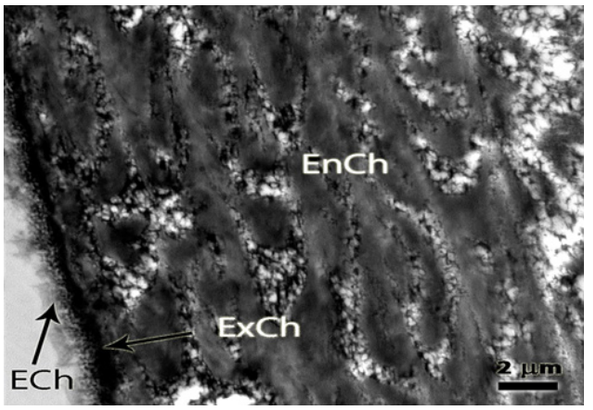

Fig. 19: Electron micrograph of 5 hrs old egg of Schistocerca gregaria showing, extrachorion (ECh),very dark exochorion (ExCh) and tubules of endochorion (EnCh)

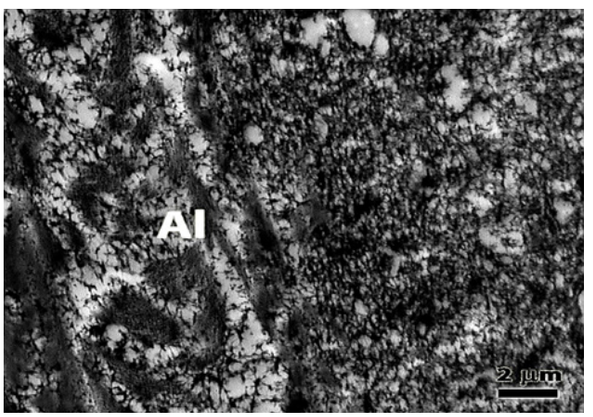

Fig. 20: Electron micrograph of 5 hrs old egg of Schistocerca gregaria showing , air layer (Al) of chorion

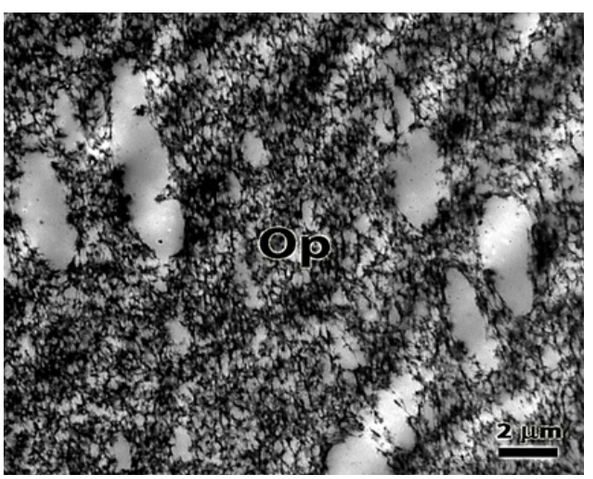

Fig. 21: Electron micrograph of 5 hrs old egg of Schistocerca gregaria showing, egg ooplasm (Op)

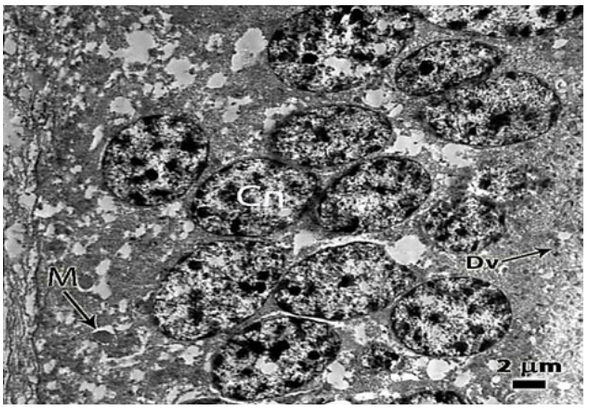

Fig. 22: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing, the cleavage nuclei (Cn), mitochondria(M), and dense vesicles (DV)

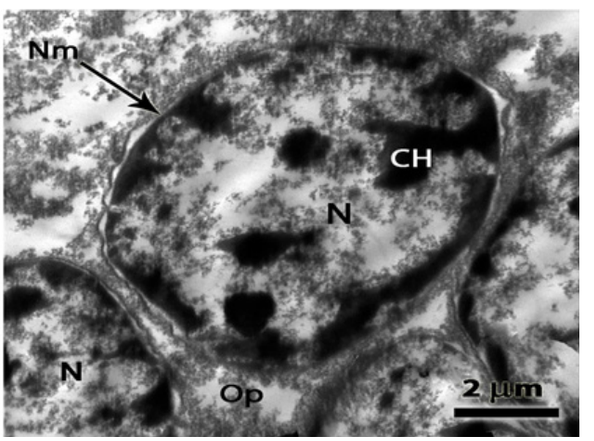

Fig. 23: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing, nucleus (N), chromatin (CH), ooplasm (Op) and nuclear membrane (Nm)

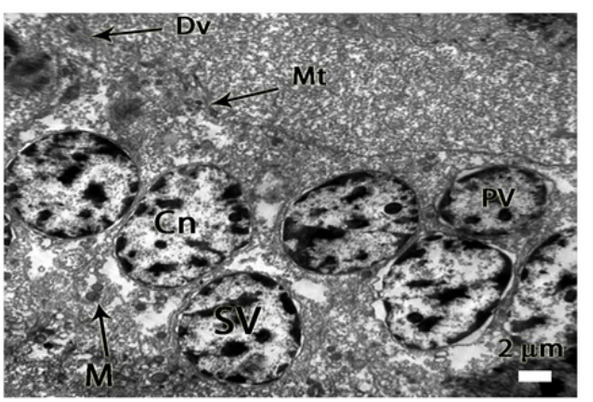

Fig. 24: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing , the cleavage nuclei (Cn), the primary vitellophages (PV), secondary vitellophage (SV), microtubules (Mt), dense vesicles (DV) and mitochondria (M)

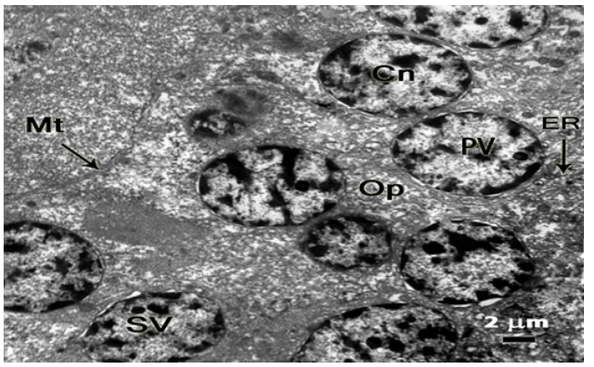

Fig. 25: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing the cleavage nuclei (Cn), microtubules (Mt), ooplasm (Op), endoplasmic reticulum (ER), primary vitellophages (PV), and secondary vitellophage (SV)

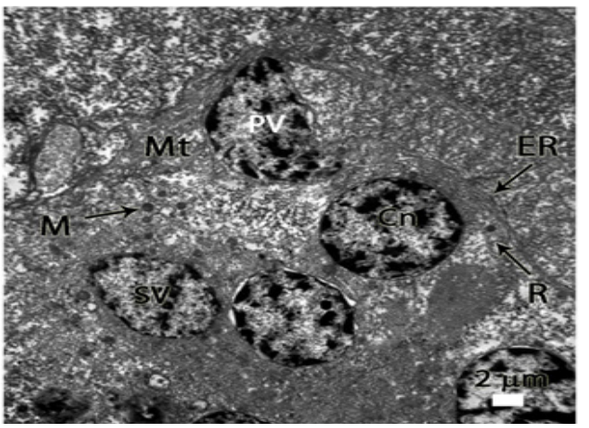

Fig. 26: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing, the cleavage nuclei (Cn),the primary vitellophages (PV), secondary vitellophage (SV), mitochondria (M) microtubules (Mt), ribosomes (R) and cisternae of endoplasmic reticulum (ER)

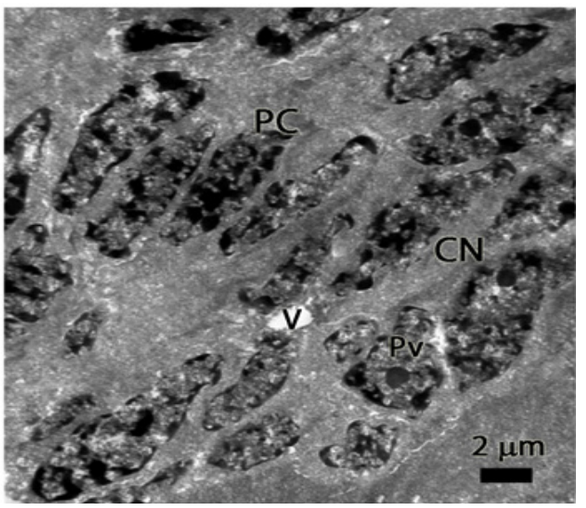

Fig. 27: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing , the cleavage nuclei (Cn), primary vitellophages (PV), spindle shape nuclei (SN), vacuoles (V) and pole cell (pc)

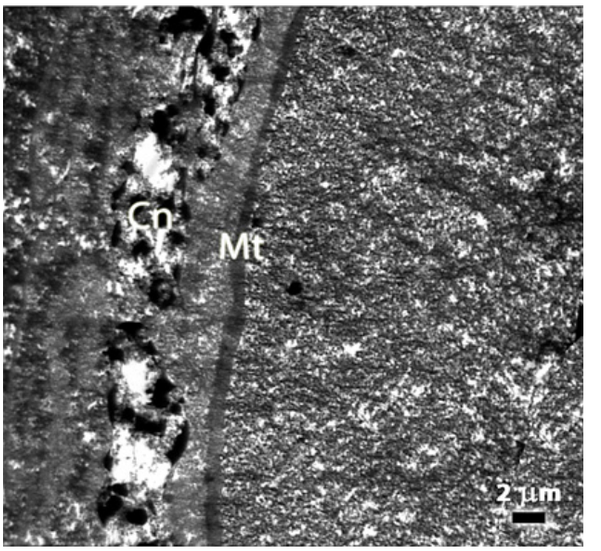

Fig. 28: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing, blastoderm having a row of elongated cleavage nuclei (Cn) and stripe of microtubules (Mt)

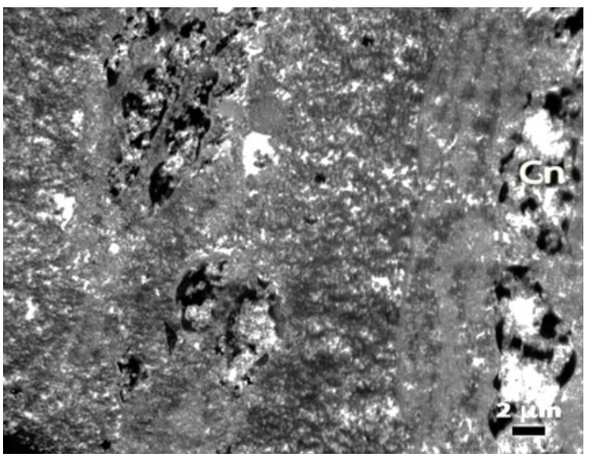

Fig. 29: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing, blastoderm stage having two rows of elongated cleavage nuclei (Cn)

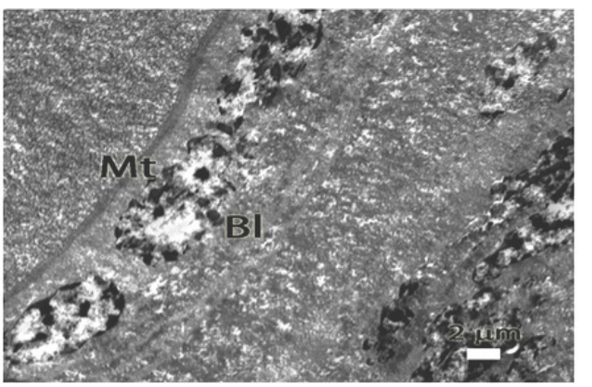

Fig. 30: Electron micrograph of 30 hrs old egg of Schistocerca gregaria showing blastoderm (Bl) and stripe of microtubules (Mt)

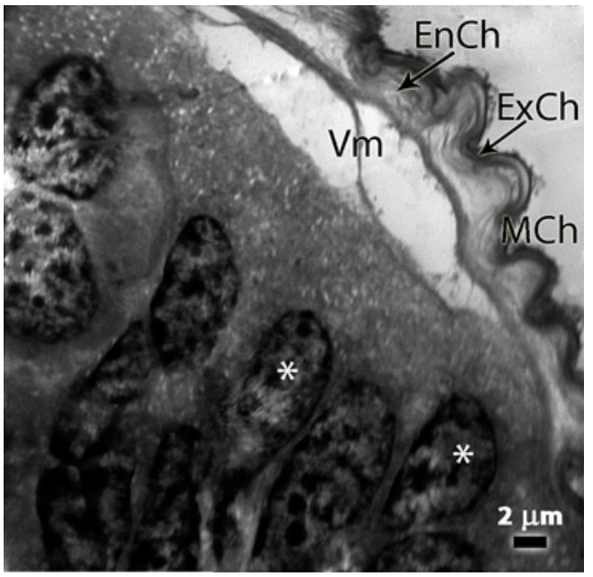

Fig. 31: Electron micrograph of 5 hrs old egg of Schistocerca gregaria affected with LC50 lufenuron treatment showing malformed chorion (MCh), (irregularity and degeneration of extra chorion (ECh), exochorion (ExCh) & endochorion (EnCh)). Irregularity & degeneration of vitelline membrane (Vm) (indicated by arrows) and malformed cleavage nuclei (Cn) (indicated by stars)

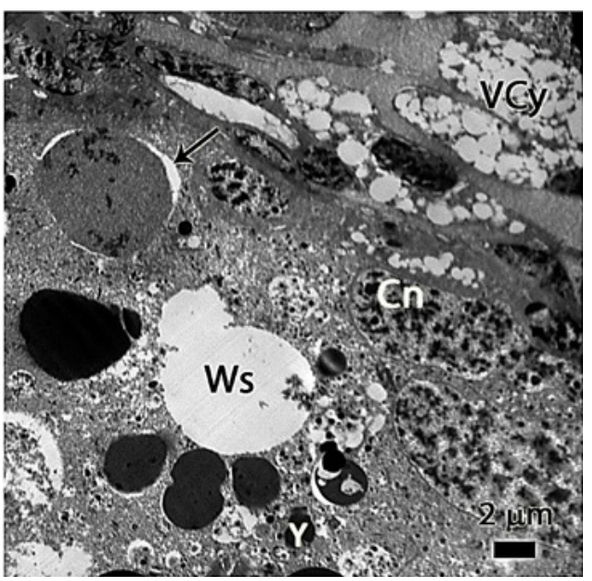

Fig. 32: Electron micrograph of 30 hrs old egg of Schistocerca gregaria affected with LC50 lufenuron treatment showing disintegration of all organelles leaving wide empty space (Ws), clear ring around the yolk (indicated by arrows) vacuolated cytoplasm (VCy), irregular shaped cleavage nuclei (Cn) and malformed yolk granules (Y)

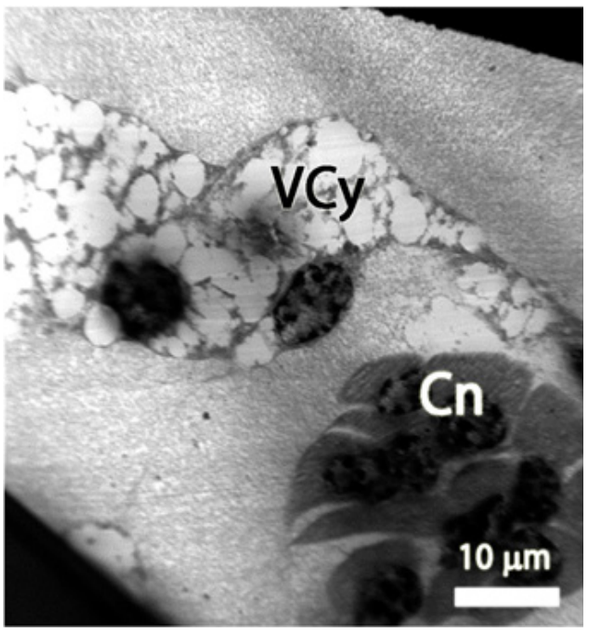

Fig. 33: Electron micrograph showing higher magnification of 30 hrs old egg of Schistocerca gregaria affected with LC50 lufenuron treatment , vacuolated cytoplasm (VCy) and irregular shape of cleavage nuclei (Cn)

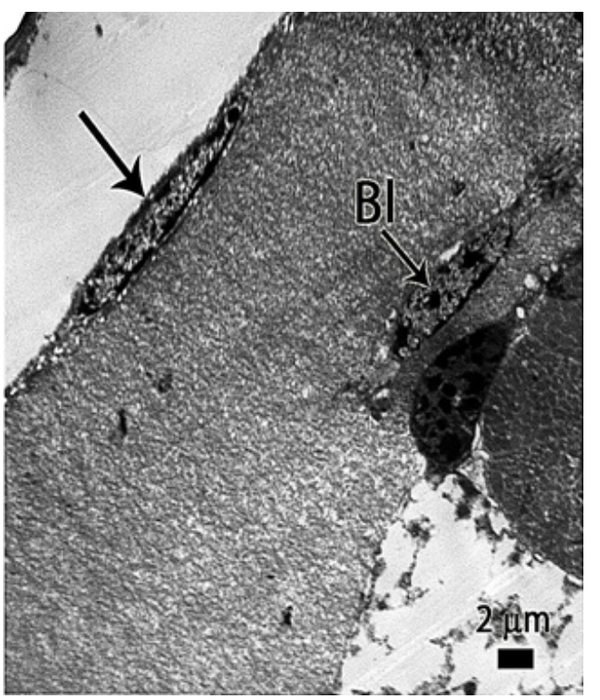

Fig. 34: Electron micrograph of 30 hrs old egg of Schistocerca gregaria affected with LC50 rice bran extract treatment, disintegration of blastodem (Bl) (indicated by arrows)

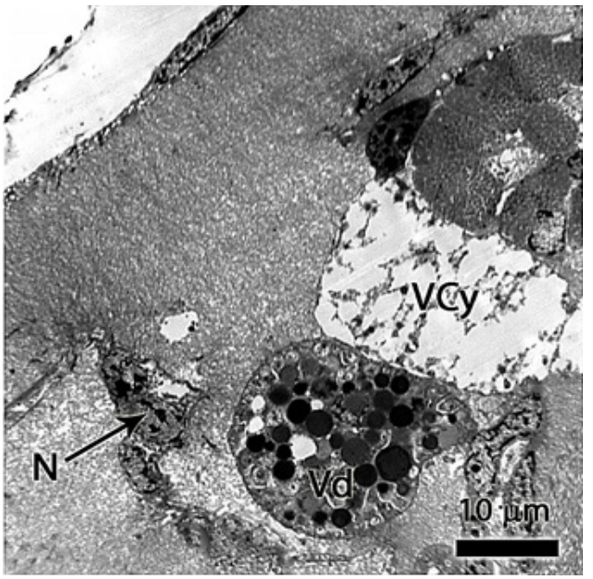

Fig. 35: Electron micrograph of 30 hrs old egg of Schistocerca gregaria affected with LC50 rice bran extract treatment showing vacuolated cytoplasm (VCy), malformed nucleus (N) (indicated by arrows) and vesicles enclosed several debris (Vd)

DISCUSSION

1. Toxicological effect of Lufenuron (CSI) and Oryza sativa bran extract (plant waste product) on embryonic development of Schistocerca gregaria

The present study indicated that, chitin synthesis inhibitors, (CSIs) and plant waste product had significant toxic and growth inhibitory effects against the desert locust, Schistocerca gregaria. The effects of these compounds depend on their concentrations and the age of the treated insects. In this study, newly moulted females of the 5th nymphal instar of Schistocerca gregaria were treated with different concentrations of Lufenuron (CSI) and Oryza sativa bran extract by feeding technique. Lethal effect of LC50 value after treatment with Lufenuron was more effective than Oryza sativa extract. The greatest mortality was recorded during ecdysis of early the 4th nymphal instar to the 5th nymphal instar of treated S. gregaria with chlorfluazuron[22]. Also chlorfluazuron induced appreciable failure in ecdysis to adult stage when applied on the last nymphal instar[23].

2- Histological and ultrastructure studies of embryogenesis

2.1- Cleavage and blastoderm formation

In the present study, cleavage started about 5 hrs post oviposition. About 1 day later, cleavage nuclei increased in number, this was also observed by[24] who found that the first four cells of the earliest cleavage stage in Locusta migratoria migratorioides occur about 5.5 hrs. The eggs of S. gregaria undergoing cleavage (4, 5, 6, 9, 15, 22 and 28 hrs after oviposition[25]. Meiosis and early syncytial cleavages occur (0 – 6 hrs) in Gryllus bimaculatus[26].

At 1 day old eggs, cleavage nuclei (energies) migrate gradually to the periphery. The formation of these energies has been described by[27] and[28]. It was also described in many other insects such as in Gryllus bimaculatus[29,26] and in L. migratoria manilensis[30].

At the same time, dark granules begin to appear in the periplasm at the posterior pole of the egg forming pole cells. Some of cleavage nuclei migrate back into yolk as secondary vitellophages. The nuclei of the primary vitellophages are not enclosed by cell membrane. The vitellophages increase in number at this stage by mitotic division. Formation of secondary vitellophages is also described in Stomoxys calcitrans[31], Phlebotomus papatasi[32] and the Red Palm Weevil[33].

The vitellophages have a variety of functions. They are concerned with breakdown of yolk at all stages of development and later, when the yolk is enclosed in the midgut, they may form part of the midgut epithelium. They are also involved in the formation of new cytoplasm and are responsible for contraction of the yolk, producing local liquefactions which are necessary for this process[34]. Some cleavage nuclei migrating to the egg periphery are prevented from entering the periplasm and they remained attached to the yolk. These are secondary vitellophages while other cleavage nuclei maintain their position in the yolk as primary vitellophages each surrounded by a halo of cytoplasm. In Pectinophora gossypiella[35] divided the vitellophages into three types on the basis of their ultrastructure and concluded that these changes represented stages in maturation of vitellophages.

Mitotic division and nucleus migration continued until the blastoderm formation about 1 day (pop). Blastoderm is formed from one layer of cells arranged in a complete ring enclosing the yolk and surrounded by cytoplasmic membrane. This results similar to[25] results on S. gregaria embryogenesis where early blastoderm begin about at 22 hrs (pop) (syncytial blastoderm formed round posterior half of the egg) and late blastoderm (cellularization of blastoderm) completed in 28 hrs (pop). This is also true in Locusta migratoria[24] Schistocerca nitens[14], the Red Palm Weevil[33] and Gryllus bimaculatus[26].

2.2. Gastrulation

Our results showed that the blastoderm thickens forming the germ band at about 2 days (pop). The cells of the blastoderm that do not take part in the germ band formation become flattened, to form the serosa, the embryonic envelopes begin to appear with differentiation of the inner layer about 2 days (pop). This is brought by inward folding of the lateral borders of the germ band. These folds including the tail fold increase in width, the edges approaching each other until they meet, losing over the germ band. The outer surface composed of the pavement cells, the serosa, which cover over of the germ band forming the amnion. Similar results were detected by[25] and[21] when they studied gastrulation in S. gregaria.

Also this process has been described in Locusta migratoria migratorioides[24], Schistocerca nitens[14], Drosophila[36], Locusta migratoria[37] and L. migratoria manilensis[30]. By the end of 2 day (pop) the embryonic envelopes, amnion and serosa are completed. In Locusta migratoria, the germ band at this stage is formed of multilayer strips of cells that are differentiated into 2 layers of ectodermal and mesodermal cells[38]. In Cimex lectularis[39] it occurs at 24-48 hrs (pop). In Manduca sexta, serosa is formed at 12 hrs (pop) (about 10 % development)[40].

2.3.Organogenesis

In the present study segmentation of the inner layer forming the head and thorax segments (mouth parts and three thoracic segments) were observed at 3 days (pop). The stomodeaum did not invaginate before presumptive anterior mid gut rudiment cells, but they nearly invaginate at the same time. This is also true in Culex fatigans[41]. In Aedes aegypti[42] by 19 hrs (pop) the stomodaeal and proctodaeal invagination were observed. By 5 days (pop) trachea appears as an ectodermal invagination. Tracheae and longitudinal trunks were well developed by 60 hour (63% of development) in Aedes aegypti[42].

In the present study by 3 days (pop) the brain appeared as two ganglionic masses separated by oesophagus. The dorsal ganglion is the superaoesophageal ganglion. It exhibits a trilobed mass. The anterior is the deutocerebrum appearing as an elongated structure. The median lobe is tritocerebrum, the smallest one of the 3 units. The largest lobe, the protocerebrum, located posteriorly. The ventral ganglion is subesophageal ganglion, which is made of neural cell aggregates of the gnathal regions. By 5 days (pop), brain appears as 2 large interconnect cerebral lobes which should be enwrapped by a neurilemma. While in Apanteles glomeratus L[43], the first sign of ventral cord begin on third day as an ectodecmal thickening extending on the mid-ventral portion of the embryo. In Phlebotomus papatasi[32] brain formation started l08 hrs (pop) with proliferation of ectodemal cells in the posterior region of blastocephalon. The brain was completely developed by 75 hour (78% of development) in Aedes aegypti[42].

In the present study electron micrograph of newly deposited eggs showed that, the chorion consists of several easily distinguiable layers. The inner one is endochorion and the outer exochorion. The vitelline membrane is the inner most chorionic layer. The endochorion consists mainly of tubules linked by strands of flocculent material and separated by large spaces. The outer layer of endochorion is denser and contains fewer tubules. The exochorion is composed of web of fibrillary substance and islands of endochorion material are present in this web adjacent to endochorion. A layer of curly fibers the extra chorion covers the outer layer of laid egg. Air layers are present at the end of chorion tubules. This is also true in Oxya hyla hyla eggs where four layers were present, the vitelline membrane, innermost chorionic layer, outer chorionic layer and air layer[44].

Insect egg envelopes are generally represented by an outer chorion and an inner vitelline envelope. The chorion is the thickest of all egg envelopes and is generally characterized by a remarkable elasticity and resistance. In addition, it controls the interactions with environment through specialized structures[45]. In the screw worm egg, the chorion consists of three structural layers[17] and in cat flea, it is made up of four distinct layers[18]. While in Melanoplus differentialis egg[46], Locustana pardalina egg[47] Eyprepocnemis plorans egg[48] and Scopura montana egg[49] egg shell consists of two layers.

At the present study, wax layer was absent in the chorion of S. gregaria. Wax layer was also absent in eggshell of Blattella germanica[50] and in some other insects such as Acheta and O. hyla hyla (Orthoptera), where there was no wax layer[44]. These characteristics could be considered as a unique feature of Orthopteran eggshell.

In electron micrographs of 30 hrs old eggs of Schistocerca gregaria, cleavage nuclei of different shapes can be observed migrating to the periphery of the egg and form a single layer called the blastoderm. These nuclei are surrounded by double layered membrane[51]. reported that migration of cleavage nuclei in the fly, Wachtliella persicariae, are surrounded by a complex multilayered membrane but such complex nuclear membrane is not found in the cleavage nuclei of the housefly egg and also in Bombyx mori[52].

3- Histopathological and ultrastructure study of affected eggs

In the present study eggs produced of one day old females resulted of the 5th nymphal instars of S. gregaria, which were treated with LC50 of lufenuron and rice bran extract (Oryza sativa), showed great effect on brain, alimentary canals and compound eyes. Other embryos were blocked at germ band formation at lufenuron treatment or at cleavage stage in rice bran extract treatment.

Fenoxycarb block embryogenesis of cat flea, Ctenocephalides felis at early blastoderm, blastokinesis and advanced larval developments up to hatching[18]. Blockage of embryogenesis by application of different plant extracts was also reported in eggs of Corcyra cephalonica after treatment with 6 plant extract and neem [53,54]. Treatment of Locusta migratoria females with teflubenzuron resulted in full ovicidal activity. It has been proposed that an inhibition of chitin synthesis in the embryo was responsible for the ovicidal properties[55].

Homalodisca coagulate eggs is inhibited by pyriproxfen, when applied to younger eggs within 48 hrs (pop) causing marked effect of embryogenesis[56]. The plant extract of Clausena dentate reduced egg hatchability and proved to be highly ovicidal against Helicoverpa armiyera[57].

In the present study electron micrographs of 5 hrs old eggs affected with LC50 of lufenuron treatment showed signs of deterioration, chorion exhibit abnormal appearance. The chorion thickness is greatly reduced and become

lighter in color than normal. The structural component of extra chorion, exochorion and endochorion are completely lysis. The chorion involved the vitelline membrane which is the most inner layer of it and has irregularities of the outlines. Some cleavage nuclei have abnormal appearance and short stripe of microtubules is obvious. Other embryos showed gross disintegration of cell organelles leaving wide empty space or cell debris, vacuolated cytoplasm. Irregular shapes of cleavage nuclei have poor chromatin and very thin nuclear membrane. The yolk granules with spherical texture are changed to become pear in shape. Yolk bodies are surrounded by relatively clear ring, others have a clear rim.

Ovicidal effects of chitin Synthesis inhibitors may be due to adverse effects of these compounds on the chorion of eggs before hatching[58]. The present CSIs penetrated the eggs and interfere with the embryonic cuticle synthesis and the malformed muscles could not enable the developed embryos to hatch as Hexaflumuron on Aubeonymus mariaefranciscae and novaluron on Tribolium castaneum[59,60].

Electron micrographs of eggs, affected with LC50 of rice bran extract treatment, displayed signs of deterioration, including vaculation and lysis of cell components leaving cavities within the ooplasm. Blastoderm is disintegrated, and failed to arrange. The nuclei have severe malformation, showing irregularity of the outlines and suffer chromatolysis, where chromatin is very poor and dispersed. The vacuolated cytoplasm are also observed, include peculiar vesicle like body filled with remnants of degeneration, involving yolk and other debris.

Similar results were detected in cat flea eggs after treatment with fenoxycarb represented in disruption of the blastoderm yielding unorganized non-cellular material[61] and in Culex pipines after female treatment with non-volatile jojoba oil[62].

A peak of free ecdysons observed during embryonic development of locusta migratoria[63] where involved in the initiation of morphogenetic movements and the induction of cuticulogenesis[64]. Therefor it seems that the compounds (CSIs) used in the present study on S. gregaria may interfere with free ecdysone, which may be bound to vitelline inside the eggs[65,66]. The Rice bran extract (Plant waste product) has major advantages of giving control. It is a chemical free and insects are not likely to develop resistant to it.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربى

التغيرات النسيجية والتغيرفى التركيب الدقيق للتطور الجنينى لحشرة الجراد الصحراوى الناتجة عن المعاملة بكل من مركب الليوفنيرون (مثبط تكوين الكيتين) ومستخلص نخالة الأرز

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الخلفية: يعتبر نتاج المخلفات من نخالة الأرز وليوفنيرون مركبات آمنة للانسان والبيئة. ويمكن استخدامهم في مكافحة الجراد الصحراوي.

هدف الدراسة: دراسة تأثير مثبط تكوين الكيتين (ليوفنيرون) ,أحد منظمات النمو الحشرية ومستخلص نخالة الأرز أحد المستخلصات النباتية على بيض حشرة الجراد الصحراوي باتباع الدراسة الهستولوجية والتركيب الدقيق للتطور الجنينى.

المواد والطرق: تمت دراسة التطور الجنينى لحشرة الجراد الصحراوى خلال مرحلة التفلج ؛ تكوين البلاستودرم ؛التبطين والتعضى. وقد تبين ان مرحلة الانقسام تبدا بعد حوالى 5 ساعات من وضع البيض حيث تتميز بوجود أحجام مختلفة من انوية التفلج حتى مرحلة تكوين البلاستودرم عند عمر يوم . وتتكون الطبقة الجرثومية عند عمر يومين حيث تتركب من عدد من الطبقات الخلوية التى تتميز إلى طبقة الاكتودرم والميزودرم. وقد ظهرت حلقات فى الطبقة الجرثومية مكونة من 3 حلقات صدرية و حلقات تمثل اجزاء الفم عند عمر 3 ايام كما يمكن أيضا تمييز قرون الاستشعار. بعد ظهور حلقات الجسم وتمييز الطبقة الجرثومية يظهر اندغام المعى الأمامى , ويمكن تمييز مبديئات تكوين المعى الامامى والاوسط والخلفى فى عمر 5 ايام ,وكدلك يمكن تمييز المخ و العيون بعمر4 ايام . عند دراسة التركيب الدقيق للبيض الناتج عن الاناث الغير معاملة وجد أن الغلاف الخارجى (الكوريون) للبيض حديث الوضع والغير معامل يتكون من عدة طبقات . وعند 30 ساعه من عمر البيضه تظهر الانويه الانقساميه بأشكال مختلفه والتى تهاجر الى حافه البيض ، وتحاط هذة الانويه بجزء من بلازما البيض والتى تحتوى على حويصلات وطبقه من الشبكه الاندوبلازميه وقليل من الميتوكندريا .وتبدأ الانويه فى الانقسام بطريقه غير مباشره لتكوين طبقه البلاستودرم ,حيث كانت انوية هذة الطبقة مغزلية الشكل.

النتائج: اثبتت الدراسات الهستولوجيه ان البيض الناتج من اناث حشرات كاملة عوملت بالتغذية لاناث حوريات الطور الخامس التى تبلغ من العمريوم واحد بالجرعة النصف مميتة لكل من مركب ليوفنيرون ومستخلص نخالة الأرز , أنه قد تأثر فى نموه الجنينى بمراحله المختلفه. وكان التأثيرواضح على تكوين المخ والقناة الهضمية والعين. كما أظهر

مركب ليوفنيرون تأثيره على تكوين الشريط الجرثومى ,بينما أدى مستخلص نخالة الأرزالى توقف النمو الجنينى عند المرحلة الأنقسامية فى بعض البيض المتأثر بالمعالجة. كما لوحظ ظهورتغير لمحتوى البيض متمثلا فى ؛تحلل لمكونات البيضه وأيضا غلاف البيضه الخارجى (الكوريون) مع ظهور فجوات فى البلازما الداخليه. دراسة التركيب الدقيق للبيض المتأثربمركب ليوفنيرون أظهرت تشوهات فى غلاف البيضة (الكوريون) من حيث تحلل لمكوناته واصبح أقل سمكا وغير منتظم,كما أمكن ملاحظة تحلل عضيات السيتوبلازم تاركاً أماكن فارغة أو بقايا خلوية. كما اظهرالبيض المتأثربمستخلص نخالة الأرز ضمور فى خلايا البلاستودرم مع توزيعها العشوائى .وأخذ الأنوية شكل غير منتظم مع تبعثر للمادة الكروماتينيه وتتحللها . كما لوحظ تحلل عضيات السيتوبلازم تاركا اماكن فارغه. وظهور أوعية تضم بقايا خلويه.

الخلاصة: المركبات المستخدمة احدثت تغيرات خطيرة في اجنة الجراد الصحراوي كما كشفت عنها الدراسات الهستولوجية والتركيب الدقيق