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Ultrastructure of the Midgut of theThird Larval Instar of *Chrysomya megacephala* (Diptera:Calliphoridae) fed on malathion treated diet

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ABSTRACT

Our study established the histopathlogical effect of different dosages malathion on the third larval instar of *Chrysomya megacephala.* The concentration of malathion increased gradually during the larval stages reaching the maximum concentration in the third instar larvae.The midgut of third larval instar was studied using transmission electron microscope. The most significant alterations were increasing damage to the larvae midgut including columnar prominent ,fat vacuoles, atrophied microvilli. These results indicate that malathion retard larval development.

Keywords*:* Malathion, *Chrysomya megacephala*, mid-gut, Ultrastructure.

INTRODUCTION

Chrysomya megacephala is commonly found in cadavers in many parts of the world (Gruner *et al*., 2007; Sukontason *et al*., 2007; Wang *et al*., 2008) and is used in forensic entomology cases, for postmortem interval determination (Goff & Odum, 1987; Goff *et al*.,1988; Goff, 1992). Entomotoxicology is a relatively new branch of forensic entomology. The potential use of necrophagous insects for detecting drugs and other toxins in decomposing carcasses have been widely demonstrated (Nolte *et al*., 1992; Goff and Lord, 2001; Campobasso *et al*., 2001; Introna *et al*., 2001; Kharbouche *et al*., 2008). The analysis of larvae found in cadavers can, therefore, contribute to the qualitative identification of drugs present in the corpse (Nolte *et al*., 1992; Kintz *et al*., 1990a, b; Introna *et al*., 1990). In addition, drugs in putrefied tissues may have an influence on the development of the necrophagous Diptera that can affect the estimation of the PMI (Goff *et al*., 1991, 1992, 1993; Bourel *et al*., 1999; Carvalho *et al*., 2001; O'Brien and Turner, 2004). Drug levels in larvae could also be correlated to drug concentrations in tissues eaten by the insects, providing valuable information to elucidate the cause of death. The larval behavior toward several substances is unsure; consequently, their use for qualitative identification and quantitative analysis of drugs or toxins are strongly limited (Sadler *et al*., 1997a, b; Tracqui *et al*., 2004). Malathion is an organophosphorous insecticide that is employed for both agricultural and medical purposes. It is used throughout the world in a variety of formulations and is widely available. Although it has relatively low toxicity in humans it is metabolised to the more toxic malaoxon in our bodies and if taken to excess can prove fatal. Owing to its ease of availability, malathion is often used as a means of committing suicide particularly among agricultural communities (Thompson *et al*., 1998; Pannell *et al*., 2001). The effect of malathion on the development of *Chrysomya megacephala* is examined by (El-Samad *et al*., 2006; Rumiza *et al*., 2008; Xiaoshan Liu *et al*., 2009), larvae from control group developed more rapidly than

larvae feeding on tissue containing malathion. The objective of this paper is to illustrate the histological effects on the third laval instar of *Chrysomya megacephala* tissus contaning malathion.

MATERIAL AND METHODS

Rearing of flies:

The colony *Chrysomya megacephala* was reared in the research laboratory of the Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt, Similar to Sukontason *et al*. (2004) and Gabre *et al*. (2005). These fly colonies were maintained at ambient temperature (25.5±2.5ºC) and natural light/dark photoperiod in a wooden box in the rearing room. Adults were reared on two kinds of food: 1) a mixture of sugar & powdered milk and 2) fresh pork liver (used as both a food source and ovipostion site). Water supply was a piece of paper as a long thread in a bottle filled with water. Small pieces of fresh pork liver and water supply were changed every 2 days. Subsequently, the ovipostion sites were observed daily for the presence of eggs; if present, the eggs were transferred into a (12 x 15 x 6 cm) transparent plastic box, and 40 g of fresh pork liver was provided as larval food. The lid of each box was rectangular, likes a fine mesh suitable for ventilation and prevention of other small insects entering the box to oviposit in it. The lid was sealed tightly with adhesive rubber cord to prevent the larvae from crawling out. Immediately after the third larval instars observed, larvae were transferred into the rearing cage (40 x 40 x 56 cm). At the postfeeding stage, dry autoclaved sawdust was added as a medium for pupation. *Ch..megacephala* reared on rabbits designated as R_0 for control colony, R_1 for colony fed on rabbits adminesterd 513 mg/kg malathion and R_2 for larvae growing on rabbits treated with 1026 mg/kg of malathion.

Histological studies:

Approximately 10 specimens of the third larval instar of *Chrysomya megacephala* were collected from the rabbit carcasses for each experiment and then placed in 3% glutraldehyde, buffered to pH 7.3 at 4 ºC. After one hour a cut was made with a sharp razor blade to divide the larvae into many portions. Then larvae were cut into small pieces, about 1 mm^2 each .The samples were placed in fresh 5% cold glutraldehyde and fixation was continued for 24 hours. Samples were then washed in two changes of cold phosphate buffer, pH 7.3, for 1 hour. The specimens were then post-fixed for 1-2 hours in buffered 1% osmium tetraoxide. They were washed twice for 15 minutes, in phosphate buffer, pH 7.3, and then dehydrated in ascending grades of ethanol to propylene oxide (30%,40%, 50%, 60%, 70%, 80%, 90%, 95% and absolute), infiltration with acetone for 30 minutes and embedded in Araldite each (Khattab *et al*., 2004). Semithin sections of 0.7m thickness were cut with glass knives on the 6000 MT RMC ultratome. Stained with 0.25% toludine blue (Davis, 1971).and examined by light microscopy. Thin sections (600-700 A) were then cut and collected on copper grids. These sections were stained with uranyl acetate and lead citrate and examined photographed in a JEOL 1200 EXIL transmission electron microscope at the Electron Microscope unit, Faculty of Science, Ain Shams University.

RESULTS

For larvae reared on control colony:

The alimentary canal consists of foregut, midgut and hindgut. The larval mid-gut of *Chrysomya megacepha* is the longest portion of the alimentary canal lying

convoluted and twisted within the larval body cavity (Worachote *et al*. 2007).

The epithelial cells of the mid gut include two types of cells, more electron dense and intestinal cells less (Fig. 1& 2). The nucleus is slightly regular, and has prominent nucleoli, with clumped eu and heterochromatin (Fig. 2). The most abundant organells are mitochondria (elongated& rounded) and small dense lysosomes that scattered in between mitochondria (Fig. 4). Long attached microvilli (Fig. 4). The cytoplasm lacked granules (fats & glycogen). The rest of cytoplasm is rarified (not compact) and containing small elements of rough & smooth endoplasmic reticulum (Fig. 1).

For larvae reared on R_1 colony:

Effect of malathiont on the third instar larvae increased with an increase of concentration. Regression analysis showed a correlation between concentration in larvae and administered dosage of malathion ($r = 0.9974$, $p = 0.046$).

The most characteristic changes in this group are: Dense compressed cells with irregular, shrinked nucleus that has fragmented nucleoli and no clumped chromatin (Figs.6 a&b). Engulfed cytoplasmic granules in prominent mitochondria located at the periphery and absence of lysosomes (Fig. 7). There are a number of fat vacuoles at the periphery in between mitochondria (Fig. 8).

For larvae reared on R_2 colony:

Most cells are lacked so, it is hardly to distinguish prominent nuclei in the most midgut intestinal cells.

The most histological alterations of this group are: No dark or light, all cells are damaged, containing prominent fat vacuoles and coagulated cytoplasmic granules & organelles (Figs.1a&b). The cells possessing short, shrinkage or atrophied microvilli (Fig. 4).

Data revealed that different toxic effects of malathion varied according to the the insecticide concentration.

DISCUSSION

Third larval instar midgut of *Ch. megacephala* is functionally the most important part of the digestive system, responsible for digestion and absorption of nutrients as in other insect larvae (Dow 1986). The midgut of *Ch. megacephala* is similar to those of other Diptera. Romoser (1996), reported that the presence of microvilli provide an enormous surface area for absorbing materials from the lumen. The thickness of the basement membrane in *Ch. megacephala* is due to the good nutrition during the larval stage (Clements, 1992) which facilitates the transport of products between the intestine and the haemolymph (Reinhardt and Hecker, 1973; Houk *et al*., 1980). The cytoplasm of mid-gut cells of *Ch.megacephala* possesses numerous vesicles of rough endoplasmic reticulum. Authors interpret this phenomenon as a transition of the cell synthetic apparatus into a more active state (Billingsley *et al*., 1983; Lehane 1976a; Staubli *et al*., 1966 and Filimonova, 1989). More mitochondria are located at the periphery of the cell more than basal part which indicated that the transport role by the apical part more functionally than the basal part (Hecker 1977and Houk, 1977). Observation of numerous secretory granules with different shapes and sizes, most of which located at the periphery and some of them project in the gut lumen. According to Chun-Nu *et al*. (2000), the release of digestive enzymes from midgut cells of oriental fruit fly is merocrine due to high concentration of secretory granules in the apical part of the cell. Also the presence of numerous secretory granules and numerous vesicles of rough endoplasmic reticulum in *Ch*,

megacephala near the periphery of the cell may account for the production of peritrophic membrane as reported by Filimonova (2005).

Malathion concentration increased gradually until the third instar larval stage after which it decreased. This phenomenon can be explained by the fact that larval stages with increased malathion concentrations had a period of rapid feeding and thus excluded the postfeeding prepupal stage (Hedouin *et al*., 1999).

The highest concentration of malathion was found in gastric content (Farago, 1967; Morgade & Barquet, 1982; Jadhav *et al*., 1992; Mahat, N.A. *et al*., 2012). According to insectidal action of malathion, the development of the third larval instar of *Ch. megacephala* was retarded**.** (Lamia M. El-Samad *et al*., 2006, Rumiza Abd. Rashid[1] *et al*., 2008, Xiaoshan Liu *et al*., 2009).

The most characteristic histopathological effects of malathion on the midgut of *Ch. megacephala* were in concurrence with those findings previously reported, (Hassan F. Dahi[1] *et al*., 2011) observed that *Spodoptera littoralis* was affected by the action of pyridalyl, the epithelial cells were completely ruptured after treatment and separated from the basement membrane. The peritrophic membrane was not closely lying to the epithelial cells and the space in between the epithelium and peritrophic membrane was filled with few cytoplasmic vesicles. (Habes Da. *et al*., 2006) stated that boric acid toxicity to midgut of *Blattella germanica*, revealed alterations in the epithelial cells and a significant increase in the epithelium thickness. Assar and El-Sobky (2003) observed that the water extract of *Eichhornia crassipes* on larvae of *Culex pipiens*, revealed drastic effect on larval midgut as the brush border and most of the epithelial cells completely degenerated and vacuolated.

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Control group

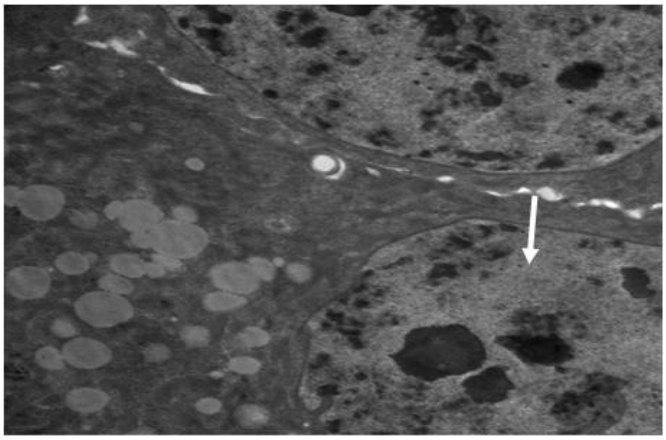

Fig. 1: Electron micrograph of a dark columnar cell (dense compressed cells) in middle mid-gut showing nucleus (arrow). Magnification X 2,000.

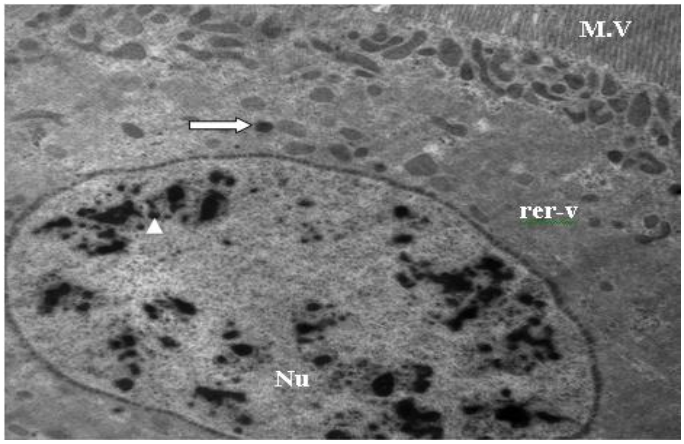

Fig. 2: Electron micrograph of a light columnar cell in middle mid-gut (less electron dense cytoplasm) showing nucleus (Nu) surrounded by numerous vesicles of rough endoplasmic reticulum (rer-v) and chromatin patches of varying densities (head arrow) lysosomes (arrow). Magnification X 1,500.

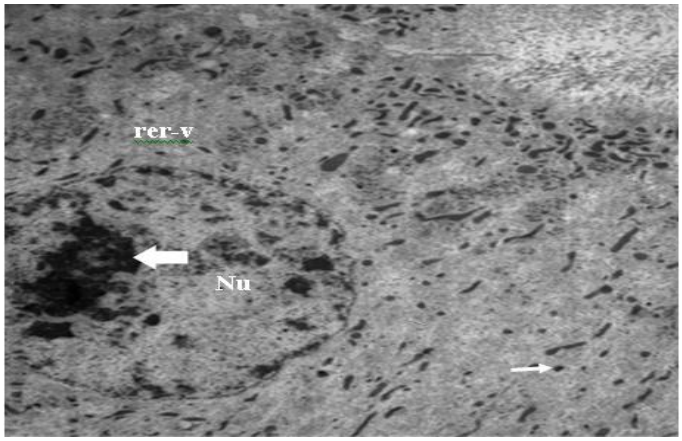

Fig. 3: Electron micrograph of a columnar cell in middle mid-gut showing nucleus (Nu) surrounded by numerous vesicles of rough endoplasmic reticulum (rer-v), lysosmes (arrow) and clumped (eu & hetero) chromatin (big arrow). Magnification X 1,000.

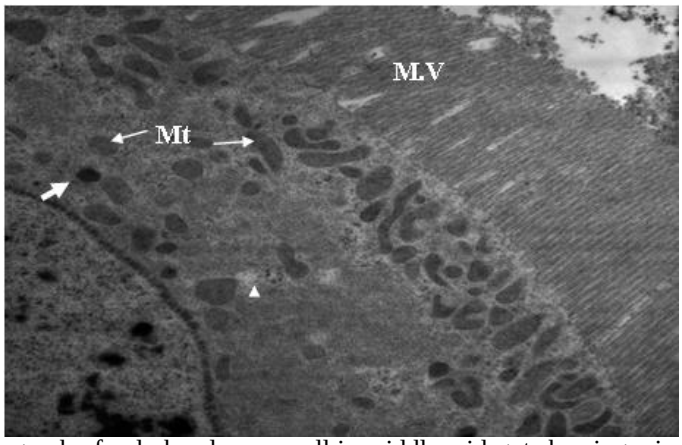

Fig. 4a: Electron micrograph of a dark columnar cell in middle mid-gut showing microvilli (Mv), small dense secretory granules (arrow), small vesicles of rough endoplasmic reticulum (head arrows) and elongated & oval mitochondria (M.t). Magnification X 2,000.

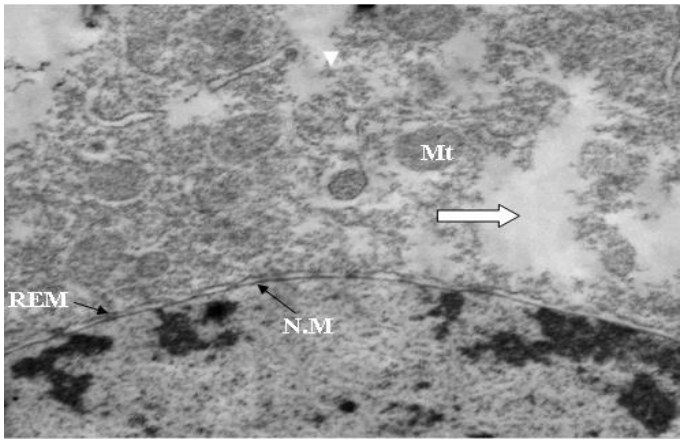

Fig. 4b: Electron micrograph of a columnar cell in middle mid-gut showing rarified mitochondria (M.t), rarified areas of cytoplasm (big white arrow), ribosomes (head arrow), rough endoplasmic membrane (REM) and Nuclear membrane (N.M). Magnification X 5,000.

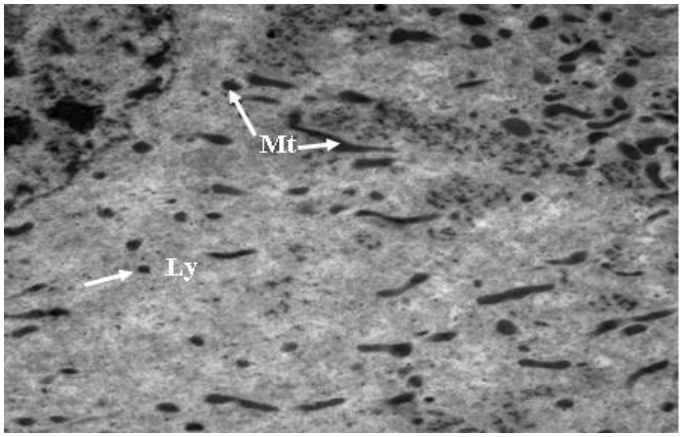

Fig. 5: Electron micrograph of a columnar cell in middle mid-gut showing rarified mitochondria (M.t) and homogenously scattered lysosomes (Ly) between mitochondria. Magnification X 2,000.

First dose group

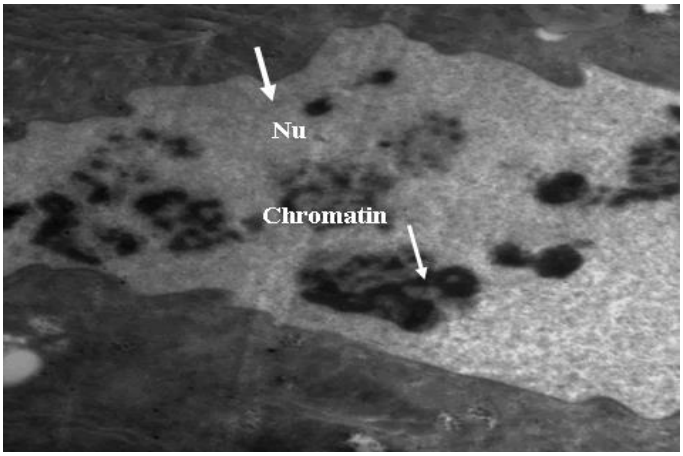

Fig. 6 a: Electron micrograph of a dark columnar cell in middle mid-gut showing irregular nucleus and lacked clumped of chromatin. Magnification X 2,000.

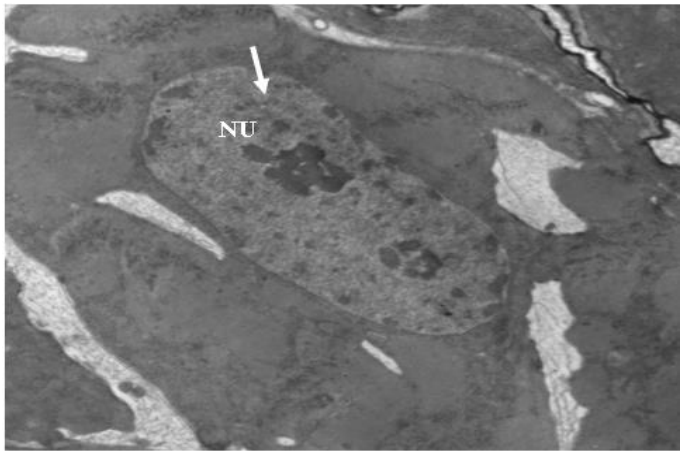

Fig. 6 b: Electron micrograph of a dark columnar cell in middle mid-gut showing irregular nucleus. Magnification X 2,000.

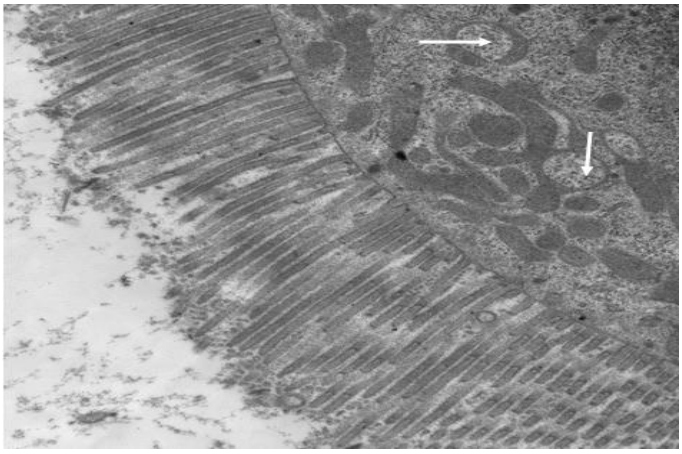

Fig. 7: Electron micrograph of a columnar cell in middle mid-gut showing significant engulfed granules in curved mitochondria. . Magnification X 4,000.

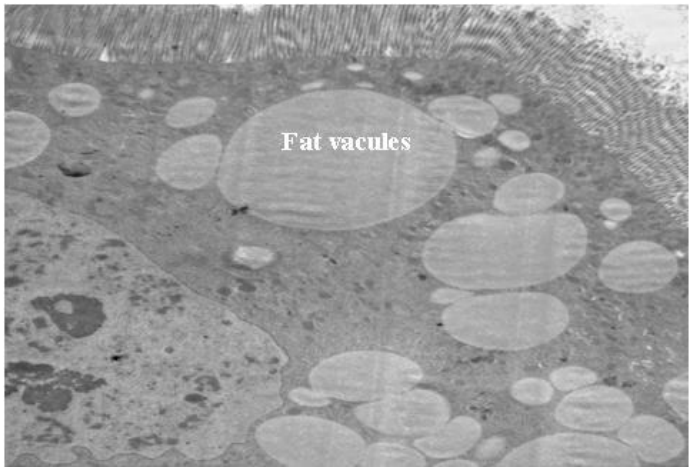

Fig. 8: Electron micrograph of a columnar cell in middle mid-gut showing accumulation of fat vacules at the periphery in between mitochondria and absence of lysosomes. Magnification X 1,200.

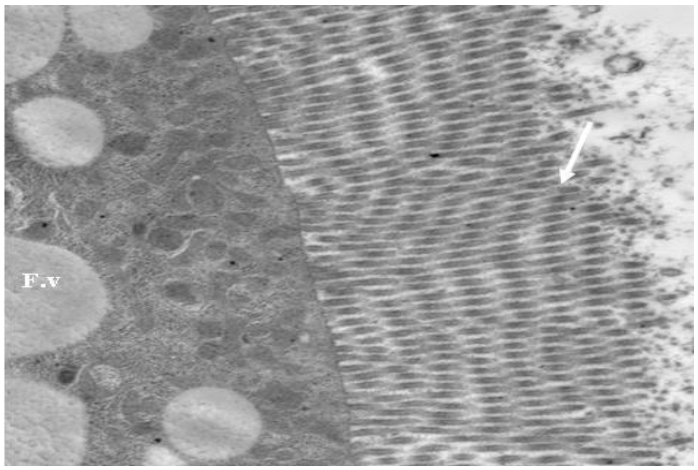

Fig. 9: Electron micrograph of a columnar cell in middle mid-gut showing no change in M.V (arrow) & fat vacuoles (F.v) in between mitochondria. Magnification X 4,000.

Second dose group

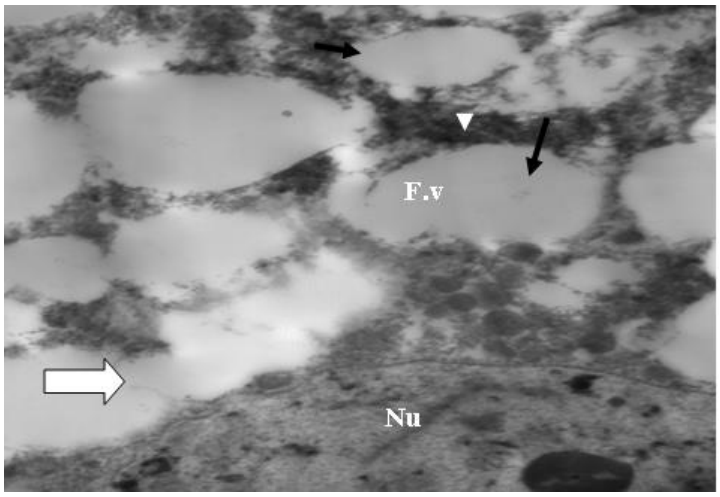

Fig. 10.a: Electron micrograph of a columnar cell in middle mid-gut showing nucleus (Nu) abundant of fat vacuoles (black arrows) and rarified areas of cytoplasm (white arrows) & coagulated cytoplasmic granules & organells (head arrow). Magnification X 2,000.

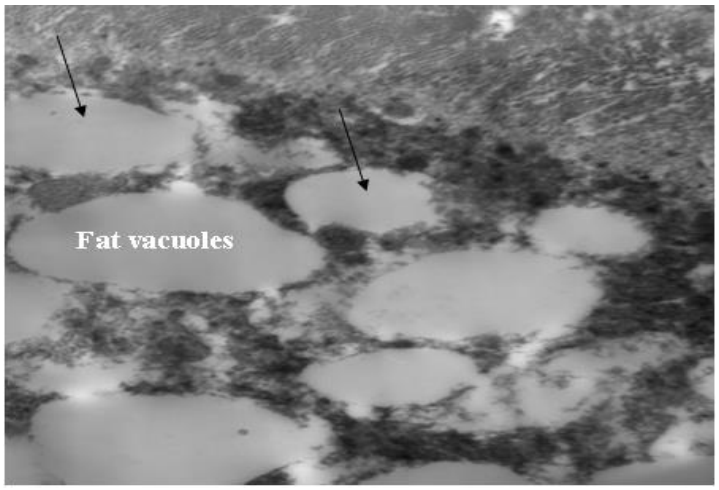

Fig. 10-b: Electron micrograph of a columnar cell in middle mid-gut showing abundant of fat vacuoles (black arrows). Magnification X 2,000.

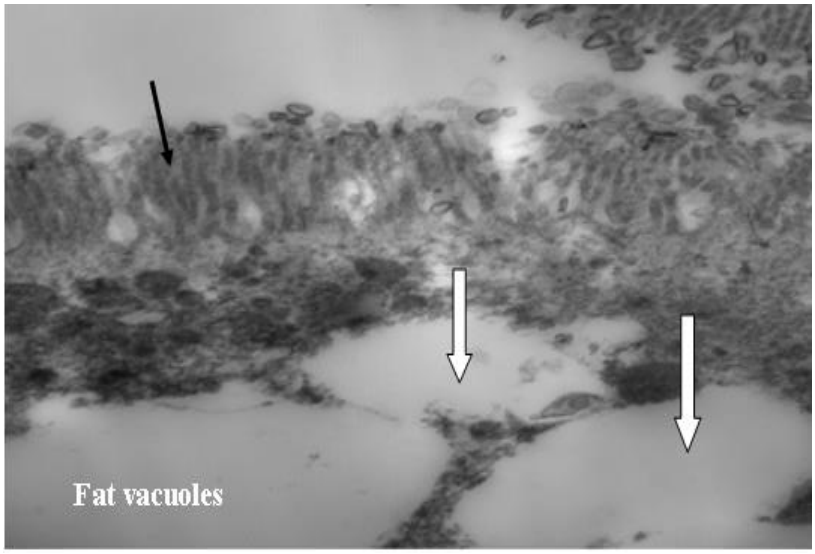

Fig. 11: Electron micrograph of a columnar cell in middle mid-gut showing abundant of fat vacuoles (white arrows) microvilli shrinkage (black arrow). Magnification X 4,000.

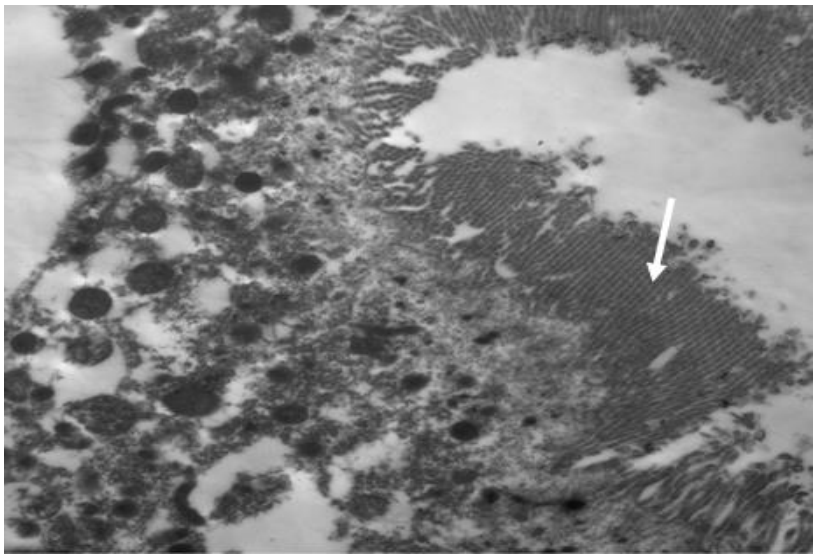

Fig. 12: Electron micrograph of a columnar cell in middle mid-gut showing coagulated and shrinkage microvilli. Magnification X 2,000.

ARABIC SUMMARY

التركيب فائق الدقة للمعى المتوسط للطور اليرقى الثالث لحشرة كريسوميا ميجاسفالا (ثنائية الاجنحة :كاليفوريدى) التى تغذت على الملاثيون

رضا فضيل على بكر[1&4] ، روحية حسن رمضان[2] ، سناء محمد عبدالقادر الصاوى[3] وسماح محمد احمد .

1- قسم عام الحشرات - كلية العلوم – جامعة عين شمس
2- قسم علم الحشرات - كلية العلوم – جامعة عين بنها
3- المعمل المركزى للمبيدات –مركز البحوث الزراعية - الدقى
4- قسم الاحياء - كلية العلوم – جامعة الملك خالد – ابها – المملكة العربية السعودية

اوضحت هذه الدراسة التأثيرات المرضية للانسجة الناتجة عن جرعات مختلفة من المالاثيون على الطور اليرقى الثالث لذبابة المراحيض الشرقية , واسفرت النتائج غلى ان التركيزات تزداد تدريجيا خلال الاطوار اليرقية حتى تصل الى الحد الاقصى فى الطور اليرقى الثالث ,

وقد تم دراسة المعى المتوسط للطور اليرقى الثالث بواسطة الميكروسكوب الالكترونى وكانت التعيرات الاكثر وضوحا على الخلايا الطلائية فى المعى المتوسط لليرقات ووجود الفجوات وضمور الزغبيات وقصرها, واشارت النتائج على ان المالاثيون سبب تأخر نمو اليرقات