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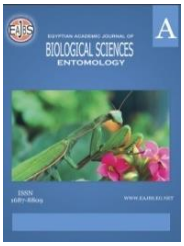

Latent Histopathological Effects of Lufenuron and Rice Bran Extract on *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

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ABSTRACT

Laboratory experiments evaluated the latent effects of lufenuron and rice bran extract on the newly hatched nymphs of *Schistocerca gregaria* produced from females who were treated as fifth nymphal instar with LC_{50} of each lufenuron and rice bran extract. Semithin sections of the newly hatchling showed great histological malformations in the structure of the brain, compound eyes, midgut, and hind leg muscles compared with control, these organs lost their normal structures, shapes, and functions. The tested compounds had serious extended histopathological effects, so they can be used to control *S. gregaria* as safe alternative agents to toxic synthetic pesticides.

INTRODUCTION

The main source of food for people and animals, cultivated crops, is severely damaged by the desert locust *Schistocerca gregaria*. Due to its two to five annual generations and the capacity of its swarms to travel quickly over huge distances, the desert locust is arguably the most deadly of the locust pests (Homberg, 2015). In a gregarious phase, the desert locust can result in up to 100% crop loss (Simpson *et al*., 1999). So, it seemed necessary to develop an effective preventive control strategy against its nymphal instar to stop the emergence of mobile swarms (Taha & El-Gammal, 1990). The main pesticides used to control locusts today are organophosphorus pesticides because of the banning of organochlorines (Lecoq, 2001); the current use of conventional pesticides in desert locust control may have direct and indirect adverse effects on human health and the environment (Garriga, & Caballero, 2011). To solve these problems, alternative agents for controlling desert locusts must be found that are economical, practical, and safe for the environment. In contrast to the dangers of toxic synthetic pesticides, which act as potential acute or chronic insecticides, insect growth regulators (IGRs) and botanical extracts have low mammalian toxicity and low environmental problems.

Chitin synthesis inhibitors are part of insect growth regulators, that effectively inhibit insect development throughout their entire life cycle (Verloop & Ferrel, 1977), the desert locust is effectively killed by these compounds (Azam & Seegh, 1993; Coppen & Jepson 1996; and Bakr *et al*., 2008). Numerous studies have proven the activity of lufenuron

(CSI) as an inhibitor of ovicidal, transovarial-ovicidal, and embryonic development of pests at the recommended dose (Fonseca *et al*., 2015 and Sampson *et al*., 2017). Also, it causes different morphological, biochemical, and histological malformation in different stages of the desert locust (Abdel Rahman, 2017; Al-Zeeb *et al*., 2018; Mahdy *et al*., 2019 & 2020 and Said, 2020). As well it has reasonable persistence and low toxicity against non-target organisms.

Botanical insecticides are expected to be potential alternatives for conventional synthetic insecticides (Abdellaoui *et al*., 2013 & Ben Hamouda *et al*., 2015). Botanical insecticides such as agricultural waste products and plant extracts degrade quickly in air and moisture by detoxification enzymes; this refers to less environmental persistence and lower dangers to organisms that aren't the target (Isman, 2008). Rice bran extract is one of the agricultural waste products that showed a serious effect on the desert locust *S. gregaria* life cycle (Mahdy *et al*., 2019 & 2020).

The purpose of the current study is to investigate the histopathological changes brought about by the latent effects of lufenuron and rice bran extract in newly hatched nymphs of *Schistocerca gregaria*.

MATERIALS AND METHODS

1- Insects Colony:

Desert locust *S. gregaria* were reared at the Plant Protection Research Institute, Agricultural Research Center according to the technique described by Abbassi *et al*. (2003), They are placed in wooden cages with a layer of sand (15 cm deep) on the bottom and a humidity of 10-15% suitable for spawning. A bulb (100 watts) was set to maintain a continuous photoperiod (12 light:12 dark). The locusts were maintained at a temperature of (32±2^{o}C). Every day, faces and dead locusts were removed and the entire cage was completely cleaned and effectively sterilized at the end of any experiment.

2- Tested Compounds:

- **a-** Lufenuron (10%) is a chitin synthesis inhibitor.
- **b-** Rice bran extract is a plant waste product, it was extracted by utilizing petroleum ether through Bakr *et al.* (2006)'s method. Then it was stored in screw-capped vials at 4° C until use.

3- Bioassays Studies:

S. gregaria fifth nymphal instar females were treated using the feeding technique with LC_{50} concentrations of lufenuron (10%) and rice bran extract which were calculated by Mahdy *et al*. (2020) and were (95 & 14.3×10^{3} ppm) respectively. Samples of newly hatched nymphs were collected from normal and affected eggs resulting from these treatments to study the latent effect of these compounds on different organs and jumping legs using the histological technique.

4-Histological Studies:

The samples of head capsules, abdominal region, and hind legs of control and affected newly hatching were moved to the initial fixative after being submerged in 3% glutaraldehyde. They were post-fixed for 1 hour in 1% osmium tetroxide in 0.1M cacodylate buffer following getting fixed overnight and washed three times in sucrose-cacodylate buffer (pH 7.2), washed three times in distilled water, followed by a graded sequence of ethanol alcohol dehydration. Then specimens were infiltrated with spur's epoxy resin in a graded series of absolute alcohol-spur's resin mixtures and then embedded in freshly prepared spur's resin at 70°C for 72hrs. Samples were sectioned with glass knives on a Reichert OM-2 ultra-microtome.

RESULTS AND DISCUSSION

The present study indicated that; the sublethal dose (LC_{50}) of each lufenuron and rice bran extract had latent histopathological effects on the newly hatching of affected *S. gregaria* eggs compared with control as the following:

The Head:

Semithin sections in the head of a control group showed the brain with its 3 lobes; protocerebrum, deutocerebrum, and tritocerebrum. Each one is pair, the protocerebrum is the largest part of the brain, and it continuous laterally with the optic lobe to the compound eye (Fig.1a). Neurosecretory cells in the pars intercerebralis and pars lateralis of the protocerebrum (Fig.1b). Similar structure and other details of the brain in the desert locust and others had been described by Bharadwaj & Banerjee (1971); Kurylas *et al*. (2008) and Chapman (2013).

The compound eyes consist of many similar units of vision called ommatidia. Each ommatidium consists of a convex thickened transparent cuticle known as the corneal lens. Below the lens, there are a crystalline cone and photoreceptor cells (retinula cells) to differentiate between color and brightness, light passes through the lens and cone into a channel, known as a rhabdom, two kinds of pigment cells; the primary pigment cells, completely surround the apex of the crystalline cone, and the secondary pigment cells encircle the crystalline cone and the retinula, these pigment cells separate ommatidium from its neighbors (Fig.1c). Our results are in agreement with the structure of the locust compound eye was described by Wilson *et al*. (1978); Homberg & Paech (2002); Chapman (2013) and Nation (2015).

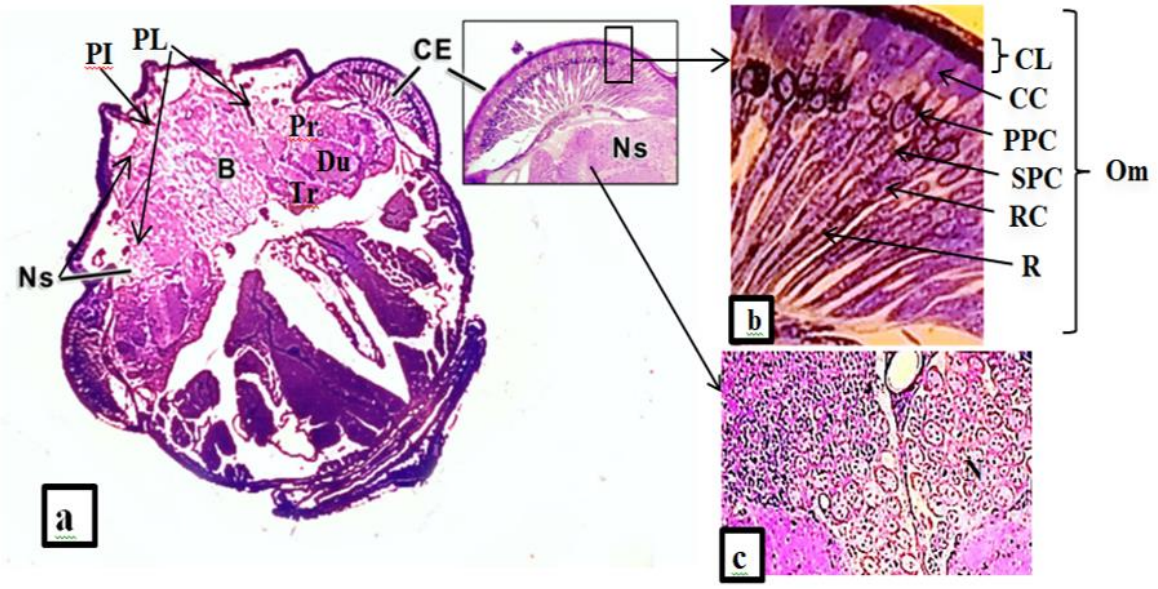

Fig.1 (a, b & c): The semithin section in the head capsule of newly hatched nymphs of normal *S. gregaria* eggs shows a normal histological structure of (a) brain [B] and its parts: protocerebrum [Pr], deutocerebrum [De], and tritocerebrum [Tr]; The neurosecretory cells [Ns] in the protocerebrum within pars intercerebralis [PI] and pars lateralis [PL]; and the compound eye [CE]; (b) the compound eye unit (ommatidium [Om]) consists of the corneal lens [CL], crystalline cone [CC], retinula cells [RC], a rhabdom [R] the primary pigment cells [PPC] and the secondary pigment cells [SPC] and (c) the neurosecretory cells have normal nuclei.

But in the affected group with lufenuron treatment, the brain deformation was observed, it lost its basic structures and integrity, and it becomes solid masses scattered, and no longer connected to sensory organs, as well as the neurosecretory cells lysed. The compound eyes showed an asymmetrical shape and unorganized histological constituents (F.2a). Also rice bran extract caused compression and loss in the brain morphic nature, and poorly developed compound eyes (F.2b).

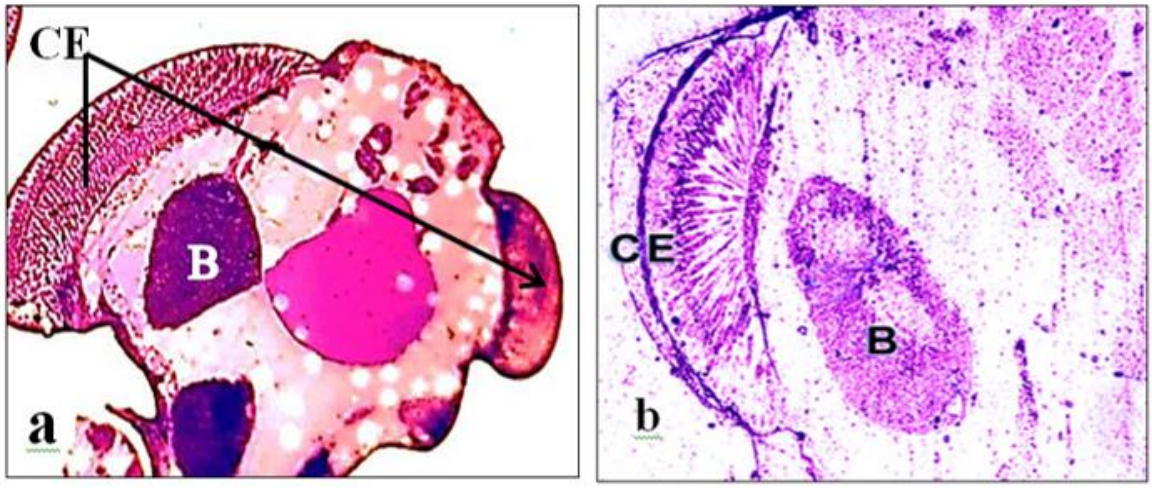

Fig.2 (a & b): Semithin sections in the head capsule of newly hatched nymphs produced by affected *S. gregaria* eggs with lufenuron (a) and rice bran extract (b) treatments showing the latent histopathological alteration in the brain [B] and compound eyes [CE].

These observations are in agreement with the findings of the brain and compound eyes damage in newly hatched nymphs of *S. gregaria* due to lufenuron treatment reported by (Abdel Rahman, 2017), also, rice bran extract and lufenuron exhibited disruption in the histological formation of the brain and compound eyes during embryonic development in the desert locust (Mahdy *et al*., 2020). Similar observations showed by different plant extracts as azadirachtin on *S. gregaria* eggs caused poorly developed brain and compound eyes of newly hatching (Ghazawy *et al*., 2010), abnormal brain cells of desert locust nymphs treated with Cestrum leaf powder were observed by Ben Hamouda *et al*. (2011).

The Midgut:

The midgut is the principal site for digestive enzyme production, food digestion, and nutrient absorption.

In the control group, the midgut is lined by normal enteric epithelium which has two main types of cells, the columnar epithelial cells, and regenerative cells, or nidi cells at the base of the midgut epithelium to renew destroyed epithelial cells. These cells cling to the basal lamina, continuing serially from the base to the epithelium apex. An inner layer of circular muscle and an outer layer of longitudinal muscle surrounds the midgut epithelium on the outside. There are vesicles filled with secretion at the ends of epithelial cells. The midgut epithelium is separated from the gut lumen by the peritrophic membrane (Fig.3). Similar structure was described by Chapman (2013), and Nation (2015) in the same insect and others.

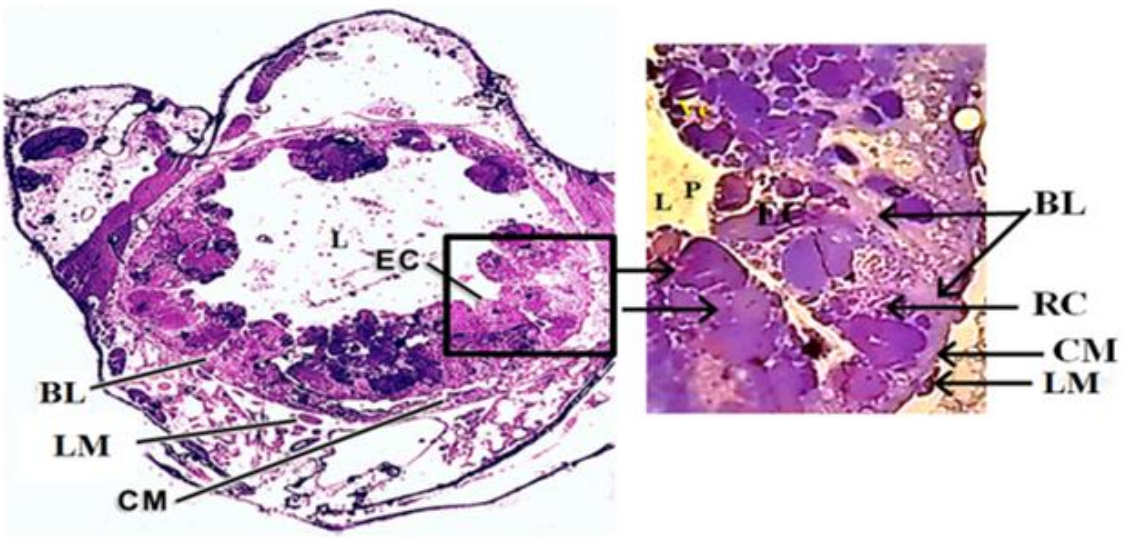

Fig.3: The semithin section in the midgut of newly hatched nymphs of normal *S. gregaria* eggs shows a normal histological structure of epithelial cells [EC], regenerative cells [RC], the basal lamina [BL], circular muscle layer [CM], longitudinal muscle layer [LM], vesicles [Ve], the gut lumen [L] and peritrophic membrane [P].

Our observation showed the latent effects of each lufenuron and rice bran extract on the midgut of newly hatching, where lufenuron caused shrinkage in the gut epithelium cells which lost its normal shape with vacuoles, rupture of the circular muscle layer, and the peritrophic membrane, also vesicles spread in the gut lumen leading to blockage of the midgut (Fig.4a).

Severe histopathological effects on midgut cells caused by rice bran extract where the gut's epithelial cells lost their shape and structure leading to stretching, and deterioration of cells, as well as the vanishing of peritrophic membrane and regenerative cells. Cell content fragmentation was observed in the midgut lumen, also circular and longitudinal muscles are obliterated (Fig.4b).

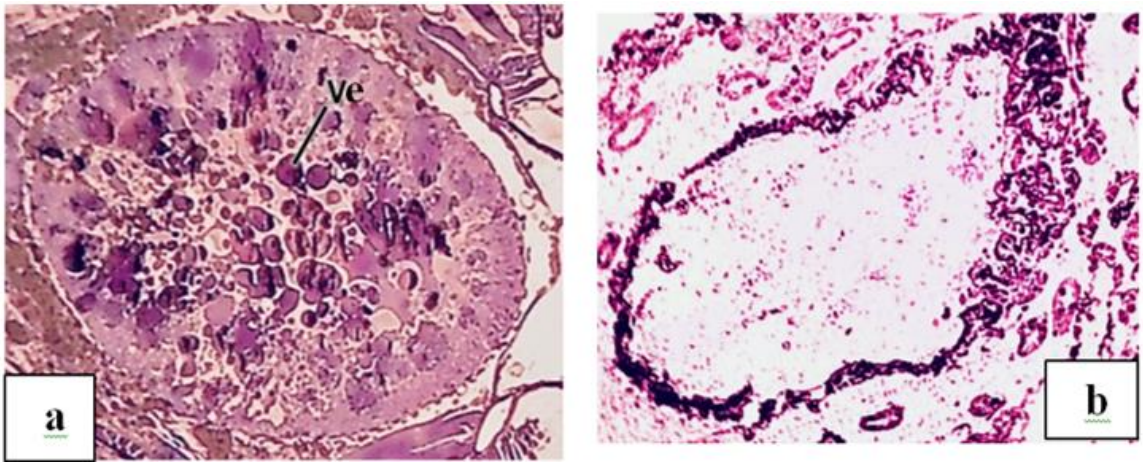

Fig.4 (a & b): Semithin sections in the midgut of newly hatched nymphs produced by affected *S. gregaria* eggs with lufenuron **(a)** and rice bran extract **(b)** showing the latent histopathological alteration. [Ve] vesicles.

Similar histopathological observations were reported by many authors by using other IGRs and plant extracts as well as Bouaichi & Chihrane (2001) showed that

diflubenzuron effect on the peritrophic membrane structure in the midgut of *S. gregaria*, Tebufenozide treatments resulted in abnormal mid-gut epithelial cells of *S. gregaria* (Ghoneim *et al*., 2008), necrosis of epithelial cells and many vacuoles resulted after treatment of *S. gregaria* and *L. migratoria* adults with azadirachtin (Nasiruddin & Mordue, 1993). The midgut of desert locusts exhibited a decrease in epithelial cell stature and muscular layer thickness after ingesting an artificial diet that included *Cestrum parquii* leaf powder (Ammar & N'cir, 2008).

These histopathological alternations agree with the observation of other species in the same order such as grasshopper *Heteracris annulosa* midgut caused by neem seed extract (Naqvi *et al*., 1994), Also alterations of the gut structure of the grasshopper *Heteracris littoralis* reported after treatment with the *Allium sativum* essential oils (Sharaby *et al*., 2012). On the other hand, lufenuron exhibited histopathological effects on other species; it caused the destruction of the epithelial cells of *S. littoralis* larvae's gut (Adel, 2012).

The Muscles of The Legs:

The striated muscle is the only type of muscle in insects. Histological study of the muscles in the femur of the jumping legs in newly hatched nymphs of control eggs showed that skeletal muscle tissue is formed of muscle fibers in bundles (fascicles) which are separated by a slightly thick connective tissue layer called perimysium, another thin layer of connective tissue called endomysium surrounds each muscle fiber which acting as a site of metabolic exchange, each muscle fiber has peripheral nuclei and surrounds by the plasma membrane (sarcolemma), capillaries and the fibroblasts appeared in the space between muscle fibers that maintains muscle structure (Fig.5). Similar basic muscle structure showed by Chapman (2013) and Nation (2015).

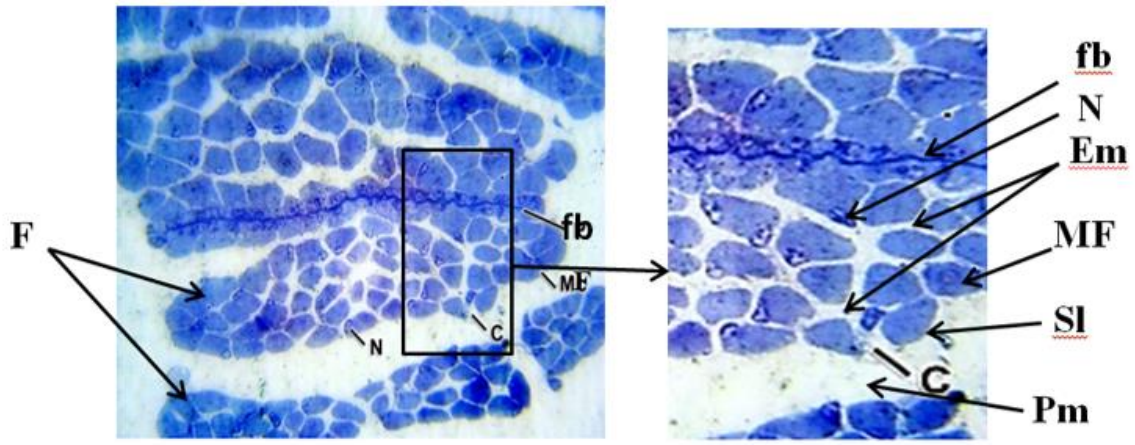

Fig.5: The semithin section in the muscles of the jumping leg femur in newly hatched nymphs of control *S. gregaria* eggs shows a normal structure of muscle tissue, muscle fiber bundles (fascicles [F]), muscle fiber [MF], perimysium [Pm], endomysium [Em], peripheral nuclei [N], sarcolemma [Sl], capillaries [C] and the fibroblasts [fb].

Histopathological examination of the leg muscles of newly hatching affected by lufenuron showed degeneration of connective tissue layers perimysium and endomysium resulting in the attachment of muscle fiber bundles, sarcolemma disappearance of some fibers, the vacuolated nuclei appears, and vanishment of other components as compared with the control muscles (Fig.6a). In affected legs by rice bran extract, complete decomposition of some fascicles and shrinkage in others occurred, the sarcolemma of some fibers, endomysium in the most bundles and some components disappeared. (Fig.6b).

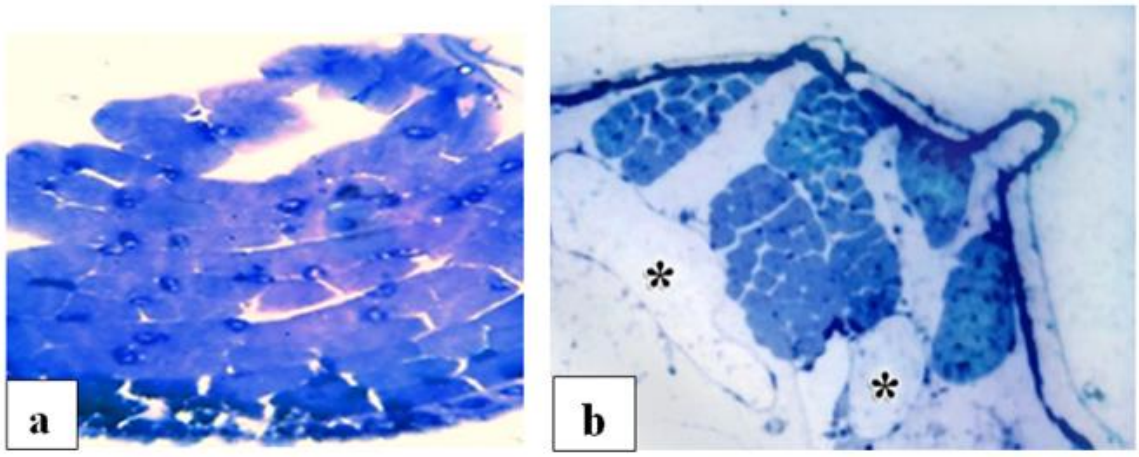

Fig.6 (a & b): Semithin sections in the muscles of the jumping leg femur in newly hatched nymphs produced by affected *S. gregaria* eggs with lufenuron **(a)** and rice bran extract **(b)** showing the latent histopathological alterations**.[*]**degenerated muscle bundles.

Our results agree with Hussein *et al.* (2007) who showed that Rice bran extract caused histopathological effects on the muscles of the desert locust, also Bakr *et al.* (2008) and Al-Zeeb *et al*. (2018) reported that Lufenuron treatment caused malformation and degradation in the desert locust muscles. On the other hand, other IGRs as tebufenozide and Flufenoxuron Indicated disrupture in the muscle structure of the desert locust (Ghoneim *et al.*, 2008 and Bakr *et al.*, 2008).

The present study revealed that The tested compounds have latent histopathological effects where they induced serious changes in newly hatching of affected *S. gregaria* eggs with lufenuron and rice bran extract treatments and these associated with their toxic effects which were reported by Mahdy *et al*. (2019 & 2020), where they caused a disturbance in the protein and DNA of eggs resulting from treated females as fifth nymphal instars with LC_{50} of them leading to histopathological variations of embryonic structure and inhibition of embryonic development and hatching in some eggs, and this explains, why some affected eggs produce abnormal newly hatched nymphs.

A hormonal imbalance especially juvenile hormone (JH) in affected adults female may be the main reason for all these problems where many authors explain that vitellogenesis and oocyte maturation is stimulated by Juvenile hormone (JH) as Wyatt & Davey (1996); Wu *et al*. (2021) and Zheng *et al*. (2022), also Luo *et al*. (2017) reported that vitellogenesis and egg formation are reportedly dependent on the 78-kDa glucose-regulated protein (Grp78) genes that JH regulates.

Conclusion:

Finally, our results suggest that lufenuron and rice bran extract are potential compounds for safe control against *S. gregaria* in integrated pest management (IPM).

Ethical Approval:

The study was approved by the ethical committee of the Institutional Animal Care and Use Committee, Faculty of Science, Ain Shams University (ASU-SCI/ENT/2023/5/4).

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ARABIC SUMMARY

التأثيرات الهستوباثولوجية الكامنة لمركب الليوفنيرون ومستخلص نخالة الأرز على حشرة الجراد الصحراوي(*شيستوسيركا جريجاريا*)

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تم التقييم المعملى للتأثيرات الهستولوجية الممتدة (الكامنة) لكل من اللوفينورون ومستخلص نخالة الأرز على حوريات الفقس حديثا للجراد الصحراوي الناتجة من اناث عوملت فى العمر الخامس بالجرعة النصف مميتة لكلا المركبين. واثبتت الدراسة حدوث تشوهات نسيجية كبيرة في بنية الدماغ، والعين المركبة، والمعي المتوسط، وعضلات الساق الخلفية للفقس مقارنة بالكنترول، وفقدت هذه الأعضاء تركيبها وشكلها ووظيفتها الطبيعية. فكان للمركبين تأثيرات هستوباثولوجية خطيرة طويلة المدى، لذا من الممكن استخدامهما للسيطرة على حشرة الجراد الصحراوي كعوامل بديلة آمنة لمبيدات الآفات الاصطناعية السامة.

الكلمات المفتاحية:

الجراد الصحراوى- دراسة هستولوجية - الليوفنيرون - مستخلص نخالة الأرز - مثبطات تكوين الكيتن- منتجات المخلفات الزراعية.