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Original Article

Histopathological studies of red palm weevil *Rhynchophorus ferrugineus*, (Olivier) larvae and adults to evaluate certain nano pesticides

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(With 8 figures)

Abstract

Rhynchophorus ferrugineus (RPW) (Olivier) (Coleoptera: Curculionidae) is one of the most destructive pest of palm according to dozens of literature, a lot of effort have been made since three decades up to date to solve this problem, one of newest solution raised is using nano pesticides. Imidacloprid and Chloropyrophos and their nano form were tested against 10 days-old larvae and newly emerged adults of the red palm weevil. The pesticides had toxicological and pathological effects on *Rh. ferrugineus* larvae and adults. The toxicity effect of nano chloropyrophos was more than others. The damage included vaculation of cytoplasm, analyzes and destroyed nuclei of the epithelial cells. The larvae were more sensitive in the total damages in comparison with adults. This investigation is the first record to the histopathological effects of nano pesticides.

Keywords: nano chloropyrophos, nano Imidacloprid, histopathology, pesticide effect *Rhynchophorus ferrugineus*.

Estudos histopatológicos de gorgulho do palmito *Rhynchophorus ferrugineus*, larvas (Olivier) e adultos para avaliar certos nano pesticidas

Resumo

Rhynchophorus ferrugineus (RPW) (Olivier) (Coleoptera: Curculionidae) é uma das pragas mais destrutivas da palmeira, de acordo com dezenas de publicações, muito esforço foi feito desde três décadas para resolver esse problema, uma das mais recentes soluções levantados está usando nano pesticidas. O imidaclopride e o cloropirofós e sua nano forma foram testados em larvas com 10 dias de idade e em adultos recém-emergidos do gorgulho da palma vermelha. Os pesticidas tiveram efeitos toxicológicos e patológicos em Rh. larvas de ferrugineus e adultos. O efeito da toxicidade dos nano cloropirofos foi maior que outros. O dano incluiu a vacinação do citoplasma, análises e núcleos destruídos das células epiteliais. As larvas foram mais sensíveis no total de danos em comparação com os adultos. Esta investigação é o primeiro registro dos efeitos histopatológicos dos nano pesticidas.

Palavras-chave: nano cloropirofos, nano imidaclopride, histopatologia, efeito de pesticida Rhynchophorus ferrugineus.

1. Introduction

The digestive system is an important device in the body where it absorbs and digests food. The digestive system of insects consists of three parts, Foregut, midgut and hindgut, however, differences exist between different species because of various in eating habits (Harris et al., 2015; Temitope, 2013). The foregut and hindgut consist of ectodermal layer. The function of the foregut is to eat the food and hindgut water absorption while the mid gut secretion enzymes and digestion food (Aljabr et al., 2014). The red palm weevil *Rhynchophorus ferrugineus* (RPW) (Olivier) (Coleoptera: Curculionidae) is one of the most serious pest of different species of palm (Habib et al., 2017; Faleiro, 2006; Murphy and Briscoe, 1999). The originality of RPW is south and Southeast Asia from coconut (El-Shafie et al., 2013; Yasin et al., 2017). In mid-1980s the Gulf region become under threat RPW which spread rapidly to many countries of Asia and Africa by transportation of ornamental palms (Faleiro et al., 2012). In 1986 the pest was first record in united Arab of Emirates and Oman (El-Ezaby et al., 1997; Kaakeh, 2006), then Kingdom of Saudi Arabia in 1987. It reached to Egypt in 1992through transportation of infested palm (Cox, 1993). recently it was moved to Tunisia in 2011 where it was record in *phoenix canariensis* (Habib et al., 2017). The risk of this pest in its hidden behavior, which makes it difficult to detect the injury in its early stages (Sayed et al., 2016; Al-Dawood et al., 2013). This behavior include feeding the larval stages with in the trunk of palm

tree which is considered the most dangerous stage which kill it in the finally (Kaakeh, 2006). Several studies have indicated that RPW prefer to have less than 20 years of palm infection (Al-Dosary et al., 2016). Faleiro (2006) and Al-Dosary et al. (2010) refer to that RPW affects about 17 species of palm all over the world. Early detection of the injury is considered to be the success of any RPW-IPM programs (Al-Dosary et al., 2016; Abraham et al., 1998). The affected palms are identified by tunneling on the stem and the presence oozing of thick yellow brown fluid and tissue around the palm trunk openings (Ul Haq et al., 2018; Kaakeh et al., 2001a). The several methods have been used to control this pest. Chemical control considered as main for rapid treatment of infected palm trees which is one of the most effective methods in pest control (Al Dawood et al., 2013; Abdel-Salam et al., 2014). Several reports indicated the success of chlorpyrifos and imidacloprid in control *R. ferrugineus* (Abuzuhairah et al., 1996; Abraham and Vidyasagar, 1992; Cabello et al., 1997; Kaakeh, 2006; Dembilio et al., 2014).While others recommended using nano imidacloprid in control some pests (Sabbour, 2015; Assemi et al., 2014). In previous investigation (Abdelfattah et al., 2019) evaluated the tested pesticides and their nanoparticles in a laboratory and field and found them effective and recommended. In this investigation we made further studying their effect on the tissues and cells of the digestive system of *R. ferrugineus* in order to explain and understand the mechanism of damage.

2. Materials and Methods

2.1. Samples

The larvae and adult of (RPW) that used for this study were reared in the laboratory. Temperature of rearing room was kept between 26± 2 °C, humidity level 60 ± 20% and. The photoperiod was 12 L:12 D. Larvae from 5th instar were used in this study to ease of dissection and observation.

2.2. Treatment

The concentration (500ppm) was used for the four tested pesticides, imidacloprid and chlorpyrifos, and there nano forms for both larvae and adults. This concentration was added to semi-artificial diet (Kaakeh et al., 2001b). Then the larvae were allowed to feed on them for a day until they lost their balance. The larvae removed to fixed solution.

Adults left on cotton full off the certain concentration of pesticides, it left till lose balance for 24 hours then placed in fixed solution.

2.3. Histological study

The mid gut of adult and larvae were separated carefully (Figure 1 and 2). Then have been fixed in Bouins solution for two days. The samples were dehydrated at serial concentrations of ethanol alcohol; 70, 80, 90 and 100%. Then, they were cleared in xylene for 30 min. The samples were embedded in paraffin wax. The sections were cut using a microtome then finally stained with Haematoxyline and Eosin (Drury and Wallington, 1980; Abdullah, 2009).

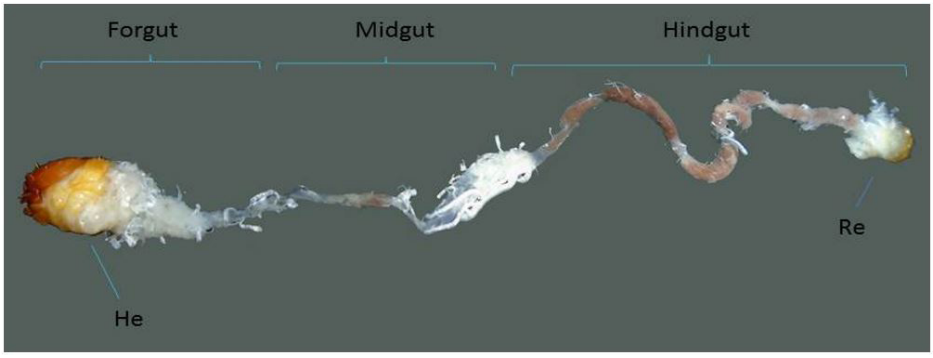

Figure 1. Alimentary canal of red palm weevil 10 days larva *Rhynchophorus ferrugineus*. Head (He); Rectum (Re).

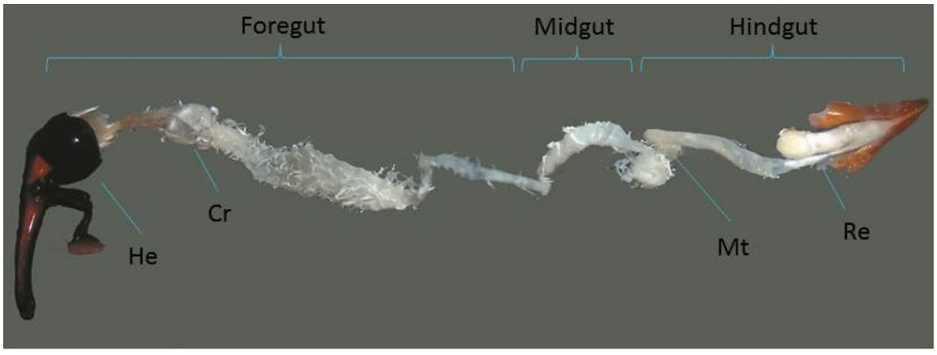

Figure 2. Alimentary canal of red palm weevil newly emerged adult, *Rhynchophorus ferrugineus*. Head (He); Crop (Cr); Malpighian tube (MT); Rectum (Re).

3. Results and Discussion

The histopathological changes were ever important; the induced changes by different plant extracts on different insects were documented by many authors (Lusis, 1963; Abo El-Ghar et al., 1994; Abdel-Ghaffar, 2004; Wanderley-Teixeira et al., 2006). However, little work has been got on the insecticidal effect against the insect's larval mid-gut. The conventional pesticides are costly and result in problems of residues, pollution, resistance, and health hazards. Therefore, the problem still exists. Nano technology recently takes place in solving the damage caused by RPW (Abdelfattah et al., 2019).

Many investigations have been conducted on the oil effects (Schmutterer and Ascher, 1984; Nassar, 1995; Bream et al. 2001; Butterworth and Morgane, 1968; Zanno et al., 1975). They may also disrupt growth; inhibit mounting (Garcia and Rembold, 1984; Dorn et al., 1986) and oogenesis (Steets, 1976; Rembold and Sieber, 1981; Senthilnathan and Saehoon, 2005). Some oils found affect the production of ecdysone and consequently disturb the moulting process and finally cause insect death (Gujar and Mehrotra, 1983; Mostafa et al., 1995). Some insecticides inhibit cholenestrase enzyme for regulate insect metabolism (Naqvi et al., 1994). The obtained results were revealed that, larval mortalities by the effect of Nano chloropyrophos more than other treated pesticides.

The midgut of the 10 days old larvae and the adults of the red palm weevil *Rhynchophorus ferrugineu*s divided into three regions (fore gut, midgut, and hind gut). Its anterior region was straight cylindrical structure located in the thorax and the posterior region was coiled shaped and lied in the abdominal segment (Figure 1 and 2). Midgut contains epithelial cells and the distinctive features of the midgut were the villi. The interior surface of the epithelial cells provided with borders. The epithelial cells rest on a basement membrane. Mid gut longitudinal muscle layer appears externally followed by an internal circular muscle layer as illustrated in (Figure 3). Where the muscle layer appeared detached from the gut wall Also, cell boundaries found absent between epithelial cells, vacuolization, shrinkage of the epithelial cells. The transverse sections of the treated mid gut, revealed that the nuclei moved toward the distal part of the epithelial cells and the nuclear membrane disappeared. Also, the cytoplasm of the columnar epithelial cells appeared less condensed at the basal part of the columnar cells (Figure 4 and 5); degeneration of the

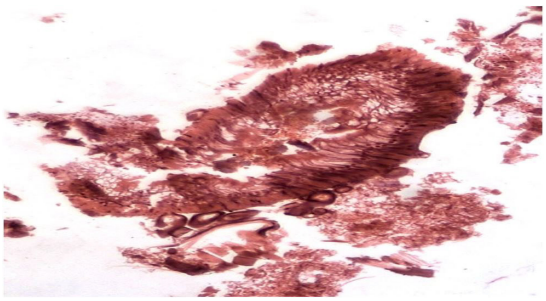

Figure 3. Cross section of untreated R ferrugineus larva midgut (H/E X200) Midgut contains epithelial cells and the distinctive features of the midgut were the villi. The interior surface of the epithelial cells provided with borders. The epithelial cells rest on a basement membrane. Mid gut longitudinal muscle layer appears externally followed by an internal circular muscle layer.

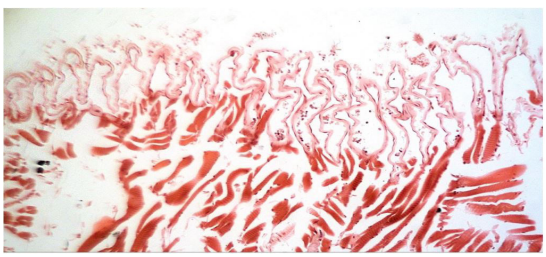

Figure 4. Cross section of R ferrugineus larva midgut treated with Imidacloprid (H/E X200) where the muscle layer appeared detached from the gut wall. Also, cell boundaries found absent between epithelial cells, vacuolization, and shrinkage of the epithelial cells.

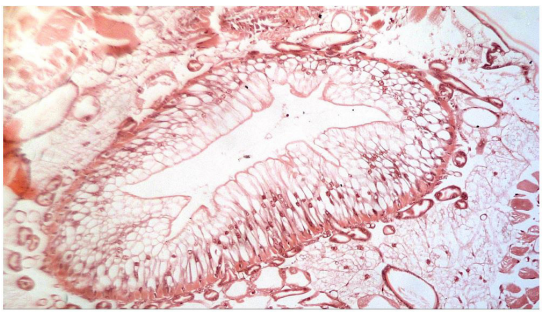

Figure 5. Cross section of R ferrugineus larva midgut treated with nano Imidacloprid (H/E X200) where the muscle layer appeared detached from the gut wall. Also, cell boundaries found absent between epithelial cells, vacuolization, and shrinkage of the epithelial cells.

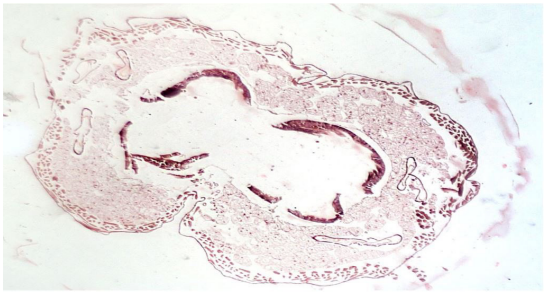

Figure 6. Cross section of R ferrugineus larva midgut treated with chloropyrophos (H/E X200) Where the transverse sections of the treated mid gut, revealed that the nuclei moved toward the distal part of the epithelial cells and the nuclear membrane disappeared. Also, the cytoplasm of the columnar epithelial cells appeared less condensed at the basal part of the columnar cells.

epithelial cells of the mid gut is clearly noted in the larvae treated with nano chloropyrophos and nano Imidacloprid. In addition the nuclei lose the nuclear chromatin granules and the peritrophic membrane appeared wrinkled and vacuolated. The present work showed that both nano chloropyrophos and nano Imidacloprid caused histopathological changes in the midgut of larvae (Figure 6 and 7). These changes included degeneration, vaculation and shrinkage of the epithelial cells, movement of nuclei towards the apical part of the cell, vaculation of peritrophic membrane and detachment of muscle layers. For adult it was difficult to obtain changes (Figure 8) which it may refer to quick death that doesn't lead to any histopathological obvious changes. The obtained results revealed that, midgut was sensitive to nano pesticides and normal pesticides. Similar result was obtained by using different pesticides against different insects (Abdel-Ghaffar, 2004). Hussein et al. (1994) mentioned that the effect on midgut may due to digestion and absorption of pesticides. On the other hand Ahmed (1995) reported that histopathological effect of certain pesticide was produced enlargement of epithelial cells, appearance of vacuoles Nasiruddin and Mordue (1993) studied the histological and ultrastructure changes caused by azadirachtin on the midgut of locust. His findings revealed necrosis of epithelial cells, enlargement of cytoplasmic inclusions and small sized striated borders. These results agree with the finding of Ruscoe (1972) and Naqvi et al. (1994). Zudaire et al. (1998) cleared that, the gut of locusts disturbed and showed disrupter in the endocrine cells as affected by food nutrient content, insect age and stage.

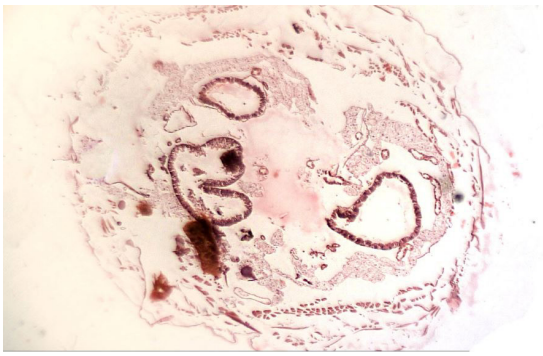

Figure 7. Cross section of R ferrugineus larva midgut treated with nano chloropyrophos (H/E X200) Where the transverse sections of the treated mid gut, revealed that the nuclei moved toward the distal part of the epithelial cells and the nuclear membrane disappeared. Also, the cytoplasm of the columnar epithelial cells appeared less condensed at the basal part of the columnar cells.

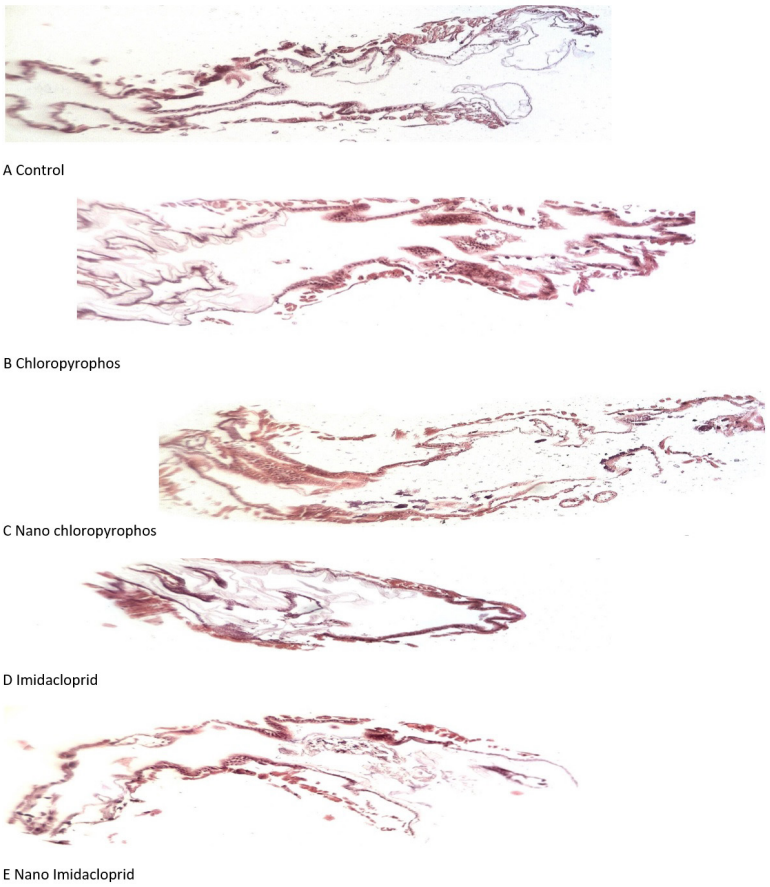

Figure 8. Cross section of untreated (a) R ferrugineus adult (H/E X200) and Treated ones (b, c, d and e).

On the other hand, Abo El-Ghar et al. (1994) provided histological effects of abmectin on mid-gut of leaf worm which had similar hazard shredding and erosion on the lining epithelium. Also, Magd El-Din (1999) showed the same previous histological alterations. This investigation revealed that nano pesticides mainly affect the mid gut in both larvae and adults causing death.

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