Effects of azadirachtin on mortality rate and reproductive system of the grasshopper Heteracris littoralis Ramb. (Orthoptera: Acrididae)

Authors: Ghazawi, N. A., El-Shranoubi, E. D., El-Shazly, M. M., and Rahman, K. M. Abdel

Source: Journal of Orthoptera Research, 16(1) : 57-65

Published By: Orthopterists' Society

Effects of azadirachtin on mortality rate and reproductive system of the grasshopper Heteracris littoralis Ramb. (Orthoptera: Acrididae)

N.A. GHAZAWI, E.D. EL-SHRANOUBI, M.M. EL-SHAZLY, K.M. ABDEL RAHMAN

Entomology Department, Faculty of Science, Cairo University, Egypt. Email: kabdelrahmn@yahoo.com

Abstract

Male and female nymphs of Heteracris littoralis were topically treated with serial concentrations of azadirachtin. Effects on mortality, development, oogenesis and spermatogenesis were observed. Mortality was dose-dependent; fourth and fifth instars died about the time of ecdysis. Overaging took place at low concentrations. Ovaries in treated adult females showed complete shrinkage with abolished oocyte growth, and the number of deposited egg pods/female decreased from 4 to 9 in normal females, to 1 to 3 pods in treated insects. Deformation in sperm tubes was observed in treated males. Electronmicrographs revealed disintegration and destruction in follicular cells and mitochondria in females. In males the testicular epithelia and the spermatids completely disintegrated. Treatment with higher doses inhibited cyst formation around the spermatogonia.

Materials and Methods

Adults and nymphs of Heteracris littoralis Ramb. were collected from Abou Rawash district (Giza Governorate), Egypt. A laboratory stock was reared in electrically heated wooden cages (25×25×25 cm) at a constant temperature of 30±1°C, with fluctuating relative humidity (50 to 70%). Insects were fed clover, Trifolium alexandrinum, from November to May and then fresh leaves of Sesbania sesban. Cages were supplied with suitable ovipositional pots for egg deposition which were kept moistened. Hatched hoppers were transferred to 20×25-cm cylindrical glass jars. After the fourth or fifth moult, hoppers were released in the large cages.

Novel problems of pest resistance to pesticides enhanced interest in botanical insecticides during recent decades. Extracts from the neem tree Azadirachta indica, of which azadirachtin is the most important active principle, received the attention of many research workers. It was found to exhibit deterrent, antiovipositional, antifeedant, growth-disrupting (growth-regulating), fecundity and fitness-reducing properties on many insect species.

The disruption of insect development and behavior by azadirachtin was observed in Orthoptera (Schmutterer et al. 1993, Linton et al. 1997), Mallophaga (Habluetzel et al. 2006), Hemiptera (Nisbet et al. 1992), Isoptera (Steets 1976), Coleoptera (Steets 1976, Klocke & Barnby 1989), Lepidoptera (Nathan et al. 2006 a, b), and Diptera (Lucantoni et al. 2006).

In addition, azadirachtin is an effective sterilant. After uptake of the active material, females of some insect pest species were sterilized to various degrees, sometimes completely (Steets 1976, Schmutterer 1987), where juvenoids and ecdyysteroids strongly affected fecundity and/or egg sterility if applied during sensitive growth phases of target insects (Karnavar 1987, Tanzubil & McCaffery 1990). On the histological level, azadirachtin was found to inhibit oogenesis (Ghosh et al. 1999, Medina et al. 2004) and spermiogenesis (Linton et al. 1997, Abdel-Rahman et al. 2004).

The aim of the present work is to investigate some effects of azadirachtin on histological and ultrastructural levels of the gonads in H. littoralis. The economic importance and the pest status of this species have been documented by El-Shazly (1991).
insects and control groups were fixed in Carnoy's Formula I for 6 h. Specimens were washed with absolute alcohol, then hydrated with a descending alcoholic series and preserved in Formal saline solution (Abdel Rahman 1995). Specimens were dehydrated in ascending alcoholic series and cleared in xylene for a few seconds, infiltrated in three changes of paraffin wax (melting point 58°C) each lasting 20 min. Specimens were sectioned serially at 6 µ thick using a rotatory microtome (American Optical) and stained with Ehrlich haematoxylin and eosin.

For TEM observations, ovarioles and sperm tubes were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.3) at 4°C for 24 h, then washed in three changes of the fresh buffer. Specimens were post-fixed in 1% osmium tetroxide in the same buffer for 2 h at 4°C. Specimens were then washed in the same buffer and dehydrated in alcoholic series up to absolute alcohol. They were then removed to absolute alcohol, then hydrated with a descending alcoholic series and preserved in Formal saline solution (Abdel Rahman 1995). Specimens were dehydrated in ascending alcohol and acetone, followed by pure acetone for half an hour each. Infiltration took place in acetone and resin (Epon 812) 2:1 and 1:1. Semi-thin sections (1 µ thick) were obtained using a glass knife and were stained with toluidene blue for a few seconds. The specimens were examined under a normal light microscope and photographed. Ultra-thin sections (90 nm thick) were obtained using a diamond knife. Sections were put on a coppergrade mesh and stained with uranyl acetate and lead (90 nm thick) were obtained using a diamond knife. Sections were examined under a Joel JEM JE 1200EXII transmission electron microscope and photographed.

Results and Discussion

Effect of azadirachtin on mortality rates and molting

Treated 4th, 5th and 6th instars of female H. littoralis displayed dose-dependent mortality rates, as demonstrated in Fig. 1 and Table 1. Females of the 4th instar were significantly more sensitive, as shown by the LC₅₀ values in Table 1. According to the values of the moments (m₁, m₃), differences in the lethal doses between instars were significant at 95% confidence limits. Nymphs of the fourth and fifth instars died at about the time of ecdysis of the control individuals. At 50 ppm, 6th instar nymphs survived for a long period as over-aged individuals. The last instar in control groups lasted 9.8 ± 0.3 d, while over-aged nymphs survived for 15.16 ± 2.35 d (6 ± 1.5 d more than the control).

Azadirachtin inhibited molting when applied within the first two days after molting onto 6th instar nymphs. Nymphs were unable to undergo or terminate ecdysis. Treated nymphs remained in this condition, unable to shed their old cuticle successfully. Weak ec dysial movements that could last for several hours led to rupturing of old skin along the dorsal midline of the meso- and metathorax and over the pronotum and head capsule, but the shedding was not continued, leading finally to death. When insects were treated 3 to 5 days after the last molt, few adults were able to ecdyse, but shrinkage of wings was observed in emerging individuals.

These results agree with those of Sieber and Rembold (1983) on the molting and mortality of L. migratoria: a dose of 2 µg/g body weight completely prevented molting and prolonged intermolt period, which ranged between 8 and 60 days, compared to 6 (4th

---

Table 1. Effect of different concentrations of azadirachtin on the mortality rate of fourth, fifth and sixth female instar nymphs of H. littoralis.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Fourth instar</th>
<th>Fifth instar</th>
<th>Sixth instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>86.66</td>
<td>80.00</td>
<td>73.33</td>
</tr>
<tr>
<td>200</td>
<td>84.61</td>
<td>60.00</td>
<td>66.67</td>
</tr>
<tr>
<td>100</td>
<td>50.00</td>
<td>50.00</td>
<td>46.67</td>
</tr>
<tr>
<td>75</td>
<td>30.00</td>
<td>37.50</td>
<td>23.07</td>
</tr>
<tr>
<td>50</td>
<td>20.00</td>
<td>20.00</td>
<td>14.28</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>LC₅₀ (ppm)*</td>
<td>101.50 ± 2.5</td>
<td>126.12 ± 1.9</td>
<td>148.22 ± 2.8</td>
</tr>
<tr>
<td>Toxicty in mg/g body weight</td>
<td>7.51 ± 0.1</td>
<td>15.76 ± 0.2</td>
<td>28.00 ± 0.2</td>
</tr>
<tr>
<td>Moments (m₁,m₃) at 95% confidence</td>
<td>6.05 – 9.31</td>
<td>10.33 – 17.09</td>
<td>20.21 – 33.21</td>
</tr>
<tr>
<td>SE Slope</td>
<td>0.14</td>
<td>0.16</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*LC₅₀ was estimated according to Abbott (1925) and Finney (1971).
** LC₅₀ values followed by different letters are significantly different from each other based on 95% confidence limits.

---

Fig. 1. Regression lines representing the mortality rate of 4th (A) 5th (B) and 6th (C) female instar nymphs of H. littoralis with increasing concentration of azadirachtin.
instar) and 9 days (5th instar) in the control groups. At lower doses of azadirachtin (1.7 mg/g body weight) adults with curled wing tips and reduced longevity were produced. Other authors support these findings: Pener & Shalom (1987), Pener et al. (1989), Van der Host et al. (1989).

On the other hand treatment with higher doses (2.9 mg/g) resulted in death during the imaginal molt. Doses ranging between 6 and 7.3 mg/g body weight caused either death prior to the molt or greatly extended instar duration. Doses of 80 mg/g body weight resulted in death within 24 h [Mordue (Luntz) et al. 1985].

Also, nymphs of S. gregaria treated with azadirachtin survived beyond 40 days without molt and generally died during ecdysis (Rao & Subrahmanyam 1986, Nicol & Schmutterer 1991). Other grasshoppers treated with neem had abnormal nymph-adult molts as in Zonocerus variegatus (Olaifa et al. 1991).

It could be concluded that treatment of H. littoralis 4th, 5th and 6th instar nymphs with high concentration of azadirachtin resulted in high dose-dependent mortality rates during the period of ecdysis in comparison with control groups. At lower concentration, it caused prolongation of the stadium, leading finally to death. In some few cases malformed adults were able to emerge (Table 1).

Effects of azadirachtin on the fecundity and fertility of H. littoralis

Treated female suffered from decrease in number of egg pods/female in a dose dependent manner (Table 2). The average number decreased from 6.47 ± 2.21 (control group) to 2.00 ± 0.82 (females treated with 25 ppm). At higher doses (100 ppm and above) no egg-pods were deposited.

Such was also the case observed in azadirachtin-treated L. migratoria adults (Rembold and Sieber, 1981) as well as in Oncopeltus fasciatus (Heteroptera: Lygaeidae) (Dorn et al. 1987), Spodoptera exempta (Lepidoptera: Noctuidae) (Tanzubil and McCaffery, 1990), and in Sesamia calamistis (Lepidoptera: Noctuidae) and Eldana saccharina (Lepidoptera: Pyralidae) (Bruce et al. 2004).

Effects of azadirachtin on the female reproductive system

**Effects of azadirachtin on the ovary of H. littoralis.**—Female H. littoralis (10-d old adults) topically treated with azadirachtin (10 µl of 25 ppm) showed shrinkage in the whole ovary, besides a partially abolished oocyte growth, as only a few eggs were developed (Fig. 2). The whole organ seemed like the developing ovary of a nymph (3.5±0.3 cm in length, compared to the control 7.4±0.2 cm), while the average length of ovarioles in treated insects was 1.5±0.12 mm, compared to 8.0±0.58 mm in the control females.

**Fig. 2.** Normal (left) and azadirachtin-treated ovary (right) of H. littoralis. A, Ventral view; B, Dorsal view; C, Common oviduct; G, Accessory gland; L, Lateral oviduct; O, Ovariole; T, Terminal filament.

**Effects of azadirachtin on the ovary of H. littoralis.**—Female H. littoralis (10-d old adults) topically treated with azadirachtin (10 µl of 25 ppm) showed shrinkage in the whole ovary, besides a partially abolished oocyte growth, as only a few eggs were developed (Fig. 2). The whole organ seemed like the developing ovary of a nymph (3.5±0.3 cm in length, compared to the control 7.4±0.2 cm), while the average length of ovarioles in treated insects was 1.5±0.12 mm, compared to 8.0±0.58 mm in the control females.

**Fig. 3.** Transverse section in normal oocyte (left) with homogenous yolk and with regular follicular epithelium; treated (right) showing yolk mass separated from follicular epithelium as well as follicular epithelium totally destroyed. F, Follicle cells; N, Nucleus; V, Vacuole; Y, Yolk.
Table 2. Effect of different doses of azadirachtin on the longevity and egg production of *H. littoralis* (mean ± SD).

<table>
<thead>
<tr>
<th>Dose (Ppm)</th>
<th>Pre-ovip. Period</th>
<th>Ovip. Period</th>
<th>Post-ovip. period</th>
<th>Total</th>
<th>No. egg-pods/female</th>
<th>No. eggs/pod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.50 ± 3.14 (15 - 25)</td>
<td>42.60 ± 6.41 (36 - 61)*</td>
<td>63.0 ± 2.11 (3 - 11)</td>
<td>70.21 ± 3.11 (62 - 90)*</td>
<td>6.47 ± 2.21 (4 - 9)*</td>
<td>31.6 ± 6.0 (26 - 45)*</td>
</tr>
<tr>
<td>25</td>
<td>22.50 ± 2.96 (17 - 28)</td>
<td>8.70 ± 6.40 (1 - 21)*</td>
<td>2.70 ± 1.1 (1 - 4)</td>
<td>36.10 ± 5.6 (18 - 53)*</td>
<td>2.00 ± 0.82 (1 - 3)*</td>
<td>29 ± 4 (21 - 33)*</td>
</tr>
<tr>
<td>50</td>
<td>22.60 ± 4.58 (17 - 27)</td>
<td>7.60 ± 6.40 (1 - 18)*</td>
<td>2.20 ± 1.1 (1 - 3)</td>
<td>33.20 ± 6.4 (18 - 48)*</td>
<td>1.85 ± 0.69 (1 - 3)*</td>
<td>27 ± 3.6 (24 - 31)*</td>
</tr>
<tr>
<td>75</td>
<td>23.70 ± 4.06 (18 - 28)</td>
<td>8.60 ± 5.40 (1 - 15)*</td>
<td>2.40 ± 1.3 (1 - 5)</td>
<td>33.60 ± 5.9 (19 - 48)*</td>
<td>1.64 ± 0.67 (1 - 2)*</td>
<td>19 ± 2.4 (17 - 24)*</td>
</tr>
<tr>
<td>100</td>
<td>30.30 ± 2.60 (24 - 36)*</td>
<td>-</td>
<td>-</td>
<td>30.30 ± 2.60 (24 - 36)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>25.20 ± 1.20 (20 - 27)*</td>
<td>-</td>
<td>-</td>
<td>25.20 ± 1.20 (20 - 27)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>22.50 ± 1.0 (18 - 24)*</td>
<td>-</td>
<td>-</td>
<td>22.50 ± 1.10 (18 - 24)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>21.30 ± 3.30 (15 - 25)*</td>
<td>-</td>
<td>-</td>
<td>21.30 ± 3.30 (15 - 25)*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values followed by different superscripts (a, b, c) in the same column are significantly different \((P < 0.05)\).

*individuals died without laying eggs. ovip., oviposition.*

**Effects of azadirachtin on the histological components of the ovarioles.** — A normal ovariole in female *H. littoralis* is surrounded by a peritoneal coat of connective tissue which contains a reticulum of muscle fibers. The walls are formed of a layer of compact cuboid epithelial cells, resting upon a basement membrane (the follicular cells) (Fig. 3) and containing a homogenous yolk material. The pronucleus is more or less located in a central position. The matrix contains large yolk spheres and clear spaces near the apices of the follicular cells. At the end of the maturation stage, the follicular epithelial cells provide the vitelline membrane and chorion around the egg. Thus in *H. littoralis* the follicular cells play an important role in incorporation of yolk granules into the oocytes during vitellogenesis.

Treatment of 10-d-old adult females with 25 ppm of azadirachtin affected the above histological components as follows: the oocytes were degenerate, with faint yolk deposition. The follicular cell layer was folded on itself and the cells partly destroyed, their compact shape lost as compared with control specimens. The yolk material was no longer homogenous and contained many vacuoles. The follicular epithelia were irregularly developed, showing clear separation of yolk. In a few cases the oocyte succeeded in reaching the egg stage, but large vacuoles appeared between the yolk spheroles, and the eggs failed to complete their development as compared with the normal compact yolk spheroles in the control groups.

Electron micrographs showed that normal follicle cells appeared with homogenous ooplasm (Fig. 4). Inside the cytoplasm, the mitochondria contained normal cristae, indicating a well-developed capability for follicle-cell protein synthesis and secretion throughout vitellogenesis (Fig. 6). During vitellogenesis the nucleus and the nuclear membrane appeared normal and the cytoplasm was homogenous and rich in mitochondria (Fig. 8).

Many changes were observed in the histological structure of treated female ovarioles as shown by electron micrograph. During vitellogenesis the follicle cells appeared degenerated, with the presence of many vacuoles (Fig. 5). The contents of mitochondria were totally disintegrated without any cristae inside (Fig. 7). The ooplasm was no longer homogenous; the nucleus appeared with many lysosome-like bodies, indicating the beginning of cell lysis (Fig. 9).

Schmutterer *et al.* (1981, 1993) reported severe damage to the female ovarian follicles of *Locusta, Nomadacris,* and *Schistocerca* after treatment with azadirachtin. Also, Rembold (1987) reported delayed vitellogenin synthesis in azadirachtin-treated *L. migratoria,* causing severe damage to the oocytes. Shalom *et al.* (1988) showed inhibition of oocyte development and oocyte resorption in female *L. migratoria* after treatment with azadirachtin. The inhibition of ovarian follicle development was also reported by Ghosh *et al.* (1999) on *Gesonula punctifrons* (Orthoptera: Acrididae) treated with azadirachtin.

The effect of azadirachtin on histological components of the reproductive system, in insects belonging to orders other than Orthoptera, reveals different degrees of damage. Schulz and Schlüter, (1983) showed that neem caused dissolving of mitochondria inside the ooplasm in the ovarioles of *Epilachna varivestis* (Coleoptera: Coccinellidae). Autophagic vacuoles were formed and the prefollicular epithelium was partly destroyed and folded on itself. Jagannadh and Nair (1997) reported that azadirachtin induced a considerable reduction in the ovariole size and a delay in previtellogenic differentiation of oocytes, in addition to apparent degeneration of ovarioles in larvae, pupae and adults of *Spodoptera mauritia* (Lepidoptera: Noctuidae). Medina *et al.* (2004) reported that azadirachtin affected the ovarioles of *Chrysoperla carnea* (Neuroptera: Chrysopidae): growing follicles in treated females were significantly smaller than those of controls.

It seems that when azadirachtin is administered at an appropriate time, it can cause severe damage to the oocytes, probably as a result of interference with the vitellogenesis process. This may explain the blocking of the developmental process of ovarioles and consequently the shrinkage of the ovary (Fig. 2). Vitellogenesis is a rather complicated process, involving the deposition of yolk in the oocyte, resulting in a very rapid increase in size. Azadirachtin may inhibit vitellogenin synthesis or absorption which eventually leads to: inhibition of both oogenesis and ovarian ecdysteroid synthesis (Rembold & Sieber 1981), inhibition of ovarian development (Karnavar 1987), delaying of the vitellogenin synthesis process (Rembold 1987) and interference with vitellogenin synthesis and its absorption by the follicles (Schmutterer *et al.* 1981, 1993; Ghosh *et al.* 1999).
The effects of azadirachtin application may resemble those of juvenile hormone and its analogues, in respect to ovarian development, time of application and vitellogenin synthesis. Several authors have reported the effects of juvenile hormone analogues/minics on the histology of the reproductive system as well as oogenesis in different insect groups. Tobe and Pratt (1975) established a link between JH and ovarian maturation in *S. gregaria*, with a peak of JH synthesis at the onset of the previtellogenic period. Previtellogenesis occurred on days 5 to 6 in adult females and vitellogenesis occurred on days 7 to 8. Application of the JH mimic was timed with respect to the gonadotrophic cycle of individual insects.

Feyereisen and Tobe (1981) showed that oogenesis and vitellogenesis are inhibited by other JH analogues such as precocene in many insects. Eid *et al.* (1988) showed that ovaries of the female *S. gregaria* treated with precocene did not exhibit the vitellogenic stage, while in the normal females vitellogenesis started earlier. Ahi (1988) used another JH mimic (aldrin) on *Poekilocerus pictus* and mentioned abnormal fragmentation of oocytes with degeneration of the follicular epithelial cells. Polivanova and Triseleva (1989) also observed inhibition of vitellogenesis and sterilization of *L. mi*-

**Fig. 4.** Electron micrograph of normal follicle cell during middle vitellogenesis showing the homogenous ooplasm. N, nucleus.

**Fig. 6.** Electron micrograph of normal follicle cell near the end of vitellogenesis showing homogenous ooplasm and abundant mitochondria (M) with normal cristae (Cr).

**Fig. 8.** Electron micrograph of normal follicle cell during vitellogenesis showing a homogenous cytoplasm, rich in mitochondria (M). N, nucleus; NM, nuclear membrane.

**Fig. 5.** Electron micrograph of follicle cell in the treated insects during middle vitellogenesis; showing bursting of the follicle cell, disappearance of its contents and appearance of many vacuoles. N, nucleus; V, vacuole.

**Fig. 7.** Electron micrograph of follicle cell in the treated insect near the end of vitellogenesis showing nonhomogenous cytoplasm with damaged mitochondria (M) without cristae inside.

**Fig. 9.** Electron micrograph of follicle cell in the treated insect showing damaged mitochondria. Appearance of nucleus with many lysosome-like bodies (Ly). M, mitochondria; N, nucleus; NM, nuclear membrane.
Fig. 10. A, Normal sperm tube B, Treated sperm tube. G, germarium; M, maturation zone; Tr, transformation zone; ve, vas efferens.

Fig. 11. Sagittal section of a normal sperm tube showing spermatocyte maturation (Sp).

Fig. 12. Sagittal section in treated sperm tube in the apical zone showing nonhomogenous matrix and appearance of vacuoles and cell swelling. dc, degenerating cells; V, vacuoles.

Fig. 13. Sagittal section of a normal sperm tube showing the transformation of spermatides into sperm (sm); sperm bundle (sb) (arrow).

Fig. 14. Sagittal section in treated sperm tube in the transformation zone showing degeneration of the sperm bundles (dsb), and appearance of large vacuolated areas (V).
gratoria when last instars were fed on treated food with precocenes. Application of precocene and methoprene to Phormia regina (black blow fly) inhibited oocyte development and lowered the amount of vitellogenin, respectively (Yin et al. 1989). The follicular epithelium surrounding the vitellogenic oocytes of L. migratoria treated with the JH analogue, methoprene, was found to develop large spaces between the cells, with an eventual decrease in their volumes (Davey et al. 1993). Pinto et al. (2000) tested pyriproxyfen on Apis mellifera and reported the inhibition of vitellogenesis.

Effects of azadirachtin on the male reproductive system

Effects of azadirachtin on the sperm tubes of H. littoralis.—Treated sperm tubes showed a swelling in the zone of growth with a slight reduction in its length (Fig. 10b). A follicle in treated males measures 5.15 ± 0.11 mm in mean length while its mean width is 0.55 ± 0.13, these values were significantly larger than the control which measures 6.25 ± 0.21 mm in average length, its average width at the maturation zone being 0.3 ± 0.02.

Effects of azadirachtin on the histological components of the sperm tubes.—Histologically, sagittal and transverse sections in the apical part of a sperm tube of males treated with low doses of azadirachtin (25 ppm), showed swelling of the dividing cells; some cells were destroyed and cells in the central part were smaller in size as compared with the control group (Figs 11, 12). In the transformation zone there was clear cell damage, total lyses, and the tissue lost its consistency with the occurrence of many vacuoles. The testicular epithelia in the transformation zone were completely disintegrated; the spermatids were degenerated leaving vacuolated areas. The control group formed normal sperm (Figs 13, 14). In the maturation zone there was disintegration of the epithelial layer, while the matrix appears with many vacuoles and totally damaged cells. In the terminal zone there is degeneration of the sperm bundles and the peritoneal membrane has become finer, with the appearance of large vacuolated areas.

Electron micrographs of follicles from untreated males showed that the spermatocytes undergo their division and development normally (Fig. 15) then complete their differentiation and transformation till sperm is finally liberated (Fig. 16). In insects treated with 200 ppm azadirachtin, the cyst formation around the spermatogonia in the gerarium was inhibited without any further development to spermatocytes and with the appearance of many vacuoles (Figs 17, 18). The electron micrographs also showed a clear separation of the basement membrane from the peritoneal membrane.

Schistocerca gregaria treated with azadirachtin suffered from arrested spermatogenic meiosis at Metaphase I (Linton et al. 1997). In Epilachna varivestis (Coleoptera: Coccinellidae) degeneration of
the sperm bundles without sperm formation has been reported by Schulz and Schlüter (1983). Disintegration of the germ cells and degeneration of the sperm bundles in the testes were also observed by Abdel-Rahman et al. (2004) after treatment of the male Pectinophora gossypiella (Lepidoptera: Gelechiidae) with azadirachtin. Shimizu (1988) showed that azadirachtin caused degeneration of spermatocytes in males of Mamestra brassicae (Lepidoptera: Pieridae), suggesting a direct effect of azadirachtin on the testicular membrane, rendering the tissue incapable of developing spermatocytes.

A review of the literature concerning the effects of some other insect growth regulators on insect gonads showed that aldrin caused pycnosis of germ cells leading to incomplete spermiogenesis in Poekilocerus pictus (Ahi 1988).

However, it should be pointed out that the factors which regulate spermatogenesis are not well understood (Dumser 1980). There is no strong evidence to indicate that hormones are generally involved (Engelmann 1970), but in some moths, the molting hormone could facilitate the process by increasing the permeability of the wall of the testis to some macromolecular factors (Wilde & Loof 1973). It seems that at higher concentrations azadirachtin prevents the spermatogonia from cyst formation and therefore the spermatocytes fail to make their normal divisions and complete spermiogenesis. On the other hand, at lower concentrations the process of spermatocyte formation proceeds till the appearance of the sperm bundles, but with aberrations and degenerations.

References


