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Biological and histopathological effects of some insecticidal agents against red palm weevil *Rhynchophorus ferrugineus*.

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ABSTRACT

The red palm weevil *Rhynchophorus ferrugineus* is the most important insect pest for the date palm trees in the middle east and Gulf states. The objective of this study was to investigate the pathogenicity of different concentrations of a natural plant extract (neem) and a synthetic insect growth regulators (IGRs) (flufenoxuron) on the histology of the ovary and testis, and on some biological parameters of the red palm weevil. Prepupae were grouped and treated with three different doses of neem extract (Neem J, 50, 100, 500 ppm) and a synthetic insect growth regulator (flufenoxuron, 0.05, 0.1, 0.5 ppm).

The present study demonstrates that both natural (Neem) and synthetic (flufenoxuron) IGRs are capable of disrupting growth, development and reproduction in *Rhynchophorus ferrugineus*. Growth and developmental disruptions are resulted from the production of high mortality rate, reduction in body length, sex ratio, and morphological malformations, in a dose-dependent fashion. The study further reveals that IGRs exert effects on histological structure of gonads, in this way, disrupt gamete productions. IGRs disrupt female gamete production through their actions on the accumulation of yolk granules (vitellogenesis) and follicular epithelial cells. Disruption of male gamete production was detected by disorganization of testicular cysts and depopulation of these cysts in addition to degeneration and necrosis in germ cell lineage.

Keywords: *R. ferrugineus*, IGRs, biological parameters, reproductive organs.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is an economically important, tissue-boring pest of date palm in many parts of the world. One of highest destructive pests in palm plantation, the larvae are responsible for damaging the palm, and once they have gained access, the death of the palm generally ensues. The female weevils lay their eggs on palms mostly in cracks, crevices and wounds. Another important site of pest entry into the palm is at the leaf axil and also from where offshoots emerge. Because of the concealed nature of the larvae, effective methods for the management of the red and other palm weevils have been difficult to develop (Murphy and Briscoe, 1999). The pest has caused large economic losses in date palms worldwide for the last 30 yr (Faleiro, 2006; Murphy and Briscoe, 1999), and still there are no effective control measures. The chemical control of this pest is undesirable, since chemicals lead to the pollution of water courses around areas with palm weevil infestation (Moura *et al.,* 1995; Abuzuhairah *et al.,* 1996). Therefore, safer and yet effective control methods should be sought (Nassar and Abdullah, 2001). Isman *et al.* (1990) reported that the natural bio-pesticides offer desirable alternatives to using synthetic chemicals in agricultural systems where protection of the environment and preservation of beneficial organisms are important. One such bio-pesticide of interest is the natural insect growth regulator (IGR), azadirachtin, (one of the most active ingredient of neem seed extract) a botanical compound that can be effective, is biodegradable, and rapidly metabolizes in the environment. This compound is a liminoid that accumulates in the seeds of the neem tree (*Azadirachta indica*), from which it can be extracted efficiently (Schroeder and Nakanishi, 1987). Beside the natural neem extract, flufenoxuron represents another safe category of synthetic insect growth regulators (IGRs), especially chitin-synthesis inhibitors, because of their selectivity and low toxicity to the non-target organisms (Mitsui, 1985).

Therefore, the present study was designed to evaluate some biological, toxicological and histopathological effects of one synthetic IGR (flufenoxuron, a chitin synthesis inhibitor), and another botanical one, neem extract (containing azadirachtin, one of the active ingredients of the neem tree *Azadirachta indica*), against prepupal larvae of *Rhynchophorus ferrugineus*.

MATERIALS AND METHODS

The experimental insect

Prepupal stage of the Red palm weevil, *Rhynchophorus ferrugineus* were collected from diseased date palm tree at Al-Manaief Al-Gharbia region, Ismaileya governorate. They were kept in the laboratory in small plastic cages approximately 5x10x13 cm. The insects were incubated at laboratory temperature of (28±2ºC) and relative humidity (80±10% R.H.). Soon after collection, the prepupae were randomly assorted in groups and topically treated individually with different doses of the plant extract (Neem J) or the insect growth regulator (flufenoxuron).

Bioassay and administration of chemicals:

Two insect growth regulators (IGRs) were used in the present study. One of them is a natural plant extract obtained from the neem tree *Azadirachta indica* (Meliaceae). Azadirachtin is one of the most important active ingredients of neem seed kernel of this tree.

The other IGR used is the chitin synthesis inhibitor (fluefenoxuron), with the formula of $C_{21}H_{11}ClF_6N_2O_3$. Its IUPAC name is 1-[4-(2-chloro-α,α,α-trifluoro-*p*-tolyloxy)-2-fluorophenyl]-3-(2,6-difluorobenzoyl) urea.

Three dose levels were prepared from neem: 500, 100, 50 ppm. On the other hand, three dose levels were prepared from fluefenoxuron: 0.05, 0.1, 0.5 ppm.

For each treatment, prepupae were topically treated with 10 µl of the plant extract or the synthetic IGR. These insecticides were topically applied onto the dorsum of the prothorax. Control insects were topically applied with acetone only. All treated and control insects were checked daily until pupation and then adult emergence. Adults were provided daily with portions of fresh sugarcane stem tissue approximately 0.5x2x2cm.

Bioassay parameters and different criteria were observed and recorded just after the prepupal treatment.

Statistical analysis of data:

Data obtained were presented as mean ± standard error of mean (SEM). Differences between every 2 groups were analyzed by the unpaired student's *t*-test. The difference was considered significant if $p < 0.05$. All graphs and statistical analysis were performed using Microsoft Excel software.

Histopathological studies:

Ovaries and testes were dissected out and fixed with the appropriate fixative to be processed for hematoxylin and eosin staining Treated larvae and the untreated larvae of the red palm weevils were dissected in Ringer's solution. was isolated and fixed in Bouin's solution then embedded in paraffin. Many sections were obtained and stained with haematoxyline and eosin according to method of Drury and Wallington, (1980).

RESULTS AND DISCUSSION

Bioassay studies:

Lethal effects

As shown in table (1) the lethal effect of neem and flufenoxuron was dose-dependent, increased with the increasing of the dose. The lethal effect of neem extract on RPW was not obvious in a distinct developmental stage while, the lethal effect of flufenoxuron on RPW was more obvious in the pupal stage. Also, topical application with neem extract resulted in reduction in the number of the emerged adults that exhibit many morphological abnormalities. The highest mortality rates attained 26.7% and 13.3% in treated prepupae with the higher concentration of neem (500 ppm) and flufenoxuron (0.5 ppm), respectively (Table 1). Whereas the highest concentration of neem and flufenoxuron caused 22.2 and 14.3% mortality of adult stage respectively compared to 0% of control group. The lower larval mortality was 6.7 and 6.7% while adult mortality was 7.7 and 0% after treatment of both stages with the lower concentration (50 & 0.05 ppm) of neem and flufenoxuron, respectively.

Table 1: Lethal effect of different concentrations of neem & flufenoxuron (ppm) applied topically onto the prepupae of the Red Palm Weevil, *Rhynchophorus ferrugineus*.

Dose (ppm)		%prepupal mortality		% pupation		% pupal mortality		% adult emergence		% adult mortality		% total inhibition of adult emergence	
(Control)		6.7		93.3		0		93.3		0		6.7	
N	F	N	F	N	F	N	F	N	F	N	F	N	F
50	0.05	6.7	6.7	93.3	93.3	7.1	0	86.7	93.3	7.7	0	20	6.7
100	0.1	13.3	6.7	86.7	93.3	7.7	21.4	80	73.3	8.3	0	26.7	26.7
500	0.5	26.7	13.3	73.3	86.7	18.2	46.2	60	46.7	22.2	14.3	53.3	60

Many authors reported toxic effects of neem tree extracts, such as Azt, against various insect species. Using another neem preparation, NeemAzal (with 20% of Azt content), Ghoneim *et al.* (2000) recorded various mortality percents among larvae, pupae and adults of the Egyptian cotton leafworm *S. littoralis.* The latter neem preparation exhibited various degrees of lethality on the house fly *Musca domestica* which decreased if the concentration decreased below 2000 ppm in the artificial diet of larvae (Mohamed *et al.,* 2000). However, so many results had been reported by several authors for Azt or Azt preparations against different species (Osman, 1993; Osman and Bradley, 1993; Linton *et al.,* 1997).

Flufenoxuron is known to be highly effective, acting against many agricultural pests with a relatively low toxicity to mammals and natural enemies (Su and Scheffrahn, 1996). Flufenoxuron has been tested on some insect species, such as *Culex quinquefasciatus* and *Aedes albopictus* (Ho *et al*., 1990); *Spodoptera exempta* and *S. littoralis* (Fisk and Wright, 1992); *Blattella germanica* (Reid *et al*., 1992);

Rhyzopertha dominica, *Sitophilus oryzae, Oryzaephilus surinamensis* and *Tribolium castaneum* (Elek and Longstaff, 1994).

Biological parameters effects:

Body length

In the present study, topical application of neem extract onto prepupae led to pronounced suppression in the maximal body length. Table 2 and Fig.1&2 summarizes the previous results, males and females of neem-treated red palm weevils groups were generally shorter and smaller than the control. The difference between males and females was similarly significant in both control and neem-treated weevils, independently from the applied dose of neem. However, in comparison to controls, both male and female body lengths were affected similarly and significantly by neem.

However. these reduction was more obvious in females than in males especially at the higher doses. This was reflected on the growth because its smallest index was calculated by the highest dose-level and *vice versa.* Also, Azt enhanced the development because the prepupal duration was significantly shortened especially at the higher dose-levels. Lastly, Neem-based products have been reported to cause toxicity and growth regulation, reductions in larval survival and weight, effects in other coleopteran insects (Schluter 1985, Schmutterer and Singh 1995, Trisyono and Whalon, 1999; Weathersbee and Tang, 2002).

Table 2: Effect of different concentrations of neem & flufenoxuron (ppm) applied topically onto the prepupae on the pupal duration, sex ratio and body length of the Red Palm Weevil, *Rhynchophorus ferrugineus*.

Dose (ppm)		Pupal duration (days ± SEM)		Emergence sex ratio (male\female)		Body length (cm ± SEM) male		female	
(Control)		27.5±0.		1:1		3.46±0.04		3.61±0.04	
N	F	N	F	N	F	N	F	N	F
50	0.05	23.4± 0.8	25.1± 0.0	1:1.38	1:1.3	3.22 ± 0.04	3.03± 0.0	3.46± 0.05	3.36± 0.0
100	0.1	21.1± 0.3	20.4± 0.0	1:1.22	1:1.8	3.14 ± 0.04	2.75± 0.0	3.38± 0.05	3.17± 0.0
500	0.5	18.1± 0.3	16.0± 0.0	1:1.33	1: 2	2.93 ± 0.03	2.6± 0.0	3.2± 0.07	3.2± 0.0

Emerged control female red palm weevils were longer and greater than males. This statistically difference was kept after treatment with flufenoxuron (Tab.2). However, the difference between males and females was not similar in flufenoxuron-treated weevils as in controls. The significance increased on increasing the applied dose of flufenoxuron.

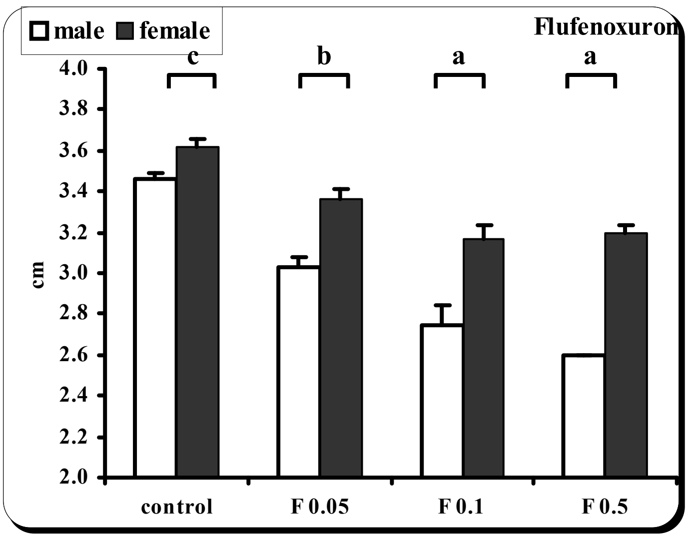

Fig.1. Effect of different concentrations of flufenoxuron (ppm) on the difference in body length (cm) between male and female RPW. Values are expressed as mean ± SEM. Statistical analysis: a = p< 0.00001; b = p<0.001; c = p<0.01.

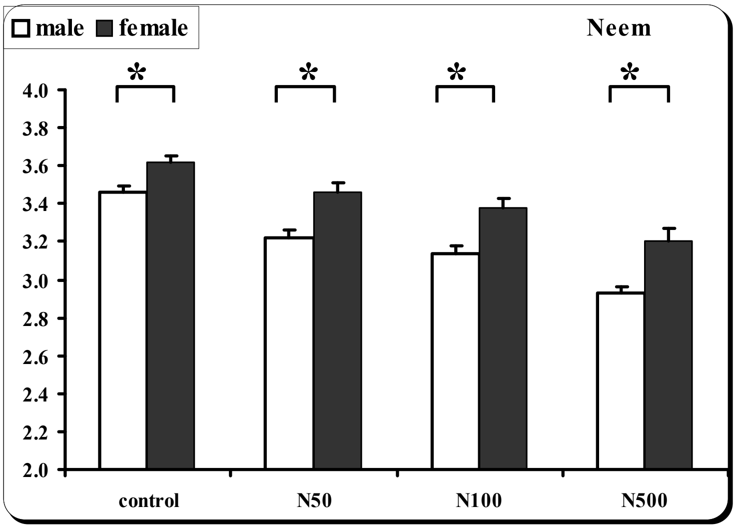

Fig. 2. Effect of different concentrations of neem, N (ppm) on the difference in body length (cm) between male and female RPW. Values are expressed as mean ± SEM. Statistical analysis: * = p<0.01 between each male and female value.

This may also show that the effect on males was higher than that on females. In the present study *R. ferrugineus*, prepupal treatment with flufenoxuron resulted in decreasing of body length after emergence. It is similar to, loss of weight by some other chitin inhibitors, in general, had been reported for several insect species, such as *M. domestica* (Bakr *et al*., 1991); *Spodoptera exempta, S. exigua, Mamestra brassicae* and *Galleria mellonella* (Smagghe and Degheele, 1994), as well as *S. littoralis* (Ghoneim *et al*., 1998). Diflubenzuron, the pioneer of benzoylphenyl ureas, affects the development, among other vital criteria, of several insect species (Soltani *et al*., 1984, 1996; Basiouny, 2000; Chebira *et al*., 2000).

Duration of pupation

Fig. 3 & 4 and table 2 shows that neem and flufenoxuron applications significantly reduced the pupation period of the red palm weevil. There was an inversely proportional relationship between neem concentration and pupation period. The period of pupation decreased as the neem concentration increased. This period reduction was significant between all treated groups.

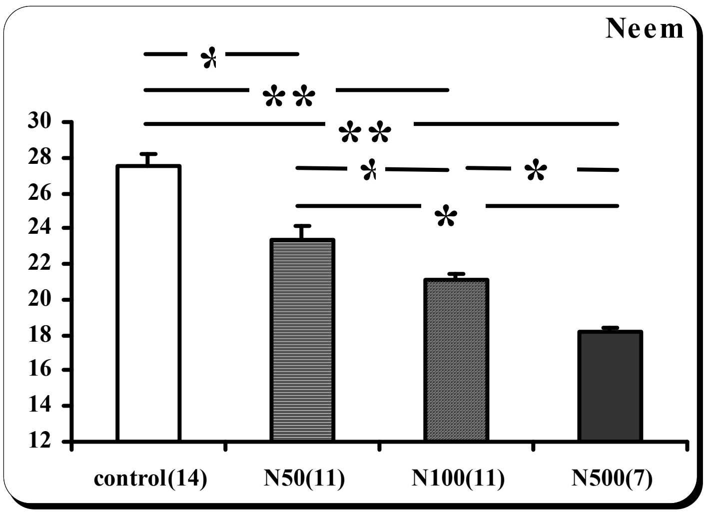

Fig. 3. Effect of different concentrations of neem, N (ppm) on the duration of pupation of RPW. Values are expressed as mean ± SEM. Number of samples (n) is shown in parentheses. Statistical analysis: * = $p < 0.005$; ** = $p<0.0001$; *** = $p<0.00000001$.

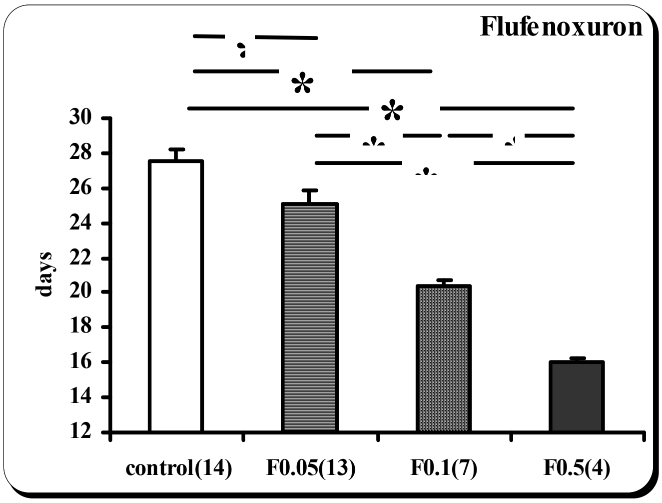

Fig. 4. Effect of different concentrations of flufenoxuron (F, ppm) on the duration of pupation of RPW. Number of insect (n) is shown in parentheses. Values are expressed as mean ± SEM. Statistical analysis: * = p< 0.05; ** = p<0.0001; *** = p<0.00000001.

Similar results had been obtained by using plant extracts and dimilin or its analogues against *S. littoralis* (Gamal *et al*., 1994), *Tribolium confusum* (El-Sayed *et al*., 1984), *M. domestica* (El-Kordy *et al*., 1989), *C. tarsalis* (Mulla *et al*., 1989), some mosquito species (Vasuki and Rajavel, 1992; Montada *et al*., 1994), some muscoids (Ghoneim *et al*., 1992; Abou El-Ela *et al*., 1995; Griffth *et al*., 1996; Basiouny, 2000).

Sex Ratio:

As clearly shown from the data in Table (2) that neem affected male more than female during their growth. The number of emerged females were more than males, an effect which did not increase with the increase of neem dose. The results of the three applied concentrations were similar.

Also It was observed that after flufenoxuron treatment, the number of emerged females were more than the number of male (tab. 2). This effect, in contrast to the results of neem, increased with the increase of flufenoxuron dose. The ratio of females increased on increasing the dose, giving an indication that the mortalities were higher among males than females. These data indicated that neem and flufenoxuron was more affected on male than female emergence. Also, treatment with hexaflumuron caused the same results against *pectinophora gossypiella* (El-Barkey *et al.* 2009).

Gonadal histopathological effects

Ovary:

As shown in table (3) some ovarian follicles in the germarium of RPW treated with lower concentrations of neem and flufenoxuron revealed atrophy and degeneration (Fig. 6). This effect was severe in some RPW treated with higher concentrations (Fig. 7). It was also associated with massive hyperplasia in the nurse epithelial cells.

Table 3: Semi-quantitative histopathological effect of different concentrations of neem & flufenoxuron on the ovary of the red palm weevil. (-) = no effect, (+) = mild effect, (++) = moderate effect, and (+++) = severe effect.

Histological changes	Control	Neem (ppm)			Flufenoxuron (ppm)		
		50	100	500	0.05	0.1	0.5
Trophocytes (Nurse cells)							
Cell shape	cuboidal	irregular	irregular	irregular	variable	variable	variable
Appearance							
▪ *Hyperplasia*	---	+	++	+++	+++	+	---
▪ *Degeneration*	---	++	++	+++	+	++	+++
▪ *Pyknosis*	---	---	---	---	---	+	++
▪ *Necrosis*	---	+	++	+++	---	+	++
Abnormalities of Ovarian follicles							
• *Atrophied*	---	---	+	++	---	---	+
• *Absorbed*	---	---	---	+	---	---	---
• *Cytoplasm heterogeneity in oocyte*	---	---	+	++	---	---	+++
Yolk granules							
▪ *Size heterogeneity*	---	---	---	---	---	++	++
▪ *Distribution heterogeneity*	---	+	++	+++	+	++	+++
▪ *Reduction in quantity*	---	+	++	+++	+	++	+++

In higher concentrations treated RPW, some oocytes appeared degenerative with abnormal distribution of yolk granules and vacuolization of cytoplasm was observed (Fig. 8). This effect was accompanied by moderate hyperplasia in the epithelial layer of nurse cells. Some of those cells were flattened instead of cuboidal.

The most outstanding results found in both of the three concentration treatments were the hyperplasia in the surrounding trophic nurse cells and the decrease in the amount of yolk granules of the mature ovarian follicles. (Fig. 9) (Fig.10). The above mentioned results were summarized in a semi-quantitative manner in table 3. The table clarifies the graduality of the histopathological effect of different concentrations of neem on the ovary of the red palm weevil.

In the present study, ovaries from newly emerged adult of *R. ferrugineus* prepupally treated with both neem and flufenoxuron exhibited ovarian developmental retardation. The prominent features of retardation involved increases in oocytes resorption, delay of follicle and oocytes development and destruction of follicular epithelium. The delay of oocytes development included abnormal distribution and size and regression in the accumulation of yolk granules. This may result in a decrease in fecundity and egg viability of the females. The destruction of follicular epithelium involved degeneration, hyperplasia and necrosis in the follicular cells.

Similar ovarian histopathological observations were reported by many investigators: Lutfallah *et al*. (1986) after irradiation on the ovary of *Agrotis ipsilon*, Shalaby *et al*. (1987) in juvenoid-treated *Spodoptera littoralis*, Younes *et al*. (1994) in botanical extracts-treated *Spodoptera littoralis*, and Mohamed *et al*. (2000) in Dimillin, Malathion and Cypermethrin-treated *Spodoptera exigua*.

On the other hand, the maturation of insect eggs is dependent, among other factors, on the materials taken up from the surrounding haemolymph (Telfer *et al*., 1981), and by materials synthesized by the ovary *in situ* (Indrasith *et al*., 1988). These materials include proteins, lipids, and carbohydrates all of which are required for embryogenesis (Kanost *et al*., 1990).

Since ovarian protein content was decreased in insect growth regulator diflubenzuron-treated female of *C. pomonella* (Soltani and Mazouni, 1992), also, significant decrease in ovarian protein content in chlorfluazuron- treated female was reported by Perveen and Miyata (2000) and in RH-0345 and pyriproxylen-treated mealworm pupae (Aribi *et al*., 2001), therefore, the ovarian and oocyte growth development and vitellogenesis retardation showed in the present study may be due to the alteration in ovarian protein content. This possibility is also suggested by Perveen and Miyata (2000) who suggested the interference of chlorfluazuron with vitellogenesis through the lack of protein in ovarioles.

Testis

In general, the testes of the neem-treated RPW were smaller in size than the control. The testicular follicles were also smaller and narrower, even in healthy appeared follicles.

In RPW treated with lower concentrations of neem and flufenoxuron some testes had narrower follicles with reduction in the cellular content (Fig. 13, 14) , with higher concentrations of neem and flufenoxuron the testicular follicles revealed pronounced disorganization characterized by decreasing of the cellular content less than previous (Fig. 16). The cysts of spermatocytes and spermatogonia were severely reduced in number (Fig.15). The above mentioned results were summarized in a semi-quantitative manner in table 4. The table clarifies the graduality of the histopathological effect of different concentrations of neem on the ovary of the red palm weevil.

Table 4: Semi-quantitative histopathological effect of different concentrations of neem & flufenoxuron on the testis of the red palm weevil in comparison to control. (-) = no effect, (+) = mild effect, (++) = moderate effect, and (+++) = severe effect.

Histological changes	Control	Neem (ppm)			Flufenoxuron (ppm)		
		50	100	500	0.05	0.1	0.5
Atrophy of testis	---	+	+	++	+	++	+++
Testicular follicles							
• *reduction of width*	---	+	+	++	---	+	++
• *reduction of cellular content*	---	+	++	++	---	++	+++
• *absence of cyst sheath*	---	+	+	++	---	+	++
Reduction of cysts							
• *Spermatogonia*	---	---	+	++	+	++	++
• *spermatocytes*	---	+	+	++	+	+++	+++
• *Spermatids*	---	---	+	++	+	++	++
• *Spermatozoa*	---	---	++	++	+	++	++
Reduction of divisions	---	+	++	++	+	+++	+++
Spermatogonia							
• *Reduction in number*	---	+	+	++	+	+	++
• *scattering*	---	---	++	++	+	++	++
• *necrosis*	---	---	---	---	---	+	+
Spermatocytes							
• *Reduction in number*	---	+	++	++	---	++	+++
• *scattering*	---	++	++	+++	---	++	+++
• *necrosis*	---	---	+	++	---	---	+++
Spermatozoa							
• *Reduction in number*	---	+	++	+++	---	+	+++
• *scattering*	---	+	++	+++	---	+	+++
• *necrosis*	---	---	+	+	---	+	+++

The present study revealed that topical application of both Azt and flufenoxuron onto prepupae of *R. ferrugineus*, resulted in testicular architectural defects, which were dose-dependent, increased with the increasing of the dose. Neem extract had the higher effect. The prominent architectural defects were germ cells degeneration, displacement of testicular cysts and depopulation of germ cells, in addition to the decrease in the number of spermatozoa especially in neem treated weevil.

The observed reduction in the spermatozoal number is in agreement with the study of Linton *et al.* (1997) who observed poisoning and arrest in spermatogenic meiosis at metaphase I in the testis of *S. gregaria* treated with azadirachtin. The histological destruction detected in the present study are similar to that demonstrated in different insects treated with other IGRs and botanical extracts (Wilson and Hays, 1969; Younes *et al.*, 1994; Mohamed *et al.*, 2000).

REFERENCES

Abou El-Ela, R. G.; Helmy, . N. M.; EI-Monairy, O. M. and. Salah, H. (1995): Effect of certain plant extracts on some biochemical aspects of house fly larvae *Musca domestica* (Dipt., Muscidae). *Bull. Ent. Soc. Egypt, 22: 17 -25.*

Abuzuhairah, R. A.; Vidyasagar, P. S. P. V. and Abraham, V. A. (1996): Integrated pest management of red palm weevil *Rhynchophorus ferrugineus* in date palm plantations of the Kingdom of Saudi Arabia. Proceedings, International Congress of Entomology, Firenze, Italy, 25-31 August 1996, p. 541.

Aribi, N.; Lakbar, S.; Smagghe, G. and Soltani, N. (2001): Comparative action of RH-0345 and Pyriproxfen on molting hormone production and protin analysis in mealworm pupae. *Med. Rijksun. Gent. Fak. Landbouwked Toegep. Boil. Wet., 66: 445-454.*

Bakr, R. F. A.; Abdel Razik, N. A.; Hamed, N . S. and Guneidy, N. M. (1991): Physiological effect of some insect growth regulators on the respirometric measurements, total protein and free amino acids of the house fly *Musca domestica. Ain Shams Sci. Bull., 28: 169-183.*

Basiouny, A. L. (2000): Some physiological effects of certain insect growth regulators (IGRs) on the false stable fly, *Muscina stabulans* (Fallen.) (Diptera : Muscidae). *Ph. D. Thesis, Fac. Sci., Al-Azhar Univ., Cairo.*

Chebira, N.; Soltani-Mazouni and Soltani, N. (2000): Evaluation of two insect growth regulators, Flucycloxuron and Diflubenzuron, affecting *in vivo* and *in vitro* the ecdysteroid production in *Tenebrio –molito.* (Coleoptera). *J. Egypt Ger. Soc. Zool., 32: 75-89.*

Drury, A.R. and Wallington, E.A. (1980): Carleton's histological techniques 5th ed.

El-Barkey, M. N.; Amer A. E. 2 and Kandeel, A. M. (2009): Ovicidal Activity and Biological Effects of Radiant and Hexaflumuron Against Eggs of Pink Bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). Egypt. Acad. J. biolog. Sci., 2(1): 23 – 36.

Elek, J. A. and Longstaff, B. C. (1994): Effect of chitin-synthesis inhibitors on stored-product beetles. *Pestic. sci 40: 225-230.*

El-Kordy, M. W.; Abbassy, M. A.; El-Gayar, F. H. and Waston, W. M. (1979): Biological activity of the chitin synthesis inhibitor, dimilin, against larval instars of Musca domestica L. *Alex. J. Agric. Res., 27: 655-658.*

El-Sayed, M. A.; Abdel Razik, F. and Kandil, M. A. (1984): Biological activity of insect growth regulator Triflumuron against *Tribolium confusum. Bull. Ent. Soc. Egypt, 14: 171-175.*

Faleiro, J. R. 2006. A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera:Rhynchophoridae) in coconut and date palm during the last one hundred years. Int. J. Trop. Insect Sci. 26: 135–154.

Fisk, T. and Wright, D. J. (1992): Response of *Spodoptera exempta* (Walk.) larvae to simulated field spray applications of acylurea insect growth regulators with observations on cuticular uptake of acylureas. *Pestic Sci., 35: 321-330.*

Gamal, E. S.; Abo Elgar, G. E.; Radwan, S. A.; EI-Bermawy, Z. A. and Zidan, L. T. M. (1994): Biological activity of Avermctin B1, β-Bxotoxin of *Bacillus thuringiensis* and Diflubenzuron against cotton leafworm (Lepidoptera: Noctuidae). *Bull. Entomol. Soc. Egypt, 21: 11-23.*

Ghoneim, K. S.; Bream, A. S. and Mohamed H. A. (1998): Bioactivity the ecdysteroid agonist. Tebufenozide (RH-5992) on the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd). (Noctuidae: Lepidoptera). *Al-Azhar Bull. Sci, 9: 947-963.*

Ghoneim, K. S.; Essa, N.; Abul-Ela; R. G.; Al-Morsy, A. A. and Nassar, M. I. (1992): Efficacy of Triflumuron (Bay Sir 8514). for remedial control of the false stable fly *Muscina stabulans* (Fallen) (Diptera: Muscidae). *Al-Azhar Bull. Sci., 3: 687-693.*

Ghoneim, K. S.; Mohamed, H. A. and Bream, A. S. (2000): Efficacy of the neem seed extract, NeemAzal on growth and development of the Egyptian Cotton Leafworm, Spodoptera littoralis (Bosid) (Lepidoptera: Noctuidea). *J. Egypt. Ger. Soc. Zool., 33: 161-179.*

Griffith, B.; Michael, B. M. and Perry, A. S. (1996): Effects of aerial application of Diflubenzuron on emergence and flight of adult aquatic insects. *J. Econ. Entomol., 89: 442-446.*

Ho, C. M., Wu, S. H. Wu, C. C. (1990): Evaluation of the control of mosquitoes with insect growth regulators. *Gaoxiong Yi Xue Ke Xue Za Zhi., 6: 366-74.*

Indrasith, L. S.; Sasaki, T. ;Yaginuma, T. and Yamashita,O. (1988): Theoccurrence of a premature form of egg-specific protein in vitellogenic follicles of *Bombyx mori. J. Comp. Physiol. 158: 1–7.*

Isman, M. B.; Koul, O.; Luczynsk, A. and Keminski, J. (1990): Insecticidal and antifeedant bioactivities of neem oil and their relationship to azadirachtin content. *J. Argic. Food Chem., 38: 1406-1411.*

Kanost, M. R.; Kawooya, J. K.; Law, J. H.; Ryan, R. O.; Van Heusden, M. C. and Ziegler, R. (1990): Insects haemolymph proteins. In Evans,P. D. and Wigglesworth, V. B. (eds.): Advances in Insect Physiology, Academic, London. pp. 299–396.

Linton, Y. M.; Nisbet, A. J. and Mordue, A. J. (1997): The effect of azadirachtin on the testes of desert locust, *Schistocerca gregaria* (Forskal). *J. Insect Phsiol., 43: 1077-1084.*

Linton, Y. M.; Nisbet, A. J. and Mordue, A. J. (1997): The effect of azadirachtin on the testes of desert locust, *Schistocerca gregaria* (Forskal). *J. Insect Phsiol., 43: 1077-1084.*

Lutfallah, A. F.; Abdel-Meneam, E. M. and Bishara, S. I. (1986): Histopathological effects of irradiation on the ovary of the cutworm *Agrotis ipsilon* (Hufn.). *Bull. Ent. Soc. Egypt., 14: 343-357.*

Lutfallah, A. F.; Abdel-Meneam, E. M. and Bishara, S. I. (1986): Histopathological effects of irradiation on the ovary of the cutworm *Agrotis ipsilon* (Hufn.). *Bull. Ent. Soc. Egypt., 14: 343-357.*

Mitsui, T. (1985): Chitin synthesis inhibitors: benzophenylurea insecticides: a review. *Jpn. Pestic. Inform. 47: 7(cf. Basiouny, 2000).*

Mitsui, T. (1985): Chitin synthesis inhibitors: benzophenylurea insecticides: a review. *Jpn. Pestic. Inform. 47: 7(cf. Basiouny, 2000).*

Mohamed, H. A.; Bream, A. S. and Ghneim, K. S. (2000): Developmental and reproductive responses of house fly Musca domistica (Diptera; Muscidae) to the neem seed extracts, NeemAzal. *J. Egypt. Ger. Soc. Zool., 31: 235-249.*

Mohamed, H. A.; Bream, A. S. and Ghoneim, K. S. (2000): Developmental and reproductive responses of house fly Musca domistica (Diptera; Muscidae) to the neem seed extracts, NeemAzal. *J. Egypt. Ger. Soc. Zool., 31: 235-249.*

Montada, D.; Rajavel, A. R. and Vasuki, V. (1994): Use of Hexaflumuron as insect growth regulator in the control of *Aedes albopictus* (Skuse). Southeast-Asian. *J. Trop. Med. Public. Health, 26: 374: 377.*

Moura, J. I. L.; Resende, M. L. B. and Vilela, E. F. (1995): Integrated pest management of *Rhynchophorus palmarum* (L). (Coleoptera: Curculionidae) in oil palm in Bahia. *Anais da Sociedade Entomoloica do Brazil 24, 501-506.*

Mulla, M. S.; Darwazeh, H. A. and Schreiber, E. T. (1989): Impact of. New insect growth regulators and their formulations on mosquito larval development and food water habitats. *J. Am. Mosq. Control Assoc., 5: 15-20.*

Murphy, S. T. and Briscoe B. R. (1999): The red palm weevil as an alien invasive: biology and the prospects for biological control as a component of IPM. *Biocontrol News and Information, 20: 35-46.*

Nassar M. and Abdullah M. (2001): Evaluation of Azadirachtin for control of the red palm weevil *Rhinchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae). *J. egy. Ger. Soc. Zool., 36: 163-173.*

Osman, M. Z. (1993): Effects of neem seed extract on growth and development of larvae of *Pieris brassicae* L. (Lep : Pieridae). *J. Appl. Entomol, 115: 254-258.*

Osman, M. Z. and Bradley, J. (1993): Effects of neem seed extract on *Pholeastor (Apanteles) glomeratus* L. (Hym., Braconidae*),* a parasitoid of *Pieris brassicae* L. (Lep., Pieridae). *J. Appl. EntomoL., 5: 259-265.*

Oxford University Press. London; pp. 140.

Perveen, F. and Miyata, T. (2000): Effects of sublethal dose of chlorfluazuron on ovarian development and oogenesis in the common cutworm *Spodoptera litura* (Lepidoptera: Noctuidae*) Ann. Entomol. Soc. Am. 93: 1131–1137*

Reid, B. L.; Appel, A. G.; Demark, J. J. and Bennett, G. W. (1992): Oral toxicity, formulation effects, and field performance of flufenoxuron against the German cockroach (Dictyoptera: Blattellidae). *J. Econ. Entomol., 85: 1194-1200.*

Schluter, U.; Bidmon, H. J. and Grewe, S. (1985): Azadirachtin affects growth and endocrine events in larvae of the tobacco horn worm *Manduca Sexta. J. Insect Physiol., 31: 773-777.*

Schmutterer, H. and Singh, R. P. (1995): List of insects susceptible to neem products, in: Schmutterer, H. (ed.): The neem tree, source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other. Purposes. VCH Publishers, Weinheim, New York; Basel Cambridge, Tokyo.

Schroeder, D. R. and Nakanishi, K. (1987) Simplified isolation procedure for azadirachtin. J. Nat. Prod. 50: 241-244.

Shalaby, A. R.; Hashem, H. D.; El-Zayat, M. R. and Swidan, M. H. (1987): Effect of the Jevenoid Zr-520 on ovarian development of *Spodoptera littoralis* (Boisd) moths. *Bull. Fac. Sci. Alex. Univ., 27: 154-168.*

Smagghe, G. and Degheele, D. (1994): Action of a novel nonsteroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. *Pestic. Sci, 42: 85-92.*

Soltani, N. (1984): Effects of ingested diflubenzuron on the longevity and peritrophic membrane of adult mealworms *Tenebrio molitor* (L.). *Pestic. Sci. 15: 221–225.*

Soltani, N., and Mazouni, N. S. (1992): Diflubenzuron and oogenesis in the codling moth, *Cydia pomonella* (L.). *Pestic. Sci. 34: 257–261.*

Soltani, N.; Mazouni, N. S. and Delachambre, J. (1996): Evaluation of Triflumuron, a benzoyphenylurea derivative, on *Tenebrio molitor* pupae (Col., Tenebrionidae): effects on cuticle. *J. Appl. Entomol. 120: 627- ٦٢٩.*

Su, N. Y. and Scheffrahn, R. H. (1996): Comparative effect of two chitin synthesis inhibitors, hexaflumuron and lufenuron, in a bait matrix against subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol. 89: 1156-1160.*

Trisyono, A. and Whalon, M. E. (1999): Toxicity of neem applied alone and in combinations with *Bacillus thuringiensis* to Colorado Potato Beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 92: 1281-1288.

Vasuki, V. and Rajavel, A. R. (1992): Influence of short-time exposure to an insect growth regulator, Hexaflumuron, on mortality and adult emergence of vector mosquitos. *Mem. Inst. Oswaldo Cruz., Riode Janeiro, 87: 275-283.*

Weathersbee, A. A. and. Tang, Y. Q. (2002): Effect of Neem Seed Extract on Feeding, Growth, Survival, and Reproduction of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *J. Econ. Entomol. 95: 661-667.*

Wilson, J. A. and Hays, S. B. (1969): Histological changes in the gonads and reproductive behaviour of house flies following treatment with chemosterilants p,p-Bis (1-aziridinyl)-N-(3-methoxypropyl) phosphinothioc amid. *J. Econ. Entomol. 62: 690-692.*

Younes, M. W. F.; Ryan, M. F. and Emara, T. E. A. (1994): Histopathological effects of gamma radiation on the testis and ovary of the greater wax moth *Galleria mellonella* (L). (Lepidoptera: Galleriidae). *J. Urion. Arab. Biol., 1: 209-238.*

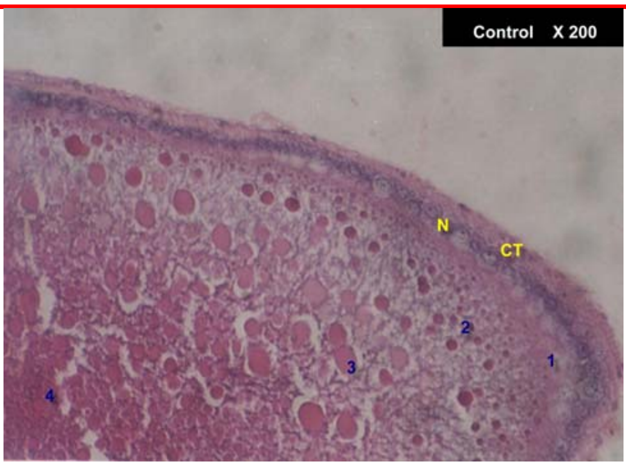

Fig. 5: Photomicrograph of longitudinal section of a vitellarium of control RPW showing heterogeneous cytoplasm and different sphere of yolk granules.

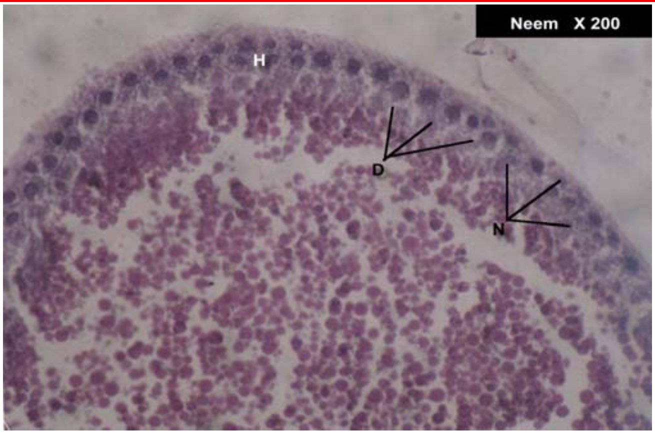

Fig. 6: Photomicrograph of longitudinal section of an ovariole of 50 ppm neem treated RPW showing mature ovarian follicle with epithelial cell hyperplasia, mild decrease in yolk granules, degenerative and necrotic cells.

Fig.7. Photomicrograph of longitudinal section of mature ovarian follicle of 500 ppm neem treated RPW showing epithelial cell hyperplasia (H) and clumped yolk granules (CY).

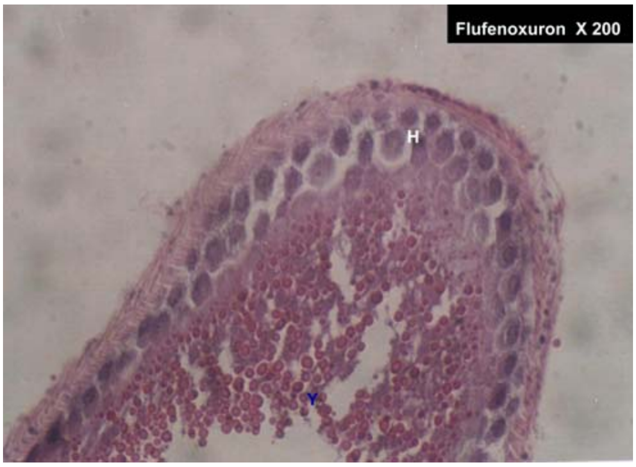

Fig.8. Photomicrograph of longitudinal section of an ovariole of 0.05 ppm flufenoxuron treated RPW showing a vitellarium with epithelial cells hyperplasia, stratified polygonal shaped cells and unequal distribution of yolk granules.

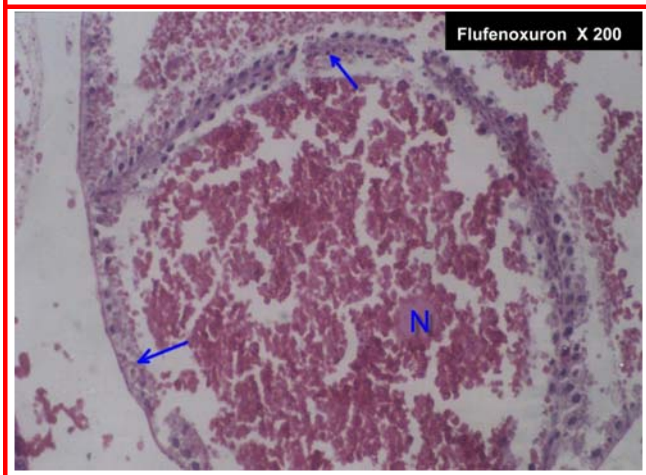

Fig.9. Photomicrograph of longitudinal section of an ovariole of 0.05 ppm flufenoxuron treated RPW showing small mature ovarian follicle with hydropic degenerative, some pyknotic and necrotic epithelial cells (arrow) and displacement of nuclei of epithelial cells. Note the healthy appearance of the nucleus, N.

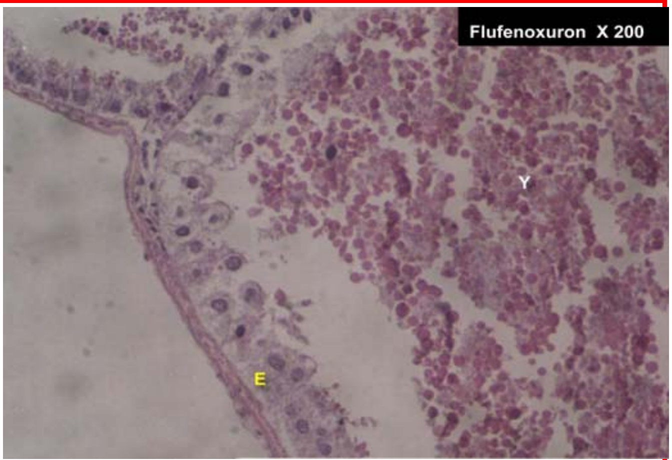

Fig.10. Photomicrograph of longitudinal section of an ovariole of 0.5 ppm flufenoxuron treated RPW showing mature ovarian follicle with moderately reduced yolk granules and degenerated, pyknotic or necrotic columnar epithelial cells.

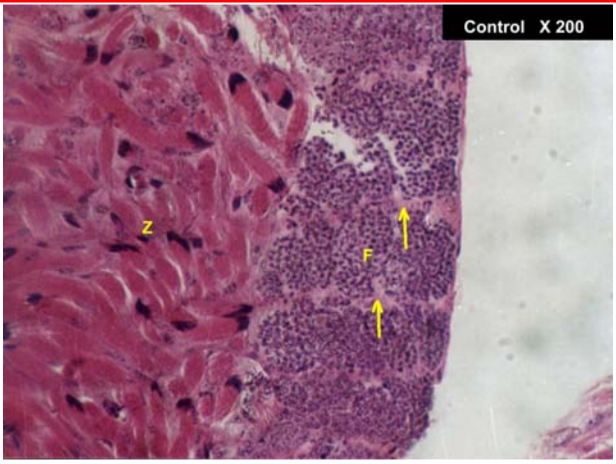

Fig. 11. Photomicrograph of longitudinal section of testis of control RPW showing testicular follicles (F) separated by septa (arrows) with the spermatogonia in the outer rim and spermatozoa (Z) towards the centre.

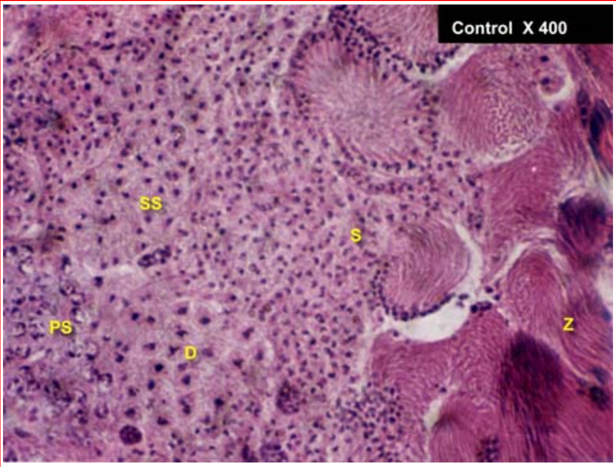

Fig.12. Photomicrograph of transverse section of testis of control RPW showing cysts with different stages of germ cells development.

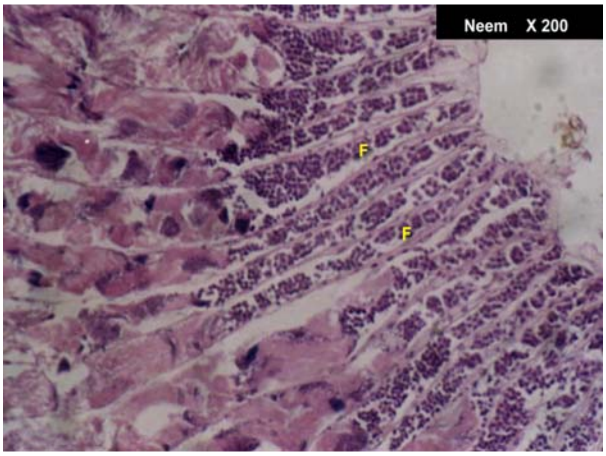

Fig. 13. Photomicrograph of longitudinal section of testis of 50 ppm neem treated RPW showing narrow, mild testicular follicles atrophy.

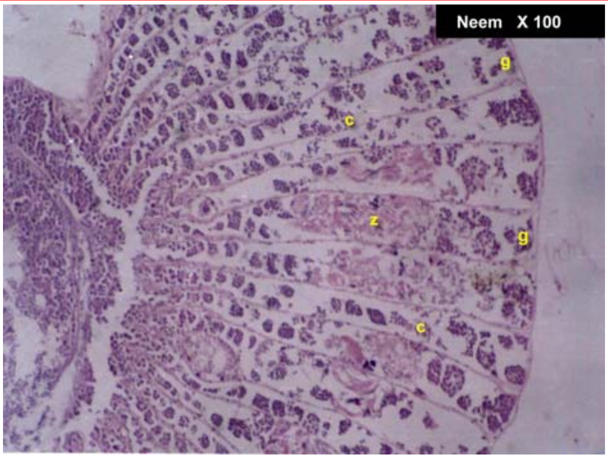

Fig. 14. Photomicrograph of longitudinal section of testis of 500 ppm neem treated RPW showing atrophied testicular follicles with few numbers of spermatogonia (g) spermatocytes (c) and spermatozoa (z) within the cysts.

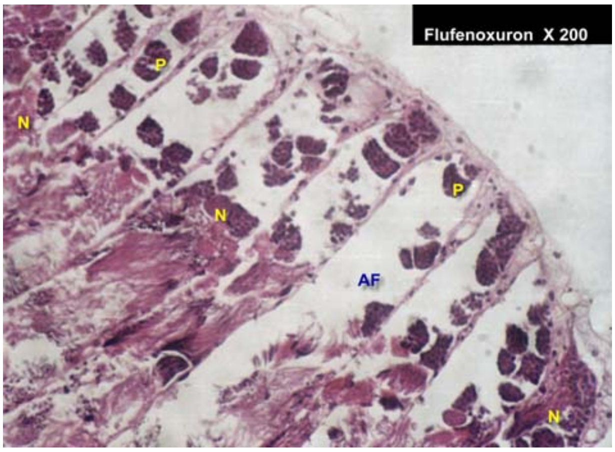

Fig. 15. Photomicrograph of longitudinal section of testis of 0.5 ppm flufenoxuron treated RPW showing testicular follicles with severe atrophy (AF), few cysts of unhealthy pyknotic(P) and necrotic spermatic cells (N)

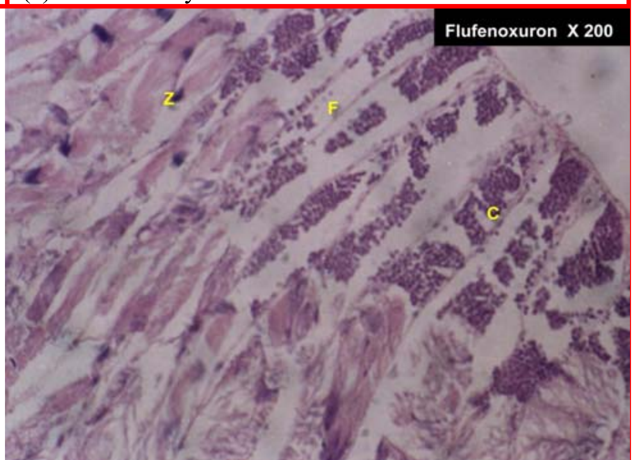

Fig. 16. Photomicrograph of longitudinal section of testis of 0.05 ppm flufenoxuron treated RPW showing narrow testicular follicles with few number of cellular contents. (F) testicular follicle; (C) spermatocytes ; (Z) spermatozoa.

ARABIC SUMMARY

التأثيرات البيولوجية والنسيجية الممرضة لبعض عوامل السمية ضد سوسة النخيل الحمراء رينكوفورس فيروجينيس.

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٣- كلية العلوم – فرع دمياط – جامعة المنصورة – مصر

تعتبر سوسة النخيل الحمراء رينكوفورس فيروحينيس أهم الآفات الحشرية لأشجار نخيل البلح فى الشرق الأوسط ودول الخليج. تهدف هذه الدراسة الى تقييم الأثر التراكمى لعاملين من عوامل السمية هما المستخلص النباتى الطبيعى للنيم (neem J) وأخر مخلق الفلوفينوكسيرون على بعض المعايير البيولوجية والتركيب النسيجى لكل من المبيض والخصية لسوسة النخيل الحمراء. وتم ذلك بمعالجة طور ما قبل العذراء بتركيزات مختلفة هى ppm ٥٠ و ١٠٠ و ٥٠٠ للنيم ، 0,5 , 0,1 ppm 0,05للفلوفينوكسيرون.

أثبتت الدراسة الحالية أن كل من مستخلص النيم والفلوفينوكسيرون أحدثا اضطراباً فى عملية نمو ونماء وتكاثر سوسة النخيل الحمراء. فظهرت اضطرابات النمو فى صورة معدل وفيات عال ، قصر طول الجسم ، زيادة فترة التعذر ، اختلال نسبة ظهور الجنس بفروق معنوية ، وكذلك التشوهات المورفولوجية وذلك فى تناسب طردى مع التركيزات المستخدمة.

أوضحت الدراسة أيضا أن كل من النيم والفلوفينوكسيرون أحدثا تأثيراً على التركيب النسيجى للمناسل ، وإنتاج الأمشاج الأنثوية والذكرية. وكان ذلك من خلال نقصان حبيبات المح وتراكمها فى مركز البويضة ، وامتصاص لبعض البويضات الناضجة وفرط تنسج فى الخلايا المغذية وقد لوحظ تأثير أكبر لمعاملات النيم عن معاملات الفلوفينوكسيرون. أما التأثير على التركيب النسيجى للخصية فقد ظهر فى صورة قلة عدد وعدم تعضى الحويصلات الخصوية ونقصان المحتوى الخلوى وعدد الحيوانات المنوية فى بعض الحويصلات.

من نتائج هذه الدراسة يتضح الدور الفعال لكل من النيم والفلوفينوكسيرون على المعايير البيولوجية والجهاز التناسلى الذكرى والأنثوى لسوسة النخيل الحمراء مما يمهد للمساهمة بهما فى برامج مكافحة هذه الآفة.