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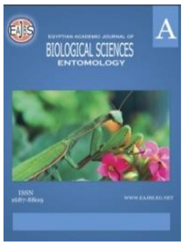

Biological and Histological Alterations in The Larvae of *Culex pipiens* L. (Diptera: Culicidae) Induced by Imidcaloproid and Tannic Acid

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ABSTRACT

The mosquito family Culicidae is the most common disease vector in the Diptera order. Almost all of them are bloodsuckers that cause a lot of major diseases, such as malaria, yellow fever and elephantiasis. The development of resistance in mosquitos to pyrethroid, organophosphate, and carbamate created the need for alternative insecticides. Therefore, 3rd instar *Culex pipiens* larvae were treated with the median lethal concentrations (LC_{50}) of imidacloprid (IMI) and tannic acid. Biological tests were performed by following up the treated insects from the 3rd larval instar till the adult stages. The biological test showed that tannic acid was more effective than imidacloprid by increasing the larval period and decreasing both pupation percentage and pupal duration. The histopathological effect of the tested compound on the larvae's midgut epithelium was studied using light and transmission electron microscopy. A histological study showed that the treated larvae had cytopathological alterations of the midgut epithelium, muscular layers, epithelial cells and internal organelles. Both IMI and tannic acid showed nearly the same effect on the larvae histology. Based on these results, tested compounds could be used in the Integrated Pest Management (IPM) programs for greater effectiveness.

INTRODUCTION

Mosquitoes are one of the most effective insect vectors, transmitting a variety of diseases that are both common and deadly. *Culex* mosquitoes (Diptera: Culicidae) are the world's most widely distributed mosquitoes. The *Culex pipiens* complex has spread through all continents, from its African origins to tropical and temperate climate zones. Lymphatic filariasis, Rift Valley Fever, West Nile encephalitis and St. Louis encephalitis are only a few of the diseases they spread (Saba *et al.*, 2018). Lymphatic filariasis threatens approximately millions of people in many countries. *Cx. pipiens* is the most widespread mosquito species in Egypt, and it creates a health risk to humans in both urban and rural areas (Zahran and Abdelgaleil, 2011).

Mosquito control has mostly relied on chemical pesticides, which resulted in the emergence of resistant mosquito strains. The mosquito's resistance to a variety of chemicals, including organochlorines, organophosphates, carbamates, and pyrethroids, necessitated the use of alternative pesticides such as neonicotinoids and tannic acid (Riaz *et al.*, 2013). Neonicotinoids, the most effective new class of synthetic insecticides

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produced in the last few decades, are used to control sucking insects on both plants and companion animals (Tomizawa and Casida, 2003).

Alternative insecticides were used, like neonicotinoids which are a synthetic analogue of nicotine. Imidacloprid (IMI) is an insecticide that prevents stimuli from being transmitted through the nervous system of insects. It is neurotoxic as it binds to the nicotinic acetylcholine receptor in the same way as nicotine (Azevedo-Pereira *et al.*, 2011). IMI was established to act as an agonist for post-synaptic nicotinic acetylcholine receptors, inducing overstimulation and thereby disrupting neuronal functions, which could result in overall impairment and even death (Matsuda *et al.*, 2001). In insects, imidacloprid acts on postsynaptic nicotinic acetylcholine receptors, which are only found in the CNS. The imidacloprid binds to the nicotinic receptor, causing spontaneous discharge leading to the neuron's inability to propagate more impulses. This binding to the receptor is irreversible manner. Imidacloprid has a large safety margin in mammals because it has a lower affinity for vertebrate nicotinic receptors than it does for insect nicotinic receptors (Dalefield, 2017).

In addition to neonicotinoids, tannic acid (tannins) is also used in controlling mosquito larvae. Tannins, also known as phenolics, are a class of secondary plant compounds that includes some of the most abundant and biologically active chemicals found in plants associated with hydro systems. Tannins have been shown to be poisonous to certain species. They are also poisonous to a lot of caterpillars. However, Only a few Culicine taxa associated with unique biotopes have been investigated for their effect on aquatic dipteran larvae (Rey *et al.*, 2000). Condensed tannins vary in quantity and type among plant species and are related to herbivorous insect defense (Forkner *et al.*, 2004). However, condensed tannins inhibit gastrointestinal nematodes in vertebrate hosts that consume them (Iqbal *et al.*, 2007). Broadleaf plants have a high percentage of tannins, but it is rare in grasses (Waghorn, 2008). Previous research on the mode of action of tannins in insects found that tannins formed complexes in the gut with digestive enzymes, lowering digestion efficiency and slowing insect growth (Chen *et al.*, 2018).

To evaluate the effect of the two insecticides on the tissues and cell organelles, biological evaluation and histological effects of IMI and tannic acid on both anterior and posterior midgut of the 3rd larval instar of *Cx. pipiens* were carried out on two levels: the regular light microscope and the electron microscope.

MATERIALS AND METHODS

Maintenance of *Culex pipiens* Larvae:

Cx. pipiens was cultured in an insectary at Entomology department, Faculty of Science, Ain Shams University. The larvae were held in plastic and enamel trays containing tap water to help the colony expand after hatching. Right up to the fourth instar, first instar larvae were provided by a tiny amount of fish food (Tetra-Min). Pupae were manually collected and placed in a glass beaker filled with distilled water. The beaker was placed in cages to allow adult mosquitos to emerge. Adult mosquitoes were fed cotton soaked in a 10% glucose solution as a glucose meal, and a pigeon was given on a regular basis to act as a blood supply for laying eggs (Mohan *et al*., 2006).

Biological Evaluation:

The biological test was conducted using protocol published in WHO (2005) on 100 third instar larvae placed in plastic cups containing distilled water. Each test was conducted in three replicates. Each container was treated with a median lethal concentration (LC_{50}) of IMI and tannic acid, 0.02 and 7842 ppm, respectively (Farahat *et al*., 2018). The larval duration was calculated for each one as the interval between the treatment day until pupation. The pupal duration was calculated for each one as the interval between the beginning of pupation and the beginning of adult emergence, and then the mean value was taken. The adult emergence percentage was calculated by using the equation of El-Sheikh (2002). The emergence of adult % = A / B × 100. Where A represents the number of emerged adults and B represents the number of tested pupae.

Histological and Ultrastructure Studies:

Studies were demonstrated using JEOL JEM1011, transmission electron microscope (TEM) at the regional center for Mycology and Biotechnology (RCMB), Al-Azhar University. Untreated and treated larvae with median lethal concentration (LC_{50}) of IMI and tannic acid were subjected to histopathological and ultrastructural studies. The larvae were fixed in 3% glutaraldehyde in 0.1M cacodylate buffer (PH 7.2) for an hour followed by an overnight wash in a fresh batch of the same buffer. Specimens were shortly washed in acetate buffer and incubated for an hour at 37˚C in a medium consisting of tablets of P-nitrophenyl phosphate disodium salt, 25mg lead acetate and 25ml acetate buffer. The incubation step was stopped by further washing in cacodylate buffer before post fixing in osmium tetroxide followed by routine dehydration and embedding in araldite. The sections were cut on a Reichert- Jung Ultra-microtome. Semi and ultrathin sections of 20-60mm & 0.5-1.0µ were cut. Semi-thin sections were stained for 1-2 minutes in toluidine blue stain, washed in tape running water, dried and mounted in dibutyl phthalate in xylene, DPX. Ultra-thin sections were stained with lead citrate and uranyl acetate before the examination (Bowen and Ryder, 1976).

Statistical Analysis:

SPSS 19 software was used to analyze the results, which was followed by a one-way analysis of variance (ANOVA) and Tukey's HSD. The findings were expressed as (means ± SE) of untransformed data, and P<0.05 was used to determine if they were significantly different.

RESULTS

Biological Evaluation of IMI and Tannic Acid on 3rd Larval Instar of *Cx. pipiens*:

Table 1 shows that tannic acid was the most effective insecticide by significantly influencing the larval duration, causing it to be delayed from 4.8±1.16 to 7.4±1.72 days. IMI, on the other hand, had no effect on the larval lifespan. Pupation was highest in IMI followed by tannic acid. Also, pupal duration decreased in tannic acid than IMI. There were no changes in the percentage of adult emergence after treatment with tannic acid or IMI compared to the control.

Table 1: Effect of median lethal concentration of IMI and tannic acid on the biological aspects of the 3rd larval instar of *Cx. pipiens.*

Tested insecticides	Mean larval periods (Days±SE)	Mean pupation %±SE	Mean pupal duration (Days±SE)	Adult emergence %
Control	4.8±1.16 a	97.1±2.17 a	2.13±1.45 a	98.95±0.36 a
IMI	4.6±1.21 a	75.48±1.74 b	2.04±1.53 a	97.76±0.41 a
Tannic acid	7.4±1.72 b	17.5±3.37 c	1.02±0.67 b	97.95±0.37 a

Data are presented as mean ± SE *Means bearing different letters within column are significantly different (P<0.05) ANOVA, Tukey’s HSD test.

Histological and Ultrastructure Studies:

Semi-Thin Sections:

Histological and ultrastructural studies were illustrated in untreated larvae of *Cx. pipiens* and in the treated larvae with a median lethal concentration of IMI and tannic acid to show their effect on the anterior and posterior midgut.

Semi-thin sections in the untreated anterior mid-gut showed that the epithelium consists of a single layer of columnar epithelial cells which are separated from the hemocoel by a basement membrane and two layers of visceral muscle fibers (longitudinal and circular). Numerous microvilli evaginate from the luminal surface of the epithelial cells, peritrophic membrane forms the cardia or proventriculus at the beginning of the mid-gut as shown in Figure (1). While the treated with IMI (Fig.1.B). showed severe histological changes as the rupturing of the cuticle and adipose tissue and destruction of the muscle layer. Gastric caeca became hollow, and vacuoles appeared, nuclei of epithelial layers were enlarged and cardia of the peritrophic membrane became destructed. The anterior mid-gut treated with tannic acid (Fig.1.C). showed the same histological changes as the rupturing of the cuticle and adipose tissue and destruction of the muscle layer. The appearance of many vacuoles, distortion and irregularities of the outline of the epithelial cells were detected. Destruction of the cardia of the peritrophic membrane and gastric caeca became destructed and unrecognized.

Semi-thin sections in the untreated posterior mid-gut show the cuticle, the adipose tissue and the five Malpighian tubules. The epithelium consists of a single layer of cuboidal epithelial cells which are separated from the hemocoel by a basement membrane and two layers of visceral muscle fibers (longitudinal and circular). Numerous microvilli evaginate from the luminal surface of the epithelial cells, peritrophic membrane also is detected as shown in Figure 2. The treated with IMI (Fig.2.B). showed several changes in the histology of sections as the rupturing and disappearing of the cuticle and adipose tissue and destruction of the muscle layer. Malpighian tubules became destructed, appearing of many vacuoles and irregularities of the outline of the epithelial cells and nuclei of epithelial layers were enlarged. The peritrophic membrane became unequal in thickness. The posterior mid-gut treated with tannic acid (Fig.2.C). showed the same histological changes as the rupturing of the cuticle and adipose tissue and destruction of the muscle layer. Appearing of many vacuoles and irregularities and destruction of the outline of the epithelial cells were detected. The peritrophic membrane became unequal in thickness. Malpighian tubules became very small in size.

Ultra-Structure Sections:

Control sample: The cuticle of the untreated samples is differentiated into epicuticle and procuticle (inner endocuticle and outer exocuticle). Also, the muscular layer is differentiated into longitudinal and circular muscles without any vacuoles and the adipose tissue is well-formed. The epithelium consists of a single layer of columnar epithelial cells with the nucleus in the base, numerous microvilli evaginate from the luminal surface of the epithelial cells (Fig.3).

Treated sample with tannic acid: Larvae treated with tannic acid suffered severe histological changes in the mid-gut. Cuticle became destructed and unequal in thickness, adipose tissue and muscular layer became destructed and vacuolated. Distortion and irregularities of the outline of the epithelial cells were detected. Enlargement of the nucleus and disappearing of the nucleolus was observed, there was a change in nuclear shape with clumping of chromatin materials (Fig.4).

Treated sample with imidacloprid: The cross section of larvae treated with IMI showed severe damage as lyses of internal organelles, destruction of the cuticle, destroying and vacuolation of adipose and muscular layers and disappearing of the peritrophic membrane and peritrophic space. Many vacuoles and degradation of the epithelial layer and Malpighian tubules have appeared. The epithelial layer became irregular and the epithelial cells had enlarged besides clumping of chromatin materials and the presence of many vacuoles (Fig. 5).

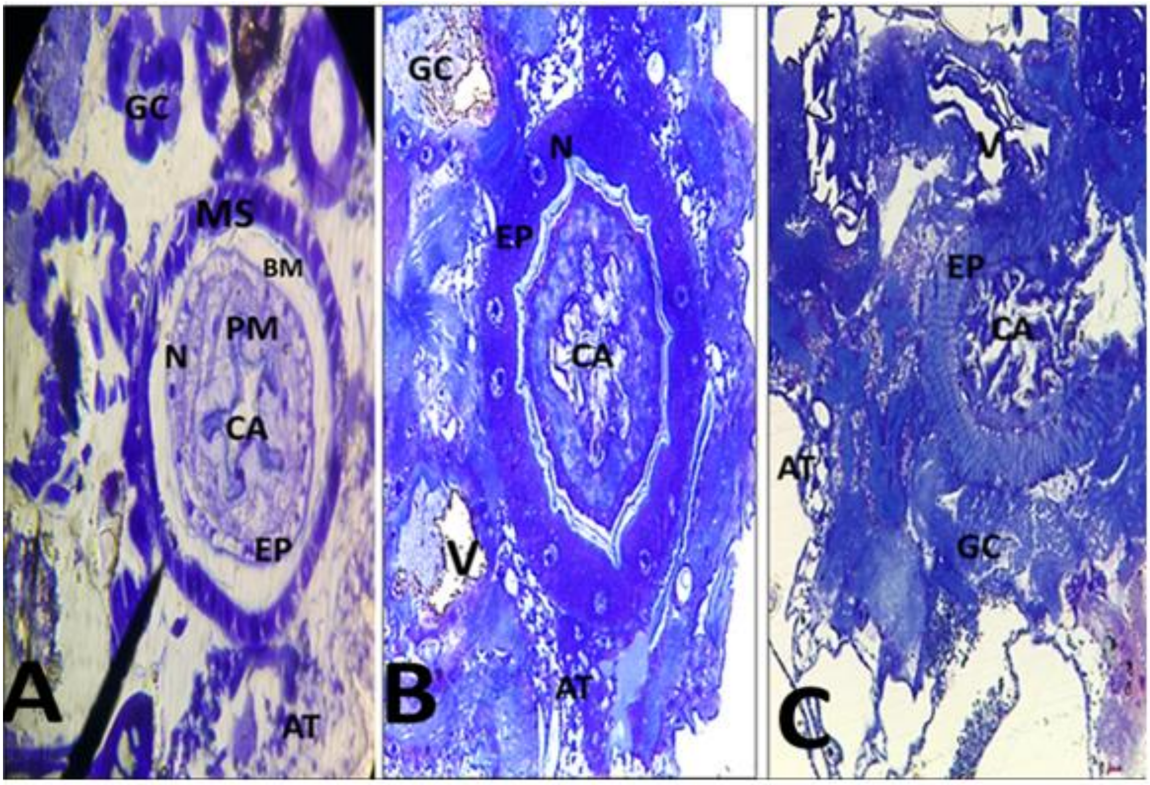

Fig. 1: semi-thin sections showing transverse sections in the anterior mid-gut of 3rd larval instar of *Cx. pipiens* in normal (A) and treated samples (B) treated with IMI and (C) treated with tannic acid, showing cuticle (C), adipose tissue (AT), muscles (MS), gastric ceaca (GC), basement membrane (BM), epithelial layer (EP), nucleus (N), peritrophic membrane (PM), vacuole (V) and cardia (CA). (40X).

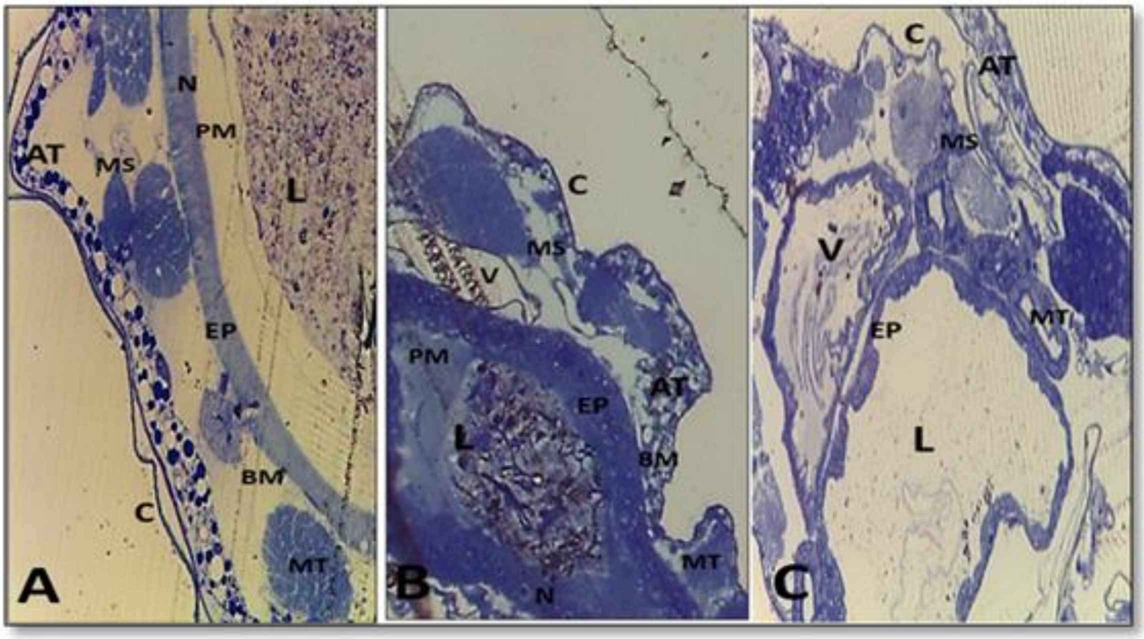

Fig. 2: Semi-thin sections showing a transverse section in the posterior midgut of 3rd larval instar of *Cx. pipiens* in normal and treated samples (B) treated with IMI and (C) treated with tannic acid, showing cuticle (C), adipose tissue (AT), muscles (MS), Malpighian tubules (MT), basement membrane (BM), epithelial layer (EP), nucleus (N), peritrophic membrane (PM), vacuole (V) and lumen (L). (40X).

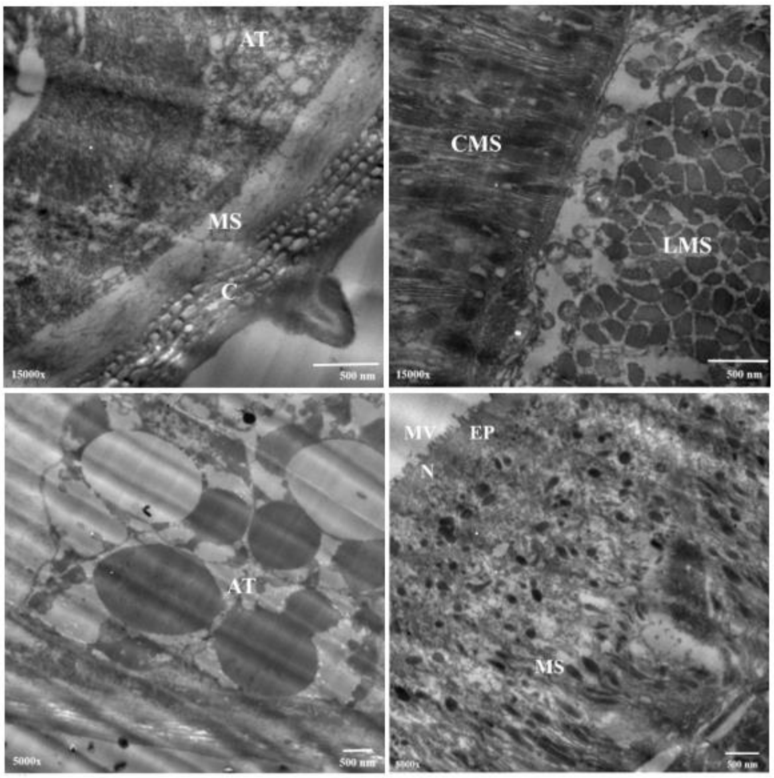

Fig. 3: Electron micrograph in the mid-gut of 3rd larval instar of *Cx. pipiens* in normal samples showing cuticle and epicuticle region (C), muscles (MS) and adipose tissue (AT); the muscles longitudinal muscles (LMS), circular muscles (CMS); epithelial layer (EP), nucleus (N) and microvilli (MV).

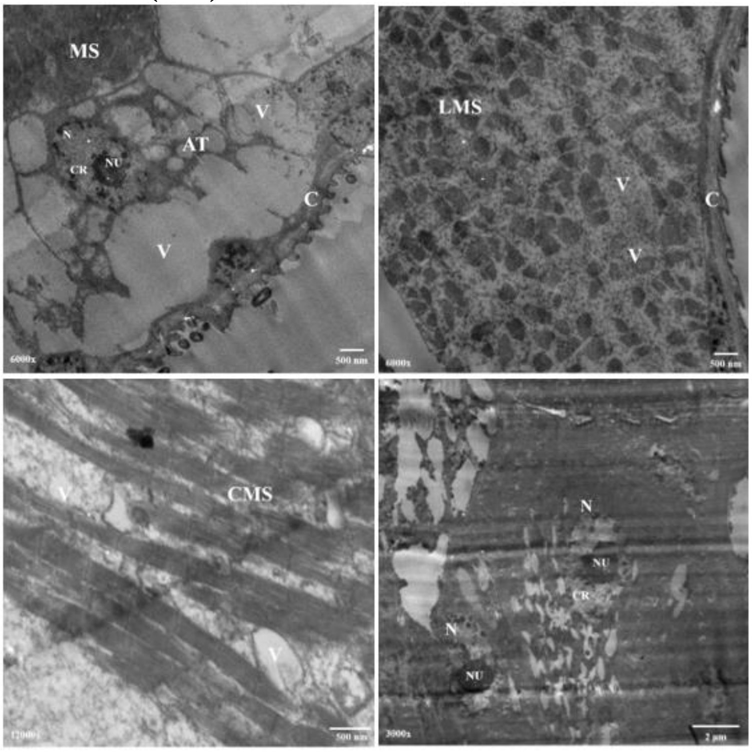

Fig. 4: Electron micrograph showing a transverse section in the mid-gut of treated 3rd larval instar of *Cx. pipiens* with tannic acid showing cuticle (C), adipose tissue (AT), muscles (MS), nucleus (N), nucleolus (NU), chromatin (CR) and vacuole (V); longitudinal muscles (LMS), circular muscles (CMS); enlargement of the nucleus and degradation of the nucleolus

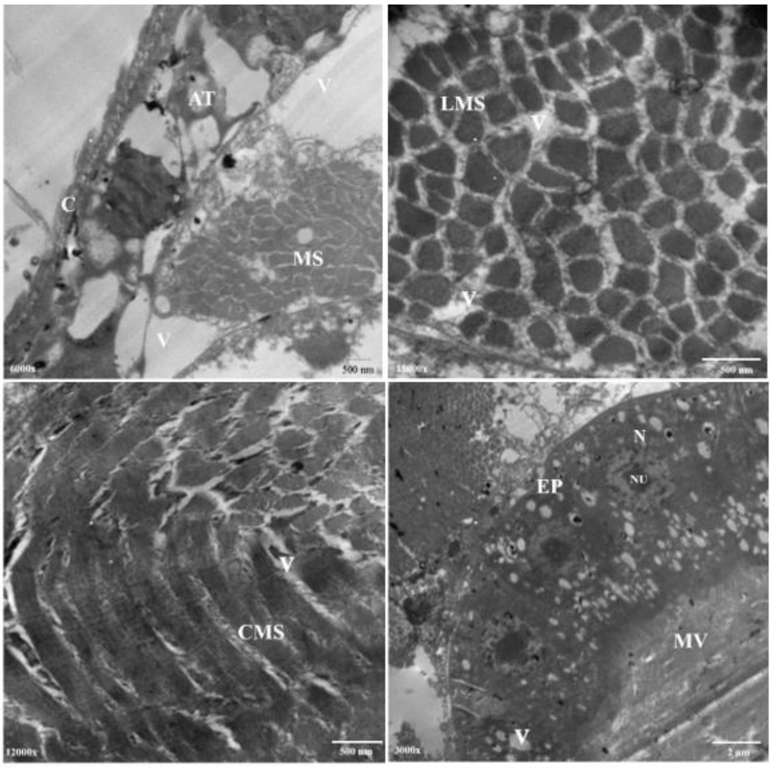

Fig. 5: Electron micrograph showing a transverse section in the mid-gut of treated 3rd larval instar of *Cx. pipiens* with imidacloprid showing cuticle (C), adipose tissue (AT), muscles (MS and vacuole (V); longitudinal muscles (LMS); circular muscles (CMS); epithelial layer (EP), nucleus (N), nucleolus (NU), microvilli (MV).

DISCUSSION

In this study, imidacloprid and tannic acid were used as alternative insecticides. IMI belongs to neonicotinoids. It directly acts on the nicotinic acetylcholine receptor (nAChR) (Suchail *et al*., 2004). IMI has been commonly used as a pest control agent on a lot of crops (Elbert *et al*., 1991). Tannic acid is a class of secondary plant compounds that includes some of the most abundant and biologically active chemicals present in vegetation-related hydro systems (Rey *et al*., 2000).

The sensitivity of 3rd larval instar *Cx. pipiens* to the toxic effects of the tested insecticides was not the only factor in determining their effectiveness. The effectiveness of the tested insecticides was influenced by their delayed effects that occurred during the developmental period. When these biological parameters were compared in treated and control insects, it was discovered that the treated insects displayed a variety of toxicity symptoms. Farahat *et al*., (2018) reported that the median lethal concentration caused a high percentage of mortality after the first day of treatment with IMI then the survived larvae completed their life cycle without any malformations. Ahmed & Saba (2014) proved that IMI became more toxic after 72 hrs. This result was achieved because the main target of the neonicotinoids group, specifically IMI, was the insect's central nervous system (CNS) as agonists of the post-synaptic nicotinic acetylcholine receptors (nAChRs) so, These compounds triggered a blockage in the neuronal pathway, enabling further acetylcholine to accumulate, resulting in paralysis as a first symptom and finally the insect's death. IMI has an imidazolidine ring which makes it a five-member (cyclic) neonicotinoid insecticide. Where cyclic neonicotinoids are more lipophilic than the other open-chain compounds so, IMI can reach the nervous system faster which explains the efficiency of

IMI and the high mortality rates over tannic acid. In addition to the high percentage mortality of IMI, it also slightly decreased the pupation period but did not affect the total developmental rate as reported in Cao *et al*. (2019).

Tannic acid induced delay in larval duration and shortened pupal duration after the exposure of the *Cx. pipiens* larvae to the median lethal concentration. This is due to the mode of action of tannins, where it can form complexes with proteins; when oxidized to quinones in the mid-gut of insect, they can produce semiquinones radicals and other forms of reactive oxygen species. This agreed with Ahmad & Pardini (1990). The relatively slow action of tannic acid helped it dramatically affect the larval and pupal duration throughout the development process. Tannins increased the mean larval period and decreased the mean pupal period. The increase of the larval period and decrease of the pupal period is due to the action of the tannic acid in the midgut. Tannic acid caused a reduction in the protein synthesis and increased simultaneously the production level of the fecal wastes which contained tannic acid. Due to this increase in faeces production, larvae treated with tannic acid hardily gained weight and barely molted to the next instar. So, the mean larval period increased, and the pupal period decreased. This agreed with Al-Izzi & Al-Maliky (1996). The results declare that both insecticides increased larval mortality, but tannic acid was the most effective. Tannic acid increased the larval duration. So, tannic acid was the insecticide that affected the biology of the 3rd larval instar of *Cx. pipiens* the most.

Histological and Ultrastructure Studies:

The effect of the two insecticides on the internal tissues of the *Cx. pipiens* larvae mid-gut was investigated using histological and ultrastructural techniques. The control *Cx. pipiens* larvae's mid-gut had been divided into two sections: the anterior mid-gut, which extends along one-third of the mid-gut, and the posterior mid-gut. Anterior mid-gut of normal *Cx. pipiens* larvae typically consist of a single layer of columnar epithelial cells which are separated from the hemocoel by a basement membrane and two layers of visceral muscle fibers (longitudinal and circular). Numerous microvilli evaginate from the luminal surface of the epithelial cells. The peritrophic membrane forms the cardia or proventriculus at the beginning of the mid-gut. Besides, gastric caeca surround the epithelial layer from the outer side. The other posterior part of the mid-gut consists of a single layer of cuboidal epithelial cells which are separated from the hemocoel by a basement membrane and two layers of visceral muscle fibers (longitudinal and circular) in addition to the adipose tissue. Numerous microvilli evaginate from the luminal surface of the epithelial cells, the peritrophic membrane lining the lumen. Besides the presence of five Malpighian tubules. This is elucidated by Al-Mehmadi & Al-Khalaf (2010).

Semithin Results:

IMI had a significant effect on the anterior mid-gut. IMI showed many histological changes as the rupturing of the cuticle and adipose tissue and destruction of the muscle layer. Gastric caeca became hollow, and vacuoles appeared, nuclei of epithelial layers were enlarged and cardia of peritrophic membrane was destructed.

IMI in the posterior mid-gut showed changes in the histology of sections as the rupturing and disappearance of the cuticle and adipose tissue and destruction of the muscle layer. Malpighian tubules were destructed, the appearing of many vacuoles and irregularities of the outline of the epithelial cells and nuclei of epithelial layers were enlarged. The peritrophic membrane became unequal in thickness.

These results are in harmony with Fernandes *et al*. (2015) who examined the IMI's median lethal effects on mid-gut growth. Third-instar larvae were exposed to various concentrations of IMI, which impaired the differentiation of regenerative cells, resulting in a significant reduction in the number of digestive and endocrine cells, as well as malformation of the mid-gut epithelium in adults. Martínez *et al*. (2019) elucidated the effect of the median lethal concentration of the IMI on the histological and cytological changes of the mid-gut of *Podisus nigrispinus* (Heteroptera: Pentatomidae). This median lethal concentration of IMI induced histological changes in the mid-gut epithelium as well as cytotoxic features, such as abnormal border epithelium, cytoplasmic vacuolation, and apocrine secretions in the first 6hrs after exposure to the insecticide. Digestive cells in the mid-gut began to apoptose after 12hrs of exposure.

The anterior mid-gut treated with tannic acid showed dramatic histological changes as the rupturing of the cuticle and adipose tissue and destruction of the muscle layer. Appearing of many vacuoles and distortion and irregularities of the outline of the epithelial cells were detected. Cardia of the peritrophic membrane and gastric caeca became destructed and unrecognized.

The posterior mid-gut treated with tannic acid also showed rupturing of the cuticle and adipose tissue and destruction of the muscle layer. Appearing of many vacuoles and irregularities and destruction of the outline of the epithelial cells were detected. The peritrophic membrane became unequal in thickness. Malpighian tubules became very small in size.

These results were also mentioned by Hussien (2001) who said that the tannic acid at LC_{50} severely damaged mid-gut epithelium (epithelial cells were swollen and lysed their content), destruction in peritrophic membrane and brush border causing large numbers of lesions in mid-gut cells. Ultrastructure studies indicated that tannic acid lysed the mid-gut epithelial cells, detachment of the cells from each other and from the basement membrane, rupturing of peritrophic membrane and microvilli and, damaging the mid-gut epithelial organelles such as, lysosomes, nucleus and mitochondria.

Ultra-Structure Studies:

The obtained studies proved that the cuticle of the untreated samples is differentiated into epicuticle and procuticle (inner endocuticle and outer exocuticle). Also, the muscular layer is differentiated into longitudinal and circular muscles and the adipose tissue is well-formed. The epithelium consists of a single layer of columnar epithelial cells with the nucleus in the base, numerous microvilli evaginate from the luminal surface of the epithelial. Rey *et al*. (1999) reported nearly the same actions of the tannic acid on Culicidae larvae mid-gut. Similar to our results Al-Mehmadi and Al-Khalaf (2010) estimated the *Cx*. larvae mid-gut and found that the larvae possessed a well-preserved layer of epithelial cells with the surrounding basal lamina. Besides a brush border membrane lining the lumen composed of many microvilli.

Larvae treated with IMI showed severe damage as lyses of internal organelles, destruction of the cuticle, destroying and vacuolation of adipose and muscular layers and disappearing of the peritrophic membrane and peritrophic space. Many vacuoles and degradation of the epithelial layer and Malpighian tubules were observed. The epithelial layer became irregular, and the epithelial cells had enlarged besides clumping of chromatin materials and the presence of many vacuoles. Fernandes *et al*. (2015) studied the effects of the IMI on the electron level to show that the cytoplasm of regenerative cells had many vacuoles and the plasma membranes in some cells were disrupted and cytoplasmic contents of the destroyed cells were released in the lumen of the mid-gut.

Larvae treated with tannic acid exhibited many histological changes in the mid-gut. Cuticle became destructed and unequal in thickness, adipose tissue and muscular layer became destructed and vacuolated. Distortion and irregularities of the outline of the epithelial cells were detected. Enlargement of the nucleus and the disappearance of the nucleolus was observed, and there was a change in nuclear shape with the clumping of chromatin materials. Results from Hussien (2001) agreed with our results, He found that the junctional complexes of mid-gut epithelial cells were disrupted. In addition to local detachment from the basal lamina and microvilli degeneration, cytoplasmic and nuclear lysis are observed. There are lysosome and mitochondrial ruptures. Intracellular vacuolization and cytoplasmic swelling were observed in the cytoplasm.

So, we can conclude from the previous results, that tannic acid was the most effective insecticide than IMI in the biological test as tannic acid delayed the larval duration and decreasing both pupation percentage and pupal duration. Both IMI and tannic acid showed deleterious effects in the midgut of the larvae. These two compounds are a promising tool for integrated pest management programs and need to be considered in the future integrated mosquito control program.

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ARABIC SUMMARY

التغيرات البيولوجية والنسيجية في يرقات كوليكس *بيبينز* (رتبة ذوات الجناحين) الناجمة عن إيميداكلوبريد وحمض التانيك

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عائلة البعوض هي أكثر ناقلات المرض شيوعًا في رتبة ذوات الجناحين. إنهم جميعًا يمتصوا الدماء تقريبًا وهم مسؤولون عن انتشار العديد من الأمراض المهمة مثل الملاريا والحمى الصفراء وداء الفيل. نتيجة لتطور مقاومة البعوض للبيرثرويد والفوسفات العضوي والكاربامات ظهرت الحاجة إلى مبيدات حشرية بديلة. لذلك تم معالجة يرقات *كوليكس بيبينز* في العمر الثالث بتركيزات مختلفة من المركبات المختبرة، إيميداكلوبريد وحمض التانيك. أجريت الاختبارات البيولوجية من خلال متابعة الحشرات المعالجة من عمر اليرقات الثالث حتى مرحلة البلوغ. أظهر الاختبار البيولوجي أن حمض التانيك كان أكثر فعالية من إيميداكلوبريد وذلك من خلال زيادة فترة عمر اليرقات وتقليل كل من نسبة التشرنق وفترة عمر العذراي. تم أيضا فحص التأثير النسيجي المرضي للمركب المختبَر على ظهارة المعى المتوسط لليرقات تحت المجهر الإلكتروني. أظهرت الدراسة نسيجية أن اليرقات المعالجة بها تغيرات مرضية خلوية في ظهارة الأمعاء المتوسطة والطبقات العضلية والخلايا الظهارية والعضيات الداخلية. أظهر كل من إيميداكلوبريد وحمض التانيك نفس التأثير تقريبًا على أنسجة اليرقات. أشارت النتائج بوضوح إلى أن إيميداكلوبريد كان أكثر سمية لـيرقات بعوضة *كوليكس بيبينز*، لكن حمض التانيك كان أكثر فاعلية في الجوانب البيولوجية. بناءً على هذه النتائج، يمكن استخدام المركبات المختبرة في برامج الإدارة المتكاملة للآفات لتحقيق فعالية أكبر.

الكلمات المفتاحية: إيميداكلوبريد ، حمض التانيك ،*كوليكس بيبينز* ، التغيير النسيجي.