ULTRASTRUCTURE AND HISTOPATHOLOGICAL CHANGES IN TESTES AND ACCESSORY GLANDS IN CALLOSOBRUCHUS MACULATUS (F) (COLEOPTERA, BRUCHIDAE) TRANSPRIRED BY SOLAR RADIATION


Entomology Department, Faculty of Science, Ain Shams University, Abbasiya 11566, Cairo, Egypt.
E. mail: dalia.salem@email.com, Cell: 01221001066

Received: March 26, 2015; Revised: April 15, 2015; Accepted: May 2, 2015

Abstract: The ultrastructure and histopathology in the testes and accessory glands were studied in normal adult males and in those developed from solar energy treated pupae (53 ºC for 15 minutes) of Callosobruchus maculatus. The adult male reproductive system consists of two pairs of testes, two vasa deferentia, vesicular seminales, an ejaculatory duct and four pairs of accessory glands. Testes are formed of numerous testicular follicles containing germ cells at different stages of development. The transformation process of spermatid to sperm involves several morphological reorganization of the cells. Cross sections of elongating sperm tail of mature spermatozoa revealed the existence of two equal mitochondria derivatives containing crystalline material and the axial filament comprises 9+9+2 microtubules. C. maculatus has four pairs of accessory glands (one pair of lateral gland, the mesadenia and three pairs of median glands the ectadenia). The accessory glands consist of glandular cells with a well developed rough endoplasmic reticulum and Golgi apparatus. Variable morphological appearance of the accessory glands secretion was present in the gland lumen. The testicular follicles of treated pupae showed structural abnormalities. The cyst cells have a severe vacuolation, degeneration of germ cells, malformation and tearing of sperm bundles. Accessory glands were the most obvious signs of damage. The three different parts of the accessory glands (muscle layer, gland epithelium and lumen) changed as a result of solar effect. Deterioration of muscular layer, cytoplasmic organelles were very poor and the glandular lumen was filled with vacuoles and malformed granules. The damage was more pronounced in the gonads of (F) progeny of the treated generation.

Key words: Callosobruchus maculatus, Male reproductive organs, Ultrastructure, Solar energy.

INTRODUCTION

Cowpea is highly susceptible infested with different species of beetles of family Bruchidae. This family is wide spread in many regions of Asia, Africa and central and South America except Antarctica [1]. The two main bruchids found in Egypt are the pulse beetle Callosobruchus chinensis and the cowpea beetle Callosobruchus maculatus [1,2]. C. maculatus is a field-to-store pest of several grain legumes that causes a substantial quantitative and qualitative lose by larval feeding which makes the seeds unfit either for planting or human consumption [3,4].

Several methods have been used to protected stored-products from pest infestation including chemical substitutes (plant extracts), exploitation of controlled atmospheres, integration of physical methods such
as heating [5,6], cooling [7] and biological agents such as parasitoids [8]. Another prospective controlling method is utilization of solar energy. Heating produced from solar radiation has been demonstrated to have a potential role in disinfestations of store insect pests [5,6]. Several studies investigated the utilization of solar heating in post – harvest cowpea weevil control [6,9-12]. All studies demonstrated the potential of solar in suppression of *C. maculatus* population in stored grains. Moreover, solar heating has many advantage as it is a safe technology, free for everyone, easy to get and not expensive.

Reproductive system and sperm formation in insects is an extremely complex process. Postmeiotic maturation of sperm in insects involves a highly ordered sequence of developmental changes. Spermiogenesis and organization of the tail complex in insect spermatozoa has been the subject of many investigations. The majority of insect spermatozoa have two central tubules, nine doublets and nine single tubules in their axoneme as found in *S. bullata*, *D. melanogaster* and *D. radicum* [13,14], *Rhynchophorus ferrugineus* [15,16] and *C. maculatus* [17] respectively.

The classic spermatozoa of coleopteran are characterized by enormous accessory bodies and large and almost fully crystallized mitochondrial derivatives in the tail region. The head region is formed by an acrosome (three-layered) and nucleus [16,18-22].

In most insects, the male genital system also consists of accessory glands of mesodermal or ectodermal origin, which open into the deferent or ejaculatory ducts. The accessory gland are of various size, shape, number and embryological origin [23-26]. The secretory cells of these accessory glands are arranged as a mono layered epithelium and contribution to the seminal fluid and activation of the spermatozoa [27,28].

Inspite of varios studied the link between reproduction and environmental signals is poorly understood at the physiological, genetic and molecular levels (27,29,30). Up to the authors’ knowledge, information on the effect of solar radiation on the male reproductive system of *C. maculatus* is not available. The aim of the present study is to examine the ultrastructural changes and histopathological alteration in the testes and accessory glands of male *C. maculatus* induced by the solar energy.

**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 (*Vigna 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. [6] 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 120º). The box was covered externally with 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. Black polystyrene sheet was held on the ground for about 10-15 minutes to collect the sun rays on the experimental spot and the box put on it during the exposure time. The temperature and relative humidity inside and outside the box were recorded during experiment using thermometer and hygrometer.

**Exposure pupal stage of cowpea weevil to solar heat:** Three pairs of newly emerged adults (0 day) from a laboratory colony were introduced into vials contain 15 gm of cowpea seeds. The females were allowed to lay eggs for 24 hours 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 minutes respectively. 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.
Figs.1-7: photographs & sections of normal male *C. maculates*: Fig 1: Photograph of the internal reproductive organs (20X), Fig.2: Sagittal section of the testis (400X), Fig.3: Electron micrograph of interstitial cell (8000X), Fig.4,5: Electron micrograph of spermatids (15000 & 20000X), Fig. 6: Electron micrograph of sperm bundle (6000X), Figs.7a,b: Transverse section of sperm (30000, 100000X). (T=Testis, CEJ = Common ejaculatory duct, MT=Muscular tube, GA=Genital aperture, M=Mesadenia, E= Ectadenia, Pm=Peritoneal membrane, TF=Testicular follicles, SG=Spermatogonia, SC=Spermatocytes, SD= Spermatids, PS= Presperm, SB=sperm bundle, IC=interstitial cell, V=vacuoles, N= Nucleus, CH=Chromatin, CY=Cytoplasm , R=Ribosomes, RER=rough endoplasmic reticulum, AX=Axoneme, SP=sperm, MS=Membranous sheath, MD=Mitochondria derivative, AB=Accessory bodies, ELM= Electron lucid material , PM= Plasma membrane ).
emergence. The malformed adults developed from solar energy treated pupae at 53 °C for 15 minutes were subjected to ultrastructural studies.

Electron microscopy studies: For electron microscopy, the males were dissected in 2.5% gluteraldehyde fixation. The testes and accessory glands were immediately removed and placed in fresh ice-cold 2.5% gluteraldehyde in 0.1M sodium cacodylate and 0.17M sucrose at PH 7.4 for 2hrs. at 4°C. The testes and accessory glands were post-fixed in 1% osmium tetraoxide in 0.1 sodium cacodylate and 0.17M sucrose at 4°C for 90 min. They were then embedded in pure Epon 812 resin, sectioned with RMC ultramicrotome, stained in 30% uranyl acetate in methanol for 30 min. followed by aqueous lead citrate for 7 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

Morphology of male reproductive organs: The internal reproductive organs of male C. maculatus consist of two pairs of translucent testes. Each pair of testes is located on each side of the gut. The testis is a rounded sac and it is connected posteriorly to a short vas deferens. The vas deferentialia of the two testes on each side open into a common vas deferens, which receives at its cephalic end a bilobed accessory gland, the mesadenia. The vas deferens communicates with the swollen bifurcation of the ejaculatory duct of its side. The latter receives three accessory glands, the ectadenia. The lateral swollen ejaculatory ducts join to form a common ejaculatory duct, a long muscular tube which opens to the exterior through the male genital aperture (Fig. 1).

Accessory glands: The male C. maculatus has four pairs of accessory glands; one pair of lateral gland, the mesadenia and three pairs of median glands, the ectadenia. The mesadenia are the largest gland. The ectadenia are tubular glands and it consists of two pairs of anterior or superior glands and a pair of posterior of inferior glands. At emergence, the accessory glands of normal males are full of milky white secretion (Fig. 1).

HISTOLOGY AND ULTRASTRUCTURE OF MALE REPRODUCTIVE SYSTEM:

Testis: The testes are enclosed in a transparent non-pigmented membrane, the peritoneal membrane. The testicular follicles are composed of aggregations of small round or oval sacs (Fig. 2). The testicular follicles are filled up with germinal cells in different stages of development. These include spermatogonia, spermatocytes, spermatids, presperm and spermatozoa. The testes of the normal male are almost occupied by mature sperm and presperm bundles and spermatids in various stages of spermiogenesis. Cysts containing spermatogonia or spermatocytes are relatively few in normal testes (Fig. 2). Electron micrographs show that cysts are formed by the grouping of germ cells of the same stage, in spherical clusters which are embedded and enclosed by interstitial tissues, the cyst cells. The nuclei of the cyst cells have polymorphic shape with irregular outlines. The cytoplasm of the cyst cell contains round mitochondria, membranes of rough endoplasmic reticulum and is rich with free ribosomes and aggregation of vacuoles of various sizes (Fig. 3).

The spermatogonia are spherical cell. They have dark stained, round or oval nuclei (Fig. 2). The spermatocytes are generally larger than spermatogonia but with less intensely stained nuclei (Fig. 2). Young spermatids are relatively small in size with intensively stainable round nuclei which are smaller than those of the spermatocytes (Fig. 2). Spermatids at different stages of spermatogenesis could be distinguished in light microscope (Fig. 2). In the electron micrograph the nucleus migrates to the anterior tip of the cell and the cytoplasm is displaced posteriorly. Small quantities of electron dense material are located in the cytoplasm. The axial filament (axoneme) appears behind the spermatid nucleus (Fig. 4). Another morphologic nucleus transformation changes process was observed in electron microscope preparation where, chromatin condenses forming an elongated dark mass in the nucleus (Fig. 5).

Sperm: Sperm are differentiated from the presperm by the great elongation of the tail. Within one bundle, sperm are aligned roughly parallel to one another and oriented in the same direction (Fig. 2). In electron micrograph, sperm in one bundle appear to be held closely together by an electron opaque material and are surrounded by a membranous sheath (Fig. 6). In cross sections individual sperm enclosed by membranous sheath (Figs. 7a,b).
The nucleus of the sperm is surrounded by a very thin layer of cytoplasm. The head tapers anteriorly where acrosome is present. Two unequal mitochondrial derivatives are located behind the nucleus. They are filled with dark granular substance and have no distinct cristae. The axoneme flanked by two dark crescent-shape accessory bodies, runs along the middle line behind the two mitochondrial derivatives. All components are spaced by an electron lucid material and are enclosed in the plasma membrane of the cell. In the sections of the sperm tail, three distinct elements can be identified: the axoneme, two mitochondrial derivatives and two accessory bodies (Figs. 7a,b).

The axoneme in the sperm of *C. maculatus* is formed of 20 fine microtubules, which are arranged as a wheel like pattern of (9+ 9+ 2). Two central microtubules, encircled by 9 doublets which, in its turn, are surrounded by 9 singlets. The 2 central and the 9 peripheral singlets have circular profile whereas the pairs of the 9 doublets are ellipsoidal. The two central tubules are of equal diameter and they are connected to the (A) subfibre of the doublets by spoke like projections or radical, links. The right subfibre (A) is smaller than the left subfibre (B) of the doublets. The adjacent doublets are linked together by interdoublet links. Each of (A) and (B) subfibre had a peripheral arm which extend outwards forming interbridges between the 9 singlets and join to them via the peripheral arm of the (B) fibre.

Peripheral to and spaced between the doublets are the 9 singlets. The singlets have equal diameter. Arising from the right side of each singlet one arm which is connected to the fore mentioned peripheral arm of the (B) subfibre. On the left side, each singlet has 2 side arms which join the interbridges between the singlets (Fig. 7b).

**The accessory glands:** The accessory glands of *C. maculatus* consist of two symmetrical groups, the mesadenia and ectadenia (Fig. 1).

**The lateral gland (Mesadenia):** Light microscopic study revealed that the gland is an elongated bilobed tube. A basement membrane surrounds the gland and within this, is situated one layer of columnar epithelial cells. The cytoplasm is granular in nature with large round or elongated nuclei. The lumen contains a homogeneous secretion (Fig. 8).

Electron micrograph revealed that the lateral gland is composed from outside to inside of the following: A basic longitudinal muscular layer that is formed of groups of fibrils. The muscles surrounding the gland are enveloped by a basement membrane. The gland epithelium is formed of one layer of the epithelial cells. The cell has cubic shape with non-distinct boundaries in-between them. The cells have a very round nuclei containing heterochromatin (Fig. 9). The cytoplasm of the epithelial cells is rich in organelles containing an extensive rough endoplasmic reticulum, free ribosomes and several Golgi elements. The endoplasmic reticulum is constituted of fine tubules; it forms ribbons which encircle the secretory granules (Fig. 10). The rough endoplasmic reticulum is highly organized and located around the nucleus in vesiculated form which has uniformly-spaced parallel cisternae (Fig. 10). In the epithelial regions secretory droplets appear throughout the cell. The Golgi vesicles or dictyosomes are several in numbers formed of aggregation of membranous vacuoles and vesicles (Figs. 9,10). The plasma membrane at the apical region appears to have several infoldings along with large microvilli which are abundantly scattered in the luminal surface (Fig. 11). Two distinct types of granules can be distinguished inside the cells, type (1) granule is the most common and constitutes homogeneous dense masses (Fig. 11) and granules of type (2) that are relatively small. The secretory granules are frequently attached at the plasmic membrane at the apex of the cells and in between the bases of microvilli. Granules seem to be secreted by exocytosis which involves the rupturing of plasma membrane enclosing the secretory granules and release of the granules in the gland lumen. The lumen of gland is distended by dense materials which are granules, containing condensed materials of variables sizes. Vacuoles of variable size are also crowded in the lumen (Fig. 12).

**The median gland (Ectadenia):** The median gland consists of a single layer of cuboidal epithelial cells. The cytoplasm is granular and nuclei are round or ellipsoid, located towards the base. The epithelial cells rest on a basement membrane which is surrounded by a circular muscular layer. The lumen contains a homogeneous secretion (Fig. 8).

Ultrastructural study revealed that the median gland is composed from outside to inside of following
Figs. 8-15: Sections of normal male *C. maculates*: Fig. 8: Longitudinal section of the accessory glands (200X), Figs. 9, 10, 11, 12: Electron micrograph of the lateral gland (8000, 3000, 3000 & 2000X), Figs. 13, 14, 15: Electron micrograph of the median gland (4000, 8000 & 30000X); (LG = lateral gland, MG = Median gland, IG = Inferior gland, EC = Epithelial cells, BM = Basement membrane, LML = Longitudinal muscular layer, N = Nucleus, L = Lumen, R = Ribosomes, RER = Rough endoplasmic reticulum, SD = Secretory droplets, GV = Golgi vesicles, MV = Microvilli, SG1 = Secretory granules type 1, SG2 = Secretory granules type 2, SV = Secretory vacuoles, Spt = Septa, CML = Circular muscular layer, CEC = Cuboidal epithelial cells, CY = Cytoplasm, GE = Golgi elements, CH = Chromatin)
structure. A continuous layer of circular muscles, which is formed of a group of muscles fibres and enveloped by a basement membrane (Figs. 13,14). The gland is composed of one row of cuboidal epithelial cells which constitute the secretory region. The epithelial cells rest on a basement membrane. At the apices of the cells, the plasma membrane is drawn into long oblique microvilli which are scattered in the luminal surface. The cell components are the nucleus, the rough endoplasmic reticulum, free ribosomes, mitochondria and a variety of granules in the cytoplasm. The nucleus is basely located and the chromatin is dispersed in the nucleoplasm (Fig. 14). The secretory granules of type (1) aggregate in between the bases of the microvilli to be released in the gland lumen as the plasma membrane is ruptured. They seem to adhere in groups localized near the periphery of the gland lumen and before they are dispersed in the lumen (Fig. 14). Round flakes are present in the lumen (Fig. 15). These flakes are permanent elements of glandular cells, they are little opaque under electron microscope and are considered as secretion type (2) in these median glands. The flakes appear to be poured out in the gland lumen as they pass through the ruptured plasma membrane in between the bases of microvilli. The secretion in the gland lumen is formed of peripheral aggregation of dense granules which may be broken to disperse in the gland lumen among the flaky globules secretion type (2) (Figs. 14,15).

The inferior gland: The inferior median gland consists of a single layer of cuboidal epithelial cells that rest on a basement membrane which is surrounded by a circular muscular layer. The cytoplasm of epithelium is granular and has apical flattened nuclei. The lumen contains a homogeneous secretion (Fig. 8). Ultrastructural study revealed that the inferior gland is composed from outside to the inside of the following structures A continuous circular muscular layer which enveloped by a basement membrane (Fig. 16). The gland is composed of one layer of cuboidal epithelial cells. The epithelial cells rest on a basement membrane. The prominent components of the cell are the nuclei which are flattened and heterochromatic. Mitochondria are round, Golgi elements are little developed. Dark granules aggregated at the bases of the microvilli. The plasma membrane at the luminal surface infolding forms short oblique microvilli. The gland lumen is filled with non-granulated homogeneous secretion (Fig. 16).

EFFECT OF SOLAR ENERGY ON THE MALE REPRODUCTIVE SYSTEM:

The internal reproductive organs of male C. maculatus developed in solar energy treated pupae (F) are markedly reduced in size as compared to that of the normal ones. The accessory glands of the (F) male appear translucent, almost devoid of secretory material which fills the lumen of these glands and make them opaque in normal male (Fig. 17).

Testis: Severe damage of the epithelial septa was observed in the testes. Most of the septa were completely destroyed leaving vacuolated areas in the organ. The testes of the (F) males were almost occupied by spermatogonia, spermatocytes, spermatids, presperm and mature sperm bundles. Most of gonial cells showed morphological changes than normal. Some groups of gonial cells were lyzed and replaced by empty areas of tissue (Fig. 18). The cyst interstitial cells were degenerated leaving vacuolated areas in the testes and polymorphic nuclei were deeply invaginated in the nuclear membrane (Fig. 19).

Sperm: Sperms of the (F) male exhibit a variety of abnormalities. Deteriorated sperm bundles were observed within the vacuolated cyst cells (Fig. 20). Sperm bundles appear to have no regular outlines as in normal. The sperm bundles irregularity distributed in lyzed cyst cell in an unusual manner. Sometimes sperms could be observed not enclosed within a relatively thin plasma membrane. The abnormal multiple-unit sperm are generally observed, a sperm with axoneme and nucleus contain pairs of malformed accessory bodies and a pair of distorted mitochondria derivatives (Figs. 21a,b,c).

Accessory glands: The lateral gland (Mesadenia): The lateral gland of (F) male consists essentially of the same elements as normal (Fig. 22). Electron micrograph revealed lysis of the epithelial cells layer. The chromatin of nucleus is very poor. Irregular invaginations in the nuclear membrane are noticeable. The cytoplasmic organelles are very poor occupied by large area of vacuolization. The rough endoplasmic reticulums which surround the nucleus in normal male are not noticeable. The plasma membrane at the luminal surface is free of microvilli. Secretary granules are not detected in the cytoplasm of the cells. The glandular lumen is condensed with secretory vacuoles and few dark secretory granules comparable to those in normal (Figs. 23a,b,c,d).
The median gland (Ectadenia): The median gland of (F) male consists essentially of the same elements as normal (Fig. 22). Ultrastructural study revealed that the circular muscular layer is not noticeable. The cytoplasm is completely devoid of any organelles and occupied by vacuoles. The plasma membrane at the luminal surface is free of microvilli. Secretory granules are not detected in the cytoplasm of the cells. The glandular lumen is condensed with deformed flakes of variable sizes and few dark secretory granules aggregate in the vacuolated sac or scattered freely in the lumen (Fig. 24a,b).

The inferior gland: The inferior gland of (F) male consists essentially of the same elements as normal (Fig. 22). Electron micrograph revealed that deterioration of microfibriles of circular muscular layer. The cytoplasmic organelles are very poor, except of few mitochondria and much reduced rough endoplasmic reticulum. The cytoplasm is filled with large vacuoles. The chromatin of nucleus is very poor. The plasma membrane at the luminal surface has few and very little microvilli. The glandular lumen is filled with vacuoles of variable sizes which spread along the non-granulated homogeneous secretions as compared to that of normal ones (Fig. 25).

DISCUSSION

The current study of the internal reproductive organs in male C. maculatus resulted from solar energy treated pupae showed that the sizes of testes and accessory glands are markedly reduced as compared to the normal. 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 [31-36]. These morphological changes include reduction or enlargement of different parts of both male and female reproductive organs. In yellow dung fly, Scathophaga stercoraria testis length decreased with increasing developmental temperatures. In contrast, sperm length increased or showed a negative quadratic relationship with increasing temperatures [37].

The gonads and accessory glands resulted from solar energy treated pupa consist basically of the same elements as normal. However, these elements may, exhibit some histological and ultrastructural changes. The testicular follicles in C. maculatus are composed of aggregations of oval or round sacs which open directly in the vas deference. The germarium in the testis of C. maculatus is followed by a series of sperm cysts in various stages of development. Testes of normal males are mostly occupied by mature sperm, presperm and to lesser extent by spermatids but spermatogonia and spermatocytes were relatively scarce. This suggests that complete spermatogenesis in C. maculatus takes place during larval and pupal stages as in many other insect species having a short life span [38-40]. In contrast to normal, the testis of (F) male C. maculatus is almost filled with different stages of spermatogenesis. Some of these gonial cells degenerate and a few of them continue developing to reach the stage of spermatid, or to the mature sperm. Retardation, degeneration of spermatogenesis occurs in sterile males which were induced by irradiation [41-43] or hybrid sterile males [44,45].

The transformation process of spermatids to sperm involves several morphological reorganization of the cells. The changes are occurred by eccentric displacement of nucleus and the caudal displacement of the cytoplasm. These structural changes of nuclear development have been described for other beetles [21,46]. In sperm the nucleus elongates, the tail develops, the two mitochondrial derivatives, which extend in front of the axial filament (axoneme). This is flanked by two crescent shaped dark accessory bodies. Baccetti et al. [18] found that accessory bodies in Tenebrio sperm, exhibit an intense UT pase and AT pase activities which suggest their involvement in supplying the energy required for the maintenance of the large waves of sperm movement. The mitochondrial derivatives are considered as the "power plant", supply the flagellum with energy. In spermatids and spermatozoa of Tenebrio molitor, [18] found that cristae of the mitochondrial derivatives are rich in cytochrome-C-oxidase. However, the authors found no ATPase activity associated with the mitochondrial derivatives. The presence of accessory bodies and mitochondria derivatives comparable to those found in the sperm of C. maculatus have been reported in several other insects such as the Lepidopteran Plodia interpunctela [41] and the Coleopteran Tenebrio molitor [18], Anthonomus grandis [47] and S. zeamais and S. oryzae [22].
Fig. 16: Electron micrograph of the inferior gland of normal male *C. maculatus* (4000X). **Figs. 17-21**: Sections of male *C. maculatus* resulting from solar energy treated pupa. **Fig. 17**: Photograph of the internal reproductive organs (20X). **Fig. 18**: Sagittal section of the testis (400X). **Fig. 19**: Electron micrograph of testis (400X), showing malformed interstitial cells. **Fig. 20**: Electron micrograph of sperm bundle (4000X). **Fig. 21**: (a,b,c) Transverse sections of sperm bundle (15000, 30000, & 40000X). HS=Homogeneous secretion, MV=Microvilli, T= Testis, CEG= Common ejaculatory duct, MT=Muscular tube, GA=Genital aperture, M= Mesadenia, E=Ectadenia, Pm=Peritoneal membrane, TF=Testicular follicles, SG=Spermatogonia, SC=Spermatocytes, SD=Spermatids, Sp=malformed Sperm, IC=malformed interstitial cells, N=deteriorated nucleus, CML=Circular muscular layer, CH= Chromatin, arrows=deteriorated sperm bundle, MD=Mitochondria derivative, AB=Accessory bodies, AX=Axoneme, ELM=Electron lucid material, PM=Plasma membrane.
The microtubules of the axoneme are considered as a framework, or cytoskeleton which processes the shaping of the cell and redistributes its contents [48]. The axoneme which is believed to be the motile element of the sperm arises behind the nucleus in the sperm head and extends longitudinally flanked by the accessory bodies along the sperm tail. The axoneme, two accessory bodies and two mitochondria derivatives are the distinct elements in the sperm tail in *C. maculatus*. This pattern similar to most Coleoptera [22]. However, the flagellum of *Coelomeralanio and Cerotomaarucauta* (Coleoptera: Chrysomelidae) have a single accessory body [21,49]. The axoneme of sperm in both normal and (F) male has (9)+9+2 configuration; with two central tubules encircled by 9 doublets which in their turn are encircled by 9 singlets. This concentric wheel-like pattern of axoneme microtubules is comparable to that described in most pterygota [50,51], in the Lepidopteran, *Heliothis virescens* and *Heliothis subflexa* [45], in *Anticarsi agemmatalis* (Lepidoptera: Noctuidae) [52]. This pattern was also described in *Tenebrio molitor* (Coleoptera: Tenebrionidae) [18]. *Anthonomus grandis* [47], *C. maculates* [7,53], *Trogoderma granarium* [43] and *Rhynchophorus ferrugineus* [6,54], *S. zeamais*, *S. oryzae* [22] and in *Heliothis* (Hymenoptera: Apoidea) [55]. Links connect the components in the centre or inner circle to those in the outer circle. Links are illustrated in electron micrographs of axoneme in *Tenebrio molitor* [18]. In insects, ATPase and UTPase activities have been detected in the coarse fibres between the accessory tubules.

Degenerating and rupturing sperm bundles were observed mostly in light or electron microscope preparations of (F) male. The lack or scarcity of the irregularity of bundles outlines, rupture and degeneration of bundles and interlocking of the plasma membranes of adjacent sperms within the same bundle in (F) male may reflect a probable interference with or reduction of sperm activity. In the sterile insects produced by irradiation treatment [56] or by interspecific crosses [57] and [17] abnormalities such as the presence of number of double, multiple-unit sperm cells rupture of bundles and dispersion of sperm were observed in (F) male.

Extragonal cells of testis of (F) male *C. maculatus* have suffered of damage. The epithelial septa in between follicles showed extensive vacuolation and degeneration. The cyst cells may supply nutrient to the developing sperm and in *Popillia* (Coleoptera), the sperm at one stage have their heads embedded in the cyst-cells, this perhaps facilitating the transfer of nutrient [58] or the interstitial cells in Heteroptera, *Oncopletus* [59], are considered as trophocytes which are involved in the transfer of nutrients to the sperm cysts. Trophic tissues degeneration and vacuolation were observed among testicular cysts of sterile insects produced by irradiation [56]. Inhibition of spermiogenesis of eupyrene spermatozoa also occurred by elevated, sublethal temperatures during pupal adult development of the bertha armyworm [60].

The male *C. maculatus* has four pairs of accessory glands; mesadenia (one pair of lateral glands) and ectadenia (two pairs of median glands and one pair of inferior gland). In most insects, the male genital system consists of accessory glands of mesodermal or ectodermal origin. Among insects, the accessory glands vary notably in size, shape, number, and embryological origin [23-26,29,61,62]. In the mesadenia and ectadenia, the secretory epithelium forms a single layer in each of the normal and (F) male. In normal male *C. maculatus*, the secretory epithelium of the two types of the accessory gland are provided with all ultrastructural characteristics appropriate to the synthesis and secretion of proteins, as has been demonstrated in other insects [63,64]. Among the functions of the secretions of these glands is the contribution to the seminal fluid and activation of the spermatozoa [27,28,61,65].

The rough endoplasmic reticulum is highly developed and consists of stacks of large flattened cisterne covered with ribosomes and occupying most of the cell in normal male *C. maculatus*, while in the (F) male the rough endoplasmic reticulum is not developed in the mesadenia and inconspicuous in the ectadenia. The rough endoplasmic reticulum is developed in cells actively engaged in protein synthesis [48]. Marchini et al. [66] found a large amount of rough endoplasmic reticulum in the cytoplasm of the male accessory glands of the medfly *Ceratitis capitata*, which is in accordance with the presence of proteins in the secretions. In *Vespula vulgaris* rough endoplasmic reticulum remains present in considerable quantity, indicates a high-secretory activity [67].
Figs. 22-25: Sections of male *C. maculatus* resulting from solar energy treated pupa. **Fig. 22:** Longitudinal section of the accessory glands (400X). **Figs. 23a-d:** Electron micrograph of lateral gland (4000, 10000, 10000 & 4000X). **Fig. 24a,b:** Electron micrograph of median gland (3000, 8000X). **Fig. 25:** Electron micrograph of the inferior gland (8000X).

(LG=lateral gland, MG=Median gland, IG=Inferior gland, HS=Homogeneous secretion, EC=lysis of Epithelial cells, CY=Vacuolated cytoplasm, V= Vacuoles, Bb= Brush border free of microvilli, N=Nucleus, L=Lumen, SG1=secretory granules type1, SV=Secretory vacuole, Spt= Septa, Bb= Brush border free of microvilli, CML=Circular muscular layer, CH=Chromatin, MF=Microfibrilles, PM=Plasma membrane, RER=Rough Endoplasmic Reticulum, m=Mitochondria, MV=Deterioration of microvilli)
The cytoplasm in accessory glands of normal male *C. maculatus* is rich with ribosomes while that of (F) male is poor. Ribosomes are organelles that are composed of ribonucleic acids and proteins and have a role in protein synthesis [48]. In (F) male *C. maculatus*, the observed reduction in the rough endoplasmic reticulum and ribosomes suggests the reduced biosynthetic capacity of proteins.

Golgi elements are numerous and distributed throughout the cytoplasm of the accessory glands in normal male *C. maculatus*, while they are not noticeable in (F) male. Golgi vesicles receive the protein synthesized at the rough endoplasmic reticulum [48]. The abundance of Golgi vesicles in accessory glands of normal male *C. maculatus* suggest a period of high activity in the synthesis of secretory granules. The accessory gland of *Achroia grisella* consisted of glandular cells with a well-developed rough endoplasmic reticulum and Golgi apparatus [68].

Mitochondria are distributed throughout the cytoplasm of the glandular epithelium of normal male *C. maculatus* and are not seen in the accessory glands of the (F) male. The abundance of mitochondria in the epithelium and their accumulation near the luminal surface may reflect the increasing demands for energy in the cells for synthesis of the secretion, transport and release into the gland lumen.

In normal male *C. maculatus*, the mesadenia secrete small and relatively large dark granules. The granules attach themselves to the plasma membrane. The plasma membrane ruptures and the secretory granules are poured into the lumen of the gland, a process which resembles exocytosis [48]. The ectadenia of normal male *C. maculatus* have dark granules and electron opaque flaky secretions. They are aggregate at the bases of the microvilli to be released in the gland lumen by exocytosis [48]. Similarly, in *Acanthoscelides obtectus* [69].

At the apices of the secretory cells of the accessory glands of normal male *C. maculatus*, the plasma membrane is drawn into microvilli which have higher density in the mesadenia than in the ectadenia. The microvilli and the infoldings of the plasma membrane increase the area of secretory surface. De Robertis et al. [48] anticipated movement of microvilli may help mixing of the secretions within the gland lumen. The secretory cells of *Achroia grisella* had poorly developed luminal microvilli [68]. The lumen of the mesadenia in normal male *C. maculatus* has secretory dark granules and vacuoles of different sizes.

In the ectadenia, the secretion in the gland lumen is formed of peripheral aggregates of dense granules which are dispersed in the gland lumen among electron-lucent flaky globules. Histochemical and ultrastructural studies suggested that the materials in the secretions within the epithelial cells and in the lumen are proteins [27,63,70-72]. The secretion in the lumen organized in globules was essentially proteinaceous for most insects [65,73,74].

In the (F) male *C. maculatus* no ultrastructural sign or indication that active production of secretion is going on within the mesadenia. The dark secretory granules are absent in the glandular epithelium. The glandular lumen is occupied by deformed vacuoles. The microvilli at the luminal surface disappeared. In the ectadenia of the (F) male *C. maculatus*, the dark granules are lacking and the glandular lumen contains only malformed flaky secretions and vacuoles. Microvilli of the luminal surface of the mesadenia and ectadenia are completely reduced in the (F) male *C. maculatus*. Hihara [76] postulated that the microvilli in the accessory gland secretion of the sterile male *Drosophila melanogaster* are probably defective. In some insects, the accessory gland secretion exerts physiological and biochemical control of the female reproductive physiology [27,30]. In *Drosophila*, the secretory product of the accessory glands is transferred to the female tract during mating where it forms a plug and modifies the behavior and physiology of the female [77-80]. The effect of temperature on the reproductive system, especially on the male accessory gland in *Drosophila*, including structure of gland of cell size and cell number, that changed when flies were exposed to different temperature [81].

Prospective controlling method is utilization of solar energy. Heating produced from solar radiation has been demonstrated to have a potential role in disinfestations by stored grain pests. Moreover, solar heating has many advantage as it is a safe technology, free for everyone, easy to get and not expensive.
REFERENCES


Khaled et al.