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Ultrastructure and histopathological alteration in the ovaries of *Callosobruchus maculatus* (F.) (Coleoptera, Chrysomelidae) induced by the solar radiation

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Ultrastructure;
Solar energy

Abstract The ultrastructure and histopathological changes in the ovarioles were tested in normal adult females and in those developed from solar energy treated pupae (53 °C for 15 min) of *Callosobruchus maculatus*. The telotrophic ovarioles of *C. maculatus* contain germarium region followed by vitellarium region which constitutes the vitellarium.

The germarium houses trophocytes and the vitellarium consists of ovarian follicles.

At the germarium region of the ovariole, the trophocyte or nurse cell nucleus contains uniformly spaced clumps of heterochromatin. The cytoplasm contains numerous free ribosomes and mitochondria.

At the vitellarium region, follicular epithelial cells form a layer around the oocyte, each cell contains a large oval nucleus with abundant heterochromatin, and the cytoplasm contains mitochondria, free ribosomes and dark spherical globules. Also the vitellarium includes the previtellogenic oocyte which is the most anterior one and two last vitellogenic oocytes at the posterior end as the yolk was deposited.

Oocyte microvilli are interdigitated with those of the follicle cells. The ooplasm consists primarily of electron-dense yolk bodies and lipid droplets.

These phases could be identified in the ovarioles of normal females and to a less extent in those of females developed from the treated pupae. In the ovarian follicles of the treated generation, degeneration of the cell components of trophocytes, follicular epithelium and oocytes were the most obvious signs of damage. Also, lacking of yolk bodies and vacuolation in the border of the ooplasm were observed.

The damage was more pronounced in the ovarioles of (F.) progeny of the treated generation.

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Introduction

The cowpea, *Vigna unguiculata* (L.), is one of the most important hay-crops.

Unfortunately, wherever cowpea is cultivated, the seed is subject to very severe attacks by the bruchid beetle commonly known as the southern cowpea weevil.

Surveys of the literatures reveal that the southern cowpea weevil, *Callosobruchus maculatus* F. is known to be occurring long ago in many parts of the world. At the present time there is every reason to believe that this bruchid has, through the channels of commerce, become of world-wide distribution. However, it can safely be stated that it occurs wherever cowpea are grown or stored.

The larvae of this bruchid feed inside the seeds, gradually rendering them unfit for planting and unsuitable for human consumption (Lale, 1998; Aly et al., 2005).

The southern cowpea weevil does not confine its attack to cowpea in storage, but also lays its eggs on the exterior of ripening pods in the field and on seeds in spilt-pods as well.

Temperature is an abiotic environmental factor which strongly influences insect physiology and reproduction (Blanckenhorn and Henseler, 2005; Weng et al., 2011). The effect of heat and heat stress on viability and performance of insects has been investigated in many studies (Mohammed, 1990; Neven, 2000; Gruntenko et al., 2003; Blanckenhorn and Henseler, 2005). Mohammed (1990) conducted histocytological studies using transmission electron microscope which revealed the effect of heat on gonads of *C. maculatus*. Several studies investigated the utilization of solar heating in post-harvest cowpea weevil control (Ntoukam et al., 1997; Chauhan and Ghaffar, 2002; Murdock et al., 2003; Mekasha, 2004; Mekasha et al., 2006). They all demonstrated the potential of solar radiation in suppression of this stored grain insect.

Extreme high temperature is a non insecticidal possibility for the control of the weevil.

This work paid special attention for utilization of solar radiation for suppression of weevil population. The present histological studies are made at both the light and electron microscope levels in an attempt to clarify the detailed structure of the female reproductive system of the southern cowpea weevil *C. maculatus* which results from solar energy treated pupa.

Materials and methods

Origin of population

The strain of cowpea weevil, *C. maculatus* (F.) was obtained from the plant protection Research Institute, Dokki, Giza, Egypt, where the colony is maintained on cowpea seeds (*V. unguiculata* L.) at temperature of 27 ± 2 °C and relative humidity of 60 ± 5%.

Sun exposure technique

Exposure to solar heat was conducted using an obtuse-base-angle box heater described by Mekasha et al. (2006) with some modifications. The box was constructed with 1 mm thick galvanized metal sheet; the upper open side of the box was 51 cm × 20 cm, length by width and the perpendicular height was 23 cm with obtuse-base angle (about 120°). The box was covered externally with a black polystyrene sheet to increase the capability to sun rays absorption. In the middle of the box a glass plate was introduced to help in raising the inner temperature during the exposure time. The black polystyrene sheet was held on the ground for about 10–15 min to collect the sun rays on the experimental spot and the box put on it during the exposure time. Temperature and relative humidity inside and outside the box were recorded during the exposure times using thermometer and hygrometer.

Exposure of the pupal stage of cowpea weevil to solar heat

Three pairs of newly emerged adults (0 day old) from a laboratory colony were introduced into vials containing 15 g of cowpea seeds. The females were allowed to lay eggs for 24 h then all insects were carefully removed from the vials. The infested seeds were then held under controlled conditions of temperature (27 ± 2 °C) and relative humidity (60 ± 5%) until treatment.

Pupal stage was exposed to sun heat about 53 °C in an obtuse-base-angle box heater for 10, 15 and 20 min respectively (this technique can be applied by special equipment through granaries). For each treatment control group was run at the same time of the experiment with 0-min exposure. After exposure to sun heat all vials were kept at controlled temperature (27 ± 2 °C) and relative humidity (60 ± 5%) until adult emergence.

The malformed adults developed from solar energy treated pupae at 53 °C for 15 min were subjected to ultrastructure studies.

Electron microscopy studies

For electron microscopy, the females were dissected in 2.5% glutaraldehyde fixation. The ovaries were immediately removed and placed in fresh ice-cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate and 0.17 M sucrose at PH 7.4 for 2 h at 4 °C. The ovaries were post-fixed in 1% osmium tetraoxide in

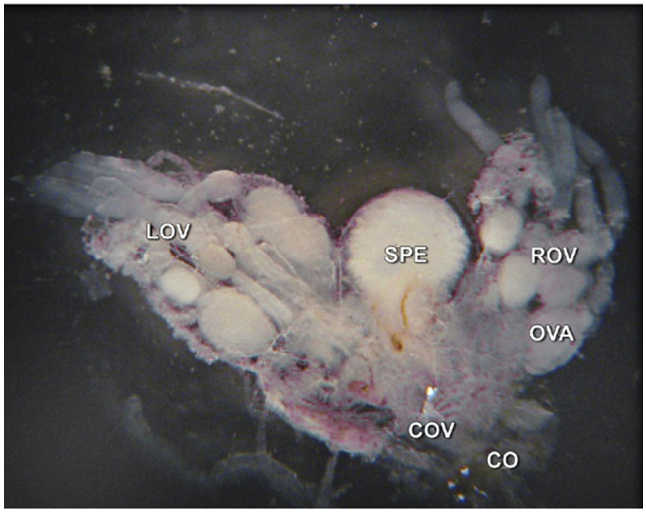

Figure 1 Photograph of the internal reproductive organs of female *C. maculatus*. Right ovary (ROV), Left ovary (LOV), Ovariole (OVA), Spermatheca (SPE), Common oviduct (COV) and Gonophore (GO) (×20).

0.1 sodium cacodylate and 0.17 M sucrose at 4 °C for 90 min. They were then embedded in pure Epon 812 resin, sectioned with RMC ultramicrotome, stained in 2% uranyl acetate in methanol for 15 min. followed by aqueous lead citrate for 3 min. and viewed in a Sumy Electron Optics PEM 100, 75 kV Transmission Electron Microscope at the Medical Military Academy. Electron micrographs were taken at several magnifications.

Result

Effect of solar energy on reproductive organs of ***C. maculatus*** *adults comparing with normal ones*

The normal female reproductive organs

The internal organs of reproduction. The female *C. maculatus* has a pair of ovaries located on both sides of the alimentary canal. Each ovary is composed of six ovarioles or egg tubes. The posterior ends of the two ovaries are connected to a pair of lateral oviducts which join to form a common oviduct. This leads to a genital chamber which opens to the exterior at the gonopore. Just posterior to the common oviduct is the duct of a single spherical spermathecal sac located ventral to the common oviduct and is connected posteriorly to a short, slender duct (Fig. 1).

The ovarioles of female *C. maculatus* are of acrotrophic type. Each ovariole is divided into four regions: the terminal filament, the germarium, the vitellarium and the ovariole stalk or pedicel.

Histological and ultrastructural studies of ovarioles. The germarium. It is the anterior part of the egg tube and contains

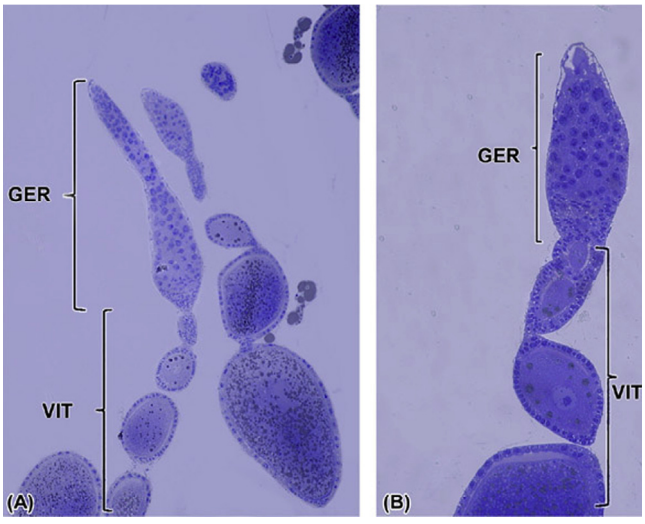

Figure 2 (A and B): Photomicrograph of ovariole of normal female *C. maculatus* showing germarium (GER) and Vitellarium (VIT) regions (A, ×100) and (B, ×200).

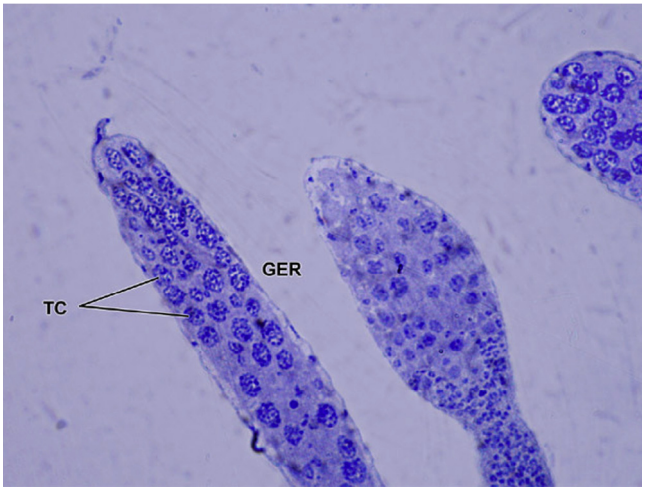

Figure 3 Higher magnification of the germarium region (GER) of normal female *C. maculatus* showing trophocyte cells (TC) (×400).

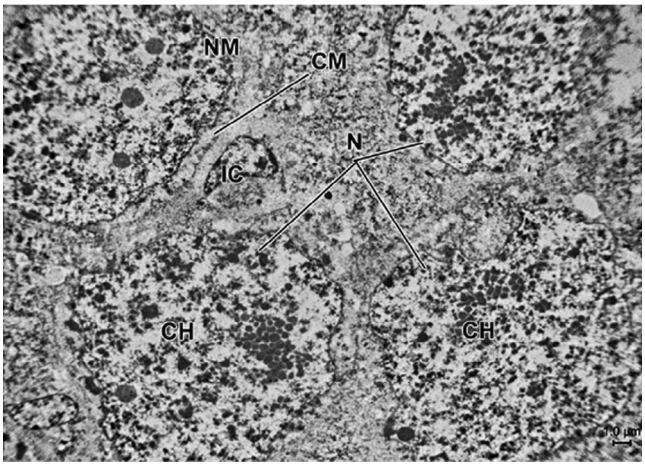

Figure 4 Electron micrograph of the trophic chamber of the normal female *C. maculatus* showing trophocytes at the distal region. Nucleus (N), Chromatin clumps (CH), Nuclear membrane (NM), Cell membrane (CM) and Interstitial cell (IC) (×3000). Scale bar: 1 µm.

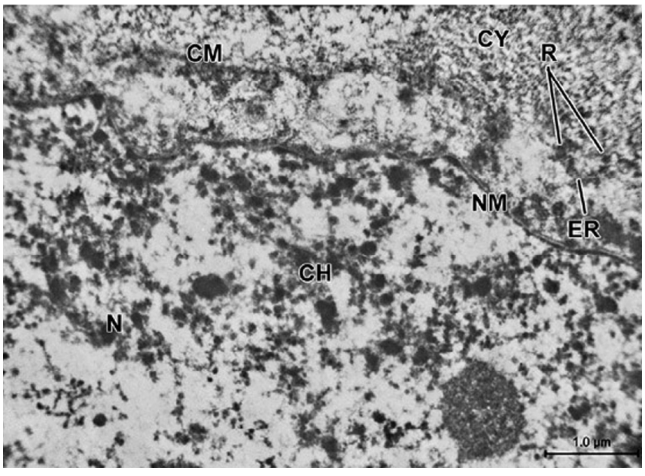

Figure 5 A higher magnification of a trophocyte (nurse cell) of normal female *C. maculatus* showing: Nucleus (N), Nuclear membrane (double) (NM), Chromatin clumps (CH), Cell membrane (CM), Endoplasmic reticulum (ER), Ribosomes (R) and Cytoplasm (CY) (×15,000). Scale bar: 1 µm.

trophocytes (nurse cells), young oocytes and prefollicular cells. The germarium is a cylindrical tube with more or less uniform diameter throughout most of its length. The proximal portion of the germarium slightly narrows (Figs. 2 and 3). The trophic zone contains large nurse cells (the trophocytes) the interstitial cells and small scattered prefollicular cells (Figs. 2, 3, 7 and 9).

The trophocytes: According to the ultrastructural studies, trophocytes of *C. maculatus* ovarioles could be divided into three types; trophocytes in the distal part, trophocytes in the middle zone and trophocytes in the basal zone of trophic chamber. In the distal region of the trophic chamber, cysts of round each trophocyte are distinguished clearly. Each trophocyte has a conspicuous spherical nucleus, surrounded by a relatively small amount of finely granular cytoplasm (Fig. 4). Nuclei in this region are heterochromatic and are surrounded by a distinct double membrane (Fig. 5). The cytoplasm of trophocytes is rich in organelles, containing ribosomes, Golgi elements and vesicles of endoplasmic reticulum (Figs. 4 and 5).

Trophocytes in the middle region of the trophic chamber are characterized by the partial reduction of their surrounding membrane and the nuclei are heterochromatic (Fig. 6). The granule aggregates are found in close association with chromatin clumps or localized free in the nucleus (Fig. 7). The cytoplasm of the trophocytes is rich with ribosomes, Golgi elements, tubules of endoplasmic reticulum and mitochondria (Fig. 6). Also, vesicular membranous aggregates are observed between trophocytes and interstitial cells (Figs. 6 and 7).

Trophocytes in the basal region of the trophic chamber form a syncytium (Fig. 8A). The cell boundaries between cells are completely abolished and nuclei are embedded in a continuous matrix of finely granular cytoplasm. The cytoplasm in the syncytium is partially rich with ribosomes and loaded with vacuoles of different size (Fig. 8A). The nuclei of the

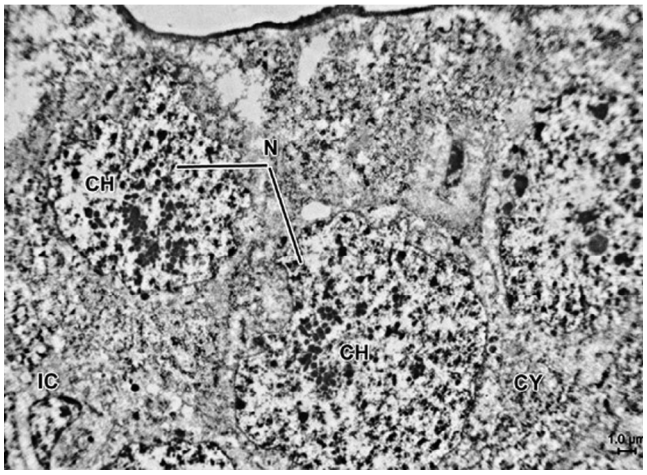

Figure 6 Electron micrograph of the trophic chamber of the normal female *C. maculatus* showing trophocytes at the middle region. Nucleus (N), Chromatin clumps (CH), Interstitial cell (IC) and Cytoplasm (CY) (×3000). Scale bar: 1 µm.

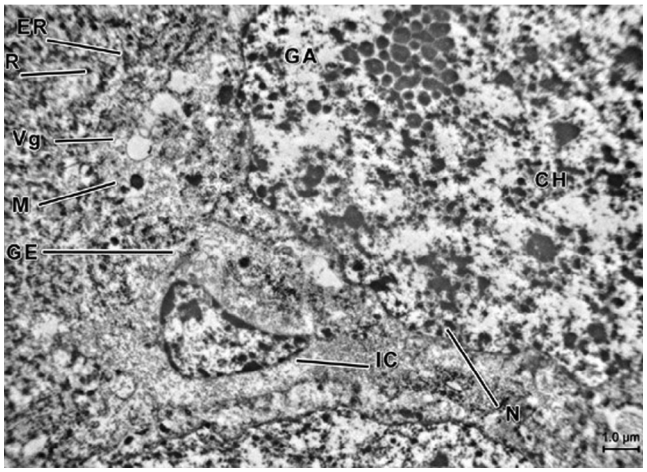

Figure 7 Electron micrograph of the trophic chamber of normal female *C. maculatus* showing Ribosomes (R), Golgi elements (GE), Endoplasmic reticulum (ER), Mitochondria (M), Vesicular membranous aggregates (Vg), interstitial cell (arrow), Nucleus (N), Chromatin (CH) and Granules aggregates (GA) (×6000). Scale bar: 1 µm.

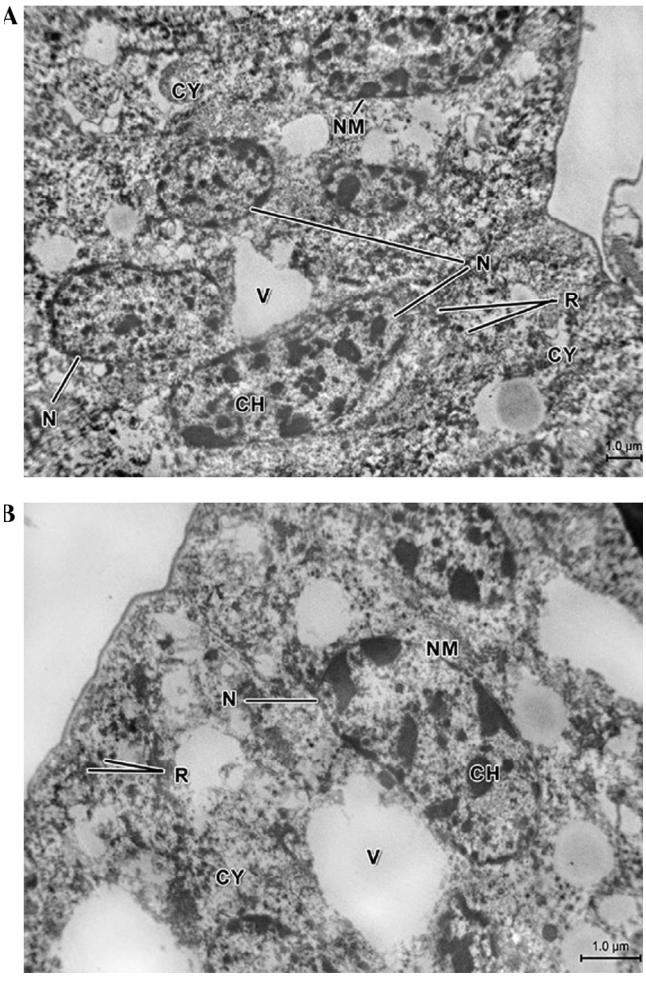

Figure 8 (A and B): Electron micrograph of the trophic chamber of normal female *C. maculatus* showing syncytium at the basal region. Nucleus (N), Chromatin (CH), Nuclear membrane (NM), Cytoplasm (CY), Ribosomes (R) and Vacuoles (V). (A, ×6000), (B, ×10,000). Scale bar: 1 µm.

syncytium are oval and heterochromatic. Chromatin tends to adhere to the nuclear membrane Fig. 8(A and B).

Interstitial cells: These are small compressed cells of irregular shape, lying in-between the trophocytes (Figs. 4, 6 and 7). The cells are almost occupied with halo nuclei. Nuclei are surrounded by a thick layer of cytoplasm. The cytoplasm is granulated, contains few membranous vesicles, and microtubules (Fig. 7). Nuclei have condensed chromatin which attaches to the nuclear membrane.

Region of oocyte differentiation: The distinction of oocyte is possible in the germarium region just posterior to the trophic chamber. In this region small compact prefollicular cells are arranged to encircle oocytes (Fig. 9).

The vitellarium. The region of the ovariole immediately lying beyond the germarium constitutes the vitellarium. It consists of a series of egg chamber or follicles; each contains an oocyte enveloped by the follicular epithelium cells (Figs. 2 and 10).

Oocytes: The oocytes are arranged in a single row along the vitellarium. The most advanced in development are usually at the posterior end. Generally, the most anterior 2 or 3 oocytes (previtellogenic) remain connected to the posterior region of the trophic chamber of the germarium. Which is the most anterior oocytes deposited Fig. 2. These early growing stages of oocytes are characterized by having granular cytoplasm and relatively large germinal vesicle (Fig. 10). The oocytes and the germinal vesicle progressively enlarge in size as they move posteriorly down the ovariole. At the basal part of the ovariole, the oocytes enter the late stages of their growth. This stage involves the most posterior one or two oocytes (vitellogenic). These oocytes have lost their connections with the trophic chamber. These are characterized by cytoplasm which is loaded with large yolk granules of different sizes (Fig. 11).

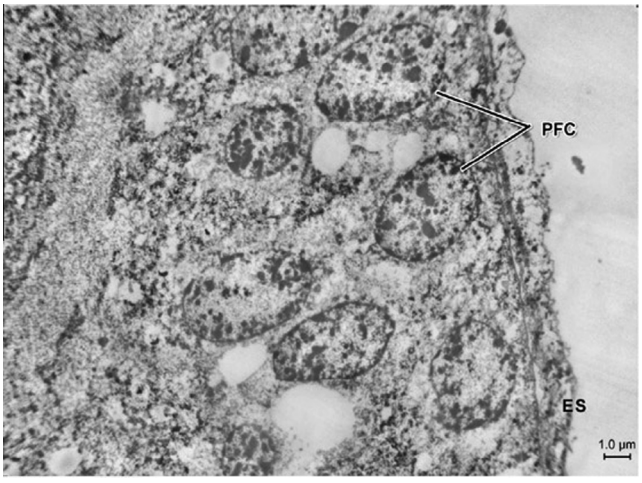

Figure 9 Electron micrograph of normal female *C. maculatus* at zone of oocyte differentiation posterior to the trophic chamber showing: Prefollicular cells (PFC) and Epithelial sheath (ES) (×4000). Scale bar: 1 µm.

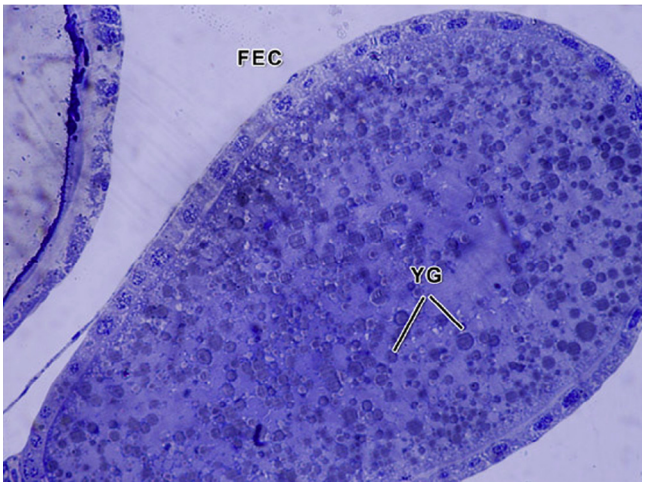

Figure 11 A higher magnification of vitellogenic oocyte of normal female *C. maculatus* showing follicular epithelial cells (FEC) and Yolk granules (YG) (×400).

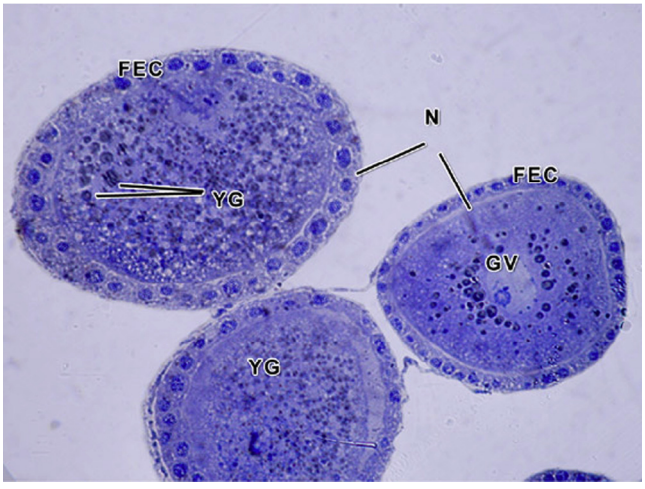

Figure 10 Higher magnification of previtellogenic oocyte of normal female *C. maculatus* showing follicular epithelial cells (FEC), Nucleus (N), Yolk granules (YG) and Germinal vesicle (GV) (×400).

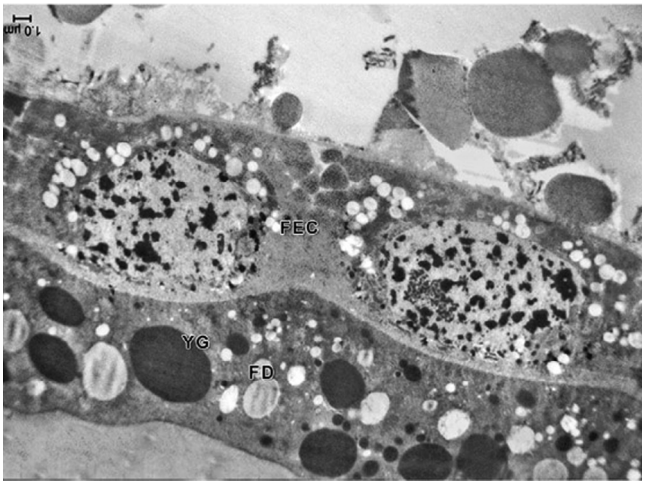

Figure 12 Electron micrograph of a vitellogenic oocyte of normal female *C. maculatus* showing: Follicular epithelial cells (FEC), Yolk granules (YG) and Fat droplets (FD) (×3000). Scale bar: 1 µm.

Follicular epithelial cells: As the oocyte passes down the ovariole, it is enclosed in a layer of elongated epithelial cells which has flattened nuclei occupying most of the cell (Figs. 10 and 11). Electron micrographs reveal that the follicular epithelium is formed of columnar epithelial cells with elongated nuclei which are heterochromatic (Figs. 12 and 13). The cells are rich with organelles including tubules of rough endoplasmic reticulum, small secretory granules and large number of round and elongated mitochondria. These latter tend to aggregate forming masses of mitochondria extending towards the follicle cells' interface with the oocyte Fig. 14(A and B). The plasma membrane of follicle cells at the side facing the oocyte is drawn into microvilli, interdigitating with those of the oocyte (Fig. 15B).

Electron micrographs of the vitellogenic oocytes revealed that the plasma membrane around the oocyte is drawn into numerous finger-like microvilli, projecting into the space between the oocyte and the follicle cells Fig. 15(A and B). The microvilli of the oocyte inter digitate with those of follicle cells. Some of the long channels between oocyte microvilli are filled with electron dense material. The tips of the invaginations are cut off forming pinosomes (Fig. 15B). The pinosomes fill the oocyte cortex and enlarge as they move towards the interior of the oocyte forming large dark spherical globules. In addition to pinosomes and dark globules, the cytoplasm contains round and elongated mitochondria, yolk granules, fat droplets and vacuoles Fig. 15(A–C).

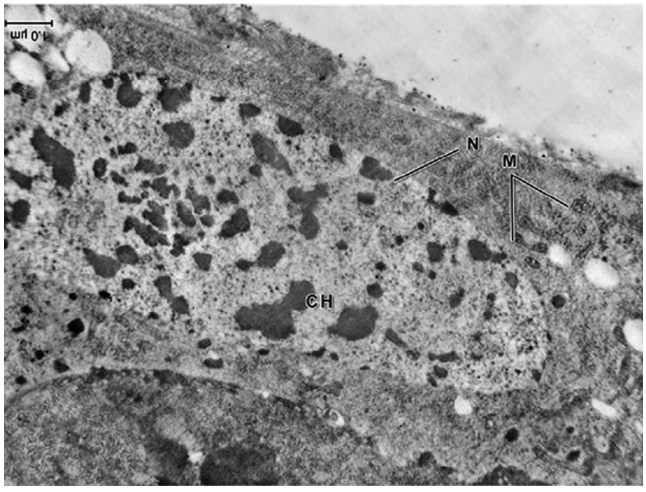

Figure 13 A higher magnification of a follicular epithelial cells showing: Mitochondria (M), Nucleus (N) and Chromatin (CH) (×8000). Scale bar: 1 μm.

Effect of solar energy on the female reproductive system

The internal reproductive organs of the adult female *C. maculatus* (F.) developed from solar energy treated pupa consist of all the essential parts as normal but in reduced number and size of oocytes, and enlargement and swelling of spermatheca (Fig. 16). The histopathological investigations of the ovarioles of adult female *C. maculatus* produced from solar energy treated pupa (F.) showed different deterioration effects.

Germarium

Trophocytes. Trophocytes can be identified in the trophic chamber of females (F.). These include trophocytes with reduced number and wide empty space in the region of oocyte differentiation Figs. 17A and 18.

Electron investigation identified two types of trophocytes in the trophic chamber. These involve trophocytes with reduced cell membrane at the middle and basal region Fig. 19(A and B) and (Fig. 21). Trophocyte nuclei have an irregular outline (Fig. 21). The cytoplasm is lighter than normal and occupies a greater area of the cell Fig. 19(A and B) and (Fig. 21). It is very poor in organelles (Fig. 20) except degenerating mitochondria and large vacuoles Fig. 19(A and B). The cytoplasm has aggregates of small dark granules at the basal zone of the trophic chamber (Fig. 21). Nuclei of most trophocytes are almost devoid of chromatin clumps, having evenly granulated nucleoplasm Fig. 19(A and B) and (Fig. 21).

Region of oocyte differentiation. In the germarium of the female (F.), prefollicular cells, also arrange longitudinally to envelop the posterior region of the trophic chamber (Fig. 22). The follicular cells seem to be degenerated and their

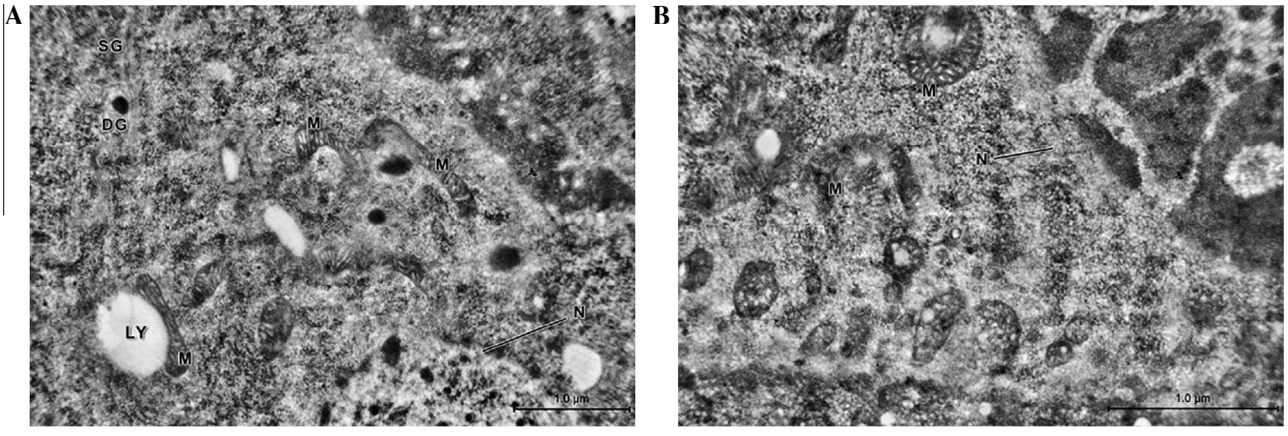

Figure 14 (A and B): Electron micrograph of a vitellogenic oocyte of normal female *C. maculatus* at the cytoplasmic region of follicular epithelial cells (FEC) showing: Nucleus (N), Mitochondria (M), secretory granules (SG), Dark granules (DG) and Lysosomes (LY). (A, ×20,000) and (B, ×30,000). Scale bar: 1 μm.

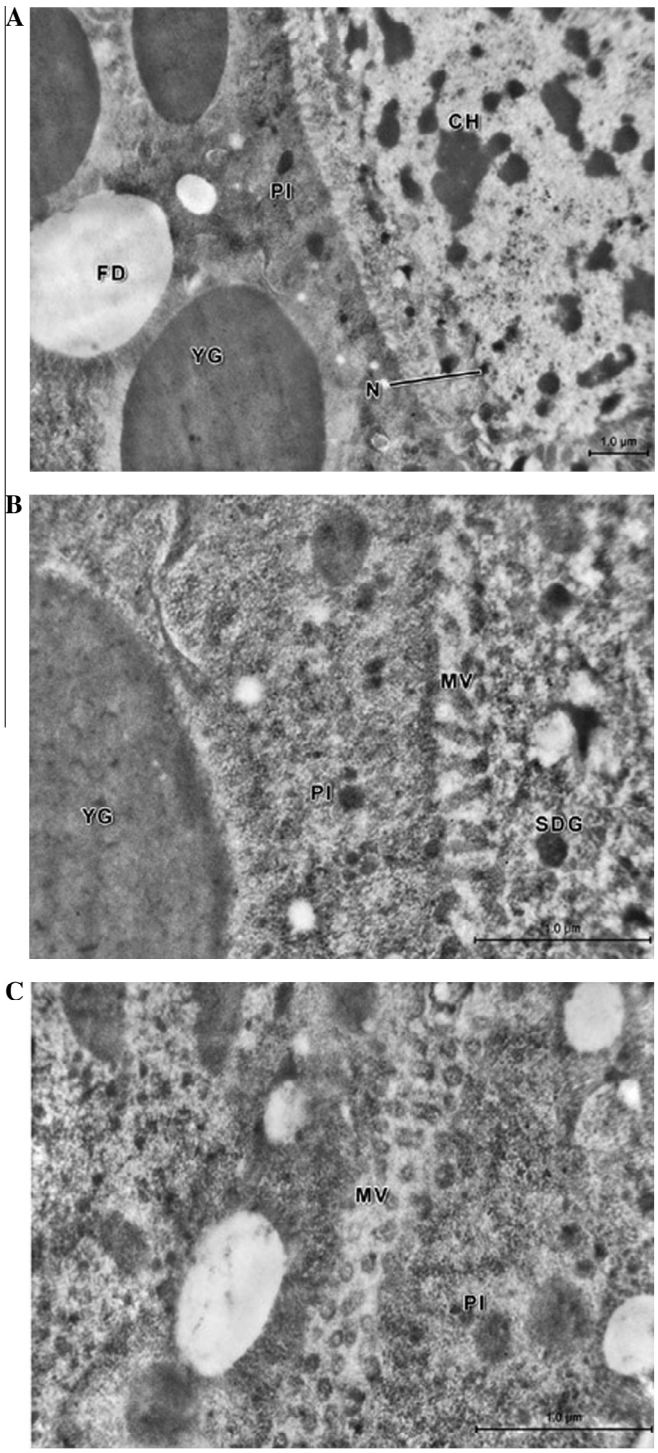

Figure 15 (A–C): Electron micrograph of a vitellogenic oocyte of normal female *C. maculatus* showing: Brush border (microvilli) (MV), Pinosomes (PI), Spherical dark globules (SDG), Yolk granules (YG) and Fat droplets (FD). (A, ×100,000), (B and C, ×30,000). Scale bar: 1 µm.

cytoplasm and organelles are lysed and having a wide space of vacuoles. The cell nucleus loses its polymorphic nature and irregularities of the outlines. Chromatin is very poor and dispersed. Some nuclei have few peculiar ring-like bodies filled with fine granules. Degeneration of ovarian sheath is also detected (Fig. 22).

Vitellarium

Histological studies of vitellarium of the female *C. maculatus* developed from solar energy treated pupa showed that it contains one or two small anterior previtellogenic oocytes and large vitellogenic one at the posterior end of the ovariole Fig. 17(A and B).

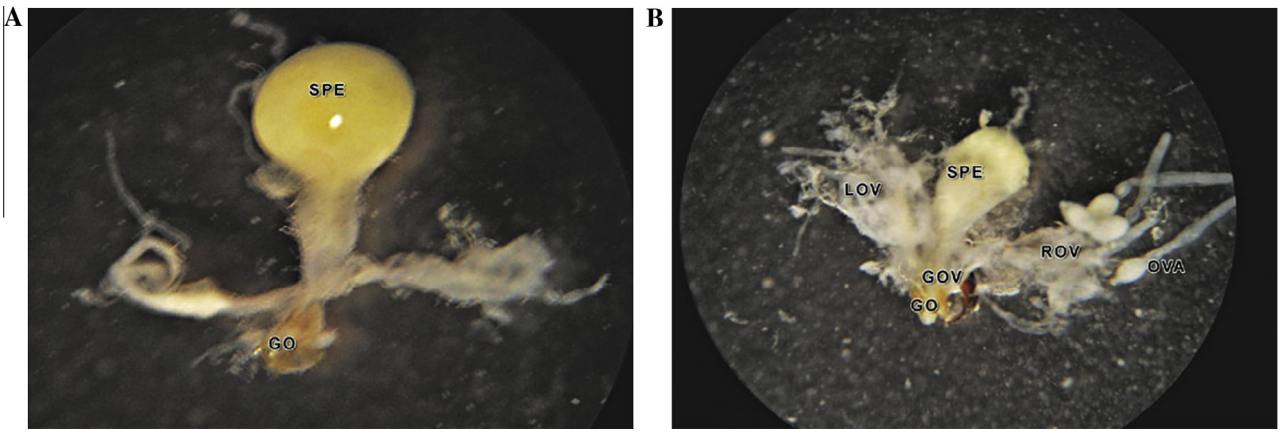

Figure 16 (A and B): Photograph of the internal reproductive organs of females *C. maculatus* resulting from solar energy treated pupa showing: Right ovary (ROV), Left ovary (LOV), Ovariole (OVA), Spermatheca (SPE), Common oviduct (COV) and Gonophore (GO) (×20).

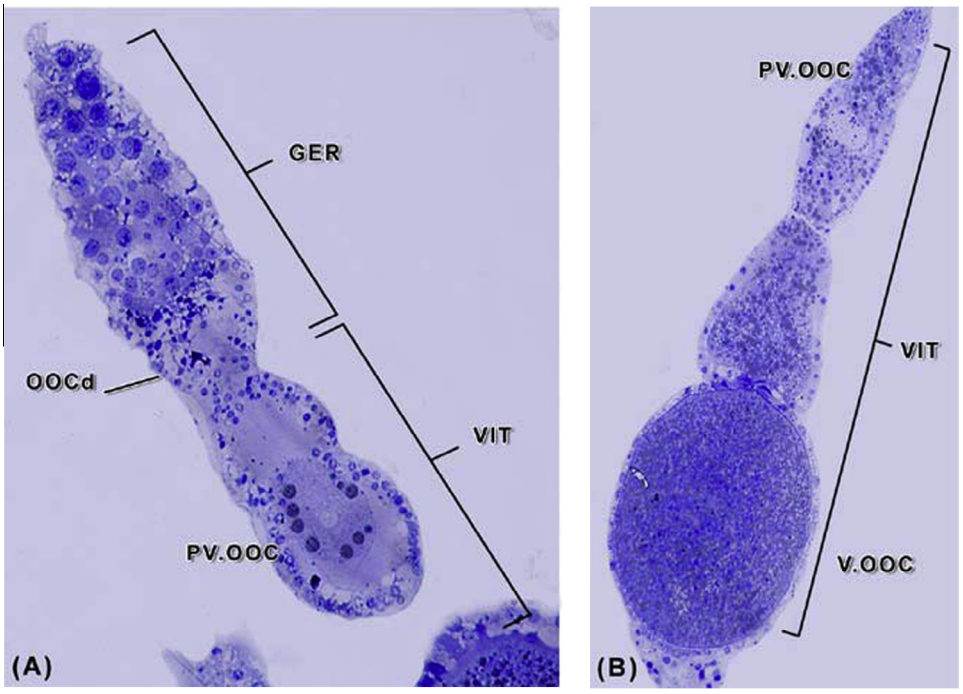

Figure 17 (A and B): Photomicrograph of an ovariole of female *C. maculatus* resulting from solar energy treated pupa showing: Germarium (GER), Vitellarium (VIT) and region of oocyte differentiation (OOCd), Previtellogenic oocyte (PV. OOC) and Vitellogenic oocyte (V. OOC) (×200).

Oocyte. Previtellogenic oocyte has an abnormal appearance (Fig. 23). Cytolysis is observed in the cytoplasm, which is empty. The germinal vesicle is severely degenerated and vacuolized. The follicular epithelial cells seem to be degenerated (Fig. 23).

In the vitellogenic oocyte, signs of deterioration are observed (Fig. 24). Electron investigation confirmed this histological study; degeneration of ovarian sheath Fig. 25(B and C), distortion and irregularities of the outlines of follicular epithelial cells Fig. 25(A–C). The follicular epithelial cells are greatly vacuolized Fig. 25(A and B). Lyses of cytoplasm and its organelles are observed Fig. 25(A–C) and (Fig. 26). Nucleus of follicular epithelial cells is small round with dispersed chromatin Fig. 25(A–C) and (Fig. 26). In the vitellogenic oocyte, signs of deterioration are observed, including vacuolation in the border of the ooplasm (Fig. 25C). Lyses and complete degeneration of cell organelles leave cavities within the ooplasm Fig. 27(A and B). Mitochondria and microvilli are also distorted and remnants of degenerated mitochondria are observed Fig. 27(A and B).

Discussion

The current study of the internal reproductive organs in female *C. maculatus* resulting from solar energy treated pupae showed

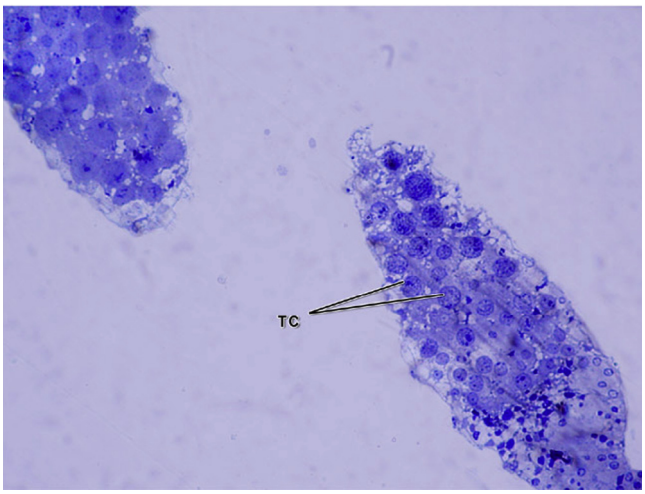

Figure 18 A higher magnification of the germarium region of female *C. maculatus* resulting from solar energy treated pupa showing reduction in trophocytes cells (nurse cells) TC and wide empty spaces (×400).

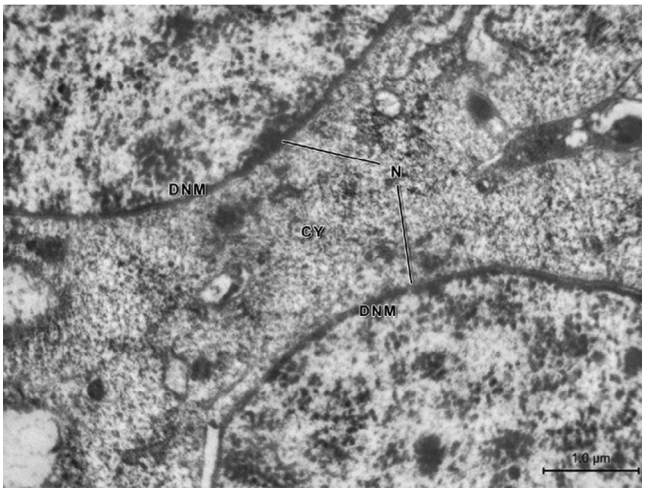

Figure 20 A higher magnification of trophocytes of adult female *C. maculatus* resulting from solar energy treated pupa showing double nuclear membrane (DNM) of the nucleus (N) and deteriorated cytoplasm (CY) (×15,000). Scale bar: 1 µm.

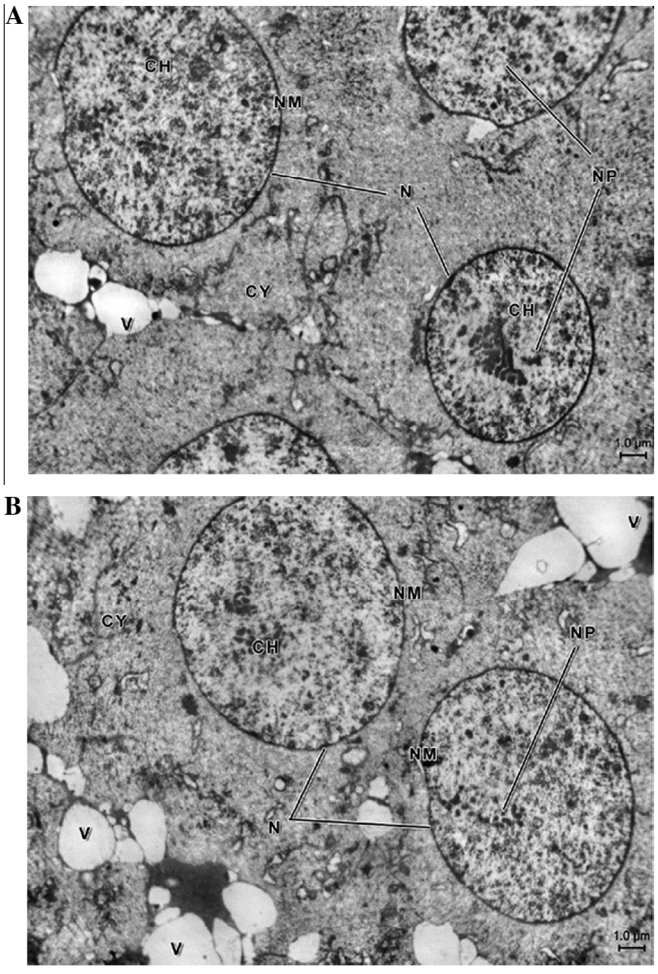

Figure 19 (A and B): Electron micrograph of the trophocytes of adult female *C. maculatus* resulting from solar energy treated pupa showing the Nucleus (N) with reduced cell membrane, Chromatin (CH), Nucleoplasm (NP), Nuclear membrane (NM) and Vacuoles (V) in the cell cytoplasm (CY) (×4000). Scale bar: 1 µm.

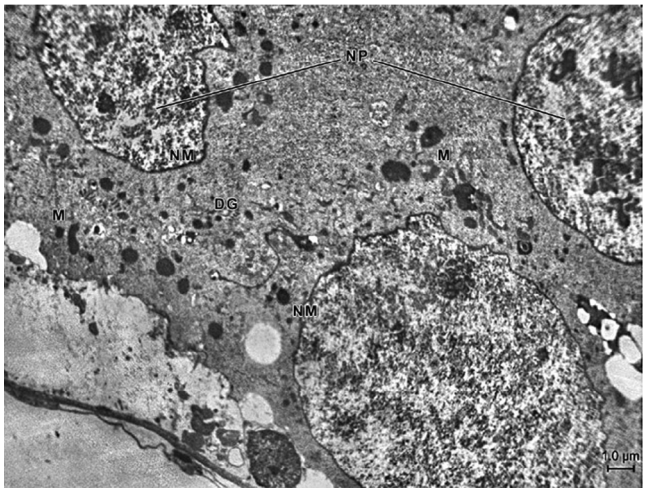

Figure 21 Electron micrograph of the trophocytes at the basal region of the germarium of adult female *C. maculatus* resulting from solar energy treated pupa showing the irregularity of the nuclear membrane (NM), Nucleoplasm (NP), Dark granules (DG) and Mitochondria (M) (×4000). Scale bar: 1 µm.

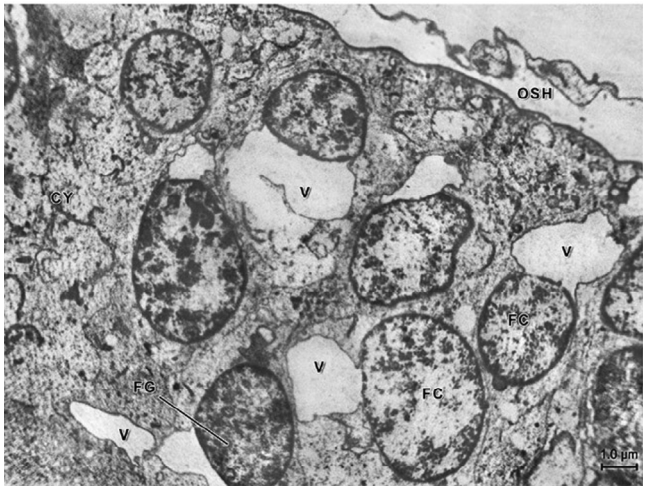

Figure 22 Electron micrograph of the region of oocyte differentiation of adult female *C. maculatus* resulting from solar energy treated pupa showing the deteriorated follicular cells (FC), detachment of ovarian sheath (OSH), vacuoles (V) scattered in the cytoplasm (CY) and Fine granules (FG) (×6000). Scale bar: 1 µm.

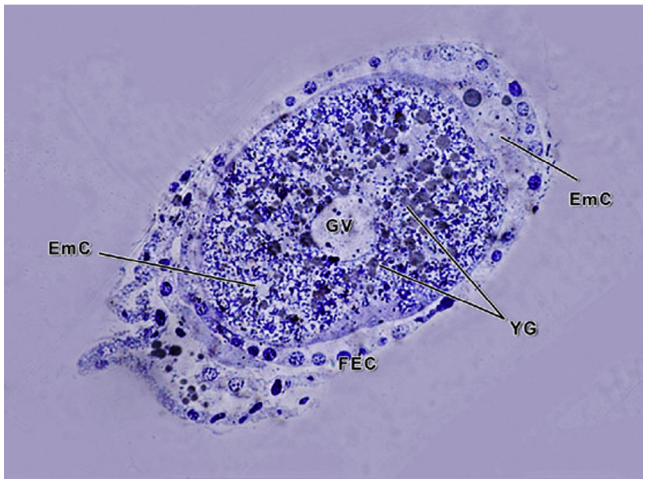

Figure 23 A higher magnification of previtellogenic oocyte of adult female *C. maculatus* resulting from solar energy treated pupa showing degenerated follicular epithelial cells (FEC), Yolk granules (YG), Germinal vesicle (GV) and Empty cytoplasm (EmC) (×400).

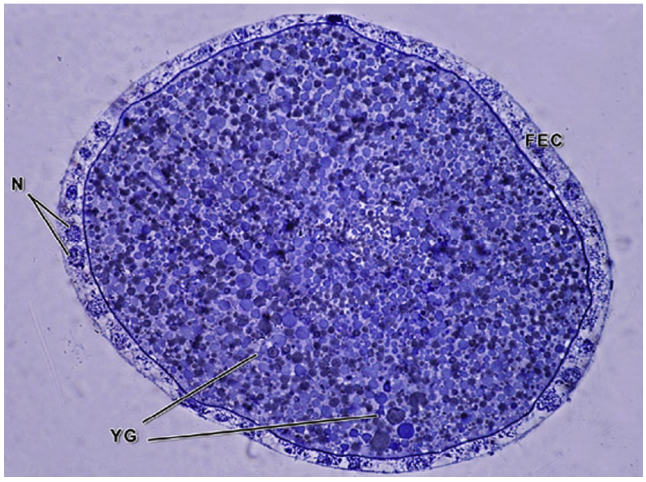

Figure 24 A higher magnification of vitellogenic oocyte of adult female *C. maculatus* resulting from solar energy treated pupa showing degenerated follicular epithelial cells (FEC), deteriorated nucleus (N) and Yolk granules (YG) (×400).

that the size of ovaries is markedly reduced as compared to the normal. These females had enlarged and swollen spermatheca. It has been reported that different abiotic factors such as heat stress, starvation, chemical stress and radiation had physiological and morphological changes on insect's reproductive organs (Gruntenko et al., 2003; Perez-Mendoza et al., 2004; Blanckenhorn and Henseler, 2005; Banu et al., 2006; Xu et al., 2009; Weng et al., 2011).

The female *C. maculatus* has a telotrophic–meroistic ovarioles in which the growing oocytes are connected by nutritive cords with an apical trophic tissue. Telotrophic–meroistic ovarioles have been found in all the previously studied polyphage Coleoptera including *Bruchus obtectus* (Buning, 1979) and *Rhynchophorus ferrugineus* (Kamel et al., 2005).

The germinal trophic tissue of the ovariole of *C. maculatus* can be distinguished into three zones according to the degree of the nurse cell membrane reduction (Buning, 1979). Trophocytes in the apical zone of the germarium have intact cell membrane and are corresponding to the primary staged nurse cells described by Buning (1979) and Kamel et al. (2005). Trophocytes in this zone appear to be actively involved in the production of materials where the granular cytoplasm of these cells contains ribosomes, relatively well developed Golgi elements and rough endoplasmic reticulum. In the germarium middle zone of *C. maculates* ovarioles, nurse cell membrane is partially reduced permitting cytoplasmic continuity between

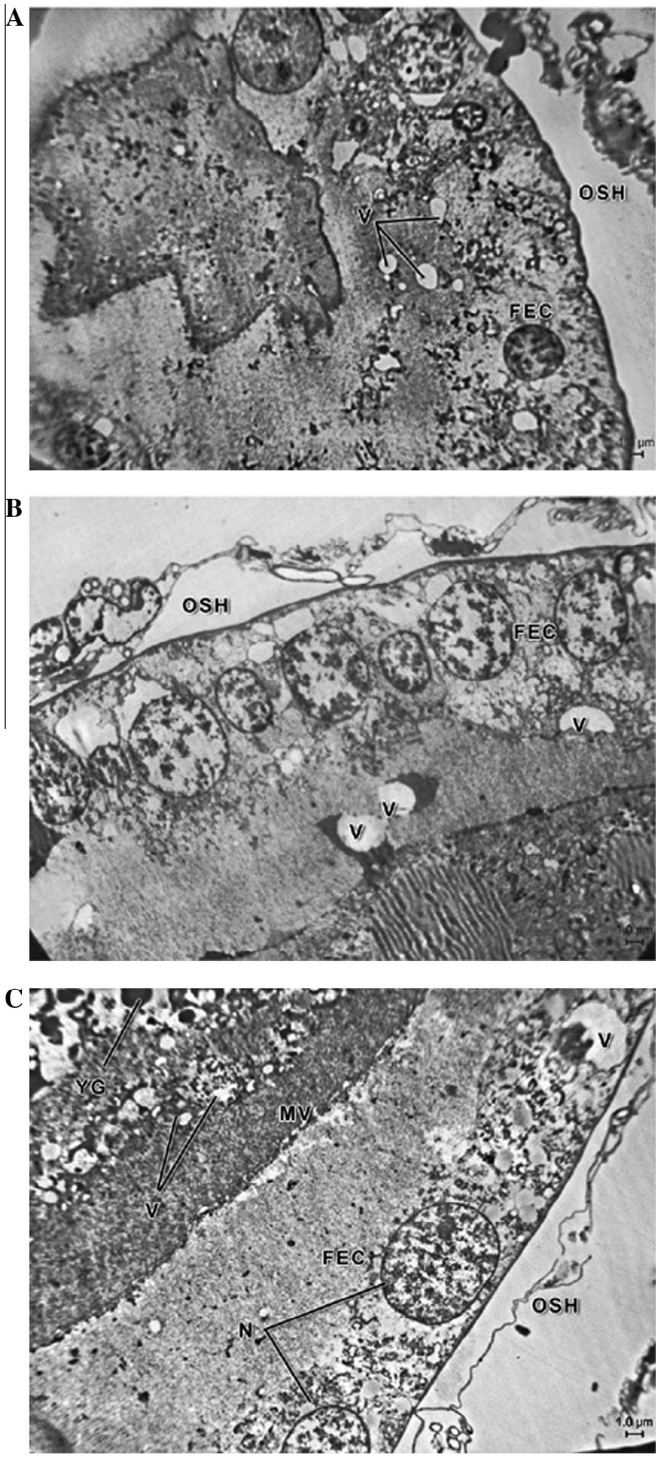

Figure 25 (A–C): Electron micrograph of a vitellogenic oocyte of adult female *C. maculatus* resulting from solar energy treated pupa showing degenerated follicular epithelial cells (FEC), deteriorated nucleus (N), Brush border with microvilli (MV),Vacuoles(V), Yolk granules (YG) and Ovarian sheath (OSH) (A, ×2000, B and C, ×3000). Scale bar: 1 µm.

trophocytes. These are corresponding to the transition staged nurse cells of Buning (1979) and Mohammed (1990).

The nuclei of trophocytes in *C. maculatus* ovarioles contain strands of black masses of chromatin which are particularly evident in the middle zone of the trophic chamber. These masses of chromatin may represent segments of the polytene chromosomes described in the polyploidy nuclei of the nurse cells of polytrophic (King and Burnett, 1959) and telotrophic (Buning, 1979) ovarioles. Ray and Ramamurty (1979) found that trophocytes in the telotrophic ovarioles of *Crynodes peregrinus*, synthesize RNA in their large polyploidy nuclei and supply the products to oocytes via trophic cords. Engelmann

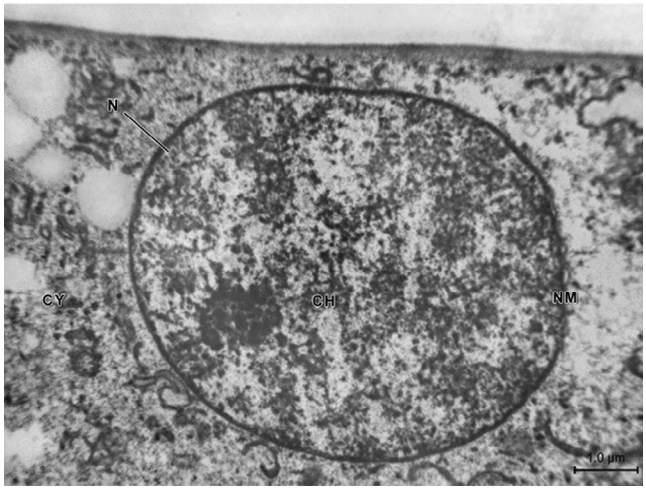

Figure 26 A higher magnification of a follicular epithelial cell (FEC) of adult female *C. maculatus* resulting from solar energy treated pupa showing deterioration of cell organelles; Nucleus. (N), Nuclear membrane (NM), Chromatin (CH) and Cytoplasm (CY) (×10,000). Scale bar: 1 µm.

(1970) reported that nutritive tissues seem to supply mostly RNA to the oocytes of telotrophic and polytrophic ovarioles. There is a high polyploidization of DNA of the nurse cell in the egg chamber of *Forficula auricularia* for supporting the developing oocyte (Jung-Kong and Joon, 1999).

Trophocytes in the basal region of the trophic chamber in *C. maculatus*, form a syncytium where cell boundaries are lacking and nuclei are embedded in a continuous cytoplasmic matrix. The syncytium contains number of vacuoles. These vacuoles are most probably lysosomes which are actively involved in the digestion and lyses of the trophocyte components to be transported via the nutritive cords to the developing oocytes. The complete reduction of membranes between all nurse cells to form syncytium has been observed in few species of polyphage including the bruchid, *B. obtectus* (Buning, 1979) and *R. ferrugineus* (Kamel et al., 2005).

The extensive abundance of lysosomal vacuoles in the germinal syncytium in *C. maculatus* suggests lyses and breakdown of trophocytes contents to be used by developing oocyte (De Wilde, 1964). Engelmann (1970) reported that, during late stages of oocyte growth or vitellogenesis, the nurse cells are broken down and the products are incorporated into oocytes

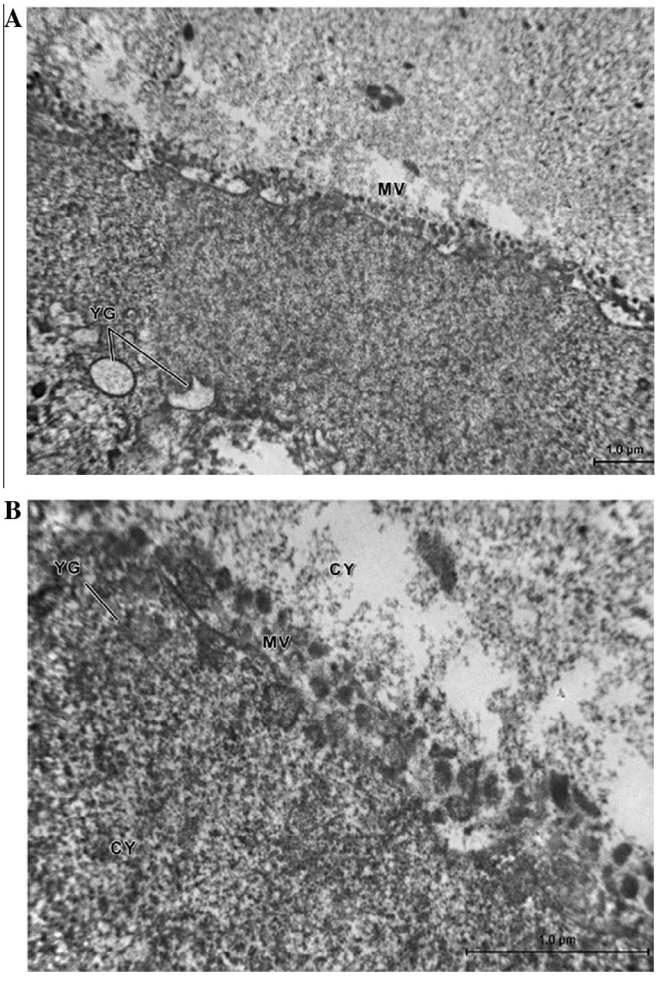

Figure 27 (A and B): A higher magnification of brush border (microvilli) of adult female *C. maculatus* resulting from solar energy treated pupa showing deterioration of microvilli (MV), Yolk granules (YG), and Cytoplasm (CY) (A, ×10000) and (B, ×30,000). Scale bar: 1 µm.

and thus constitute an additional source of oocyte protein, RNA and other materials.

Ovarioles of the female *C. maculatus* resulting from solar energy treated pupa (F.) are poorly developed. The germarium region in these females, consists essentially of the same elements as normal. However, the complete reductions of nurse cell membrane and syncytium formation have been observed in the germarium of (F.) female ovarioles. Trophocytes appear suffering irregularity of outlines. Black masses of chromatin have disappeared from the nuclei in most trophocytes. This disappearance of nucleolar granules may suggest an interference with RNA synthesis in most trophocytes in (F.) females. This suggestion agrees with the observations of (Klug, 1968) on sterile mutant of *Drosophila melanogaster*, SU^2-HW. Cells in all regions of germaria of (F.) females *C. maculatus* have suffered degeneration and lyses where nutritive cells contained several phagocytic vacuoles. The cytoplasm of cells has lost its finely granular texture and appeared faint with no distinct organelles except degenerating mitochondria and lysosomal vacuoles.

As many other short lived insects (Engelmann, 1970); *C. maculatus* emerges with ovarioles containing a full complement of oocytes required for oviposition during the female life span (Mohammed, 1984). Two main stages of oocyte growth are recognized in the ovariole of normal females, the early or the previtellogenic stage and late or vitellogenic stage. Previtellogenic oocytes are generally devoid of yolk globules and are surrounded by a single layer of cuboidal cells. The vitellogenic oocytes in the ovarioles of *C. maculatus* have excessive development of microvilli on the inner surface of follicle cells which interdigitate with those on the surface of developing oocytes also suggesting, the active involvement of follicle cells during vitellogenesis.

The occurrence of pinocytosis at the oocyte surface and the pinching of pinosomes into the oocyte suggest the transfer of material from haemolymph and possibly follicle cells into oocytes. The yolk proteins synthesized in the fat body are released into the haemolymph (Isaac and Bownes, 1982), and those produced in the follicle cells are secreted towards the oocyte membrane (Brennan et al., 1982; Isaac and Bownes, 1982; Butterworth et al., 1992). The crowding of mitochondria in the follicle cells near the interface with the oocyte may reflect the increasing demand for energy during the transport of materials. Pinocytosis in insect oocyte was first shown by Roth and Porter (1962, 1964) in *Aedes aegypti* and afterwards in several other insects (Engelmann, 1970; Bruce and Colleen, 1995; Gilberto and Milvia, 1999; Kamel et al., 2005).

Vitellogenic oocytes in *C. maculatus* are full of yolk globules which start deposition at the cortex of the oocyte and proceed towards the centre. Yolk globules have been observed to result from fusion of pinosomes (Telfer and Smith, 1970; Kamel et al., 2005). The yolk proteins from fat bodies and follicle cells are taken up into oocytes by receptor-mediated endocytosis (Giorgi, 1979; Giorgi and Postlethwait, 1985; DiMario and Mahowald, 1987; Giorgi et al., 1993; Schonbaum et al., 1995, 2000). Peripheral ooplasm of growing eggs is enriched by microvillar projections which presumably act as a guide-system for vitellin envelope deposition in *Sitophilus granarius* (Elda and Attilia, 1995).

In female *C. maculatus* which resulted from solar energy treated pupa the vitellarium region contains one or two previtellogenic oocytes which have an abnormal appearance. Vitellogenic oocytes have been observed in (F.) females, where oocytes are devoid of any yolk globules. The follicular epithelial cells showed signs of deterioration. The cytoplasm of the cells appeared with no distinct organelles. Vacuolation appears in the border of the ooplasm. Mitochondria and microvilli are also distorted.

High temperature resulted in delayed oocyte maturation, early degradation of vitellogenic egg chamber, inhibition of yolk protein gene expression in follicle cells and accumulation of mature oocytes in *Drosophila virilis* (Gruntenko et al., 2003).

Warmer ambient temperature during the adult stage of yellow dung fly *Scathophaga stercoraria* resulted in smaller ovarioles and final egg size. Temperature effects on gonad and gamete maturation are strong when temperatures become stressfully high (Blanckenhorn and Henseler, 2005). The effects of heat shock on ovary development and hsp83 expression in *Tribolium castaneum* showed that the beetles of the heat shock line had a longer pre-oviposition period and smaller ovariole size than those of the control line. Heat shock led to higher hsps83 expression in offspring of the control line than in offspring of heat shock line. (Xu et al., 2009). Heat stress mainly produced high quantities of HSPs in treated females which delayed the ovarian development and resulted in ovarioles smaller in size. Heat stress and HSPs decreased the average number of eggs of female *T. castaneum* after heat treatment (Weng et al., 2011).

Changes in insect development and reproduction may result from changes in the endocrine system. In this situation the endocrine system could have been affected by the high temperature and prevented the maturation of germ cells and perhaps inhibited the deposition of vitellin in the eggs (Neven, 2000).

The utilization of solar heat for bruchid control is based on the knowledge that insects die when exposed to high temperature because of their limited physiological capacity to thermoregulate.

Death at high temperature may result from various factors besides the fact that insects have a limited physiological capacity to regulate their body temperature, proteins may be denatured or the balance of metabolic processes may be disturbed so that toxic products accumulate (Chapman, 1998). High temperature also causes a number of adverse biochemical changes in insects (Beckett et al., 2007).

Solar heating has major advantages of giving complete disinfestation while being comparatively rapid. It is chemical free, and insects are not likely to develop resistance to it.

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